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Sciatic Nerve Crush in Rats and its Impact on Soleus Muscle

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Abstract: Nerve injuries have different consequences on the structure and function of their target muscles. The present research was designed to investigate effects of sciatic nerve crush on the histological structure of soleus muscle in adult male rat. The sciatic nerve was crushed at one cm proximal to its trifurcation. The histological structure of soleus muscle was examined at two, four and eight weeks after crushing the sciatic nerve. Scattered areas of muscle degeneration were observed in the sections of rat's soleus muscle at two weeks post-crush. Marked degenerative changes with loss of architecture and extensive inflammatory cell infiltration were observed at four weeks post-crush, whereas, the affected muscular areas were replaced by fibrous tissues with many blood vessels in-between. Regenerative changes and many fibroblasts were seen in the sections of soleus muscle at eight weeks after nerve crush. crushing the sciatic nerve induced degenerative changes in the soleus muscle detected at 2 weeks post-crush. The muscle fiber degeneration was more evident and more extensive at 4 weeks post-nerve crush. Evidences of regeneration with reduction of inflammatory cell infiltration were seen at eight weeks after sciatic nerve crush. It was concluded that the degenerative changes of soleus muscle progress rapidly from the second to the fourth week after sciatic nerve crush. Eight weeks after sciatic nerve crush the muscle fibers start to regenerate as a few fibroblasts are observed in the degenerated areas.

Key words: Sciatic Nerve Crush · Soleus Muscle · Muscle Atrophy · Rats

INTRODUCTION

Peripheral nerve injuries compose a major medical difficult [1]. It was reported that the peripheral nervous injury is different from central nervous system injury, whereas, the peripheral nervous system has been stipulated with a significant ability for natural regeneration in reaction to traumatic injury [2, 3].

Animal models are routinely used to improve our understanding of injures of peripheral nerves. The most commonly model used for motor and sensory nerve evaluation is the rat sciatic nerve model. Nerve injury models can be classified into mild (needling), moderate (crush) and severe (sectioning) [4].

The sciatic nerve is formed in the pelvis from the ventral roots L4-S3 of the lumbosacral plexus [5]. Sciatic nerve crush is a commonly used nerve injury (by different crush techniques) as it is considered a very well established axonotmetic model. This model is not expensive and easy to handle. Moreover, the rat sciatic nerve model of crush injury is widely used to assess the post-traumatic impairment of motor function offering some advantages over the nerve transaction model [6].

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The skeletal muscle is a specialized tissue formed by elongated cells. These cells, when observed in cross section, have a polyhedral or hexagonal shape with peripheral flattened multiple nuclei [7]. Permanent loss of innervation induces alterations of function and structure of muscle, since the maintenance of internal structure of muscle fibers is strictly dependent upon their innervation and a minimum level of activity [8]. Muscle denervation is accompanied by atrophy and a decline in oxidative capacity. Short period of time after denervation results in reversible changes in muscle tissue, while a long period of denervation results in irreversible severe atrophy of muscle fibers [9]. Muscle fiber death and fibrosis occur as continued denervation progresses to muscle fiber atrophy and loss of contractility [10].

In the denervated muscle, different patterns of atrophy may result according to the type of muscle fibers. Muscle fiber atrophy proceeds faster in soleus muscle (composed of slow muscle fibers) than in muscles composed of fast fibers like the extensor digitorum longus. In muscles which consist of more fast than slow fibers, atrophy progresses faster in type-II than type-I muscle fibers[11,12]**.** Under the electron microscope, both soleus and gastrocnemius muscles share the same general arrangement of the nerve-muscle junction. However, differences exist between the two muscles regarding the number and depth of the subneural infoldings, the number of motor end plates, number and size of mitochondria and glycogen content, as reported by Duchen [13].

Magill *et al*. [14] reported 100% denervation of the motor end plates one week post-sciatic nerve crush injury, partial reinnervation at two weeks post-injury, hyper-innervation at three and four weeks post-injury and restoring of the axons to motor end plate relationship by six weeks post-operative.

