PERIPHERAL NERVOUS SYSTEM INVOLVEMENT IN HUMAN AND EXPERIMENTAL CHRONIC AMERICAN TRYPANOSOMIASIS

By R. E. P. SICA, S. M. GONZALEZ CAPPA, O. P. SANZ & G. MIRKIN

Abstract: An electrophysiological and histological study of the muscle and the peripheral nervous system (PNS) was carried out in chronic human American trypanosomiasis (Chagas' disease) and in an experimental Chagas' disease (Chd) mouse model. Altogether 995 patients with chronic Chd and 261 mice, experimentally infected with RA and CA-I parasite strains, were investigated. Results were compared with matched controls. Techniques employed in humans were: clinical assessment, conventional electromyography (EMG), estimated number of motor units, motor and sensory nerve conduction velocities, repetitive nerve stimulation and muscle and sural nerve biopsies. In mice conventional EMG, sciatic nerve conduction time, sciatic nerve action potential amplitude, in vitro miniature end-plate potentials (MEPPs) and end-plate potentials (EPPs) recordings, muscle, nerve and spinal cord histology and identification of cell phenotypes within the inflammatory infiltrates were the employed procedures. Out of 511 patients submitted to clinical examination, 52 disclosed signs and symptoms of mixed peripheral neuropathy. By employing electrophysiological techniques, it could be shown that about 30% of the investigated patients had one or more of the following features: diminished interference pattern, most of the remainder motor units potentials being (MUPs) polyphasic; reduced number of functional motor units in the thenar, hypothenar, soleus and/or edb muscles; slow sensory and motor nerve conduction velocities; low sensory action potential amplitude and impairment of neuromuscular transmission.
In mice, MUPs duration and amplitude were increased at later stages of the infection, nerve conduction was slow, nerve action potentials were of low amplitude, mepps were of low amplitude and double eps were frequently found. Muscle histology in humans with chronic Chd showed type I and type II grouping, atrophic angular fibers and targetoid muscle fibers. In mice perivascular mononuclear cells infiltrates, small round fibers, muscle fibers necrosis, atrophic angular fibers, type II muscle fibers grouping and grouped muscle fibers atrophy were found. Sural nerve samples showed segmental and paranodal demyelination and axonal loss. The same features were observed in mice nerves, also in this model mononuclear cells infiltrates at the nerve, dorsal root ganglia and meninges surrounding the spinal cord were observed. Muscle and nervous tissues infiltrates were mainly composed of T lymphocytes with predominance of CD8 or CD4 subsets according to the parasites strain employed for infecting the animals. These findings suggest that the skeletal muscle and the PNS may be involved in chronic american trypanosomiasis.

INTRODUCTION

American trypanosomiasis, or Chagas' disease (Chd), is a parasitic illness caused by a flagellate protozoan, *Trypanosoma cruzi*. The parasite is transmitted to humans by triatomine bugs of the order of Hemiptera, family of Reduviidae, at the time when they pierce the skin to suck blood. It is not inoculated directly by the insect, but it is passively deposited in the bug's faeces on the skin and penetrates into the receiver through the bite wound. Other routes for infection are blood transfusion, the placenta or contaminated organ transplants.

The parasite, *T. cruzi*, belongs to the Mastigophora subphylum of the phylum Sarcomastigophora, order Kinetoplastida, which comprises flagellar organisms with a kinetoplast.

Certain strains display particular tropism for macrophages, others for liver or muscle or the nervous system (27).

Once the parasite has entered a human, three stages of the disease can be recognized, the acute, the indeterminate and the chronic stages.

The acute stage is characterized by general malaise of variable intensity and different clinical manifestations. The symptoms may be mild enough and not recognized at this stage. However, in other circumstances, a local overt inflammation appears at the site of the bite which is associated with local lymphadenitis. General symptoms include fever, generalized edema, vomiting, diarrhoea, enlargement of the liver and spleen and swollen lymph-nodes. Occasionally, myositis and myocarditis could be seen. Less often, and as the most serious complication of this stage, meningoencephalitis may develop, mainly in children under 2 years of age, the mortality in this case can reach 50%. Parasitaemia is always present during this period. After some weeks all the symptoms subside, and the patient approaches the indeterminate stage.

