ICHTHYOPHONUS HOFERI, PLEHN and MULSOW, A FLOUNDER PARASITE NEW TO NORTH AMERICAN WATERS:—Studies from the Department of Zoology, Dalhousie University, and the Atlantic Biological Station, St. Andrews, N. B., By MARJORIE F. ELLIS, B.A., Dalhousie University, Halifax, N. S.

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ABSTRACT.

The form *Ichthyophonus hoferi*, Plehn and Mulsow, is reported as occurring in a marine form, the winter flounder, *Pseudopleuronectes americanus*, (Walbaum). This is the first report of this organism in other than European waters and the first instance of its occurrence in salt water.

Nodular masses ranging in size from 2 mm. to 5 cm. in diameter were found in the coelomic cavity and viscera.

Microscopic examination revealed an encysted organism which, when placed on the slide, sent out protoplasmic processes resembling the plasmodia of the Myxomycetes.

In stained preparations the striking reactions set up in the host tissue are apparent.

Certain infection experiments on other flounders are described.

The obscure micro-organism, *Ichthyophonus hoferi*, Plehn and Mulsow, was first described by Hofer as the cause of "*Taumelkrankheit*", "dizziness disease," in trout and salmon. Since then several cases have been reported in fishes in fresh waters of Europe.

The disease has been found to attack practically all the body organs of the host, and in some cases the brain has been the major seat of infection.

As far as can be ascertained the organism has never been reported in other than European waters and there only in fresh water.

The purpose of the present paper is to report the occurrence of this disease in an American food fish, the winter flounder, *Pseudopleuronectes americanus* (Walbaum). The infected hosts were taken in commercial fishing operations in Passamquoddy Bay and were discovered by the writer in the course of pathological examinations of the landed catch at St. Andrews, New Brunswick. The fish had been dead several hours and were partially eviscerated before they came under observation.

Large creamy-white masses approximately 5 cm. in diameter adhered to the body wall. The parts of the viscera remaining in the animal were invaded with numerous smaller nodular masses of the same appearance and about 2 mm. in diameter.

Fresh smears of these nodular masses were microscopically studied. They were found to be composed of colonies of organisms of a most peculiar appearance. When first placed on the slide these organisms were inclosed in a transparent colorless cyst, about 50μ. in diameter. Almost immediately long amoeboid-like pseudopodia, closely resembling the plasmodium of a myxomycete, appeared leaving the empty cyst-case behind. These protoplasmic protrusions, some of which reached a length of 100μ appeared to emerge through a single opening in the cyst wall from 4 to 5μ. in diameter. These processes were filled with granular protoplasm, the granules of which were 2μ in diameter. Although the material was kept on the slide for some hours no further change took place. Plehn and Mulsow in 1911 described the protoplasm as migrating to the ends of the pseudopodia, and there forming spherical masses which divided into smaller cysts with a firm shell, and resembling the common cyst in color. They considered this to be a resting stage and called them the secondary cyst fruiting bodies. These were not observed in the present case. A block of the tissue was kept in running sea water and examined two days later. Only empty cyst cases remained.

Material for stained preparations was fixed in Bouin's picroformal solution and stained with Heidenhain's Iron Alum and Orange G.

In stained sections the protoplasm was found to contain numerous deeply staining granules 1.5μ in diameter and surrounded by a clear membrane about 1μ in diameter. Plehn and Mulsow consider these structures to be nuclei, and the dark granule in the center the nucleolus. Other granules embedded directly in the protoplasm have been described by Plehn and Mulsow in 1911 and Laveran and Pettit in 1910.

In the present material they were observed in a single case in which the other granules were absent. It is impossible at present to state the significance of this fact.

The host tissue shows very striking reactions to the parasite. In the case of sections made from diseased kidney the cells of the kidney tubules have become greatly modified and together with the hypertrophied adjoining connective tissue make up a wall surrounding the encysted parasite. Examination of the tissue had disclosed only one cyst not surrounded by a wall. This would strongly indicate that the avenue of infection in the kidney is through the uriniferous tubules. However, the freed plasmodia of the parasites occur as naked structures directly in the host tissues, showing clearly that, whatever the avenue of infection may be, the released organisms, far from being restricted to the lumina of the uriniferous tubules occur, in the majority of cases not in the lumina but scattered freely in a connective tissue matrix.

