BLACKHEAD IN CHICKENS AND ITS EXPERIMENTAL PRODUCTION BY FEEDING EMBRYONATED EGGS OF HETERAKIS PAPILLOSA.

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The occurrence of blackhead or typhlitis in chickens has been noted by several observers. To the meager literature reported by Smith in 1915 the following may be added.

In 1907 Theobald reported the occurrence of the disease in England. In 1908 Milks observed it in four widely separated localities in Louisiana. It was not seen in birds more than 6 weeks old. The course of the disease was rapid and the mortality 30 to 50 per cent. Higgins found in one chicken ulceration of one cecum which he describes as typical of enterohepatitis or blackhead in turkeys. Tyzzer observed the disease in two chickens. In a chick 4 weeks old there was found a slight invasion of the cecum and extensive involvement of the liver. A slight involvement of one cecum was later found in a hen 2 years old.

During the past 3 years a few cases of disease in chickens associated with *Amoeba meleagris* were brought to the laboratory. In the brief notes given below Nos. 18 and 19 were from one flock, and Nos. 91 and 92 from another. These flocks were over 50 miles apart.

Chicken 18, White Leghorn, brought alive Apr. 18, 1917. Said to have been hatched in Feb. At the autopsy there was found an intussusception of the small intestine of which about 7 cm. were involved. This portion was in a hemor-

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rhagic condition. Each cecum contained a consistent fecal mould coated with a thin but tough, partly white, partly grayish exudate. The walls of the ceca were thickened, opaque. The liver contained quite small, scattering, indistinctly outlined, yellowish foci. Sections of the ceca showed complete loss of the mucosa which was replaced by a dense layer of fibroblastic tissue. Below this layer in the muscular coat and extending to the serous covering, there were numerous vacuoles of various sizes in the tissue which contained each from one to a dozen or more roundish bodies about 6μ in diameter. Each contained a nucleus with a minute deeply stained caryosome in state of binary division in some of the organisms. In the liver sections the foci seen at autopsy were about one-third the diameter of a lobule. They consisted of necrotic liver cells, parasites like those of the cecal walls, chiefly within giant cells, and many lymphoid elements.

Chicken 19, White Leghorn, received at the same time and from the same flock. The ceca were affected as in No. 18, but the liver was free from foci. Sections of the ceca showed the same kind of changes as in No. 18, but portions of the mucosa were still present. Parasites were rare and seen only in some of the many vacuoles scattered through the superficial fibroid substitute for the destroyed parts of the mucosa. These vacuoles probably lodged parasites now destroyed.

Chicken 91, Rhode Island Red, had been killed before reaching the laboratory. Female, weighing 500 gm. Both ceca contain consistent cores of fecal material which are not adherent to the wall. The underlying mucosa is smooth and free from necrotic areas. The liver is enlarged and covered with opaque whitish foci 6 to 7 mm. in diameter. Some contain minute yellowish specks in the whitish ground. In teased preparations from these foci numerous homogeneous, roundish bodies, about 12μ in diameter, are seen. These are not distinguishable from *Ameba meleagridis* found in diseased turkeys. In sections of the ceca, the thickening of the wall is due to a marked increase of plasma-like cells in the intertubular tissue. Small groups of *Ameba meleagridis* are scattered through the mucosa and one large group is located in the muscular portion of the intestinal wall. Coccidia are scattered through the core. Sections of the liver contained large numbers of *Ameba meleagridis*. This organ was involved as extensively and intensively as in fatal cases of blackhead in turkeys, while the lesions of the ceca were slight.

Chicken 92, Rhode Island Red, was received dead from the same flock. In this bird coccidiosis predominated and the only traces of blackhead found were two microscopic foci in the liver tissue crowded with *Ameba meleagridis*.

From the preceding quotations and studies it is obvious that fatal blackhead is relatively rare in chickens. The data indicate that it may be a quite common disease which passes unnoticed because partial or total recovery is the rule. The injury to the mucous membrane as shown in Cases 18 and 19 may prevent the recovered bird from reaching normal full development. The lesions and the asso-
ciliated parasite appear to be identical with those observed in turkeys. The disease shows, however, greater variations from case to case. In some the liver is the chief seat and the ceca almost intact. In others the reverse is true.

The Experimental Production of Blackhead.

