Morphological Properties of Skeletal Muscle in Spastic Paraplegia

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Abstract

Needle biopsies from the quadriceps femoris and soleus muscle of fifteen young male patients with spastic paraparesis due to traumatic complete spinal cord transection (lesional level T1-T11) were studied.

At 7-12 months after cord lesion, ingravescent muscular atrophy with fatty infiltration accompanied with myopathic changes and patterns of denervation atrophy were observed. The quantitative analysis of muscle fibre types revealed a type I fibre preferential atrophy and reduction; a significant number of type IIB fibres and some regenerating type IIC fibres were also evident.

The main ultrastructural changes were characterized by myofibrillar alterations and dilatation of the sarcoplasmic reticulum. The mitochondrial content decreased in paraplegics and changes in mitochondrial morphology were seen in the degenerating muscle fibres. A significant increase of sarcoplasmic lipid droplets was seen in all cases.

In comparison with the normal capillary bed, the morphometric analysis of the intramuscular capillaries showed reduction of the average number of capillaries per fibre and thickening of the wall with reduplication of the basal lamina.

The possible influence of spasticity on fibre type modifications seen in paraplegics is reviewed and discussed with reference to morphological findings.

Key words: fibre types, ultrastructural changes, muscle capillaries, paraplegia, skeletal muscle.

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The upper motor neuron lesions that produce hemiplegia or paraplegia result in atrophy of skeletal muscle in both experimental animals and humans. In the last years the muscle atrophy and spasticity in paraplegic patients from complete traumatic spinal cord transection are conditions that excited increasing interest for the continuous improvement of the rehabilitation procedures.

Pharmacology, functional electric stimulation of skeletal muscle and clinical experimentations of mechanical orthoses, tend to the recovery of the muscle contractile properties and of the standing up position or walking.

These conditions are important in the preservation of some functions as the blood circulation in the paretic limbs, Ca content in bones, renal function and reduction of spasticity and painful contractions in paraplegics [3, 27]. Moreover the spasticity seems to be stimulus for specific slow to fast muscle fibre transformation in paraplegics [20, 22, 23, 28].

These conditions are interesting in the physiopathological interpretation of the plastic modifications of the skeletal muscle in different conditions, with particular regard to the functional recovery of paraplegics.

The present study was carried out to determine the morphological, enzyme-histochemical and ultrastructural changes in soleus and quadriceps femoris muscles from 15 young spastic patients with 7-12 months paraplegia from traumatic complete cord transection (lesional level Tl-T11). Particular attention was paid to the analysis of the fibre type morphology and composition, to the ultrastructural profile of muscle mitochondria and to the quantitative analysis of the intramuscular capillaries.

Short reports of some of this material have been previously published.

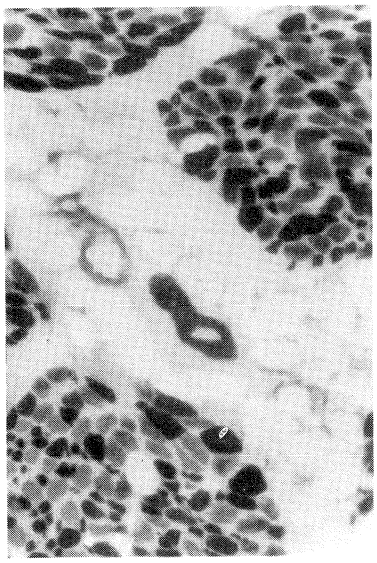


Figure 1. Evident reduction of muscular fascicles dimensions and wide interfascicular fatty infiltration. 10 months paraplegia. ATPase pH 4.6; x 40.

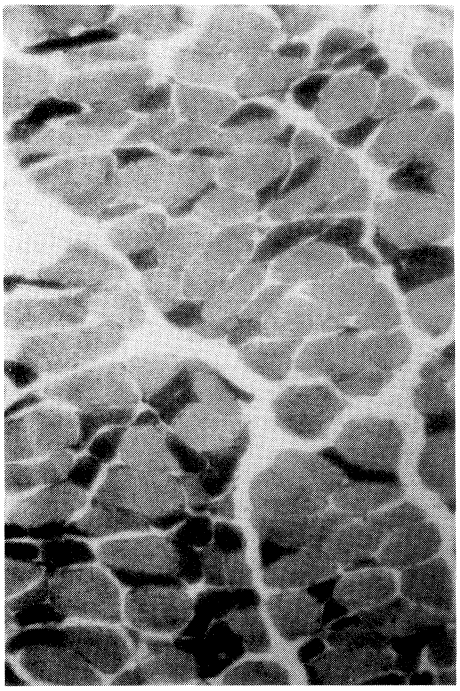


Figure 2. Numerous small atrophic type I fibres with dark appearance. 7 months paraplegia. NADH; X100.

