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Research Note

ENDOCYTOSIS OF ALBUMIN-GOLD PARTICLES BY *TRYPANOSOMA CRUZI* INFECTED AND NON INFECTED HEART MUSCLE CELLS

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Trypanosoma cruzi, an obligatory intracellular parasite infects eucaryotic cells through an endocytic process involving formation of a membrane bound vacuole, where the parasite is initially located (N. Nogueira & Z. A. Cohn, 1976, *J. Exp. Med. 143:* 1402-1420; T. A. Maria et al., 1982, *Acta Tropica, 39:* 99-109; M. N. L. Meirelles et al., 1986, *Eur. J. Cell Biol., 41:* 198-202). Several ultrastructural cytochemical approaches have revealed that selection of plasma membrane regions occurs during this process. Cytochemical characterization of plasma membrane markers, such as adenylate cyclase, Ca2+ Mg2+ ATPase and anionic sites, have shown that these components are not found in the parasitophorous vacuolar membrane of macrophages as well as muscle cells (M. N. L. Meirelles & W. De Souza, 1986, *J. Submicrosc. Cytol., 18:* 99-107). Several markers have been used to label lysosomes in mammalian cells and to follow the process of lysosome-phagosome fusion, such as thorotrast particles, cationized ferritin and native ferritin, horseradish peroxidase and recently albumin-gold particles (T. C. Jones & J. G. Hirsch, 1972, *J. Exp. Med. 136:* 1173-1194; P. J. Edelson & Z. A. Cohn, 1974, *J. Exp. Med., 140:* 1364-1385; J. Alexander & K. Vickerman 1975, *J. Protozool. 22:* 502-508; R. Milder & J. Kloetzel, 1980, *Parasitol., 80:* 139-145; M. N. L. Meirelles et al., 1984, *J. Submicrosc. Cytol., 16:* 533-545; M. N. L. Meirelles et al., 1987, *Cell Struct. and Funct., 12:* 387-393); J. D. Berman et al. (1981, *J. Protozool., 28:* 239-242) described the use of colloidal gold as an appropriate label for ultrastructural investigation of the interaction of *Leishmania* with lysosomes within macrophages, both *in vivo* and *in vitro.* It has also been shown that albumin-gold labeling can be used as a probe to label macrophage lysosomes and to follow the lysosome-phagosome fusion process in phagocytic cells (T. U. Carvalho et al., 1988 *J. Submicrosc. Cytol. Pathol., 20:* 773-776). Besides the advantage of the high inherent electrodensity of this probe, proteins can be associated with different diameters of gold particles, allowing double labeling.

The aim of our study was to analyse the process of endocytosis of bovine albumin, adsorbed to colloidal gold particles, by non professional phagocytic cells. For this purpose we worked with primary cultures of heart muscle cells (HMC) and followed the interiorization of *T. cruzi* in these cells.

HMC were obtained from eighteen days old mouse embryos as previously described (M. N. L. Meirelles et al., 1986, *loc. cit.*). *T. cruzi* metacyclic trypomastigotes of the Dm 28c clone were obtained using a chemically defined differentiating medium as described by V. T. Contreras et al. (1985, *Mol. Biochem. Parasitol., 16:* 315-327).

Coloidal gold particles with a mean diameter of 15 nm were made according to the method of Frens (G. Frens, 1973, *Nature (Phys. Sci.) 241:* 20-22). Albumin at a concentration of 25 ug/ml was adsorbed to colloidal gold particles at pH 5.5 according to Bendayan (M. Bendayan, 1984, *J. Electron Microsc. Technique, 1:* 243-270). For the experiments, the stock solution was diluted 10 times in Eagle medium.

Seven days old cultures were washed twice with PBS and incubated at 37 °C in the presence of 15 nm albumin-gold particles for periods varying from 30 min to 24 h. In some cases, after a 2 h incubation with the albumin gold complex, HMC were washed and infected

