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Structural Manifestations of Mitochondrial Dysfunction in Skeletal Muscles of Early Aging OXYS Rats

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Changes in the mitochondrial compartment are the central element in the morphogenesis of musculoskeletal abnormalities in early aging OXYS rats. Compensatory hyperplasia and hypertrophy with enlargement of the working surface area are seen in these organelles at the age of 2 months. The mitochondria are characterized by polymorphism, compact packing of cristae. By the age of 9 months destructive changes and sharp reduction of the mitochondrial compartment are observed is many myocytes. Disorders in the mitochondrial structure and function and oxidative stress can be among the causes of degenerative changes in the myofibrillar system and other structures of muscle fibers, including those resultant from activation of apoptosis.

Key Words: *skeletal muscles; OXYS rats; mitochondria; electron microscopy*

Primary and secondary mitochondrial dysfunction and energy deficit associated with it, impairment of ionic homeostasis, and oxidative stress belong to the basic mechanisms of cell injury and death. Recent studies showed that oxidative stress ("mild" uncoupling) results in opening of a nonselective cyclosporin-sensitive pore in the inner mitochondrial membrane with the release of Ca^{2+}, cytochrome C and other proapoptotic protein activators of effector proteases and endonucleases into the cytoplasm [10,13]. The central role of mitochondria in cell death processes is not doubted, but many aspects still remain unclear. For example, one of poorly studied problems is the realization of these mechanisms in the unique cell system of the skeletal muscles, *i.e.* in multinuclear muscle fibers, where individual nuclei control successive segments of the fiber, while mitochondrial compartment is characterized by qualitative and quantitative heterogeneity [15].

Studies of these and other fundamental patho- and morphogenetic aspects were difficult, because of the absence of adequate experimental models of primary mitochondrial dysfunction [12].

Early aging OXYS rats (at first registered as W/SSM) were bred at Institute of Cytology and Genetics, Siberian Division of Russian Academy of Medical Sciences [8]. These animals are characterized by short life span, high incidence of malignant tumors, early involutional changes in the viscera, including cardiomyopathies [6]. The key role in the pathogenesis of early aging of OXYS rats is played by disorders in the mitochondrial structure and functions augmenting with age: changed cytochrome ratio in the inner membrane, increased cytochrome b5 content in the outer membrane, low activity of F_1F_0-ATP-synthetase, and reduced respiratory control and phosphorylation rate [2,9]. Accumulation of oxidative injuries of proteins and lipids, premutation abnormalities in DNA are indi-

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rect signs of oxidative stress [3,4]. Though ROS production in liver mitochondria decreased because of disorders in the electron transfer chain and poor conjugation of the oxidation-phosphorylation processes [5], the balance in the pro- and antioxidant system in tissues easily shifted towards prooxidants in the presence of energy deficit augmenting with aging, which results in oxidative stress [2].

Since skeletal muscles are normally exposed to high metabolic stress, they are very sensitive to mitochondrial disorders; hence, we studied structural reactions of somatic muscles of OXYS rats with primary disorders of oxidation metabolism.

MATERIALS AND METHODS

The study was carried out on 38 male OXYS rats aging 2 and 9 months; 15 Wistar rats of the same age served as controls. The preparations of the crural muscles in the "tension at rest" status were made as follows: after decapitation the left hind limb was amputated at the level of the hip joint, the skin was separated, and the limb was put into cold (4°C) fixative (4% paraformaldehyde solution on Millonig phosphate buffer, pH 7.4). The diaphragm was fixed together with the costal ring. After 24-h preliminary fixation, fragments of the gastrocnemius muscle and diaphragm were cut from the macropreparations for further processing.

Tissue samples for light microscopy were fixed in 12% neutral formalin. Histological preparations were stained with hematoxylin and eosin in combination with Perls reaction for detecting iron ions, by Van-Gieson's method with poststaining of elastic fibers with Weigert's resorcin-fuchsine, with colloid iron and periodic acid Schiff reagent (PAS) hematoxylin, and PAS test was carried out.

For electron microscopy the fragments were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and after standard processing were embedded in epon-araldite. Semithin sections were stained with 1% Azur II, PAS reaction, and examined under a Docuval light microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a JEM 100 B electron microscope.

