UNUSUAL MITOCHONDRIAL CRISTAE IN THE VINEGAR EELWORM

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INTRODUCTION

Mitochondria with cristae which are triangular in cross section have been reported from certain specialized cells of several organisms (1, 3, 4), but not previously from nematodes. This paper reports on the appearance and distribution of triangular cristae in mitochondria of the vinegar eelworm, *Turbatrix aceti* (Nematoda), and of a previously undescribed cristal configuration.

MATERIALS AND METHODS

Stock cultures of *T. aceti* were grown axenically at 27°C in a liquid medium described by Castillo and Krusberg (2). Female nematodes from two cultures were examined, one of which had been maintained at East Wareham for 4 yr and the other obtained from the Department of Cell Physiology, Boston Biomedical Research Institute. Age at sacrifice for electron microscopy was determined by utilizing procedures developed to study aging of another nematode (7). Several combinations of fixatives and embedding media were utilized. Since technique influenced visualization of the cristal configurations, each method and the results obtained are given in Table I. Sections were cut with a diamond knife at 50–90 nm on an LKB Ultratome 1 (LKB Instruments, Inc., Rockville, Md.) or with a glass knife on a Danon Ultramicrotome (Miles-Yeda Ltd., Kiryat Weizmann, Rehovot, Israel), then stained with uranyl acetate followed by lead citrate, and examined either with a Philips 200, JEM-7 or JEM-T-7 electron microscope.

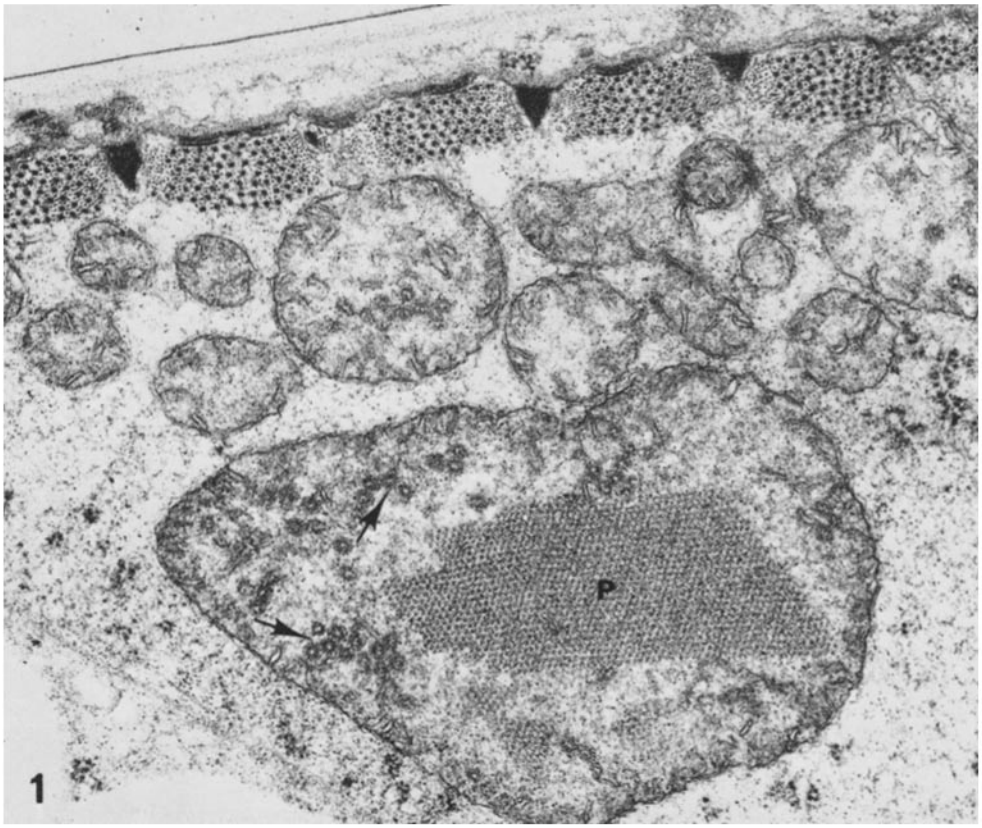
OBSERVATIONS

Mitochondria with triangular cristae and cristae in the form of doughnuts occurred within the hypodermal tissues, the somatic muscle cells, the

