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Structural Reorganization of the Adrenal Cortex in OXYS Rats during Ontogeny

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Study of the structural reorganization of the adrenal cortex in OXYS and Wistar rats during aging showed that age-associated reorganization of the adrenal cortex in these rats consisted in atrophy, particularly pronounced in OXYS rats. Age-specific changes in the architectonics of the organ were noted mainly in the zona glomeruloza and zona fasciculata. The vessel-parenchyma relationships play the key role in the spatial reorganization of the adrenal cortex in OXYS and Wistar rats. The volume density of the sinusoidal capillaries in the zona fasciculata and zona reticularis increases during the late ontogeny, particularly so in Wistar rats, causing, in turn, an increase of the stroma/parenchyma volume proportion.

Key Words: *OXYS rats; gerontogeny; adrenal cortex; morphometry; stereology*

Study of the structural and functional reorganization of organs and tissues during gerontogeny is essential for understanding the mechanisms of many diseases characterized by early aging. These studies are expected to clear out the possibility of inducing the regeneratory reactions supporting the functioning of various body systems, corresponding to the biological age. The aging process, its velocity and intensity are determined by genetic polymorphism and effects of numerous unfavorable factors, damaging the cellular and subcellular structures and modifying the molecular biological reactions [2,3,12].

The molecular genetic mechanisms of gerontogeny and specific features of morphogenesis and regeneratory processes in senile age are usually studied on conventional laboratory animals and on specially bred animal strains. Among these latter ones are SAM mice (more than 10 substrains are known) [11-13] and OXYS rats, bred in Russia, which are characterized by genetically determined metabolic defects [9]. Recent studies revealed shortening (by 28%) of the maximum life span in these animals, high incidence of "diseases of elderly age", early emergence of involution changes in the viscera, and cognitive deviations [1,4-8].

Study of the compensatory adaptive reactions and spatial reorganization of the adrenals is important, as adequate functioning of the adrenals maintains the regulation and interactions of many systems responsible for vital activity [10]. Despite the significance of age-specific reorganization of the adrenals and their individual structural and functional compartments, this aspect remains little studied.

We compared the specific features of structural reorganization of the adrenal cortex in Wistar and OXYS rats in the course of gerontogeny.

MATERIALS AND METHODS

Comparative study of age-specific reorganization of the adrenal cortex was carried out in 14 male

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OXYS rats, which were decapitated at the age of 5 (150-200 g), 14 (380-460 g), and 26 months (230-410 g). The reference group consisted of 12 male Wistar rats of the same age groups. All animals were kept under standard vivarium conditions with free access to water and food.

Specimens of the adrenals were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and dehydrated, after which they were embedded in epon and araldite mixture. Semithin sections were stained with 1% Azur II solution. Photo-optic study and morphometrical analysis were carried out under a universal Leica DM 4000B microscope. Microphotographs were made with a Leica DFC 320 digital photocamera and Leica QWin V3 software.

Tissue stereological analysis of the adrenal cortex was carried out at magnification ×1320 (linear magnification of the objective ×106) using a multipurpose test system of short fragments (n=36, P_T=72, L_T=192.3 μ). Visual fields, not overlapping each other, were analyzed separately for the zona reticularis and zona fasciculata; the number of the test system superimpositions (sample volume) varied from 40 to 60 for each animal.

The following stereological parameters were analyzed as the basic ones: volume and surface densities of adrenocorticocytes (ACC), their nuclei, sinusoids, volume density of cells, fibers, and main substance of connective tissue. Secondary parameters (surface/volume proportions for ACC and their nuclei, sinusoids, sinusoid/ACC, nucleus/cytoplasm, and stroma/parenchyma volume proportions) were calculated from the basic stereological parameters. At least 20 measurements of the adrenal cortex width were made for each case. Statistical processing of the results included calculation of the means, evaluation of dispersions and errors in the means, and comparison of the means using Student's test.

