

EVOLUTION OF SARCOMA 180 IN MICE INFECTED WITH
TRYPANOSOMA CRUZI

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*Mice infected with Trypanosoma cruzi were challenged with 2×10^6 cells of sarcoma 180 (ascites tumor) by i.p. route, on day seven post infection. Tumor development was followed by evaluation of weight gain, by measurement of ascitic fluid produced and enumeration of tumor cells in ascitic fluid. Infected mice were more resistant to tumor development as demonstrated by reduction in ascites formation and by reduction in the number of tumor cells in ascitic fluid, at different time intervals after tumor challenge. The number of peritoneal cells exsudated after tumor inoculation was greater in infected mice than in controls. This increased resistance of mice infected with *T. cruzi* to tumor development could be due to the action of macrophages activated by the infection and by the action of endotoxins absorbed from the gut or produced by the own parasite.*

Trypanosoma cruzi infection in mice induces humoral and cell mediated immunosuppression (reviewed by Krettli & Pereira, 1981). In addition it increases macrophage activity as demonstrated by the increase in colloidal carbon clearance (Ortiz-Ortiz et al, 1976), phagocytic activity of peritoneal macrophages (Pereira & Sassine, 1976; Nogueira, Gordon & Cohn, 1977) and carbon uptake by liver macrophages (Pereira, 1977).

On the other hand, there is evidence that *T. cruzi* or *T. cruzi* extracts have an antitumoral activity (Galliard, Brumpt & Martinez, 1950; Coudert, 1956, 1958; Kagan, Norman & Hall, 1968), although these data have been in discussion. Amato Neto (1963) did not observe any effect of an associated *T. cruzi* infection in a woman with an advanced ovarian adenocarcinoma. In an area where Chagas' disease is endemic (Uberaba, Minas Gerais State, Brazil), Chapadeiro et al (1964) and Lopes, Pereira & Chapadeiro (1967) did not find differences between the frequency of malignancies in patients with the chronic form of trypanosomiasis and in the general population.

In this paper we showed that previous *T. cruzi* infection in mice reduces the development of Sarcoma 180 (S-180), as evaluated by measurement of ascites formation and by enumeration of tumor cells in ascitic fluid.

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MATERIAL AND METHODS

Male, outbred, albino mice, weighing 25 to 27g were used in the experiments. *T. cruzi* infection (10^4 circulating trypomastigotes of the Y strain) was performed by i.p. route. The parasitaemia was determined on day seven post infection, in a drop of blood collected after tail section and covered with a 20x20mm coverslip. Control mice received an i.p. injection of homologous blood without parasites. Seven days later, *T. cruzi* infected and control mice received by i.p. route, 0,2ml of a suspension with 2×10^6 S-180 cells, diluted in cold Ringer solution, and removed recently from a mouse with ascitic tumor. At the moment of inoculation the viability of tumor cells was higher than 95%, confirmed with the Trypan blue exclusion test.

At intervals of 48 h all the mice were weighed during the period of evolution of the tumor. *T. cruzi* infected mice, non infected with S-180 cells, were weighed for control. The difference between the weight on the first day of S-180 inoculation (Po) and the weight in following days (P) indicates the development of ascites. A group of *T. cruzi* infected and control mice were killed at different intervals (2, 3, 6, 8 and 12 days after tumor implantation) for ascites volume measurement, evaluation of the number of tumor cells and the number of peritoneal exsudate cells in ascitic fluid. The mice were killed, after ether anaesthesia, by axilar vein section. The peritoneal fluid was collected with a syringe armed with a 28 gauge needle, after injection of a known volume of cold Ringer solution. Cell counts were performed in a Neubauer chamber, after dilution in a 0,1% gentian violet solution in 5% acetic acid. The number of peritoneal cells was easily recorded because of the large difference in size between these and the tumor cells.

