HEMATOLOGICAL CHANGES IN MICE EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CRUZI

JARBAS E. CARDOSO* Z. BRENER*

Mice inoculated with Trypanosoma cruzi display an intense thrombocytopenia which is more severe in animals infected with the Y than CL strain. In animals inoculated with a T. cruzi strain which induces chronic infection (Colombiana), the number of platelets decreases as parasitemia ascends, and then reverts to normal values as soon as the acute infection merges into the chronic phase. The mechanisms involved in the thrombocytopenia are still obscure and are likely to be related to more general phenomena affecting the host rather than to direct damage of platelets or precursor cells by parasitism. Anemia and leukopenia are also present in T. cruzi infected mice.

Anemia and thrombocytopenia have been described in a number of experimental trypanosomiasis. Severe thrombocytopenia has been reported in rats infected with *Trypanosoma rhodesiense* (Davis et al., 1974) as well as in *Trypanosoma congolense* infected cattle (Wellde et al., 1978). Erythrocyte destruction causing anemia has been detected in mice inoculated with *T. congolense* and *Trypanosoma brucei* (Ikede, Lule & Terry, 1977). In this paper we describe severe hematological changes occurring in experimental Chagas' disease induced by different *Trypanosoma cruzi* strains.

MATERIAL AND METHODS

T. cruzi strains — Y (Pereira da Silva and Nussenzweig, 1953) and CL (Brener and Chiari, 1965). Both strains are being maintained since their isolation by intraperitoneal blood passages performed at the 7th and 12-14th days of infection for, respectively, Y and CL. Some aspects of the infection induced in mice by the two strains have been described by Brener (1977) and Mello and Brener (1978). In one series of experiments mice were inoculated with the "Colombiana" strain (Andrade et al., 1975).

Infection of mice — male albino mice, 18 - 20g, were inoculated with 100,000 bloodstream forms by intraperitoneal route. The number of parasites was determined according to Brener (1972).

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^{*}Centro de Pesquisas René Rachou, FIOCRUZ, Department of Parasitology and School of Pharmacy, University of Minas Gerais, 30000 — Belo Horizonte — Brazil.

Hematological methods — red cell counts were performed through an electronic particle counter (Coulter Counter). The number of leukocytes was determined in standard Neubauer hematocytometers. Leukocyte differential counting was carried out in May-Grunwald Giemsa stained smears. Reticulocytes were detected by supravital staining with brillant cresyl blue. Hemoglobin concentration was determined using the method of cyanometahaemoglobin.

Thrombocytes were counted by phase microscopy according to Brecher and Cronkite (1950). In some experiments normal and splenectomized mice inoculated with *T. cruzi* according to the experiments described by Brener et al. (1979) were used.

The hemolytic effect of plasma from *T. cruzi*-infected mice on mouse red blood cells (MRBC) was investigated according to Fiennes (1954) and Ikede, Lule & Terry (1977): 0.2ml of a 5% suspension of normal MRBC in 0.015M PBS at pH 7.4 was added to 0.1ml of plasma from normal as well as infected mice, the optical density being determined by a colorimetric procedure after 30 minutes incubation at 37°C.

The presence of platelet aggregation induced by bloodstream trypomastigotes was investigated according to Davis et al. (1974). Parasites were harvested from blood collected at the peak of parasitemia from mice inoculated with the Y strain. The defibrinated blood was centrifuged at 100g for 8 minutes at 4°C; the supernatant containing the parasites was then centrifuged at 1,000 for 15 minutes at 4°C and the pellet used for the experiments. 0.1ml of blood collected from normal mice using EDTA was mixed with 0.1ml of a suspension of 1×10^7 bloodstream forms and then shaken for 30 minutes at room temperature. Platelet countings were performed after 30, 60 and 90 minutes of contact between the whole blood and the concentrated parasites.