RESULTS

The width of the adrenal cortex reduced significantly during gerontogeny in OXYS and Wistar rats: from 1037.8±23.7 μ at the age of 5 months to 862.1±14.9 and 881.4±181.4 μ at the age of 14 and 26 months, respectively, in Wistar rats and from 1173.4±14.0 μ at the age of 5 months to 923.5±2.2 and 637.4±57.7 μ at the age of 14 and 26 months, respectively, in OXYS rats (p<0.01). The most significant (45.6%) shrinkage of the adrenocortical width was observed in OXYS rats at the age of 26 months.

Tissue architectonics of the adrenal cortex of Wistar and OXYS rats during different periods of ontogeny corresponded to that in mammals, with easily discernible zona glomerulosa, zona fasciculata, and zona reticularis. On the other hand, the strains differed by the structure of adrenocortical zones (particularly the zona glomerulosa) at different age. A cambial layer (layer of small polymorphic ACC, parallel to the organ capsule) was well seen between the zona glomerulosa and zona fasciculata of Wistar and OXYS rats at the age of 5 months (Fig. 1, *a*, *b*). Uneven thickening of the connective tissue capsule was seen in OXYS rats. ACC of the zona glomerulosa formed mainly typical arcade structures. The cytoplasm of these ACC contained numerous small-vesicular lipid incorporations. The structure of the zona fasciculata was typical, but was not always subdivided into external and internal subzones. ACC of the zona fasciculata were represented mainly by "dark" cells and differed by the content of lipid incorporations. Uneven dilatation of sinusoidal capillaries in the zona fasciculata was noted in Wistar and OXYS rats. The sinusoidal capillaries in the zona reticularis were markedly dilated and plethoric, ACC were smaller and number of lipid incorporations in them was lower than in ACC of the zona fasciculata.

Thickening of the connective tissue capsule was noted at the age of 14 months in Wistar and OXYS rats, which was more pronounced in OXYS rats (Fig. 1, *c*, *d*). The glomeruli forming the zona glomerulosa varied much in size; there was virtually no clear-cut cambial layer between the zona glomerulosa and zona fasciculata. The zona glomerulosa was represented by small ACC, containing solitary lipid incorporations. OXYS rats of this age developed changes in the architectonics of the zona glomerulosa: arcade structures alternating with irregularly disposed ACC. Importantly that ACC of the zona glomerulosa in OXYS rats contained more lipid incorporations than these cells in Wistar rats; higher lipid content rendered a "foamy" appearance to the cells (spongiocytes). The zona fasciculata was clearly subdivided into external and internal subzones. The external subzone was characterized by pronounced polymorphism of ACC and reduced number of liposomes in their cytoplasm in comparison with the internal zone. The sinusoids in the zona fasciculata were unevenly dilated, due to which it looked like the zona reticularis.

Uneven thickening of the connective tissue capsule of the adrenals was observed at the age of 26 months (late ontogeny) in Wistar and OXYS rats. In Wistar rats, arcade structures of the zona glomerulosa greatly varied in size, though the architectonics of this zone was unchanged; the cam-

