COLLAGEN AND FIBRONECTIN IN A HEALING SKELETAL MUSCLE INJURY

AN IMMUNOHISTOLOGICAL STUDY OF THE EFFECTS OF PHYSICAL ACTIVITY ON THE REPAIR OF INJURED GASTROCNEMIUS MUSCLE IN THE RAT

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The presence of the connective tissue components fibronectin and the different types of collagen was demonstrated by histological and immunohistological methods in the granulation and scar tissue of a healing injury in rat muscle. The effects of physical activity on granulation tissue production, scar formation and muscle regeneration at various stages of healing were studied. It was shown that immobilisation after injury accelerates granulation tissue production, but if continued too long, leads to contraction of the scar and to poor structural organisation of the components of regenerating muscle and scar tissue. However, a certain period of immobilisation, about five days for rat muscle, is required to allow newly-formed granulation tissue to cover the injured area and to have sufficient tensile strength to withstand subsequent mobilisation. This mobilisation, at the correct interval, seems essential for the quicker resorption of scar tissue and the better structural organisation of the muscle.

It is generally accepted that during the healing of a muscle injury two processes are competing, the regeneration of disrupted muscle and the production of a connective tissue scar. Such scarring may inhibit the complete regeneration of muscle fibres by excessive formation of granulation tissue (Allbrook 1973; Burry 1975; Järvinen 1975). Collagen is thought to be necessary for muscle regeneration, forming a sheath around fusing myoblasts during myotube formation (Allbrook 1973; Bailey, Shellswell and Duance 1979), but when a large volume of muscle is devitalised by major trauma, proliferation of fibroblasts can rapidly lead to the formation of excessive scar tissue, and this may form a dense mechanical barrier to the regenerating muscle fibres (McMinn 1967).

Rerupture after muscle injury commonly results from over-vigorous and intensive physical activity after prolonged immobilisation (Jackson and Feagin 1973), but Woodward (1954) claimed that rerupture can be avoided when early mobilisation is used as treatment. The timing of mobilisation after injury has an obvious effect, both on the speed of muscle repair and on the structural organisation of the reparative tissue elements.

Early mobilisation induces vast production of granulation tissue and parallel orientation but poor penetration of muscle fibres through the connective tissue scar. Conversely, immobilisation produces more pronounced penetration of a reduced connective tissue scar by inter-lacing and irregularly-orientated muscle fibres (Järvinen 1975).

Mobilisation also induces more rapid and intensive capillary ingrowth from all borders of the injury, especially during the early stages of healing (Järvinen 1976a). In addition, there is rapid return of the tensile properties of injured muscle to the level of uninjured muscle, whereas immobilisation tends to decrease the tensile properties throughout the period of splintage (Järvinen 1976b).

Local response to injury includes the stimulation of cell proliferation and the synthesis of extracellular matrix components. During the formation and evolution of granulation tissue, cells produce collagen very actively (Bazin and Delaunay 1964). It is well established that in the early stages of tissue repair, synthesis of Type III collagen increases even before any mature fibroblasts can be detected. The assumed source being primitive, multipotent cells (Bailey et al. 1975; Gabbiani et al. 1976; Gay et al. 1978). As repair progresses there is a return to a marked predominance of Type I collagen (Bailey et al. 1975; Gay et al. 1978).

In healing wounds, immunohistologically detectable fibronectin appears as early as one to five hours after wounding. At this early stage fibronectin is distributed along strands of fibrin in the clot (Fujikawa et al.)
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1981; Grinnell, Billingham and Burgess 1981; Viljanto, Penttinen and Raekallio 1981). The fibrin-fibronectin linkage is thought to be an important factor in subsequent events, since adhesion of fibroblasts to fibrin has been shown to be maximal if it is cross-linked to fibronectin (Grinnell et al. 1980). The fibrin-fibronectin network and its association with pre-existing collagen forms a scaffolding for platelet adhesion and aggregation and provides anchorage for the invading fibroblasts (Kurkinen et al. 1980).

It has been consistently found that fibronectin precedes the appearance of collagen deposition in young granulation tissue (Kurkinen et al. 1980; Grinnell et al. 1981; Viljanto et al. 1981). Later, fibronectin is closely associated with the newly synthesised collagen fibrils (Grinnell et al. 1981), which in this initial phase of wound healing have been shown to be of Type III collagen (Gay et al. 1978).