In the present study the histo-morphological changes that take place in the soleus muscle after two, four and eight weeks of sciatic nerve crush, were investigated.

MATERIALS AND METHODS

Animals & Experiment Design: 30 Adult Spargue-Dawley rats (150-200g) were used. The rats were obtained from the Animal House, King Fahd Research Center at KAU University. The rats were kept in cages in a temperature-regulated environment (~20°C) and provided with 12-hour light & dark cycles. Food and water were available *ad libitum*. The rats were grouped at random into the following groups: a control group (n=6); a sham operation group (n=6) and a sciatic nerve crush group (n=18). The latter was further subdivided into 3 subgroups; two weeks post-crush (n=6); four weeks post-crush (n=6) and eight weeks post-crush (n=6).

Sciatic Nerve Crush:

- Under ether inhalation anesthesia animals were put in the prone position.
- Skin of the back of the right thigh was shaved.
- The skin was sterilized and an incision was made high in the back of the right thigh.
- The muscles of the back of the thigh were separated so as the sciatic nerve is exposed.
- The sciatic nerve was crushed by applying continuous pressure for 25 seconds on the sciatic nerve one cm above it trifurcation, using a needle holder forceps.
- The muscles were approximated by chromic cat gut sutures (2/0).
- Skin was closed by interrupted silk suture (2/0).
- The rats were observed for 2 h after recovery from anesthesia and then kept in cages (5rats/cage).
- The rats were observed for general health (weight, gait, position and right hind paw, skin and hair).
- Specimens were taken from soleus muscles at 2, 4 and 8 weeks post-operative.

Then, the specimens were prepared for light microscopic examination.

Sham Operation: The same above-mentioned surgical procedure was performed, except that the sciatic nerve was left in place after being exposed, then the muscle and skin were sutured [15, 16].

Light Microscopy: At 2, 4 and 8 weeks post-operative rats were subjected to operation of scarification. Specimens from each soleus muscle were fixed in 10% formalin solution for 48h and dehydrated in ascending concentrations of ethyl alcohol. Sections were then cleared in xylol and embedded in paraffin blocks. Serial sections (3-5 μm) were cut and received in a water bath, then left in the oven for dewaxing. Thereafter, the sections were stained with hematoxylin and eosin for histological examination. Also, Masson's trichrome staining was performed to visualize the connective tissue [17]. The stained tissue-slides were mounted with DPX and covered with cover slips.

The study was carried out in agreement with the guidelines of the National Institute of Health regarding the use of experimental animals, after the approval of the Ethical Committee of the Faculty of Medicine, king Abdulaziz University, Jeddah, KSA.

RESULTS

Morphology of the Control Soleus Muscle: Longitudinal sections of soleus muscle from the control and sham-operated groups showed normal longitudinally-arranged multinucleated and parallel muscle fibers enclosed by a thin perimysium. The nuclei were flattened and peripherally located. Normal transverse and longitudinal striations of the muscle fibers were seen (Fig. 1). In Masson's trichrome stained section, a minimal amount of collagen fibers was observed in the thin perimysium (Fig. 2).

Morphology of the Soleus Muscle Two-weeks after Sciatic Nerve Crush: The soleus muscle of the operative group, two weeks after crush showed scattered areas of degenerated muscle fibers with inflammatory cells infiltration within and around the affected areas. In addition, loss of cross striations and reduction of the diameter of the muscle fibers were noticed. Many blood vessels with thick wall were observed within and around the degenerated areas (Fig. 3). In Masson's trichrome stained sections collagen fibers were seen. These fibers were concentrated mainly within the degenerated areas, in-between the muscle bundles and in the perimysium and epimysium layers (Fig. 4).

