METHODS

OXYS Rats as a Model of Senile Cataract

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Changes in the lens appear by the age of 2 months in early aging OXYS rats, at the age of 6 months these changes are detected in 100% animals, and by 12 months both eyes are involved. According to biomicroscopy findings, predominant forms of cataract are vesicular, annular, and dendritic, located in the cortical layer and/or nucleus of the lens. Light and electron microscopy showed morphological changes involving all structural components of the lens: epithelial degeneration progressing with age, deformation of fiber architectonics, and appearance of uneven condensations. Cataract in OXYS rats corresponds to senile cataract, which makes this rat strain a unique model for studies of the pathogenesis of involutive cataract and elaboration of methods for its prevention and correction.

Key Words: *OXYS rats; senile cataract model; ocular lenses; biomicroscopy, ultrastructure*

Lens opacity is the main cause of deterioration of vision and in many cases of its loss in elderly people. This condition is often regarded as an inevitable manifestation of aging [1]. However in recent years we observed a stable trend to "rejuvenation" of cataract. There are no effective means for preventing cataract, while the presence of overt changes in the lens corresponds to irreversible stage of the disease. Biological models help to investigate the pathogenesis of cataract, its genetic and biochemical bases, develop methods for effective therapy and prevention of the disease. Studies aimed at creation of cataract models were carried out for many years, but few of the known models can be considered as truly senile: sometimes cataract development is provoked by chemical or radiation exposure [15]. Several models of senile cataract are acknowledged and widely used: on Emory [9] and SAM mice (SAM-R/3 substrain) [8] and on UPL Sprague-Dawley [14] and SCR (Shumiya Cataract Rat) rats [13].

No cataract models were created in Russia. At the same time a strain of early aging OXYS rats was created by selection and inbreading of Wistar rats sensitive to the cataractogenic effect of galactose in 1970s at the Institute of Cytology and Genetics by a group of scientists headed by R. I. Salganik [5,11]. Recent studies confirmed that the intrinsic genetic defect of metabolism in these animals (increased sensitivity to oxidative stress [12]) leads to the formation of a complex of characteristics which can be regarded as syndrome of accelerated aging. These animals are characterized by short life span and at young age develop cataracts, involutive changes in the viscera, and deviations in the emotional and cognitive spheres typical of aging humans and animals [2-4]. Early cataract remains the key sign used for the control of the state of this strain, but changes in the lens were never specially investigated. The aim of this study was more profound selection of rats by this sign, analysis of cataract development in OXYS rats of generations 55-68, evaluation

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of changes in the lens at the ophthalmoscopic and ultrastructural levels, and comparison of all these parameters with those in parental Wistar rats.

MATERIALS AND METHODS

The incidence of cataract in OXYS and Wistar rats was evaluated directly at the Laboratory for Animal Breeding, Institute of Cytology and Genetics. OXYS rats of generations 55-68 (1122 animals of both sexes) were examined. Cataract development was evaluated using SL-30 slit lamp (Opton) with a automated image recording system. The animals were instilled with 1% homatropine hydrobromide for pupil dilatation. Wistar rats of the same age served as the control.

Light and electron microscopy of the lenses were carried out in 11 OXYS and 11 Wistar rats aged 10 and 14 months. The lenses were isolated immediately after sacrifice, the material was fixed in cold (4°C) 4% paraformaldehyde and 1% osmium tetroxide and 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. The results were statistically processed by unifactorial and bifactorial analysis using STATGRAPHICS software.

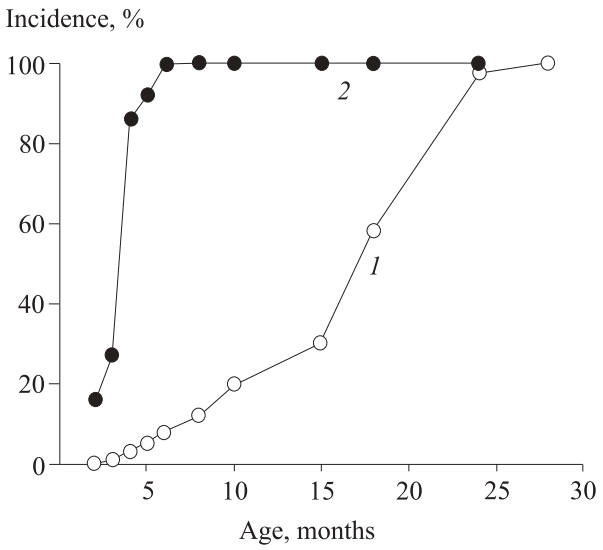

Fig. 1. Incidence of cataract in Wistar (*1*) and OXYS (*2*) rats of generations 61-68.

