Electron microscope observations of *Trypanosoma cruzi* during development inside the haemocytes of Triatominae

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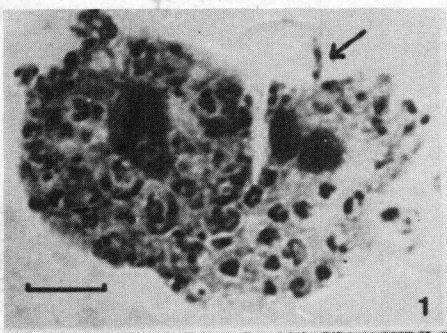
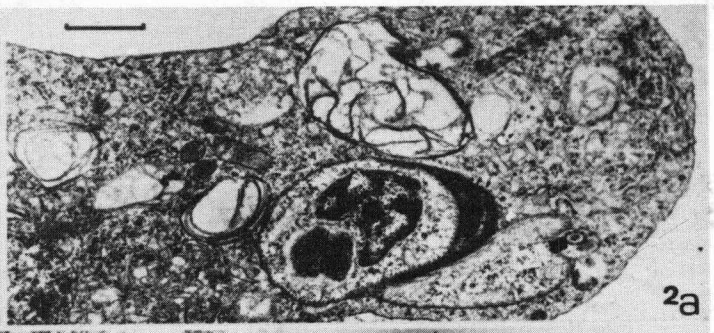
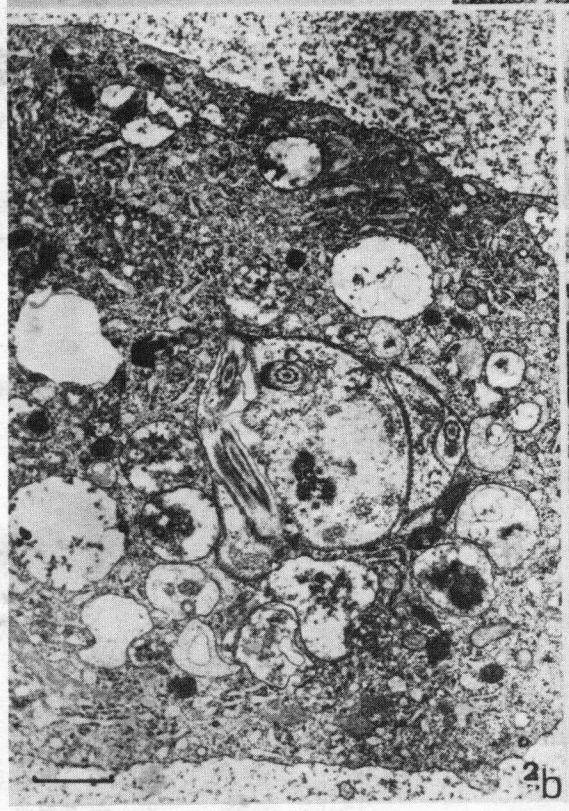
Abstract

The haemolymph of *Panstrongylus megistus*, infected by *Trypanosoma cruzi*, was examined by light and electron microscopy. Numerous parasites lie inside the haemocytes, each one in a vacuole. Their well-preserved morphological features support the concept of parasite multiplication in cells of the insect haemolymph.