Following the successful attempts to produce blackhead in turkeys by feeding embryonated eggs of *Heterakis papillosa*, similar experiments were made on chickens.

*Experiment 1.*—The chickens were hatched in an incubator. The eggs before incubation had been washed to remove any adhering dirt, placed in 0.5 per cent bichloride of mercury for 30 seconds, washed again, and dried. During the experiment the chicks were kept in brooders within isolation units, protected against vermin and infectious material likely to reach them if kept in the open. The only source of infection to which they were exposed was the grain and sour milk fed regularly with cooked food.

The worms were obtained from the ceca of two adult hens penned with old turkeys in an outdoor enclosure since the fall of 1919. To liberate the ova, the females were cut up in a Petri dish containing a shallow layer of physiological salt solution. In this they were incubated at room temperature. The feeding was done Mar. 1, when the cultures were 14 days and the chicks 32 days old. Four White Leghorns, four Plymouth Rocks, and four Rhode Island Reds were fed the ova mixed with the food in cages. After the feeding their feet were thoroughly washed and they were placed in a brooder in an isolation unit. From the same hatch nine chickens were held as controls in another unit. The chickens were killed and autopsied at certain intervals after the feeding. In case the ceca were diseased, one was opened and examined as to contents and condition of the mucosa and the other simply incised and then placed in Zenker's fluid for future study.

Chickens killed respectively 1, 2, 3, and 7 days after feeding were found normal, except for weakness of the legs, due presumably to confinement. Sections of one cecum and of liver tissue showed normal conditions. The contents of the ceca consisted of bacteria, a few food remnants, and some *Heterakis* larvae. The walls were free from infiltrations and contained only the usual number of lymphoid cell groups. In the livers a few small compact groups of cells resembling lymphocytes were present.

In No. 279, killed 10 days after feeding, both ceca were distended and contained a firm reddish core replacing the usual soft contents. The walls of the non-villous portion were quite uniformly thickened to about 1 mm. Larval worms

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(Heterakis papillosa) were found in the lumen. The liver was of normal appearance. Transections of one cecum fixed without disturbing the core showed a number of changes. The core, replacing the normal fecal mass, consisted almost wholly of red blood cells, only a few of which had retained the hemoglobin. There were also some necrotic tissue cells in the mass. Throughout the clot were small colonies of bacteria and a few larval forms of Heterakis. The mucosa was greatly altered. In several places it was completely destroyed. Elsewhere, the tubules were scarce. They were distorted and oblique to the surface. The mucosa and submucosa contained large groups of lymphocytes and strands of the same were lying in the muscular tissue. Parasites resembling Amoeba meleagridis were abundant in the mucosa and submucosa. Many were within giant cells. The muscular coats were free. Sections of a small nematode were found in the intertubular tissue, measuring about 0.05 mm. in diameter. In the liver there were fairly numerous dense collections of lymphocytes up to 0.15 mm. diameter. Parasites could not be detected in these foci.

No. 280, killed 11 days after feeding, presented the same lesions. Neither this nor the preceding bird had shown evidences of disease. The core in the cecum was like that of No. 279. The wall presented certain differences, however. The epithelium and the tubules were normal and intact. The submucosa was markedly edematous and the lymphoid tissue somewhat increased. The blackhead parasite had permeated quite generally and distended the intertubular tissue of the mucosa and had penetrated into the submucosa in large numbers. Worms were seen in the mucosa and the lumen. The cell foci as described under No. 279 were present in the liver of this case.

Chicken 281 was killed 14 days after feeding ova. It had not shown signs of illness. The contents of one cecum were normal and contained larval worms. The wall was possibly slightly thickened and the mucosa sprinkled with minute hemorrhages. Sections of the other cecum showed conditions differing both from the preceding and the following case. The contents were normal. A small portion of the wall was normal, the rest thickened. The tubules and surface epithelium were intact. One larval worm was found partly embedded in a tubule. The increased thickness of the wall was due chiefly to a great increase in lymphoid cell groups in the submucosa. In the mucosa there was a slight diffuse infiltration of plasma cells. The muscular coat was not involved. Amoeba meleagridis occurred in groups of two to six or more individuals in tissue spaces. The parasites were relatively scarce as compared with No. 279. The liver contained minute collections of lymphoid cells, from one to two in a field of the 16 mm. objective.

