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ON THE TISSULAR PARASITISM OF TRYPANOSOMA CRUZI Y STRAIN IN SWISS MICE

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SUMMARY

A review of the tissular parasitism of **Trypanosoma cruzi** Y strain in Swiss mice was carried out. This strain parasitized preferentially smooth, skeletal and cardiac muscle fibers, with low transitory spleen and liver parasitism, as previously found by some Authors, although differing from other reports. These results can be related to the host genetical constitution and/or the degree of the strain virulence at the time of this study. Furthermore, we discuss that the high macrophagotropism reported for this strain in some instances could be an artificially induced condition resulting from its serial maintenance in mice, either for a longer time and/or by using young animals. The heavy parasitism and inflammation observed in the bladder, pancreas and spermatic duct of some inoculated mice, as well as the testis parasitization, were also noteworthy findings.

INTRODUCTION

Trypanosoma cruzi Y strain, isolated in 1950 by Dr. V. Nussenzweig through xenodiagnosis from an acute human case (SILVA & NUSSENZWEIG [22]), has been largely used as a parasite sourche in biological, biochemical, chemotherapic and immunological studies; its morphobiological characterization was carried out by some Authors [4,5,21,22]. REGO & GARNHAM [20], in 1956, were the first to report a histopathological study of this strain, emphasizing its preferential multiplication in the mouse spleen; however, these Authors did not observe organ invasion of mice inoculated with this strain following its passage through **Triatoma infestans**, even during subsequent inoculations in mice. REGO [19] also reported that the spleen parasitization had been uncommon in previous observations, this strain intensely multiplying in cardiac and skeletal muscle cells. Otherwise, CARVALHEIRO & COLLARES [6] observed that reticulotropism of this strain was drastically reduced after passage through **T. infestans** or culture medium, this condition remaining during sucessive passages in mice. ANDRADE & ANDRADE [2] reported very low spleen and liver parasitism of mice inoculated with this strain, whereas the muscle cells, mainly of the intestines, were chiefly parasitized; however, it became preferentially reticulotropic in infected mice treated with cortisone [2] or following higher number of serial passages in mice [3]. MELO & BRENER [17] found remarkable multiplication of this strain in the liver, spleen and bone marrow of inoculated mice, both normal and X-irradiated, while the muscle parasitization was lower; these Authors also suggested the use of the term "macrophagotropic" instead "reticulotropic" for describing strains presenting this parasitism pattern. In our experiments using the Y strain, including the study of its histopathological behaviour in 10g male albino mice on days 4,6 and 9 postinoculation [24], such striking parasitism of spleen and liver was not observed, which led us to a review of the pro-

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blem. The strain used through this work presents the morphological pattern, parasitemic levels and profiles, as well as high lethality for mice as previously reported [4,5,8]. Moreover, since 1980, its k-DNA profiles have remained unimpaired, being also in accordance with the Y pattern [8,12,13].

MATERIAL AND METHODS

T. cruzi Y strain used through this work was supplied by Dr. Zigman Brener (René Rachou Research Center, Belo Horizonte, Brasil) in 1978 in LIT medium. Thence, it has been weekly passed through 20g male albino mice (Swiss-Webster) from the Oswaldo Cruz Institute colony, by intraperitoneal inoculation of 10^5 trypanosomes/mouse; parasite counts were done with a Neubauer hemocytometer according to the method described by HOFF [15]. The conditions used for the strain maintenance were followed through the present study. Groups of six mice, randomly chosen among the inoculated ones, were killed on days 7, 10 and 12 postinoculation, the following organs and tissues being removed and fixed in 10% formalin: liver, spleen, inguinal lymph node, kidney, bladder, esophagus, stomach small and large intestines, pancreas, diaphragm, abdominal wall muscle, ventral muscular mass of the thigh, heart, lung, tongue, adipose tissue (around the kidney and lymph node), central nervous system (CNS), testicle and spermatic duct. After paraffin inclusion, these organs and tissues were sectioned at 5 μm thickness (at least two sections from each organ or tissue/mouse), stained by Hematoxylin and Eosin (H.E.), and exhaustively examined under light microscope, usually at X 400 magnification. At the time of the present work, this strain had about four years of serial passages in mice.

RESULTS

On the 7th day of infection, the parasitism of the spleen and liver was very low and comparable to that observed in the myocardium and muscle fibers of the stomach, spermaic duct and thigh. Inflammation occasionally occurred in these muscles, as well as in the bladder musculature, diaphragm and adipose tissue; myocarditis occurred rarely, being always mild. The liver of the infected mice generally presented sparse small inflammatory foci, while the spleen and lymph node were hyperplasic.