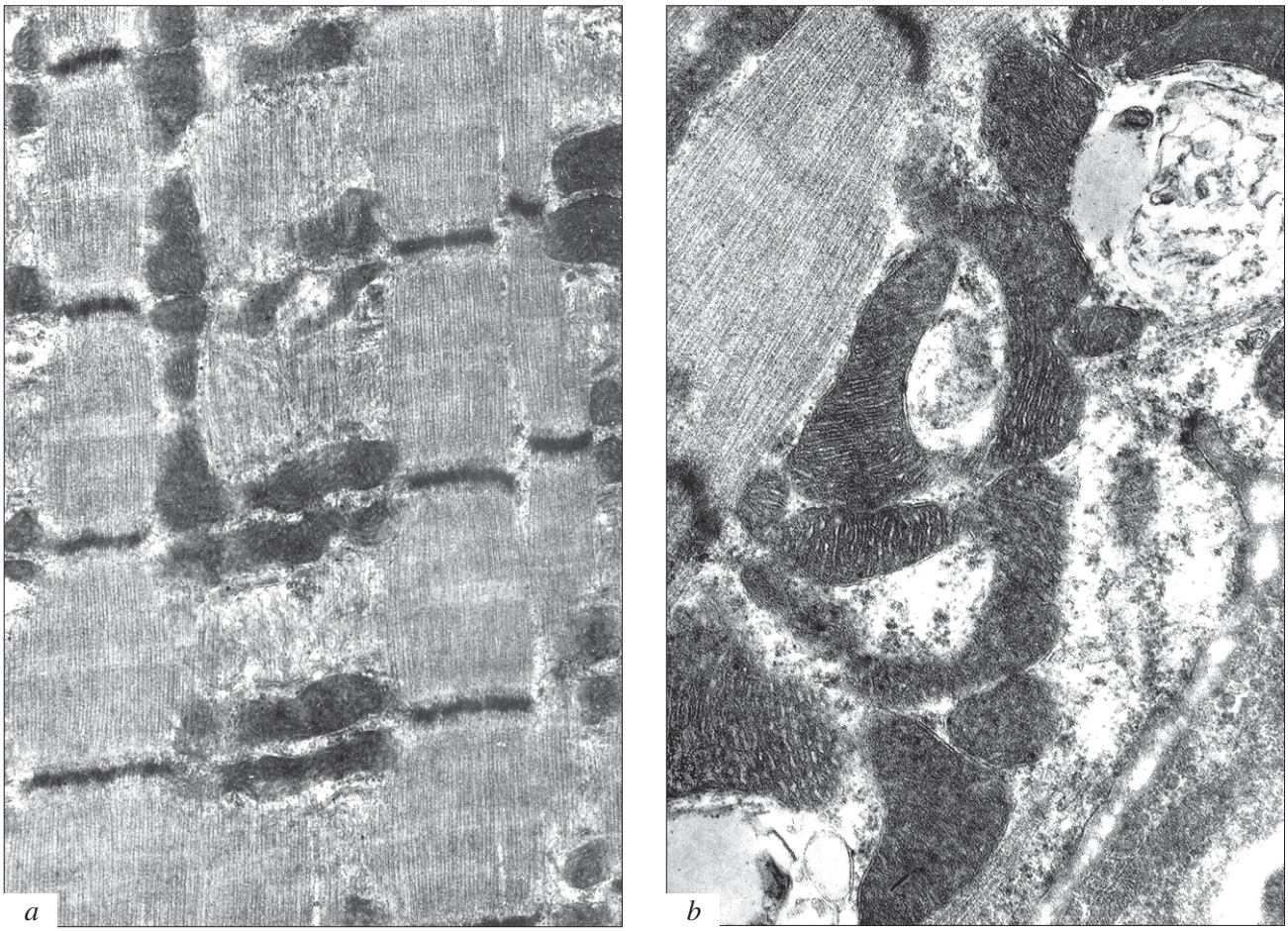

Fig. 1. Ultrastructural changes in skeletal muscles of 2-month-old OXYS rats. *a*) mitochondrial polymorphism, high electron density of the matrix. I-II-degree contractures of myofibrils, ×8000; *b*) initial stage of myofilament regeneration: ribosomes and polysomes in zone of myocytolysis; large polymorphic mitochondria, ×20,000.

