# THE EFFECT OF OVERSTRAINING THE SENSORY EXCITATION PROCESS ON THE REGENERATION OF MUSCLE TISSUE IN WHITE RATS

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The task of this work was to clarify the effect of a pathological condition in the process of cortical-stimulation within the cerebral hemispheres of the brain on the regeneration process in skeletal muscle. This question has still not been illuminated in the literature.

In experimental animals, under conditions overtaxing the strength and adaptability of the cortical excitation process, we studied the basic phases of regeneration in muscle tissue following partial transection of the muscle: The course of the inflammatory reaction, the destructive and proliferative phenomena, and the dynamics of growth and differentiation in the connective and muscle tissues.

## EXPERIMENTAL METHOD

The experiments were carried out on 42 white rats, 300 to 400 g in weight, of which 20 served as controls and 22 as experimental animals. Before the operation the experimental rats were prepared according to the motor-alimentary conditioned reflex method (the task was carried out with each rat individually). The conditioned reflexes were worked out in special chambers with a moveable floor. The magnitude of the conditioned reflex was gauged by two indicators: the rapidity of the run to the food and the force with which the rat depressed the disk with the food, tripping the pneumatic system. A kymograph record reflected the following experimental data: the free motion of the rats in the chamber, the conditioned signals, the moment that the food was taken and the time in seconds.

Initially, positive motor-alimentary conditioned reflexes were worked out in the rats in response to an electric tone of average intensity and to a light from a 10 w electric lamp. After fortification of the conditioned reflexes we used an electric tone of greater intensity and a 150 w electric lamp. The number of combinations was increased, in one experiment, to 15 to 20, and the pauses between the conditioning signals shortened to 20 to 180 sec. Sterotype was excluded both in the order of the afforded stimuli and in their number or the pauses between them. From the conditions of the experiment it is apparent that the strength of the process of excitation in the rats was overtaxed, as was its mobility in both analyzers, visual and auditory. The indicated activities were applied for 20 days prior to the operation on the muscle and continued after the operation up to the time the material was taken for histological investigation.

Trauma was inflicted on the anterior tibial muscle, not including the basic nerves and vascular trunks. The muscle was cut with scissors through 2/3 of its thickness The operation was performed under strict aseptic conditions using ether anaesthesia. Material was fixed in Zenker formol, 12, 24, and 48 hr, and 5, 8, 12, 16, 20, and 30 days, after the operation. Serial paraffin sections were stained with appropriate histologic stains.

## EXPERIMENTAL RESULTS

Upon working out the conditioned reflexes, differences were detected in the type-specific properties of the rat nervous system. Two basic groups appeared: the first with a predominance of the excitatory process, the excitable type; the second with predominantly inhibitory processes, the inhibited type. Both groups included variations which we did not separate out here.

With prolonged application of only intense positive stimuli in the majority of the rats in the first group the excitatory process acquired a pathologically inert character that, apparently, set the conditions for a strong excitation of the motor analyzer and a deficiency in the inhibitory process. In rats of the second group there was observed a temporary disappearance of the conditioned reflexes in the presence of a constant fortification of the conditioning signal, an incompletion in the conditioned reflex act, and a stiff posture, in which the rat remained over the course of several minutes, not reacting to the conditioning signals. In the given case, obviously, there occurred an intense inhibition of the motor component in the alimentary reflex. Phase phenomena were noted in the rats of both groups. Disruption of the vegetative functions was manifested by urination and defecation during the experiment. Several of the rats showed remarkable work capacities, manifesting them in prolonged maintenance of a high level of effort, even when we applied up to 20 combinations in one experiment.

In the rats of the control series 12 hr after injury the muscle fibers markedly swelled, lost their specific structure, and fell into fragments of different sizes.

An intense accumulation ofleukocytes occurred in the wound exudate and between the injured muscle fibers; the predominant forms at this time were neutrophils. After 24 hr an active process of differentiation of ceil forms from hematogenous and tissue origin into macrophages was noted, and along with this many neutrophils degenerated. After 48 hr the dead cells and necrotized tissue were almost completely resorbed; the fibrin was partially resorbed. In the central portion of the wound (the site of direct injury) the appearance of a thin latticework was maintained for 6 days. The wound filled in with granulation tissue; in this tissue the predominant forms were fibroblasts.