Materials and Methods

Patients

Fifteen young male patients aged 20-30 years, suffered from complete traumatic transection of the cord dorsal segments (lesional level Tl-Tll), were studied. After 7-12 months, when the spastic paraparesis was firm, the patients had, with appropiate written consent, open or needle muscle biopsies from the quadriceps femoris muscle. Some of them received a needle biopsy from the soleus muscle that shows a predominance of type I slow fibres.

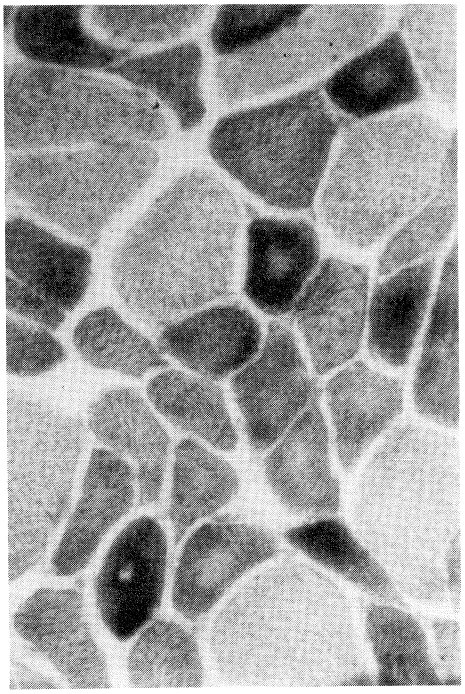


Figure 3. Targetoid fibres as sign of denervation. 7 months paraplegia. NADH; X250.

The patients had a normal rehabilitative physiokinesiological therapy for the control of the muscle hypertonia and the avoidance of pressure sores. None of the examined patients showed ectopic calcifications.

Coeval control muscle biopsies were obtained from normal subjects undergoing orthopedic surgery or from healthy volunteers.

Histology and enzyme-histochemistry

Three specimens obtained under local anaesthesia from the mid-portion of the selected muscle were fixed in 10% neutral formalin, in Karnovsky fluid for ultrastructure and frozen in isopentane cooled in liquid nitrogen for enzyme-histochemistry.

Paraffin-embedded transverse sections were stained with hematoxilin and eosin and transverse epoxy-resin embedded semithin sections were treated for the Gomori's silver stain for reticulin and microvascular profiles. For histochemical and enzyme-histochemical investigations, serial transverse cryostat sections were stained with the modified Gomori's trichrome, PAS and Oil Red O stains, ATPase at pH 9.4 - 4.6 - 4.3, nicotinamide nucleotide dehydrogenase (NADH), as previously described [10]. For electron microscopic studies, ultrathin sections were stained with uranyl acetate and lead citrate as previously described [29, 35, 36].

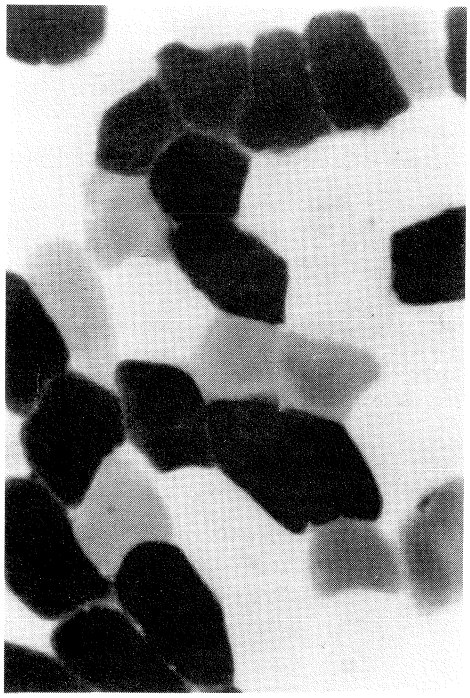


Figure 4. Normal appearance of fibre types in normal muscle. Type I fibres are dark, type IIA are light and type IIB show an intermediate grey staining. ATPase pH 4.6; X 250.

