

SOME OBSERVATIONS ON THE REPRODUCTIVE CYCLE OF THE INFUSORIAN, *ICHTHYOPHTHIRIUS MULTIFILIIS*

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A year ago the writer¹ described an epidemic of ichthyophthiriasis which had appeared among the fishes in an Indianapolis pond. The disease was attributed to the holotrichous infusorian, *Ichthyophthirius multifiliis* Fouquet. This fish parasite is common in Europe where it frequently causes serious epidemics among freshwater fishes in aquaria and hatchery ponds. In this country the disease has also appeared from time to time in widespread localities, and the parasite causing it has been identified as the same species as the one producing the disease in Europe. It may have been introduced with some of the imported fishes. The European carp is a possible source.

There are a number of papers in various European publications which relate to the disease; a few are concerned with the nuclear changes during the reproductive cycle. I have found no record of a study of these changes in American material. The purpose of this paper is to record the results of observations that were made on the life history of material that was collected during the above mentioned epidemic, and to call attention to the interesting life cycle of this parasitic infusorian. The work is incomplete in respect to details because of the lack of material which can be collected successfully only when the disease appears in epidemic form. The work of Buschkiel² contains the most satisfactory account of the reproductive cycle. This study shows some differences in the nuclear cycle from that reported by Buschkiel.

The typical infusorian carries on the reproductive process by binary fission which is interrupted from time to time by a sexual process. *I. multifiliis* may divide by binary fission; this is followed by sporulation, a process of multiple division, during which autogamy occurs. Sporulation ends in the production of the reinfecting spores.

The Adults. In epidemic form the adults are found in abundance in the gills and skin of many fishes. They have a characteristically large macronucleus and a micronucleus which was found to migrate into the macronucleus during the earlier parasitic stages. A short time after this migration the micronucleus was seen with difficulty and later not at all. For a microscopical study of these nuclei pieces of gill tissue containing an abundance of parasites were fixed with saturated

¹ Pearson, N. E. Ichthyophthiriasis among the fishes of a pond in Indianapolis. Proc. Ind. Acad. Sci. 41:455-463. 1931 (1932).

² Buschkiel, A. L. Beiträge zur Kenntnis des Ichthyophthirius multifiliis Fouquet. Arch. f. Protistenk. 21:61-102, 1910.

"Proc. Ind. Acad. Sci., vol. 42, 1932 (1933)."

mercuric chloride without acetic, Zenker's, Flemming's strong solution, or Kleinenberg's micro-sulphuric. Sections were stained with Delafield's hematoxylin or Heidenhain's iron-hematoxylin, and counter-stained with eosin or acid fuchsin. By none of these methods could the micronucleus be located in the later adult stages. A characteristically small spindle shaped body which took the counter-stains was present, but a study of later stages showed this to be a different structure.

A few observers have reported that the adults may undergo binary fission in the tissues of the host. Although a few hundred specimens of variable sizes were studied in the gills, none were found dividing. If division occurs in the tissues of the host it probably occurs only in mature adults which for some reason fail to free themselves at the time they would normally begin the sporulation process.

The mature adults migrate from the tissues of the host and may settle down directly and begin the process of sporulation, or they may swim actively for a time, divide by transverse fission and then settle down. A temporary gelatinous cyst is secreted outside of the cilia. Within the cyst multiple division occurs during which all cells are kept in constant rotation by actively moving cilia.

Sporulation. The adults may attain a length of 0.8 mm., but many smaller ones escape and undergo sporulation. The smallest one observed in this laboratory was 0.14 mm. The smaller individuals produce fewer re-infecting spores than the larger ones, but the nuclear changes are the same in both. The divisions within the cyst are regular, all of the daughter cells being found in the same stage of development. Sporulation was usually completed within 22 to 30 hours in the larger adults whereas the smaller ones usually completed the process in less time. The nuclear changes in the reproductive cycle occur in the cells which follow the second from the last division; consequently, the material for the study of the nuclear changes should be taken toward the close of the sporulation process.

Micronuclear Cycle. The material that was used for this study was fixed in Zenker's and stained with Delafield's hematoxylin or Heidenhain's iron-hematoxylin, and counter-stained with eosin or acid fuchsin. Iron-hematoxylin was not satisfactory inasmuch as it had a tendency to stain cytoplasmic structures in the same manner it did the micronuclei.

During the process of multiple division each cell was found to divide by ordinary fission, until each attained a diameter which averaged 0.035 mm. During these cleavages the macronucleus was constricted in two equal parts, but no micronucleus could be discovered. As yet no worker has been able to locate a micronucleus during these divisions. When the above mentioned cell size was reached the sexual cycle was initiated by the migration of a micronuclear body from the macronucleus. This first detached micronuclear body was first observed just as it began to migrate from the macronucleus. At that time it stained slightly deeper than the macronucleus, its outlines were only fairly sharp, and the chromomeres making up the structure appeared the same as those of the macronucleus. It measured 0.00348 mm. in

diameter. As the body migrated from the macronucleus a trail of chromatin followed it. During the migration the body enlarged, at the same time gradually lost its staining capacity, became ellipsoid and formed into a spindle on which there were many thick thread-like chromosomes. Then followed a division of the spindle into two parts; the daughter nuclei moved to opposite ends of the cell, at the same time contracting and consequently taking more stain. During this contraction the division of the cell took place so that each daughter cell received one of the micronuclear bodies. After the division of the cell the micronuclear body continued to contract until it became an intensely staining nucleus.

