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MORPHOLOGY AND LEUKOCYTE VALUES IN THE EXPERIMENTAL CHAGAS DISEASE.

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ABSTRACT

Morphological alterations of leukocytes were studied in three groups of mice: one injected intraperitoneally with trypanosome infected blood, another that was injected with normal blood, and a third group that was injected with normal saline solution.

On the 5th day after inoculation, leukopenia was observed in the infected mice and leukocytosis in those that received normal blood, in comparison with the group that only received saline. Both findings were more accentuated on the 10th day; but on the 15th day the leukocyte counts reached normal values in the infected group, remaining high in the blood injected one.

The differential formula showed lymphocytosis and neutropenia in the two groups injected with blood, but a high increase of leukocyte debris was only observed in the infected group. This finding coincided with the lower neutrophil levels and the time of maximum parasitemia.

The presence of the parasite in peripheral blood may be correlated with an increase of cellular lysis. In fact, an increase of leukocyte debris was observed when the parasitemia and leukopenia were greater.

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INTRODUCTION

It has been shown that in Chagas disease hematological alterations are frequent. It is generally accepted that mild leukocytosis with lymphocytosis and eosinopenia are present in the acute phase of the disease.

Dias[2] studied human infections and found slight leukocytosis with intense macrolymphocytosis in one patient in the acute phase of the disease and three cases with less than three months with the infection. He also found a tendency to leukopenia with eosinophilia and moderate increase of basophils. Mazza et al[7-39] confirmed Dias' results.

Niño[43] studied the experimental disease in mice and other laboratory animals infected with **Trypanosoma (Schizotrypanum) cruzi** and observed initial lymphocytosis followed by neutrophilia.

Mazza et al[39] and Romaña[47] pointed out that, in some acute cases, beside lymphocytosis and neutropenia there was intense leukocytosis with relative eosinophilia at the end of this stage. In several observations, Mazza found more than 20% of monocytes and considered that this finding should be regarded as characteristic of the disease.

Wood[53] found in experimentally infected mice macrolymphocytosis and eosinopenia in the late stage of the acute infection. He also found "young lymphocytes" and hipersegmented neutrophils in peripheral blood.

Cardoso and Rosenfeld[1], Pinto[46] and Tálice[51] reported monocytosis. Cardoso and Rosenfeld[1], Pessoa et al[44] and Pessoa and Spinelli[45] found in acute human cases atypical lymphocytes ranging from 0.4 to 1%.

Segura et al[48] observed leukocytosis and an increase of neutrophyls in children under 2 years. Normal leukocyte counts were found in older children; and lymphocytosis was frequent in children above 6 years of age.

Jamra et al[5] found marked lymphocytosis with atypical lymphocytes and observed that neutrophils and eosinophils increased with the progress of the infection.

The above studies mainly referred to quantitative leukocyte changes and variations of their differential counts but scarce information is available in relation with morphological changes of the white cells.

It is well known that bacterial and viral infections cause morphological alterations of the blood cells: appearance of normally absent elements like plasma cells, changes in the chromatin and staining characteristics of the cells and general signs that suggest an increase in cellular lysis[4,6,40,41,50,52]. Since T.(S.) **cruzi** produces quantitative variations of white cells, it seems logical to think that they could be accompanied by morphological alterations of variable degree as it happens in the mentioned infections.

The purpose of this paper was to study the leukocyte alterations in mice with acute experimental Chagas disease emphasizing those aspects that could suggest cellular damage.

MATERIAL AND METHODS

The Brazil strain of T.(S.) **cruzi** isolated in 1953 by Silva and Nussenzweig[49] from a patient with Chagas disease was used for these studies. The strain is maintained in mice by successive intraperitoneal, inoculations, every 10 days.

White male albino mice, about 2 months old of the MRT strain were used for the experiments. Sixty normal animals served as controls to 60 infected ones. All of them were injected intraperitoneally. Sixty animals were injected with 0.1 - 0.2 ml of total blood (containing 110-125 trypanosomas) withdrawn from infected mice; thirty animals received the same volume of total blood, obtained from normal mice, and another group of thirty only received normal saline solution (0.1 - 0.2 ml).

