

## FATE OF MITOCHONDRIAL BODIES IN *ACANTHAMOEBA CASTELLANII*: AN ULTRASTRUCTURAL STUDY

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### ABSTRACT

Electron dense intramitochondrial bodies are induced in *Acanthamoeba castellanii* and followed ultrastructurally by transmission electron microscopy. The spherical masses are very small or non-existent in trophozoites but enlarge under encystment conditions often reaching 500 nm in diameter. Each body develops as an intracristal mass near the center of its host mitochondrion. Under starvation conditions for *Acanthamoeba*, an intracristal body along with its host mitochondrion, may become enclosed within a large digestive vacuole where the mitochondrion but not the intracristal mass appears to be digested. Under excystment conditions, intramitochondrial bodies may be extruded from their mitochondrial hosts and move freely in the cytoplasm.

### INTRODUCTION

*Acanthamoeba castellanii* is a small soil amoeba which, until 1974, was considered an innocuous protozoan in the human environment. In 1974, Culbertson reported that this cyst-forming strain of amoeba was pathogenic in mice. A flood of reports followed linking *Acanthamoeba* with meningoencephalitis in humans in the USA (Martinez, 1975; Grunnett, 1981; Seidel, 1985), in Nigeria (Lawande, 1979), in England (Rowan-Kelly, 1984; Rutherford, 1985), in South Korea (Ho-Joon, 1985), in Costa Rica (Chinchilla, 1979), in Canada (Scholten, 1979), in Austria (Thong, 1980), in Australia (Carter, 1981), in Hungary (Matyi, 1985), osteomyelitis in the USA (Borochovitz, 1981), keratitis of the eye in the USA (Gullett, 1979; Blackman, 1984; Moore, 1985; Meisler, 1986), in Germany (Witschel, 1984), in Belgium (Hanssens, 1985), in England (Wright, 1985), pneumonia in the USA (Martinez, 1982), lung infections in the USA (Grunnett, 1981), granulomatous brain tumors in the USA (Ofori-Kwakye, 1986), and as the "KPN" agent in Viluy encephalomyelitis (Chumakov, 1986). The organisms have been reported as contaminants of drinking water supplies in West Germany (Janitschke, 1983), human contact lenses (Moore, 1985; Ludwig, 1986; Silvano,

1987), commercial vegetables (Rude, 1984), hot tubs (Samples, 1984), surgical instruments (Meisler, 1985), dental treatment rinsing units (Michel, 1984), human tissue culture cells (Visvesvara, 1983), and air supplies in Mexico (Rivera, 1987), and Australia, (Walker, 1986). Finally, and very ominously, *Acanthamoeba* has now been linked with *Legionella* infections in Israel (Henke, 1986; Rowbotham, 1986), in England (Anand, 1983), and with acquired immune deficiency syndrome (AIDS) (Rolston, 1986; Wiley, 1987). Furthermore, the capacity to form highly resistant cyst walls has enabled *Acanthamoeba* to be transmitted from one person to another via air, soil, including the frigid soil of Antarctica, and even bottled and treated mineral water (Janitschke, 1982; Rivera, 1981; Brown, 1982).

Since the capacity of *Acanthamoeba* to form a cyst wall appears to be central in its survival, transmissibility and, perhaps, pathogenicity, the encystment process in this organism is the primary focus of research in this laboratory. In this report, specific ultrastructural changes in the mitochondria of *Acanthamoeba* which occur when the organism is induced to encyst are reported and the fate of such changes are followed through induced excystment.

### MATERIALS AND METHODS

*Acanthamoeba castellanii*, cloned from a single isolated cell, were grown and maintained in sterile culture media utilizing glucose and proteose peptone as carbon and nitrogen sources. One liter of growth medium (GM) adjusted to pH 5.5 contained 10.0 g proteose peptone, 10.0 g glucose, 20.0 ml of 0.1 M phosphate buffer, 2.0 mg Ca<sup>++</sup>, 20.0 mg Mg<sup>++</sup>, 1 µg vitamin B12, 1.0 mg vitamin B1, and 0.1 ml of 0.01 M ferric citrate. The cultures were aerated continuously at the rate of 3 cu ft per hr per liter.

