FREE RADICALS AND THEIR ACTION IN THE MIDDLE INTESTINE STRUCTURES DURING THE METAMORPHOSIS OF *RANA TEMPORARIA TEMPORARIA* (LINNAEUS, 1758)

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Abstract: The action of free radicals in the middle intestine structures during the metamorphosis of anura amphibians represents a hint to some profound structural and ultrastructural reshuffling. By following the values of the free radicals one can observe that between the XX and XXII stages there is an explosion at the level of free radicals, which corresponds to the destruction of the larval epithelium at structural level.
The oxidative explosion takes place with the contribution of macrophages, whose number in the chorion of the middle intestine grows during this period of time.

INTRODUCTION

The middle intestine wall of Rana temporaria temporaria in adults is formed of mucous, muscles, and serum. The epithelium is simple cilia type with cubical cells in the premetamorphical stages, and with columnar cells in the prometamorphical stages. At the epithelium base there are basal cells whose divisions will form the juvenile epithelium, after the larval epithelium is destroyed during the metamorphic climax.

The processes of cellular lysis in the digestive tract structures during the metamorphosis of the anura amphibians contribute to replacing the larval structures with the juvenile ones.

Free radicals play an important role in the structural reshuffle. They are involved in the redox reactions or in other intermediary reactions, in the rapid structure change of some substances that change their metabolic way or that become more water-soluble. The role of free radicals in the processes of destroying the cellular structures is well known, but there are no references to their action in the metamorphosis process of anura amphibians.

MATERIAL AND METHODS

The analyzed material includes spawns of *Rana temporaria temporaria* collected in spring and hatched in the laboratory. The larval development stages were established according to Taylor and Kollros' table for *Rana pipiens*.

The level of free radicals was evaluated by dosing the reduced gluthathione (GSH), the dismutase superoxide (SOD), the SH-, SH protein, and SH non-protein groups, and the malonic dialdehyde (MDA).

For optical microscopy the specimens where fixed in 10% formic aldehide, and for electronic microscopy in 1% osmium tetraoxide 2% glutaric aldehide. The tissue where stained with hemalaun eosina and counterstained with uranil acetate.

RESULTS AND DISCUSSIONS

In order to compare the action of free radicals and the self-defense capacity of the anura amphibians body during the processes of structural reshuffle of the metamorphosis, we dosed and compared the values of SOD, GSH, the total SH groups, protein and non-protein SH, and DAM, in the structures of the middle intestine (Table 1).

Table 1. The Gr SHt, GSH, SHnp, SHp, DAM and SOD values, in Rana temporaria metamorphosis.

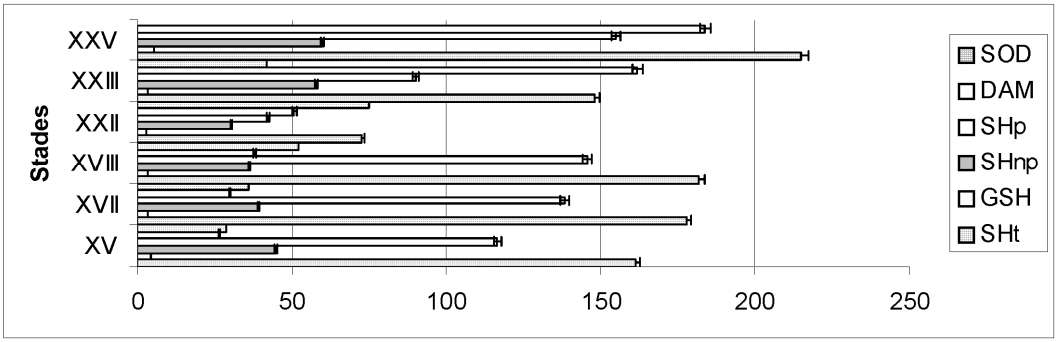
Stades	SHt	GSH	SHnp	SHp	MDA	SOD
XV	$161{,}25x10^{-6}$±0,10	$4{,}154x10^{-2}$±0,20	$44{,}65x10^{-6}$±0,51	$116{,}60x10^{-6}$±0,10	2,7±0,23	28,95±0,25
XVII	$177{,}78x10^{-6}$±0,21	$3{,}65x10^{-2}$ ±0,30	$39{,}28x10^{-6}$±0,32	$138{,}50x10^{-6}$±0,10	6,7±0,24	35,85±0,21
XVIII	$181{,}92x10^{-6}$±0,12	$3{,}38x10^{-2}$ ±0,21	$36{,}38x10^{-6}$±0,30	$145{,}54x10^{-6}$±0,12	9,6±0,22	52,09±0,14
XXII	$72{,}76x10^{-6}$	$2{,}83x10^{-2}$	$30{,}43x10^{-}$	$42{,}33x10^{-6}$	16,6±0,11	74,88±0,15

