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SYNOPSIS. The kinetoplast of *L. tarentolae* remains attached to the basal body upon cell rupture by detergent lysis, sonication, or hypotonic lysis in 0.02 M Tris buffer (pH 7.9) at 0-4 C. Hypotonic lysis in 0.02 M Tris-HCl-2 mM EDTA at 0-4 C and application of mild shearing forces bring about release of most of the swollen kinetoplasts. The kinetoplast DNA can be seen in phase contrast microscopy as a dark mass contiguous to the kinetoplast membrane directly opposite the basal body. Upon return to isotonic media, the kinetoplast shrinks; the membranes of such kinetoplasts are impermeable to added DNAase.

THE kinetoplast of the hemoflagellates is part of the mitochondrion in which a large amount of DNA is concentrated. It is probable that there is only one mitochondrion per cell thruout the hemoflagellates. The mitochondrial nature of the kinetoplast was established by electron microscopy(9,4,16,17,21,25), Janus green staining(18) and histochemical staining for NADH diaphorase(22). The existence of DNA within the organelle was revealed by Feulgen staining(3), acridine orange staining(2), deoxyribonuclease digestion(5), and incorporation of H_3-thymidine into deoxyribonuclease-sensitive material(8,23). The kinetoplast DNA seems to be concentrated in a fibrous, elongated structure situated in the anterior portion of the organelle(14,15). The kinetoplast DNA is double-stranded and differs in buoyant density from the nuclear DNA(6,13,19,20); its molecular weight is unknown.

Historically, the term "kinetoplast" stood for the darkly stained granule seen at the base of the flagellum at the light microscope level (Fig. 1). On the electron microscope level the kinetoplast is a slightly concave, disc-shaped structure containing typical mitochondrial cristae and the fibrous DNA mass. In general, the disc-shaped portion of the chondriome is a permanent fixture of the cell, whereas the mitochondrial ramifications are variable, both in extent and appearance(16,26).

This communication describes the appearance of the kinetoplast in *Leishmania tarentolae* and the behavior of the kinetoplast upon cell rupture. Evidence will be presented for the existence of a connection between the kinetoplast and basal body and for the attachment of the kinetoplast DNA to the kinetoplast membrane.

MATERIALS AND METHODS

Cells. A strain of *Leishmania tarentolae* was used which has been growing continuously in a defined medium(24) since 1959. The cells were grown at 27 C in the dark in 30 ml of Medium C in stationary 250 ml Erlenmeyer flasks equipped with cotton plugs wrapped in gauze and covered with parafilm.

Detergent lysis. Cells were washed 3× in 0.25 M sucrose at 0-4 C. The pellet (0.1 ml) was resuspended for 45 sec in 5 ml of 0.25 M sucrose-2% Tergitol TP-9†-1% bovine serum albumin by use of a test tube mixer. The suspension was kept on ice for 10 min and then "buzzed" again for 45 sec. $CaCl_2$ was added to a final concentration of 1.5 mM.

Hypotonic lysis.

a. *Tris.* Cells were washed 3 times in 0.25 M sucrose-0.02 M Tris-HCl (pH 7.9) and kept on ice for 3-5 hr with occasional "buzzing."

b. *Tris-EDTA.* Cells were washed as above and resuspended in 10 volume of 0.02 M Tris-HCl-0.002 M EDTA (pH 7.9) at 0-4 C. The suspension was kept on ice for 3-5 hr with occasional "buzzing" and then passed 3-4 times thru a #26 needle. The free swollen kinetoplasts were centrifuged down at 4000 *g* for 20 min.

Fluorescence microscopy. Lysates were stained for 10 min at 27 C with 10 μg acridine orange (Allied Chemical Corporation, National Aniline Division) per ml in the lysis medium or in 0.25 M sucrose-0.02 M Tris-HCl (pH 7.9). The stained material was examined in a Zeiss fluorescent microscope using excitor filters BG-3 and BG-12 and barrier filters 50-47.

RESULTS

The entire chondriome of *L. tarentolae* (Fig. 1) can be seen *in vivo* by phase contrast microscopy as a thread-like structure extending the length of the cell (Fig. 2).

Upon cell rupture by a number of means, the chondriome, or at least the disc-shaped portion which contains the DNA, usually remained attached to the basal body. This is illustrated in Fig. 3, which shows a Giemsa-stained preparation produced by lysis with the non-ionic detergent, Tergitol TP-9. Lysis was inhibited by Ca^{++} or Mg^{++}, and therefore the addition of 1.5 mM $CaCl_2$ was used to stop the reaction. The kinetoplast remained next to the basal body even tho the cell membrane ruptured and the nucleus and much cytoplasmic material dispersed. The kinetoplast membrane, however, in this case had been rendered permeable to added DNAase.

