THE CHONDRIOME OF SELECTED TRYPANOSOMATIDS

A Three-Dimensional Study Based on Serial Thick Sections and High Voltage Electron Microscopy

JEROME J. PAULIN

From the Department of Zoology and the Central Electron Microscope Laboratory, University of Georgia, Athens, Georgia 30602

ABSTRACT

The unitary nature of the chondriome of two species of trypanosomatids, *Blastocrithidia culicis* and *Trypanosoma cruzi*, has been demonstrated by utilizing serial thick-sectioning techniques combined with high voltage electron microscopy. Profiles of mitochondrial elements seen in thin sections and suspected to be parts of a continuum were confirmed by serial thick sectioning (0.25–0.50 μm thick) and stereopair analysis to be parts of the same mitochondrion. Three-dimensional models obtained from tracings of mitochondrial profiles on cellulose acetate reveal the mitochondrion of *B. culicis* to consist of a posterior mass with six tubular extensions extending upward and terminating in the anterior apex. The kinetoplast was found suspended between two of the tubular extensions, or less frequently, protruding as a nodule from one of the extensions. A bifurcation of one of the extensions was found in some specimens. The mitochondrion of *T. cruzi* consists of a triangular-shaped convoluted tubule, the base being the kinetoplast portion while the apex is directed posteriorly. The mitochondrion bifurcates behind the flagellar pocket, lateral to the kinetoplast, sending two entwined extensions into the tenuous anterior apex. Whether the mitochondrion of *T. cruzi* is unitary in the trypomastigote form was not determined in this study, since only epimastigote forms were used.

For over a decade it has been speculated that the chondriome of the trypanosomatids is unitary, i.e., morphologically either a simple vermiform organelle or a highly branched reticulum. This hypothesis is based on random electron micrographs of fortuitous sections or light microscopical observations demonstrating NADH-tetrazolium reductase activity (17). These techniques provide only limited data, for the former is abstracting a three-dimensional object from two dimensions while the latter suffers from lack of resolution. Vickerman (18, 19) and Brown et al. (4) have postulated that in the *T. brucei* group of trypanosomes pleomorphism may be the result of changes in mitochondrial configurations in response to metabolic changes elicited by host and vector environments. Culture or insect forms have reticulated mitochondria while their blood stream counterparts have simple tubular-shaped mitochondria. Growth and/or regression of the mitochondrion elicit morphotypic epimastigote forms, respectively.

The purpose of this investigation is to show that

the mitochondria of two species of trypanosomes *Blastocrithidia culicis*, a monomorphic insect parasite, and *Trypanosoma cruzi*, a pleomorphic mammalian form, are indeed unitary. Three-dimensional models have been constructed from micrographs, taken in the high voltage electron microscope, of serially thick-sectioned organisms as well as from stereopair analysis of the mitochondrion. A portion of this work has been published elsewhere (15).

MATERIALS AND METHODS

Axenic cultures of *B. culicis* (with symbiont) were maintained in 50 ml of a modified Cowperthwaite media (6) in 125-ml Erlenmeyer flasks at 24–26°C. Epimastigotes of *T. cruzi* (Goble, Brazilian strain kindly supplied by Dr. William Hanson, Veterinary School, University of Georgia, Athens, Ga.) were grown in LIT medium (13) in 125-ml Erlenmeyer flasks. Cells harvested in the logarithmic phase of growth were concentrated by light centrifugation and fixed for 30 min in 1.5% glutaraldehyde buffered in 0.1 M cacodylate (pH 7.2), washed in buffer, and postfixed for 1 h in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2). Alternately, in lieu of double fixation, *B. culicis* were fixed for 1 h in diluted 2% Veronal-acetate-buffered osmium tetroxide (1 part buffer, 3 parts distilled H_2O pH 7.2); this fixative swells the cells slightly, which facilitates the study of the mitochondria. Regardless of the fixative, the cells were dehydrated in ethanol and embedded in a mixture of Epon (Shell Chemical Co., New York) and Araldite (Ciba Products Co., Summit, N. J.) (14). Thin sections were cut on a Porter-Blum MT_2 microtome, stained with 7.5% uranyl magnesium acetate (distilled H_2O) for 15 min followed by lead citrate (3 min), and viewed in a Siemens 101 or Elmiskop 1 electron microscope.