RESULTS

Lens opacities were detected in OXYS rats at a much younger age than in Wistar rats. Factor analysis showed that cataract development in OXYS rats did not depend on sex, but depended on animal age: F(10.897)=

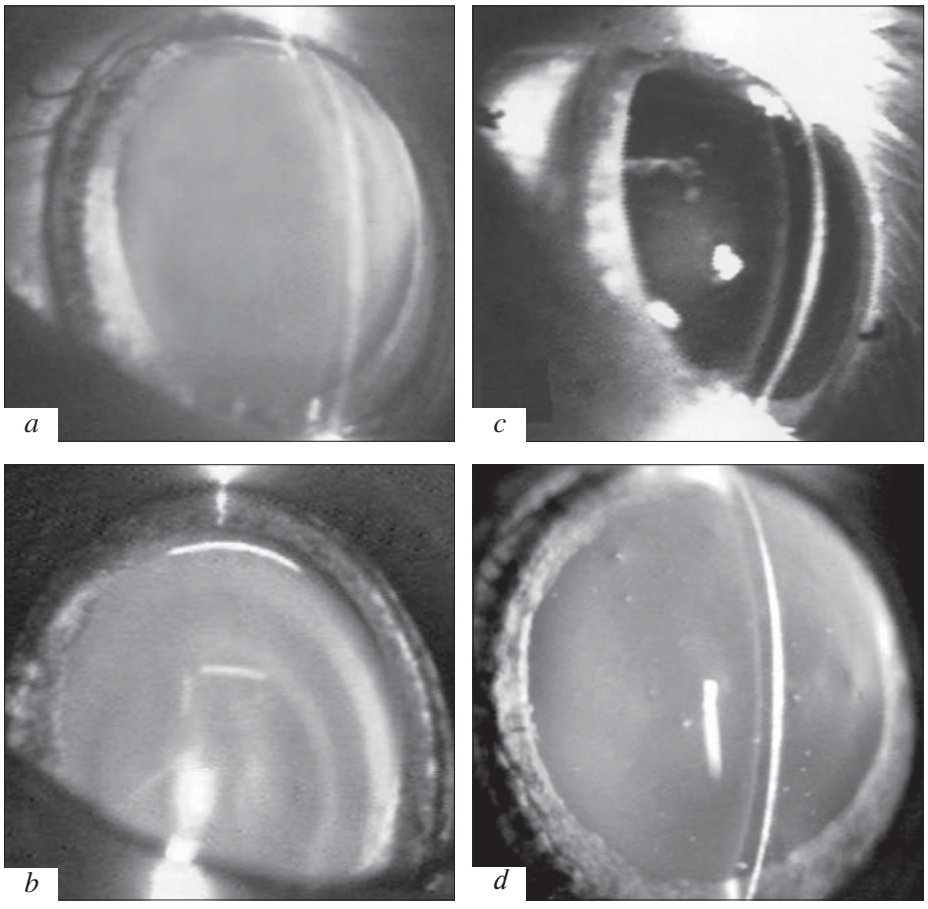

Fig. 2. Biomicroscopy of lenses from OXYS and Wistar rats aged 10 months. *a*) lens from Wistar rat (no changes); *b*) zonular cataract in the lens of OXYS rat; *c*) nuclear spindle-shaped cataract; *d*) spot cataract.

