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Investigations of ultrastructure of damaged and regenerated skeletal muscle fibers

Loss of muscle power closely depends on muscle atrophy, thus immobilization and inactivity of knee joints rapidly result in quadriceps muscle degeneration. The majority of hitherto prevailing investigation done on volunteer patients had dealt with only changes concerning the peripheral part of extremities and concerned muscle thickness, measured with help of CT or ultrasound, or disturbances in electric conductivity and loss of muscle power, as well as with some of the morphological and biochemical parameters.

Our investigations concerned the head of the parietal part of *quadriceps femoris*, and were based on observations of the ultrastructure of muscle fibers using an electron microscope. We observed tissue samples taken from patients (10 men) 25–35 years old, who had old damage of knee joints (after about 6 weeks' immobilization). Legal consent was obtained from each individual tissue donor.

MATERIAL AND METHODS

In the first group, segments of tissue of parietal head of *quadriceps femoris* were taken inter-operationally from patients in whom there was found old damage of knee joint ligament. The second group were of tissue segments of this muscle after surgical repair of knee and rehabilitation, which consisted in power training using resistance machines. Specimens of muscle tissue from both two groups were fixed with glutaraldehyde and 1% OsO_4 solution. After dehydration in successively higher alcohol concentrations and propylene oxide, the specimens were embedded in "Spurr" resin. The sections used for electron microscope examination were cut using an ultramicrotom (Reichert Ultracut S). Ultrathin sections were contrasted with uranyl acetate and lead citrate plumb. Ultrastructural observations and electron micrographs were taken using a Tesla BS-500 transmission electron microscope.

RESULTS

The muscle fiber samples of *quadriceps femoris* which were taken from patients during the first operation, showed big changes in their ultrastructure. These changes included: myofibrils disintegration; disturbance of regularly arranged striation in sarcomers; dissappearance of Z line. In the sarcoplasm, we observed large vacuolisation, and in the interfibrillar spaces – an accumulation of exudate and morphotic elements of blood outside the capillary vessels. After surgical repair of their knees, patients were submitted to rehabilitation, which involved resistance training. After 12 months, during the operation to remove metal elements used to repair the knee, samples were taken from the same patients, for the next investigation phase (the second group).

Observations of muscle tissue after regeneration, showed a big improvement in the muscle cell's ultrastructure – the myofibrills were regularly arranged, and the sarcomers striations showed no deviations from the normal structure. We also observed a considerable increase in the number of properly formed ultrastructure mitochondria when compared with the first group. Beside normal tissue, foci with the irregular structure were present, in which we observed numerous satellite cells.

