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Histochemical and ultrastructural study of skeletal muscle in patients with sepsis and multiple organ failure syndrome (MOFS)

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Summary. Muscle biopsies for histochemical and ultrastuctural analysis were obtained from seven critically ill patients admitted to the Intensive Care Unit of the "Domingo Luciani" Hospital, Caracas, Venezuela. The sample included two patients with sepsis of abdominal origin, and five that presented sepsis/MOFS, with renal, hepatic, and respiratory disturbances and muscular weakness. Sections were examined for myosin adenosine triphosphatase (ATPase) after pre-incubation with both acid buffer (pH 4.37 and 4.6) and alkaline buffer (pH 10.3), for reduced nicotinamide dinucleotide diaphorase (NADHd), and for α-glycerophosphate dehydrogenase (α-GPDH). Sections were stained with hematoxilin and eosin to look for pathological changes and examined with a transmission electron microscope. Skeletal muscle of patients in early stage of sepsis showed a normal aspect with light microscopy, but at the ultrastructural level some of the fibres showed atrophy and some capillaries looked altered. Patients with sepsis/MOFS exhibited an evident muscle disorder with oedema, infiltrate, atrophy and segmental necrosis. All fibre types showed decrease in diameter; specially fibre types IIA and IIB. Intramuscular capillaries were thickened and occluded, indexes of capillarity were slightly reduced, and fibre oxidative activity was decreased. At ultrastructural level fibres showed severe atrophy, contractile system disorganization and segmental necrosis. Capillaries were also altered and the mononuclear cell infiltrate was abundant and represented by macrophages, lymphocytes and mastocytes.

Key words: Sepsis, MOFS, Skeletal muscle, Ultrastructure, Histochemistry

Introduction

To date, sepsis is defined as the presence of a confirmed infection process with a systemic response which includes two or more of the following conditions: hyper or hypothermia, tachycardia, tachypnea and altered white blood cell count (Bone et al., 1992; Bone 1995; Rangel-Fausto et al., 1995). The final stage of this process is the development of multiple organ dysfunction which can include the complete failure or the chemical failure of an organ that may or may not result in clinical findings (Bone, 1991; Bone et al., 1992; Beal and Cerra, 1994). This condition has been called multiple organ failure syndrome (MOFS) (Eiseman et al., 1977), critical illness (Zochodne et al., 1987) and multiple organ dysfunction syndrome (MODS) (Bone, 1991; Beal and Cerra, 1994). We will use the first denomination. Patients with sepsis and MOFS exhibit after a specific insult a general hypermetabolic state characterized by an increase in oxygen consumption, tachycardia, tachypnea and fever (Cerra, 1987; Beal and Cerra, 1994). In addition, they develop muscle weakness and wasting (Wokke et al., 1988; Helliwell et al., 1991). Light microscopic studies of skeletal muscle have shown denervation atrophy (Zochodne et al., 1987, Wokke et al., 1988; Bolton, 1993), occasional fibre necrosis (Zochodne et al., 1987; Wokke et al., 1988) and necrotizing myopathy of up to 95% of the fibres (Helliwell et al., 1991). Cell infiltration was observed only in the cases with necrotizing myopathy (Helliwell et al., 1991). The only ultrastructural study of muscle pathology in this condition showed marked muscle atrophy with well preserved end-plates. Massive necrosis and cell infiltration were also observed (Wokke et al., 1988).

In this work we report a histochemical and ultrastructural study on skeletal muscle pathology in patients with sepsis and sepsis/MOFS.

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Materials and methods

Muscle biopsies were obtained from seven critically ill patients (four females and three males) admitted to the Intensive Care Unit of the "Domingo Luciani" Hospital, Caracas, Venezuela. Patients, from 15 to 45 years old, included two with sepsis of abdominal origin one week after the injury, and five presented sepsis/MOFS with

Table 1. Clinical data of patients.

PATIENT	SEX/YEARS OLD	DIAGNOSIS	DAYS AFTER INJURY	MICROORGANISM FOUND
1	m/15	sepsis	5	*E. coli, Enterococcus*
2	f/15	sepsis	6	*E. coli*
3	f/39	sepsis/MOFS	13	*Ps. aeuroginosa, Serratia sp., E. coli*
4	f/34	sepsis/MOFS	10	nd
5	m/58	sepsis/MOFS	30	*Serratia sp., Streptococcus, C. albicans*
6	f/58	sepsis/MOFS	24	*E. coli*
7	m/27	sepsis/MOFS	24	*Enterobacter*

nd: not determined.

