

FURTHER OBSERVATIONS ON "BLACKHEAD" IN TURKEYS

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The investigation of blackhead, continued during the summer of 1920, has yielded additional data which, although contributing to the general knowledge of the disease, leave certain practical questions still unanswered. Blackhead was transmitted by subcutaneous and intramuscular inoculation throughout the season, thus confirming previous results.¹ The resistance of the virus to variations of temperature, and the question of its discharge from the mucous membranes were studied. Attempts to produce the disease other than by the previously employed method of inoculation of acute blackhead lesions have yielded some facts concerning the characteristics and distribution of *Histomonas meleagridis*, the parasite of blackhead. A study was also made of the relative frequency in normal and infected turkeys of the parasitic round-worm *Heterakis papillosa*.

On account of the varied character of the experiments, the data obtained will be summarized under separate headings.

EFFECT OF TEMPERATURE ON VIRUS

The resistance of the virus, as it occurs in the lesions of acute blackhead, was determined by inoculating turkeys with infectious material after its exposure to different temperatures. Livers showing lesions characteristic of acute blackhead were removed under aseptic precautions from freshly killed young turkeys and portions of the infected foci were distributed in sterile Petri dishes. Bits of these lesions after being subjected to freezing, refrigerator, or room temperature for definite periods of time, were then subcutaneously inoculated into turkeys from 3 to 6 weeks old. The results are given in table 1.

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¹ Tyzzer, E. E., and Fabyan, M.: Jour. Infect. Dis., 1920, 27, p. 207.

It is obvious from these results that the active virus is present in the lesions of a freshly killed turkey with acute blackhead, and that it probably deteriorates more rapidly at room temperature than at lower temperature. At refrigerator temperature—5 C.—it may remain alive

TABLE 1
EFFECT OF TEMPERATURE ON VIRUS

Blackhead Virus	Date	Treatment of Virus	No. of Turkey Inoculated	Age in Days	Weight in Grams	Result
Turkey 20.86..... (Spontaneous) Liver lesions	June 28	None. Used at once	4	21	260	Died of inoculated blackhead on 12th day
Ibid.	48 hours at 5 C.	5	23	280	Died of inoculated blackhead on 13th day
Ibid.	48 hours at 22 C.	6*	23	222	No infection
Ibid.	7 days at 5 C.	7*	28	280	No infection
Ibid.	7 days at 22 C.	8*	28	300	No infection
Turkey 4..... (Inoculated) Liver lesions	July 10	None. Used at once	10	24	255	Died of inoculated blackhead on 11th day
Ibid.	4 days at 5 C.	12	28	310	Died of inoculated blackhead on 14th day
Turkey 12..... (Inoculated) Subcutaneous and liver lesions	July 28	5 days at 5 C.	13*	47	340	No infection
Turkey 10..... (Inoculated) Liver lesions	July 20	None. Used at once	2	44	530	Died of inoculated blackhead on 10th day
Ibid.	Frozen for 5 minutes after 12 hours at 1 C.	6*	45	500	No infection
Turkey 20.101..... (Spontaneous) Liver lesions	July 26	5 days at 5 C.	8*	54	730	No infection (The fresh virus was proved virulent by control inoculation)
Turkey 2..... (Inoculated) Subcutaneous and liver lesions	July 31	48 hours at 5 C.	6*	56	730	No infection
Turkey 19-12..... (Inoculated) Subcutaneous lesion	Oct. 9	Frozen for 5 minutes	19-13	104	2,050	No infection (The control inoculation of the opposite breast with untreated virus produced infection)

* Died of spontaneous blackhead later on, showing turkey to be susceptible to the disease.

for at least 4 days, but it is quickly killed by actual freezing. Whether more resistant forms are present, which are not infective when injected into the tissues of the turkey, or whether such forms are produced elsewhere than in the blackhead lesions, are questions on which these data have no bearing.

INTRAVENOUS INOCULATION

Turkeys were inoculated intravenously to determine whether certain organs or tissues were more favorable for the development of *Histomonas* than others. Certain of these turkeys also received injections of Niagara blue and India ink, but these dyes did not appreciably affect the results of the inoculation.

There were three objects in injecting these pigments: to stain the lesions vitally; to stain the parasites vitally, and to determine whether these dyes would have any deterrent effect on the growth of the parasite. The lesions were always vitally stained, but in such an irregular manner that no definite conclusions could be drawn as to the origin of the cellular reaction to the virus; the ink distribution was particularly irregular. Niagara blue was present in most of the phagocytic cells in the lesions, and it was possible to demonstrate granules of the dye occupying the same vacuole with one or more *Histomonas*. These granules were often grouped in two masses at the poles of a parasite, but always outside its body. The parasites were never vitally stained, and the presence of the dye in the lesions had no apparent effect on their growth.

Portions of liver or subcutaneous lesions from a freshly killed case of acute blackhead, were ground in a mortar with a small amount of normal salt solution. The resulting suspensions (in doses of 0.5 to 2 c.c.) were injected into a wing vein, usually the large superficial one extending across the elbow-joint at its inner surface. No immediate effect was noted, except in one instance (No. 30) in which a suspension of lung lesions was employed. This injection produced immediate death, attended by rapid clotting of the blood in the vessels. This phenomenon has already been noted by others in work on poultry.² The experiments are briefly tabulated in table 2.