Figure 5. Type I atrophy and numerical reduction and type II predominance. Numerous type IIB fibres are seen. 12 months paraplegia. ATPase pH 4.6; X 250.

The method proposed by Siperstein [41] with modifications was used to quantify the capillary basement membrane width.

Overall capillary studies were performed on transverse formalin-fixed sections stained with Gomori's silver stain and on transverse cryostat sections treated for the immuno-cytochemical demonstration of endothelin that has specific sites in the vascular wall [16].

Morphometry

For quantitative analysis of muscle biopsies, the fibre diameter was determined by measuring the shortest diameter of 100 type I, type IIA and type IIB fibres stained with ATPase at pH 4.6 and 4.3. The morphometric evaluation of fibre type diameter and percentage was performed using an automatic Interactive Image Analysis System IBAS I-II (Kontron,Bildanalyse, Munich).

Each specimen was placed under a TV camera and its image was shown on a TV screen. The image was elaborated according to a program menu of the IBAS system. All measurements were calculated in microns. Overall capillary measures were performed on sections stained with Gomori's silver stain and the following values were determined: capillaries per fibre (C/F) and capillary density (CD) per mm^2 of muscle fibre, according to the method of [2, 8], with the modifications in [38].

Quantitative evaluation of mitochondria and lipid droplets were performed with the IBAS system. Five micrographs for each subject were taken from ultrathin longitudinal sections of the central and peripheral part of the fibre; the total number of micrographs was 210 at a final magnification X 12,000. In our measurements we selected three parameter: area, diameter (of area equivalent circle) and area percentage. The area percentage was automatically assessed as the ratio between the area occupied by the structures and the field where these structures were contained. This field was selected by a determined window inside the fibre [46]. All measurements were calculated in microns.

Statistical analysis

Data were stored on a floppy disk for the statistical evaluation by means of the IBAS I system. Data expressed as mean + SEM and/or percent frequency distribution. To test the significance of differences between control and paraplegic muscles with regard to the structural parameters studied and measured, analysis of variance (T test) was used [29].

Results

Morphology of muscle fibres

Soleus and quadriceps femoris muscle biopsies from young paraplegic patients with high dorsal cord transection (lesional level Tl-T11) were studied by light and electron microscope at 7-12 months following traumatic lesion.

Generalized and progressive decrease in the fibre diameter, with reduction of fascicular dimensions was seen. The atrophy grade increases with the age of the lesion

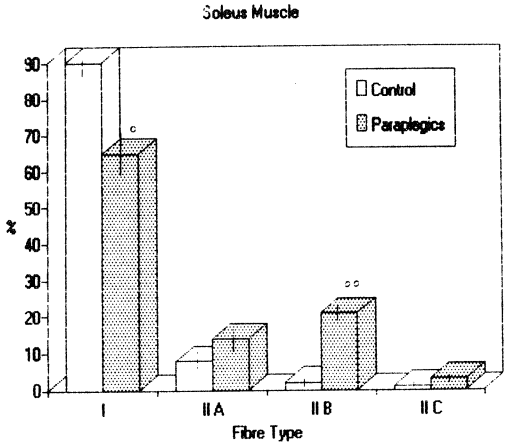


Figure 6A. Fibre type composition in soleus muscle from normal controls and paraplegics. Bar represents standard error of the mean (SEM). Statistical level of significance: °P < 0.01; °°P < 0.001.

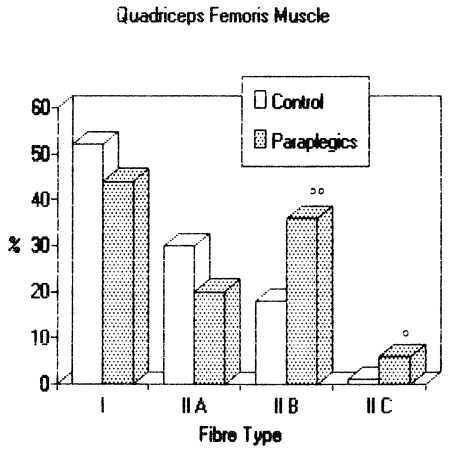


Figure 6B. Fibre type composition in quadriceps femoris muscle from normal controls and paraplegics. Bar represents standard error of the mean (SEM). Statistical level of significance: °P < 0.25; °°P < 0.001.

