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# AGE DEPENDENT REPAIR OF MUSCLE RUPTURE

## *A Histological and Microangiographical Study in Rats*

MARKKU JÄRVINEN[1,2,3], ALLAN J. AHO[2], MATTI LEHTO[1] & HANNU TOIVONEN[1]

[1]Sports Medical Research Unit, Department of Physiology and [2]Department of Surgery, University of Turku, Turku, Finland

Healing of contusion injury in the gastrocnemius muscle was studied in young adult and old rats. A standard blunt muscle contusion was induced to the left calf of each animal and studied histologically and microangiographically 2–21 days after the injury. The inflammatory cell reaction was more intense, the haematoma was larger and the proliferation of fibroblasts and production of collagen scar more pronounced in the young rats. The sprouting of new capillaries and regeneration of ruptured muscle fibres occurred more rapidly and intensively in the young animals, and the resorption of haematoma and phagocytosis of necrotic tissue occurred later in the old rats.

The decreased repairing capacity in muscles of older animals resembles that seen earlier in immobilized muscles of adult rats, indicating that the response to the stimulus of injury decreases with advancing age.

*Key words:* age, injury; striated muscle; healing; histology; microangiography

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The limited exercise capacity of older persons is a great clinical problem after trauma or surgical procedures and severe complications are often followed by long-term bed rest. Operative or accidental injuries to muscles are followed by a decreased working capacity and strength of the affected muscles, leading to a limited rehabilitation potential. It is known that the healing of many tissues, e.g. skin wounds (Leaming 1963, Holm-Pedersen & Viidik 1972, Goodson & Hunt 1979) and fractures (Aho 1966) is impaired with age, but no investigations on the effect of age upon the healing of striated muscle have been previously published.

The repair of striated muscle includes the simultaneous regeneration of muscular tissue and the formation of connective scar tissue (Millar 1934, Allbrook et al. 1966, Carlson 1968, Järvinen 1975), the latter limiting the regeneration process. The speed and intensity of these processes are dependent on many factors, e.g. denervation of affected muscle (Saunders & Sissons 1955, Schick & Jerusalem 1973), physical activity or inactivity (Kvist et al. 1974, Järvinen 1975), irradiation (Reznik 1970) and steroid hormones (Sloper & Pegrum 1967). The purpose of this study is to compare the healing of a crushed gastrocnemius muscle in old and young adult rats.

## MATERIAL AND METHODS

Forty-eight male rats of Wistar strain were used in this study. At the time of traumatization the age of the 24 old rats was over 2 years and that of 24 young rats was 4

[3]Present address: Institute of Clinical Sciences, University of Tampere, Teiskontie 35, 33520 Tampere 52, Finland.
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*Figure. 1. The site of injury (→) in the left calf of the rat.*

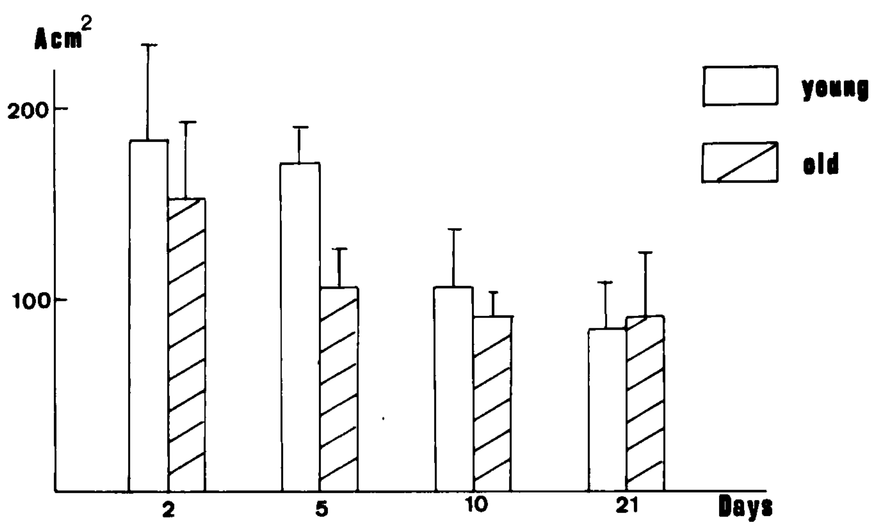



*Figure 2. The size of injured area measured planimetrically from the microangiograms. The measurements indicate the more extensive contraction of connective tissue scar in young rats.*