Table 2. Results of histochemistry in percentage of fibres.

PATIENT	FIBRE TYPE			OXIDATIVE CAPACITY (NADH-d)			GLYCOLYTIC CAPACITY (αGPDH)		
	I	IIA	IIB	High	Medium	Low	High	Medium	Low
Control*	48	22	30	39±5	32±4	29±6	47±13	26±4	27±13
1	48	38	14	38	17	45	26	62	12
2	37	38	25	16	42	42	42	29	29
3	43	45	12	22	41	37	43	30	27
4	-	-	-	-	-	-	-	-	-
5	56	33	11	18	51	31	44	33	23
6	-	-	-	-	-	-	-	-	-
7	48	36	16	52	24	24	28	46	26

*: control for fibre types from Dubowitz (1985) and control for oxidative and glycolytic capacity from Hernández (1994).

renal, hepatic, and respiratory disturbances and muscular weakness, 10-30 days after the injury. *E coli* was present in 57% of the cases, and other microorganisms found were *Candida albicans*, *Enterococcus*, *Streptococcus*, *Pseudomona aureoginosa*, *Serratia sp.* and *Enterobacter* (Table 1).

Needle biopsies were taken from the quadriceps femoris muscle. Upon collection, the muscle sample was divided in two portions, one for histochemical and the other for electron microscopic analysis. For histochemical analysis the sample was covered with O.C.T. and frozen in isopentane cooled with liquid nitrogen.

Histochemical analysis

Transverse sections (10 μm width) were cut on a cryostat-microtome at -20 °C and mounted on coverslips for staining. Sections were examined for myosin adenosine triphosphatase (ATPase) (Brooke and Kaiser, 1970), after pre-incubation with acid buffer (pH 4.37 and 4.6) and alkaline buffer (pH 10.3), for reduced nicotinamide dinucleotide diaphorase (NADHd), (Novikoff et al., 1961) and for α-glycerophosphate dehydrogenase (α-GPDH), (Wattemberg and Leong, 1960). The percentage of fast and slow fibres was determined by the comparative analysis of sections stained for ATPase activity at different pH (Brooke and Kaiser, 1970). From the sections stained for NADHd, the percentage of high and low oxidative fibres was determined. From the sections stained for α-GPDH, the percentage of high and low glycolytic fibres was determined. Sections were stained with hematoxilin and eosin to look for pathological changes.

Capillary density, capillary/fibre index and capillaries adjacent to each fibre type were determined on the sections stained by the α-amylase-periodic acid-Schiff (PAS) technique (Andersen, 1975). Atrophy factor was calculated on ATPase-stained sections according to Dubowitz (1985). To compare diameters of fibres, control values were taken from results of Dubowitz (1985). Student *t* test was applied.

Electron microscopical analysis

For electron microscopical analysis, pieces of muscle, 2 mm in diameter, were fixed in 3% glutaraldehyde in phosphate buffer for 45 min at pH 7.4 and 320 mOsmol, postfixed in 1% OsO_4 for 1 h and embedded in epon. Sections were cut with a diamond knife in a Porter-Blum MT2-B ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed in a Hitachi H-500 transmission electron microscope, at an accelerating voltage of 100 kV.

Results

Light microscopy

Skeletal muscle structure of patients in early stage of sepsis showed a normal aspect with hematoxilin and eosin stain. Fibre type percentual distribution was not altered when compared with normal values, except for a decrease in IIB fibre type proportion in one patient (Table 2). Fibre type diameters did not exhibit

Fig. 1. Cross section from a patient with sepsis/MOFS showing oedema, abundant infiltrate, internalization of nuclei and necrotic areas. x 250

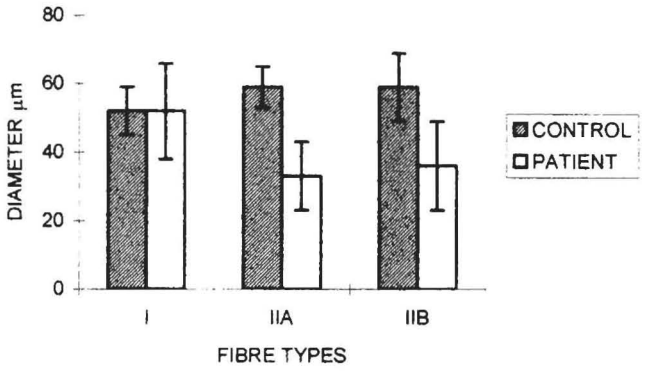

Fig. 2. Diameters of the fibres types from a patient with sepsis/MOFS. IIA and IIB $p \leq 0.05$.

significant differences when compared with the normal values of Dubowitz (1985), showing very low atrophy factors. Microvessels did not seem to be altered with the PAS-amylase staining.