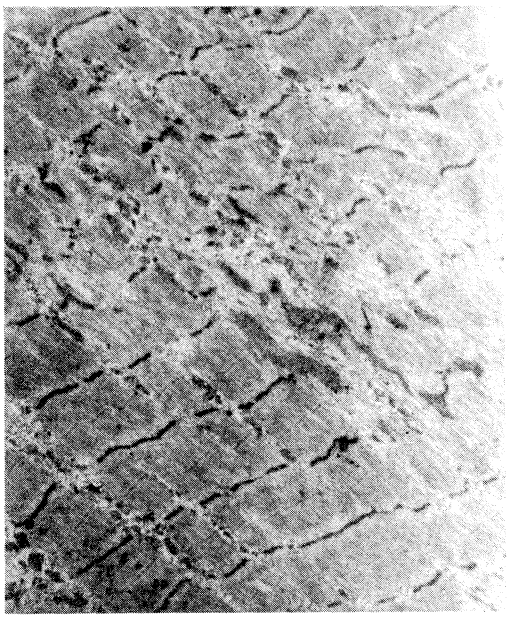


Figure 7. Disorganized myofibrils with loose myofilaments and smearing of Z line. 10 months paraplegia. Electron micrograph; X 10.000

and cytoarchitectural changes of muscle fibres are characterized by two main types of alteration:

a) myopathic changes as internal nuclei, degenerative phenomena and sometimes phagocytic activity in necrotic fibres. In some cases scattered small mononuclear infiltrates were observed. The increase of the interstitial connective tissue and fatty infiltration of muscle was an ingravescent phenomenon, particularly evident in long term paraplegia (Fig 1).

b) denervation atrophy changes as small isolated or grouped small angular atrophic fibres with a dark appearance due to an increased reaction for oxidative enzyme activities, and target-targetoid fibres are sometimes observed (Figs 2, 3).

Quantitative analysis of the fibre types

After fibre type characterization by ATPase at pH 4.6 and 4.3, individual fibres of normal muscle are categorized as type I (slow-twitch), type IIA (FR fast resistant) and type IIB (FF fast fatigable fibres (Fig 4). Type IIC regenerating fibres are also observed in particular conditions.

At 7-12 months after cord lesion, the spastic paraplegic patients show a significant striking decrease in fibre diameter. In some patients there is a type I preferential atrophy. In this advanced stage of paraplegia it is evident a significant reduction of type I fibres; a significant number of type IIB fibres accompanied with some type IIC regenerating fibres is also evident near a lot of type IIA fibres (Fig 5).

Table 1. Morphometric data and variance analysis of mitochondria in quadriceps femoris muscle from controls and paraplegics. Mean ± standard error of the mean (SEM). Statistical level of significance: °P < 0.25; °°P < 0.001.

	Mean mitochondrial size (μm^2)	% mitochondria per fibre area
Controls	0.14 ± 0.09	2.53 ± 0.3
Paraplegics	0.08 ± 0.04°°	1.70 ± 0.5°

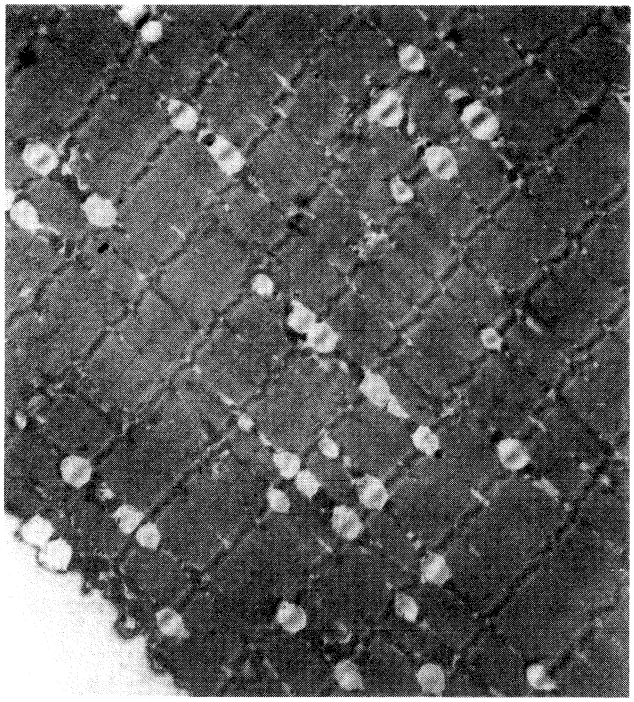


Figure 8. Electron micrograph of longitudinal sections from paraplegic muscle fibre showing lipid droplets accumulation between myofibrils. X 10.000

The Figs 6a and 6b show the morphometric findings of fibre type distribution in soleus and quadriceps femoris muscle from control and paraplegics. The increase of type IIB fibres is particulary evident in the soleus muscle that show in normal healthy subjects a striking predominance of type I slow fibres.

