PELOMYXA PALUSTRIS GREEFF

II. ITS ULTRASTRUCTURE*

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Summary. The Pelomyxa palustris amoebae used in this study were multinucleate, herbivorous protozoans. All nuclei within a single organism were similar, but several types of nuclei were seen in different amoebae. These nuclei might represent various stages of mitosis although metaphase and anaphase stages were never seen. Rod-shaped bacteria within vesicles characteristically surrounded the nuclei. Bacterial rods of this as well as another type also occurred within vesicles in the cytoplasm. The nuclear envelope contained annuli and it was covered externally by minute vesicles. Nucleoli and micronucleoli were most frequently located along the inner surface of the nuclear envelope. Clusters of electron-opaque spheroids were found within the nucleoli; sometimes, they existed free in the nucleoplasm. Intranuclear globules of lipid-like material were often seen.

Mitochondria, Golgi bodies, contractile vacuoles, and crystal vacuoles were definitely absent in P. palustris. The cytoplasm contained many food vacuoles and clear vacuoles of various sizes. Vacuole-like aggregations, probably containing glycogen, were present.

The cytoplasm of the giant amoeba, Pelomyxa palustris, characteristically contains sand grains which prevent thin sectioning for electron microscopy. We recently discovered many sand-free P. palustris from which we have obtained thin sections of high quality. Their ultrastructure is presented in this paper. Earlier work with this amoeba (Part I) was published by KUDO (1957).

Material and Methods

Our first collection of sand-free Pelomyxa palustris was obtained on November 4, 1964 from a stagnant pond four miles southeast of the Biology Building at Argonne National Laboratory. It is one of several ponds in an abandoned limestone quarry region where collections for P. palustris have been made several times per year since 1955 (DANIELS, 1956; KUDO, 1957). The amoebae used in the present work were obtained on the south edge of the pond where the depth of the water measured 0.5 meters. A large cottonwood tree annually shed many leaves into the water at this location. Throughout the year the temperature of the water, taken at the time of each collection, ranged from 10° to 22° C; the pH was between 7.1 and 7.8.

The number of amoebae declined in collections older than 1 to 3 days. When the amoebae were separated from the debris (organic sediment without sand or mud) and placed in clear

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1 These structures are designated nucleoli because of their negative response to the Feulgen nuclear reaction (KUDO, 1957), and their morphological similarity to the nucleoli of other large, free living amoebae. They also may be called endosomes.

2 We were not able to make thin sections of amoebae (from previous collections) with glass, quartz, or diamond knives because of very hard particulates in the protoplasm. Other investigators have used the term sand grains to describe these particulates.
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pond water, most of them died within a day or two. Therefore, we usually did not disturb fresh collections, but placed them in a constant-temperature room at 21°C. After the material in the collection had settled overnight, we examined the surface of the debris for amoebae and fixed them at once. Many algae of different kinds, including *Spirogyra* and diatoms, were found in the collections along with invertebrates such as midge-fly larvae, copepods, rotifers, etc.

Most amoebae were fixed in 2% unbuffered, aqueous osmium tetroxide for a minimum of 0.5 hours. A few individuals (5 to 10%) were fixed in a sodium veronal buffered, 1% osmium tetroxide solution. After fixation, the amoebae were rinsed in Ringer's solution to remove excess osmium, dehydrated in a graded series of ethanols followed by three changes of propylene oxide, then embedded in Epon 812. Sections were cut with a diamond knife on a Porter-Blum microtome and examined with an RCA EMU-3F electron microscope. A total of 80 amoebae were studied. About 20% of them had apparently engulfed material that would not infiltrate properly, which made them extremely difficult to section. Upon examination, the sections were found to contain holes and scattered debris caused by the removal of this material from cytoplasmic vacuoles during sectioning and its distribution across the surface of the sections. The sections were not stained.

Results

The Nuclear Envelope and Adjacent External Structures

*Pelomyxa palustris* was characteristically multinucleate. Although the size and structure of the nuclei often varied from one amoeba to another, all of the nuclei within a single organism were similar, an indication of synchrony (Figs. 1, 11). Several types of nuclei were found repeatedly in different amoebae from each collection.

The nuclear envelope of *P. palustris* was composed of two 70 Å electron-opaque layers separated by an electron-lucent area which varied in thickness from about 150 to 230 Å (Fig. 3, NM). The nuclear envelope contained many annuli (Figs. 3, 5, A) which averaged 68 mμ in diameter and 185 mμ from center to center. In very thin sections (80—110 mμ) examined at high magnifications, several minute vesicles, each separated by a space, could be seen in the wall of an annulus. At low magnifications, the annuli appeared similar to those in the nuclear envelope of *Pelomyxa illinoisensis* (Daniels and Roth, 1964). Andreesen et al. (1965) have shown annuli in the nuclear envelope of *P. palustris*.

Bacterial rods, 3 μ × 0.3 μ, usually surrounded the nucleus of *P. palustris* (Figs. 1—5, 7, 8, 13, 14, Bn). These bacteria were generally separated from the nucleus by (1) relatively clear cytoplasm; (2) minute, irregular vesicles (Figs. 2 and 3, V); and (3) a complex, somewhat opaque zone adjacent to the nuclear envelope (Fig. 3). The latter sometimes contained an electron dense layer located midway between the nuclear envelope and the vesicles (V in Figs. 2 and 3) and which was concentric with the nuclear envelope.

Instead of the structures just mentioned, a lamellated band, surrounded by electron-opaque, variable-stellate bodies within vesicles, was found around each nucleus in about 10% of the amoebae (Figs. 11, 12, LB, S). In some amoebae, both empty and nearly empty vesicles encircled the lamellated band instead of the variable-stellate forms.

In 7% of the amoebae studied by us, the nuclear envelopes were inwardly serrated (Figs. 7—9, NM) as in *Amoeba proteus* (Pappas, 1959; Mercer, 1959; Roth et al., 1960; Cohen, 1957; and Greider et al., 1958). Minute vesicles, already