

## STRUCTURAL-FUNCTIONAL PECULIARITIES OF THE URINARY BLADDER IN POSTNATAL ONTOGENESIS

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**Abstract.** It is known about the high incidence of the urinary bladder lesions in the structure of urological pathology regardless of age; however, according to the analysis of world literature sources, data on postnatal ontogenetic transformations of its wall constituents are poorly studied. Therefore, the purpose of our work was to study the morphofunctional peculiarities of the urinary bladder during sexual formation and puberty in parallel with research of pro- and antioxidant systems, as these processes are interrelated and interdependent.

In compliance with bioethical principles, in the experiment on 20 immature and 20 mature white outbred male rats using a complex research methods (injection, histological, immune-histochemical, electronic-microscopic and biochemical) the structural changes in the wall of the urinary bladder and pro- and antioxidant systems in stages of postnatal ontogenesis were studied.

Vascular transformations in investigated age periods occur in parallel with the transformations of cellular and non-cellular elements of the urinary bladder wall, ensuring adequacy of tissue homeostasis in ontogenesis. All this is associated with the processes of peroxidation and antioxidant systems operation, indicating their interrelationship and interdependence and strict control of the whole hierarchical system of regulation.

Taking into account the peculiarities of the structural elements of the urinary bladder wall at these stages of postnatal ontogenesis, clarification of the dynamics in other age groups and under the influence of various factors is promising.

**Keywords:** urinary bladder; postnatal ontogenesis; pro- and antioxidant systems.

**Introduction.** The problem of the study of age patterns of development is the subject of one of the most important areas of modern morphology – auxology (age anthropology) [1, 3, 11]. Permanency of the development in ontogenesis is conditioned by asynchrony and heterochrony of the components of body organs and tissues [13]. With age, there is a quantitative and qualitative change in the integrity of the body systems as a result of their structure reorganization and, consequently, a specific reaction to external and internal influences, and in terms of age disintegration of centers regulating homeostasis these changes can be a trigger mechanism of various diseases development [8]. However, according to the analysis of world literature, data on postnatal ontogenetic transformations of the components of urinary bladder wall are poorly studied, while it is known about the high incidence of its injuries in the structure of urological pathology, regardless of age [5, 9, 15].

**Objective.** Based on the above-said, the purpose of our work was to study morpho-functional peculiarities of the urinary bladder during sexual formation and puberty in parallel with the research of pro- and antioxidant systems, as these processes are interrelated and interdependent.

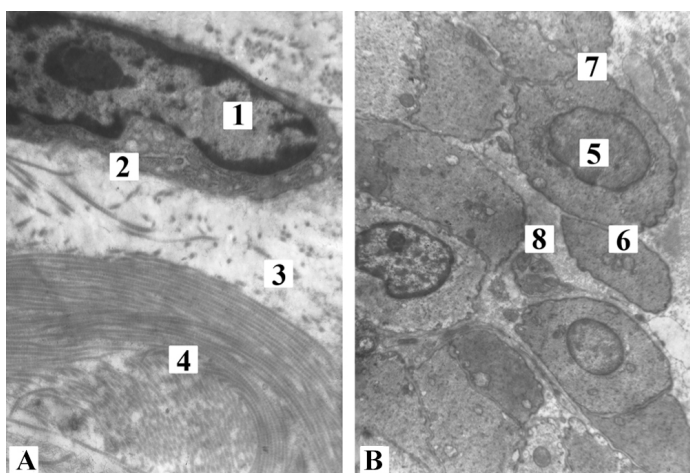
### Material and methods of the study

To achieve this goal there were used 20 immature and 20 mature white outbred male rats, weighing 120-150 g and 160-180 g, respectively. All animals were kept in normal conditions in vivarium with adequate nutrition without restrictions in drinking water [6]. Euthanasia of animals – was performed by an overdose of ether anesthesia. There were used: a thin blood vessel injection with Parisian blue, histological (hematoxylin and eosin staining, fuxeline staining after Hart (identification of elastic fibers), trichrome staining after Mason (identification of collagenous fibers), alcian blue after Stidman (detection of glycosaminoglycans), toluidine blue (visualization of mast cells)); immunohistochemical (for selective staining of synaptic structures there were used polyclonal antibodies and imaging systems Poly Vue Mouse/Rabbit HPR Kit); electronic-microscopic and biochemical methods. State of processes of lipid peroxidation (LPO) was evaluated according to the dynamics of accumulation of the primary – diene conjugates (DC) and secondary TBA-active products (TBA-AP) (those that react with thiobarbituric acid). To determine the state of antioxidant protection (AOP) the activity of glutathione peroxidase (GP), glutathione reductase (GR) was studied with the help of kinetic spectrophotometric methods. As a result of oxidation of proteins, aldehyde and ketone groups of amino