Morphology of the Soleus Muscle Four Weeks after Sciatic Nerve Crush: Scattered areas of extensive degeneration were observed in denervated areas of the soleus muscle. Atrophy of the muscle fibers with wide interfascicular spaces was observed within the structure of the muscle. Reduction of inflammatory cells infiltration

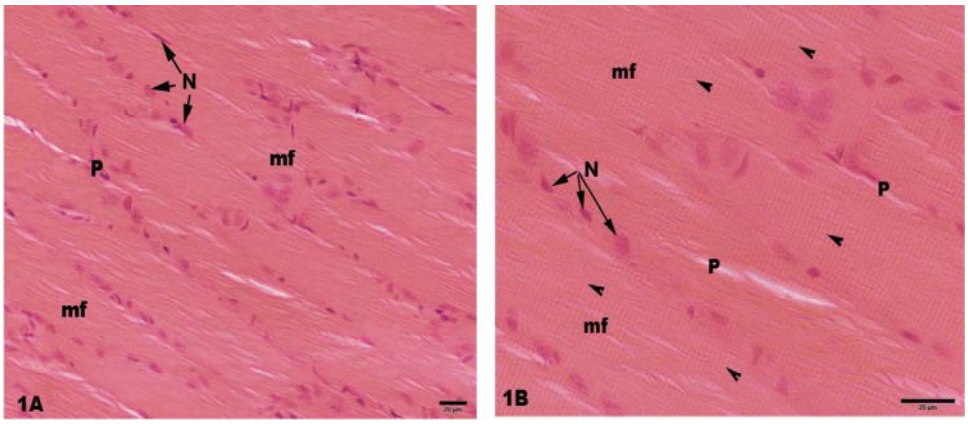

Fig. 1: Light micrographs of longitudinal sections of soleus muscle of the control rat showing normal architecture of skeletal muscle; muscle fibers (mf) are parallel to each other with transverse striations (arrow heads). The nuclei (n) of the muscle fibers appear flat and have marginal position (N). Narrow perimysium (P) is seen between the bundles of muscle fibers. H & E stain (1A= X200 Bar 20; 1B=X400 Bar20).

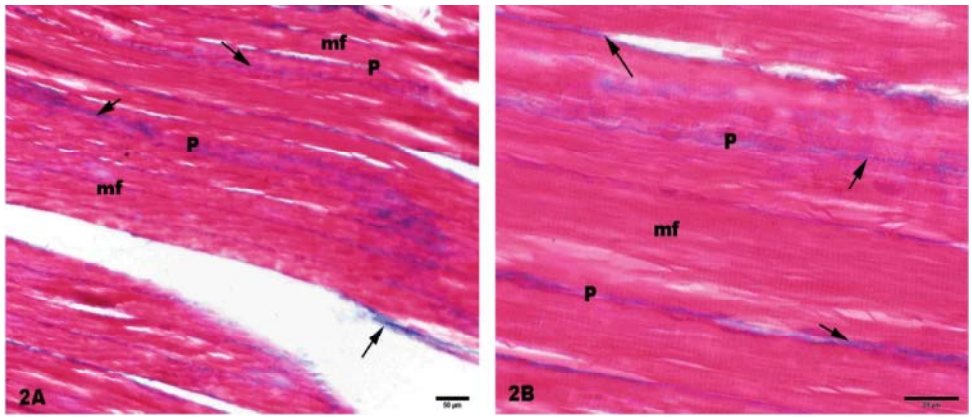

Fig. 2: Light micrographs of control rat soleus muscle showing the bundles of the myofilaments (mf) having thin perimysium (P) in-between. The perimysium contains little mount of collagen fibers (arrow). Masson's trichrome stain (2A=X100 Bar 50; 2B=X400 Bar 20).