RESULTS

A marked thrombocytopenia was observed in animals inoculated with *T. cruzi*, which was much more severe in those infected with the *Y* strain than the *CL* (Fig. 1). The number of platelets was also determined in a group of animals inoculated with the "Colombiana" strain which, conversely to what happens with the highly lethal *Y* and *CL* strains, permits the animals to merge into a chronic phase after a gradual fall of the number of parasites. In this case the intense thrombocytopenia which occurred when the parasitemia was increasing, reverted to normal levels as the number of parasites gradually declined (Fig. 2), an evidence that the decrease in the number of platelets is closely related to the number of bloodstream forms. Previous splenectomy has not prevented the development of thrombocytopenia in mice inoculated with the *Y* strain, which presented a number of platelets much lower than normal controls and splenectomized uninfected animals (Fig. 3). No aggregation or destruction of platelets could be observed after incubation with the bloodstream forms suspension.

A similarly intense leukopenia was detected in the animals inoculated with both the Y and CL strains (Fig. 4). The number of red blood cells started to decrease a few days after inoculation of the Y strain and reached the lowest level at the peak of parasitemia whereas only mild anemia was observed in the CL-inoculated mice (Fig. 5). The levels of hemoglobin parallel the numbers of red blood cells in both groups of animals. The percentage of reticulocytes in the group of mice inoculated with the Y strain ascended during the course of infection (Fig. 6), suggesting that precursors of red blood cells had not been demaged by the infection. No hemolytic effect of the plasma from mice infected with the Y strain on red blood cells could be observed.

