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Light and Electron Microscope Studies of the Autonomic Nervous System in Experimental and Human American Trypanosomiasis

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Summary. Light and electron microscope studies of lesions of the autonomic nervous system (ANS) in experimental Chagas' disease showed them to display irregular and unpredictable distribution, severely injured ganglia being found side by side slightly degenerated or even morphologically normal ones. The lesions were seen to present the following characteristics: 1. parasitism of the capsular fibroblasts and of the Schwann and satellite cells; 2. vacuole formation, in the host cells, induced by the presence of living parasite, no changes in the neighbouring tissue being observed; 3. acute focal inflammation (periganglionitis and ganglionitis) resulting from parasite of host cell degeneration and affecting the neighbouring structures (neurons and nerve fibers); 4. eventual organ denervation (already evidenced by optical microscopy) directly or indirectly triggered by multiple pathogenetic mechanism, no electivity of the *Trypanosoma cruzi* for the autonomic nervous system having been observed.

In human trypanosomiasis cruzi, the ANS ultrastructural lesions (Auerbach plexus) in the megaesophagus, megacolon and jejunum showed to be essentially equal and similar to those observed in experimental infection, just differing in their intensity, being more discrete in the jejunum. All ganglia examined presented lesions in the neurons, in the Schwann cells and in the nerve fibers. In the same ganglion, degenerated neurons could be found side by side with normal healthy ones. The latter displayed some aspects that suggested the existence of a compensating mechanism. It was observed, for instance, that, in the megaesophagus, both granular and non-granular vesicles, seat of biogenous amines, were in large number than those present in the normal esophagus. This findings seems to indicate that peristaltic disturbances of the megaesophagus and megacolon are partially due to some change in the synthesis and in the liberation of biogenous amines from the nervous plexus of those organs.

Chagas' disease, identified and extensively studied since 1909, is a chapter of marked interest in the field of tropical pathology, especially in Brazil, where its high incidence and gravity place it among medico-social problems.

It is well established that, both in man and in other animals, *T. cruzi* causes lesions in the Autonomic Nervous System (ANS), such phenomenon being so common and characteristic as to lead Köberle (1956, 1959) to regard American trypanosomiasis as a disease of neuro-vegetative periphery, or, rather, as a parasympatheticoprivic disorder. Expanding his concept to the pathology of megas, Köberle postulates that, in Brazil, megas are from chagasic etiology.

Experimental American Trypanosomiasis

In the acute and chronic stages of the infection, the ANS lesions are essentially similar, just differing in their degree of parasitism and inflammatory process. Under light microscopy, the lesions can thus be described:

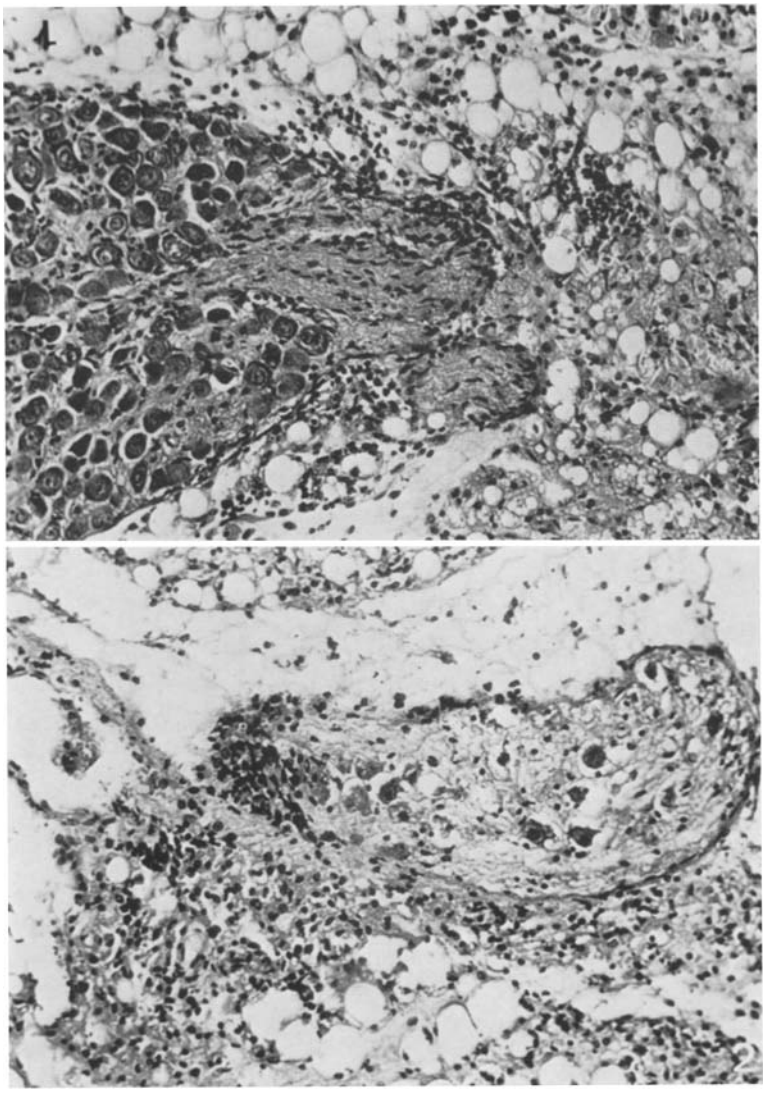

Fig. 1. a Experimental Chagas' disease. Mouse with 9-day infection. Celiac ganglion. Acute periganglionitis with granulo-histiolimphocytic exsudation. H.E. × 200. b Experimental Chagas' disease. Mouse with 15-day infection. Sympathetic lumbar ganglion. Necrosing acute periganglionitis and ganglionitis

