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Inhalt:

I. Wissenschaftl. Mittheilungen.

1. Calkins, *Lymphosporidium Truttae*, nov. gen. nov. sp. The cause of a recent Epidemic among Brook Trout, *Salvelinus fontinalis*. (With 6 figs.) p. 513.
2. Latzel, Zwei neue Myriopoden aus dem Mittelmeergebiete. (Mit 1 Fig.) p. 520.
3. Suschkin, Weitere systematische Ergebnisse vergleichend-osteologischer Untersuchungen der Tagraubvögel. p. 522.

II. Mittheilungen aus Museen, Instituten etc.

1. Congrès International de Zoologie. p. 528.

III. Personal-Notizen.

(Vacat.)

Litteratur. p. 429—444.

I. Wissenschaftliche Mittheilungen.

1. Lymphosporidium Truttae, nov. gen. nov. sp. The cause of a recent Epidemic among Brook Trout, Salvelinus fontinalis.

By Gary N. Calkins, Columbia University, New York City.

(With 6 figs.)

eingeg. 26. Juli 1900.

In October of the last year (1899) my attention was called to the great mortality among the Brook Trout of a private hatchery on Long Island, New York. Since the middle of May 1899, the fish had been dying by thousands and by fall it appeared that every fish in the hatchery was doomed.

The disease was first noticed in May when a young individual was taken out dead, and the Superintendent of the hatchery observed a small, clean-cut hole in the side. Thinking that the hole was made by some birds beak, and that the yearling had been killed in this way, the fish was thrown away without further thought. When however, others were observed from time to time with similar holes and the death rate became noticeably large, steps were taken to ascertain the nature of the disease and the cause of the trouble.

In October the fish appeared sluggish and to have lost considerable vitality. They were unable to withstand handling of the gentlest kind and hundreds of them died every day. The dead ones as a rule,

floated to the surface of the water and were removed and thrown away. Some of these had no external marks whatever to indicate the disease, but were plump and apparently sound, the cause of death apparently being some internal organic trouble. Others however, had numerous round clear-cut holes or ulcers in the sides or back; in others great patches of the skin and underlying muscle tissue had fallen out leaving large irregular, but shallow pits sometimes two or three square inches in area; in others the flesh had fallen away in spots leaving the back bone visible, and in others still, the clear-cut holes went entirely through the body wall and not unfrequently the internal organs e. g. the testis or liver could be seen, even in the living fish, hanging out of the openings thus made. Again the eyes would be entirely gone, or the lower jaw would be eaten away, and many instances were seen in which the tail and one or more of the fins had disappeared.

In looking over the available literature I could find no record of an epidemic of like nature among Brook Trout. Inquiry however led to the discovery that about twelve years ago a very disastrous epidemic had carried away most of the Brook Trout in a neighboring hatchery. Unfortunately no report was written upon this epidemic and the cause of the trouble was not located. From one or two eye-witnesses I was able to gather that the diseased fish at that time had similar holes or ulcers in the body wall and that various parts were eaten away in a similar manner. I do not doubt that the epidemic of twelve years ago and the one of last summer were due to the same cause.

A careful examination of the surroundings of the fish left no reason to believe that the disease was due to the external conditions. The water of the ponds is supplied from springs having a constant flow of cold and clear water which is free from suspended matter and rarely exceeds a temperature of 60 degrees F. The bottoms are of mud and gravel with comparatively few weeds.

On the other hand the wide distribution of the diseased spots about the body of the fish led to the belief that the cause of the trouble lay in some parasite small enough to be carried about in the blood to all parts of the animal.

The large number of reported cases of Myxosporidiosis in fish of various kinds led me at first, to think that I should find the cause of the epidemic to be some member of the order Myxosporidiida of the Sporozoa. With this in mind the various organs of diseased fish were fixed in different killing agents and carried to the laboratory for examination. The material thus collected consisted of digestive tract, liver, kidney, gall-bladder, testis, swimming-bladder, gills, muscle and

ulcerated spots in the body wall. These were fixed in Flemming's fluid (stronger formula), sublimate acetic, sublimate, and picro-acetic (Boveri). They were sectioned and stained in iron haematoxylin, Flemming's triple stain, and in thionin with a counter stain of eosin.

