



## Muskrat's (*Ondatra Zibetica*) endocrine regulation organs as bioindicators for evaluation of ecological conditions in Baikal Region

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### Abstract

The article describes the morphometric measurements of the adrenal cortex functional zones and analyzes the results of these measurements and the percentage correlation of the zones to the total adrenal cortex area. Besides, an analysis of the functional activity of the adrenal cortex zona fasciculata is presented. The research has shown that the highest tension of the adrenal gland function occurs in the animals inhabiting the north-west outskirts of Irkutsk (Novo-Lenino district). They have a thicker adrenal cortex zona fasciculata and reticular zone as well as a higher functional activity of the reticular zone in comparison to other study groups. This is probably indicative of an intensive production of glykocorticoid, androgens, and estrogens. On these grounds we can assume that this district is the most hazardous for the existence of living organisms which we believe to be a sign of unfavorable ecological conditions. The use of the muskrat as a bioindicator for the evaluation of the ecological conditions in different city districts and the Selenga River delta is unprecedented.

**Keywords:** muskrat, test object, bioindicator, adrenal cortex, nuclear-cytoplasmic ratio

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### INTRODUCTION

The study of the morphofunctional aspects of biological indicators is often used in ecological-physiological research. The morphological study methods are highly informative and they allow the researcher to acquire objective information about the reactions of the living organism to certain climatic and anthropogenic factors. The change in the morphofunctional characteristics of the endocrine regulation organs is connected, as a rule, with some metabolic shifts in the animals that occur due to certain environmental conditions.

In our geo-ecological studies of Baikal region we have used the muskrat (*Ondatra zibetica*) as a morphofunctional bioindicator of the ecological conditions. This small mammal species is widespread, numerous, and easy to work with. This is why it can be successfully used as a test object in the course of different ecological studies in our region. The muskrat leads a semiaquatic life which means that it has direct contact both with water and soil. Consequently, it is the first animal to come into contact with harmful agents that are accumulated in the substances. Naturally, it reacts to impacts caused by this or that agent and thus it can be used as a biological indicator. The use of the muskrat

as a morphofunctional indicator of the ecological conditions yields objective results. This animal also inhabits city areas and co-exists with humans and this makes it acquire some new ecological characteristics and develop certain adaptations.

### AREAS OF STUDY

The material was collected during field expeditions from healthy mature individuals in different parts of the city of Irkutsk (Russia) along the banks of the Angara River, where the populations of muskrat (*Ondatra zibetica*) and near the Delta of the Selenga river of Kabanskiy district of the Republic of Buryatia (Russia) were observed. Angara is the only river flowing from Lake Baikal on the banks of which the city of Irkutsk is located. The Selenga is the major waterway flowing into the lake and provides up to half of the annual inflow to the lake, at the confluence into the lake forms a vast Delta with an area of 680 km<sup>2</sup>.

Data on areas of production, quantity and sexual status of animals are given in **Table 1**.

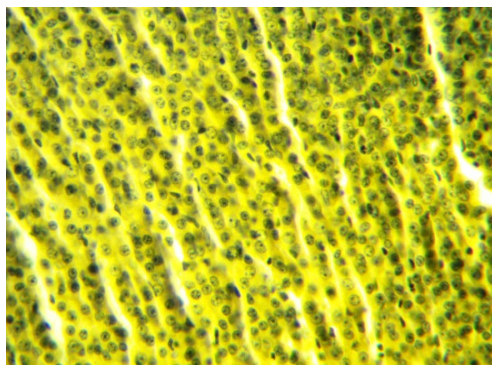
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**Table 1.** Information about the research material

Areas of collection of experimental material	Number of animals (n=)		
	Males	Females	
Districts of Irkutsk	Novo-Lenino	9	6
	Horse Island	6	5
	The Solar Neighborhood	10	7
Kabanskiy district, Republic of Buryatia	The Selenga river Delta	27	33