It is apparent from these results that the injection of blackhead virus directly into the blood stream produces disease in only a relatively small proportion of cases, in striking contrast to subcutaneous inoculation, which practically always causes infection. In those cases in which infection follows intravenous inoculation (see table 2, nos. 26, 28 and 29) one or two large initial lesions usually develop in the lungs and smaller secondary lesions distributed in various organs, in order of frequency as follows: lung, liver, kidney, proventriculus, pancreas, small intestine, spleen, cecum and ovary. Occasionally, however, as wide a dissemination of lesions may be associated with either subcutaneous blackhead or the natural disease. The process may extend from the initial lesion of the lung through the thoracic wall, destroy-

² Bang, Oluf: *Centralbl. f. Bakteriöl.*, I, O., 1908, 46, p. 468.

TABLE 2
INTRAVENOUS INOCULATION

Turkey	Intravenous Injection *	Findings
7	Aug. 21. 2.5 c.c. Suspension of liver lesions Aug. 23. 2 c.c. Suspension of liver lesions (48 hours in refrigerator)	Spontaneous blackhead Aug. 21. Symptoms noted before injected Aug. 26. Died (5th day) no lesions except in caeca and liver (Injections caused no appreciable change in course of disease)
14†	Aug. 6. 1 c.c. Suspension of liver lesions (Some of suspension escaped into surrounding tissues)	Subcutaneous blackhead Aug. 19. Killed (13th day) Local lesion on wing and secondary lesions in lungs and liver Spontaneous blackhead (early) Both caeca show early lesions
15†	Aug. 6. 1 c.c. Suspension of liver lesions (Most of suspension escaped into surrounding tissues)	Subcutaneous blackhead Aug. 17. Killed (11th day) Local lesions on wing, numerous small secondary lesions in lungs
16†	Aug. 6. 0.5 c.c. Suspension of liver lesions	Failed to infect Aug. 20. Killed; normal
17	Aug. 6. 1 c.c. Suspension of liver lesions Aug. 21. 1.5 c.c. Thick suspension of liver lesions	Both intravenous injections failed to infect Aug. 28. Breast inoculated with virus Sept. 7. Killed (10th day) Subcutaneous blackhead Local lesion on breast, secondary lesions in lungs and liver (Susceptibility demonstrated)
26	Sept. 9. 2 c.c. Suspension of liver lesions	Visceral blackhead Sept. 24. Died (15th day) No lesion on wing Extension of process from primary focus in lung through wall of thorax Smaller lesions in lungs, liver, kidney, ovary, pancreas, proventriculus, small intestine and one cecum
28	Aug. 26. 1.5 c.c. Suspension of liver lesions	Visceral blackhead Sept. 9. Moribund and killed (14th day) No lesion on wing Primary lesion replacing much of right lung Secondary lesions in liver and kidneys
29	Aug. 26. 2 c.c. Suspension of liver lesions Sept. 9. 2 c.c. Suspension of liver lesions Sept. 24. 2 c.c. Suspension of subcutaneous lesion Oct. 8. 2 c.c. Rich suspension of subcutaneous lesion	Visceral blackhead Oct. 25. Died (17th day after last in- jection) No lesion on wing Primary lesion in lung, extending through thoracic wall with destruction of intervertebral ligament and in- volvement of periosteum Secondary lesions in liver, spleen, kid- neys, proventriculus and duodenum
30	Aug. 28. 2 c.c. Suspension of liver lesions Sept. 9. 2 c.c. Suspension of liver lesions Sept. 13. 1 c.c. Suspension of lung lesion	No infection from first or second in- jection Third injection caused sudden death owing to use of lung tissue
31	Sept. 9. 2 c.c. Suspension of liver lesions Sept. 24. 2 c.c. Suspension of subcutaneous lesion Oct. 8. 1.7 c.c. Thick suspension of subcutaneous lesion	Visceral blackhead Oct. 19. Breast inoculation with virus Oct. 30. Killed (22d day after last in- jection) Subcutaneous blackhead Local lesion on breast Lesions of lungs, pancreas, and cecum of similar size

* The material used in each intravenous injection, with the exception of those injected on Oct. 8 was proved virulent by control subcutaneous inoculation of other turkeys.

† Injected with Niagara blue and India ink at intervals.

ing muscles, ligaments and other tissues. Although the skeletal muscles are readily invaded, no metastatic lesions have thus far been found in them.

IMMUNITY

The frequent failure of intravenous inoculation to produce any evidence of infection led us to consider the possibility of obtaining an artificial immunity by this procedure. It was found, however, that if the intravenous injections were repeated a sufficient number of times, the disease was eventually produced (table 2, no. 29). Furthermore, a negative intravenous inoculation failed to protect the turkey against later subcutaneous inoculation, and the disease resulting from the latter ran its usual fatal course. Intravenous inoculation of a turkey ill with spontaneous blackhead did not appreciably alter the course of this disease or produce additional lesions.