TABLE I

Influence of Fixation and Embedding on Visualization of the Described Cristal Structures

Fixation	Embedding medium	Cristal structures: Visualized	Cristal structures: Not visualized
1. 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, + 1 or 2% osmium tetroxide	Epon 812	×	
2. As above	Spurr's medium (5)	×	
3. 4% glutaraldehyde + 1% acrolein (5 parts) in 0.1 M phosphate buffer, pH 7.2, + 20% dimethylsulfoxide (1 part)	Spurr's medium		×
4. 1% osmium tetroxide	Spurr's medium		×



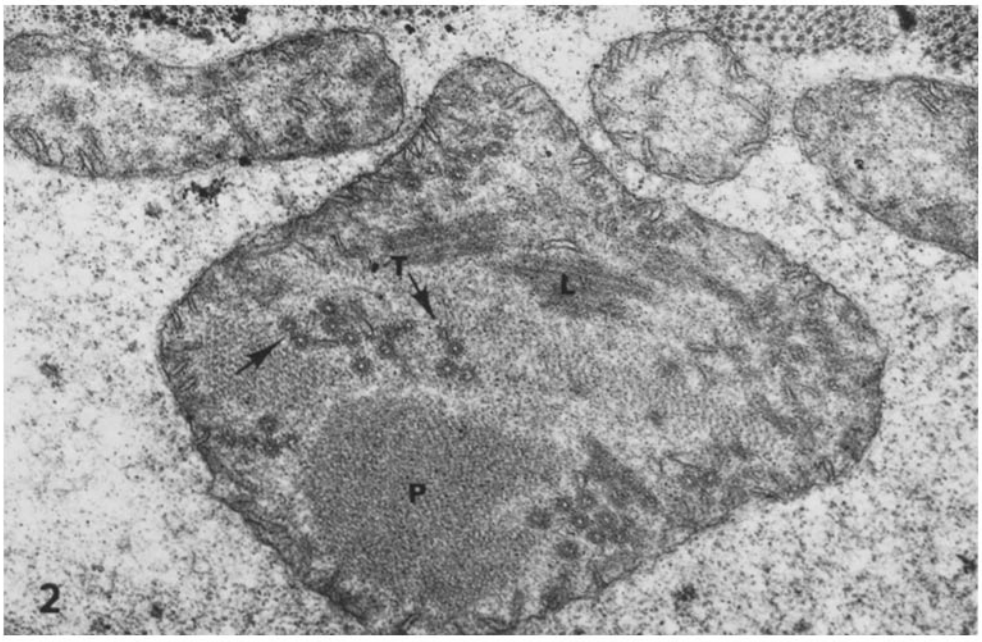
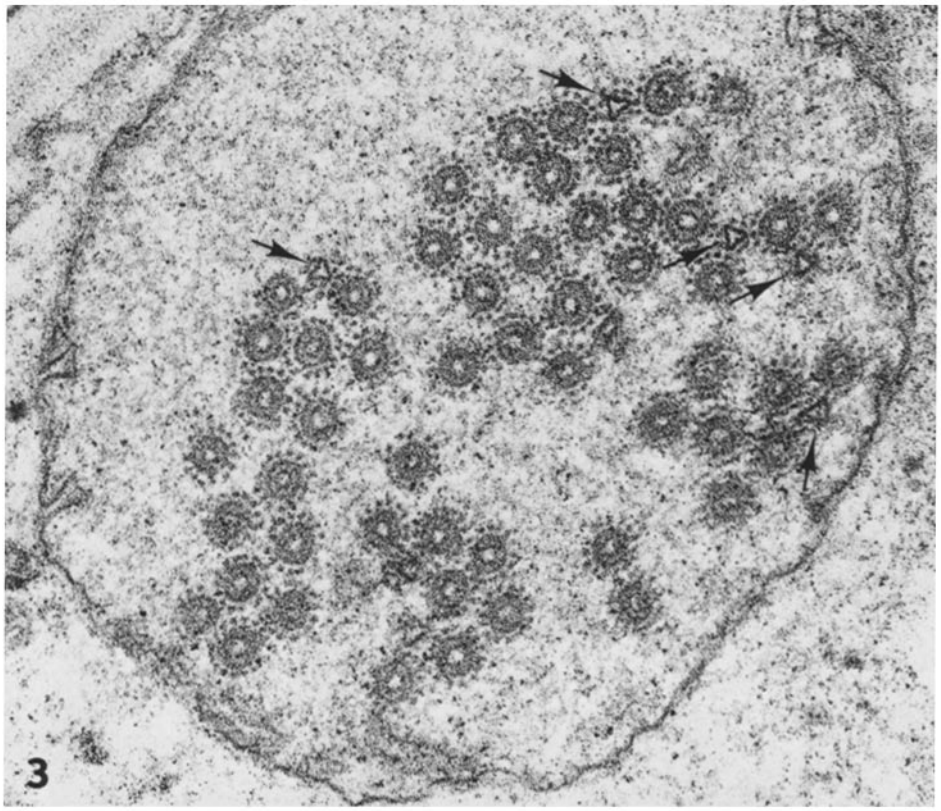


