REPARATIVE REGENERATION OF SKELETAL MUSCLE IN MAMMALS RECEIVING VITAMIN B_{12}

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With the increasing clinical use of vitamin B_{12}, its application is no longer confined to disorders of hemopoiesis, but it has been extended to the treatment of of many other diseases, and, in particular, of neuromuscular dystrophies [12] and diseases of the peripheral nervous system [6, 9]. Several workers have reported [1, 2, 4, 7, 8] that vitamin B_{12} accelerates the regeneration of peripheral nerves after trauma, which is bound to have a benefical effect on reparative processes in damaged muscle. As Charkey and his co-workers [11] have shown, the administration of vitamin B_{12} to an animal causes an increase in the synthesis of tissue proteins, especially in skeletal muscle.

In view of these findings, we considered it desirable to study the effect of vitamin B_{12} on the regeneration of skeletal muscle tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male white rats, weighing from 200 to 250 g. Vitamin B_{12} was injected intramuscularly in a dose of 20 μg to each animal. For the first 20 days the injections were given daily, and thereafter on alternate days.

In selecting this dose we were guided by the findings of Leroy and Robin [12], who showed that high doses of vitamin B_{12} were therapeutically effective in neuromuscular disorders. In addition to vitamin B_{12}, one group of experimental animals was given thyroidin by mouth. A group of animals acted as controls.

The object selected for study was the tibialis anterior muscle in both hindlimbs. Operations were performed under ether anesthesia in strictly aseptic conditions. After exposure of the muscle, a small hole was excised in its center with a special round punch (5 mm in diameter) at an equal distance from the point of attachment of the tibialis anterior muscle to the crest of the tibia. To prevent injury to the underlying tissues, a thin metal plate was introduced beneath the muscle. Sutures were inserted into the skin at the wound edges. The muscles were fixed in Zenker formol at different times during the month. Sections in paraffin wax were cut to a thickness of 6-8 μ, and stained with Carazzi's hematoxylin, azure II-eosin, Mallory's mixture and iron hematoxylin.

EXPERIMENTAL RESULTS

The distinguishing feature of the reparative manifestations in the control rats was the slight spread of the destructive processes in the injured muscle. In the proximal portion of the wound, for instance, only the ends of the divided muscle fibers underwent degenerative changes; in the distal portion the muscle fibers immediately injured were in process of destruction.

On the fourth day after operation the products of destruction had completely resorbed. The defect in the muscle was filled with an exudate containing fibrin, effused blood and numerous cells of tissue and vasogenic origin; granulation tissue was developing along the periphery of the wound. At this stage, signs of regeneration of the muscle tissue were already well marked. In the proximal portion of the wound, muscle buds were growing out from the viable ends of the muscle fibers, and becoming outstretched into myosymplasts. The absence of conditions favoring stretching in this area, however, led to a haphazard arrangement of the developing buds and myosymplasts. Their volume increased as a result of the growth in the mass of the cytoplasm, but their increase in length was slowed down. At the distal end of the wound, the newly formed muscle cells mainly had the form of thin, stretched myosymplasts, arranged along lines of tension arising in this particular area of the muscle, functioning more actively. Myofibrils appeared in the sarcoplasm of the myosymplasts; differentiation into Q and I disks could be observed in some of them.

Seven days after operation the center of the defect still contained much exudate, with fibrin and blood cells. At the periphery there was intensive proliferation of granulation tissue, undergoing differentiation into loose connective tissue. At this stage the myosymplasts continued to develop into muscle tubes. On the tenth day the nuclei in the muscle tubes began to be displaced beneath the sarcolemma, which was due to the process of formation of the muscle fibers. At the end of the wound nearest the tendon, more fibers were formed. The number of cells in the connective tissue decreased, and dense collagen bundles appeared.

Fifteen days after operation, adipose tissue could be seen in the center of the defect, replacing the round-cell infiltration. Differentiation of the muscle tubes into young muscle fibers continued. On the twentieth day the processes of their formation were largely terminated; the newly formed muscle fibers were distinguishable from the old by the larger number of nuclei and their smaller diameter. From 25 to 30 days after operation, the defect in the muscle was filled with a chaotic disarray of muscle fibers (Fig. 1). Much of the regenerated tissue consisted of connective tissue, which had acquired the character of compact fibrous tissue.

