

GASTROCNEMIUS SKELETAL MUSCLE MICROVASCULATURE AND NEUROMUSCULAR JUNCTION ALTERATIONS IN MICE WITH EXPERIMENTAL ACUTE CHAGAS INFECTION

Alteraciones en la microvasculatura y unión neuromuscular del músculo esquelético *Gastrocnemius* de ratones con infección experimental de Chagas agudo

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ABSTRACT

A light and transmission electron microscopy study was performed in skeletal muscles (SM) *Gastrocnemius* (G) from mice experimentally infected with *Trypanosoma cruzi* to determine changes on microvessels (MV) and neuromuscular junction (NMJ) of G. In this study 10 male (*mus musculus*) (20 g) were infected subcutaneously with 1.10^4 bloodstream trypomastigotes M/DID/Ve/02/DSM strain. Five mice were kept as uninfected controls. The parasites induced a complete paralysis of the rear limbs and death while still in the acute Chagas' disease. The histopathology of SM showed inflammatory cell infiltration by mononuclear and polymorphonuclear leukocytes associated with marked parasitism in the muscle fibers of G. Indirect immunofluorescence revealed interstitial IgG deposit as bands regularly spaced along the nerve terminals at 40 days post-infection (pi). At this time *T. cruzi* antigens and intracellular amastigotes nests were also observed. The marked inflammatory response and morphological changes in the SM were confirmed by transmission electron microscopy. Capillary ultrastructure was seen to be altered, with points of cell cytoplasm discontinuity that appear to represent holes in the microvessel walls. This finding coincided with amastigote nests in myofibers, close contacts between trypomastigotes and endothelial cells and marked thickening of the basement membrane of the muscle vessels. Loss of capillary lumen and a process of ischemia also were observed in the SM of infected mice. The neuromuscular junction showed degeneration of intramuscular nerve fibers, reduction in the axon caliber, swollen mitochondrial, increase in the actin filaments and microtubules in the

axoplasm, and swelling of the Schwann cells. Increase in the nerve terminal perimeter and most of the synaptic vesicles were localized near the presynaptic active zones and scarce in the axoplasm. At this stage of infection the changes findings in MV and NMJ of G infected with *T. cruzi*, as well as ischemia and alterations in the presynaptic membrane densities in the active zones, shows that the abnormal mice NMJ is associated with an activity dependent modulation of the neurotransmission, producing abnormal motor activity and paralysis of the rear limbs mice while still in the acute Chagas' disease.

Key words: *Trypanosoma cruzi*, acute Chagas' disease, skeletal muscle, microvasculature, ischaemia, neuromuscular junction.

RESUMEN

Un estudio con microscopía de luz y electrónica de transmisión fue realizado en muestras del músculo esquelético (ME) *Gastrocnemius* (G) de ratones experimentalmente infectados con *Trypanosoma cruzi*, a fin de determinar las alteraciones en la microvasculatura y unión neuromuscular (UNM) de G durante la infección chagásica aguda. Un grupo de 10 ratones machos (*mus musculus*) NMRI (20 g), fueron infectados subcutáneamente con $1,10^4$ tripomastigotes sanguícolas de la cepa M/DID/Ve/02/DSM. Cinco ratones NMRI no infectados fueron usados como control. Estos parásitos produjeron completa parálisis de las patas posteriores y muerte de los ratones durante la infección aguda. La histopatología del ME mostró infiltración de células mononucleares y leucocitos polimorfonucleares asociados con marcado parasitismo en la fibra muscular de G. La inmunofluorescencia indirecta reveló IgG a manera de bandas sobre el nervio terminal a los 40 días post-infección (pi).

En este tiempo, antígeno y grupos de amastigotes de *T. cruzi* fueron observados en los cortes del ME. La marcada respuesta inflamatoria y las alteraciones morfológicas en el tejido muscular fueron verificadas por microscopía electrónica de transmisión. La ultraestructura de los capilares estuvo relacionada con puntos de discontinuidad en la microvasculatura. Este encuentro coincidió con la presencia de grupos de amastigotes dentro de las miofibrillas, estrecho contacto entre los tripomastigotes y las células endoteliales y marcado adelgazamiento de la membrana basal de los vasos sanguíneos. La pérdida del lumen capilar y un proceso de ischemia también fue observado en el G de los ratones infectados. La unión neuromuscular mostró degeneración de la fibra nerviosa intramuscular, reducción en el calibre del axón del nervio motor determinada por una retracción de la vaina de mielina, inflamación mitocondrial, aumento en los filamentos de actina y microtúbulos en el axoplasma e inflamación de las células de Schwann. Aumento del perímetro del nervio terminal y vesículas sinápticas fueron observadas cerca de las zonas activas presinápticas y escasas en el axoplasma. En este estado de la infección, los cambios observados en la MV y UNM de G infectado por *T. cruzi*, tales como isquemia y las variaciones en la densidad de vesículas sinápticas en las zonas activas de la membrana presináptica pudieran estar relacionadas con la modulación neurotransmisora, produciendo la pérdida de la actividad motora y parálisis de las patas traseras de los ratones durante la infección chagásica aguda.

Palabras clave: *Trypanosoma cruzi*, infección chagásica aguda, músculo esquelético, microvasculatura, isquemia, unión neuromuscular.

INTRODUCTION

The protozoa parasite *Trypanosoma cruzi*, is the etiological agent of Chagas' disease, and represent an important public health problem in American continent [39]. The parasite is naturally transmitted to humans by of the blood-sucking bug hemiptera of Triatominae sub-family producing variable clinical manifestations. In Venezuela *T. cruzi* has been found infecting the rural and suburban human host and a large variety of mammalian reservoirs and triatomine vector species [8]. It is widely known that one of the most notable characteristics of Chagas' disease in humans is general damage to the cardiac tissue, and histological lesions observed in other tissues as intestinal tract and nervous system are qualitatively similar to those found in acute chagasic experimental models, as well as the destruction of nerve cells in the vicinity of ruptured pseudocysts with disintegration of parasites and host cells inducing inflammatory reaction [37]. Similar events occur in animals infected with *T. cruzi* from different geographical regions, where the parasite circulates between wild and domestic reservoirs and triatomine vectors [11]. Following the discovery of the intracellular forms of the parasite, a large number of studies have been carried out to analyze the lesions produced by *T.*

cruzi, as in the cardiac tissue, nervous, digestive and muscle systems of experimentally infected animals [9, 19]. In both human and experimental models of Chagas' disease, the clinical and pathogenic studies involving the trypanosome heterogeneous population, tissue colonization and the establishment of lesions caused by direct action of parasites produce initial alterations to the mononuclear phagocytic system, followed by a process of progressive damage of the muscle fiber, their enervation, neuromuscular disorders and also neurological structures [12, 13, 30]. There have been reports on microvascular compromise, as a factor implicated in the etiology of the cardiomyopathy associated with acute murine Chagas' disease, and are attributed to both host and parasite characteristic [1]. These changes disturb the microcirculation and appear to be associated with a marked parasitism of myofibers, and with the role of endothelin in the pathogenesis of Chagas' disease [24].

During the acute infection, the target structures compromised may be the muscle itself and the peripheral nerve, neuropathic features, as expressed by type fiber grouping and grouped muscle fiber atrophy and axonal degeneration as observed in skeletal muscle in mice chronically infected with *T. cruzi* [14]. Degenerative and inflammatory processes in the affected tissues as motor neuron, parietal cortex of the brain and thoracic and lumbar levels of the spinal nerve of mice and rats may also occur [3, 38]. In this study was used a murine experimental model of Chagas' disease, infected with bloodstream trypomastigotes, isolated from wild animal *Didelphis marsupialis* captured in a endemic region in Barinas State, Venezuela, where most cases have been detected in many Municipalities with natural acute infection, with relatively high frequency showing sign of the Chagas' disease [8]. In this study the G muscle of mice was used, to look into why the animals were affected in the locomotor activity during infection with *T. cruzi*. This G muscle is a strong superficial muscle located with the soleus muscle at the superficial posterior compartment of the leg and is vital at walking and running, during knee extension and flexion performed on the leg. It is a powerful plantar flexor of ankle inserted on the middle 1/3 of the posterior calcaneal surface and is supplied by a sural branch of the popliteal artery. The observations included a parasitological evaluation, a description of the capillary abnormalities and neuromuscular disorders in the G of mice during the acute phase of Chagas' disease using light and transmission electron microscopy.

