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# *Histological and ultrastructural changes of the structure of rabbit's skeletal muscle cell in experimental alloxan-induced diabetes*

The aim of the work is to evaluate the influence of the experimental diabetes on the changes of the histological and ultrastructural structure of rabbit'skeletal muscle. The model of alloxan-induced diabetes was used to this purpose.

## MATERIAL AND METHODS

A total of 56 male, approx. 3 kg, sexually mature New Zealand rabbits were used in this study. Diabetes was induced by the intravenous injection of 10% alloxan solution (100 g/kg i.v.) As the criteria of diabetes presence the glycaemia level of 11.1 mmol/l (200 mg/dl) was taken. The animals were divided into five experimental groups: control (group 1), 21-day diabetes (group 2), 42-day diabetes (group 3), 90-day diabetes (group 4), 180-day diabetes (group 5).

The taken fragments of muscle were fixed for 2–5 days in 10%. indifferent formalin. It was dehydrated in increasing sequence of the concentrations of alcohols and x-rayed in xylol. The prepared fragments of tissue were embedded in paraffin. About 7 μm thick sections were cut and placed on a slide. Typical methods of histological investigations were applied in the light microscope: staining with hematoxylin and eosin (H + E), Masson as well as PAS (periodic acid Schiff reaction) according to McManus (12, 13). Ultrathin sections were contrasted with the uranyl acetate and lead citrate according to Reynold's method and were evaluated with the use of transmission electron microscope.

## RESULTS

Group 1 (control group). The histological structure of muscular cross striated tissue in preparations of animals of the control group stained with the method H+E as well as the Masson method was in the accordance with descriptions in the accessible literature. Muscle fibres as structural units were joint in bundles surrounded by loose connective tissue. A very gentle connective endomysium tissue, covering directly the muscle fibre, was not very well visible in histological staining. Perfectly visible, however, was perimysium joining fibres in bunches. This somewhat stronger tissue connection membrane contained abundant blood vessels, well visible both in specimens stained with the Masson and H+E method. The muscle fibres making up the largest part of the muscle cross-section in the transverse cross-section created the characteristic Conheim areas. Muscle fibre constructs the regular architectonics of skeletal muscle surrounded by sarcolemma, with numerous oval cores being directly under it. In sarcoplasma regularly arranged contractile elements – myofibrils are in surroundings of mitochondrions and sarcoplasmic reticulum. In preparations of this group H+E staining demonstrated a very regular arrangement of numerous oval nuclei under sarcolemma. In eosin absorbent sarcoplasma a regularly arranged

myofibrils were observed, which created something like columns that once were called "Leydig columns". In preparations stained with the H+E method a characteristic striation was visible. In preparations stained with the PAS method the presence of the PAS-positive substance was affirmed, which was most strongly marked in sarcolemma areas. Locally in sarcoplasma the accumulations of PAS-positive grain-like structures arranged within the areas of T channels were observed.

Group 2 (21-day diabetes). In staining with the H+E method, muscular tissue of animals of this group, after a short time of duration of diabetes differed to a slight degree from the healthy animals' muscular tissue. Locally, however, next to the correct fibres, the disturbed organization of muscle fibre structure was observed. Fibres with some signs of disorganization showed also necrotic features, the fibre was losing its continuity, and in the vicinity of sarcolemma there were no correct nuclei. In the Masson method staining the strongly marked quantity of connective tissue was observed more clearly. Within the perimysium some broadened, full of blood vessels were affirmed. Endomysium, almost invisible in specimens of control group, after 3 weeks of the duration of disease, was clearly perceptible. The staining of the PAS-positive substances showed a considerable growth of tinge intensity within the sarcolemma as well as the connective tissue. Staining with the PAS method demonstrated the striation of muscular tissue, showing also the structure disorganization of some fibres. In muscle fibres of the Group 2 animals' skeletal muscle, after 3 weeks of the disease duration, the ultrastructural changes of muscle fibres were not affirmed. The correct picture of sarcomeres and contractile filaments was kept. Only the insignificant swelling of mitochondrions was observed, as well as cell nuclei with the indent nuclear areola.