*Figure 3. Muscle injury in young rats 5 days post-traumatization. Intense granulation tissue formation with numerous fibroblasts in the centre of the rupture (left in the Figure) and some myotubes (→) sprouting from the ends of disrupted muscle fibres towards the centre of injury are seen. Van Gieson-haematoxylin × 200.*

months. The body weight of the old rats ranged from 275 to 470 g (mean 377 ± 7 g) (± s.e.m.) and that of the young rats ranged from 200 to 340 g (mean 257 ± 6 g) (± s.e.m.). The animals were housed in wire mesh cages and received laboratory chow (Hankkija, Finland) and water *ad libitum.*

The constant contusion injury was induced using a blunt spring-loaded hammer on the left calf of each animal under light ether anaesthesia (Figure 1) as described in detail earlier (Järvinen & Sorvari 1975). The time of traumatization is designated Day 0 and the consecutive days Days 1, 2, etc.

On Days 2, 5, 10 or 21 (six animals in each group) the animals were anaesthetized with intraperitoneal pentobarbitone (Nembutal®) and microangiography was performed by the technique described in detail earlier (Järvinen 1976).

Chromopaque® (Damancy Co., Ltd. England) was used as a contrast medium. After formalin fixation the triceps surae muscles were removed and embedded in a medium containing paraffin and plastic polymers (Fibrowax, supplied by Bethlehem Trading, Gothenburg, Sweden). Serial 500-µm sections were then cut sagittally and placed on Kodak High Resolution photographic plates (Eastman Kodak, Rochester USA).

A Machelett AEG-50 röntgen tube with a 1.5 mm beryllium window was employed at 30 kV. The film-focus distance was 50 cm and the exposure time was 30 min.

Sections measuring 6 µm obtained from the sagittal

*Figure 4. Muscle injury in old rats 5 days post-traumatization. Early phase of granulation tissue formation with invasion of fibroblasts are seen in the centre of the injury (left in the Figure). Vacuolar degeneration in the stump of disrupted muscle fibres, but no myotubes indicating muscle regeneration are seen. Van Gieson-haematoxylin × 200.*

midline of the injured area were prepared and stained according to the van Gieson-haematoxylin method for histological examination.

The size of the injured area and the location of the capillary pattern were identified in the microangiograms, which were projected at constant magnification on to a screen on which the width of the injured area was planimetrically measured.

In the histological sections the inflammatory cells, fibroblasts, myoblasts, myotubes and capillaries were studied without knowledge of the group to which the specimen belonged and estimated semiquantitatively by scoring from 3 (abundant) to 0 (none) as previously (Järvinen 1975, 1976).

## RESULTS

The animals in both groups lost some body weight (about 10 g) after the injury. The weight had returned to the initial level by Day 10 and was about 10 g higher at the end of the observation period.