1. Presence of apparently healthy leishmanias (abundant in the acute phase and scarce in the chronic one) in the cytoplasma of the Schwann and satellite cells, of the ganglion capsular fibroblast, of the sheath envolving intra- and extra-ganglionic nerve fibers, as well as in the histiocytes adjacent to the ganglia, with no surrounding phlogistic process.

2. Focal or diffuse periganglionitis and ganglionitis.

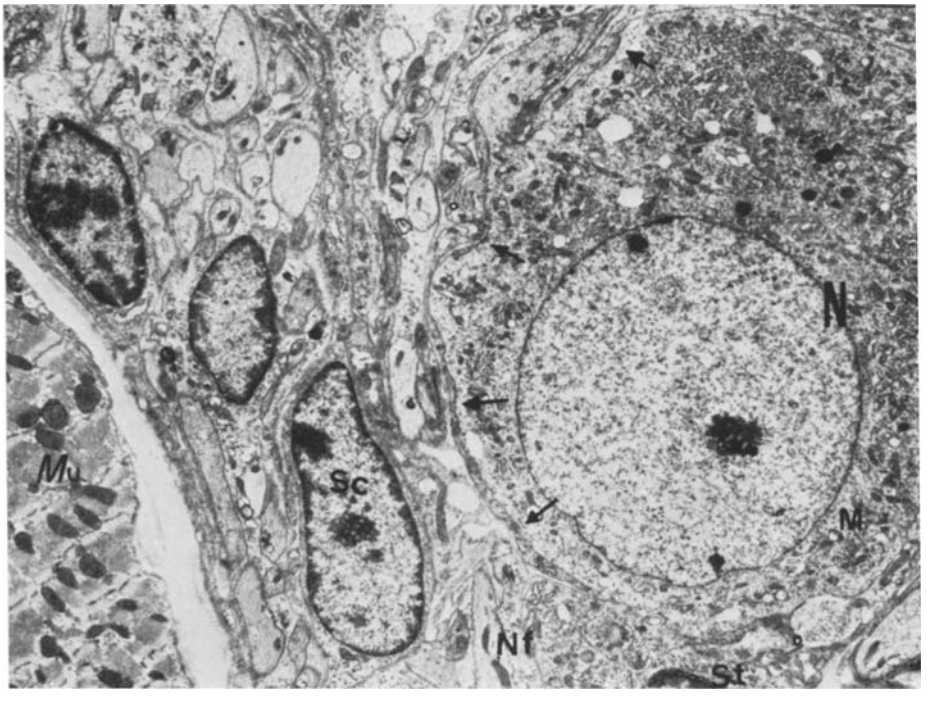

Fig. 2. Experimental Chagas' disease. Mouse with 9-day infection. Subepicardiac ganglion. Slightly injured neuron (*N*) swelling of some mitochondria (*M*) and scarcity of ribosomes in the peripher.—Arrows. Degenerated unmyelinic nerve fibers (*Nf*) by the side of normal ones. Schwann cells (*Sc*) and satellite cell (*St*). Muscular fibers (*Mu*). × 5600

3. Intense regressive phenomena involving the neurons (chromatolysis, acute swelling and karyolysis) and eventually leading to necrosis. Disorderly and unpredictable distribution of lesions, apparently normal ganglia being often found side by side with slightly injured or utterly destroyed ones, the former being always related to the inflammatory phenomenon—focal ganglionitis, periganglionitis, cellulitis and myositis (Fig. 1).

Electron microscope data were seen to support the findings by light microscopy. In the injured ganglia, the Schwann cells, the neurons, the nerve fibers and the periganglionic connective tissue undergo focal or diffuse alterations which are always related to the leishmanias degenerated inside or outside the cells or to the phlogistic process (Figs. 2 and 3). In cases of focal ganglionitis, injured neurons are found by the side of normal ones. It is quite frequent the presence of leishmanias in the Schwann cells (Fig. 4) in the capsular fibroblasts, in the interstitium of the ganglion itself (Fig. 3), and in the periganglionic connective tissue.