Chicken 282 was killed 15 days after feeding. It had not appeared quite normal. Both ceca were distended and firm to the touch, owing to the presence of hemorrhagic cores replacing normal feces. The walls were 2 to 4 mm. thick. Larval worms were present. The core was made up of a mass of red corpuscles embedded in a homogeneous feebly stained matrix. In one spot it was incorporated with the wall and here the mucosa was destroyed. The rest of the mucosa was covered with epithelium but the tubules were partly destroyed, partly dis-
torted. The intertubular tissue was infiltrated with lymphoid cells and amebae.
The submucosa was markedly edematous. At irregular intervals it contained
large dense collections of cells enclosing amebae. In a few places the muscular
coad was infiltrated with lymphocytes.

Chicken 285, killed 18 days after feeding, had not shown symptoms of illness.
It was normal as to ceca. There were a few whitish foci 1 to 2 mm. in diameter
in the liver. Transverse sections of fixed and hardened tissue from four different
levels of one cecum showed the same changes. There was a cellular infiltration
and increase of lymphoid tissue in the mucosa and a few tubules were markedly
distended. Just below the muscularis there was a dense zone or layer of cells,
probably lymphocytes, and roundish masses of the same, suggesting newly formed
follciles. There was no core or mass of exudate in the lumen and there were no
parasites in the tissues. In the liver sections, besides the minute collections of
lymphoid cells, there are a few larger groups of the same type of cells enclosing
necrotic liver tissue. Heterakis larvae were in the ceca.

Chicken 286, killed 23 days after feeding, had not shown symptoms. The con-
tents of the ceca were normal and larval worms were present. There was evidence
of increased numbers of cells, resembling plasma cells in the mucosa. Lymphoid
follciles were more numerous than normally in the submucosa, but the diffuse
infiltration of lymphocytes was lacking.

Chicken 287 was killed after 28 days. It had not shown symptoms. One
cecum contained some Heterakis larvae, but contents were normal. Sections of the
other cecum showed slight irregularity and occasional loss of tubules, their places
being filled with cells of lymphocyte type. In the intertubular tissue there was
an increase of plasma-like cells. The submucosa contained more than the normal
number of lymph follicles. There was one group of eighteen follicles. In the
liver certain whitish foci, 1 mm. in diameter, seen at the autopsy, were found to
be made up entirely of cells of lymphocyte type. A central mass about 0.5 mm.
diameter was enclosed in a ring of follicles. Many small foci of similar cells,
0.1 to 0.15 mm. diameter, frequently filling out vessels were also present.

Chicken 288 was killed after 29 days. Heterakis larvae in ceca. Although the
animal had shown no distinct signs of disease and the organs appeared normal
with the exception of a few whitish areas on the liver 1 to 2 mm. in diameter,
the walls of the ceca were not normal. The mucosa had the usual number of
rightubes with the exception of about one-tenth of the circumference, in which the
tubules were replaced with lymphoid cells. There was a general increase of cells
of plasma type between the tubules and the number of follicles in the submucosa
was increased. In a section of the liver there were two foci, one consisting of a
plug of cells in a vessel, the other a group of cells simulating four lymph follicles
surrounding a small vessel also plugged with lymphoid cells.

Putting the data of this experiment together, we observe that as a
result of hemorrhages the contents of the ceca appeared as firm cores
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on the 10th to the 15th day inclusive, but not before the 10th, nor after the 15th day. Protozoan parasites were in the walls of the ceca within the same period. A larger number of cases examined at shorter intervals will define these limits more accurately.

Of the controls, which had remained well with the exception of some lameness due to confinement, two were killed 17 days after the other lot had been fed, and one 30 days after. None showed signs of lesions and worms were not found.

Experiment 2.—In this experiment, which is similar to Experiment 1, certain controls were introduced. The chickens were obtained from a poultry farm just after they had been hatched and placed in a brooder in an isolation unit. They were fed as were those in Experiment 1. The worms used were obtained from the same source as those used in Experiment 1. The cultures were prepared in the same way as heretofore. Three groups were included, each consisting of four White Leghorn chickens. Group I was fed with cultures made by cutting up adult female Heterakis to permit eggs to escape. Group II received cultures prepared by cutting up only males. Group III was fed with sediment from the washed contents of the ceca after the worms had been removed and the contents run through a wire screen of No. 300 mesh which did not permit the few free ova to pass through. At the time of feeding, the cultures were 20 days and the chickens 18 days old. All of the chickens in Group I were diseased, as the following notes show.