High Voltage Microscopy

The procedures for fixation and embedding are as described above. Ribbons of serial thick sections (0.25 μm, 0.50 μm, and 1 μm, based on interference colors and microtome settings) were picked up on Formvar films (Belden Mfg. Co., Chicago, Ill.) (0.5% in ethylene dichloride), mounted on copper wire loops (5 mm diam), and transferred to slot grids (1 × 2 mm rectangle). The sections were stained for 4 h at 50°C in 7.5% uranyl magnesium acetate, followed by 20 min in stock Reynolds's lead citrate solution. After staining, a thin layer of carbon was evaporated over the grids.

The AEI EM7 high voltage electron microscope operated at 1,000 kV with a 25-μm objective aperture was used in taking the micrographs. All micrographs were taken on Kodak Estar base cut film (Eastman Kodak Co., Rochester, N. Y.) (4,489), at initial magnifications of X 4,000, X 6,300, or X 8,000.

Three-Dimensional Models

Mitochondrial profiles and appropriate reference structures (e.g., flagella, cell membrane profiles, or adjacent cell profiles), used to prevent positional errors, were traced directly from the negatives on thin sheets of cellulose acetate (0.005 inches thick) with a rapidograph pen (size 00). The thin cellulose acetate sheets were sandwiched between 0.02-inch thick cellulose sheets; this procedure was repeated until all profiles in the series had been included. The entire assembly was flooded with acetone, gluing the sheets together. With the aid of a jeweler's saw, the model was cut from the cellulose acetate and smoothed and sprayed with white paint. Tracings were also made directly on the 0.02-inch acetate sheets, and these in turn "glued" with acetone. To facilitate cutting, a series of tracings were broken up into units, e.g., 6 units of three each in an 18-section series. Each unit was cut out, leaving narrow attachments between reference structures and mito-

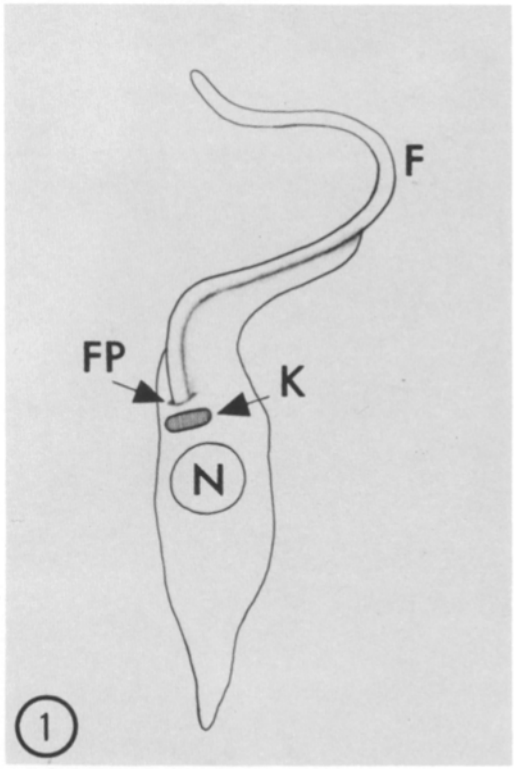
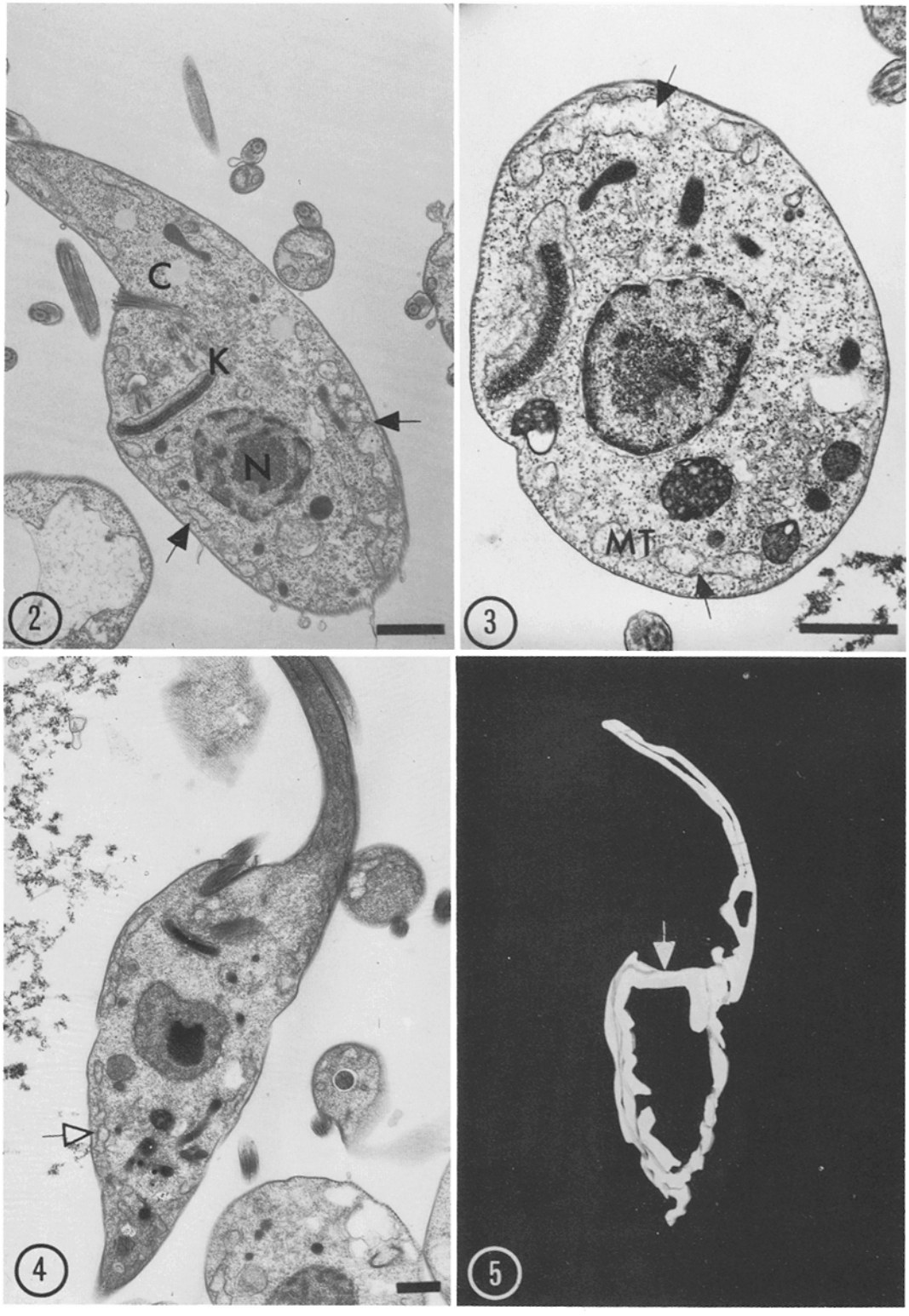