There is little doubt that recovery sometimes occurs in natural blackhead, notwithstanding its usual fatal course. Turkeys showing all the characteristic signs of blackhead and derived from flocks in which blackhead was present have been known to recover. One turkey (no. 25) which showed weakness, loss of weight and sulphur droppings late in November, at the age of 5½ months, improved later on and when killed on Dec. 22, had healing lesions in one cecum and scattered depressions with some scar tissue in the liver.

The immunity in such a recovered case of spontaneous blackhead has not been tested as yet by subsequent subcutaneous inoculations. That immunity follows recovery from blackhead is indicated, however, by the failure of a subcutaneous inoculation of active virus to infect a turkey (43) which had recovered (whether or not because of special treatment) from inoculated blackhead.

The occurrence of a small localized cecal lesion and a few liver lesions in a turkey killed late in Dec., which had not shown any symptoms of blackhead, suggests the possibility that slight attacks may pass unrecognized. The disease, however, may prove fatal even in mature birds. We have recently received a report of the disease occurring in several adult hen turkeys, two of which died. The liver of one of these submitted for examination showed characteristic lesions of blackhead. A year old male said to have lost weight and shown sulphur droppings at about the same time, now appears normal. It is thus quite apparent that those turkeys which fail to become infected in early life may, contrary to popular belief, later on develop blackhead. It is probably true, however, that resistance increases with age so that the outlook is better both in respect to the frequency of blackhead and the prognosis of the cases that occur.

EMETIN TREATMENT

The efficiency of emetin in the treatment of entamebiasis of man suggested its use in this disease. Of three turkeys 72 days old, inoculated on Sept. 24 with bits of an active subcutaneous lesion, 2 received subcutaneous injection of emetin hydrochloride at intervals, while the third served as a control. Emetin treatment was commenced in one turkey (43) on the day following inoculation and was repeated at intervals of 24 or 48 hours, the dose being sufficient to cause loss of appetite and weakness. A total of 9 mg. of the drug was given in 10 injections during a period of 11 days. This treatment did not prevent the development of the disease, the local lesion developing to considerable size—2.5 x 1.5 x 1.0 cm.—but later regressed, and the turkey was shown by subsequent subcutaneous inoculation to be immune to blackhead. A similar course of treatment was commenced in the other turkey (42) 3 days after inoculation. The disease was not appreciably affected in this case, death occurring 14 days after inoculation. The control or untreated turkey (41) on account of weakness, was killed on the 11th day, and showed typical inoculated blackhead. It is evident that early sublethal doses of emetin hydrochloride fail to prevent the development of subcutaneously inoculated blackhead. While the recovery of one of the treated turkeys may have been due to the treatment, this isolated instance would not warrant such a conclusion at present, for one of the turkeys inoculated earlier in the season showed a spontaneous regression of the disease when killed.

SUBCONJUNCTIVAL INOCULATION

To ascertain whether flagellated forms of *Histomonas* escape from lesions in a readily accessible mucous membrane, a turkey (21) was inoculated beneath the conjunctiva near the anterior angle of the eye. Eight days after the inoculation there was a local swelling composed of opaque, whitish tissue beneath the conjunctiva, accompanied by a watery discharge from the eye. The swelling of the subconjunctival tissues increased and the eyeball was gradually covered by the edematous nictitating membrane. There was no ulceration up to the 12th day, when a slight abrasion appeared, possibly due to rubbing the affected side of the head against the feathers. Careful microscopic examination of the discharge showed an occasional rounded organism, but none that assumed the appearance of an active flagellate. Scrapings of the lesion after killing the turkey showed large numbers of *Histomonas* present.

ATTEMPTS TO PRODUCE BLACKHEAD BY INTESTINAL ROUTE

With the view of determining the source and distribution of the blackhead parasite, apart from the lesions of the disease, various materials were fed and injected.

Ingestion of Virus.—Since the alimentary tract is always primarily affected in the naturally acquired disease, it might be expected that feeding of the lesions would result in infections. Three turkeys 2 weeks old were fed repeatedly with fresh subcutaneous, liver and lung lesions, but none developed blackhead as the result of this procedure. One of these on two occasions was placed in a cold room and was thoroughly chilled by partial immersion in ice-water on the first day for four minutes and on the following day for 8 minutes. This turkey apparently thrived on the treatment and grew faster than any of the others hatched from the same setting.

Injection of Virus into Cecum.—That the virus, under normal physiologic conditions, might be destroyed in passing through the alimentary tract before reaching the ceca, was considered as a possible basis for the failure to infect by feeding fresh lesions. Accordingly, laparotomy was performed on 3 turkeys and a suspension of ground subcutaneous and lung lesions was injected directly through the wall of the cecum into the lumen. Precautions were taken to avoid undue trauma of the cecal wall, and the introduction of the virus into the tissue. In no instance did the injection of such virus alone result in infection.

That some form of *Histomonas*, not found in acute lesions, might be necessary for the penetration of the cecal mucosa before the natural infection could be initiated, was considered a possibility. It might be expected that such forms would occur in the cecal "core," which is of the nature of a cast of the cavity of the cecum, being composed chiefly of exudate deposited in successive layers. Accordingly, a suspension of ground-up cecal "core" taken directly from a freshly killed case of blackhead, was injected into the ceca of two turkeys, but in neither did blackhead develop.

