

INTRODUCTORY STUDY OF THE EFFECT OF ENHEPTIN (2-AMINO, 5-NITROTHIAZOLE)
ON THE HOST-PARASITE RELATIONSHIP IN BLACKHEAD

by

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Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

1953

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I

INTRODUCTION

In 1895 Theobald Smith published an account of a highly infectious and fatal disease of turkeys which he named infectious enterohepatitis. The usual symptoms of the disease are a ruffling of the feathers, a rather dejected appearance, lack of appetite, leg weakness, and sulfur colored droppings. As the disease progresses the birds become weaker and show a disinclination to move about or even stand. Although the disease is commonly called blackhead, in reality darkening of the head is not a constant symptom; this usage, however, is firmly established in the literature.

The etiologic agent, Histomonas meleagridis, enters the body of the host in food or water contaminated by the droppings of affected or carrier birds. The site of primary attack is one or both ceca. The protozoon produces extensive ulcerations of the cecal wall and is presumably carried via the blood to the liver, where secondary foci are established.

Previously unexposed turkeys of any age are susceptible to fatal infections, however, flocks which have been exposed to contaminated surroundings without medication usually show a high proportion of individuals having various degrees of immunity.

Chickens are not, as a rule, subject to fatal infections regardless of previous experience; age, however, is of some importance. Birds from 3 to 6 weeks old are most susceptible.

Several drugs have been found useful in the treatment of blackhead, the one in widest use today is enheptin (2-amino,5-nitrothiazole). This drug is of greatest value when fed continuously at a prophylactic level (0.05 percent), incorporated in the mash. Several undesirable side effect have been noted; there is a slight growth retardation and fertility and hatchability are lowered.

Although numerous investigations have confirmed the prophylactic value of enheptin, little is known concerning its effect on the development of immunity. The phenomenon of development of immunity during prophylactic treatment has been observed to occur in avian coccidiosis; the possible absence of this mechanism in blackhead of turkeys was suggested by certain observations of DeVolt and Tromba (1950). In an experiment attempting to ascertain whether increased resistance to blackhead could be shown by poultts which had been exposed while being treated with enheptin, they found that after medication was removed and a second exposure effected, these poultts showed clinical symptoms and death before identically exposed controls.

In attacking the problem, it seemed advisable at first to study the effect of enheptin on Histomonas meleagridis in the cecal contents. This could best be done by using chickens, which are not usually subject to a fatal case of blackhead and thus would provide suitable hosts for extended observations.

After these preliminary observations the exposure of groups of turkeys to blackhead both by means of contaminated soil, and culture inoculation would serve a twofold purpose; the use of large numbers of birds under controlled conditions of medication would permit a critical evaluation of the immune mechanism during treatment; further

data could be gathered on the effect of enheptin on the organisms in the cecal lumen.

Apart from the main problem several correlated experiments were performed to determine whether other prophylactic levels of enheptin could be established which would eliminate or minimize undesirable side effects. In addition the prophylactic value of another drug was evaluated.

II

HISTORICAL REVIEW

A. Etiologic agent

A disease known as blackhead, typhlitis, or infectious enterohepatitis was reported from turkeys by Cushman in 1893. Theobald Smith (1895) described as Ameba meleagridis a protozoan which he found in cecal and liver lesions of turkeys affected with this disease. Cole, Hadley, and Kirkpatrick (1910) believed that the organism seen by Smith was the schizogony stage of a coccidium. Jowett (1911) came to the conclusion that the lesions in blackhead were due to a Trichomonas which had become aflagellate in the tissues. Hadley (1916) stated that he had seen flagella on some of the tissue forms and considered them to be Trichomonas. Tyzzer (1919) returned to Smith's original view that the organism is actually an ameba. In a later investigation Tyzzer (1920) showed that the amoeboid bodies were capable of a distinctive jerky motion when seen alive. In sections and smears Tyzzer demonstrated the presence of axonemes originating at a blepharoplast, and the formation of a paradesmose or fibril between daughter blepharoplasts at division; these observations suggested flagellate affinities. Tyzzer, however, rejected the idea that they were Trichomonas and instead regarded them as aberrant flagellates. He proposed Histomonas meleagridis as the name for this organism originally described by Smith as Ameba meleagridis.

Much of the confusion in the early description of H. meleagridis was due to its variability in form when seen in liver and cecal

lesions. Tyzzer (1919) in conjunction with his studies on the etiology of the disease described three stages which may appear in lesions. The first or invasive stage is found most frequently in early lesions. The organisms are distinctly amoeboid and measure from 8 to 17 microns in diameter in fixed tissue. They occur at the periphery of involved areas but are not usually found in older lesions. They may be found in the gland epithelium of the ceca, in the goblet cells, occasionally in the lumen of the glands, between the muscle layers of the ceca, and, in general, in almost any part of the liver or ceca involved in the disease. Tyzzer found that these forms were basophilic when stained with eosin and methylene-blue or Giemsa. The ectoplasm is abundant and clear while the endoplasm contains a number of stainable particles which may represent ingested food material.

The second or vegetative stage (Tyzzer) appears in older lesions and measures from 12 to 21 microns in diameter in fixed tissue. These organisms have a basophilic cytoplasm and lack the inclusions noted in the invasive stage. Groups of these organisms occur tightly packed together so that their surfaces seem to form a reticulum in which the interstices are relatively clear (Fig.8). Globular or ring-like bodies may appear in the cytoplasm of such forms but they are not accompanied by any nuclear changes which would suggest division nor are they consistent as to size or number.

The third or resistant phase, according to Tyzzer, is characterized by the development of a refractive cell membrane and an increase in coagulable material. They are the smallest of the three phases,

measuring from 4 to 11 microns in fixed material. Groups of these organisms may be found enclosed in spaces by the reaction of the tissue or engulfed by phagocytic cells. Groups of organisms taken up by phagocytes might simulate the schizogony stages described by Smith (1895, 1915) and Hadley et. al. (1910). Tyzzer, however, pointed out that there is no consistency in number of organisms, nor is there any residual material associated with such groups.

Free flagellated forms were first seen by Tyzzer and Fabyan (1922) in material from the ceca of infected turkeys. Tyzzer (1934) described the motility of H. meleagridis in cecal discharges and found that at about 42 C rhythmic rotary movements and active amoeboid motion were apparent. A characteristic counter-clockwise motion turning the organism one-fourth to one-third of a full rotation is produced at each beat of the flagella.

Although Tyzzer's work apparently settled the problem of the etiologic agent, reports ascribing the disease to other organisms subsequently appeared. Menzani (1933) and Enigk (1933) described a budding fungus of the blastocystis type as the sole organism isolated from the liver in certain cases of blackhead. Allen (1936) found a Pentatrichomonas in liver lesions of guinea fowl and reported a successful infection in turkeys with organisms cultured from these lesions. DeVolt and Davis (1936) isolated Trichomonas from liver lesions on several occasions but did not consider them to be primary invaders. Niimi (1936) reported that he isolated 1 strain of Trichomonas and 5 strains of Chilomastix from the livers of chickens naturally infected with blackhead. Bishop (1938) cultivated Trichomonas from hepatic lesions but could demonstrate

only Histomonas in sections of these lesions and concluded that the Trichomonas was a secondary invader. Allen (1941) reported macroscopic differences in lesions due to Trichomonas which distinguished them from those caused by Histomonas. Unequivocal proof of the identity of Histomonas meleagridis as the principal if not the sole etiologic agent has been furnished by the recent work of Harrison, Hansen, DeVolt, Holst, and Tromba (1953). Typical liver lesions were produced by intrahepatic inoculations of bacteria free suspensions of Histomonas meleagridis, while similar inoculations of protozoan-free filtrates of this suspension uniformly failed to produce any evidences of blackhead.

B, Mode of transmission

Under natural conditions the transmission of blackhead may occur in two ways; directly by trophozoites in feces without the interposition of a resistant form, or indirectly by histomonads incorporated in cecal worm eggs. Experimentally it has been transmitted by inoculation of suspensions of infected organs, or culture material. Possible mechanical transmission by arthropods has been noted by DeVolt and Davis (1936).

Curtice (1907b) showed that young turkeys could be infected by exposure to contaminated soil. Smith (1915) reported the production of blackhead in turkeys reared in close proximity to a hen-yard and also the infection of turkeys by feeding the ceca and liver of diseased birds. Cysts were noticed in the feces of diseased turkeys and presumed to be encysted amoebae although their relation to the tissue parasites was not determined. Tyzzer(1919)

states that these forms are undoubtedly blastocysts. Tyzzer and Fabyan (1920) confirmed transmission by infected soil as did also DeVolt and Davis (1936).

Tyzzer and Fabyan (1920) disproved the existence of a resistant state by showing that histomonads in tissue lesions and feces were found to remain viable for only 24 hours at room temperature. Bishop (1938) reported that cultures of H. meleagridis did not survive exposure to room temperature of (18-22 C) for more than 24 hours. Lower temperatures appeared to be equally detrimental; no living organisms being found after 24 and 48 hours at 5 C. This evidence, however, failed to explain the observation of soil borne infections.

Graybill and Smith (1920), however, showed that blackhead could be produced by feeding the embryonated eggs of the cecal worm "Heterakis papillosa" = Heterakis gallinae to healthy turkeys. Tyzzer and Fabyan (1922) collected cecal worms from chickens and treated them with 1.5 percent of nitric acid until the eggs became embryonated (usually about 13 days). Tests at the end of 3 days showed this material to be bacteriologically sterile. Ova treated in this manner regularly produced blackhead when fed to incubator reared poults. In order to demonstrate again the absence of a resistant form, liver lesions, cecal lesions, and cecal cores were immersed in 1.5 percent nitric acid for an equal period. Materials so treated uniformly failed to produce infection, as did the feeding of unembryonated eggs or of male Heterakis. Tyzzer (1934) observed and figured H. meleagridis in the gut epithelium of 11-day-old Heterakis gallinae and also of Heterakis exposed to winter temperatures in soil. Although no worker has reported the culture of Histomonas from sterilized Heterakis ova Niimi (1937) claims to have seen the

causative organism in the eggs of "Heterakis papillosa" = Heterakis gallinae. He stated that it measured 1 to 1.4 microns and was in the form of a mononuclear round cell; this report is as yet unconfirmed. Bishop, while recognizing the transmission of infection by the cecal worm egg concluded, "There is obviously still some factor in the method of transmission of blackhead which is not yet clearly understood."¹

Another source of infection, from carrier chickens, has long been recognized. Curtice (1907a, 1907b) noted that the chicken is occasionally subject to blackhead and observed the causative organisms in the tissues of chickens. He stated that the ordinary fowl was a carrier and recommended that turkeys be kept away from other domestic fowl. Smith (1915) and Tyzzer and Fabyan (1920) infected turkeys by exposure to chickens. Reports of blackhead in chickens are not uncommon and have come from many localities. Since chickens are also commonly infected with cecal worms they are regarded as an important factor in the transmission of blackhead.

Experiments to determine arthropods and other organisms as possible vectors have mostly proven negative. Smith (1895) observed that not all flocks were infected as would be expected if insects were involved. Curtice (1907b) stated that poults which he raised disease-free had an opportunity to eat many kinds of insects. Tyzzer and Fabyan (1920) fed a turkey crickets, grasshoppers, and also flies which had fed on lung lesions from a case of blackhead. These birds showed no evidences of disease. However, DeVolt and Davis (1936) exposed 67 poults to flies

¹Bishop, A. 1938. Histomonas meleagridis in domestic fowls (Gallus gallus). Cultivation and experimental infection. Parasitology 30(2):192.

and obtained 5 cases of blackhead; they concluded that flies may act as mechanical vectors. Cram (1927) reported the earthworm to be a mechanical vector for the eggs of Heterakis gallinae. Wild birds, especially sparrows, which are commonly found around poultry yards may also serve as accidental vectors. Such examples of accidental transmission are by themselves of minor importance; however, they form part of the total picture of the epidemiology of blackhead.

