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AMŒBÆ OF THE CÆCA OF THE COMMON FOWL AND OF THE TURKEY. — *Entamœba gallinarum*, SP. N., AND *Pygolimax gregariniformis*, GEN. ET SPEC. NOV.*

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The co-existence of several species of parasites in the cæca of the turkey has apparently led to the description of two or more species as developmental stages of the protozoön of the disease popularly known as "blackhead." It, therefore, appears important to facilitate the recognition of the various species of protozoa present in the turkey's cæca by a description of each. The cæcal fauna of the domestic fowl and that of the turkey are so similar that they may be discussed very well together. The organism causing "blackhead," *Amœba meleagridis*, was described by Smith in 1895. Four species of flagellates from the cæca of fowls have also been described by Martin and Robertson (1911). The prevalence of blastocysts in the cæca of both the turkey and the fowl have already been alluded to in a previous paper (Tyzzer, 1919).

Several species of amœbæ have been cultivated by Walker (1908) from the intestinal tract of the turkey. A contractile vacuole was described in each of the three species obtained, and this, together with other characteristics, makes it appear practically certain that none of these represents a strictly parasitic species. Hadley (1916) in an article on the subject

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of " blackhead " mentions two associated species of amœbæ which he identifies as *Amœba intestinalis* and *Amœba gallo-parvonis*, both described by Walker. It is obvious from Hadley's notes on one of these species, and especially from his illustrations (Hadley, 1916, Figs. 21 and 39) that he has mistaken a true entamœba for the culturable species *A. intestinalis*, described by Walker. Hadley states that this species commonly undergoes " autogamous spore formation " in the cæcum, with the production of seventy or more daughter cells in a single cyst, and that this appears to be the chief mode of reproduction. The encysted stages are described as somewhat polygonal in shape with the cytoplasm in contact with the cyst wall only at the angles of the cell. These forms are said to be readily distinguished from the encysted flagellates which produce not more than twelve daughter cells. It is evident that this author has interpreted the large blastocysts as the encysted forms of the amœba observed by him, and smaller blastocysts as encysted flagellates. An entamœba evidently identical with the one shown in Hadley's illustrations has been observed both in common fowls and turkeys by the writer, who proposes for this species the name *Entamœba gallinarum.*

This organism appeared during the month of August in the cæcal excrement of young turkeys hatched two months previously. It was found in great numbers in the hen with which these turkeys were reared, and later on it was noted as well in turkeys from another source.

Entamœba gallinarum, n. sp. — The living trophozoites of this species have a dense appearing cytoplasm with a well-differentiated hyaline ectoplasm which is abundant in amount. The endoplasm has a distinctly greenish tinge and usually shows inclusions of considerable size. The nucleus is relatively large, in fact is readily distinguishable during life and is very frequently situated near the surface of the organism. Most of the trophozoites found in the cæcal discharge are continuously motile, the rate of movement being somewhat slower than that of *E. histolytica* and considerably more rapid

than that of *E. coli*. At room temperature these organisms continue to move actively for many hours after the material is collected. The pseudopodia are broad and blunt, and before a given one disappears new ones are frequently protruded. Their formation is gradual rather than eruptive in character. The trophozoites vary from 9 to 25μ in diameter but the majority measure from 16 to 18μ. There is no contractile vacuole. Eight nucleated encysted forms were observed on different occasions in fresh material.

In stained sections this amœba shows a cytoplasm having a dense hyaline appearance; the endoplasm stains intensely and is thus sharply demarcated from the paler ectoplasm. The former usually contains numerous relatively large food globules. A variety of materials are ingested, such as cell fragments, flagellates and other material from the cæcal contents, including a small amœba to be described later on. In case the material is not fixed at once upon the death of the host, intestinal epithelium is frequently taken up by these entamœbæ. Bacteria are apparently not utilized as food by this species.

The nucleus is usually spherical. It varies from 3μ in the smallest forms to 5μ in the largest, and averages about 4.5μ. It is frequently situated so near the periphery that it appears to bulge into the ectoplasm. The chromatin, which is usually distributed peripherally in a dense layer against the nuclear membrane, is more abundant than in the entamœbæ of the human being. There is a centrally situated endosome practically devoid of chromatin, and within this is a very definite central granule. Several trophozoites with two nuclei have been observed, but none have been found in the process of nuclear division.

