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# Features of Muscle Tissue Microstructure of Cattle in Industrial Agglomerations under the Environmental Pressure Conditions

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## Abstract

The intensive development of the industrial sector, intensification of the agro-industrial complex, associated with the use of various fertilizers, active use of modern household chemicals lead to the constant increase in xenobiotics in the environment in both rural and urban agglomerations. There are settlements and farms within the industrial areas, therefore, the issue of accumulation of ecotoxicants in the organs and tissues of an animal, as well as the impact on the state of its health, is of particular importance. In the regions with tough environmental situation associated with anthropogenic contamination, xenobiotics of anthropogenic origin can directly or indirectly modify the activity of various body systems. The integral characteristic that reflects adaptive modifications of biota is the morphological and functional status of organs and tissues of an animal, including the state of muscle tissue. An analysis of the regenerative plastic potential of muscle tissue allows finding innovative approaches to assessing the effects of environmental impacts on animals. Up to the present day, the morphological and functional characteristics of muscle tissue in young animals and adult cattle have not been sufficiently studied in the conditions of the tough environmental situation of the Central Federal District of the Russian Federation. The issue of ecological pathologies of organs in productive animals is quite urgent for the territory of the Central Federal District with its developed agro-industrial complex and industry. The study of animals from the agglomeration of the large chemical plant showed that cattle react differently to pollutants. The animals demonstrated changes not only in hematological and biochemical parameters, but also in the morphological and functional status of muscle tissue.

**Keywords:** environment, ecotoxicants, cattle, microstructure, muscle tissue

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## 1. Introduction

The deterioration of the environmental status and the imbalance of the equilibrium between the environment and the body can lead to both violation of the adaptation mechanisms and the appearance of pathologies not typical of the population. The rapid growth of the industrial sector, the intensification of the agro-industrial complex, accompanied by the use of various fertilizers, active use of modern household chemicals lead to a constant increase in xenobiotics in the environment in both rural and urban agglomerations [1].

There are settlements and farms within the industrial areas, therefore, the issue of accumulation of ecotoxicants in the organs and tissues of an animal, as well as the impact on the state of its health, is of particular importance [2]. Under prolonged environmental pressure, the alternative effects of complex xenobiotics on the animal's body increase. At the same time, the formation of natural-technogenic provinces with excessive content of heavy metals and radionuclides takes place. In regions of unfavorable environmental conditions associated with anthropogenic contamination, xenobiotics of anthropogenic origin can directly or indirectly modify the activity of various body systems [3--5]. The integral characteristic that reflects adaptive modifications of biota is the morphological and functional status of organs and tissues of an animal, including the state of muscle cells [6--9].

Monitoring of the formation of muscle tissue optimizes the search for innovative approaches to assessing damage from environmental effects on a living organism [10]. Up to the present day, the morphological and functional characteristics of muscle tissue in young animals and adult cattle have not been sufficiently studied in the conditions of the tough environmental situation of the Central Federal District of the Russian Federation [11].

## 2. Methods and Equipment

### 2.1. Methods

#### 2.1.1. Diagrammatic representation

The classical histomorphological methods were used for the present research.

The process of preparation of a histological specimen for light and electron microscopy includes the following key steps:

1. taking the samples and their fixation;
2. sample compaction;
3. slice preparation;
4. slice coloration.

For light microscopy, another step is necessary -- putting the slices in a balm or other transparent media [12].

Fixation ensures the prevention of autolysis processes, which helps preserve the integrity of the structures. The tissue fixation was achieved by the fact that a sample taken from an organ was immersed in a fixative -- neutral 10 % formalin. Under the influence of the fixative, complex physical and chemical changes occur in tissues and organs. The most significant of them is the process of irreversible coagulation of proteins, as a result of which vital activity ceases, and the structures become dead, fixed. Fixation leads to compaction and a decrease in the volume of pieces, as well as to improvement in the subsequent coloration of cells and tissues. Further processing consisted of preliminary dehydration of the samples by successive immersion in ascending concentrations of alcohols, starting from 70 percent. The compaction of the pieces necessary for the preparation of the slices was carried out by impregnating the previously dehydrated material with the specialized Histomix medium.

The slice preparation for light microscopy was carried out on a rail microtome MS-1.

The next step was coloration of the slices with hematoxylin-eosin to increase the contrast of the image of the individual structures of the studied tissue. For long-term preservation, the dehydrated histological slice was enclosed between the subject and the cover glass in Canadian balsam.

For electron microscopy, slices obtained by the LKB ultramicrorotome were placed on special grids, contrasted with manganese salts, after which they were examined with TESLA microscope and photographed. The obtained micrographs served as the study object along with histological preparations [13].

## 3. Results

The industrial sector contributes to the accumulation of heavy metal salts in the fertile soil layer. High content of cadmium, nickel, zinc, copper, lead, and mercury was found in some areas of the Voronezh region was noted [14].

At the moment, only in the Voronezh region the volume of ecotoxicants has increased significantly. The key causes of contamination were stationary sources (15--17 % of pollution), mobile sources, that is, vehicles (83--85 % of pollution) (Figure. 1).