The healthy intracellular leishmanias induce partial lysis of the cytoplasmic organules, which, around the parasite, take the form of vacuoles with no density detectable by electron microscopy (Figs. 4 and 5). At this stage the parasitized cells do not present any morphological signs of death, nor inflammatory reaction around them. However, when the parasite and/or parasitized cells degenerated

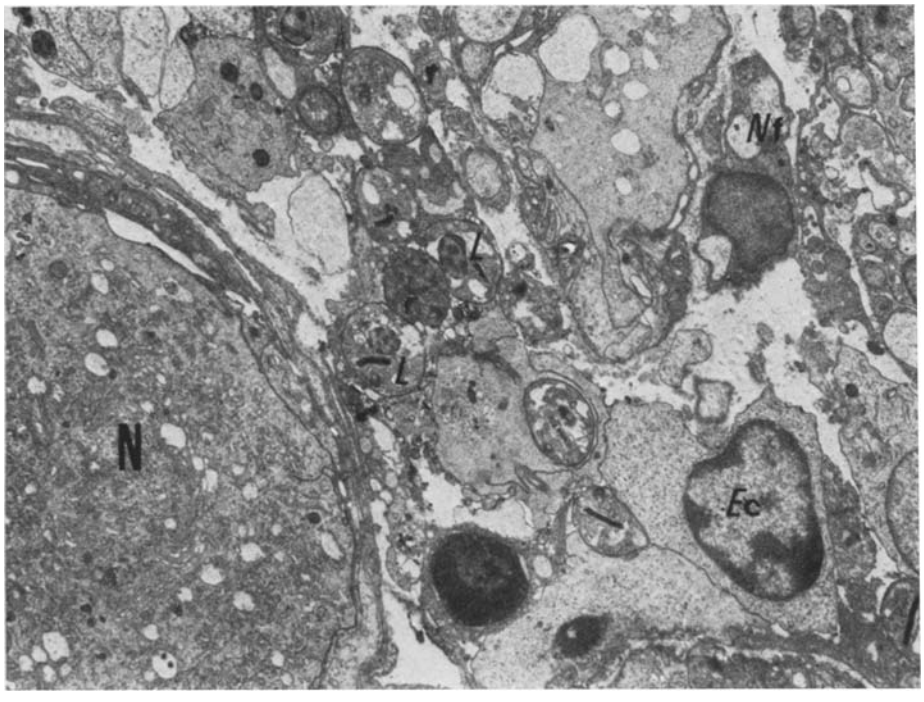

Fig. 3. Experimental Chagas' disease. Mouse with 12-day infection. Subepicardiac ganglion. Acute ganglionitis. Degenerating interstitial and intracellular leishmanias (*L*). Exudation cells (*Ec*), interstitial edema presenting dissociation of partially injured unmyelinic nerve fibers (*Nf*). Neuron (*N*) with numerous swelling and vacuolated mitochondria. × 4700

(Fig. 6), there develops, in their vicinity, a focal phlogistic process characterized by an exsudate, predominatly granulocyto-histiocytic. The nerve and the satellite cells, as well as the nerve fibers close to the degenerated leishmanias or to the inflammatory focus also undergo severe degenerative process. It seems obvious, therefore, that the inflammatory phenomenon be elicited by the phlogenic products liberated by the cells and/or by the degenerated leishmanias. The inflammatory process that is then brought about an aggravating influence on the ganglia regressive lesions and on the nerve fibers. As cellular parasitism is processed by chance, in random foci, we easily understand the irregularity observed in the distribution of the lesions in the ANS ganglia, as in any other system.

Although the morphological changes undergone by the ANS in Chagas' disease are well known, there is great controversy as regards the pathogenetic mechanisms responsible for the denervation of the hollow organs. According to Köberle (1960) destruction of the neurons is caused, during the acute stage of the disease, by the liberation of a neurotoxin or enzymes from the degenerated leishmanias in the inflammatory focus. Lisboa (1960) believes the neuronal destruction to be triggered by a direct mechanism (ganglionitis) and not by an indirect remote process as reported by Köberle. Lopes (1965) thinks the heart neuronal destruction to be partly due to the inflammatory periganglionitis of the subepicardiac

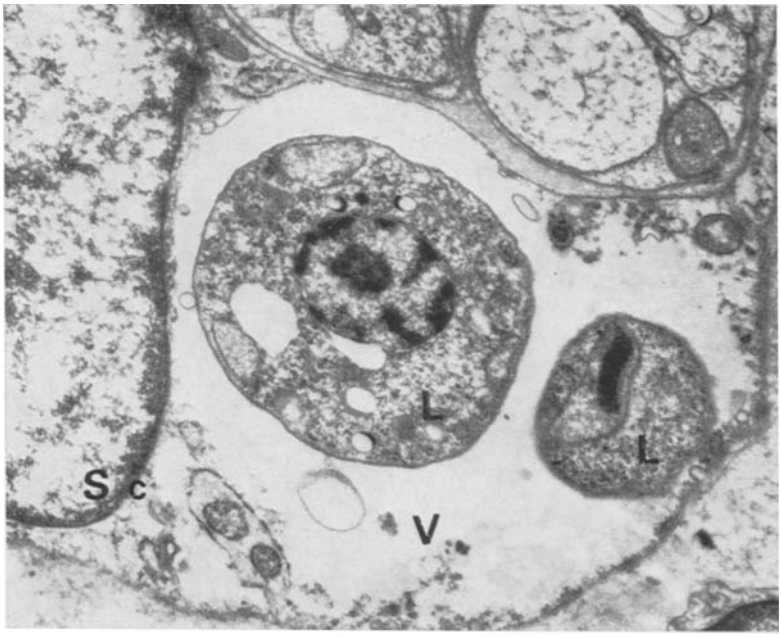

Fig. 4. Experimental Chagas' disease. Mouse with 9-day infection. Cardiac plexus. Schwann cell (*Sc*) with two normal *T. cruzi* leishmanias (*L*) in the cytoplasm. See halo (*V*) presenting no electronic density around the parasites. × 22000

adipose tissue. Likewise, Andrade and Andrade (1966) also believe the inflammatory to have a preponderant role in such destruction. As to Okumura (1966), denervation results from direct action of the parasite in the neuron.