FIGURE 1 Diagram of the epimastigote form of *T. cruzi* and *B. culicis*, indicating position of kinetoplast (*K*), nucleus (*N*), flagellum (*F*), and flagellar pocket (*FP*).


C
K
N
2
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5

chondrial profiles, and assembled with adjacent units; the attachments were removed after final assembly. Mitochondrial profiles were traced from 14 specimens of *T. cruzi* either from 0.25-μm or 0.50-μm thick serial sections. 12 separate *B. culicis* mitochondria were traced from 0.50-μm thick serial sections. Due to the thin vermiform mitochondrial extensions found in the anterior extremities of *T. cruzi*, 0.25-μm thick sections were utilized; thicker sections included large portions of the mitochondrion, making reconstructions difficult.

RESULTS

Both species examined are epimastigote forms and are shown diagrammatically in Fig. 1. In these, the kinetoplast (*K*, Figs. 1, 2) and flagellar apparatus are anterior to the nucleus. A short undulating membrane is formed by the juxtaposition of the flagellum to the cell body; approximately one-half of the flagellum extends freely from the anterior apex of the cell. The flagellar pocket, a small depression in the cell surface, forms an atrium for the cryptic cytostome (*C*, Fig. 2) and emerging flagellum (Figs. 1, 2, 7). A single file of microtubules, running parallel to long axis of the cell, is found beneath the cell membrane in both species (Figs. 3, 6, 7).

In random thin sections of *T. cruzi*, some indication of a possible continuous mitochondrial reticulum can be observed. Irregular beadlike mitochondrial profiles are seen in longitudinal (arrows, Figs. 2, 4) and cross sections (arrows, Fig. 3). This is not the case in *B. culicus* where the mitochondrial profiles appear as discrete ovoid or elliptical bodies (Figs. 6, 7). An indication of an anastomosis of a portion of the chondriome is suggested in fortuitous sections through the posterior portion of *B. culicis* (Fig. 8). In this organism, where mitochondrial profiles are appressed to the cell membrane the subpellicular microtubules are generally absent. This is true only of *B. culicis* and of no other trypanosomatid studied to date.