RESULTS

All the infected mice developed parasitaemia seven days post infection and 90% of them died within three weeks. The *T. cruzi* infected mice which received S-180 showed a survival time shorter than that of the infected mice without tumor implantation (Fig. 1).

The control mice that received S-180 showed a progressive rate of weight gain, four days after tumor inoculation, and all the mice developed market ascites (Figs. 2 and 3).

In *T. cruzi* infected mice the weight gain was lower than in controls. There was weight loss in the initial phase after tumor implantation and only a small number of mice developed ascites, but lower than in controls (Fig. 2). The volume of ascitic fluid produced and the number of tumor cells was significantly lower in infected mice than in controls (Fig. 3 and Table I).

The number of peritoneal cells among tumor cells, evaluated two, three and six days after tumor inoculation was higher in infected mice. In both control and infected animals the number of peritoneal exsudate cells increased after tumor challenge. Approximately 85% of these cells were mononuclear and 15% were polimorphonuclear.

DISCUSSION

The development of S-180 has been quantitated in various experiments by several investigators. Donneli, Rosso & Garantini (1969) showed that the S-180 tumor mass during a fixed time interval was approximately a linear function of the initial dose of tumor cells. Lubiniecki & Cypess (1975) in quantitative studies showed that the evolution of S-180 in mice can be separated into distinct phases: incubation period (time after inoculation until the beginning of ascites formation), duration of illness (period after the beginning of ascites formation until the death) and survival time (period between the

inoculation and death). The survival time and the incubation period were functions of the initial dose of tumor cells, while the duration of illness and the relative rate of weight gain were constant.

Our results showed in control mice that the rate of weight gain and of ascite volume development were approximately a linear function of time, four days after tumor inoculation. The incubation period was around four days. In *T. cruzi* infected mice there was weight loss due to the infection, making it difficult to follow the weight gain after tumor challenge. Only a few mice developed ascites (four among ten animals in one experiment) and the greater number of infected mice died with little or no ascites.

Although we could not study all the phases of tumor evolution in infected mice due to the weight loss and short life span induced by the infection, the ascites volume measurement and the enumeration of tumor cells in ascitic fluid showed clearly that a reduction occurred in tumor development in *T. cruzi* infected mice.

A possible explanation for these results is that activated peritoneal macrophages in infected mice killed more tumor cells than occurred in controls. Thus, the inoculum was reduced and the greater exudation of peritoneal cells in infected mice rendered the peritoneal cavity adverse for tumor cells. In fact there are many reports that peritoneal macrophages can kill tumor cells "in vitro" and "in vivo" (reviewed by Otter, 1981) and in *T. cruzi* infected mice the peritoneal macrophages are activated (Pereira & Sassine, 1976; Nogueira, Gordon & Cohn, 1977).

This tumoricidal effect of peritoneal macrophages in mice infected with *T. cruzi* could be due to the activation induced by the parasite. On the other hand, endotoxins produced by the parasite itself or absorbed from the gut could enhance the tumoricidal effect of activated macrophages, inducing the production of tumor necrosis factor (Alexander & Evans, 1971; Currie & Basham, 1975; Carswell et al, 1975; Doe & Henson, 1978 and Meltzer et al, 1979).

There is evidence that *T. cruzi* infected mice have high levels of circulating endotoxins. Infected mice are more sensitive to exogenous endotoxin and the suppression of intestinal flora by antibiotic treatment increases the survival time of infected animals (Bambirra et al, 1979). Also the parasite has lipopolysaccharide in its structure (Goldberg et al, 1979), although there is not evidence of any effect of this lipopolysaccharide on the mice (Oliveira Lima, personal communication). Therefore, the tumoricidal activity of peritoneal macrophages in mice infected with *Trypanosoma cruzi* could be explained by the action of endotoxins on those activated cells that generate the tumor necrosis factor.

Another possible explanation for this tumor resistance would be a lessened production of blocking antibodies, due to the humoral immunosuppression that accompanies *T. cruzi* infection in mice. Experiments are being planned to study the effect of *T. cruzi* infection on the immune response to tumor cells in mice.