Patients with sepsis/MOFS presented an evident muscle disorder with different degrees of oedema, infiltrate and some muscle fibres were pleomorphic in cross sections and showed decreased diameter. Also, internalized nuclei and segmental necrosis were seen (Fig. 1). In fibre types IIA and IIB the decrease in diameter was significant when compared with normal values from Dubowitz (1985), and the atrophy factor was elevated (>50%) (Fig. 2). Intramuscular capillaries were thickened and occluded. Indexes of capillarity were slightly reduced (Table 3).

Table 3. Capillarity indexes.

PATIENT	1	2	3	5	7	CONTROL*
Cap/mm	267	278	319	309	261	367±74
Cap/Fib	1.35	10.3	1.18	1.17	1.31	1.4±0.2
Cap/I	3.87	3.25	3.76	3.29	2.95	3.9±0.2
Cap/IIA	3.42	3.04	3.48	1.83	2.37	3.8±0.2
Cap/IIB	3.5	2.62	1.8	2	1.85	3.0±0.7

*: control from Torres et al. (1990).

Electron microscopy

In patiens with sepsis, some of the fibres showed atrophy with widening of the intermyofibrillar spaces and foldings of sarcolemma (Fig. 3) at the ultrastructural level, despite the normal appeareance of skeletal muscle fibres under the light microscope. Some muscle fibre sections presented disorganized and contracted myofibrils and alterations of the mitochondria (Fig. 4). Sarcotubular system elements were swollen and disorganized in some areas. Additionally, glycogenosomes and other autophagic vacuoles were also seen. Capillaries showed thickened basement membrane and endothelial cell wall and partial occlusion (Fig. 5).

Muscle fibres from patients with sepsis/MOFS showed severe atrophy (Fig. 6), different degrees of contractile system disorganization and segmental necrosis (Fig. 7). Some areas contained myelinated nerve debris surrounded by contractile elements suggesting regions of broken neuromuscular contacts (Fig. 8). Capillaries were also altered showing thickened basement membrane, endothelial infoldings to the

Fig. 3. Longitudinal section of a muscle fibre from a patient with sepsis showing intermyofibrillar spaces widened (asterisk), pleomorphic mitochondria with a very electron dense matrix (arrows) and foldings of sarcolemma (arrowhead). x 15,000