On the 9th day after feeding No. 290 was not quite normal. Its head was drawn back and its wings drooped slightly. It was chloroformed next day. The body was in good condition and only the ceca were affected. They were distended to about 1 cm. in diameter, firm to the touch, and hemorrhagic at the distal end. One, cut open, contained a spongy pink and reddish core slightly adherent to the hemorrhagic mucosa. The liver showed some whitish specks. Transverse sections from the other cecum showed the presence of a core made up of a meshwork of a homogeneous substance containing some red corpuscles. It was attached to the greater part of the wall and the mucosa was here destroyed. The remaining mucosa was low, with continuous epithelium and distorted tubules. The submucosa was broadened by infiltration with large numbers of Amoeba meleagridis and lymphoid cells. The muscular layers were also infiltrated with cells, even into the mesentery. Larvae were present in the mucosa and lumen. In the liver sections only minute groups of lymphocytes were present.

Chicken 295 was not quite normal on the 11th day, when it was chloroformed. The gross appearance of the ceca and the condition of the wall and core of one cecum as shown in sections were so like those of No. 290 that a detailed statement is omitted. In the liver a necrotic focus with a few Amoeba meleagridis in it deserves mention.
Chicken 296 was killed on the 12th day. It had not shown any distinct signs of illness. Both ceca were distended and firm. One was opened and a long, cylindrical, non-adherent core, gray and brown in color, removed. The wall was about 2 mm. thick. In stained sections, the core was found composed chiefly of exudate cells (lymphocytes). It was attached at one spot to the wall and here the mucosa had been destroyed. Elsewhere the low mucosa was covered with epithelium. Normal tubules were scarce. The rest were much dilated and filled with cell debris. The mucosa, submucosa, and muscular layers were indistinguishable, owing to a general invasion of Amoeba meleagridis and a general infiltration of cellular elements, lymphocytes, and plasma cells. There were also in the muscular coat masses of cells simulating lymph follicles. In the liver there were the usual minute collections of lymphocytes and some necrotic foci, but parasites were not seen in them.

The 4th and last chicken of Group I, No. 298, was killed 13 days after feeding. It had been clinically normal. Both the macroscopic and microscopic pictures were so like those of No. 296 that no detailed description is given. The invasion of Amoeba meleagridis was slight below the submucosa, but the infiltration of the musculature with large masses of lymphocytes, even into the mesentery, was striking.

In three of the foregoing cases larval worms were found in the contents of one cecum when examined fresh. In the fourth they were demonstrated in transections of the core of the other cecum.

The four chickens of Group II remained normal until they were killed on the 10th, 11th, 13th, and 13th day, respectively. No lesions were found and worms could not be detected. The same was true for the chickens of Group III, which were killed on the 10th, 11th, 12th, and 13th day, respectively.

DISCUSSION.

The foregoing experiments demonstrate that a disease of the ceca very closely resembling that of the enterohepatitis or blackhead of turkeys can be produced in chickens still in the brooder by feeding an overdose of embryonated eggs of Heterakis papillosa. The lesions appeared on or about the 9th day after feeding. Chickens killed after 15 days showed, in spite of an intact mucosa, signs of a past inflammation in the presence of large numbers of cells of the lymphocyte type in mucosa and submucosa and in the increased number of roundish accumulations of similar cells resembling lymph follicles.

The active disease manifests itself in a pouring out of blood into the lumina of the ceca, which coagulates into a firm spongy core. Later the red corpuscles disappear and lymphocytes emigrate from the
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injured mucosa to form the outer zone of the core. This is attached in part to the wall and here the mucosa is necrotic. The walls of the ceca are thickened up to 4 mm. in diameter. The thickening is due to edema, infiltration of lymphocytes and of *Amoeba meleagris* in large numbers. The mucosa is more or less injured in addition to the necrosis where the core is adherent. The tubules are distorted, dilated, and in part missing. Some are filled with cell debris. In all cases there is a diffuse infiltration of plasma-like cells between the tubules. Occasional hemorrhages into the substance of the mucosa are present.

The liver, besides containing in all cases microscopic focal collections of lymphocytes, shows rather infrequently barely visible yellowish specks which consist either of necrotic foci in which giant cells and cells of endothelial type are replacing the liver cells or else of collections of lymphocytes in the form of roundish follicles or within vessels. *Amoeba meleagris* was seen in one case in a necrotic focus. On the whole, the changes in the liver so formidable in turkeys are insignificant.