During the development and organisation of granulation tissue, when collagenous proteins are organised into bundles, fibronectin is co-distributed with Type I collagen; the fibronectin gradually diminishes during the maturation of granulation tissue (Kurkinen et al. 1980). It has been postulated that during remodelling of granulation tissue, when collagen turnover is high, fibronectin may play an important role in the removal of collagen by binding to collagen fragments generated by collagenase digestion (Gay and Miller 1978) and promoting their phagocytosis by macrophages and fibroblasts (Hopper et al. 1976; Grinnell 1980; Grinnell et al. 1981).

The aim of the present investigation was to examine the process of repair of a standard experimental muscle injury at various levels of physical activity and to follow the sequential appearance of different types of collagen and of fibronectin in the extracellular matrix. The effects of immobilisation and mobilisation on granulation tissue production and scar formation, and how these processes influence the regeneration of muscle fibres, were also studied.

MATERIALS AND METHODS

The animals used in this study were 96 male rats of Sprague-Dawley strain and 36 of Wistar strain, aged 12 to 16 weeks. All the animals used in the mobilisation experiments were taught to run on a treadmill before they were injured.

A standard transverse contusion injury was inflicted to the left calf of each rat, under light ether anaesthesia, using a blunt spring-loaded hammer. The skin remained intact, so infection was avoided (Järvinen and Sorvari 1975). Animals were divided into four treatment groups.

No specific treatment (Group NT). These rats received no treatment after injury, and were left to move freely in their cages.

Immobilation (Group IM). A padded plaster was applied to the injured leg while the rats were still under anaesthesia immediately after injury. The maximal period of immobilisation was 21 days, after which the rats were allowed to move freely in their cages.

Mobilisation after 2 days (Group IM2-MO) or after five days (Group IM5-MO). In these two groups of rats the injured leg was immobilised in a plaster cast for either two days (IM2-MO) or five days (IM5-MO). The casts were then removed and the rats were placed at intervals on a motor-driven treadmill, with an upward inclination of 15°, running at a constant speed of 40 cm/sec. All the animals were seen to use all four legs when running. On the first day after plaster removal the rats were exercised for two periods of 20 minutes with a 60-minute interval. On the second day they were exercised for two 30-minute periods and thereafter once daily for 60 minutes (Järvinen 1975).

Preparation of antigen and antibodies. Collagen Types I, III, IV and V were extracted from newborn rat skin and human placenta and purified by fractional salt precipitation as described by Bailey et al. (1979b). All collagen-extracted specimens were passed through a DEAE-cellulose column to remove contaminating glyco-proteins before use as antigens. Antibodies were raised in rabbits or goats using an immunisation schedule described by Duance et al. (1977). They were made type-specific by passage through immuno-absorbent columns (March, Parikh and Cuatrecasas 1974). The activity and specificity of the antibodies were tested by an ELISA technique (Voller, Bartlett and Bidwell 1978). The antibodies used were rabbit anti-rat Types I and V collagen, rabbit anti-human Type IV and goat anti-human Type III collagen. The specificity of human fibronectin antibodies, which cross-react with rat fibronectin (Kuusela et al. 1976), has been described by Stenman and Vaheri (1978); these antibodies were a kind gift from Professor A. Vaheri of the University of Helsinki, Finland.

Immunohistology and histology. Animals from the treatment groups were killed at the times after injury indicated in the results section. Specimens for indirect immunofluorescence studies were obtained from the gastrocnemius muscles immediately after death. Muscles were cut in the sagittal midline and the medial half was snap-frozen in isopentane cooled in liquid nitrogen. Serial sections 6 μm thick were cut on a cryostat, and sections were incubated for 1 hour with 1% testicular hyaluronidase (Sigma, 1:100 in 0.1 mol/l phosphate buffer, pH 5.0) to remove the masking glycosaminoglycans. After washing in PBS for 30 minutes the sections were stained for 20 hours in a humidified chamber at room temperature with the antibodies or with normal rabbit serum as control.

The concentration of the antibodies for tissue staining was kept constant throughout the whole series of experiments. The sections were washed in PBS for 30 minutes, air-dried and then stained for 30 minutes with either fluorescein isothiocyanate conjugated anti-rabbit IgG (Wellcome Reagents Ltd, UK) or anti-goat IgG (Miles-Yeda Ltd, Israel) depending on the origin of the
antibody. The stains were diluted 1:20 in PBS. After washing for one hour in PBS the sections were then mounted with glycerol jelly (10 g gelatin, 70 ml glycerin, 60 ml distilled water, 0.25 g phenol).