This second stage begins about 3 months after the acute episode, whether there have been clinical manifestations or not, and may last several years or indefinitely. The patient, during this period, is free of clinical symptoms. The only abnormalities are the positiveness of serum tests for trypanosomiasis and the presence of few parasites within the blood.

The third or chronic stage develops 10 to 20 years after the triatomas bite and it is estimated that about 30% of the patients will suffer from digestive, cardiac or neurological involvement.

The neurological complications are, currently, the least studied, and may involve the central, peripheral or autonomic nervous systems.

Update discussions about the pathogenesis of the complications which develop in the chronic stage of the infection signal that, most probably, immunological disturbances are the main mechanisms underlying the development of the complications (4, 19, 21, 26).

The geographical distribution of the disease extends from the south of USA (41° N latitude) to the south of Argentina (46° S latitude) (23). Among an estimated total population in the endemic area of about 360 millions inhabitants, 90 millions people (25%) are considered at risk of infection and 17 millions people are known to be infected (23). As mentioned earlier, it is accepted that about 30% of persons infected who reach the chronic stage will develop overt clinical manifestations of cardiac, digestive or neurological damage (23). So, it can be estimated that 5.1 millions people will have clinical disorders attributable to the trypanosomiasis.

Since the earliest reports of the disease, it has been claimed that the peripheral nervous system (PNS) may be involved in human chronic Chd (2). However, definitive proofs of this damage have been presented more recently (3, 7-9, 14, 15, 19, 21, 25, 26, 28-33, 35-37).

During the last years, we pursued a combined clinical, electrophysiological and anatomical study of these patients looking for PNS involvement and, simultaneously, developed and experimental mouse model for the same purposes (7-9, 14, 15, 19, 25, 28-31, 33, 35).

MATERIAL AND METHODS

Human studies

The study involved altogether 995 patients with the diagnosis of chronic Chagas' disease. Coincidental causes of neurological disorders were eliminated from the study by rejecting patients over the age of 60 years (34) and those who had had toxic or metabolic disorders or genetic diseases or other parasitic illnesses known to induce nervous system damage. Special care was taken in selecting patients who did not have any intestinal abnormality.

Two hundred and ninety-three healthy subjects served as controls for one or more investigations.
Clinical assessment

Five hundred and eleven patients were admitted within the protocol. A complete neurological examination was carried out in all of them, emphasizing on the PNS functions.

Electrophysiological techniques

Conventional electromyography (EMG) was performed with concentric needle electrodes in muscles of the upper and lower limbs.

The number of functional motor units (MUs), and their sizes, were estimated in the thenar, hypothenar, soleus and extensor digitorum brevis (edb) muscles by using techniques described previously (16, 32, 34).

Motor nerve conduction studies were conducted in the ulnar, median and deep peroneal nerves by employing surface stimulating and recording electrodes. Within the ulnar nerve, whose results will be presented in this paper, maximal conduction velocity was measured at the arm and at the forearm. Proximal conduction velocity was estimated by the « F » wave latency, applying conventional formula (5, 13). Slow nerve fibers conduction velocity was calculated using the HOPF method (11).

Sensory nerve fibers were investigated in the median and sural nerves by employing both surface and needle recording electrodes. Median nerve results will be presented in this paper.

Neuromuscular transmission studies were performed by supramaximal repetitive stimulation of the ulnar nerve at 3 and 10 Hz during 2 and 1 seconds respectively. Recordings were done with surface electrodes at the end plate area of the hypothenar muscles. Other method employed was the quantification of the end-plate noise by employing an insulated tungsten electrode inserted within the edb muscle end-plate area (24).

Histological techniques

In 8 subjects, who gave their consent, medial gastrocnemius muscle specimens were obtained by open biopsy and stained with hematoxylin-eosin, periodic acid Schiff, modified Gomori trichrome, NADH, ATPase at pH 9.4 and 4.6, alkaline phosphatase and non-specific esterase.

