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Ultrastructure of murine cardiac ganglia in experimental Chagas' disease

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Summary. Albino mice, infected with *Trypanosoma cruzi* (Tulahuen strain) were sacrificed on days 7, 9, 12, 14, 16, 18, 21, 32 and 39 following infection. Transmission electron microscopic examination of the cardiac ganglia revealed no ultrastructural change at day 7. At day 9 there was peri- and intraganglionic monocytic infiltration but parasites were absent. Between days 12 and 16 there was intense monocytic infiltration, with intra-ganglionic presence of parasites within fibroblasts, monocytes and macrophages. None were seen within capsular cells, endothelial cells, Schwann cells, statellite cells and ganglion cells. The Schwann cells and satellite cells, however, showed phagocytic activity. Satellite cells were also reactive with proliferative pseudopodia which encircled neuronal processes. By day 18, parasites were absent in the ganglia. But monocytes were still present up to day 39, some of them still engulfing satellite cell and neuronal processes. Satellite cells continued to be reactive and Schwann cells phagocytic. Ganglion cells remained normal throughout the experiment.The results suggest that infection of Schwann cells, satellite cells and ganglion cells may depend upon the tissue tropism of the strain of the parasite used and its concentration in the inoculum. The results are consistent with the view that any parasympathetic dysfunction in experimental Chaga's disease in the mouse may be of a transient nature.

Key words: Cardiac, Ganglia, Chaga's disease

Introduction

In experimental Chagas' disease, the mouse has been used extensively to study the structural effects of infection by the parasite *Trypanosoma cruzi* on various organs and systems. Among these may be mentioned the heart, its working muscle *in vivo* (Grimaud and Andrade, 1984; Andrade and Freitas, 1987; Rowland and Lavy, 1987) and *in vitro* (Meirelles et al., 1986) and its conducting system (Molina et al., 1988); the mammary gland (Ribeiro et al., 1988); the spinal cord, peripheral nerves and skeletal muscle (González et al., 1987; Molina et al., 1987; Lasavio et al., 1989); and the thymus and lymphoid organs (Da Costa et al., 1991; Leite de Moraes et al., 1991).

In the autonomic nervous system, Tafuri (1970, 1971) described the ultrastructural alterations in the coeliac, myenteric and cardiac ganglia. However, these descriptions were confined to the first two weeks post infection. The present study was undertaken to provide data on the sequential changes in the cardiac ganglia from the first to the seventh week post infection.

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Materials and methods

Trypanosoma cruzi (Tulahuen strain) were maintained in the laboratory by syringe passage in mice. One drop of blood was withdrawn from the tail vein of the mouse to which one drop of saline was added and the blood examined microscopically for *Trypanosoma cruzi* parasites. If parasites were seen, the mouse was sacrificed and blood was collected by cardiac puncture using a heparinised syringe. 0.05 ml of the infected blood was injected intraperitoneally into each «clean» mouse.

For the experiments, three week old male Swiss albino mice weighing 20 - 28 g were inoculated intraperitoneally with 0.05 ml of blood containing 250 of trypomastigotes of *Trypanosoma cruzi* (Tulahuen strain). The animals were sacrificed on days 7 (4 mice), 9 (3), 12 (2) 14 (2), 16 (2), 18 (3), 21 (2), 32 (1) and 39 (1) following infection. Age matched controls were sacrificed at the corresponding stages.

Under chloral hydrate (0.5 ml of 7% solution) anaesthesia, the animals were perfused through the left ventricle with 50 ml of Ringer's solution followed by 50 ml of an aldehyde fixative (3% glutaraldehyde + 2%

paraformaldehyde) in 0.1 M cacodylate buffer (pH 7.2 - 7.4). The atria with a ring of ventricle were removed whole, immersed in fresh fixative and kept at 4º C overnight. The following morning, thin slices of the atria were trimmed from the specimens and placed in ice cold 0.1 m cacodylate buffer (pH 7.2 - 7.4) containing 5% sucrose. After two further changes of buffer at intrevals of 10 mins, the tissue slices were post-fixed in 1% osmium tetroxide (containing 1.5% potassium ferrocyanide) for 2 h at 4º C, dehydrated in an ascending series of ethanol and embedded in Araldite.

Semithin sections of 1 µm were cut on a Reichert-Jung Ultracut and stained with 1% methylene blue. Selected areas of the blocks containing cardiac ganglia were trimmed for ultrathin sectioning. The ultrathin sections were doubly stained in uranyl acetate and lead citrate and examined in JEOL 1200 CX and Philips CM12 electron microscope.

Results

At day 7 after inoculation the cardiac ganglia of *Trypanosoma cruzi* infected mice could not be distinguished from those of control animals. The capsular cells and the fibroblasts in the intervals between their laminae were normal. Within the ganglia, the ganglion cells and their satellite cells were not altered. Myelinated and unmyelinated nerve fibres and their associated Schwann cells were not different from those in control animals.

At day 9, however, there was a moderate periganglionitis and ganglionitis with monocytic infiltration (Fig. 1). Monocytes were located within the lumen of the intraganglionic capillaries or dispersed among the bundles of nerve fibres. Occasionally, a monocyte was adjacent to the surface of a neuron from which it was separated by a lamina of satellite cell cytolasm. The ganglion cells appeared normal. No parasites were observed within the ganglion, although in the extraganglionic interstitial space an occasional fibroblast was colonised by a nest of amastigotes.