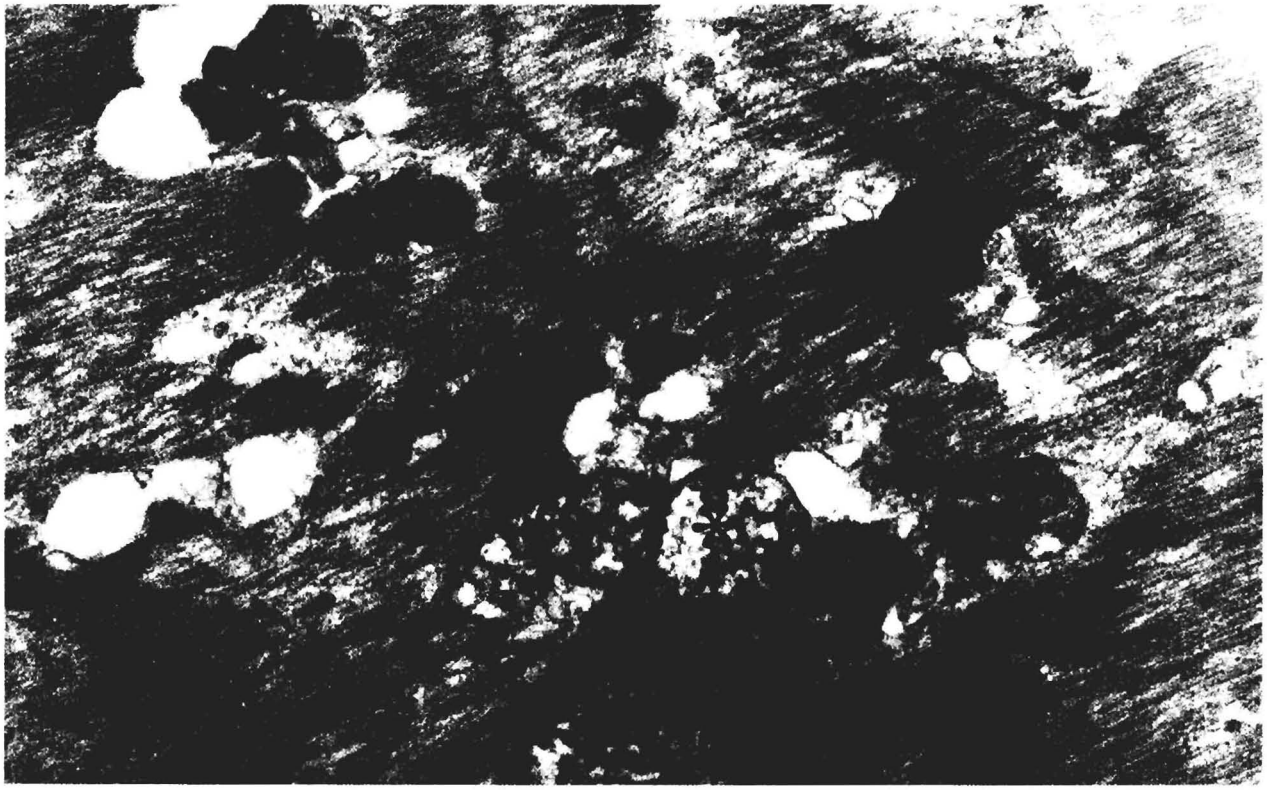

Fig. 4. Section of muscle biopsy from a patient with sepsis exhibiting hypercontracted myofibrils (star) lacking defined sarcomeric bands and lines, sarcotubular elements are swollen (arrowhead) and mitochondria are altered (asterisk). x 18,000