Infected blood used for inoculation was obtained by cardiac puncture with heparinized syringe at the 10th day of evolution of the experimental disease. Previous studies in our laboratories, showed that parasitemia was at its maximum at this time and that infected animals died between 2 and 2-1/2 weeks after the inoculation. The number of parasites were counted with a hemacytometer. Blood withdrawn from the tail of different groups of 40 mice (20 that had received infected blood, 10 that received blood from apparently normal mice and 10 that received saline) was used for blood smears on the 5th, 10th and 15th day after inoculation.

The smears were stained with Giemsa stain and studied with optical microscope, by using immersion objective. One hundred cells were observed in every smear. White differential count was made on each case, taking note of the number of vacuolated cells and leukocyte debris per hundred of white cells.

RESULTS

White blood cells.- On the 5th day after inoculation, there was a statistically significant reduction of leukocytes in the infected animals ($p<0.05$) as compared with the saline injected group, and there was a sharp rise of leukocytes in the mice injected with apparently normal blood ($p<0.001$). Both events were more pronounced on the 10th day ($p<0.001$ and $p<0.001$ respectively), but while the infected mice returned to normal values on the 15th day, the blood injected group remained high (Fig. 1).

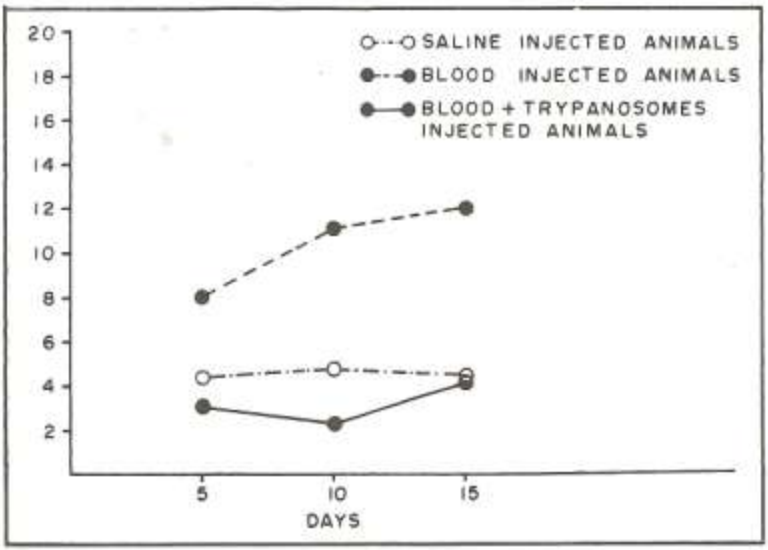

Fig. 1.– Variations of leukocyte values with the evolution of the infection. (Each point represents the mean of the observations).

Leukocyte formula.- Figure 2 shows that on the 5th day of infection, there were no significant variations of the differential formula among the three groups. However, at the 10th day an accentuated lymphocytosis was observed ($p<0.001$), with the corresponding reduction of neutrophils in the groups that received blood, the lymphocytosis being more evident in the group that was not infected. The difference was not significative; but there was a $p<0.01$ between these two groups and the one injected with saline. Fifteen days after the inoculation, the lymphocytes reached normal values in all groups. There were no significant variations in the other cells of the differential formula.