The encysted forms occur in the cæcal contents. Eight delicate nuclei in which a small amount of chromatin is visible are produced, but incompletely developed cysts are encountered with four nuclei. The cysts are spheroidal in shape and measure on the average 12 x 15μ. They present a delicate limiting membrane and a large, deeply stained central

mass around which the nuclei are distributed (Figs. 7 and 8). The nuclei are relatively small, measuring only 2μ.

Habitat: This species has been found in the crypts and gland lumina as well as upon the surface of the cæcal mucosa of both the common fowl, *Gallus domesticus*, and the domestic turkey, *Meleagris galloparvo*, and it is quite possible that it occurs also in other hosts. In case the cæca are left for a short time after death, this organism migrates through the tissue and is found in great numbers throughout the mucosa and submucosa.

No development was obtained on planting fecal material containing the trophozoites on Musgrave-Clegg medium. This species was readily transmitted to young chicks by feeding fecal material from an infected fowl.

Pygolimax gregariniformis sp. n. — A small amœba showing characteristics quite distinct from those of the above species has been observed in the diseased cæca of turkeys, and also in the cæca of normal fowls. In material examined at room temperature, organisms of this species show at most only sluggish motility, amounting to slight changes of outline. They are of ovoid shape, show no distinct differentiation of ectoplasm and endoplasm and no readily distinguished structural features. There is no contractile vacuole present. The smallest forms observed measured only 3.9μ. In stained sections, the great majority of these organisms are of ovoid shape, with a deeply stained protuberance at one extremity which is evidently the posterior one, since the cytoplasm at the opposite end is somewhat clearer and has the appearance of a rounded pseudopodium. There is no distinct differentiation of ectoplasm and endoplasm. The cytoplasm is somewhat basophilic, and usually shows a variable number of food vacuoles in which various types of bacteria and occasionally other larger bodies are enclosed. Apparently a great variety of bacteria are devoured by this amœba.

The largest form measured was 12.3μ in length, and 6.3μ in breadth. The average of measurements was 8.75μ for length, and 5.3 for breadth.

The nucleus is distinctly vesicular in type and shows a large, deeply staining, centrally situated karyosome into which is incorporated apparently all the chromatin. The nucleus measures from 1.5 to 2μ, and the karyosome from .8 to 1.3μ in diameter. It is thus more strictly " limax " in type than the nucleus of *Endolimax* (*Entamœba*) *nana* of the human being.

Rounded forms of this species are observed enclosed in a delicate but slightly uneven membrane. As far as has been observed, these show only a single nucleus. This species is devoured in great numbers by the larger amœba, *E. gallinarum* with which it is associated in the cæca. After inclusion within the latter, it appears to serve as food material rather than to behave as a parasite, for many examples are observed which are degenerated and partially digested. There is also no evidence of increase in size or of multiplication of this organism when ingested by *E. gallinarum*, but many of the ingested forms show a distinct limiting membrane as though they had become encysted under these conditions.

No examples of nuclear division have been observed, although several organisms were found with two nuclei. Only a single nucleus was found in rounded, apparently encysted, forms. Attempts to cultivate this amœba on Musgrave-Clegg medium have invariably failed.

This species is readily transmitted experimentally from adult fowls to young chicks, by the feeding of small amounts of fecal material. Multiplication is evidently rapid, for the chicks show myriads of the small amœbæ in their cæcal discharges four days after the ingestion of such material.

The characteristic ovoid shape of this amœba when at rest, the posteriorly situated protuberance, the lack of differentiation of ectoplasm and endoplasm, its small size, together with the character of its nucleus, serve in distinguishing it from other parasitic amœbæ. Since its mode of reproduction has not yet been followed out, it is at present only possible to furnish a tentative classification. No encysted forms of this species with more than a single nucleus have been found.

This, together with the fact that it also differs from *Endolimax* (*Entamœba*) *nana* (Wenyon and O'Connor, 1917), Brug, 1918, with respect to type of nucleus, makes it appear preferable to place it, provisionally at least, in a distinct genus for which the name *Pygolimax* is proposed. Since there are so many intermediate forms between the species showing the extremes of difference with respect to morphology and development, it is perhaps an open question whether it is possible to divide the parasitic amœba into clearly defined genera. Although the name proposed is obviously not in all respects appropriate, it is at least in conformity with that applied to the above mentioned genus, *Endolimax*. The specific name *gregariniformis* here offered appears to be applicable on morphological grounds.

