ULTRASTRUCTURAL ASPECTS OF EQUINE EXERTIONAL RHABDOMYOLYSIS

Aspectos ultraestructurales de la rabdomiólisis por ejercicio en el equino

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ABSTRACT

An electron microscope study was carried out with left *Gluteus medius* muscle samples, of fifty-one (51) mares (3-4 years old). Sixteen (16) of these were inactive (approximately 4 months), and with no signs or symptoms of exertional rhabdomyolysis ER (control group), the other thirty-five (35) mares with (ER), were in training at the "La Rinconada" Racetrack (Caracas-Venezuela). Fifteen (15) had sub-clinical phases of the disease, three (3) showed clinical episodes, and seventeen (17) showed recent episodes (approximately 8 days) of ER (chronic periods of the syndrome). Three phases of the process were observed in the left *Gluteus medius muscle*: Phase 1 atrophy, phase 2 segmental necrosis and phase 3 regeneration. In some cases all three phases were found in the same animal. Ultra-structural aspects of these three phases are discussed and related to some possible etiologic mechanisms.

Key words: Thoroughbred racehorses, skeletal muscle, ultrastructural changes, exertional rhabdomyolysis.

RESUMEN

Se realizó un estudio de microscopía electrónica, con muestras musculares del *M. G. medius* izquierdo, de cincuenta y un (51) yeguas de edades comprendidas entre 3-4 años. Dieciseis (16) de estos animales se encontraban inactivos (aproximadamente 4 meses) y sin signos o síntomas de rabdomiólisis por ejercicio RE (caballos control), las otras treinta y cinco (35) yeguas con (RE), se encotraban entrenando en el Hipódromo "La Rinconada" (Caracas-Venezuela). Quince (15) de estos animales, presentaron episodios subclinicos de la enfermedad, tres (3) mostraron signos de episodios clínicos y diecisiete (17) con un episodio reciente (aproximadamente 8 días) de RE (período crónico del síndrome). Tres (3) fases del proceso fueron observadas en el músculo glúteo medio izquierdo: Fase 1 atrofia, Fase 2 necrosis segmental y Fase 3 de regeneración. En algunos casos las tres fases se encontraron en el mismo animal. Los aspectos ultraestructurales de las tres fases son discutidos y relacionados a algún posible mecanísmo etiológico.

Palabras clave: Caballo Pura Sangre de Carrera, músculo esquelético, cambios ultraestructurales, rabdomiólisis por ejercicio.

INTRODUCTION

Rhabdomyolysis in association with exercise has been recognised as a major cause of disability in horses for more than a century [80]. Exertional rhabdomyolysis in horses is characterised by signs of muscular pain, stiffness, and cramping. Moreover, it has been described exercise intolerance, poor performance, and high serum levels creatine kinase (CK) and aspartate transaminase (AST) activities (enzyme markers of skeletal tissue damage). In extreme instances, it has been reported reluctance to moving (during or after mild to moderate exercise) and visible myoglobinuria [5, 8, 9, 34, 40, 53, 70, 71, 73, 74, 77, 78, 79, 80, 81].

The aetiology of rhabdomyolysis in horses have generated numerous and conflicting opinions and hypotheses, probably owing to the complexity of this disease, and very likely its multifactortial origin, therefore, in many cases the cause and pathogenesis are unknown 8, 9, 40, 70. However exertional rhabdomyolysis in equine has been associated with certain causes for the condition: basis genetic [8, 77], vitamin E and selenium deficiency [8, 35], electrolyte imbalance [8, 78],

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abnormal calcium regulation [54], and stages of oestrus cycle 33 among of other causes.

Different authors [5, 53, 62, 79, 80] have described morphological alterations of horse skeletal muscle. Valberg et al [80] correlated the severity of histological alterations with the presence of elevated amounts of aspartate aminotransferase (AST: EC 2.6.1.1) and creatine kinase (CK: EC 2.7.3.2), and myoglobin in urine. Unfortunately the histopathological findings of these authors do not provide a systematic description of ultrastructure changes in muscle fibres from the horses with rhabdomyolysis, and neither correlated them with the different phases disorder.

The purpose of this study was to describe the evolution process of exertional rhabdomyolysis in its different phases, in the *Gluteus medius* muscle of Thoroughbred racehorses, and to correlate the histological alterations severity with clinical sings severity.

MATERIALS AND METHODS

Horses

Fifty-one (51) mares between 3-4 years-age, were used in this study, sixteen (16) were inactive (approximately 4 months) and no signs or symptoms of exertional rhabdomyolysis (ER) was the control group. These animals were, scatted in a farm near Belen (Carabobo-Venezuela). The other thirty-five (35) ER were evaluated at the race track "La Rinconada" (Caracas-Venezuela), from which fifteen animals (15) had subclinical episodes, three (3) showed clinical signs (clinical episodes), and seventeen (17) horses had a recent (approximately 8 days) history of ER (chronic period of the syndrome).

Biopsies

Biopsies were obtained percutaneously from the left middle gluteal muscle with a needle [10], according to technique described by Lindholm Piehl [52], taken at a depth (6 cm) and at a standard site (18 cm along a line from the dorsum of the tuber coxae to the base of the tail) [55].

Transmission Electron Microscopy technique:

Muscle blocks of approximately 2mm diameter were fixed in cold glutaraldehyde phosphate buffer (pH 7.4 and 320mOsmol), postfixed in 1% OsO4, ethanol dehydrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed in a Hitachi H-500 transmission electron microscope, with 100kV acceleration voltage. Thick sections (1-2 m) were stained with toludine blue for light microscopy.