Even though we did not measure the parasitaemia on different days after infection, it is possible that the tumor enhanced the infection, considering the shorter life span in infected mice that received tumor implantation. In fact it has been demonstrated that tumor cells inhibit the microbicidal effects of macrophages within the first 24 h after implantation (North, Kirstein & Tuttle, 1976).

RESUMO

Camundongos infectados com o *Trypanosoma cruzi* foram inoculados, por via intraperitoneal, com 2×10^6 células do sarcoma 180 (tumor ascite). O desenvolvimento do tumor foi acompanhado pela avaliação do ganho de peso, do volume de líquido ascíti-

co produzido e do número de células tumorais no líquido ascítico. Os camundongos infectados foram mais resistentes ao desenvolvimento do tumor, como demonstraram a redução da produção de líquido ascítico e do número de células tumorais nesse líquido, em diferentes dias após a inoculação do tumor. O número de células peritoneais exsudadas após a inoculação do tumor foi maior nos animais infectados do que nos controles. O aumento de resistência ao desenvolvimento do tumor, apresentado pelos camundongos infectados pelo *T. cruzi*, estaria relacionado à ação de macrófagos ativados pela infecção e pela ação de endotoxina absorvida do intestino ou produzida pelo próprio parasita.

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TABLE I

Evolution of sarcoma 180 in mice infected with *Trypanosoma cruzi*. Number of tumor cells in the peritoneal cavity of control mice and of mice infected with 10^4 trypomastigotes of *T. cruzi* (day 7 post infection) at different time intervals after the inoculation of $2 \cdot 10^6$ sarcoma 180 cells.

Days after tumor inoculation	2	3	6
Control	3.94±0.65	14.76±2.95	178.28±34.50
Infected	1.67±0.87	6.18±2.68	90.12±19.28

The results are the mean ($\times 10^6$) \pm one standard deviation of five mice per group. The differences observed are significant ($p < 0.05$).

TABLE II

Number of cells exsuded into the peritoneal cavity in different time intervals after implantation of 2.5×10^6 cells of the sarcoma 180 in control mice and in mice on day seven after infection with 10^4 trypomastigotes of *T. cruzi*.

Days after tumor implantation	2	3	6
Control	8.66±2.64	14.77±3.86	26.65±5.92
Infected	11.21±3.83	19.65±4.95	31.46±7.69

The results are the mean ($\times 10^6$) \pm one standard deviation of five mice per group. The differences observed are significant ($p < 0.05$).