The possibility of transmission through the turkey egg is negative as shown by Curtice (1907a), Smith (1915, 1917), and Tyzzer and Collier (1925). All of the eggs from which poults hatched came from infected flocks, yet none of the control birds used in their infection experiments developed blackhead.

Experimental production of blackhead by other than natural routes has been reported: Tyzzer (1920) subcutaneous inoculation of minced liver lesions; DeVolt and Davis (1936) subcutaneous and intrasinal inoculation with minced liver lesions; and Harrison, Hansen, DeVolt, Holst, and Tromba (1953) intrahepatic inoculation with H. meleagridis in bacteria-free suspensions.

C. Pathology

In the typical case of blackhead primary lesions occur in one or both ceca. Pathology in naturally occurring cases is usually limited to the ceca and liver, however, lesions in other organs have been reported. Smith (1915) found lesions in the proventriculus, small intestine, and kidney; Levine (1947) also reported kidney lesions.

Smith (1895, 1915) first described the gross and microscopic appearance of the lesions in blackhead and regarded the site of primary invasion

as the blind end of the cecum. Hadley (1917) believed that invasion was through the goblet cells in the crypts of Lieberkuhn. Tyzzer (1919) also reported invasion through the goblet cells. Jowett (1911) advanced the theory of a secondary invasion of the protozoan following damage by helminths or other organisms. Farmer, Hughes, and Whiting (1951) found no conclusive evidence as to the mode of primary invasion of the ceca.

As the disease progresses inflammatory and ulcerative changes appear in parts or most of the ceca. In most cases the organ is enlarged and indurated and may contain a core of sloughed cells and exudate. In some cases the disease terminates fatally at this point but in most instances metastasis occurs to the liver. A diseased liver is enlarged and spotted with areas of necrotic and degenerative tissue. The lesions are roughly circular, up to one centimeter or more in diameter, are usually somewhat depressed, and may exhibit a yellowish cast. In acute cases in young birds the lesions may be small and numerous, while in older birds the lesions are often merged or replaced by scar tissue. Microscopically there is no sharp demarcation between diseased and healthy tissue. In sections of liver lesions extensive destruction of tissue in the necrotic areas coupled with an invasion of leucocytes and giant cells is seen. Histomonads in this area are usually found in groups or enclosed in giant cells, (Tyzzer's resistant phase). At the edges of the lesion or in younger lesions the parasites are found to be more scattered and lying between the intact cells of the liver cords. Farmer, Hughes, and Whiting (1951) studied the pathology of the artificially induced disease; their findings were substantially in agreement with those of Smith (1915) and Tyzzer (1919).

Johnson and Lange (1938) studied the blood picture in artificially

infected poult and reported marked changes. These were: a sudden heterophilia beginning within 24 hours after infection and continuing until death; an irregular monocytosis in some cases; myelocytosis and anemia in moribund birds. McGuire and Cavett (1952) reported that the blood non-protein nitrogen and uric acid at first decrease, but return to normal before death. They also observed that blood glucose increases during the disease and eventually a severe hypoglycemia develops, which these authors consider may be the immediate cause of death.

D. Medicinal treatment

The practical administration of drugs to poultry is limited by certain organizational and economic factors. Because of the low monetary value of the individual, treatment of a single sick fowl is impractical. The poultryman can accept with equanimity a certain percentage loss which in other fields of animal husbandry would be ruinous. Furthermore when flocks numbering in the thousands are to be treated the drug must be one that can be used with a minimum of effort. These conditions practically eliminate the use of any medicinal agent which must be given parenterally and limit the field to those compounds which are effective through the digestive tract and can be dissolved in water or mixed with feed. A further limitation is that of price; since mass treatment is the method of choice the drug must not add materially to the cost of marketing the individual. The force of this economic mandate is evident.

Early attempts to find a specific for the treatment of blackhead were somewhat influenced by the assumed resemblance of Histomonas meleagridis to Endamoeba histolytica. Tyzzer and Fabyan (1920) found

that an infusion of the drug "chaparro amargosa" (prepared from the plant Castela nicholsoni, Simarubaceae) an effective therapeutic for amoebic dysentery, had no value against enterohepatitis. Tyzzer and Fabyan (1922) also found ipecac, emetine (the active principle of ipecac), and sulfur to be ineffective. Tyzzer (1923) conducted preliminary tests on the value of neoarsphenamine, arsenious acid, atoxyl, and tryparsamide; of the four tryparsamide alone showed enough promise to be recommended for further work. Eriksen (1925) also showed neoarsphenamine to be ineffective. Bolin and Vardiman (1941) claimed activity for mapharsen but their data were insufficient. A more critical evaluation by Jaquette and Marsden (1947) showed mapharsen to have no value. Clover (1948) found mapharsen and Formula 1144 (a quaternary ammonium compound) ineffective. Sautter and Pomeroy (1950) tested various arsenicals and found that stovarsol and tryparsamide had a marked prophylactic effect. DeVolt and Holst (1948) found that vioform lowered the death rate significantly. DeVolt and Holst (1949) also found clcioform to be effective against artificially induced blackhead but of no value in the naturally occurring disease. McGregor (1949) found metachloridine, bacitracin, tetramethylthiuram disulphide, tetraethylthiuram disulphide, hexachloroethane, sulphaquinoxaline, compound P-196, and compound P-29 of no value. Waletsky, Clark, and Marson (1950) were first to find enheptin (2-amino,5-nitrothiazole) effective in treating blackhead. Jungherr and Winn (1950) confirmed the work of Waletsky et. al. as did DeVolt, Holst, and Tromba (1951). McGregor (1951) reported that antrycide and vioform did not produce significant lowering of the death rate in turkeys infected by contaminated drinking water.

Farmer (1950) tested arsenious trioxide, neoarsphenamine, sulph-

arsphenamine, arsphenamine diglucoside, acetarsol, vioform, clioform, a formaldehyde sulfathiazole condensation product, iodobismuthate of quinine, a bismuth salt of ethylhydrogen cyclohexane, and mepacrine hydrochloride. Of these only acetarsol, vioform, and arsphenamine diglucoside were effective in preventing blackhead. Swales (1950) evaluated the action of chloroquine diphosphate, dihydroxyquinoline, phenyl mercuric nitrate, streptomycin, acetarsol, mercuric nitrate, carbarsone oxide, l-pentane arsonic acid, arsphenamine diglucoside, 8-amino-5,7-diiodoquinaldinic acid, neoarsphenamine, 4-(3'4'-dichlorobenzylamino)-benzoic acid, 1 percent solution of arsenic, arsenious acid solution, arsenic diethyldithiocarbamate, bismuth dithiocarbamate, sodium diethyl dithiocarbamate, and mapharsen. The lowest mortality recorded for any of these compounds was 40 per cent.

Berks and Neal (1952) used an in vitro method for testing enheptin, vioform, tryparsamide, reduced tryparsamide, acetarsol, penicillin, and streptomycin. Enheptin and vioform prevented growth at a dilution of 10^{-4} but were ineffective at higher dilutions. None of the other compounds tested were as effective, although penicillin and streptomycin apparently inhibited the growth of H. meleagridis by acting on the associated bacterial flora.

E. Methods of experimental infection

Experimental infections with Histomonas meleagridis may be produced in two ways; by exposure to contaminated soil or by inoculation or feeding of infective material. The former method has been employed by Curtice (1907a, 1907b), Smith (1915), Tyzzer and Fabyan (1920), DeVolt and Davis (1936), and others. It is a simple straightforward procedure

having the advantage of closely approximating natural conditions of infection. However, since morbidity and mortality may be highly variable it is often desirable to employ other methods.

Tyzzer and Fabyan (1920) first demonstrated inoculated blackhead by using an intramuscular injection of minced liver lesions. Six serial passages were made with a fatal termination in each case. Lesions were demonstrable at the site of inoculation and also in the lungs and liver. This method was not particularly applicable to drug screening, however, and other methods were devised by subsequent workers. Tyzzer (1934, 1936) successfully infected chickens and turkeys by inoculation with histomonads cultured at 37 C. McGregor (1949) fed a mixture of embryonated Heterakis ova, strained chicken feces, and earth from a run in which blackhead was known to be enzootic. Equal amounts were given in the drinking water every day for 16 days, or until blackhead began to produce deaths in the flock. The highest mortality produced by this method was 77.1 per cent. Farmer and Stephenson (1949) reviewed several methods of infection and recommended rectal inoculations with 2.5 ml of a suspension of finely chopped cecal lesions obtained from birds dying of blackhead. Using this method (preceded by an enema of 5 ml of Ringer's solution) these authors reported an 87.5 per cent mortality. DeVolt and Holst (1948, 1949) achieved high mortality (92.9 per cent) by rectal inoculation of cultured histomonads. Using this method a pre-treatment with Ringer's was not necessary and the amount of inoculum could be reduced.

F. The cultivation of Histomonas meleagridis

Histomonas meleagridis has been cultured in several different media with varying degrees of success.

Urbohlav (1924) employed a Locke-egg-serum medium utilized in the study of Endameba histolytica. While this medium was successful, a heavy overgrowth of bacteria usually occurred after several days. Tyzzer (1934) used an agar slant prepared with egg albumin and buffered to pH 7.2. The overlay consisted of normal saline containing 5 per cent sterile horse serum. Sterile rice starch was added just before use. This medium was reported to give good growth and to be less favorable for the growth of associated bacteria. Bishop (1938) cultivated the protozoon in a medium developed by Dobell and Laidlaw (1926) for the cultivation of E. histolytica, however attempts to use the liver medium of Cleveland and Sanders (1930) failed due to heavy overgrowth of bacteria. Bishop also cultured the organism in the horse serum-saline mixture employed by Laidlaw, Dobell, and Bishop (1928) in the study of E. histolytica. DeVolt and Davis (1936) used a Locke-egg-serum medium containing turkey serum and albumin, or serum alone, with subsequent additions of sterile rice starch. DeVolt (1943) reported a new monophasic medium consisting of 2 per cent turkey serum in Locke's solution adjusted to pH 9.0 by the addition of N/20 sodium hydroxide. The medium is tubed and autoclaved; sterile rice starch being added just before use. Best growth is obtained at 42 C; cultures have been successfully maintained in this medium by the writer for 18 months with transfers being made twice weekly. The chief advantage of this medium is the elimination of the diphasic system with its attendant problems of contamination.

III

MATERIALS AND METHODS

A. General techniques

White Holland turkeys, and Rhode Island Red chickens were used in these experiments. Day-old poults were obtained from several commercial hatcheries and reared in brooders. Chicks were furnished by the Poultry Department of the University of Maryland. All birds were wing banded for identification and at about 4 weeks of age were removed to wire floored cages in an isolated building.

Two methods of exposure were used: rectal inoculation with Histomonas meleagridis from culture; and exposure to contaminated soil.

New cultures of H. meleagridis were isolated at intervals and maintained for varying periods using DeVolt's medium. Three cultures were used in these investigations and are designated cultures 1, 2, and 3. Culture 1 was isolated September 30, 1949 by H.M. DeVolt; the writer isolated culture 2 on July 24, 1951, and culture 3 on June 6, 1952. By 1951, when this study was begun, culture 1 had become avirulent as determined by its failure to produce mortality in young turkeys. Cultures 2 and 3, however, were fully virulent at the times they were used.

