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AN ELECTRON MICROSCOPE STUDY OF *TRYPANOSOMA CRUZI* INTRACELLULAR FORMS IN MICE TREATED WITH AN ACTIVE NITROFURAN COMPOUND

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SUMMARY

An electron microscope study of *T. cruzi* intracellular parasites was performed on mice experimentally infected and treated with an active nitrofuran compound. Degenerated parasites highly predominated in the treated animals although a certain number of degenerated forms has been also observed in the untreated controls. The sequence of nuclear and cytoplasmatic parasite alterations following the drug administration was described.

INTRODUCTION

Investigation performed on tissue cultures experimentally infected with *T. cruzi* has demonstrated some nitrofuran compounds to be active against the parasite intracellular forms, BRENER [4]; BAYLES et al. [1]; MIETH & SEIDENATH [5]. Nevertheless, some important phenomena such as immunity, inflammatory process as well as drug metabolic transformation not being observed in these experiments, data obtained in tissue culture may not be considered as truly representative of the drug action in the living host.

The present paper shows an electron microscope study of the intracellular parasites performed on mice experimentally infected with *T. cruzi* and treated with an active nitrofuran compound.

MATERIAL AND METHODS

Male albino mice weighing 18-20 g were intraperitoneally inoculated with about 150,000 blood forms of "MR" strain, BRENER[3]. Counting of parasites was performed according to a technique previously described, BRENER [2]. Nitrofurazone (5-nitro-2-furaldehyde semicarbazone(was given, by oral route, in the dose of 100 mg/kg, from the 12 th day of infection on. Treated and control mice were sacrificed 24 and 48 hours after treatment.

The mice were intraperitoneally anestethized with thionembutal and the heart, removed "*in totum*" was fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4). The organ was then sectioned at the level of the atrioventricular septum and the atria were kept for 4 to 5 hours at 4°C in the same solution. After fixation, the material was washed for twelve hours, at the refrigerator temperature, with 0.3 M sucrose in 0.1 M phosphate buffer, with two or three changes of this solution. After being rinsed in distilled water, the material was dehydrated in acetone series and embedded in westopal W at 60°C for 48 hours. Sections were performed with a MTI Porter-Blum ultramicrotome. The ultrathin sections, stained for 40 minutes in uranil acetate and 10 minutes in lead citrate, were examined in a Zeiss EM 9A electron microscope.

RESULTS

Normal and degenerated leishmaniae have been found in the heart myofibers, histiocytes, fibroblasts as well as the intersticial

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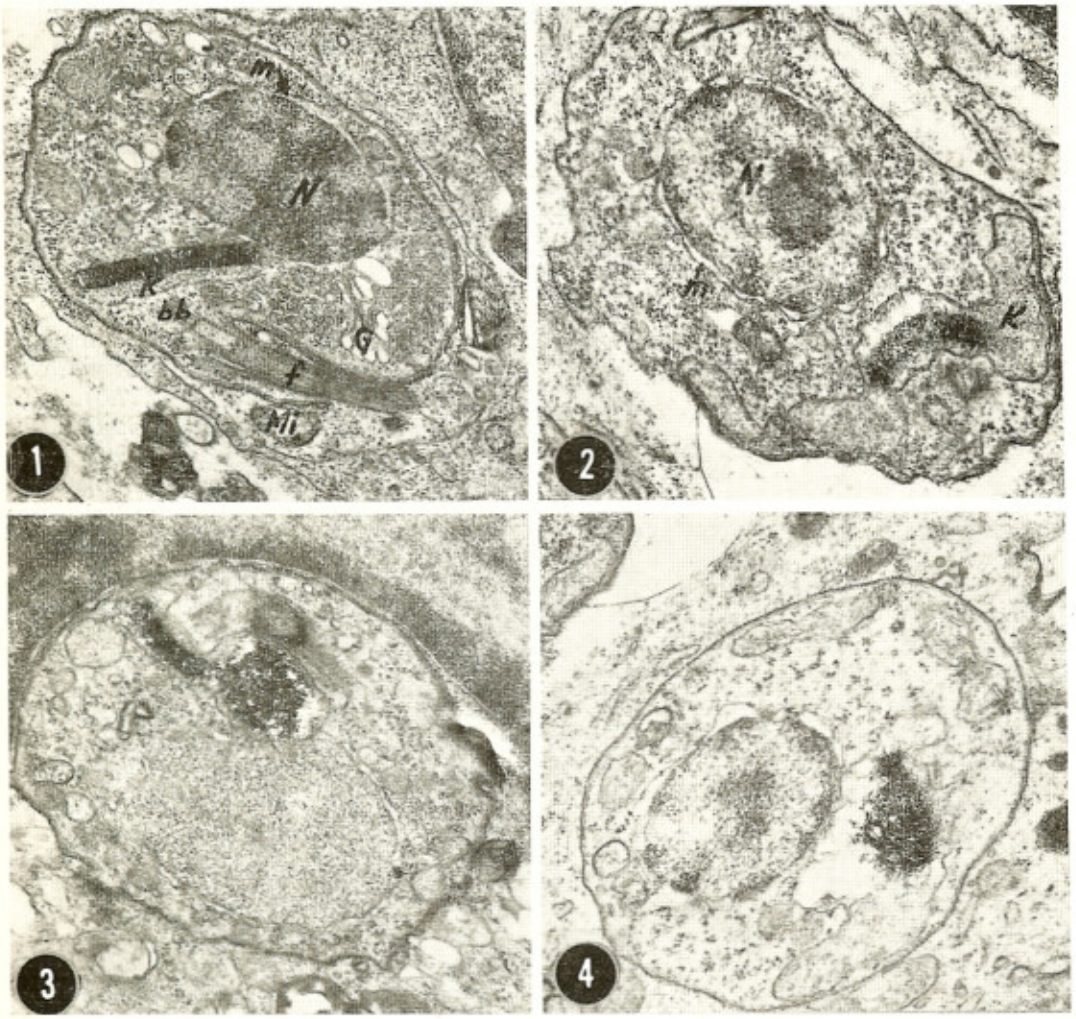
spaces in control and treated mice. In the latter group of animals, however, degenerated parasites highly predominated whereas in the untreated controls, most of the parasites were normal. In 25 electron micrographs, taken from the myocardium of treated animals, were found 58 degenerated and 9 normal leishmaniae (86.5% and 13.5%, respectively); in the myocardium of control animals, examined in the same way, 14 leishmaniae were found to be degenerated and 66, normal (17.5% and 82.5%, respectively).