Morphometric methods

Measurements of thickness of Z line and mitochondria means area were carried out on each micrograph electron by using image-analysis system LADD Microcomputer (Graphic Data Analyser System).

Statistical analysis

Quantitative results of means mitochondria area and thickness of Z line are expressed as means (SD). Student's *t* test 63 was used to compare group of horses (Group I and II).

RESULTS

Ultrastructural aspects of skeletal muscle of healthy equine

The ultrastructural studies of the left*Gluteus medius muscle* from control Thoroughbred racehorses allowed to identify three types of skeletal muscle fibres (I, IIA, and IIB). The criterions used for identify these types of fibres, were the proposed by Finol 32, such criterions are: trace and thick of Z line, presence of M line, disposition of thin filaments around thick filaments, in the level of A band, development of sarcotubular system, number of mitochondria, and presence of lipid droplets. The statistical analysis of Z line thickness of, showed Z line of fibre Type I was 140 35 nm of thick, Type IIA of 110 29 nm, and IIB presented 70 18 nm. Additionally other differences between fibres Type IIB and IIA, were that fibre Type IIA, showed mitochondria in intermyofibrillar and subsarcolemmal spaces, FIG. 1. The presence of lysosomal structures in these specimens (lipofuscine granules and myelin like figures) was evident see FIG. 1.

Ultrastructural aspects of equine with exertional rhabdomyolysis

The ultrastructural study of *Gluteus medius muscle* from ER Thoroughbred racehorses showed that extreme physical exercise produces similar alterations to those seen in other cases of skeletal breakdown atrophy, degeneration and regeneration of affected muscle fibres. The histopathologic findings in acute and chronic rhabdomyolysis allowed three phases to be separated: phase 1 corresponding to the subclinical rhabdomyolysis, phase 2 Rhabdomyolysis with necrosis, and the phase 3 o regenerative observed in the chronic period of the syndrome.

The initial phase is characterised by atrophy, FIGS. 2 and 3. In areas devoid of myofibrils were located mitochondria with different densities, shape of crests, and size, surrounded by abundant glycogen particles, FIGS: 2, 3, 4, 5, TABLE I. Moreover it was observed in some cases, intramitochondrial granules, FIG. 6. Lysosomes were abundant including autophagic vacuoles, FIG. 2, both primary and secondary lysosomes, FIG. 4, glycogenosomes, residual bodies, and lipofuscine granules, FIG. 7, and myelin-like figures, FIG. 6. The terminal cisterns showed swollen, FIG. 5. Atrophy occurred frequently without any visible disorganisation of sarcomeric struc-

ture FIGS. 3,4 and 7. However in some cases irregularities of the Z line were observed, FIGS.4 and 7.

Capillaries showed, in this phase, an endothelial proliferated cytoplasm with abundant surface enfolding to the lumen being present, FIG. 8, endothelial cell surface and almost occulted lumen FIG. 9. It was also observed, thickening of basement membrane, FIG. 9.

Phase 2 was characterised by segmental necrosis, FIGS. 10, 11, 12, and 13, it is which was associated with hypercontraction FIG. 10, 11, 12 and 13, and loss of sarcolemma, FIGS. 11, 12 and 13. Necrotic fiber areas were usually found next to degenerative capillaries, FIG. 12. Plasmocytes, macrophages and lymphocytes formed the infiltrate, FIGS. 14 and 15.

In phase 3, a process of partial regeneration is seen in areas of previous necrosis. In these areas it was possible to locate a proliferation of myofilaments in association with free polysomes, FIG. 15. Additionally, activated satellite cells were also observed, FIG. 16.

DISCUSSION

Coinciding with clinical alterations, horses were observed having different ultrastructural abnormalities, which included changes in sarcotubular and contractile systems. These were descriptions made for sub-lethal 34, 74 and lethal 6, 17, 18, 22, 39, 45, 53, 57, 60, 84 myodegeneration post-exercise. In the present study alterations varied from mild to complete segmental necrosis of both system. Interestingly, in some horses three phases of the syndrome were present in the same animal. This finding confirms previous observations 4, 6, 22 that support the conclusions that the appearance of three phases in the same horse respond to the presence of myofibrils more susceptible to exercise-induced damage (stress-susceptible) 22, and of fibres with a history recent of injury pre-exercise 4, 6.

Ultrastructural changes of contractile system (loss of myofilaments, Z line streaming, myofiblillar disruption) observed in this study, FIGS. 2, 3, 4, 5, 6, 10, 11, 12 and 13, have been described in exertional rahdomyolysis by other authors in humans 4, 6, 22, 34, 39, 61, 84, rats [49, 76] and horses 5, 8, 53, 74, 80. Similar observations have been described experimentally in different reactions of skeletal muscle, such as: amyotrophic lateral sclerosis 1, denervation atrophy 14, 19, 31, 67, tenotomy 83, simultaneous effect of denervation and tenotomy 28, simultaneous effect of denervation and chloroquine 51, 82, experimental ischemic myopathy 46, induced thyrotoxic myopathy 48, in experimental myopathy induced by *Mycobacterium leprae* 12, in experimental myopathy induced by castration 44], and in experimental myopathy induced by energy deficient diets [59]. Similar alterations in the contractile system have been described in diseases such as: Cussing´s syndrome 68, hyperthyroid patients 30, 56, during paranoplastic phenomen 23, 24 myopathy by distance effect by *Trypanosoma evansi* 69, rheumatoid myositis [29], murine dystrophy 64, deficiency of vitamin E and selenium [35, 47].