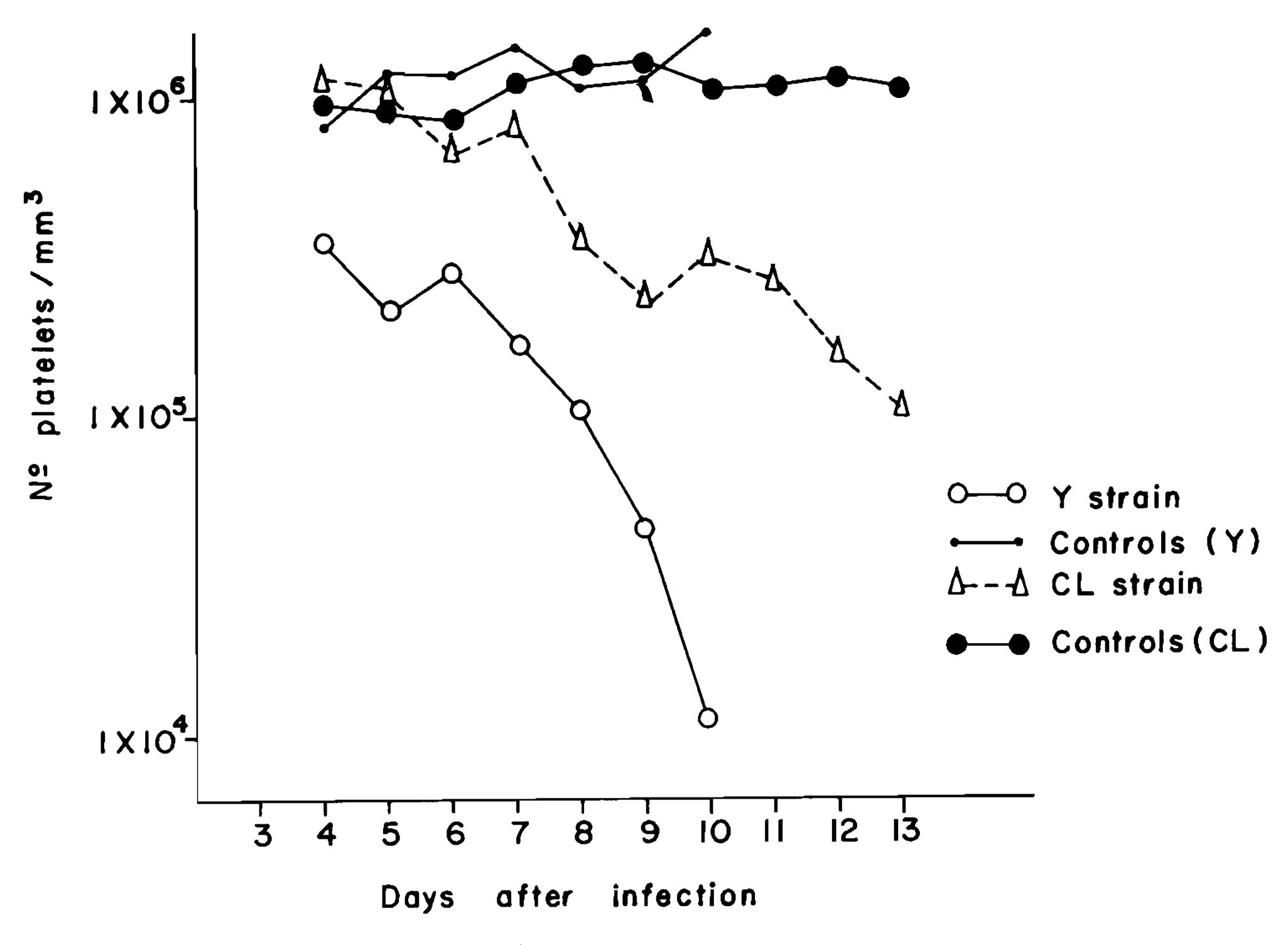


Fig. 1. Number of platelets in groups of mice inoculated with parasites from the *T. cruzi Y* and *CL* strains as well as normal controls.

