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Ultrastructural alterations of the nucleus and the kinetoplast of *Trypanosoma cruzi* exposed to ethidium bromide

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ETHIDIUM bromide (EB) is a phenanthridinium compound, which has been widely used against trypanosomiasis of cattle in Africa, given intramuscularly as a single dose of 1 mg/kg.[1] EB has been tried for *T. gambiense* infections of man but was only moderately effective.[2] Evidence concerning its probable mode of action *in vivo* has been reviewed by Newton,[3] leading to the conclusion that the action of the drug on living organisms is correlated to interfere extensively with nucleic acid,[4, 5] and it has been concluded that this drug is capable of intercalative binding between DNA base pairs causing an unwinding of the duplex structure. When trypanosomes are exposed *in vitro* to EB at low concentration (0·5–5 μg/ml) the drug is specifically bound to the kinetoplastic DNA;[6] observed under an u.v. microscope, the kinetoplast is highly fluorescent.[7] When trypanosomes are cultured in presence of EB (0·5–1·5 μg/ml) the kinetoplastic DNA is progressively lost during successive trypanosomes divisions.[7] The nucleus and the mitochondrial apparatus which is associated to the kinetoplast are unaffected.[8] Dyskinetoplastic trypanosomes are not viable; they can only survive for 4 weeks, if they are transplanted each week in a new medium free of EB.[9] In this paper we report observations concerning the ultrastructure of trypanosomes growing for 48 hr in the presence of high concentration of EB.

Trypanosomes (*Trypanosoma cruzi*, strain Institut Pasteur Paris) were grown as described in a previous work.[6] After 3 days' culture, during the exponential phase of trypanosomes growth, a sterile EB stock solution (1 mg/ml) was added to the culture medium to obtain a final concentration of 20 μg/ml. After 48 hr, trypanosomes were collected by centrifugation at 1000 *g* and processed for electron microscopy as described in a previous work.[10]

Observations with light microscope reveal that the trypanosomes still moving are alive after 48 hr treatment with EB (20 μg/ml). When colored by Giemsa's stain, a few trypanosomes (about 2 per cent) appear attached to each other by the kinetoplast at the last stage of cytokinese. Treatment with EB results in the formation of vacuoles within the cytoplasm of the trypanosomes. These lesions are not observed in trypanosomes growing without or with low concentration of EB. Electron microscope studies allow us to elucidate the nature of lesions observed by light microscopy. As the cultured trypanosomes do not divide synchronously, several more or less advanced lesion stages can be observed. A few trypanosomes with normal ultrastructure are found as shown in Fig. 1. The nucleus (N) with its nucleolus (Nu) is set in the center of the cell. Below, the kinetoplast (K), which appears elongate or rodlike, contains the DNA typically disposed in a double row. On the right of the cell the mitochondrion (M) and two digestive vacuoles (va) can be seen.

The most typical lesions observed after EB treatment are:

(1) Alteration of the nuclear structure: the normal granular structure and the nucleolus have disappeared. The chromatin is irregularly condensed (Fig. 2) then disappears; the nucleus appears such as a clear mass with many fine filaments (Figs. 3 and 3a).

(2) Alteration of the kinetoplast: the structure of the kinetoplastic DNA is quite disturbed. The DNA is fractionated in numerous fibrillar spheres (Fig. 2). Such a structure is usually obtained with low concentration of EB (0·5 μg/ml).[8] But the alterations usually observed consist in a typical swelling of the kinetoplast (Fig. 3). Its DNA is condensed in a globular mass① in the middle of the swollen kinetoplast (Figs. 3 and 3b). A rod shaped DNA② persists near the flagellar basis (Fl in Figs. 3 and 3b). This kinetoplastic DNA is strongly connected to the kinetoplastic membranes by numerous filaments③.

(3) Inhibition of the kinetoplastic division: in normal trypanosomes the first stage of trypanosomes division begins with the apparition of a second basal body and a second flagellum, after the kinetoplast divides, then lastly the nucleus. After EB treatment the nucleus divides while the division of the kinetoplast is blocked and the cytokinesis is stopped. The kinetoplast makes a bridge between the two trypanosomes (arrows in Fig. 4).

Other drugs are known to cause active swelling of mitochondria.[11] Beaudoin *et al.*[12] have shown that primaquine induces swelling of *Plasmodium fallax* mitochondria and not those of the host cells. Trager *et al.*[13] in studying dyskinetoplasty induction by acriflavin treated trypanosomes, noted that the mitochondria appeared swollen, with their cristae replaced by circular profiles. The severe alterations produced by EB in the nucleus and mitochondrial apparatus of trypanosomes account for the strong trypanocidal action of this drug.

