

7.—ON CERTAIN WART-LIKE EXCRESCENCES, OCCURRING ON THE SHORT MINNOW, *CYPRINODON VARIEGATUS*, DUE TO PSOROSPERMS.

BY EDWIN LINTON.

[Plate XXXV.]

On August 20, 1889, while at the U. S. Fish Commission laboratory, Wood's Holl, Mass., I obtained a specimen of the short minnow (*Cyprinodon variegatus*), having upon its body several fungoid, wart-like excrescences, which, upon examination, proved to be occasioned by the presence of psorosperms. These parasitic protozoans are regarded as related to the Gregarinidæ on account of their resemblance to the pseudonavicellæ of that family, but their exact nature is not yet well understood. They have been recorded by naturalists from a number of European fishes, among which are several species of perch (*Cyprinus rutilus*, *C. erythrophthalmus*, and *C. leuciscus*), other perch-like fishes (*Lucioperca*), the white-fish (*Coregonus fera*), and some of the fresh-water minnows (*Cyprinodon*). They have also, doubtless, been observed hitherto on kindred American fishes, but so far I have been unable to find any literature describing their occurrence in America.

The specimen of *Cyprinodon variegatus* which I had the opportunity of examining at Wood's Holl, had three of these fungoid masses on the left side of the body and one on the right. The latter is shown in the sketch, Fig. 1.

One of those on the left is also shown in part in the same sketch, where it projects above the line of the back. This mass was about 6 millimeters in diameter; it was situated a short distance behind the eye and above the operculum; another behind the gill-slit and extending diagonally backward and downward was 10 millimeters long and 4 millimeters broad; another behind the latter and near it was 4 millimeters long and 2½ millimeters broad. These masses are irregular in outline and elevation; they protrude as much as 3 millimeters above the general surface of the body; they do not appear to consist of closed cysts.

The abnormal growth is apparently confined to the superficial muscular and subcutaneous tissue. Sections carried through one of the masses reveal clusters of psorosperms lying in the interstices of the connective tissue, and patches of dark pigment, with a few capillary vessels. The skin of the host overlying these tumors is more or less cracked and broken, and the scales scattering.

When a piece of one of these abnormal growths was placed on a slide in water and gentle pressure applied myriads of oval bodies, such as are shown in Figs. 2-4, were set free. These spore-like bodies when liberated lay motionless at the bottom of the water. During all my observations on them no movements were discovered.

They are of very uniform size and shape. In their usual position they appear to be flattened or disc-shaped; when turned on one edge they are seen to be lenticular. The outline is always elliptical, the longer diameter being about 0.0139 millimeter, the shorter, 0.0110 millimeter. Their thickness was not so certainly made out, but in one individual, which appeared to be standing on one edge, it was about 0.008 millimeter. Fig. 5 is a sketch of an ideal section along the shorter diameter.

Near one end of each there are two transparent, pyriform, refractile bodies, their smaller ends converging and directed toward the nearest border. These are the twinned vesicles of Balbiani and the polar capsules of Bütschli. In many cases smaller supplemental refractile bodies were seen at the base of the pyriform bodies. This phenomenon is shown in Fig. 2. These have the position but not the appearance of Bütschli's dark granules. The remainder of the interior is filled with a clear viscid fluid, which in some cases has a few small refractile particles in it. The walls are rather thick and quite firm, with a sharp, clear, entire outline. In optical section there is often the appearance of a third refractile body behind the pyriform vesicles. This is due, in the fresh specimens, to the thick transparent walls and the viscid refractile fluid interior. When treated with certain reagents this viscid fluid seems to separate from the wall so as to appear as a nuclear body. This appearance is shown in Fig. 7, which represents one of the psorosperms after having been treated with acetic acid. The two filiform appendages, said to be characteristic of these animals, were not seen satisfactorily in fresh specimens. Some specimens were placed in one-half per cent. osmic acid for a few minutes and afterward examined with a high magnifying power, but no appendages were distinguishable. It is likely that a longer continuance in osmic acid would be followed by better results, as the material in the walls is but slowly attacked by even concentrated sulphuric acid.

Other examples after being fixed to a slide by means of alcohol were stained with methyl green, and subsequently with eosin. The pyriform vesicles were not stained deeply, while the walls were deeply stained and differentiated from the plastic, homogeneous material which fills the interior.

In some of the individuals that had been subjected to the action of osmic acid and were viewed under especially favorable conditions a small pore was discerned at the apex of each of the pyriform vesicles. These are evidently the orifices from which the filiform appendages issue. The osmic acid preparations also enabled me to perceive for the first time a feature that was afterwards seen in specimens treated with sulphuric acid and which appears to be constant, viz, a low rounded ridge which extends along the edge of the animal from tip to tip, Fig. 6. This feature is noticed and figured by Bütschli in his account of myxosporidæ from the gills of certain fresh-water cyprinoids.

Specimens were kept in sea water for about ten days and observed from time to time, but no noteworthy changes were observed to take place. Although the connective tissue of the mass underwent maceration, the psorosperms showed but little indication of the effect of maceration. At the end of the eighth day a few were noticed in which the walls seemed to have given way, in which case the pyriform bodies were liberated. The latter were still intact.