FIGURE 1 Mitochondrion within a somatic muscle cell of *T. aceti* with the "doughnut" cristal configuration (→) and a paracrystalline array (*P*). Other mitochondria in the cell contain normal appearing cristae. ×33,000.

FIGURE 2 Cristae, doughnut-shaped, in cross section surrounded by opaque dots (→), triangular cristae (T→), parallel tubules (*L*), and paracrystalline arrays (*P*) within a large mitochondrion. ×33,000.



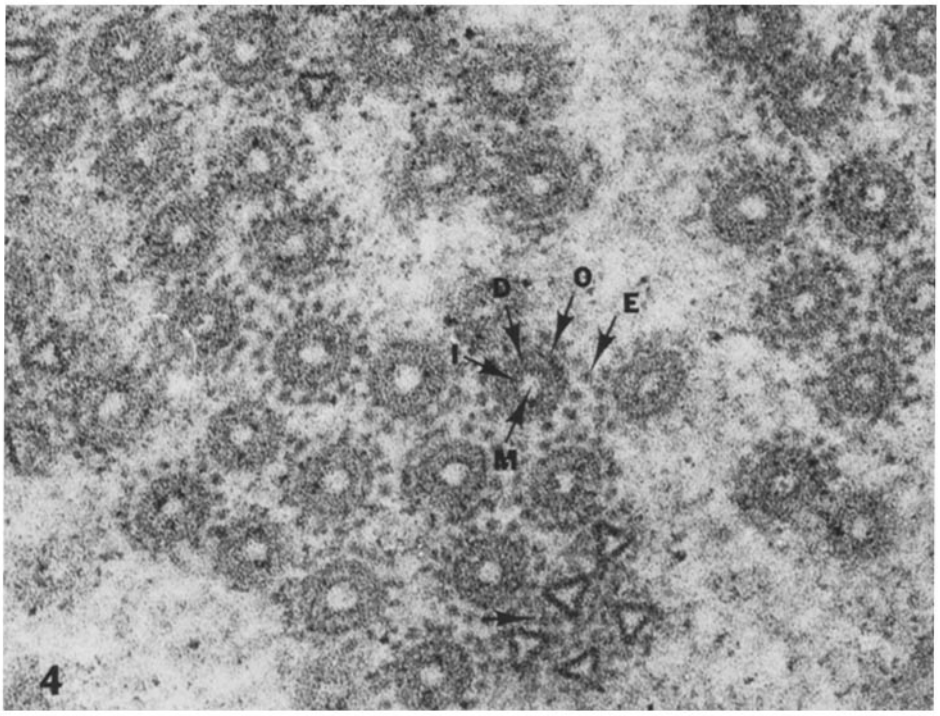


FIGURE 3 Several triangular cristae (→) amidst numerous doughnut-shaped cristae. ×100,000.