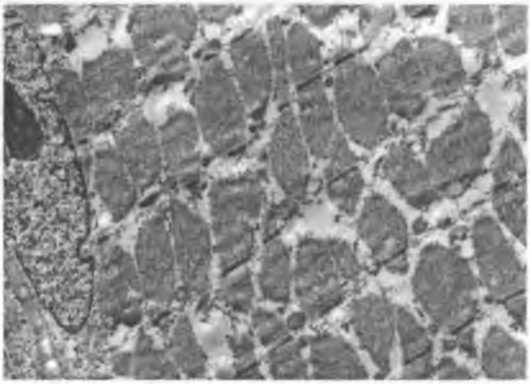

Fig. 1. Experimental group I. Magn. 4 000x. TME

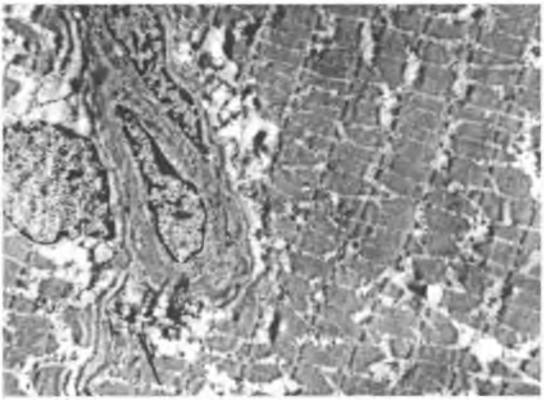

Fig. 2. Experimental group I. Magn. 3 000x.TME

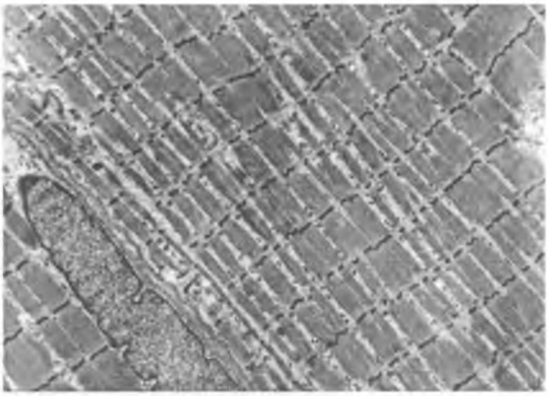

Fig. 3. Experimental group II. Magn. 3 000x. TME

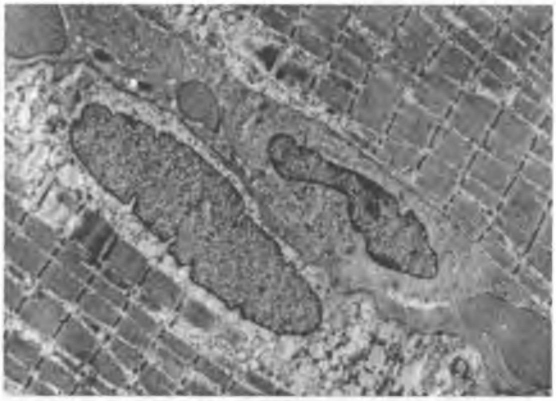

Fig. 4. Experimental group II. Magn. 3 000x.TME

DISCUSSION

After 5 week s immobilization with plaster cast of extremities of patients who were operated on for damage to the anterior *sacralis ligamentum*, (11) affirmed 21.4% atrophy of *quadriceps femoris*. Real muscle atrophy on account of immobilization is yet bigger than tomography investigations showed. Intermuscular oedema and hypertrophy of connective tissue cover also occurs, and muscle fibers atrophy. The unavoidable result of muscle fiber atrophy is loss of their power, which in the first week of immobilization, results in a loss amount of 40–50% (17). In the first week of immobilizing, particularly in the first four days, most pathological changes occur (21). In this time, atrophy occurs, first of all in the muscular fibers type I, which have considerably more mitochondria, particularly small subsarcolemmal mitochondria. In electron microscope, after some of hours of immobilizing, we observed progressive striations atrophy; myofibrils undergo decomposition on a big area of muscle fibers. In mitochondria gradual fragmentation was observed and sarcotubular system was destroyed. From about the seventh day of immobilization the regeneration process began with help of satellite cells – possibly the new "young" muscle fibers rise through mitoses from this satellite cells (2). The progressive process which is observed during immobilization is hypertrophy of collagenous fibers in the spaces between muscle fibers (3).

Muscle exercises after the immobilization period, are known as "reconstruction training". This, in the first seven days, has the effect that new muscle fibers are developed very fast, and full contraction ability is re-established. The resistance exercises are an organism effort, in which energetic processes in muscle cells happen in the oxidative respiration manner. This oxidative respiration depends on a number of mitochondria which contain the enzymatic system of cell oxidative respiration. Resistance training favours the neogenesis of vessels and the restablishment of muscle capillarization (13), so the oxidative capacity of muscle is corrected, resulting in increasing number of muscle fibers type I and mitochondria in all kinds of muscle fibers. After resistance training, there were changes in the ultrastructure and biochemical properties of mitochondria, and there was increased almost twice (2x) the number of small, subsarcolemmal mitochondria, which were aligned near the capillary vessels.