### INDUCTION OF ENCYSTMENT

Trophozoites of *Acanthamoeba* were induced to encyst by suspending 10<sup>6</sup> cells per ml in a non-carbon, non-nitrogen containing encystment medium (EM) consisting of 7.4 g KCl, 20 mg Mg<sup>++</sup>, 2.0 mg Ca<sup>++</sup>, and 20.0 ml of 0.1

M phosphate buffer. The solution was made up to 1 liter with distilled water and adjusted to pH 6.8 with NaOH.

#### INDUCTION OF EXCYSTMENT

Cysts of *Acanthamoeba* were induced to excyst by suspending  $10^5$  cells per ml in growth medium (EM + 1% glucose and 1% proteose peptone) and aerating at 3 CFH with filtered air.

#### COLLECTION OF SAMPLES FOR ANALYSIS

Aliquots were collected from a single culture of encysting or excysting *Acanthamoeba* as follows: A 2-liter culture of encystment medium or growth medium was inoculated and control samples removed. At intervals thereafter, duplicate aliquots of  $10^7$  cells were removed aseptically and concentrated by centrifugation for subsequent fixation.

#### PREPARATION FOR ANALYSIS BY ELECTRON MICROSCOPY

*Acanthamoeba* were fixed for electron microscopy in 2% glutaraldehyde for 1 hr at 4°C. Specimens were dehydrated in ethanol using two washes of 50%, 70%, 95% and absolute, embedded in Epon 812 and sectioned on an LKB ultratome with Dupont diamond knives. Specimens were then stained on grids with 1% uranyl acetate for 10 minutes and examined in a Philips 200 transmission electron microscope operated at 40 KV to 100 KV with double condenser.

#### RESULTS

The mitochondria of trophozoite *A. castellanii* may be elongate and measure  $0.6 \mu\text{m} \times 1.5 \mu\text{m}$  or spherical and measure about  $1.0 \mu\text{m}$  in diameter. They are surrounded by the classical unit membrane and possess tubular cristae which are formed by branching, anastomosing, fingerlike