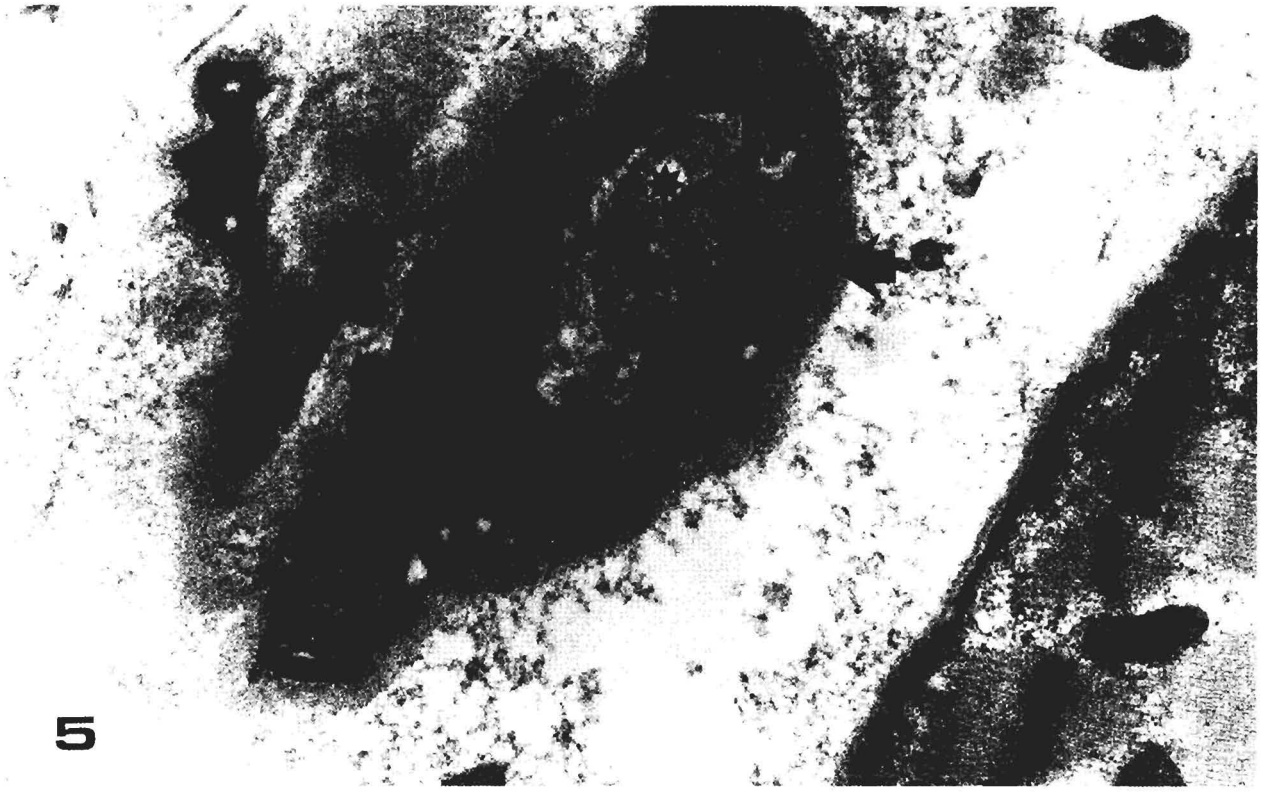

Fig. 5. Intramuscular capillary from a patient with sepsis showing basement membrane (asterisk) and endothelial cell wall (arrow) thickened and lumen partially occluded (star). x 24,000

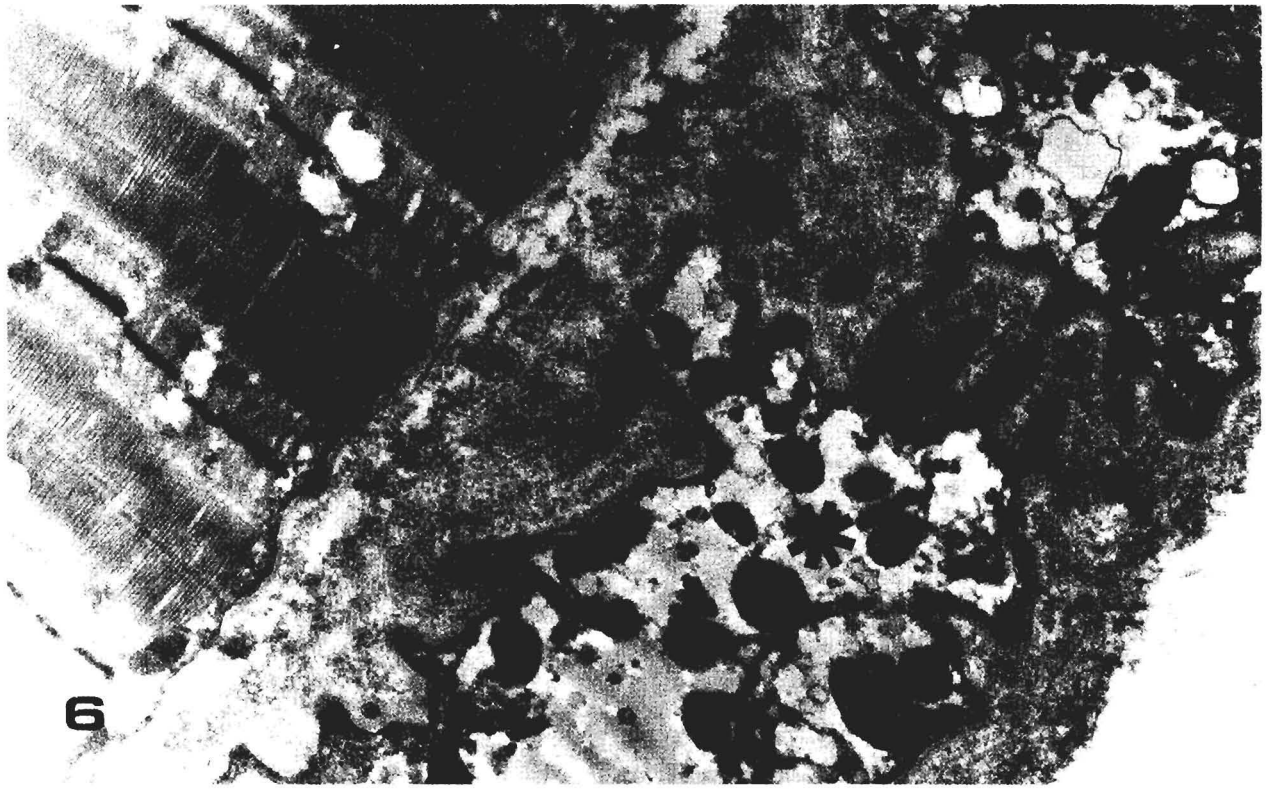

Fig. 6. Muscle section from a patient with sepsis/MOFS showing sarcoplasmic lack of myofilaments (asterisk). x 15,000