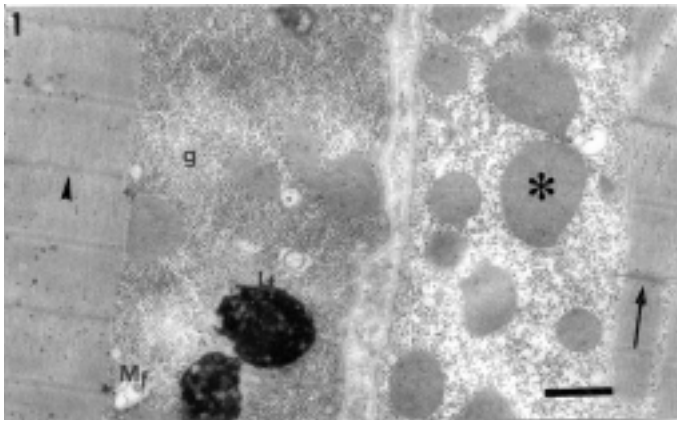

FIGURE 1. ELECTRON MICROGRAPH, OF A LONGITUDINAL SECTION OF MUSCLE FIBRE, SHOWING TWO TYPE OF FIBRES. IN THE RIGHT SIDE IT IS EXHIBITS A FIBRE TYPE IIA. NOTE THE PRESENCE OF Z LINES WITH STRAIGHT AND THIN TRACE (arrows). IN THE LEFT SIDE IT IS EXHIBITED A FIBRE TYPE IIB. NOTE THE PRESENCE OF Z LINE, WITH STRAIGHT AND VERY THIN TRACE (arrowhead). ADDITIONALLY, IN SUBSARCOLEMMAL SPACES, CAN BE LOCATED LIPOFUCSIN GRANULES (Lf), MYELIN-LIKE FIGURE (Mf), MITOCHONDRIA (asterisk), AND ABUNDANT GLYCOGEN PARTICLES (g)
MAGNIFICATION 16.800 X.
IN THIS AND ALL FIGURES BAR = 1m.

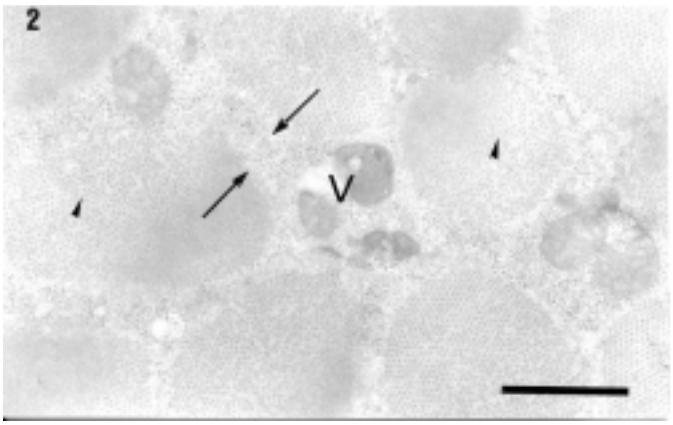

FIGURE 2. ELECTRON MICROGRAPH, SHOWING A CROSS SECTION OF FIBRE TYPE IIA. POINTING WITH ARROWS, A WIDE INTERMYOFIBRILLAR SPACE. WHILE THAT POINTED WITH ARROWHEAD, SPACES WITHOUT MYOFILAMENTS. NOTE THE PRESENCE OF SOME AUTOPHAGIC VACUOLES (V).
AGNIFICATION 22.480X.

It is noteworthy to mention that the decrease of contractile elements happened in phase 1, occurred without disorganisation of I band, as in amyotrophic lateral sclerosis 1, neurogenic atrophy 67, or with the loss of A band, and the conservation of I band and Z line, as in the hypokalemic periodic paralysis 41.

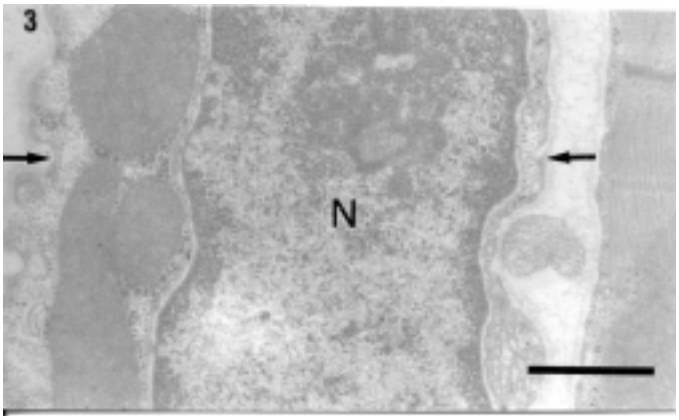

FIGURE 3. ELECTRON MICROGRAPH, SHOWING A LONGITUDINAL SECTION OF AN ATROPHIC SKELETAL MUSCLE FIBRE (between arrows). NOTE THE PRESENCE OF A MYONUCLEUS (N).
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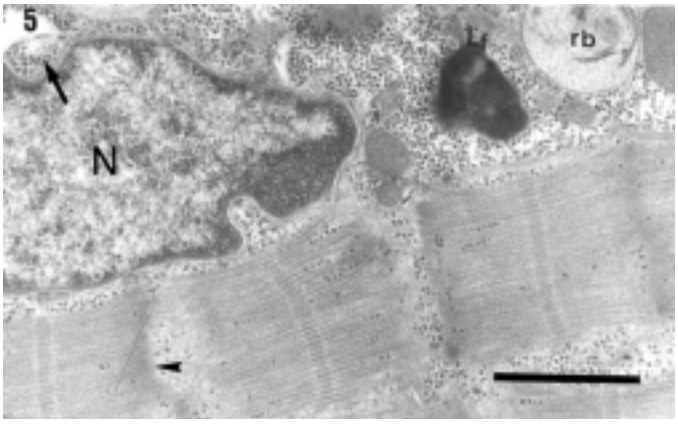