All pathogenetic interpretations aforementioned were based on studies performed under Light microscopy. Our optical and electron microscope studies (Tafuri *et al.*, 1962, 1966, 1967, 1969, 1970) have demonstrated the pathogenesis of ANS lesions to related to several contributing factors:

1. Acute-Ganglionitis and Periganglionitis which, although with varying intensity, are always present and lead to the degeneration of a certain number of neurons.

2. Parasitism of the Schwann and Satellite Cells. These cells, also liable to ganglionitis and periganglionitis, suffer, as the neurons do, the consequences of such process. In some of the parasitized cells, part of the cytoplasma has been observed to be normal, despite the presence of the parasite; in others, both the parasite and the cellular body show deep alterations, or even signs of death. Although it cannot be stated, in each individual case, whether the parasite's death precedes that of the cell or vice-versa (both possibilities being viable), it seems obvious that parasitism alone, independently of inflammation, may induce changes in the Schwann and satellite cells. These cells, therefore are affected in two ways: by the phlogistic process and by the parasitism.

3. Alterations of the Ganglionic Cells. Although these cells are seldom parasitized (their parasitism being less frequent than that of the Schwann and satellite cells),

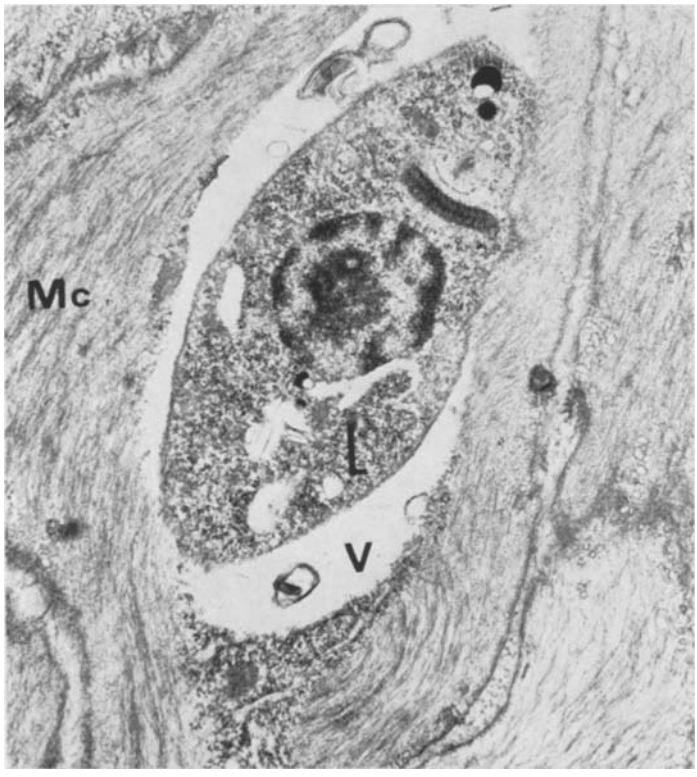

Fig. 5. Experimental Chagas' disease. Mouse with 9-day infection. Healthy *T. cruzi* leishmanias (*L*) in the cytoplasm of smooth muscular cell. Halo (*V*) around the parasite. The remnant of the cytoplasm of the muscle cell (*Mc*) displaying normal aspect. × 28000

their neurons may also undergo changes, either directly induced by the inflammation or indirectly elicited through the lesions of the Schwann and satellite cells and through axonal degenerations.

4. Lesions of the Nerve Fibers, also subject to degeneration owing to multifold causes such as: a) ganglionitis and periganglionitis; b) inflammation of the myocardium and of the subepicardiac adipose tissue (see item 6), reaching the neural network at any spot (in the intestine, the myositis); c) parasitism of the ganglionic cells; d) changes in the ganglionic cells induced by lesions of the Schwann and satellite cells; e) lesions of the Schwann and satellite cells, not elicited by those mechanisms usually causing them, thus being licit to admit their influence to be felt not only in the intraganglionic nerve fibers but also in the extraganglionic neural network. Electron microscopy has indeed shown, practically all over the heart, a large number of unmyelinic and myelinic nerve fibers to be affected, even when far away from the inflammatory foci.

