

## EFFECT OF THE HOST SPECIFIC TREATMENT IN THE PHAGOCYTOSIS OF *TRYPANOSOMA CRUZI* BLOOD FORMS BY MOUSE PERITONEAL MACROPHAGES

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*Single doses of drugs active against Trypanosoma cruzi (megazol, nifurtimox and benznidazole) induce a rapid clearance of the blood parasites in experimentally infected mice. Furthermore, the in vitro phagocytosis and intracellular destruction by mouse peritoneal macrophage of blood forms collected from the treated animals is strongly enhanced as compared with parasites from untreated controls. The uptake of the blood forms by macrophages is significantly higher with megazol than with benznidazole and nifurtimox, a finding that concurs with data showing that megazol is also the most active compound in the living host. The possibility that macrophages participate in a synergic effect between the host immune response and chemotherapeutic effect is discussed.*

Key words: *Trypanosoma cruzi* – host treatment – phagocytosis – mouse – macrophages

Administration of single doses of compounds active against *Trypanosoma cruzi* induces in infected mice, within 4 to 6 h, a marked decline in the parasitemia levels (Filardi & Brener, 1984). Such results show a fairly good correlation with those obtained with long-term treatment involving administration of drugs for at least 20 consecutive days. The mechanism by which the parasite are so rapidly removed from the blood has not yet been investigated. In this paper we suggest that mouse peritoneal macrophages and probably other cells of the mononuclear phagocytic system may participate in the parasite clearance. We also discuss the possible existence of a cooperation between drug effects and immune response which has already been identified in a number of parasitic diseases (review: Target, 1985).

### MATERIALS AND METHODS

**Mice infection** – Swiss male albino mice were inoculated with *T. cruzi* Y strain isolated from an acute case of Chagas' disease (Pereira da Silva & Nussenzweig, 1953). Parasitemia of

the animals was determined as described by Brener (1962).

**Treatment of infected mice** – Mice at peak of parasitemia, which usually occurs at the 7th day of infection, were treated with a single dose of 500 mg/kg, oral route, of the following drugs: 3-methyl-4 (5'-nitrofurfurylidene-amino)-Tetrahydro-4H-1,4-thiazine-1,1-dioxide ("nifurtimox"). (N-benzyl-2-nitro-1-imidazolacetamide ("benznidazole") and 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole ("megazol, CL 64'855").

**Isolation of *T. cruzi* blood forms (BTry)** – Blood collected from the infected mice was defibrinated with glass beads, diluted (v/v) with Tc199 medium (Difco Laboratories, Detroit, USA) supplemented with 10% fetal calf serum (Tc-FCS) and centrifuged at 100 g for 10 min at room temperature. The BTry from the supernatant were collected and washed twice with Tc-FCS at 4 °C, 1000g for 15 min.

**Macrophage infection** – Mouse resident peritoneal macrophages were cultivated at 37 °C, 5% CO<sub>2</sub> in Tc199-20% FCS. The purified BTry were added to the macrophage monolayer at a parasite: cell ratio of 5:1 and the preparations incubated at 37 °C, 5% CO<sub>2</sub>. After 3 h the monolayers were repeatedly washed with Hanks balanced salt solution (HBSS) to remove

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adherent BTry. Part of the preparations were fixed with Bouin solution, water washed and stained with Giemsa whereas the remaining preparations were reincubated for further 24 h in the same conditions and then stained. To determine the percentage of macrophage infection, 500 cells were examined at random ( $\times 1000$ ). The percentage of infection was expressed by the ratio T/C in which T = % macrophages infected with BTry from treated animals and C = % macrophages infected with BTry from untreated control animals. The mean number of intracellular parasite stages was determined in 100 infected cells.

*Effect of megalol on intracellular stages of T. cruzi* – Forty-eight hours infected macrophages were incubated with sera from normal mice treated 3 h before with a single dose of megalol, 500 mg/kg. The preparations were maintained for 24 h at 37 °C, 5% CO<sub>2</sub>, washed, fixed with Bouin solution and stained with Giemsa.