The fibrillar system of muscle cells was evaluated by examination of the paraffin sections (stained and unstained) with longitudinally oriented muscle fibers in polarized light. Phase contrast studies were supplementary to polarization microscopy.

RESULTS

Light microscopy revealed predominance of moderate nonspecific myopathic changes reflecting the balance between alternative and compensatory processes in somatic muscles under conditions of chronic disorders in cell homeostasis [1]: variability of the diameter, longitudinal splitting of muscle fibers, and uneven distribution of glycogen granules. Roughening of the connective tissue skeleton in adult animals was determined by accumulation of collagen bundles.

A characteristic electron-microscopic sign in 2-month-old OXYS rats was heterogeneous organization, consisting in less ordered packing of myofibrils, frequent splitting of myofibrillar bundles, irregularities, displacement, and transverse deformations of Z lines. Small sites (1-2 sarcomers) of myofibril disaggregation and lysis were seen.

However, changes in the mitochondrial compartment were of priority significance. Numerous mitochondria were compactly grouped in spaces between the bundles and under the sarcolemma. The mitochondria of OXYS rats were much more numerous and were characterized by more variable size and shape, more osmiophilic matrix, and more compact but disordered packing of cristae (Fig. 1, *a*), in comparison with Wistar rats, which attested to proliferation of mitochondria and enlargement of their surface area. The inner space of the cristae was extended in some mitochondria.

Signs of regeneration were seen: myofilament synthesis on polyribosomes with the formation of small bundles of protofibrils. The focus of regeneration was characterized by a moderate number of glycogen grains, was surrounded by large polymorphic mitochondria with compactly packed parallel cristae and homogeneous matrix of high electron density (Fig. 1, *b*).

In older age group of experimental animals the ultrastructural picture differed from the above description mainly quantitatively, except some features (higher variability in the width of myofibrillar bundles and their more often splitting with contracture changes). Focal lysis and disaggregation of myofibrils usually involved one or several sarcomers with denudation of T-system tubules and dilation and vacuolation of the Golgi complex profiles. Floccular substrate was seen in these foci.

The mitochondrial compartment was characterized by heterogeneity and its status correlated with other signs reflecting the general status of muscle fibers, for example, number of glycogen granules. Hyperplasia and grouping of mitochondria in spaces between the bundles and under the sarcolemma were seen in muscle fibers with high content of glycogen; however, these phenomena were less pronounced than in 2-month-old animals. Many mitochondria, particularly in foci of myocytolysis, were characterized by larger size, pronounced polymorphism, disorganization of the internal structure with reduction of the cristae, heterogeneity, and moderate electron density of the matrix (Fig. 2, *a*).

Appreciable reduction of the mitochondrial compartment was usually associated with almost complete absence of glycogen granules. In these cases "empty" spaces between the bundles contained, apart from solitary retained mitochondria, numerous polymorphic destructed organelles and myelin figures (Fig. 2, *b*). Mitochondria with pronounced changes, including sharp condensation of some cristae, focal edema and matrix clarification, electron-dense incorporations, signs of destruction of the outer membrane were often seen in zones of myofibril disaggregation and lysis (Fig. 2, *c*). The number of autophagosomes increased, some of them contained fragments of degenerated mitochondria, lipid droplets, and sharply osmiophilic residual bodies.

Proliferation and structural abnormalities of mitochondria (enlargement, polymorphism, incorporations) are the most frequent signs revealed in more than half patients with primary mitochondrial diseases during morphological analysis of skeletal muscles. These changes are most typical of states associated with respiratory chain deficiency and impaired oxidation-phosphorylation coupling and are rarely observed in disorders of substrate transport and utilization [14].

This complex of mitochondrial changes is in line with previously detected changes [2,9] attesting to possible location of the primary defect in the respiratory chain with the development of "mild uncoupling" status.

Experimental study enabled us to trace the adaptive reaction of the mitochondrial compartment. Deviations in the functioning of the respiratory chain in liver mitochondria of OXYS rats are recorded as early as at the age of 2-3 months, but ATP synthesis is not inhibited [7]. A possible explanation is compensatory hyperplasia of mitochondria with enlarged working surface previously detected for the liver [2] and confirmed in this study. Signs of structural "decompensation" of mitochondria augmented in the skeletal muscle fibers of 9-month-old OXYS rats.