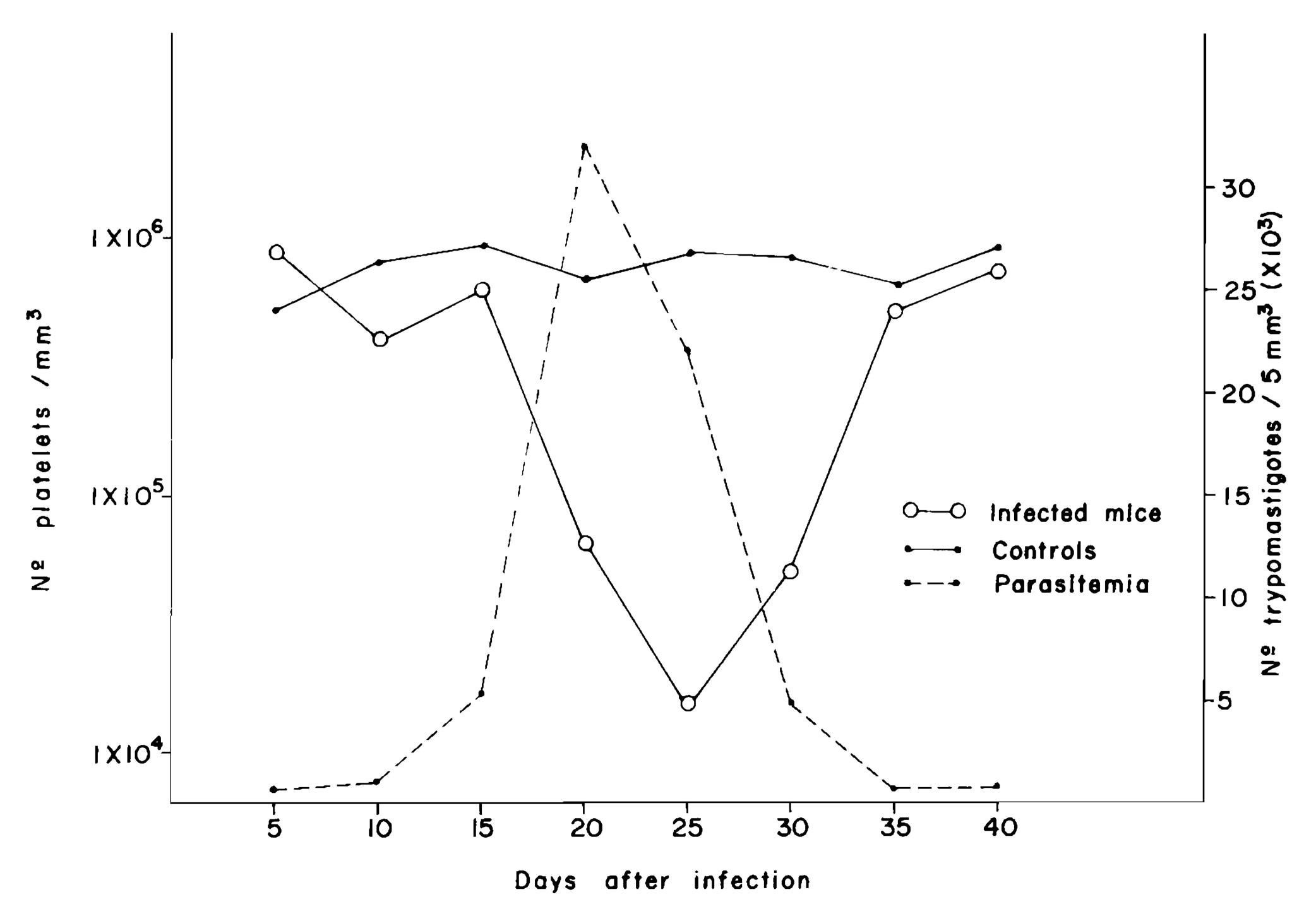


Fig. 2. Number of platelets and parasitemia in mice inoculated with the T. cruzi Colombiana strain.

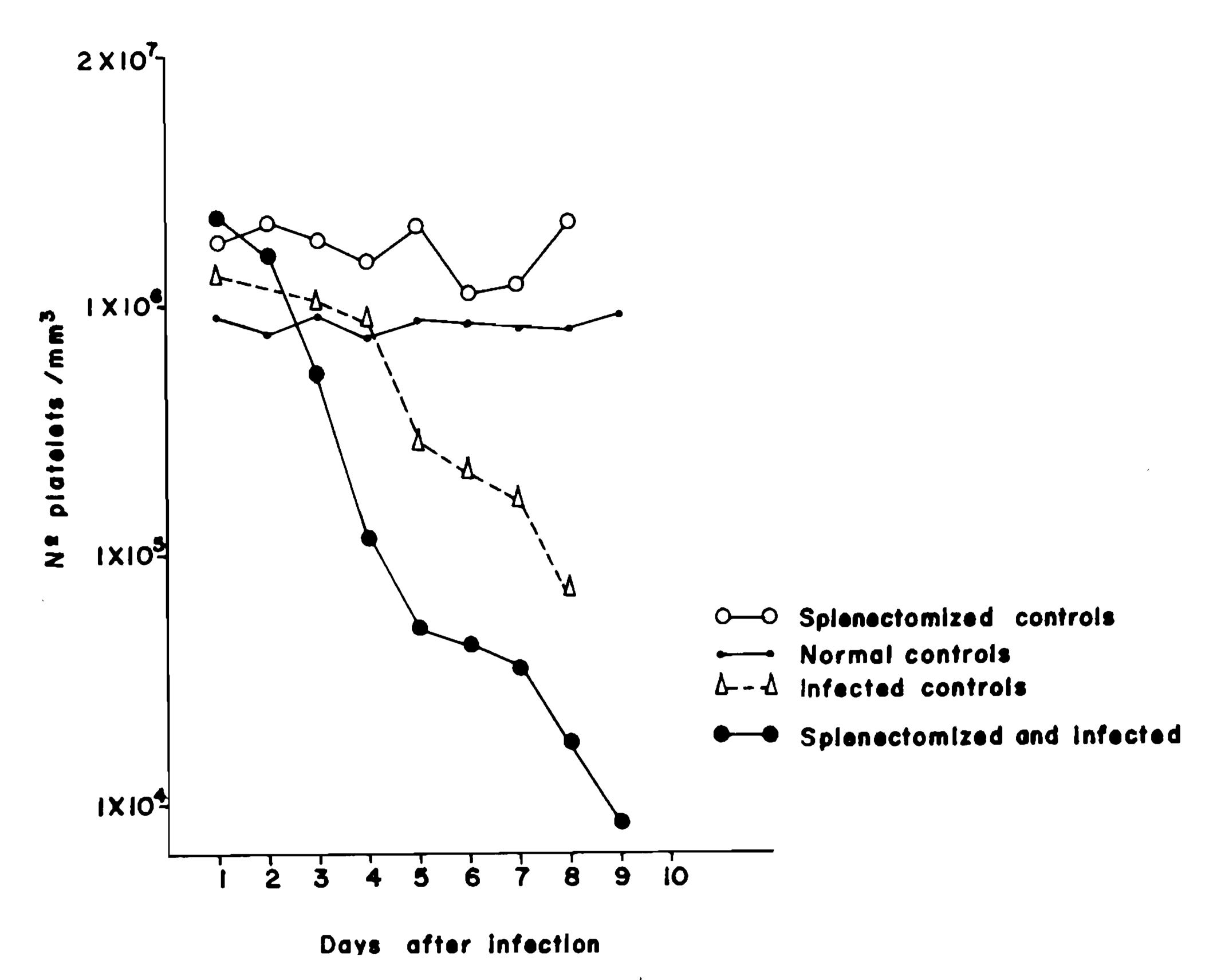


Fig. 3. Number of platelets in groups of splenectomized and normal mice inoculated with the T. cruzi Y strain.