5. Lesions of the Muscle Cells of the Heart and of the Intestine. Under light microscopy, chagasic myocarditis and myositis (esophagitis, enterocolitis, etc) were seen to present as acute, subacute or chronic inflammation induced by the

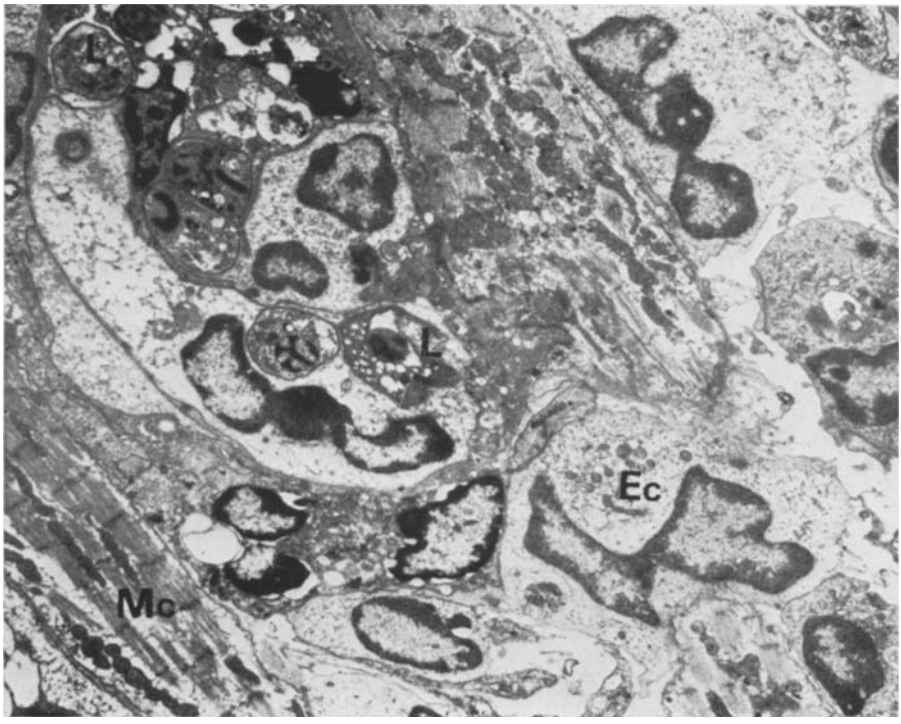

Fig. 6. Experimental Chagas' disease. Mouse with 15-day infection. Myocardium. Acute myocarditis. Degenerated intra- and extracellular *T. cruzi* leishmanias (*L*). Exudation cells (*Ec*); edema of the interstitium and of the sarcoplasmatic reticulum of the heart muscular cells (*Mc*). × 5600

parasitism of the muscular and intersticial cells. Besides undergoing changes, the paratized muscular cells may rupture, this depending on the number of parasites they hold. In the acute stage, inflammation is generally intense, the exsudate being predominantly granulocyto-histiocytic; in the subacute and chronic phases, the exsudate is seen to be mostly histio-lymphoplasmocytic, there appearing productive phenomena (neo-formation of fibroblasts, collagen and reticulin) leading to fibrosis, its intensity varying in accordance with the severity of the phlogosis.

Electron microscopy has demonstrated that, in the acute stage of trypanosomiasis, the lesions are always circumscribed by groups of muscular cells. Among the degenerated cells, one can see others, more numerous and quite healthy. Two groups of enterirely different lesions were observed: one directly related to the cellular parasitism and another displaying more severe injury and affecting a larger number of cells, seldom parasitized, which was seen to be both directly and indirectly related to the phlogistic process (Figs. 5–7).

The parasite, when in the cytoplasm of muscular and interstitial cells, induces the formation of vacuoles presenting the characteristics already described with regard to the Schwann and satellite cells and to the fibroblasts (Fig. 5). Around the degenerated parasites, on the contrary, no vacuoles could be seen, the host

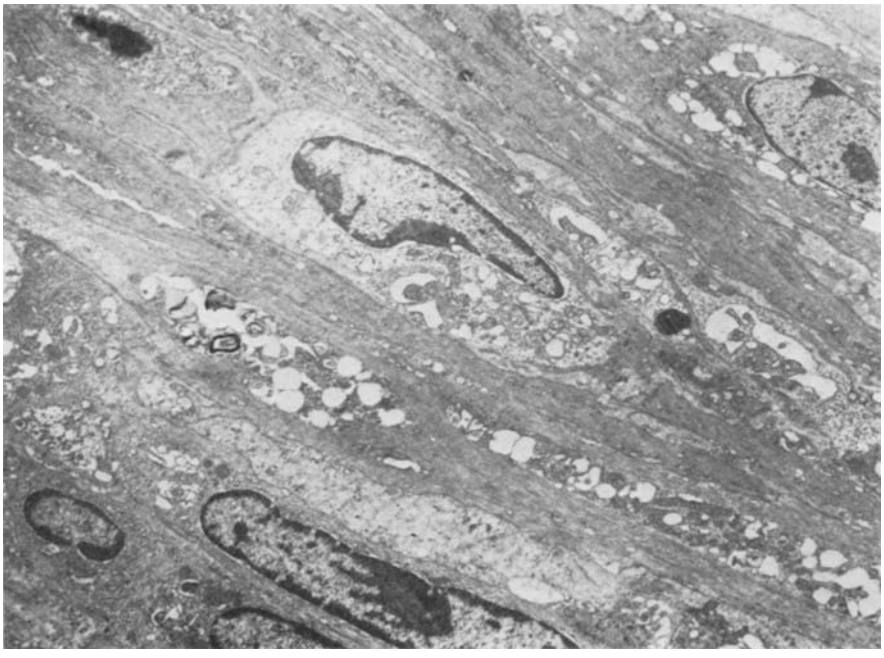

Fig. 7. Experimental Chagas' disease. Mouse with 12-day infection. Colon. Acute myositis. Intense regressive phenomena of the smooth muscular cells and of the interstitium components ×7200