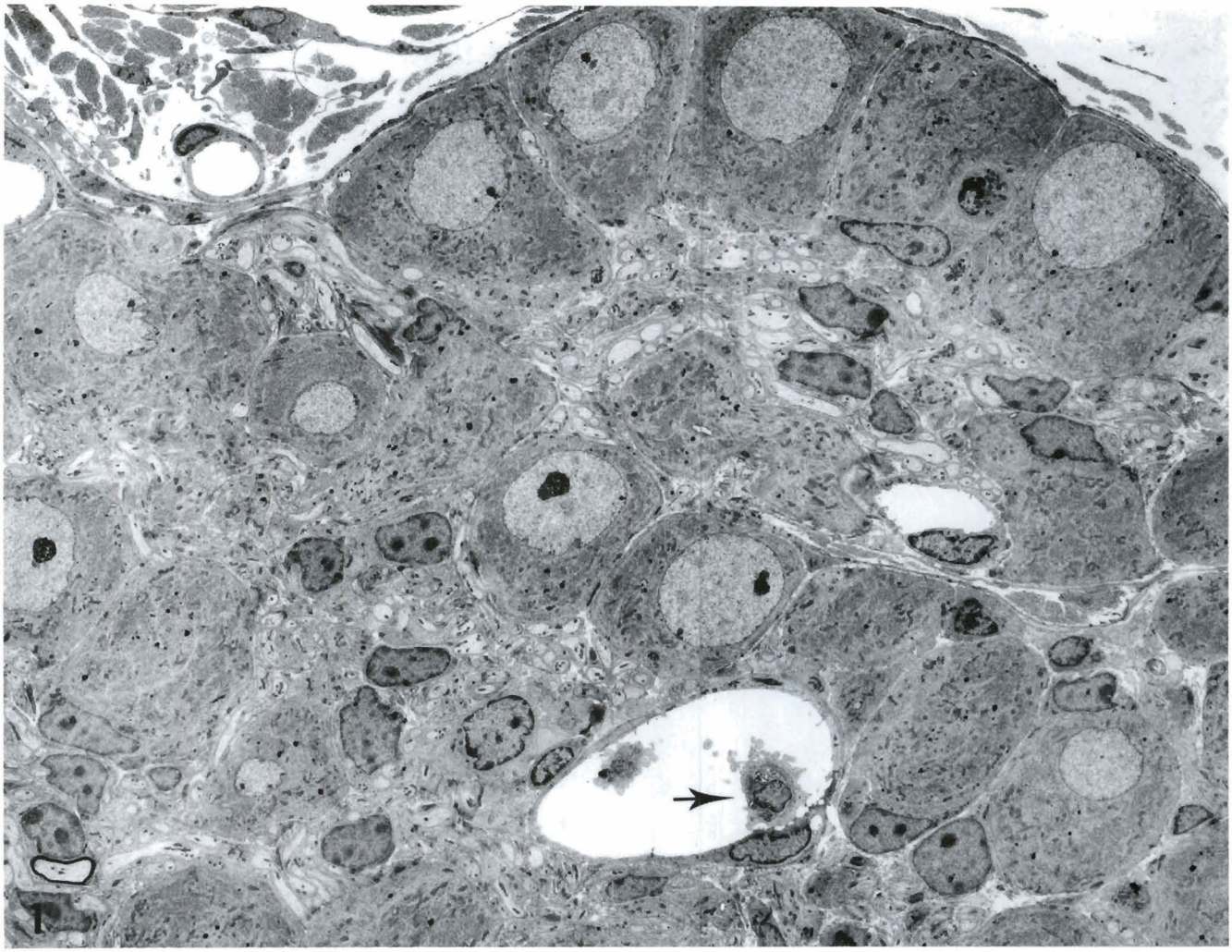
Between days 12 to 16, there was intense periganglionitis and ganglionitis with infiltration of monocytes and parasites (Fig. 2). The fibroblasts in the interlaminar spaces of the capsule, within the ganglion and within large nerve bundles were parasitised (Figs. 2 - 4). The parasites displayed various degrees of disintegration within the cytoplasm of the parasitised fibroblasts, which may be vacuolated with accumulation of cellular debris. In some cases, the plasmalemma of the infected fibroblast was partially disrupted with extrusion of cellular contents into the interstitial space (Fig. 4). Monocytes were now abundant within the ganglia, either lying free or associated with the surface of neurons; in the latter instance, they either indented or were separated from the neurons by a thin lamina of satellite cell cytoplasm. The cytoplasm of some monoctyes contained sequestered parasites (Fig. 5) while that of others was uniformly electron dense (Fig. 2). Occasionally, a degenerating monocyte may be internalised by a macrophage (Fig. 6). Macrophages were also abundant and contained within their cytoplasmic parasites undergoing various degrees of degeneration, as well as other cellular debris. The great majority of Schwann cells, readily identified by their close association with nerve fibres and the possession of a basal lamina, were normal. But in the cytoplasm of some Schwann cells, there was sequestration of electron dense degenerating debris (Fig. 7). An occasional Schwann cell was seen to be phagocytic as it sent its pseudopodia round cellular debris in the interstitial space (Fig. 8). Rarely, a Schwann cell showed mitosis. Satellite cells were also readily identified by their close association with ganglion cell bodies and the possession of a basal lamin. While most satellite cells appeared normal, the cytoplasm of some contained membrane-bound electron dense phagosomes (Fig. 9). Other satellite cells showed focal reactivity. Many fiolopodial extensions from the cell surface enclosing neuronal elements were seen (Fig. 10). When compared with the control animals, the internal structure of the ganglion cells appeared unaltered during this stage of the infection. No parasite has been observed within capsular cells, Schwann cells, satellite cells or ganglion cells.

By day 18 and subsequently, no parasites were seen within and immediately adjacent to the ganglia although monocytes were still frequently encountered in close apposition to satellite cells (Fig. 11). In some cases, no basal lamina was observed to intervene between the monocyte and the satellite cell, although the adjacent neuronal elements may be partially engulfed by monocytic pseudopodia (Fig. 12). Monocytes, with pseudopodial extensions, were frequently present among the nerve fibres (Fig. 13). Most Schwann cells appeared normal. In a few instances, the cytoplasm of Schwann cells contained a membrane-bound electron dense phagosome undergoing degradation (Fig. 14). The cytoplasm adjacent to the phagosome appeared to be undergoing liquefaction. Some satellite cells were still focally reactive with numerous filopodial processes. The internal structure of the ganglion cells were unaltered during these stages.

Discussion

The results of the present study showed that in mice inoculated with the Tulahuen strain of *Trypanosoma cruzi* there was a selective parasitism of the cellular elements in the cardiac ganglia. Although the latter were free of parasites at day 9 after infection, there was a moderate inflammatory response as evidence by a monocytic infiltration. The observation of monocytes within the lumen of the capillaries, in perivascular positions and adjacent to satellite cells may be a response to circulating or leaked macromolecules from the parasites.