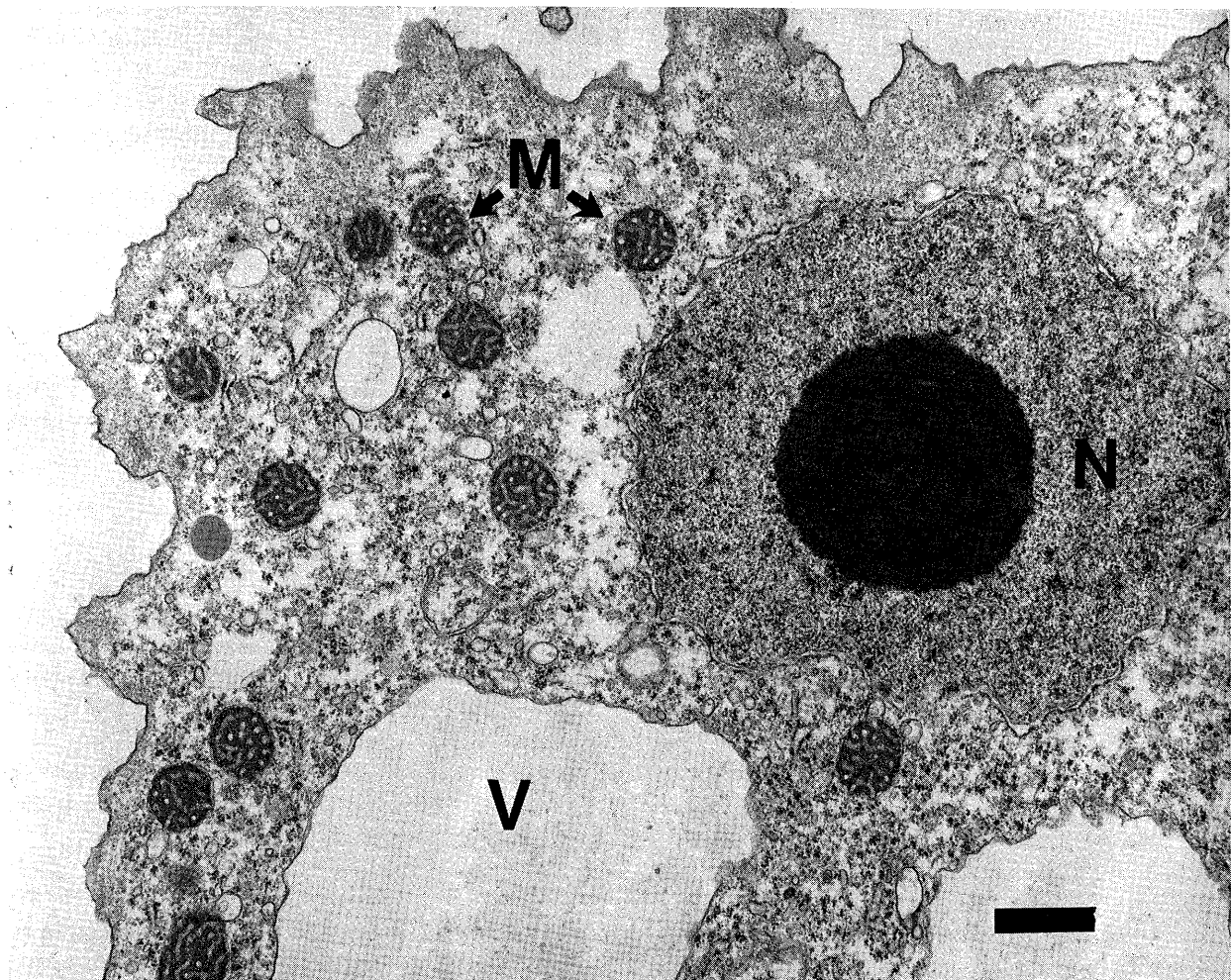


Figure 1. Trophozoite of *Acanthamoeba castellanii*. Nucleus, N; vacuole, V; mitochondrion, M.  $\times 26,250$ .