Stades	SHt	GSH	SHnp	SHp	MDA	SOD
	±0,31	±0,20	^{6}±0,21	±0,10		
XXIII	148,10x10^{-6}±0,20	3,58x10^{-2} ±0,32	58,10x10^{-6}±0,50	90,00x10^{-6} ±0,22	15,8±0,11	42,03±0,26
XXV	215,03x10^{-6}±0,20	5,58x10^{-2} ±0,23	59,95x10^{-6}±0,40	155,08x10^{-6}±0,14	16,00±0,10	0,02±0,23

SHtx10^{-6}γ/g.t.u., GSHx10^{-2}γ/g.t.u., SHnpx10^{-6}γ/g.t.u., SHpx10^{-6}γ/g.t.u., MDA µmol/g.t.u. and SOD u/g.t.u.
N=10

Observing the SOD values, one can notice that they form a curve opposite to the GSH one, with minimal values in the XV and XXV stages, and maximal values in the XXII stage, which expresses an oxidative explosion (Graphic1).

Graphic 1. The Gr SHt, GSH, SHnp, SHp, DAM and SOD values, in *Rana temporaria* metamorphosis



The increasing SOD values are due as well to the migration towards the apoptotic cells of the larval epithelium of the macrophages, eosinophiles and neutrophiles of the lamina propria of the middle intestine's, which will produce the epithelium heterolysis. The non-phagocyted elements and the macrophages full of phagocyted material are released in the intestine lumen.

Before the metamorphic climax, in the lamina propria there can be seen numerous capillaries and extravasate sanguine elements. The number of extravasate sanguine elements is larger in lamina propria and smaller in the larval epithelium. In the incipient stages of the climax, the number of macrophages and eosinophiles grows among the larval apoptotic cells. The presence of cellular remains and degraded lipids induces the increase of the number of macrophages (Fig.1).

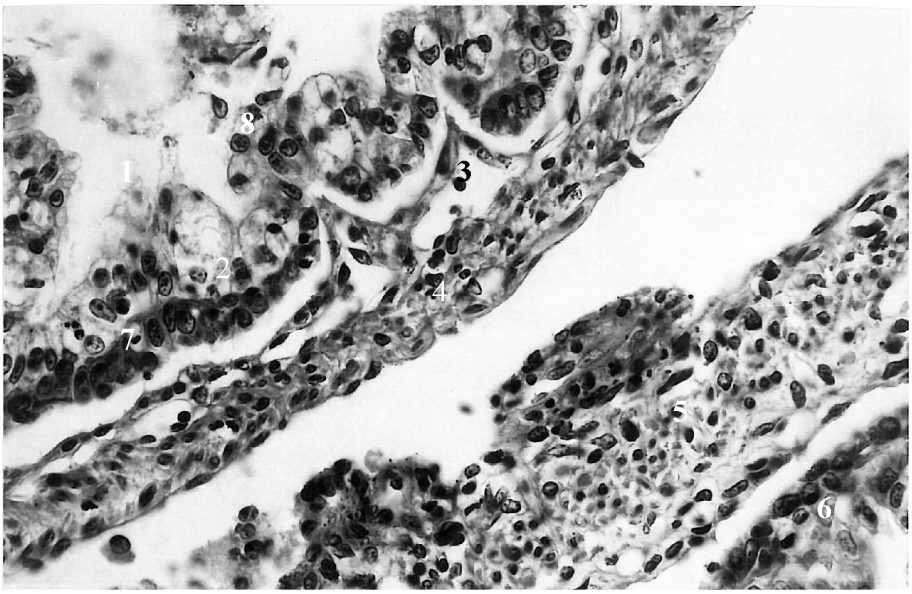


Fig.1 Middle intestine, cross section, Rana temporaria temporaria, stade XXII, 1.larvar epithelium,2. juvenile epithelium, 3.blood capillaries, 4.connective tissue, 5.muscularis mucosae, 6.serosa, 7.macrophage, 8.leucocite, hemalaun eosine, o.m.10x40.