## RESULTS

Figure 1 illustrates the decline of parasitemia induced in *T. cruzi* infected mice within 6 h after the administration of a single dose of benznidazole. As shown in Fig. 2A, the phagocytosis of BTry collected from infected mice 3 h after megalol administration is significantly enhanced as compared to the parasites collected from untreated mice or those obtained 1 h after treatment. Moreover, the macrophages display a clear trypanocidal effect as demonstrated by the dramatic fall in the percentage of infected cells after 24 h incubation (Fig. 2A). These results are confirmed by the decrease in the number of intracellular stages observed in the preparations examined 3 h after the parasite uptake and 24 h thereafter (Fig. 2B). BTry from untreated mice readily multiplied within 24 h whereas multiplication of treated parasites collected 3 h after drug administration was abrogated. Nifurtimox and benznidazole, although less active than megalol, induced similar patterns of phagocytosis and parasite destruction (Figs 3A, B). Fig. 4 shows aspects of macrophages infected with BTry collected from mice treated with benznidazole.

Experiments with BTry collected from animals treated 1 or 3 h before with megalol, suspended in Tc-FCS and incubated in the absence of macrophages at 37 °C, 5% for up

to 3 h, show that the parasites, although less motile, remain viable for the whole period of time used to infect the macrophages (Fig. 5).

The Table shows that sera from normal mice previously treated with megalol exhibit a strong trypanocidal effect upon *T. cruzi* stages from infected macrophages.