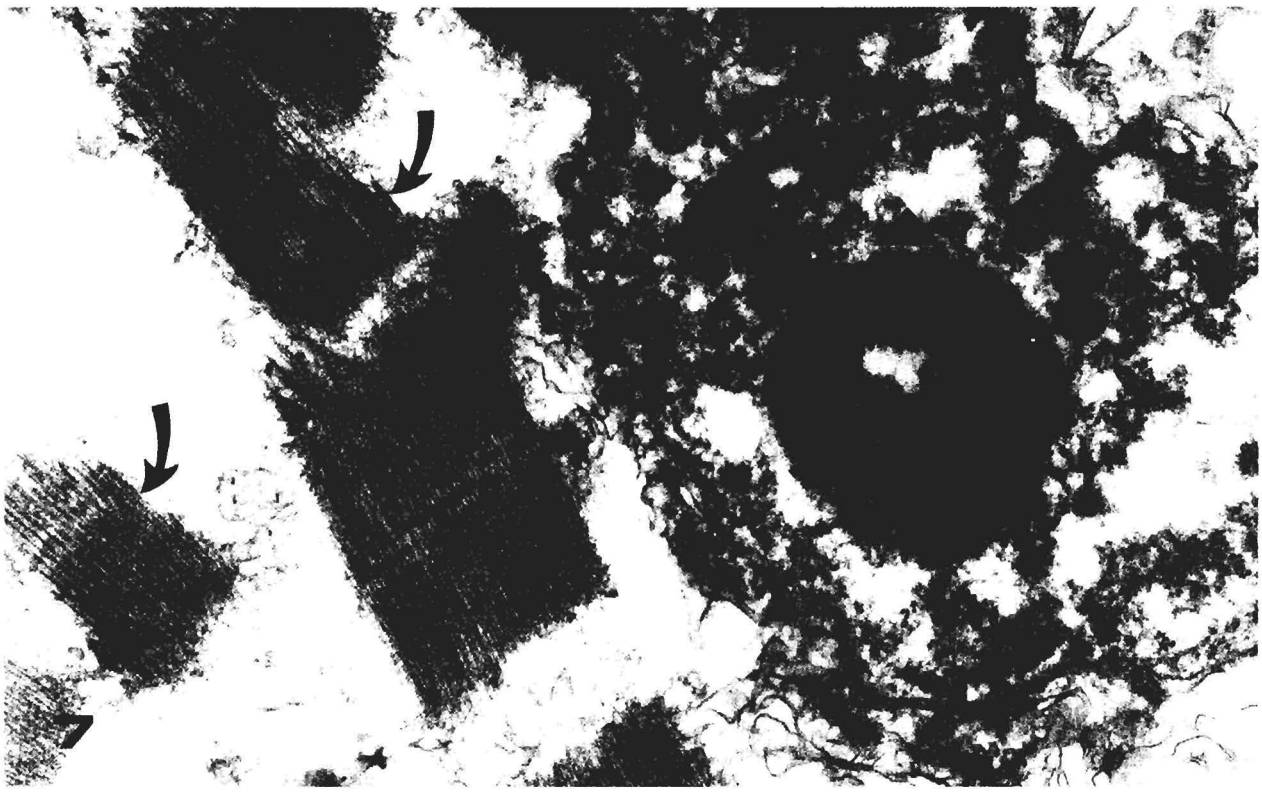

Fig. 7. Section of muscle fibre from a patient with sepsis/MOFS showing myofibrillar (arrows) and nuclear rest (triangle). x 20,000

