Distribution of glycogen particles in muscle cells infected by *Trypanosoma cruzi*

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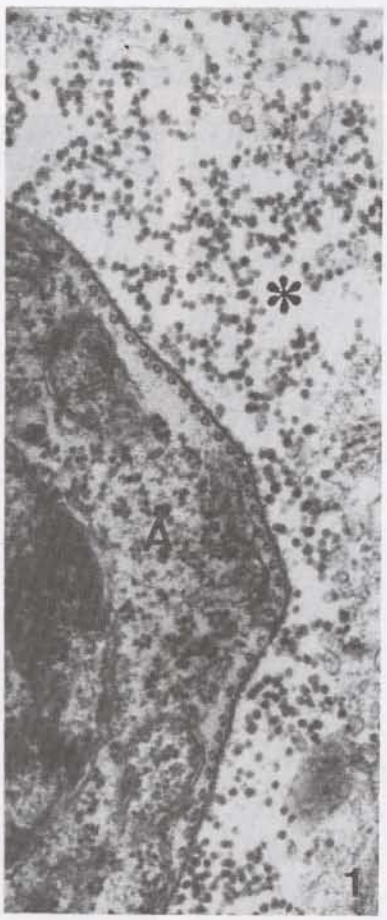
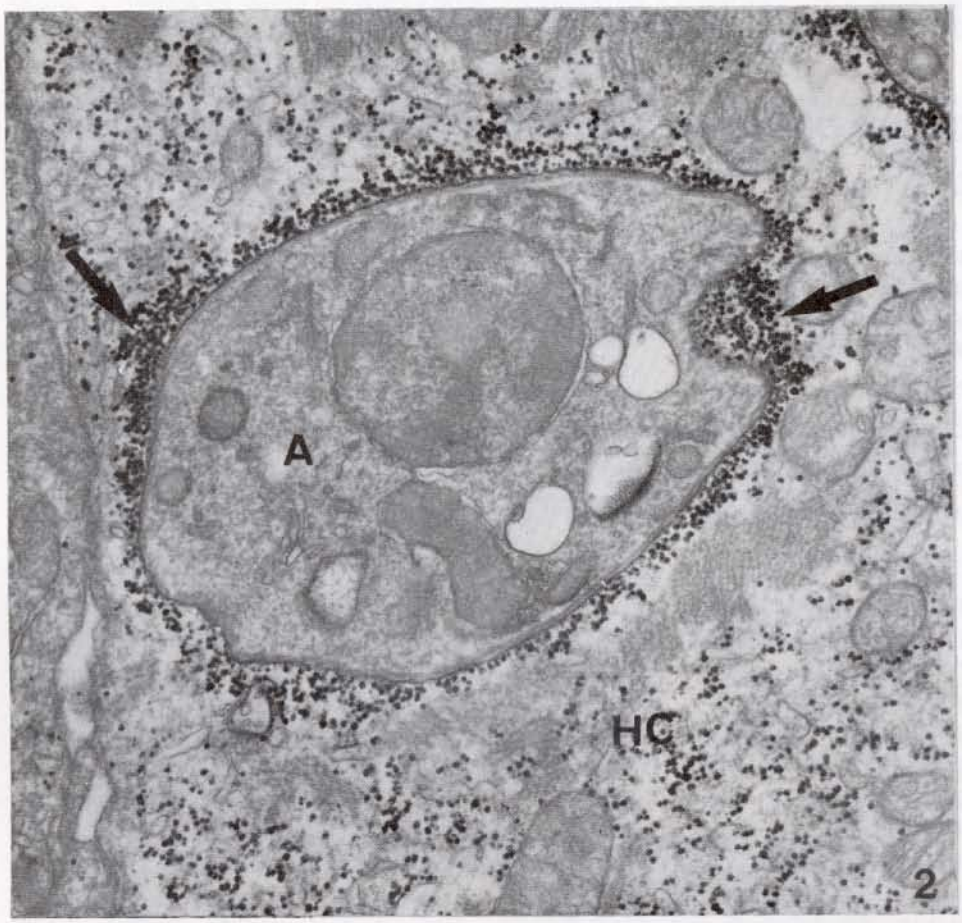
The infective trypomastigote form of *Trypanosoma cruzi*, the causative agent of Chagas'disease, is able to penetrate a large number of cells through an endocytic process with formation of a parasitophorous vacuole. A few hours after penetration the trypomastigote form gradually transformates into the spherical amastigote form (Review in De Souza 1974). During this process a porin-like macromolecule is released by the parasite (Andrews et al. 1990) gradually lysing the membrane lining the vacuole so that after a few hours the amastigote form is in direct contact with the structures and organelles of the host cell (De Carvalho and De Souza 1994). There are very few information on the changes which take place in the host cell cytoplasm during the evolution of the intracellular parasitism. Here I report observations made on the distribution of glycogen particles of heart muscle cells infected with *T. cruzi*.

