Dynamics of Structural and Functional Changes in Hepatocyte Mitochondria of Senescence-Accelerated OXYS Rats

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Electron microscopy showed signs of degenerative dystrophic changes in hepatocytes and structural decompensation of mitochondria in senescence-accelerated OXYS rats in comparison with Wistar rats. These signs were detected in the presence of liver mitochondria dysfunction in OXYS rats: reduced oxygen consumption rate in all metabolic states, respiratory control volume, ADP/O ratio. transmembrane potential, and phosphorylation rate. The revealed disorders in mitochondrial structure and function are the key factors in the pathogenesis of accelerated aging in OXYS rats and visceropathies characteristic of these animals.

Key Words: *OXYS rats; hepatocyte mitochondria; electron microscopy; energy metabolism of liver mitochondria*

Rat model of accelerated aging, OXYS rat strain (initially registered as W/SSM) was created at the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, by selection and inbreeding of Wistar rats sensitive to cataractogenic effect of galactose [6,8]. Apart from early development of cataract, these animals are characterized by decreased life-span, high incidence of malignant tumors, early involution changes in the viscera, including cardiomyopathies [3], and impaired cognitive function [2]. Hyperproduction of oxygen radicals in the liver and heart detected by electron paramagnetic resonance [14] is believed to underlie the pathogenesis of these symptoms; this hyperproduction was the key characteristic for registration of this mouse strain as OXYS [13].

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The most probable causes of intracellular oxidative stress are disorders in the electron transporting chain of mitochondria, characteristic of OXYS rats, which can be detected as early as at the age of 2-3 months and then progress [7]. However chemiluminescent analysis showed that generation of active oxygen forms by enzymatic systems of liver mitochondria in OXYS rats is even lower than in Wistar rats of the corresponding age [4]. These results seem to be unexpected, as the involvement of free radicals in changes leading to cell and tissue aging has been persuasively demonstrated [9,15,16]. Moreover, it is known that imbalance in the generation of active oxygen forms and energy metabolism can be both the cause and result of aging [12].

We investigated structural and functional changes in liver mitochondria of OXYS rats characterized by accelerated aging.

MATERIALS AND METHODS

The study was carried out on 23 male OXYS rats aged 4, 12, and 24 months and weighing 287±48, 332±30,

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and 352±23 g, respectively. Control group consisted of 22 Wistar rats of the same ages weighing 340±60, 379±72, and 396±42 g, respectively. The rats were from Breeding Laboratory of the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences.

For light microscopy, the samples were fixed in 10% neutral formalin and treated routinely. Paraffin sections were stained with hematoxylin-eosin in combination with Pearls reaction, by Van Gieson method with poststaining of elastic fibers by Weigert resorcin-fuchsin, and periodic acid Schiff reaction was carried out. Liver fragments (no more than 1 mm^2) for electron microscopy were fixed in 4% paraformaldehyde and postfixed in 1% OsO_4. After standard treatment for electron microscopy, the tissue was embedded in epon-araldite mixture. Semithin sections were stained with 1% Azur II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

Biochemical studies were carried out on 4- and 12-month-old rats. Mitochondria were isolated from the liver by standard differential centrifugation in the following medium: 250 mM sucrose, 1 mM EDTA, 10 mM Tris-HCl (pH 7.4); the mitochondria were repeatedly precipitated in the same medium without EDTA. Mitochondrial protein was measured by the Lowry method with BSA as the reference. Oxygen consumption rate was evaluated by polarography at 25°C using a Clarke electrode. The mitochondria were incubated under the following conditions: 100 mM sucrose, 50 mM KCl, 20 mM Tris-HCl (pH 7.4), 5 mM K_2PO_4, 1 mM $MgCl_2$, 1 mM EGTA. Oxidation substrate: 10 mM succinate with rotenone 2 μg/mg mitochondrial protein. Transmembrane potential ($\Delta\Psi$) was measured with a fluorescent probe 4-n(diaminaminostyryl)-1-methylpyridinium (Zonde) [1]. The results were statistically processed using Statsoft software.