FIGURE 5. ELECTRON MICROGRAPH, SHOWING A LONGITUDINAL SECTION OF SKELETAL MUSCLE FIBRE. NOTE THE PRESENCE OF LIPID DROPLETS (L), MYELIN-LIKE FIGURE (Mf), AND INTRAMITOCHONDRIAL GRANULES (arrows).
MAGNIFICATION 28.100X.

Alterations in sarcotublular system dilatation, FIG. 6, fragmentation, and vesiculisation, FIG. 12, showed in this study, coincide with descriptions of these changes in other such myodegenerative processes of different aetiology such as: dermatomyositis in childhood 7, 17, diseases of collagen 17, systemic lupus erythematosus 27, some autoimmune nervous diseases 26, hyperthyroid patients 30, and hypokalemic periodic paralysis 41. Additionally, there have been reported such damages, in experimental myopathy induced by *Mycobacterium leprae* 12.

The presence of mitochondria with different electron density, (electron dense and electron lucid: swollen mitochondria), FIGS. 4, 5 and 11, and sizes (swollen mitochondria, were larger (P<0.0025) than mitochondria of medium size correspond to Group I), TABLE I, in the material, could be linked to:

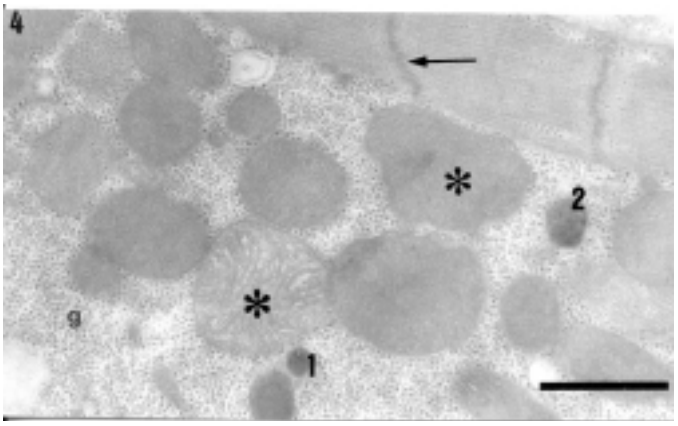

FIGURE 4. ELECTRON MICROGRAPH, IT IS EVIDENT A DRASTIC DIMINUTION OF MYOPHILAMENTS. IN THE WIDE SARCOLEMMAL SPACE, THERE ARE LYSOSOMES IN DIFFERENT LEVELS OF DIGESTION (PRIMARY-1 AND SECONDARY-2 LYSOSOMES). NOTE THE PRESENCE OF ABUNDANT GLYCOGEN PARTICLES (g), AS WELL AS, MITOCHONDRIA WITH DIFFERENT DENSITIES AND CRESTS (asterisks). SOME IRREGULARITIES OF Z LINE TRACE ARE EVIDENT (arrow).
MAGNIFICATION 22.480X

TABLE I
MEAN SD, MEAN AREA OF MITOCHONDRIA, AND MEAN AREA OF DIFFERENT TYPES OF MITOCHONDRIA BY SIZE (m²) EXISTENT IN *Gluteus medius* MUSCLE OF A SAMPLE FROM FIFTY-ONE (51) VENEZUELAN THOROUGHBRED RACEHORSES (3-4 years old). Group I, HEALTHY CLINICALY AND INACTIVE. Group II WITH EXERTIONAL RABDOMIOLISIS

	Mitochondria		
Groups of horses	Mean area of large size (µm²)	Mean area of medium size (µm²)	Mean area of small size (µm²)
Group I (n= 16)	0.90 ± 0.26	0.42 ± 0.08	0.58 ± 0.08
Group II (n= 35)	1.68 ± 0.75 **	0.75 ± 0.36 **	0.61 ± 0.06

**P<0.025

normal metabolic changes (reversible ultrastructural changes in metabolic steady states oscillating between orthodox and condensed conformations) 37, and in mitochondrial myopathy of different aetiology 3, 17, 45, 78, 79. Alteration in electron density in mitochondria has been described also in: tenotomy 83, ischemia 42, 46, thyrotoxic myopathy 48, myopathy by effect at distance of *Trypanosoma evansi* 69, experimental denervation 31, 67, steroid myopathy 68, myopathy associated to paraneoplastic phenomenon 23, 24, in hypertrophic cardiomyopathy [20], and lactic acidosis induced by exercise 8, 15, 53, 62, 80. Valberg et al 80, have indicated that some changes of electron density in mitochondria may occur in biopsy speci-

