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LIVER PIGMENT CELLS OF THE GREEN FROG (PELOPHYLAX ESCULENTUS COMPLEX) ARE INVOLVED IN HEMATOPOIESIS AND LIVER PARENCHYMA REPAIR.

Zoology

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ABSTRACT

The article examines the factors influencing hematopoiesis in the liver of green frogs under conditions of anthropogenic pollution. For this, the experiment compared the indicators of animals from groups with maximum and minimum signs of the liver damage taken from a contaminated biotope. It has been shown that there are no differences between the groups in the indices of hematoposis, but there are significant differences in the content of pigments in the liver. Also, significant correlations were found between the amount of pigments and indicators of erythropoiesis and myelopoiesis. It was concluded that the reserves of pigments in the liver of frogs are involved in compensatory reactions.

KEYWORDS

amphibians, liver, pigment, hematopoiesis

INTRODUCTION

The presence of cells that contain pigment is a characteristic feature of the liver of poikilothermic vertebrates (Fenoglio e.a., 2005, Akulenko, 2020). Despite numerous studies, their functions in normal and pathological conditions are not entirely clear. However, it can be assumed that liver pigments are involved in compensatory reactions to environmental pollution (Loumbordis, Vogiatzis, 2002, Fenoglio e.a., 2005, Akulenko, 2016, Jayawardena e.a., 2017, Franco-Belussi, 2020)

We have described typical signs of pathological and compensatory changes in the liver of tailless amphibians in anthropogenically polluted landscapes (Akulenko, 2015, 2019). In addition to the pathological changes described in mammals, extensive zones of hepatocyte regeneration after necrosis were found in the liver of amphibians (Akulenko, 2015, Akulenko e.a., 2019). Since the liver of amphibians is involved in the processes of hematopoiesis (Akulenko, 2012), chemical pollution of biotopes is one of the factors that can also affect erythro- and myelopoiesis.

Materials And Methods

The object of the study was sexually mature green frogs (Pelophylax esculentus complex) from the agrocenosis (29 males and females, weight 12-50 g, body length 6-12 cm). The animals were sacrificed using ether anesthesia. Histological preparations of the liver, as well as smears-imprints of the liver, were made according to the technique we modified (Akulenko, 2020). Liver myelograms were counted on smears-prints. Then, integral indicators were calculated for each animal. The indicator "immature erythroid cells" was calculated as the sum of erythroblasts, basophilic and polychromic normoblasts. The indicator "immature myeloid cells" was defined as the sum of myeloblasts, myelocytes and metamyelocytes of eosinophilic and heterophilic lines. The indicator "lymphoid cells, sum" was calculated as the sum of lymphocytes, plasma cells and plasmablasts. The indicator "blasts, sum" was defined as the sum of erythroblasts, myeloblasts, lymphoblasts and undifferentiated blasts of an unexplained nature.

To determine the indicator "The number of pigment inclusions in the liver" in each animal on histological sections using an eyepiece grid in 35 fields of view, the percentage of the area occupied by pigment cells was calculated and the average value for the group was calculated.

Correlation coefficients were determined for the total sample (29 frogs) (see table). Based on the histological study of liver sections, two samples of green frogs were isolated from the total number: one in which the manifestations of protein dystrophy of hepatocytes were minimal (10 specimens), and the second, with strongly pronounced protein and hydropic dystrophy, passing into zones of necrosis (9 specimens). For each selected sample, using the Student's test, the mean values of the indicators, the error of the mean, reliability of differences between the samples with the minimum and maximum lesions of the liver parenchyma were calculated.

Results And Discussion.

The quantitative analysis of leukogram and myelogram data in poikilothermic tetrapods is complicated by the fact that the concept of norm and pathology in them is very vague (Sykes, Klaphake, 2008). Animals in natural biotopes are affected by many factors, such as dietary habits, the presence of infections or invasions, and the temperature of the environment. In natural populations, mature individuals of green frogs, after spawning, suffer to some extent from exhaustion. This affects the intensity of hematopoiesis and the number of pigment inclusions (Akulenko, 2016).

To consider the effect of toxic damage to the liver on its other functions, regardless of external factors, it is necessary to compare animals that are in the same conditions. The best way out was to compare samples of animals taken from the same population (Table).

Table. Indicators of liver myelograms in samples of green frogs, grouped depending on the degree of damage to the liver parenchyma (in%)

indicators	Weak lesions of the parenchyma		Strong lesions of the parenchyma		Significance of differences	Correlation with the indicator "area of pigment inclusions"
	M	m	M	m		
Erythroid cells immature, sum	22	4,6	26	5,7		-0,6 p<0,01
Myeloid cells, sum	18	1,2	15	2,3		
Immature myeloid cells, sum	4,6	0,7	4,9	0,6		0,5 p<0,01
Lymphoid cells, sum	37	4,5	32	3,2		
basophils	3,6	0,9	3,5	1,2		0,5 p<0,01
Immature macrophages	2,1	0,5	3,6	1,3		
Active macrophages	13	3,1	15	2,9		
Blasts, sum	7,9	1,1	8,5	1,5		- 0,5 p<0,01
Mitotic index	0,8	0,5	0,5	0,2		
Area of pigment inclusions	3,83	1,15	0,85	0,16	p<0,01	

To analyze the effect of changes in the liver on hematopoiesis, in order to simplify the comparison of the two samples, we considered integral indicators such as "immature erythroid cells, sum", "immature myeloid cells, sum".