Isolations were made by placing several loopfuls of scrapings from the ceca of an infected bird into tubes of medium. Although an initially large growth of bacteria accompanies this method, it is more successful than isolations attempted from liver lesions which according to

Harrison (1952) are often free from bacteria.

A culture was considered established after 3 weeks of successful transfers; it could then be used for inoculum. This period of 3 weeks was necessary in order to eliminate the possibility of confusing hold-over forms with a propagating culture. Usually 12 tubes of a culture were carried from one transfer to the next. However, when material for inoculation was needed the number of tubes was increased so that adequate numbers of organisms were on hand, both for inoculum and for continuance of the culture. Three-day-old cultures were always used for inoculation; a pool of all the tubes was made, transfer into fresh medium was then made and the remainder used for inoculum.

The method employed by DeVolt and Holst (1948, 1949) for rectal inoculations was used for the most part; several modifications were introduced in some cases and will be described in part B. of this section.

After the number of organisms per ml was determined by hemocytometer count, a rectal inoculation of 0.1 ml was administered. In most cases a second and third inoculation of 0.1 ml was given from the same pool, an hour elapsing between doses. During inoculation the material was agitated frequently to insure a uniform suspension. In order that cloacal contractions would not expel the inoculum, the birds were held for about a minute following administration of the dose. If expulsion occurred another 0.1 ml was given immediately.

In the natural exposure groups the birds were placed in wooden poultry houses each divided in half by a screen partition and given free access to communicating screened cages. A layer of contaminated soil taken from nearby poultry yards was distributed to a depth of

3 to 4 inches on the floor of both house and cage. The dirt was well mixed before placing it in the house, and was stirred on the floor at 1 to 2 week intervals. In order to provide the maximum opportunity for infection, the houses were not cleaned during the course of the experiment.

In all experiments the liver and ceca of birds dying or sacrificed were examined for gross lesions and the cecal contents was examined microscopically for the presence of H. meleagridis. Cause of death was recorded as blackhead or "other causes". It may be noted here that the experimental birds were almost entirely free of cecal worms; the few infected individuals being all from the natural exposure groups.

Methods of drug administration were by mixing with a standard growing mash or by dissolving the compound in the drinking water. To medicate feed an appropriate amount of a 20 per cent pre-mix was first thoroughly mixed with 10 pounds of mash. This was then added to 40 pounds of mash and mixed for 5 minutes in an electric mixer. The medicated feed was then stored in labeled, covered cans.

Medicated drinking water was prepared fresh daily and was placed before the birds in an earthenware container as their sole source of water. On particularly warm days consumption was checked about 3 P.M. and, if necessary, more medicated water was added.

In all experiments in which medication was used, a 24 to 48 hour preliminary feeding period of the medicated group was employed prior to exposure.

B. Establishing and evaluating carriers

1. Inoculation of experimental birds. In attempting to establish

carriers some variation in number of inoculations, concentration, and culture strains was employed.

In experiment 1 a single group of 20 Rhode Island Red chicks was used. These birds each received an initial inoculation of 3.8×10^3 histomonads from culture 1. Seven days later each bird was dosed with 1.2×10^4 organisms from culture 2. A third inoculation was given 1 week later and consisted of 1.5×10^4 histomonads from culture 2.

In the second experiment 2 groups of Rhode Island Red chicks were established and designated groups A and B. Each bird in group A received 1.2×10^5 histomonads from culture 1, while the chickens in group B were inoculated with 1.2×10^5 histomonads of culture 2.

In experiment 3 the effect of serial dilution of inoculum was tested on 20 White Holland turkey poults and 20 Rhode Island Red chicks divided into 10 groups of 4 birds each. A pool of organisms was made from culture 2 and the concentration was determined by hemocytometer count to be 14.5×10^5 histomonads per ml. A dilution series of 10 tubes was set up; tube 1 received 6 ml. of the pooled inoculum and tubes 2 through 10 received 2 ml of sterile culture medium. Using a 10 ml pipette, 2 ml of the inoculum was withdrawn from tube 1 and introduced into tube 2. Mixing was effected by drawing up and releasing the suspension 3 times. A 2 ml aliquot was then withdrawn and transferred to tube 3. In a like manner, using the same pipette the procedure was repeated through tube 10. This resulted in a series of dilutions ranging from the undiluted pool to a dilution of 1 to 19,683 at which point the concentration had diminished to 73 organisms per ml. Several of the dilution were checked by hemocytometer count and a close agreement with the calculated dilutions was found.

A 1 ml pipette was placed in each tube of inoculum and was thereafter removed only for inoculation. A series of 3 rectal inoculations of 0.1 ml each was given at intervals of 1 hour to the 2 chickens and 2 turkeys of each group.

2. The treatment of carriers. Carrier birds established were removed to clean wire floored cages and divided into 2 groups. The medicated group was placed on a ration containing 0.05 per cent en-heptin; the controls were fed a standard growing mash.

3. The examination of cecal droppings. In these and in other experiments it was necessary to make daily examinations of cecal droppings to determine the presence or absence of H. meleagridis. Cecal droppings can be distinguished from main bowel evacuations by their color, consistency, and form. Material from the ceca is usually light to chocolate brown in color, finely granular, and unformed. That from the main bowel is variable in color, coarse or fibrinous, and usually cylindrical. It was found convenient to collect droppings in small (25x65 mm) jars. These jars were fitted with cork stoppers through which was driven a length of stiff 22 gauge wire bent into a loop at one end. Each bottle was labeled to correspond with the wing band number of an experimental bird. Each bottle with its accompanying cork and wire was thoroughly washed and dried before each re-use.

Procedures for collections and examinations were standardized. Collections were made at about 9 A.M. each morning. This time was chosen because cecal evacuations usually occur before 9 in normal birds, and fresh specimens could then be collected. As much of the dropping as could be gathered from the pan was transferred to the appropriate

bottle by the wire loop. To each bottle 2.5 ml of Ringer's solution had been added previously. The droppings were then thoroughly mixed in the solution and placed in an incubator set to 42 C; after 30 minutes specimens were withdrawn for examination. Each bottle was again thoroughly shaken to insure a uniform suspension and a loopful of this suspension was removed, placed on a clean glass slide and covered with a 22 mm No. 1 cover slip. Examinations were made with a compound binocular microscope at a magnification of 440 x. A specimen was not declared negative until 100 fields had been examined. Positives were recorded after finding at least one organism showing typical morphology and motility. Findings were recorded as either positive or negative for H. meleagridis. The presence of other protozoa such as coccidia, trichomonads, Chilomastix sp. or ameba was also recorded. In some cases not every bird had passed cecal droppings at the time of collection. In that event their pans were checked several more time during the morning. If a dropping had not appeared by noon an absence was recorded for that day. Usually at the conclusion of the days collections the dropping pans were scraped clean, although on occasion several days droppings were allowed to accumulate.

C. Immunity trials

1. Natural exposure experiments. Four experiments employing 240 poults were performed, 3 used contaminated top soil placed on the floor of the house and cage and 1 employed exposure to contaminated yards and houses. In all experiments 1 medicated and 2 control groups each consisting of 20 birds were established. The first control group, designated infection controls, was introduced at the beginning of each experiment to indicate the degree of blackhead exposure to which the medicated group was

subjected. The second control group, designated immunity controls, consisted of poult from the same brood held in reserve and neither medicated nor exposed. This group was introduced into the experimental house after cessation of medication to indicate the degree of resistance to blackhead that could be expected in poult neither previously exposed to the disease nor treated with the drug. In addition to wing banding, different colored leg bands were used for ready identification of the 3 groups. In all experiments a standard growing mash, medicated with enheptin at a concentration of 0.05 per cent was fed to the medicated groups. A preliminary drug feeding period of 48 hours was employed for the medicated groups in all experiments.

The natural exposure experiments were divided into 2 periods of 2 months each. During the first period both medicated and control groups were exposed to blackhead. During the second period drug feeding was discontinued. Then, previously medicated poult, survivors from the infection control group, and the added immunity control group were simultaneously exposed to blackhead. An equal degree of exposure for all groups during the second period was obtained by removing the wire partition and allowing the poult free access to all parts of the house and cage. In the case of the experiment in which the birds were exposed in yards, the gates were wired open and the poult allowed to range freely in the 2 yards.

2. Exposure by rectal inoculation. The experiments employing rectal inoculation were divided into 2 periods of 15 days each. As in the case of the natural exposure experiments 1 medicated and 2 control groups were used. Initially both medicated and control groups received a rectal inoculation of histomonads from culture, while the immunity controls

were held in reserve. At the end of the first period half of the medicated birds were placed in the cages previously occupied by the infection controls and half were left in their original cages. The immunity controls were also divided into 2 groups, half occupying cages previously used by infection controls and half entered cages previously occupied by medicated birds. No further inoculations were given during the second period and all birds received unmedicated mash. The immunity controls served a somewhat different purpose in these experiments since they were not exposed by inoculation as were the medicated and infection control groups. However, since they were exposed to the same environment as the medicated group they would indicate whether cases of blackhead appearing in the medicated group were due to latent foci or to contamination of the environment.

In the first experiment 30 White Holland poultts were divided into the 3 designated groups. The medicated and infection control groups were each inoculated with 3.6×10^5 histomonads from culture 3. During the first 15 day period the cecal droppings of the medicated group were examined daily. At the end of the period the immunity controls were introduced in the manner outlined above.

A second experiment employed similar techniques. Culture 3 was again the source of inoculum; each poult in the medicated and infection control groups received 1.5×10^5 histomonads. Daily cecal dropping examinations of the medicated poultts were carried out during the first period. At the end of this period 5 medicated birds were sacrificed and pieces of cecal and liver tissue from each were fixed in Bouin's fluid. The tissues were dehydrated and embedded in paraffin in the usual manner; then sectioned on a rotary microtome at 6 microns, and stained in

Delafield's hematoxylin-eosin-azure II. The remaining medicated poultts and introduced immunity controls were observed for the second 15 day period.

D. Low level and intermittent feeding of enheptin

1. Low level feeding of enheptin. Feed medicated with enheptin at a concentration of 0.025 per cent was prepared in the manner previously described and fed to a group of 23 poultts housed in wire floored cages. After 48 hours they were placed, together with a control group of 22 poultts, in house 5 which was divided in the usual manner. Exposure was by contaminated soil. The duration of the experiment was 3 months, at the end of this period all survivors were sacrificed.

2. Intermittent feeding of enheptin. Two groups of 20 poultts each were placed in house 9. Separation and exposure were by the usual methods. On the medicated side 2 feed hoppers were provided; one contained mash medicated with enheptin at a concentration of 0.05 per cent, the other held regular mash. Each hopper was provided with a hinged lid so that the type of mash fed could be readily controlled. The medicated group was allowed to eat medicated mash half of the time and plain mash for an equal period. The control group received only unmedicated feed. The duration of the experiment was 4 months, at the end of this period the survivors were sacrificed.

E. Medicated drinking water

1. Medication with Hopkincide. Hopkincide is a soluble iodine preparation which is stated by the manufacturer to contain 5 per cent iodine in solution as Lugol's iodine and another 5 per cent bound by a

plasma substitute. The recommended prophylactic level of this preparation was 2 grams per gallon (0.05 per cent).

Two groups of poults were exposed to contaminated soil in house 1. One group, 19 poults, received water medicated with Hopkincide at 0.05 per cent, the other group, 18 poults, served as controls. The experiment was terminated after 51 days.

