DEVELOPMENTAL PHASES OF THE PROTOZOOON OF "BLACKHEAD" IN TURKEYS.*

ERNEST EDWARD TYZER, M.D.

(George Fabyan Professor of Comparative Pathology, Harvard University Medical School.)

Since the causal agent of a serious disease in turkeys, commonly known as "blackhead," was described by Theobald Smith (1895) under the name *Ameba meleagridis*, conflicting views have been expressed as to its nature and classification. It appears therefore desirable to review in a somewhat critical manner the various observations and views which have been published on this subject. Motility, characteristic of an amöeba, has been noted only on a single occasion by Smith (1915) in 1910, when very slight changes of form were observed. Although most amöebæ ingest solid particles as food, the manner by which this parasite obtains nourishment is not discussed. Smith used the generic name *Ameba* because the parasite appeared to differ from amöebæ less than from other protozoa. The possibility of its relationship to the flagellates of the turkey's cæca is suggested in a footnote appended to the original description (Smith, 1895). He has explained (1915) that the name "*Ameba*" is employed tentatively and that it may be necessary to change this when the nature of the parasite is better understood.

* Received for publication, May 1, 1919.
While no very definite opinion is furnished of the life cycle of this parasite or of its multiplication in the tissue, Smith in his original description stated that there is no evidence of endogenous segmentation but that multiplication takes place by simple division. In a later paper (1915) attention is called to forms "which probably represent such a multiple agamic division." The ring-like bodies seen within certain forms are believed to be concerned in this process of multiplication. Host cells were also observed containing small spherical bodies which were thought to be probably young parasites. A tentative interpretation is offered that the multiplication of the parasite may take place within phagocytic cells. Passage of the parasites to the liver is explained by embolism of the parasites "or, what is more likely, of phagocytes filled with young forms" through the portal circulation. It is stated that the parasites do not invade epithelium either in the cæca or in the liver, and attention is called to the extent of their degeneration in the tissues. — These various points will be discussed later in connection with the findings in the present investigation.

"Blackhead" in turkeys has been investigated by Hadley (1908, 1909) who concluded that this disease is caused by a coccidium of which the parasite described by Smith represents only a portion of the life cycle. — Smith (1895) had already called attention to the occurrence of coccidia in turkeys. — Hadley concluded that this coccidium causes not only blackhead in turkeys but also white diarrhea in chicks and some cases of roup in fowls. Later on, Hadley (1916) became convinced that blackhead is of the nature of an infection of the cæca and liver of the turkey with a flagellate, "Trichomonas." Although four different species of trichomonads have been described from the cæca of the common fowl, "Trichomonas" is discussed merely as a flagellate found in the intestine of all poultry as well as of most other animals. No attempt is made to identify the parasite with known species or establish a new species. He states that the "nature of the infective process . . . makes it clear that the intestinal flagellatosis cannot be regarded as an infectious disease,"
successful infection depending upon the factors present in
the host rather than to virulence on the part of the infecting
organism. It is obvious that this investigator does not
consider the organism in question as primarily pathogenic in
nature, but as a normal inhabitant of the alimentary tract of
turkeys and fowls, which may invade the tissue under con-
ditions which lower the resistance of the host. Thus he
says (Hadley, 1917),—"It is here that we recognize Trichomonas
in a new rôle. Having experienced its first taste of blood its whole
nature is changed; it becomes another animal, raging through the tissues and impeded by no protective
action that the host organism is able to muster in its defense.
Here, then, we must recognize Trichomonas as a cell parasite,
an organism that has the power to actively invade living cells
and to bring about their destruction." Apparently this
author attaches no importance to the fact that the disease
may be produced in healthy flocks by the introduction of
infected birds.

Hadley supports his view by morphological evidence which
he believes shows intermediate stages between the tricho-
monads which occur within the cæca and forms found in the
infected tissue. The organisms, shown as intermediate forms,
present unmistakable trichomonad features, and do not
resemble the organism of blackhead. The presence of tricho-
monads beneath the epithelium as well as in the goblet cells
of the cæcal glands appears to this author also to be evidence
of tissue invasion, although the difficulty of obtaining intact
intestinal epithelium in preserved material, and the frequency
with which the intestinal flora and fauna become disseminated
beneath the epithelium, either through post-mortem migra-
tion or by even slight manipulation, is quite generally recog-
nized. This author claims that "Trichomonas" after distend-
ing the crypts of the cæcum, penetrates through the epithelium,
thereby separating it from the underlying tissue, and also
that following their multiplication in the tissue, these organ-
isms escape into the cæcum by lifting the epithelium from the
tips of the villi. The fallacy in such reasoning is quite ap-
parent when the facts of the case are considered. There are
no villi in the portion of the caecum commonly involved in blackhead. In one case the separation of the epithelium is taken as evidence of invasion, in the other it is taken as evidence of the escape of the flagellates from the tissue. It is said that the epithelium becomes separated by the multiplication of the flagellates between it and the underlying tissue but, in a figure illustrating a normal gland without flagellates, the epithelium is also separated,—here explained as an artefact. The organisms interpreted by Hadley as encysted forms of the flagellate being discharged from the tissue are evidently blastocysts derived from the caecal contents. Their situation beneath the epithelium may be accounted for by a disturbance of relations in the course of the preservation of the tissue. Although such organisms have frequently been mistaken for the developmental stages of Trichomonas (Per- roncito 1888, Schaudinn 1903, Prowazek 1904, Doflein 1911 and others), there is now quite general agreement that they represent a distinct type of organism, i.e., Blastocystis enterocola, Blastocystis hominis, etc., probably of a vegetable nature (Dobell 1909, Wenyon 1910, Alexeieff 1911, Bruun 1912). The cysts, noted by Smith (1915) in the faeces of the turkey and regarded by him as probably encysted amœbæ, although their relation to the tissue parasites was not understood, were evidently of similar nature. The observation of Kofoid and Swezy (1915) that trichomonads sometimes ingest small blastocysts probably accounts to some extent for the confusion of the two species.