9.6, p=0.001). Selection of animals through 55-68 generations led to appreciable rejuvenation of cataract: ophthalmoscopic examinations revealed changes in the lens in 30-40% animals of generations 55-56 at the age of 6 months, and in generations 61-68 these changes were observed in 100% OXYS rats of this age

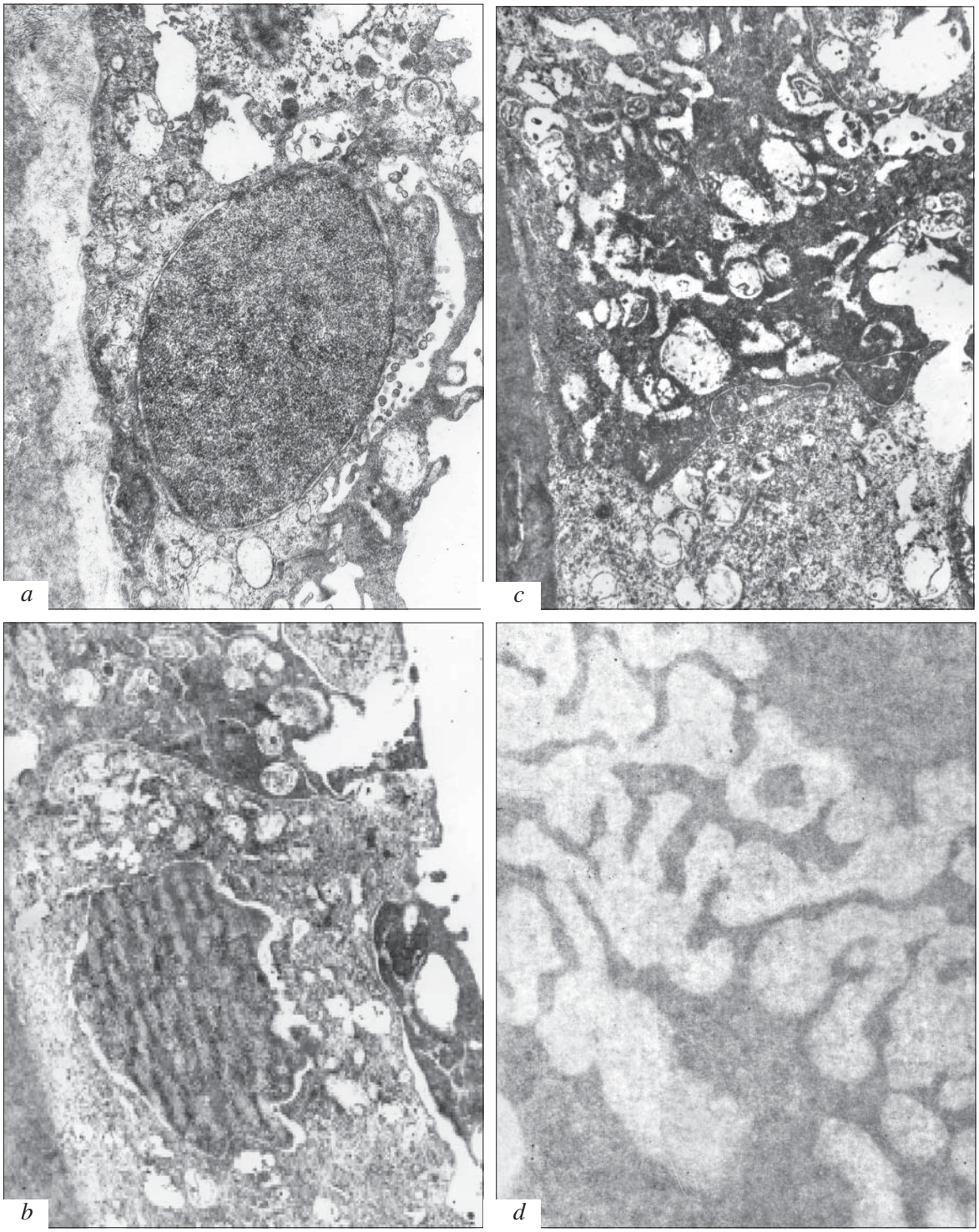

Fig. 3. Ultrastructural changes in equatorial zone of the lens in control Wistar and OXYS rats with zonular cataract. *a*) epitheliocyte of the lens in a 10-month-old Wistar rat: euchromatic nucleus, ribosomes and polymorphic vesicles in the cytoplasm, electron-light basal membrane, ×13,500; *b*) fragment of eye lens epithelium of a 10-month-old OXYS rat: flattened nucleus, heterogeneous cytoplasmatic matrix, vacuolation of membrane organelles, electron-dense basal membrane, ×13,500; *c*) fragments of lenticular epithelium of a 14-month-old OXYS rat: highly osmiophilic cytoplasm and basal membrane, alteration of organelles, ×18,000; *d*) fragment of the cortical layer fiber of the eye lens of a 14-month-old OXYS rat: markedly heterogeneous electron density, ×45,000.