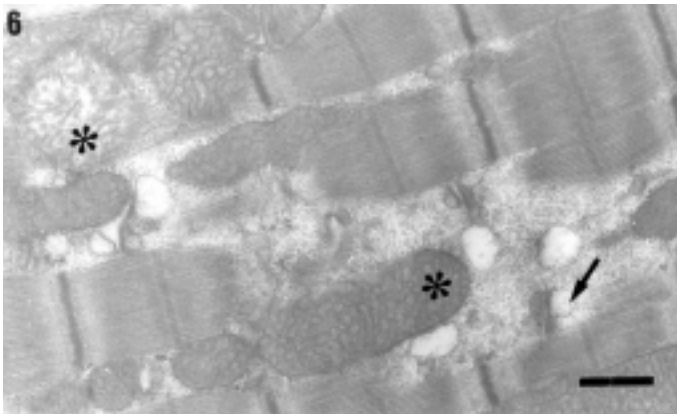

FIGURE 6. ELECTRON MICROGRAPH, SHOWING A LONGITUDINAL SECTION OF SKELETAL MUSCLE FIBRE. IN SUBSARCOLEMMAL SPACE, CAN BE SEEN LIPOFUSCIN GRANULES (Lf), GLYCOGENOSOME (arrow), RESIDUAL BODY (rb), AND NUCLEUS (N). THE ARROWHEAD SHOWS IRREGULARITIES OF Z LINE TRACE.
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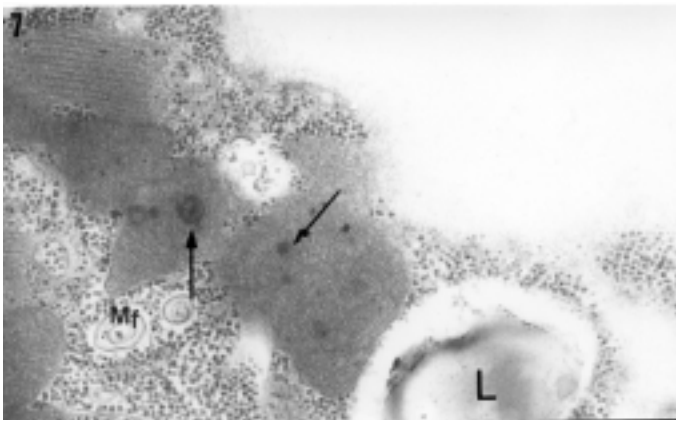

FIGURE 7. ELECTRON MICROGRAPH, SHOWING A LONGITUDINAL SECTION OF SKELETAL MUSCLE FIBRE. IN SUBSARCOLEMMAL SPACE, CAN BE SEEN LIPOFUSCIN GRANULES (Lf), GLYCOGENOSOME (arrow), RESIDUAL BODY (rb), AND NUCLEUS (N). THE ARROWHEAD SHOWS IRREGULARITIES OF Z LINE TRACE.
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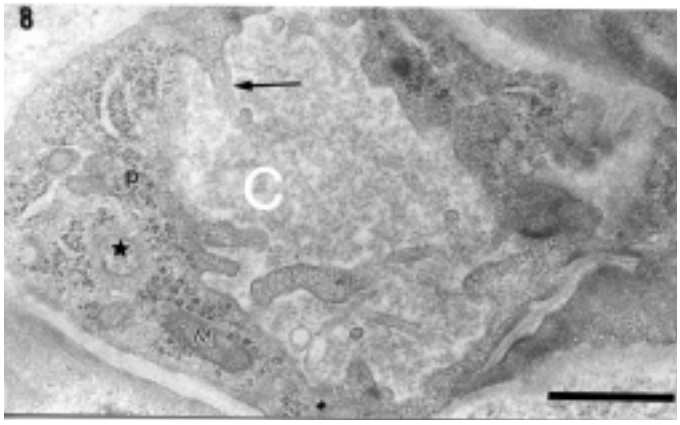

FIGURE 8. ELECTRON MICROGRAPH, THE CAPILLARY (C), IS SHOWING SOME ENDOTHELIAL CYTOPLASMATIC PROLONGATIONS IN TO WARD THE LUMEN (arrow). THE ENDOTHELIA CELL CYTOPLASM EXHIBITS A MULTIVESICULAR BODY (star), NUMEROUS POLYSOMES (P), AND MITOCHONDRIA (M).
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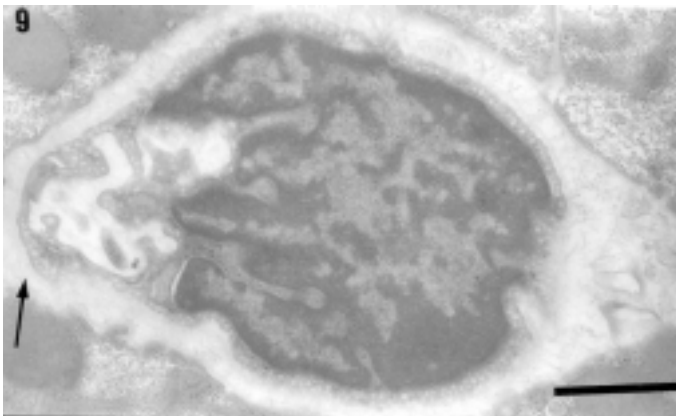

FIGURE 9. ELECTRON PHOTOMICROGRAPHY, A PARTIAL OCCLUSION OF CAPILLARY´S LUMEN CAN BE SEEN. NOTE THE THICKENING OF THE BASEMENT MEMBRANE (arrow).
MAGNIFICATION 22.480X.

mens prepared for electron microscopy (such as it is exhibited in a study of Nimmo and Snow 62). In samples of skeletal muscle from horses after exercise, as in the present study, this could not account for the lesions observed in horses with rhabdomyolysis, since little fixation artefacts occurred in biopsies from control horses, FIG. 1. Moreover the buffer used in present study, was pH 7.4 with an osmolarity of 320mOsmol.