The protozoan parasites presented the same morphological characters shown in the turkey's tissues. They occupied the tissue spaces in mucosa and submucosa and more rarely in the muscular coat. As a rule, the bodies of the parasite appeared as if in a state of disintegration except in No. 281, in which they had a homogeneous cytoplasm. In some cases most of them were within phagocytic cells. In one case (No. 290) the protozoa set free from teased portions of the mucosa showed, without the use of a warm stage, finger-like pseudopodia in continual change.

The genesis of the clot in the ceca is not clear. The cases studied were not timed so as to encounter it in its formation. This gap must be filled before any basis for a discussion of the nature and significance of the cecal lesions can be found.

In general the larval stages of *Heterakis* were present but in small numbers. In four cases individuals were detected in the mucosa itself.

Flagellates so common in the tubules of the turkey were entirely absent in the two series of chickens.
The injurious effects of feeding embryonated eggs of *Heterakis papillosa* to young chickens are no less definite than those observed after feeding them to turkeys. There are, however, certain distinctions to be drawn between the disease in turkeys and in chickens. The turkeys were found much more susceptible since the feeding produced uniformly a severe disease probably fatal in all birds if those that were chloroformed had been allowed to live longer. The effect on the condition of the young chickens was slight and they would probably have all survived.

The lesions due to the feeding differ materially as regards the liver. In turkeys this organ is almost uniformly invaded by *Amoeba meleagridis* and the resulting foci of multiplication lead to a destruction of a variable amount of liver tissue, often over 50 per cent. In chickens the invasion is so slight that when it does take place the resulting lesions are scarcely more than microscopic in size. The liver is not wholly immune, however, as spontaneous cases now and then prove.

As to the ceca, the lesions induced in chickens appear formidable enough, but they probably undergo speedy resolution and the destroyed mucosa is covered with epithelium in due time. The after effects of the partial destruction of the mucosa may be more serious and tend to interfere with the normal growth of the chicken. This destruction may be permanent and lead to a replacement of the mucosa by scar tissue, as shown in the two spontaneous cases described above. In general it may be assumed that injury to the ceca due to *Heterakis* and *Amoeba meleagridis* is not uncommon and its causal relation to other pathological conditions of poultry may be far reaching. Not until this worm has been largely suppressed can the extent of the injury due to it be inferred in retrospect. The degree of injury inflicted appears to be largely a question of dosage. The more ova ingested the more widespread and intensive the lesions, whereas the ingestion of a few does not appear to be dangerous even to turkeys.

Indications of a tendency of the nematodes to encyst in the walls of the ceca, as observed by Letulle and Marotel7 in a pheasant, were wholly absent in the cases examined. The presence of *Amoeba meleagridis* in chickens still in the brooder after they have been fed ova either free or still within fragments of

7 Letulle, M., and Marotel, Arch. parasit., 1908, xii, 361.
worms incubated in physiological salt solution for 15 to 20 days at room temperature points to the presence of *Ameba meleagridis* in the cultures. This cannot be confirmed or disproved until some method other than feeding *Heterakis* eggs is found which will induce conditions favorable to the invasion of *Ameba meleagridis* into the walls of the ceca. So far it has been impossible to start the disease by feeding incubated feces or cultures from which the worms and ova had been removed. It should be stated that protozoa resembling amebae and flagellates have been found in the cultures of ova fed and a study of these is now under way. The relation of *Heterakis papillosa* to typhlitis in turkeys and chickens and the slightly varying morphology of the invading protozoa present for consideration the possibility that the latter may not necessarily belong to one species.

**CONCLUSIONS.**

Feeding embryonated eggs of *Heterakis papillosa* to brooder chickens led to a disease of both ceca, characterized by the presence of a core consisting of fecal matter, coagulated blood, and emigrated cells from the mucosa. The walls of the ceca were thickened as a result of cell invasion and multiplication, invasion and multiplication of *Ameba meleagridis* or allied parasites, and more rarely hemorrhage and edema. The respective parts played by *Heterakis papillosa* and the protozoa in starting the lesions and the source of the protozoa remain to be defined. The invasion of the liver by the protozoa was insignificant.