

2023 年臺灣國際科學展覽會 優勝作品專輯

作品編號	050019
參展科別	動物學
作品名稱	Study of regenerative and ontogenetic processes under the influence of EHF EMR.
得獎獎項	
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關鍵詞	<u>regeneration、 development、 electromagnetic</u>

作者照片







Abstract. The increased sensitivity of aquatic organisms to the effects of EMF has been proven by numerous experimental studies. It has been repeatedly noted that exposure to EMF of certain frequencies and intensities leads to disruption of physiological functions, orientation in time and space, changes in the behavior of organisms, suppression of motor activity. Other ranges of electromagnetic radiation, on the contrary, can cause the effects of increased regeneration, growth rate and survival. In connection with these trends, the purpose of our research is to analyze the effects of the influence of electromagnetic radiation of extremely high frequency on the development of the *Xenopus laevis* and the regeneration of newts and planarians.

Objective: to study the impact of electromagnetic environmental factors on model objects and processes by biotesting with the possibility of subsequent application of our results and developments in biotechnology, medicine and ecology.

Tasks:

To study the effect of millimeter waves on the course of the stages of embryonic development of amphibians as a biotest.

To study the regeneration processes of planaria under the influence of electromagnetic factors.

To study the processes of amphibian limb regeneration, using the example of the Urodela group, under the influence of electromagnetic factors.

Methods

Electromagnetic radiation is an abiogenic factor. There are several ranges of the electromagnetic spectrum - gamma, X-ray, UV, visible light, IR, HF, radio. The EHF band was chosen for the study, since millimeter waves have intensively advanced into medicine and have taken firm positions there as a therapeutic agent, in ecology - a new environmental factor, and in biotechnology - a new tool of work.

EHF Impact :

For therapeutic effects in EHF therapy, electromagnetic radiation with an intensity of less than $10 \text{ MW} / \text{cm}^2$ with a frequency from 30 to 300 GHz (length waves from 1 to 10 mm). Electromagnetic waves of EHF have an extremely low penetrating power. Heating of tissues by EHF radiation is insignificant, it does not have a thermal effect on the body.

We also used the method of computer morphometry to measure the area of blastemes in planarians. We used the TopView program to create snapshots and the ImageJ program for measurements.

The main part

The reaction of frogs was studied at the early stages of development, since they are the most sensitive to the modifying effect of factors, which makes it possible to trace the presence or absence of the studied dependencies. Before starting the experiment, we injected *Xenopus laevis* with Gonadotropin, a hormone that stimulates the maturation of oocytes. The irradiation was carried out remotely, the distance between the water surface and the radiating horn did not exceed 7 mm. The water level in the experimental and control tanks was 5 mm. At stage 2-4 of the blastomer, we divided the eggs of the *Xenopus laevis* into two groups. Some were irradiated with EHF, and the others were left in standard conditions (control group). The irradiation time for *Xenopus laevis* caviar was ten minutes. We placed the caviar in a photometric vial with 10 ml of water and lucigenin. Then they put the vial in a photoelectronic multiplier and irradiated it. Schedules were drawn up for both groups. The EHF received a graph with a peak at the early gastrula. (Fig. 1)

