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Muscle regeneration after exercise-induced myoglobinuria: an electron microscopic study

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Abstract. Muscle regeneration was studied by light and electron microscopy in a case of exercise-induced acute myoglobinuria in a young patient with carnitine-palmityl-transferase deficiency. Various stages of regeneration existed in the foci of necrosis scattered throughout apparently normal muscle. Activated satellite cells, myoblasts and myotubes were found, some of them containing myofibrils. Among the cells accumulating in the necrotic fibres, some apparently contained surviving myonuclei. In some fibres of normal size, developing myofibrils were abundant. Surviving myonuclei may be of significance in the reaction of muscle cells after injury.

Key words: Muscle regeneration – Myonuclei – Electron microscopy

Introduction

We have followed the progression of muscle necrosis and regeneration after a significant exercise-induced acute myoglobinuria in a young boy with a carnitine-palmityl-transferase defect, using light and electron microscopy. Myolytic foci (microinfarcts) were disseminated throughout apparently normal tissue (Mantz et al. 1992) and in these foci, regeneration at different stages coexisted with more or less complete necrosis. Two weeks later, the muscle was pratically normal.

Since muscle regenerative processes remain a subject of interest and controversy (Carlson 1973; Mastaglia et al. 1975; Allbrook 1981; Cullen and Mastaglia 1982; Carlson and Faulkner 1983; Plaghki 1985), we now report a follow-up of the morphological process of regeneration in this patient. The role of satellite cells is clear but, once more, the problem of the fate of the myonuclei and their possible participation in the process of repair is raised.

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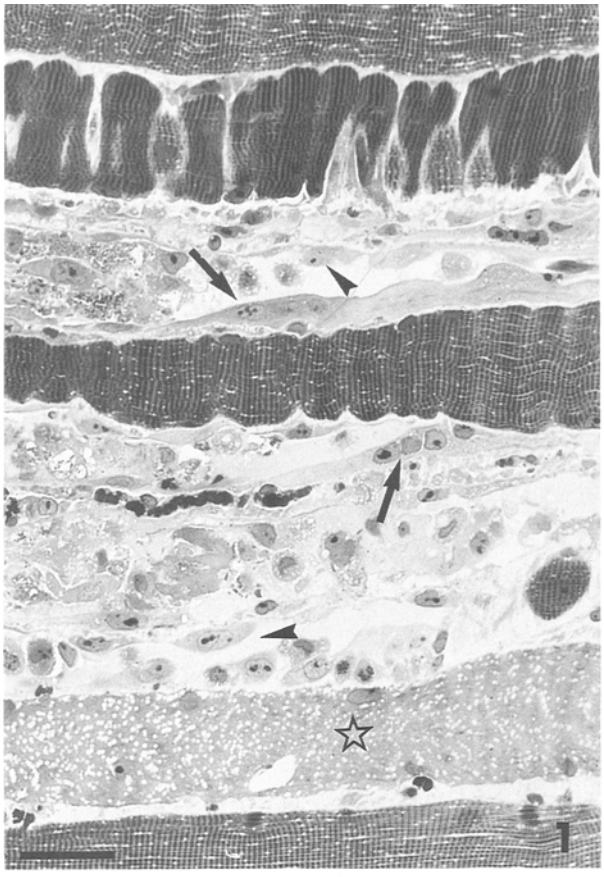
Materials and methods