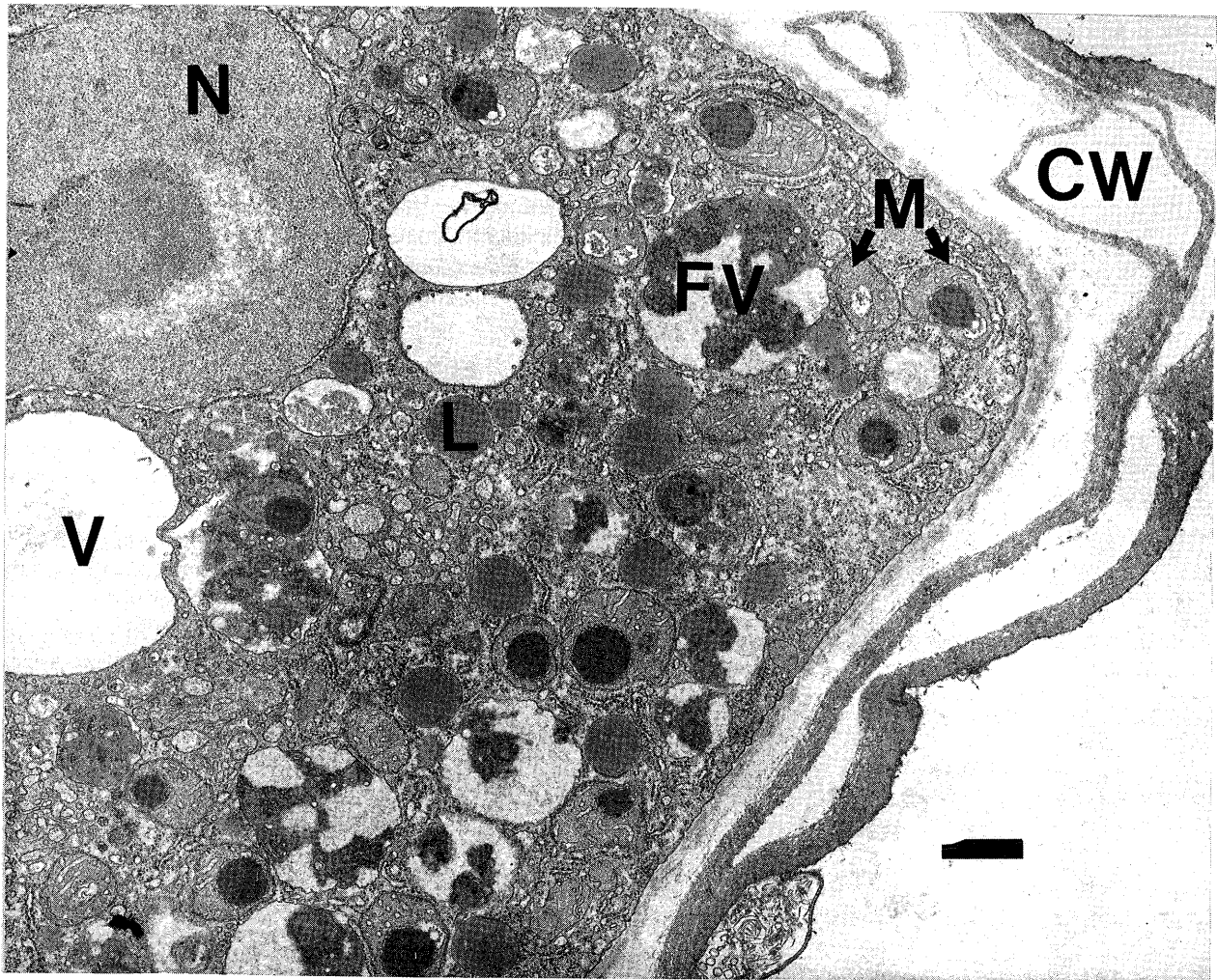


Figure 2. Cyst of *Acanthamoeba castellanii* showing numerous mitochondrial bodies and cups (double arrow). The bar represents 1.0  $\mu\text{m}$ . Mitochondrion, M; nucleus, N; vacuole, V; cyst wall, CW; food vacuole, FV.  $\times 26,250$ .

extensions of the inner mitochondrial membrane. Such tubular cristae are 60 nm in diameter and lie in a heterogeneous matrix that contains filamentous patches and includes granules which resemble ribosomes (Figure 1). When trophozoites are induced to encyst, small intracristal mitochondrial granules 80 nm in diameter become visible and increase in size as encystment proceeds to become large intramitochondrial bodies 500 nm in diameter. In addition, round or oval vacuoles bounded by minivesicular units 25 nm in diameter also appear in mitochondria. These "empty" vacuoles are similar in size to intracristal bodies but lack their staining properties. Such intramitochondrial vacuoles appear to result from cup-shaped mitochondria which are formed via an invagination of both mitochondrial outer membranes and are sectioned transversely. It is not uncommon for 70% of the

mitochondria in cysts to contain one or both of these inclusions. With time, mitochondria appear to be subjected to autodigestive forces in which case they become engulfed in large food vacuoles. Such mitochondria are slowly degraded except for the darkly staining intracristal body which remains undigested in the cell (Figure 2). Under excystment conditions, mitochondria which contain intracristal bodies appear to extrude their intracristal inclusions which become free-floating bodies in the cytoplasm of the cell. Such bodies retain their dark staining characteristics, morphology, and appear to resist further degradation in the cytoplasm of the cell. Most mitochondria are enlarged and spherical in such cells. Additionally, numerous lipid globules are present in cysts even though the cell may have been under starvation conditions (Figures 3 and 4).

