Citation:

N.H. Swellengrebel, Pleistophora gigantea Thelohan, a parasite of Crenilabius melops, in:
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formation of carbonic and organic acids, but without any production of alcohol neither in the air nor in a hydrogen atmosphere.

Destruction of the cellular structure and treatment with alcohol or acetone do not therefore inactivate the respiratory enzymes in the present case, their power of decomposing sugar remains very marked.

In the same way a crude enzyme is obtained from the leaves of Sauromatum, which is similar, but has a weaker action.

In the ether extract of the acid liquid citric acid was demonstrated, which acid very probably must be formed by the respiratory enzymes at the expense of the glucose.

Amersfoort, September 1911.

Zoology. — "*Pleistophora gigantea Thélohan a parasite of Crenilabrus melops.*" By N. H. SWELLENGREBEL. (Communicated by Prof. MAX WEBER.)

(Communicated in the meeting of September 30, 1911).

Among the neosporidia the microsporidia distinguish themselves by their spores, which are smaller than those of the allied myxosporidia and do not possess such distinct polar capsule and polar filament as the spores of the latter group.

According to MINCHIN (1903) the microsporidia are divided into:

1. *Polysporogenea*; the trophozoite (i. e. the vegetative generation) forms many pansporoblasts, each of which contains many spores.

2. *Oligosporogenea*; the trophozoite transforms itself entirely or partly into one single pansporoblast. Each pansporoblast contains 4, 8 or many spores.

The parasite that I wish specially to describe here has the following life-history. Trophozoites with one or more nuclei are found in the connective tissue of the skin and in the mesenterium of *Crenilabrus melops*. After encystment the trophozoites form by successive division an unequal number of sporoblasts, each containing two nuclei. These sporoblasts become spores by the formation of a thick membrane. The spores have one or two nuclei, whilst there is nothing that points to the existence of a polar capsule with polar filament.

THÉLOHAN (1895) described a microsporidium: *Glugea gigantea*, found in the abdomen of *Crenilabrus melops*. He has not been able to investigate the development of this parasite, but only states that by none of the expedients known the polar filament could be indicated in the spores. It is difficult to decide whether the parasite described here is the same as that of THÉLOHAN, but as in neither of them a polar capsule is found, and they have the same host, in whom, according to AUERBACH, (1910) no other microsporidium lives, it is very likely that these two parasites are identical. The microsporidium that I intend to describe here is however no Glugea, because the trophozoite is directly transformed into a pansporoblast, and produces an unequal number of spores, consequently it belongs to the genus *Pleistophora* and I propose to call it *Pleistophora gigantea*. If *P. gigantea* is identical with *Glugea gigantea* then the latter name must be dropped, if not, the two names continue to exist beside each other. It will however be hardly possible to decide if this identity exists or not. For this THÉLOHAN's description is too incomplete.

2. *Material and methods.*

The different stages of the development of *P. gigantea* were found in a big tumour, situated at the ventral side of the head and the thorax of *Crenilabrus melops*. The tumour extended from the posterior margin of the gills along the pectoral fins to the ventral fin. The tumour was caused by hypertrophy of the connective tissue; it did not show any inclination to infiltration, neither the gills and the organs of mouth and thorax, nor the parts of the skeleton were effected by it, it was a pure outgrowth of the skin. The tumour was so heavy as to deprive the fish of its hydrostatic equilibrium.

Portions of the periphery and the centre of the tumour were fixed

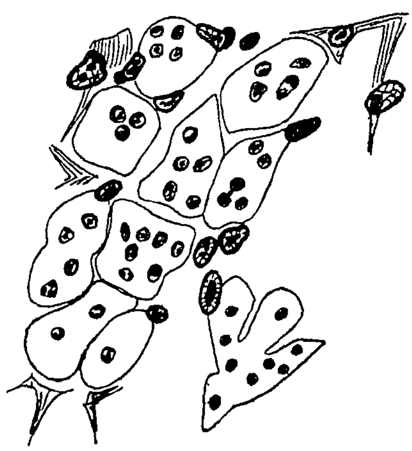

Fig. 1.

