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The presence of basic proteins associated to the kinetoplast-DNA of *Trypanosoma cruzi* epimastigotes

Short communication

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Trypanosoma cruzi epimastigotes possess a single mitochondrion with a differentiated DNA containing region, which is termed the kinetoplast. Steinert (1965), based on cytochemical techniques has suggested that, like mitochondrial DNA, the kinetoplast DNA is not associated with basic proteins. The existence of a highly compact, well-structured association of molecules raises the question of stability. One wonders how the negatively charged DNA molecules can remain in such close proximity without any basic proteins to neutralize the charge (Simpson, 1972). Evidence of the existence of basic proteins in the kinetoplast has been presented by Souto-Padrón and De Souza (1978). However, the possibility that these basic proteins are not associated with DNA has not been definitely ruled out. One way of demonstrating whether these basic proteins are associated with K-DNA is to examine their alterations after inducing the loss of K-DNA with ethidium bromide (EB). *T. cruzi* epimastigotes (Y strain) were grown in Warren's liquid medium (Warren, 1960) for 4 days either in the presence or absence of 0.5 μg/ml ethidium bromide. The concentration of EB used induced 50% diskinetoplasty of the parasite population as determined by light microscopical observation of Giemsa stained cells. The cells were harvested and washed twice by centrifugation in 0.15 M NaCl and prepared for electron microscopy (Souto-Padrón and De Souza, 1978). For cytochemistry, the ethanolic phosphotungstic acid (E-PTA) method to detect basic proteins was employed (Souto-Padrón and De Souza, 1978). As described previously (Delain and Riou, 1969) the initial effect of growth in a low concentration of EB on the ultrastructural appearance of K-DNA in *T. cruzi* culture forms is a disruption of the orderly double-layered arrangement of DNA fibrils. This occurred in several locations along the DNA band as seen in longitudinal section,