Partial or temporary obstruction of the cecum, as suggested by the large cores frequently found in spontaneous blackhead, was simulated by injections of melted paraffin (melting-point 42 C.) into the lumen. One turkey received an intracecal injection of paraffin and after a brief illness discharged this material in the form of a cast of the cecum. Two turkeys were given an intracecal injection of paraffin followed by an injection of virus. The larger of these (83 days old) showed

no evidence of infection as the result of the procedure. The smaller turkey, 30 days old (weighing 280 gms.), developed blackhead. However, as the initial lesion in the cecum appeared at the point of puncture, it seems probable that infection resulted from the introduction of virus into the tissue where the cecal wall was punctured. It would not be safe to conclude that obstruction was the determining factor in this single positive case for the delicacy of the ceca in so small a turkey makes it almost impossible to inject into the lumen without some leakage.

Ingestion of Heterakis Ova.—In preparation for this experiment, turkeys hatched in an incubator were transferred to a freshly white-washed brooder. Attached to the brooder was a cage, which, except for the first two or three days, was covered with mosquito netting to keep out flies and other insects. Owing to cold weather and the failure of the brooder lamp, many of these specially hatched turkeys succumbed before they could be utilized. We were obliged therefore, to employ smaller groups than was originally intended, and to include for one group, older turkeys that had been fed blackhead lesions previously. On Sept. 22nd the turkeys were separated into 3 groups, each group being placed in a screened coop on clean grass:

Group A: Two turkeys (48 and 49, hatched Aug. 20) were fed large amounts of fresh blackhead lesions on three different occasions (Sept. 22, 24 and Oct. 5). These turkeys, although exposed to cold, dampness and accumulating filth, remained normal up to Nov. 11, and were then used for other experiments, and subsequently proved to be susceptible to inoculated blackhead. At necropsy they showed no evidence of cecal disease.

Group B: Three turkeys (50, hatched Aug. 20, and 51 and 52, hatched Aug. 31) were fed ripe ova of *Heterakis papillosa* on Sept. 22 and 24. One died of blackhead in 18 and 2 in 19 days after the first feeding of *Heterakis* ova, and each showed typical lesions of spontaneous blackhead.

Group C: This group consisted of 3 older turkeys, hatched Aug. 3, that had been fed blackhead lesions without apparent result. One of these (47, that had failed to show the normal weekly increase in weight) was killed on Sept. 24 and showed no lesions of blackhead and no *Heterakis* present. The other two (45 and 46) were fed ripe *Heterakis* ova and blackhead lesions on Sept. 24 and Oct. 5. One died of blackhead in 22 days and the other in 23 days after the first feeding of *Heterakis* ova.

The results of this experiment confirm those of Graybill and Smith,³ and indicate clearly that a disease in every respect identical with spontaneous blackhead may be experimentally produced by feeding the ripened ova of *Heterakis papillosa*. Apparently it is not necessary, under ordinary conditions, to furnish blackhead virus in addition to the worm ova, for brooder turkeys (Group B) placed on clean ground, without access to insects, contracted the disease when fed the worm eggs alone. It must be noted, however, that the precautions for the comparative isolation of these young turkeys from the time of hatching, did not prevent the appearance of protozoa in their cecal discharges. Although the brooder had been thoroughly whitewashed, the young turkeys which succumbed to cold and exposure showed myriads of intestinal flagellates, chiefly *Tetratrichomonas gallinarum*⁴ with fewer *Eutrichomastix gallinarum*.^{4 5} Several also showed numerous oöcysts of *Eimeria avium*.⁶

Whether or not the material containing *Heterakis* ova represents a source of the blackhead virus under the given circumstances may possibly be answered by more carefully conducted experiments. On account of the lateness of the season few newly hatched (and hence presumably clean) turkeys were available for subsequent experimentation.

Two of these were fed ripe *Heterakis* ova on Oct. 11, the day after they were hatched. They were then transferred to a clean chamber which served as a brooder, and were provided with sterilized food, grit and water. One of these, a weakling, died 10 days after the ingestion of *Heterakis* ova. Necropsy showed no evidence of blackhead and strange to say, no worms. Both ceca showed rather soft, friable cores which were found on microscopic examination to be composed of fibrin, cellular exudate and bacteria. A careful examination failed to reveal any protozoa.

The other turkey remaining apparently normal, was again fed *Heterakis* ova on Oct. 22 and 23. On account of increase in size it became necessary to remove him from the above chamber and on Nov. 1 (19 days old), he was placed with other slightly younger turkeys in a brooder outdoors. This turkey now failed to gain in weight and was killed on Nov. 8. There was a core in one cecum, the walls of both ceca were somewhat thickened, and numerous ill-defined,

³ Jour. Exper. Med., 1920, 31, p. 647.

⁴ Martin, C. H., and Robertson, M.: Quart. Jour. Micr. Sc., 1911, Part 1, 57, p. 53 and p. 58.

⁵ Kofoed, C. A., and Swezy, O.: Proc. Am. Acad. Arts and Sciences, 1915, 51, p. 289.

⁶ Silvestrini and Rivolta: Giorn. Anat. Fisiol., 1873.

small, grayish lesions were present in the liver, which proved to be blackhead on histologic examination. Only 2 immature worms were found on careful search of the cecal contents.