Between days 12 to 16 after infection, there was intense inflammation of the ganglia with parasites



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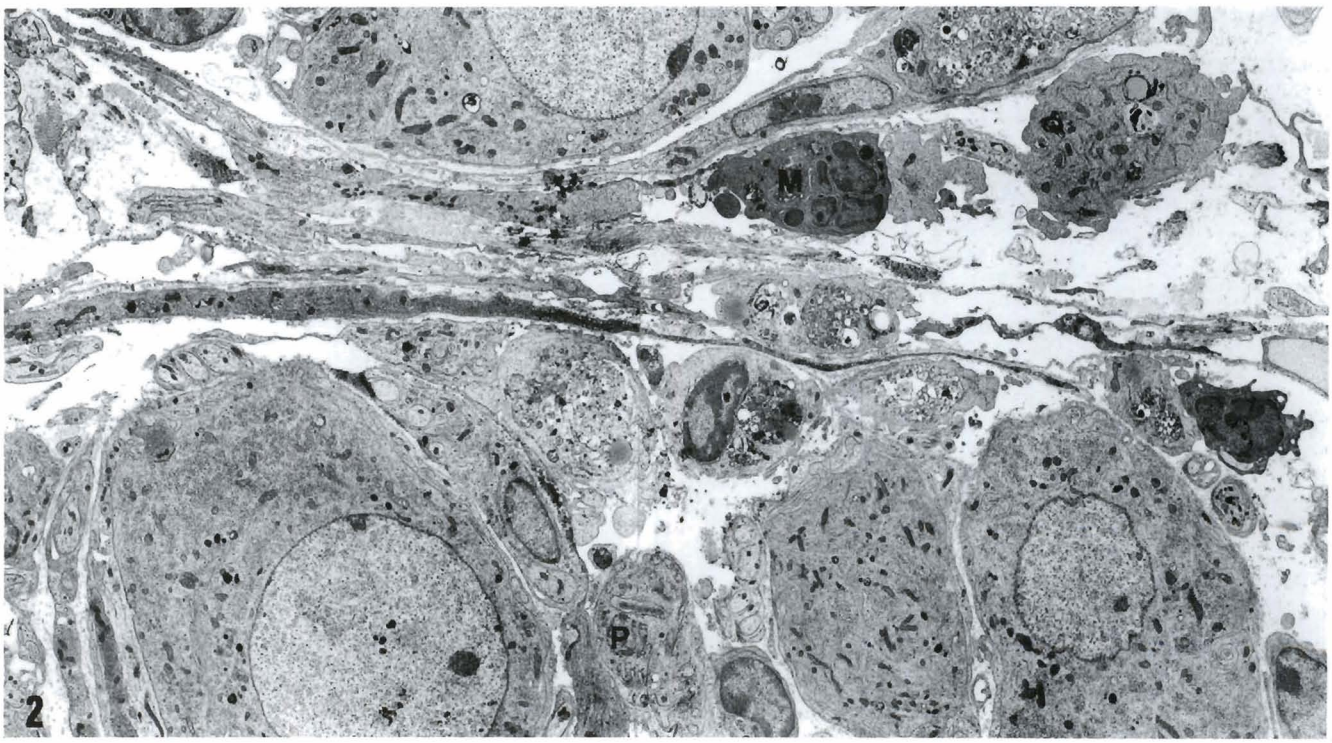

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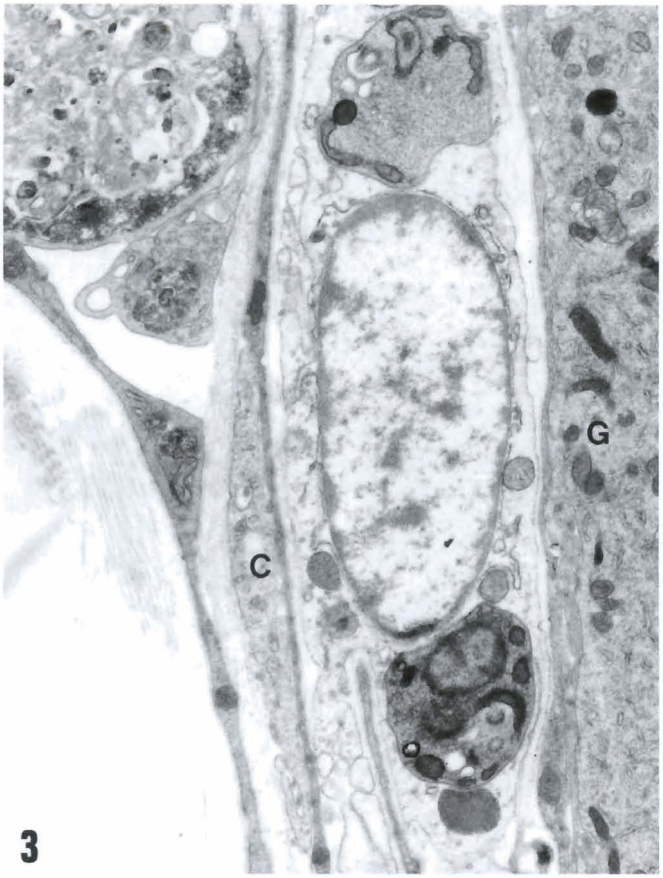

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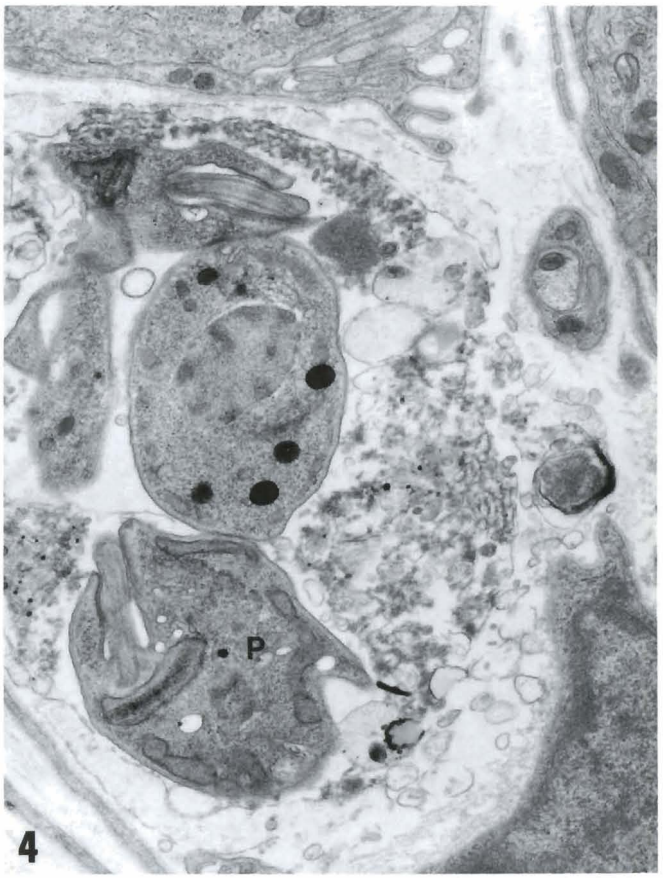