Upon treating a small piece of the abnormal tissue with sulphuric acid brisk effervescence ensued. As the psorosperms remained with but little change under this severe treatment, the effervescence was plainly from some other source. Another piece

of the tissue was subjected to the action of strong potassic hydrate. This dissolved out the connective tissue and left a residue which consisted of the psorosperms, still unaffected by the reagent, and small calcareous particles of extremely irregular shape. Some of the latter are shown in Fig. 14, and three of the calcareous particles more highly magnified, along with some of the psorosperms, in Fig. 15. The walls of the psorosperms withstand the action of concentrated sulphuric acid and of a saturated solution of potassic hydrate for a long time. When treated with iodine they stain yellowish brown. When placed in glycerine the walls of the psorosperms collapse.

The action of sulphuric acid was most successful in bringing out certain details of structure which had otherwise escaped detection. One of the first effects of concentrated sulphuric acid which was observed, and which resulted almost immediately after application of the acid, was to cause the protrusion of two filamentary appendages from the anterior end, *i. e.*, the end at which the polar vesicles lie. In some cases these threads are nearly straight, in others they are undulate, and a few were thrown into a more or less close spiral.

The latter gives some hint of the retracted condition of these threads, and confirms Balbiani's view that the twinned vesicles which are found in all the psorospermæ serve normally as sheaths for the threads which, according to that author, always issue from the end of the animal near which the converging ends of the vesicles lie.

Zschokke (9) figures a psorosperm from *Coregonus fera*, which bears a very close resemblance to these from the short minnow. The two filiform appendages, however, appear to be exceptional, in that they issue from the end opposite the polar vesicles. The psorosperms described by Zschokke occur in cysts from the size of a pea to that of a walnut, fixed firmly among the muscles.

These cysts are white, oval, inclosed in a thick envelope without apparent structure and containing a whitish liquid of milky appearance. Examined under the microscope thousands of psorospermæ may be seen disposed among the granular protoplasm. The psorospermæ described by Bütschli from the fresh-water cyprinoids were also inclosed in a cyst, in which were calcareous particles.

In some cases the psorosperms which have been treated with concentrated sulphuric acid have ejected the polar vesicles bodily with the filaments extruded (Figs. 12, 13). The threads are of the same diameter throughout and are not extremely slender. The distal ends are truncate. The organs, therefore, do not resemble flagella. The appearance of one of these vesicles with its thread is strikingly suggestive of the nematocyst of a cœlenterate. The threads stain yellowish-brown with iodine.

One psorosperm was observed which appeared to have three threads, two normal and a third at the opposite end. This appearance was at first thought to be due to one of the free vesicles getting behind the body of a psorosperm. Such a posterior thread has been noticed by other observers and is figured by Bütschli (8).

When treated with acetic acid a nuclear vesicle was clearly defined in the apparently homogeneous tissue behind the polar vesicles. A similar appearance was noticed in a few that had lain in sea water for a period of eight days. None of the reagents employed brought out the diagonal striæ represented by Bütschli in his figures of the polar vesicles of the myxospores from the fresh-water cyprinoids, and which are due to the thread coiled up in a spiral within the vesicle. In other respects Bütschli's figures of cyprinoid myxospores bear a very close resemblance to these psorosperms from *Cyprinodon variegatus*.

In some seasons the short minnows are quite commonly affected with this parasite, as I have observed in previous years, and as I am informed is the case by Mr. Vinal N. Edwards, who has had a long and extended experience in observing our coast fishes. I am indebted to Dr. E. L. Mark for assistance in obtaining some of the literature of this interesting subject, especially Bütschli's excellent paper.

The following is a partial bibliography of the *Psorospermia*:

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EXPLANATION OF PLATE XXXV.

1. *Cyprinodon variegatus*, with excrescences caused by Psorosperms, one on right side and another on left side showing above outline of back. x 1½.
- 2-3. Psorosperms with supplemental refractile bodies behind the polar vesicles. In Fig. 3 there are a few small refractile globular masses near the posterior end.
4. An individual treated with osmic acid, showing pores at the apices of the polar vesicles.
- 5-6. Specimens lying on the edge, showing the rounded elevated ridge which follows the edge.
7. Specimen treated with acetic acid. A nuclear body is defined back of the polar vesicles.
8. Diagram of transverse section, showing lenticular shape of psorosperms.
- 9-11. Specimens treated with concentrated sulphuric acid; 9, with a few refractile bodies and one thread exerted; 10, an example with both threads exerted and a number of small refractile globules; 11, a specimen in which the plastic fluid interior is aggregated into a single refractile body; a thread also appears at the end opposite the polar vesicles.
- 12-13. Polar vesicles with their threads, liberated from the body of the psorosperms after treatment with concentrated sulphuric acid. Nos. 2 to 13 all highly magnified.
14. Calcareous bodies found in the abnormal tissue associated with the psorosperms. x 200.
15. Three of the same, with a few psorosperms. Sketch made from material that had been subjected to the action of potassic hydrate. x 400.
16. Psorosperms in place. (a) nests of psorosperms; (b) section of a blood capillary; (c) connective tissue. Sketch made from a section of decalcified abnormal tissue.