Parasites of the small amœba of the cæca of the turkey and common fowl. — In the course of a search for nuclear division in *Pygolimax gregariniformis* described above, nuclei were found which showed deeply stained bodies in addition to the karyosome. Such nuclei are somewhat distended, and the karyosome is usually pushed to one side by the inclusions. The latter are stained bluish both by the Giemsa method and by the eosin and methylene blue method, while the karyosome is stained purplish. These intranuclear inclusions appear as rounded coccus-like bodies, although some appear nearly twice as long as they are broad. These bodies vary in size, but the elongated forms average .8 x .4μ and the rounded forms not more than .5μ in diameter. The number in a single amœba varies from four or five to several dozen, and the nucleus of the latter is distended in a corresponding degree (Figs. 12, 13 and 14). In certain instances the nucleus appears to have ruptured and allowed the bodies to escape into the cytoplasm. The karyosome is frequently distinguishable after the nuclear membrane has disappeared. These bodies are morphologically indistinguishable from certain of the bacteria which are seen in the cæcal contents.

Inclusions of quite another type were also found in this amœba (Figs. 15 and 16). These occur in the cytoplasm in the vicinity of the nucleus, and appear as rounded masses

measuring from 1 to 1.5μ in diameter, with numerous very minute granules distributed at regular intervals in their surface. These inclusions are quite uniform in type although in certain amœbæ they appear somewhat larger and show more numerous peripheral granules than in others. With the great variety of ingested material present in the cytoplasm, it has been impossible to distinguish anything that may be interpreted as earlier stages of their development. From the characteristics of these two types of inclusions, it appears probable that they represent two distinct species of parasites.

A brief survey of the literature on the subject indicates that parasites occur rather frequently in various species of free living amœbæ. *Sphærita endogena*, a fungus of the group *Chytridiaceæ*, first reported by Dangeard (1886 and 1889), has been observed repeatedly as a parasite of amœbæ. Sporangia are developed which contain a variable number of spherical spores measuring 1.5μ in diameter. A similar type of organism occurs also in the nucleus and is known as *Nucleophaga amœbæa* Dangeard (1895). It does not differ essentially from the preceding except by its position — Grüber (1904), Penard (1905), Elpatiewsky (1907). This form appears first in the karyosome, but increases in size so that the nucleus becomes greatly distended. Doflein (1907) observed a similar organism which developed between the karyosome and the nuclear membrane so that the former was pushed to one side. This author was the first to establish the uniflagellate nature of the zoöspores and the fusion of these in pairs, with the formation of a copula. Prowazek (1907) includes certain of the parasites of amœbæ in the Chlamydozoa. From a discussion by Chatton and Brodsky (1909) on the subject of the occurrence of parasites in amœbæ, it would appear that organisms agreeing more or less closely with the *Nucleophaga* have been found in the following species: *Amœba verrucosa* (*A. proteus?*) Ehrbg. by Dangeard (1895), *A. viridis* Leidy by Grüber (1904), *A. terricola* Greef by Penard (1905), *A. vespertilio* Penard by Doflein (1907), *Endamœba blattæ* Bütschli by Mercier (1910), and in *Arcella vulgaris* Ehrbg. by Elpatiewsky (1907). Nägler (1909–1910) has reported the infection

of an amœba of the " limax " type by a micrococcus which is generally distributed throughout the cytoplasm. Mackinnon (1913–1914) found a micrococcus in the parasitic amœba *Loschia* (*Entamœba*) *hartmanni* Mackinnon, and Wenyon (1907) noted what might be considered as an instance of parasitism of *Entamœba muris* by a coccus.

The appearance of the two types of inclusions found in *Pygolimax gregariniformis* here described, as well as indications of multiplication and their association with degenerative changes in this amœba, lead to the conclusion that they represent parasites. The intranuclear form does not agree with the micrococci of Nägler and Mackinnon, since it is frequently elongated and is situated in the nucleus rather than in the cytoplasm. Furthermore, it does not correspond with *Nucleophaga amœbœa* Dangeard, since its elements are considerably smaller, measuring on the average only .5μ and the elongated forms measuring not more than .8μ in their greatest diameter as compared with the spores measuring 1.5μ in the above-named species. The number of inclusions in the nuclei of infected amœbæ vary very greatly, but there was no evidence of any other phase of development. The staining peculiarities of the intranuclear form, together with the apparent method of its multiplication within the nucleus, indicate that it is probably not a protozoön.