### *Inflammatory cell reaction and haematoma formation (Day 2)*

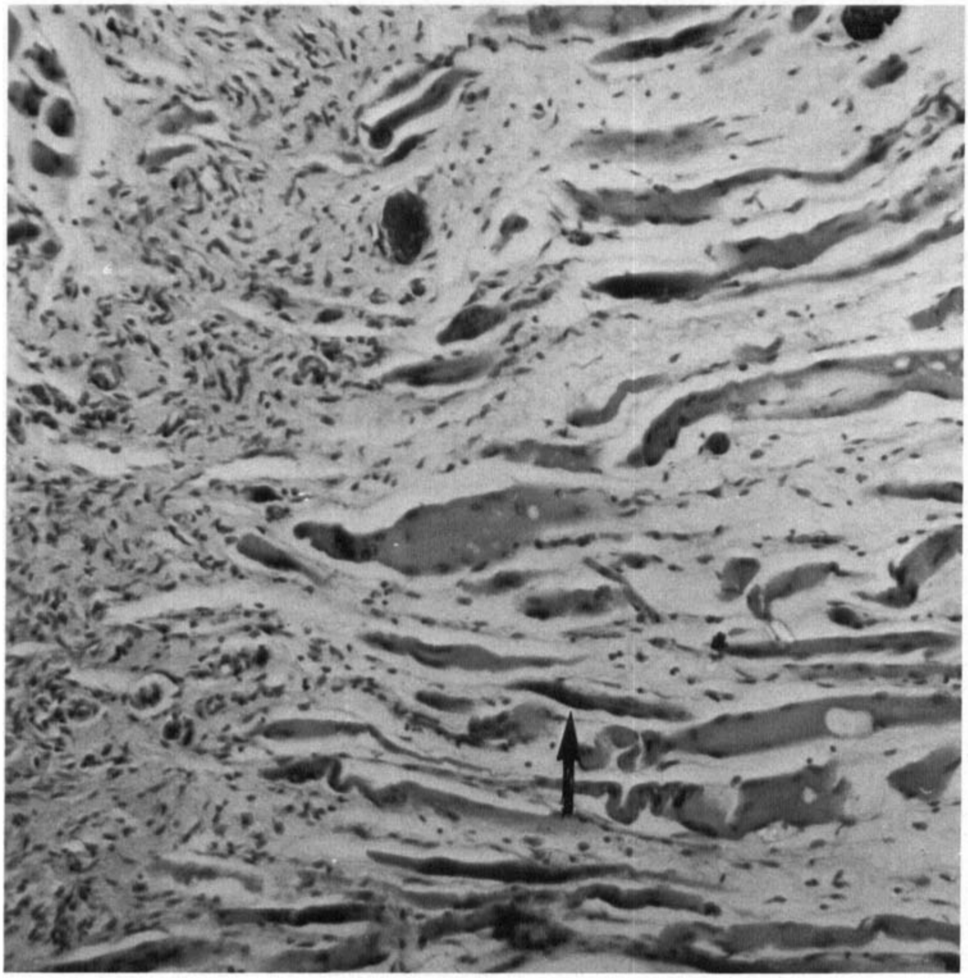
Both in the histological sections and in the microangiograms the injured area was quite constant

*Figures 5 and 6. Muscle rupture 10 days post traumatization in young (Figure 5) and old (Figure 6) rats. In young rats (Figure 5) a large amount of myotubes (→) and thin new myofibres are seen among the new connective scar tissue. In old rats (Figure 6) only a few myotubes (→) and new regenerated muscle fibres are seen. Vacuolar degeneration in the disrupted muscle fibres is still seen. Van Gieson-haematoxylin × 200.*

(Figure 2) and the rupture contained crushed, totally disrupted necrotic muscle fibres, a large amount of erythrocytes and fibrin clots. In the young rats the haematoma was larger and the inflammatory cell infiltration more pronounced, consisting mainly of polymorphonuclear leucocytes and macrophages around the stumps of surviving muscle fibres. In the semiquantitative estimation of inflammatory cells in young and old rats, the mean score values were 1.5 and 1.0, respectively. No myotubes were found and no sprouting of new capillaries was seen in this phase of healing. In the microangiograms the vessels near the rupture contained more contrasts medium than those far from it.

### *Proliferation and muscle regeneration phase (Days 5 and 10)*

By Day 5 the amount of inflammatory cells especially leucocytes, and the size of the haematoma had markedly decreased in both groups and by Day 10 they had disappeared nearly totally. There was less necrotic tissue in the young ani-



mals with denser infiltration of fibroblasts (mean scores 2.7 and 2.1) around the stumps of the disrupted muscle fibres.

Myoblasts and myotubes, representing muscle regeneration, were found in the transitional zone of the rupture in both groups, but in greater numbers in the young animals. The presence of new thin collagen fibres in the middle of the injury and of capillary sprouting, particularly from the proximal border of the injury, were both more pronounced in the young rats (Figures 3, 4, 7, and 8).

On Day 10 the inflammatory cells had nearly totally disappeared. Muscle regeneration, identified as a myotube formation, was more intense and the production of new connective tissue in the middle of the injury was more prominent in the young rats (Figures 5 and 6).

The area without capillaries in the centre of the injury had nearly disappeared in the young and markedly diminished in the old rats (Figure 2).