RESULTS

Light microscopy showed no marked differences in the liver structure between 4-month-old experimental and control rats, except slightly increased glycogen content in centrolobulbar hepatocytes and moderate peripolesis (lymphocyte migration into interhepatocellular spaces) in OXYS rats. Comparative electron microscopy of hepatocyte ultrastructure in 4-month-old OXYS rats (Fig. 1, *a*) showed a higher α-glycogen content and numerous mitochondria compactly grouped in various parts of the cytoplasm, often near the granular cytoplasmatic reticulum. In comparison with hepatocyte mitochondria of Wistar rats (Fig. 1, *b*), they were much more abundant, smaller in size, and had more osmiophilic matrix, the structure and density of cristae being similar. These changes indicated mitochondrial proliferation and increase in their surface area.

Moreover, hepatocytes of 4-month-old OXYS rats contained free ribosomes and polysomes; sinusoidal and lateral hepatocyte plasmalemma formed numerous microvilli. Generally, this complex of ultrastructural signs corresponded to the juvenile phenotype (probably due to delayed liver maturation or adaptive restructuring of the liver parenchyma).

Light microscopy showed that the liver structure in 12-month-old OXYS rats differed from that of Wistar rats of the same age by a higher glycogen content accumulating in the portocentral direction, moderate mononuclear infiltration of portal tracts, Ito cell hyperplasia, and changed tinctorial characteristics of hepatocyte cytoplasm. Electron microscopy showed progressive degenerative processes in the liver parenchyma of OXYS rats, much more advanced than in the control group. The mitochondrial compartment during this period was characterized by polymorphism of its numerous organelles (Fig. 2, *a*): the mitochondria were enlarged (compared to 4-month-old animals), had clarified matrix and reduced, thinned, and chaotically oriented cristae. Hepatocyte cytoplasm had lipofuchsin granules (markers of cell hypoxia and/or aging), polymorphic residual bodies, and solitary lipid inclusions. Foci of intracellular regeneration were not so numerous as in Wistar rats of the same age.

Liver structure of 24-months-old OXYS rats was characterized by moderately expressed dyscomplectation of hepatic trabecules and parenchymatous cell polymorphism due to uneven glycogen content in the lobules, acidophilic and lipid degeneration of periportal and pericentral hepatocytes, devastation of cytoplasmatic matrix in some hepatocytes. Moderate proliferation of biliary ducts, focal mononuclear infiltration, and solitary lymphoid aggregations were seen in portal tracts; central vein fibrosis was observed. The same changes, but less pronounced, were seen in Wistar rats.

Electron microscopy showed that the number of mitochondria and glycogen content were decreased in the majority of hepatocytes of 24-month-old OXYS rats; numerous lipofuchsin granules (Fig. 2, *b*), autophagosomes containing fragments of degenerated mitochondria, and polymorphic residual bodies were seen. Many hepatocytes contained abundant small mitochondria with rare cristae and homogenous moderately electron-dense matrix, sometimes with osmiophilic granules (pathognomonic for hypoxia and intoxication). This indicated heterogeneity of parenchymatous liver cells maintaining the adaptive compensatory potential in these animals surviving until the critical age (maximum life-span of OXYS rats is 24.6±4.3 months