In other group of 6 patients, who also gave their consent, sural nerve specimens were obtained by open biopsy. The samples were fixed in 3% glutaraldehyde in cacodylate buffer, embedded in paraffin and epony and stained with hematoxylin-eosin, PAS, luxol fast blue, Ziehl-Nielsen and toluidine blue methods. Teasing of single myelinated fibers was stained with 2% osmium tetroxide. Morphometric studies were performed in prints enlarged 1,000 times.

All patients were males, with ages ranging between 20 and 37 years and all of them had one or more evidences of clinical or electrophysiological denervation.

Mice studies

Two hundred and sixty-one C3H mice were infected with RA and CA-I tryptomastogotes strains and they were studied at 15 days, 1, 3, 4, 6, 9 and 12 months post-infection (pi). Techniques employed were hamstring muscles conventional EMG; in vivo sciatic nerve conduction time and action potential amplitude measurements; in vitro end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) recordings in the phrenic-diaphragm preparation; muscle, nerve, dorsal root ganglia and spinal cord histology and identification of cell phenotypes within the inflammatory infiltrates.

RESULTS

Human results

Clinical assessment

Fifty-two (10.2%) out of 511 patients submitted to clinical examination showed the following features: a) sensory impairment, in the form of paresthesias and distal limb hypoesthesia (33 patients); b) diminished tendon jerks (42 patients), either in isolation (19 patients) or combined with sensory impairment (25 patients). Most frequently abnormalities were limited to the lower limbs (57% of the symptomatic patients) or could involved the four limbs (33%); seldom, they were found only in the upper limbs (8%).

Electrophysiological findings

EMG diminished interference pattern in one or more muscles, which signals a reduced number of MUs recruited voluntarily, was found in 54% of a sample of 80 patients, mainly in distal lower limbs muscles. Most of the remainder MUs were fragmented or polyphasic (Table I).

The number of functional MUs were reduced (p < 0.001) in 51% of 187 studied patients in the thenar, 18.4% in hypothenar, 35.5% in the soleus and 20.4% in the edb muscles (Table II). In table II, patients have been divided into 2 groups, group I shows reduced number of functional MUs, while group II has normal number of MUs. It can be seen that group I significantly differs from controls and group II, while these last two groups do not differ between them. Subjects with diminished number of functional MUs showed increased size of the remainder units (Table III).

Ulnar motor nerve conduction velocity was slow (p < 0.001) in 33% of a sample of 55 patients with involvement of large and small fibers. Slowness was more conspicuous at distal segments.
Tab. I. — Human electromyographic findings.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Percentage of patients (n 80) with reduced interference pattern (pri)</th>
<th>Percentage of polyphasic MUPs in pri</th>
<th>Percentage of MUPs with increased duration &amp; enlarged amplitude in pri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deltoid</td>
<td>8.8</td>
<td>61.2</td>
<td>17.5</td>
</tr>
<tr>
<td>Biceps</td>
<td>17.9</td>
<td>62.7</td>
<td>23.9</td>
</tr>
<tr>
<td>a.p.b.</td>
<td>8.5</td>
<td>65.8</td>
<td>24.4</td>
</tr>
<tr>
<td>vast. med.</td>
<td>34.3</td>
<td>85.7</td>
<td>54.3</td>
</tr>
<tr>
<td>tib. anter.</td>
<td>32.4</td>
<td>88.7</td>
<td>35.2</td>
</tr>
<tr>
<td>e.d.b.</td>
<td>54.2</td>
<td>93.5</td>
<td>34.7</td>
</tr>
</tbody>
</table>

a.p.b.: abductor pollicis brevis; vast. med.: vastus medialis; tib. anter.: tibialis anterior; e.d.b.: extensor digitorum brevis