The following parasite alterations have been observed in the treated animals:

Nuclear alterations — The range of nuclear changes can be described as follows: 1) the chromatin is finely granulated, homogenously distributed and presents low density; 2) this chromatin, then strongly osmiophilic, is

PLATE I



Figs. 1, 2 — Normal leishmaniae of *T. cruzi*. Nucleus (N), nuclear membrane (m), Kinetoplast (K), Golgi apparatus (G), mitochondria (Mi), flagellum (f), basal body (b b). 19,400 X. Fig. 3 — Leishmania degeneration. Nucleus with finely granulated chromatin with low density. 19,400 X. Fig. 4 — Leishmania early alterations. Irregular distribution and decrease in the number of ribosomes. Kinetoplast alterations. 19,400 X

PLATE II

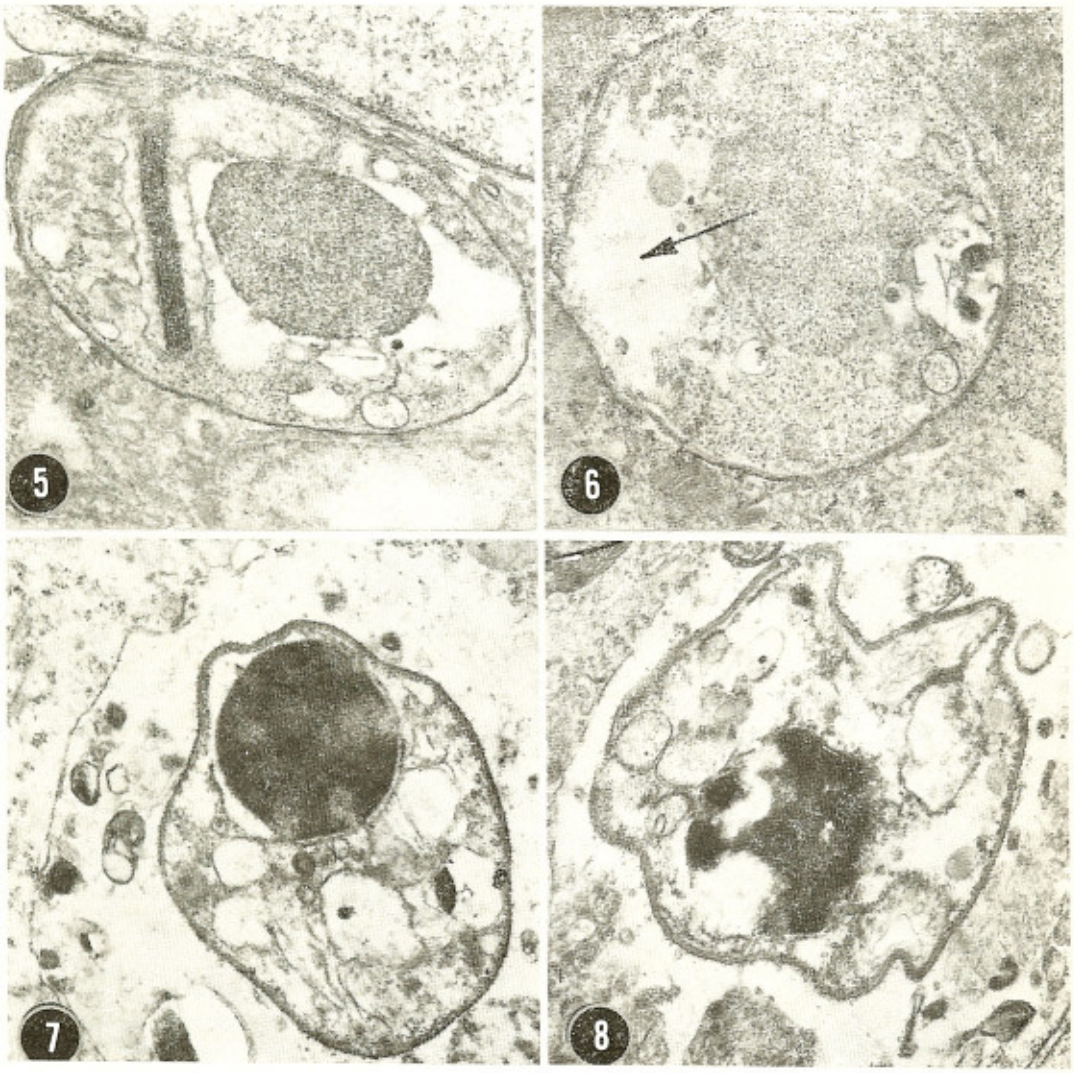
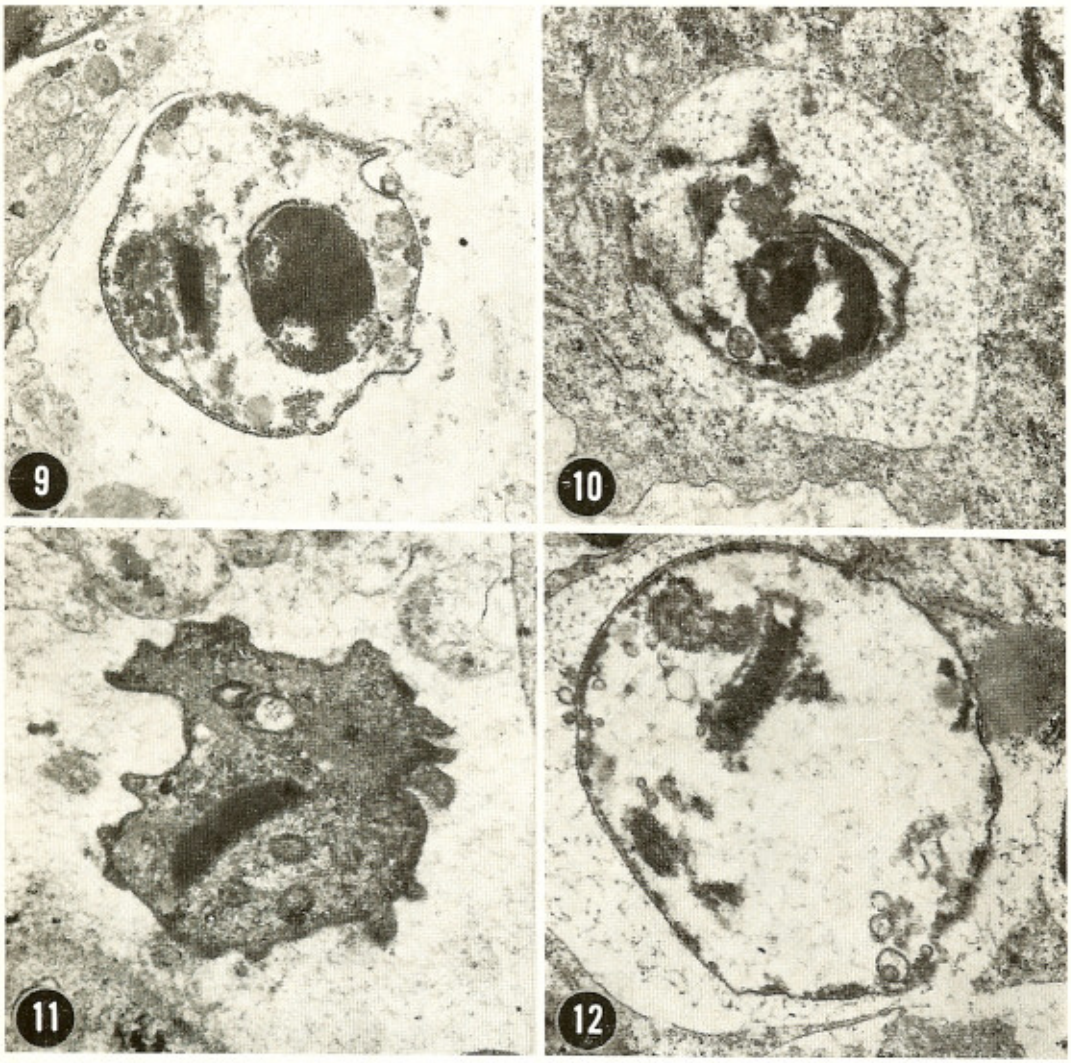