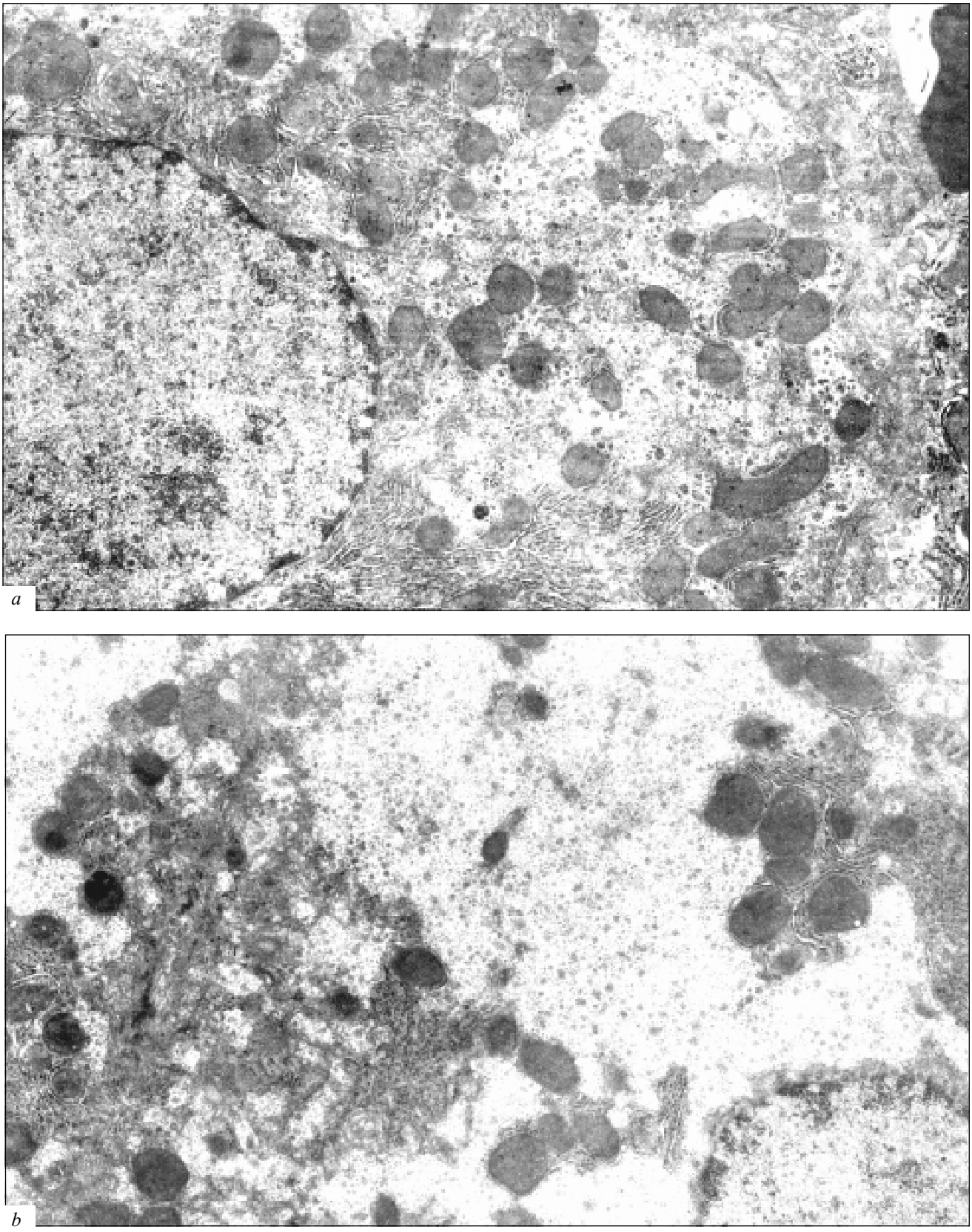

Fig. 1. Ultrastructural characteristics of hepatocytes in 4-month-old OXYS (*a*) and Wistar (*b*) rats, ×4000. *a*) hyperplasia of mitochondria and granular cytoplasmatic reticulum; *b*) scanty mitochondria and elements of cytoplasmatic reticulum.