*Maturation and scar formation*

Day 21. The injured area was nearly vascularised in both groups. There were fewer myotubes in the young rats as many had matured to normal muscle fibres. The collagen scar produced in the

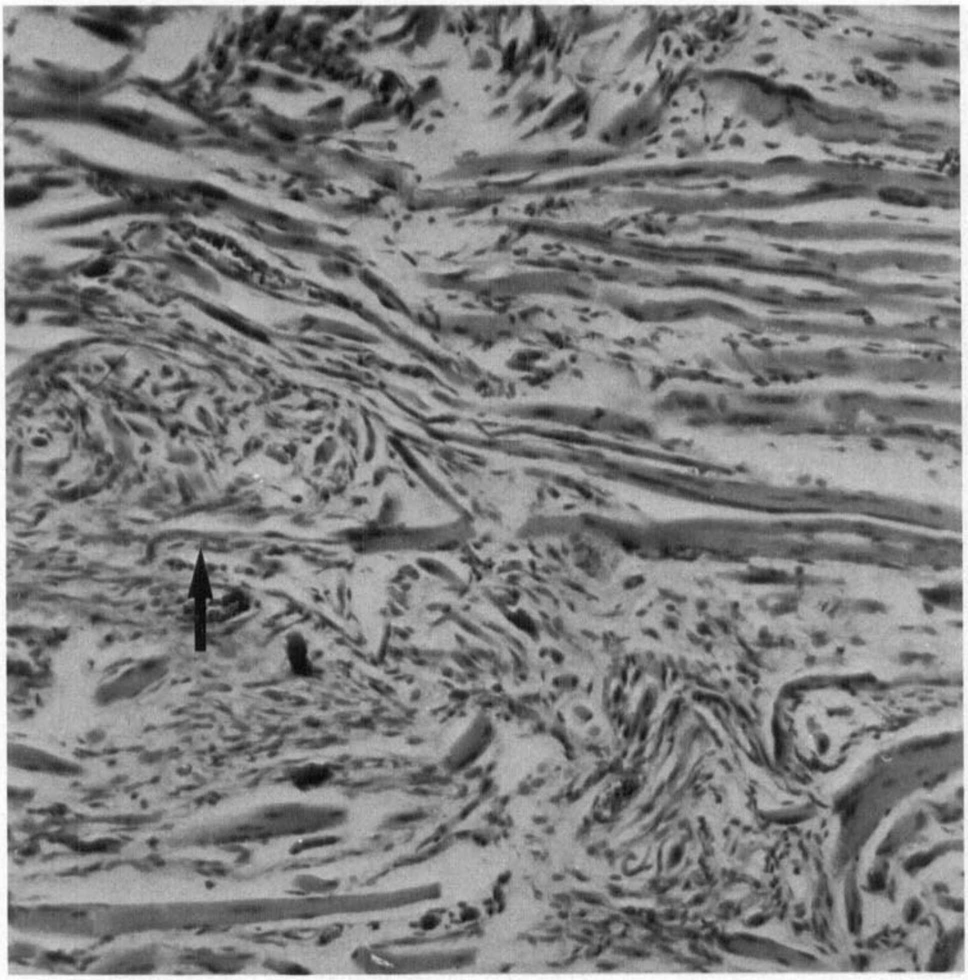


*Figure 7. Microangiogram of the muscle rupture 5 days post-traumatization in a young rat. The ingrowth of new capillaries (→) from all borders towards the non-vascularized centre of the injury is found × 42.5.*

centre of the injured area seemed to be more compact in the young rats. The area of neovascularity was smaller in the young animals and the new vessels were more tortuous, indicating a more pronounced contraction of the connective scar tissue in the young rats (Figures 2, 9 and 10).

## DISCUSSION

"Young rats" in this study are represented by young adult animals aged 4 months, because the speed of healing in striated muscle can be considered normal in rats of this age (Allbrook 1973).