Relative intensities of indirect immunofluorescence in the injured area were estimated visually, using a scoring system (Duance et al. 1977; Gay et al. 1978; Viljanto et al. 1981). The staining intensity was scored from "", intense; "", moderate; "", faint to 0, none.

RESULTS

The gastrocnemius muscle injury was above the level of the underlying plantaris muscle and was such that the functional capacity of the leg remained intact (Järvinen and Sorvari 1975). The body weights of the rats in all groups initially decreased. The injured but untreated rats (NT) showed a gradual increase in weight after one week, while the other three groups, the immobilised (IM) and the mobilised groups (IM2-MO and IM5-MO), increased in weight after three weeks, the two mobilised groups gaining more weight than the immobilised group.

Day 2, Groups NT and IM. Histology. There was a haematoma in the centre of the injured area. Disruption and necrosis of the muscle fibres were seen, with inflammatory cells—polymorphs and macrophages—around the damaged fibres in both groups but more conspicuously in Group NT. Spindle-shaped cells, regarded as fibroblasts, were seen in both groups.

Immunohistology. In Group NT fibronectin was detected along the strands of fibrin (Fig. 1), whereas in Group IM the fibronectin was evenly distributed throughout the injury (Fig. 2). Types I and III collagen were similarly distributed in both groups and were also seen in the endomysial structures of the surviving muscle fibres. In some specimens there was slight staining with Type III collagen antibodies in the inner zone of the injured area, especially in Group IM. Types IV and V collagen antibodies stained only the muscle endomysium (Fig. 3).

Day 5, Groups NT, IM and IM2-MO. Histology. The haematoma was still present in Groups NT and IM2-MO, but had almost completely disappeared from Group IM. The inflammatory cells had nearly disappeared and numerous new myotubes and myoblasts were seen in all groups. Fibroblasts were seen throughout the injured area except in the area of the haematoma.

Immunohistology. Fibronectin antibodies stained the newly-formed granulation tissue in all groups, and in Group IM2-MO it was also detected in the centre of the injured area lying along the fibrin strands (Fig. 4).

Type I collagen was detected in Group IM generally as a fibre-like structure except in the areas of haematoma where it was seen more as occasional spots along the strands of fibrin (Fig. 5). Type III collagen was seen in the granulation tissue in all groups (Fig. 6). Types IV and V collagen were located in the endomysium of muscle fibres and also to some extent in the newly formed granulation tissue (not shown).

Day 7, all Groups (NT, IM, IM2-MO and IM5-MO). Histology. In Groups NT and IM2-MO the haematoma was still present, but smaller in size than on Day 5, and in Groups IM and IM5-MO it was not detected. There was little variation in the numbers of myotubes present. The regenerating muscle fibres had commenced penetration of the granulation tissue and appeared to be more interlaced in Group IM than in other groups (Fig. 7).

Immunohistology. Fibronectin was detected in all groups as a fine network filling the injured area and also staining the contours of muscle fibres (Fig. 8). In Groups NT and IM2-MO it was seen in the haematoma as a brightly stained area. Type I collagen was detected throughout the granulation tissue in Groups IM and IM5-MO and throughout the injured area in Group IM2-MO excluding the haematoma, where parallel structures were seen against a dark background (Fig. 9). Type III collagen was seen in the same distribution as Type I except that it did not stain any structures within the haematoma in Group IM2-MO (Fig. 10). Types IV and V collagen were seen in the endomysium as fine circular structures within the granulation tissue in the innermost zone of the injured area.

The sequential appearance of different collagen types and fibronectin, estimated by the relative intensities of indirect immunofluorescence during the first seven days after injury in Groups NT and IM is shown in Figures 11 and 12. The early changes seen in wound healing are of importance for subsequent events. Fibronectin is present at an early stage in both Groups NT and IM, probably being derived from serum. The synthesis of new fibronectin may lag behind in the control group as the initial quantity reduces temporarily. Types I and III collagen showed only small differences between Groups NT and IM with Type III preceding Type I in both. Subsequent changes in quantity of the various components of the matrix were in general relatively small, the most important feature being their differing distribution.