Ultrastructure of muscle fibres

Many ultrastructural changes have been observed in paraplegics. In most cases the sarcolemma of atrophic fibres was arranged in multiple projections and folds containing packed mitochondria and lipid droplets; dilated

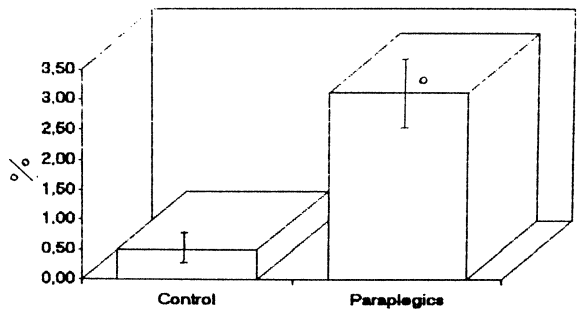
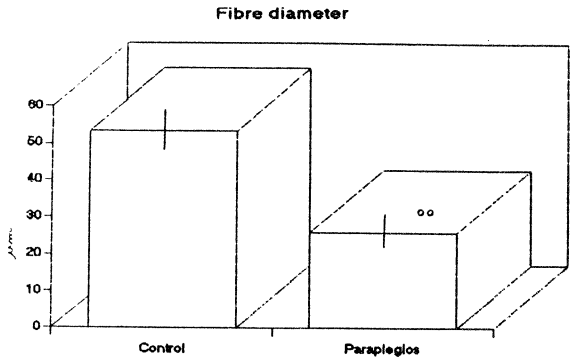
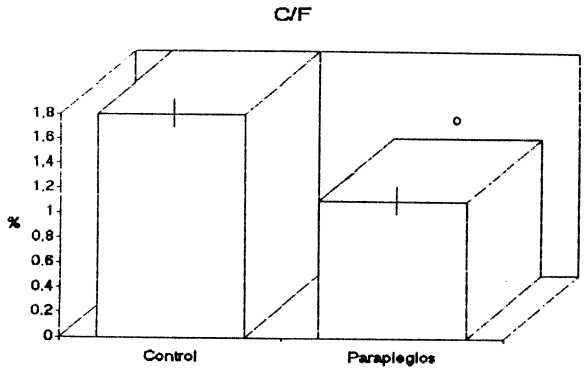


Figure 9. Percentage of lipid droplets per fibre area in quadriceps femoris muscle from controls and paraplegics. Bar represents standard error of the mean (SEM). Statistical level of significance: P < 0.001



10 A



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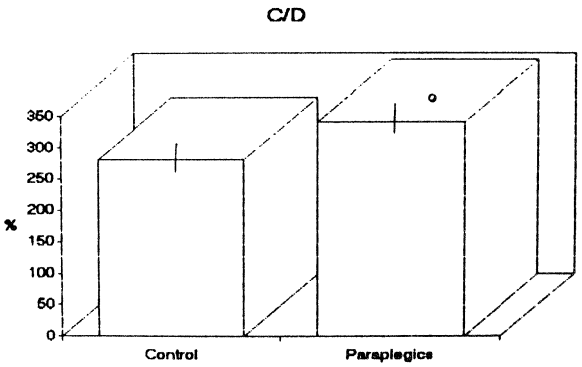

10 C

Figure 10. Quantitative measurement of: A, the fibre diameter; B, the capillary number per fibre (C/F); C, the capillary density (CD) in quadriceps femoris muscle from controls and 12 months paraplegia. Bar represents standard error of the mean (SEM). Statistical level of significance: °P < 0.01; °°P < 0.001

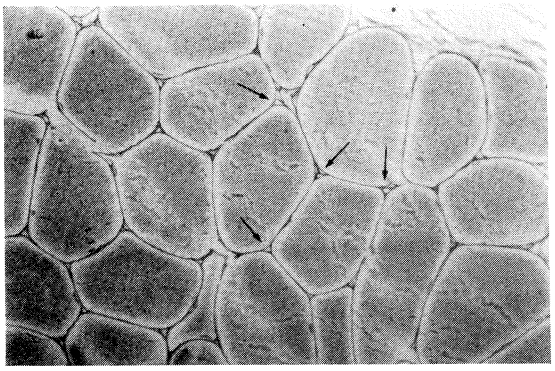

Figure 11. Resin-embedded transverse section of muscle fibres from a normal subject showing the capillaries around single fibres (arrows); Gomori's silver stain. X 400.