Repair in striated muscle comprises regeneration of disrupted muscle fibres, simultaneous production of connective scar tissue and capillary sprouting from the borders of the injured area (Millar 1934, Allbrook et al. 1966, Carlson 1968). This study demonstrated a decreased repair capacity in striated muscle with advanced age, a phenomenon which has been described in the context of healing in other tissues, e.g. fractures of bones (Aho 1966) and skin wounds (Leaming 1963, Holm-Pedersen & Viidik 1972, Viljanto & Raekallio 1976, Goodson & Hunt 1979). A decrease in repair similar to that seen in the old rats was demonstrated in our earlier

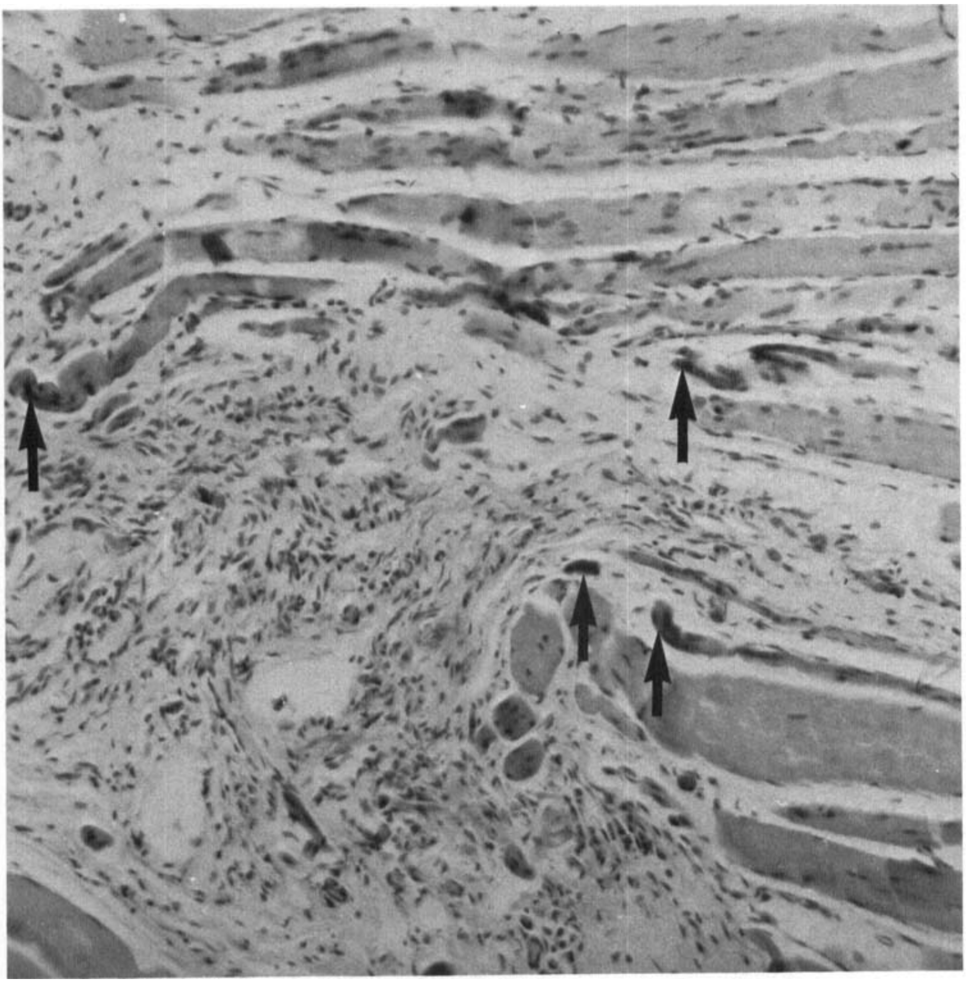


*Figure 8. Microangiogram of an old rat 5 days post-tramatization. The ingrowth of new capillaries from the borders of injury is not demonstrable and the injured area is nonvascularized × 42.5.*

studies by immobilizing the injured muscle. In the immobilized muscles the blood circulation is markedly decreased, especially in the beginning (Hudlicka 1973). In these studies the blood flow was not measured but the decrease in the sprouting of new capillaries in old rats was similar to that observed earlier in the immobilized muscles. The decreased capillary production in immobilized muscles is apparently due to the lack of stimuli, usually provided by normal muscle movements. Increased capillary production was seen in the mobilized muscles during the repair period (Järvinen 1976). Lack of oxygen and low oxygen tension are considered to stimulate the production of new capillaries (Remensnyder & Majno 1968). The tissue oxygen tension decreases strongly in the centre of a muscle injury similar to that used in our study (Józsa et al. 1980). The oxygen tension in the muscle injuries of old rats is unlikely to be greater than in young rats, but the response of nearly all biological tissues to different stimuli diminishes with advancing age. In skin wounds, the $PO_2$ tension in the dead space of a wound was lower in young animals (Holm-Pedersen & Zederfeld 1972), which may be caused by the higher oxygen consumption of the wound tissue of younger rats. The observation of Viljanto & Raekallio (1976) and