Day 21, all groups. Histology. In all groups and especially Group IM, the number of myotubes was further decreased. Muscle giant cells were conspicuous in Group IM, but were less so in other groups. Regenerating muscle fibres were seen in the scar tissue in all groups; their orientation was less interlaced in Group IM5-MO than in other groups.

Immunohistology. In all groups fibronectin and Type III collagen showed a very similar distribution within scar tissue; they were evenly spread throughout the injured area and the nearby thickened endomysial and perimysial structures (Fig. 13). In addition, fibronectin antibody stained the contours of the muscle fibres. In Groups IM2-MO and IM5-MO the intensity of the fibronectin antibody staining was slightly decreased when compared to that seen in Groups NT and IM.

Antibodies to Types IV and V collagen strongly stained the endomysium of the cross-sectioned regenerating muscle fibres within the scar tissue of the injured.
Two days after the muscle injury. Figure 1 In Group NT, fibronectin antibodies stain along fibrin strands (FS) and weakly within the haematoma. Figure 2 In Group IM they stain throughout the injured area. Figure 3 Antibodies to Type IV collagen stain the surviving and disrupted endomysial structures (En) in Group IM (x 90).

Five days after the muscle injury in Groups IM2-MO (Fig. 4), Groups IM (Fig. 5) and NT (Fig. 6). Antibodies to fibronectin (Fig. 4) and Type III collagen (Fig. 6) show staining in the net-like newly-formed granulation tissue (G) in the haematoma (H). Type I collagen is located along the fibrin strands (FS), except in the haematoma (H) where it appears as spots (Fig. 5) (x 90).
Seven days after the muscle injury. Figure 7—In Group I haematoxylin and eosin staining shows abundant myotubes (M) in the regenerating muscle fibres with numerous fibroblasts and some inflammatory cells (IC) covering the injured area. Figure 8—Fibronectin antibodies show similar staining of both the fibrillar connective tissue matrix and the fine endomysial structures (En) in all groups. Type I collagen is evenly distributed through the injured area in Group IM5-MO (not shown), but in Group IM2-MO (Fig. 9) only a few larger bundles are seen in the haematoma (H). Type III collagen is evenly distributed through the injured area in all groups except IM2-MO, where in Figure 10 it is seen to be absent from the haematoma (× 90).

area. However, the intensity of antibody staining of both Types IV and V collagen was much brighter in the uninjured fibres than those which had been injured (Fig. 14).

In Groups IM2-MO and IM5-MO Type V collagen was seen around more numerous regenerating muscle fibres than in Groups NT and IM.

Day 56, all groups. Histology. Fibrous scar was present in all the groups examined, most prominently in Group IM2-MO (Fig. 15). A large number of regenerating muscle fibres were seen within the scar tissue in all groups, a great proportion of them being smaller in diameter than those in uninjured areas, especially in Groups IM and IM2-MO. No muscle giant cells or myotubes were seen in any group. The organisation of the regenerating muscle fibres was complex and interlaced in Groups IM and NT, and generally parallel in Group IM5-MO.

Immunohistology. Fibronectin and Type III collagen were widely distributed throughout the scar tissue and the adjacent thickened endomysial and perimysial tissue in all groups. The fibronectin staining was most intense and showed a denser meshwork in Group IM (Fig. 16), and was much reduced in Group IM5-MO (Fig. 17). Type I collagen was widely distributed within the scar tissue in Group IM and also in the thickened nearby endomysial and perimysial tissue. In Groups NT, IM2-MO and IM5-MO. Type I collagen was more clearly localised in the scar tissue of the injured area and showed a lower staining intensity in adjacent endomysial structures (Fig. 18). In the same groups some of the perimysial structures, especially those that were parallel to the original muscle fibres, showed strong staining with Type I collagen antibodies (Fig. 18).

Types IV and V collagen were detected in the endomysium around the regenerated and the uninjured muscle fibres. Small regenerating muscle fibres were seen within the scar tissue in all groups, most abundantly in Groups IM and IM2-MO (Fig. 19). In all groups, some of the endomysial structures of the muscle fibres in the injured area were curved in shape, where they clearly related to the connective tissue scar.