The information content obtained from a given section increases considerably with an increase in section thickness, particularly form and spatial relationship between organelles. This becomes evidently clear in stereopair analysis, which eliminates overlap of structures, and in reconstructions obtained from micrographs of serial thick sections taken with the high voltage microscope. Fig. 4 is a micrograph of a 0.25-μm thick section of *T. cruzi*. A reticulated mitochondrial network, not unlike that seen in thin sections (≤800 Å), can be observed in anterior and posterior portions of the cell (cf. Fig. 2 with Fig. 4) without loss of resolution and contrast. However, a 0.5-μm thick section (Fig. 9) of *B. culicis* clearly shows the continuity of the chondriome only suggested in thin sections. The elliptical mitochondrial profiles seen in thin sections (Figs. 6, 7) are observed in thick sections as tubular extensions of the posterior anastomosis. Stereopair analysis reveals the continuity of several mitochondrial extensions in the cell body (Fig. 10).

Reconstructions of the mitochondrion of *T. cruzi* (Fig. 5) clearly indicate the unitary nature of this organelle. A single loop studded with villus-like projections courses through the body proper, while two entwined finger-like projections extend into the anterior apex of the cell. The mitochondrial wall is folded, not smooth and uniform as in *B. culicis*, which may explain its beadlike appearance in thin sections. The model (Fig. 5) and illustration (Fig. 11) clearly reflect the irregular nature of the chondrial wall. The kinetoplast is contained within the anterior portion of the somatic loop (arrow, Fig. 5).

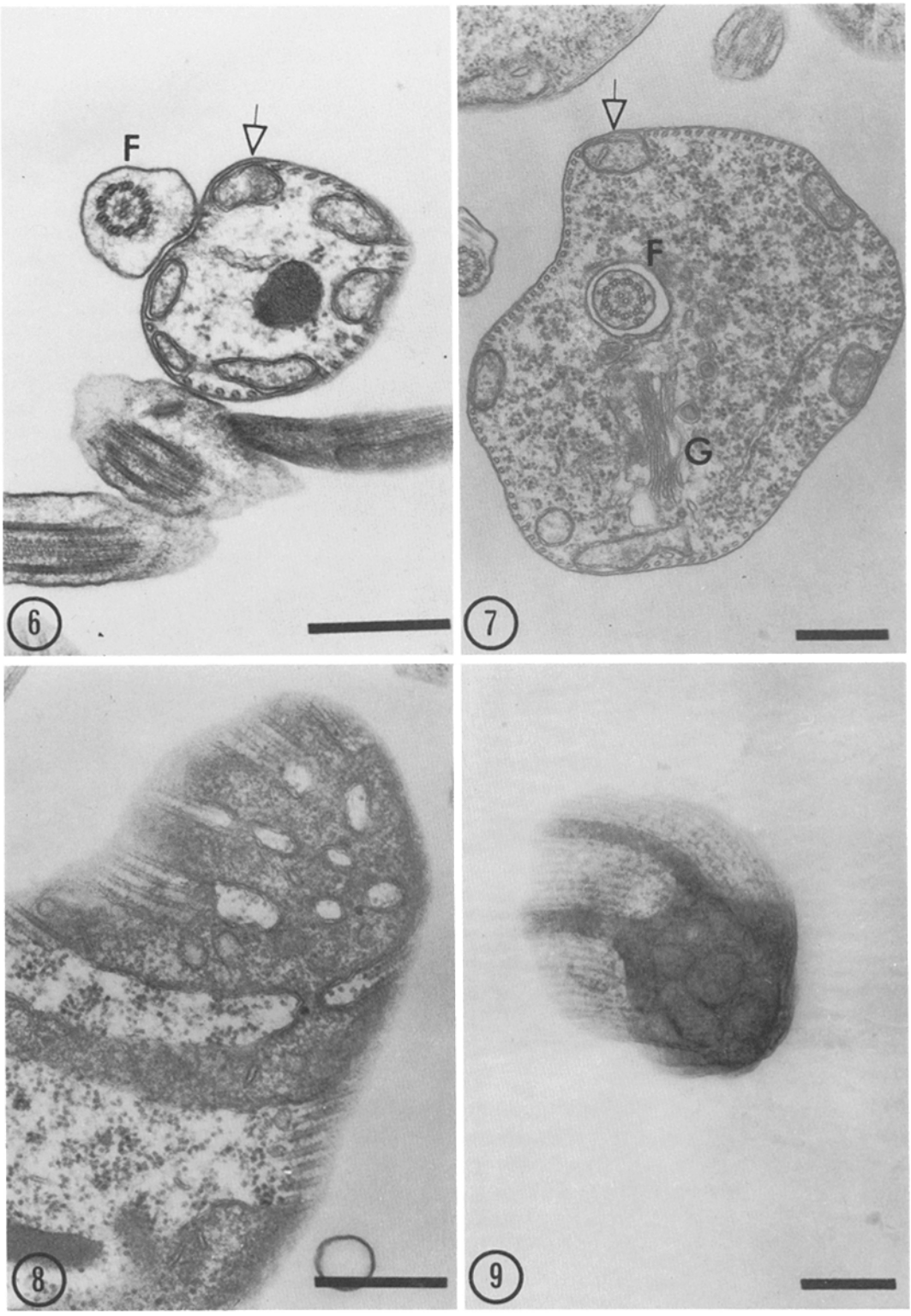
The mitochondrion of *B. culicis* is strikingly different from that of *T. cruzi*. In this species, six