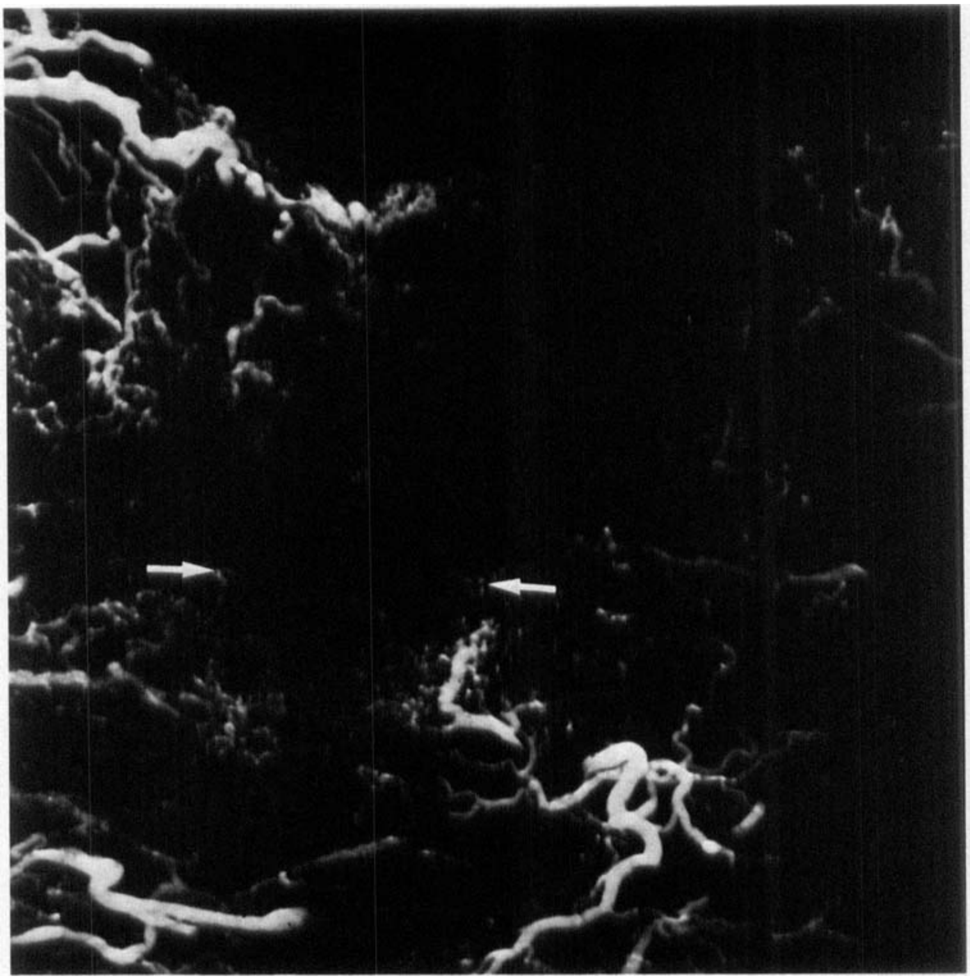


*Figure 9. Microangiogram of the muscle rupture 21 days post-traumatization in a young rat. Completely vascularized injury with radial growth of vessels is seen in the middle of the Figure. × 42.5.*

Heikkinen et al. (1971) of an increased activity of some enzymes necessary for energy metabolism in the wounds of young individuals indicates their greater metabolic capacity. The more pronounced infiltration of inflammatory cells and fibroblasts observed in skin wounds of younger individuals (Viljanto & Raekallio 1976) was also clearly demonstrated in this study. The connective scar tissue formation was less intense in the injuries of old rats, a phenomenon which parallels our earlier observations in immobilized muscle (Järvinen 1975) with a marked decrease in the tensile properties (Järvinen 1976). This decrease in tensile properties has been observed in skin wounds and attributed to the smaller degree of cross-linking in the wound collagen of old rats (Holm-Pedersen & Viidik 1972).