Conjugation in threes as well as in pairs, which according to Hadley occurs in the trichomonad state, has not been definitely shown to occur in the genus Trichomonas or other related flagellates. Furthermore, the process described as conjugation is not conjugation according to the ordinary usage of this term by protozoologists. Multiplication in the tissues is said to take place by "autogenous spore formation" without making clear just what is meant by this process. Parasites which were formerly thought to be stages in the development of the coccidium found in turkeys are now considered as stages in the life cycle of Trichomonas. Not only may the
disease be complicated by coccidiosis but also by the presence of two species of amœbæ which appear to this author to be identical with certain of those cultivated by Walker (1908) from turkeys' intestine. The measurements and other characteristics furnished for one of these do not, however, coincide with any one of those described by Walker, but appear to apply to a true non-pathogenic Entamoeba which occurs commonly in turkeys and fowls and which will be discussed elsewhere. Space will not permit of a more extended discussion of the subject matter of Hadley's various papers. Whether or not the hypothesis of the trichomonad nature of the parasite of blackhead will be found to apply, this author has thus far failed not only to establish its identity with any species of Trichomonas but also to demonstrate any features characteristic of this genus.

The view that the parasite of "blackhead" is a trichomonad has found support in observations made in South Africa by Jowett (1911), who identifies it as Trichomonas eberthi Kent, although he finds this flagellate in healthy as well as in diseased birds. Two other flagellates were also observed in turkeys; one with two and one with a single flagellum. He claims that the organism as it occurs in the infected tissues possesses two nuclei which he pictures as differing in size and situated at a considerable distance one from the other. Jowett states that the word "infectious" as applied by Smith to this disease is a misnomer, since the condition is not readily communicated from the diseased to healthy birds and should not be regarded as infectious. As measures for the prevention of the disease, however, he advocates the isolation or, better, the killing of all birds in which the disease appears, the free use of quicklime about the quarters occupied, or the burning of the soil of the latter!

It may appear that the above discussion concerning the etiology of blackhead is unduly critical of the findings of other investigators. The confused state of this subject, however, appears to warrant rather drastic methods, and the singling out of various misinterpretations and inconsistencies, for it
is quite evident that the enthusiasm of certain investigators for their views has caused them to neglect important facts. The author has no hypothesis to defend concerning the nature of the parasite in question, but in presenting the following observations hopes to contribute certain facts that may assist in the solution of this difficult problem.

Material. — The tissues employed in the present study were derived from five infected turkeys of different ages, killed at various stages of the disease. In each case the tissue was fixed in either Zenker's fluid or Schaudin's fixative immediately on killing the bird. The most useful stains employed for sections were Mallory's phosphotungstic hematoxylin, and eosin followed by Unna's methylene blue. Films were stained by McJunkin's modification of the Giemsa method and by both Heidenhain's and Delafield's hematoxylin after fixation in Zenker's fluid. Although an abundance of tissue derived from dead specimens was available, this was not considered as especially suitable for the present morphological study and was used only for general comparative purposes.

Occurrence of the parasite in various hosts. — The parasite under discussion, commonly known as *Amoeba meleagridis* Smith, has been found by the author in several species of birds in addition to the turkey. Numerous specimens of the ruffed grouse, *Bonassa umbellus*, which had been reared in captivity, by the Massachusetts Fish and Game Commission, were found to have succumbed to this infection. The disease has also been noted in a number of quail, *Colinus virginianus*, from the same source, sent here for examination. A slight infection of the cæcum and extensive involvement of the liver was found in May, 1908, in a common chick not more than four weeks old. The disease in this instance was complicated by bacillary infection. Slight involvement of one of the cæca was recently found in a two-year-old hen from a flock which had never been exposed to turkeys and to which no fowls had been added for a period of somewhat over a year and a half. The fact that the lesion in this case was
minute suggests that the parasite may occur in common fowls without producing symptoms.

Attention should be called to the similarity of the fauna of the turkey's caeca and that of the common fowl. Martin and Robertson have described four species of trichomonads in the latter, — *Chilomastix gallinarum*, *Trichomonas* (properly *Tetratrichomonas*) *gallinarum*, *Trichomonas eberthi* and *Trichomastix* (properly *Eutrichomastix*) *gallinarum*. (For changes in generic names see Kofoid and Swezy, 1915.) A large *Entameba* and a minute oval-shaped amœba with a large karyosome occur in the caeca of both the turkey and the common fowl. Blastocysts which have been confused with other organisms, as already noted, occur also in both these hosts.

The parasite of blackhead presents such a variety of forms in stained preparations that, if it were not for certain constant features, it would be difficult to prove that we are dealing with one and not with several species of parasite. Certain forms which have irregular amœba-like outlines and occur free in tissues more especially in early lesions and at the periphery of infected areas, are interpreted as trophozoites representing the invasive phase in the development of this parasite. Such organisms after accumulating in great numbers so that they distend the host tissues, apparently lose their motility and enter upon what may be interpreted as a vegetative phase of development. Multiplication in the tissues terminates in the resistant phase in which the organisms are of relatively small size, show a dense acidophilic cytoplasm and develop a resistant membrane. The invasive and resistant phases show pronounced characteristics, while the vegetative phase is less definite and might well be considered as representing a period of transition. While in these different phases are found the extremes of variation, the latter are connected by complete series of intermediate forms. Other investigators have apparently either not observed certain of the forms present or have failed to recognize their significance so that it appears desirable to furnish a more detailed description of the parasite.
Description. — The appearance of the living parasites as observed in fresh preparations corresponds very closely with Smith's descriptions. The only forms thus far recognized appear as nearly spherical clear bodies, each having a small nucleus which becomes more apparent in material which has been kept for twenty-four hours. Smith has described the nucleus as being "somewhat eccentric" in position, but, since it is usually found near the center and rarely near the periphery, its usual position may be more properly defined as paracentral. The cytoplasm does not appear perfectly homogeneous, however, for granules are distinguishable in its interior.