Progressive dysfunction of mitochondria (because of accumulation of mutation and oxidative injuries) leads to energy deficiency, shift in the pro- and anti-

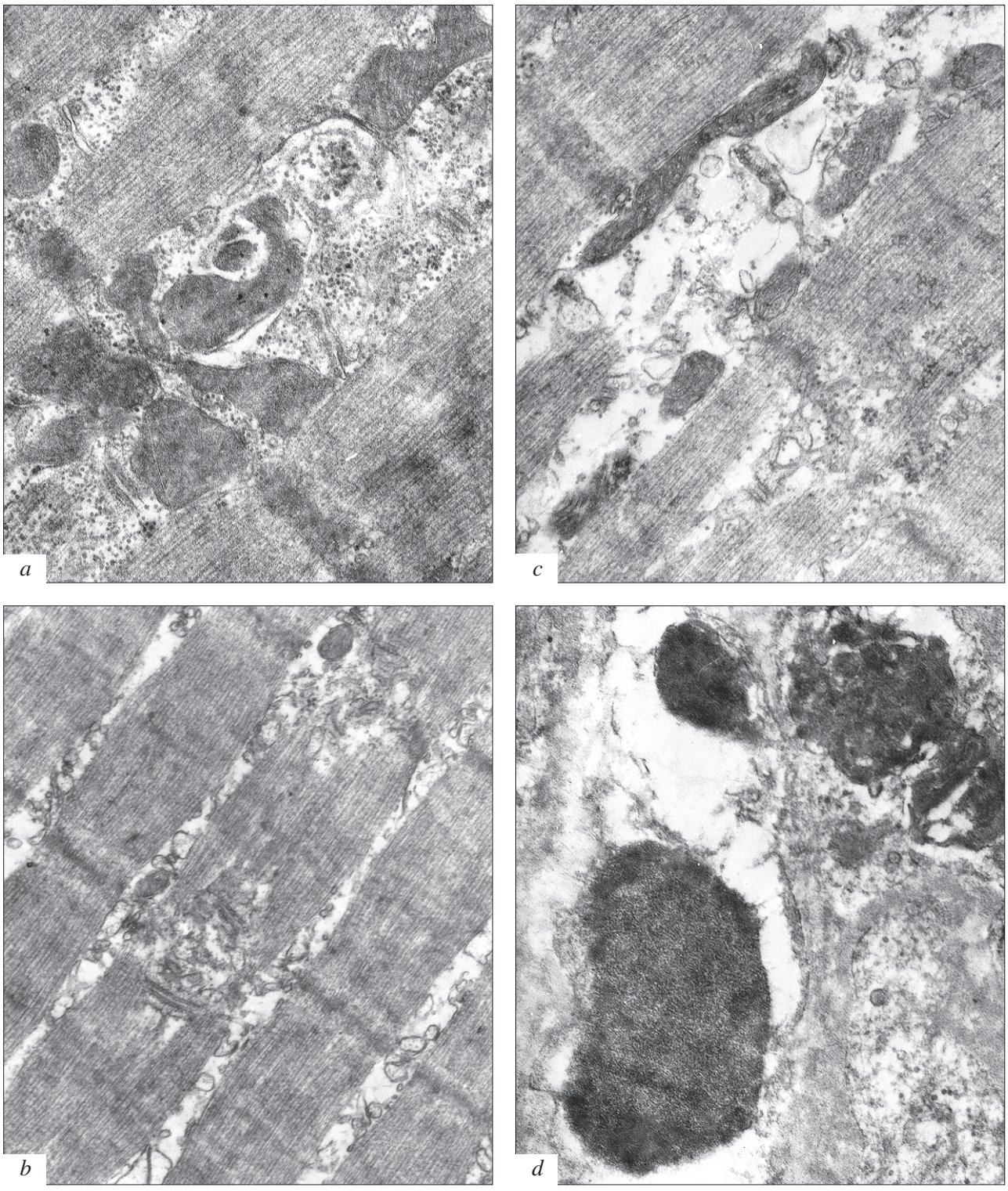

Fig. 2. Ultrastructural changes in skeletal muscles of 9-month-old OXYS rats. *a*) large polymorphic mitochondria in focus of myocytolysis: disorganization of the inner structure, reduction of cristae, heterogeneity and moderate electron density of the matrix; *b*) destructurized mitochondria and myelin figures in spaces between the bundles. Myofilament lysis and destruction of Z line. Absence of glycogen granules; *c*) destructive changes in the mitochondria in myocytolysis zone: focal edema and matrix clarification, electron-dense incorporations, destruction of outer membrane; *d*) degeneration of the myocyte nuclear compartment (apoptotic transformation). *a*, *c*, *d*: ×20,000; *b*: ×15,000.

oxidant system, development of oxidative stress, which, in turn, can be a cause of changed morphology and function of the mitochondria and of degenerative changes in the myofibrillar system and other structures of the muscle fibers. These latter can be partially due to mechanisms of apoptosis (which can be activated because of pathogenetic characteristics of this model).

Since apoptotic changes in muscle fibers are mainly local with rapid elimination of unviable segments [15], their detection by electron microscopy is very difficult. Changes in the nuclei characteristic of the final stage of apoptosis were detected in only one of the studied samples (Fig. 2, *d*), but small sites of myofibril disaggregation, surrounded by normal myofibrillar structures and including mitochondria with signs of degeneration were frequent ultrastructural findings (Fig. 2, *a*, *c*). This picture was described previously as changes in the zone of apoptotic nucleus [11].