Exposure to ethidium bromide (20 μg/ml) for 48 hr causes lesions in the nucleus and the kinetoplast of *Trypanosoma cruzi* grown *in vitro*. The electron microscope reveals alterations in the organization of the nuclear and kinetoplastic DNA and swelling of the mitochondrial apparatus. The kinetoplastic DNA has lost its typical ultrastructure and is condensed such as a globular mass with many filaments attached to the membrane of the swollen kinetoplast. The nucleolus disappeared while the nuclear DNA gets a filamentous structure. Lastly, the altered kinetoplast cannot divide and blocks the separation of trypanosomes.

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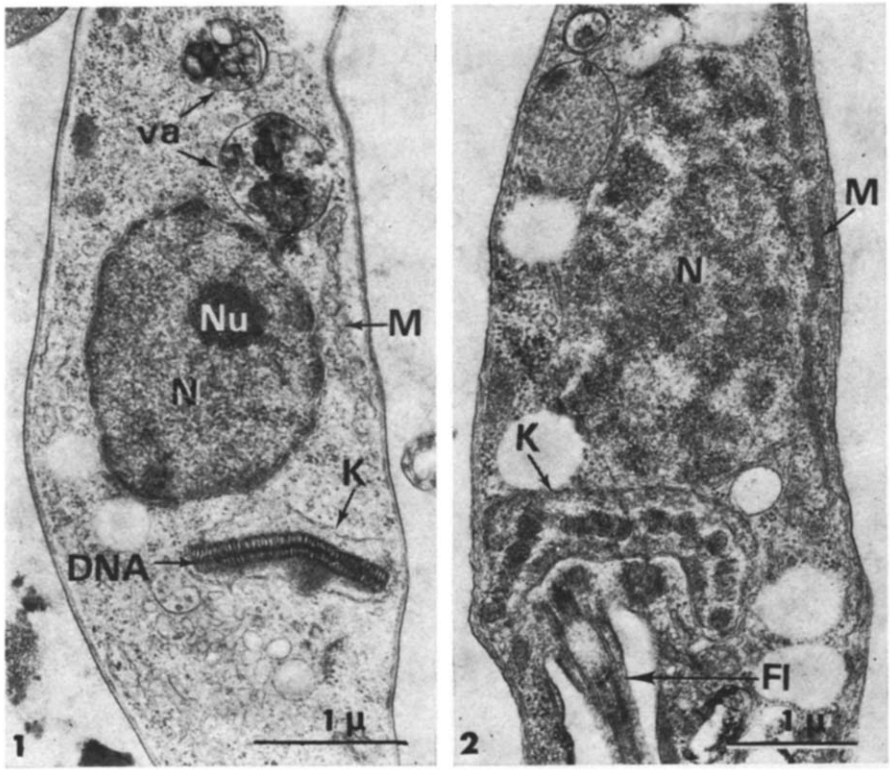

FIG. 1. Electron micrograph (× 20,500) of normal *T. cruzi*—N = nucleus, Nu = Nucleolus, K = kinetoplast, M = mitochondrion, va = digestive vacuole.

FIG. 2. Electron micrograph (× 17,300) of *T. cruzi* treated with EB (48 hr, 20 μg/ml). Fl = Flagellum.