FIGURE 2 Longitudinal thin section through *T. cruzi* showing extensive beadlike mitochondrial profiles (arrows), *K*, kinetoplast; *N*, nucleus; *C*, cytostome; × 10,800; calibration bar, 1.0 μm.

FIGURE 3 Oblique thin section through *T. cruzi* revealing the subpellicular microtubular system (*MT*) and mitochondrial ramifications (arrows); × 15,300; calibration bar, 1.0 μm.

FIGURE 4 0.25-μm thick section cut longitudinally through *T. cruzi*. The extensive mitochondrial reticulum is evident, particularly in the posterior portion of the cell (arrow); × 6,400; calibration bar, 1.0 μm.

FIGURE 5 Cellulose acetate model of *T. cruzi* mitochondrion derived from serial thick sections; arrow indicates position of kinetoplast.


F
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F
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tubular extensions course in a helical spiral through the cell body from the posterior mitochondrial mass and terminate in the anterior apex. These extensions lack the villus-like projections seen in *T. cruzi*, but on occasion, one of the extensions is bifuricated (Fig. 12); thus, when viewed in cross section, seven profiles would be seen. During fixation and processing for electron microscopy, *B. culicis* became pyriform shaped rather than the more characteristic lancet-like shape seen in living cells. Fig. 12, an illustration of a mitochondrion, was taken from a reconstruction of a near normal-shaped cell. In this illustration, one of the mitochondrial arms is bifurcated, and the kinetoplast is suspended by two mitochondrial extensions. The stereophotographs in Fig. 13 were taken of a cellulose acetate model reconstructed from a pyriform-shaped cell, the cell being compressed on the vertical axis. The illustration in Fig. 14 is based on the same cell and shows, like the model, a disjunction between the kinetoplast and one mitochondrial extension.

DISCUSSION

Reconstructions based on serial thick sections and stereoscopic photography confirm the unitary nature of the chondriome of *B. culicis* and *T. cruzi* epimastigotes. Morphotypic differences in the mitochondria of these species probably reflect generic differences. *B. culicis*, a monomorphic form, showed only minor differences in chondriome anatomy, these being occasional bifurcations of one of the tubular extensions or a discontinuity between one of the extensions and nodular kinetoplast. These could be interpreted as possible early stages of replication of the chondriome. Replication of this organelle has not been studied, but it is well established in other trypanosomotids that kinetoplast replication precedes karyokinesis, temporally and spatially, during the cell cycle (5). Therefore, autonomous replication can be ruled out; genesis of this complex organelle during division is an intriging problem still to be investigated. Atkinson et al. (1) have shown by three-dimensional reconstructions that the chondriome of *Chlorella fusca* is a reticulated unitary organelle and that during cell division the mitochondrial branches are pinched off by the developing septum. The corrugated-appearing and convoluted mitochondrial loops seen in *T. cruzi* appear to be characteristic of this species, if compared to those described in other published work (2). The irregular beadlike configurations may be an indication of the plasticity of this dynamic organelle. In *T. cruzi*, extensive changes in mitochondrial morphology and physiology are not profound between host-vector or culture forms as in the *T. brucei* group of trypanosomes (2, 4, 18, 19). However, *T. cruzi* is pleomorphic, and if Vickerman (18) is correct, changes in cell shape are at least in part associated with growth and/or regression of the mitochondrion, which alter the position of the kinetoplast, e.g., anterior to the nucleus in epimastigotes, posterior in trypomastigotes. Quantitative morphometric data obtained from *T. brucei* indicate that absolute mitochondrial volume increases fourfold from trypomastigote to stumpy form (9, 10). Although no quantitative morphometric data are available for *T. cruzi*, Brack (2) reports that trypomastigote forms have dense, slender mitochondrial profiles compared to epimastigote counterparts.