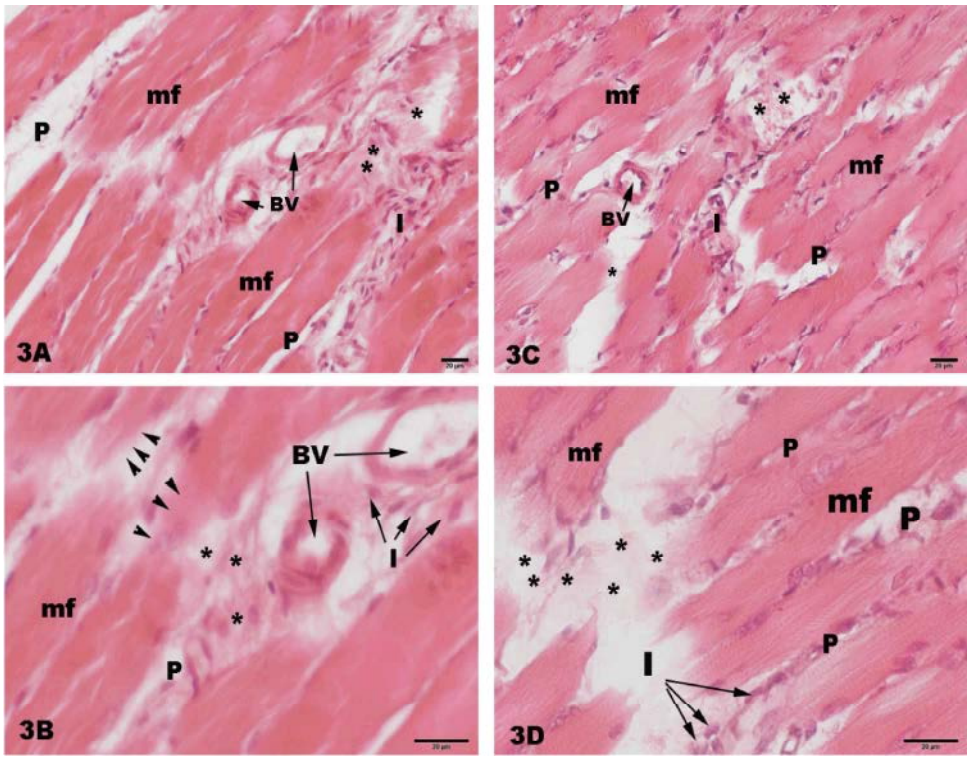

Fig. 3: Light micrographs of a longitudinal sections of soleus muscle of the operative group two weeks after sciatic nerve crush showing scattered degenerated muscular areas (*) of different size with extensive inflammatory cells infiltration (I) around them. A wide perimysium (P) and blood vessels (BV) are observed in-between the myofilaments (mf) beside the degenerated areas. Loss of cross striations and reduction of diameter (arrow heads) is noticed at the affected areas as well. H & E stain (3A= X200 Bar 20; 3B= X400 Bar 20; 3C= X200 Bar 20; 3D= X400 Bar 20).

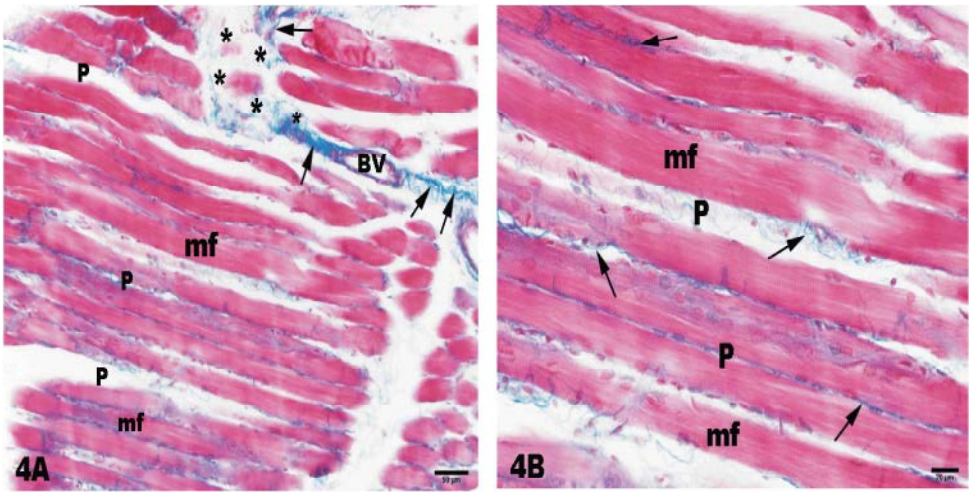

Fig. 4: Light micrographs of rat soleus muscle two weeks after crush showing the distribution of the collagen fibers (arrow) within the degenerated area (**) of the muscle fibers (mf). Wide perimysium (P) is seen between the bundles of myofilaments (mf). Masson's trichrome stain (4A= 100 Bar 50; 4B=200 Bar 20).