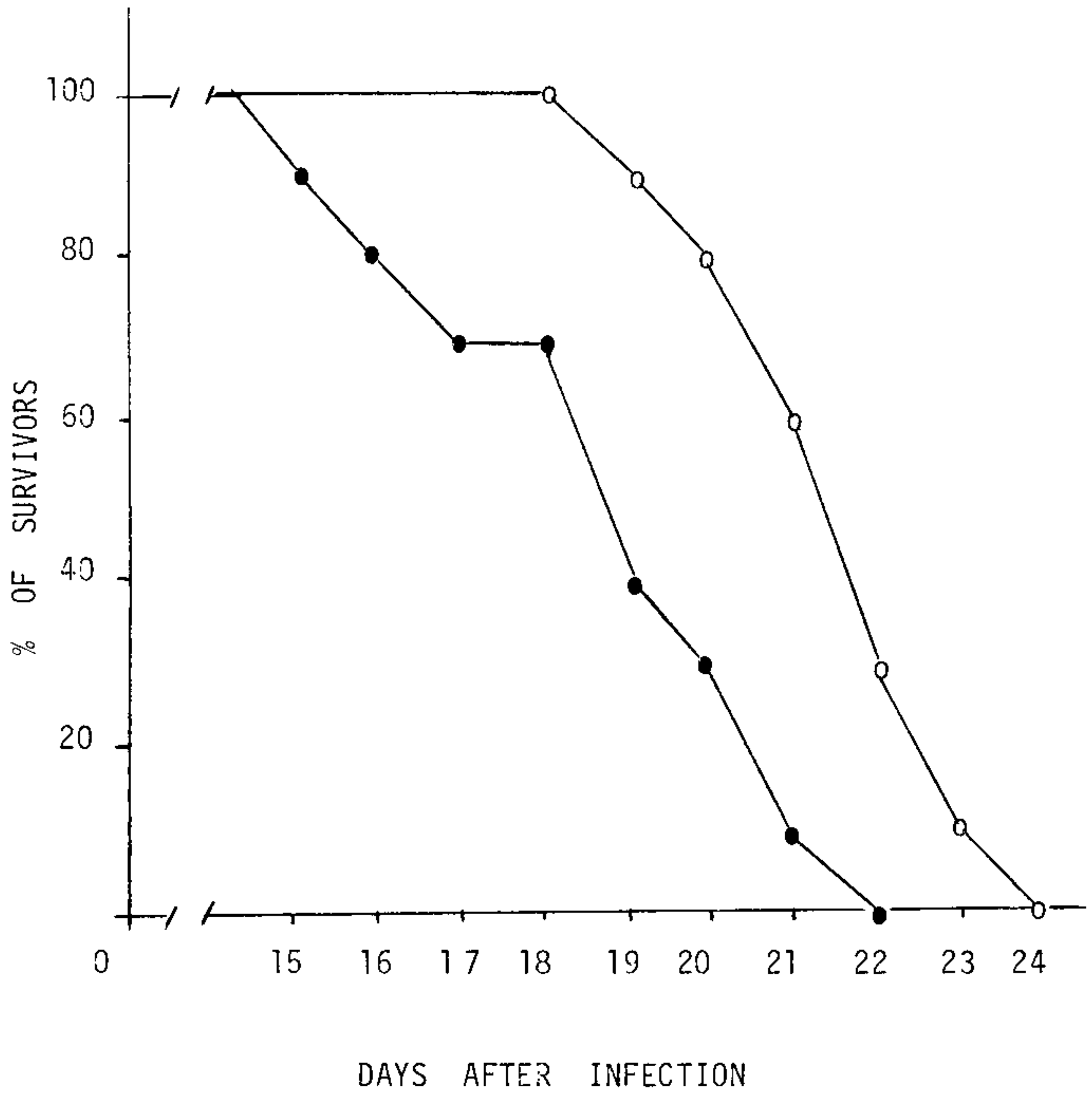


Fig. 1 – Mortality of mice infected with 10^4 trypomastigotes of *Trypanosoma cruzi* and inoculated with 2.5×10^6 cells of sarcoma 180, by i.p. route.

- — ○ - *T. cruzi* infected, without sarcoma 180
- — ● - *T. cruzi* infected, with sarcoma 180