2. Medication with liquid enheptin. The compound tested in this experiment was supplied by the manufacturer in the form of water soluble tablets. A concentration of 0.025 per cent was obtained by dissolving 16 tablets in a gallon of water. Twenty-one poults were placed in one side of experimental house 1 and received medicated water during the period of the experiment. Twenty controls of the same brood were placed in the other side. Exposure and separation were by the usual methods. The experiment was terminated after 5 weeks.

IV

RESULTS AND DISCUSSION

A. Action of enheptin on Histomonas meleagridis in the cecal lumen of carriers

1. Results of the attempt to produce chicken carriers. In the 3 experiments designed to produce carriers for further study, 60 chickens and 20 turkeys were used. Chickens proved quite refractory to infection by the methods employed and not a single carrier of lasting duration was produced. Infections were established in a number of birds, but they always proved to be transitory. For example in Table 1 is recorded the numbers of positive cecal droppings found after each series of inoculations in experiment 1. After the last series 16 chicks had shown at least 1 positive cecal dropping, however, at a subsequent examination 2 weeks later all droppings were negative.

A similar situation was encountered in the following experiments. In experiment 2 a considerable amount of cecal blockage was produced but the birds eventually recovered and could not be demonstrated to be carriers.

In experiment 3 serial dilution of inoculum did not produce any significant differences in the number of positive cecal droppings from chickens, but it did have some effect on the turkeys (Figure 1). Although no chicken carriers were produced, 6 turkey carriers were established.

It had been assumed at the inception of this problem that chicken carriers would be relatively easy to establish. Tyzzer (1934, 1936) reported the experimental production of chicken carriers by rectal

TABLE 1

A comparison of the number of positive cecal droppings after 3 series of inoculations

Band number	Positives after series 1	Positives after series 2	Positives after series 3	Final examination
111	0/6	2/4	0/9	negative
161	0/6	0/4	0/9	negative
3851	0/6	0/4	2/9	negative
3852	0/6	0/4	1/9	negative
3853	0/6	0/4	0/9	negative
3854	0/6	0/4	3/9	negative
3855	0/6	3/4	3/9	negative
3856	0/6	0/4	0/9	negative
3857	0/6	0/4	1/9	negative
3860	0/6	1/4	3/9	negative
3862	0/6	2/4	1/9	negative
3863	0/6	0/4	2/9	negative
3864	0/6	0/4	2/9	negative
3866	0/6	0/4	5/9	negative
3868	1/6	1/4	4/9	negative
3869	0/6	2/4	2/9	negative
3870	1/6	0/4	2/9	negative
3871	0/6	0/4	2/9	negative
3872	1/6	0/4	1/9	negative
3874	0/6	2/4	1/9	negative

Numerator of fraction is number of positives recorded.
Denominator is number of examination days

inoculation of histomonads cultured at 37 C. Although Tyzzer cited several examples of birds which remained carriers for as long as 7 months, the majority of his chickens were sacrificed within 14 days after inoculation. If this criterion is applied to the birds dealt with in this problem, many carriers were produced. However, a period of only 14 days was not considered adequate for evaluation of the action of the drug.

Although several attempts were made to duplicate the conditions under which Tyzzer established carriers, H. meleagridis could not be grown at 37 C. Both primary isolation and stepwise alteration of temperature from 42 to 37 C failed.

It is interesting to note in connection with the failure to establish carriers, that not a single cecal worm was found in the chickens at autopsy. Since under natural conditions chickens are commonly infected with Heterakis, and one of the proven routes of transmission is through the cecal worm egg, this observation may be of some significance. It is possible that under natural conditions the role of the chicken as a carrier is almost entirely dependent on the presence of cecal worms. It appears likely that the presence of histomonads in the cecal lumen is transitory; the continuance of the chicken as a carrier being due to the protozoon finding female Heterakis and maintaining itself in the nematode host rather than in the cecal lumen.

2. The treatment of turkey carriers with enheptin. Unlike the chickens, the turkey carriers established in experiment 3 were found to retain their infection. Periodic cecal dropping examinations during a period of 81 days elapsing between inoculation and medication were positive for H. meleagridis. The result of treatment with 0.05 per cent enheptin

TABLE 2

The effect of 0.05 per cent enheptin on turkey carriers of *Histomonas meleagridis*

Band number	Group	Positive droppings	Autopsy findings
3827	Medicated	0/9	Small healed lesions in ceca; contents of ceca negative for <u>H. meleagridis</u>
3831	Medicated	0/9	Normal. Negative for <u>H. meleagridis</u>
3833	Medicated	3/9	Small healed lesions in ceca; contents of ceca negative for <u>H. meleagridis</u>
3832	Control	5/9	Small lesions in ceca; contents of ceca positive for <u>H. meleagridis</u>
3826	Control	9/9	Small healed lesions in liver; lesions in ceca, contents of ceca positive for <u>H. meleagridis</u>
3836	Control	4/9	Small lesions in ceca; contents of ceca positive for <u>H. meleagridis</u>

Numerator of fraction is number of positives recorded
Denominator is number of examination days

is shown in Table 2. Cecal dropping examinations were begun 3 days after medication commenced. Enheptin caused the disappearance of the protozoon from the cecal droppings within 3 days in the case of 2 turkeys and within 6 days in the remaining poult. In all, 9 examinations were made the last being 3 weeks after inception of treatment. At autopsy the cecal contents of the medicated birds were negative, although healed lesions were found in 2 cases.

B. The effect of enheptin on the production of immunity in turkeys exposed to blackhead

1. Natural exposure groups. The results obtained in these experiments are given in Tables 3,4,5, and 6; and Figures 2,3,4, and 5. A summary of the 4 natural exposure experiments is given in Table 7 and Figure 6.

The initial exposure to blackhead during the first 2 month period, as judged by the mortality rates of the infection controls, varied in severity from a low of 45 per cent to a high of 80 per cent. No deaths occurred in the medicated groups during the first 2 month period in any of the experiments.

During the second period the severity of exposure, as judged by mortality in the introduced immunity controls, was also quite variable. Mortality figures ranged from 15 per cent to a high of 36.8 per cent. Losses in the medicated group varied from 15 per cent to 47.3 per cent.

On the whole, losses during the second period of experiments 3 and 4 were less than those occurring in the same period in experiments 1 and 2. These former periods fell partly within a 2 month drought which decreased blackhead losses generally throughout the area.

In all experiments the average duration of life in the infection

TABLE 3

Average duration of life in 3 groups of poult
exposed to contaminated soil (exp. 1)

Group	Average duration of life (days)	Mortality (per cent)		
		60 days	120 days	Total
Immunity controls	87.4	0	25	25
Infection controls	63.5	60	20	80
Medicated group	81.7	0	47.3	47.3

TABLE 4

Average duration of life in 3 groups of poult
exposed to contaminated soil (exp. 2)

Group	Average duration of life (days)	Mortality (per cent)		
		60 days	120 days	Total
Immunity controls	79.7	0	35	35
Infection controls	55.3	45	20	65
Medicated group	79.1	0	40	40

TABLE 5

Average duration of life in 3 groups of poult
exposed to contaminated ground (exp. 3)

Group	Average duration of life (days)	Mortality (per cent)		
		60 days	120 days	Total
Immunity controls	81.7	0	36.8	36.8
Infection controls	45.1	80	0	80
Medicated group	82.7	0	15	15

TABLE 6

Average duration of life in 3 groups of poult
exposed to contaminated soil (exp. 4)

Group	Average duration of life (days)	Mortality (per cent)		
		60 days	120 days	Total
Immunity controls	100	0	15	15
Infection controls	30	55	0	55
Medicated group	86.7	0	20	20

TABLE 7

Summary of 4 experiments showing average duration
of life in poult exposed to contaminated
surroundings

Group	Average duration of life (days)	Mortality (per cent)		
		60 days	120 days	Total
Immunity controls	87.5	0	27.5	27.5
Infection controls	48.5	60	8.75	68.75
Medicated group	82.6	0	30	30

controls was considerably shorter than in either of the other 2 groups. The differences between the medicated group and the immunity controls varied between experiments but the average showed that duration of life was longer in the latter.

Figures on average duration of life in the infection controls are not entirely accurate since some poultz were sacrificed during the period of clinical symptoms several days before death would normally be expected to occur. Total mortality figures, however, were not affected since at autopsy all such poultz showed extensive cecal and liver lesions which were judged sufficient to have caused death had the disease been allowed to run its course.

The most significant finding revealed by the data for the 4 experiments is that there was little difference between the medicated groups and the immunity controls in regard to duration of life and mortality. As a matter of fact the immunity controls had a slightly lower death rate as well as a longer duration of life. It seems reasonable to assume that had any immunity been formed during treatment, it would have been reflected in a lower mortality and longer duration of life in the medicated group. Therefore it would appear on the basis of this evidence that poultz exposed to blackhead and receiving a prophylactic level of enheptin (0.05 per cent), do not form immunity.

One difference which appeared to be of doubtful significance at this time, is shown in Figures 2,3, and 5. In these 3 experiments losses from blackhead, after cessation of treatment, began in the medicated groups sooner than in the immunity controls. In experiment 1 (Figure 2), 5 previously medicated poultz died of blackhead before a single loss occurred in the immunity controls. These observations

were similar to those noted by DeVolt and Tromba (1950) in a parallel experiment utilizing exposure by rectal inoculation of histomonads from culture. A discussion of this phenomenon will be deferred (p. 36) until after presentation of further data on exposure by rectal inoculation.

2. Exposure by rectal inoculation of histomonads from culture.

Although 2 experiments were performed utilizing rectal inoculation, only the first is tabulated, Table 8 and Figure 7. In the second experiment data were incomplete since half the medicated group was sacrificed for histological study.

The culture used was highly pathogenic, as shown by the 100 per cent mortality in the infection controls. This high mortality resulting after rectal inoculation of culture material is in agreement with reports by DeVolt and Holst (1948, 1949).

During the first 15 day period daily examinations of cecal droppings of the medicated birds were negative. In the second 15 day period symptoms appeared in the medicated group 2 days after cessation of treatment. The first death occurred 5 days after removal of medication and, in all, 8 deaths occurred within 11 days after the beginning of the second period. The 2 survivors never showed symptoms and were negative for histomonads at autopsy. No deaths or symptoms appeared in the immunity controls and all were negative when sacrificed.

In the light of these findings the observations of DeVolt and Tromba (1950), and the differences noted between immune controls and medicated groups in the contaminated soil exposures, are of significance. It is evident that not only had the immune mechanism failed, but in addition latent foci of infection had been maintained.

TABLE 8

Average duration of life in psulks exposed
to blackhead by rectal inoculation

Group	Average duration of life (days)	Mortality (per cent)		
		15 days	30 days	Total
Immunity controls	30	0	0	0
Infection controls	10.3	100	0	100
Medicated group	21	0	80	80

There is a striking similarity between human malaria and blackhead in turkeys in regard to these two points. Clinical malaria in exposed persons will not appear as long as a prophylactic level of atabrine or quinine is maintained. However, on cessation of treatment clinical manifestations will often appear, even if the person is not in a malarial area. Shortt and Garnham (1948) found that the site of exo-erythrocytic development in human malaria was the liver. In blackhead, although any body tissue could conceivably be the site of latent foci, it seemed reasonable to assume that the 3 most likely places would be the cecal lumen, the cecal tissues, and the liver. This assumption was strengthened in part by Tyzzer's observation (1934) that occasionally microscopic foci of infection could be found in the cecal tissues of chickens which appeared normal on gross examination. Cecal dropping examinations conducted on poultts medicated with enheptin had already shown that histomonads could not be demonstrated in the cecal contents, it remained therefore to investigate the tissues histologically.