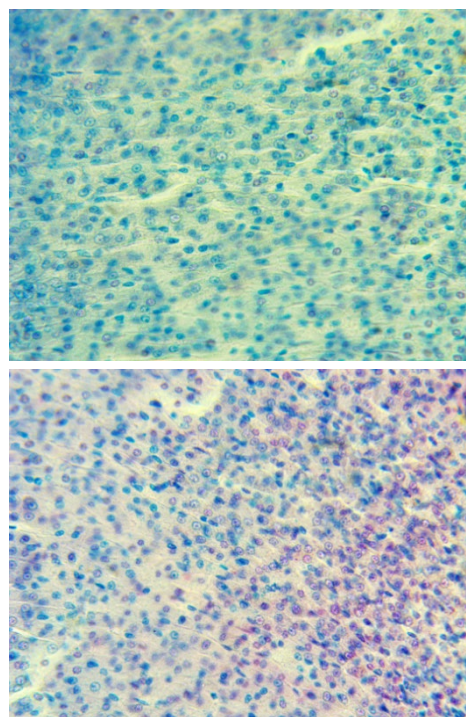
**Fig. 1.** The cells of the fascicular zone of the adrenal cortex of animals. Van Gieson method of painting (40×86)

## MATERIALS AND METHODS

Histological studies were carried out in the laboratory of clinical diagnosis and pharmacology of the Department of special veterinary disciplines of the Irkutsk state agrarian University named after A. A. Yezhevsky. The age of animals was determined by appropriate methods (Vodopyanov and Solovarov 2001). For histological studies, the adrenal gland was fixed in 10% neutral solution of formalin, a neutral locking of a mixture of Sabadasha and fluid Carnoy, paraffin sections of 5-7  $\mu\text{m}$  thickness were prepared on a sledge microtome "C. Reichert wien", were stained with hematoxylin-eosin and van Gieson method (**Fig. 1**). Micro-morphometric measurements were made on the microscope "Levenhuk" using the software "Levenhuk C 510 NG 5 M pixels" given classical methods.

When selecting morphological criteria for the assessment of the functional state of the organ measured the absolute and the relative thickness of the glomerular, fascicular and reticular zones of the cortex, as well as the diameter and volume of cell nuclei, their nuclear-cytoplasmic ratio, fascicular zone of the adrenal cortex. The thickness of various zones of cortical substance measured in 10 fields of view on gettoproperty from each animal.

The functional activity of cell nuclei granulozitov identified by a special method of coloring histotypeats (Yatzkovsky 1987). The nuclei containing euchromatin and stained with alzian blue at pH-4.8 and aqueous solution of safranin in equal proportions with resorcinol in blue or blue-red color, considered active. Inactive nuclei containing heterochromatin were colored red (**Fig. 2**).

**Fig. 2.** The cells of the beam zone of the adrenal cortex of the muskrat living in the area of Novo-Lenino and cells of the beam zone of the adrenal cortex of the muskrat living in the Delta of the Selenga river. Colouring according to the method of Yatzkovsky (40×86)

## RESULTS AND DISCUSSION

The adrenal glands of the muskrat are dark cherry-colored, located at the level of 1-2 lumbar vertebrae, closely adjacent to the cranial surface of each kidney. Anatomically, they have a characteristic shape: left-bean-shaped, right-heart-shaped or trapezoidal.

By histological structure they are no different from the adrenal glands of most mammals. Outside, the organ is covered with a connective tissue capsule. Directly under the capsule in the composition of the cortical part is the glomerular zone, which consists of narrower and smaller, compared to other cell zones. In the glomerular zone, epithelial strands are turned under the capsule in the form of glomeruli, and in the beam they go parallel to each other (**Fig. 1**). Large polygonal cells form parallel strands of the beam zone (**Fig. 1**). Further, the correct course of the strands is disturbed in the mesh zone, where epithelial strands form anastomoses on the border with the brain substance.

As a result of our studies of morphometric parameters of the adrenal cortex of the muskrat living in different areas of the city of Irkutsk and the Delta of the Selenga river, it was shown that the greatest thickness ( $M \pm m; \mu\text{m}$ ) of the beam (males –  $752.5 \pm 15.6$  and females –  $810.4 \pm 16.75$ ) and the mesh zones (males –  $576.0 \pm 17.42$  and females –  $587.3 \pm 15.42$ ) of the adrenal cortex reach individuals living in Novo-Lenino

(North-Western margin cities), and the smallest thickness ( $M \pm m; \mu m$ ) beam pipe (male –  $617.2 \pm 18.23$  and females  $635.4 \pm 14.75$ ) and net areas (males –  $436.2 \pm 12.46$  and females  $476.4 \pm 16.48$ ) individuals living in the Delta region of the Selenga river. As a percentage of the total area of the adrenal cortex, no significant differences were found in the study groups.