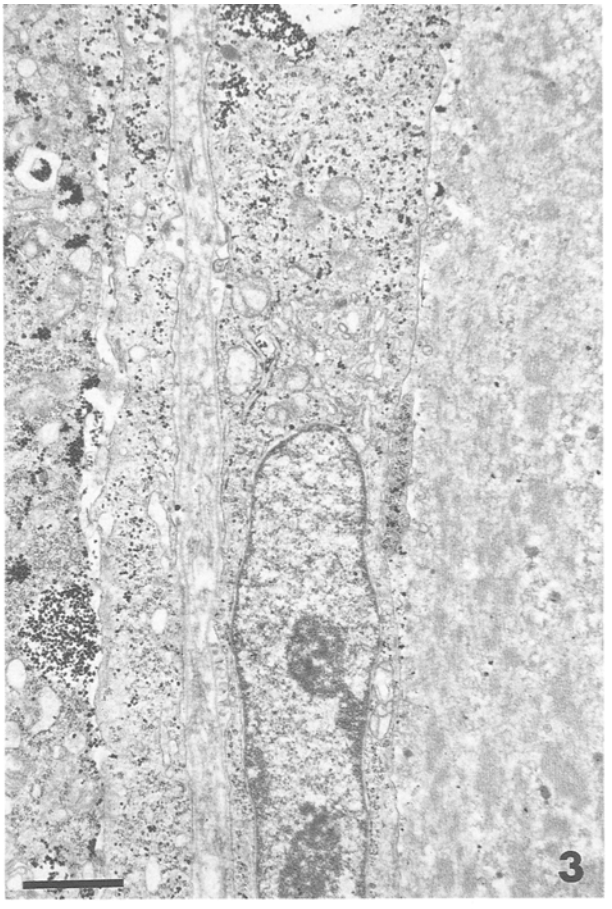
Muscle samples were taken from a biopsy taken from the extensor digitorum of the left forearm, 11 days after causal exercise, with the informed consent of the child's parents. The samples were immersed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed for 1 h with osmium tetroxide in the same buffer, dehydrated in graded alcohols and embedded in an araldite epon mixture. For light microscopy, samples were examined on semi-thin sections (1 μm), stained either with toluidine blue in 5% sodium borate, or with periodic acid-Schiff, acid fuchsin or ferric haematoxylin after removal of the embedding medium with sodium methoxide. For electron microscopy, thin sections were contrasted with uranyl acetate and lead citrate, and examined under a Jeol 100 CX electron microscope.

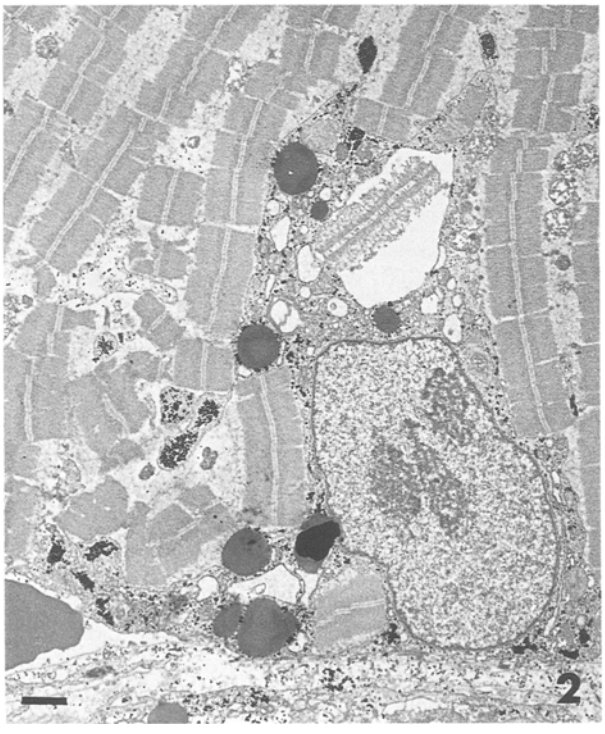
Results

Using light microscopy, myolytic foci with vascular alterations were disseminated throughout normal tissue. In these foci, fibres were at various stages of necrosis. On longitudinal views the fibre lesions were segmental. Inside the most affected fibres, myofibrils were fragmented and a varied cell population was seen: macrophages, erythrocytes, and basophilic cells with a pale nucleus were present and spindle-shaped cells were lying on the internal surface of the myocyte. In addition, myotubes were observed (Fig. 1). Some fibres or segments of fibres were totally empty.

