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# ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

## III. ON THE CHLOROMYXUM CLUPEIDAE OF CLUPEA HARENGUS (YOUNG), POMOLOBUS PSEUDOHARENGUS (YOUNG), AND P. AESTIVALIS (YOUNG)

C. W. HAHN

The attention of the writer was drawn to some very common white pseudocysts in the body muscle of the young herring by Dr. Edwin Linton in July, 1910. He had previously (1891) identified the contents of these cysts as myxospores of a myxosporidian. An effort was made at once to trace the life-history of this organism, also to learn its method of infection, the pathological condition induced, and the effect upon the vitality of the host. Several hundred herring, ranging in size from 1½ to 5 or 6 inches have been examined. About 54 per cent of them had buried in the body muscle clusters of myxospores (pseudocysts) large enough to be visible to the unaided eye. These spores are brought to light by cutting the flesh lengthwise of the body on each side of the backbone. As will be evident after reading the following pages, it is certain that a microscopic examination of fish in which no pseudocysts can be seen by ocular examination, would greatly raise the proportion of fish which harbor such parasites. Only small fish under 4 or 5 inches are known to be infected.

The pseudocysts are sometimes as large as a grain of wheat. They are usually white or cream colored, soft or creamy in structure, and spindle shaped, especially when small. Small pseudocysts cannot be distinguished at first sight from worm cysts, but the latter, when pressed with the tip of a scalpel, resist and regain their shape when the pressure is withdrawn. The cysts mash up just like a bit of soft cheese. Usually the pseudocysts lie between the bundles of fibers. Large masses occur in pockets just beneath the integument, which is slightly mounded over them. A pin-prick brings forth a pus-like fluid. The large cysts appear to make their way from deep-seated positions to the surface. A small hole then forms in the integument, through which the mass escapes. No case of the complete discharge of myxospores from such a pore has yet been observed. The process has been observed in its initial stages, and many cases have been observed of worm cysts which were just escaping or had just left pores identical with those just described.

The pseudocysts of this Chloromyxum occur throughout the body musculature. It is very common to find several just at the base of the caudal fin rays. They are also frequent just posterior to the skull and branchial cavity. It is remarkable that a fish can retain life with its flesh so burdened with the cysts, in some cases so abundant that it is impossible to count them. The large ones coalesce and form huge cavities filled with a pus-like fluid. A fish an inch and a half long may contain several hundred pseudocysts and continue for a time to hold its place in a school of several hundred fish against the incessant attack of numerous enemies.

The pseudocysts are composed almost entirely of mature myxospores. When muscle tissue adjacent to the cysts is examined under the microscope, it is found to contain myxospores in masses of all sizes and numerous isolated myxospores or chains of spores between the fibrillae. These aggregations of myxospores vary in size from one spore to two or three times the size of a grain of wheat. The shape is determined by physical conditions. In small cysts it results in long or fat oat-shaped structures.

It is not possible by direct observation to attribute any evil effects upon the host to the presence of these numerous passive pseudocysts. The fish give no visible evidence of inconvenience. But when one takes into consideration the ravages of the trophic stages which must have preceded the harmless myxospores and the toxic substances secreted during the process of sporogenesis, it is very probable that some considerable injury has already been inflicted upon the host before the myxospores develop. My statistical studies prove conclusively that the pseudocysts are in reality more or less injurious.

The trophic stages of the gall-inhabiting species of this genus are known through the researches of Erdman (1910), Auerbach, and Léger (1906). No reference to the multiplicative stages of flesh-inhabiting species has yet been found. The occurrence of myxoplasms in both inter- and intra-cellular positions in muscle tissue has been described by Thélohan (1891, 1893) for *Glugea destruens* Thél. in *Callionymus lyra* and by Gurley (1893) for *Pleistophora typicalis* Gurley in *Cottus scorpius,* and by both Pfeiffer (1891) and Keysselitz (1908) for *M. pfeifferi* in the barbel. The pathological conditions in most of these cases are practically identical to those which I have found in Fundulus. As for details regarding the trophic stages of the parasites themselves, they are scanty and cannot be satisfactorily correlated with life histories as they are now known.

The origin of the multiplicative trophoplasts is still somewhat obscure. It is probable that large myxoplasts like that shown in Figure 15 undergo schizogony and set free the small trophoplasts which occur so abundantly in newly infected tissues. The smallest of these is about 2$\mu$ in diameter. They are very closely scattered in patches throughout the myoplasm of infected muscle fibers, sometimes so close together as to be almost in contact. The fact that the size is not uniform would lead to the conclusion that they multiply by fission, since it is the habit of most myxosporidia to advance from stage to stage simultaneously. But one never meets with couplets in the same cavity in the myoplasm, as would be the case if fission were common.