DISCUSSION

Hematological alterations are a common finding in experimental infections induced by salivarian trypanosomes. Anemia was described in mice inoculated with *T. brucei* and *T. congolense* (Ikede, Lule & Terry, 1977). Thrombocytopenia was detected in rats infected with *T. rhodesiense* (Davis et al., 1974) and cattle experimentally inoculated with *T. congolense* (Wellde et al., 1978). In this paper we describe in *T. cruzi-*infected mice a marked decrease of the number of thrombocytes, red blood cells and leukocytes whose mechanism will be discussed.

Thrombocytopenia was the most striking finding in the inoculated animals. A 100-fold decrease in the number of platelets was often found in groups of animals infected with the T. cruzi Y strain whereas a milder thrombocytopenia was present in the CL-inoculated animals. Since previous work demonstrated that Y strain parasites affect selectively the spleen, liver and bone marrow of infected mice (Mello & Brener, 1978), a possible explanation for the intense thrombocytopenia induced by this strain would be an injury of precursors cells by the heavy parasitism of the bone marrow and spleen. As the fall in the number of platelets is frequently very rapid, occurring even before the peak of parasitemia (which is at the 7th day of infection), and since thrombocytopenia is also found with the CL strain which causes a negligible parasitism of those organs, this presumable harmful effect on precursor cells is not likely to be the only mechanism involved in the platelets depletion. Nevertheless, possible disturbances in the thrombocytes turn-