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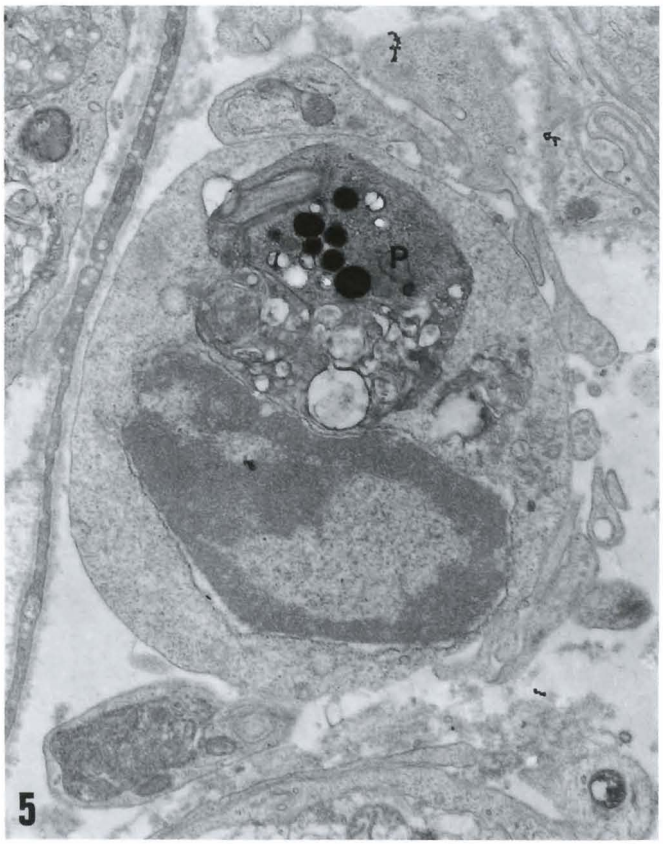

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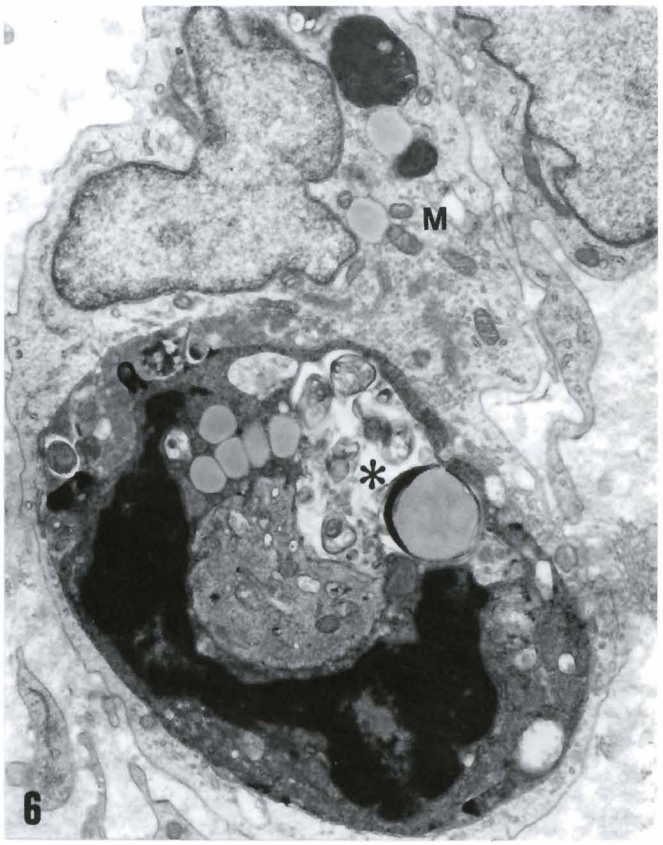