In the other hand, alterations of cellular homeostasis due to an increment in cytoplasmic calcium concentration could have produced, the presence of intramitochondrial granules 74, (such as exhibited in the FIG. 6). This alteration of homeostasis has been associated with its abnormal regulation 54, maybe due to ultrastructural damage of sarcolemma after exercise 17, 39, 45, 61, 65, 66, 80, 84, as well as dysfunction of the sarcoplasmic reticulum (by depletion of ATP, pH and temperature), leading all these events to an increment in cytoplasmic [Ca^{+}] and eventually muscular necrosis 80, 86. Similar observations have been reported in: ischemic myopathy 46, deficiency of vitamin E and selenium 35, and exercise in horses 53, 74.

It is noteworthy the finding of giant mitochondria, in samples of skeletal muscle from horses of Group II, FIG. 10. These mitochondria, were larger ($P<0.0025$) than mitochondria of big size corresponding to Group I, TABLE I. Giant mitochondria (megaconial mitochondria), were first described by Pellegrini et al 66 in the myopathy after his name. They have been also identified in tyrotoxic myopathy 50, equine myopathy by distance effect by *Trypanosome evansi* 69, experimental myopathy associated with deficiency of energy diets 59, in ischemic

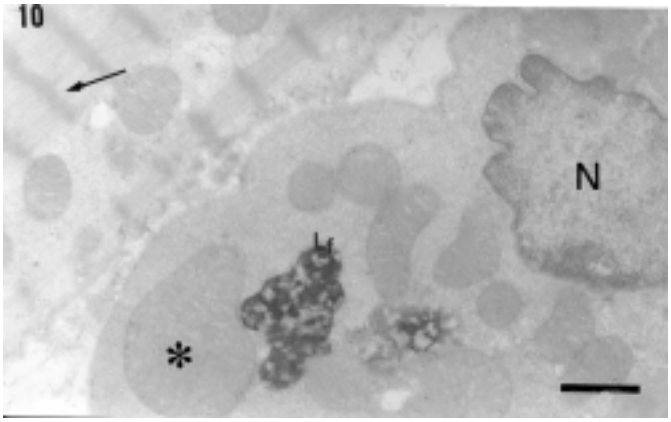

FIGURE 10. ELECTRON MICROGRAPH, IT IS SHOWING A WIDE SUBSARCOLEMMAL REGION, WITH GIANT MITOCHONDRIA (asterisk), LIPOFUSCIN GRANULES (Lf) AND A MYONUCLEUS (N). NOTE THAT MYOFIBRILS ARE HYPERCONTRACTED (arrow)
MAGNIFICATION 14.050X.

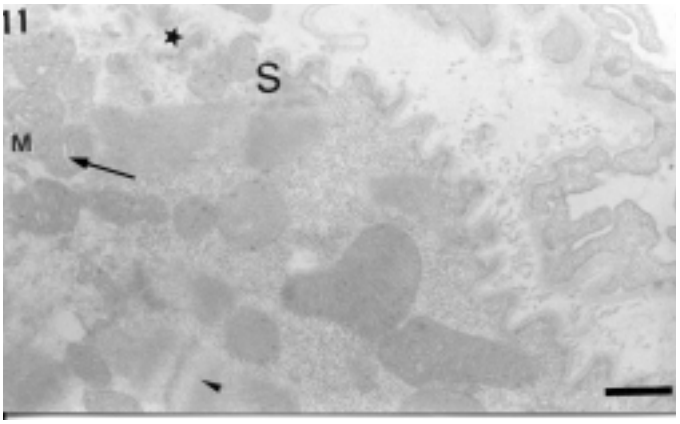

FIGURE 11. ELECTRON MICROGRAPH, IT IS EVIDENT A MORE ADVANCED DEGREE OF THE PROCESS OF MUSCULAR DEGENERATION. MYOFIBRILS ARE HYPERCONTRACTED (arrowhead), MITOCHONDRIA (M) EXHIBIT DIFFERENT DENSITIES, AND SWOLLEN CRESTS (arrow). IN THE SARCOLEMMA (S), IT IS NOTABLE THE DIFFERENT LEVELS OF FOLDING AND LOSS OF IT IS STRUCTURE (star).
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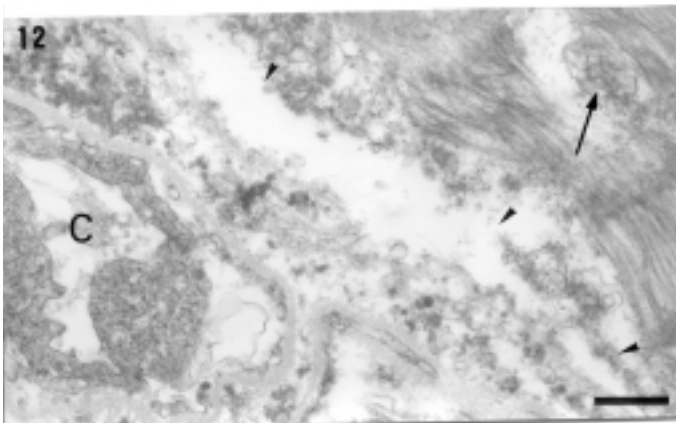