3. Histological study of exposed medicated poultts. A uniform exposure to the highly virulent organisms of culture 3 resulted in 100 per cent mortality in the infection controls. During the period of medication the exposed medicated group was checked daily for histomonads in the cecal droppings; all examinations were negative. At the cessation of prophylaxis 5 medicated poultts were sacrificed; no lesions were apparent on gross examination of the ceca and liver; the cecal contents were negative. Within 8 days after removal of the drug 4 of the surviving medicated poultts had died; the survivor was negative at autopsy. No deaths occurred in the immunity controls and all were negative when

sacrificed.

Sections prepared from the livers of the 5 medicated poultts sacrificed at cessation of treatment were negative for H. meleagridis. The tissue was normal and exhibited none of the changes which Farmer, Hughes and Whiting (1951) have regarded as indicative of infection in artificially induced blackhead.

Sections of the ceca, in 4 cases, showed an invasion of the glandular epithelium by organisms measuring, on the average, 9 microns in diameter (Figure 9). The cytoplasm of these forms was quite basophilic with numerous inclusions while the nucleus showed a deeply stained red karyosome surrounded by a clear nucleoplasm (Figure 10). Comparison of these organisms with the descriptions and figures of Tyzzer (1919) and with sections prepared from the ceca of fatal cases of blackhead proved that these organisms were the invasive phase of Histomonas meleagridis.

No great concentration of organisms was seen and penetration was limited to the glandular epithelium. Clear areas were observed to occur surrounding many of the histomonads; this may be taken as the result of lytic action by the parasite or it may be a fixation artifact. No significant host tissue reaction was noted, although a slight eosinophilia may have been present.

The finding of H. meleagridis in the cecal tissues of turkeys receiving a prophylactic feeding of enheptin during exposure explains the earlier deaths observed in such birds when medication is removed. It also reinforces the data on the failure of immunity, although the explanation for this failure is not clear. It may be that the immune process in blackhead is dependent on metastasis to the liver via the

blood, which when it occurs in the course of the disease in unmedicated turkeys results in the production of some immune individuals. This implies that in medicated individuals a histomonocidal level of enheptin is maintained in the blood. Limited support is given this hypothesis by the fact that liver sections of medicated poults were negative for H. meleagridis. Clarification of this point must await further investigation.

C. Low level and intermittent feeding of enheptin

1. Prophylactic effect of low level enheptin. The results of this experiment are given in Table 9. The rate of susceptibility includes birds dying of blackhead and birds found to have lesions when sacrificed. Since the susceptibility rate in the medicated group was higher than that in the controls it is apparent that 0.025 per cent enheptin has no value as a prophylactic.

2. Intermittent feeding of enheptin. Results obtained are given in Table 10. As in the previous experiment susceptibility figures reflect fatal cases and those having lesions when sacrificed. The average duration of life in the control group was 37 days as compared with 70.9 days in the medicated group, however, no significant difference was found in susceptibility rate. Although the drug succeeded in prolonging life, its failure as a prophylactic when fed intermittently makes this procedure of little value.

D. Prophylaxis by medicated drinking water

1. Medication with Hopkincide. The results of this experiment are given in Table 11. Although the susceptibility rate was higher in the

TABLE 9

Death rates from blackhead after exposure to
contaminated soil in poults treated with
0.025 per cent enheptin and controls

Group	Number	Deaths		Having lesions when sacrificed	Susceptibility	
		No.	Per cent		Number	Per cent
Medicated	23	8	34.7	10	18	78.2
Control	22	9	40.8	7	16	72.7

TABLE 10

Death rates from blackhead after exposure to
contaminated soil in poults treated with
0.05 per cent enheptin intermittently
and controls

Group	Number	Deaths		Having lesions when sacrificed	Susceptibility	
		No.	Per cent		Number	Per cent
Medicated	20	9	45	1	10	50
Control	20	11	55	1	12	60

control group the difference was not significant. It is doubtful that Hopkincide has any value as a prophylactic in blackhead.

2. Medication with liquid enheptin. The results of this experiment are given in Table 12. As in previous experiments susceptibility includes poultz dying as well as those having lesions at autopsy. Since the mortality in the medicated group closely approximated that of the control group and the susceptibility rates in both groups were equally high it is apparent that this preparation of enheptin had no value as a prophylactic.

TABLE 11

Death rates from blackhead after exposure to
contaminated soil in poultts treated with
Hopkincide and controls

Group	Number	Deaths		Having lesions when sacrificed	Susceptibility	
		No.	Per cent		Number	Per cent
Medicated	19	9	47.3	5	14	73.6
Control	18	11	61	5	16	89

TABLE 12

Death rates from blackhead after exposure to
contaminated soil in poultts treated with
Liquid enheptin and controls

Group	Number	Deaths		Having lesions when sacrificed	Susceptibility	
		No.	Per cent		Number	Per cent
Medicated	20	12	60	7	19	95
Control	21	14	66.6	5	19	90.4

V

SUMMARY

Establishment of chicken carriers was attempted by multiple, serial, rectal inoculations with histomonads cultured at 42 C. Sixty Rhode Island Red chickens so inoculated failed to pass the organism in the cecal droppings for longer than 10 days. Because of the negative result of these inoculations, and since Heterakis gallinae was not recovered from any of the experimental birds, it is suggested that under natural conditions the carrier state in chickens is prolonged by the maintenance of Histomonas meleagridis in the tissues of the cecal worm.

Twenty White Holland turkey poults inoculated rectally with histomonads from culture produced 6 carriers which remained positive for 81 days. Treatment of 3 of these with enheptin (2-amino,5-nitro-thiazole) caused the disappearance of H. meleagridis from the cecal droppings.

In 6 experiments on immunity, White Holland turkey poults were divided into 3 groups; medicated, infection controls, and immunity controls. Exposure to blackhead was by contaminated top soil, contaminated ground, and by rectal inoculation of cultures. Medication in all cases was by incorporation of enheptin in a standard growing mash at a concentration of 0.05 per cent.

Medicated birds in the natural exposure groups suffered a 30 per cent loss after removal of the drug. Immunity controls lost 27.5 per cent while the infection controls suffered a 68.75 per cent loss. Immunity to blackhead was not developed during treatment with enheptin

in birds^s exposed by contaminated soil.

In exposure by rectal inoculation of culture, mortality in the infection controls was 100 per cent while the medicated group suffered an 80 per cent loss after removal of the drug. The immunity controls experienced no losses at any time. As in the preceding groups, immunity was not developed during prophylactic treatment.

In 4 of 5 turkeys sacrificed after exposure and during treatment with enheptin, H. meleagridis was demonstrated in sections of the glandular epithelium of the ceca even though the droppings had been negative for 15 days previous to autopsy. Sections of the livers of these birds were uniformly negative.

The presence of microscopic foci of infection, which have been hitherto observed only in untreated chickens, explains the relapses suffered by turkeys when prophylactic treatment is removed.

The use of other prophylactic levels of enheptin, 0.025 per cent fed continuously or 0.05 per cent fed intermittently failed to protect poults against fatal infections of blackhead. Attempts to apply prophylaxis by means of drinking water medicated with Hopkincide, or by the use of liquid enheptin were unsuccessful.

VI

CONCLUSIONS

1. Chicken carriers of Histomonas meleagridis were not established by inoculation with the parasites cultured in a Locke's alkaline serum medium at 42 C.

2. Losses from blackhead in turkeys often occur rather quickly after cessation of treatment with enheptin.

3. In a limited number of turkey carriers 0.05 per cent enheptin terminated patency.

4. During simultaneous treatment with 0.05 per cent enheptin and exposure to the disease, immunity to blackhead did not develop.

5. Although enheptin at 0.05 per cent terminated patency, it failed in some cases to eliminate histomonads from the cecal glands of turkeys.

6. While treatment with enheptin places blackhead under control, losses are often resumed rather quickly after cessation of treatment because of latent foci of infection in the glandular epithelium of the ceca.

7. Enheptin at the 0.025 per cent level, intermittent feeding at the 0.05 per cent level, and soluble iodine (Hopkincide) all proved ineffective as blackhead preventives.

VII

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VIII

FIGURES

FIG. 1

THE EFFECT OF SERIAL DILUTIONS ON NUMBER
OF POSITIVE CECAL DROPPINGS

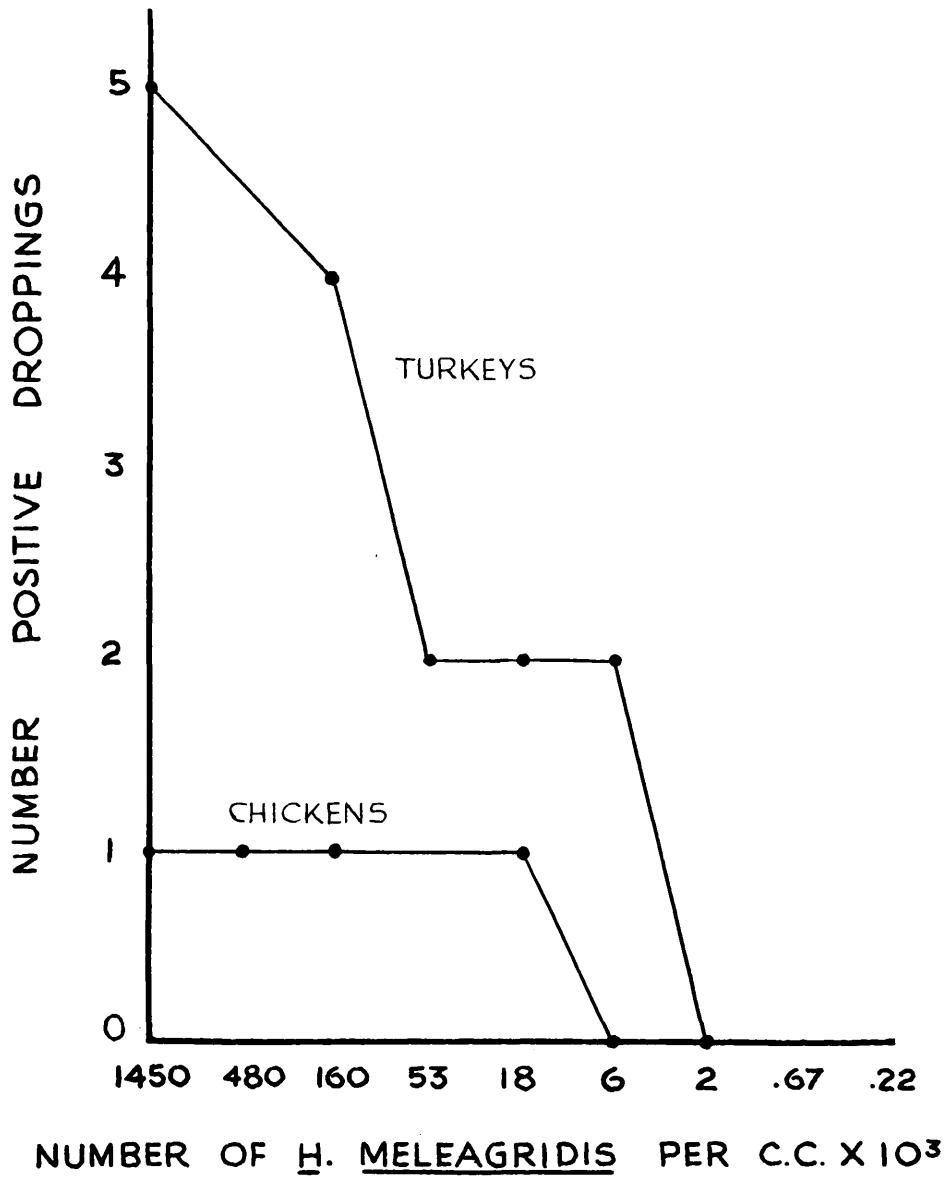


FIG. 2

COMPARISON OF DEATH RATES IN 3 GROUPS OF POULTS EXPOSED TO CONTAMINATED SOIL.