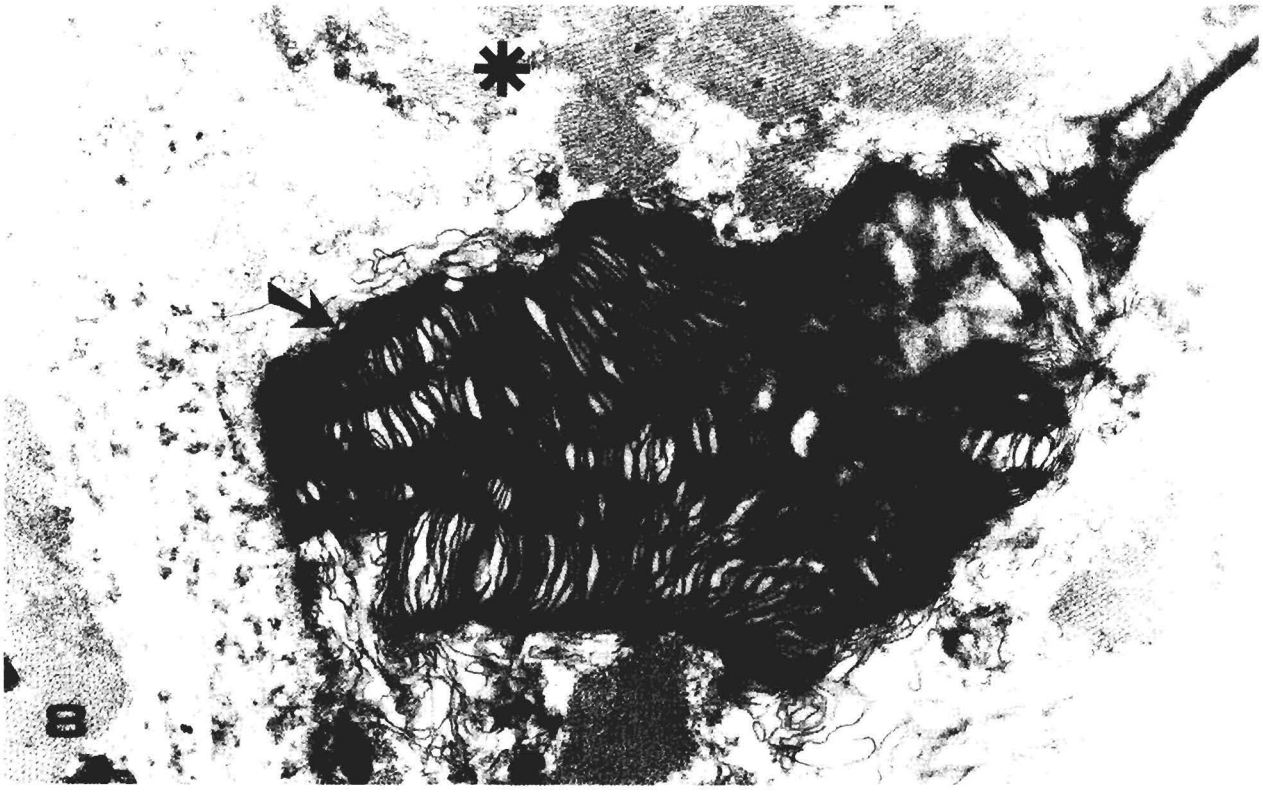

Fig. 8. Muscle section from a patient with sepsis/MOFS with myelin (arrow) and myofibrils rests (asterisk). x 20,000

lumen, occlusion and even necrosis, and some pericytes looked hypertrophied (Fig. 9). The mononuclear cell infiltrate was abundant and represented by macrophages (Fig. 9), lymphocytes and mastocytes.

Discussion

To our knowlegde, alterations in the muscles of patients in early stage of sepsis have not been described. It is possible that the lack of clear evidence of muscle changes by light microscopy in the early stages of sepsis did not stimulate the study of muscle ultrastructure in these patients. In our cases, we investigated muscle ultrastructure in the early stages of sepsis because of the possibility of finding muscle injury even if it appeared normal with light microscopy. The reported changes in the capillaries like the widening of basement membrane and endothelial cell wall, and occlusion of the lumen would correspond to an inflammatory process (Finol et al., 1990, 1994). In experimental septic shock produced by *E.coli* endotoxin widening of the endothelial cell was found without thickening of basement membrane (Hauptmann et al., 1994). That is opposite to several observations obtained from other inflammatory myopathies (Finol et al., 1990, 1994).