FIGURE 12. ELECTRON MICROGRAPH, THE DEGREE OF THE PROCESS OF MUSCULAR DEGENERATION (SEGMENTAL NECROSIS), SHOW IT SELF-MORE EVIDENT THAN IN THE PREVIOUS ELECTRON PHOTOMICROGRAPHY. NOTE THE DISORGANISATION OF SARCOMERA, AND LOSS OF SARCOLEMA (arrowheads). IT IS NOTABLE THE PRESENCE OF INTRAMUSCULAR CAPILLARIES (C) AND VERY DEGENERATED MITOCHONDRIA (arrow).
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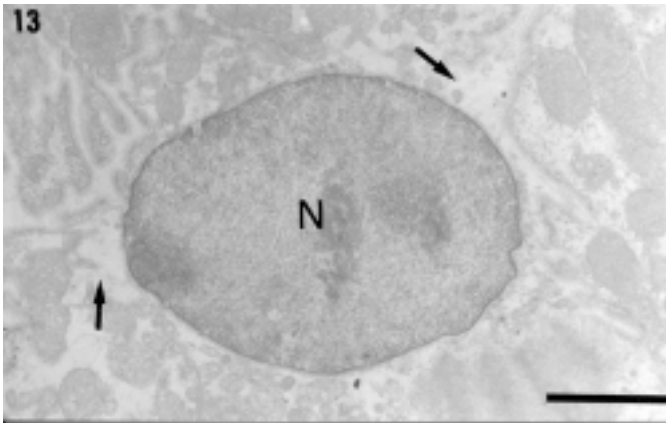

FIGURE 13. ELECTRON MICROGRAPH, THERE CAN BE SEEN A NECROTIZED AREA OF A MUSCULAR FIBRE, IN WHICH IT IS OBSERVED A ROUND NUCLEUS. THE ARROWS POINT TO THE LOSS OF SARCOLEMMA.
MAGNIFICATION 22.480X.

myopathy 38, and experimentally induced in pancreatic exocrine cells by effects of ethanol and iron [75].

Changes in the sarcoplasmic structure were accompanied by presence of primary and secondary lysosomes, FIG. 4, Myelin-like figure, FIG. 6, and vacuoles formed with mitochondrial debris, FIG. 2, and glycogen particles, FIG. 7, and have been described by others 11, 12, 13, 26, 36, 51, 72, 82, 83. In the case of autophagic vacuoles formed with particles or glucogenosomes, FIG. 7, they have been considered as a predisposing factor of rhabdomyolysis in human 13 and horses 8, 77, 78. Additionally, glucogenosomes have been observed in different pathological conditions, in experimental simultaneous denervation and tenotomy 28, in experimental reinnervation 36, and some autoimmune nervous diseases 26, all of which suggest that its abundance could represent an unspecific response to muscle aggression. The finding of such structures has been considered pathologic 13, 18, 67, although according to Márquez and Finol 58, presence of glucogenosomes does not represent a criterion of pathological condition, as it could just represent the abundance of theses organelles.

Lipofuscine granules have been described in normal skeletal muscles, and have been associated with age 87, and with lipid peroxidation production after strenuous exercise 50, 74. The presence in this study of lipofuscine granules and other lysosomal structures in control horses, FIG. 1, as well in

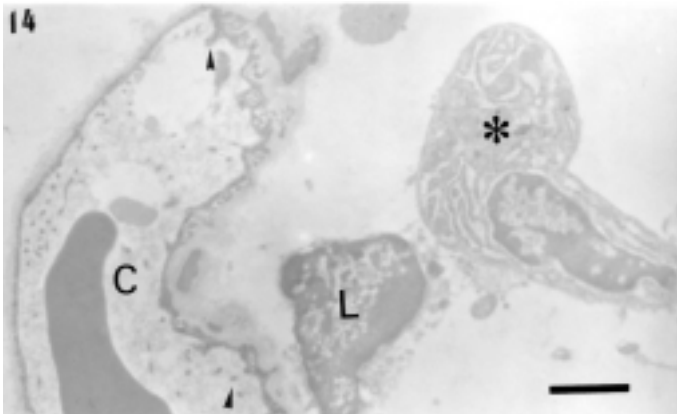

FIGURE 14. ELECTRON MICROGRAPH, NEXT TO THE CAPILLARY (C) A NORMAL PLASMOCYTE (asterisk) AND A LIMPHOCYTE (L), ARE EXHIBITED. NOTE THE PRESENCE OF ENDOTHELIAL CELL SURFACE PROLONGATIONS TO THE LUMEN (arrowheads).

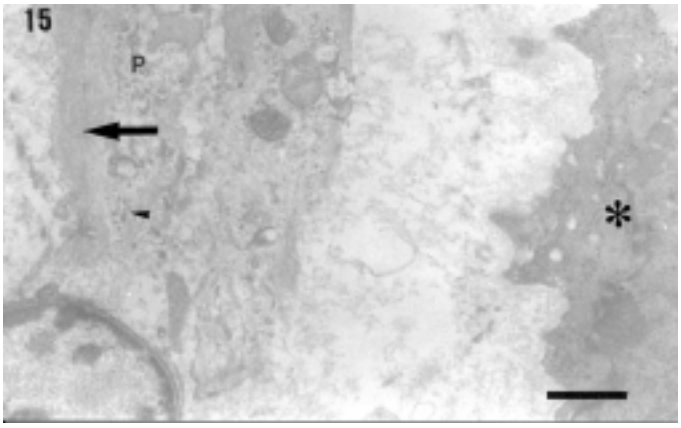

FIGURE 15. ELECTRON MICROGRAPH, OF A MUSCULAR SECTION IT IS OBSERVED AN AREA OF REGENERATION. THE PROLONGATION OF MUSCULAR FIBRE IN REGENERATION SHOWS GROUPS OF MYOFILAMENTS (arrow), FREE POLISOMES (P) AND ROUG ENDOPLASMIC RETICULUM CISTERNAE (arrowhead). NOTE THE PRESENCE OF A MACROPHAGE (asterisk).
MAGNIFICATION 14.050X.