MATERIALS AND METHODS

Mice

A total of 15 male mice two months-old NMRI 20g body weight were used for this study. They were divided into two groups. The first group of ten animals was infected with *T. cruzi*. The second group of five animals was used as uninfected controls. Mice were maintained under balanced food and water *ad libitum*.

***T. cruzi* strain and experimental infection**

Trypomastigotes M/DID/VE/02/DSM strain, isolated from a wild animals (*Didelphis marsupialis*) captured in the Obispos Municipality, Barinas State, Venezuela, was used. Parasites were characterized by enzyme profiles [20] and maintained in male mice NMRI by alternate passages. The test mice were inoculated subcutaneously with $1 \times 10^4/0.1$ mL bloodstream Trypomastigotes collected by bled via cardiac puncture [19]. The inoculum was prepared of parasitized blood, which was diluted using phosphate-buffered saline pH 7.2 and counted in a Neubauer chamber by direct microscopic examination [4]. Uninfected mice were injected with saline solution. Parasitemia determination was done daily from 5 to 40 days post-infection (pi), using 10 μ L of blood collected cutting the tail tip of each mouse, put on slides for to count parasite numbers.

Sections *Gastrocnemius* processing

Skeletal muscle was dissected out of male mice that had died at 40 days pi, and divided in two pieces. One was mounted onto the specimen holder using OCT Tissue-Tek mounting medium and frozen in liquid nitrogen, transversally cut in sections of 7 μ m cut at -20°C and stained to perform routine histological with hematoxylin and eosin methods and immunocytochemical studies. Another piece was processed for electron microscopy.

Immunofluorescence antibody test

Cryostat sections of G of 7 μ m were fixed in cold acetone (4°C) and processed as follows: a) washing for 30 min with solution A (0.15 M NaCl, 50 mM phosphate buffer with 0.1% Triton X-100, pH 7.4); b) quenching of nonspecific protein binding sites with solution B (20% normal goat serum in solution A) for 30 min; c) overnight incubation at 4°C with a rabbit anti-*T. cruzi* antibody diluted 1:32 with solution B; d) washed with solution A for 30 min; e) 2 hr incubation in goat anti-mouse IgG-fluorescein conjugated diluted in 1:100 with solution B; f) washed with solution A for 2 h. Sections were mounted in 90% glycerol/solution A and examined using confocal laser scanning microscopy (CCD System, Olympus Optical CO., Japan) to search for trypomastigotes antigens.

Ultrastructural studies

Another piece of G muscle was fixed by immersion in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, on ice for 2 h, post-fixed with 1% osmium tetroxide in the same cacodylate buffer, dehydrated through graded ethanol solutions and embedded in epoxy resin. Ultrathin sections from G both the infected mice as uninfected control group, were double-stained with 2.5% uranyl acetate and 0.5% lead citrate and examined in a Hitachi H-500 electron microscope.

RESULTS AND DISCUSSION

Parasitemia and clinical finding

Parasitemia patents during the course of the infection with *T. cruzi* was observed in all infected mice. The parasitemia peaked between 5th through the 40th days pi, was of 304.20 ± 55.32 and 283.0 ± 45.10 trypomastigotes/ mm^3 blood. At this time the animals showed loss of mobility of the rear limbs and the infected mice died spontaneously while still in the acute phase Chagas' disease. The necropsy revealed a marked abdominal swelling with abundant peritoneal infiltrates, swollen urinary bladder with a bit of urine, continuous diarrhea, loss of weight and hepatosplenomegaly. Parasitemia produced by *T. cruzi* may be considered as a parameter of comparison between the virulence, tropism, initial inoculation site and route of the parasite both in infected animals and humans. Apparently some differences in the parasite strain and in the host's genetic background seem responsible for such a wide spectrum of clinical manifestations. In addition, the host-parasite interaction may be related to the quality of the immune response against the parasite, and the onset of the animals death during the acute phase [21].

Histopathologic findings

Skeletal muscle fiber showed inflammatory process, consisting of lymphomononuclear cells and polymorphonuclear leukocytes, macrophages and plasma cells in the muscle fiber atrophy. Group of *T. cruzi* amastigotes were seen concentrated near inflammatory foci (FIG. 1 A) and some sections of G showed prominent parasitism associated with destruction of muscle cells close to at blood vessel walls. Sections of SM also showed IgG-*T. cruzi*, antigens and attached fluorescent parasites inside of the skeletal muscle fibers. Pathological studies of the acute phase of Chagas' disease have showed that the initial tissue alteration by *T. cruzi* affects the mononuclear phagocytic cells. This is followed by an invasion of the remaining organs, attributed to both host characteristics and virulence of the parasite, where macrophages and muscle cells are the main targets for invasion of *T. cruzi* during acute infection [22].

Ultrastructural finding

This study showed changes in the infected muscular fiber with alterations in the vessels and neuromuscular junctions. Parasite group into muscle fiber (FIG. 1 B) was followed by an inflammatory response and skeletal muscle damage. It was characterized by marked swelling of the interstitium, with lymphocytic foci and degenerated fibroblast together with free red blood cells as compared with G of uninfected mice. Inflammatory cell infiltration was predominantly monocytes, neutrophils, fibroblast and macrophages in phagocytosis processes. This infiltrate formed large areas of expansion with local disin-

tegration of myofibrils, as well as a large increase of mitochondria frequently occupying interfibrillar spaces in these areas and in the vicinity of the walls of the vessels of atrophic muscle fibers, together to amastigotes nests. Free parasite also frequently appeared in the swollen interstitium together with cellular debris (FIG. 2 A-C). It is possible that the mechanism of injury may be due to continuous parasitic invasion of the muscle fibers, release of cytokines, or both, susceptibility to parasite

antigens, similar to the chronic cardiomyopathy of Chagas' disease [2, 26]. In these conditions cellular damage may be attributed to early parasitism, which may occur 3-4 months pi or in a few days as in humans, who have become infected with *T. cruzi* through an insect vector, involving morphological changes in the skeletal muscle, as well as its nerve supply [5], as observed in the sections of SM, in which the microvasculature showed holes in the endothelial layer usually associated