A control turkey, hatched Oct. 12, and kept in the outdoor brooder, received a single dose of the same *Heterakis* material on Oct. 22. This bird died on Nov. 5, of blackhead at a much more advanced stage than was found in the previous turkey, which received its last dose of *Heterakis* ova on Oct. 23, and was killed Nov. 8. Forty-two worms were counted, without a very complete search, from one cecum of the control turkey.

While the result is not wholly conclusive, the early stage of the disease seen in the "clean chamber" turkey strongly suggests that blackhead was contracted after the bird had been transferred to the outdoor brooder. It is a peculiar fact that only two worms developed in the "clean chamber" turkey, which received 3 doses of *Heterakis* ova, whereas the control turkey showed 42 worms in one cecum alone as the result of a single feeding of the same *Heterakis* material.

The subsequent feeding of *Heterakis* ova to several other turkeys resulted in the production of blackhead. The data of all the *Heterakis*-fed turkeys are collected in the accompanying table.

The attempt to eliminate all sources of blackhead infection other than the *Heterakis* material has not yielded definite results, so that it will be necessary to repeat this experiment.

INOCULATION TEST FOR PRESENCE OF HISTOMONAS

In order to test the possible infectiousness of various materials, including certain intestinal protozoa and *Heterakis* material, a series of turkeys was inoculated subcutaneously or intramuscularly.

Intestinal Protozoa.—Cecal contents of turkeys containing various species of protozoa were diluted with salt solution and inoculated into the breast. There were usually two or more species of protozoa in each cecal discharge used, but as a number of samples were employed, all the known cecal parasites of the turkey, with the exception of *Trichomonas eberthi*⁴ were available. The turkeys inoculated with cecal material invariably showed toxic symptoms during the first 24 hours after inoculation. There was local swelling at the site of inoculation, but this subsided in all instances without ulceration, and in no case did blackhead develop. The following protozoa were present in great numbers in the various samples injected: *Tetrachilomastix*

TABLE 3
FEEDING OF HETERAKIS OVA

Experiment	Turkey	Hatched	Treatment	Result
Group A Fed blackhead	48	Aug. 20	Sept. 22. Fed subcutaneous and lung lesions	Remained normal Nov. 26. Died (Inoculated blackhead of Nov. 11)
	49	Aug. 20	Sept. 24. Fed lung lesions Oct. 5. Fed subcutaneous and lung lesions	Remained normal Dec. 20. Killed (Inoculated blackhead of Dec. 11)
Group B Fed ripe Heterakis ova	50	Aug. 20	Sept. 22. Fed Heterakis ova collected Sept. 7 from chickens Sept. 24. Fed Heterakis ova collected Sept. 10 from chickens	Cecal infection Oct. 5. Sulphur droppings Oct. 11. Died Blackhead lesions left cecum and liver 125 immature Heterakis
	51	Aug. 31		Cecal infection Oct. 10. Died Blackhead lesions left cecum and liver 42 immature Heterakis
	52	Aug. 31		Cecal infection Oct. 11. Died Blackhead lesions left cecum and liver 128 immature Heterakis
Group C Fed blackhead and ripe Heterakis ova (Group C had previously been fed blackhead lesions on Aug. 17, 18 and 19)	45	Aug. 3	Sept. 24. Fed fresh blackhead lesions (subcutaneous and Heterakis ova collected Sept. 10 from chickens	Cecal infection Oct. 8. Sulphur droppings Oct. 17. Died Blackhead lesion right cecum and liver (Secondary mycosis) 73 immature Heterakis
	46	Aug. 3	Oct. 5. Fed fresh blackhead lesions (lung and subcutaneous and Heterakis ova collected Sept. 10 from chickens	Cecal infection Oct. 8. Sulphur droppings Oct. 16. Died Blackhead lesions left cecum, liver and kidney 18 immature Heterakis
Control for Group C	47	Aug. 3	None	Control Sept. 24. Killed No lesions No Heterakis found
"Clean chamber"	58	Oct. 10	Oct. 11. Fed Heterakis ova collected Sept. 7 from chickens	No infection Oct. 21. Died Both ceca contained delicate cores. Microscopically no evidence of blackhead No Heterakis found
	59	Oct. 10	Oct. 11. Fed Heterakis ova collected Sept. 7 from chickens Oct. 22. Fed same material Oct. 23. Fed same material Nov. 1. Moved to outdoor brooder	Cecal infection (early) Nov. 8. No gain in weight. Killed Blackhead lesions both ceca and liver 2 immature Heterakis
Control for No. 59	60	Oct. 12	Oct. 22. Fed Heterakis ova collected Sept. 7 from chickens	Cecal infection Nov. 5. Died Blackhead lesions both ceca and liver 42 immature Heterakis (one cecum)
	63	Oct. 12	Nov. 18. Fed Heterakis ova collected Sept. 10 from chickens	Cecal infection Dec. 1. Sulphur droppings Dec. 4. Killed Blackhead lesions both ceca and liver 87 immature Heterakis

gallinarum,^{4, 7} *Eutrichomastix gallinarum*,^{4, 5} *Tetratrichomonas gallinarum*,⁴ *Entamoeba gallinarum*,⁸ *Pygolimax gregariniformis*,⁸ and *Eimeria avium*.⁶

Contents of Diseased Ceca.—This material was collected from a case of blackhead by searing through the wall of the diseased cecum in order to avoid contamination with *Histomonas* from the infected tissue. The injection on two separate occasions of such material into the breast of a normal turkey failed to produce blackhead.