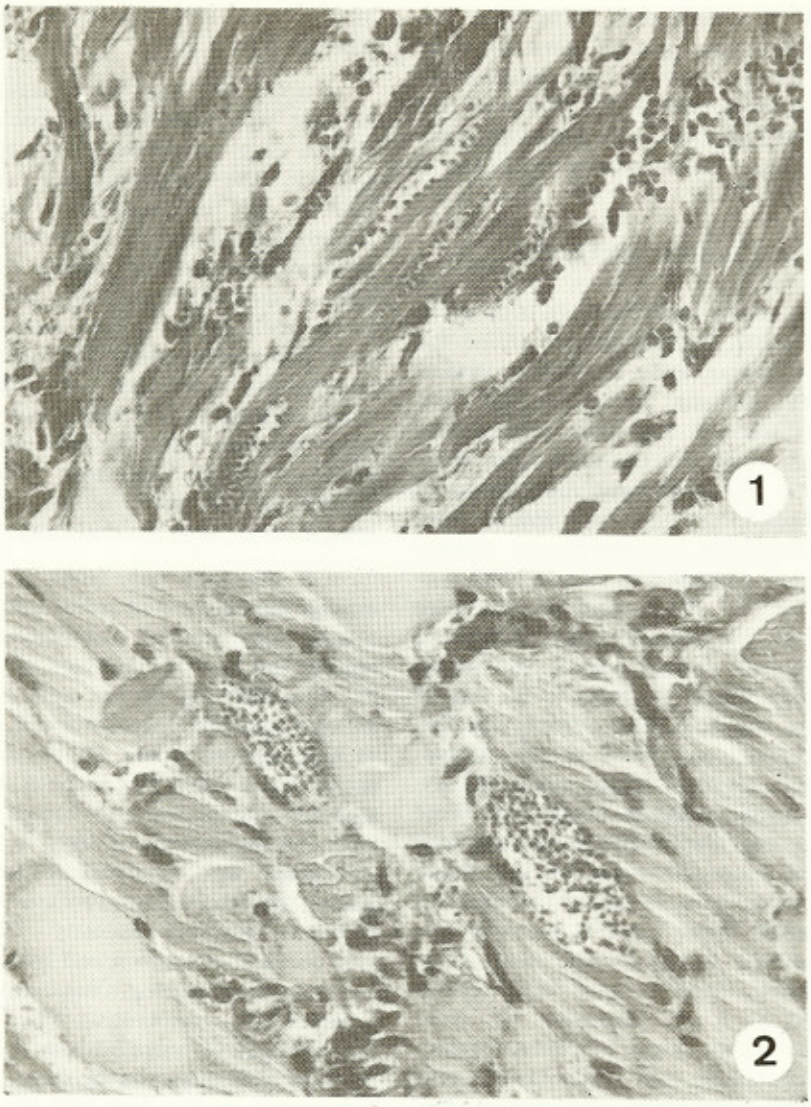
On the 10th day, the muscular layer of the bladder was preferentially parasitized and inflamed (Fig. 1). Amastigote nidi could also be seen in the myocardium, pancreas, musculatures of the spermatic duct, small intestine, stomach, thigh, abdominal wall, diaphragm and adipose tissue. The liver and spleen remained scarcely parasitized; some forms seen in the spleen and lymph node rather appeared degenerating amastigotes. Inflammatory processes of different degrees could be seen in the mentioned organs and tissues, whereas necrotic foci were sometimes observed in the pancreas. Histopathological aspects of the liver, spleen and lymph node remained as above mentioned.

On the 12th day, time of high mortality among the inoculated mice, several organs and tissues were heavily parasitized and inflamed, chiefly the striated muscles of the thigh (Fig. 2), musculatures of the spermatic duct (Fig. 3) and bladder, myocardium, pancreas (Fig. 4) and adipose tissue. Other parasite multiplication sites were the smooth muscles of the stomach, intestines and blood vessels of the lung and esophagus, as well as the diaphragm, abdominal wall musculature and testis (albuginea); inflammatory processes in such tissues displayed variable intensity. The rare forms seen in the liver, spleen and lymph node appeared to be degenerating amastigotes. Necrosis associated with pancreatitis (Fig. 4) and hyaline degeneration of muscle fibers of the thigh (Fig. 2) were also observed in some mice. Focal inflammation of the liver and hyperplasia of the spleen and lymph node were more pronounced.

In this study, we did not find parasites in the CNS, kidney and tongue of the inoculated mice.