cells showing severe regressive process and, sometimes, obvious signs of necrosis. Around the leishmanias and the degenerated cells, inflammation, sometimes extensive, could be detected, the presence of the exsudate cleary disturbing the relations-connective-vessel-muscle cells. It can then be easily understood how muscle cells non-parasitized but related with the phlogistic process may be deeply affected (Fig. 7).

Despite the random distribution of parasitism in the heart, in the esophagus-gastrointestinal tract, as well as in any others organ, there are many parasitized muscular and intersticial cells in the vicinity of the intramural nervous plexuses. The phlogosis elicited in these regions, reaches the ganglia, often destroying them. Ganglionic lesions, therefore, do not depend on a special electivity of the trypanosoma for such process; it is merely a consequence of the swelling of the intermuscular interstitial connective tissue.

The data obtained by light and electron microscopy-suggest the pathogenetic mechanisms responsible for the ANS lesions in the myocardium and in the intestine to be multiple various factors concurring for the same results: the severe denervation of such organs.

Megaesophagus, Megacolon and Jejunum in human Trypanosomiasis cruzi

The finding of just two cases of megaesophagus in 60,000 necropsies carried out at the Pathology Institute of the University of Viena (Köberle, 1963) demon-

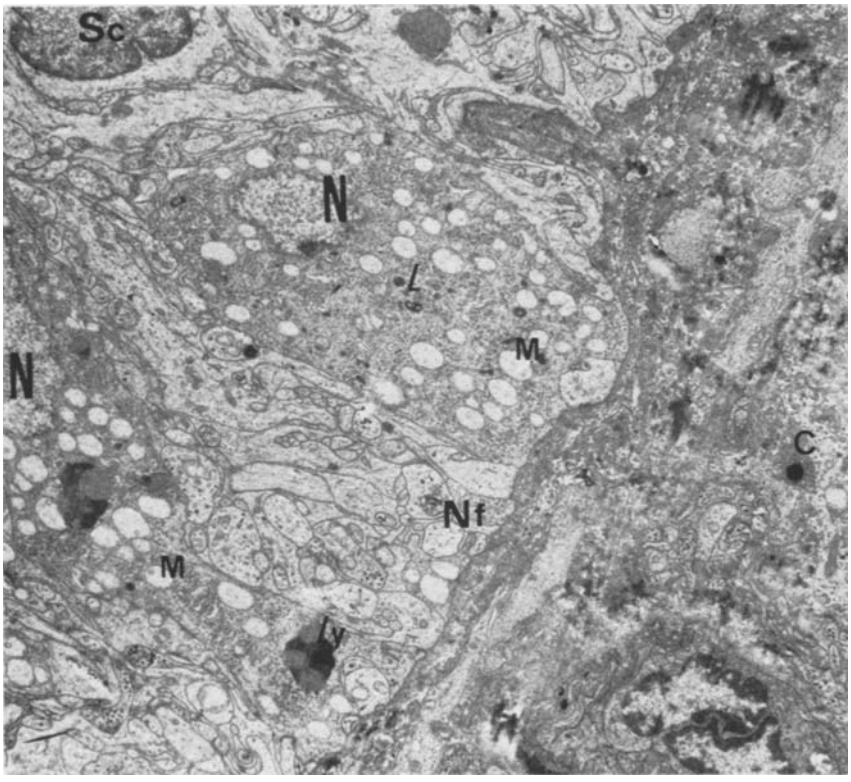

Fig. 8. Human megaesophagus in chronic chagasic infection. Auerbach ganglion. Neurons (*N*) showing swelling and vacuolization of mitochondria (*M*), rarefaction of ribosomes, presence of liposomes (*L*), and lysosomes (*Ly*). Unmyelinic nerve fibers (*Nf*) with focal lysis of the neurofilaments, swelling of mitochondria and presence of amorphous osmiophilic granules inside it. Schwann cell (*Sc*). Capsule (*C*) displaying marked fibrosis. × 5600

strated that, in Europe, megas are scarce. In Brazil, on the contrary, the megaesophagus is a commom occurrence, especially in places exhibiting a high incidence of Chagas' disease. Chapadeiro *et al.* (1964) reported megaesophagus and megacolon in 35% of the necropsies performed. The frequent concomitance of Chagas' disease and megas led the Brazilian Authors to admit the existence of cause-effect relationship. Barring rare cases of congenital disease, the megaesophagus and the megacolon, in Brazil, are of chagasic etiology, and the lesions of the myenteric plexus constitute the substratum of the alterations in motility, or, better, of the intrisic and extrinsic peristaltic reflexes of the hollow organs, especially of the esophagus and of the colon.