Hence, the model of prematurely aging OXYS rats offers unique possibilities for complex studies of the fundamental mechanisms of patho- and morphogenesis associated with oxidative stress and dysfunction of mitochondria. Utilization of these potentialities is a prospective trend in studies of the skeletal muscle abnormalities.

REFERENCES

1. M. A. Bakarev, L. M. Nepomnyashchikh, and V. G. Tsimmerman, *Byull. Eksp. Biol. Med.*, **127**, No. 6, 700-705 (1999).
2. N. G. Kolosova, S. V. Aidagulova, G. I. Nepomnyashchikh, *et al.*, *Ibid.*, **132**, No. 8, 235-240 (2001).
3. N. G. Kolosova, T. V. Shcheglova, T. G. Amstislavskaya, and L. V. Loskutova, *Ibid.*, **135**, No. 6, 696-699 (2003).
4. Zh. S. Krysanova, O. I. Sinitsina, and N. G. Kolosova, *Free Radicals, Antioxidants, and Human Diseases* [in Russian], Smolensk (2001), pp. 42-43.
5. E. B. Men'shchikova, I. G. Shabalina, N. K. Zenkov, and N. G. Kolosova, *Byull. Eksp. Biol. Med.*, **133**, No. 2, 207-210 (2002).
6. L. M. Nepomnyashchikh, R. I. Salganik, G. I. Nepomnyashchikh, *et al.*, *Morphology of Genetically Determined Common Pathological Processes in the Myocardium of W/SSM Rats* [in Russian], Novosibirsk (1995).
7. R. I. Salganik, I. G. Shabalina, N. A. Solov'eva, *et al.*, *Byull. Eksp. Biol. Med.*, **119**, No. 6, 628-631 (1995).
8. N. A. Solov'eva, T. S. Morozkova, and R. I. Salganik, *Genetika*, **11**, No. 5, 63-71 (1975).
9. I. G. Shabalina, N. G. Kolosova, A. Yu. Grishanova, *et al.*, *Biokhimiya*, **60**, No. 12, 2045-2052 (1995).
10. P. Bernardi, *Ital. J. Neurol. Sci.*, **20**, 395-400 (1999).
11. J. Chae, N. Minami, Y. Jin, *et al.*, *Neuromuscul. Disord.*, **11**, 547-555 (2001).
12. P. F. Chinnery and D. M. Turnbull, *Mol. Med. Today*, **6**, 425-432 (2000).
13. A. L. Nieminen, *Int. Rev. Cytol.*, **224**, 29-55 (2003).
14. S. Rollins, R. A. Prayson, J. T. McMahon, and B. H. Cohen, *Am. J. Clin. Pathol.*, **116**, 26-30 (2001).
15. D. S. Tews, *Basic Appl. Myol.*, **13**, 181-190 (2003).