Fig. 5 — Nuclear changes and lysis of perinuclear structures. 19,400 X. Fig. 6 — Degenerated leishmaniae. Nuclear and cytoplasmatic structures becoming indistinct. Focal lysis of cytoplasmatic components (at arrow). 19,400 X. Figs. 7, 8 — Desintegrated leishmaniae. Chromatin strongly osmiophilic forming masses separated by clear areas. Cytoplasmatic vacuoles. 19,400 X

gathered into masses situated at the center of the nucleus or close to the nuclear membrane and separated by clear areas; 3) the nuclear membranes become indistinct or thickened; 4) small, pycnotic and osmiophilic nuclei are observed; 5) Kariolysis and Karyorrhexis were also observed (Plates I, II, III).

Cytoplasmatic alterations — The changes in the cytoplasm components followed, as regards intensity, those alterations occurring in the parasite nucleus. In general, the last structures to be affected and destroyed were the membrane, the periplast and the Kinetoplast. The following changes have been observed in the treated parasites: 1) irre-

PLATE III



Figs. 9, 10, 11, 12 — Leishmaniae in the final stages of degeneration. Nuclear pyknosis, Kariolysis and Karyorrhexis. Lysis of the cytoplasmatic structures and shrinking of the parasite membrane. 19,400 X

gular distribution of ribosomes which were seen to be rare or even absent in certain areas; 2) complete lysis of perinuclear structures; 3) numerous vacuoles of variable size and shape appear, limited by a single and strongly osmiophilic membrane; 4) presence of membrane debris, lamelar imbricated figures and amorphous masses; 5) mitochondria lysis; 6) the Kinetoplast loses its fibrillar structure and becomes strongly osmiophilic; 7) the flagellum and the basal body become indistinct; 8) the cytoplasm membrane becomes thickened, osmiophilic and sometimes presents ruptures.

DISCUSSION

This paper describes the occurrence of a number of *T. cruzi* degenerated leishmaniae

in the tissues of untreated animals, the percentage of these forms, however, being much more than those found in treated animals. The presence of a certain percentage of degenerated intracellular forms during the normal course of infection seems to be a general phenomenon. Fifty-seven electron micrographs taken from the myocardium of mice inoculated with "Y" strain have shown 237 leishmaniae to be normal and 79, degenerated (75% and 25%, respectively). It is difficult to distinguish between the alterations found in treated parasites from those observed in the untreated ones, but the lesions intensity was seen to be higher in the former group.

The parasite lesions in the treated animals appear, apparently, very soon after the drug administration, maked degeneration being detected as early as 24 and 48 hours after the beginning of treatment. This seems to be in agreement with the findings observed in experimentally infected animals as well as in tissue culture, BRENER [4].

TAFURI [6], after an electron microscope study, demonstrated, in experimentally infected animals, normal uninjured leishmaniae being unable to trigger any inflammatory reaction which, however, develops as soon as the parasites or the parasitized host cells degenerate. It then could be assumed that sudden massive intracellular parasite destruction would increase the inflammatory reaction. On the other hand, however, it is worthwhile reminding that at least experimentally, active drugs may very rapidly cut off the high parasitemia and cellular parasitism occurring in the acute phase and, therefore, avoid the harmful effects of a more prolonged cellular destruction.

It would be of great interest investigating whether other compounds, active against intracellular forms of *T. cruzi* in tissue culture, would also be active against those forms occurring in the living host. This seems to be of primary importance for establishing that the drug action in tissue culture is indeed a dependable picture of the phenomena taking place in infected animals.

RESUMO

Estudo ao microscópio eletrônico das formas intracelulares do T. cruzi *em camundongos tratados com compostos ativos de nitrofurano*

Foram estudadas, ao microscópio eletrônico, em camundongos experimentalmente inoculados com *Trypanosoma cruzi* e tratados com um derivado nitrofurano ativo, as alterações da ultra-estrutura das formas intracelulares do parasita. Parasitas degenerados predominavam nìtidamente nos animais tratados embora formas intracelulares degeneradas fôssem também encontradas nos animais contrôles não tratados. Foi descrita a seqüência das alterações nucleares e citoplasmáticas dos parasitas nos animais tratados.

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