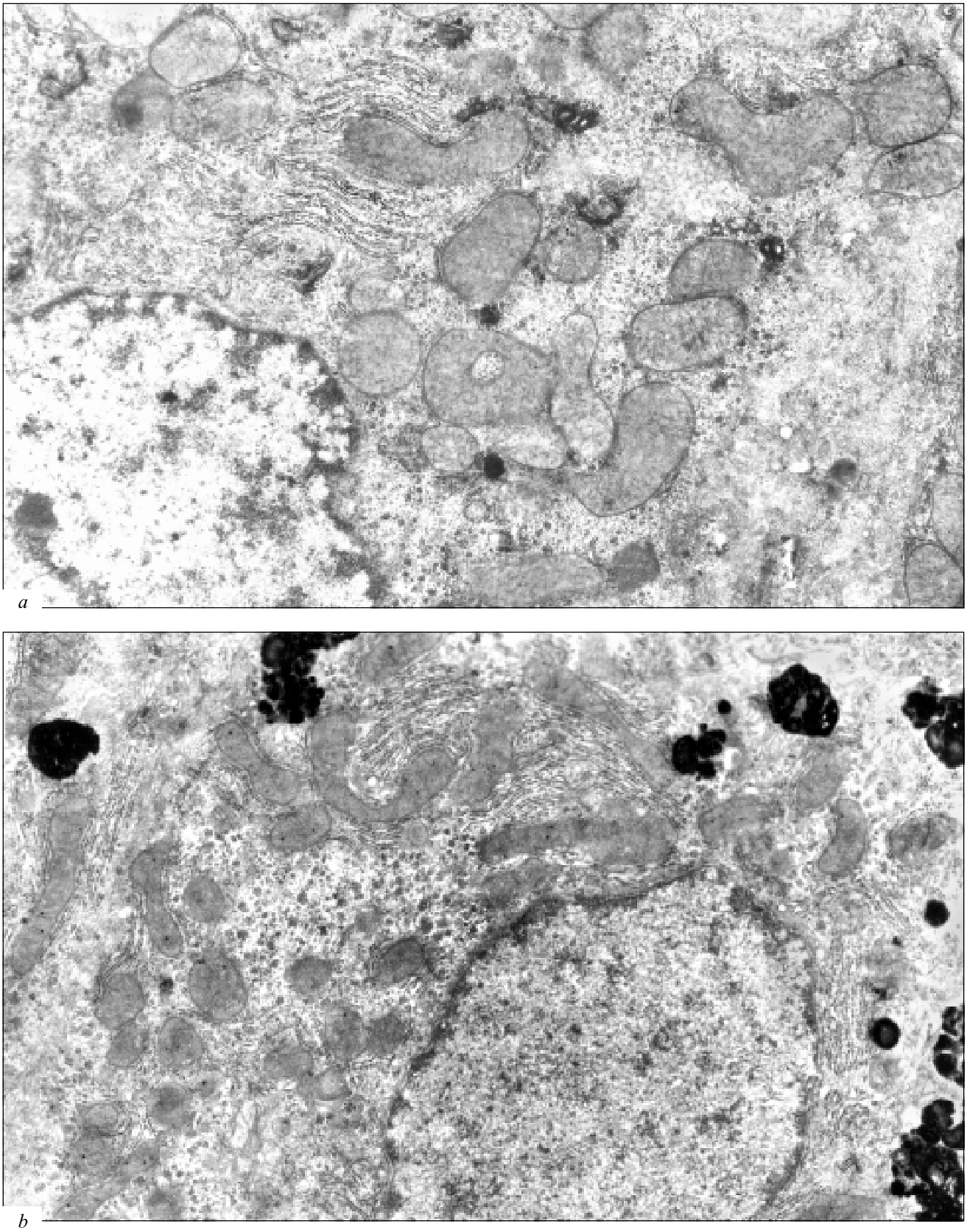

Fig. 2. Fragments of hepatocytes in OXYS rats, ×4000. *a*) pronounced mitochondrial polymorphism, formation of residual bodies at the age of 12 months; *b*) signs of mitochondrial destruction and lipofuchsin granules at the age of 24 months.