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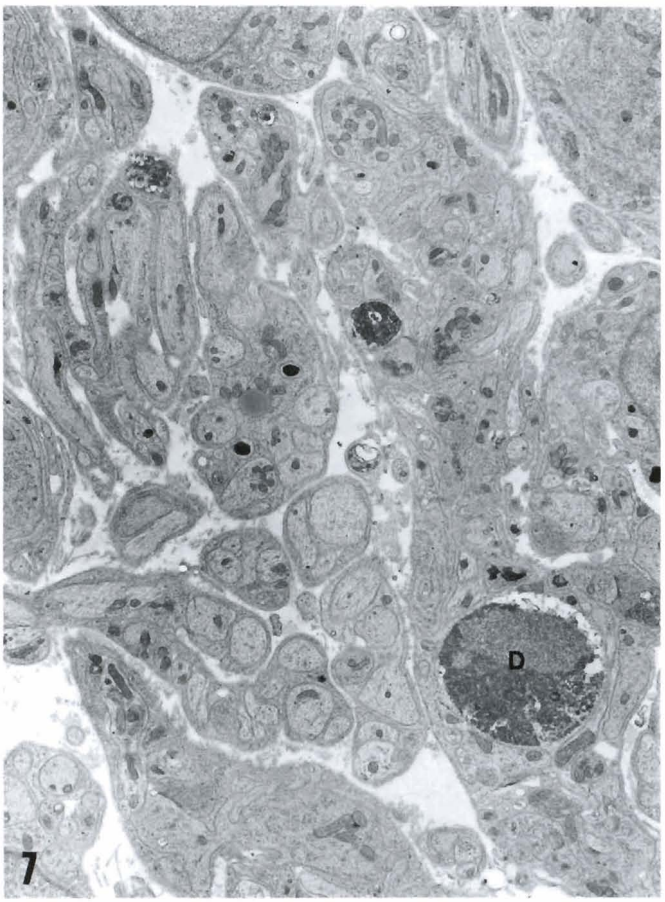
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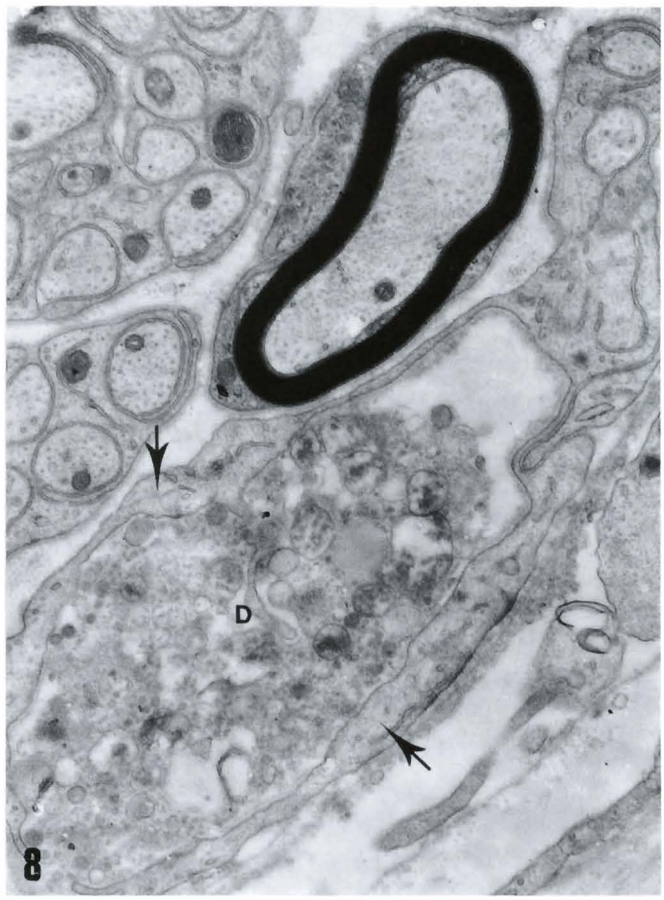
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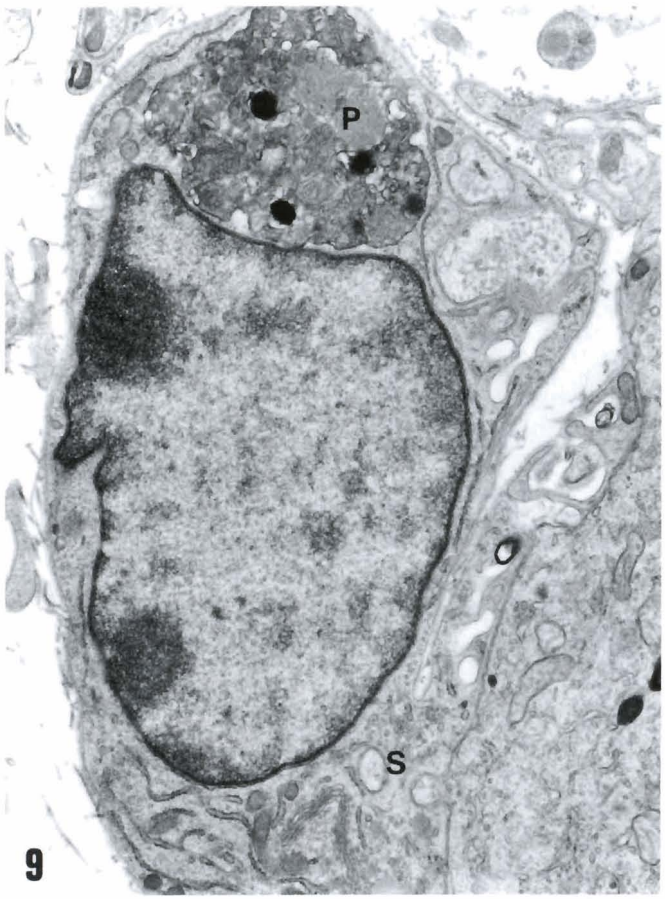
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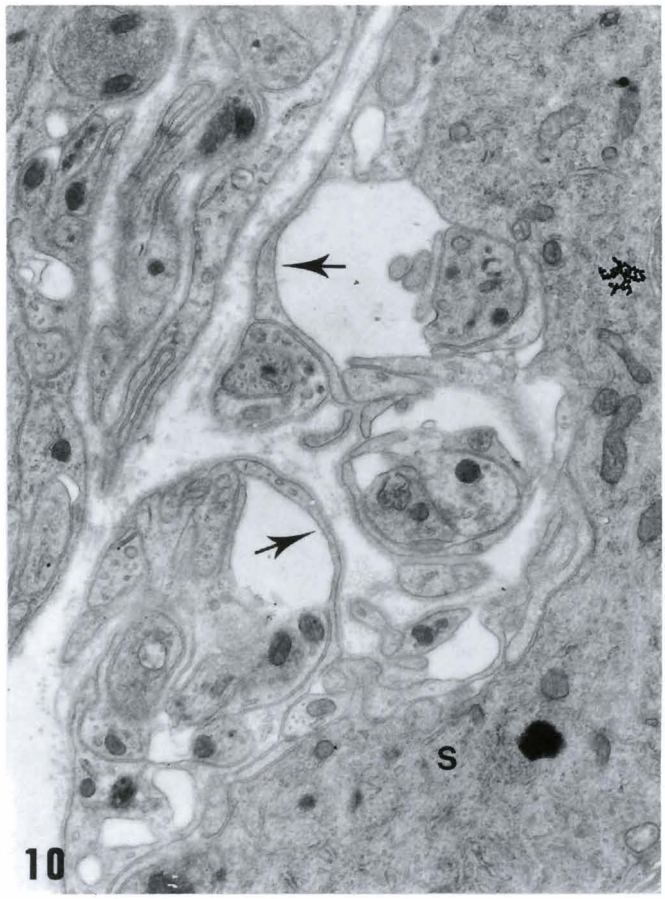
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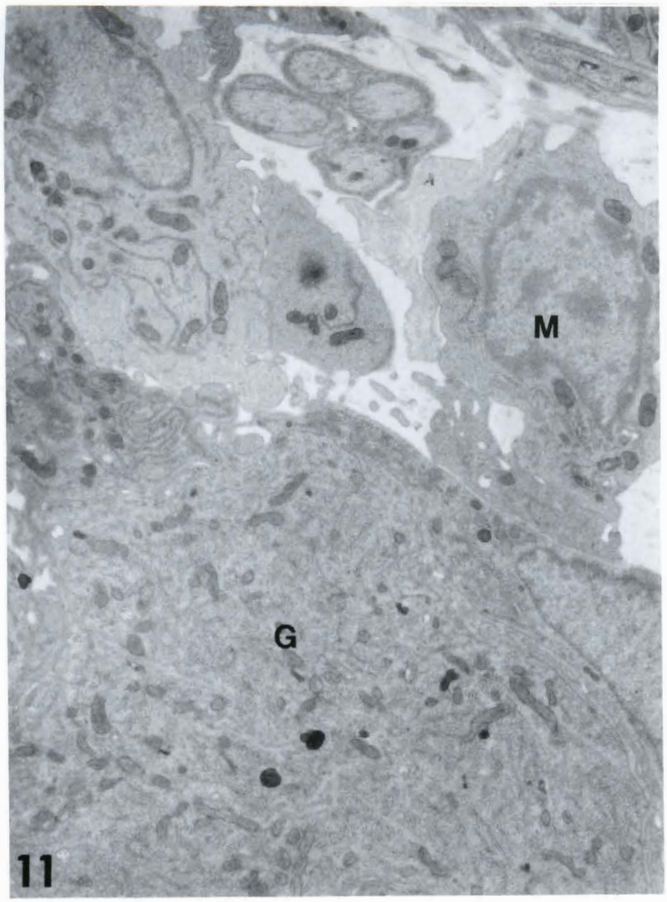
