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## Electron and Light Microscope Studies of *Endamoeba terrapinae*

MARILYN DRIML<sup>1</sup>

**Abstract.** This study was restricted to the trophozoite stage of *Endamoeba terrapinae*. The most striking feature found was lack of cellular components such as are found in *Amoeba proteus* and the giant amoeba. No structures resembling mitochondria, Golgi bodies, or fat globules were observed. The only defined structures with the exception of the nucleus were vesicles containing bacteria and electron-dense granules. These cytoplasmic components stained lightly with hematoxylin.

Saunders and Cleveland (1) first described *Endamoeba terrapinae*, a small parasitic species of amoeba. This parasite is present as a small, actively motile trophozoite in *Chrysemys elegans*, the common laboratory turtle.

The growth cycle of this amoeba includes encystation; the mature cyst contains four nuclei. At temperatures below 25°C the organism remains in the cyst stage, but excystation occurs above this temperature. The uninucleate trophozoite, which is 10-15  $\mu$  in diameter, is typical of *Endamoeba* in that it consists of a central mass of chromatin material surrounded by a clear halo. The peripheral chromatin of the nucleus is beaded. The trophozoite ingests starch and bacteria. The present study was restricted to the trophozoite stage of *E. terrapinae*.

Electron microscope studies demonstrated the presence of cytoplasmic granules. Such granules in other species of amoebae have demonstrated a basophilic property. Palade (2) suggests a relationship in cells between electron-dense granules in the cytoplasm and cytoplasmic basophilia, a property known to be due to a large extent to ribonucleic acid (RNA).

The object of the experiment was to observe the fine structures of *E. terrapinae* with the electron microscope and to determine the nature of cytoplasmic granules seen under the electron microscope.

### METHODS AND MATERIALS

**Electron microscopy.** Because of the small size of *E. terrapinae*, a method was devised to concentrate these organisms

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<sup>2</sup>Cultures of *Endamoeba terrapinae* were obtained from the General Biological Supply House, Chicago, Illinois.

during fixation and processes leading toward embedding. A cotton plug was inserted into one end of a 7-cm piece of glass tubing. The solution containing the amoebae was transferred to the tube, and the supernatant fluid was allowed to filter through the cotton plug into a beaker. When most of the liquid filtered from the tube, Palade's fixative, osmium tetroxide buffered to pH 8 with veronal acetate (3), was added. All changes of solutions thereafter were accomplished in this manner.

After fixation the organisms were dehydrated in a series of alcohols (10 minutes in each) and placed in a methacrylate mixture. The latter was composed of 60% *n*-butyl methacrylate (by volume), 40% ethyl methacrylate (by volume), and 1.5 gm of 2,4-dichlorobenzoyl peroxide (4). The cells were then transferred with a micropipette from the glass tube to gelatin capsules filled with the methacrylate mixture. The amoebae settled to the bottom of the capsules, and the methacrylate polymerized at 60° C for at least twelve hours.

The embedded specimens were sectioned at 100  $m\mu$  and placed on carbon-coated copper grids. The mounted sections were stained for one hour with 1% uranyl acetate (5). Observations were made with an RCA EMU-3 electron microscope.

*Light microscopy.* *Endamoeba terrapinae* were fixed with Lo Bianco's fluid for 10 minutes. Dehydration was accomplished in the same manner as in the preceding experiment. When the organisms were in 95% ethanol, eosin was added. This stain made them visible. After they were embedded in paraffin, they were sectioned at 8  $\mu$ . The sections were then stained with the basic stain, hematoxylin.

#### RESULTS AND DISCUSSION

The electron micrographs revealed the fine structures of *E. terrapinae*. The most striking feature of this parasitic amoeba is the lack of cellular components such as are found in *Amoeba proteus* and the giant amoebae. No structures resembling mitochondria, Golgi bodies, or fat globules were observed. The absence of such structures may be the result of the stage in which the amoeba was studied. The only defined structures with the exception of the nucleus are vesicles containing bacteria (Fig. 1). Starch and bacteria are the food materials of this organism, but it may be debatable as to whether all bacterial organisms are utilized as food. There is little evidence that bacteria are being digested within the amoeba's cytoplasm.