Group 3 (42-day diabetes). In muscular tissue of that experimental group, after 42 days of the disease duration, in the review staining with the H+E method, considerable changes were observed. The decrease of the muscle fibres thickness was clear. Nuclei in the areas of sarcolemma were more densely arranged. The atypical shape of nuclei was observed, often there were two nuclei next to each other as after the division. In addition to a considerable decrease of the fibres thickness the disorganization of their structure was more often observed. In the areas of the considerably altered architectonics of the fibre the large quantities of the infiltrating cells were observed. Some areas of the entirely destroyed muscle structure were replenished with the connective tissue. This was perfectly visible while the Masson staining method was used. Both in staining with the H+E method and the Masson method the observed focuses of destruction were being replaced with the connective tissue, next to which the fat cells appeared. Both staining methods showed the anisochromia of muscle fibres. PAS method staining also showed some considerable morphological differences in comparison to the control group. Next to the fibres with the large quantity of mucopolysaccharides there were fibres where even sarcolemma had the trace amounts of PAS-positive substances. The strongly PAS-positive grains were not clearly visible in specimens of the control group. After 6 weeks of the course of experience in the animals' skeletal muscles some changes of the ultrastructure of muscle fibres were affirmed. They consisted in the disintegration of the contractile fibrils, as well as in the disorder of the correct arrangement of stripes in sarcomeres. Often the atrophy of Z stripe or its incorrect course was observed. Within the sarcoplasma the considerable vacuolisation was observed, as well as the large degree of swelling of mitochondrions. Cell nuclei showed the considerably indent nuclear areola.

Group 4 (90-day diabetes). Both staining with the H+E method and the Masson method in the specimens of this group enabled to observe, as in the earlier group, the clear disorganization of the contraction apparatus. The muscle fibres were clearly separated by the connective tissue, more abundant than in the control group. Also, more often there were irregular and whirled myofibrils. Fibres with the irregular structure were next to fibres with the regular architectonics. Considerably more often than in the previous experimental groups the decrease of the thickness of fibres was observed. However, the accumulation areas of nuclei creating

something like "the chainlet" were observed. PAS method staining showed considerable differences in the amounts of mucopolysaccharides within the separate fibres. Within the fibres of the smaller thickness considerably smaller quantities of PAS-positive substances were observed. In the accumulation areas of nuclei creating "the chainlet" in the subsarcolemmal area the more intense tinge was observed. That tinge meant the presence of considerably larger amounts of PAS-positive substances. In fibres, whose architectonics underwent considerable changes, there was no reaction. The structure of muscle fibres of the rabbits' skeletal muscle after 3 months of the diabetes duration did not show changes in the ultrastructure. The correct picture of sarcomeres and contractile fibrils was kept. Within sarcoplasma some single vacuoles were observed. Cell nuclei were oval, with the correct utrastructural build.

Group 5 (180-day diabetes). While staining with the H+E method in the specimens of muscular tissue after 6 months of the disease duration the weak eosin absorptiveness of somehow homogene cytoplasm was observed, striation of the myofibril was invisible. There were some muscle fibres with no nuclei as opposed to polynuclear fibres from the control group. Fibres being next to such a fibre had "chainlets" of nuclei well seen under the sarcolemma. In their neighbourhood there were fibres with disorganized, faintly stained somehow stretched myofibril structure observed. Staining with the Masson method showed the presence of clear cords of connective tissue more massive than these observed in the control group. With the use of the PAS method the anisochromia of individual fibres was demonstrated. Fibres with described disturbed architectonics showed a reduced colourful reaction to mucopolysaccharides. The observed animals' muscular tissue after 6 months of the course of experience showed the correct picture of the cell ultrastructure. Contractile fibrils had the regular arrangement, sarcomeres stripes did not show any form of deviations. In comparison to Group 2 (animals after 3 weeks of the disease duration) and Group 3 (animals after 6 weeks of the disease duration) a considerable increase of the amount of mitochondria with the correct utrastructural build was observed.

## DISCUSSION

In the accessible literature there is a lack of reports about the influence of experimental diabetes on the histological structure of the skeletal muscle. We found only one work on the changes in utrastructural and histochemical structure with the use of the experimental diabetes in mice. Considerably more often, but still seldom, are presented the results of observation on the influence of diabetics on the change in the structure of a heart muscle (1, 2, 3, 4, 6, 8, 9). Those works most often concentrate on the structure of calcium channels changes. In 1990 Watanabe described changes in the utrastructural structure of mouse's cross striated muscle using the model of 1, 3, 6 and 9 weeks' diabetes. He noticed the atrophy of muscle fibres in the early stage of the duration of that disease. With the progress of the disease those changes intensified, and much wider separation of the individual myofibril from each other as well as the presence of incorrect mitochondria and massive glycogen inclusions were described (11).

In our experience just after 21 days of the diseases duration there were changes perceptible in the morphological structure, showing the occurrence of muscle fibres with the disturbed organization of their build. The damaged fibres showed the presence of the necrotic features, the loss of their continuity was observed as well as the decrease in the number of nuclei with the correct structure. The enlarged amount of connective tissue in muscle fibres as well as broadened blood vessels were significant. During utrastructural investigations of muscle fibre there were no considerable changes observed at the beginning of the experience. The picture of sarcomeres and contractile fibrils was correct. The only changes concerned cell nuclei, with the locally indent nuclear areola and mitochondria with the noticed insignificant swelling in their structure.