acid residues can form, that interact with 2,4-DPH (dinitrophenylhydrazine). Optical density was measured on a spectrophotometer Specord M-40. Depending on the predominance in the protein molecules of amino acids of neutral (valine, leucine, isoleucine, and others) or basic (lysine, arginine, and others) nature in molecules, aldehyde-dinitrophenylhydrazines or ketone- dinitrophenylhydrazines of neutral or basic character are formed (ADPHNC, KDPHNC and ADPHBC, KDPHBC in accordance). They have different absorption spectrum ranges. ADPHNC were measured at a wavelength of 356 nm, ADPHBC – 430 nm, KDPHNC – 370 nm and KDPHBC – 530 nm. Definition of the level of medium molecular weight peptides (MMWP) is based on direct spectrophotometry of the deproteinized supernatant of blood, obtained after precipitation of proteins by 10% solution of trichloroacetic acid. At a wavelength of 254-258 nm nucleoprotein component MMWP (MMWP<sub>254</sub>), in 278-282 – protein component (MMWP<sub>280</sub>) are determined. Nucleoprotein index – is determined due to metabolic products of nucleoproteins, protein – due to protein products of proteolysis. The metabolism of collagen is assessed according to the content of serum oxyproline, which is its typical biochemical marker. Oxyproline is oxidized by chloramine followed by paradimethylaminobenzaldehyde condensation, with the formation of red coloured chromogen. Analysis of morphometric parameters was performed by nonparametric statistics methods using the Mann-Whitney factor.

### Results and discussion of the study

Urinary bladder is a pear-shaped organ with enlarged cranial pole – top, elongated caudal body, which goes to the bottom and narrow neck. In blood vessels injection with the solution of Parisian blue the dye admission is marked into major artery-sources of blood supply of the urinary bladder (paired cranial and caudal bladder arteries). Their main branches are well visualized in the wall of the organ in the transmitted light, and near convoluted veins, that accompany these arteries, are quickly injected, which may indicate the functioning of arterial-venous anastomoses. As a result, the wide-loop reticula are formed in adventitia, and soon in the muscular membrane there is polygonal volume lattice. Later, when mass injection enters into blood vessels of mucous membrane, adjacent to each other ring-shaped rims are traced. So, the dense network of arteries of different diameter penetrates the bladder wall, settling in layers of connective tissue. Arteries are spin-like curved; anastomose each other, forming dense multishaped plexus. The used injection and histological methods of study helped to



**Fig. 1. Submicroscopic organization of the structural elements of the urinary bladder wall of immature rats.** 1 – fibroblast nucleus, 2 – canaliculi and cisterns of granular endoplasmic reticulum, 3 – basic substance, 4 – multidirectional bundles of collagenous fibers, 5 – smooth myocyte nucleus, 6 – mitochondria, 7 – myo-myocyte contacts, 8 – non-myelinated nerve fiber. Magnification: A – 8000, B – 4000

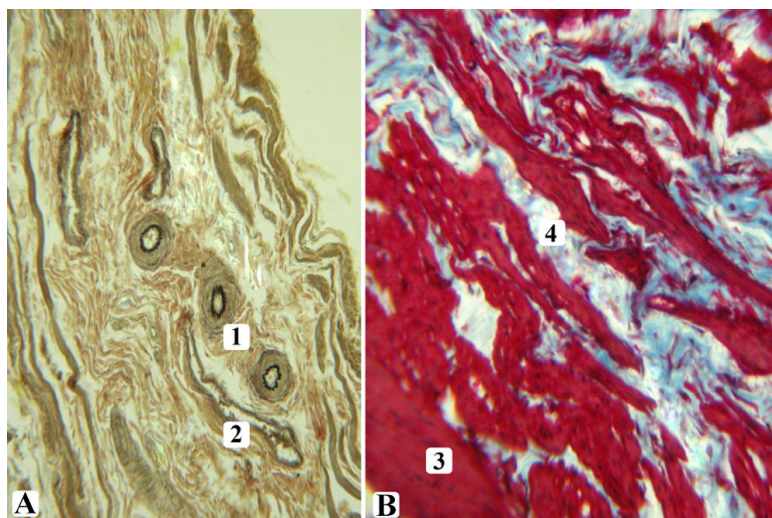
clarify angioarchitectonics and to identify the components of the vascular bed with their subsequent morphometric analysis. In immature animals sections of hemomicrocirculatory bed have already definitive structure with characteristic typical and organ-specific signs. Thickening of the arteries' walls is due to their medium membrane; structure of elastic frame arteries and veins is complicated.

Mucous membrane is folded except the area of the bladder triangle. Urothelial cells (in transitional epithelium) forming three layers are well visualized. In the basal layer they are small, in the interim – polygonal, and in the superficial – large with rounded nucleus. Intercellular connections are represented by tight junctions and desmosomes. In the middle membrane of the urinary bladder of immature animals the number of smooth myocytes increases in parallel with the increase of collagenous and elastic fibers; so it is well expressed and presented by collagenous-elastic frame with plaited in three layers of smooth myocytes. Organelles of biosynthesis are reduced gradually and contractile apparatus elements are increased, intercellular contacts are complicated in these cells. In the outer membrane there are many collagenous fibers (Fig. 1). In staining with alcian blue in the basic substance of connective tissue, we have found glycosaminoglycans. The processes of synthesis and breakdown of collagen on the stages of postnatal ontogenesis are manifested on the content of oxyproline blood serum, which in immature rats is  $(76.2 \pm 1.59)$  mcmol/l, while in mature ones –  $(72.8 \pm 2.52)$  mcmol/l. Equally important is the structural-functional condition of those connective tissue cells that synthesize large amounts of biologically active substances, they are active participants in the exchange, assimilation processes in the body. So, according to our data, in the immature rats mast cells density per area unit of the urinary bladder wall is  $128.49 \pm 29.63$ , of which  $32.64 \pm 1.85$  in the state of degranulation. In mature animals these figures are  $75.97 \pm 16.04$  and  $20.92 \pm 6.09$  respectively. They are identified in all membranes of the urinary bladder wall, mainly around blood vessels. Mast cells are polymorphic. Their population is represented with different saturation