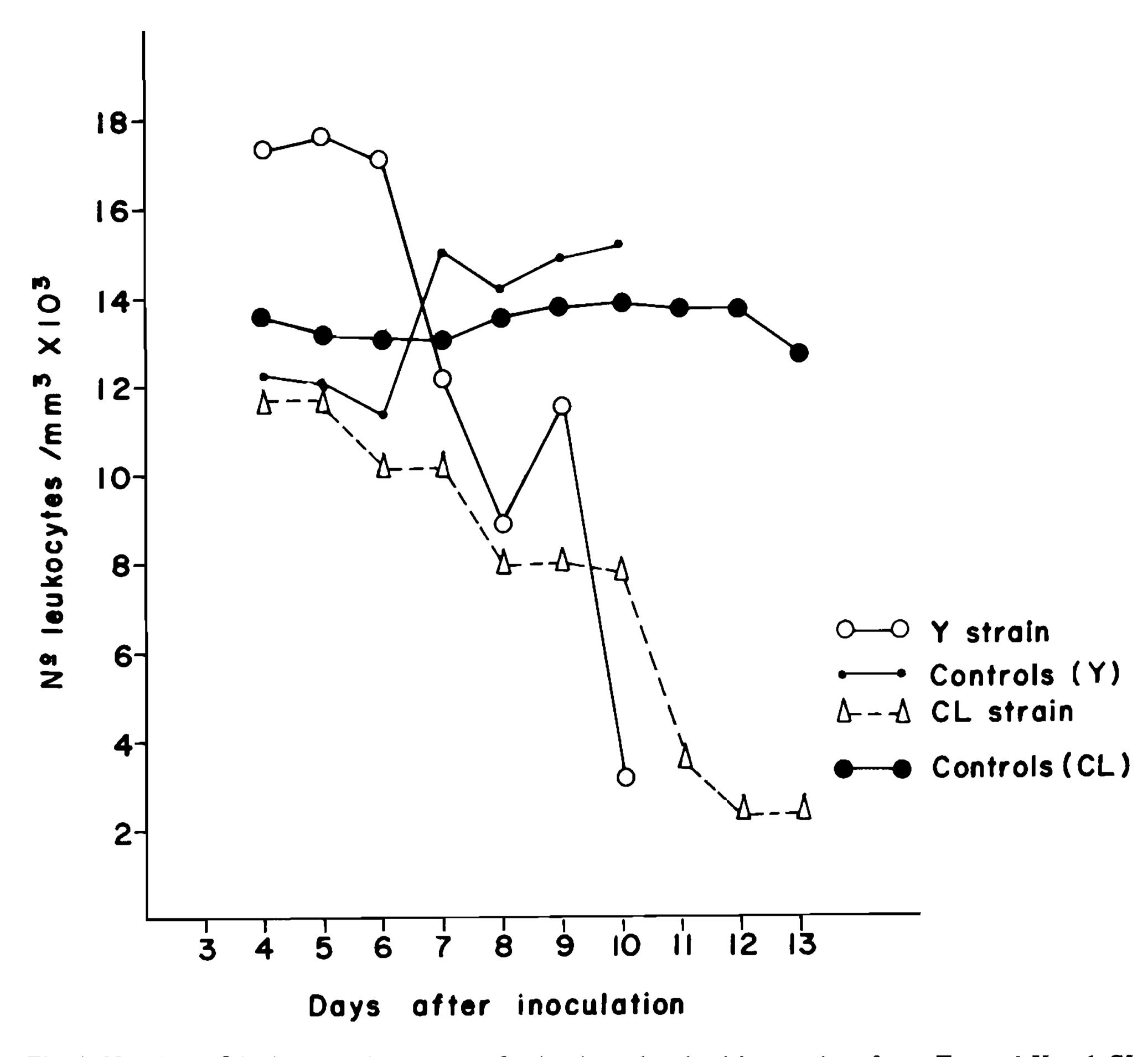


Fig. 4. Number of leukocytes in groups of mice inoculated with parasites from T. cruzi Y and CL strains as well as in controls.

over could only be identified by studies with 51 Cr-labelled platelets which have not been yet performed in animals inoculated with T. cruzi.

Although a close relationship could be observed between parasitemia and throm-bocytopenia, which was evident in animals inoculated with the three *T. cruzi* strains, no direct effect of isolated trypomastigotes on the platelets, such as the aggregation and lysis induced by *T. rhodesiense* blood forms (Davis et al., 1974), could be observed in our material. Alternative causes for the phenomenon could be primary sequestration of platelets in the spleen, disseminated intravascular coagulation and splenic pooling (reviewed by Davis et al., 1974) or destruction of thrombocytes mediated by immune-complexes (Schulman, 1964). Since the mechanism of thrombocytopenia in *T. cruzi* infections, similarly to what occurs in the salivarian trypanosomiasis, remains obscure, there is still room for specific investigations such as the presence of circulating fibrin degradation products and intravascular fibrin deposition indicative of disseminated intravascular coagulation, or the participation of immune-complexes and complement in the process of platelets sequestration.

The mechanisms participating in the anemia which occurs in experimental trypanosomiasis are also not yet completely known. Even in livestock infected with the so-