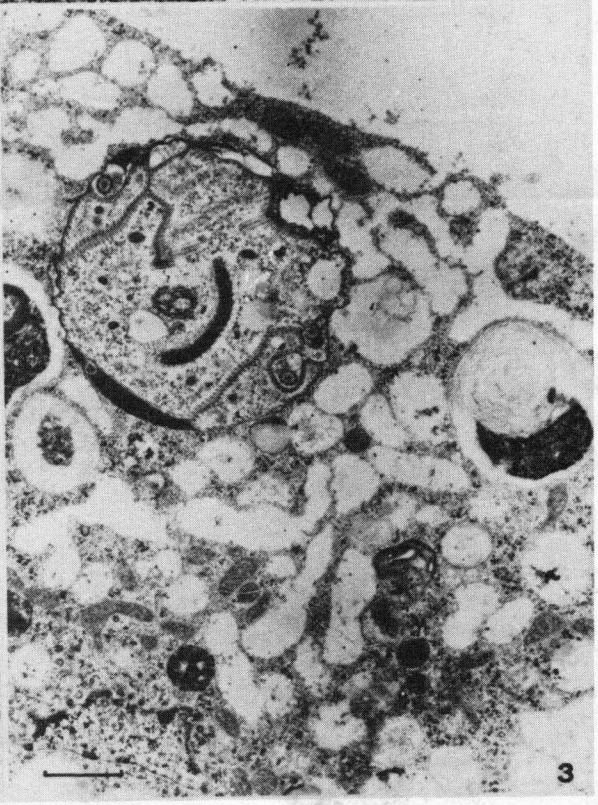


Fig. 1. Parasites inside and outside (arrow) a haemocyte (bar=10μm)

Figs. 2a and 2b. Electron micrographs of *T. cruzi* inside non-ruptured young haemocytes (bar = 1 μm)

Fig. 3. Electron micrograph of a parasite inside an old haemocyte (bar = 1 μm)

Introduction

The occurrence and development of *Trypanosoma cruzi* in the coelomic cavity of some Triatominae were noticed by DIAS (1932), NÁQUIRA (1962), RIBEIRO *et al.* (1977a, b) and CAMARGO *et al.* (1980). The last authors used the immunofluorescence technique to demonstrate the presence of parasites. Detailed histological observations on the extra-intestinal cycle of *T. cruzi* were presented by LACOMBE (1979). Later LACOMBE & SANTOS (1984) emphasized the multiplication of parasites in insect haemocytes. This stage of parasite development corresponds to the amastigote form. Light-microscopic observations show that the parasites lie inside vacuoles, taking an elongate epimastigote form after release from these cells. To obtain better information about the parasites inside the haemocytes, electron-microscopic observations were made.

Materials and Methods

Culture form *T. cruzi*, strain Y, were injected into the haemocoel of 100 individuals of *Panstrongylus megistus*. Control smears and fresh preparations were made daily until the 30th day after inoculation. The level of parasitaemia was recorded. At the same time permanent histological preparations of the haemolymph, digestive apparatus and Malpighian tubes were made by classical methods (LACOMBE & SANTOS, 1984). At the 5th day, when numerous round parasites were easily recognizable inside vacuoles in haemocytes (Fig. 1), preparations for electron microscopy were obtained by fixation of the haemolymph in 2% glutaraldehyde in 0·1 M phosphate buffer pH 7·2, dehydration by increasing ethanol concentrations, three intermediate baths of propylene oxide, and embedding in Epon.

Results

By electron microscopy, globular parasites can be observed inside vacuoles that may also contain other elements such as multi-membranous vesicles. The parasites occupy the least space possible (Fig. 2), so that several sections of the flagella are usually seen and no extended images of them may be obtained. Comparison with light microscope observations suggests that the transparent vacuolar area around the included parasites may be an artifact of fixation. Kinetoplast, microtubules and paraxial body of the flagellum are also shown in Fig. 2, besides haemocyte organelles such as vacuoles, lysosomes, smooth and rough endoplasmic reticulum, mitochondria and other vesicles. Inside the irregularly shaped young haemocytes with finger-like projections of the plasma membrane, the aspect of the parasites is similar to that observed inside older haemocytes, which may be more or less ruptured or entirely destitute of plasma membrane. The well developed kinetoplast at this last stage of the parasite, inside an almost disintegrated haemocyte (Fig. 3), supports the concept of persistent parasite viability and the role of the haemocyte in parasite multiplication in the insect.

A report of further electron microscope investigations during the presence of the parasites in the Malpighian tubes after the 7th day of inoculation (LACOMBE, 1979; LACOMBE & SANTOS, 1984) is in preparation.

Acknowledgement

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