Cecal Mucosa.—The subcutaneous injection of scrapings of the cecal mucosa of several old turkeys also resulted negatively.

Heterakis.—*Heterakis* freshly obtained from cases of blackhead were washed several times in salt solution and injected subcutaneously into a turkey with negative result.

Heterakis Ova.—Material containing ripe *Heterakis* ova, which when fed to turkeys produced blackhead, was injected into the breast of a normal turkey with negative result.

Cultures of Ameba and Flagellates.—Cultures obtained from the *Heterakis* material containing a species of ameba and a flagellate not yet classified, but belonging to the *Bodonidae*, were also injected into turkeys with negative result.

Tracheal Washings.—The washings from the trachea in a case of inoculated blackhead with extensive lung involvement, were inoculated subcutaneously without effect.

Sequestrum.—In order to determine to what extent *Histomonas* survives in the necrotic material in a subcutaneous lesion a turkey was inoculated with a bit of the necrotic portion. No infection resulted. Later the sequestrum and surrounding tissue from a healing subcutaneous lesion was implanted in the breast of a normal turkey with negative result.

Table-4 gives the data of these various experiments.

It is possible that blackhead virus, even if present, might have been incapable of developing in the presence of the acute inflammatory reaction resulting from the injection of the associated material. Unfortunately the control, that is, the inoculation of a mixture of blackhead virus and cecal contents, was omitted, but will be carried out in future work.

⁷ La Fonseca: Brazil Medico, 1915, 29, p. 281.

⁸ Tyzzer, E. E.: Jour. Med. Res., 1920, 41, p. 199.

TABLE 4
INOCULATION EXPERIMENTS

Tur- key	Hatched	Injection		Result
		Date—Dose	Material	
2	June 7	June 23 1 c c left breast	Scrapings cecal mucosa from an old turkey	No reaction
3	June 7	June 23 1 c c left breast	Ibid.....	No reaction
7	June 7	July 30 1 c c left breast	Ibid.....	No reaction
20	June 16	Aug. 13 1 c c right and 5 c c left breast	Cecal contents..... <i>Tetrachilomastix gallinarum</i> <i>Eutrichomastix gallinarum</i> <i>Entamoeba gallinarum</i>	Acute inflammation Healing without ulceration
21	June 16	Aug. 13 5 c c right and 1.5 c c left breast	Cecal contents..... <i>Eutrichomastix gallinarum</i> <i>Entamoeba gallinarum</i> <i>Pygollmax gregariniformis</i>	Extensive induration involving muscle Healing without ulceration
22	June 16	Aug. 14 5 c c right and 1 c c left breast	Cecal contents..... <i>Tetrachilomastix gallinarum</i> <i>Eimeria avium</i>	Slight local inflammation
23	June 16	Aug. 17 5 c c right and 1 c c left breast	Cecal contents..... <i>Eutrichomastix gallinarum</i> <i>Entamoeba gallinarum</i>	Local inflammation Healing without ulceration
		Sept. 13 0.1 gm. left breast	Necrotic material from subcu- taneous lesion	No reaction
24	June 16	Aug. 20 5 c c right and 1 c c left breast	Cecal contents..... <i>Tetratrichomonas gallinarum</i> Few <i>Eutrichomastix galli- narum</i>	Slight local reaction Healing within 3 days
		Oct. 11. 3 separate in- oculations in left breast	Washed <i>Heterakis</i> Fresh from diseased ceca 3 cases of blackhead	Slight foreign-body reaction at site of each inoculation
		Oct. 11 1 inoculation right breast	Washed <i>Heterakis</i> Fresh from normal ceca 3 cases of blackhead	
25	June 16	Aug. 21 5 c c right and 1 c c left breast	Cecal contents..... (Blackhead case) <i>Eutrichomastix gallinarum</i> Blastocysts (Possibly a few <i>Tetrachilo- mastix</i> present)	Extensive induration Healing without ulceration
		Oct. 17 3 c c left breast	Cecal contents..... (Blackhead case)	Moderate induration Healing without ulceration Nov. 24. Symptoms of blackhead Nov. 30. Improvement Dec. 22. Killed Spontaneous blackhead Recovery indicated by scars
26	June 16	Aug. 24 0.1 gm. left breast	Sequestrum and tissue from healing subcutaneous lesion	No reaction Susceptibility shown by subse- quent inoculation (table 2)
48	Aug. 20	Oct. 14. 5 c c right, 1 c c left breast	Cultures of amoebae and flag- ellates from <i>Heterakis</i> ma- terial	No reaction
		Oct. 25 2 c c right breast	Washings from trachea of case of inoculated blackhead	No reaction
49	Aug. 20	Oct. 14 1 c c left breast	<i>Heterakis</i> ova..... Collected from chickens Sept. 10	No reaction Same material produced black- head when fed to young turkeys

ASSOCIATION OF HETERAKIS PAPILLOSA WITH SPONTANEOUS
BLACKHEAD

This worm appears to be very generally distributed in common fowls and turkeys. Absent in newly-hatched stock, the prevalence of these parasitic worms depends on the environment in which the birds are reared. Turkeys have been kept entirely free from worms for several months after hatching, by isolation on clean grassed-over areas. When reared on ground previously occupied by turkeys, or when allowed access to hen-coops, young turkeys soon acquire considerable numbers of these worms. It is probable that the warmth of the summer months favors the development of the *Heterakis* ova so that greater infestation occurs as the season progresses. During the past summer a record was kept of the number of *Heterakis* found in practically all turkeys necropsied.

