Hartmannella Culbertsoni as Revealed in Scanning Electron Microscopy

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HARTMANNELLA CULBERTSONI AS REVEALED IN
SCANNING ELECTRON MICROSCOPY

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Abstract. An axenic culture of Hartmannella culbertsoni obtained from the American Type Culture Collection was used in this study. Amoebas maintained in a trypticase soy broth medium were concentrated, fixed and dehydrated before being transferred drop-wise to round microscope cover classes for critical point drying in liquid CO₂. Dried specimens were coated with carbon and palladium-gold and studied with an SEM. H. culbertsoni showed extreme polymorphism. Surface specializations or microappendages, not previously demonstrated in the light microscope, were observed. Some of the amoebas possessed smooth cellular surfaces with scattered bleb-like and bulbous ectoplasmic projections and others had irregular surface contours with thread-like processes of varying lengths. Other cells exhibited finger-like projections which formed a fringe over most of the surface. Such advanced pleomorphism, under identical environmental conditions, depends on the phase of growth, differential adhesion and locomotion of cells.

Free-living soil amoebas or Acanthamoeba (Butt, 1966) have been difficult to describe by light microscopy, and as a result disagreement and confusion concerning their taxonomy prevail in the voluminous literature. The disagreement is due mainly to different views about taxonomic criteria (F. C. Page, personal communication). According to Page (1967b), the erosion of the taxonomic line between the free-living and parasitic amoebas has been responsible for the difficulty encountered in dealing with the subclass Rhizopoda (phylum Protozoa).

The evolution of the taxonomic problem began when Jahnes, Fullmer and Li (1957) found a small amoeba classified as Acanthamoeba in monkey tissue culture cells. Later Culbertson, Smith and Minner (1958) observed a similar amoeba in tissue culture fluid suspected of having an unknown simian virus. When this tissue culture fluid was injected into the brains of mice and monkeys, only free-living amoebas identified as Acanthamoeba were found in resulting lesions of severe primary meningoencephalitis. These motile amoebas were designated as Hartmannella by Singh and Das (1970). After finding several other hartmannellid amoebas which were identical, they proposed the new species Hartmannella culbertsoni.

The discovery of the pathogenicity of some free-living amoebas (Butt et al., 1968; Callicott, 1968; Callicott et al., 1968; Cerva et al., 1969; Culbertson, 1971) resulted in the division of the superclass Sarcodina into two groups based on their habitats (Cotter, 1973). One group is made up of free-living amoebas which increase in number at the expense of other small organisms, usually bacteria. The other group exists in some type of symbiotic relationship with other eukaryotic organisms. The fact that the so-called free-living group includes members which are pathogenic under certain conditions has further complicated the taxonomy of amoebas.

The two schemes of classification found in the literature (Singh, 1952; Page, 1967a, 1967b) overlap, and as a result it is difficult to assign names to the amoebas. Singh (1952) suggested a scheme of classification based upon the type of mitosis. He proposed two large families, Hartmannellidae and Schizopyrenidae, the main difference being the dissolution of the

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nucleolus in the former, and the persistence and division of it in the latter family. Page (1967a, 1967b) modified this scheme with a greater emphasis upon morphological features. According to Page's criteria, the A-l Strain is Acanthamoeba, while by Singh's it is Hartmannella.

Page (1967a, 1967b) described these pathogenic amoebas in detail but an accurate demonstration of their surface morphology was beyond the resolving power and depth of field of the light microscope. Two scanning micrographs (SEM) of H. culbertsoni, without a detailed interpretation, were included in the study on cystforming filose amoebas by Sawyer and Griffin (1975). Since no other description of H. culbertsoni has been reported using this technique, it was deemed worthwhile to examine the surfaces of H. culbertsoni, regarded as one of the etiological agents in primary amoebic meningocencephalitis, to better depict its morphological features during active (log phase) growth.

**METHODS AND MATERIALS**

An axenic, concentrated suspension of H. culbertsoni was obtained from the American Type Culture Collection for use in this study. Stock suspension transfers to a trypticase soy broth nutrient medium as suggested by Culbertson (1971) resulted in more satisfactory maintenance of the amoebas when kept at 37°C and pH of 7.2. Hartmannella was selected over Naegleria, the other well known amoebic pathogen, primarily because the H-A amoebas do not require living cells for satisfactory growth from small inocula.

Amoebas were concentrated by centrifugation (300-400 rpm), separated from the supernatant nutrient medium, re-suspended in a sodium cacodylate-sucrose rinse (Sabatini el al., 1963) at pH 7.3, before being fixed with a cacodylate-buffered paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965). The fixed samples were dehydrated through a graded series of ethanol and alternately concentrating the amoebas by centrifugation and re-suspending in the next higher grade of ethanol. Amoebas in absolute ethanol were transferred drop-wise to chemically clean, round cover glasses before being dried in a Denton Critical Point Drying System using liquid CO2. The specimen cover glasses were mounted on SEM stubs with an electrically conductive adhesive (Electrodag) and coated with carbon and palladium-gold in a Denton DV-502 Vacuum high vacuum evaporator. Specimens were viewed in an ETEC Autoscan scanning electron microscope using 20 kV and photographed on 4 x 5 Kodak Commercial Estar Thick Base Film (No. 4127).