Whether the unitary nature of the chondriome is a common feature among protists remains to be seen. Leedale and Buetow (12) have shown by phase-contrast microscopy that the chondriome of *Euglena gracilis* is a dynamic, ever-changing organelle. Three-dimensional reconstructions of yeast mitochondria reveal conflicting results. Hoffman and Avers (11) demonstrated in *Saccharomyces cerevisiae* a unitary chondriome.

FIGURES 6 and 7 Thin sections through the anterior apex of *B. culicis* above the kinetoplast. Six discrete mitochondrial profiles can be seen in each micrograph. Notice the absence of subpellicular microtubules at points where the mitochondrial extensions are appressed to the cell membrane (arrows); *F*, flagellum; *G*, Golgi apparatus. Fig. 6, × 44,000; Fig. 7, × 28,600; calibration bars, 0.50 μm.

FIGURE 8 Glancing thin section through the posterior mitochondrial mass. × 40,500; calibration bar, 0.50 μm.

FIGURE 9 0.50-μm thick section through the posterior portion of *B. culicis*. The large mitochondrial mass and two extensions can be seen. × 14,800; calibration bar, 1.0 μm.

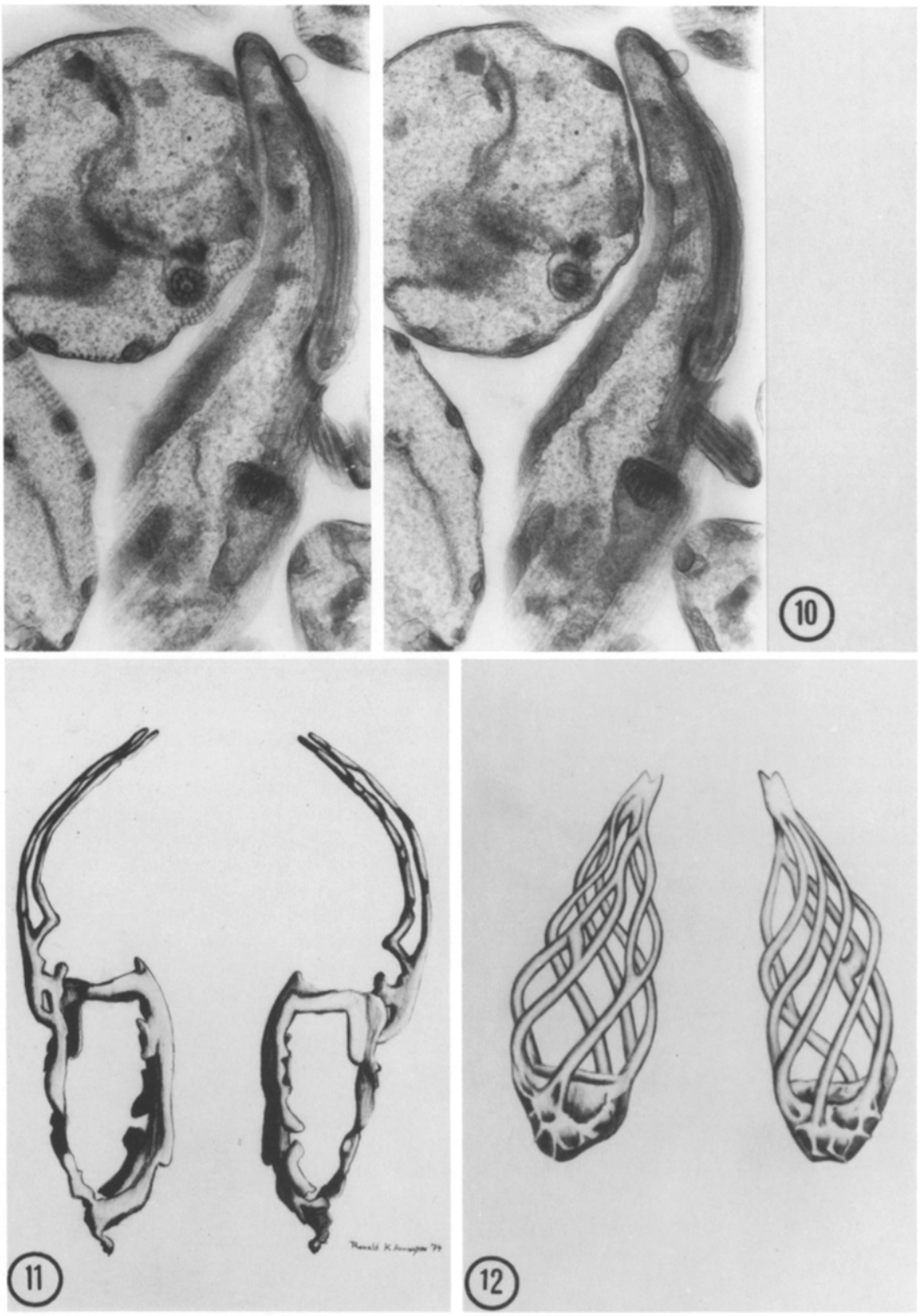
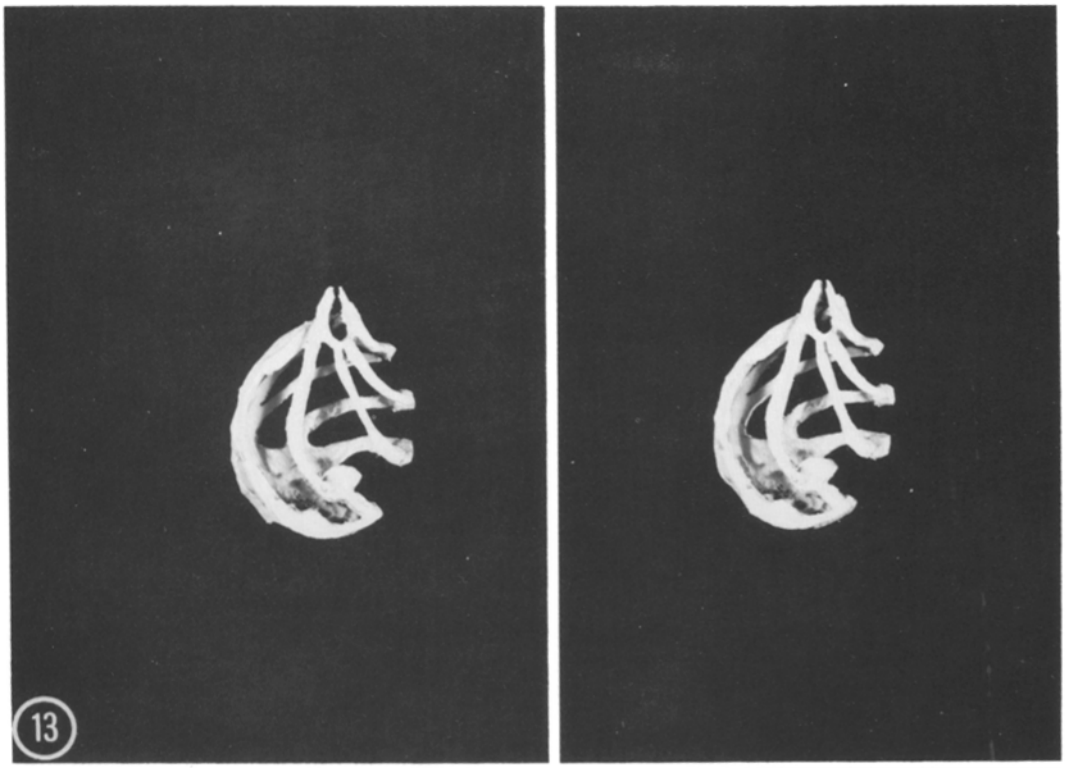


FIGURE 10 Stereopair of 0.50-μm thick sections of *B. culicis* showing portions of the mitochondrial extensions. Kinetoplast is at viewer's lower right; × 13,000.

FIGURE 11 Artist's illustration of the mitochondrion of *T. cruzi*.