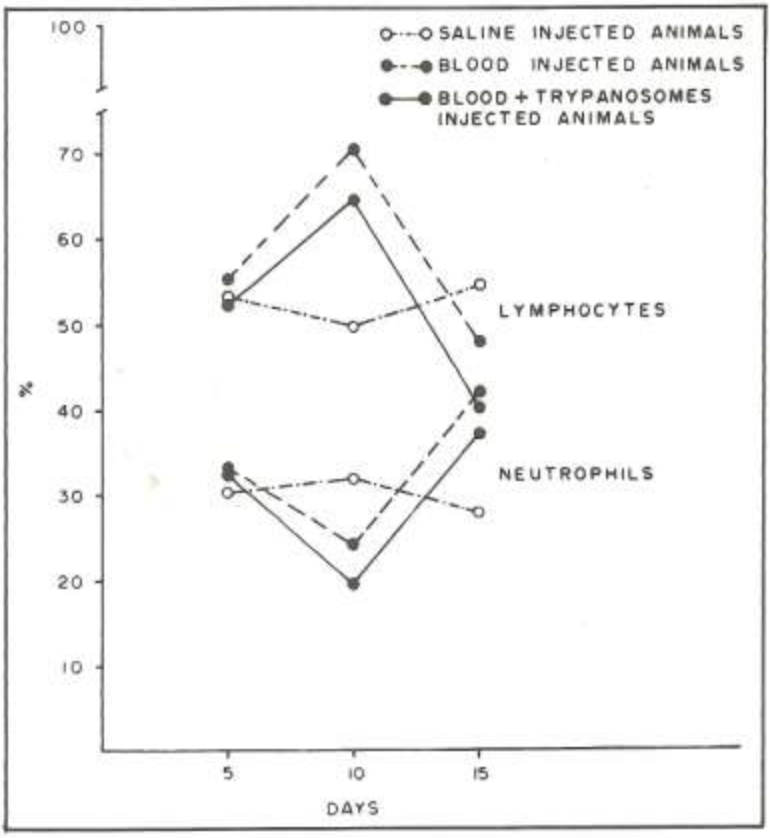

Fig. 2.– Variation of leukocyte formula with the evolution of the infection. (Each point represents the mean of the observations).

Leukocyte debris and vacuolated cells.- The leukocyte debris were classified according to Negrette[42] as follows: a) cells in lysis (Fig. 3) b) nuclear debris (Fig. 4) and c) "basket cells" (Fig. 5). Five days after the inoculation, no significant variation in the amount of leukocyte debris was observed among the groups. However, on the 10th day, a significant increase was found in the infected mice ($p<0.001$) in comparison with the other two; this change was mainly due to increased nuclear debris and the "basket cells". On the 15th day the leukocyte debris reached normal levels (Fig. 6).

No statistical difference was encountered in the number of vacuolated cells.

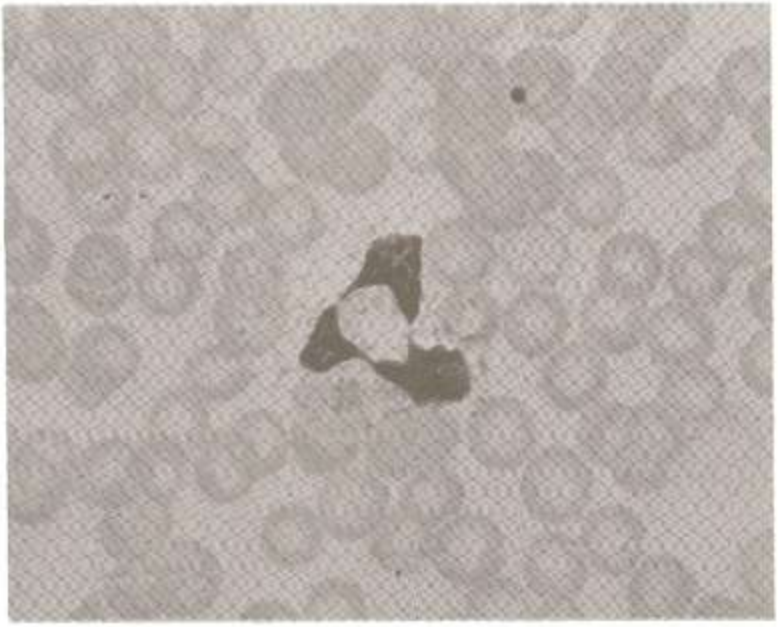

Fig. 3.– Cell in lysis.

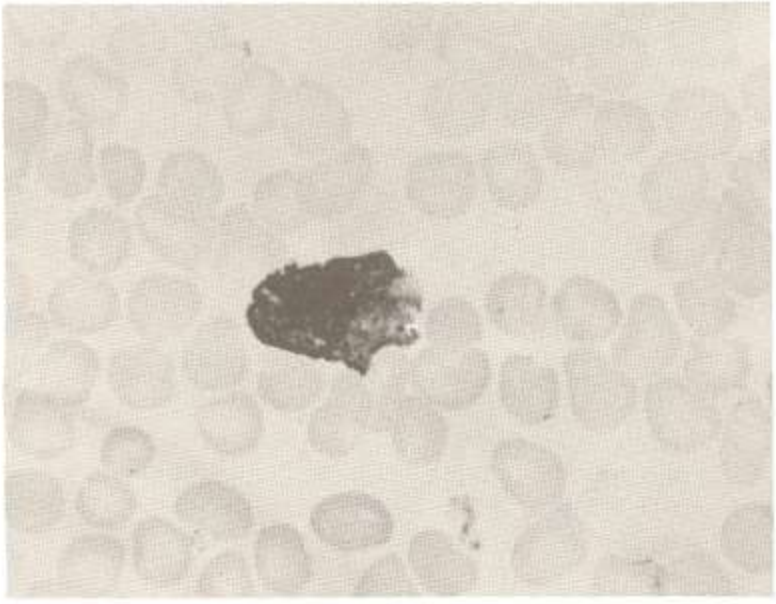

Fig. 4.– Nuclear debris.