At the stage of 4 days, in the experimental animals receiving vitamin B_{12}, more cells had accumulated in the region of the wound. Granulation tissue filled nearly the whole of the defect, and only in the center of the cavity were exudate and fibrin still present.

The reparative processes in the muscle tissue of the experimental animals were characterized by sharply defined reactive changes in those muscle fibers not directly damaged. These changes took the form of increased destruction of part of the muscle fibers, and especially of the intensive morpho-physiological reorganization of other muscle fibers, with the active formation of young cells. In spite of the widespread nature of the destructive changes, on the fourth day after operation the zone of injury was completely free from products of destruction, which was evidence of the high intensity of the processes of resorption. At the site of the resorbed necrotic masses, many myosymplasts had arisen from the remaining viable fragments of muscle fibers. In the experimental animals, in contrast to the controls, the processes of dedifferentiation (the formation of sarcoplasm rich in nucleic acids) and the regenerative increase in the number of nuclei extended to a considerable number of muscle fibers, especially in the lateral and distal portions of the muscle. Such "excited" fibers became the source of formation of an abundance of myosymplasts (Fig. 2). The latter arose as the result of longitudinal splitting of muscle fibers with the isolation of long bands of basophilic cytoplasm from the nuclei, as we have previously described in hyperthyroid animals [5]. This method of formation of the myosymplasts brings about their parallel arrangement, which also provides for preservation of the endomysium.

In the experimental rats at this stage, just as in the control animals, differentiation of the sarcoplasm began in the myosymplasts. The development of muscle cells was most intensive in the areas where they were formed by cleavage from the "excited" muscle fibers of long myosymplastic bands. Mitosis was more often found in the myosymplasts in rats receiving vitamin B_{12} than in the control animals.

Subsequently, on the 7th-10th day, some of the myosymplasts had become converted into muscle tubes which, in turn, developed into young muscle fibers. On histological examination of the regenerating tissue 10 days after injury, attention was drawn to the active growth of the muscle tubes and fibers. The distal and lateral

Fig. 1. Defect in the muscle 25 days after operation. Control. Zone of repair nearer the proximal end of the wound. The defect in the muscle is filled with a chaotic disarray of muscle fibers. Stained by Carazzi's hematoxylin. Magnification: ocular 10×, objective 8×.

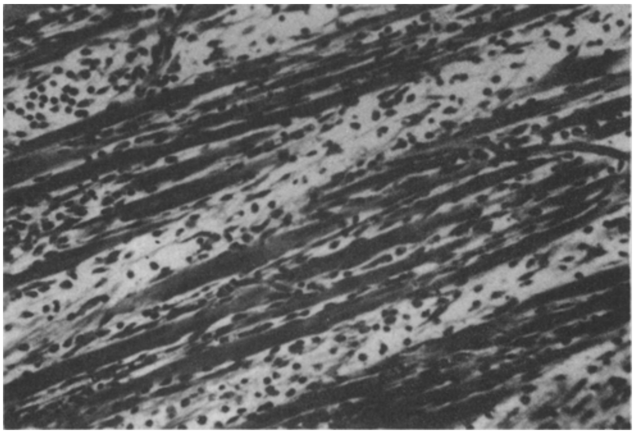

Fig. 2. Defect in the muscle 4 days after operation. Experiment using vitamin B_{12}. Growth of myosymplasts in areas of reactive reorganization of muscle fibers at a distance from the wound. Stained by Carazzi's hematoxylin. Magnification: ocular 7 × objective 20 ×.