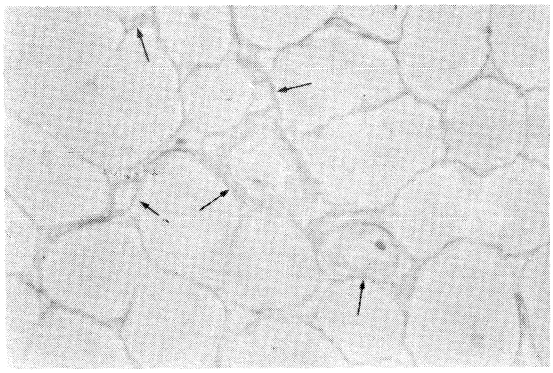

Figure 12. Reduction and dilatation of capillaries in 12 months paraplegia. Some capillaries are thickened. Gomori's silver stain. X 400.

structures of sarcoplasmic reticulum were observed. In numerous cases the nuclei showed a vescicular appearance and their migration inside the fibre was a phenomenon of the advanced paraplegia. However the most remarkable pathologic changes have been observed in the myofibrillar apparatus, with loss or disruption of myofilaments, changes in the normal banding pattern and streaming or smearing of the Z line (Fig 7). Considerable proliferation of T-tubules occurs in isolated cases with formation of tubular aggregates.

Morphometry of mitochondria

Changes in mitochondria following paraplegia have been analyzed by morphometry. Changes in mitochondrial morphology were seen in some atrophic and degenerating fibres; the alterations consisted of swelling, cristal degeneration and occasional presence of intracristal paracristal line inclusions. In most fibres, mitochondria are

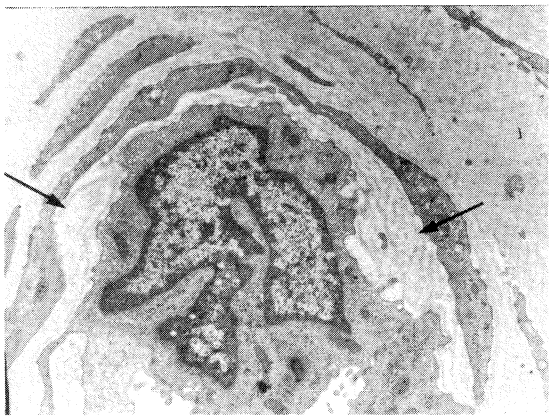

Figure 13. Electron microscopic appearance of a thickened capillary with reduplication of the basal lamina (arrows). X 18.000

generally rare and small in size. As shown in Table 1 the mitochondrial amount decreases in paraplegics; the comparison between paraplegics and normal controls shows a significant difference for mitochondrial size.

These data are referred to both centrally and periferically located mitochondria.

Morphometry of lipid droplets in the sarcoplasm.

Between the myofibrils and beneath the plasma membrane of muscle fibres from paraplegics, round or oval lipid vacuoles occur and their amount is remarkable in the majority of fibres (Fig 8). The results of the morphometry concerning the lipid droplets percentage per fibre are reported in Fig 9. In paraplegics, a significant increase of lipid droplets widely distributed in the sarcoplasm, is seen.

Size of fibres and capillary morphometry

In normal biopsies, most capillaries were 3-5 microns in diameter and none was less than 1 nor more than 7 microns. In paraplegics, the capillaries were 5-9 microns. Mean fibre diameter is significantly reduced in long term paraplegia, if compared with controls (Fig 10a). This important fibre atrophy is accompanied by significant reduction of the average number of capillaries per fibre (Fig 10b). The capillary density increases in relation to the reduction of the fibre area (Fig 10c). It may be related to the reduction in the fibre diameter in paraplegics.