DISCUSSION

Studies on the effects of different treatment programmes on the healing of muscle must start with a standard injury in respect to size and location. Muscle ruptures in athletes are not usually associated with skin tears; therefore experimental injuries should ideally leave the skin intact. In our series, the blunt contusion caused partial rupture of rat calf muscle without tearing the skin and
left enough functional capacity in the affected muscle to allow immediate mobilisation of the injured leg. The constant nature of the injury and the effectiveness of the two experimental methods of treatment, immobilisation in a padded plaster cast and mobilisation on a treadmill, have been previously documented (Järvinen and Sorvari 1975; Järvinen 1975, 1976a, b).

Immunohistological methods for locating different collagen types and fibronectin in tissues have been widely used in recent years, although some disadvantages have been discussed (Timpl, Wick and Gay 1977). The main difficulty is that of producing non-specific antisera to native collagens, yet this has been achieved by exhaustive purification of the antisera by immuno-absorption (Duance et al. 1977; Timpl et al. 1977). The methods employed can be considered to be only semi-quantitative (Duance et al. 1977; Gay et al. 1978; Viljanto et al. 1981) even though constant conditions were maintained.

It is widely accepted that physical activity and exercise improve the recovery of injured tissue although little experimental investigation has been carried out (O'Donoghue 1970; Järvinen 1976c; Tipton et al. 1977).

In our study four different schemes of treatment, based on clinical practice, were used after a standard muscle injury in rats.

In the untreated group fibronectin was detected in the injured area as early as 24 hours after trauma. This parallels those findings reported for wound healing, in which fibronectin was observed within a few hours of wounding (Fujikawa et al. 1981; Grinnell et al. 1981; Viljanto et al. 1981). We found fibronectin distributed along the fibrin strands as part of the fibrin clot. It is likely that this fibronectin is derived from plasma (Kurkinen et al. 1980; Grinnell et al. 1981). The role of fibronectin in these early stages may be the formation of a primary matrix by cross-linking with fibrin. This then acts as a scaffold to which the invading fibroblasts may anchor (Mosher 1975; Kurkinen et al. 1980). It is very interesting that the intensity of fluorescence of anti-fibronectin staining decreased in untreated rats after the first day. A possible explanation is that the normal physical activity seen immediately after injury causes the muscle pump mechanism to extract wound fluid containing the fibronectin from the injured area. This hypothesis is supported by the finding that in the immobilised group the intensity of fluorescence with anti-fibronectin was considerably higher during the first five days than in the control group (NT) which was allowed free physical activity and had no specific treatment.

Type III collagen antibody staining showed increasing intensity in the injured area during the first three days, especially near the muscle stumps. On Day 5 in the untreated group (NT), Type III collagen was present in the young granulation tissue throughout the injured area, except for the haematoma. Type I collagen, although distributed similarly to Type III collagen, was not arranged into bundles or a network-like matrix. It also was absent from the haematoma.

The sequence of appearance of fibronectin, Type I and Type III collagens observed in this study is similar to that reported to occur in wound healing (Gay et al. 1978; Viljanto et al. 1981) and in experimental granulation tissue (Kurkinen et al. 1980). The diminution in the intensity of antibody staining of fibronectin during the later stages of healing also agrees with previous studies (Gay et al. 1978; Kurkinen et al. 1980; Fujikawa et al. 1981; Grinnell et al. 1981). Although the appearance of Type III precedes that of Type I collagen in the injured area of the untreated group, their similar distribution and localisation suggests that they may be synthesised by the same cells, as has been shown for isolated human skin fibroblasts (Gay et al. 1976). It also indicates that the injured area of muscle may not gain significant tensile strength until one week after injury in the absence of treatment. This hypothesis takes into account the knowledge that Type I collagen forms thicker fibres than the fine reticular fibres of Type III collagen (Wiedemann et al. 1975).

The effect of physical activity on the sequence of the appearance of connective tissue components in healing.
At 21 days after injury. Immunofluorescent staining in Group NT. Figure 13—Antibodies to fibronectin and Type III collagen (not shown) stain the connective tissue matrix (G) in the injured area. Figure 14—Antibodies to Type IV collagen stain the endomysial structures of old (En→) and regenerating (En►) muscle fibres. The new fibres are smaller than the old fibres (×90).