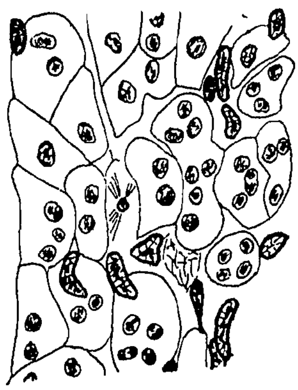

Fig. 2.

in corrosive alcohol of SCHAUDINN; washed in water, alcohol and iodine, hardened in absolute alcohol, mounted in paraffine, and cut into sections of 3—5 μ, which where coloured with haematoxyline of EHRLICH, DELAFIELD or HEIDENHAIN or with GIEMSA's solution. In order to discover polar filaments living spores were examined. With none of the reagents used (caustic soda and potash, sulphuric-acid, hydrochloric acid, nitric-acid, methyl-alcohol, ether, iodine, distilled water), was it possible to demonstrate a polar filament.

3. *The life-history of Pleistophora gigantea.*

The youngest stages of the development of the parasite that I could discover, are uni- or multi-nucleate cells 9.8—11.2 μ long and 4.6—6.3 μ broad, situated between the connective tissue of the tumour (fig. 1). Probably these cells multiply by schizogony, it seems at least that stages as those of fig. 1 point to this fact; they are each surrounded by a thin membrane. These cells represent evidently the vegetative stages of the development, they are the trophozoites. In the beginning these cells are quite diffusedly spread between the fibrils of the cellular-tissue. Afterwards they unite into smaller or larger groups (fig. 2) and surround themselves at last with a membrane, which, though very thin, is after all thicker than that of the separate individuals.

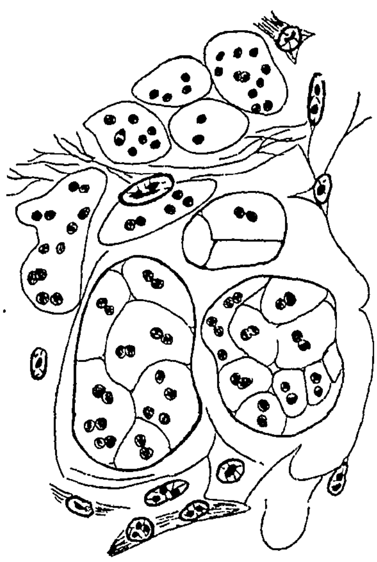

Fig. 3.

In the individuals that have united into groups and are surrounded by a cyst-wall (the pansporoblasts) the nuclei range themselves into groups of two nuclei. This is occasioned by the division of the nuclei whilst the daughter nuclei continue to lie against each other. The encysted individuals are now divided into a number of smaller cells (fig. 4—5) containing each two nuclei lying close together. The formation of these cells does not take place by schizogony of the originally encysted individuals, they separate, by the formation of buds, repeatedly part of their protoplasma from

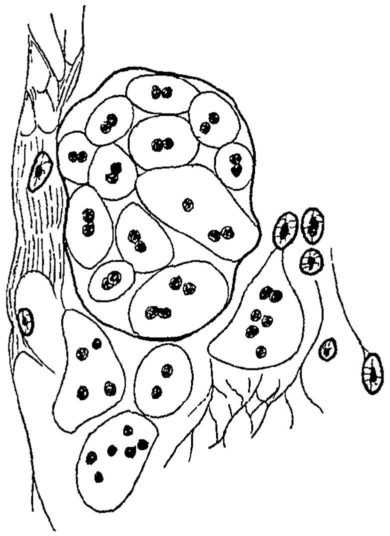

Fig. 4.

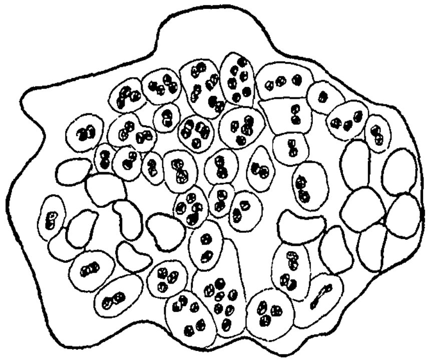

Fig. 5.