Similar phenomena were observed upon mild sonication in 0.25 M sucrose-1.5 mM $CaCl_2$-1% bovine serum albumin, or upon hypotonic lysis in dilute Tris buffer (pH 7.9) at 0-4 C. As shown in Fig. 4, the Tris-lysed cells ruptured and released the nucleus, but retained the swollen kinetoplast (K). There was some heterogeneity in the size of the swollen kinetoplasts (Fig. 4A). It is likely that the swollen kinetoplast included the entire chondriome, but this was not proven.

Often, as in Figs. 4B, C & E, the "ghost" inverted and yet the kinetoplast apparently remained attached to the basal body. Occasionally the kinetoplast broke free as in Fig. 4E. Upon return to isotonic media (0.25 M sucrose)

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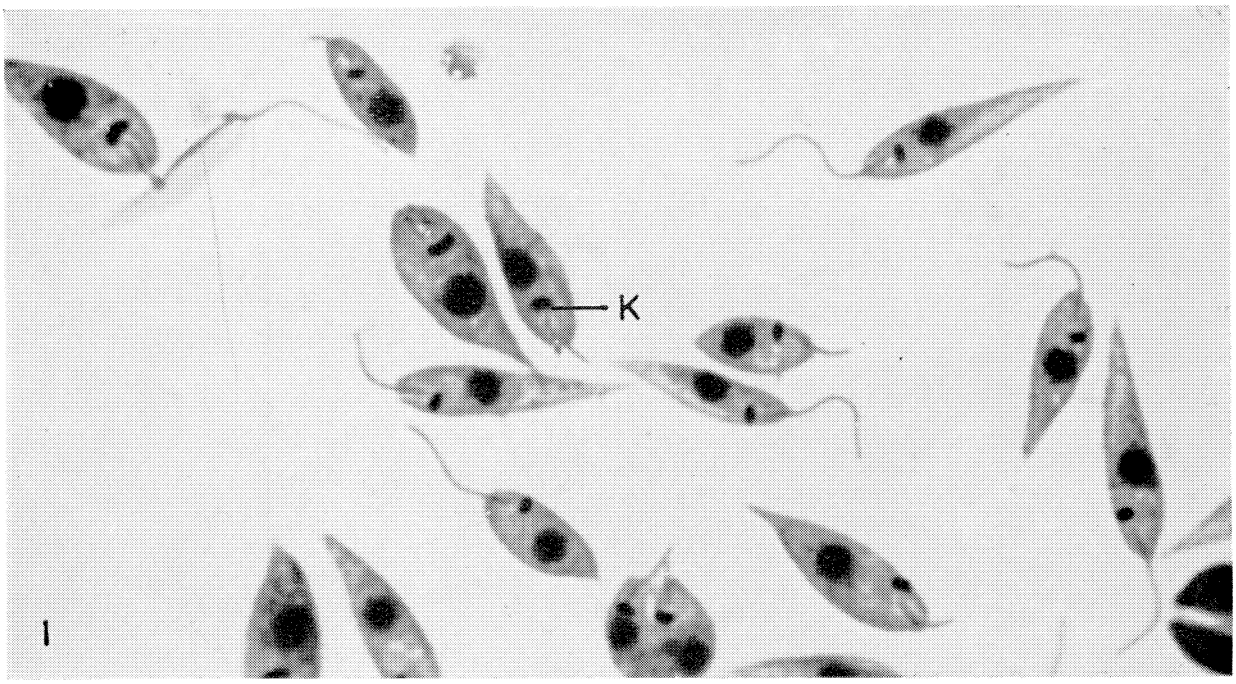
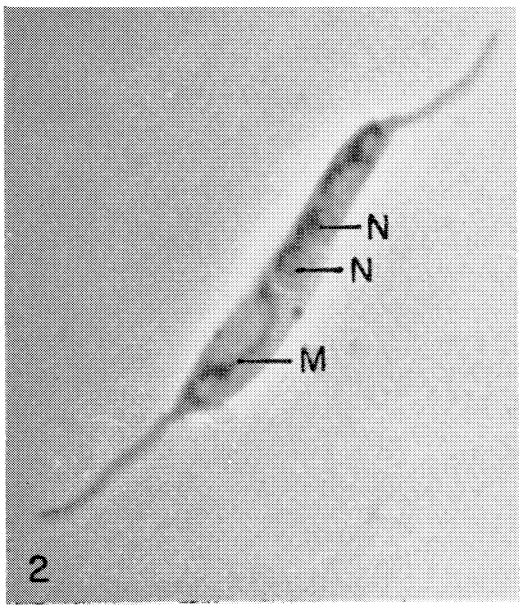
† Union Carbide Chemical Company