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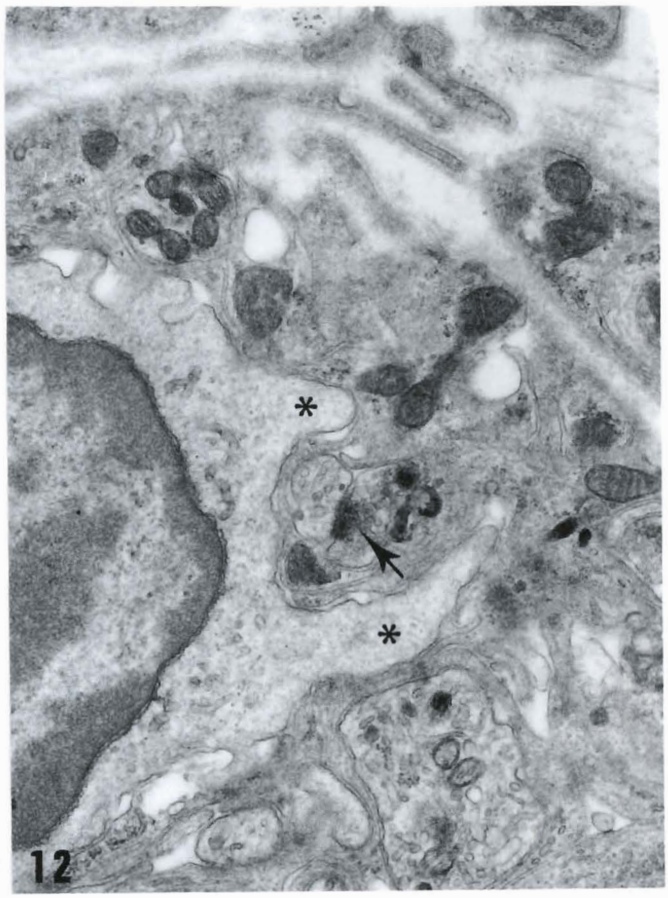
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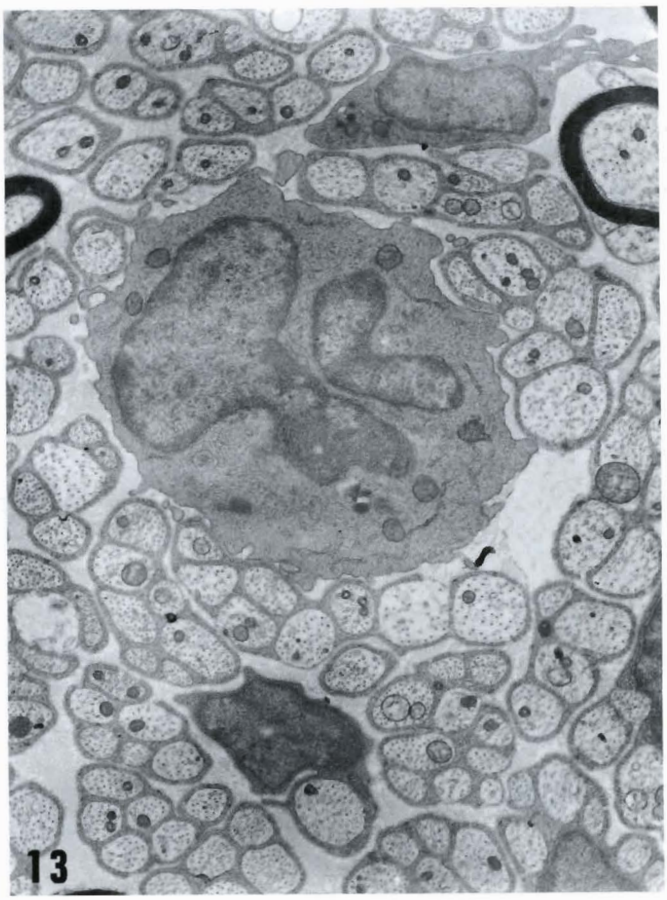
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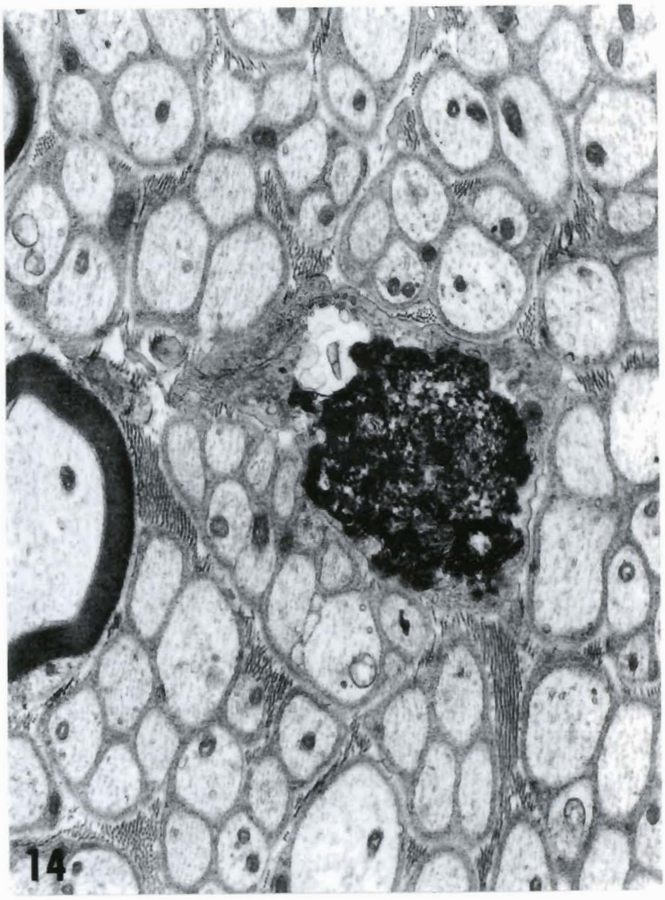
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Fig. 1. Low power survey of the cardiac ganglion showing normal-looking ganglion cells. No parasite was observed. Two monocytes (arrow) were present in one of the interganglionic capillaries. (9 days postinfection). x 1,750