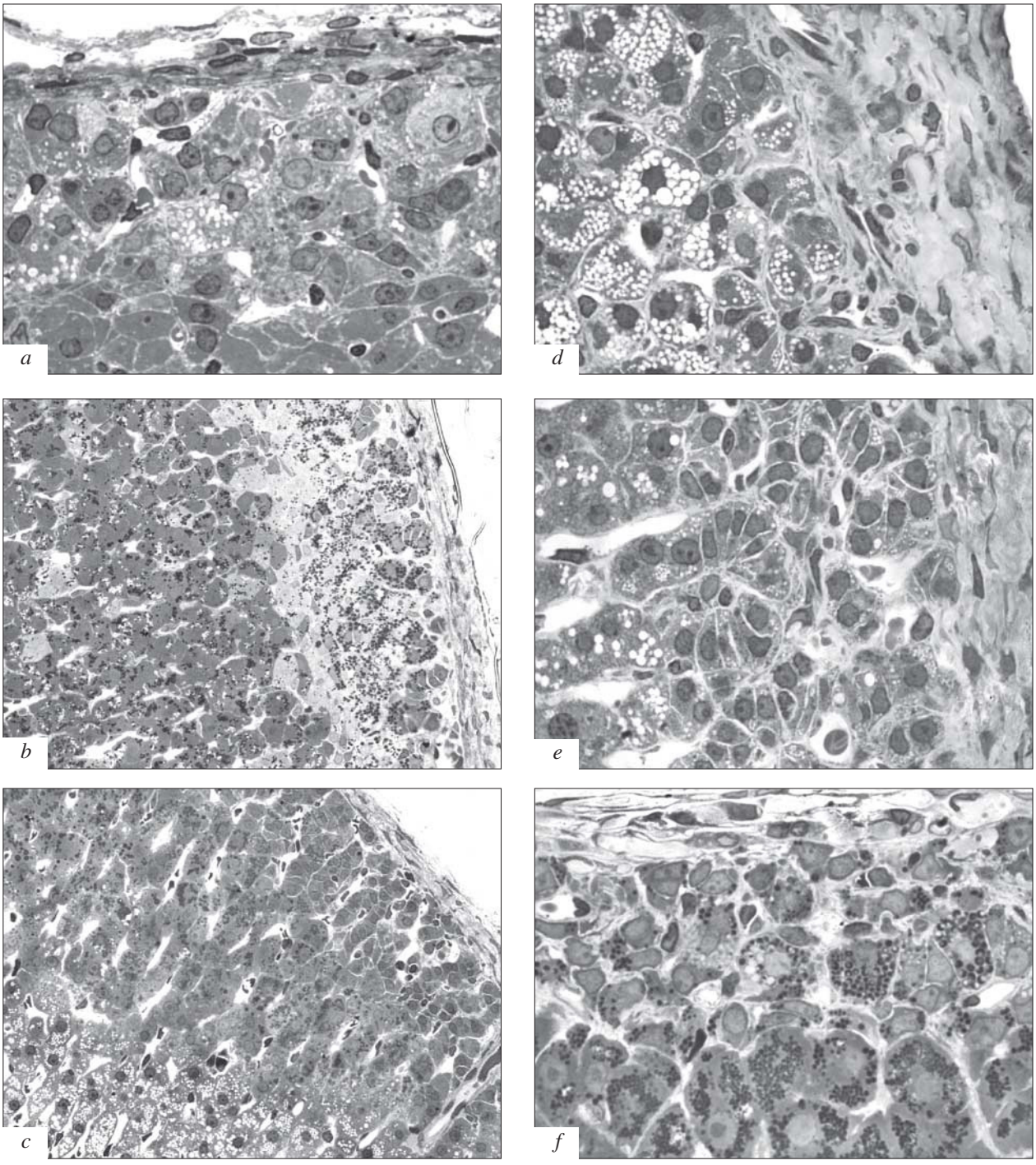

Fig. 1. Age-specific reorganization of the adrenal cortex in Wistar and OXYS rats. Semithin sections, azur II staining. *a*) pronounced polymorphism of zona glomerulosa ACC in Wistar rats aged 5 months, ×1000; *b*) significant number of ACC with lipid incorporations in the zona glomerulosa of OXYS rats aged 5 months, ×400; *c*) clear-cut division of the zona fasciculata into external and internal subzones in the adrenal cortex of Wistar rats aged 14 months, ×400; *d*) significant thickening of the adrenal capsule in OXYS rats aged 14 months, ×1000; *e*) arcade structures from small ACC in the zona glomerulosa of Wistar rats aged 26 months, ×1000; *f*) pronounced reduction of the zona glomerulosa in OXYS rats aged 26 months, ×1000.