By means of complex reorganization, accompanied by dedifferentiation of the muscle fibers, accumulation of basophilic sarcoplasm, and multiplication of nuclei, the ends of the injured muscle fibers were converted into muscle buds and myosimplasts. They appeared to be the basic origins of muscle tissue reconstruction. On the fifth day there was observed an active formation of muscle buds and myosimplasts, growing into the granulation tissue in the form of long basophilic projections, tapered or swollen at the free end. The process of differentiation of the myosimplasts in the muscle tubes occurred most intensively on the 6th to 8th days. On the 12th day the undifferentiated myosimplasts remained very few in number, and the granulation tissue was replaced by loose connective tissue. Further development of the regenerative process was accompanied by the formation of collagen bundles, and by the growth and final differentiation of the young muscle fibers. After 20 days the process of collagen formation diminished at the same time that a marked increase in growth was noted in the regenerating muscle tissue. On the 30th day the defect was filled in with young muscle fibers, scattered in a disoriented fashion in the central portion of the wound, and small layers of connective tissue. The amount of connective tissue progressively decreased with the further formation of the regenerate.

Corresponding with the observations of Z. P. Ignat'eva [5] on the results of the reconstruction of skeletal muscle in rats followed for some time, after more than 2 months the site of the defect is just barely recognizable by the noticeable diversion in the path of the muscle fibers.

In the experimental rats of the excitable type with overstraining of the cortical process of excitation the extent of the destructively altered portions of injured muscle fibers was significantly greater than in the control rats. The inflammatory reaction was accompanied by copious exudation and more abundant transudation of leukocytes from the blood vessels. The increased exudation set up conditions inhibiting the phagocytic activity of the macrophages. Despite the vast numbers of the latter, after 2-3 days there was still much unresorbed detritus in the wound. In the maturing granulation tissue there was a multitude of polyblasts and newly formed capillaries, the calibre of which was significantly increased by the 5th day (Fig. 1 a, b). The regenerative processes in the connective tissue at that period were manifested more more weakly than in the control rats. The fibroblasts had the form of small cells with poorly developed extensions, loosely disposed. An increased proliferation of nuclei was noted in the muscle buds and myosinplasts. On the 8th day a shift occurred in the regeneration reaction of the connective tissue toward an increase in collagen formation, as a result of which the defect quickly filled in with loose connective tissue. On the 12th day a considerable amount of differentiated connective tissue was formed, which had a negative effect on the growth of the newly formed muscle. There was especially large amounts of connective tissue in the central portion of the wound, where the young muscle fibers underwent complete atrophy. In addition, local growth of the intermuscular connective tissue and fascia took place.

These formations were made up of collagen fibrils, cellular elements, blood vessels, and thin (4 $\mu$ in diameter) but completely differentiated muscle fibers.

On the 16th day the composition of the scar components pointed to a profound trophic disturbance in the regenerating muscle tissue. Chaotically growing massive collagen bundles compressed the atrophically altered young muscle fibers. Granular clumps were visible—remains of the destruction of the latter, surrounded by lymphoid elements (Fig. 2a, b). On the 30th day the muscle wound was filled in with well-developed collagen bundles, forming complex interweavings with the poorly developed young muscle fibers. The latter varied in diameter (from 4 to 20 $\mu$) and in the state of their differentiation, while the fibers with the smallest diameter had the most clearly manifested transverse striations.

Thus, in the excitable rats, the regeneration of muscle tissue is characterized by the following fundamental features: intense development of the inflammatory process, increased regenerative reaction on the part of the blood vessel system, increased collagen formation after the 8th day of healing and inhibition of growth of the young muscle fibers.

In regard to the experimental rats of the inhibited type, overstraining of the process of excitation reflected itself on the regenerative process in a different way. The inflammatory reaction in the work-capable rats of the inhibited type ran its course in a manner similar to the controls, which, apparently, was related to the development of a protective inhibition in them. In rats of the extremely inhibited type, in which the process of excitation was also weak, the inflammatory reaction distinguished itself in its sluggishness and its lingering character. Twelve hours after the operation there were very few leukocytes in the wound; at the end of the first day the inflammatory picture had almost not changed, inhibition being noted in the differentiation of the cellular elements into macrophages. On the 8th day the edema was still not resorbed, the fibroblasts were poorly differentiated, and many underwent degenerative changes. The myosimplasts were vacuolated, and stained poorly as a result of cloudy swelling. These disturbances appeared to be a result of the extreme debilitation of cortical activity, caused by overstraining of the excitatory process.