DISCUSSION

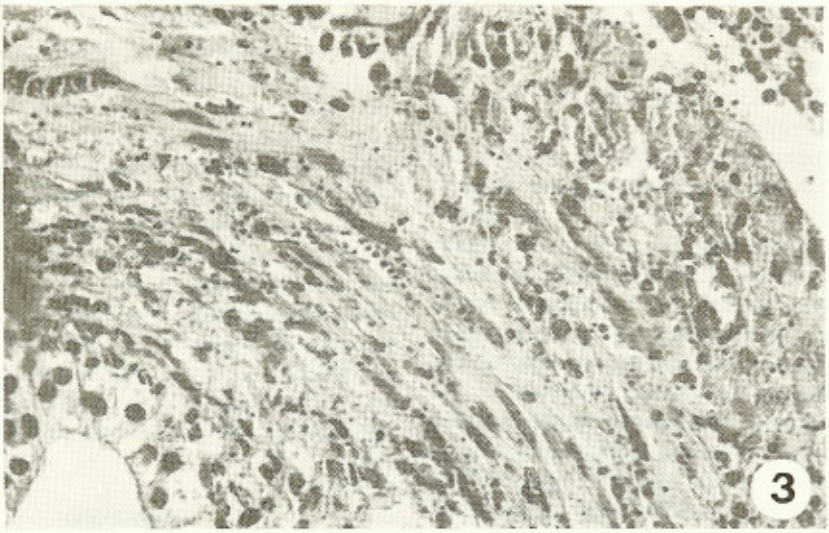
Throughout this study parasites of the Y strain preferentially multiplied within smooth, skeletal and cardiac muscle fibers. Low initial multiplication in the liver and spleen rather appeared a transitory event, possibly favoured by the phagocytosis of parasites and the capacity of a few blodstream trypomastigotes to develop in macrophages. However, this capacity does not endure, since forms that

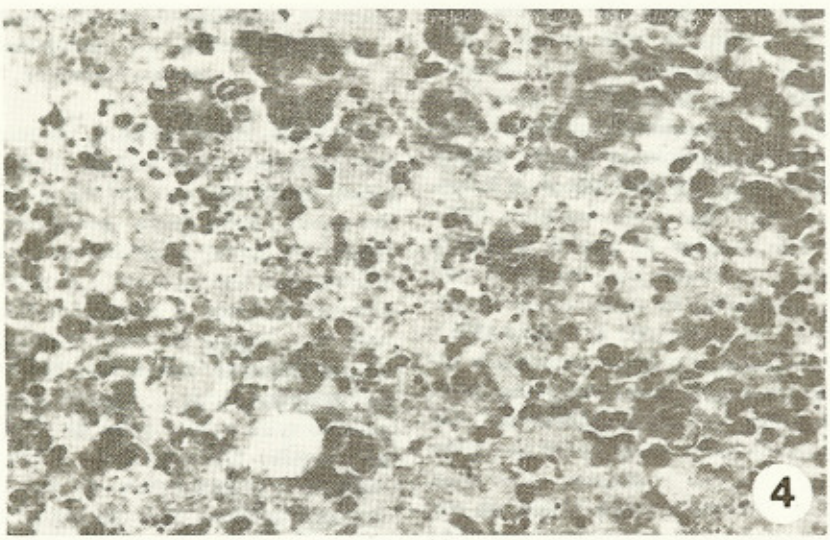


Figs. 1,2 — 1) Parasitism and inflammation of the bladder musculature on the 10th day of infection. 2) Musculature of the thigh on the 12th day of infection presenting amastigote nidi, inflammatory infiltrate and hyalin degeneration of some muscle fibers. H.E., 500 X

suggest degenerating amastigotes were subsequently seen in these organs, as well as in lymph nodes, most probably on account of the host immunological response. Initial and transitory multiplication of this strain in the liver and spleen of inoculated mice had been previously reported [2,16,17], as well as its preferential parasitism in muscle cells both of mice [2,19] and rats [23]. On the other hand, despite the remarkable macrophagotropism reported "in vivo" for the Y strain [3,16,17,19,20], "in vitro" studies by infecting peritoneal macrophages with bloodstream forms of this strain only displayed very low rates of parasitized cells, even at 5:1 parasite/macrophage ratios [1,18].

