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HISTOLOGICAL CHANGES IN COMMON TOAD, *BUFO BUFO* (ANURA, BUFONIDAE), LIVER TISSUE UNDER CONDITIONS OF ANTHROPOGENICALLY TRANSFORMED ECOSYSTEMS

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Histological Changes in Common Toad, *Bufo bufo* (Anura, Bufonidae), Liver Tissue under Conditions of Anthropogenically Transformed Ecosystems. Akulenko, N. M., Dziubenko, N. V., Marushchak, O. Yu., Nekrasova, O. D., Oskyrko, O. S. — In the article the histological changes in the liver of the common toad, *Bufo bufo* (Linnaeus, 1758), under conditions of moderate anthropogenic pollution (a vast park surrounded by urban areas) are examined. In the liver parenchyma, numerous changes characteristic of toxic damage were found: hepatocyte necrosis, fatty dystrophy, protein (and hydropic) dystrophy, signs of inflammation. An analysis of the number of pigment inclusions in the liver shows hyperpigmentation in some specimens and hypopigmentation in others. The presence of hyperpigmentation indicates a moderate degree of damage and activation of compensation processes in animals. This is confirmed by a large population of common toads in this biotope. It is concluded that in anthropogenically modified biotopes some deterioration of animal health can be compensated by the absence of predators and the reduction of food competition.
Key words: liver, anthropogenic pollution, amphibian.

Introduction

The analysis of the anthropogenic pollution biological effects on vertebrates in natural landscapes is a complicated issue. Synergistic effect of many diverse and unpredictable factors is the main difficulty (Akulenko, Nekrasova, 2008). Therefore, methods that allow evaluating the anthropogenic pollution cumulative physiological effects directly on the vertebrates' organisms, including humans, are of great importance. From this point of view, the study of wild animals caught in affected biotopes does not have a satisfactory alternative. Such studies are important in terms of conducting the environmental monitoring. It is equally important to study the physiological state of animals in natural populations for the studied species' protection and the preservation of biodiversity. In the anthropogenically transformed biotopes, the environmental conditions for certain species differ from those in the wild. Under such conditions, trophic links often change for tailless amphibians: decrease in numbers of predators and food competitors, increase in the number of suitable shelters and the amount of food. As a result, the requirements for physical activity are significantly reduced. Our data shows

that in anthropogenically transformed biotopes, a perfectly prosperous amphibian population may consist of unhealthy individuals and be subjected to a rigorous selection for resistance to toxic pollution (Akulenko et al., 2012). Of course, if the pollution intensifies, the adaptation possibilities will reach their limit and the species will disappear (Pysanets, 2007; Litvinceva et al., 2010; Nekrasova et al., 2010).

In previous publications the possibility of using of green frogs as an indicator of water pollution was analyzed (Akulenko, 2015). It was shown that histological changes in animal liver are the most reliable indicators of toxic pollution presence. It is shown that the occurrence of foci of necrosis, fatty and protein dystrophy of hepatocytes are specific for animals from contaminated biotopes. In pure biotopes, these changes are not detected. At the same time, it was found that in such studies, it is necessary to take into account the specifics of reparative reactions of this species, which are much more powerful than in mammals (Akulenko, Zhalai, 2011; Akulenko, 2015). Common toads, *Bufo bufo* (Linnaeus, 1758), are terrestrial animals. They find shelters under the roots and trunks of fallen trees, winter in non-freezing shelters — rodents' holes, etc. Due to their lifestyle, common toads are a suitable target indicator for soil contamination monitoring. *B. bufo* is listed in Appendix III of the Berne Convention, and is mentioned in the IUCN protection lists under the category Least Concerned (LC). In Ukraine, it is listed in the regional Red Lists of Kharkiv, Mykolaiv, Dnipro, Kherson and other regions. The purpose of this work is to identify the features of histological changes in the liver of the common toads from an anthropogenically transformed biotope that can be used for pollution detecting and environmental state monitoring.

Material and methods

For the study, sexually mature males of common toads were taken (n = 9, mean snout-to vent length is 7–8 cm, mean weight 45–55 g). The animals were captured in early April 2016, during the end of the spawning season (approximately 2 weeks after the end of the hibernation) near Didorivka lake (National Nature Park (NPP) "Holosiivskyi", Kyiv). The park territory is located within Kyiv City and is surrounded by residential areas. The spawning territory for this part of *B. bufo* population is situated in a recreational zone of the NPP, which indicates the presence of anthropogenic transformation.