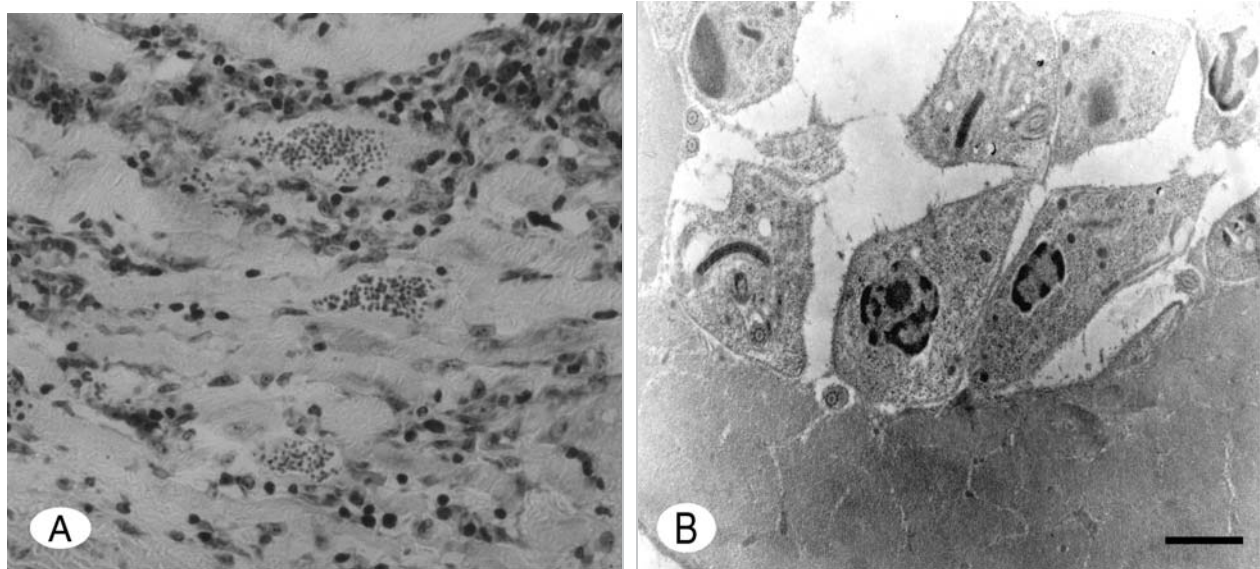


FIGURE 1. A) HISTOLOGICAL SECTION OF *Gastrocnemius* OF INFECTED MICE WITH *T. cruzi* THAT SHOW INFLAMMATORY CELLS AND NEST OF PARASITES. HEMATOXILIN-EOSIN 400X. B) ELECTRON MICROGRAPHS THAT PRESENT A PARASITE GROUP AND LOSS OF MUSCLE MIOFIBRILS. SCALE BAR: 1 μ m / A) SECCIÓN HISTOLÓGICA DE *Gastrocnemius* DE LOS RATONES INFECTADOS CON *T. cruzi* QUE MUESTRA CÉLULAS INFLAMATORIAS AND GRUPOS DE PARÁSITOS. HEMATOXILINA-EOSINA 400X. B) FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA QUE PRESENTA GRUPO DE PARÁSITOS Y PÉRDIDA DE FIBRAS MUSCULARES. ESCALA DE LA BARRA: 1 μ m.

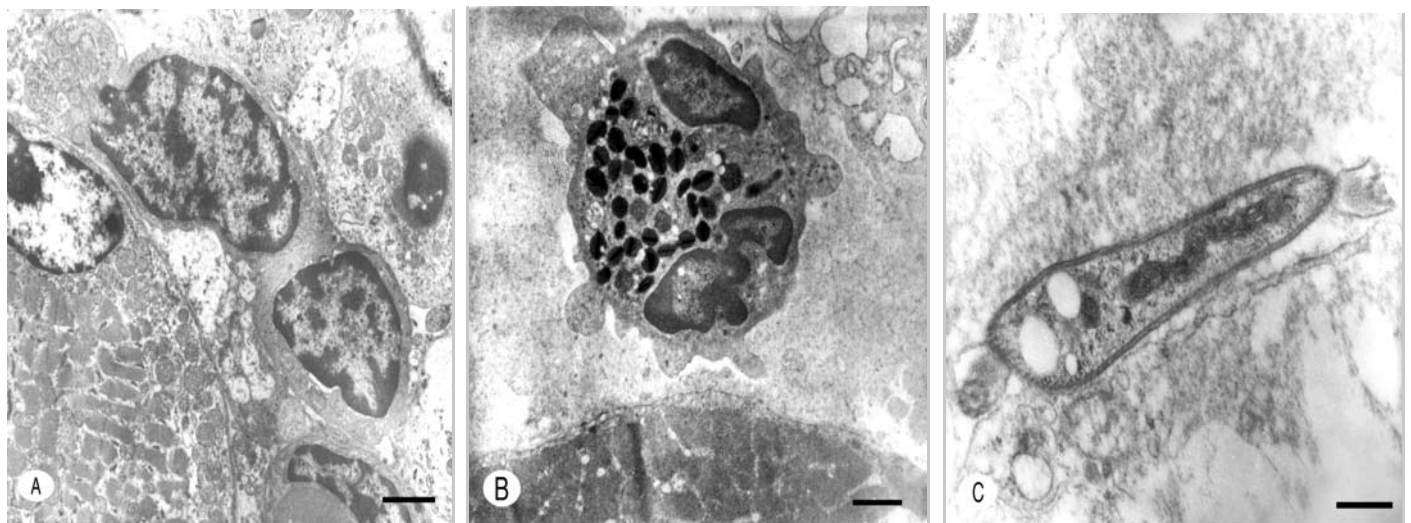


FIGURE 2. ELECTRON MICROGRAPHS THAT SHOW AREA OF INFLAMMATION IN THE MUSCLE INTERSTITIUM. A) CONTAINS INFLAMMATORY INFILTRATE THE POLYMORPHONUCLEAR CELLS. B) ACTIVATED EOSINOPHIL. C) TRYPOMASTIGOTE AND CELL DEBRIDS. SCALE BARS: 1 μ m / FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA QUE MUESTRAN ÁREA DE INFLAMACIÓN EN EL INTERSTICIO MUSCULAR. A) CONTIENE INFILTRADO INFLAMATORIO DE CÉLULAS POLIMORFONUCLEARES. B) EOSINÓFILO ACTIVADO. C) TRIPOMASTIGOTE Y DETRITUS CELULARES. ESCALA DE LA BARRA: 1 μ m.

with platelet-fibrin aggregates at the point of rupture. In the vicinity of intramuscular amastigotes pseudocysts, the endothelial cells showed ultrastructural alterations characterized mainly by various degrees of swelling and hypertrophy, a large increase in cytoplasmic vesicular structures and a reduction in the size of intercellular specializations and increase of the electron density lumen. Besides, large amount of extracellular cell-debris were always observed in associated with altered endothelial cells, mainly inflammatory response, swelling of endothelial cells, altered mitochondrias, and capillary occlusion. The capillary endothelial cytoplasm abnormalities play a significant role in the pathogenesis of Chagas' disease and have been compromised with abnormalities of the coronary microcirculation in acute murine Chagas' disease [7, 10]. Some reports have shown changes in the intramuscular vessels of animals infected with *T. evansi*, *T. cruzi* and *Toxoplasma gondii*, represented by capillary endothelial cytoplasm abnormalities that induced proliferation of organelles, decrease of pinocytic vesicles, degenerative changes and necrosis of the infected skeletal muscle [35, 36]. In this study the endothelial hypertrophy, progressive reduction of the vascular lumen and total luminal obliteration observed, giving rise to blood vessel ischemia (FIG. 3 A-D), as compared with G the non-infected mice (FIG. 4 A, B). In this muscle tissue the parasites also were found inside active macrophage and the altered endothelial cells (FIG. 5 A-C). This reduction of the capillary lumen have been associated [25] with processes of autoimmunity in *T. evansi* parasitized horse and with ischemic mechanisms, at least in part in the chronic phase of experimental cardiomyopathy in rabbits infected with *T. cruzi* [28]. Other authors have found endothelial layer cells discontinuity reduce the protective role of the endothelium, participating in the genesis of vasospasm and usually with platelet fibrin aggregates at the point of rupture, and leading to the focal pathology as in acute chagasic myocarditis and segmental vasospasm and expansion in the microvascular bed in dogs, rats and acute murine *T. cruzi* infection when studied the microcirculation flow [27, 29, 32].