Tab. II. — Estimated mean number of functional motor units.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Controls (n 263)</th>
<th>Patients I (n 187)</th>
<th>Patients II (n 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thenar</td>
<td>318 ± 70</td>
<td>168 ± 29*</td>
<td>307 ± 66</td>
</tr>
<tr>
<td>Hypothenar</td>
<td>379 ± 79</td>
<td>208 ± 33*</td>
<td>364 ± 89</td>
</tr>
<tr>
<td>Soleus</td>
<td>841 ± 183</td>
<td>420 ± 85*</td>
<td>846 ± 226</td>
</tr>
<tr>
<td>e.d.b.</td>
<td>215 ± 69</td>
<td>85 ± 23*</td>
<td>245 ± 83</td>
</tr>
</tbody>
</table>

Patients I: infected subjects with number of motor units below the lowest limit of the control range. Patients II: infected subjects above the lowest limit of the control range. ( ): number of subjects; *: p < 0.001. Controls and Patients II do not differ significantly between them.

Median sensory nerve conduction velocity was reduced (p < 0.001) in 47.8% of a sample of 23 patients and sensory action potential amplitudes were low (p < 0.001) in 72% of those subjects showing slow conduction velocity. Also here, slowness of conduction was more evident at distal segments of the nerve.

Repetitive nerve stimulation showed decremental response (> 25% of the first potential amplitude) in 10 of 58 patients submitted to this investigation. End-plate noise frequency was increased in 8 tested patients showing denervation by other methods.

Histological findings

Muscle fibers type (I and II) grouping and isolated angular fibers were the main findings in the medial gastrocnemius muscle. Occasionally, some targetoid fibers were also seen.

On sural nerve samples, diminished number of myelinated fibers, mainly those of larger and smaller diameters, was found on cross sections. Also axonal clusters and signs of remyelination were noted. On teased fiber preparations, paranodal and segmental demyelination was observed.

Mice results

Electrophysiological findings

On EMG recordings, spontaneous activity, in the form of fibrillations and positive sharp waves, was seen after 15 days pi. A diminished voluntary interference pattern was detected within the first month pi as well as an increment of the percentage of polyphasic MU potentials amongst the remainder MUs (Table IV). MUs potentials of enhanced amplitude and duration could be found mainly at the latter stages of the infection, nevertheless others showed diminished amplitude and duration when compared with controls.

Regarding the end-plate recordings, mean MEPPs amplitude for the infected mice was lower (p < 0.001) than the controls as from day 37 pi until

Tab. III. — Estimated mean size of functional motor units.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Controls (n 263)</th>
<th>Patients I (n 187)</th>
<th>Patients II (n 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thenar</td>
<td>0.33 ± 0.24</td>
<td>0.77 ± 2.48*</td>
<td>0.32 ± 0.18</td>
</tr>
<tr>
<td>Hypothenar</td>
<td>0.28 ± 0.20</td>
<td>0.47 ± 0.24*</td>
<td>0.29 ± 0.15</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.14 ± 0.20</td>
<td>0.26 ± 0.18*</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>e.d.b.</td>
<td>0.49 ± 0.35</td>
<td>1.2 ± 0.92*</td>
<td>0.46 ± 0.30</td>
</tr>
</tbody>
</table>

The mean motor unit sizes have been expressed as % of the amplitude of the maximal M wave.

Patients I: infected subjects with number of motor units below the lowest limit of the control range; Patients II: infected subjects above the lowest limit of the control range; ( ): number of subjects; *: p < 0.001. Controls and Patients II do not differ significantly between them.
Tab. IV. — Electromyographic characteristics of mice chronically infected with Trypanosoma cruzi.