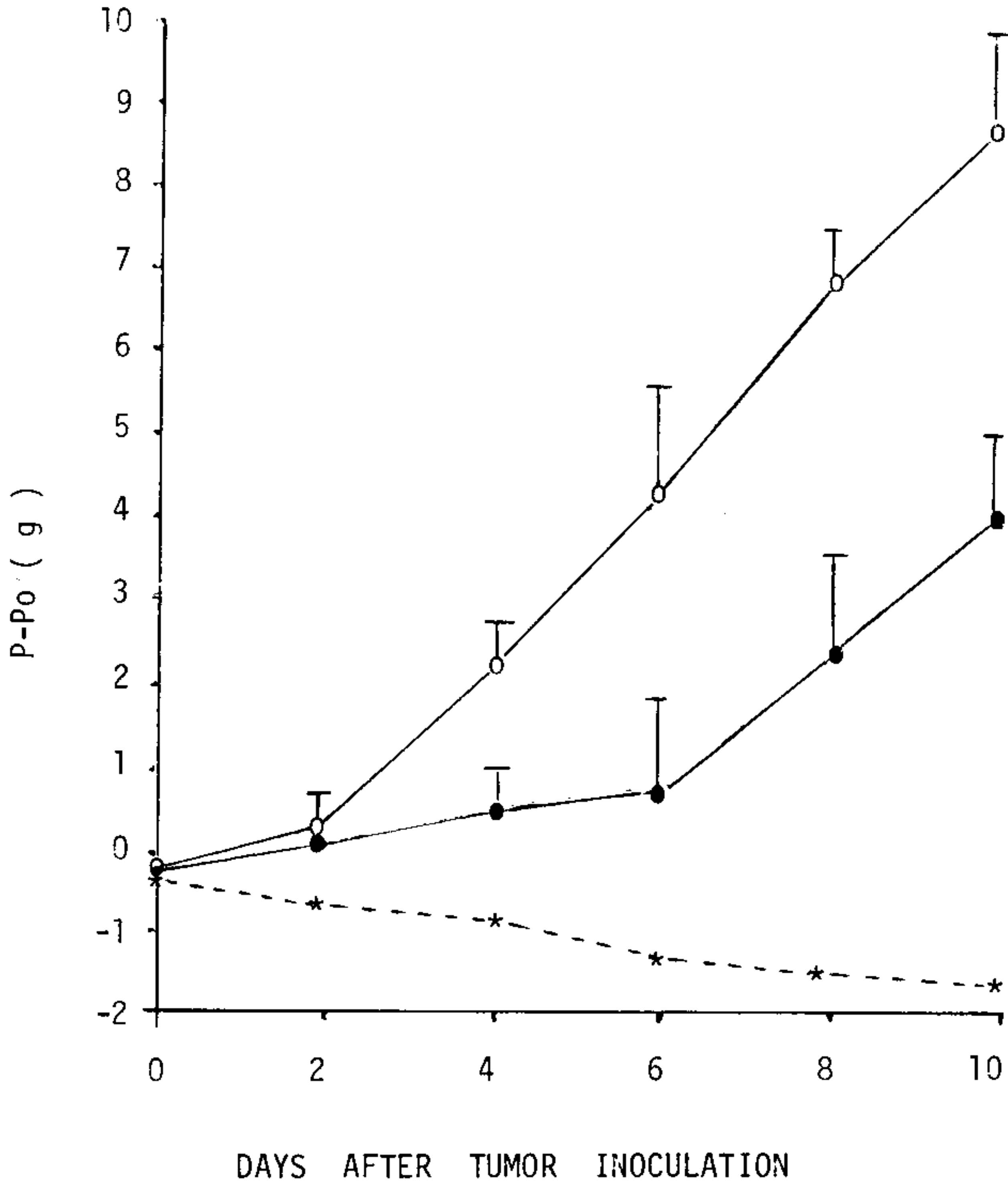


Fig. 2 – Evolution of sarcoma 180 in mice infected with 10^4 trypomastigotes of *Trypanosoma cruzi* and inoculated with 2.5×10^6 tumor cells by i.p. route on day seven after infection. The tumor evolution was evaluated by the weight gain ($P - P_0$; P_0 = weight on day of tumor implantation and P weight on following days). The results are the mean \pm one standard deviation of ten mice per group.