The distribution of the small and large trophoplasts is very irregular, there being frequent isolated individuals. The muscles of the head region, especially those around the branchial arches and the eye and jaw muscles, are frequently riddled with these parasites. They also occur abundantly in the striated muscle of the digestive tract (the herring has ribbon-shaped striated fibers in the wall of the intestines) and less frequently in the body muscle near the backbone. They are both inter- and intra-cellular (Fig. 8).

When fresh muscle is examined, the trophoplasts appear as almost invisible homogeneous droplets. Stains have no greater tendency to take hold of them than they do the trophoplasts of *M. musculi.* Anilin stains reveal them as white spaces in the myoplasm. Hematein gives to them a homogeneous clouded appearance as shown in Figure 13. The shape in the very small individuals is rounded or ovate. Larger ones are irregular in shape, but in fixed preparations have always an entire contour. Rarely a medium-sized trophoplast has the nucleus faintly stained (Fig. 13).

There is the same evidence that these bodies are not artifacts that has been given for the trophoplasts of *M. musculi.* The appearance of the older stages of the multiplicative trophoplasts is almost identical with that of the young. But the size, shape, and distribution gradually change. Some are evidently motile, judging from their shape (Fig. 13). A few individuals of this type occur in rather isolated positions. The muscle is taken from the body near the backbone. Adjacent tissues are liberally sprinkled with smaller trophoplasts. Older stages than that in Figure 13 are usually still more isolated from the more gregarious young stages. It is probable that the schizogony sets free innumerable multiplicative spores which throughout their growth migrate from atrophied toward normal tissue and become quiescent in the mature schizont (Fig. 15). The latter are very large (sometimes 40 to 50 by 50 to 60$\mu$), and almost spherical in form. They are usually in tissues which are comparatively free from trophoplasts of small size, and also free from evidences of infection. Rarely one can discern a faintly stained material within which is probably the nucleus. Another large body which is almost identical in appearance, but which is not associated with the smaller trophoplasts, nor connected with them by any link as yet discovered, is of much greater size. It reaches 890$\mu$ in length by 30$\mu$ in width. The outline is sharp and the slightly opaque cytoplasm reveals no trace of the internal structure. These bodies lie between the muscle fibers, sometimes in rows. Around and between them is a granular deposit which runs into the masses forming faint partitions. It gives the appearance of a large number of schizonts which have more or less fused. Separate large myxoplasts do occur in exactly similar positions. This form and position in the muscle fibers is also reproduced in a striking way by masses of sporoblasts and myxospores in tissues which lack all of these earlier stages. It is therefore apparent that the large elongated myxoplasts represent aggregations of some kind of migratory trophoplasts. That they do not grow in situ is shown by the uninjured condition of the tissues. They are too large to represent the adult of any single one of the largest myxoplasts without a vigorous consumption of host tissue of which there is negative evidence. The sporoblasts and myxospores occur in chains and smaller groups such as to indicate that there are numerous small clusters of sporoblasts which are not gathered together as in the cases above cited. It is altogether probable that either mechanically or through their own activity, the propagative myxoplasts, having migrated deeper into the tissues of the host, become assembled into larger or smaller groups. In this condition the sporoblast cells are formed and sporulation takes place.

In these two kinds of presporulating cells one evidently has the multiplicative and propagative schizonts. The former represented in Figure 15, is always found in tissues adjacent to the young multiplicative trophoblasts. The latter is never to be found in tissues which contain multiplicative stages, but always in the very presence of sporoblasts and myxospores. The multiplicative stages and schizonts alone are encountered in the muscle of the digestive tract and its vicinity. The propagative schizonts are always in the tissues of the body muscle. These facts prove (1) that the trophoplasts migrate from centers of infection to parts free from previous attack; (2) that the general trend of the migration is from the digestive tract into the body muscle. (3) that the initial infection takes place through the digestive tract. It is probable that this occurs throughout the entire length of the digestive tube, because there is no very marked superiority in the number of myxospore cysts of the anterior body muscle over the posterior region. However, this equality of distribution may be due to transit through the blood vessels.

In the large schizont cysts described above one can occasionally find the contents divided into hundreds of irregular-shaped cells whose cytoplasm is so clear and structureless that the cell boundaries are almost invisible. They contain conspicuous masses of varying size and shape and intensity of stain. The two upper cells of Figure 1 are almost identical with the above, but were drawn from a group that had been set free from the cyst. These are sporocysts. The deeply stained portion represents the developing myxospores and is not a nucleus as one might suppose.

