EFFECT OF THE ANODE OF AN INTERMITTENT DIRECT CURRENT ON REPARATIVE REGENERATION OF SKELETAL MUSCLE

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Systematic polarization of a region of mechanical injury to the soft tissues of animals with the anode of a direct current accelerates the healing of the wound, and on this basis the use of anodization in clinical conditions has been proposed [4]. However, the assessment of healing in these conditions has been based purely on macroscopic evidence.

The object of the present investigation was to make a histological study of the effect of anodization on posttraumatic regeneration of mammalian skeletal muscle tissue.

EXPERIMENTAL METHOD

The experimental animals were 50 male guinea pigs weighing 350-400 g.

In sterile conditions and under ether anesthesia the skin near the shoulder was incised, the triceps muscle was exposed, and a piece of muscle tissue was taken from it with a punch 6 mm in diameter. The wound was sutured. Operations were performed on both limbs. For the next 30 days the region of injury on the left limb was polarized daily with the anode of an intermittent direct current for 15 min. Nonpolarizing electrodes of Dubois-Reymond type were used; the method of polarization was unipolar. The frequency of the direct current pulses supplied by a stimulator was 60/min, and the duration of each pulse 0.3 sec. The pulses were rectangular in shape. The strength of the polarizing current was just below threshold, and it thus varied from one animal to another and at different periods of the experiment (the limits of variation were from 0.6 to 2.0 mA).

Only the left triceps muscle was polarized and the right acted as control. As an additional control, the triceps muscles of other guinea pigs undergoing the operation but not treated by polarization were used.

The animals were sacrificed 4, 8, 15, and 30 days after the operation and the muscles were fixed with Zenker-formol. Paraffin sections 7-8 μ in thickness were cut and stained with hematoxylin-eosin, azure-2-eosin, by Mallory, and with iron hematoxylin by Heidenhain's method.

Besides the ordinary histological preparations, the area of connective tissue developing in the region of injury was calculated by means of a drawing apparatus and planimeter. The area was determined at 10-12 levels of the zone of injury, starting at its surface and ending at maximal depth. The numerical results obtained were analyzed by statistical methods.

EXPERIMENTAL RESULTS

On the 4th day after the operation in the polarized muscle as a rule no muscle fibers were found in a state of Zenker's necrosis. In the distal zone of injury an intensive phagocytosis and lysis of the injured tissues were present (Fig. 1, a). The ends of some muscle fibers were dedifferentiated and formed basophilic pools with many nuclei. Among the intensively dividing fibroblasts and histiocytes, forming the granulation tissue, thin myosyncytial structures and newly formed capillaries were seen.

In the right (control) muscles, as in both muscles of the animals not treated by polarization, on the 4th day after the operation the zone of necrosis in the distal part of the wound was more extensive (Fig. 1, b). The muscle buds and myosyncytia were fewer in number than in the polarized muscles.

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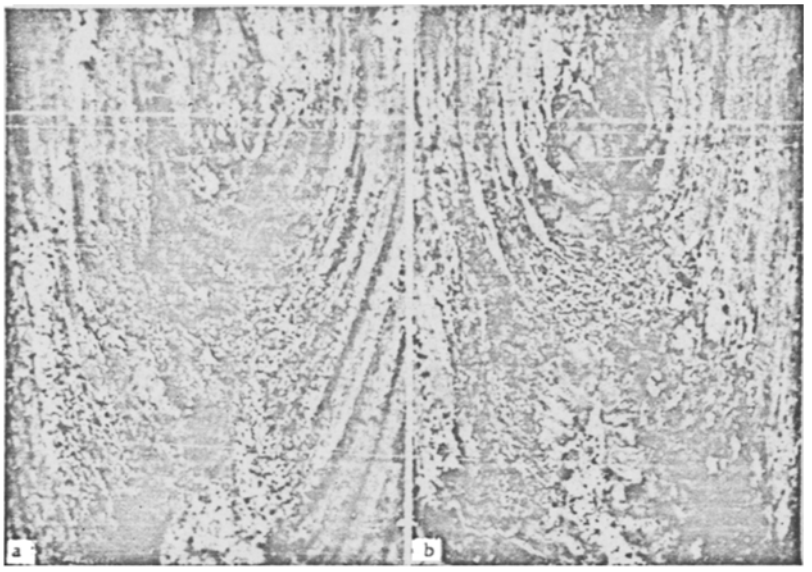

Fig. 1. Region of injury on the 4th day after operation. a) Left limb; b) right limb. Fixation in Zenker-formol, stained with hematoxylin-eosin. Objective 9 ×, ocular 7×.