FIGURE 4 The doughnut-shaped cristae: encircling electron-opaque particles (*E*→), external membrane (*O*→), electron-dense area between two membranes (*D*→), internal membrane (*I*→), material encircled by the internal membrane of about the same density as the matrix (*M*→). Also a cluster of five triangular cristae (→). The micrograph shows the greater thickness of the triangle sides as compared to the internal or external membranes. ×150,000.

intestinal epithelial cells, and the gonad epithelium cells of *T. aceti* (Fig. 1). In some tissues mitochondria with these abnormal cristae were abundant. For example, 6 of 12 mitochondria within one section of an intestinal epithelial cell contained cristae of the type described herein. These structures were not observed within esophageal tissues. The doughnut-shaped structures consisted of two concentric membranes ~200 Å apart and having an outer diameter of 800 Å (Fig. 4). The internal membrane surrounded material of about the same appearance as the matrix, whereas between the two concentric circles the material was of greater electron density. The external membrane was encircled by electron-opaque particles, ~100 Å in diameter, regularly spaced ~125 Å from each other and ~100 Å from the external membrane. These opaque particles are identical in location and appearance to the "structural material" surrounding prismatic cristae described by Korman et al. (3) and Morales and Duncan (4). Triangular cristae most frequently occurred singly in about a 1:8 ratio to the doughnuts (Fig. 3); however, in some sections they occurred in small clusters (Fig. 4). Electron-opaque particles were not clearly seen as encircling the triangular cristae, but similar particles were frequently seen in less ordered arrangement around the triangles (Fig. 4). The triangles appeared to be equilateral, with each side being ~500 Å in length. The sides of the triangles were of greater thickness than either the internal or the external membrane (Fig. 4). Atypical cristae were observed in nematodes from both cultures examined and in 12-day-old and 37-day-old nematodes.

Paracrystalline array patterns were also observed in a few giant mitochondria, most frequently occurring in conjunction with triangular and doughnut-shaped cristae (Figs. 1–2) but, in one case, occurring alone. Parallel tubules characterized by dark parallel lines separated by lines of lesser density were also observed (Fig. 2).

The described structures were visualized in both Epon 812 and Spurr's (5) embedding media only when glutaraldehyde followed by osmium tetroxide was used as a fixative. These structures were not seen in tissues fixed with a combination of glutaraldehyde, acrolein, and dimethylsulfoxide nor in those fixed with osmium tetroxide alone.

DISCUSSION

One explanation for the doughnut-shaped structures is that they represent cross sections through cristae with invaginated membranes as shown in Fig. 5. If this is the case then the area surrounded by the internal membrane consists of matrix material, and the area between the two concentric membranes is intracristal space. The triangular structure (Fig. 5 B-1), in the authors' opinion, is a view near the apex of the membrane invagination where the internal and external membranes are appressed (Fig. 5 A-1). This opinion is supported by the observed greater thickness of the triangle sides as compared to the internal and external membranes (Fig. 4). Watson (6) made electron microscope observations of *T. aceti* mitochondria from the hypodermal and somatic muscle tissues, but did not describe unusual cristae. This may be explained by her use of osmium tetroxide alone as a fixative, since in the current study the cristal configurations were not visualized when this fixation procedure was used (Table I).

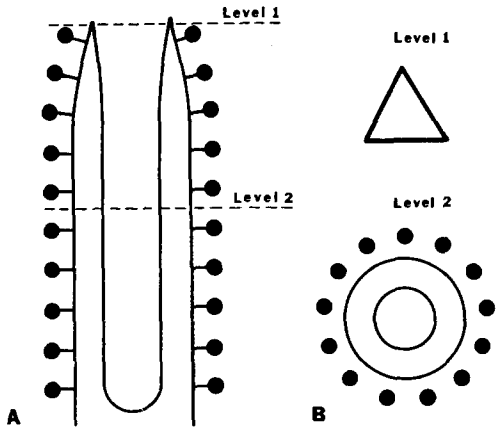


FIGURE 5 Schematic interpretation of the cristal configurations in mitochondria of *T. aceti*. (A) A longitudinal view. A cross section through level 1 where the membranes are appressed yields a thick-walled triangle (B-1), whereas a cross section through level 2 yields two concentric membranes (B-2).

CONCLUSION

Mitochondria with unusual cristae are described from the vinegar eelworm. In cross section the cristae are most frequently doughnut-shaped and are encircled by electron-dense particles. Less frequent are cristae which are triangular in cross section. These configurations are believed to derive from invaginations of the cristal membrane. Parallel tubules and paracrystalline arrays also occur in these mitochondria.

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