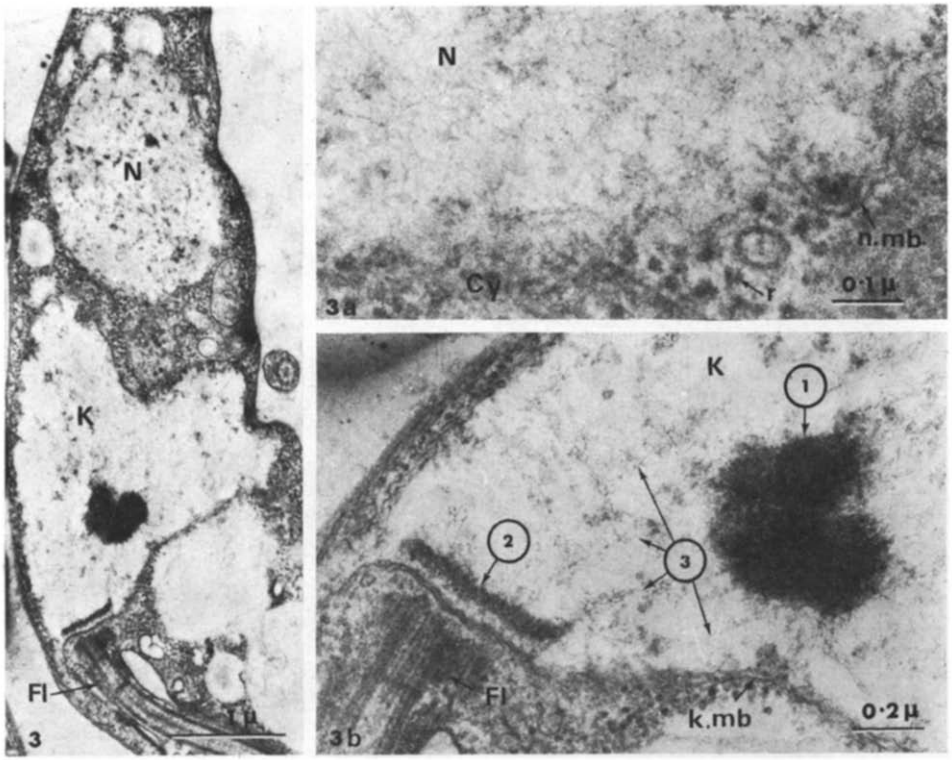

FIG. 3. Electron micrograph (× 21,000) of typical alterations of *T. cruzi* after EB treatment. Figure 3 (a) (× 129,000) and (b) (66,000) are magnifications of nuclear and kinetoplastic regions of Fig. 3. Cy = cytoplasm, n.mb = nuclear membrane, r = ribosome, k.mb = kinetoplastic membrane. In Fig. 3b, globular mass of kinetoplastic DNA ①, rod shaped kinetoplastic DNA ②, DNA filaments connected to the kinetoplastic membrane ③.

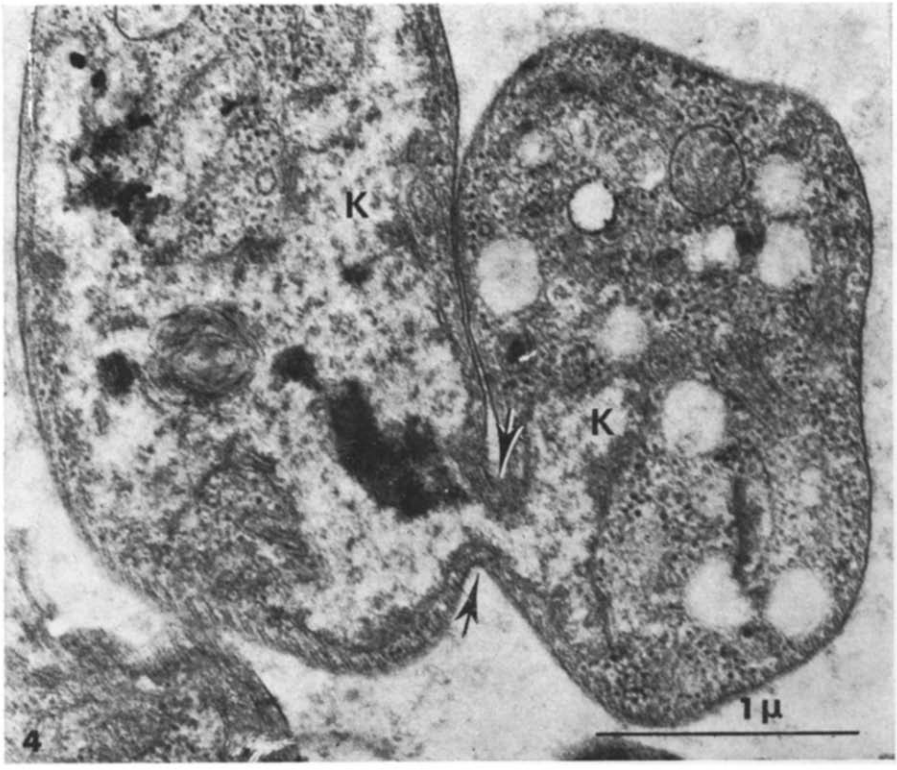

FIG. 4. Electron micrograph (× 35,200) of two trypanosomes with blockage of the cytokinesis.