The captured animals were euthanized using ether anesthesia. Histological slices of 5 μm thickness were made from liver samples dehydrated in alcohols and embedded in paraffin. Stained with hematoxylin-eosin, balsam embedded liver tissue specimens were examined under a light microscope. Changes in the histological structure were taken into account as qualitative signs based on the presence-absence principle. The presence of such features as hepatocyte necrosis, fatty dystrophy, protein (and hydropic) dystrophy, inflammation, hyperpigmentation and hypopigmentation were considered. Statistical processing was performed by the standard method adopted for alternative features (Lakin, 1973: 149–157).

Results and discussion

In previous publications changes in the liver tissue of green frogs (*Pelophylax esculentus* complex) under conditions of anthropogenic pollution were studied (Akulenko, Zhalai, 2011; Akulenko, 2015). In green frogs, violations of the normal histological structure were found. The same changes typically occur in the mammalian liver tissue under conditions of chronic toxicosis (Miadelets, Lebedeva, 2018). These are inflammations, foci of necrosis, adipose, protein and protein-hydropic degeneration of hepatocytes. These changes are easy to identify. Their causes are not difficult to determine, because the indicated pathologies are well studied in the context of medicine. These results do not contradict the data of other authors (Loumbourdis, 2007; Falushinnska et al., 2008). At the same time a specific complex of histological changes in the frog liver parenchyma were described too. They indicate the phenomenon of the so-called "urgent regeneration", characteristic of green frogs and, probably, other tailless amphibians. This reaction allows rapid liver tissue restorqtion after severe necrotic changes. The "urgent regeneration" is indicated by characteristic changes in histological structure: unusually small hepatocytes have an elongated shape and lie in strands, without forming the usual hepatic beams. Anisocytosis is often observed with the appearance of binuclear cells (Akulenko, 2015).

The histological structure of the liver tissue of healthy animals within the order of tailless amphibians differs very slightly (Franco-Belussi et al., 2012; Akiyoshi, Inoue, 2012). The list of pathological reactions to toxic impacts mentioned above is also generally found in other tailless amphibians (Çakıc, 2015.) Changes in the liver of the common toad under the conditions of the NPP "Holosiivskyi" reiterate signs of liver toxic damage that was

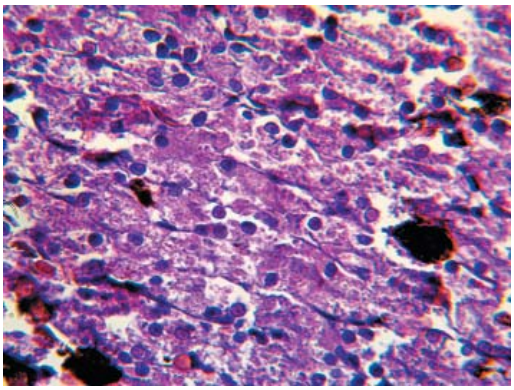

Fig. 1. Protein dystrophy in common toad. Protein granules are visible inside hepatocytes. Hematoxyline-eosine staining, ×200.

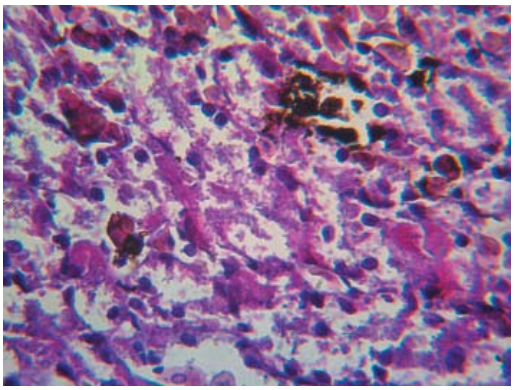

Fig. 2. Foci of necrosis in common toad liver tissue. Destructed cells are seen. Hematoxyline-eosine staining, ×200.

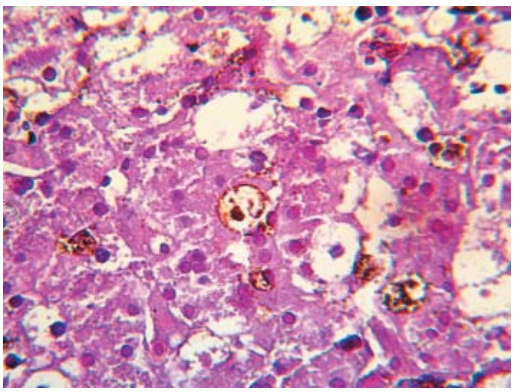

Fig. 3. Adipose dystrophy and hypopigmentation of common toad's liver. Vacuol and pigment remainings are seen in melano-macrophagal aggregations. Hematoxyline-eosine staining, ×200.