A superficial examination did not reveal any cysts or aggregates of spores so characteristic of the majority of the Myxosporidiida and in the more careful subsequent examinations although innumerable quantities of spores were found distributed throughout the body, they were not aggregated in cysts, while in no case did I succeed in finding thread-bearing capsules which distinguish the spores of the Myxosporidiida from those of all other Sporozoa. The various methods recommended by Gurley (1893)[1], and Thélohan (1892) for demonstrating the presence of the capsule and its contained thread were tried with no result.

The spores were found in all parts of the fish including all of the organs selected for examination. In the intestine they formed

Fig. 1. Spores and sporozoite-formation. *A* typical spores; *B* Sporozoite-formation; *C* Isolated sporozoites. Camera lucida $\times$ 2000.

large masses together with the intestinal bacteria; they were found in the lymph spaces surrounding the intestine, liver, kidney, and other organs of the viscera; in the muscle bunoles of the body wall; in the mesenteries and connective tissue throughout the body; in the cavities of the gall- and swimming-bladder, and in the lumina of blood vessels. They were, in short, universally distributed about the cavities and spaces of the diseased fish. They were not seen however in the glandular tissues in general, although they were occasionally met with in the kidney. The testis however, seemed to be the main seat of the spores, and was literally crowded with them, especially in fish from four to five inches in length.

The spores (fig. 1*A*) are very small (2 to 3 μ), pyriform in shape and of homogeneous appearance. The substance of which they are composed shows a decided affinity for the chromatin stains, especially

[1] U. S. Fish Commission, Report 1893.

saffranin, methyl green etc. In two places, viz. the intestine and the testis, the homogeneous appearance of the spores is lost and various stages in the formation of exceedingly minute germs which I have interpreted as sporozoites, are found. Owing to the extremely small size of the spores the process of sporozoite-formation is almost impossible to follow and the following results may not be entirely free from errors. The material of the spore segregates into a comparatively thick shell about the periphery of the spore but within the spore membrane. It then breaks or divides by multiple division into eight minute parts all contained within the spore membrane which at this period for the first time, can be distinctly seen (fig. 1 *B*). The sporozoites then leave the spore, by what means I do not know, and, in the intestine are lost sight of among the bacteria; in the testis they collect around the various lobes as minute spherical granules which stain less intensely than the original spores. The empty spore cases are frequently seen with a rupture or a crack at some part through which the sporozoites evidently made their exit (fig. 1 *B*, *C*).

The next stages of the young sporozoites rest in considerable obscurity. Lost in the hordes of intestinal bacteria the naked germs

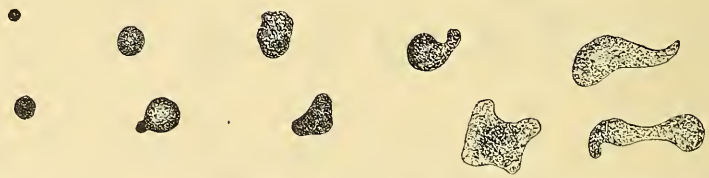

Fig. 2. Development of the sporozoite in the lymph. Camera lucida × 2000.

could not be followed. The mere fact that they are naked however, makes it probable that they do not leave the intestine for an extra-corporeal mode of life but that their further history takes place in the epithelial cells and in the lymph spaces. The only positive evidence that I can bring forth to show an intra-cellular stage is the fact that minute granules, apparently foreign to the epithelial cells are frequently found in the cytoplasm. While I cannot be certain that these granules are young phases of the parasite, no such doubt rests upon the small spherical bodies which were frequently found in considerable abundance in the lymph which surrounds the digestive tract. Various stages were found here from small granules scarcely larger than the youngest sporozoites, to the adult amoeboid organisms 25 to 30 microns in length.

