ON THE LIFE-HISTORY OF *GIARDIA*

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This paper is based upon the examination of 220 mammals, gray and white culture mice (98), culture rats (42), and wild *Peromyscus maniculatus gambeli* (Baird) (59), *Microtus californicus californicus* (Peale) (6), and coyotes [Canis ochropus ochropus (Peale)] (2) trapped near Berkeley. About 13% of the total were infected, 22 of the mice, and 4 of the *Peromyscus*, with *Giardia muris* and three of the *Microtus* with *G. microti* sp. nov. Several hundred slides prepared by the Schaudinn-iron haematoxylin wet method were examined and stages in binary and multiple fission and in encystment obtained in quantity.

In the last edition of Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen* Jollos (1913) denies the existence of multiplicative phases in *Lamblia* in the free stage in the intestine of the host. Rodenwaldt (1911) had also held this view in Prowazek's *Handbuch der pathogenen Protozoen* and Alexeieff (1914) and Prowazek and Werner (1914) as the result of recent investigations have confirmed it. Castellani and Chalmers in the last edition (1913) of their *Manual of Tropical Medicine* accept Noc's (1908) conclusion, seemingly made without study of comparative material, that there is only one species concerned in the intestinal infections alike of mice, rats, cats, and man, and make the inference that such vermin become sources of infection to man by the contamination of his food and water with their cyst-bearing faeces.

It comes thus to be of interest to human and comparative medicine to learn whether or not these intestinal flagellates which produce a chronic enteritis in young mice and are under suspicion of similar activity in man, may multiply in the free state in the intestine, or whether infection is limited to the number of ingested spores, and especially to learn whether there are separate species restricted to given host species or groups of species of rodents, and another species found in man. If the latter be the case, the probability of these mammals being sources of infection is reduced considerably, unless it be that the parasites transform from one 'species' to another with change of host. The biological problems of host specificity, and transformation by environment are thus involved in this question.

It is the purpose of this paper to demonstrate the occurrence of binary and multiple fission in *Giardia* (=*Lamblia*), in both the free (hitherto denied) and encysted stage, to describe mitosis in these minute organisms, to offer confirmatory evidence for the presence of several species instead of one only in the genus, to add to the skepticism regarding autogamy and the existence of an *Octomitus* stage in the life-history, advanced by Hartmann (1911), and to offer some inconclusive evidence of maturation in the so-called conjugation cysts. The paper will be published in full in the University of California Publications in Zoology.

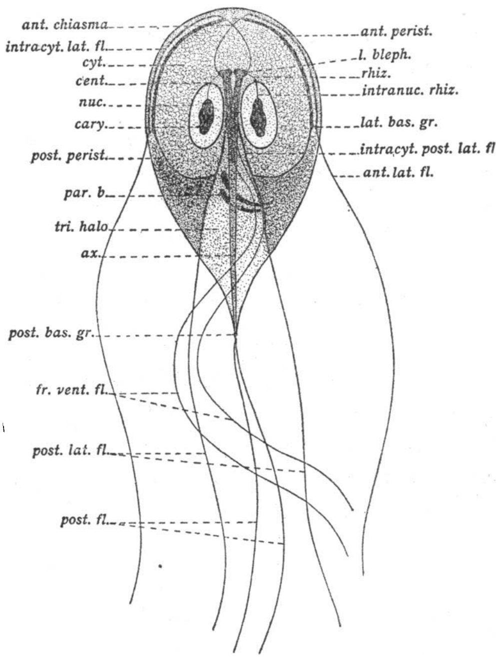

Fig. 1. Ventral view of *Giardia microti* sp. nov. in early prophase of mitosis, somewhat diagrammatic. × 3400. Abbreviations: ***ant. chiasma***, anterior chiasma; ***ant. lat. fl.***, anterior lateral flagellum; ***ant. perist.***, anterior peristome; ***ax.***, axostyle; ***cary.***, karyosome; ***cent.***, centrosome; ***cyt.***, cytostome; ***fr. vent. fl.***, free ventral flagellum; ***intracyt. lat. fl.***, intracytoplasmic part of the antero-lateral flagellum; ***intracyt. post. lat. fl.***, intra-cytoplasmic part of the postero-lateral flagellum; ***intranuc. rhiz.***, intranuclear rhizoplast; ***lat. bas. gr.***, lateral basal granule of antero-lateral flagellum; ***l. bleph.***, left blepharoplast; ***nuc.***, nucleus; ***par. b.***, parabasal body; ***post. bas. gr.***, posterior basal granule of posterior flagella; ***post. fl.***, posterior flagella; ***post. lat. fl.***, posterolateral flagellum; ***post. perist.***, posterior peristome; ***rhiz.***, rhizoplast joining nucleus to blepharoplast; ***tri. halo:*** triangular halo about posterior part of the axostyle.

