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AMOEBA, EXTERNAL PARASITES ON HYDRA

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In the fall of 1930 and again in 1933, the presence of amoeba on the tentacles of *Pelmatohydra oligactis* was observed in our laboratory. At the latter date six of these fresh water hydra were placed in a small jar with a few strands of *Chara.* A few days later only two specimens could be found in the jar; not wishing to lose these, they were killed and mounted. Fig. 1 of the plate shows clusters of amoeba on the tentacles.

Last September, *Elodea canadensis* and *Chara fragilis* were collected from the fresh water lake Sagatagan for the purpose of obtaining hydra for class study. The weeds were put into various aquaria. One of these contained many amoebae of different species. It was hoped that some of these would parasitize the hydra. The following day there was a good supply of *Hydra americana.* Daily observations were made with a low power binocular microscope for the appearance of amoeba on the hydra. On the fifth day, and again a few days later, expectations were realized. For the purpose of investigating this interesting phenomenon, the parasitized hydrae were put into small Petri-dishes and into Syracuse watch crystals, using all precautions to avoid what is known as "depression." This is a condition in which the hydra loses its tentacles, as a result of an unnatural environment, such as stagnant water, fresh tap water, or too small a quantity of water. Sudden temperature changes, as well as changes in acidity, also seem to play a part in causing the condition.

This report contains a summary of findings, based on the observations made on some fifty odd parsitized hydrae, in the course of two and a half months. The accompanying plate of photomicrographs of whole mounts and of sections illustrates some of the findings.

The method employed to preserve parasitized hydrae consisted in transferring amoeba-free hydra to a dish containing hydra with amoebae. On the second day after the transfer the tentacles were usually infected; in a few instances however, the amoebae appeared first on the aboral end. Their ability to cling to the host is very remarkable. Rather large specimens have been observed hanging on the tentacle of a hydra with one small pseudopod, as an acrobat would on a trapeze, while the tentacle was being moved about freely. No evidence was found that the nematocysts play any part in this phenomenon, nor that the hydra uses them at any time in defense against the parasite. The amoeba is usually very active, and can

be seen crawling, using its short pseudopods as "toes." Killing and fixing reagents (heated picro-formol being usually employed) do not separate host and parasite; the same is true during the process of dehydration and clearing for parafin imbedding.

The question arises whether we are dealing with a case of true parasitism. Dr. F. Doflein in his Lehrbuch der Protozoonkunde, 4th edition, 1916, states that there is only one amoeba known to be an external parasite and that only a facultative one on the gills of some marine fish. Dr. W. A. Riley, University of Minnesota, has called my attention, after the presentation of this report, to a reference by G. Entz, 1912, "Ueber eine neue Amoeba auf süsswasser Polypen (*Hydra oligactis,* Pall)" Arch. f. Protistenkunde 27: 19–47, and there is a reference to it by Hegner and Talaiferra, 1924. From observations made, and from the study of sections, there seems to be no doubt that the amoeba is a parasite, though it may be only a facultative one. The size and behavior agree fairly well with Schaeffer's *Amoeba (Mayorella) bigemma.* The peculiar nucleus resembles that of the genus *Entameba.* Further studies must be undertaken to establish its definite systematic position.

During the first two or three days, the hydra, with only a countable number of amoebae, behaves normally, is fully distended, parks on the side of the dish or on a leaf of Elodea, and feeds when a Daphnia or other small crustacean is encountered. Thereafter one notices tentacles of variable size, mere stubs, partly reduced and normal ones. The reduced tentacles do not give the appearance of being contracted; the shortening apparently begins at the distal end, as if wearing away. Pieces of tentacles have never been found in the dish nor any other evidence that the tentacles are being cut off. As many as five amoebae are often seen at the end of the stubs. On several occasions hydra with four or five amoebae were isolated in a dish of amoeba-free water. The next day the number of amoebae had doubled, showing that they grow and multiply on the host. When the tentacles are gone the amoeba shows a preference for the oral end, where they are densely clustered, one often partly overlapping the other. The floating of the protoplasm in the formation of short pseudopods occurs almost continually, although the amoeba does not readily alter its location on the hydra. The hydra at this stage will not readily distend.