Fig. 2. A low power view showing intense periganglionitis and ganglionitis. Note the infiltration of monocytes (M) and parasites (P). (16 days postinfection). x 3,150

Fig. 3. Electron micrograph of a parasitised fibroblast between a capsular cell (C) and a ganglion cell (G). (12 days postinfection). x 10,500

Fig. 4. Electron micrograph showing partially extruded cellular debris and parasite (P) from the cytoplasm of a fibroblast. (12 days post-infection). x 14,000

Fig. 5. Electron micrograph of a monocyte containing sequestred parasite (P). (16 days postinfection). x 14,000

Fig. 6. Electron micrograph of a degenerating monocyte (*) being phagocytosed by a macrophage (M). (14 days postinfection). x 10,400

Fig. 7. A low power view showing degenerating debris (D) being sequestered by a Schwann cell. (16 days postinfection). x 5,250

Fig. 8. Cellular debris (D) in the interstitial space being engulfed by Schwann cell processes (arrows). (16 days postinfection). x 17,500

Fig. 9. A satellite cell cytoplasm (S) containing phagosome (P). (14 days postinfection). x 14,000

Fig. 10. Electron micrograph showing multiple filopodial extensions (arrows) from the surface of a satellite cell (S). (16 days postinfection). x 14,000

Fig. 11. A lower power view showing a monocyte (M) still in close apposition to a ganglion cell (G). Note the absence of parasites. (21 days). x 6,650

Fig. 12. Electron micrograph showing a monocyte with pseudopodia (*) partially engulfing some neural elements (arrow). (28 days postinfection). x 21,000

Fig. 13. Electron micrograph showing a monocyte among nerve fibres. (39 days postinfection). x, 8,750

Fig. 14. Schwann cell cytoplasm an electron-dense phagosome. (18 days postinfection). x 11,025

frequently found in fibroblasts, monocytes and macrophages. The parasites displayed various degrees of degeneration. Ganglion cells, satellite cells, Schwann cells and capsular cells were not parsitised. These results differ from the report of Tafuri (1970) who described frequent parasitism of satellite cells and Schwan cells and a single instance in a neuron. The differences may be provisionally attributed to the different strains and concentration of the inoculum used in the two studies. In Fafuri's (1970) study the Y strain (4,000 parasites) was used and in the present study the Tulahuen strain (250 parasites). In this regard, it is intresting that Camargos and Machada (1988) using the Y strain (300,000) in the rat could not detect any amastigote pseudocysts in the superior cervical ganglion even at stages in which the myocardium was heavily infected with parasites. On the other hand, Molina et al. (1987) using the Tulahuen strain (50 parasites) found no amastigote nests in the murine sciatic nerve but three instances of amastigote nests in the lumbar spinal cord. These examples suggest that in specific cases interpretation of differences of results attributed to unlike strains used should be cautiously drawn even though it has been shown that different strains of *Trypanosoma cruzi* have different tissue tropisms (Zeledon and Ponce, 1972; Melo and Brener, 1978). The issue may be resolved in a comparative study of the effects of infection by *Trypanosoma cruzi* on various organs and systems, where the strains, route of infection, concentration of inoculum, time course and cytological techniques are standardised. Although Schwann cells and satellite cells were not parasitised, the presence of phagosomes undergoing various degrees of degradation and the observation of pseudopodial engulfment of cellular debris in the former and of neural elements in the latter suggest that these cells may become phagocytic under pathological conditions. Such activity may be a response to the presence of debris or macromolecules in the microenvironment. These observations compare with those of Tay et al. (1984) who reported similar phenomena in the monkey cardiac ganglia following vagotomy. On the other hand, monocytes and macrophages were frequently infected with parasites which underwent different degrees of internal degeneration. Some of the infected monocytes were in turn engulfed by macrophages. In this way, infected monocytes may be removed from the interstitial space.