bial layer was poorly discernible. ACC forming the arcades and glomeruli were usually very small and contained scanty lipid incorporations (Fig. 1, *e*). By the age of 26 months, OXYS rats developed pronounced atrophy of the zona glomerulosa, which was presented predominantly by small ACC without lipid incorporations (Fig. 1, *f*). Mononuclear infiltration of the zona glomerulosa was observed. The

zona fasciculata of Wistar and OXYS rats was not always subdivided into external and internal subzones; its cords partially lost their parallelism. ACC of the zona fasciculata were stained unevenly and contained small lipid incorporations sometimes occupying the entire volume of the cell. The appearance of binuclear ACC in the zona glomerulosa and zona fasciculata is worthy of note. The parallelism of cell cords in the zona fasciculata was disturbed because of uneven dilatation of the sinusoidal capillaries. The architectonics of the zona reticularis did not change much in Wistar rats aged 26 months. The zone was formed by large heterogeneous ACC with the cytoplasm containing numerous optically transparent vesicular structures. The sinusoidal capillaries were unevenly dilated. The width of the zona reticularis in OXYS rats of the same age was reduced significantly. ACC of this zone (similarly as in the zona fasciculata) contained lipid incorporations; the sinusoids were significantly dilated and plethoric.

Tissue stereological analysis showed that aging was associated with reduction of the volume density of ACC in the zona fasciculata and zona reticularis of Wistar rats (by 9 and 13%, respectively, by the age of 26 months; $p<0.05$; Fig. 2 *a*), while their surface density increased (by 65 and 47%, respectively; $p<0.05$). The ACC surface/volume proportion in the zona fasciculata and zona reticularis increased by the age of 26 months (by 82 and 89%, respectively; $p<0.01$), this indicating shrinkage of ACC in both zones. In OXYS rats the volume density of ACC in the zona fasciculata and zona reticularis did not change with age, while the surface density increased (by 41 and 32%, respectively; $p<0.05$). The ACC surface/volume proportion in the zona fasciculata and zona reticularis also increased by the age of 26 months (by 48 and 44%, respectively; $p<0.05$), this indicating the predominance of small ACC.

The ACC nucleus/cytoplasm volume proportion in 26-month-old Wistar rats did not change in comparison with 5-month-old rats, while in OXYS rats this parameter decreased by 17% in the zona fasciculata and by 39% ($p<0.05$) in the zona reticularis in comparison with the age of 5 months (Fig. 3, *a*). The most pronounced differences in the ACC nucleus/cytoplasm volume proportion between Wistar and OXYS rats were detected in the zona reticularis: at 5 months this parameter was higher in OXYS (by 28%; $p<0.05$), while at 14 and 26 months it was higher in Wistar rats (by 18 and 27%, respectively).

The volume density of sinusoidal capillaries in Wistar rats increased by the age of 26 months by