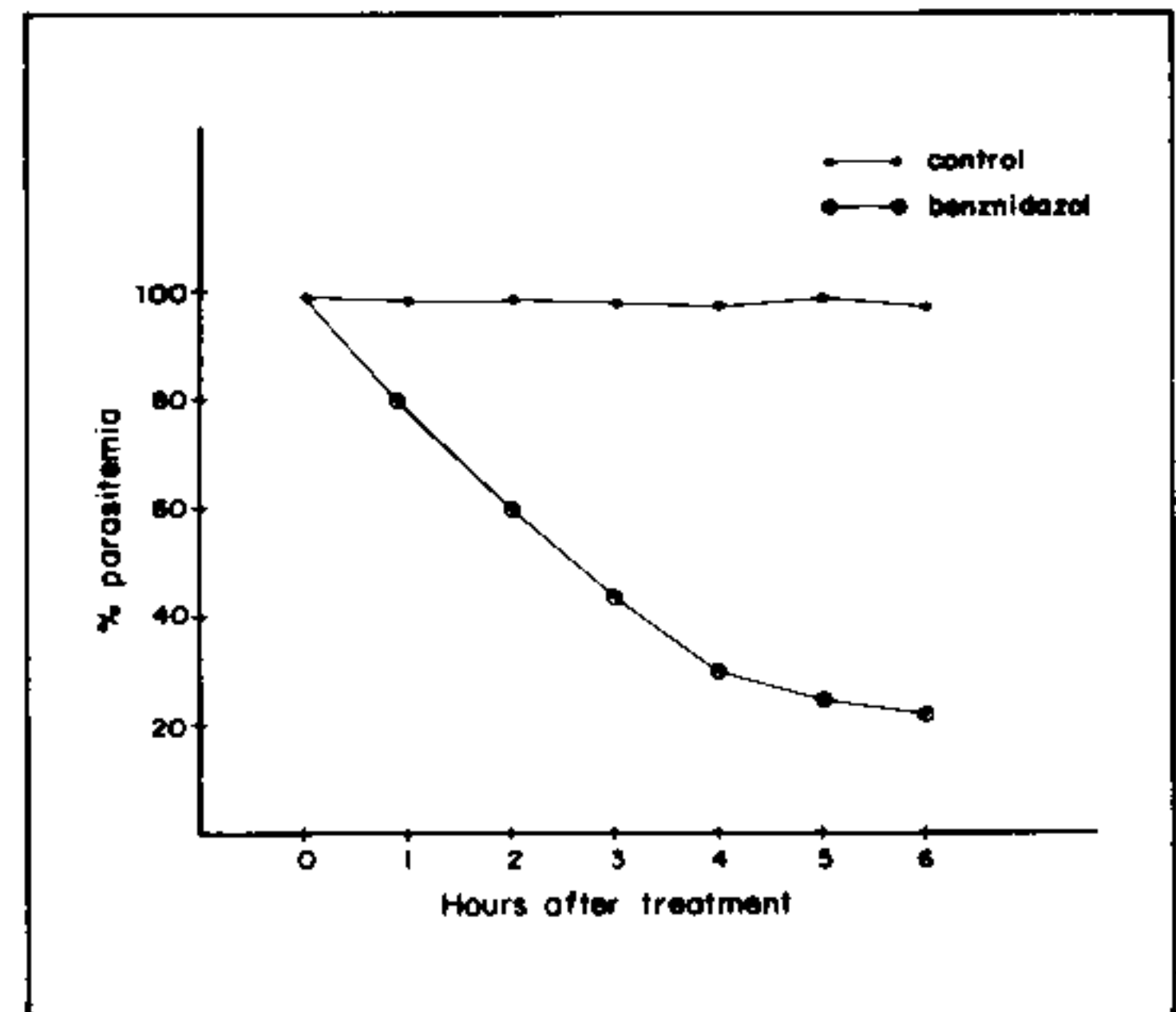


Fig. 1: parasitemia levels in mice infected with *Trypanosoma cruzi* Y strain and treated with a single dose of benznidazole (500 mg/kg, p.o.) as well as untreated infected control.