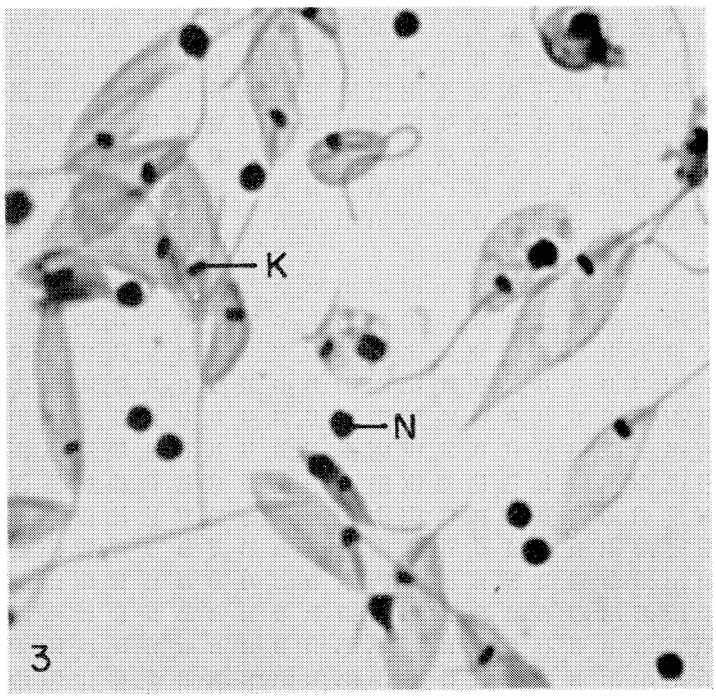


Fig. 1. *Leishmania tarentolae* cells showing the stained kinetoplast (K). Giemsa stain. × 1,250.

Fig. 2. A dividing *L. tarentolae* cell. The thread-like mitochondrion (M) and two nuclei (N) can be seen. Phase contrast. × 1,250.

Fig. 3. Detergent-lysed preparation of *L. tarentolae*. Free nuclei (N) and ghosts containing stained kinetoplasts (K) are shown. Giemsa stain. × 1,250.

the kinetoplast shrank to nearly its original size and shape (Fig. 5).

Addition of 2 mM EDTA to the Tris buffer and application of mild shearing forces, such as several passages thru a #26 needle, brought about the release of a good percentage of the swollen kinetoplasts from the basal bodies. This possibility indicates that the attachment involves divalent ions which are removed by the chelating agent, but other explanations are also possible.

These kinetoplasts were impermeable to DNAase (20 μg/ml-30 min-27 C-in 0.25 M sucrose-1.5 mM MgCl). The DNA of the liberated and lysed nuclei, however, was destroyed by such treatment, as verified by acridine orange staining.

The DNA in the swollen kinetoplasts obtained by Tris or Tris-EDTA lysis appeared in phase contrast microscopy as a clumped mass situated next to the kinetoplast membrane directly opposite the basal body (Fig. 4). Evidence

that this material was kinetoplast DNA was obtained by fluorescence microscopy of acridine orange-stained lysates. Also, the dark mass was not present in swollen kinetoplasts obtained by Tris-EDTA lysis of acriflavin-induced, dyskinetoplastic cells (Fig. 6), which are known to lack kinetoplast DNA(20,25). It is of some interest that the kinetoplast DNA always remained adjacent to the membrane at a certain location. It is likely that the DNA structure is membrane-bound.