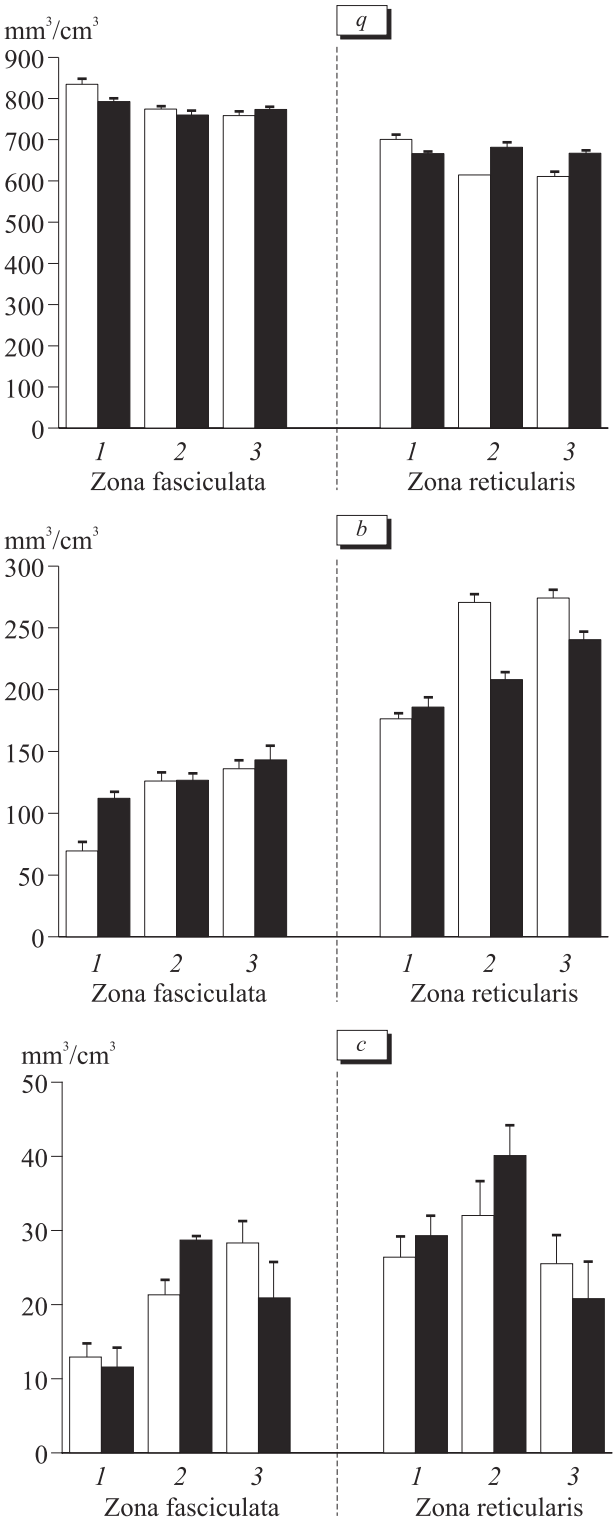

Fig. 2. Changes in volume density of ACC (*a*), sinusoidal capillaries (*b*), connective tissue components (*c*) in the adrenal cortex in the course of gerontogeny in Wistar (light bars) and OXYS rats (dark bars). Here and in Fig. 3: *1*) 5 months; *2*) 14 months; *3*) 26 months.

96% in the zona fasciculata ($p<0.01$) and by 55% in the zona reticularis ($p<0.05$; Fig. 2, *b*); the surface density increased by 24 and 42%, respectively

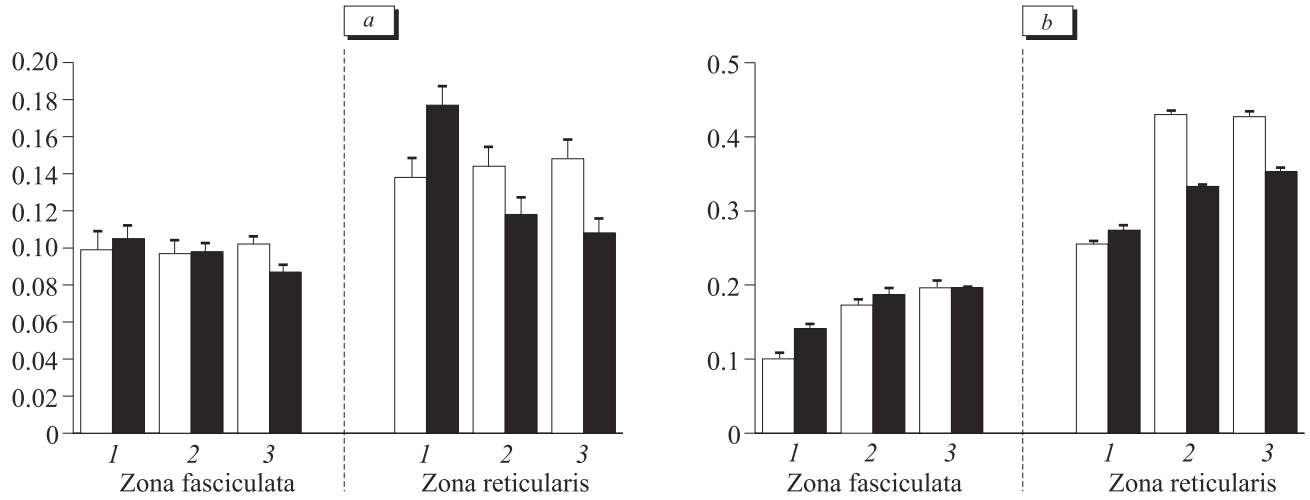

Fig. 3. Changes in the ACC nucleus/cytoplasm proportion (*a*) and in the stroma/parenchyma volume proportion (*b*) in the adrenal cortex over the course of gerontogeny in Wistar (light bars) and OXYS rats (dark bars).