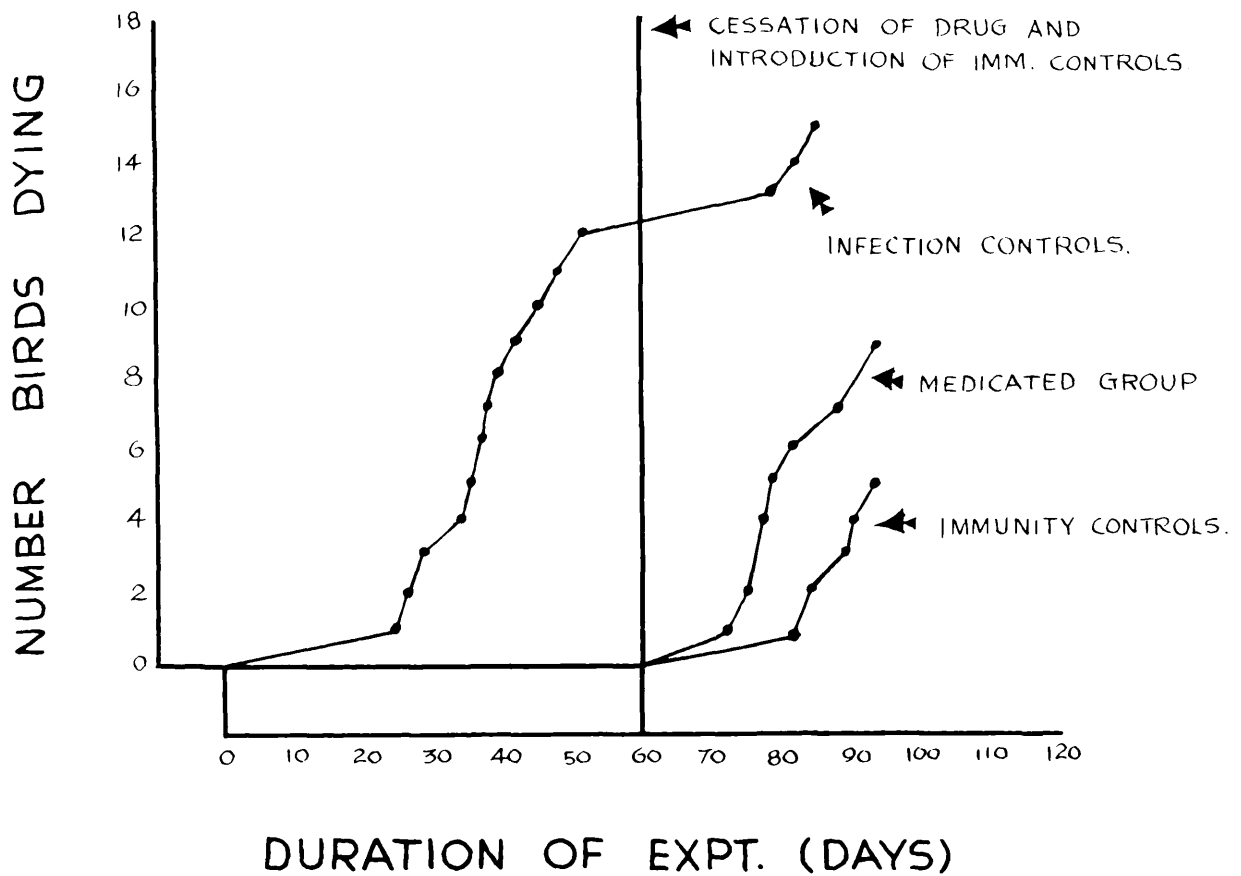


FIG. 3

COMPARISON OF DEATH RATES IN 3 ADDITIONAL
GROUPS OF POULTS EXPOSED TO CONTAMINATED
SOIL.

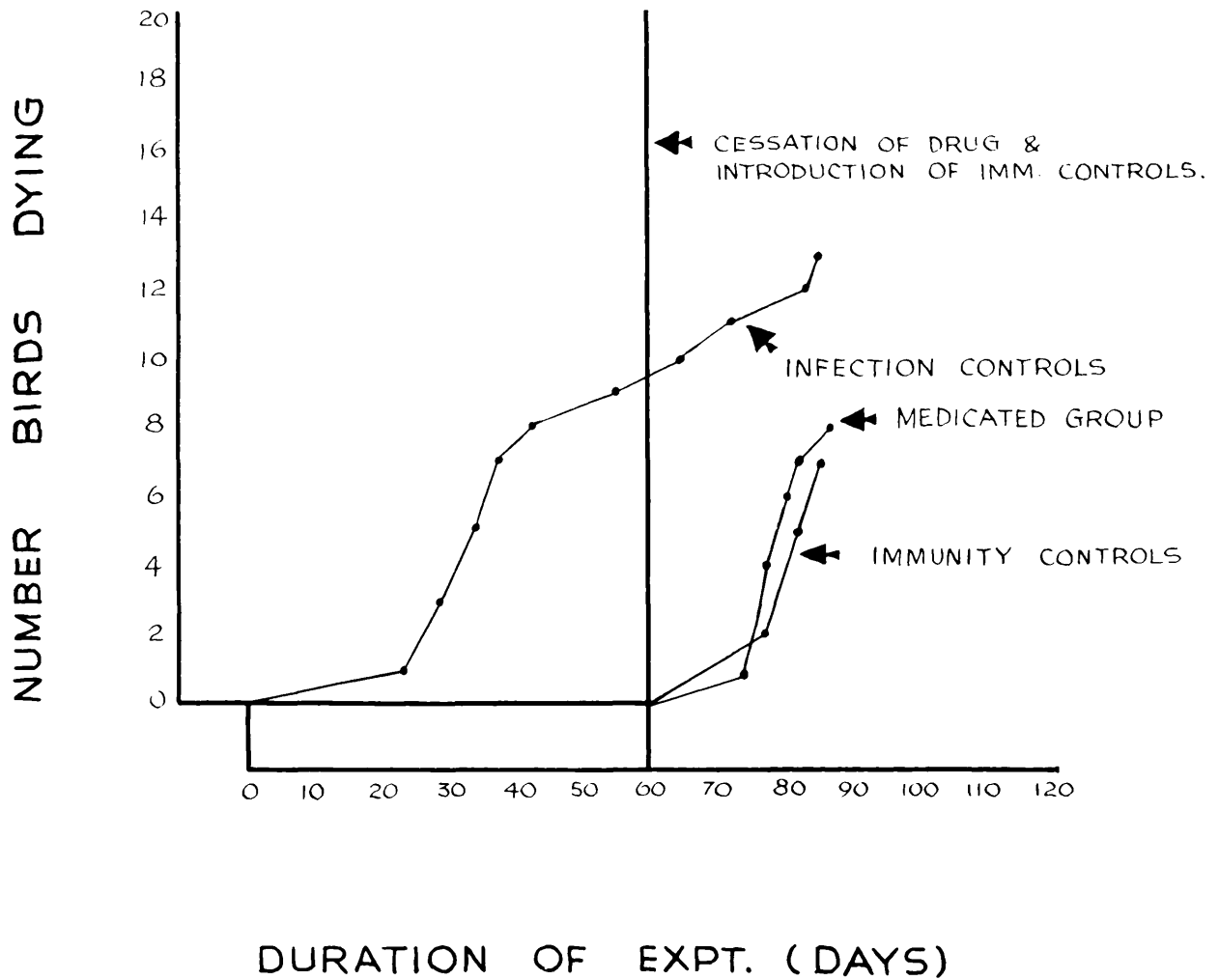


FIG. 4

COMPARISON OF DEATH RATES IN 3 GROUPS OF POULTS EXPOSED TO CONTAMINATED GROUND.

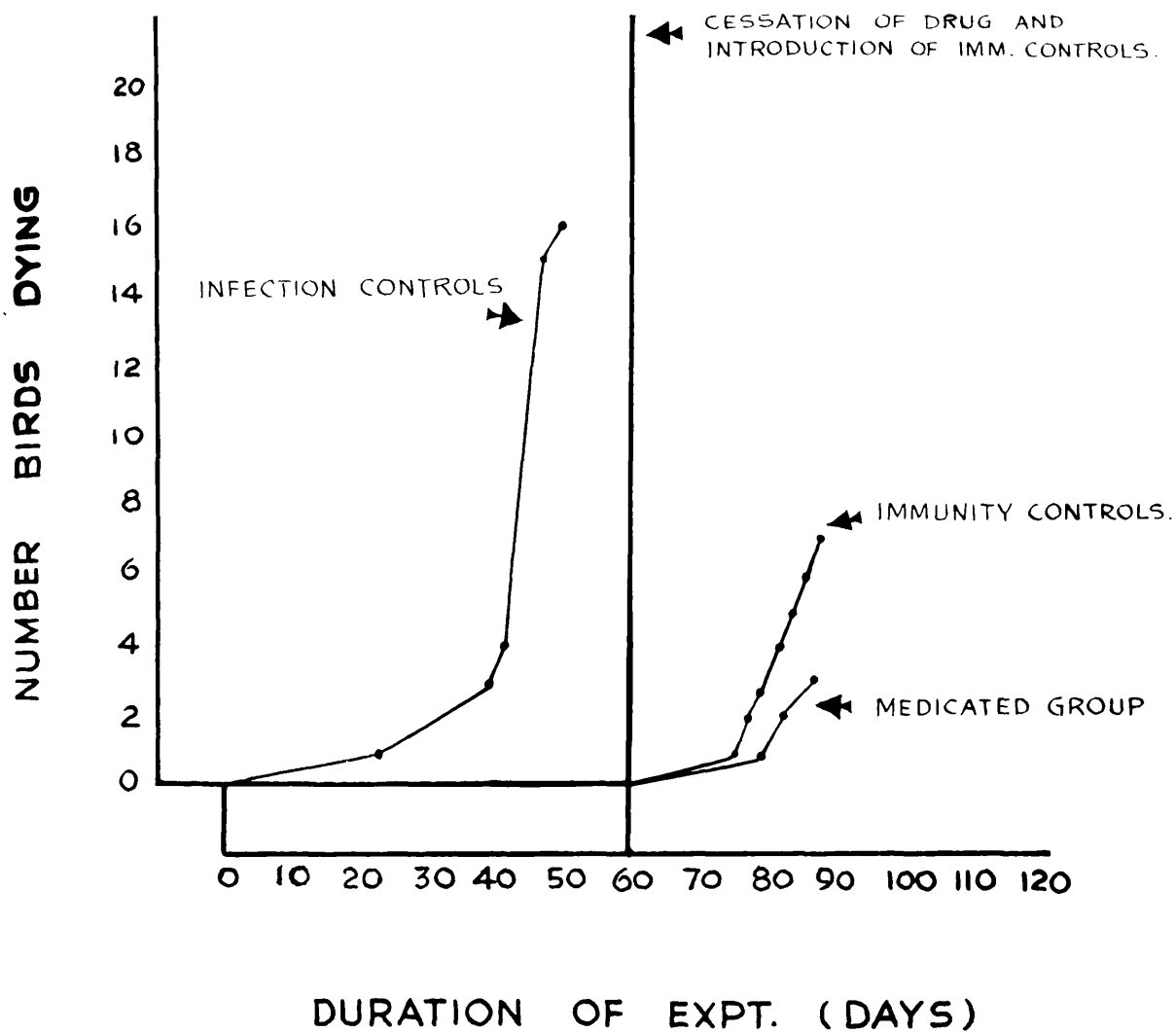


FIG. 5

FURTHER COMPARISON OF DEATH RATES IN 3 GROUPS OF POULTS EXPOSED TO CONTAMINATED SOIL.

