The Impact of the Medium-Wave UV Radiation on the State of Embryonic Membrane of Amphibian

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Abstract. The relation between the destruction of tertiary egg membrane of an amphibian embryo irradiated during the neurula stage and the dose of radiation was revealed. In order to determine the degree of membrane destruction methylene blue was used which made it possible to evaluate its structural and functional integrity level depending on the dye penetration rate. The longer the exposure time was, the more considerable the destruction of tertiary egg membrane turned out to be: it was gradually losing its shape, structure, and was getting more permeable to dye. These changes provoked the release of the amphibian embryos that started to lose their suspension properties and fell on a Petri plate, yet some of them being of a great vitality.

1. Introduction
The biodiversity conservation is regarded as one of the most urgent problems of modern times. Different points of view (e.g. UV radiation, injuries, parasitic contamination, anthropogenetic impact, and in particular, pharmaceutical preparations, agricultural runoffs containing herbicides, pesticides, metals, and other chemically active substances) are put forward in order to explain the environmental unsustainability and the amphibian population decline.[1,2]

In the case of UV radiation, the influence of both direct (causing different pathologies in amphibian embryos and their death) and indirect (resulting in greater sensitivity of embryos to various adverse environmental conditions) sun rays is explained. [3] Earlier in some of our works, it has already been shown that along with their species sensitivity to the medium-wave UV rays the amphibians are also characterized of having the individual sensitivity to this very factor. [4, 5]. To determine the reasons of individual sensitivity of embryos to UV radiation the research on the analysis of the degree of sustainability of embryonic membrane was initiated. It was shown that when amphibian embryos were directly exposed to the medium-wave UV rays for 90 seconds their tertiary membrane started to deteriorate.

The aim of the study: to identify the level of destruction of the tertiary membrane (slime) of amphibians being exposed to the medium-wave UV radiation of varying intensity.

2. Material and methods
The research was performed on the embryos of Rana macrocnemis Boulenger and Rana ridibunda Pallas. The embryological material was gathered in the areas where they were the most widespread in the territory of the Republic of North Ossetia-Alania. During the research 3 clusters of eggs were used and 4 experiments were carried out on each of them. The embryos were irradiated with the help of
DRT lamp 240-1C, wave length 280-320 nm. The intensity of exposure was identified by its duration (180, 240, 360 and 420 seconds).

In order to visualize the degree and dynamics of membrane destruction the methylene blue was used. The exposure of embryos was carried out in the following way: the clusters of eggs were grouped depending on the species isolated from each other, then the stage of development was determined and only the embryos at the late neurula stage were selected (28 phase, 50 embryos on every Petri plate). As in previous experiments, the embryos were placed in the dechlorinated mains water. The overall quantity of water used was 30 ml [6-13]. 0,1% methylene blue solution was added into Petri plates with irradiated embryos on them. Every experimental series consisted of 4 experiments of different duration times (90,180,240 and 360 seconds); the embryos from only one cluster were used. The embryos were under observation for 6-7 days before the commencement of histogen- and organogenesis periods. The methylene blue was also added into the control group of unirradiated embryos (neurula stage). The method of photography (with the help of digital cameras SAMSUNG and Nikon COOLPIX L12) was applied to record the changes observed.

3. Results and discussion
In the control group of unirradiated embryos on the first day of observation the germs and their membrane remained achromatic, their spherical form and the embryos inside of them could be visualized clearly (Figure 1).

![Image 1](image1.png)

Figure 1. The state of embryonic membrane of *Rana macrocnemis Boulenger* in the conditions of methylene blue coloring.

During the next several days of embryonic development of amphibians the membrane was gradually getting colored and reached its maximum intensity on the 6th day. The solution itself on a Petri plate went paler and eventually turned pale blue. The embryos during the whole observation were progressively released from the embryonic membrane and on the 7th day the hatching took place; the embryonic membranes retained their initial state, form, and remained in close contact with each other.

In the experimental (irradiated) groups of embryos the degree of embryonic membrane destruction was evaluated per unit UV exposure (Table 1).

| №  | Object of the research     | Duration of UV exposure in sec. |
|----|----------------------------|---------------------------------|
|    |                            | 90    | 180   | 240   | 360   | 420   |
| 1  | Rana macrocnemis Boulenger | -     | 18%   | 38%   | 82%   | 96%   |
| 2  | Rana ridibunda Pallas      | 96%   | 96%   | 100%  | 100%  | 100%  |

N.B. The table shows the percentage of amphibian embryos with damaged tertiary membrane
The comparative analysis of the number of damaged tertiary membranes and embryos without any membranes at all demonstrates the increase in the number of damaged embryonic membranes of amphibians depending on the level of UV exposure. Thus, if irradiated for 180 sec. the number of damaged membranes and therefore membraneless embryos was 18%, then when being irradiated for 360 – 420 sec. a large part of embryos turned out to be membraneless (82 and 96%). This means that the exposure of higher intensity caused the greater degree of slime destruction, practically all the membranes lost their initial spherical forms, the links between them were destroyed, and they have actively been losing their structures (Figure 2).

A - Rana macrocnemis Boulenger

| Duration of UV radiation (180’s) | Duration of UV radiation (240’s) | Duration of UV radiation (360’s) | Duration of UV radiation (420’s) |
|---------------------------------|----------------------------------|----------------------------------|----------------------------------|

B - Rana ridibunda Pallas

| Control | Duration of UV radiation (180’s) |
|---------|----------------------------------|

**Figure 2.** The embryonic membrane state in the conditions of methylene blue coloring (experimental groups). A - Rana macrocnemis Boulenger, B - Rana ridibunda Pallas. In the given photos one can observe the increase in the degree of membranes destructions and their number connected with an increased dose of exposure.

It should be noted that the embryos with membrane completely destroyed died right away or during the following 2-3 days [5]. When exposed to UV radiation of lower intensity the survival rates of species was considerably higher and amounted to % (). The patterns described were typical of Rana macrocnemis Boulenger. As for the Rana ridibunda Pallas, they were of a higher sensitivity to UV exposure. For that reason, the starting dose of radiation in this experimental series was 90 seconds. Figure 2 presents the embryos of Rana ridibunda Pallas in the control and irradiated groups. In the control group the integrity of tertiary membrane is saved throughout the entire experiment. Whereas being exposed to UV radiation for 90 seconds it gets completely destroyed.

Summing up the data obtained, the following particularities of state dynamics of slime and amphibian embryos in the conditions of UV radiation of different intensity must be emphasized. The longer the exposure time was, the more considerable the destruction of tertiary egg membrane turned out to be: it was gradually losing its shape, structure, and was getting more permeable to dye. These changes provoked the release of the amphibian embryos that started to lose their suspension properties and fell on a Petri plate, some of them being of a great vitality.
4. Conclusion
The existing to date information on the embryo membranes allows us to treat them as multifunctional barriers possessing numerous protective, shape forming, bactericidal, optical, and other properties. Membrane destruction of that kind results in unbalancing of certain structural and functional properties of embryo and has a great influence on its further development. On the other hand, the fact of embryos (with damaged membranes) survival in the conditions of UV radiation of low intensity is of a great interest. It goes without saying, that the changes were observed in the laboratory conditions far from any external factors. However, the fact of the embryos survival without any membrane at all dictates the need of more detailed consideration of the tertiary membrane’s role in the regulation of processes of development of amphibian embryos. It has already been discussed that the destruction of secondary membrane can lead to death or destructive changes in the structure. On the other hand, during the special experimental series of similar conditions of observation the death of amphibian embryos without membranes was shown.

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