In comparison with normal capillary bed, morphological analysis of the intramuscular capillaries showed thickening of the vessel walls and abnormal enlargemet of some capillaries (Figs 11, 12). Ultrastrutural studies showed enlarged endothelial cells with preponderance of micropynocytotic vescicles and evident thickening and reduplication of the basal lamina (Fig 13).

Discussion

After traumatic spinal cord lesions the acute response to the destruction of pyramidal tract fibres in humans is paralysis and profound hyporeflexia for the innervated muscles. After few weeks the majority of paraplegics developed exageration of some proprioceptive reflexes of the limb producing spasticity. The basis of hypertonia and hyperreflexia of spasticity are complex and have been discussed extensively [6].

Our results indicate that progressive and ingravescent muscle fibre atrophy in the affected muscles is the indirect effect of complete spinal cord transection. A 50% decrease in the fibre diameter is present in early stages of paraplegia [4, 23, 36]; the most remarkable ultrastructural changes in the atrophic fibres have been observed in the myofibrillar apparatus with loss or disruption of the myofilament and abnormalities in the banding pattern [36]. Lipid accumulation is another alteration seen in muscle fibres, mainly in the advanced stages of paraplegia. The muscle atrophy is accompanied by focal proliferation of the fibrous interstitial tissue and by fatty infiltration related to the muscle wasting. The described changes characterizing the so called central muscle atrophy, are due to the disuse and immobilisation [13, 22] and to loss of inputs from the descending motor patways [36, 37].

A progressive ingravescent muscle fibre atrophy and patterns of plastic structural reorganization with slow-to-fast fibre type transformation are the response to long-term spastic paraplegia from complete spinal cord transection, although in some patients muscular neurogenic changes, such as small groups of angular dark atrophic fibres, fibres with targetoid appearance and with proliferative changes of the sarcoplasmic reticulum, were observed [36, 39].

These denervation patterns might reflect some coincidental alterations in the peripheral nervous system, as hypothesized in parkinsonism and in different upper motor neuron diseases. There are also reports of neurogenic EMG patterns in patients with upper motor neuron diseases suggestive of anterior horn cell reduction for suppression of upper connections and suggestive of secondary disturbances of electrogenesis [25, 48].

Trans-synaptic degeneration in the lower motor neuron due to a drop-out of trophic influences from the upper motor neuron or to a hypothesized derangement of the upper motor neuron control on axoplasmic flow of the lower motor neuron [11, 23] is another possible pathophysiologic mechanism of denervation in spastic paraplegia.

Human skeletal muscle contains at least three different fibre types (I, IIA and IIB) which can be identified on the basis of their physiological, enzyme-histochemical and morphological characteristics. Each of these fibres was found to correspond to a particular physiological unit.

The type FF (fast fatigable) muscle units consist of type IIB muscle fibres, type FR (fast resistant) units consist of type IIA muscle fibres and type S (slow) units consist of type I muscle fibres.

The interconvertibility of fibre types has been demonstrated in animals following cross-innervation and training [1, 10]; it has been conclusively demonstrated that prolonged endurance and continuous low frequency nerve stimulation transforms a fast into a slow muscle in animals and in humans [9, 12, 31, 32, 33, 40, 42]. Following spinal cord injury, if the alpha motor neurons are not damaged due to the primary lesion, the motor neurons remain healthy and can contract when excited with electrical stimulation.

Although these motor neurons remain healthy, there is widespread disuse atrophy of skeletal muscle [14, 43]. Mainly the fast motor twitch units show a reduction of their contraction speed and an increase in the oxidative capacity [5, 44]. In the present investigation we studied muscles with different fibre composition; the soleus muscle that show a striking predominance of type I slow twitch fibres with predominant oxidative activity, and the quadriceps femoris muscle composed by similar percentages of slow and fast fibres. In long-term spastic paraplegia, a preferential atrophy and reduction of type I slow fibres were seen in both paretic muscles. In this stage, a significant percentage of type IIB fibres, with intermediate ATPase activity were observed [20, 22, 23, 28]. This pattern may be interpreted as a loss of type I fibres or as a fibre type conversion from slow to fast. The last hypothesis may be confirmed by studies on experimental spinal cord transection, in that the rat and cat skeletal muscle properties would change with almost complete type I slow to type II fast fibre transformation [21, 47]. These findings are strikingly different from those seen in simple immobilisation in which large increases in the ratio for fast resistant and slow units are seen [24]. At last, studies on gastrocnemius muscle motor units in cats with long-term complete low thoracic spinal cord transection showed a greater proportion of type F motor units and the corresponding type IIB muscle fibres than expected in normal samples [24]. It is hypothizable that in long-term paraplegia the loss of the upper motor neuron control and spasticity may induce the described plastic muscular changes different from those seen in simple muscle immobilisation.