Macroscopically, the chagasic megaesophagus and megacolon present the following characteristic: 1) varying degree of dilatation, with or without organ lenghtening; 2) wall thickening, especially in correspondence with that of the muscules; 3) absence of mechanical obstruction; 4) apparently normal segment immediately beyond the dilated part.

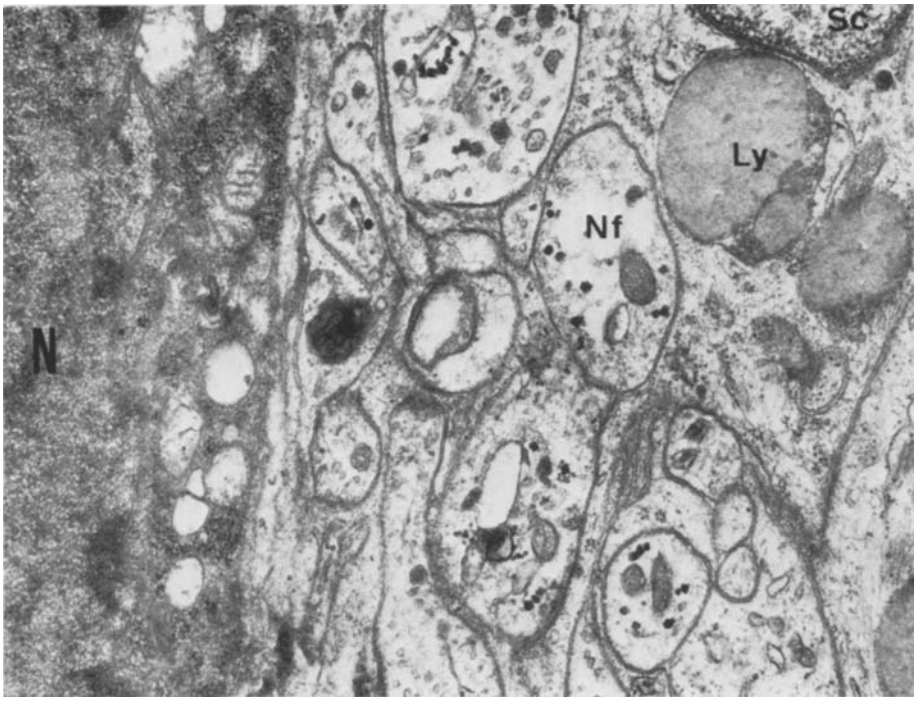

Fig. 9. Human megaesophagus in chronic chagasic infection. Auerbach ganglion. Neuron (*N*) showing nearly complete fading of the cytoplasmatic structures and of the nuclear membrane by the side of unmyelinic nerve fibers (*Nf*) with lysis of the neurofilaments and neurotubules, as well as presence of amorphous osmiophilous particules, inside it. Schwann cells (*Sc*) with voluminous lysosomes (*Ly*) in the cytoplasma. × 23000

Under optical microscopy, the following may be observed: chronic inflammatory infiltrate, predominantly lymphocytic, in regularly distributed foci in the muscular mucosa, in the submucosa and, mainly, in the muscular propria; 2) injury of the intramural nervous system, especially of the Auerbach plexus (focal or diffuse periganglionitis and ganglionitis, with intense regressive phenomena involving the neurons, leading to ganglion destruction and inducing consequent fibrosis of the same; 3) in the most severe cases, regressive lesions of the mucosa with ex-ulcerations and consecutive secondary inflammation which may be focal, or, more frequently, diffuse, and then extending to the submucosa; 4) intermuscular interstitial fibrosis, resulting from myositis, periganglionitis and ganglionitis; 5) quite exceptional presence of parasites at this stage of the disease.

As far as we known, no studies on the ultra-structural changes in the autonomous nervous system in Chagas' disease, can be available in the pertaining litterature. At present, our Departament has been studying, under electron microscopy the magaesophagus, the megacolon and the jejunum of chagasic patients (Tafuri *et al.*, 1970, 1971). In these segments, the ultra-structural alterations of the myenteric plexus are similar, except for their intensity. In all ganglia, of the