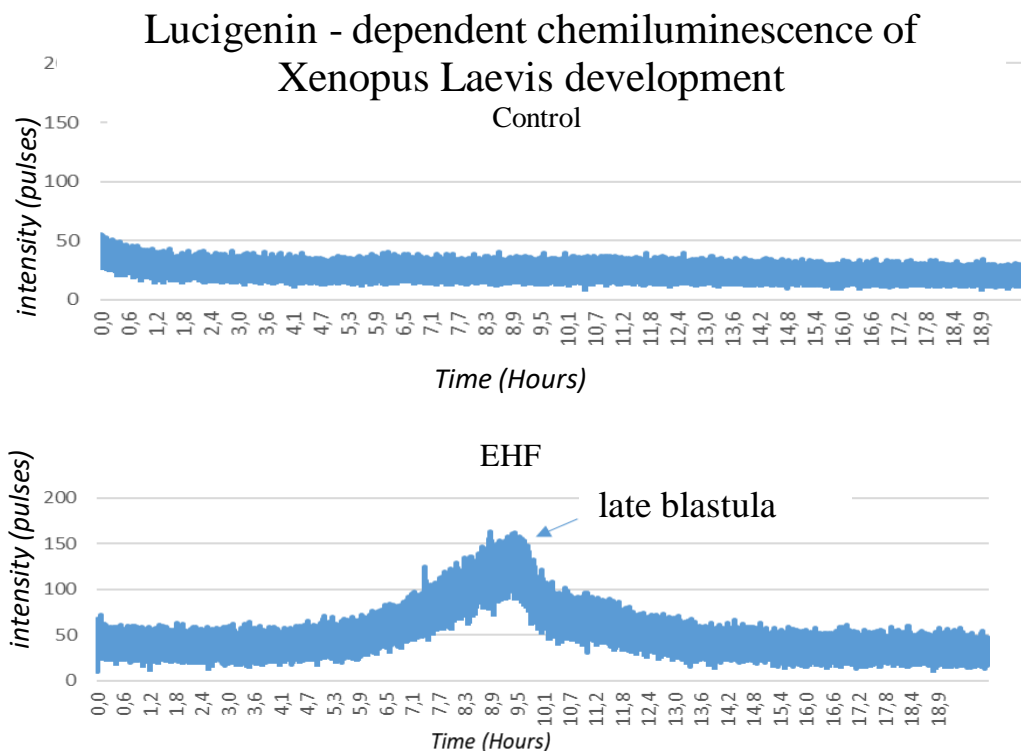


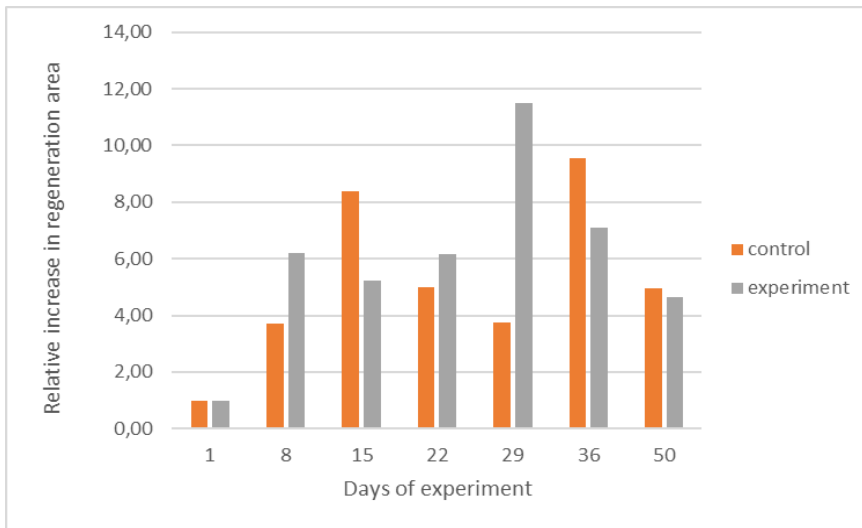
Figure 1

This means that there was a violation of the cell cycle and the eggs began to divide asynchronously. When comparing the mortality rate in the 2 groups, no major differences were observed.

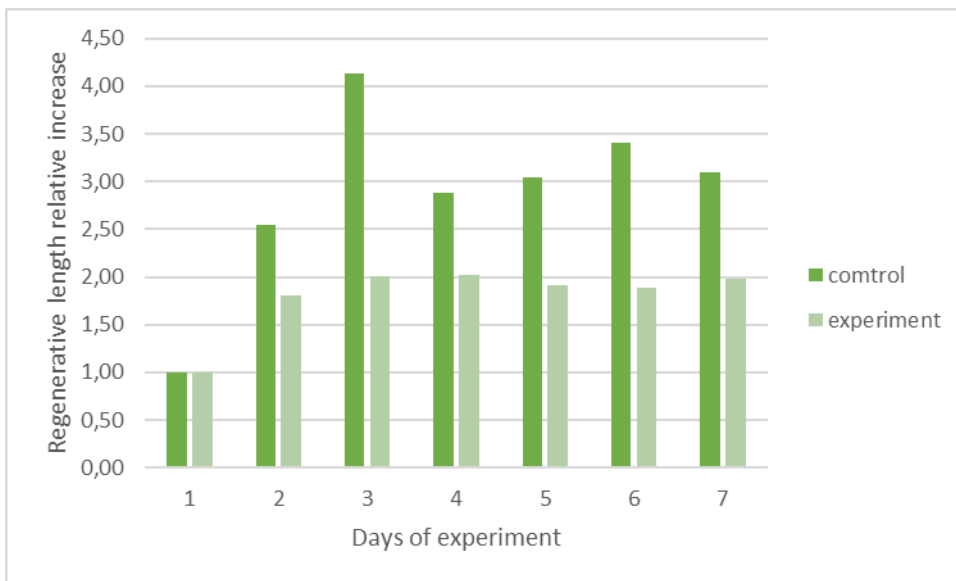
People have repeatedly been interested in the nature and mechanisms of such a process as regeneration. Since about the 1930s, this topic has become particularly important. The appeal to the models of repeated regeneration was due to the relevance of information for establishing the mechanisms of development and restoration of organs and tissues in humans. Amphibians of the Urodela group were used as the first model objects. Later experiments were carried out on invertebrate flatworms – planarians. thanks to their unique ability to restore lost organs. Based on the data of the article by E. N. Grigoryan, Yu. V. Markitantova, P. P. Avdonina, E. A. Radugina "RESEARCH of regeneration in amphibians in the era of molecular genetic approaches and methods", we learned that as a result of numerous experiments it was found that the process of limb regeneration in newts is due to the secondary activation of the embryonic development program. There is a dedifferentiation of cells of damaged tissue, the formation and accumulation of blastema (regenerate), the effect of expression factors on it, which as a result leads to the restoration of lost tissues.