The nuclei vary in shape and form. An electron-dense mass is observed in the center of the nucleus. This is designated as

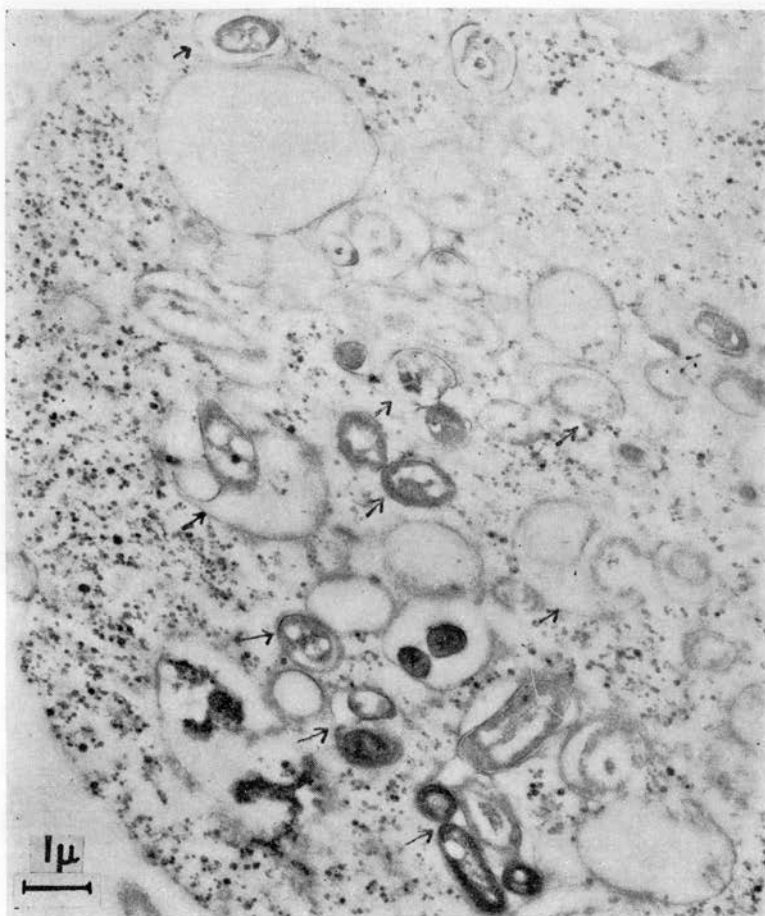


Figure 1. Bacterial organisms enclosed in vesicles (arrows).

the endosome, or inside body. This corresponds in position to the Feulgen-positive area in *Amoeba proteus* which has been identified as desoxyribonucleic acid material (5). Dense areas are also seen around the periphery just inside the nuclear membrane. These might be analogous to the material in other amoebae that exhibits a negative Feulgen test and is assumed to be ribonucleic acid (7).

Electron-dense granules fill the cytoplasm of *E. terrapinae* (Fig. 2). These granules are observed in other species of amoebae but are not so numerous. In other amoebae these have been defined as granules of RNA (8).

Light microscope studies revealed the cytoplasm of this organism was stained lightly by the basic dye, hematoxylin. It was