DISCUSSION

The existence of one large, convoluted mitochondrion per

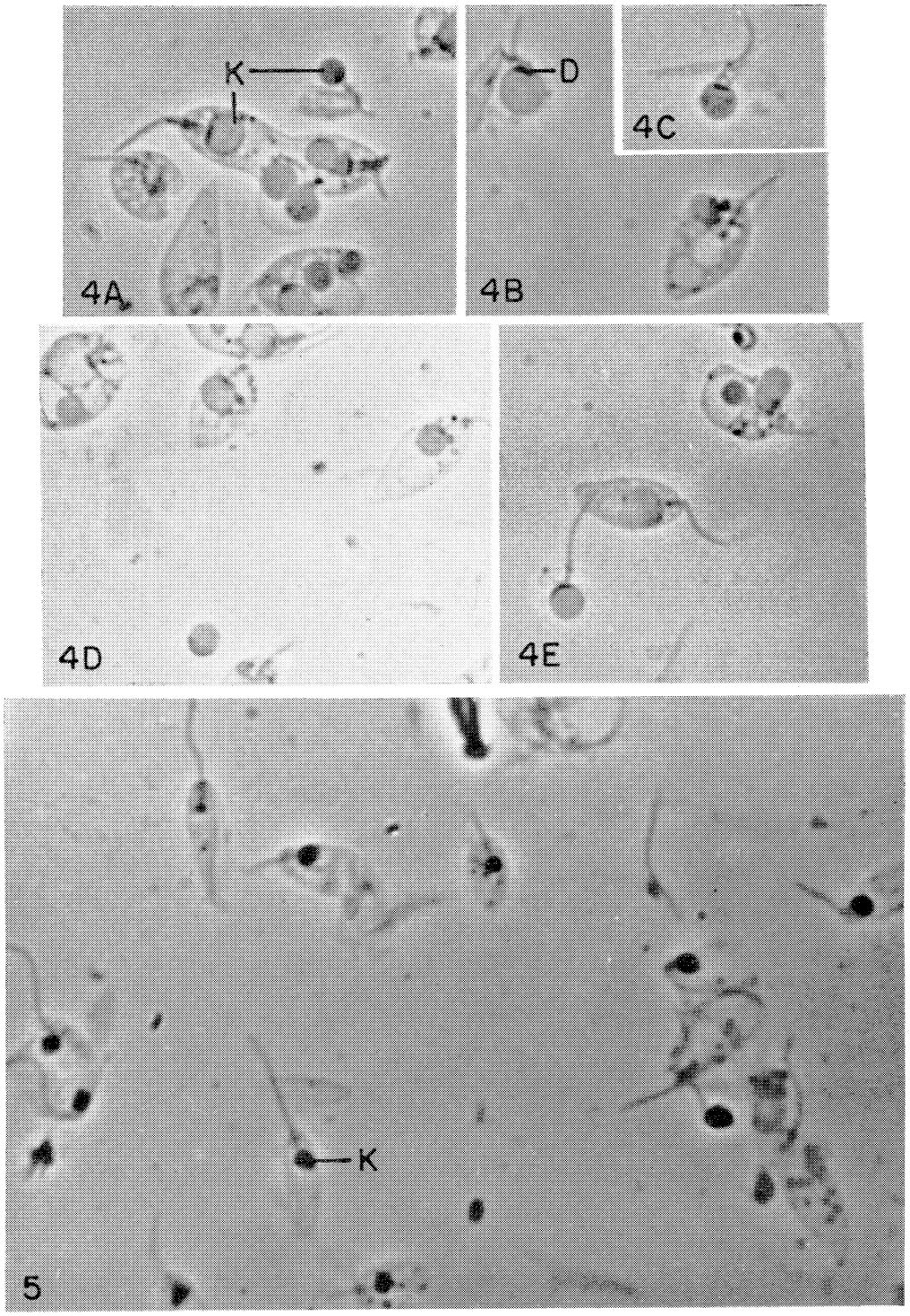


Fig. 4. Hypotonic Tris-lysed preparation of *L. tarentolae*. The kinetoplast (K) is the swollen vesicle at the base of the flagellum. The kinetoplast DNA (D) is the dark clump next to the membrane directly opposite the basal body. Phase contrast. × 1,250.

Fig. 5. Tris-lysed ghosts of *L. tarentolae* after resuspension in 0.25 M sucrose-1.5 mM $MgCl_2$. The kinetoplast (K) is the dark structure at the base of the flagellum. Phase contrast. × 1,250.