TABLE 1. Functional State of Liver Mitochondria in Wistar and OXYS Rats (*M*±*m*)

Parameter	Wistar		OXYS	
	4 months (*n*=7)	12 months (*n*=7)	4 months (*n*=9)	12 months (*n*=8)
Oxygen consumption rate, ng atom O/min/mg mitochondrial protein				
metabolic state 3	158.6±22.0	158.8±18.4	147.0±14.6	83.8±8.7*
metabolic state 4	41.6±6.1	49.0±5.0	47.0±6.8	34.70±2.35*
uncoupling state	212.9±20.0	224.0±17.0	212.0±12.5	155.2±12.9*
Respiratory control	4.00±0.43	3.64±0.26	3.4±0.4	2.50±0.33**
ADP/O	1.65±0.09	1.68±0.07	1.50±0.09	1.37±0.12**
Phosphorylation rate, nM ADP/min/mg mitochondrial protein	202.0±15.0	207.0±24.0	238.0±21.0	122.0±21.0*
$\Delta\Psi$, mV	194.0±7.8	198.0±1.9	200.0±3.4	187.00±2.12*

Note. *$p<0.01$, **$p<0.05$ *vs.* Wistar rats of the same age. Metabolic state 3 (after addition of 150 μM ADP in the presence of oxidation substrate succinate); metabolic state 4 (after complete conversion of added ADP into ATP); uncoupling (after addition of 0.5 μM chlorocarbonylcyanide phenylhydrasone).

vs. 36 months in Wistar rats). Hepatocyte mitochondria in the control varied in size and degenerative changes were less expressed.

Polarographic analysis revealed no differences in the function of liver mitochondria of 4-month-old OXYS and Wistar rats. In 12-month-old OXYS rats liver mitochondria differed from those of Wistar rats by lower rate of oxygen consumption in all metabolic states (state 3, state 4, and uncoupling), lower respiratory control and ADP/O coefficient, and lower $\Delta\Psi$ values (Table 1). The decrease of the ADP/O coefficient and oxidation rate in active metabolic state resulted in an essential decrease of phosphorylation rate in liver mitochondria of OXYS rats.

According to modern concepts, mitochondrial changes play the key role in cell aging [9]. The relative number and quality of mitochondria (*i.e.* their capacity to energy transformation) change during ontogeny [12]. Mitochondria play an important role in the triggering of the mechanism of programmed cell death (apoptosis), a process playing an important role in involution changes in tissue cell population [10].

Oxidative stress inhibits mitochondrial respiration and induces their swelling [11]. Our results indicate that oxidative stress can be the cause, at least partially, of changes in the mitochondrial morphology and function. The most important event in changing ultrastructure of OXYS rat hepatocytes at the age of 4 months is mitochondrial hyperplasia with increase in the area of their working surface — an adaptive reaction of the mitochondrial compartment. Previously we observed deviations in the function of mitochondrial respiratory chain in the liver of OXYS rats at the age of 2-3 months, but they were paralleled by a compensatory reaction (no decrease in ATP production) [5].

The absence of disorders in the mitochondrial respiratory chain in 4-month-old animals can result from active compensatory processes, which is proven by increased number of small mitochondria in OXYS rats, seen under electron microscope. Degenerative dystrophic changes in hepatocytes and structural decompensation of the mitochondria, more pronounced in OXYS rats in comparison with Wistar rats, were observed starting from the age of 12 months.

Hence, our results demonstrated a correlation between changes in mitochondrial structure and function in the liver of OXYS rats: the mitochondria increased in size and their structure and functions were impaired in 12-month-old animals. Damage to mitochondrial cristae characteristic of "old" mitochondria can be responsible for decreased transmembrane potential [15]. Progressive disorders in the mitochondrial function leads to development of energy deficit, when the balance of the pro- to antioxidant system in the tissues of OXYS rats easily shifts towards prooxidants, which leads to the development of oxidative stress. Presumably, progressive mitochondrial dysfunction are the key factor in the pathogenesis of accelerated aging and visceropathies in these rats.

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