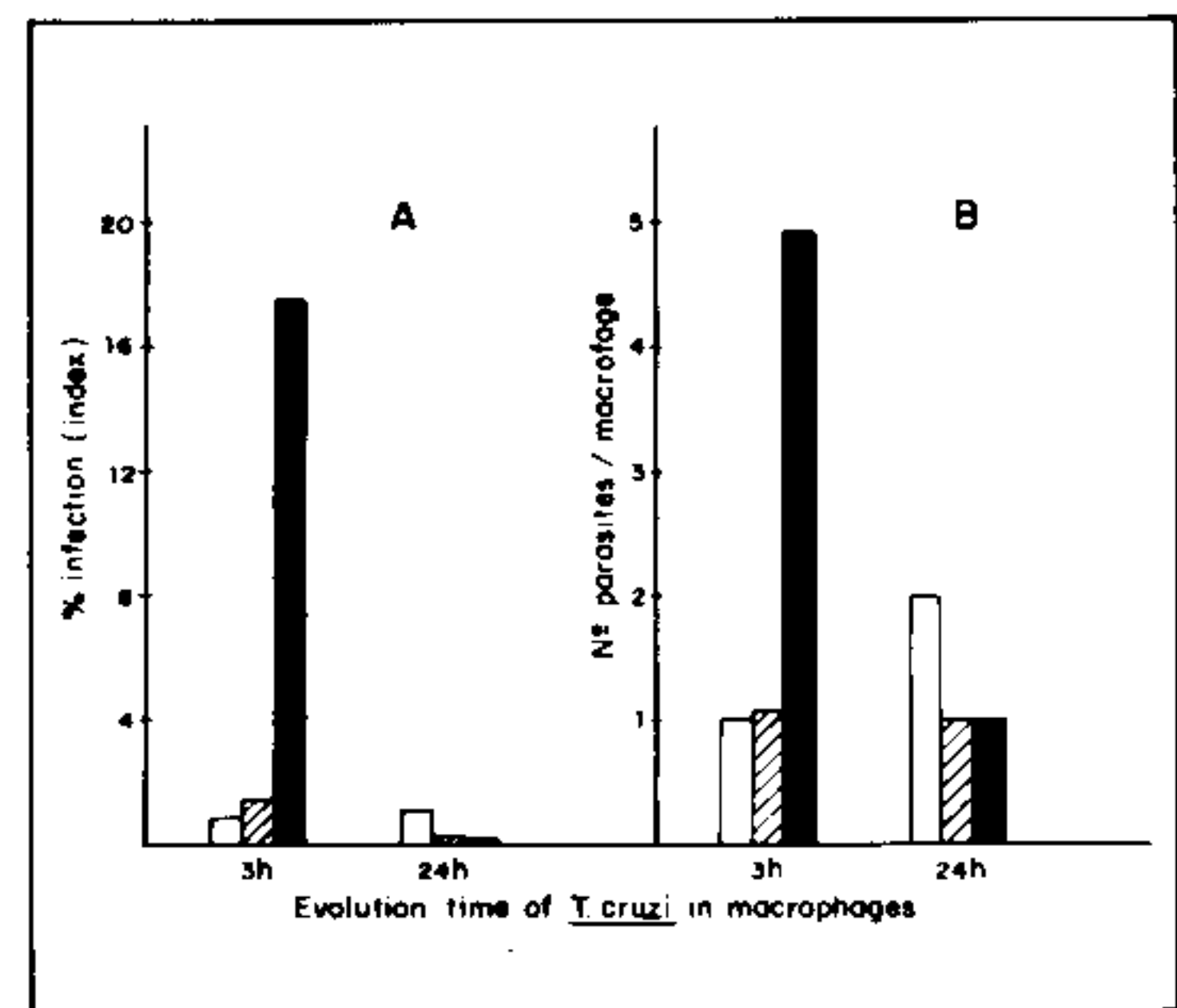


Fig. 2: effect of the specific treatment with megalol (500 mg/kg, p.o.) on the *in vitro* interaction between *Trypanosoma cruzi* BTry and mouse peritoneal macrophages; observations at 3 and 24 h of contact. A: index of macrophage infection. B: mean number of intracellular parasites/macrophages. □ BTry collected from untreated *T. cruzi* infected mice. ▨ BTry collected from infected mice 1 h after treatment ▤ BTry collected from infected mice 3 h after treatment ■ BTry collected from infected mice 3 h after treatment.