<table>
<thead>
<tr>
<th>Months</th>
<th>Groups (n mice)</th>
<th>% Spontaneous muscle activity</th>
<th>% Reduc. interfer. pattern</th>
<th>% MUP polyph (n MUPS)</th>
<th>Amplitude $\bar{x} \pm SD$ (mV) (n MUPS)</th>
<th>Duration $\bar{x} \pm SD$ (ms) (n MUPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>I (11)</td>
<td>64.0</td>
<td>44.0</td>
<td>62.8 (95)</td>
<td>1.85 (92) $\pm 0.85^*$</td>
<td>3.66 (92) $\pm 0.90^*$</td>
</tr>
<tr>
<td></td>
<td>C (9)</td>
<td>0</td>
<td>0</td>
<td>15.2 (100)</td>
<td>0.66 (100) $\pm 0.30$</td>
<td>3.00 (100)</td>
</tr>
<tr>
<td>12</td>
<td>I (18)</td>
<td>11.1</td>
<td>83.2</td>
<td>28.6 (175)</td>
<td>0.91 (175) $\pm 0.42^*$</td>
<td>3.57 (177) $\pm 0.60^*$</td>
</tr>
<tr>
<td></td>
<td>C (9)</td>
<td>0</td>
<td>0</td>
<td>24.0 (129)</td>
<td>0.70 (130) $\pm 0.29$</td>
<td>2.97 (130) $\pm 1.07$</td>
</tr>
</tbody>
</table>

I : infected; C : matched controls; MUP : motor unit potential; $^* : p < 0.01 - 0.001$ (Student's t-test)

the end of the experimental period. MEPPs frequency and acetylcholine quantum content were of similar value to those obtained in controls at any time pi. The number of double EPPs increased significantly after the third month pi and remained so until the end of the experimental period (Table V).

Tab. V. — MEPP's and EPP's recorded from the diaphragms of mice infected with Trypanosoma cruzi (CA-I strain).

<table>
<thead>
<tr>
<th>Days post-infection</th>
<th>MEPPs</th>
<th>Number of EPPs in 500 impulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amplitude (mV)</td>
<td>Frequency (sec$^{-1}$)</td>
</tr>
<tr>
<td>7</td>
<td>1.50 (4) $\pm 0.25$</td>
<td>0.79 (4) $\pm 0.3$</td>
</tr>
<tr>
<td>37</td>
<td>1.06 (4)$^*$ $\pm 0.14$</td>
<td>0.94 (4) $\pm 0.5$</td>
</tr>
<tr>
<td>90</td>
<td>1.25 (8)$^*$ $\pm 0.17$</td>
<td>1.36 (8) $\pm 0.9$</td>
</tr>
<tr>
<td>120</td>
<td>1.23 (4)$^*$ $\pm 0.15$</td>
<td>0.96 (4) $\pm 0.3$</td>
</tr>
<tr>
<td>360</td>
<td>1.30 (5)$^*$ $\pm 0.11$</td>
<td>0.85 (5) $\pm 0.2$</td>
</tr>
<tr>
<td>controls</td>
<td>1.63 (16) $\pm 0.10$</td>
<td>0.84 (16) $\pm 0.4$</td>
</tr>
</tbody>
</table>

(): number of muscles; MEPPs : miniature end-plates potentials; EPPs : end-plate potentials; $^* : p<0.001$; $^*^* : p<0.002$; $^*^*^* : p<0.005$; m : acetylcholine quantum content.

Observations made on the functional changes of the sciatic nerve in mice chronically infected showed that the nerve action potential amplitudes were significantly reduced and their latencies significantly prolonged when they were compared with controls (Table VI).

Histological findings

Muscle histology at different times pi showed inflammatory changes with perivascular, perimysial and endomysial mononuclear cells infiltrates, also intramuscular neuritis was present. Myopathic changes, in the form of small round fibers, fibers necrosis and fibers replacement by connective tissue, side by side with denervatory images, in the form of type II muscle fibers grouping, grouped muscle fibers atrophy and isolated angular fibers, were also seen. The sequence of these events are depicted in table VII.

The sciatic nerve showed epi, peri and endoneural vasculitis with mononuclear cells, mainly macrophages, from 15 days pi. Semi-thin cross sections disclosed loss of myelinated fibers and thin myelin layers surrounding axons of large diameter. Seared fibers showed paranodal and segmental demyelination.

Dense mononuclear vascular infiltrates were observed in spinal roots and dorsal root ganglia as well as in the meninges surrounding the lumbar spinal cord.