On the other hand, the capillary vessels damage in skeletal muscle of a patient with advanced Chagas' disease with cardiac involvement, could eventually lead to deficient blood perfusion through the muscle and cause anaerobic metabolic changes affecting muscle function; other muscular abnormalities are probably due to reduced oxygen in the abnormal muscle microvasculature. These changes could affect oxygen extraction affecting the functional capacity of the infected mice [18]. In the animals infected with DSM-strain the nerve-muscle preparations exhibited marked changes, as the axonal degeneration as shown by myelin digestion chambers, was present together with mitochondrial swelling, signs of Schwann cells damage indicating a demyelinating process and the presence of onion figures in the Schwann cells cytoplasm of the infected nerves (FIG. 6 A-C). The nerve terminals from nerve-muscle preparations showed changes in the distribution of subcellular organelles, axoplasm becomes more electron

dense and there was pronounced mitochondrial swelling. Most of the synaptic vesicles were closely packed at the synaptic side, near the active presynaptic zone and scarces in the axoplasm (FIG. 7 A-C). None of the features described above could be observed in the non-infected control group. Based on typical clinical findings related to decreased motor activity, common symptoms observed in the all infected mice with *T. cruzi*, included progressive neuromuscular junction disorders, which may become associated with decreased muscular function, as consequence of the micropathological changes of the intramuscular nerve fibers [31]. This alterations in NMJ of G affect the presynaptic membrane densities in the active zones and the postsynaptic membrane densities become larger, which shows, that in the normal adult mammal NMJ there is an activity-dependent modulation of the neurotransmission-related structures in response to slight physiological functional demands as observed in normal adult rats trained to walk, indicating a precise inverse relation between the amount of locomotor activity and the complexity finally attained by the motor nerve terminals [34]. In this study, it is possible that alterations in the skeletal muscle fibers and motor nerve terminal of the NMJ at 40 day pi with DSM strain, may be at least due in part to strain behavior [6], which produced irreversible decrease of motor activity and paralysis in the rear limbs in the experimental model used. It is possible that inflammation and parasites rupturing and releasing antigen in infected skeletal muscle, become associated with the membrane surface and development of inflammatory neuropathy [16, 17]. On the other hand, it is possible the existence of plastic changes in the branching pattern, as revealed by studies in the *extensor digitorum longus* muscle of adult rats housed in metallic cages or trained to walk, indicating a precise inverse relation between the amount of locomotor activity and the complexity finally attained by the motor nerve terminals [15, 33].

Based in other findings, the results have showed that is possible that clinical presentation findings during early *T. cruzi* infection, can be at least in part due to both motor endplates and nerve terminals of the neuromuscular junction disorders affecting neurological structures [23], reflected in the progressive muscular dystrophy, with inactivation and loss of mobility in the rear limbs of the infected mice, as occurred in infected mice with DSM-strain isolated the wild mammalian that live in the geographic area where Chagas' disease is endemic.

CONCLUSIONS

In this work, it was described the effect of *Trypanosoma cruzi* infection with DSM-strain in the model murine, on microvasculature, neuromuscular junction and motor nerve terminals lesions of *Gastrocnemius* muscle. The common pathological clinical finding in mice acutely infected was the change in the locomotor activity. The results of the present study show ultrastructural changes in the skeletal muscle fiber, affecting normal function of the G muscle on infected mice. It determines the

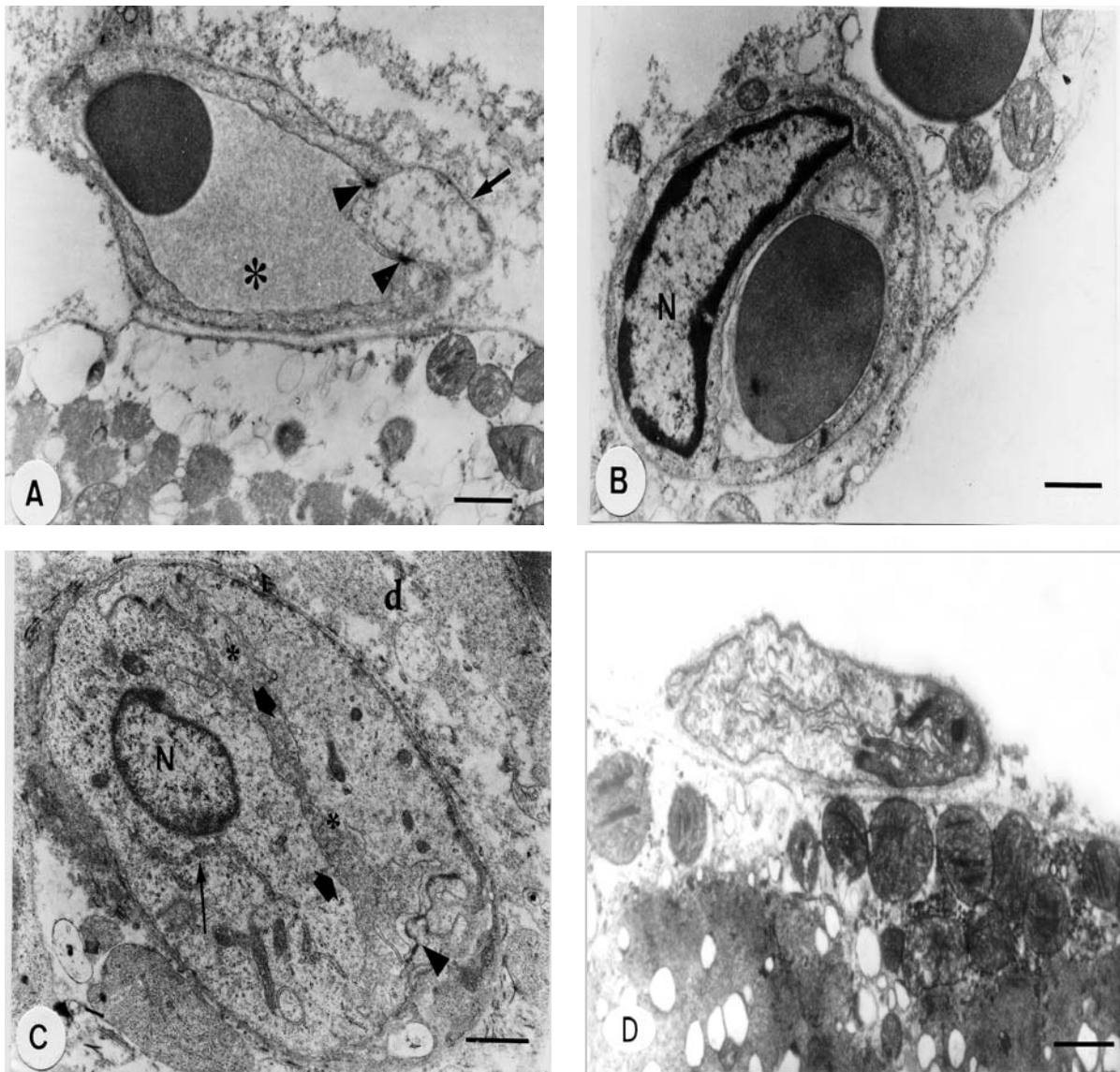


FIGURE 3. ELECTRON MICROGRAPHS OF BLOOD VESSELS IN TRANSVERSE SECTIONS FROM *T. cruzi* INFECTED-MUSCLE. A) ONE OF THE THREE ENDOTHELIAL CELLS THAT FORM THE VESSEL IS SWOLLEN (SMALL ARROW). THE INTERCELLULAR SPECIALIZATIONS ARE PUNCTUATED AND HIGHLY ELECTRON DENSE (ARROWHEADS). THE CAPILLARY LUMEN IS FILLED WITH A GRANULAR MATERIAL (ASTERISK). B) ALTERED BLOOD VESSEL SHOWING A LARGE NUCLEUS (N) OCCUPYING ALMOST ALL THE ENDOTHELIAL CYTOPLASM. NOTICE THE REDUCTION IN THE CAPILLARY LUMEN, SUCH THAT, ONLY ONE ERYTHROCYTE IS ABLE TO PASS THROUGH IT (C AND D). COMPLETELY OBLITERATED BLOOD VESSEL AND NO LUMEN CAN BE OBSERVED (SMALL ARROWS) GIVING RISE TO ISCHEMIA. NOTICE AN INCREASE IN THE ROUGH ENDOPLASMIC RETICULUM (ARROW), FREE POLYRIBOSOMES, AS WELL AS AN INCREASE IN ENDO-EXOCYTIC VESICLES AT THE LUMINAL SIDE OF ENDOTHELIAL CELLS. THE INTERCELLULAR SPECIALIZATIONS BETWEEN ENDOTHELIAL CELLS ARE SEEN PUNCTUATED AND FRAGMENTED (ARROWHEAD). CELL DEBRIS AND IN IT D) ARE CAPILLARIES. SCALE BARS: 1 μ m. / FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA DE SECCIONES TRANSVERSALES DE VASOS SANGUÍNEOS DEL MÚSCULO INFECTADO CON *T. cruzi*. A) UNA DE LAS CÉLULAS ENDOTELIALES ESTÁ INFLAMADA (FLECHA PEQUEÑA). LAS ESPECIALIZACIONES INTERCELULARES ESTÁN PUNTEADAS Y MUY ELECTRÓN DENSAS (CABEZA DE FLECHA). EL LÚMEN CAPILAR ESTA LLENO CON UN MATERIAL GRANULAR (ASTERISCO). B) VASO SANGUÍNEO ALTERADO MOSTRANDO UN GRAN NÚCLEO (N) OCUPANDO GRAN PARTE DEL CITOPLASMA ENDOTHELIAL. SE NOTA LA REDUCCIÓN EN EL LÚMEN CAPILAR, DE TAL FORMA QUE, UN SOLO ERITROCITO PUEDE PASAR A TRAVEZ DEL LUMEN. (C Y D) VASO SANGUÍNEO CON EL LÚMEN COMPLETAMENTE CERRADO (FLECHA PEQUEÑA) DANDO LUGAR A UNA ISQUEMIA. SE OBSERVA UN AUMENTO EN EL RETÍCULO ENDOPLÁSMICO RUGOSO (FLECHA), POLIRRIBOSOMAS LIBRES, ASÍ COMO UN AUMENTO DE LAS VESÍCULAS ENDO-EXOCÍTICAS EN EL LADO LUMINAL DE LAS CÉLULAS ENDOTELIALES. LAS ESPECIALIZACIONES INTERCELULARES ENTRE LAS CÉLULAS ENDOTELIALES ESTÁN PUNTEADAS Y FRAGMENTADAS (CABEZA DE FLECHA). RESTOS CELULARES Y EN (D) ESTOS SON OBSERVADOS EN EL ESPACIO EXTRACELULAR DE LOS CAPILARES ALTERADOS. ESCALA DE LA BARRA: 1 μ m.