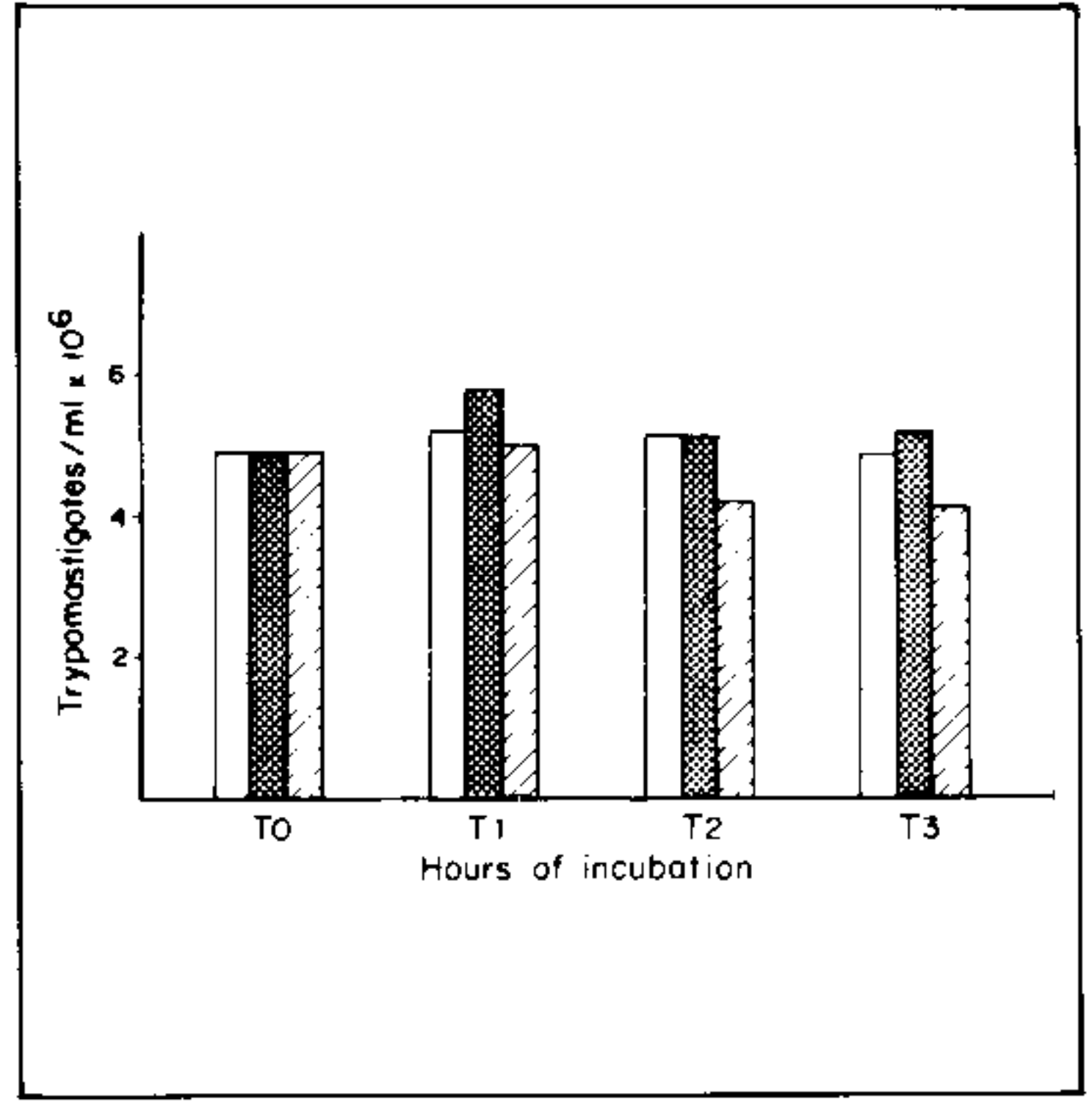
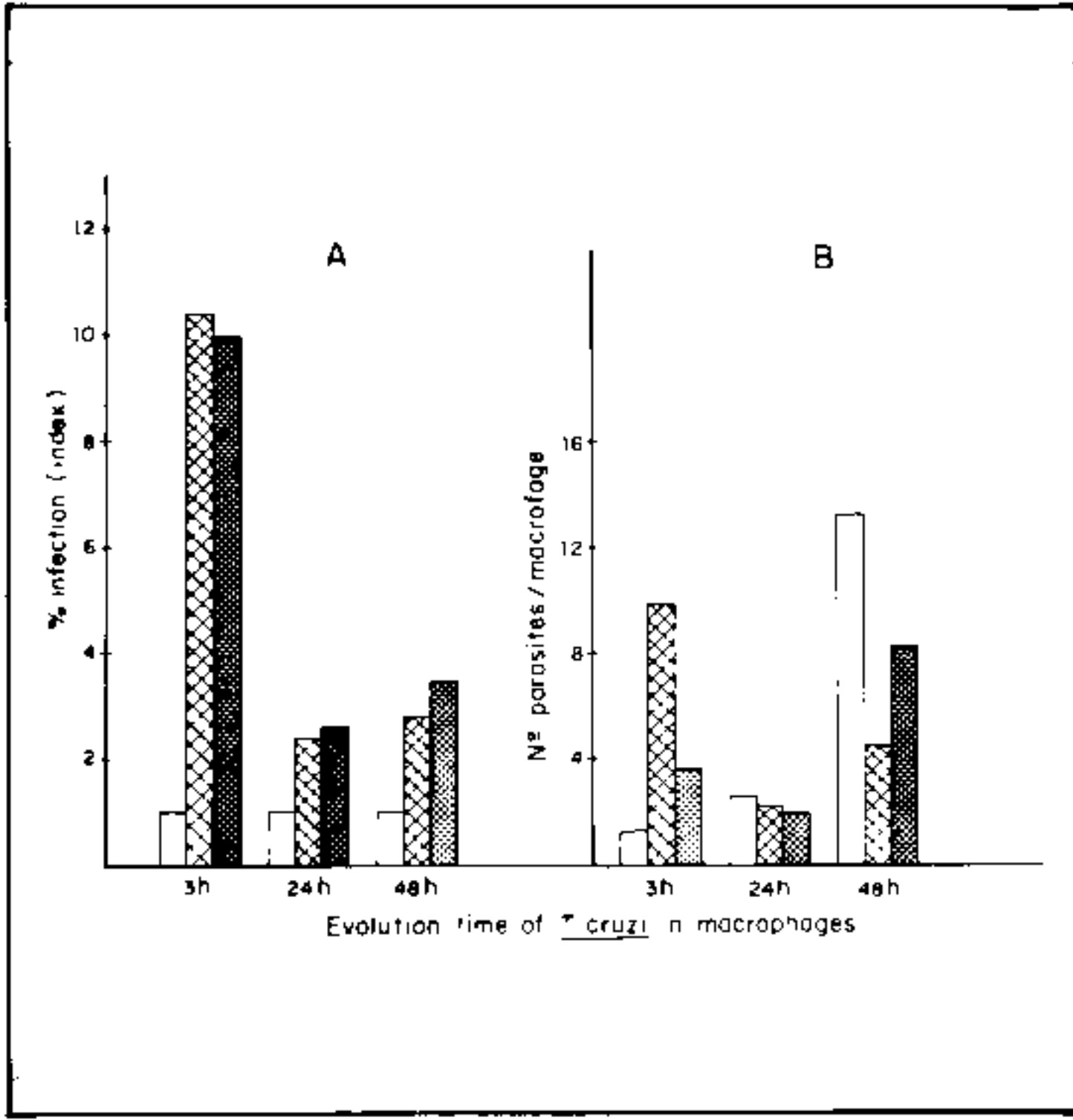