Whether the division of this micronuclear body represented a reductional division could not be determined from a study of the chromosomes; but the chromosomal condition was suggestive of the so considered reductional division in *Paramoecium*.

A second micronuclear body then became detached from the macronucleus in the same manner that the first body became detached. It was slightly smaller than the first measuring approximately 0.003 mm. in diameter. As it migrated from the macronucleus with a trail of chromatin following, it became ellipsoid and formed into a spindle. While this change was progressing the first detached body likewise formed a spindle.

The structure of the chromatin on these spindles and the nature of the division that followed could not be determined, because Zenker's reagent was not an ideal fixative for the spindles and the material for the study of this particular stage was not abundant. However four nuclei, all of the same size, resulted from the change. Two of these nuclei fused while still ellipsoid. The fusion nucleus which resulted contracted, exhibited a deeply staining chromatin network during the early stages, and later became an intensely staining nucleus measuring approximately 0.0025 mm. in diameter. The remaining two nuclei became greatly enlarged and gradually lost their staining capacity during the contraction of the fusion nucleus. The two structures then fused into a single spherical body that filled the central half of the cell. The material in this body fixed and stained very poorly thereby indicating disintegrative changes. This material finally intermingled with the cytoplasm of the cell.

The final division in the process of sporulation followed. In this division the micronucleus became spindle shaped and took up a position at the side of the cell between the macronucleus and the cell wall. Its division just preceded the cytoplasmic cleavage. The macronucleus divided amitotically as in earlier divisions. The resultant daughter cells then became the reinfesting spores by slight cytoplasmic changes.

From the above described changes it is seen that the spores which initiate a new cycle in the life history contain a macronucleus and a single micronucleus; the former derived from the original micronucleus out of which micronuclear material had migrated, the latter from the fusion of two micronuclei which, it is reasonable to conclude from the degenerating nuclei, had undergone divisions in which the chromosomal number had been reduced.

A third body about one-fourth to one-third the size of the other detached bodies was observed to separate from the macronucleus in some of the cells following the migration of the second detached micronuclear body. It became spindle shaped as the preceding two; but its changes could not be followed. Whether this third detachment was present in all cells could not be determined; however, a small micronuclear body was observed in a few cells that contained the fusion nucleus, and a few of the mature reinfecting spores were observed to contain a small micronuclear body in addition to the micronucleus. The function of this detached body is not understood unless it represents micronuclear material that failed to migrate from the macronucleus during the earlier migrations.

The small, usually spindle shaped structures that were referred to in connection with the macronucleus of the adults were also present in the cells resulting from multiple division. Some of the macronuclei contained several, others a single one. They were observed to increase in size and to be cast out of the macronucleus from time to time. The macronucleus of the cell undergoing the final micronuclear changes contained a single, yet the most characteristic of these bodies. These structures may have something to do with the metabolic activity of the cell.

The new micronucleus was found to migrate into the macronucleus some time after the parasitic spores had entered the tissues of the host. It entered the macronucleus while still a deeply staining body and then enlarged exhibiting a chromatin network apparently continuous with that of the macronucleus. At this time the parasite measured approximately 0.13 by 0.06 mm.

The free swimming adults which measure about 0.14 mm. in diameter undergo the process of sporulation slowly if at all. This seems to indicate that the micronucleus bears a relationship to the macronucleus which is necessary to initiate the process of cell division and this relationship is not reestablished until some time after the micronucleus has entered the macronucleus.

SUMMARY

Mature adult parasites have a micronucleus that is concealed in the macronucleus during the greater part of the vegetative stage, and also during the greater part of the sporulation process. It migrates from the macronucleus in two separate migrations that take place in the first two of the last three cells of the sporulation process. The first detached body divides and the daughter nuclei are distributed to daughter cells. The daughter nuclei of the first detached body and the second detached body divide forming four nuclei of equal size. Two of these fuse (presumably daughter nuclei of the first and second detached bodies) and the remaining two degenerate. A single cell division follows the formation of the fusion nucleus; thus giving rise to the reinfecting spores which penetrate the tissues of the host and enter into the vegetative state. After a short period of time the micronucleus migrates into the macronucleus.

The fusion of the two nuclei is to be interpreted as the fusion of "gametes"; but what represents the meiotic divisions is problematical. The answer to the question as to whether each of the two detached bodies represent reduced chromatin, or whether the divisions of these bodies represent meiotic divisions must be held in abeyance until a careful examination of the chromosomes can be made. The third detached body further complicates the problem.