The histological investigation of muscle in two critically ill patients with generalized weakness showed muscle fibre atrophy of both type I and II fibres (Wokke et al., 1988). This result was supported by the work of Helliwell et al. (1991), who documented muscle fibre atrophy in 12 patients. In the present work in sepsis/MOFS cases, atrophy affected both type I and II fibres, although in two patients fibre II atrophy was more severe than the atrophy of type I fibres. Our results on segmental necrosis in muscle fibres from patients with sepsis/MOFS support previous observations in this condition (Zochodne et al., 1987; Wokke, 1988; Helliwell et al., 1991). Occasional fibre necrosis in critically ill patients was found by Wokke et al. (1988). However, the extension and intensity of necrosis varied in different reports. Helliwell et al. (1991) evidenced in most patients an important necrosis process, which allowed them to coin the denomination of necrotizing myopathy for the condition of these patients. In this work only one patient presented severe necrosis, the others, presented areas of segmental necrosis. We agree with Helliwell et al. (1991) in connection with the origin of muscle fibre segmental necrosis. In effect, our results

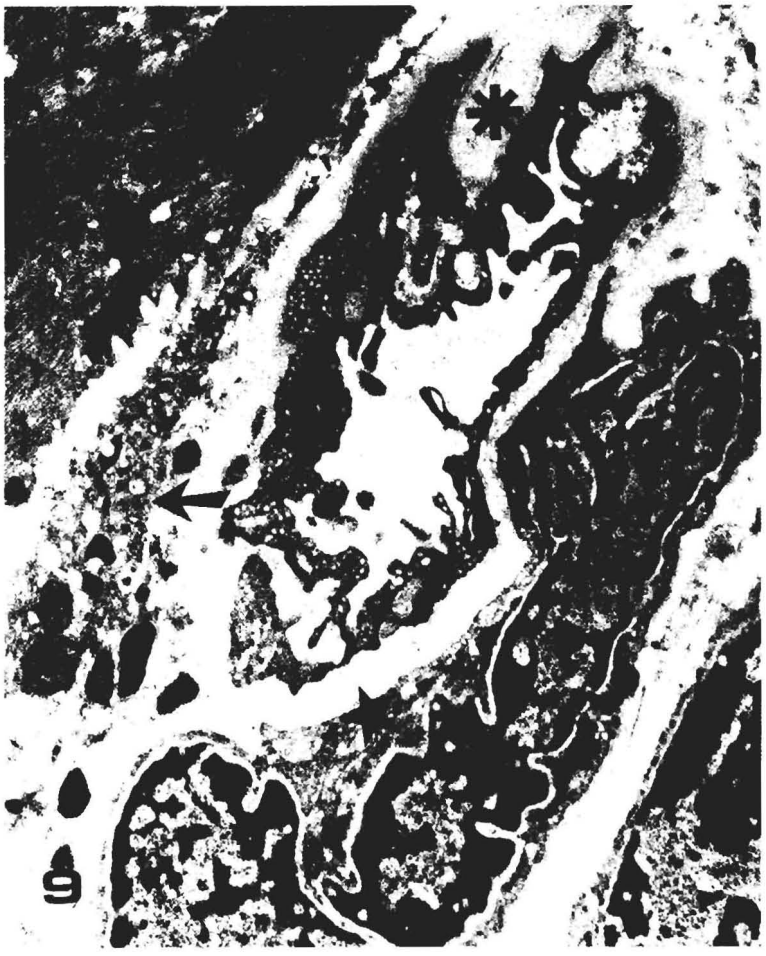

Fig. 9. Section of muscle biopsy from a patient with sepsis/MOFS showing capillary endotelial cell infoldings to the lumen (arrowheads), basement membrane thickened (asterisk), pericyte hypertrophied (star) and a macrophage prolongation (arrow). x 10,000

suggest that necrosis is produced in association with capillary abnormalities including oclusion and necrosis. However, some authors attribute necrosis to nerve lesion (Zochodne et al., 1987; Wokke, 1988). We have only found one patient with nerve injury, in contrast with capillary lesions in all the patients. This would suggest that muscle changes are secondary to circulatory problems.

As in the work of Helliwell et al. (1991) and similarly to other inflammatory myopathies (Finol et al. 1990, 1994), in our case the cell infiltrate was abundant.

In conclusion, this study shows that patients with sepsis/MOFS exhibit muscle disorder characterized by oedema, atrophy, segmental necrosis and infiltrate, as the histopathological bases for the muscular weakness and wasting in these patients.

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