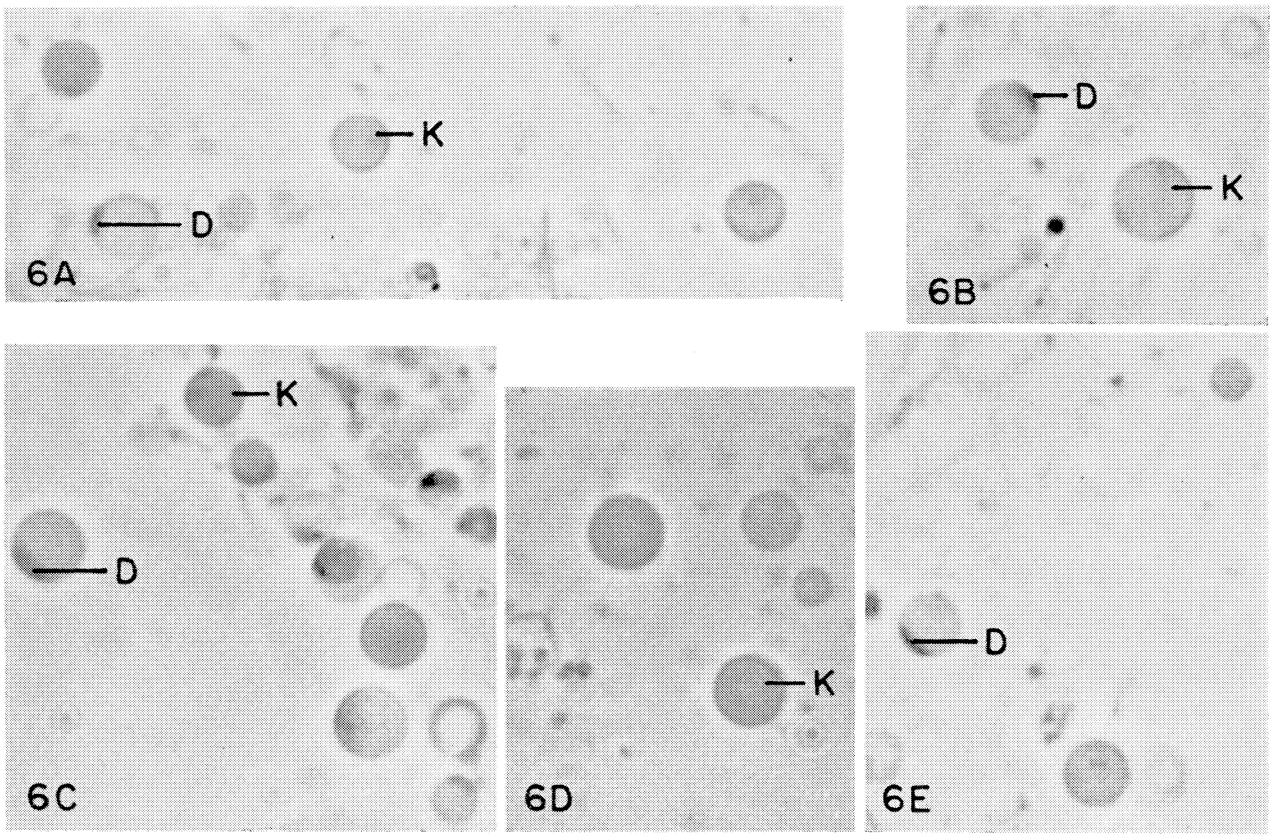


Fig. 6. Hypotonic Tris-EDTA-lysed preparation of acriflavin-induced 50% dyskinetoplastic *L. tarentolae*. The kinetoplast DNA (D) is absent from many of the swollen kinetoplasts (K), which were released from the ghosts by passing the suspension thru a #26 needle.

cell appears to be a general phenomenon thruout the hemoflagellates. This organelle has been found by several workers to be intimately involved with morphogenetic changes that occur during the life cycles of the hemoflagellates(16,26). The apparent attachment of the disc-shaped portion of the chondriome to the basal body may be significant in this regard. Vickerman(26)found that a *posterior* outgrowth of new mitochondrial tubules from the kinetoplast accompanies, and is presumably responsible for, the *anterior* movement of the kinetoplast-basal body complex during the cellular morphogenesis of a trypanosome. The basal body may be carried along by virtue of its attachment to the kinetolpast.

No electron-dense attachment of the kinetoplast to the basal body has ever been seen in the electron microscope, but this does not mean that an EDTA-sensitive "cement" of some sort could not be present. Attempts to isolate the kinetoplast have to date been frustrated by the varying amount of membrane that remains bound to the organelle after cell rupture. It is possible that the Tris-EDTA-shear method will be of use in obtaining a pure kinetoplast fraction.

The impermeability of the kinetoplast membranes to exogenous DNAase is in agreement with studies on *Neurospora* mitochondria(11) and on water-lysed ghosts of *Leishmania enrietti*(6).

The apparent membrane-bound state of the kinetoplast DNA is in agreement with observations of Nass(12) on mitochondrial DNA. This fact may have some significance for the replication of the kinetoplast DNA and of the organelle itself, in view of work on the relevance of this phenomenon in bacterial systems(7,10).

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