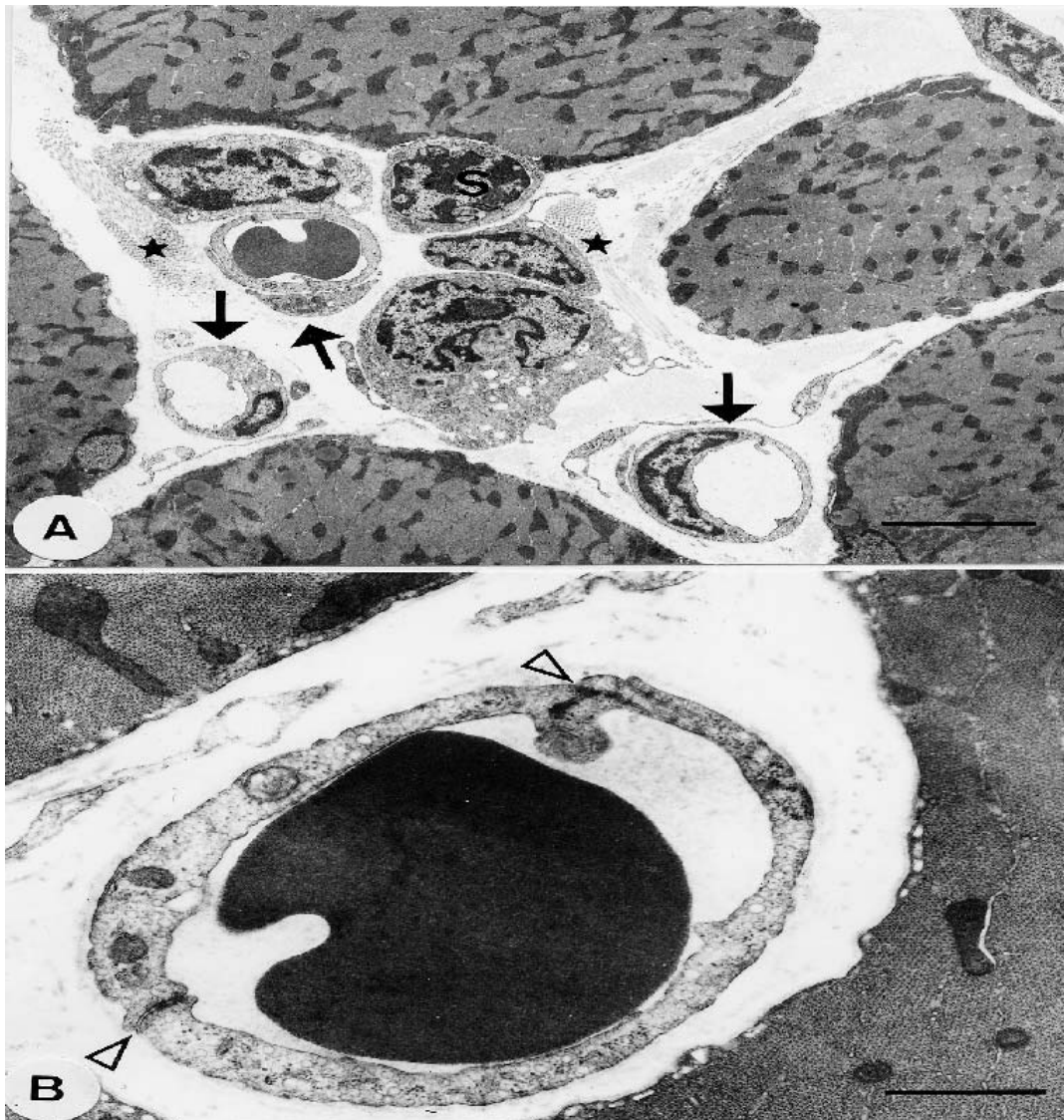


FIGURE 4. ELECTRON MICROGRAPHS OF SKELETAL MUSCLE FROM NON-INFECTED MOUSE (CONTROL).
A) LOW MAGNIFICATION VIEW OF THE INTERSTITIAL SPACE BETWEEN FIVE MUSCLE BUNDLES WHERE THREE BLOOD VESSELS (ARROWS), FEW COLLAGEN BUNDLES (STARS) AND FIBROBLASTS ARE PRESENT AS WELL AS A SATELLITE CELL (S). NOTICE THAT THE ENDOTHELIAL CELLS AND THE VASCULAR LUMEN SHOW NORMAL ULTRASTRUCTURAL CHARACTERISTICS. AT HIGHER MAGNIFICATION. B) THE ENDOTHELIAL CELLS OF A BLOOD VESSEL SHOW FEW MITOCHONDRIA, A LARGE NUMBER OF ENDO-EXOCYTOTIC VESICLES AND THE CHARACTERISTIC INTERCELLULAR SPECIALIZATION (ARROWHEADS). SCALE BARS: A= 1 μ m; B= 5 μ m. / FOTOGRAFÍAS DE MICROSCOPÍA ELECTRÓNICA DE MÚSCULO ESQUELÉTICO DE RATÓN NO INFECTADO. A) VISTA CON BAJO AUMENTO DEL ESPACIO INTERSTICIAL ENTRE 5 HACES MUSCULARES DONDE TRES VASOS SANGUÍNEOS (FLECHA), POCO COLÁGENO (ESTRELLA) Y FIBROBLASTOS ESTÁN PRESENTES ASÍ COMO UNA CÉLULA SATÉLITE (S). SE OBSERVA LAS CÉLULAS ENDOTELIALES Y EL LUMEN VASCULAR CON CARACTERÍSTICAS ULTRAESTRUCTURALES DE ASPECTO NORMAL. A MAYOR AUMENTO. B) LAS CÉLULAS ENDOTELIALES DE UN VASO SANGUÍNEO MUESTRA POCAS MITOCONDRIAS, UN GRAN NÚMERO DE VESÍCULAS ENDO-EXOCITÓCICAS Y CARACTERÍSTICAS ESPECIALIZACIONES INTERCELULARES (CABEZA DE FLECHA). ESCALA DE LA BARRA: A=1 μ m; B=5 μ m.

course of earlier stages of pathological processes represented by irreversible decrease of the locomotor activity and paralysis of the rear limbs of the mice during the course of infection.