Fig. 3: effect of the specific treatment with benznidazole and nifurtimox (500 mg/kg, p.o.) on the *in vitro* interaction between *Trypanosoma cruzi* BTry and mouse peritoneal macrophages; observations at 3, 24 and 48 h of contact. A: index of macrophage infection. B: mean number of intracellular parasites/macrophage. □ BTry collected from untreated *T. cruzi* infected mice. ▨ BTry collected from infected mice 3 h after benznidazole treatment. ▩ BTry collected from infected mice 3 h after nifurtimox treatment.

Fig. 5: survival of *Trypanosoma cruzi* BTry incubated at 37 °C, 5% CO<sub>2</sub> for up to 3 h in the absence of macrophages. □ BTry collected from untreated *T. cruzi* infected mice, ▨ BTry collected from infected mice 1 h after treatment with megazol (500 mg/kg, p.o.). ▩ BTry collected from infected mice 3 h after treatment with megazol (500 mg/kg, p.o.). T0, T1, T2, T3: number of parasites after respectively 0, 1, 2 and 3 h of incubation.



Fig. 4: macrophages infected with BTry collected from mice 3 h after treatment with benznidazole (500 mg/kg, p.o.). A: stained after 3 h incubation. B: stained 24 h after incubation.

TABLE

Percentage of infection and mean number of intracellular stages in mouse macrophages infected with *Trypanosoma cruzi* and incubated for 24 h with sera from normal mouse and sera collected from normal mice 3 h after being treated with megazol, 500 mg/kg, p.o.

	Sera	
	Normal mouse	Megazol-treated mice
% infected macrophages	2.56	0.85
No. parasites/macrophage	7.4	3.4

## DISCUSSION

The data presented in this paper indicate that the *in vitro* phagocytosis of *T. cruzi* BTry by mouse macrophages is significantly increased by previous specific treatment of the infected host. Whether the role played by the macrophages in the parasite clearance is to scavenge damaged parasites or represents an active participation of the immune response in the chemotherapeutic effect is still unclear. In this respect Gee et al. (1983) reported that an antibody response to surface antigens was necessary to remove African trypanosomes from the blood of infected mice treated with DL- $\alpha$ -difluoromethylornithine (DFMO). On the other hand, the efficacy of at least three schistosomicidal agents (hycanthon, oxamniquine and praziquantel) active against *Schistosoma mansoni* was reduced in T-cell deprived mice as compared with immunologically intact animals (Sabah et al., 1985). More recently, Brindley & Sher (1987) treated with praziquantel intact and B-lymphocytes depleted mice infected with *S. mansoni* and concluded that the chemotherapeutic effect depends on a synergic interaction between the drug and the host immune response. Interestingly, the effector antibodies involved in this phenomenon start recognizing schistosome surface antigens which were exposed only after the drug administration.

In our experiments the highest percentage of phagocytosis and intracellular destruction of *T. cruzi* BTry has been caused by megazol treat-

ment, a finding that concurs with previous data showing that this compound is much more effective against this parasite than nifurtimox and benznidazole (Filardi & Brener, 1982). These data may suggest that the enhanced parasite uptake and destruction result from specific and early surface membrane alterations induced by the drugs. However, experiments carried out in our laboratory demonstrated that the treatment of *T. cruzi* infected mice with nifurtimox and benznidazole has not affected the distribution in the BTry of membrane surface carbohydrates detected by a number of fluorescein-labelled lectins (Fontes, 1989).

Recent published data indicate that the yield of oxyradicals currently implicated in the trypanocidal effect of nifurtimox (Docampo & Moreno, 1984), is not apparently applicable to megazol (Tshako et al., 1989) and that this compound and benznidazole seem to differ in their mode of action. It would be worth to investigate further alternative mechanisms, including membrane alterations, that may explain the trypanocidal effect of those nitroheterocyclic derivatives.

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