- — ○ = control mice with sarcoma 180
 ● — ● = *T. cruzi* infected mice with sarcoma 180
 x ---- x = *T. cruzi* infected mice without sarcoma 180

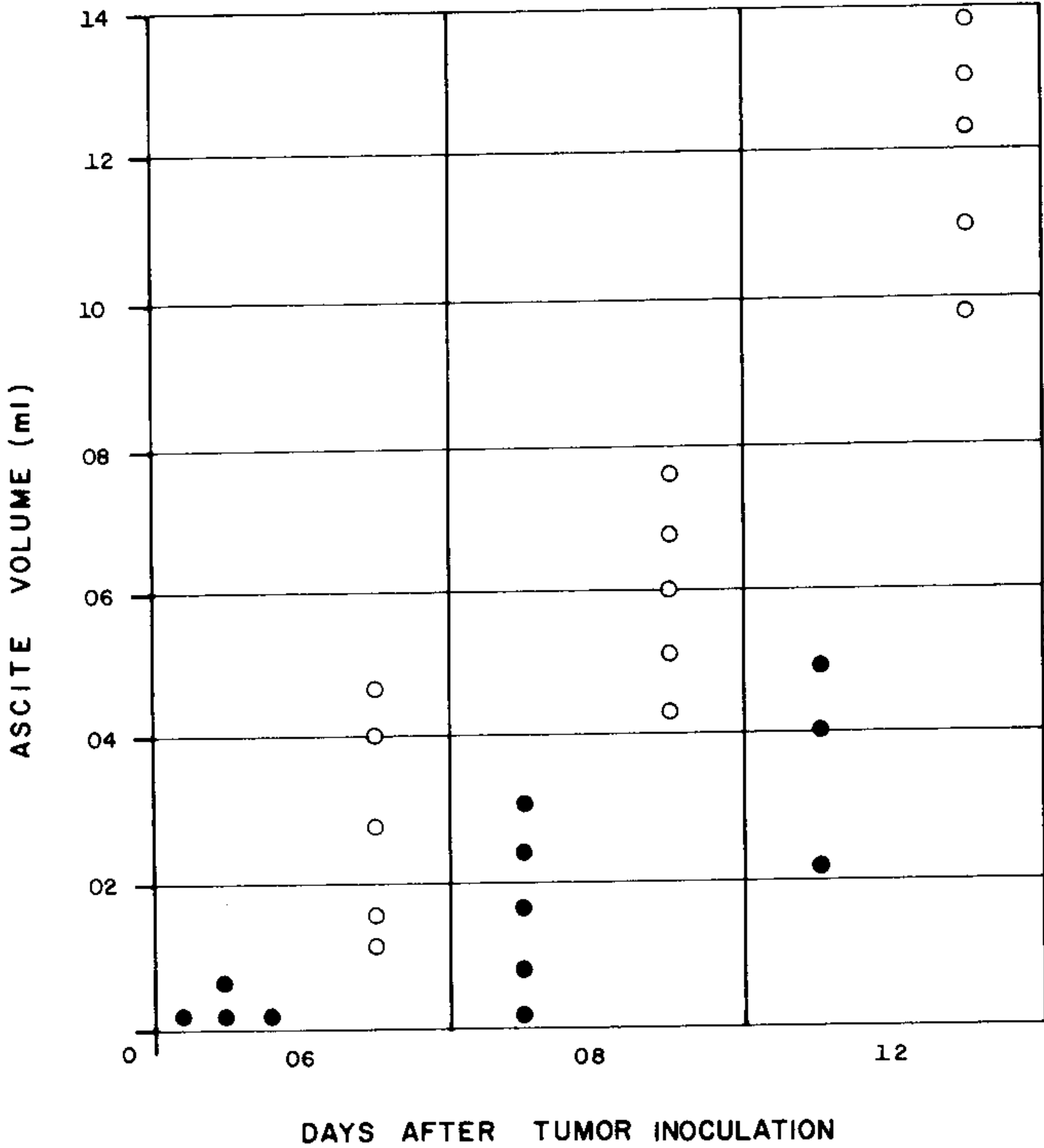


Fig. 3 – Evolution of sarcoma 180 in mice infected with 10^4 trypomastigotes of *Trypanosoma cruzi* and inoculated with 2.5×10^6 tumor cells, by i.p. route on day seven post infection. The tumor evolution was evaluated by the measurement of the ascitic fluid volume.

○ = control ● = *T. cruzi* infected, with sarcoma 180