metachromatic granules. Submicroscopically the structure of nucleus is homogeneous, in the cytoplasm there is a great number of granules of different electronic density, in the intergranular intervals there are membranous organelles, and mitochondria are often localized at the nucleus. In mature animals configuration of structural elements in the membranes of the urinary bladder wall is complicated (Fig. 2). According to the morphometric data its thickness in immature animals is  $(371.39 \pm 14.85)$  mcm, particularly membranes: mucous –  $(169.44 \pm 10.17)$  mcm, muscular –  $(184.17 \pm 12.89)$  mcm and adventitial –  $(17.78 \pm 1.24)$  mcm; in mature –  $(386.18 \pm 23.17)$  mcm,  $(179.49 \pm 7.18)$  mcm,  $(187.99 \pm 13.16)$  mcm and  $(18.70 \pm 1.31)$  mcm, respectively.

The wall of the urinary bladder in all its anatomical parts is saturated with components of autonomic nerve plexus. In immunohistochemical detection synaptophysinpositive terminals are visualized in round shape granules, chains in close relationship with smooth myocytes, blood vessels. Area of synaptophysin expression in  $1 \text{ mm}^2$  of the urinary bladder wall in immature animals is  $(11421.36 \pm 456.85) \mu\text{m}^2$ , in mature ones –  $(9931.55 \pm 695.10) \mu\text{m}^2$ . The nuclei of neurons of autonomic plexus are round or oval, chromatin is evenly distributed; in the cytoplasm of perikaryon there are membranous and non-membranous organelles. We have also found both undifferentiated nerve cells with large nuclei (usually in immature animals) and glial cells. Mitochondria, neurofibrils and neural microtubules are visualized in axoplasm. In the wall of the urinary bladder of mature rats myelin fibers with inherent structural elements often become apparent, which is consistent with the concept about peculiarities of ontogenetic changes of visceral nerve myelin fibers [7, 10]. The stimulating effect of afferent innervation is also confirmed, thus providing the processes of growth and development, according to the functional needs [14].

The above mentioned structural changes in the urinary bladder wall are accompanied by biochemical reactions – lipid peroxidation, which is a normal physiological process and is performed under the control of enzymes of antioxidant system [2]. Thus, we have found that in immature rats the level of HP and MDA is 1.3 times higher than in mature ones. This is explained by metabolic activity in this age group, which is associated with the formation of morphofunctional peculiarities of the organs and systems (Table 1).



**Fig. 2. Histostructure of the urinary bladder wall of mature rats.** 1 – artery, 2 – vein, 3 – smooth myocytes, 4 – intermuscular connective tissue layers. Staining after Hart (A), after Mason (B). Magnification: eyeglass 10, objective 20 (A), 40 (B)

**Table 1. Indexes of the system of LPO-AOP, OMP, MMWP in animals of immature (A) and mature (B) age in norm, M±m**

	GP mmol/min/mg	GR mmol/min/mg	TBA-AP mmol/l	DC mmol/l	ADPHNC un/ml	KDPHNC un/ml	ADPHBC un/ml	KDPHBC un/ml	MMWP <sub>254</sub> c.u.	MMWP <sub>280</sub> c.u.
A	0.24 ±0.01	0.34 ±0.01	3.806 ±0.009	0.804 ±0.001	1.776 ±0.001	1.886 ±0.001	0.781 ±0.001	0.192 ±0.001	0.242 ±0.001	0.270 ±0.001
B	0.18 ±0.01	0.44 ±0.01	5.130 ±0.007	0.603 ±0.001	2.992 ±0.001	2.926 ±0.001	0.968 ±0.001	0.204 ±0.001	0.304 ±0.001	0.311 ±0.001

### Conclusions

Vascular bed undergoes changes due to age restructuring and changing need for blood supply and is transformed along with the development and growth of urinary bladder wall membranes. Analyzing the pro- and antioxidant protection in rats of these two age groups, we have noted that in each of the studied developmental stages there is its own background level of their indexes. It was determined that the processes of lipid and proteins peroxidation and antioxidant defense form an integrated system, that being in a state of dynamic equilibrium, provides adequacy of metabolic reactions to morphofunctional needs at each stage of development.

### Prospects for further developments in this direction

Taking into account the peculiarities of the incipience of structural elements of the urinary bladder wall at these stages of postnatal ontogenesis, promising is to clarify their dynamics in other age groups and under the influence of various factors.

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