The motor vehicle segment is extremely large in the overall structure of environmental pressure, while there is a slight decline, which is due, on the one hand, to the improvement in the quality of modern fuel, and, on the other hand, to the development of industry and the low efficiency of its treatment facilities.

The amount of ecotoxicants in the industrially developed areas of the Voronezh region is quite large. The critical situation with dusting the air was noted in a number of districts of the Voronezh region and amounted to 0.30--0.40 mg/$m^3$[14].

The rapidly changing total mass of xenobiotics in recent years has been characterized by decrease in the accumulation of pollutants, which can be explained, first of all, of all by a change in the structure of production, reconstruction of enterprises and their treatment facilities.

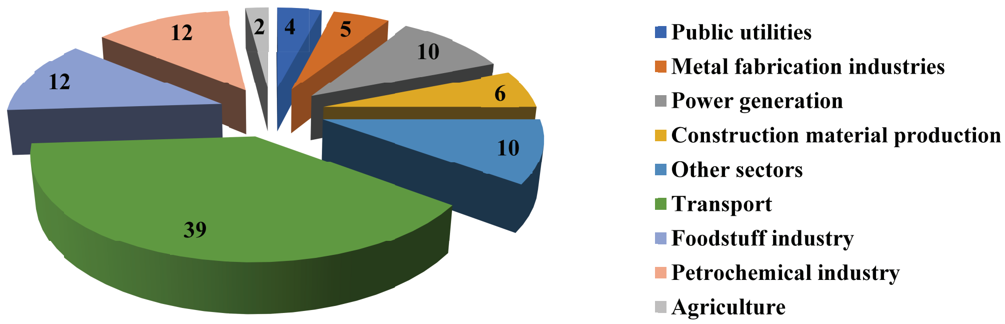


**Figure** 1: Sectoral structure of xenobiotic sources, %.

The Voronezh region, in addition to technogenic pressure, is also experiencing radiation one. The radiation situation in the region is characterized by both natural sources of radiation and the activities of the nuclear energy complex.

The result of the technogenic accident at the Chernobyl nuclear power plant (NPP) was pollution of a significant part of the former Russian Soviet Federated Socialistic Republic. Voronezh region was also within the zone of the radioactive trail, which provoked the contamination of biogeocenoses. The leading role in the radiation contamination was played by iodine-131 and cesium-137. The main harm was caused by iodine radionuclides. Monitoring of the radiation contamination data indicates the increase in the content of cesium-137 in soils compared with 1964 (the year of nuclear testing) due to the Chernobyl nuclear power plant. Moreover, during the observation period, the cesium-137 content in the soil was 0.3--2 curie/$km^2$. The main sources forming the radioactive harm to the population are radionuclides of global and Chernobyl origin. The activity of these radionuclides in the environment is several times higher than the activity of radionuclides coming from the operation of the Novovoronezh NPP. Consequently, the radiation pressure of the Novovoronezh NPP does not have significant consequences for the biogeocenoses of the region. That is, there is rather tough situation in relation to environment in the Voronezh region. In the agglomeration of industrial centers, specific geochemical provinces are formed; this entails degradation in the quality of agricultural products, as well as the accumulation of xenobiotics in living organisms.

Thus, the study of the impact of anthropogenic xenobiotics on the morpho-functional state of organs and tissues of animals, including cattle of various age groups in the tough environmental situation, is relevant in the scientific and practical sense.

The aim of this research was to carry out morpho-functional studies and assess the physiological parameters of animals in areas with different technogenic loads, in particular, based on the example of the state of muscle tissue in cattle.

The study was carried out for cattle muscle tissue samples from agglomerations with tough environmental situation for a number of indicators, such as heavy metals and radionuclides.

In the studied preparations of muscle tissue, bundles consisting of muscle fibers, elements of the connective tissue stroma, blood vessels, and areas of adipose tissue were clearly visible.

Muscle tissue was characterized by longitudinal fibers densely spaced to each other with thin layers of loose connective tissue stroma. The thickness of the muscle fibers was not uniform. In muscle fibers, transverse striation was clearly visible. Individual muscle bundles were divided into thinner ones with connective tissue layers (Fig. 2). The nuclei had an elongated ovoid shape and were located on the periphery of the muscle fibers. There were areas of loose connective tissue. The structural differences of the studied muscle samples consisted in the thickness of the muscle fibers and the relative amount of the connective tissue stroma forming the muscle layer (Figure 2).

The cross-striated muscle tissue of an animal from the ecologically favorable area comprised many muscle cells, which are its structural units (Figure. 3).

The cells were cylindrical and parallel to each other. Groups of muscle cells are surrounded by stromal elements. The connective tissue elements formed tendons on the terminal part of the muscles (Figure 4). Each fiber is surrounded by sarcolemma.

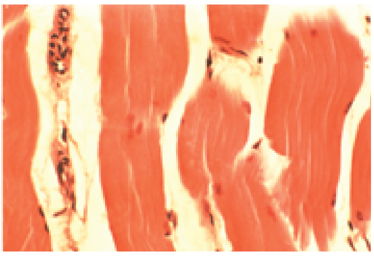

**Figure** 2: Muscle tissue, consisting of longitudinal, tightly spaced fibers with thin layers of loose connective tissue stroma. Clinically healthy animal. Hematoxylin-eosin coloration. Magn. ×400.