No attempt will be made to classify the second type of inclusions found in the cytoplasm of *Pygolimax gregariniformis* except to point out their probable parasitic nature. No intermediate stages were found between these rounded cytoplasmic forms with numerous peripheral granules and the bacteria-like intranuclear forms already discussed, and they are probably not related one to the other. Parasites producing composite spores having a rosette-like appearance have been described by Grüber (1904), but these occurred in the nucleus rather than in the cytoplasm of an amœba. While there is little doubt concerning the parasitic nature of these inclusions, their small size and infrequent occurrence make the study of their development difficult.

LITERATURE.

Brug, S. L. (1918.) Enkele opmerkingen over de nomenclatuur van eenige nieuwere parasieten uit den menschelijken darm. Geneesk Tijdschr. v. Nederl.-Indië, 1918, lviii, 283.

Chatton, E., and Brodsky, A. (1909.) Le parasitisme d'une Chytridinée du genre *Sphærita* DANGEARD chez *Amœba limax* DUJARD. Étude comparative. Arch. f. Protistenkunde, 1909, xvii, 1.

Dangeard, P. A. (1886.) Sur un nouveau genre de Chytridiacées parasite des rhizopodes et des flagellates. Bull. Soc. Bot. France, 1886, xxxiii, 240–241.

Dangeard, P. A. (1889.) Memoires sur les Chytridinées. Le Botaniste, i, 39–74, pl. ii and iii.

Dangeard, P. A. (1895.) Memoire sur les parasites du noyau et du protoplasma. Ibid., iv, 199–249.

Dangeard, P. A. (1897.) Contribution à l'étude des Acrasiees (*Sappinia pedata* n. gen., n. sp.). Ibid., v, 1–20.

Dangeard, P. A. (1901.) Le caryophyseme des Eugleniens. Ibid., viii, 358–360.

Doflein, F. (1907.) Studien zur Naturgeschichte der Protozoen. Arch. f. Protistenkunde, 1907, ix, 250.

Elpatiewsky, W. (1907.) Zur Fortpflanzung von *Arcella vulgaris* Ehrbg. Arch. f. Protistenkunde, 1907, x, 441.

Gläser, H. (1912.) Untersuchungen über die Teilung einiger Amöben, u. s. w. Arch. f. Protistenkunde, 1912, xxv, 28–152.

Grüber. (1904.) Über Amœba viridis LEIDY. Zoöl. Jahrb., Festschr. f. Weismann, Suppl. 7, 1904, 67.

Hadley, P. B. (1916.) The avenue and development of tissue-infection in intestinal trichomoniasis. Bull. 168, Agricultural Experiment Station of the Rhode Island State College, November, 1916.

Janicki, v. C. (1909.) Über Kern und Kernteilung bei *Entamœba blattæ* Bütschli. Biologisches Centralblatt, 1909, xxix, 381.

Kuenen, W. A., and Swellengrebel, N. H. (1917.) Korte beschrijving van enkele minder bekende protozoön uit den menschelijken darm. Geneesk Tijdschr. v. Nederl.-Indië, lvii, 496.

Mackinnon, Doris L. (1913–1914.) Observations on amœbæ from the intestine of the crane-fly larva. *Tipula* sp. Archiv. f. Protistenkunde, 1913–1914, xxxii, 267.

Martin, C. H., and Robertson, M. (1911.) Further observations on the cæcal parasites of fowls, with some reference to the rectal fauna of other vertebrates. Part I. Quart. Journ. Micr. Sci., 1911, lvii, 53–78.

Mercier, L. (1910.) Contribution à l'étude de l'amibe de la Blatte (*Entamœba blattæ* Bütschli). Arch. f. Protistenkunde, 1910, xx, 143.

Nägler, K. (1909.) Entwicklungsgeschichtliche Studien über Amöben. Arch. f. Protistenkunde, 1909, xv, 1.

Nägler, K. (1910.) Fakultativ parasitische Micrococcen in Amöben. Archiv. f. Protistenkunde, 1910, xix, 246.

Nägler, K. (1912.) Die Kern-und Centriolbildung bei Amöben, eine Entgegnung an Gläser, u. s. w. Arch. f. Protistenkunde, 1912, xxvi, 435–442.