However, after 42 days of diabetes, both in light microscope and in electron microscope considerably larger changes were noticed. The decrease of the thickness of muscle fibres was obvious, considerably more fibres showed the disorganization of the structure, nuclei in the area of sarcolemma were much more densely arranged. The atypical shape of nuclei was observed, they

were often arranged in pairs, somehow imitating the situation as would be after the division. There were also some areas of almost entirely destructed structure of muscle, replaced with the connective tissue, next to which the fat cells appeared. In that experimental group, after 6 weeks of diabetes duration, the most intensive changes occurred in utrastructural build. Contractile fibrils underwent the disintegration, clear disorders of arrangement of stripes were observed in sarcomeres. Cell nuclei in many areas showed the considerably indent nuclear areola. Within the sarcoplasma a considerable vacuolisation was observed, as well as a large degree of swelling of mitochondria. Such a picture visible in the electron microscope speaks for developing the basic form of tissue reaction on the harmful activity, the adaptation (3).

After 90 days of the disease duration to investigation in the light microscope enabled to observe the disorganization of the contraction apparatus. The muscle fibres were clearly separated by the connective tissue, more often than in the previous group the decrease of the thickness of fibres was observed. There were places of the distinct accumulation of nuclei, nuclei were not arranged so regularly as in the control group. The investigation with the use of the electron microscope did not show such strong changes as in the previous experimental group. Sarcomeres as well as contractile fibrils presented the correct utrastructural build. Oval cell nuclei did not show indent nuclear areola, the amount of vacuole in sarcoplasma decreased obviously. Such a state most probably speaks for starting the adaptive defence mechanisms of muscular tissue. The observations confirm the decrease of glucose to glycogen storing in the observed tissue (5, 7, 10).

After 180 days of the experiment, while using the light microscope the consolidation of the destructive changes of muscular tissue was observed. The striation of myofibril was faintly visible, sometimes even invisible, the presence of muscle fibres with the considerably reduced amount of nuclei was observed. In some fibres nuclei were not observed at all. While using the electron microscope, almost correct arrangement of the cell ultrastructure was showed. The swelled mitochondria were less numerous, the increase of the amount of mitochondria with the correct ultrastructural build was observed. Such a picture of morphological changes in the course of experience testifies to large adaptive possibilities of the investigated muscular tissue.

Clear changes in the course of experiment were noticed just after 21 days of the disease, with its maximum intensification observed in the group of animals with the 42-day diabetes. Those observations were proved both in the light microscope and in the electron microscope. In the following experimental groups, after 90 and 180 days of the disease duration, the consolidation of the morphological changes visible in the light microscope occurred. The more exact electron microscopy, however, showed the regression of the earlier observed destructive changes.

## CONCLUSIONS

1. In the course of the experimental diabetes the morphological build of the rabbit's skeletal muscle showed less or more intensified changes, depending on the duration time of the disease, perceptible just after 21 days of the experiment.

2. The largest disorganization of cellular structures occurred in the animals' muscles after 42 days of the disease. It concerned both cell nuclei and the remaining elements of the sarcoplasma.

3. In the remaining two experimental groups, among animals with 90- and 180-day diabetes, while using the light microscope the consolidation of the observed changes was visible. However, while using the electron microscope the regression of the earlier observed destructive changes was observed which testifies to large adaptive possibilities of the investigated muscular tissue to harmful agents as well as to its potential abilities to regenerate on the utrastructural level.

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## SUMMARY

A total of 56 male, approx. 3 kg of weight, sexually mature New Zealand rabbits were used in this study. The animals were divided into five experimental groups: control (group 1), 21-day diabetes (group 2), 42-day diabetes (group 3), 90-day diabetes (group 4), 180-day diabetes (group 5). Samples of the back surface muscle, after preparation, were examined with the use of light and electron microscope. The obtained results help to formulate thesis that experimental diabetes causes deep cell changes also on the utrastructural level which, during the disease, become subjected to reconstructive and adaptive mechanisms.

### Zmiany budowy histologicznej i ultrastrukturalnej komórki mięśnia szkieletowego królika w przebiegu cukrzycy doświadczalnej

Do badań przeznaczono króliki rasy nowozelandzkiej białej, samce dojrzałe płciowo o masie ciała około 3 kg. Zwierzęta podzielono na następujące grupy doświadczalne: grupa 1 – kontrolna, grupa 2 – cukrzyca 21-dniowa, grupa 3 – cukrzyca 42-dniowa, grupa 4 – cukrzyca 90-dniowa, grupa 5 – cukrzyca 180-dniowa. Pobrane fragmenty mięśnia powierzchownego grzbietu po odpowiednim przygotowaniu poddano ocenie w mikroskopie świetlnym i elektronowym. Uzyskane wyniki obserwacji pozwalają na sformułowanie tezy, że doświadczalnie wywołana cukrzyca powoduje głębokie zmiany komórkowe, również na poziomie ultrastrukturalnym, które z czasem trwania choroby zostają poddane mechanizmom naprawczym i adaptacyjnym.