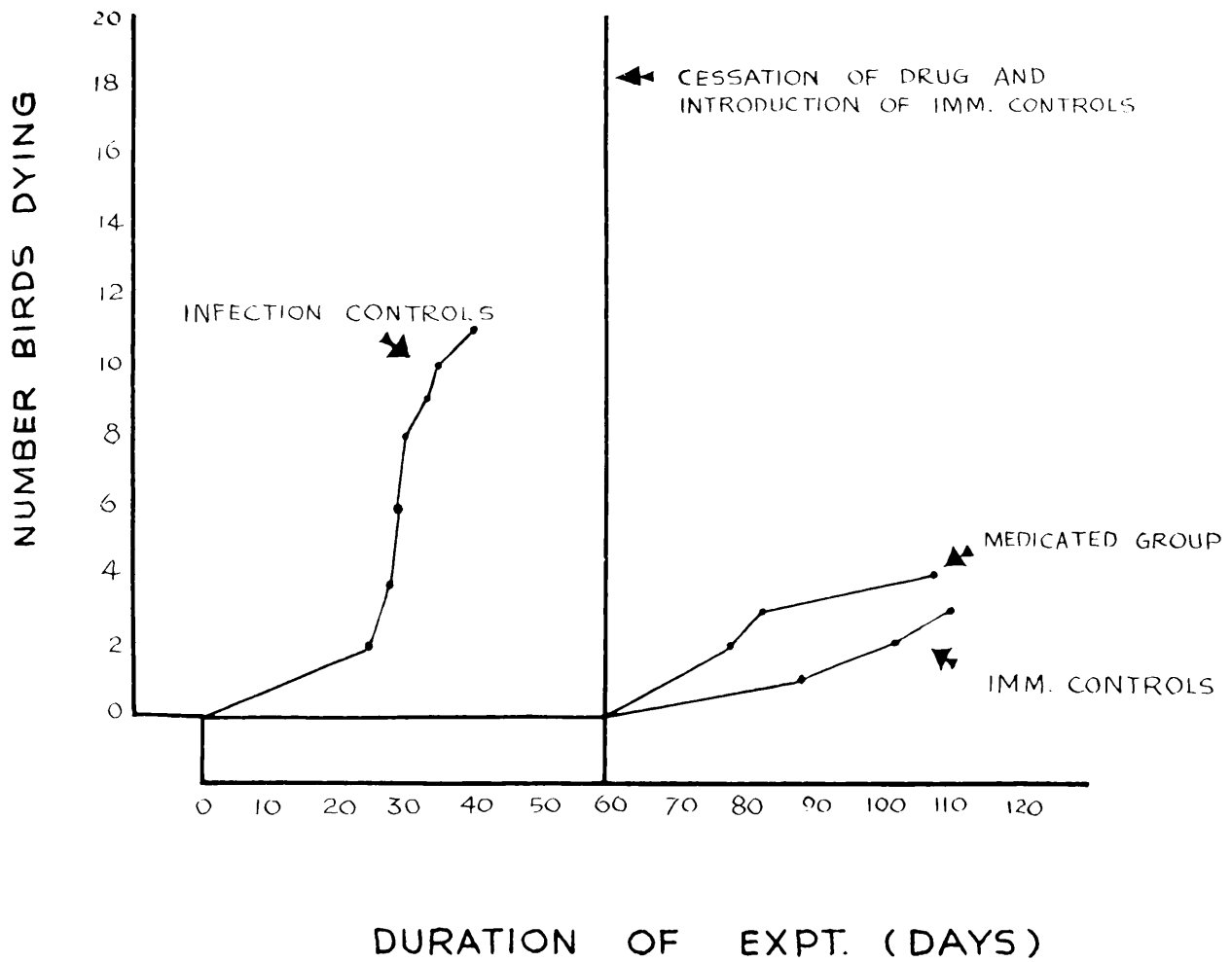
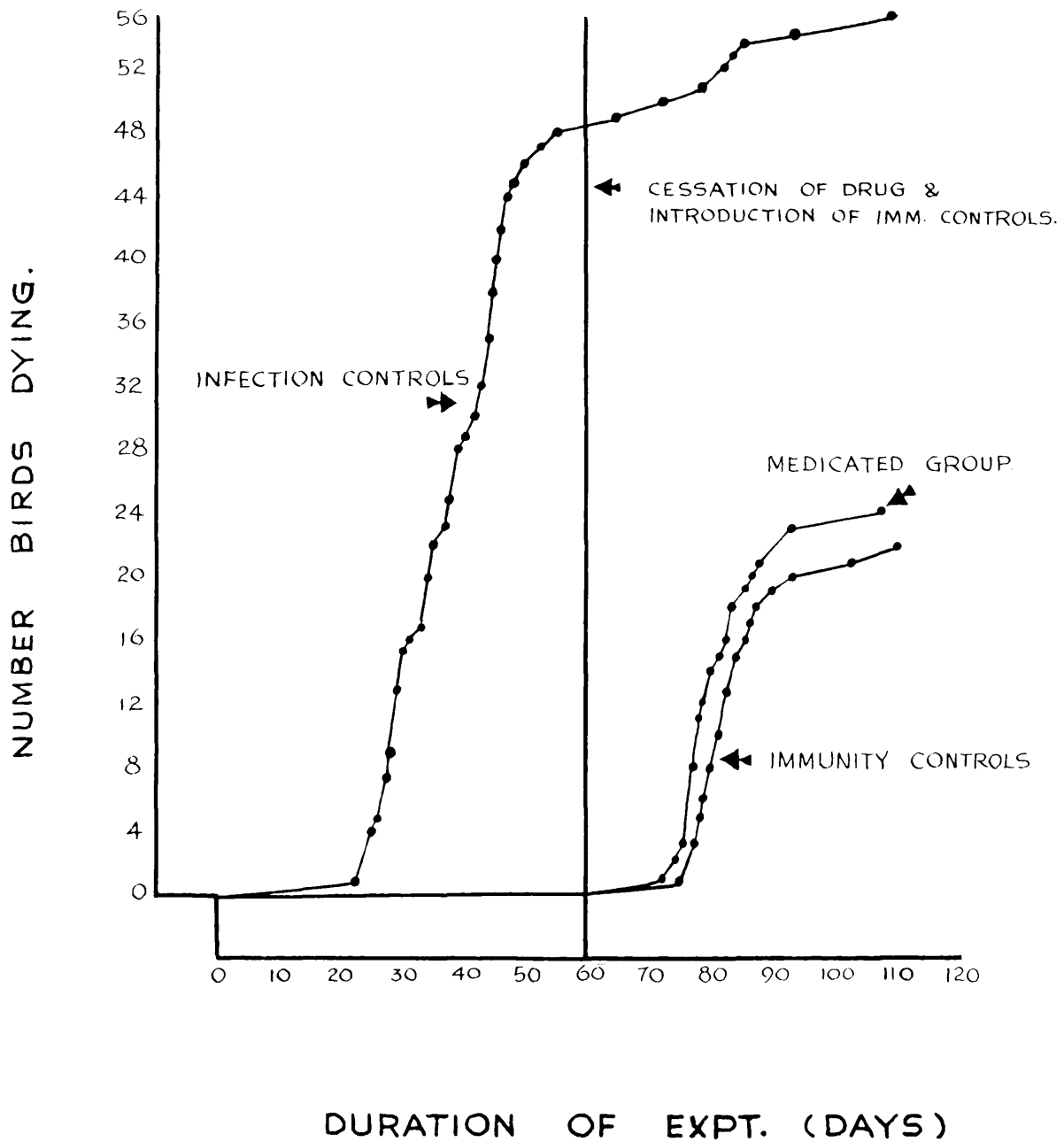


FIG. 6

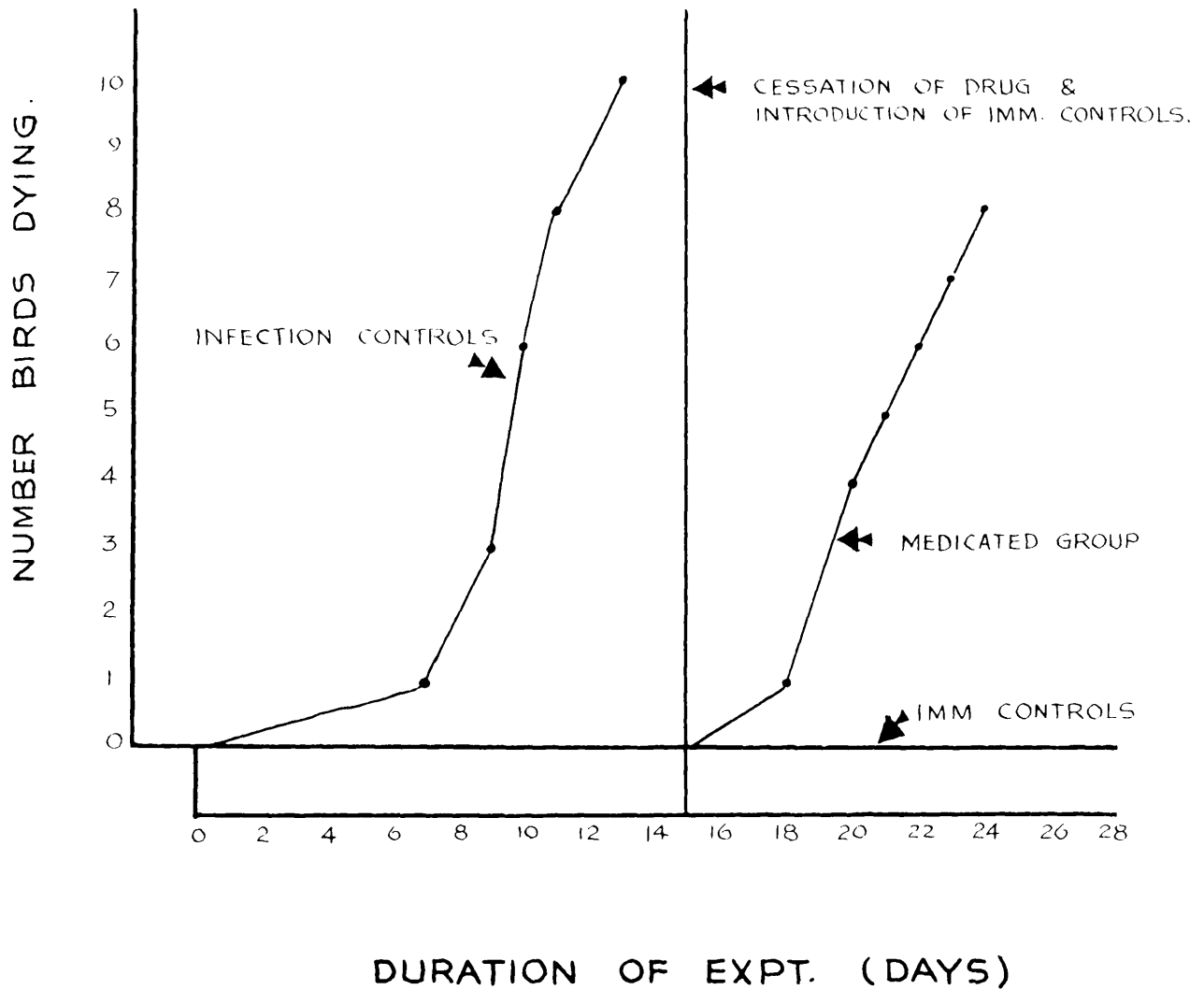
SUMMARY OF 4 EXPERIMENTS COMPARING THE DEATH RATES OF 3 GROUPS OF POULTS EXPOSED TO BLACKHEAD UNDER NATURAL CONDITIONS.