In sections from the large mass which adhered to the body wall the host tissue has formed connective tissue rings around one or more of the encysted parasites and, as before, the freed plasmodia appear directly embedded in the host tissue.

It is interesting to compare these reactions of the host tissue with the reactions set up by the nematode worm, *Trichinella spiralis* (Owen). In this case, after the connective tissue walls have been formed a calcareous deposit is laid down by the host. This process results in the formation of a hard calcareous nodule enclosing the parasite.

Some attempts were made to infect other flounders. Two young flounders were infected, one by scraping the walls of the branchial cavity and rubbing in some of the diseased tissue; the other by inserting some diseased tissue into the coelomic cavity through an incision in the body wall. The flounder infected in the branchial cavity gave negative results. The animal infected in the coelom died six days later. Careful examination was made for traces of infection. On the walls surrounding the incision some empty cyst cases and round protoplasmic spherules, much smaller than the original cyst,

were observed. Some of these had a large central granule and resembled the fruiting bodies described by Plehn. This material was used to make further infection on a fresh flounder with negative results. Owing to the fact that the material was kept on the slide for some hours the organisms may have died before the infection was made.

Hofer in 1893 first described this form without naming it or attempting to give it any place in the system of classification.

In 1910 the disease was reported by Laveran and Pettit. It appeared in the establishment of a fish culturist in France and was causing a high mortality among the fishes. These writers described the organism as reproducing by means of small secondary cysts which were derived from a division of the primary protoplasmic body and were set free by a splitting of the cyst wall. These secondary cysts were observed in the excretions of diseased trout. They consider this to be the ordinary means of propagation of the parasite. These writers did not name the organism. They stated that they believed it had affinities with the protozoa, especially the Haplosporidia, but that certain details of structure seemed to be of a plant nature.

In December 1910 two dead rainbow trout came under the observation of Plehn and Mulsow and further investigations were carried on. These writers came to the conclusion that the organism did not belong to the sporozoa nor to the animal kingdom at all; but that it was one of the lower fungi. They named it *Ichthyophonus hoferi*, Plehn and Mulsow, and classed it in the Phycomycetes in the neighborhood of the Chytridinae.

They found it possible to grow the organism on a gelatine medium but were unable to trace fully its life history. They describe the formation of fruiting bodies which are in all probability identical with the secondary cysts described by Laveran and Pettit.

In response to correspondence Henry O'Malley, Commissioner of the United States Fisheries, (which organization carries on extensive researches in fish pathology throughout

North America) states that his investigators have found no instances of the occurrence of this disease.

The conclusion reached by the writer is that the form discussed in the present paper is identical with the form described by Hofer, Plehn and Mulsow, and Laveran and Pettit as the etiological agent in "dizziness disease." The occurrence of the form reported here is in a marine fish. The diseased animals exhibited an extensive and profound character of infection and bodily change. Microscopic study revealed an extensive permeation of the host tissues by the parasites outside the cysts themselves. Certain infection experiments were carried out on fresh fish which were not attempted by previous investigators on the organism. The result of these was the formation of protoplasmic spherules which resembled the fruiting bodies described by Plehn and Mulsow. The form is particularly interesting because of the obscure affinities of the parasite itself and the profound disturbance produced in the host.

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LITERATURE.