Motility. — Sluggish amœboid activity has been observed in fresh liver material examined on the warm stage. Observations have been made with respect to movement on three different occasions. The first attempt was made with material taken from liver lesions and examined in .8 per cent salt solution at room temperature. Numerous organisms were present but all retained their spherical shape while under observation. The second observation was made with similar material examined on the warm stage immediately on killing the turkey. Amœboid motion was observed in what were thought to be parasites, but, as the matter was considered after the observation, there remained the possibility that wandering host cells had been mistaken for motile parasites. The third observation was made under ideal conditions. Material was taken from the liver lesions immediately on killing the bird and, after being mounted in tissue fluid, was at once placed in a carefully regulated warm chamber. A field was chosen showing numerous parasites, many of which showed no change from the spherical shape during a period of several hours. Certain organisms, however, showed a very sluggish amœboid motion. This movement was so slow that it was only distinguished by drawing the outline of a given parasite and then noting its change of shape at intervals of several minutes. By this method rounded forms were found to
Protozoan of "Blackhead" in Turkeys.

Develop broad processes and to change position. Changes of shape observed in two such parasites are illustrated by the freehand sketches, Figure 1.

Extranuclear body.—Before passing to the description of the various forms encountered in the different phases of development, attention should be called to an extranuclear body from which are derived the division centers in nuclear division and which is readily demonstrable in the various types of organisms found. This body commonly presents a deeply stained double or dumb-bell-shaped granule situated near and in some instances almost in contact with the nuclear membrane, with its long axis either perpendicular or parallel to the surface of the latter. In a certain proportion of the organisms the simplicity of this structure is lost in a complex of less definite granules. Radiating from this body are deeply staining lines which vary from delicate rays extending over the surface of the nuclear membrane to coarse flagellalike filaments passing over the surface of the nucleus or outward into the cytoplasm. Whether this body was observed by Jowett is open to question—at least he has furnished no adequate description. It should not be confused with the structure described by Smith as the "karyosome," which is distinctly intranuclear in position. Furthermore, both this structure and the extranuclear body coexist in the same parasite. If it were not for the constant presence of this extranuclear body throughout the development of the parasite, it would be often impossible to distinguish certain forms of the latter. It is difficult with our limited knowledge of this parasite to find a suitable name to apply to this structure. If we call it the "blepharoplast," we imply that it is concerned in locomotion; if it is called the centrosome, it will then be inferred that it has no other significance than this name implies. It, therefore, appears preferable, for the present, to employ provisionally the non-committal term "extranuclear body."
Invasive phase (Figs. 27 and 28). — Organisms at this stage of development are distinctly amoebiform, and vary considerably in size. They measure, when rounded, from 8 to 17 microns in diameter in fixed tissue. Long forms may measure 30 microns in length. They occur in early lesions at the periphery of the involved areas, unassociated with the forms constituting the later phases of development. Away from the periphery they are found intermingled with other forms but tend to disappear from older portions of the lesion. They are found stretching through the interstices of the tissues and present blunt, rounded processes, evidently pseudopodia. They are found passing through the gland epithelium, in the goblet cells and occasionally in the gland lumina, between the fibers of the smooth muscle layers of the cæca, between the liver cells, and, in fact, in all the tissues involved in this disease. The cytoplasm of such forms is distinctly basophilic either in sections stained by the eosin and methylene-blue method or in smears stained by the Giemsa technic and its modifications. The ectoplasm is relatively abundant, and in stained sections appears quite clear, so that the periphery of the organism appears to be separated from the granular portion by a clear space, and this is especially evident in the pseudopodia. The shape of the organism is modified by the tissue through which it wanders. Long forms with a single pseudopodium are found stretching through the distended tissue of the cæcal mucosa (Fig. 28), while broad forms with sheet-like pseudopodia are seen between the muscle bundles (Fig. 32). The position of the extranuclear body with respect to the nucleus has no constant relation to the chief pseudopod which presumably indicates the direction in which the organism was moving at the time of the fixation of the tissue. It may be situated either anterior or posterior to the nucleus and also frequently lateral to the latter.

The endoplasm contains a variable number of deeply stained particles which evidently represent ingested material. These particles are in general considerably smaller than the nucleus but vary greatly in size as well as in number and are situated in vacuoles. In many organisms the uniformity of
size and the regularity of their distribution suggests very strongly a multinucleated stage in development, but in all such forms the single nucleus and the extranuclear body with its radiating lines is present. These cytoplasmic inclusions of the parasite are not of uniform appearance, and, although the more prominent ones take the nuclear stain, others occur which take the diffuse stain, and some are composed in part of deeply stained and in part of faintly stained material. Since the nucleus and extranuclear body show no characteristic changes in parasites which show these cytoplasmic bodies, and since similar particles are frequently encountered free in the involved tissue, it is evident that we are dealing with ingested material probably derived from the tissue cells. Bacteria have not been observed in these organisms. The parasite during its invasive phase is thus apparently holozoic in habit and lives upon small particles taken up in its migration through the tissues.

Vegetative phase (Figs. 29 and 4, 6, 8 and 23). — Organisms of large size (from 21 x 15 to 12.5 x 12 microns in fixed tissue) and having a basophilic cytoplasm, in which the inclusions encountered in the invasive phase tend to disappear, accumulate in great numbers and distend the host tissues which have not yet reacted to their presence. It may be inferred from their rounded outlines that active movements have practically ceased. The cytoplasm of such forms is clear and transparent as though distended with water and shows a minimum amount of coagulable material either in granular or reticular form. The outline is the more prominent feature in such forms, so that where large numbers are closely packed together their surfaces are molded by mutual pressure so as to appear in outline as a reticulum. The cytoplasm shows a small amount of reticular or granular material distributed around the nucleus.

Faintly stained globular or ring-like bodies such as have already been noted by Smith occur in the cytoplasm of many such organisms. Although in a single organism they may be of such uniformity and appearance as to simulate the
nuclei in encysted amœbæ, they do not occur in any characteristic number and vary in size from barely visible granules to globules nearly as large as the nucleus. Parasites in which such bodies are found show no changes in the nucleus or extranuclear body. While it seems most probable that they represent shadows of the globules ingested earlier in development, it is possible that they are related to the globules of the coagulable material which is found in abundance in later stages of development. There is no evidence that they represent either nuclei or spores or are concerned in the multiplication of the parasite.