Analysis of the data given in the table shows that there are practically no differences in the myelograms of the liver of green frogs between samples with strong and weak lesions of the parenchyma. The only reliable, pronounced difference lies in the amount of pigment inclusions (Table, Fig. 1, 2). It was preliminarily assumed that in the liver of green frogs, pigment cells are involved in the processes of repairing damaged liver areas and a significant amount of pigments is consumed for this (Akulenko, 2016). From this point of view, it is quite understandable why in animals with extensive foci of necrosis the amount of pigment inclusions in the liver is very small (Fig. 1). The data in the table show that the presence of pigment reserves in the liver of amphibians partly smoothes out the negative effect of necrosis foci on hematopoiesis.

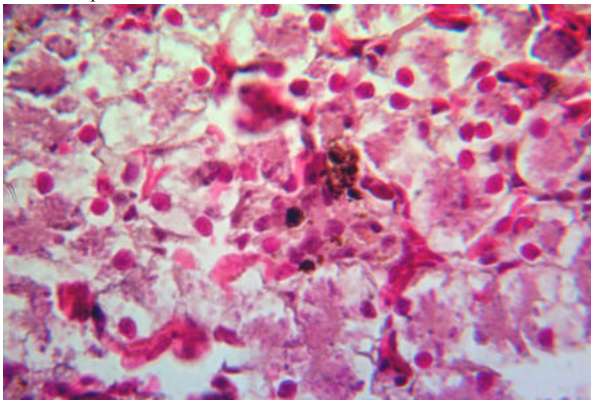

Fig. 1 Liver Of A Green Frog With Pronounced Manifestations Of Protein Dystrophy And Necrosis. A Devastated Melanomacrophage Cluster With Remnants Of Pigment Can Be Seen.

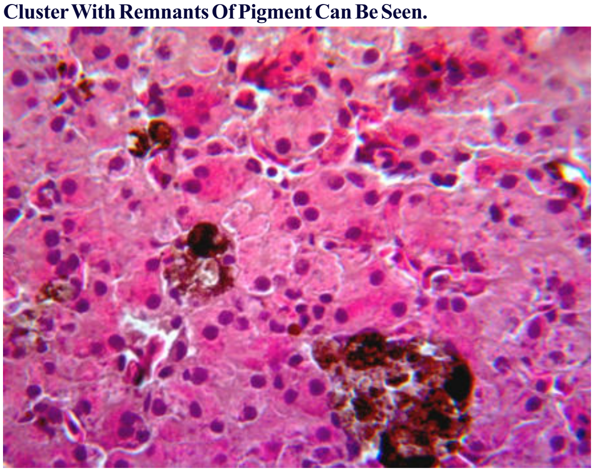

Fig. 2 Liver Of A Green Frog With Minimal Histological Changes. Melanomacrophage Clusters With Pigment Cells Are Visible.

The veracity of this statement is confirmed by an analysis of the correlation coefficients between indicators reflecting the activity of hematopoietic processes and the number of pigment inclusions. The indicators of erythrocyte differentiation in this experiment (Table) and in other samples of green frogs from contaminated biocenoses (Akulenko, 2010) are associated with the amount of pigments in the liver by a negative correlation. The correlation coefficient is reliable in all cases and has values from -0.6 to -0.9, depending on the degree of heterogeneity of the sample. Obviously, in frogs, the synthesis of pigments in the liver and erythropoiesis are competing processes, since they use gem for the synthesis of hemosiderin (in pigment cells) or hemoglobin (in erythrocytes). Indicators of differentiation of granulocytes are associated with the amount of pigments by a significant positive correlation, although the values of the coefficients are usually somewhat lower (Table). The difference is that erythroid cells mature intravascularly and assimilate plastic resources directly from blood plasma. Thus, they directly compete with pigment cells for substances dissolved in the blood. At the same time, maturing granulocytes come into contact with pigment cells as part of melanomacrophage clusters and can use their contents as a trophic resource.

There were no significant differences in the number of macrophages and their activity in two samples. However, it should be noted that in the myeloharmma of the liver of frogs from ecologically clean biotopes, the indicator "active macrophages" is about 5% (Akulenko, 2012), while in samples from the agrocenosis it is 13-15%. Obviously, in green frogs, even insignificant liver lesions cause a significant increase in the differentiation and functional activity of macrophages.

The myelograms of green frogs from other anthropogenically transformed biotopes show signs of stimulation of erythropoiesis during liver regeneration (Akulenko, 2010). This observation can be explained by the non-specific effect of hepatocyte growth factors on erythropoiesis, which was shown by Koibuchi e.a., (2004). Thus, the processes of regeneration of the liver parenchyma are accompanied by the stimulation of local erythropoiesis as a side effect. However, in this sample, which was taken from the agrocenosis, there are no signs of mass regeneration of hepatocytes (Akulenko, 2015).

Conclusions

In green frogs, the effect of hepatocyte necrosis on hematopoietic processes is greatly mitigated by the presence of pigment inclusions in the liver. In moderate liver damage, the primary reaction is to reduce the reserves of pigments, which are consumed in the process of repairing damage.

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