From the above and further details of sporogenesis which follow, it will be seen that sporoblasts may not necessarily occur free, the large presporulating masses being composed of many assembled myxoplasts of comparatively large size. Within the schizont, there are no doubt many stages of sporogenesis as yet concealed because of inability to stain them. While it is known that the sporocysts arise from some sort of sporoblast or gametoblast cells, the method of origin of said cells is absolutely unknown, whether it be by a continuous process of internal budding or a simultaneous schizogony.

The earliest condition of the spore which I am able to identify with any degree of certainty is shown in Figure 16. It is a sporocyst composed of cytoplasm that is identical in properties to that of the multiplicative trophoplasts. The nuclei do not stain. What appears to be a large nucleus at the center is really the early condition of a myxospore. It is irregular in shape and at first discloses no nuclei. Sometimes these spore fundaments are encountered free from the sporocystplasm as shown above in Figure 16. The rectangular form of the myxospore is assumed later (Fig. 1).

In the homogeneous stainable portion of the sporoblast which later becomes the myxospore, there at first appears a large, more densely staining portion, which, by its behavior, proves to be the nucleus (Fig. 1, upper sporoblast). The nucleus becomes more concentrated (the two lower left-hand cases), and by some method of fission not yet clear, it is divided into as many as nine fragments (Fig. 11). In some cases the sporoblasts contain all of the nine nuclei before there is any evidence of polar capsules. In others the polar capsules appear in the presence of only four or five nuclei (Fig. 1, the right-hand sporoblast). Myxospores with one nucleus opposite the large end of each polar capsule are very common. The others may occupy almost any position in the free periphery of the sporoplasm. In Figure 11 there are two nuclei opposite each polar capsule. Five of these are the generative and wall nuclei which have not yet left their central position. With some stains the polar capsules are conspicuous and the nuclei almost invisible (Fig. 10).

The mature myxospore (Fig. 10) is more or less square with bulging sides. The polar capsules are pointed at the inner end and have a very short, tapered neck. The myxospores of *C. clupeidae* measure on an average $7\mu$ across from one side to the other. The polar capsules are a trifle over $1\mu$ in diameter and about $2\mu$ in length. I have examined many hundreds of these myxospores and have never discovered any indication of valves in the spore wall.

When compared with the *C. funduli* (Hahn, 1913: 205) it is readily distinguished. The latter is circular when viewed from the polar end and tapers with an incurved outline from the antipolar region to the polar end. The polar capsules are therefore drawn out and curved to correspond to the exterior. In *C. clupeidae* the profile from the polar end is square with rounded corners. It is not drawn out at all on the polar end, but is shaped like a very low conical pyramid with a round base. The profile is, therefore, that of a hemisphere on the polar side and of an oblate spheroid on the antipolar side.

As far as I have been able to discover, there is no Chloromyxum answering to the above description which has previously been described, unless it is *C. quadratum* from the muscles of *Callionymus lyra* (Thélohan, 1895). This is a very similar parasite, though by no means identical. It forms small pseudocysts and masses of myxospores in the muscles. The myxospores occur in small groups or bundles of ten to twelve which are massed together into secondary groups of three to thirty or more. The primary groups of myxospores are probably derived from a single propagative myxoplast. The propagative myxoplasts, having been gathered into masses are thus responsible for the primary and secondary groups above mentioned. No such limitation of myxospore groups has been observed in *C. clupeidae*. Otherwise the conditions of spore formation are apparently the same.

The myxospores of *C. quadratum* are much longer than those of *C. clupeidae*, being $7\mu$ in length along the polar axis and $5\mu$ in diameter, while the myxospore of *C. clupeidae* is $7\mu$ in diameter and not over $5\mu$ in length. *C. quadratum* is deeply incurved on the sides and has a long polar apex with very small polar capsules.

The myxospore of *C. mucronatum* (Gurley, 1893) differs in shape from *C. clupeidae* in a very distinctive way. The profile, as seen from the polar end, is similar to that of the latter, but is circular in outline. The profile from the view at right angles to the polar axis is relatively shorter in *C. clupeidae* than in *C. mucronatum*, otherwise they are very similar. The polar capsules of the latter are relatively a little smaller and shorter. The difference between published figures of the two species may be due to a difference in relative maturity, but *C. mucronatum* is a free-living form from the gall and is polysporous.

The most obvious pathological change which is induced by the *C. clupeidae* is the degeneration of the muscle fibers. As in the invasion of fundulus muscle by *M. musculi,* the early trophic stages cause the myoplasm to hypertrophy. But I have never encountered tissues in the herring that had suffered in a way comparable to those of Fundulus. Parts of the musculature and connective tissue of the intestines of the former are completely disintegrated, while the parasites occur in herring flesh by hundreds of thousands; no gross hypertrophy is ever to be observed. In the muscle of the head the fibers are sometimes riddled with holes containing the parasites. Atrophied muscle fibers are also to be encountered in the body muscle. As such fibers occur in tissues having only multiplicative stages, it is quite certain that the greatest injuries to the body muscle are not caused by the propagative stages. This conclusion is confirmed by the location and habits of the propagative stages themselves.