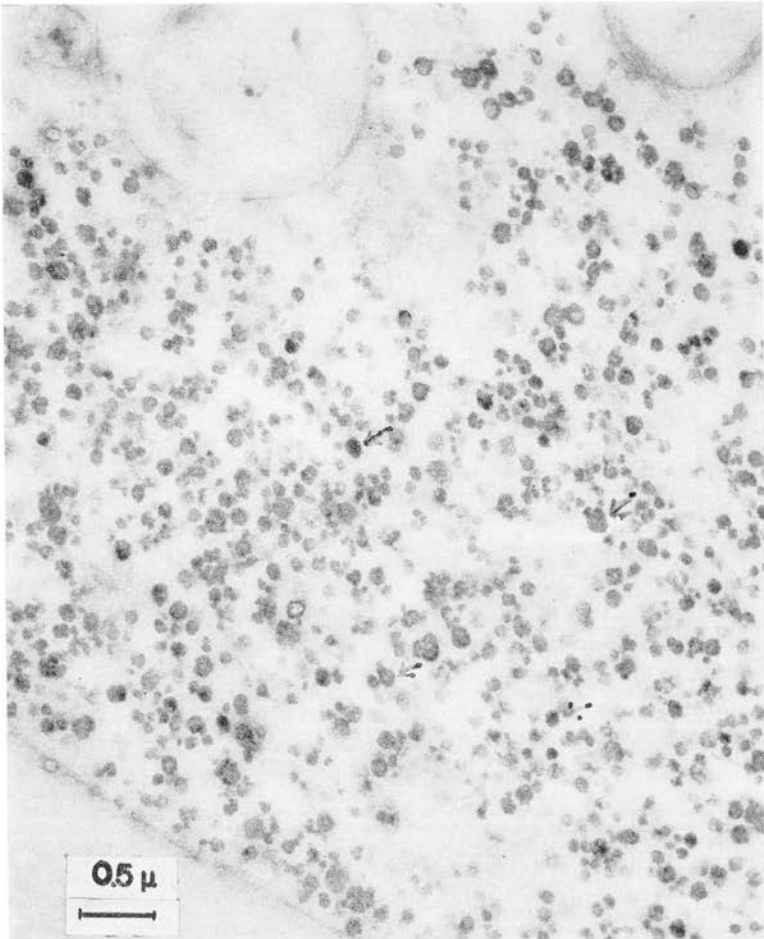


Figure 2. Electron-dense particles found in the cytoplasm.

impossible to determine if the particles so stained were the electron-dense granules or other cytoplasmic components.

#### ACKNOWLEDGMENTS

I wish to thank Dr. Edward W. Daniels for his greatly appreciated help in initiating and supervising this research project. Had it not been for his patience and advice concerning the electron microscope and protozoa the research could not have been carried out. I also wish to thank Mr. O. T. Minick of the same laboratory for his instruction in electron microscope techniques.

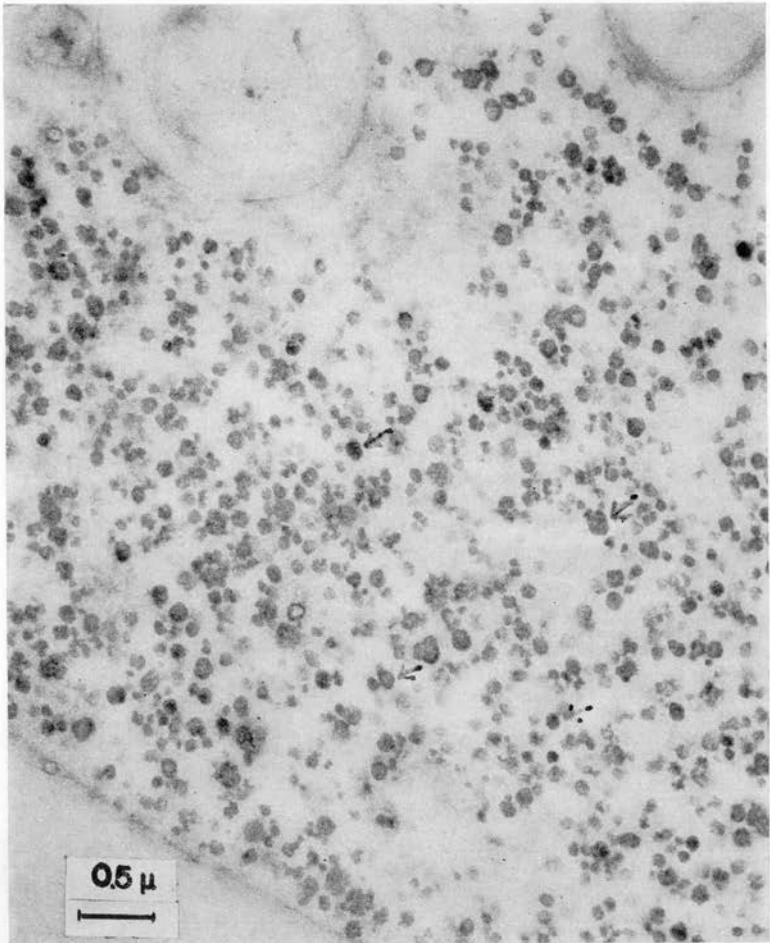


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