(differences between generations are significant: F(13.976)=9.6, p=0.001).

In generations 65-68 these changes were detected as early as at the age of 2 months, and by 4 months they were detected in 86% animals (in 41% rats both eyes were involved). No more than 5% Wistar rats at this age had cataract and in none of them both eyes were involved. By the age of 12 months both lenses were usually involved in OXYS rats, while in Wistar rats aged 1 year cataract was detected in 20-28% animals (Fig. 1). Only by the age of 28 months virtually all Wistar rats developed changes in the lens, but even in this age group some animals had cataracts on only one eye.

Biomicroscopy of the lenses showed predominance of annular, vesicular, and dendritic forms of cataract in OXYS rats (Fig. 2). In annular cataract the opacities were located in the cortical layer and/or nucleus of the lens, in vesicular cataract changes were seen in the anterior subcortical layer, and in dendritic cataract — near lens nucleus. Annular cataracts were detected most often; opacity progressed from the cortical layer towards the nucleus. Opacity increased with age, but cataract involving all layers of the lens did not always developed. It seems that this is senile cataract typical of rats; such changes were also observed in Wistar rats including old (2-3-year-old) animals. Our findings are in line with previous data on changes in the lens rapidly aging rodents: C57BL/6, (C57BL/6×DBA/2)F_1, and (C57BL/6×C3H)F_1 mice [10] and Norwegian brown and Fisher 344 rats [16].

Light microscopy showed that the development of cataract in OXYS rats involves morphological changes in all structural components of the lens including degenerative atrophic changes in the lenticular epithelium typical of senile cataract and augmenting with age. On semithin sections of lenses from 10-month-old OXYS rats epitheliocytes of the equatorial zones were characterized by pronounced polymorphism, were often flattened and had numerous invaginations of the lateral plasmalemma. Condensation of the subepithelial layer attracted special attention. Electron microscopy showed pronounced heterogeneity of structural organization and osmiophilia of the cytoplasmatic matrix of lenticular epitheliocytes and vacuolation of membrane organelles in the lenses from OXYS rats (Fig. 3, *b*), in contrast to Wistar rats (Fig. 3, *a*). Some epitheliocytes still produce structural components of secretory material. Moreover, the apical and basal cytolemma formed deep branched invaginations serving for connection between the lens epithelium and its capsule and substance.

By the age of 14 months degenerative changes in the lenticular epithelium of OXYS rats were most pronounced, which was indirectly confirmed by detachment of the capsule during treatment of the preparations for electron microscopy. In many cases the epithelium was sharply flattened in the equatorial zone of the lens, cell nuclei were in a state of degradation; vacuolation and alteration of cytoplasmic organelles (mainly mitochondria) and formation of residual bodies were seen (Fig. 3, *c*). The lenses of OXYS rats at this age group were characterized by pronounced disorders in the architectonics and organization of fibers: deformation, homogenization, or appearance of focal condensations of peculiar shape (determined by uneven osmiophilia of the cytoplasm) (Fig. 3, *d*).

Fourier analysis of the lens electronograms carried out by our colleagues from the Universities of Northern Carolina and Oakland [7] confirmed that the changes detected in our study corresponded to structural manifestations of cataract in humans. These findings indicate that the cytoplasm of lenticular fibers of OXYS rat was characterized by pronounced changes in homogeneity and compactness, completely corresponding to those observed in human lenses removed from patients with senile cataract. This heterogeneity is determined by changed chemical characteristics and/or redistribution of cytoplasmatic components of lens fibers determining disorders in light scattering and formation of cataract.

Thus, impairment of draining and plastic functions of the lenticular epithelium caused by degenerative and atrophic changes underlies the pathogenesis of cataract in OXYS rats. This can be seen from the predominance of annular cataract, for which such changes in the epithelium are typical [6]. However high incidence of vesicular and dendritic cataracts in OXYS rats do not permit us to rule out the possibility of focal changes in the epithelium, when other mechanisms can be involved in the pathogenesis.

Hence, the results of ophthalmoscopic and morphological studies indicate that early cataract developing in OXYS rats corresponds to senile cataract, which makes this rat strain a unique model for studies of the pathogenesis of involutive cataract and for developing methods for its prevention.

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