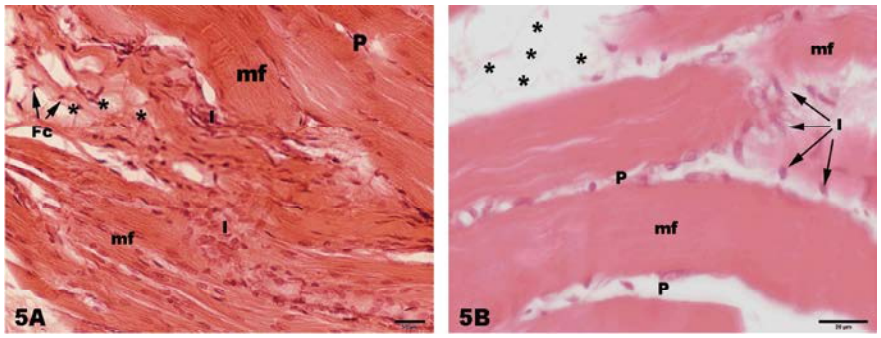

Fig. 5: Light micrographs of the longitudinal sections of soleus muscle of the operative group, four weeks after crush showing an extensive degeneration and atrophy of the muscle fibers (*) with collagen fiber replacement in the denervated areas. Less infiltration with inflammatory cells (I) and many fibroblasts (FC) are observed in the affected areas. H & E stain (5A= X200 Bar 50; 5B=400 Bar 20).

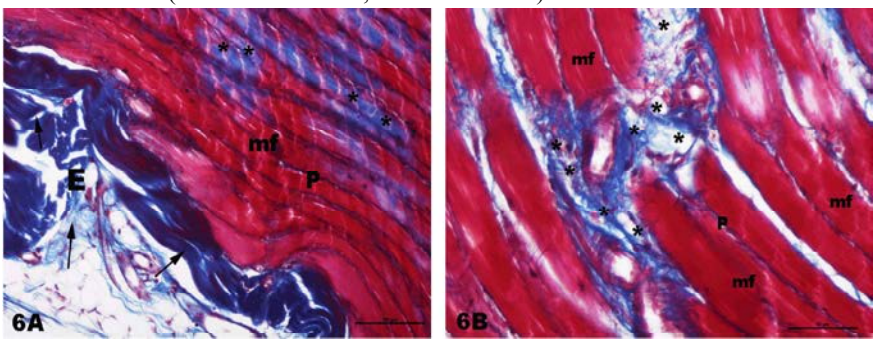

Fig. 6: Photomicrographs of rat soleus muscle four weeks after sciatic nerve crush showing an excessive amount of the collagen fibers (arrows) within the degenerated areas (**), epimysium (E) and in-between the muscle fibers (mf) within the perimysium (P). Masson trichrome stain (6A= X200 Bar 100; 6B=400 Bar 50).