In the work-capable rats of the inhibited type the regenerative reaction of the muscle tissue showed itself as an intense accumulation of sarcoplasm at the ends of the old injured muscle fibers. This gave rise to massive beadings, yielding a weakly basophilic reaction. The differentiated structures appeared in them on the 8th to 12th days, in the form of loosely scattered myofibrils. Depression of the neurotonic state by means of systematic overstraining of the excitatory process led to inhibition of the differentiation in the regenerating tissue. On the 16th day portions of unreplaced granulation tissue remained

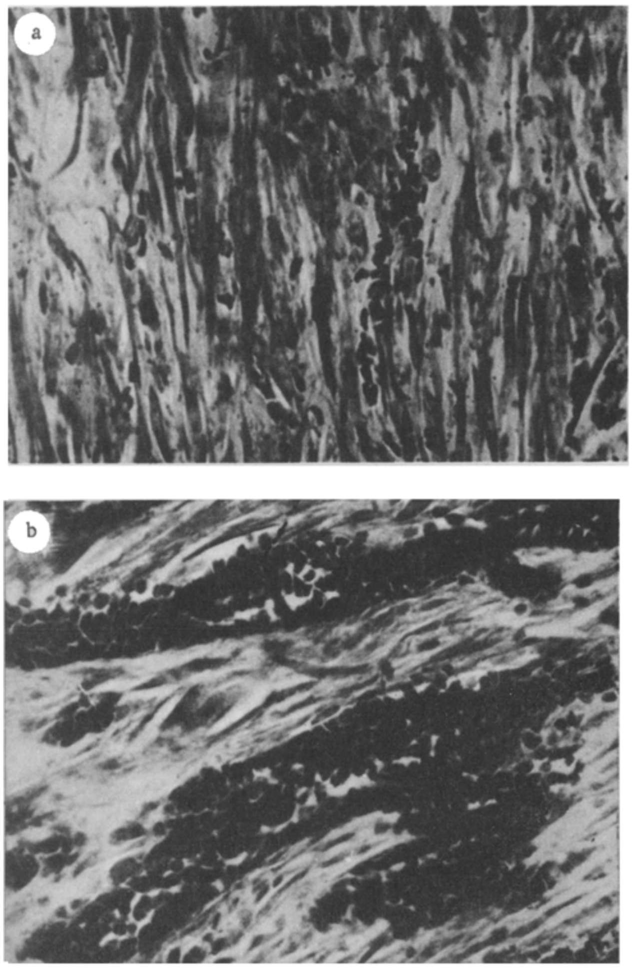

Fig. 1. Photograph of the healing muscle injury on the fifth day after operation. a) In the control rat. The wound is filled in with granulation tissue and the myosimplasts growing in it. The newly formed blood vessels are visible; b) in the rats of the excitable type, with overstraining of the excitatory process. The granulation tissue is intensively vascularized, and the c calibre of the newly formed blood vessels is greatly increased. Stained with azure-II-eosin. Magnification: Objective 40 ×, ocular 7 ×.