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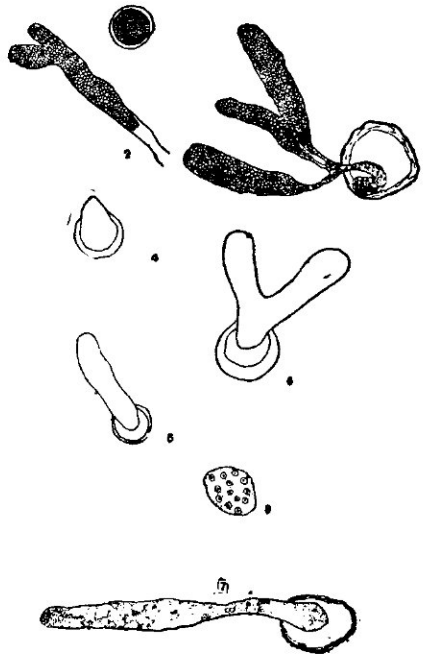
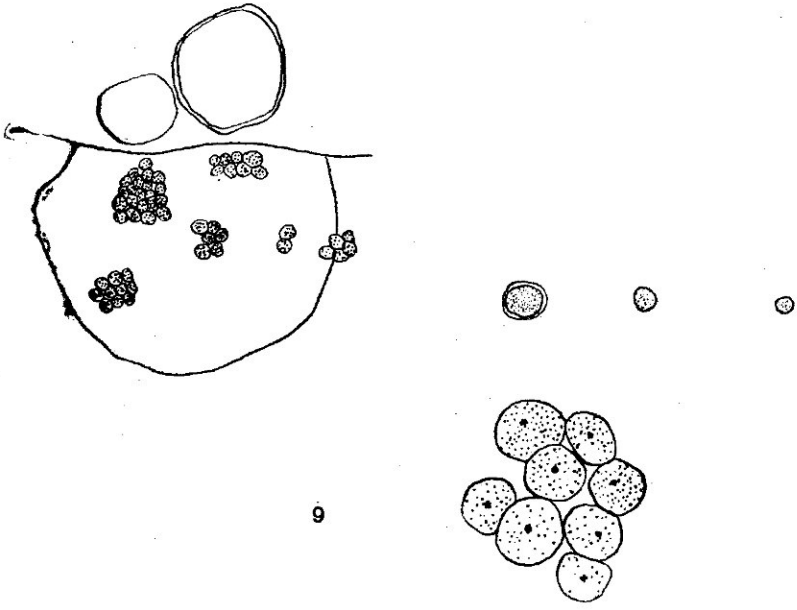
Fig. 1 Encysted animal. Fresh preparation. x 475.
Fig. 2 Pseudopodium with the protoplasm beginning to migrate to the end. Fresh preparation. x 475.
Fig. 3 Complete animal with extended psuedopodia. Fresh preparation. x 475.
Figs. 4, 5, & 6. Outlines of organisms showing the pseudopodia in different stages of growth.
Fig. 7 Organism drawn from stained preparation showing granules embedded directly in the protoplasm. x 475.
Fig. 8 Section through a pseudopodium showing the granules surrounded by a clear membrane. x 475.
Fig. 9. Cyst case enclosing protoplasmic spherules and escaped spherules showing central granules. Fresh preparation. x 475.
Figs. 10 & 11 Photomicrographs of individuals from smears stained with Heidenhain's Iron-Alum. x 100.
Fig. 12 Photomicrograph of section of nodule found in the kidney, showing modified uriniferous tubules. x 220.





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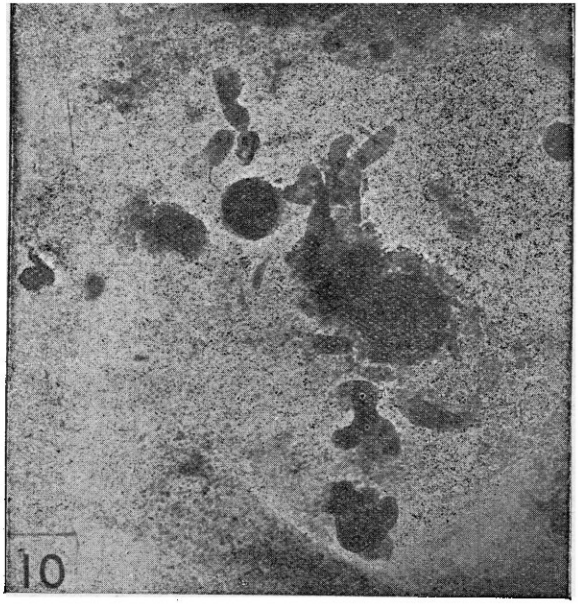

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