This finding was attributable to presence of amastigotes and inflammatory infiltrates associated with tissue destruction,

as well as to capillary alterations that could eventually lead to deficient blood perfusion through the muscle and cause anaerobic metabolic changes affecting muscle function. Other observed changes in the same muscle had been associated with morphologic alterations of nerve terminal motor of the neuromuscular junction of G muscle [15]. Particularly, the axonal

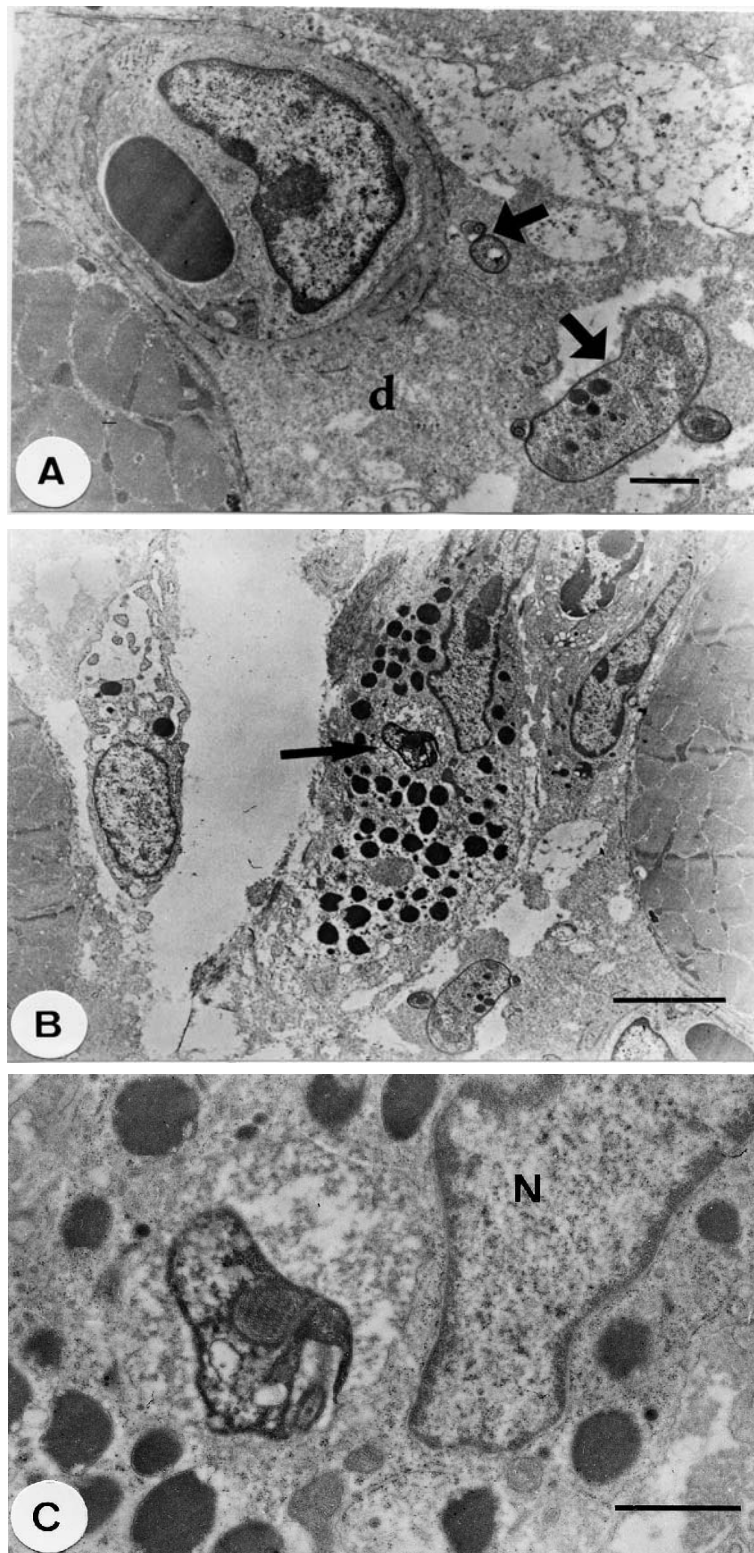


FIGURE 5. ELECTRON MICROGRAPHS SHOWING: A) *T. cruzi* (ARROWS) IN THE MUSCULAR INTERSTITIUM NEAR THE ALTERED BLOOD VESSEL AND ASSOCIATED WITH A LARGE AMOUNT OF CELL DEBRIS (D). B) A PARASITE INSIDE THE ACTIVE MACROPHAGE (ARROW). C) INSET OF (B) WHICH SHOWS DETAILS OF THE PARASITE NEAR THE MACROPHAGE NUCLEUS (N). SCALE BARS: A, C= 1 μ m; B= 3 μ m / FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA MOSTRANDO: A) *T. cruzi* (FLECHA) EN EL INTERSTICIO MUSCULAR CERCA DE UN VASO SANGUÍNEO ALTERADO Y ASOCIADO CON UNA GRAN CANTIDAD DE RESTOS CELULARES (D). B) UN PARÁSITO DENTRO DE MACRÓFAGO ACTIVADO (FLECHA). C) INSERTADO DE (B) EL CUAL MUESTRA DETALLES DEL PARÁSITO CERCA DEL NÚCLEO DEL MACRÓFAGO (N). ESCALA DE LA BARRA: A, C= 1 μ m; B= 3 μ m.

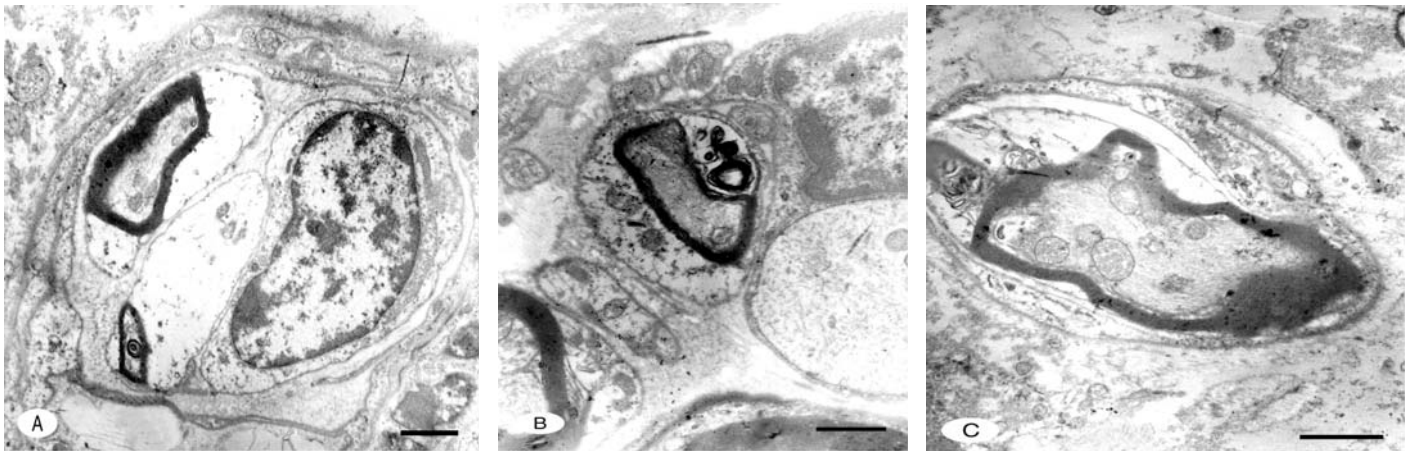


FIGURE 6. ELECTRON MICROGRAPHS SHOWING THE NERVE FIBER OF *Gastrocnemius* FROM INFECTED MICE WITH *T. cruzi*. (A, B) PRESENT REDUCTION IN THE AXON CALIBER, SWELLING OF THE SCHWANN CELL AND CYTOPLASMIC UNION FIGURES. (C) DETAIL OF AN AXON SHOWING MITOCHONDRIAL SWELLING IN THE AXOPLASM. SCALE BARS: 2 μm / FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA QUE MUESTRAN LA FIBRA NERVIOSA DE *Gastrocnemius* DE RATONES INFECTADOS CON *T. cruzi*. (A, B) PRESENTAN REDUCCIÓN EN EL CALIBRE DEL AXÓN, CÉLULA DE SCHWANN INFLAMADA Y FIGURAS UNION CITOPLASMÁTICAS. (C) DETALLE DE UN AXÓN MOSTRANDO MITOCONDRIAS INFLAMADAS EN EL AXOPLASMA. ESCALA DE LA BARRA: 2 μm .