Composition of the inflammatory lesions

In mice infected with the RA strain, phenotypic analysis of inflammatory lesions showed a consistent predominance of CD8 T-cells in nerve, ganglia and spinal cord leptomeninges. In hamstring muscles natural killer cells were identified at 120 and 270 days pi. In those mice infected with CA-I strain, a predominance of CD8 T-cells in nervous tissues was demonstrated only at the earlier stages pi, while those cells

Tab. VI. — Mean (± SD) amplitude and latency of mice sciatic nerve action potential.

<table>
<thead>
<tr>
<th>Controls (n 13)</th>
<th>Infected (n 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (in micro V)</td>
<td>3347 $\pm 1971.7$</td>
</tr>
<tr>
<td>Range</td>
<td>1000 - 8300</td>
</tr>
<tr>
<td>Latency (in ms)</td>
<td>0.52</td>
</tr>
<tr>
<td>Range</td>
<td>0.3 - 0.8</td>
</tr>
</tbody>
</table>

n : number of mice; $^* : p < 0.001$
Tab. VII. — Histological findings on skeletal muscle at different times post-infection (parasites of CA-I strain).

<table>
<thead>
<tr>
<th>Signs</th>
<th>Days post-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Inflammatory changes</td>
<td>▲</td>
</tr>
<tr>
<td>Parasites</td>
<td>-</td>
</tr>
<tr>
<td>Myopathic changes</td>
<td>-</td>
</tr>
<tr>
<td>changes</td>
<td>FG</td>
</tr>
<tr>
<td>Neuropathic changes</td>
<td>-</td>
</tr>
<tr>
<td>changes</td>
<td>FG</td>
</tr>
</tbody>
</table>

▲ scattered infiltrates ; ▲▲ non-coalescent nodular infiltrates ; ▲▲▲ coalescent nodular infiltrates ; IAF : isolated atrophic angular fibers ; FG : type II fibers grouping ; MGA : muscle fibers group atrophy.

predominated in muscles just at the latter phases of the chronic infection. In between, either in nervous tissues or in muscles, a larger proportion of CD4 T-cells could be observed. In mice infected with this strain, B lymphocytes, bearing surface IgM, were present in all investigated tissues at 270 days pi.

DISCUSSION

The purpose of these investigations have been to examine the PNS involvement in human and experimental chronic Chagas’ disease. A multiple approach has been used by employing clinical, electrophysiological and histological techniques, both in humans and mice.

The clinical observations made in patients showed that 10.2% of the explored people had signs or symptoms of PNS involvement. Most of the affected subjects disclosed a combination of sensory impairment and diminished tendon jerks which mainly involved the lower limbs. The paramount manifestations of this neuropathy were sensory disturbances. It is worth to note that muscle weakness was not noted in these patients. The sensory impairment predominance is also in line with the findings done in the electrophysiological and histological assessments of these patients where it was observed overt compromise of sensory fibers and milder involvement of motor axons (30, 31). NASCIMENTO et al. (22) also found a predominant clinical sensory neuropathy, involving the lower limbs, in 4 chronic chagasic patients whose sural nerve biopsies showed mainly axonal damage.

In summary, the clinical findings showed that about 10% of the subjects with chronic Chagas’ disease may develop a mild and predominantly sensory neuropathy which is scarcely troublesome and do not preclude them from doing their usual activities; nevertheless, a shortcoming of this clinical study is that it constitutes a transversal analysis and gives no clues of the outcome of the affected persons.