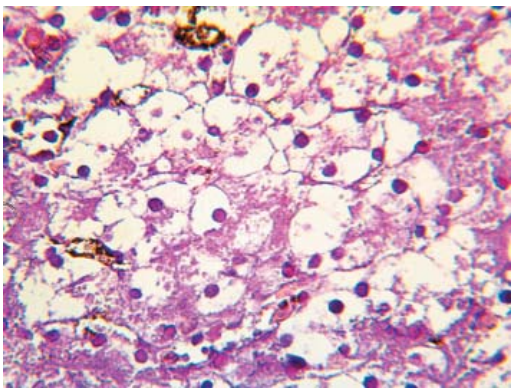

Fig. 4. Well-seen adipose dystrophy combined with protein dystrophy and a foci of necrosis in the middle of the picture. Destroyed cells and pigment remainings are seen in melano-macrophagal aggregations. Hematoxyline-eosine staining, ×200.

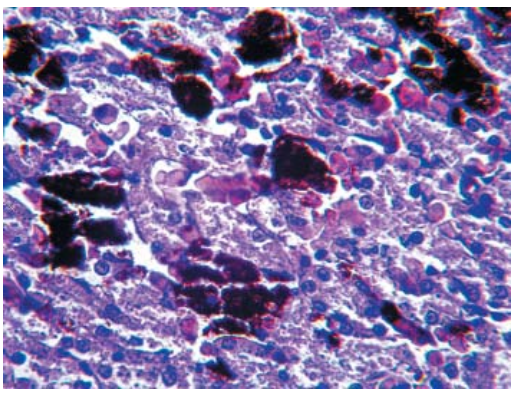

Fig. 5. Manifestation of protein dystrophy and hyperpigmentation in common toad liver tissue. Hematoxyline-eosine staining, ×200.

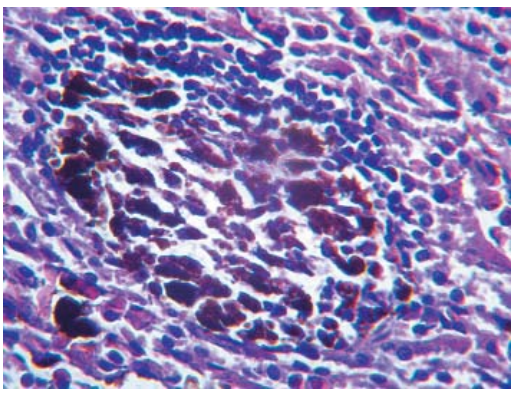

Fig. 6. Abnormally big melano-macrophagal aggregation surrounded by lymphoid infiltrate (inflammation) and hyperpigmentation in common toad's liver tissue. Hematoxyline-eosine staining, ×200.

observed in green frogs (Akulenko, Zhalai, 2011; Akulenko, 2015). The main violation of the normal structure of hepatocytes, protein dystrophy, was found in all captured individuals (figs 1–5). Protein dystrophy has the following characteristics: small protein granules instead of a homogeneous cytoplasm are visible in the hepatocytes, which lie in optically empty space. With a strong lesion in the liver, there are foci of necrosis — zones of destroyed hepatocytes (fig. 2), which were found in 5 cases. Signs of adipose dystrophy of hepatocytes were found in 5 animals. Large fat vacuoles are formed inside the cells, as a clear sign of adipose dystrophy. In the histological wiring, the fat dissolves, and large, optically empty vacuoles in the cytoplasm are visible on the preparations (figs 3, 4). Large adipose dystrophy loci also contain foci of necrosis (fig. 4). Inflammation is one of the pathological changes in the liver, without being a specific manifestation during toxic effects. Most often, hepatitis has an infectious nature. In our study, mild signs of inflammation we found in 2 animals. In one case, a small lymphoid infiltrate was located on the border of an abnormally large melanomaphagic aggregation, which in our opinion indicates the infectious nature of this formation (fig. 6).