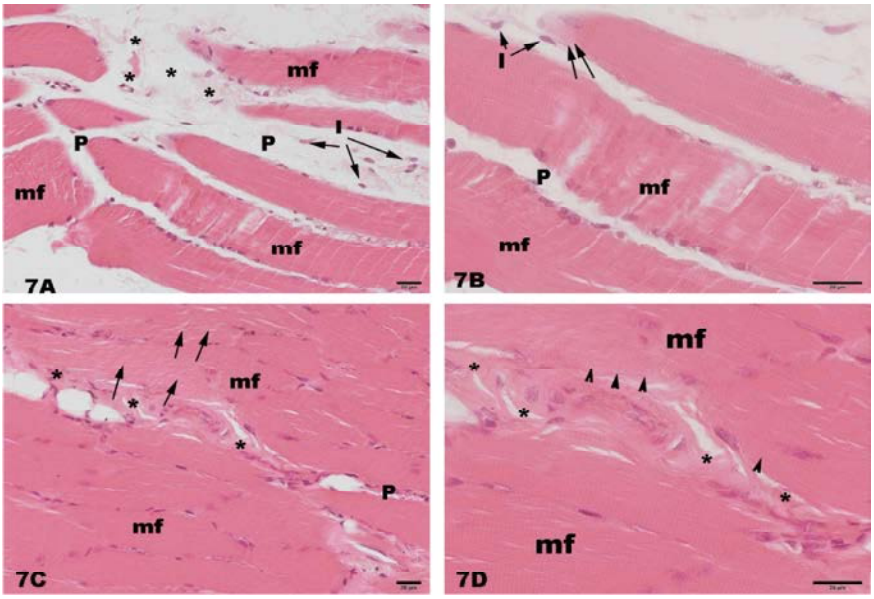

Fig. 7: Photomicrographs of longitudinal sections of rat soleus muscle eight weeks after sciatic nerve crush showing areas of regenerative changes (*) between normal muscle fibers (mf). These areas show atrophy of the muscle fibers (mf), some inflammatory cell infiltration (I) and fibroblasts (Fc). Some fibers have wavy architecture (arrow), while others show loss of cross striations (arrow head). H & E stain (7A= X200 Bar 20; 7B= X400 Bar 20; 7C= X200 Bar 20; 7D= X400 Bar 20).

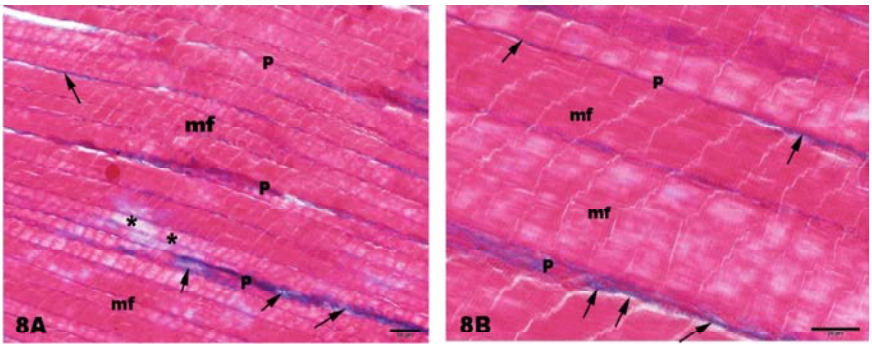

Fig. 8: A light micrograph of rat soleus muscle eight weeks after sciatic nerve crush showing little amount of collagen fibrous tissues (arrow) in-between the bundles of muscle fibers (mf) within the perimysium (P) and within the degenerated muscular areas (*). Masson's Trichrome stain (8A= X100 Bar 50; 8B= X400 Bar 20).

was observed with more fibroblasts within and around the degenerated areas (Fig. 5). In Masson's trichrome stained section, an excessive amount of the collagen fibers was observed within the degenerated areas, epimysium and in-between the muscle fibers within the perimysium (Fig. 6).

Morphology of the Soleus Muscle Eight Weeks after Sciatic Nerve Crush: Eight weeks after sciatic nerve crush, the degenerated areas revealed some regenerative changes with the reduction of inflammatory cells infiltration and number of fibroblasts. Some fibers showed wavy orientation, while others lost their cross striations. The number of the nuclei of some fibers was increased (Fig. 7). Minimal amount of fibrous tissue was observed in the regenerated areas and within the perimysium (Fig. 8).