FIGURE 12 Artist's illustration of the mitochondrion of *B. culicis*. In this illustration the continuity between two extensions of the mitochondrion and kinetoplast is evident. Note the bifurcation of one of the mitochondrial extensions.



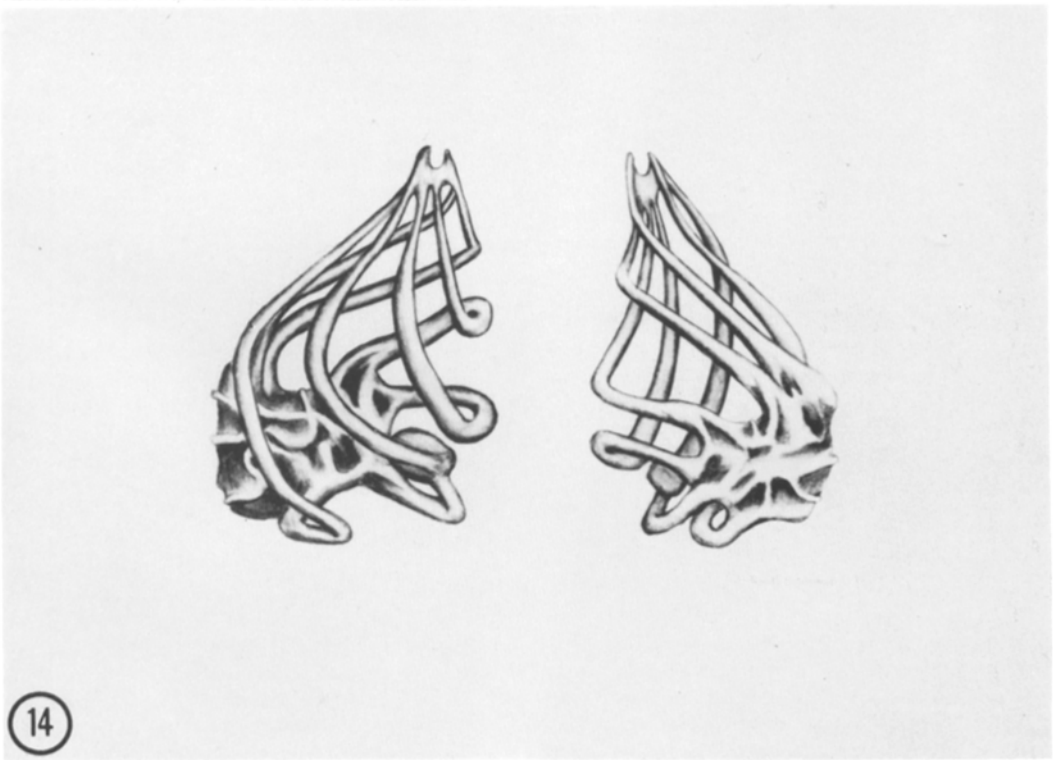


FIGURE 13 Stereopair of mitochondrial model of *B. culicis*. The cell is pyriform shaped rather than the typical elongate form seen in Fig. 12.

FIGURE 14 Artist's illustration of *B. culicis* model showing ventral surface (viewer's left) and dorsal aspects. The kinetoplast is in the lower right of the left illustration. The mitochondrial mass shows the convolutions seen in Fig. 9.

Grimes et al. (8) found that, in four strains of *S. cerevisiae* grown on lactate in the absence of catabolite repression, the chondriome was not unitary but fragmented. Davison and Garland (7) concluded that mitochondrial profiles seen in thin sections of *Candida utilis* represented parts of a mitochondrial reticulum when viewed in thick sections (0.5 μm) in the high voltage electron microscope. The water mold, *Blastocadiella emersonii*, has a single unitary cup-shaped mitochondrion in the zoospore stage, fragmenting into several separate mitochondria during germling development (3).

We can conclude, at this point, by indicating that in the case of *C. fusca* and the trypanosomatidae studied to date, the chondriome is unitary under the physiological conditions at the time of fixation. Variations in nutrition, position in the cell cycle, and/or life cycle will be reflected in chondriome morphology as also will be the effects of fixation and subsequent handling.

Two factors, electron penetration and adequate contrast obtainable in thick biological specimens (16), make the high voltage electron microscope a useful tool to probe not only the anatomy of an organelle or its spatial relationships within the cell, but also its morphogenesis in the cell cycle. This is particularly true in the trypanosomatids.

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