Blackhead cases appeared spontaneously among the turkeys kept in the "old yard," that is, the enclosure which had been used for the two previous summers for experimental work on blackhead. The majority of the cases (8) occurred in July and August, but there were also 2 cases in December. None of these cases showed over 23 worms, and in 2 of the earlier cases none could be found on careful search of the entire cecal contents.

A comparison of the number of worms found in our 8 cases of spontaneous blackhead, and 38 turkeys without cecal infection, shows no predominance in the former series. The examination of 14 cases of blackhead from outside sources also showed some in which no worms could be found, others with few, and yet others in which worms were numerous. It is evident that there is no close relationship between the incidence of blackhead and the number of worms present in our series of turkeys.

It appears probable that a pathologic condition is necessary for the production of blackhead, a condition which may result not only from overwhelming invasion by *Heterakis*, but also from other, at present, unknown causes. We have noted rudimentary casts or cores in the ceca of young turkeys in which there was microscopically no evidence of blackhead, but it is possible that such conditions, with *Histomonas* present in the cecum, may lead to the invasion of the tissues by this organism. The histologic study of such cores, developing in the absence of blackhead, demonstrates their pathologic character—since they consist chiefly of a fibrinous and cellular exudate intermingled with bacteria, but occasionally may also show red blood corpuscles. On

cross-section their laminated structure indicates that they are formed by successive deposits of exudate. The mucous membrane may show no more than slight infiltration and degeneration of the surface epithelium in such instances.

EXPOSURE TO COMMON FOWLS

One turkey was kept in the care of a common hen from the time of hatching on September 22. When 18 days old it was placed in a hen-yard for a short time each morning, and when 54 days old it was kept constantly with a small flock of hens. Symptoms of blackhead appeared 13 days later at the age of 67 days. Considerable resistance was shown by this turkey, as it lived 14 days after showing symptoms. Dying at the age of 83 days, chronic blackhead lesions were found at necropsy. *Heterakis* was present in greater numbers (279) in this bird than in any of the turkeys to which the parasite had been fed experimentally. A large proportion of the worms were fully matured. This indicates that young turkeys may gradually acquire many times the number of *Heterakis* than would certainly suffice to produce blackhead when fed experimentally on a single occasion. The result of this experiment also confirms our previous findings as to the danger of exposing young turkeys to the haunts of common fowls. Isolation thus appears to furnish the only practical means at present available of limiting blackhead.⁹

EXPOSURE TO TURKEY DROPPINGS OF PREVIOUS SEASON

Three turkeys 9 days old were confined in a cage for 47 days on the accumulated droppings and soil of the night-quarters of the previous season's flock. The attempt was made to exclude flies and insects from this cage by screening but, on the contrary, the cage proved itself a very effective fly-trap on warm days, catching swarms of blue-bottle flies on which the young turkeys gorged themselves. Although this cage became exceedingly filthy, so that the turkeys were at all times dirty and bedraggled in appearance, a much more rapid gain in weight was made than in a control turkey which was hatched on the same date and had the range of a large yard of clean grass. The phenomenal growth of these turkeys closely confined in filthy quarters is somewhat surprising, but may be accounted for by the abundance of insect food available.

⁹ Tyzzer, E. E., and Fabian, M.: Commonwealth of Mass., Dept. of Agri., Dept. Bull. No. 15, March, 1921.

INOCULATION OF THE TURKEY EMBRYO WITH BLACKHEAD VIRUS

Four turkey eggs which had been incubated for 12 days were inoculated with active blackhead virus, 2 through the upper and 2 through the lower surface, with the intention of inoculating the amniotic and yolk sacs. The examination of 3 of these, 6 days after inoculation showed one to be sterile, one with a dead embryo, and the third with a living, apparently normal embryo. The fourth egg, on further incubation, failed to hatch, but when opened showed a full-sized, normally developed, turkey chick. In none of these eggs was there any evidence of blackhead.

SUSCEPTIBILITY OF OTHER SPECIES TO BLACKHEAD

As stated in a previous paper,¹ it was found possible to infect newly-hatched chickens by inoculation. The local lesions were self-limited, although in one instance there were secondary lesions in the lungs. It was also demonstrated that a certain proportion of pigeons (30%) were susceptible to the subcutaneous inoculation of blackhead. The lesions regressed, however, after the ninth or tenth day.

An attempt has been made this season to infect other species by inoculation. Many European sparrows were inoculated, but without result. Two ducks (Indian runners) were inoculated without result.