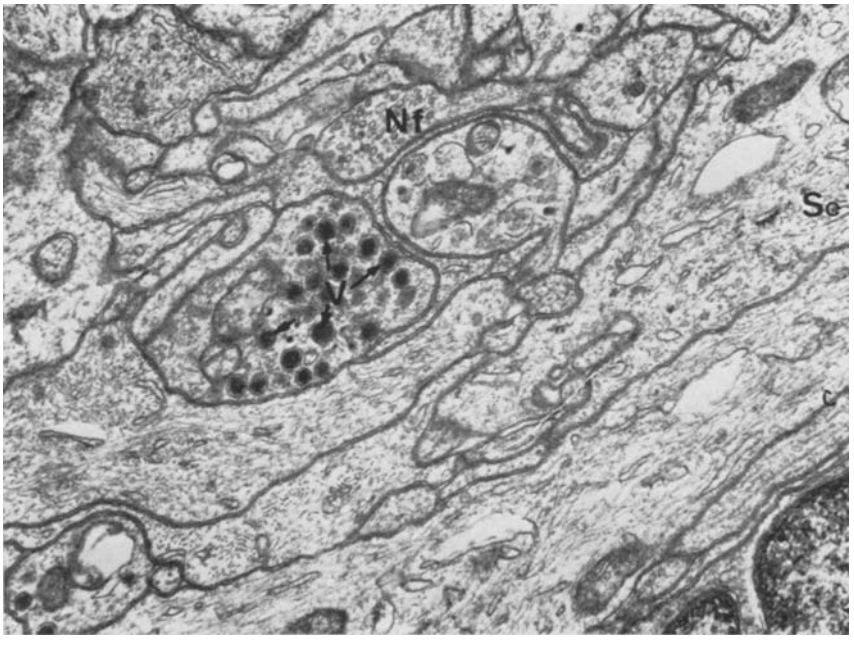

Fig. 10. Human jejunum in chronic chagasic infection. Extraganglionic unmyelinic nerve fibers (*Nf*) displaying normal aspect, some of them holding granular and nongranular vesicles (*V*). Schwann cells (*Sc*). × 23000

myenteric plexus examined there were observed focal lesions of the neurons, of the Schwann cells, of the nerve fibers and of the capsular components, by the side of healthy normal neurons (Figs. 8–11).

It is not rare the presence of a large number of wholy fibrosed ganglia (Fig. 11). As regards this particular point, the findings by optical microscopy are seen to be in agreement with those by electron microscopy.

In the megaesophagus and megacolon the lesions in the Auerbach plexus are seen to be more intense and extensive than in the jejunum. Their distribution is irregular and unpredictable, thus emphasizing the facts of the trypanosome being able to parasitize, at random, any economy organ as well as any of its parts.

In the chagasic megaesophagus and megacolon stasis and discinesia are always observed. Stasis appears just when alterations in the sphincter opening mechanism arise; discinesia, by itself, does not induce stasis, according to Habr Gama (1966). Supposing stasis to be one of the important factors of the mechanism of "megas" formation, we could easily understand the reason why plexular lesions are more severe in the intestinal tract, where stasis occurs, for the following reasons: 1) the alimentary and fecal contents, on accumulating in the lumem, compresses its mucosa and thus induces organs dilatation; 2) the compressed mucosa undergoes alterations (ischemia, and consecutive regressive lesions); 3) the degenerated mucosa is liable to get inflamed; 4) inflammation (which is frequent) may reach the submucosa and somewhat aggravate the already developed lesions of the

Fig. 11. Megaesophagus in chronic chagasic infection. Intense fibrosis among the bundles of degenerated nerve fibers (*Nf*) enveloped by collagen (*c*) in the region of the Auerbach plexus. Fibroblast (*Fi*) and Mastocyte (*Ma*). × 5600

Meissner plexus as demonstrated by the presence of *T. cruzi*; 5) The Auerbach plexus, in its turn, undergoes the consequences of the Meissner plexus lesions because of the synaptic relations between the two; 6) the phlogistic process, secondary to the stasis and to the inflammation induced by the destruction of the plexuses and of the interstitial components themselves, leaves sequels (interstitial fibrosis) which constitute one of the factors responsible for the modification of the components of the intermuscular interstitium; 7) the muscle cells, in their turn, undergo the consequences of greater contracting effort due to greater environmental resistance (fibrosis) which eventually brings about hypertrophy and/or regressive alterations; simultaneously can also be observed differences in the metabolic changes consequent to the inflammation, which modifies the relation between the interstitium components, vascular lesions, changes in the ground substance, circulating toxic products; 8) as the Auerbach plexus is in intimate relation with the muscle cells, it is easy to understand how myositis and its sequels, when in the vicinity of the ganglia, may more severely injure them; 9) et last, it is reasonable to admit that the plexus lesions would be aggravated in accordances with the duration and intensity of the mega.

In a comparative study of the neurons of the Auerbach plexus that remained healthy, in cases of megaesophagus, and of those of the control esophagus with the injured ones, it seemed to us that the nondegenerated neurons in the megaesophagus, and in the megacolon undergo a compensation process. The following are the findings supporting such hypothesis: a) probable increase in the volume of the nucleoli; b) volumetric and numerical increase of ribosomes, thus resulting an electronically very dense pericarium; c) hypertrophy of the Golgi complex and presence of several vesicles with dense granules in the vicinity; d) the fact of both granulated and non-granulated vesicles seat of biogenous amines, undergoing changes in their number of vesicles per 100 μ^2 of axonal area (930 in the megaesophagus and 585 in the control esophagus) and in their diameters (mean diameter: 950 Å in the megaesophagus and 850 Å in the control esophagus). Based on these findings, there could be raised a hypothesis holding that the disturbances of peristalsis of the megaesophagus and of the megacolon must be partly due to the hypersecretory activity of the neuron, especially then resulting from a disturbance of the synthesis and, perhaps, in the liberation of biogenous amines.

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