The various stages in the development of the sporozoite into the adult organism can be seen at a glance in the accompanying figure 2,

where all of the stages are drawn with the aid of a camera lucida to the same scale. The small rounded sporozoite grows until about the size of a spore, and at this stage appears very much like a spore although it does not have the same densely homogeneous structure. It

Fig. 3. Young sporozoites entering muscle bundles about the intestine. Camera lucida $\times$ 2000.

soon becomes amoeboid and, when slightly larger than a spore, is variable in shape (fig. 2). At this stage or slightly later, the amoeboid organisms begin to penetrate the muscle bundles surrounding the intestine and other organs. By amoeboid movements they work their

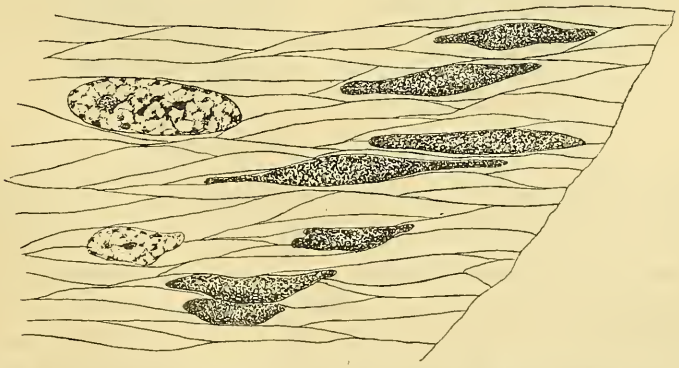

Fig. 4. Adult amoeboid individuals in the muscle bundles. Camera lucida $\times$ 2000.

way between the muscle cells where they lie until mature growth is reached and they are ready for spore-formation (fig. 3).

The adult organism has few characteristic marks of structure.

There is a very slight indication of a division into ectoplasm and endoplasm and the entire cell has a well-defined reticular appearance, which upon closer examination is undoubtedly due to the optical section of the walls of alveoli (fig. 4 and fig. 5). The most significant feature about the adult structure, and indeed throughout the entire life-history of the parasite, is the fact that there is no well-defined nucleus.

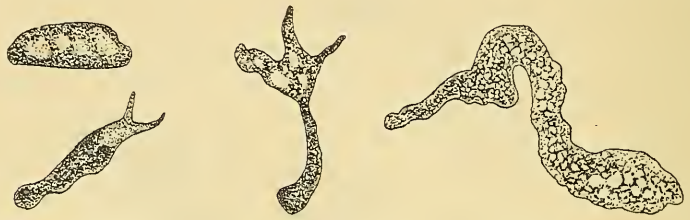

Fig. 5. Adult individuals found in the lymph. Camera lucida × 2000.

The body of the cell is well filled with distinctly-staining granules, and for a long time the animals were overlooked because of their striking resemblance to the nuclei of muscle cells (fig. 4). Apart from the developmental phases which show that they cannot be muscle-cell nuclei, these cells stain more intensely than do the tissue nuclei,

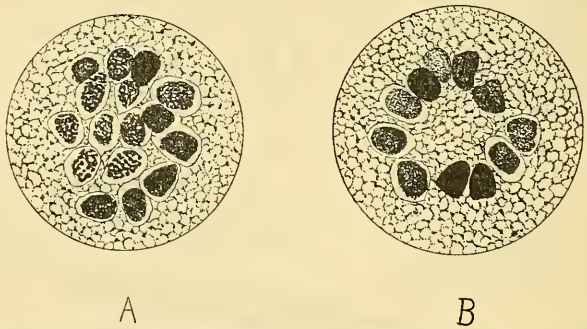

Fig. 6. Spore-forming individuals from the lymph-spaces. *A* Groups of deeply-staining granules aggregating to form spores; *B* Later stage. Camera lucida × 2000.

and possess no karyosomes or nucleoli, while their position is further evidence of their independent organization.

So far as I am aware the distributed nucleus, although described n some rhizopods, flagellates, and ciliates, has never been recorded for a Sporozoon. In the present case I have observed no concentration of the granules of chromatin as in the flagellate *Tetramitus*[2], nor any

[2] cf. Calkins, Annals N. Y. Acad. Sc. 1898.

division of the granules as described by Schewiakoff (1893) in *Achromatium*[3]. When however the organisms prepare for spore-formation these deeply staining granules disappear from the outer portions of the protoplasm and apparently collect in several masses in the interior of the cell. These masses are the spores (fig. 6).