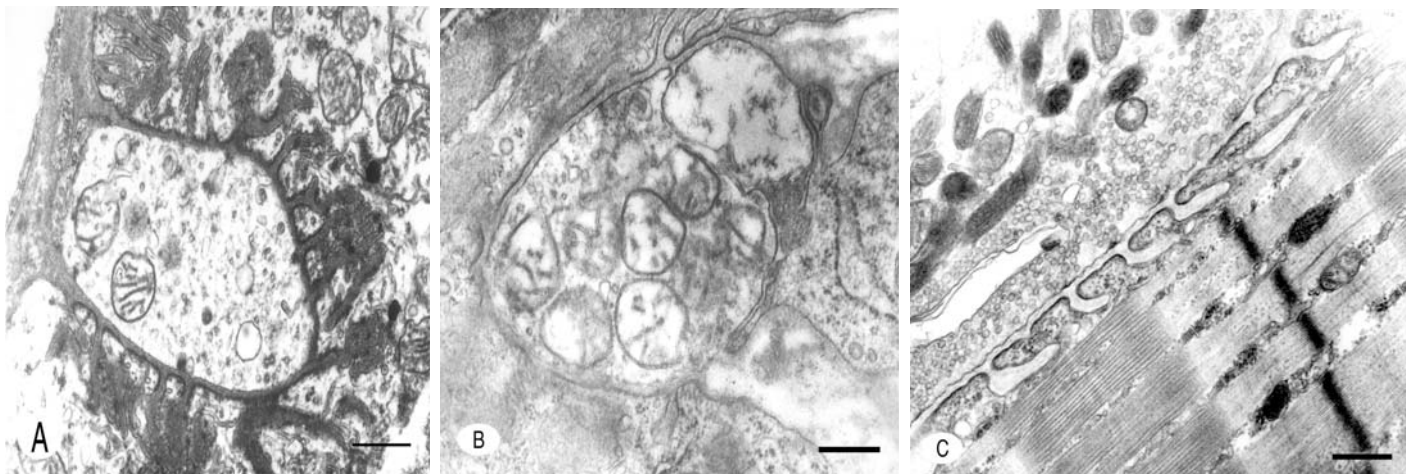


FIGURE 7. ELECTRON MICROGRAPHS OF NEUROMUSCULAR JUNCTION FROM *T. cruzi*-INFECTED SKELETAL MUSCLE. (A, B) SHOWN THE NERVE TERMINAL WITH SCARCES SYNAPTIC VESICLES AND MITOCHONDRIAL SWELLING IN THE AXOPLASM. (C) PRESENT NEUROMUSCULAR JUNCTION OF *Gastrocnemius* HEALTHY MOUSE WITH LARGE AMOUNT OF SYNAPTIC VESICLES REGULARLY DISTRIBUTED IN THE AXOPLASM AND NORMAL MITOCHONDRIAL ASPECT. SCALE BARS: 1 μm / FOTOGRAFÍAS DE MICROSCOPIA ELECTRÓNICA DE UNIÓN NEUROMUSCULAR DEL MÚSCULO ESQUELÉTICO INFECTADO CON *T. cruzi*. (A, B) MUESTRAN EL NERVO TERMINAL CON ESCASAS VESÍCULAS SINÁPTICAS Y MITOCONDRIAS INFLAMADAS EN EL AXOPLASMA. (C) PRESENTA LA UNIÓN NEUROMUSCULAR DE *Gastrocnemius* DE RATÓN SANO CON ABUNDANTES VESÍCULAS SINÁPTICAS REGULARMENTE DISTRIBUÍDAS EN EL AXOPLASMA Y MITOCONDRIAS DE ASPECTO NORMAL. 1 μm .

elimination, swelling of the Schwann cells, changes in synaptic morphological parameters as the presynaptic membrane densities in the active zones, producing neuromuscular disorders in skeletal muscle fiber modifying the motor activity of the mice with acute Chagas infection.

Emphasized the need for further studies of some aspects of the biology and behavior of *T. cruzi* strains with high virulence and isolated from the same geographic area, mainly in central region of Venezuela, where *T. cruzi* frequently circulates be-

tween triatomine bugs, peridomestic animals and the human population exposed to infection and to a clinical presentation similar as showed by experimental animals used in this study.