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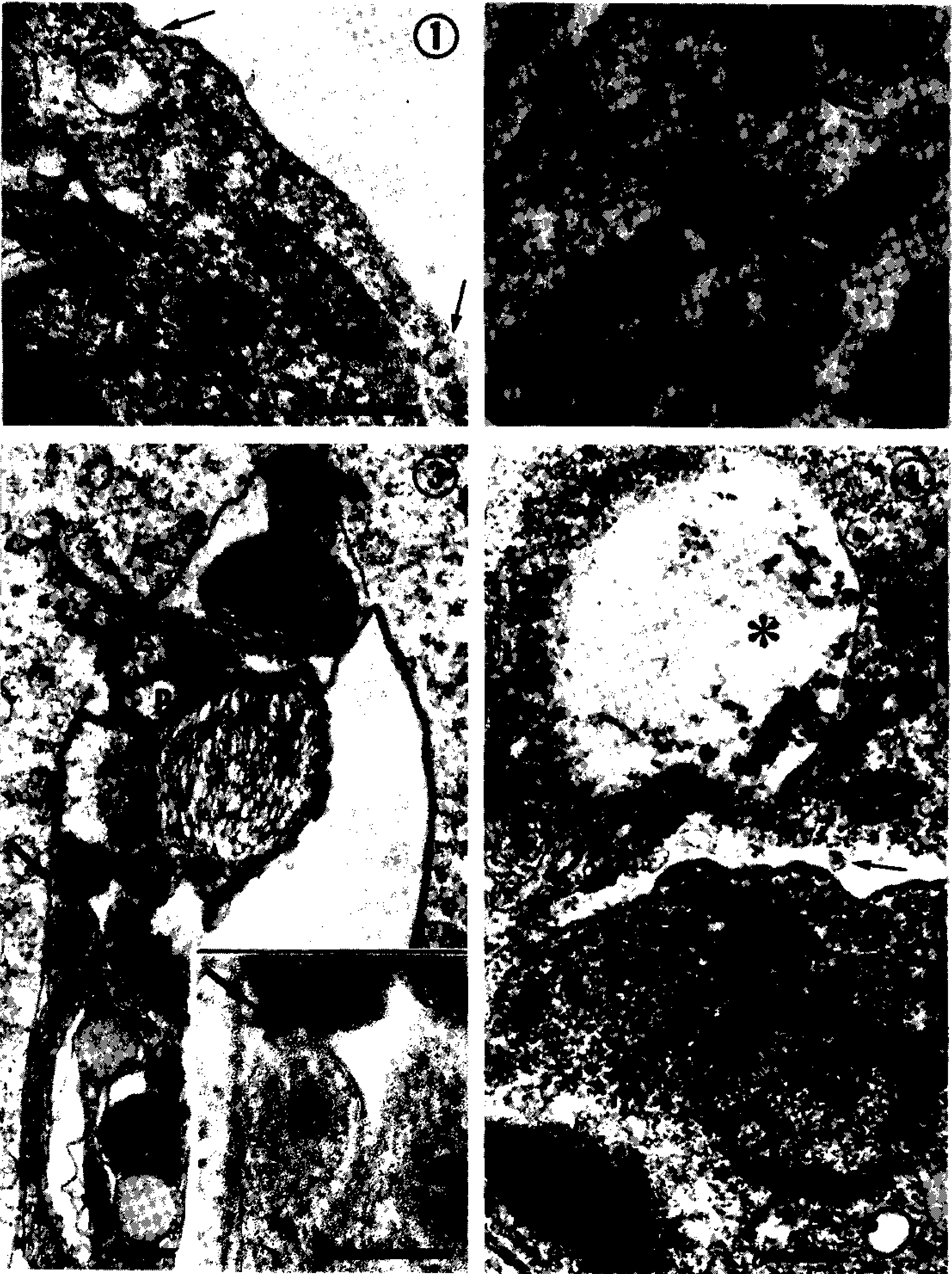

Fig. 1: 30 min incubated HMC with albumin colloidal gold particles displayed discrete labeling in the plasma membrane and in small vesicles (arrows). Fig. 2: heart muscle cells after 24 h incubation time with albumin-gold particles showed endosomes containing the marker (arrows). Fig. 3, 3a: fusion of the gold labeled lysosomes with the phagosome containing *Trypanosoma cruzi* in HMC. Inset: higher magnification of the gold particles seen inside the parasitophorous vacuole (arrows), parasite (P). Fig. 4: 24 h infected HMC displayed vesicles with albumin-gold particles close to *T. cruzi*, which was free in the cell cytoplasm (arrows) and nearby large endosomes with gold complexes were observed (asterisk), parasite (P). All bars: 0.2 µm.

with metacyclic trypomastigotes (5:1 ratio parasite/HMC) for 2 and 6 h, at 37 °C in the absence of serum.

After these incubations, the cultures were rinsed twice to remove extracellular parasites, fixed in 2.5% glutaraldehyde, postfixed with 1% OsO_4, dehydrated in acetone, embedded in Epon and observed in a EM 10C Zeiss electron microscope.

Our observations demonstrated that after 30 min of incubation, colloidal gold particles were found at the cell surface, and in small vesicles near the periphery of the cell (Fig. 1). After longer incubation times at 37 °C, endosomes containing gold particles, and in some cases membranous material, were seen both on non infected and infected cells (Figs 2 and 4). There was fusion of gold labeled vesicles with the parasitophorous vacuole (Fig. 3, 3a) and after 24 h of incubation we also observed vesicles displaying gold particles near parasites free in the cytoplasm (Fig. 4).

Endocytosis seems to be a basic mechanism for parasite interiorization by host cells. Our data confirms that, in the case of *T. cruzi*, albumin-colloidal gold complexes can be used as a good tracer for phagolysosomal fusion in non professional phagocytic cells, as heart muscle cells.