**RESULTS**

Observations were made on 8 microscope slide cover glasses containing a total of approximately 30,000 H. culbertsoni amoebas (30,000/ml). It was apparent immediately that this H-A organism exhibited polymorphism. The cell surface and the micro- and filiform extensions of ectoplasm (pseudopodia) as revealed in light microscopy (fig. 1) appear more complex and varied in the scanning electron microscope. Surface specializations, not previously seen in the light microscope, characterize H. culbertsoni (figs. 2-6). These include short microvillous-like, filiform, bleb-like and finger shaped appendages. The amoeba in figure 2 has several long filiform (thread-like) microappendages (arrows)

**EXPLANATION OF PLATE**

**FIGURE 1.** A light micrograph of an unstained, fixed H-A (Hartmannella culbertsoni) amoeba. ×1,250.

**FIGURES 2-6.** Hartmannella culbertsoni SEMs studied from above so as to be directly comparable with the light photomicrograph (viewed at an angle of 45°).

**FIGURE 2.** Spherical amoeba with several long filiform microappendages (arrows) and a comparatively smooth surface with small bleb-like ectoplasmic projections. ×2,300.

**FIGURE 3.** Ovoid cell which appears to have been in locomotion when fixed. The long filiform processes projecting primarily from one area suggest locomotion in that direction. Some of the long processes possess terminal swellings (arrow). ×2,800.

**FIGURE 4.** Slug-shaped amoeba with finger-like projections that form a fringe over most of the surface, and probably represents the more active locomotive form. ×2,300.

**FIGURE 5.** Two rounded, non-locomotive forms of H. culbertsoni. These two cells are morphologically different from each other and from the other amoebas (figs. 2-4). Neither possess long filiform extensions. The cell on the right possesses a flower, bleb-like surface contours; the one on the left is covered with microvillus and short filiform projections. ×2,800.

**FIGURE 6.** Higher magnification of an area of the cell in fig. 5 (left). Note irregular surface with numerous short microappendages. Most of which have terminal swellings. One finger-like extension is also discernible (arrow). ×8,400.
Figures 1-6
and a comparatively smooth cellular surface with scattered bleb-like and bulbous ectoplasmic projections. Figure 3 shows an ovoid cell with irregular contours and long filiform processes projecting primarily from one area. Most of the long processes possess terminal swellings. The cell in figure 4 exhibits finger-like (fimbriae) projections which form a fringe over most of the surface. These processes are stouter and do not possess bulbous terminations. Figure 5 shows two cells that are also morphologically different from each other and from the other amoebas (figs. 2–4). Neither of these amoebas possess long filiform extensions. The cell on the right side possesses short microappendages, most of which appear bulbous, while the one on the left is covered with microvillous-like and filiform projections of ectoplasm. A higher magnification of an area of the latter amoeba (fig. 6) shows an irregular cell surface having numerous microappendages with terminal swellings and one finger-like extension (arrow) is discernible in this micrograph.

**DISCUSSION**

*Hartmannella culbertsoni* exhibits pleomorphism, a characteristic of most amoebic cells. The number, shape and size and arrangement of its processes vary from cell to cell even under identical conditions (age of culture, method of preparation, etc.). Because of the pleomorphic nature of these cells and their processes, a classification scheme based on external morphology alone is not practical. The observations reported herein confirm Culbertson's (1971) light microscopic findings in which he concluded that morphological features were rather inconstant in both the H–A and N (*Naegleria*) groups. The conclusion that morphology alone is not sufficient for taxonomic distinctions does not appear to be premature on the basis of previous work (Wilkins and Thompson, 1974; Rajaraman *et al*, 1974; Sawyer and Griffin, 1975) and current work in our laboratory.

Some of the microappendages (microvilli, blebs, etc.) appear to be emerging or forming while others appear fully formed or in the process of pinching off from the surface. These microappendages may have the ability to emerge rapidly from the cell surface and disappear into the cell as demonstrated for microvilli on cultured cells (Gey, 1955), and as suggested for certain bleb-like structures on the ependymal lining of intact mammalian tissues (Allen, 1975). Culbertson (1971), in his study of pathogenic soil amoebas, observed that after certain stimuli the *Naegleria* could generate a varying number of temporary flagellate forms (trophozoite). These flagella were, in turn, usually lost after a few hours, and the amoebic form assumed again. Possibly the polymorphism is related to efforts of recently suspended and centrifugally washed amoebas to re-establish surface contacts as indicated by Rajaraman *et al* (1974) when studying the processes of cell adhesion and spreading in human WI–38 tissue culture cells.

Scanning electron microscopic observations present us with the realization that this pathogenic protozoan is not only slug-shaped (limax) with numerous ectoplasmic extensions, but that its surface has a variety of specializations. The observed polymorphism may depend upon conditions of growth, cell adhesion, cell spreading, locomotion and parasitic nature as affected by the surrounding environment. There is no doubt that *H. culbertsoni* taken from the same culture under identical osmotic conditions exhibits polymorphism. The application of SEM to protozoa is of demonstrated value and should be exploited fully. Currently we are refining our techniques to use SEM combined with transmission electron microscopy and cytochemistry as both an experimental and a diagnostic tool for identifying pathogenic soil amoebas in cerebro-spinal fluid from animals having been subjected to them.

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**LITERATURE CITED**