It is much more difficult to interpret the significance of this phase of development than either of the other two. The pale, flimsy appearance of such accumulated forms, as though they were distended with water, gives the impression of widespread degeneration of the parasite. However, the nucleus of such clear organisms is frequently found dividing in a normal manner, so that it would appear that at least a considerable proportion survive to pass into the resistant phase.

Resistant phase (Figs. 30 and 8, 10 and 11).—This is characterized by the development of a dense surface membrane, by an increase in the amount of coagulable material and probably by a loss of water on the part of the cytoplasm. Such forms are comparatively small, varying in section from $11 \times 9$ to slightly less than $5$ microns in diameter. The nucleus in stained sections appears compressed and irregular and the chromatin granules may appear scattered. It is not improbable that such appearances with respect to the nucleus may represent artefacts, for the nucleus generally appears rounded in such forms in stained films. The cytoplasm now stains chiefly with the diffuse or acid stains from the presence of material distributed in granules, globules or coarse reticulum, which is not seen in the earlier phases. In Giemsa-stained films this material appears in the form of reddish granules in a bluish matrix. Crystalloid material also appears in the cytoplasm and may increase to such an extent that other cell structures are largely obscured.
The forms which constitute this phase are eventually either enclosed in definite spaces by the reaction of the tissue or are taken up by phagocytic cells. In such spaces they may lie either disassociated from one another when they are spherical in shape, or compressed in masses when their shape is modified by contact. The latter arrangement might suggest that such forms have been produced by multiple division, but all available evidence is contrary to this view. The number of organisms in such groups is not constant but varies from two to several dozen; no organism is found of a size even approaching that of the larger groups; no multinucleated organisms have been found; and no residual material is found associated with such groups. A similar grouping of cells, observed in the embryonic development of metazoa, is associated with binary and not multiple division of cells. The size of the groups appears to be determined by their distribution at the time that they are enclosed by the reaction of the host tissue. For example, a grouping of such forms is observed in the caecal mucosa, which is not reduplicated in the liver. Binary division of the nucleus is occasionally observed in this phase, but the irregularity of the nucleus so frequently encountered is not interpreted as concerned with nuclear division.

Frequently organisms at different stages of development occur in a single group (Fig. 8). This difference in the character of associated organisms may be explained either on the hypothesis that the reaction of the tissues has enclosed organisms at different stages of development or that motile invasive forms have broken into spaces enclosing organisms in the rounded quiescent phase. There is evidence that the latter occurs frequently, and irregular forms are not only found in the act of passing into such spaces but they also occasionally penetrate giant cells. The parasite after it has become definitely enclosed by the reaction of the tissue develops a resistant membrane. The first indication of this is a rather thick transparent layer at the periphery of the organism and this eventually becomes a dense membrane having in sections a yellowish tint. It appears more distinct both in stained films and in sections than the cyst membrane in *Entamoeba coli*. 
Nucleus. — As it appears in stained sections, the nucleus shows wide variation with reference to the distribution of its component parts. To what extent the differences observed represent cyclic changes and to what extent artefacts of fixation, it is difficult to determine. The various types of nuclei encountered in this parasite are illustrated in Fig. 2, in which the sketches represent a magnification of about 2,000.

Granular nuclei, i.e., with the chromatin distributed in small grains as represented in Fig. 2a, are found in a great proportion of organisms in the invasive phase and to some extent in later phases of development. Such a distribution of nuclear material cannot be readily accounted for by artefact, so that it would appear that this type at least constitutes a normal nucleus for the parasite under discussion. Neither linin nor nucleolus is apparent in such nuclei.

Nuclei in which the chromatin granules are distributed in a mass of achromatic material (linin), which appears to have shrunken slightly from the nuclear membrane, may be considered as semivesicular in type (Fig. 2b). Such nuclei are found in both invasive and vegetative forms.

Vesicular nuclei (Fig. 2c), with the chromatin and linin material forming a dense central mass, which is separated by a clear space from the nuclear membrane, occur with greatest frequency in large, clear forms of the vegetative phase. Such nuclei are frequently of extremely small size as compared with the organisms in which they are found. The central mass apparent in the vesicular nuclei evidently constitutes the karyosome as described by Smith. In nuclear division the daughter nuclei are at first vesicular but subsequently become granular in type (Plate Figs. 15, 16, 20, 21).

Occasionally nuclei are found in which there is a definite globule of material staining less intensely than the chromatin grains. This occurs in both granular and vesicular nuclei as illustrated. (Fig. 2d.) Although this may be regarded as a nucleolus, it constitutes in this parasite an unusual rather than a constant nuclear structure.

In certain nuclei the chromatin appears in the form of deeply stained masses resembling chromosomes (Fig. 2e).
Eight such masses are usually present, and these vary considerably in size. It appears probable that this arrangement of the chromatin precedes nuclear division.

In the resistant phase both granular and vesicular nuclei are replaced by shrunken, distorted nuclei. The nuclear membrane is often not apparent, so that the chromatin grains appear scattered (Fig. 2f). To what extent this appearance represents degeneration or artefact in the fixation of encysted forms is not at present clear.

That variation in the structure of the nucleus as it appears in stained sections is in some way associated with the physiological activity of the organism does not seem improbable. The granular type of nucleus, since it prevails in the invasive phase of development, and has obviously not been produced by artefact, should, at least, be considered normal for this species.

Multiplication. — Nuclear division of a type which apparently is not found in true amœbæ occurs regularly in this species. Organisms with two nuclei occur not infrequently but none with more than two nuclei have been observed. In one instance four double nucleated parasites were noted in a small portion of an oil-immersion field. While the successive phases of nuclear division are difficult to follow in so small a nucleus, the general character of the process is quite apparent. It is best seen in the clearer forms, where it is not obscured by cytoplasmic granules and inclusions. It occurs, however, in each of the phases of development already discussed and shows the same general features irrespective of the variety of forms in which it is found. On account of the inability to determine certain details of the process, the present account of nuclear division is necessarily somewhat incomplete.