Researchers have yet to explain when muscle can recover their full efficiency, but muscle mass and contractile proteins reconstruct very fast. G r i m b y (10) observed that limb muscle, after knee joint operations in sportsmen, recovered about 80–90% of their power after only 14 months of performance training. So, in programs of planned post-operative treatment, post-operative resistance training which makes muscles regain full mobility, should be undertaken.

CONCLUSIONS

From the above observations we have made the following conclusions: Damage of knee joint ligaments cause in *quadriceps femoris* fibers, changes of the ultrastructure such as atrophia, which are visible after 6 weeks' immobilization. One year after operation to repair the knee and after rehabilitation in young men in whom full reconstruction of clinical efficiency was obtained, on the level of the ultrastructure, considerable improvement was evident, particularly in the picture of myofibrils. We believe however, that the improvement process is not finished yet because numerous satellite cells are present.

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SUMMARY

Our investigations concerned the head of the parietal part of *quadriceps femoris*, and we based our investigation on observations of the ultrastructure of muscle fibers using an electron microscope. We observed tissue samples taken from patients (10 men) 25–35 years old, who had old damage of knee joint ligament (after about 6 week' s immobilization). In the first group, segments of tissue of parietal head of *quadriceps femoris* were taken inter-operationally from patients in whom there was found old damage of knee joint ligament . The second group was of tissue segments of this muscle after surgical repair of knee and rehabilitation, which consisted in power training using resistance machines. The muscle fiber samples of *quadriceps femoris* which were taken from patients during the first operation, showed big changes in their ultrastructure. These changes included: myofibrils disintegration; disturbance of regularly arranged striation in sarcomers; dissappearance of Z line. In the sarcoplasm, we observed large vacuolisation, and in the interfibrillar spaces – an accumulation of exudate and morphotic elements of blood outside the capillary vessels. Observations of muscle tissue after regeneration, showed a big improvement in the muscle cell's ultrastructure – the myofibrils were regularly arranged, and the sarcomers striations showed no deviations from normal structure. We also observed a considerable increase in the number of properly formed ultrastructure mitochondria when compared with the first group.

Badania ultrastruktury uszkodzonych i regenerujących włókien mięśni szkieletowych

Badania dotyczące tkanki głowy przyśrodkowej mięśnia czworogłowego uda oparliśmy na obserwacjach ultrastruktury włókien mięśniowych w mikroskopie elektronowym. Obserwowano wycinki pobrane od pacjentów (10 mężczyzn) w wieku 25–35 lat, u których występowało zadawnione uszkodzenie stawu kolanowego (około 6 tyg. unieruchomienia). Wycinki tkanki głowy przyśrodkowej mięśnia czworogłowego uda pobrane śródoperacyjnie od pacjentów, u których występowało zadawnione uszkodzenie więzadeł stawu kolanowego, stanowiły pierwszą grupę badaną, drugą grupę stanowiły wycinki tkanki mięśniowej tego mięśnia po operacji chirurgicznej naprawy kolana i rehabilitacji, polegającej na prowadzeniu treningu wytrzymałościowego z elementami treningu siłowego. We włóknach mięśniowych mięśnia czworogłowego uda u pacjentów, pobranych podczas pierwszej

operacji, przed operacją stwierdziliśmy znaczne zmiany ultrastruktury włókien mięśniowych. Polegały one przede wszystkim na dezintegracji włókienek kurczliwych oraz na zaburzeniu prawidłowego układu prążków w sarkomerach, często obserwowano zanik prążka Z. W sarkoplazmie obserwowaliśmy znaczną wakuolizację, zaś w przestrzeniach międzywłóknistych – nagromadzenie wysięku i elementów morfotycznych krwi poza naczyniami włosowatymi. Obserwacje tkanki mięśniowej po regeneracji wykazały dużą poprawę obrazu ultrastruktury komórkowej. Włókienka kurczliwe miały regularny układ, prążki sarkomerów nie wykazywały odchyleń od normy. W porównaniu z pierwszą grupą badaną stwierdzamy znaczne zwiększenie ilości mitochondriów o prawidłowej budowie ultrastrukturalnej.