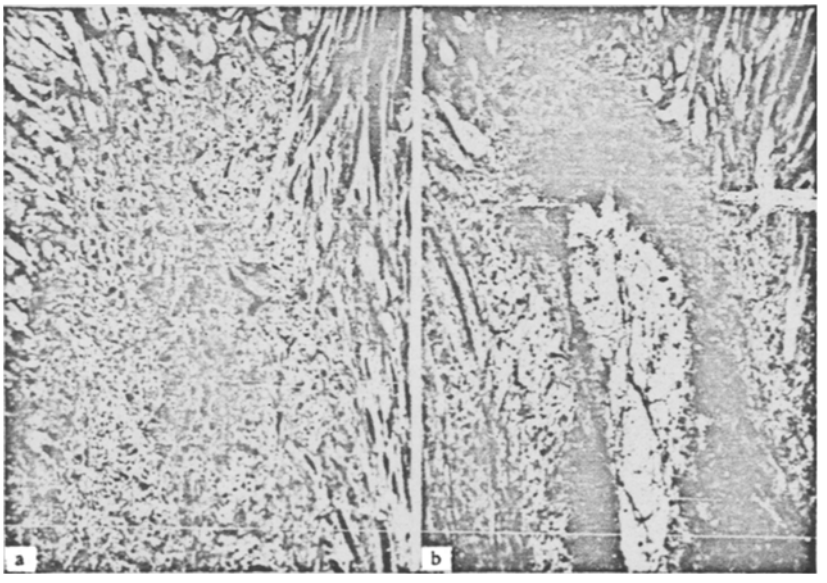

Fig. 2. Region of injury on the 9th day after the operation. a) Left limb; b) right limb. Fixation in Zenker-formol, stained by Mallory's method. Objective 9×, ocular 7×.

In the right (control) muscles, as in both muscles of the animals not treated by polarization, on the 4th day after the operation the zone of necrosis in the distal part of the wound was more extensive (Fig. 1, b). The muscle buds and myosyncytia were fewer n number than in the polarized muscles.

On the 8th day after the operation the processes of phagocytosis and lysis of the necrotic tissues in the polarized muscle were largely completed. In these circumstances as a rule the region of the defect was filled by granulation tissue (Fig. 2, a).

In the control muscles at the same period granulation tissue was found only at the edges of the wound, and in its centrai part remains of escaped blood and threads of fibrin persisted (Fig. 2, b).

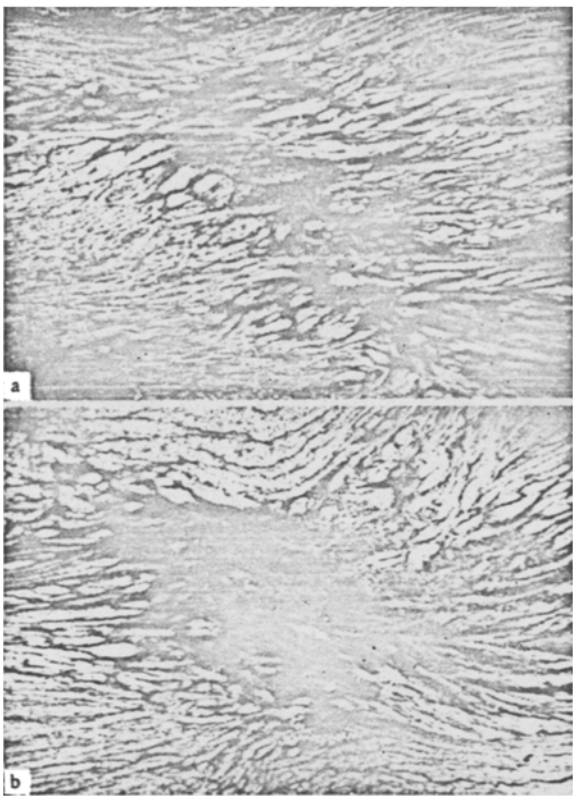

Fig. 3. Region of injury on the 15th day after the operation. a) Left limb; b) right limb. Fixation in Zenker-formol, stained by Mallory's method. Objective 9×, ocular 5×.

Effect of Polarization on the Development of Connective Tissue in Regenerating Muscle (15th day after operation)

Animals No.	No. of measurements	Arithmetical mean area of connective tissue in regenerating muscles	
		experimental	control
1	12	7,7	15,18
2	10	6,25	8,6
3	10	5,59	11,99
4	12	8,5	13,6
5	10	6,5	12,63
		$M_1=7,03$ $m_1=\pm0,491$	$M_2=12,04$ $m_2=\pm1,092$
		$P<0,001$	

The difference between the experimental and control wounds on the 15th day after the operation were that in the polarized muscles the myosyncytia and young muscle fibers had invaded the granulation tissue to a considerable distance. Accordingly, the amount of connective tissue in the region of injury was less than in the control wound (Fig. 3, a, b, and table). As a result of the heterogeneity of the histological pictures in the region of injury in different guinea pigs, these differences were seen particularly clearly when the regenerating muscles of the left, polarized limbs and the right, control limbs of each animal were compared.

On the 30th day the differences between the process of regeneration in the experimental and control muscles were less marked. The results thus showed that systematic polarization by the anode of an intermittent direct current facilitates the regeneration of muscle fibers.

It may be assumed that the more intensive resorption of necrotic masses observed in these experiments was connected with the removal of the state of depression or parabiosis of the tissues in the zone of injury, as has been suggested elsewhere [3, 4]. In this case the histiocytes became active sooner and began to perform their phagocytic function earlier. The relatively early liberation of the ends of the muscle fibers from the necrotic masses facilitated the appearance of young muscle cells in these zones.

It is possible that the anti-parabiotic action of the anode [1-2] on the functional state of the injured nerve and muscle fibers facilitated the process of regeneration.

The disappearance of the difference between the control and experimental muscles on the 30th day after the operation was probably associated with the ineffectiveness of the action of the anode on the nerve and muscle cells which had emerged at this time from their state of parabiosis (as proved by the diminishing thresholds of stimulation).

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