A considerable proportion of the parasites show the chromatin distributed as definite granules in the nucleus, and, although it is difficult to determine the number of these except in favorable instances, it is frequently possible to enumerate eight such granules which probably represent chromosomes.
The occasional occurrence of four larger masses may be accounted for by the association of the chromosomes in pairs. Nuclear division is initiated by the appearance of two division centers derived from the extranuclear body (Fig. 32). Connecting the two is a deeply staining rod, the subsequent behavior of which shows it to be of the nature of a paradesmose (Figs. 8, 13, 14 and 19 to 24). It is frequently difficult, however, to demonstrate a granule at both its extremities. The lines which radiated from the extranuclear body become less apparent and usually entirely disappear. The growth of this rod carries the two division centers to opposite poles of the nucleus, while it is now bent like a bow over the surface of the nuclear membrane. The chromatin apparently takes the form of chromosomes during division (Fig. 13), and from the subsequent grouping of the chromatin it is evident that the latter are divided equally (Fig. 14).

Following the division of the nuclear chromatin, the two resulting groups of chromosomes appear to be drawn apart and the paradesmose both lengthens and straightens out in the later phases of division, so that the material going to form the daughter nuclei are drawn far apart, frequently to opposite sides of the parent cell. The nuclear membrane is no longer apparent. The chromosomes now appear to flow together and form a dense mass which lies in a clear vesicle surrounded by a delicate membrane. At this stage there is a suggestion of two sorts of deeply stained nuclear material; at least this is indicated in certain instances (Fig. 23). In subsequent stages the chromatin becomes distributed through the daughter nuclei in small granules. The paradesmose fades from view and the division center associated with each daughter nucleus appears as a double granule.

The length of the paradesmose varies in different instances but is commonly of considerable length, approaching the diameter of the parasite when the latter is of rounded shape (Fig. 23). In long forms the paradesmose extends longitudinally (Figs. 21, 22). While the purpose of this account is to furnish an outline of what appears to be the usual steps constituting nuclear division, it is possible, when the variety
of forms presented and the probability of their being affected by adverse conditions is considered, that this process may lack strict uniformity and that there may be also a simpler process, more of the nature of a direct nuclear division. The features of the later stages of division are, however, remarkably constant. Cytoplasmic division presumably follows nuclear division but is only occasionally demonstrable, since it is difficult to determine whether we are dealing with aggregations of organisms or with groups of organisms resulting from division. Stretched-out forms such as are shown (Figs. 22 and 24) probably divide by the drawing apart of two approximately equal portions of the cytoplasm. Binary nuclear division as described above is encountered most frequently in the invasive phase of development, less frequently in the vegetative phase and more rarely in the resistant forms.

Discussion.—The present morphological study of the parasite found invading the tissue in blackhead has revealed an extranuclear body which has apparently been overlooked by other investigators. From this body are derived granules, representing division centers, and paradesmose, both of which are concerned in nuclear division. The general character of the process of nuclear division is similar to that found quite generally in trichomonads, as will be seen from a brief survey of the literature on this subject.—(Martin and Robertson 1911, Kuczynski 1914, Kofoid and Swezy 1915, Entz 1917–18.) That this peculiar type of nuclear division should have been overlooked by previous investigators is probably accounted for either by the failure to recognize as parasites the forms in which nuclear division occurs most frequently, or by the fact that the dividing nucleus is somewhat obscured by the food particles ingested by such forms.

The significance of certain phases of development as shown by morphological differences in various groups of the parasites has evidently not been understood. The recognition of these phases furnishes at once an explanation of the dissemination of the parasite through the tissues. There is definite evidence that the parasite migrates through the tissue by
means of its amöeboid motion and ingests solid particles as food during this phase of its development. Taking nuclear division as in index, the rate of multiplication appears to be at its maximum in the migrating forms. Since these active forms are found beneath the endothelium of the veins of the cæcum (Fig. 31) it is no longer necessary to consider their hypothetical transportation by phagocytic cells as suggested by Smith. Several small resistant forms have been observed within phagocytes in blood spaces near the surface of the cæcal mucosa, but it is questionable whether such organisms are capable of further development in the same host.

The interpretation of the so-called vegetative phase is perhaps less certain, but it is evident that the parasites, after accumulating in great numbers in the tissue spaces and becoming less mobile, are still able to multiply without the ingestion of visible particles of food. While the lack of solid food particles is readily explained by loss of motility, there is no apparent way of determining definitely whether the latter is developmental or due to adverse conditions appearing in the host. In either case the changes taking place may be considered as preparatory to the development of resistant forms. This phase of development produces great distention and destruction of the tissues through the swelling of the closely packed parasites.

The significance of the third phase of development is somewhat clearer. During this phase there is evidently a loss of water from the cytoplasm and an increase in coagulable material and crystalloids which are distinctly acidophilic. Such changes are observed to begin in parasites which are not yet phagocyted. In fact, phagocytosis is probably favored by loss of motility and decrease in size. The extent to which the development of the parasite is influenced by conditions such as food supply and the development of a humoral immunity by the host is at present only a matter of conjecture. As soon as it becomes enclosed in the giant cell it diminishes in size, probably from loss of water, and encloses itself in a dense membrane. Nuclear division in such intracellular forms is extremely rare. Resistant forms escape in small
numbers, at least, enclosed in portions of giant cells both from the caeca and from the bile ducts, but they are probably for the most part destroyed in the body by these cells.

Multiplication, of the parasite in the tissues is brought about by binary division which, as here shown, resembles that which occurs in trichomonads. Neither multinucleated organisms nor evidence of any other than binary division has been found. Smith has called attention to the difficulty of accounting for the rapid increase in the parasite except by multiple division. Amœbæ which multiply by binary division are found to increase very rapidly in culture media even though dividing forms are rather difficult to demonstrate. Entamœeba histolytica, a parasite which may multiply either by binary division or division into four, when inoculated into kittens may appear in great numbers within forty-eight hours and be associated with symptoms. In the present case, however, the parasite is not eliminated from the body to any extent during its multiplication, and there is a relatively long incubation period with this infection. The increase may be accounted for by binary as well as by multiple division if the rate of the former is sufficiently rapid. Dividing nuclei are found with greater frequency than in many other parasites.