Embryonic chicken heart muscle cells were maintained as previously described (Meyer and Xavier de Oliveira 1948). After cultivation for 7 days the cultures were allowed to interact for 4 hours with bloodstream trypomastigotes of the Y strain, obtained as described previosuly (Loures et al. 1980) using a 20:1 parasite host cell relationship. After interaction the cultures were washed with Hank's solution to remove extracellular parasites. Some were then fixed for 1h with 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2. Others were incubated at 37°C for 24,48 or 72 hs after which were fixed as described above. After glutaraldehyde fixation the cultures were rinsed twice in cacodylate buffer, post-fixed for 1h at 4°C in 1% OsO_4 in 0.1 M cacodylate buffer, dehydrated in acetone and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate. Other sections were collected on gold grids and stained by the periodic acid thiollsemicarbizide-silver proteinate technique, as recommended by Thiery (1967). In this method ultrathin sections are first oxidized by 1% periodic acid to convert adjacent hydroxyl or -amino alcohol groups into aldehydes. These aldehydes are then condensed with thiosemicarbazide to yield thiosemicarbazones which are powerful reducing agents. Thus after exposure to silver proteinate these thiosemicarbazones are finally revealed in the electron microscope as silver deposits at the reactive sites. Control sections were treated similarly to these experimental sections except for the omission of one of the steps.