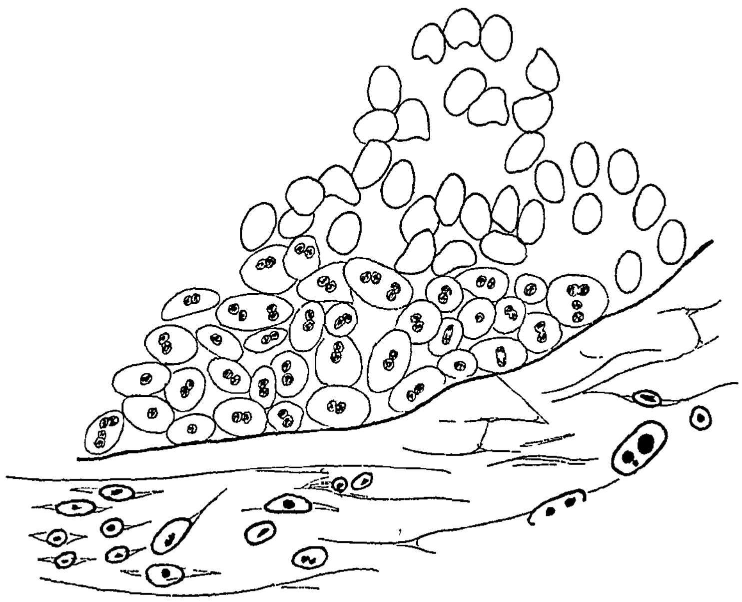

Fig. 6.

the mother cell, and with it at the same time one of the groups of two nuclei.

The cysts formed in this way containing the bi-nucleate sporoblasts are of very different sizes. The smallest have a diameter of 11—49 μ, the largest which usually contain only ripe spores and show only at the periphery a layer of sporoblasts (fig. 6 represents part of the periphery of such a cyst), can become 0.49 by 0.36 mm. to 1.44 by 3.82 mm. and even reach still larger dimensions.

By the formation of a thick cyst-wall the sporoblasts, i.e. the round bi-nucleate cells change into the real spores, which have also two nuclei. Besides the bi-nucleate spores some are found with a single large nucleus, so that it is probable that the latter is formed by the fusion of the two sporoblast-nuclei. If this were the case, then we should have to regard this phenomenon

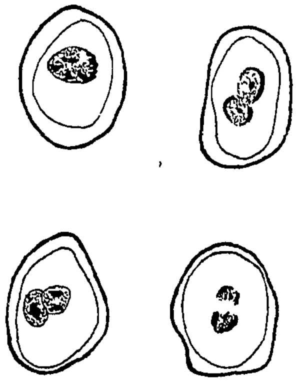

Fig. 7.

as an autogamy. The spores are 5—6 μ long and 4—5 μ broad. Besides the single or double nucleus no other organs as polar capsules and the like are to be observed in the plasma; in and beside the nucleus however sometimes chromatoïd granules are found, which show in their microchemical reactions distinct affinity to volutine.

As I remarked before the spores formed in this way are united into smaller or larger cysts. Besides these however spores are found that are diffusedly spread between the connective tissue. It is likely that these spores originate in trophozoites that were not united into groups and surrounded by a cyst-wall. As I have however never been able to observe the formation of these diffusedly spread spores in particulars, I cannot decide with certainty on this point.

The reaction of the host's tissue against the parasitic invasion is

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very different. Small isolated individuals containing few nuclei are sometimes found surrounded by thick layers of multinucleate connective tissue. Round larger aggregations of multinucleate trophozoites only an insignificant growth of connective tissue is to be observed, and round the larger cysts the connective tissue is paucinucleate. On the spots where spores are diffusedly spread between the connective tissue frequently multinucleate giant-cells are found. They contain 2--15 nuclei and obtain a dimension of 28—43 μ.

Sometimes a large cyst is found surrounded by many smaller cysts; this phenomenon is most likely to be regarded as a secondary infection, of which the large cyst is the primary seat.

It is a pleasant duty for me to pay my sincere thanks to Dr. Kerbert, Director of the "Kon. Zoöl. Gen. "Natura Artis Magistra", for his kindness of placing the material for this investigation at my disposal.

EXPLANATION OF THE FIGURES.

Fig. 1. Trophozoites in diffused infiltration (nuclei of connective tissue between the parasites).

Fig. 2. The trophozoites unite into small aggregations.

Fig. 3. Encystment of small aggregations of trophozoites.

Fig. 4. Pansporoblasts divide into sporoblasts.

Fig. 5. Small cysts with sporoblasts, pansporoblasts and spores.

Fig. 6. Periphery of a large cyst with sporoblasts and spores (in figs. 5 and 6 only the circumferences of the spores are sketched).

Fig. 7. Ripe spores.

The figures have been drawn with Zeiss's camera: fig. 1—6 with oil immersion 2 m.m. ocul. 4; fig. 7 with ocul. 18.

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