Monocytes continued to be a feature in the cardia ganglia of mice sacrificed between days 18 and 38 following infection. They were most commonly closely associated with the satellite cells or directly with the ganglion cells. Occasionally, a monocyte could be seen to to engulf neuronal and satellite cell elements. Monocytes were also observed to insinuate themselves among the nerve fibres. These observations compare with similar phenomena reported in a study of human cardiac ganglia in chronic Chagas' disease (Almeida et al., 1988) as well as the study by Wong et al. (1987) of monkey cardiac ganglia during the third and fourth weeks following vagotomy. The results of the present study suggest that the micro-environment may still contain residues of parasites or their antigens (see Petry and Eisen, 1989). This notion is borne out by the behaviour of Schwann cells and satellite cells which continued to exhibit a phagocytic tendency.

The relatively normal appearance of the ganglion cells during the whole course of the present study, despite the obvious inflammatory response in the ganglia, suggests that they were preserved from the infective process. Any parasympathetic dysfunction in such cases may be of a transient nature (Gottberg et al., 1988) since there was no evidence in the present study of extensive neuronal degeneration. This would suggest that the neurogenic theory which states that there is a

selective para-sympathetic denervation during the acute phase to account for the myocardial damage in Chaga's disease (see Oliveira, 1985) may require some modification in specific cases (see Davila et al., 1991).

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References

Andrade S.G. and Freitas L.A.R. (1987). Trypanosoma cruzi: cardiac myocells alterations due to spontaneous or therapeutically induced intracellular parasite disintegration. Cell. Mol. Biol. 33, 797-805.

Almeida H.D.O., Teixeira U.D.P.A. and Araujo M.B.M. (1988). Estudo comparativo das lesoes das ganglios e nervos atriais em Chagasicos cronices. Arq. Bras. Cardiol. 50, 159-162.

Camargos E.R. and Machado C.R. (1988). Morphometric and histological analysis of the superior cervical ganglion in experimental Chagas' disease in rats. Am. J. Trop. Med. Hyg. 39. 456-462.

Da Costa S.C., Calabrese K.S., Bauer P.G., Savino W. and Lagrange P.H. (1991). Studies of the thymus in Chagas' disease: III. Colonization of the thymus and other lymphoid organs of adult and newborn mice by Trypanosoma cruzi. Patho. Biol. (Paris) 39, 91-97.

Davila D.F., Donis J.H., Torres A., Gottberg C.F. and Rossell O. (1991). Cardiac parasympathetic innervation in Chagas' disease. Med. Hypoth. 35, 80-84.

González S.M., Sanz O.P., Muller L.A., Molina H.A., Fernández J., Rimoldi M.T. and Sica R.E. (1987). Peripheral nervous system damage in experimental chronic Chagas' disease. Am. J. Trop. Med. Hyg. 36, 41-45.

Gottberg C.F., Donis J.H., Torres A., Fuenmayor J.A. and Davila D.F. (1988). Heart rate changes in rats with acute chagasic myocarditis. Trans. R. Soc. Trop. Med. Hyg. 82, 851.

Grimaud J.A. and Andrade S.A. (1984). *Trypanosoma cruzi*: intracellular host-parasite relationship in murine infection. Cell. Mol. Biol. 30, 59-65.

Leite de Moraes M.C., Hountebeyrie-Joskowicz M., Leboulenger F., Savino W., Dardenne M. and Lepault F. (1991). Studies on the thymus in Chagas' disease. II. Thymocyte subset fluctuations in Trypanosoma cruzi-infected mice: relationship to stress. Scand. J. Immunol. 33, 267-275.

Losavio A., Jons M.C., Sanz O.P., Mirkin G., González Cappa S.M., Muchnik S. and Sica R.E. (1989). A sequential study of the peripheral nervous system involvement in experimental Chagas' disease. Am. J. Trop. Med. Hyg. 41, 539-547.

Meirelles M.N., de Araujo-Jorge J.C., Miranda C.F., de Souza W. and Barbosa H.S. (1986). Interaction of Trypanosoma cruzi with heart muscle cells: ultrastructural and cytochemical analysis of endocytic vacuole formation and effect upon myogenesis in vitro. Eur. J. Cell. Biol. 41, 198-206.

Melo R.C. and Brener Z. (1978). Tissue tropism of different *Trypanosoma cruzi* strains. J. Parasitol. 64, 475-482.

Molina H.A., Cardoni R.L. and Rimoldi M.T. (1987). The neuromuscular pathology of experimental Chagas' disease. J. Neurol. Sci. 81, 287-300.

Molina H.A., Milei J., Rimoldi M.T., González Cappa S.M. and Storino R.A. (1989). Histopathology of the heart conducting system in experimental Chagas' disease in mice. Trans. R. Soc. Trop. Med. Hyg. 82, 241-246.

Oliveiro J.S.M. (1985). A natural human model of intrinsic heart nervous system denervation: Chagas' cardiopathy. Am. Heart. J. 110, 1092-1098.

Petry K. and Eisen H. (1989). Chagas' disease: a model for the study of autoimmune diseases. Parasitology To-day 5, 111-116.

Ribeiro R.D., Lopes R.A., García T.A. and Campos A. (1988). Histopathological study of the mammary gland in Trypanosoma cruzi-infected mice. Parasitol. Res. 74, 290-292.

Rowland E.C. and Lavy R.L. (1987). Cardiac histology of mice with experimental Chagas' disease. J. Parasitol. 73, 240-241.

Tafuri W.L. (1970). Pathogenesis of lesions of the autonomic nervous system of the mouse in experimental acute Chagas' disease. Am. J. Trop. Med. Hyg. 19, 405-417.

Tafuri W.L. (1971). Light and electron microscopic studies of the autonomic nervous system in experimental and human American trypanosomiasis. Virchows Arch. 354, 136-149.

Tay S.S.W., Wong W.C. and Ling E.A. (1984). An ultrastructural study of the non-neuronal cells in the cardiac ganglia of the monkey *Macaca fascicularis* following unilateral vagotomy. J. Anat. 138, 411-422.

Wong W.C., Ling E.A., Yick T.Y. and Tay S.S.W. (1987). Effects of bilateral vagotomy on the ultrastructure of the cardiac ganglia in the monkey *Macaca fasciularis*. J. Anat. 150, 75-88.

Zeledom R. and Ponce C. (1972). Neurotropism in Costa Rican strains of *Trypanosoma cruzi*. J. Parasitol. 58, 180-181.

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