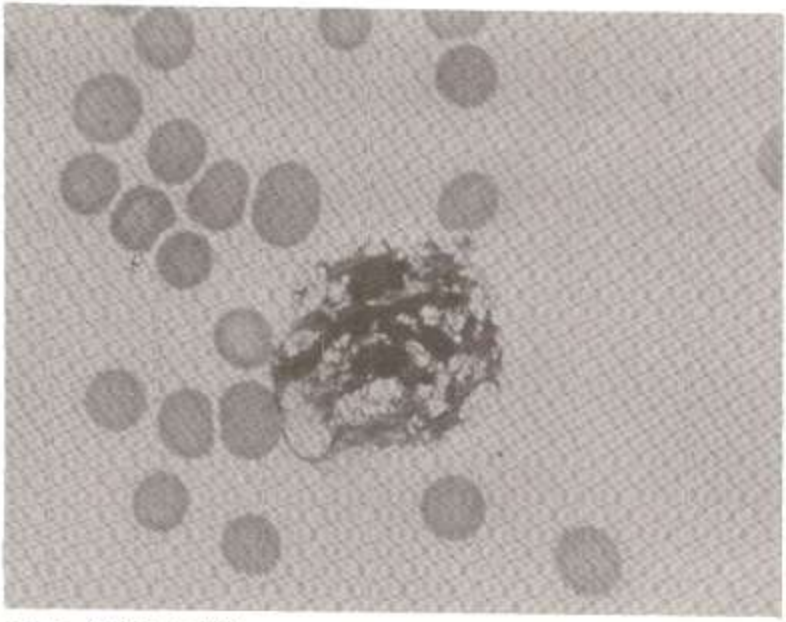

Fig. 5.– "Basket cell".

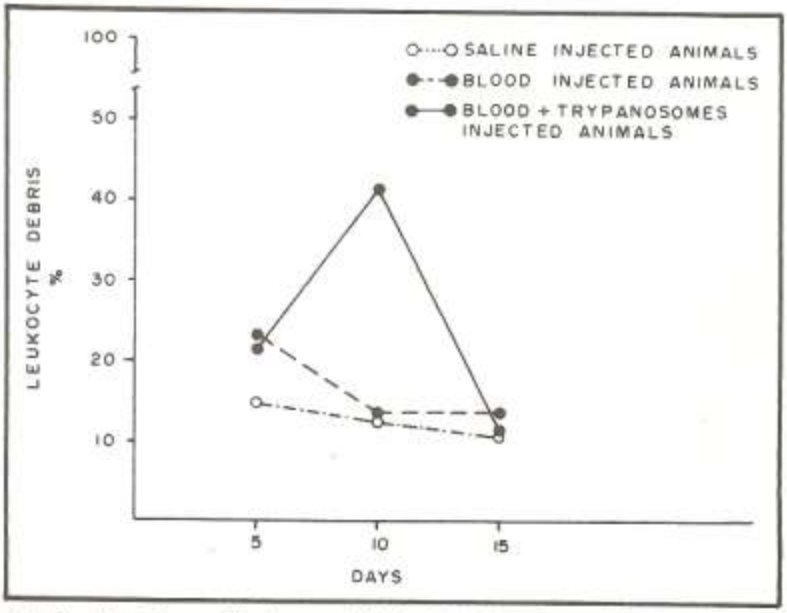

Fig. 6.– Variations of leukocyte debris with the evolution of the infection. (Each point represents the mean of the observations).