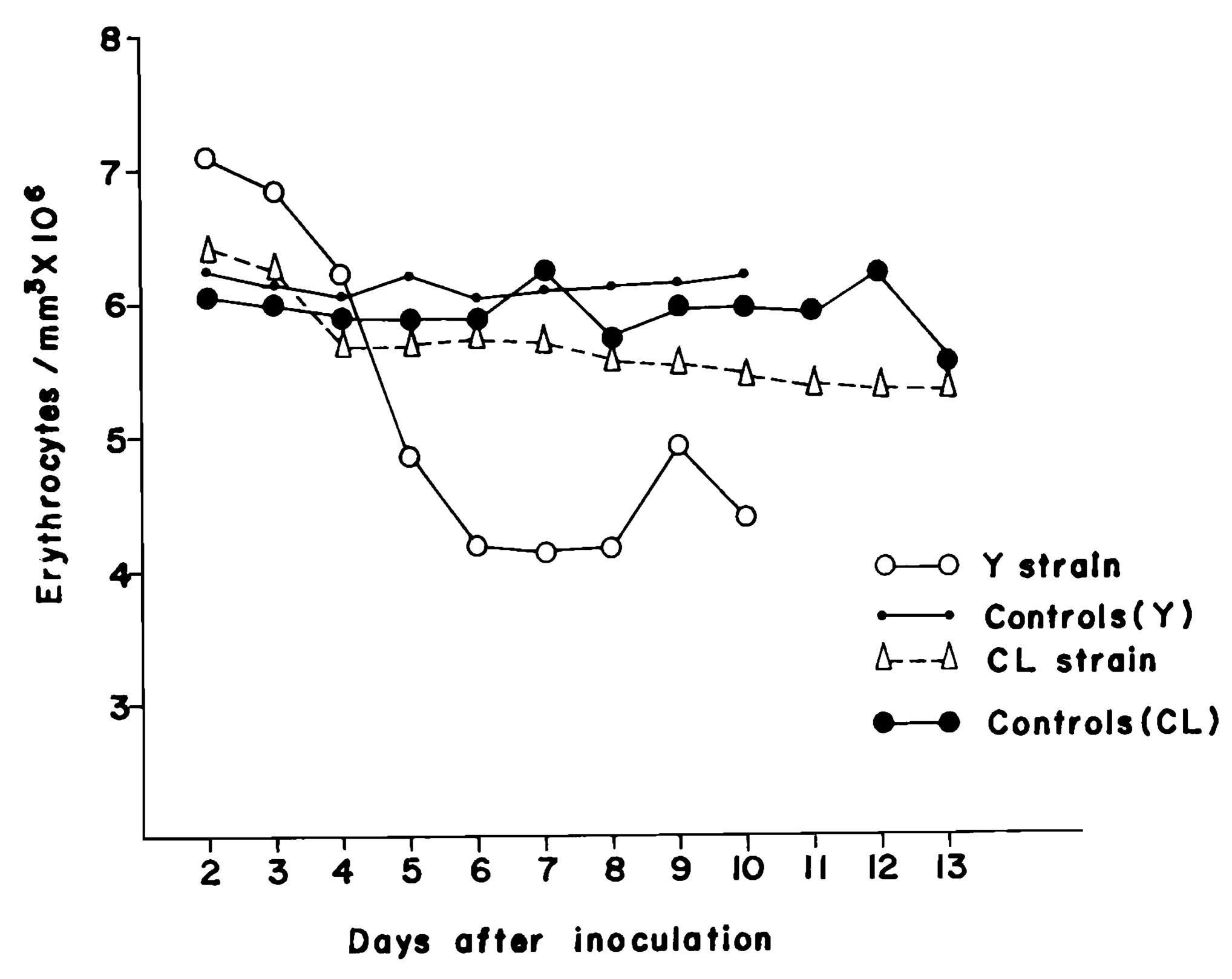


Fig. 5. Number of red blood cells in groups of mice inoculated with parasites from T. cruzi Y and CL strains as well as in controls.

called "haematic group" of trypanosomes (Trypanosoma vivax, T. congolense) the pathogenesis of the anemia is not clear and has been related to different factors such as increased erythrocytes destruction, bone marrow injury and haemodilution (Ikede, Lule & Terry, 1977). In this case of T. cruzi, no haemolytic effect of plasma from Y strain infected mice could be detected. The possibility of increased erythrophagocytosis in the spleen or hemolysis mediated by auto-immune reactions have not been investigated.

The quantitative differences in the studied hematological data between animals inoculated with the Y and CL strains should be considered as expressing an intraspecific variation which should be added to a number of differences already reported for the "polar" populations, related to morphology of the parasites, distribution of intracellular parasites, susceptibility to chemotherapeutic agents, etc. (Brener, 1977).

The relevance of our experimental findings to the human acute phase of Chagas' disease is not clear. In most acute cases haematological data are not very much altered, lymphocytosis being the most typic event at this stage of the disease (Rassi, 1979). Nevertheless, it is possible that in a few lethal acute cases such haematological disorders may occur as happens in some rare severe clinical forms of malaria and African trypanosomiasis presenting coagulation disorders.