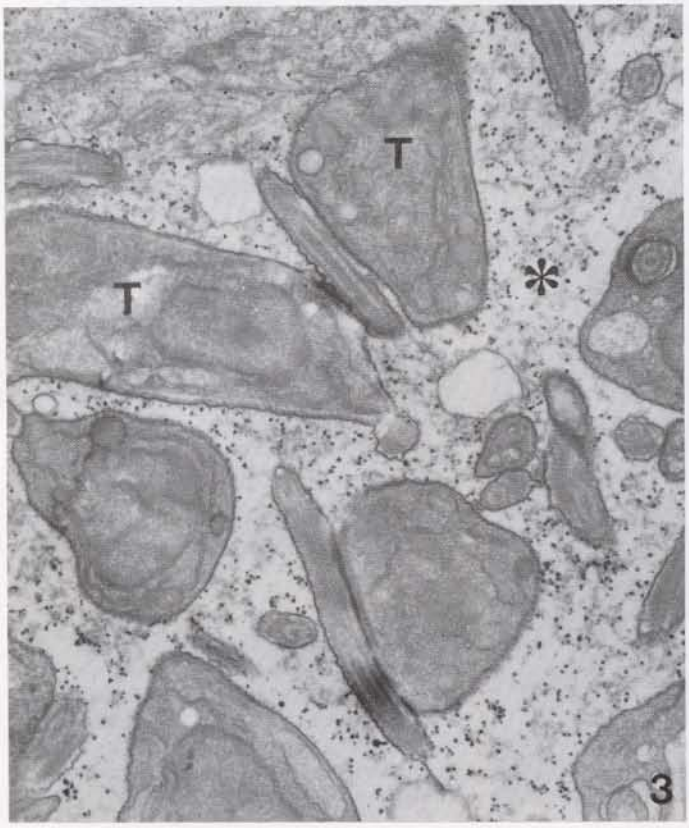


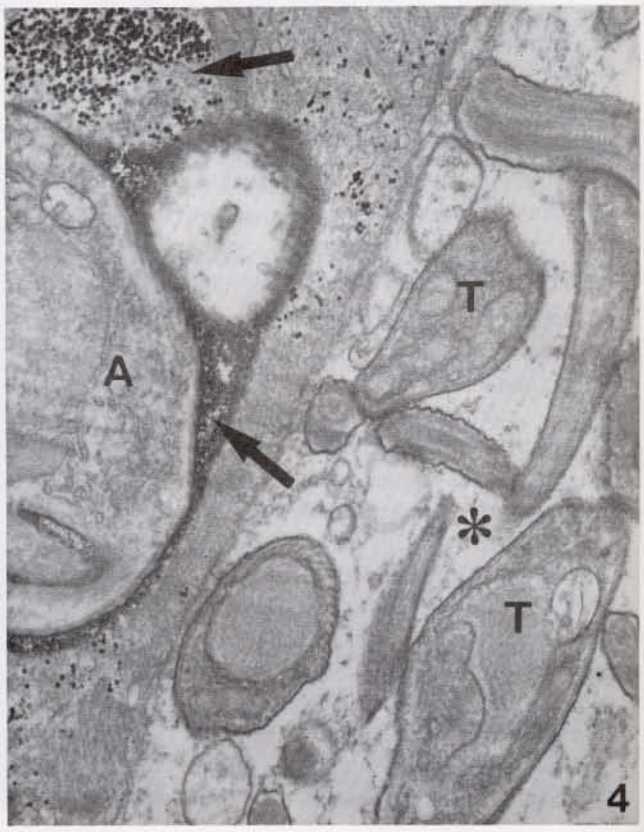
Recently transformed amastigote forms are immersed in a portion of the cytoplasm which gradually became less electron-dense. A large number of dense particles with a mean diameter of 20 nm were seen surrounding the parasite (Fig. 1). Gradually these particles concentrated around the parasite. Using the Thiery technique they were

Fig. 1. *Conventional thin section of a cell infected with T. cruzi and stained with uranyl acetate and lead citrate. An amastigote form (A) is seen in direct contact with the cytoplasm of the host cell. A large number of particles with a mean diameter of 20nm is seen in the area surounding the parasite. X 50,000.*



***Figs. 2-4**. Thin sections treated for localization of carbohydrates, as recommended by Thiery (1967). Glycogen particles are clerarly identified. In a recently infected cell containing an amastigote form (A in Figs 2 and 4) glycogen particles concentrate around the parasite (arrows). These particles are less abundant in cells containing transitional forms between amastigote and trypomastigote (T) (asterisc in fig. 3). Very few or no glycogen particles are seen at the end of the intracellular cycle when only trypomastigote (T) form are seen (asterisc in fig. 4). Fig. 2: X 30,000; Fig. 3: X 17,000; Fig. 4: X 20,000.*





identified as ß glycogen particles (Fig. 2). With subsequent division of the amastigotes there was a gradual decrease in the density of glycogen particles in areas surrounding the parasites (Fig. 3). At the end of the intracellular cycle and with the appearance of trypomastigotes very few or no more glycogen particles were seen. This significant decrease in the number of glycogen particles was more evident when two host cells in different phases of the parasite intracellular cycle were seen in the same section (Fig. 4). These observations show clearly that during the intracellular parasitism of *T. cruzi* in muscle cells glycogen particles gradually disapear. It is possible that the glycogen particles are hydrolysed into glucose which can then be used by the parasite. It is well know that trypanosomatids efficiently consume glucose through a glycolytic pathway, which takes place in a cytoplasmic organelle known as glycosome which is a special type of peroxisome (Review in Michels and Opperdoes 1991).

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