In the literature there are reports indicating that the Y strain under maintenance in mice initially appears as attenuated and mainly myotropic, and only subsequently becomes highly virulent and macrophagotropic [2,3,19,20]. Furthermore, REGO & GARNHAM [20] reported





Figs. 3,4 — 3) Parasitism and inflammation of spermatic duct. 4) Intense inflammation and necrosis of the pancreas. Both figures were from the 12th day of infection. H.E., 500 X

that the highly viscerotropic Y strain when passed through **T. infestans**, only determined mild infections in baby mice, even in the subsequent inoculations of these animals. Also, CARVALHEIRO & COLLARES [6] observed that the reticulotropism of this strain was drastically reduced during sucessive passages in mice following its passage through **T. infestans.** These findings lead us to suppose that the striking macrophagotropism reported for the Y strain in some instances could be an artificially induced feature resulting from a marked increase of its virulence following its serial maintenance in mice, which is commonly used, but does not represent the usual maintenance condition of **T. cruzi** in nature. On the other hand, ANDRADE & ANDRADE [2] observed that this strain became highly reticulotropic in infected mice treated with cortisone, although it was preferentially myotropic in non-treated animals. All these data corroborate the previously reported assumption that the macrophagotropism (reticulotropism) of a **T. cruzi** strain indicates increased parasite virulence, high sus-

ceptibility of the vertebrate host or both factors [2].

Although we had not observed the high macrophagotropism described for the Y strain [3,16,17,19,20], the experimental conditions used in the experimental conditions used in the present work were similar to those adopted by MELO & BRENER [17], regarding the strain maintenance in 20g male albino mice at weekly intervals, the inoculum/mouse and the inoculation route; moreover, the animals were also necropsied on the 7th day postinoculation on account of the intense macrophage parasitism reported to this strain at this time [16,17]. However, in the present work the six mice necropsied/day were always randomly chosen, while MELO [16] allowed the inclusion of one mouse with very high parasitemia among the three animals killed/day, which could have favoured the selection of highly susceptible mice in that study. Also, the albino mice we used were from a different colony than MELO & BRENER [17] used, and thus they could be genetically different and consequently less susceptible or simply display another parasitism pattern with the same **T. cruzi** strain. On the other hand, our results could be derived from the passage of the Y strain in acellular culture medium (as supplied us) before its re-inoculation in mice, since as demonstrated by CARVALHEIRO & COLLARES [6], passage of this strain in culture drastically reduced its reticulotropism, even following several sub-inoculations in mice. Moreover, the strain used in the present study could be under maintenance in mice for a shorter time than in the MELO & BRENER work [17] and on account of this could be less virulent and also mainly myotropic; however, such as previously observed by other Authors [2,3,7,19,20], this strain being passed serially in mice for a longer time, most probably would modify its parasitism pattern, showing higher multiplication in the liver and spleen of infected animals, unless the mouse genetical constitution hindered this.

A remarkable finding from the present study, of which we are not aware of previous reports, was the parasitism and inflammation observed in the spermatic ducts of inoculated mice (Fig. 3). The testis parasitism is also noteworthy, however this was previously observed by VIANNA [25] in experimentally infected guinea-pigs and by CHAGAS [7] and HARTZ & TOLEDANO [14] in human cases. Furthermore, it deserves mention that experimental works reporting functional and histological alterations of reprodutive organs of male rats and guinea-pigs were done with the **T. cruzi** Y strain [9,10,11]. Other remarkable findings from the present work were the heavy parasitization and inflammation observed in the bladder and pancreas of some inoculated mice; MELO [16] also reported multiplication of the Y strain in these organs, emphasizing the bladder parasitism. Regarding the severity of the pancreatic lesions sometimes observed (Fig. 4), we think that probably they also play a marked role in the pathogenicity of this strain for the inoculated mice.

RESUMO

Sobre o parasitismo tecidual da cepa Y do Trypanosoma cruzi **em camundongos albinos (Swiss-Webster)**

Através deste trabalho fizemos uma revisão do parasitismo tecidual da cepa Y do **Trypanosoma cruzi** em camundongos albinos (Swiss-Webster). Esta cepa parasitou preferencialmente as fibras musculares lisas, esqueléticas e cardíacas, sendo baixo e transitório seu parasitismo do baço e fígado, conforme já observado por alguns Autores, embora diferindo de outros achados. Estes resultados podem estar relacionados com o padrão genético do hospedeiro e/ou com o grau de virulência da cepa por ocasião deste estudo. Além do mais, discutimos a possibilidade de que o intenso macrofagotropismo descrito para esta cepa em algumas ocasiões possa ser uma condição artificialmente induzida através de sua manutenção seriada em camundongos por tempo prolongado e/ou pelo uso de animais jovens. Também são dignos de nota, o intenso parasitismo e inflamação da bexiga, pâncreas e canal espermático de alguns animais inoculados, assim como, o encontro de ninhos de amastigotas no testículo.

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