Conventional EMG showed that a proportion of patients had an old and mild denervation which affected muscles of the upper and lower limbs. This observation is similar to the one done in the mouse model, where diminished interference patterns were seen in the hamstring muscles (9). In both cases, humans and mice, some of the remaining MUs were polyphasic. These observations are also supported by the counts of functional MUs in different muscles; with these techniques, in humans it were found losses of MUs in the different muscles investigated; a distal muscle group, the thenar, and a more proximal muscle, the soleus, were partially denervated in a larger proportion of patients, while the hypothenar and edb muscles were compromised in a rather smaller number of subjects. This finding proves that distal as well as proximal muscles can be affected, albeit with a different severity. The enlarged size of many of the remaining MUs have special significance, for such enlargement could only have resulted if muscle fibers, which had lost their parent innervation, subsequently acquired a satisfactory nerve supply coming from an axonal sprout of a healthy motor-neurone (10). Therefore, the enlarged size of some MUs seen in partially denervated patients should be attributed to healthy neurones which have adopted previously denervated muscle fibers. This behaviour was also noted in mice. However, in these animals, it could be observed that some other MUs were smaller (14); also in man it was found that there were units which did not enlarge their territories (33). Taken together, these observations suggest that there is an other population of neurones which are unable to send axonal sprouts to muscle fibers relinquished by their original innervation. This « sick » motor-neurone population probably is also responsible for the decremental muscle response to supramaximal repetitive nerve stimulation (17) observed in some patients who had not peripheral motor neuropathy. Also it may explain the targetoid appearance of some muscle fibers seen in the histological studies. This last histological fact and the presence of grouped fibers atrophy in muscles of mice chronically infected (14) suggest that the PNS damage is not the sequelae of an acute insult produced at the starting of the infection, but rather an ongoing slowly progressing process leading, probably, to spinal anterior horn motor-neurones depopulation, suggesting that, somehow, the spinal alfa motor-neurones soma are involved in the disease; definitive proofs of this were given by MOLINA et al. (20) who found 21% loss of spinal motor-neurones in mice infected with the Tulahuen strain, other strain than the ones employed in this investigation.

The diminished amplitude of MEPPs, beside their
normal frequency and quantum content, observed in chronically infected mice is a rather intriguing finding and suggest a reduced number of acetylcholine receptors at the post-synaptic level; this might be the sequelae of the muscle inflammatory process occurring at the acute stage of the infection. If something similar occurs in humans, this may contribute to the decremental response seen in some patients when their ulnar nerve was supramaximally repetitively stimulated.

Other findings signal that the ongoing process might be very active, for the presence of increased frequency of the negative small monophasic potentials recorded at the edb end-plate area means that multinnervation may occur in a single fiber; this human finding also received support from the experimental side, for significantly increased number of double end-plate potentials were found in the in vitro phrenic-diaphragm preparations of chronically infected mice (14). These observations suggest that an ill motor-neurone may lose its muscle fibers, which are taken over by an other motor-neurone, but lately the sick neurone may recover sending back its axon to the original muscle fibers which will accept it having, then, to independent innervations, both active (6, 18). These findings lead to accept the possibility that the ongoing destructive process is not a steady one, rather it may develop by waves of damage followed by waves of recovery. Therefore, the patients state would be the result of the number of neurones definitively lost after each insulting wave.

The peripheral nerves are also involved in this disease, for either the motor or the sensory fibers may be damaged. The predominant distal slowness of conduction suggest that the neuropathy may be mainly of the axonal-neuronal type. This behaviour, in sensory fibers, was accompanied by reduced amplitude of the SAP all along the nerve trunk, probably due to loss of functional conducting axons within the nerve trunk. The reduced conduction velocity at the distal segments of the nerve might reflect some fiber demyelination and also, a disturbance of some neurones within the dorsal root ganglia (1, 12), which may explain the selective loss of large and small diameter fibers seen in sural nerve specimens and suggests selective involvement of some type of neurones within the dorsal root ganglia. The histological study of the sural nerve confirmed most of the electrophysiological findings, for loss of nerve fibers, axonal degeneration and foci of paranodal and segmental demyelination were found.

The human nerve trunk observations also received support from the experimental model. In mice it could be demonstrated slowing of conduction velocity and reduced nerve action potential amplitude in the sciatic nerve of chronically infected mice (15). Also, on histology it was found diminished number of nerve fibers and segmental and paranodal demyelination. Furthermore, most of the dorsal root ganglia were damaged in the model (14).

The identification of T-cells within nervous and muscle tissue infiltrates (19) suggests that immunological mechanisms are at work and also shows that Trypanosoma cruzi strain-dependent affinity are involved in the development of neuromyopathic injury.

Taken together, all these findings strongly indicate that the PNS can be compromised in some patients who have entered the chronic stage of the American trypanosomiasis.

REFERENCES