DISCUSSION

It is generally accepted that in acute American trypanosomiasis there is a moderate leukocytosis. However, in the present work a light leukopenia wes found on the 5th day of infection being more accentuated on the 10th day. The same pattern was found with the percentage of lymphocytes, which were higher on the 10th day when neutrophils were at their lower levels. The result is more significative if we consider that the animals that only received blood showed striking leukocytosis and lymphocitosis. This finding may be explained in terms of the type of strain of T.(S.) cruzi used in this work. As a matter of fact, Niño in 1929[43] working with several experimental animals and the Argentine strain of the parasite, observed a postlymphocytic neutrophilic leukocytosis which persisted until the death of the animals. However, this neutrophilic leukocytosis was not observed when the California strain was used[53]. On the other hand, normal leukocyte counts have been reported in Chagas disease by some authors[5,48,53], while others have found leukopenia, although in a little percentage of the patients[48]. Leukocytosis with lymphocytosis and neutropenia, have also been found by other authors[2,39,47]. It is necessary to point out, that in the present work leukocytosis and lymphocytosis was observed in the group that received uninfected blood; although the infected animals showed lymphocytosis, it seems to be of relative nature, since there was also an important leukopenia.

Unlike some authors[1,5,44,45] no statistical difference in the number of atypical lymphocytes was demonstrated in any of the different periods of observation.

A slight increase of monocytes in the infected mice was observed on the 15th day of the infection but the difference with the other groups was not significative. These results disagree with the monocytosis reported by Cardoso[1], Mazza[39], Pinto[46] and Tálice[51] but agree with the results of others[5,48,53].

It is interesting to notice the striking increase of cellular debris, only found in the infected group. This peak occurred at the moment when both parasitemia and leukopenia were at maximum. On the other hand, mice that received uninfected blood, showed leukocytosis and lymphocytosis and did not increase their number of leukocyte debris.

The several types of cellular debris seem to represent different stages of evolution of the leukocyte lysis[42]. According to Duncan[3] the "basket cells" are injured cells during the aspiration or in the moment of making the smear, and their presence in it has no importance except when they

coincide with lymphocytosis. Other authors have not found correlation between lymphocytes and leukocyte debris[42]. In this study, the same potential danger was present in the infected and control animals, but an explanation for the high amount of debris, in the former, could be that the trypanosome caused a dimminished resistance in leukocytes, probably due to toxic effect; if this is true, the leukocytes from infected mice, were at higher risk, in the process of making the smear, and those with higher fragility were destroyed. However, this theory does not explain the leukopenia since the counting of white cells was not made on a smear, but in the standard chamber.

It has been postulated that vacuoles found in leukocytes of patients with Venezuelan Equine Encephalitis seem to reflect a smaller grade of lesion caused by the virus, and this lesion may be the beginning of the cellular destruction which is reflected in a increase of leukocyte debris[42]. This assumption, seems to be a logical one, and may be applied to this trypanosomiasis, since in the present case it was observed, that the number of vacuolated cells in the infected animals tended to increase on the 5th day. However, on the 10th day there was no increase of vacuolated cells ($p<0.3$), but the nuclear debris ($p<0.001$) and the "basket cells" ($p<0.01$), did in fact increase, which as has been pointed out, could reflect more advanced grades of the leukocyte lysis[42]. Taking in account the fact that the higher quantity of leukocyte debris and parasites occured on the 10th day of infection, we can relate the two facts. Nevertheless, further studies on osmotic, and mechanical fragility of leukocytes in Chagas disease, need to be done.

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Morfología y valores leucocitarios en la Enfermedad de Chagas Experimental.

Bonilla L, Diez-Ewald M y Negrette A (Instituto de Investigación Clínica, Universidad del Zulia, Apartado 1151, Maracaibo, Venezuela). **Invest Clín** 14(3): 129-142, 1973.– Se estudian las alteraciones morfológicas leucocitarias en tres grupos de ratones: uno inyectado por vía peritoneal con sangre infectada con **Trypanosoma (Schizotrypanum) cruzi**, otro inyectado con sangre normal y un tercero con solución salina normal.

A los 5 días después de la inoculación se observó leucopenia en los animales infectados, y leucocitosis en los ratones inyectados con sangre aparentemente normal, cuando ambos grupos se comparan con el grupo que solo recibió solución salina. Ambos hallazgos se acentuaron a los 10 días; pero a los 15 días, los contajes leucocitarios se normalizaron en el grupo infectado, permaneciendo alto en el grupo inyectado solo con sangre.

Se observó linfocitosis y neutropenia en los 2 grupos de ratones que recibieron sangre, observándose además un gran incremento de restos leucocitarios en el grupo infectado. Este hallazgo coincidió con la neutropenia y la época de máxima parasitemia.

La presencia del parásito en sangre periférica, pudiera ser determinante del aumento de lisis celular, como lo indica el aumento de restos leucocitarios, cuando la parasitemia y la leucopenia eran mayores.

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