When comparing the studied parameters of morphofunctional characteristics of the cells of the beam zone of the adrenal cortex of the muskrat living in the conditions of the city of Irkutsk, it was noted that the highest rate for all the studied parameters was observed in individuals of the population living in the area of Novo-Lenino (North-Western outskirts of the city). So the average cell diameter ( $M \pm m; \mu m$ ) was: (males- $15.4 \pm 0.12$  and females- $16.3 \pm 0.15$ ) and the volume of cell nuclei ( $M \pm m; \mu m^3$ ), respectively: (males –  $202.5 \pm 5.63$  and females –  $210.4 \pm 6.75$ ), which is 1.5 times higher than the populations of species living in the Delta region of the Selenga river (males and  $8.6 \pm 0.05$  and females –  $9.5 \pm 0.15$ ) and (males –  $132.7 \pm 4.25$  and females  $137.5 \pm 4.35$ ), respectively.

As for the percentage ( $P\% \pm m_p\%$ ) number of granulocytes, nuclei that contain euchromatin (Fig. 2), it is slightly higher in individuals of the population living in the area of Novo-Lenino (males –  $83.7 \pm 3.52$  and females –  $86.5 \pm 3.27$ ) compared with those in the Delta of the Selenga river (males –  $60.5 \pm 3.28$  and females –  $65.5 \pm 3.48$ ).

The highest index of nuclear-cytoplasmic ratio of cells ( $M \pm m; unit$ ) of the beam zone of the adrenal cortex reached in the muskrat, living in the area of Novo-Lenino (males –  $15.0 \pm 0.12$  and females –  $17.3 \pm 0.12$ ) and the lowest in the Delta of the Selenga river (males –  $10.5 \pm 0.12$  and females –  $11.7 \pm 0.15$ ), indicating a high functional activity of cells in individuals of the population living in the area of Novo-Lenino.

In assessing the results obtained in connection with the sexual status of the studied individuals, it was found that the morphofunctional activity of the cells of the adrenal cortex beam zone is higher in female muskrats in all studied areas of the city of Irkutsk and the Delta of the Selenga river.

## SUMMARY

- In the normally functioning adrenal gland of mammals, the glomerular zone accounts for 8-10%, the beam – 50%, the mesh – 30-35% (Kirillov 1994). The data obtained by us with minor deviations correspond to the physiological norm of the adrenal gland in mammals;

- A significant increase in the thickness of the beam and mesh zones of the adrenal cortex in individuals living in the North-Western outskirts of the city of Irkutsk (Novo-Lenino) is noteworthy in comparison with other areas of the study);

- Our studies have revealed the tension of the function of the adrenal gland of the muskrat living in the conditions of the city of Irkutsk, due to the influence on the animals of their habitat, which is expressed in increasing the thickness of the beam and mesh zone of the adrenal cortex aimed at maintaining cell function in changed conditions, as numerous studies have shown, that exceeding the norms of concentration of chemical elements included in the diet of animals living in anthropogenically contaminated areas and in conditions of natural biogeochemical anomalies can cause an increase in the beam and mesh zones of the adrenal cortex, morphological and functional parameters of the nuclei and cells in the beam zone of the adrenal cortex;

- Since the important physiological role of the adrenal gland on a variety of effects plays a genetic determination of the body's response to stress, which is important for survival and the ability of animals to reproduce in natural populations (Markel and Borodin 1981), it can be assumed that the area of Novo-Lenino is the most environmentally disadvantaged, then the city center (Horse island), and the most prosperous in this respect is the district of Sunny (South-Eastern outskirts of the city) and the Delta of the Selenga river.;

- When analyzing our studies on the sex of individuals, it was found that in females the studied parameters are slightly higher than in males, which is confirmed by numerous studies, as it is known that androgens and estrogens can cause adrenal hypertrophy, the latter being more effective (Ray and Maiti 2002).

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