In order to kill the hydra in a distended condition, choral hydrate was used as a narcotic. Frequently the well known ciliates, *Kerona* and *Trichodina pedunculus,* were apparently playing a game of hide and seek, all their own, on the hydra. A weak solution of chloral hydrate caused these almost instantly to leave the hydra, go into a "tail spin" and burst. The amoeba under this treatment showed no ill effects. This method was used to rid the hydra of ciliates whenever they were encountered.

When the hydra is completely surrounded by its parasites it remains in a contracted state, it detaches itself, drops to the bottom

of the dish and soon disintegrates. Ten days is the longest time on my records, for the amoeba to accomplish its work of destruction. At one time, a heavily infested hydra trunk, which measured only two mm. in length, was taken from the bottom of a dish, placed in a watch crystal with clean water. After half an hour it disintegrated and sixty amoebae were counted leaving the battlefield. The hydra remnants appeared as if an excellent dissociating fluid had been at work.

The haematoxylin-stained sections show that the amoeba sends its pseudopods at times through the cuticle of the hydra. In a heavily infested area the tissue is broken up; in one instance the mesogloea has disappeared in a small portion, and an amoeba is making its way to the interior. Hydra cells cannot be recognized in the interior of the amoeba. Two nuclei are frequently seen in the amoeba. The nucleus is spherical, with a well defined nucleolus. A few specimens show the chromatin distinctly beaded. Four sectioned hydra show the amoebae thriving also in the gastrovascular cavity. Entrance is probably through the oral cavity.

Attempts have been made to grow the amoeba in a pure rice-agar medium. This was checked with an *Amoeba proteus* control culture. During the first week there were many actively crawling amoebae on the agar. Then they became scarcer, and what seemed to be cysts became numerous. After a few weeks no actively crawling forms could be found, but instead there appeared very numerous smaller amoebae, with longer and more pointed pseudopods radiating from a central mass of protoplasm. Three attempts gave the same picture. Hydra could not be kept alive in the amoeba culture. After such cultures were more than a week old, we did not succeed in parasitizing hydra by transferring some of the material to a hydra culture.

Many interesting problems are suggested in this study. As parasites, the amoebae do not confine themselves to the interior of the host, as we read in our texts; they may be external parasites as well, at least facultatively. Then we read that the amoeba has only intracellular digestion. The feeding of the amoeba on the hydra and particularly the penetration of the cuticle by the pseudopods can be accounted for only by the assumption, that some digestive fluid is secreted. The study of this parasitism should be of interest not only to the physiologist but also to the cytologist; he has here a means of getting good sections of amoeba for the study of nuclear structure and cell division. Further study may throw some light on the study of other amoebic diseases especially that of dysentery caused by Entameba.

EXPLANATION OF PLATE

Fig. 1. Shows clusters of amoeba on the tentacles of Pelmatohydra oligactis.
Fig. 2. Hydra with tentacles greatly reduced by action of amoeba.
Fig. 3. Transverse section through hydra body showing amoeba at work.
Fig. 4. Transverse section of body of hydra showing eight amoeba with nuclei.
Fig. 5. Locomotion of amoeba.
Fig. 6. Amoeba in gastrovascular cavity, showing peculiar beaded nucleus.

FIG. 1

FIG. 2

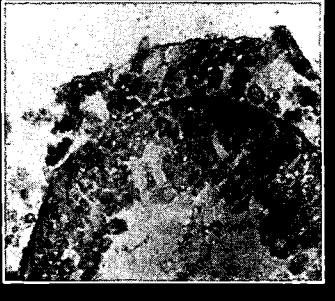

FIG. 3

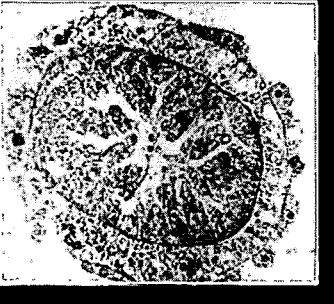

FIG. 4

FIG. 5

FIG. 6