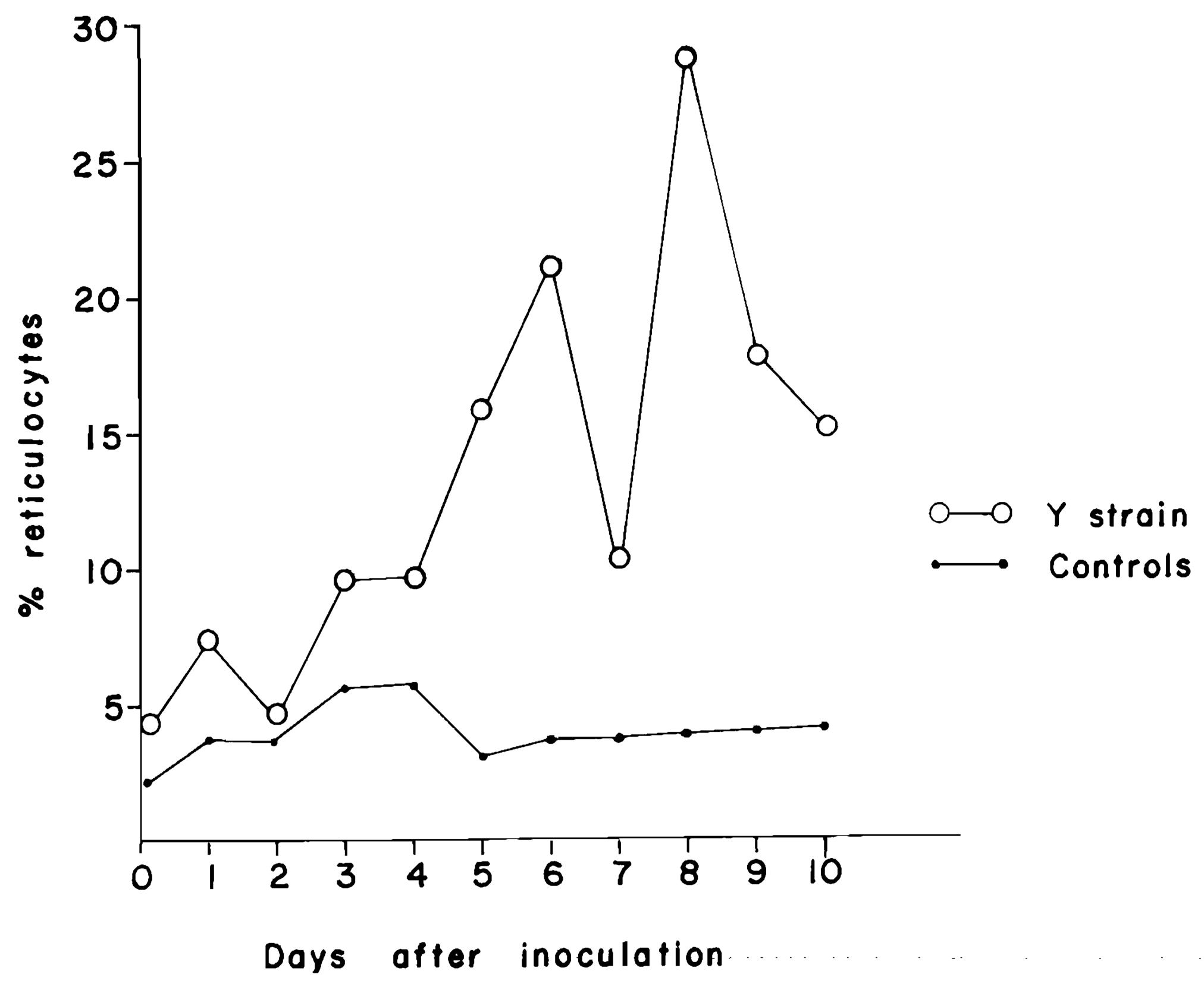


Fig. 6. Number of reticulocytes in mice inoculated with T. cruzi Y strain as well as in controls.

RESUMO

Camundongos inoculados com *Trypanosoma cruzi* mostram uma trombocitopenia intensa que é mais severa em animais infectados com a cepa Y de que com a cepa CL. Nos animais inoculados com uma cepa de *T. cruzi* que induz uma infecção crônica (cepa Colombiana), o número de plaquetas diminui enquanto a parasitemia aumenta, voltando a valores normais quando a infecção passa da fase aguda para a fase crônica. Os mecanismos envolvidos na trombocitopenia são ainda obscuros e possivelmente mais relacionados a fenômenos gerais afetando o hospedeiro de que a danos diretos causados pelo parasitismo às plaquetas ou às células precursoras. Anemia e leucopenia encontram-se também nos camundongos infectados com *T. cruzi*.

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