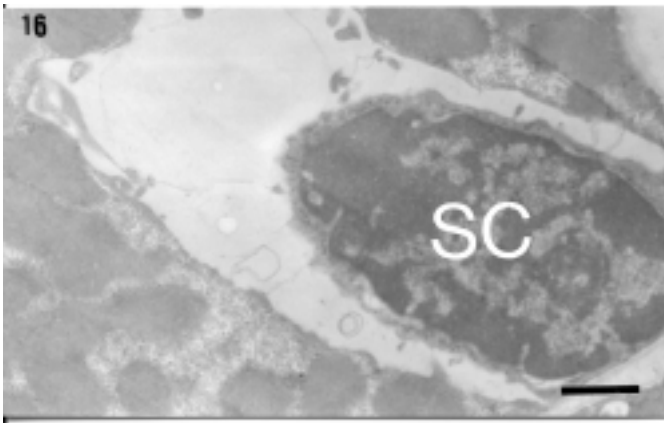

FIGURE 16. ELECTRON MICROGRAPH, IT IS FOUND A ACTIVE SATELLITE CELL (SC), PARTIALITY SEPARATED FROM SKELETAL MUSCLE FIBRE.
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horses with rhabdomyolysis, FIGS. 7 and 10, confirms previous observations 11 that indicate that only in cases where considerable increments of the presence of these elements, could be considerate pathological.

The existence of segmental necrosis has been associated in human patients with exertional rhabdomyolysis, with loss of sarcolemmal integrity 4, 6, 39, 65, 84. Sarcolemmal discontinuities have been observed in many myodegenerative processes including ischaemia 46, murine dystrophy 64, and selenium and vitamin E deficiencies 35, and phase 2 of equine exertional rabdomyolysis, FIGS. 11, 12 and 13. In this last case, necrosis was probably related to ischaemia, because myodegenerative areas were formed next to damaged capillaries, FIG. 12.

The level of severity of alterations in intramuscular capillaries was similar to the observed in muscular fibres. Among of the changes showed, is noteworthy to mention reduplication and thickening of basement membrane, FIG 9, proliferation of capillary endothelium, FIG. 8, endothelial cytoplasmatic prolongation to the lumen, FIGS. 8, 9 and 14, and necrosis of capillaries, FIG: 12.

The reduplication and thickening of the basement membrane, have been described in autoimmune diseases with muscular compromise as: rheumatoid myositis 29, paraneoplastic phenomenon 23, 24, systemic lupus erythematosus 25, systemic sclerosis inflammatory myopathy 25, dermatomyostis of childhood [7] and dermatomyositis in adult [21], polymyositis [43], and atrophy by denervation with an autoimmune component 13.

The proliferation of capillary endothelium in some capillaries observed in this study coincides with its description in other pathological processes in muscle such as: equine myopathy by *Trypanosome evansi* 69, thyrotoxic myopathy 30.

Regarding endothelial cytoplasmaic prolongations to the lumen, and the partial occlusion of it, coincides with previous 88 observations of samples of skeletal muscle taken from rats submitted to high intensity exercise.

The finding of necrosis capillary it a finding coincided with segmental necrosis in muscle fibres. Also it has been observed in other myodegenerative processes as: systemic lupus erythematous 27, rheumatoid myositis 29, atrophy by denervation with an autoimmune component 13, paraneoplastic phenomen 23, 24, equine myopathy by *Trypanosome evansi* 69, and thyrotoxic myopathy 30.

In equine skeletal muscle damage by exercise, macrophages, lymphocytes and plasmocytes, FIGS. 14 and 15, formed cellular infiltrate. This finding is similar to that reported in acute rhabdomyolysis in humans [4, 6, 22]. Such cellular infiltrate has been described in different inflammatory and autoimmune disorders in humans [23, 24, 25, 27,29, 30]. However,

in most of the mentioned works mast cells were often found, while in the equines with exertional rhabdomyolysis they were not identified.

It´s well documented that exercise-induced damage and reparation, are adaptations of skeletal muscle to exercise [16, 22, 57, 60, 64, 81, 84]. The electron microscopy studies have centred its analysis on identifying areas with the presence of polysomes in zones of myofilament formation [2, 45, 57], and satellite cell activation [60, 85]. In acute humans rhabdomyolysis a great amount of muscle fibres are in all stages of segmental necrosis and regeneration [45, 57]. Muscle fibre regeneration seen in the present study was only partial, FIGS. 15 and 16. It could be possible that a complete regeneration was avoided by a compression effect of underlying connective tissue as suggested by Allbrook [2]. But it has also been suggested that the continuing effect of a noxious influence might cause partial muscle regeneration [45]. This situation could be similar to that provided in the equine with excessive exercise.

CONCLUSIONS

Pre race physical inspection findings (particularly abnormalities of musculoskeletal system) and the suitable selection of intensity of racing and training schedules, represent methods for preventing severe musculoskeletal injuries. Related with these last asserts, results reported here suggest to possible existence of a direct relationship between response of skeletal muscle with intensity of exercise and training schedules. Finally, skeletal muscle in Thoroughbred racehorses may provide a convenient model for further studies in skeletal muscle injury and repair.

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