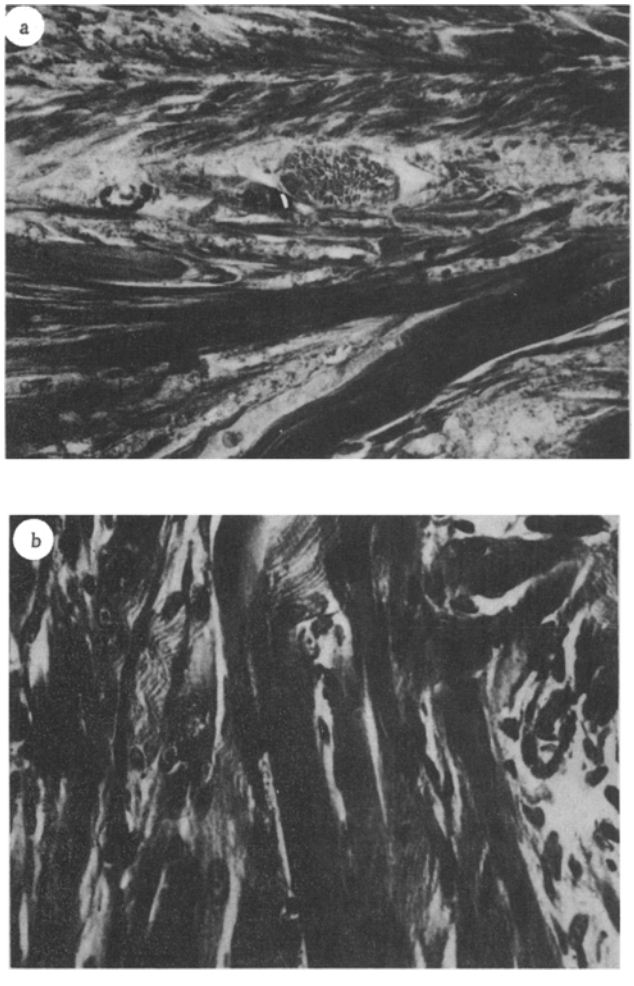

Fig. 2. Healing of the muscle wound on the sixteenth day after operation. a) In the control rat. The wound is filled in with regenerated muscle fibers and sheets of loose connective tissue; b) in the rats of the excitable type with overstraining of the excitatory process. Coarse fibered buds of connective tissue are visible in the wound, as well as atrophic changes in the yound muscle fibers and granular clumps, traces of their destruction. Stained according to Mallory. Magnification: objective 40 ×, ocular 7 ×.

in the wound; on the 20th day there were many young muscle fibers that did not have cross striations, although they surpassed the old fibers in diameter (26 μ against 22), and, in addition, a vigorous multiplication of nuclei occurred in them. Myosimplast formation was encountered among the young muscle fibers, as well as many vessels and large groups of lymphoid cells.

On the 30th day the wound defect was filled in with young muscle fibers, 24 to 30 μ in diameter (the old fibers had a diameter of 22-24 μ), which contained many nuclei, oval in form. The connective tissue was more poorly developed than in the control rats. Far from the wound it was possible to see fine regeneration foci, arising, obviously, as a result of secondary necrosis.

Our data shows that the regenerative process in the muscle of white rats subjected to overstraining of the cortical process of excitation runs its course in a varying fashion, depending on its type-specific characteristics. Thus, in lower mammals the inherent functional structure of the cortical cells holds a significance in the reaction of the organism to external influences.

The basis of the changes in the picture of injured muscle regeneration in the experimental rats lies in the trophic disturbances caused by overstraining of the excitatory process.

According to the learned K. M. Bykov, disturbances originating in the neurotonic state protect not only the cells of the cerebral cortex but also the subcortical centers, and thus influence the metabolism, alter the tissue properties, the tissue properties, the permeability of blood vessel walls , etc.

This hypothesis has been supported numerous times in the literature [2, 10, 12]. A close relationship has been established between the functional state of the cerebral cortex and the separate components of the inflammatory reaction: exudation, transudation of leukocytes, alterations in the permeability of blood vessels [3, 4, 7, 8, 11].

Our data shows that disruption of the normal cortical activity in animals influences the course of the inflammatory reaction and the entire intricate complex process of traumatic regeneration in skeletal muscle.

## SUMMARY

The author investigated the regeneration of the anterior tibial muscle in white rats the nervous system of which was subjected to stimulation.

In overstraining the cortical process of excitation in white rats with an excitable type of the nervous system the inflammatory reaction of the anterior tibial muscle is accompanied by extensive disintegration of the injured tissues, increased exudation and infiltration of the area of injurity with leukocytes. The granulation tissue shows marked vascularization. Restoration of the muscular tissue is inhibited by the active connective tissue development. The regenerative reaction in rats with a weak excitation process,i.e., in the animals with extreme inhibition, is depressed. In rats with a stronger nervous system (inhibitable, but capable of functioning) the inflammatory reaction follows the course similar to that in control animals. Intensification of the neurotic condition induces a delayed differentiation process in the connective and muscular tissues.

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