Penard, E. (1905.) Observations sur les amibes à pellicule. Arch. f. Protistenkunde, 1905, vi, 175.

Prandtl, H. (1907.) Der Entwicklungskreis von *Allogromia* sp. Arch. f. Protistenkunde, 1907, ix, Suppl. 1.

Prowazek, S. (1907.) Chlamydozoa. Arch. f. Protistenkunde, 1907, x, 336.

Smith, T. (1895.) An infectious disease among turkeys caused by protozoa (infectious enterohepatitis). Bull. 8, Bureau of Animal Industry, U. S. Dept. of Agriculture, Washington, D. C., 1895.

Tyzzer, E. E. (1919.) Developmental phases of the protozoön of "blackhead" in turkeys. Journ. Medical Research, 1919, xl, 1.

Walker, E. L. (1908.) The parasitic amœbæ of the intestinal tract of man and other animals. Journ. Medical Research, 1908, xvii, 379–459.

Wenyon, C. M. (1907.) Observations on the protozoa in the intestine of mice. Arch. f. Protistenkunde, 1907, Suppl. I, 169.

Wenyon, C. M., and O'Connor, F. W. (1917.) Human intestinal protozoa in the Near East. Wellcome Bureau of Scientific Research, London, 1917.

DESCRIPTION OF PLATE XXII.

FIGS. 1 to 8, inclusive, illustrate forms of *Entamœba gallinarum* and are drawn at 1,400 diameters. In Figs. 9 to 16 are shown forms of *Pygolimax gregariniformis* at a magnification of 2,100 diameters.

FIG. 1. — A trophozoite of *Entamœba gallinarum* showing numerous food vacuoles containing partially digested flagellates. Ectoplasm and endoplasm distinct. x 1400.

FIG. 2. — A large trophozoite with numerous food vacules and pseudopodium. x 1400.

FIG. 3. — A small form of *E. gallinarum*. x 1400.

FIG. 4. — A trophozoite of *E. gallinarum* in which are included two limax amœbæ in addition to other food material. Nucleus of large amœba not apparent in section shown. x 1400.

FIG. 5. — A four-nucleated cyst of *E. gallinarum*. x 1400.

FIG. 6. — An encysted form showing five nuclei. x 1400.

FIGS. 7 and 8. Mature cysts of *E. gallinarum* showing eight nuclei and deeply stained central mass. x 1400.

FIG. 9. — *Pygolimax gregariniformis* of cæca of turkeys and fowls. Deeply stained projection at posterior extremity. Nucleus vesicular with a large karyosome. x 2100.

FIG. 10. — An extended form of the same species. x 2100.

FIG. 11. An amœba showing bacilli and other material in food vacuoles. x 2100.

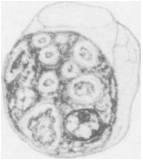
FIG. 12. — *P. gregariniformis* showing an early stage of infection with the nuclear parasite. The latter appears somewhat smaller than in later stages. x 2100.

FIG. 13. — A form of same species with nucleus distended with coccoid nuclear parasites. Karyosome pushed to one side. x 2100.

FIG. 14. — Late infection with nuclear parasite. Nucleus greatly distended. In the cytoplasm large bodies representing food material to the right of the latter. x 2100.

FIG. 15. — Same species showing cytoplasm invaded with rounded parasites, each with numerous peripherally arranged granules shown rather faintly by McJunkin's stain. x 200.

FIG. 16. — Chromatin granules of cytoplasmic parasite rather more clearly. Mallory's phosph. hem. x 2100.



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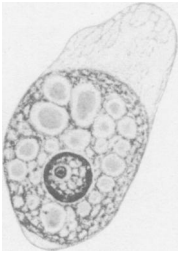

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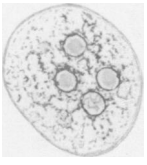

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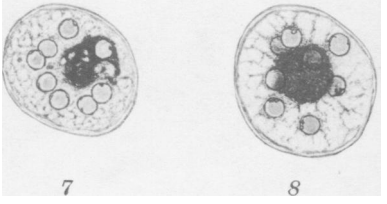

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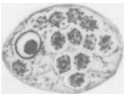

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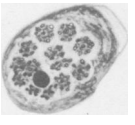

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Tyzzer. Entamaeba gallinarum.