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BIBLIOGRAPHIC REFERENCES

- [1] ANDRADE, Z.A.; CORREA, R.; SADIGURSKY, M.; FERRANS, V.J. Myocardial changes in acute *Trypanosoma cruzi* infection. Ultrastructural evidence of immune damage and the role of microangiopathy. **Am. J. Pathol.** 144:1403-1411. 1994.
- [2] ANDRADE, Z.A.; ANDRADE, S.G.; SADIGURSKY, M.; WENTHOLD, R.J.; STEPHEN, L.H.; FERRANS, V.J. The indeterminate phase of Chagas' disease: Ultrastructural characterization of cardiac changes in the canine model. **Am. J. Trop. Med. Hyg.** 57:328-336. 1997.
- [3] ARAUJO, S. Alteraciones del sistema nervioso central de ratones con infección aguda producida por *Trypanosoma cruzi*. Dpto de Biología, Facultad de Ciencias, Universidad de los Andes, Mérida, Venezuela. (Trabajo Especial de Grado). 68 pp. 2002.
- [4] BRENER, Z. Observações sobre a imunidade a superinfecções em camundongos experimentalmente inoculados com *Trypanosoma cruzi* e submetidos a tratamento. **Rev. Inst. Med. Trop. São Paulo.** 4:119-123. 1962.
- [5] GONZALEZ C, S.M.; SANZ, O.P.; MULLER, L.A.; MOLINA, H.A.; FERNANDEZ, J.; RIMOLDI, M.T.; SICA, R.E.P. Peripheral nervous system damage in experimental chronic Chagas' disease. **Am. J. Trop. Med. Hyg.** 36:41-45. 1987.
- [6] GONZALEZ C., S.M.; MIRKIN, G.A.; SOLANA, M.E.; TEKIEL, V.S. Patología por *Trypanosoma cruzi*. ¿Cepa dependiente? **Med.** 59:69-74. 1999.
- [7] FACTOR, S.M.; CHO, S.; WITTNER, M.; TANOWITZ, H. Abnormalities of the microcirculation in acute murine Chagas' disease. **Am. J. Trop. Med. Hyg.** 34:246-53. 1985.
- [8] FELICIANGELI, M.D.; CAMPBELL-LENDRUM, D.; MARTINEZ, D.; GONZALEZ, P.; DAVIES, C. Chagas' disease control in Venezuela lessons from the Andean region and beyond. **Trends Parasitol.** 19:44-49. 2003.
- [9] GUILLEN-PERNIA, B.; LUGO DE Y., A.; MORENO, E. Dilatación del tracto digestivo de ratones infectados con *Trypanosoma cruzi*. **Kasmera.** 42:195-209. 2001.
- [10] HIGUCHI, M.L.; DE BRITO, T.; REIS, M.M.; BARBOSA, A.; BELLOTTI, G.; PEREIRA-BARRETO, A.C.; PILEGGI, F. Correlation between *Trypanosoma cruzi* parasitism and myocardial inflammatory infiltrate in human chronic chagasic myocarditis: Light microscopy and immunohistochemical findings. **Card. Pathol.** 2:101-106. 1993.
- [11] HOARE, A. **The trypanosomes of mammals.** Blackwell Sci. Publ. Oxford & Edinburg, UK, 334 pp. 1972.
- [12] KIM, C.T.; STROMMEN, J.A.; JOHNS, J.S.; WEISS, J.M.; WEISS, L.D.; WILLIAMS, F.H.; RASHBAUM, I.G. Neuro-muscular rehabilitation and electrodiagnosis. 4. Pediatric issues. **Arch. Phys. Med. Rehabil.** 86:28-32. 2005.
- [13] LEON S, F.E.; PRADA, D.G.; BAYONA P, J.; VALDERRAMA, V.; GARCIA, I.; LEON, M.E.; SUNNEMARK, D. Neurological effects of American tripanosomiasis: clinical aspects. **Biomed.** 23:462-475.
- [14] LOSAVIO, A.; JONES, M.C.; SANZ, O.P.; MIRKIN, G.; GONZALEZ-CAPPA, S.M.; MUCNIK, S.; SICA, E.R.P. A sequential study of the peripheral nervous system involvement in experimental Chagas' disease. **Am. J. Trop. Med. Hyg.** 41:59-547. 1989.
- [15] LUGO DE Y., A.; GUTIERREZ, R.; COLASANTE, C. Ultrastructural changes in neuromuscular junction of skeletal muscle of mice infected with *Trypanosoma cruzi*. **Rev. Ecol. Lat. Am.** 6:1-11. 1999.
- [16] MIRKIN, G.A.; JONES, M.; SANZ, O.P.; REY, R.; SICA, R.E.P.; GONZALEZ, S.M. Experimental Chagas' disease: Electrophysiology and cell composition of the neuromyopathic inflammatory lesions in mice infected with a myotropic and a pantropic strain of *Trypanosoma cruzi*. **Clin. Immunol. Immunopathol.** 73:69-79. 1994.
- [17] MOLINA, H.A.; CARDONA, R.L.; RIMOLDI, M.T. The neuromuscular pathology of experimental Chagas' disease. **J. Neurol. Sci.** 81:287-300. 1987.
- [18] MONTES DE O., M.; TORRES, S.H.; LOYO, J.G.; VASQUEZ, F.; HERNANDEZ, N.; ANCHUSTEGUI, B.; PUIGBO, J.J. Exercise performance and skeletal muscle in patients with advanced Chagas' disease. **Chest.** 125:1306-1314. 2004.
- [19] MORENO, E., ANEZ, N., SCORZA, C., LUGO DE Y, A.; BORGES, R. Efecto de inóculos bajos en la infección experimental por *Trypanosoma cruzi*. **Bol. Dir. Malariol. San. Amb.** 39:70-77. 1999.
- [20] MORENO, E.; GONZALEZ, N.; RIVERA, I.; GUILLEN-PERNIA, B.; LUGO DE Y, A. Caracterización biológica e isoenzimática de aislados de *Trypanosoma cruzi*. **Bol. Dir. Maral. San Amb.** 52:17-28. 2002.
- [21] MÜLLER, L.A.; ANASCO, N.; GONZALEZ CAPPA, S.M. *Trypanosoma cruzi*: isolated dependence in the induction of lytic antibodies in the mouse and rabbits. **Exp. Parasitol.** 61:284-293. 1986.
- [22] PEREIRA, V.L.; ZAMORANO, M.M.B.; BOAINAIN, E. Estudo do comportamento biológico de tres amostras de *Trypanosoma cruzi* isoladas de paciente de Instituto Dvorak Pazzanese de Cardiología. **Rev. Inst. Med. Trop. Sao Paulo.** 29:155-161. 1987.
- [23] PRAKASH, Y.S.; ZHAN, W.Z.; MIYATA, H.; SIECK, G.C. Adaptations of diaphragm neuromuscular junction following inactivity. **Acta Anat.** 154:147-161. 1995.

- [24] PETKOVA, S.B.; HUANG, H.; FACTOR, S.M.; PESTELL, R.G.; BOUZAHZAH, B.; JELICKS, L.A.; WEISS, L.M.; DOUGLAS, S.A.; WITTNER, M.; TANOWITZ, H.B. The role of endothelin in the pathogenesis of Chagas' disease. **Int J. Parasitol.** 31:499-511. 2001.
- [25] QUIÑONES, M.E.; FINOL, H.J.; SUCRE, L.; GARCÍA, F.; ORTEGA, A. Anormalidades en los capilares intramusculares del equino infectado con *Trypanosoma evansi* (Steel, 1885). **Memorias de IV Jornadas de Microscopía Electrónica. SVME.** Cumana, July 16-18. Venezuela, 10 pp. 1990.
- [26] RIBERIO DOS SANTO, R.; HUDSON, L. *Trypanosoma cruzi* binding of parasite antigens to mammalian cell membranes. **Parasit Immunol.** 2:1-10. 1980.
- [27] ROSSI, M.A.; GONCALVES, S.; RIBEIRO DO SANTOS, R. Experimental *Trypanosoma cruzi* cardiomyopathy in BALB/c mice: the potential role of intravascular platelet aggregation in its genesis. **Am. J. Pathol.** 114:209-216. 1984.
- [28] ROSSI, M.A. Microvascular changes as a cause of chronic cardiomyopathy in Chagas' disease. **Am. Heart. J.** 120:233-236. 1990.
- [29] ROSSI, M.A. Aortic endothelial cell changes in the acute septicemic phase of experimental *Trypanosoma cruzi* infection in rats: scanning and transmission electron microscopic study. **Am. J. Trop. Med. Hyg.** 57: 321-327. 1997.
- [30] SICA, R.E.; FILIPINI, D.; PANIZZA, M.; FUMO, T.; BASO, S.; LAZZARI, J.; MOLINA, H.A. Involvement of the peripheral sensory nervous system in human Chagas' disease. **Medicine (B. Aires).** 46:662-668. 1986.
- [31] STROMMER, J.A.; JOHNS, J.S.; KIM, C.T.; WILLIAMS, F.H.; WEISS, L.D.; WEISS, J.M.; RASHBAUM, I.G. Neuromuscular rehabilitation and electrodiagnosis. 3. Diseases of muscles and neuromuscular junction. **Arch. Phys. Med. Rehabil.** 86:18-27. 2005.
- [32] TANOWITZ, H.B.; KAUL, D.K.; CHEN, B.; MORRIS, S.A.; FACTOR, S.M.; WEISS, L.M.; WITTNER, M. Compromised microcirculation in acute murine *Trypanosoma cruzi* infection. **J. Parasitol.** 82:124-30. 1996.
- [33] TOMAS, J.; FENOLL, R.; SANTAFE, M.; BATLLE, J.; MAYAYO, E. Motor nerve terminal morphologic plasticity induced by small changes in the locomotor activity of the adult rat. **Neurosci. Lett.** 106:137-140. 1989.
- [34] TOMAS, J.; SANTAFE, M.; LANUZA, M.A.; FENOLL-BRUNET, M.R. Physiological activity-dependent ultrastructural plasticity in normal adult rat neuromuscular junctions. **Bio. Cell.** 89:19-28. 1997.
- [35] TONINO, P.; FINOL, H.J.; CAMPO-AASEN, I. Un modelo murino para el estudio de los efectos a distancia del *Toxoplasma gondii* sobre el músculo esquelético. **Memorias del Primer Congreso Atlántico de Microscopía Electrónica. SVME,** Mérida. 24-29 de mayo. Venezuela, 68-69 pp. 1992.
- [36] TONINO, P.; FINOL, H.; MARQUEZ, A. Skeletal muscle capillary ultrastructure in *Toxoplasma gondii* parasitized mice. **Acta Cientif Venezol.** 44:349-354. 1993.
- [37] VIANNA, G. Contribuições para o estudo da anatomia patológica de Carlos Chagas. **Mem. Inst Oswaldo Cruz.** 3:276-294. 1911.
- [38] VÍCHI, F.L. Destruição de neuronios motores na medulla espinal do ratos na fase aguda da molestia de Chagas. **Rev. Inst. Med. Trop. Sao Paulo.** 6:150-154. 1964.
- [39] WORLD HEALTH ORGANIZATION. Chagas' disease. Thirteenth programme report of the UNDP/World Bank/WHO special programme for research and training in tropical disease. 112-123 pp. 1996.