Electron microscopy revealed various stages of necrosis and regeneration. Severely damaged fibres were filled with a disorganized contractile material mixed with remnants of cell organelles. Several types of cells were present including erythrocytes, rarely leucocytes, and numerous macrophages. The cells displaying phagocytic activity were mononucleated with a pale nucleus and prominent nucleolus. They were variable in size and contained dispersed or aggregated glycogen, lipid, granulo-membranous or amorphous inclusions and often membrane-bound fibrillar material (Fig. 2). Elongated cells were observed along the internal side of the basal lamina: the cytoplasm contained lipid droplets, free ri-







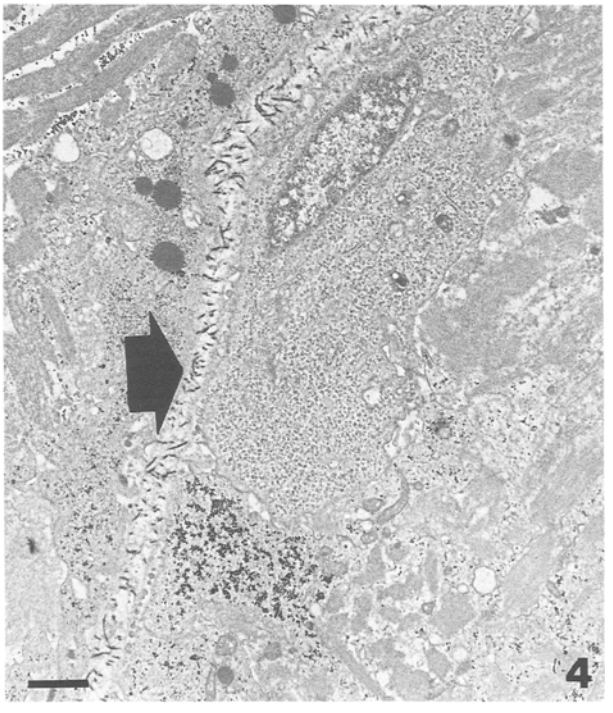


Fig. 1. Longitudinal semi-thin section of a necrotic focus: various stages of myolysis and regeneration are present in the necrotic muscle fibres which contain various types of cells: macrophages, elongating myoblasts *(arrowheads)* extending myotubes *(arrows)*. At the bottom, a fibre (*) has lost striations and contains abundant lipid droplets. The ultrastructure of this fibre is shown in Fig. 8. Toluidine blue, × 800 (*bar* = 50 μm)

Fig. 2 Macrophage under the basal lamina, phagocytosing recognizable myofibrillar debris. × 6,000 (*bar* = 1 μm)

Fig. 3. Cell lining the basal lamina of a necrotic fibre, containing abundant glycogen and a nucleus evoking a myonucleus. × 13,000 (*bar* = 1 μm)

Fig. 4. Activated satellite cell, containing numerous ribosomes, located between the basal lamina and the necrotic debris. × 7,800 (*bar* = 1 μm)

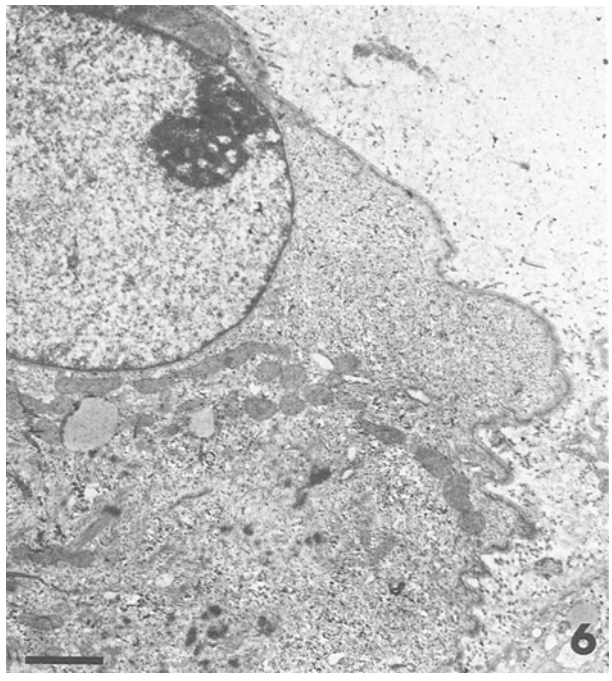


Fig. 5. Myoblasts elongating in oblique position in an otherwise empty necrosed muscle fibre outlined by its basal lamina. × 3,00 (*bar* = 1 μm)