Giardia microti sp. nov. (fig. 1) in common with the other species, is a diplozoic flagellate, a binucleate somatella with eight flagella in four pairs, antero-lateral, postero-lateral, free ventral, and posterior, the last emerging from the posterior tip of the axially located axostyle, the ventrals from the ventral surface, and the others from the lateral margins. The flagella form a part of an integrated neuromotor system of fibrils which unite the two component cells by a single chromatic system staining more or less deeply with iron haematoxylin. This system consists of a rhizoplast from the centrally located karyosome of each nucleus which passes through the centrosome on the nuclear membrane to the blepharoplast on its side at the head of the axostyle.

From the two blepharoplasts the single axostyle (not two as heretofore reported) passes posteriorly to emerge as the posterior pair of flagella. From the blepharoplast or near it, also arise the other flagella. The antero-lateral passes forward within the cytoplasm, crosses its mate of the opposite side uniting at the crossing in a node, the anterior chiasma, and thence curves posteriorly along the margin of the cytostome to emerge from the lateral basal granule as a free antero-lateral flagellum. The postero-lateral passes obliquely posteriorly within the cytoplasm to emerge near the margin, and the free ventrals emerge at once on the ventral surface. The blepharoplasts of the two sides of the axostyle are united across its head by a transverse bridge commissure. The antero-lateral flagella run in close conjunction with the thickened chromatic anterior peristome and the postero-laterals are in contact with the posterior arc of the peristome. This thickened chromatic margin lies in the rim of the deeply cupped ventral cytostome, the organ of attachment of the parasite.

Dorsal to the axostyle is a pair of chromatic bodies, the parabasals which contain stainable material whose volume fluctuates with the conditions of metabolism and which may function as reservoirs of cytoplasmic chromidial material. They appear to have a characteristic shape in each species. In *G. muris* they are stout ellipsoidal, and nearly fused. In *G. microti* (fig. 1) they are slender, curved, tapering, parallel structures. In *G. muris* each is connected with the axostyle near the blepharoplast by a slender chromatic fibre.

These organisms multiply in both the free and the encysted stages, by simple binary fission, and by multiple fission leading to the formation of a 16-nucleate, 8-zooid plasmodium or somatella, or a 16-nucleate, potentially 8-zooid cyst. Binary fission occurs by a well defined mitosis followed by a delayed plasmotomy.

Mitosis in *Giardia* is much like that described by Kofoid and Swezy (1915) for *Trichomonas*. It begins in the blepharoplast whose division is followed by that of the axostyle which splits distally to form two on the anterior ends of which the daughter blepharoplasts are attached. The centrosome at the upper pole of the nucleus divides and the daughters assume polar positions on the membrane of the elongating nucleus, spinning out a temporary paradesmose between them upon the outside of the persistent nuclear membrane. The nucleus, from which an intranuclear chromidial cloud had emerged, with subsequent increase of cytoplasmic chromidia, exhibits an enlargement of the karyosome, its breaking up into eight chromatic granules probably representing four precociously split chromosomes which form in parallel rows and merge into a split skein from which four chromosomes emerge in the equatorial plate at the metaphase. These constrict, the daughter chromosomes migrate toward the poles of the intranuclear spindle, and the nuclear membrane constricts the parent into two daughter nuclei.

Plasmotomy is long delayed. The separation is longitudinal, the two daughter nuclei of each side forming the nuclei of the daughter zooids, which hang together assuming a great variety of positions before the protoplasmic bridge finally parts.

Multiple fission is of three types. The first is in free individuals in which three mitoses follow in succession before plasmotomy occurs and each zooid during the process acquires its full complement of organelles before the next division ensues. The result is a 16-nucleate, free-moving plasmodium composed of 8 fully equipped zooids which they separate singly. These small individuals are not *Octomitus*-like and are not to be confused with *Hexamitus muris* Grassi (= *Octomitus*), which species occurs in the same host with *G. muris*. *Giardia*, according to our evidence, does not pass through an *Octomitus* stage as Hartmann (1911) proposed.

Multiple fission in free forms occurs also by nuclear division with which the extranuclear organelles do not keep pace in multiplication, so that free individuals with the normal single equipment of these of extranuclear organelles are crowded with 16 nuclei in a bilateral or ultimately an irregular arrangement. The later history of such individuals has not been traced.