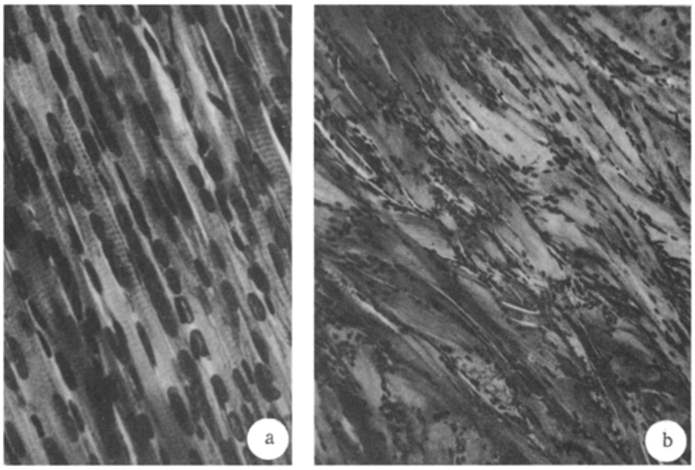

Fig. 3. Regeneration of muscle tissue 25 days after operation. Experiment using vitamin B_{12}. a) Proximal part of the regenerating tissue; the arrangement of the young muscle fibers is less chaotic than in the controls; b) regenerative reconstruction of the muscle fibers in the distal parts of the muscle (multiplication of nuclei, cleavage of myosymplasts). Stained with Carazzi's hematoxylin. Magnification: ocular 7× , objective 40 × .

portions of the muscle were in a highly "excited" state: the cytoplasm of the "old" (uninjured) muscle fibers was basophilic; the number of nuclei was increased, and they occupied the central, axial part of the fiber or were arranged in a chain beneath the sarcolemma.

At later stages (15th-20th day), these distinctive features of regeneration which have been described were preserved in both the proximal and distal parts of the wound, in the newly formed muscle cells, longer than in the controls. On the 20th day, the regenerated muscle tissue consisted mainly of differentiated muscle fibers. In the distal part, however, the regenerative reconstruction of the muscles had not come to an end. On the 25th day after operation, in this zone it was possible to see extensive areas of newly formed muscle tubes and young fibers containing an abundance of nuclei (Fig. 3 a). The center of the defect, just as in the control animals, was filled with fibrous tissue, interspersed with adipose tissue. In the proximal part of the regenerating tissue the arrangement of the young muscle fibers was less chaotic than in the control animals (Fig. 3b).

The large defect in the muscle in these rats was thus replaced by regenerating muscle and connective tissue. By comparison with the controls, in the experimental animals the reactive reconstruction of the muscle fibers was more pronounced, especially in the lateral and distal portions of the muscle. The high "densitivity" of the latter during administration of vitamin B_{12} may be due to the greater functional loading of these portions of the muscle following excision of its central area. Another important factor was presumably the partial denervation of the lateral and distal portions of the muscle as a result of injury. The preliminary results of a study of the restoration of the nerve connections in the region of the regenerating muscle showed that it was only on the tenth day that young nerve fibers grew into the region of the defect and reached its distal end.

As a result of the active regenerative reconstruction of the muscle fibers in the rats receiving vitamin B_{12}, a more intensive formation of new muscle cells was observed in these animals. The changes observed in all the experimental animals receiving vitamin B_{12}, either alone or in conjunction with thyroidin, were uniform in type and coincided with the individual stages of the process of repair. It can be assumed that during the combined action of these drugs, that of vitamin B_{12} was predominant. Evidence in favor of this is given, in particular, by the fact that in the rats receiving vitamin B_{12} and thyroidin large numbers of fat cells were deposited in the region of the wound, and there was active proliferation of connective tissue; this was not at all typical of the regenerating tissue which developed in purely hyperthyroid animals [5]. The inhibitory action of the thyroid gland hormone on the collagen-forming function of the fibroblasts was noted by V. G. Eliseev [3] and Moltke [13] in their investigations. According to the findings of Asboe-Hansen [10], this depression of collagen formation is explained by the fact that thyroxin causes a marked decrease in the content of mucopolysaccharides in the connective tissue.

SUMMARY

The author studied the effect of vitamin B_{12} on the reparative regeneration of skeletal muscle tissue in sexually mature male rats . A standard injury was inflicted on all the animals. In comparison with control animals reactive changes in the injured muscle were more pronounced in rats which received vitamin B_{12}. These changes involved increased disintegration of one part of the muscle fibers situated at some distance from the wound, as well as progressive morpho-physiological reconstruction of a large number of muscle fibers with abundant formation of myosymplasts. In rats which received vitamin B_{12} the formation of young muscular elements was intensified owing to the active regenerative reconstruction of muscle fibers.

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