DISCUSSION

The functional and structural damage of skeletal muscles induced by injury of their nerve supply is an important clinical problem [18]. Skeletal muscle is a greatly elastic tissue that unusually adapts to varied incentives including workout and injury [19]. There are two types of skeletal striated muscles in the body; red and white. The embryological and morphological differences between these two types of muscles were investigated by Patterson *et al*., [20], who reported that the skeletal muscles have two embryological origins and that muscles which develop from branchial arches respond in a different manner from the skeletal musculature developing from myotomes.

Peripheral nerve crush induces mechanical damage of the intraneuronal microcirculation [21]. Sciatic nerve crush is a widely used model of peripheral nerve injury [14]. Because of the wide availability and great similarity of sciatic nerve lesions in human, rats are generally preferred models to investigate consequences of nerve crush [22].

Different methods are used to induce a nerve crush, e.g. hemostatic forceps, vascular clamps, chilled forceps or a needle holder forceps [23]. Schwann cell proliferation in peripheral nerve injury improves axonal renewal compared to central nerve injury. Though, even in peripheral nerve injury, long-term nerve harm without repair induces deteriorating of neuromuscular junctions and muscle atrophy outcomes in permanent dysfunction [24]. In spite of the interruption of axons after crush, optimal regeneration of the nerve can occur due to the preservation of the basal lamina of Schwann cell [25]. The normal fascicular architecture of fibers persists after nerve crush, but is lost after nerve transection. Motor fibers are usually scattered within small regenerating fascicles through the nerve [26]. Nerve renewal after an injury must happen in a well-timed fashion for work to be restored [27].

The present results show increased inter-fascicular spaces among the muscle fibers, together with intrusion of connective tissue within such spaces. Connective tissue deposition was more emphasized in the 4-week post-crush group. These findings are in accordance with those reported by Borisov and Carlson [28] and Rodrigues and Schmalbruch [29], who experimented on slow and fast muscles. Davatz *et al.* [30] reported that following denervation, the loss of

muscle fiber activity played a vital role in the development of the cascade of events which eventually lead to cell death.

In the present study, the degenerative structural changes and muscle fiber atrophy were more clearly noticeable at four weeks after crushing the sciatic nerve than in the other groups. In accordance with the results of our study, Pinte´r, Mendler, Dux [31] revealed that the muscle bulk of re-innervated soleus did not decrease in weight as anticipated, on the basis of the extensive decrease in the number of muscle fibers. Connective tissue deposition also increased and was accumulated in a distinctive pattern around single muscle fibers [32]. Morphologically, these data indicate an altered, less effective regeneration in re-innervated soleus.

The present results are in agreement with the findings of previous works [33]. The latter authors reported that the most common variations associated with myonuclei in pathological activities including muscle fibers include central migration, degeneration and nuclear inclusions. Migration of nuclei to the center of the muscle fiber is a usual finding in all muscle disorders and also happens during denervation-induced atrophy as detected throughout the present study.

In the present study, the perimysium of denervated muscle was thickened. This was accompanied by thickening of the epimysium and endomysium. In addition, larger areas occupied by adipose tissue were detected. As explained by Ashley *et al.* [34], who experimented on denervated rabbit muscle, this was most probably due to fat deposition around neurovascular bundles and in the perimysium.

The results of the present study show generalized atrophy of muscle fibers and fibrosis in the denervated muscle. Other abnormalities, such as the migration of myonuclei to the center of the muscle fibers and development of vacuoles were seen throughout the muscle sections. These alterations were variable and not uniform in the same section or between the denervated groups. Abnormal arrangement and marked reduction in the diameter of the denervated muscle fibers were observed as well. Although most of the fibers were atrophic, normal-sized fibers were often detected surrounded by atrophic fibers. Similar findings were reported by Borisov and Carlson [28].

CONCLUSION

The degenerative changes of soleus muscle progress rapidly from the second to the fourth week after sciatic nerve crush. Eight weeks after sciatic nerve crush the muscle fibers start to regenerate as a few fibroblasts are observed in the degenerated areas. Thus, the findings of the present study can give an important idea about the course of the effect of the nerve injury on the structure of its target muscles.

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