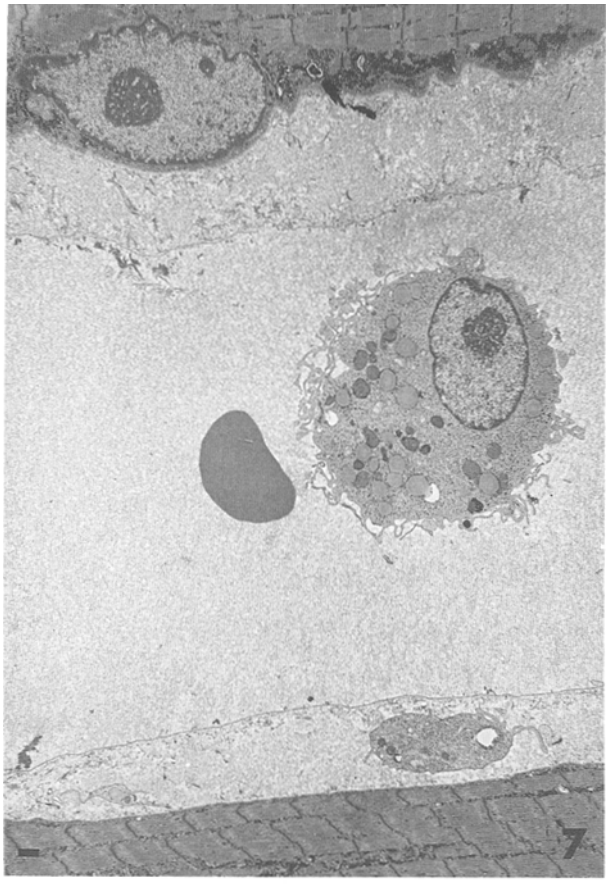
Fig. 6. Disoriented regenerating myofibrils in a myotube. × 10,000 (*bar* = 1 μm)

bosomes, scattered or gathered abundant glycogen particles and occasional membranous inclusions; the elongated, ellipsoid nucleus, parallel to the axis of the fibre, exhibited evenly dispersed chromatin and conspicuous nucleoli which are characteristics of myonuclei (Fig. 3). We did not find altered or necrotic myonuclei anywhere in these injured fibres. Activated satellite cells were found along the internal side of the basal lamina; they displayed a vesicular nucleus and the cytoplasm contained abundant ribosomes, short rough endoplasmic reticulum profiles but no glycogen (Fig. 4). Some of these cells seemed to be migrating inside the fibre. No satellite cell was present outside the muscle fibres in the interstitial space. In completely necrosed fibres, delimited by the basal lamina, myoblasts appeared to be elongating at an angle to the axis of the lamina (Fig. 5). Myotubes were observed and early myofibrils were seen in the larger ones (Fig. 6). Fibres or segments of fibres were empty, or filled with a poorly electron dense substance where only macrophages or an erythrocyte persisted. The fibres were recognizable by the surrounding basal lamina (Fig. 7). In some fibres of normal size that were more distant from the centre of the necrosis, no striation was seen and lipid droplets were abundant. The myonuclei, sometimes internal, were surrounded by disorganized slender myofibrils and numerous mitochondria (Fig. 8).

Discussion

Study of the localized foci of necrosis scattered throughout morphologically normal tissue has permitted us to make a detailed study of muscle regenerative processes. Regeneration was extremely active: in a second biopsy 25 days after the attack of acute myolysis, the muscle was practically normal, with only some scattered fibrous scars.

Regeneration in mammalian muscle remains a subject of controversy in spite of numerous investigations (see Landon 1982; Engel and Banker 1986). The "discontinuous form" of regeneration involving undifferentiated subsarcolemmal satellite cells is supported by tissue culture studies and autotransplantation of minced skeletal muscle. The "continuous form" of regeneration involv-