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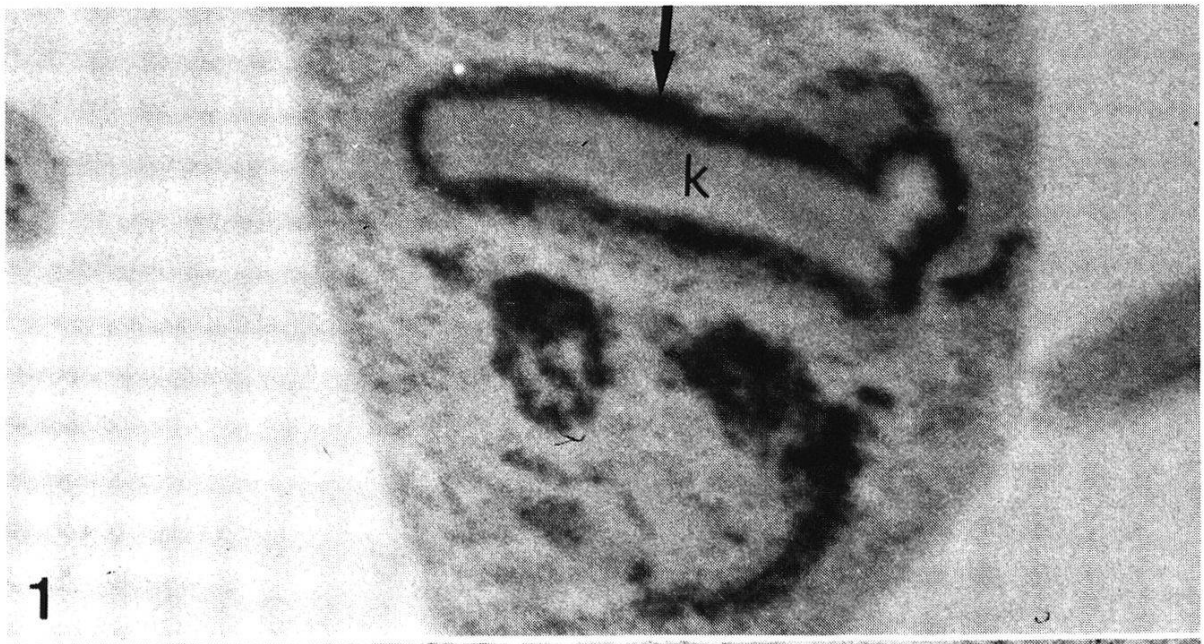
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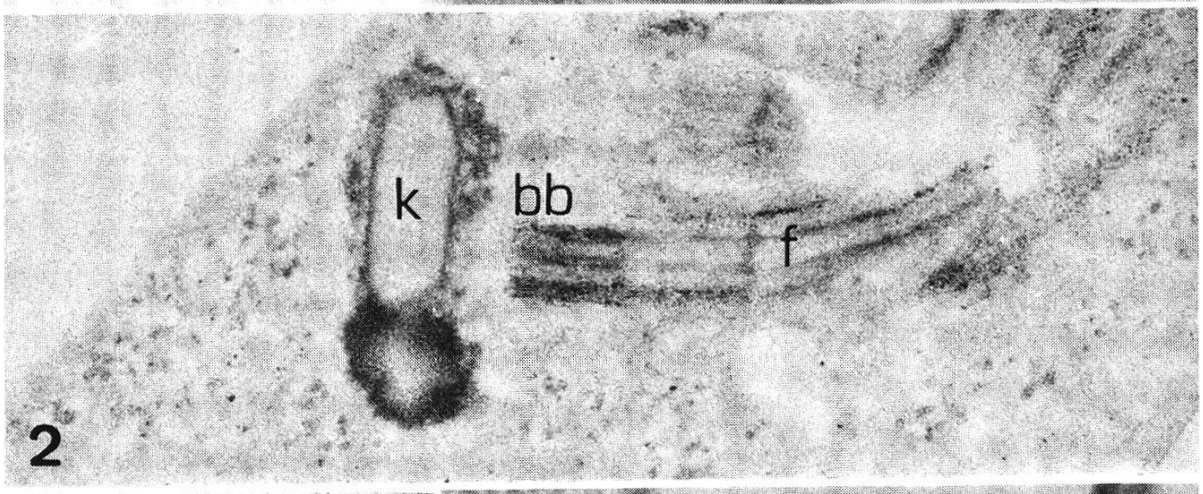
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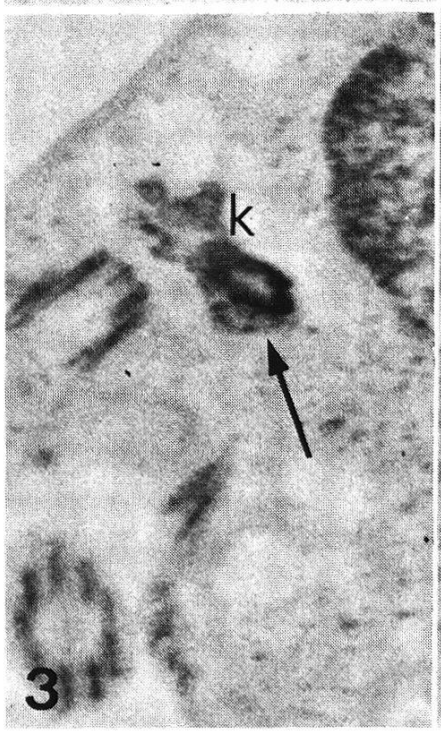
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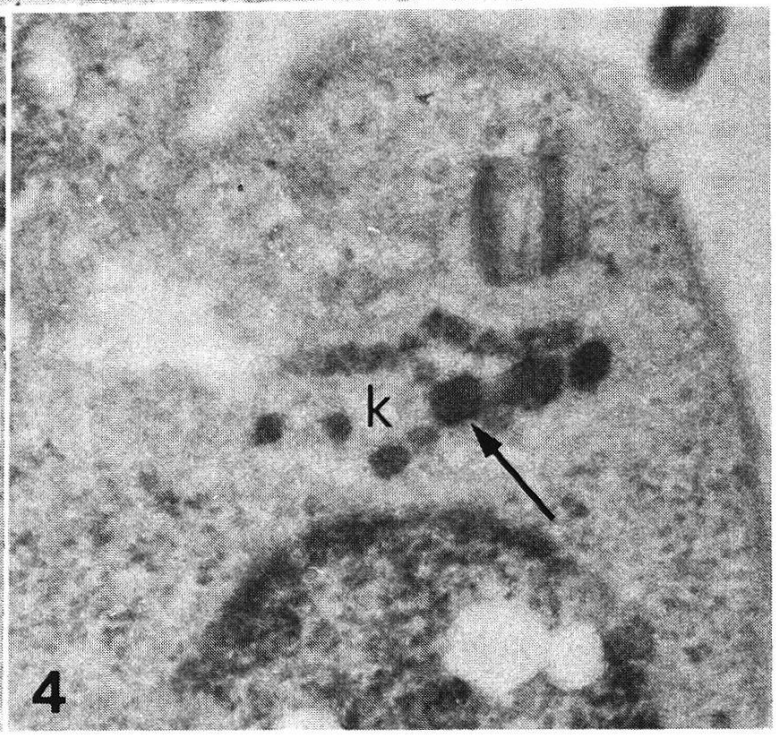
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producing several "arcs" of fibers. Then the DNA band either fragmented or retracted from the membrane, or formed a globular mass with a filamentous structure. In both cases the DNA mass remained attached to the membranes by fibers. The final phase of the process involved a further clumping of the DNA and finally an apparent decrease in the amount of DNA present.

T. cruzi epimastigotes stained with E-PTA displayed a homogenous electron-dense reaction in the nucleus, on some microtubules (basal body and peripheral doublets of the flagellum) and at the periphery of the kinetoplast DNA (Fig. 1). A similar clumping and retraction and eventual decrease in quantity of the material stained with E-PTA was observed in epimastigotes grown in EB and stained with E-PTA, thus indicating a close relationship between K-DNA and basic proteins (Figs. 2–4).

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Fig. 1. Control cell showing PTA reaction at the periphery (arrow) of the kinetoplast (k). ×45,000.

Figs. 2–4. Ethidium bromide-treated cells showing reaction product in the basal body (bb), flagellar microtubules (f) and in the kinetoplast (k). In some cells both strongly and less intensely reacting areas can be seen (Figs. 2–3). In other cells the reaction product appears on electron-dense spherical bodies (Fig. 4, arrow). ×45,000.