The GSH values form a curve with maximal values at the beginning of the prometamorphosis and at the end of the climax and with minimal values in the middle stage. This proves that the capacity of resistance of the larval epithelium to the action of free radicals decreases, which corresponds to the processes of cellular lysis.

The apoptotic cells of the middle intestine epithelium have a clear aspect, with a slightly dense nucleus. In the cytoplasm there are free micronuclei, they are frequent in apoptotic bodies (Fig.2).

The minimal value of the GSH values is due to the almost complete destruction of the larval epithelium cells. The GSH decreasing values are due to the development of the juvenile epithelium between the XXIII and XXV stages, which develops its own defense capacity against the action of free radicals.

The SHt groups emphasize a slight increase of the values up to the XVIII stage due to the synthesis of protein substances in the larval epithelium cells, followed by a considerable decrease of values until the XXII stage, due to the cellular lysis phenomena. Between the XXII and XXV stages there is an increase of the values of SHt groups, as the cells in the nests increase the division rhythm and will form the juvenile epithelium.

There is no difference of value between the two groups Sh p and Sh np, depending on the considered stage. Until the XXII stage the SH np groups have decreasing values and the SHp groups have increasing values. Between the XXII and XXV stages, both groups have increasing values.

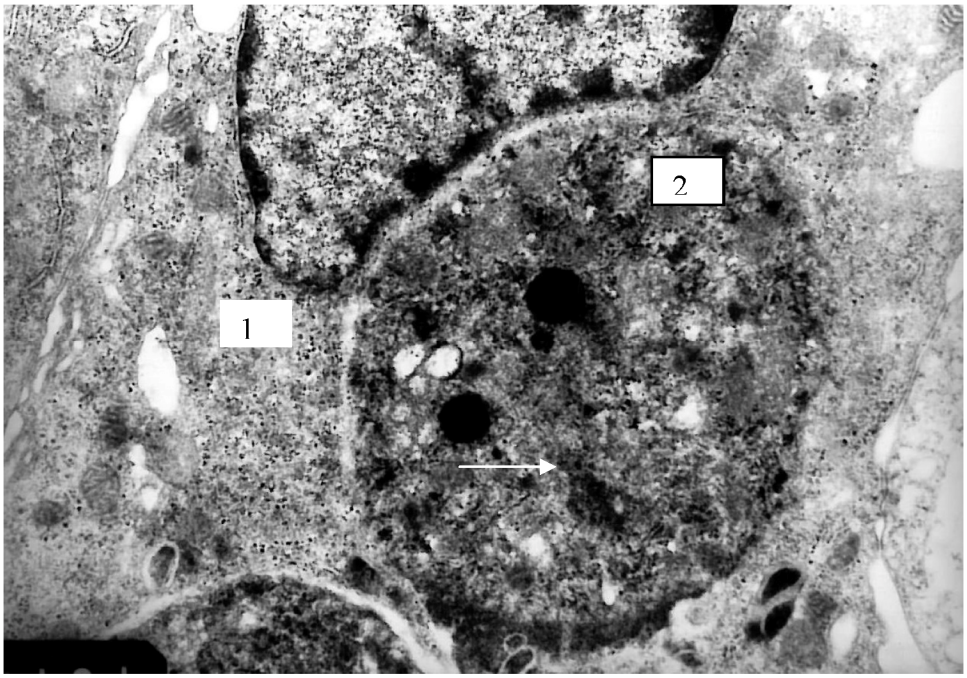


Fig.2 Middle intestine, Rana temporaria temporaria, stade XXII, arrow-micronucleu, 1.apoptotic cell, 2. apoptotic body, e.m.x20000.

The MDA values increase until the XXII stage, after which there is a slight decrease.

The values increase indicates processes of peroxidation of the lipids and degradation of cellular elements. The decrease of the analyzed values after the XXII stage is due to the release of the apoptotic remains in the digestive tract and then to the exterior.

CONCLUSIONS

The action of free radicals in the middle intestine structures during the metamorphosis of anura amphibians represents a sign of some deep structural and ultrastructural reshuffles. The values of free radicals increase until the climax, which is represented by the destruction of larval structures in the middle intestine, especially of the epithelium. The reconstruction of the intestinal epithelium in the postclimax stages leads to the re-establishment of the tissue defense capacity and the decrease of the values of free radicals.

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