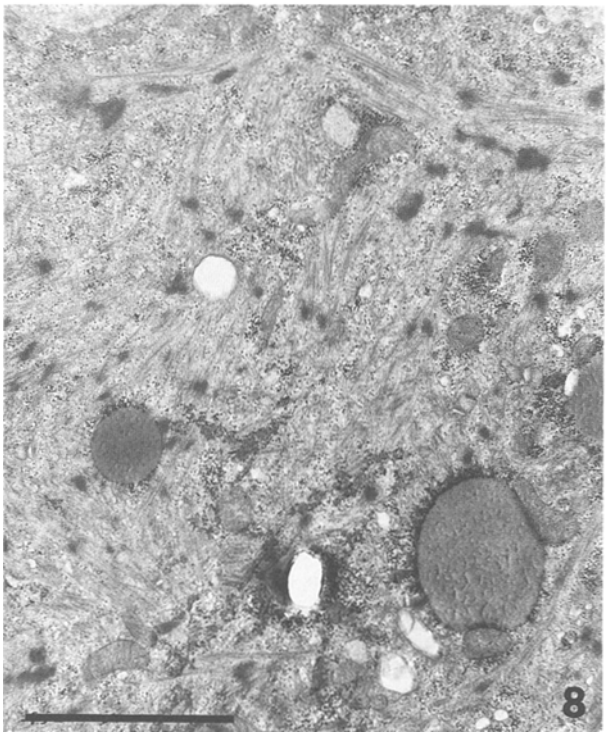


Fig. 7. Completely necrosed fibre, outlined by the basal lamina and containing an erythrocyte and a macrophage, between two apparently normal fibres. × 2,500 (*bar* = 1 μm)

Fig. 8. Disorganized slender myofibrils, probably involved in regeneration, in the large fibre labelled by an asterisk in Fig. 1. × 27,500 (*bar* = 1 μm)

ing surviving myonuclei that have gathered plasma membrane around fragments of remaining cytoplasm is supported by ultrastructural examination of partly damaged muscle fibres in situ (Shafiq and Gorycki 1965). The observations to date are generally considered to be inconclusive in deciding between these two models. Two conditioning factors are worth pointing out in the argumentation of the two types of regeneration: firstly, the intensity of necrosis and, secondly, the completeness of separation of muscle fibres from their vascular and nervous connections.

In our study, the importance of the severity of lesions in the regenerative process of the fibre is clear. In completely necrotic fibres or segments of fibres, nothing remained inside the basement membrane: the fibrous scars, eroding the fibres found in the second biopsy (Mantz et al. 1992), defined these sites. In less severely damaged fibres, activated satellite cells, some of them migrating, were always located inside the fibre. Satellite cells are known to be resistant to noxious influences that damage muscle fibres (Mauro 1961; Terravainen 1970; Snow 1977; Lipton and Schutz 1979; May 1981; Campion 1984; Schutz et al. 1986; Morgan et al. 1987; Ground and McGeachie 1992). They can proliferate in vitro, in autotransplanted minced muscle samples, and in grafts of cellular suspensions into X-irradiated ischaemic muscles (Alameddine et al. 1989). In the present study, myoblasts were observed inside the basal lamina, myotubes were present and the largest ones contained myofilaments. The situation was more complex in the case of partly damaged fibres in segments where myofibrils were fragmenting and where several types of cells – including activated satellite cells and macrophages – coexisted. Interestingly, we did not find any pyknotic nuclei. However, another type of cell which might be derived from a myonucleus which had gathered up plasma membrane was seen. Some authors suggest that these myonuclei can recover their mitotic potentialities (Shafiq and Gorycki 1965) but others have drawn attention to the possible origin of macrophages from such myonuclei in damaged fibres (Reznik 1969; Plaghki 1985). Our observations do not clarify this problem. However, it is interesting to recall that phagocytosis by smooth and striated muscle cells has been observed in vitro (Garfield et al. 1975). It would be of interest to study the fate of labelled myonuclei in vivo under experimental conditions of vascularization and innervation allowing their survival after controlled ischaemia.

In addition, we observed some regenerating fibres or segments of fibres which were of normal size, mainly located at the periphery of the necrotic foci. On light microscopy their striation was lost and lipid droplets were seen to be numerous. On electron microscopy, disorganized slender myofibrils surrounded myonuclei which were more or less centrally located. We did not find any transitional forms between these fibres and the myotubes observed in the necrotic zones. These observations suggest the possibility of recovery of muscle fibres after a slight injury without the help of satellite cells.

In conclusion, the myogenicity of satellite cells was clearly seen in the damaged fibres, but the fate of the

myonuclei after muscle injury remains obscure and their potentialities may be underestimated. It would be of interest to re-investigate their role in phagocytic processes and also in myofibrillar regeneration after slight experimental injury and controlled ischaemia, applying immunohistochemical techniques we could not use in this case.

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