The ring-like bodies, which occur frequently in the cytoplasm of the parasite and which have been interpreted by Smith as probably concerned in reproduction, may be accounted for either as the remains of material ingested in the preceding phase of development or as globules of coagulable material possibly associated with degeneration. While these inclusions occasionally show a certain degree of similarity to the nuclei of encysted amœbæ, they show no uniformity with respect either to size or to number. Furthermore, the nucleus and extranuclear body show no characteristic change in the parasites which present these globular inclusions, and there are no intermediate stages connecting these bodies with any recognized form of the parasite.

Smith has called attention to small bodies which occur in considerable number in phagocytic cells. Neither the origin nor the fate of such bodies is satisfactorily accounted for.
I am not convinced of the parasitic nature of these inclusions since what appear to be bodies identical with those described by Smith have been observed in the phagocytic cells but from their staining reaction and their relationship to the inflammatory process present appear to be phagocyted cell fragments. In the course of the destruction of the parasites by the giant cells, the former may be reduced to small hyaline masses, but there appears to be no basis for concluding that these represent a stage of development. Binary division is, therefore, the only form of multiplication which it has thus far been possible to demonstrate.

The following interpretation of the course of the infection is based on the study of certain early lesions of the caeca in which the topography is clear. The central portion of the involved area may be presumed to be the portion earliest invaded, and is often marked by a mass of exudate at the surface of the mucosa. Here, in the portion of the lesion longest involved, the tissue is infiltrated with great numbers of giant cells; further out the tissue has reacted sufficiently to enclose the parasites in definite spaces, and near the periphery of the invaded area the tissue shows no reaction but is forced apart by the swarming parasites. The distribution of the various forms of the parasite with respect to the successive stages of the pathological process indicates the course of events in this infection. At the periphery of the lesions the trophozoites are found migrating freely through the tissues. Passing toward the center of the lesion the clear, swollen forms of the vegetative phase are encountered. These are present in great numbers and are associated with distention and disruption of the tissues. Further on these are gradually replaced by the red-staining forms of the resistant phase which become enclosed in spaces by the reacting tissues and are eventually all gathered up by giant cells. The parasites occur in greatest number in the zones occupied by the pale vegetative forms and the red-staining resistant forms, while the central portion of the lesion may show relatively few of the latter. This numerical distribution of the parasites may be accounted for both by their destruction in the older
portions of the lesion and by a more active peripheral migration in the early stages of the process. If there were no other factors concerned, the infection might continue to extend indefinitely, but in cases showing more resistance there is an extensive infiltration not only of the invaded but also of adjacent parts chiefly with lymphoid and plasma cells.

The injurious effect of this parasite on its host consists primarily, as Smith has already indicated, of a distention of the invaded tissues with the multiplying parasites. This infection indeed furnishes a striking example of a form of parasitism in which there is an extensive destruction of tissue as the result of the pressure of a growing parasite. The effects of the invasion of tissue with this parasite are essentially identical with those produced by the invasion of normal tissue with tumor. The propensity of the invader to grow tends both to isolate and to compress the structural constituents of the tissue including the cells (Figs. 28 and 29). Further indirect effects result from the compression of vessels and their obliteration through thrombosis.

Contrary to Hadley's contention, the protozoon of blackhead is in no sense a cell parasite. The invasive forms migrate freely through the tissues and they are of such size as to preclude their entering the host cells among which they live (Figs. 27, 28, 29, 31 and 32). While it is possible that the smaller invasive forms might be included within an epithelial cell, they are found between rather than in these cells. Certain forms, it is true, occur in great number within giant cells, and in them an occasional nuclear division may be found (Fig. 8), but the small size, the density of the cytoplasm, and the formation of a resistant membrane as well as the evidence of their degeneration and destruction under these conditions, indicate that the reaction of such included parasites is purely a defensive one.

It is a difficult matter to determine whether certain appearances in the parasite represent degeneration or phases of development. Appearances suggesting degeneration are much less frequent in stained films than in stained sections, so that it is probable that the technic employed for the latter produces
more or less artefact in these organisms. For example, the acidophilic forms enclosed in tissue show quite generally a shrunken and distorted nucleus when studied in section, while in stained films their nuclei appear globular. No evidence of degeneration has been noted in the invasive phase of the parasite's development, but it is not improbable that many of the pale, distended organisms of the vegetative phase disintegrate in the tissues. It is quite evident, however, that the parasites after being enclosed in the tissue are destroyed in great numbers by the giant cells. Within the latter the parasites become more and more dense until they are finally represented by hyaline masses which are finally completely absorbed.

The contents of infected caeca are remarkably free from the parasite. It has thus far been impossible to identify it in the discharges examined during the life of the infected bird. In fluid taken from the caeca of such birds immediately after they are killed, an occasional example may be identified among the myriads of other parasites present. As judged by stained sections they are extremely rare in the fibrinous exudate which accumulates on the surface of the more seriously involved mucosa. The encysted forms, however, are found in the lumina of glands and occasionally on the surface of the mucosa. In such situations they are frequently enclosed by portions of giant cells which have become diassociated from the fixed tissue. In the bile ducts also giant cells distended with parasites are found extending through defects in the epithelium and for a short distance along the lumen of the duct. It is possible, therefore, that a certain number of the parasites are eliminated from the host tissue through the extrusion of portions of the giant cells in which they have become incorporated. Whether this relationship serves as a protecting covering for the organisms is not known. It appears probable that the irregularity of results attending experimental attempts to transmit the disease are dependent at least in part upon the paucity of the organisms in the discharges, and it is quite probable that elimination is less marked in the acute stages of the disease than during recovery.
From the present study it is evident that we are dealing with an unusual type of organism. As it occurs in infected tissue its morphological similarity to an amœba is unquestionable, the evidence of amœboid motion and the ingestion of solid particles being rather striking characteristics. The nucleus also being of small size, spherical in form and showing rather striking cyclical changes, resembles in these respects the nucleus of an amœba. On the other hand, its marked pleomorphism, the presence of an extranuclear body resembling the blepharoplast of flagellates and a type of nuclear division characteristic of certain flagellates, sets it apart from all known amœbae. It is evident that this organism does not belong in the genus Entamœba.