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FIG. 7

COMPARISON OF DEATH RATES IN POULTS EXPOSED BY RECTAL INOCULATION OF HISTOMONADS FROM CULTURE.



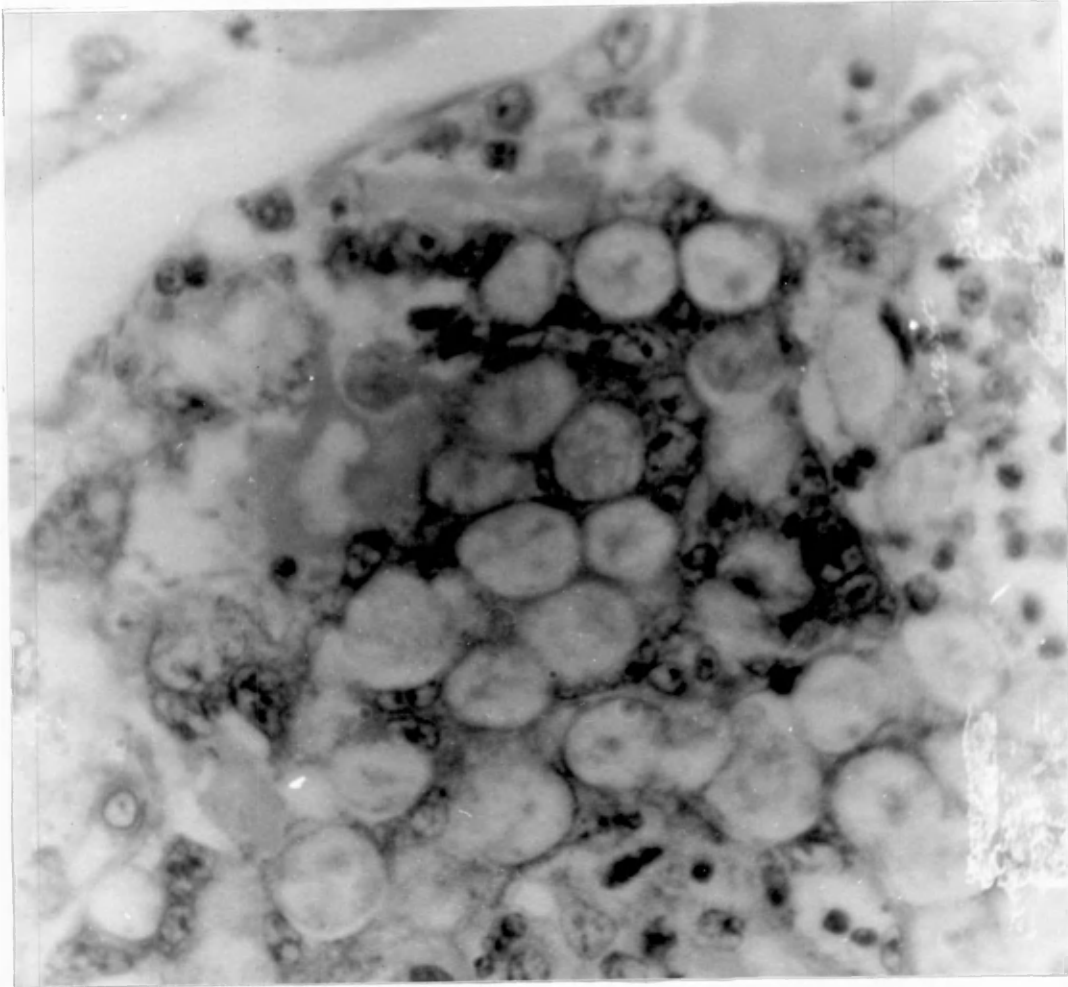


Figure 8

Section of liver of turkey poult showing histomonads in the vegetative phase. Delafield's hematoxylin-eosin-azure II. x 900

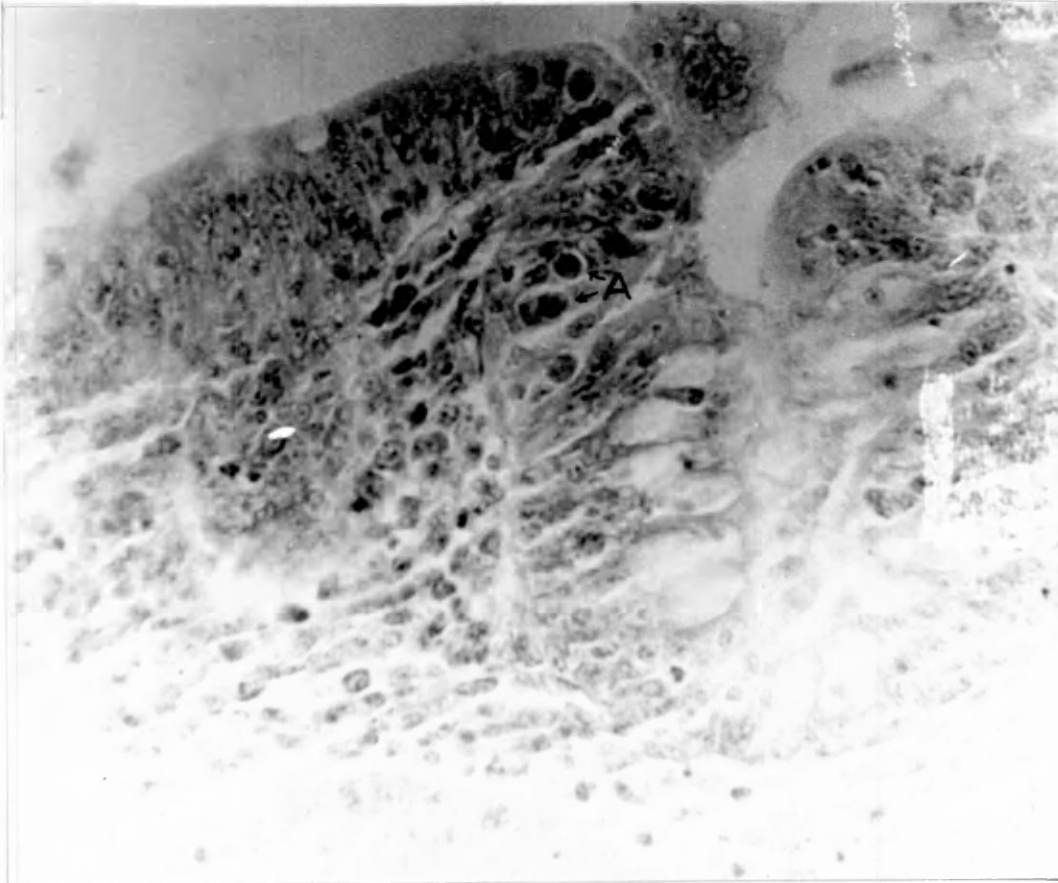


Figure 9

Cecal gland of medicated turkey poult exposed to blackhead and sacrificed during medication. Histomonads shown at A. Delafield's hematoxylin-eosin-azure II. x 450.

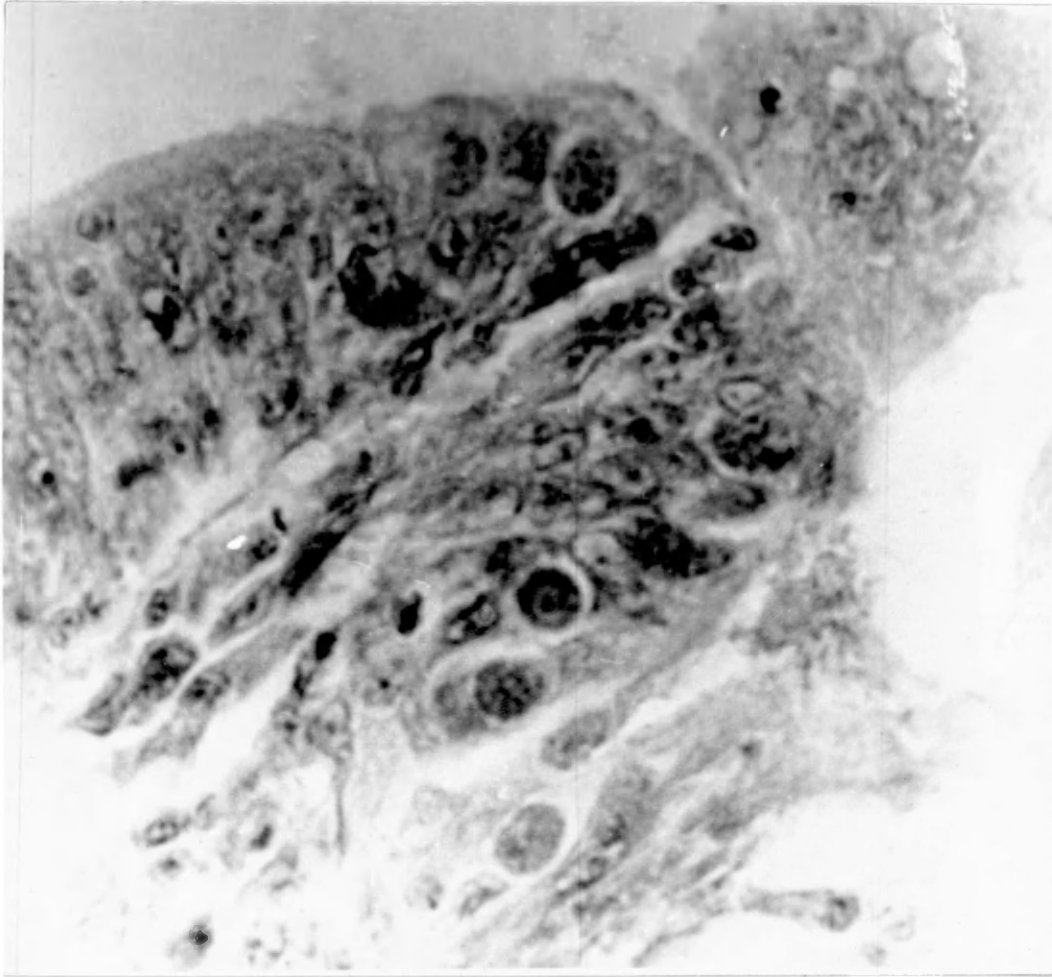


Figure 10

Enlarged view of a portion of the cecal gland
shown in Fig. 9. x 900

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ACKNOWLEDGMENTS

I wish to thank Dr. H.M. DeVolt, Poultry Pathologist at the Livestock Sanitary Service Laboratory, for his counsel and guidance both in the experimental work and in the preparation of the manuscript.

I am indebted to other members of the staff for their generosity in lending equipment, to Dr. A.L. Brueckner who graciously permitted the use of facilities, and to Mrs. Anita Holst who helped on many occasions with the handling of the experimental birds.

Although my association with Dr. George Anastos did not begin until this work had been started, I benefited greatly by his suggestions during the course of the problem and his careful review of the manuscript.