($p<0.05$). As a result, the surface/volume proportion of sinusoidal capillaries in the zona fasciculata decreased by 36% ($p<0.01$), while in the zona reticularis it remained at the level of that in 5-month-old animals. Volume density of sinusoidal capillaries of OXYS rats also increased by the age of 26 months: by 28% in the zona fasciculata and by 29% in the zona reticularis ($p<0.05$). Surface density of sinusoids remained at the same level in both zones, which led to reduction of their surface/volume proportion: by 12% in the zona fasciculata ($p<0.05$) and by 10.7% in the zona reticularis. It is noteworthy that the volume density of sinusoidal capillaries in the zona fasciculata was 61% higher in OXYS rats at the age of 5 months *vs.* Wistar rats ($p<0.05$), while at 26 months these parameters were virtually the same in both strains (Fig. 2, *b*). By contrast, the sinusoidal volume and surface densities at the age of 5 months were virtually the same in both rat strains, while by 26 months the sinusoidal volume and surface densities of OXYS rats were 12 and 25% lower, respectively ($p<0.05$) in comparison with those in Wistar rats of the same age.

The sinusoidal capillary/ACC surface/volume proportion increased by 36% ($p<0.05$) in the zona fasciculata and by 73% in the zona reticularis ($p<0.05$) in 26-month-old Wistar rats. In OXYS rats the parameter increased by 19% in the zona fasciculata and by 19% in the zona reticularis at the age of 26 months.

Aging was associated with an increase in the volume density of cells, fibers, and main substance of the connective tissue (*in toto*) in Wistar and OXYS rats: by 119% ($p<0.01$) in the zona fasciculata of 26-month-old Wistar and by 80% in OXYS rats (Fig. 2, *c*). The value at the age of 26 months was lower in OXYS rats than in Wistar rats: by 26% in the zona fasciculata and by 18% in the zona reticularis.

These changes led to an increase in the stroma/parenchyma volume proportion with age. In Wistar rats aged 26 months, this parameter increased by 118% ($p<0.01$) in the zona fasciculata and by 68% ($p<0.01$) in the zona reticularis (Fig. 3, *b*). In OXYS rats of the same age, the stroma/parenchyma volume proportion increased by 39% ($p<0.05$) in the zona fasciculata and by 29% ($p<0.05$) in the zona reticularis. At the age of 5 months the stroma/parenchyma volume proportion in the zona fasciculata of OXYS rats was 57% higher ($p<0.05$), in the zona reticularis 7.5% higher ($p<0.05$) in comparison with Wistar rats (Fig. 3, *b*). At the age of 26 months this parameter was lower in OXYS rats only in the zona reticularis (by 17%, $p<0.05$) in comparison with Wistar rats.

Hence, complex morphological study showed that aging of Wistar and OXYS rats was associated with significant morphofunctional restructuring of the adrenal cortex. This process is more pronounced in OXYS rats, which develop atrophy of the zona glomerulosa and cortex in general, with the pool of cambial cells notably exhausted. In addition, OXYS rats develop pronounced disorders in the architectonics of the zona glomerulosa (focal discomplectation of the glomeruli and predominance of small ACC with solitary lipid incorporations). Changes in the architectonics of the zona reticularis in these animals manifest by pronounced dilatation of the sinusoidal capillaries and elimination of ACC. Spatial reorganization of the zona fasciculata and zona reticularis in Wistar and OXYS rats is characterized by reduced volume density of ACC and increased volume density of sinusoidal capillaries.

These changes and increased volume density of connective tissue components, particularly in the zona fasciculata, underlie the increase of the stroma/parenchyma volume proportion in Wistar and OXYS rats. The detected unidirectional age-associated changes in the spatial reorganization of the adrenal cortex reflect genetically programmed species-specific strategies of the organ restructuring, aimed at compensation and adaptation. Differences in the degree of these changes in Wistar and OXYS rats are presumably explained by differences in metabolic reactions of ACC and in regeneratory potential of these cells (capacity to cellular and intracellular regeneration).

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