That the parasite of blackhead represents a protean development, in the tissues, of a trichomonad which under ordinary circumstances lives in the intestine of turkeys and fowls without producing disease, as suggested by Hadley, is not proved. Neither is there, at this time, evidence sufficient to warrant its classification in the genus Trichomonas. Attention has already been called to the fallacy of regarding the distribution of such flagellates in fixed material as proof of their ability to invade the tissues. The intestinal epithelium of birds is of a delicate character and is easily displaced. The following observation will show the ease with which errors may arise. A fowl with a presumably normal alimentary tract was killed and the caeca were fixed two hours after death. Trichomonads and bacteria were found in great numbers beneath the epithelium, and a large non-pathogenic amœba which is commonly found in fowls had invaded the mucosa and submucosa as far as the layer of annular muscle fibers. Spiral organisms were also found deep in the tissue. It is a matter of common occurrence to find trichomonads beneath the intestinal epithelium fixed immediately on killing normal laboratory animals, so that it will be seen that position alone is of little significance concerning the question of invasion. True invasion by a parasite will be shown by some reaction, however slight, on the part of the host tissue.
Trichomonads are occasionally found in the tissue of the mucosa involved in the lesions of blackhead. In one case portions of the involved caecal mucosa showed considerable numbers of trichomonads intermingled in the tissue with the parasite under discussion. With the latter occurring free in the glands and with trichomonads distributed in the tissue, no more ideal conditions could be imagined for the observation of transitional stages, if such are to be found. In this instance as well as in others where trichomonads have been found in the tissue, neither has *A. meleagridis* shown any additional trichomonad features, nor have the trichomonads shown any resemblance to this parasite. (See Figs. 1 and 5.) Such flagellates are promptly phagocyted, appear always to decrease rather than increase in size in the tissue, and their trichomonad features are preserved as long as the organism can be recognized. Their presence in the tissue may be accounted for by the involvement of the glands in which they occur in the inflammatory process present. It is well known that trichomonads may invade necrotic tissue since they are reported as occurring in abscesses and in gangrenous conditions. That young turkeys may harbor trichomonads in great numbers and at the same time be subjected to unfavorable conditions such as great fluctuations of temperature, wetting and confinement to a small enclosure without developing blackhead, has been shown by experiments to be reported elsewhere. On the other hand, when these turkeys were exposed to infected birds, the disease appeared in a given number after a definite period of incubation.

The reclassification of the parasite of blackhead should not be attempted until additional facts have been obtained concerning its life history. It is evidently not related to parasitic amœbæ of the genus *Entamoeba*. Chaparro amargosa, a drug which rids the alimentary tract of entamœbæ, fails to prevent blackhead when fed daily to turkeys. The presence of an extranuclear body in the resting stage and the character of the nuclear division which is similar to that which occurs in trichomonads suggests strongly that we are dealing with a species of flagellate. The lack of intermediate stages, its
Protozoon of "Blackhead" in Turkeys.

relatively large size, the double character of the extranuclear body, the spheroidal form and paracentral position of the nucleus, and the lack of organelles and such structures as chromatin blocks, axostyle and parabasal body, make it impossible at the present time to include this parasite in the genus *Trichomonas*. While the present name, *Ameba meleagridis*, is probably somewhat of a misnomer both with respect to the generic name *Ameba* and the specific name *meleagridis*, further facts are required in order to establish the true systematic position of this parasite.

Summary.—The parasite, *Ameba meleagridis* Smith, presents a variety of forms which it would be difficult to identify except for the constant presence of an extranuclear body. From this are derived the division centers and a well-developed paradesmose which may stretch from side to side of the dividing cell. Binary nuclear division of a character similar to that described in trichomonads is encountered frequently. No indication of any process of multiplication other than binary division has been found.

Motility, as observed in the warm chamber, is of the nature of amœboid motion of a slow rate. A large proportion of the organisms examined show no motion.

The various forms assumed by the parasite and their relationship to the pathological process indicate distinct phases of development. In the invasive phase, the parasite may ingest solid particles as food, and migrates freely through the tissues. The vegetative phase is characterized by the loss of motility and by the absorption of fluid in the place of ingesting solid material. The cytoplasm in both these phases is distinctly basophilic. The resistant phase is characterized by its small size, acidophilic cytoplasm and by encystment.

Multiplication by binary division is most active in the invasive phase, continues in the vegetative phase and ceases in the resistant phase.

Contrary to Hadley's claim, *Ameba meleagridis* should not be regarded as a cell parasite. Although it migrates freely through the tissues, it does not occur within cells except after
motility is lost, when it is soon phagocyted. Within cells the reaction of the organism is purely defensive; — multiplication ceases, it decreases in size and develops a resistant membrane.

As Smith has pointed out, this infection furnishes a remarkable example of the production of extensive destruction of the tissues of the host through pressure exerted by a rapidly growing parasite.

Supplementing the presence of morphological characteristics not seen in any of the amœbae, the daily feeding of Chaparro amargosa, a drug having definite amœbal properties, has failed to prevent infection in young turkeys when exposed to infected birds.

While the parasite shows a type of nuclear division similar to that of trichomonads and an extranuclear body resembling a blepharoplast, various other features characteristic of trichomonads have not been demonstrated. No intermediate forms connecting this parasite with the trichomonads associated with it in the cæca have been observed.

LITERATURE.


Dobell, C. (1914.) Cytological studies on three species of Amœba — A. lacertæ Hartmann, A. globæ n. sp., A. fluvialis n. sp. Archiv. für Protistenkunde, xxxiv, 1914, 139.