A lot of lipid droplets are normally seen in human skeletal muscle. Lipids are utilized by mitochondrial enzymes that mediate the aerobic oxidation of pyruvate, fatty acids and amino-acids to release energy for all metabolism and for muscle contraction. Lipid droplets increase with aging [29, 35], in hemiplegic muscle atrophy after cerebrovascular accidents [37] and in different pathological and metabolic conditions; therefore, they are expression of abnormal mitochondrial function [26]. As shown in the results, the lipid droplet percentage per fibre area significantly increases in paraplegics. This result may be related to the decrease in mitochondrial size and percentage in paraplegic muscle and it may be attributed to a low utilisation of the lipid energy source following muscle inactivity [36].

In the few published studies investigating the effects of immobilisation and muscle atrophy on the mitochondrial content and metabolism in human muscle fibres, the results consisted of decrease of the mean mitochondrial size and of the percentage of mitochondria per fibre area [18, 45] and of impairment of the mitochondrial enzyme activities [30]. The loss of the functional activity of muscle mitochondria is therefore accompanied by morphological damage of mitochondria [45]. Similar results have been obtained in studies on sedentary old people, in which the decrease in mitochondrial size and percentage may represent a response to a reduced metabolic demand in relation to diminished energy requirement in old age [29, 35].

The data regarding the mean mitochondrial size and percentage per fibre area in paraplegics showed a significant decrease in the mitochondrial size, while the mitochondrial percentage per fibre area was moderately affected. These results are explained in relation with the progressive muscle fibre atrophy and inactivity and with an obvious adaptation of the oxidative metabolic function to a lesser metabolic demand. Whereas some authors suggested that mitochondria in atrophic muscles are morphologically damaged [45], in the present investigation the morphological alterations of mitochondria are confined to some atrophic degenerating fibres corresponding to fibres with high oxidative enzyme activities [36].

Clinical evidence for vasomotor disturbances in paretic limbs of paraplegics is reflected in the alterations of the muscle circulation [23, 26]. In recent cord lesions, when the acute response to destruction of pyramidal tract fibres is paralysis with profound hypotonic flaccidity, interstitial vasogenic edema and capillary dilatation were observed.

In the later stage of the disease, when spasticity become evident, paraplegic patients showed a reduction of the capillary percentage and thickening of the arteriolar and capillary walls [23, 36, 39]. Primary studies on microvasculature from human skeletal muscle concerned normal healthy adults, athletes and normal subjects trained in endurance or strenght [1, 2, 17]. They demonstrated a closed relationship among the muscle fibre diameter, fibre type and capillary number. The greatest number of capillaries surrounds those fibres with the largest diameter and the highest level of oxidative enzyme activity i.e. the type I slow fibres.

More recently, studies on inflammatory myopathies, muscular dystrophies and mitochondrial myopathies showed a reduction in muscle capillarity [8, 18, 19, 38]. Moreover, studies on muscle inactivity which causes marked muscle atrophy, showed reduction and morphological damage of mitochondria associated to reduction of the capillarity [17, 30, 45]. At least, overall capillary loss and necrosis occurs in recent and long standing denervation atrophy where the capillaries are actively lost to match the reduced metabolic demand of the inactive atrophic fibres [7]. On the basis of the reported observations, the capillary reduction in paraplegics may be due to the results of an interaction between the fibre atrophy, overimposed denervation, disuse and reduction in the muscle volume. Moreover the significant loss of type I slow fibres that usually are surrounded by a greater number of capillaries, may contribute to the overall capillary reduction in paraplegic muscle.

Reduplication of the basal lamina in capillaries is a complex phenomenon described in dystrophia myotonica, diabetes mellitus and other microvascular diseases [34, 41]. Some authors interpreted the reduplication of the basal lamina in capillaries as expression of vascular degeneration [15, 18, 19]. In paraplegia, the thickening of some microvascular walls could be interpreted as a negative phenomenon possibly inducing the degenerative myopathic changes in muscle fibres in advanced paraplegia.

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