Parallel to these observations is the fact that the fibrillogenesis and differentiation rate of the fibroblasts in experimental callus is more intense in young, growing rats (Aho 1966). The results of this study indicate the decreased response of old animals, and apparently also of older persons, to traumatic lesions of striated muscle followed by a delayed healing. This leads to a limited working capacity of the affected muscles with aging for a relatively longer time after trauma. The physiologically impaired healing capacity of tis-

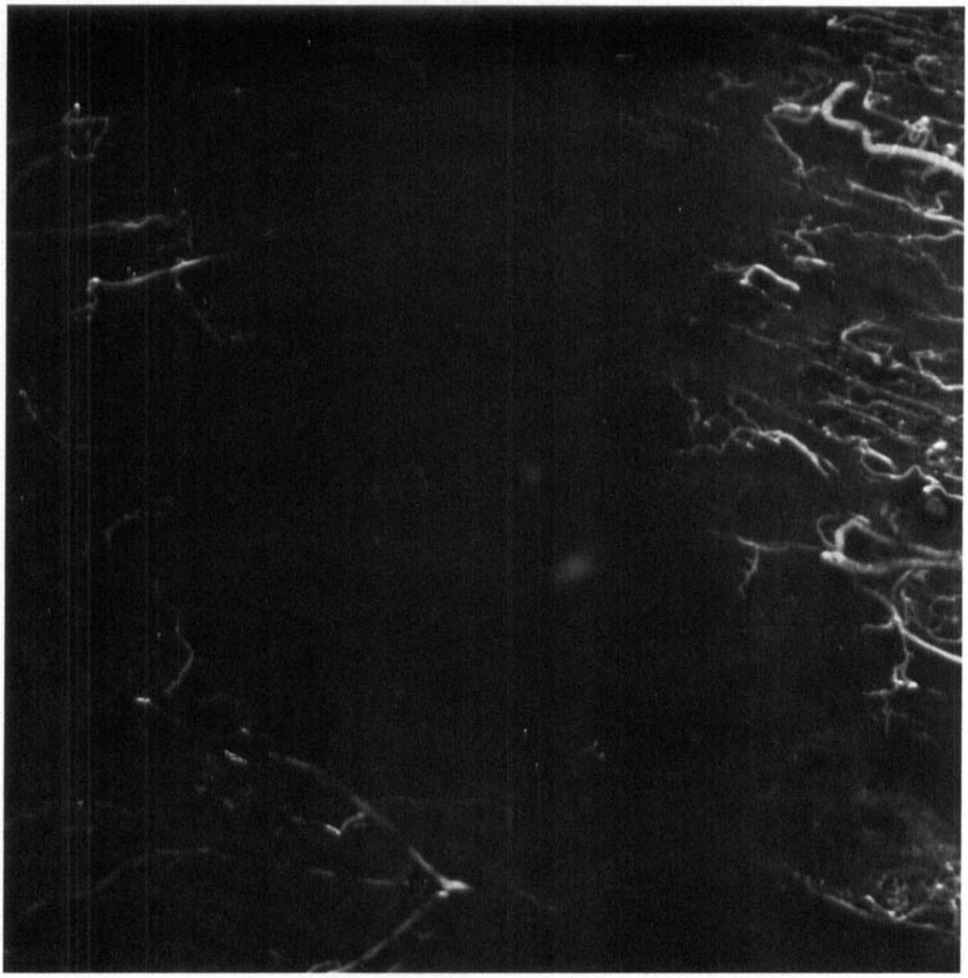


*Figure 10. Microangiogram 21 days post-traumatization in an old rat. In the middle of the injured area there is still a small nonvascularized area (→), (× 42.5) and the vessels are less tortuous and branched compared with those of the young animal (Figure 9).*

sues, and the increased risk of complications and muscle atrophy due to the immobilization should be taken into account when older individuals are treated.

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Correspondence to: Markku Järvinen, Institute of Clinical Sciences, Teiskontie 35, 33520, Tampere 52, Finland.