Doflein, F. (1911.) Lehrbuch der Protozoen-kunde. 3d. ed. (G. Fischer, Jena), xii, 1043 pp., 951 figs. in text, 1911.


Hadley, P. B. (1916.) The rôle of the flagellated protozoa in infective processes of the intestines and liver. Bulletin 166, Agricultural Experiment Station of the Rhode Island State College, June, 1916.


Jowett, W. (1911.) Note on certain protozoan organisms observed in the rectal and caecal contents of the turkeys and fowl. Journ. of Comp. Path. and Therap., xxiv, 1911, 303.


DESCRIPTION OF PLATES I. AND II.

The figures shown in the two plates illustrating this paper were drawn with a camera lucida at a magnification of 1,400 diameters. Unless stated otherwise, the figures represent organisms stained with phosphotungstic hematoxylin.

PLATE I.

Fig. 1. — A profile view of a trophozoite of Ameba meleagridis which is applied to the surface of the epithelium of a caecal gland. The ectoplasm extends in a sheet from the rounded mass of endoplasm. The extranuclear body is situated beneath the nucleus. Several globules are present in the cytoplasm. Stain—eosin and methylene blue.
Fig. 2. — A trophozoite showing numerous food globules, each within a vacuole. The nucleus shows a central mass in which the chromatin is incorporated, i.e., the karyosome.

Fig. 3. — A clear form of the vegetative phase with small nucleus and deeply stained lines radiating from the extranuclear body. Several rings are apparent in the cytoplasm.

Fig. 4. — A large organism with no cytoplasmic inclusions. The nucleus is large and coarse rays radiate from the extranuclear body.

Fig. 5. — A phagocyted trichomonad found in the involved tissue, illustrating distinctive flagellate characteristics and lack of similarity to the parasite of blackhead. Compare with Fig. 1.

Figs. 6 and 7. — Rounded forms of the vegetative phase of A. meleagridis. Note difference in size and granular type of nucleus as well as the appearance of extranuclear body.

Fig. 8. — An organism showing binary nuclear division, included in a giant cell with two organisms of a distinctly later phase of development.

Fig. 9. — A parasite with dense cytoplasm approaching in character that of the resistant forms. The appearance of the nucleus suggests changes preparatory to division.

Fig. 10. — Resistant forms isolated in the cytoplasm of a giant cell.

Fig. 11. — A group of resistant forms with surface modified by mutual contact.

Successive stages in nuclear division are illustrated in Figs. 12 to 16 inclusive.

Fig. 12. — The nucleus presents eight deeply stained granules, evidently chromosomes.

Fig. 13. — Chromosomes (about one half actual number seen in focus) separated into two groups with a deeply stained rod, paradesmose, extending longitudinally over them. No nuclear membrane apparent.

Fig. 14. — Lengthening of paradesmose and further separation of the two groups of chromatin granules. The paradesmose terminates in a granule evidently of the nature of a division center.

Fig. 15. — An organism of relatively large size, showing a late phase of nuclear division. The chromatin is now massed within a vesicle at each end of the paradesmose. Nuclear membrane reappearing.

Fig. 16. — Only a trace of the paradesmose is apparent. The daughter nuclei show a distribution of the chromatin characteristic of the granular resting nucleus, and each is associated with an extranuclear body.

Figs. 17 and 18. — Organisms with two nuclei, each associated with an extranuclear body in the form of a double granule.

Fig. 19. — A trophozoite occurring in an irregular space in glandular epithelium and showing nuclear division. Stain — eosin and methylene blue.

Fig. 20. — Nuclear division in a trophozoite packed with food globules. Stain — eosin and methylene blue.

Fig. 21. — Terminal stage of nuclear division. Paradesmose no longer rigid and stains faintly.

Fig. 22. — An organism found stretching through a space in the glandular epithelium. In this, as in the forms shown in the three preceding
figures, the paradesmose extends longitudinally so that when nuclear division is completed the cytoplasm probably is drawn apart into two portions each with a nucleus. Stain — eosin and methylene blue.

**FIG. 23.** — A rounded vegetative form in process of division. The deeply stained material of each daughter nucleus appears in two masses.

**FIG. 24.** — Terminal phase of nuclear division, paradesmose faintly stained. Stain — eosin and methylene blue.

**FIG. 25.** — Small two-nucleated organism with rather dense cytoplasm.

**FIG. 26.** — Late stage of nuclear division in a large organism with dense cytoplasm of a character indicating approach of resistant phase.

**PLATE II.**

**FIG. 27.** — A portion of a cæcal gland showing several of the parasites lying in fistulous spaces in the epithelium and one lying free in the lumen. The cytoplasm in these forms is colored an intense blue. The nuclei of certain of the parasites are not shown in this section.

**FIG. 28.** — Reticular tissue of the cæcal mucosa distended with trophozoites containing numerous food globules. Many of the parasites show a clear process or pseudopod at one extremity. To the right are three red-stained parasites of the resistant phase.

**FIG. 29.** — Large vegetative forms found near the periphery of a liver lesion. Only a trace of the liver parenchyma remains. These parasites show no food globules but appear to have absorbed a large amount of fluid so that there is relatively little coagulable substance in the cytoplasm. The various constituents of the nuclei are apparent.

**FIG. 30.** — Parasites in the resistant phase of development enclosed by the reacting host tissue. The cytoplasm takes the eosin stain and appears dense as though containing a relatively large amount of coagulable material. The nucleus is indistinct and distorted. The organisms are each enclosed in definite membrane.

**FIG. 31.** — A parasite with blue-stained cytoplasm, which has invaded the wall of a small vein where it lies between the endothelium and the media.

**FIG. 32.** — A large trophozoite without food globules situated between the fibers of muscular layers of the cæcum. Two granules (division centers) connected by a paradesmose are seen lying against the nuclear membrane. The pale area possibly indicates the site of the extranuclear body from which they have arisen. The ectoplasm is seen extending outward in sheet-like processes.
Tyzzer.

Blackhead.