Three guinea-chicks were inoculated; local lesions developed in each one, but regression followed after the 12th day. Microscopic examination of the lesions showed extensive cellular reaction, moderate numbers of parasites, but no necrosis.

One pheasant was inoculated when 39 days old. A small local lesion developed slowly and persisted until the bird was killed on Sept. 7, 17 days after inoculation. The local lesion measured 1.3x0.5x0.5 cm. and showed microscopically a marked cellular reaction and numerous parasites, but no evidence of necrosis. No other lesions were noted.

SUMMARY

The virus of blackhead, as found in turkey liver lesions, survives for at least 4 days at 5 C., deteriorates more rapidly at 22 C. and is immediately destroyed by freezing.

As compared with subcutaneous inoculation, intravenous inoculation results much less frequently in infection. The failure of an intravenous inoculation to cause infection is not due to an insusceptibility of the turkey employed; for if this procedure is repeated a

sufficient number of times, infection follows. Furthermore, turkeys that have been inoculated intravenously with negative result may now be successfully inoculated subcutaneously. That intravenous inoculation should so frequently fail to produce infection is the more remarkable since dissemination of the virus by the blood stream almost invariably occurs in infected turkeys.

These results clearly show that immunity is not produced by negative intravenous inoculation; it has been found impossible, however, to infect a turkey that has recovered from inoculated blackhead. Although not yet demonstrated, it may be expected that turkeys recovering from spontaneous blackhead will also prove immune. Resistance to blackhead seems to increase with age, so that fewer maturing birds show symptoms of infection and recovery is evidently more frequent. It is not improbable that mild attacks may pass unrecognized in large turkeys.

The treatment of the turkey by repeated subcutaneous injections of emetin hydrochloride in doses sufficiently large to produce toxic symptoms, fails to prevent the development of subcutaneous blackhead, even though commenced immediately after inoculation. Of two cases thus treated, one recovered; but spontaneous recovery from blackhead occasionally occurs.

Repeated injections of Niagara blue and India ink (whether before or after inoculation), do not apparently alter the course of the disease.

The study of the conjunctival discharge in subconjunctival blackhead furnishes no evidence that *Histomonas* escapes from a mucous surface in flagellated form. This is consistent with the findings in previous histologic studies of cecal lesions in which no intermediate stages were found between *Histomonas* and any of the flagellates in the cecal glands or contents.

Attempts to produce blackhead by the intestinal route show that the disease is not caused by the mere feeding of infected tissues. The injection into the lumen of the normal cecum of either virus from active lesions, or the substance of "cores" from ceca infected with blackhead, may not result in blackhead. Temporary obstruction has been experimentally produced by the injection of melted paraffin into the lumen of the cecum. This alone, or in conjunction with blackhead virus, fails to cause cecal infection.

The feeding of large numbers of ripe ova of *Heterakis papillosa* to isolated groups of turkeys has resulted in typical blackhead. It was noted, however, that turkeys raised in fairly strict isolation acquired

great numbers of intestinal protozoa before leaving the brooder. Apparently ripe *Heterakis* ova may fail to develop when fed to young turkeys kept in a clean chamber and furnished with sterilized food and water, but they readily develop in those having access to the soil. The reason for this is not apparent.

The subcutaneous inoculation of various materials—cecal discharges containing various species of cecal flagellates and amebae; blastocytes, cultures of protozoa of cecal origin, exudate from a cecum showing blackhead lesions, ripe *Heterakis* ova and washed *Heterakis* from several cases of blackhead—have all failed to produce blackhead. It is possible that the acute inflammation resulting from the injection of cecal contents may interfere with the development of *Histomonas*, were the latter present in the material used. Although this organism is sometimes found in considerable numbers in the air-spaces, in stained sections of pulmonary lesions of inoculated turkeys, the injection of tracheal washings from a single case resulted negatively. The results of the inoculation of necrotic portions of subcutaneous lesions, or material from healing lesions indicate that *Histomonas* does not persist long in dead tissues.

While blackhead appears to develop quite constantly as the result of feeding ripe *Heterakis* ova, the study of a large number of turkeys indicates that the disease may occur when this worm is rare or entirely absent from the ceca. It is also apparent that young turkeys do not develop blackhead as readily from the slow acquisition of *Heterakis* ova, as they do from large overwhelming doses, such as those given experimentally. These findings suggest that the invasion of the tissue by *Histomonas* in spontaneous blackhead is not dependent solely on the presence of *Heterakis* but on pathologic conditions that may occur not only in association with this parasite worm but also quite independent of it.

Soil contaminated with the droppings of turkeys of the previous season may not of itself furnish the conditions which would cause young turkeys kept thereon to develop blackhead.

A self-limited infection, similar to that produced in young chickens and pigeons, follows the inoculation of blackhead into young pheasants and guinea-chicks. All of these species show a much more extensive tissue reaction to the virus than that which occurs in the more susceptible turkey. Both the European sparrow and Indian Runner duck appear to be nonsusceptible.

The turkey embryo is apparently unsuitable as a medium for the development of *Histomonas*; for incubated turkey eggs were inoculated with blackhead virus without result.

Blackhead has followed the exposure of a young turkey to common fowls, confirming the experience of the previous years and emphasizing the importance of isolation in rearing turkeys.