The amount of pigment in specific formations — melano-macrophage aggregations — is a significant marker of the liver state in tailless amphibians (Akulenko, 2013; Santos et al., 2014; Jantawongsri et al., 2016, Pérez-Iglesias et al., 2016). The accumulations inside the liver include pigment cells and macrophages. In a healthy liver, pigment inclusions are used during blood formation and in non-specific immunity reactions (Akulenko, 2016). It was shown that the inclusion of pigments is also used in the course of hepatocyte repaire in the foci of necrosis. Therefore, the amount of pigments in the liver can also be an indicator of toxic impacts on the animal. According to our data, under conditions of moderate anthropogenic pollution, the synthesis of pigments in the liver of frogs and at the same time their use in protective reactions is stimulated (Akulenko, 2013). Therefore, in some animals, the content of pigments in the liver increases, in others, on the contrary, it is reduced (Akulenko, 2013, 2016). Fenoglio with co-authors (2005) noted the intensification of the synthetic processes in the liver melano-macrophages under conditions of anthropogenic pollution. Çakıc (2015), de Gregorio e. a. (2016) Jantawongsri et al. (2016) noted an increase in melano-macrophagal clusters in response to toxic effects. The depletion of the pigment system in adverse conditions was noted less frequently (Santos et al., 2014). In our case, 4 of 9 specimens' livers showed obvious hyperpigmentation (inclusions greater than 10 % of the total area of the slice) (fig. 5.) Another 3 pigments in the liver were found

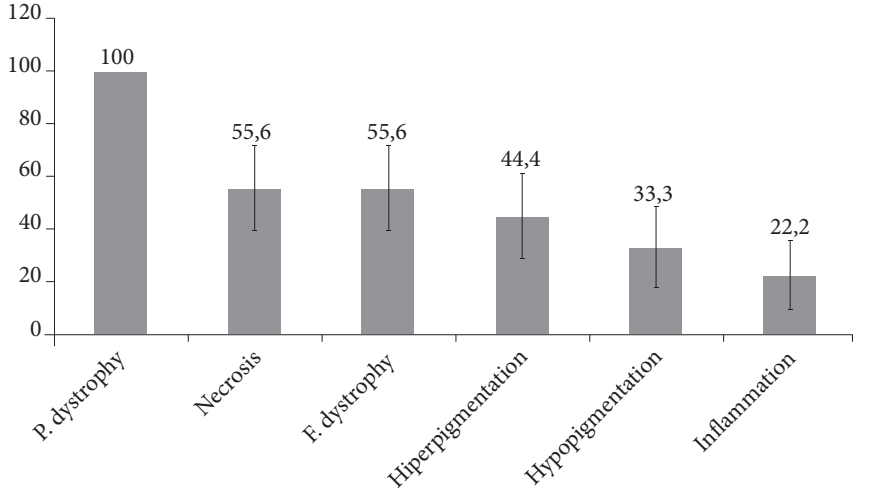

Fig. 7. Histological changes in liver tissue of common toads from breeding population in NNP "Holosiivskyi", %.

only an in trace amount, which suggests hypopigmentation (figs 3, 4). In general, the reactions of the common toad liver indicate the average degree of contamination of the chosen biotope, which causes mainly compensatory reactions in most animals (fig. 7). At the same time, there were no traces of "urgent regeneration" in the studied animal livers. However, green frogs with well seen protein dystrophy of the liver showed almost no signs of "urgent regeneration" (Akulenko, Zhalai, 2011). A large number of pigment inclusions in the liver of animals in the springtime, immediately after hibernation and participation in reproduction, also deserve attention. This shows that in the considered biotope animals eat well and do not suffer from a lack of resources.

Detected contamination has a moderate effect on the population of the common toads. Changes in the parenchyma of the liver are obvious, but in most animals, it is precisely the compensatory reactions. This degree of anthropogenic pollution does not prevent the survival of most animals and the biological prosperity of the population. In fact, information about the faunistic survey of NPP "Holosiivskyi" confirms the safe levels of pollution and a good forage base. Common toads form a fairly stable breeding population here. Spawning places are situated along the entire length of the Holosiivskyi ponds. Other amphibians also spawn here, for example *Rana temporaria* Linnaeus, 1758, the marsh frog — *Pelophylax ridibundus* (Pallas, 1771) and others (Nekrasova, 2007, 2017). At the same time, in recent years, an increase in the occurrence of external morphological anomalies has been registered in the population (up to 19 % of individuals had different variations of morphological deviations) (Marushchak, Muravynets, 2018). However, as noted above, when there are no predators and there is an abundance of food, the presence of morphological abnormalities and liver diseases does not preclude the survival of the population. The number of common toads remains high here. During the breeding season in spring, accumulations of these animals are observed yearly and the absolute number of animals at the spawning site was up to 600 individuals in some years (2016).

Conclusions

The list of histological changes in the liver of the common toads under conditions of anthropogenically contaminated biotopes does not differ from those of the green frogs. Therefore, proposals on the use of green frogs as indicators of anthropogenic pollution are quite applicable to the common toads. Our data show that these animals are quite sensitive to toxic pollution in anthropogenically modified biotopes. Anthropogenic pollution can be an important factor reducing the number and range of this species that is not clearly seen so far.

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