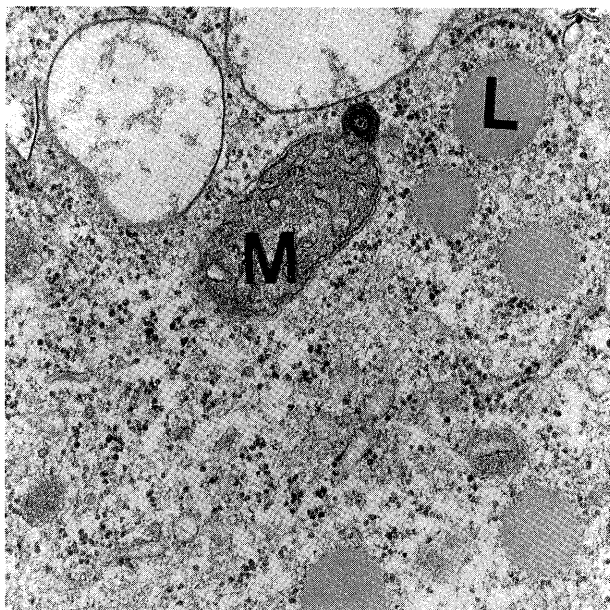


Figure 3. Mitochondrion of *Acanthamoeba castellanii* extruding an intracristal mitochondrial body. Mitochondrion, M; lipid globule, L.  $\times 70,000$ .

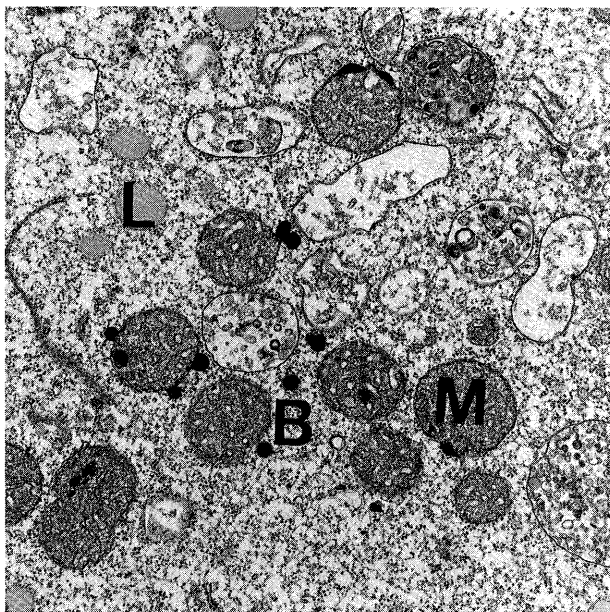


Figure 4. Enlarged, spherical mitochondria of *Acanthamoeba castellanii* along with intracristal mitochondrial bodies which have been extruded and move freely in the cytoplasm. Mitochondrion, M; mitochondrial bodies, B; lipid globule, L.  $\times 35,000$ .

#### DISCUSSION

The chemical composition of intracristal bodies in mitochondria of *Acanthamoeba* has not been determined. Concentration of  $\text{Ca}^{++}$  has been postulated as a "trigger"

for sequential changes which occur in *Acanthamoeba* mitochondria during encystment (Tomlinson, 1985). If such intracristal bodies consist of water-insoluble precipitates of inorganic calcium compounds, this could account for the apparent indigestibility of the bodies in food vacuoles and in the cytoplasm of cells. Such a "trigger" would also be consistent with the appearance of intracellular microfilaments which also appear in *Acanthamoeba* under induced encystment conditions (Tomlinson, 1984; 1986).

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