Single encysted individuals may also undergo multiple fission. The extranuclear organelles tend to disappear, the axostyle and posterior peristome lingering longer than the other chromatic parts as the nuclei go through three succeeding divisions producing a 16-nucleate mass within the cyst. Such cysts seem not to undergo further development in the rectum of the host in which they are found.

We find no evidence that the so-called conjugation cysts contain two sister individuals, and that therefore autogamy occurs. Pairs of individuals in end-to-end back-to-back position as in cysts are found free without evidence of their origin by fission. This condition may precede encystment. The two individuals which may simulate maturation divisions in so far that the first division of each nucleus gives rise to two nuclei, one with abundant chromatin, and one (first polar nucleus?) with little chromatin. The second division occurs in both of these daughter nuclei. The smaller nucleus divides forming two still smaller nuclei and the larger one forms one nucleus rich in chromatin (the gamete nucleus?) and one with little chromatin (the second polar nucleus?). However, no evidence of reduction in number of chromosomes is demonstrable. It is obvious that, since these divisions occurred in both nuclei of the somatella, we might have a binucleate gamete, and should the two 'conjugants' in the cyst unite, a binucleate zygote, a condition highly improbable. The alternative explanation of this simulation of maturation is that we have here some form of degenerative multiple fission but the evidence of this is not conclusive.

We find evidence of groups of structural characteristics which are diagnostic of the several species in the genus. The same organelles with the same fundamental relations of position occur in all of the species at comparable stages. The specific differences consist in modifications in shape, size, proportions, relative size and shape of the cytostome and its relation to the margin of the body, in the size, shape, and position of the parabasal bodies, in general stainability, and in relations to hosts. It is possible thus to characterize *G. agilis* Alexeieff from the tadpole, *G. sanguinis* (Gonder) from *Elanus*, a South African falcon, *G. duodenalis* (Davaine) from the European rabbit, *G. muris* (Grassi), from *Mus*, *Epimys*, *Arvicola* (?) and *Peromyscus*, *G. microti*, sp. nov. from *Microtus*, and the species widely known as *Lamblia intestinalis* (Lambl) from man. Conclusions as to the identity of *Giardia* (= *Lamblia*) of man and rodents now generally accepted are of doubtful validity and require critical verification.

The generic name *Lamblia* Blanchard 1888 should give way to *Giardia* Kunstler 1882 on ground of priority since species of the two are morphologically so alike that no generic distinction can be made between them.

Our work has shown that *Giardia muris* and *G. microti* produce a readily recognizable enteritis in mice, and that both binary and multiple fission take place in the free non-encysted stage. It may be found abundantly in material well smeared out from the intestinal epithelium. Multiple fission results in a 16-nucleate, 8-zooid plasmodium which later undergoes disintegrative plasmotomy. There is no *Octomitus* stage in the life-history of *Giardia*. There is typically one axostyle in the trophozoite. Prophase stages in which this has divided to form two axostyles are often seen. The so-called 'Rätzelkörper' are homologues of the parabasals of the trichomonads. The extranuclear organelles are united with the karyosome, centrosome, and blepharoplast in an integrated neuromotor apparatus. Mitosis is intranuclear, with precocious splitting of the four chromosomes which subsequently fuse in four in the equatorial plate. Free pairs of individuals are found united in back-to-back position as in the so-called conjugation cysts. Nuclei in these cysts undergo two divisions simulating reduction divisions in which, however, chromosomes reduction has not been demonstrated. No evidence in support of autogamy and no proof of sexual reproduction has been discovered.

Morphological characters separate six species in *Giardia*. The parasite in mice appears to be distinct from that in man. The generic name *Giardia* Kunstler should supersede *Lamblia* Blanchard on grounds of priority.

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THE INORGANIC CONSTITUENTS OF ALCYONARIA

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The zoantharia, madreporaria, or stony corals have been repeatedly analyzed, and with generally concordant results. Thirty analyses, made in the course of the present investigation, of which this paper is a preliminary notice,[1] have confirmed the older data. These corals consist mainly of calcium carbonate, with one or two per cent of minor impurities, and a little organic matter. The same is true of the coralline hydrozoa, of which six analyses, representing the genera *Millepora* and *Distichopora*, have also been made. The alcyonaria, however, which include the red corals, the gorgonias, and other fan-like or branching forms, are quite different; and they are generally characterized by the presence in them of magnesium carbonate, and often of calcium phos-