Because of the pathological condition one finds in the muscle fibers of infected herring, one would expect this disease to be very destructive to the fish. But when caught the fish are in apparent good health. The enormous masses of pseudocysts in the flesh do not inconvenience the locomotion of the herring so far as one can observe by watching schools of young herring as they dart about escaping from their enemies above and below. However, weak and unfit fish would undoubtedly be overtaken by such swift enemies as squid, mackerel, bonito, etc., with which they are constantly beset in the open sea. Those which were severely injured by the multiplicative stages have no doubt already been eliminated from the schools one observes in open water, the survivors having myxospores only. One can only speculate upon the possible mortality of a disease which, having passed through its most virulent stage, leaves considerably over 50 per cent of the survivors infected. Additional observations along this line will appear in a later paper.

## References Cited


Adami, J. G. 1910. The Principles of Pathology, vol. 1. New York.

Cohn, L. 1896. Uber die Myxosporidien von *Esox lucius* und *Perca fluviatilis.* Zool. Jahrb., Anat., 9: 227-272; 2 pls.

Doflein, F. 1898. Studien zur Naturgeschichte der Protozoen, III. Ueber Myxosporidien. Zool. Jahrb., Anat., 11: 281-350; pls. 18-20.

1911. Lehrbuch der Protozoenkunde. III. Aufl. Jena.

Erdman, Rh. 1910. Zur Lebensgeschichte des *Chloromyxum leydigi,* einer mictosporeen Myxosporidie, Theil I. Arch. f. Protistenk., 24: 149-162; 3 pl.

Gurley, R. R. 1893. On Classification of the Myxosporidia. Bull. U. S. Fish Com., 2: 407-420.

Keyesselitz, G. 1908. Die Entwickelung von *Myxobolus pfeifferi* Thélohan. Arch. f. Protistenk., 11: 252-275; 276-308.

Laveran, A., et Mesnil, F. 1902. Sur la multiplication endogène des myxosporidies. C. R. soc. biol., Paris, 54: 469-472.

Leger, Louis. 1906. Sur une nouvelle Myxosporidian de la Tauche commune, et de la Truite indigène, *Chloromyxum cristatum* n. sp. C. R. acad. sci., Paris, 142: 655; 1097-1098.

Linton, E. 1891. *Myxobolus lintoni.* Bull. U. S. Fish Com., 11: 414.

Mercier, L. 1908. Sur le développment et le structure des spores de *Thelohania giardi* Henneguy. C. R. acad. sci., Paris 146: 34-38.

Minchin, E. A. 1903. Protozoa. Vol. 1 of A Treatise on Zoology, E. R. Lankester, Editor. London.

Nemeczek, A. 1911. Beiträge zur Kenntnis der Myxo- und Microsporidien der Fische. Arch. f. Protistenk., 22: 143-169; 2 pls.

Pfeiffer, L. 1891. Die Protozoen als Krankheitserreger, sowie der Zellen und Zellenkernparasitismus derselben bei nicht-bakteriellen Infektionskrankheiten des Menschen. 216 pp. 91 figs. Jena.

1893. Die Parasitismus der Epithelialcarcinom sowie der Sarke-Mikrosporidien und Myxosporidien in Muskelgewebe. Centralbl. Bakt. Parasit., 14: 118-130; 1 pl.

Plehn, Marianne. 1905. Ueber die Drehkrankheit der Salmoniden (*Lentospora cerebralis*). Arch. f. Protistenk., 5: 145-166; 1 Taf.

Schröder, O. 1907. Beiträge zur Entwickelungsgeschichte des Myxosporidien, *Sphaeromyxa sabrazesi* (Lav. et Mesnil). Arch. f. Protistenk., 9: 359.

1910. Ueber die Anlage der Sporocyste bei *Sphaeromyxa sabrazesi* (Lav. et Mesnil). Arch. f. Protistenk., 19: 1-5.

Thélohan, P. 1891. Sur deux sporozoaires nouveaux parasites des muscles des Poissons. C. R. acad. sci., Paris, 112: 168-171.

1893. Alterations due tissue musculaire dues a la présence de Myxosporidies de microbes chez le barbeau. C. R. soc. biol., Paris, 5: 267-270.

1895. Recherches sur les Myxosporidies. Bull. sci France et Belg., 26: 110-394; 3 pls.