At 56 days after muscle injury. Figure 15—Group IM2-MO. Numerous small regenerated muscle fibres (MF) are seen within the injured area in a section stained with haematoxylin and eosin. Figure 16—In Group IM, fibronectin antibodies and Type III collagen antibodies (not shown) stain the connective tissue both in the injured area (G) and nearby thickened endomysial structures (En). Figure 17—By contrast, in Group IM5-MO this staining is much reduced. Figure 18—Type I collagen staining was localised to the scar tissue of the injured area (G), and showed strong staining of perimysial structures (P) but with lower staining intensity in nearby endomysial structures (En) in most groups. Figure 19—Type IV collagen staining in all groups delineates the endomysium of the old (En→) and the smaller regenerated muscle fibres (En►) (×90).
tissues has not previously been investigated. We found that five days' rest in a padded plaster cast was followed by the accelerated appearance of Type I collagen in the injured area. Early immobilisation may therefore allow the wound to gain sufficient tensile strength to reduce the risk of rupture of the injured muscle when mobilisation is started. In the animals treated by immobilisation for five days and then by mobilisation (IM5-MO), the injured area at Day 7 contained Types I and III collagen and fibronectin. Signs of rupture in the form of an invasion of erythrocytes or the reappearance of the fibrin clot were not detected. When the rats had only two days' immobilisation (IM2-MO), a fibrin clot containing fibronectin was still detectable at Day 7, indicating that an injured muscle immobilised for only two days cannot withstand the mechanical forces of mobilisation because of the lack of connective tissue components in the injured area. This leads to the formation of a larger area of granulation tissue. Leakage of plasma-derived fibronectin into the site of rupture forms a fibrin-fibronectin clot which traps the fibroblasts responsible for local synthesis of collagen in this additional area of damage. This increased damage can be avoided if an adequate period of immobilisation is allowed before mobilisation is started.

A muscle injury is repaired by the formation of granulation tissue in the injured area, and the simultaneous regeneration of muscle fibres (Allbrook, Baker and Kirkaldy-Willis 1966; Carlson 1968). The immunohistological studies appear to show that Type V collagen could be related to the regeneration of muscle fibres, as has been reported in the development of skeletal muscle during embryogenesis in the chick (Bailey et al. 1979a). During the growth of myoblasts Type V collagen has been shown to appear before myotube formation in vivo (Sasse, von der Mark and von der Mark 1978) and in vitro (Bailey et al. 1979a). In the present study, the regenerating muscle fibres were defined by conventional histological methods. Some regenerating muscle fibres showed bright immunofluorescent staining with anti-Type V collagen preceding Type IV collagen. In some cross-sections the muscle fibres within the granulation and scar tissue were stained with Type V but not with Type IV collagen antibodies, while in later stages of healing all the cross-sections of regenerating muscle fibre stained with both Types IV and V collagen antibodies.

The two different periods of immobilisation before mobilisation was started resulted in considerable differences in the remodelling and resorption of connective tissue in the later stages of healing. In the group treated by five days of immobilisation before mobilisation (IM5-MO), connective tissue was almost totally resorbed during the eight-week follow-up period, and only scanty deposits of Types I and III collagen and fibronectin were observed in the thickened endomysial and perimysial structure of the injured area. However, in the group which had only two days' immobilisation before mobilisation (IM2-MO), an area of fatty necrosis was seen in the centre of the injured area three weeks after trauma. This necrotic area was surrounded by loose connective tissue which in turn was surrounded by a more dense collagenous network. Eight weeks after injury the deposition of connective tissue in the endomysial and perimysial structure of the injured area was still considerably greater when compared to Group IM5-MO. This finding suggests that rupture of the injured muscle had occurred, resulting in the deposition of additional matrix within the pre-existing and more mature connective tissue. This may also explain the differences in the penetration of muscle fibres through the connective tissue in the two groups treated by mobilisation. Although regenerated muscle fibres are more in alignment with the uninjured ones after mobilisation (Järvinen 1975), poor resorption of connective tissue and the formation of true scar tissue prevents the full penetration of the injured area by regenerated muscle fibres in Group IM2-MO.

The trend of the results in this study is clear; immobilisation after injury accelerates the formation of granulation tissue matrix. However, later mobilisation is required to improve the penetration and orientation of regenerating muscle fibres, and the resorption of the connective tissue scar. Another important object of mobilisation, especially in sports injury practice, is to avoid the atrophy of muscle produced by prolonged immobilisation.

REFERENCES