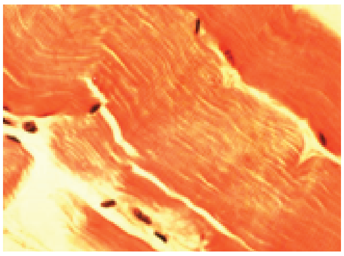

**Figure** 3: Cross striation of cattle muscle tissue. Clinically healthy animal. Hematoxylin-eosin coloration. Magn. ×400.

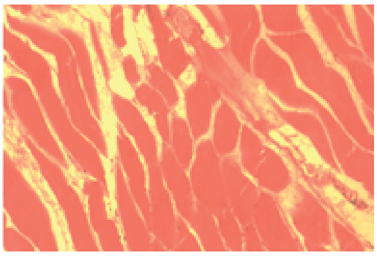

**Figure** 4: Connective tissue layers in the muscle tissue of cattle. An animal from the area with tough environmental situation. Hematoxylin-eosin coloration. Magn. ×200.

The muscle tissue cells of the checked animals presented significant accumulations of myofibrils, which had characteristic striated striation. The structure of myofibrils included both actin and myosin structures. A significant amount of mitochondria was also noted (Figure 5).

Using light microscopy, only the transverse striation of myofibrils is visualized, including light disk I, dark disk A and Z-line dividing zone I. At a much higher magnification, which electron microscope provides, it is clear that this pattern of banding is due to a certain arrangement of actin and myosin filaments (Figure 6).

## 4. Discussion

The muscle fiber cytoplasm contained sarcoplasmic reticulum. Perpendicular to the muscle fibers between the myofibrils, a tubular system was revealed, which demonstrated sarcolemma formation called the T-system.

The structures consisting of single T-tubes and two cisterna located close to it, as a rule, represented a triad. Tubules and cisterna were connected by transverse membrane bridges.

One may note as a significant fact the difference between the myofibrils of animals from the provinces in which tough ecological situation was observed. At the same time, the prevalence of violations of ultrastructural elements was noted, resulting in a decrease in the technological parameters of the muscle of the animal, which is the main

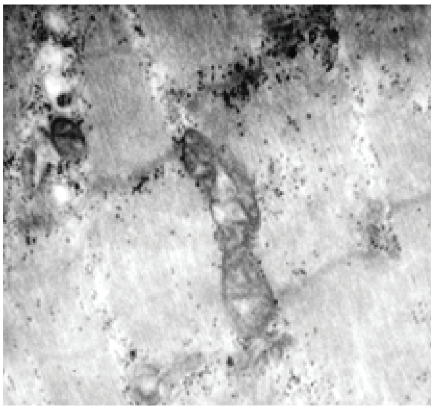

**Figure** 5: A clear matrix of mitochondria of muscle tissue of clinically healthy cattle from agglomeration distant from potential xenobiotic sources. Lead salt contrasting. Magn. ×10000.

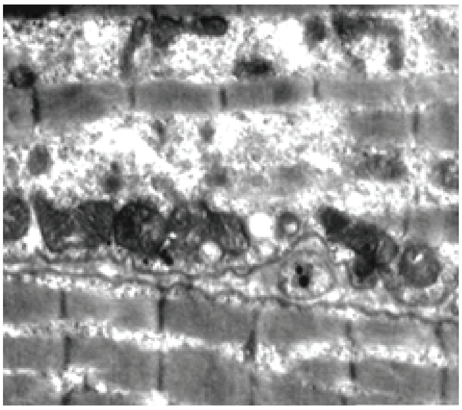

**Figure** 6: Destructive changes in myofibrils and mitochondria of muscle tissue of clinically healthy cattle from agglomeration in the area of a large chemical plant. Magnesium salt contrasting. Magn. ×6000.

raw material of meat processing enterprises. We should also mark out the destruction of both fragments of myocytes and whole organelles.

## 5. Conclusion

As a result of the generalization of the studies, it can be concluded that the intensive development of natural reserves, growth of industry and other types of human impacts on the environment pose a number of tasks for the integrated use of the biosphere, the protection of the flora and fauna, and the production of safe food for people. In this regard, a comprehensive system of assessment and control of the environment condition, including the countryside, is becoming increasingly relevant.

At present, science and practice do not have any methodological approach to studying and assessing the toxicological and ecological state of the agro-industrial complex, in particular animal husbandry, and the use of monitoring the formation of muscle tissue optimizes the search for innovative approaches to assessing damage from environmental effects on a living organism.

We determined that the borderline level of exposure to anthropogenic ecotoxicants in the early stages leads to destructive changes both at the ultrastructural level, in relation to individual cell organelles, and subsequently at the tissue level, causing a disproportion between the specific organ tissue and connective tissue cells, which in dynamics affects the functionality of a particular tissue and a decrease in its functional and technological properties.

## Conflict of Interest

The authors have no conflict of interest to declare.

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