Spore-forming individuals are frequently found in the lymph surrounding the various organs, and are quite numerous in the cavity of the gall-bladder and in the intestine. They are of relatively large size (25 to 30 μ) and may be formed from the union of two or more individuals although save for the large size I have no evidence at all to indicate that such is the case.

The young spores at first consist of granules which stain intensely like the distributed chromatin granules of the adult organism (fig. 6*A*), but as they grow older, they become more dense and, while still within the body of the parent, they assume the homogeneous character of the spores as described above (fig. 6*B*). The spores may be liberated in the body cavity and so give rise to the large numbers which are found distributed about the body, or in the intestine, where passing to the outside with the faeces they may be one cause of the spread of the disease.

Briefly summarizing the above account of the life-history of the parasite while within the body of the fish host, it appears that the organism gets into the fish probably by means of the digestive tract and while in the spore stage. In the intestine the spore completes its development and forms sporozoites which penetrate the epithelial tissues lining the wall of the gut, and get into the lymph. Here they grow into amoeboid organisms which penetrate the muscle bundles and live until maturity as inter-cellular or possibly as intra-cellular parasites. When mature they withdraw from the muscles and form spores, possibly after conjugation. The spores are liberated into the body cavity or into the lymph and are carried to all parts of the body. In the muscles of the body wall they may accumulate until the lymph passages are fairly blocked with them, and the tissues are unable to get sufficient nourishment, ultimately dying and falling out thus leaving the sores and ulcers so characteristic of the diseased or dead fish.

It is evident that, although closely analogous to the Myxosporidiida in mode of life, these organisms cannot be classified with the latter common fish parasites. Nor can they be grouped wits the Coccidiida, or Sarcosporidiida. The Haemosporidiida are likewise entirely different.

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[3] Hab. Schrift. Heidelberg, Winter.

There is however, a striking similarity in the spores of this form and of those of *Serumsporidium* as described by Pfeiffer (1895)[4]. *Serumsporidium* is a parasite infesting the body cavities of fresh water Entomostraca, filling them up until they resemble the testis of an infested Brook Trout. The spore-forming individuals are somewhat similar to the parasites described by Thélohan and Henneguy (1892)[5] in the Crayfish, the spores of which measure only 2 or 3 μ. Although the life history of these forms is not fully known I venture to place the present parasite with them under the generic name *Lymphosporidium*; and from the habitat it gets the specific name *L. truttae.*

Two very important points in connection with the epidemic have not been touched upon. These are 1) the origin of the disease, and 2) the remedy. On neither point can I throw any light. The parasite may be present in small numbers in the Brook Trout at all times, becoming epidemic only under unfavourable circumstances. Or it is possible that like the Malaria germ its normal habitat may be in some other and lower form of life than the trout. This question can be settled only by careful examination of many normal individuals, and may never be settled. The second point is no nearer settlement, for all of the fish in the hatchery died before the cause of the epidemic was discovered, and there was no time for experiment. I hope that sometime in the near future both points may be satisfactorily determined.

June 1900.

2. Zwei neue Myriopoden aus dem Mittelmeergebiete.

Von Director Dr. R. Latzel in Klagenfurt.

(Mit 1 Figur.)

eingeg. 11. August 1900.

Unter einer größeren Menge von Myriopoden, welche Prof. Dr. Oskar Schneider (Blasewitz) in den letzten beiden Frühjahren bei San Remo, Bordighera und Ajaccio gesammelt hat, fanden sich die folgenden neuen Arten. Die Veröffentlichung der ganzen Ausbeute soll in einem Nachtrage zu Schneider's Arbeit »San Remo und seine Thierwelt im Winter« in den Jahresberichten der Isis in Dresden erfolgen.

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[4] L. Pfeiffer, Die Protozoen als Krankheitserreger. Suppl. 1895.
[5] C. R. Soc. Biol. V. 44.