We decided to conduct an experiment on the effect of extremely high-frequency radiation (EHF) on the intensity of the regeneration process. We took 6 model objects – newts *Pleurodeles waltl*. We divided them into control and experimental groups of 3 animals each. The amphibians' right forelimbs were amputated. The animals were placed in containers with an aqueous medium. Once a week we observed the surfaces of amputated limbs and took measurements of the regenerate. On the first day, it was noticed that Control 2, 3 and Experiment 6 had no regenerate. We took water samples from the containers of these samples and by light microscopy found infusoria in them. In this regard, the containers of all model objects were treated with boiling water. On the third week, flowering began in containers 4 and 5, green euglena was found on the walls. In animals 4 and 5, the value of the regenerate gain did not change. On the seventh week, experimental sample 4 died. As a result, data were obtained on the value of the area and length of the regenerate throughout the experiment. They were designed in the form of bar charts.

As a result of the experiment, we found out that under the action of EHF, the maximum value of the blastema area in the experimental group is shifted to a later time (day 29), while in control samples, the largest value of the regenerate area increases twice on days 15 and 36. There is a change in the length of the regenerate in the control and experimental groups: the control is characterized by a prolonged fluctuation of the length value, for the experiment – its relative constancy. We assume that these changes are associated with an increase in the concentration of ROS occurring in connection with the action of EHF.



Regenerative area change values



Regenerative length change values

According to our results, the formation of limbs and fingers in newts was faster in the control group.

We also investigated the effect of EHF on the regeneration of planaria. Planaria belong to the type of Plathelminthes, a class of Turbellaria. The special significance of planariums is determined by their ability to regenerate the whole organism from the smallest fragments of the body and the relatively simple structural and functional organization. (fig.2)

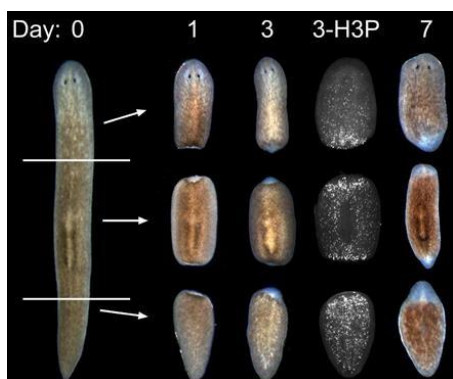


Figure 2

Traumatic regeneration of planarians is started in the experiment by cutting the body into two (as in our case) or more fragments and is accompanied by wound healing, and then the growth of regenerative blastema, from which amputated tissues and organs are restored. In about 14 days, the front part grows out of the back, and the back part grows out of the front. Neoblasts are the only proliferating cells in the planarian body. They are totipotent, that is, they can differentiate into all types of cells, and regeneration occurs due to them.

During the experiment, we cut the planarium into two parts and divided it into two groups, one was a control and was in standard conditions, and the other was irradiated with EHF. According to the logic of our biotesting, regeneration is a process that can be influenced by external environmental factors of a biogenic and abiogenic nature. Then both groups were placed in a chemiluminometer. Chemiluminescence of biological objects is usually the emission of light from electronically excited states (EMU) arising during chemical reactions of free radical oxidation. We have AFCS in such a state. By the method of lucigenin-dependent chemiluminescence, we obtained data on the dynamics of changes in the number of pulses over time. (fig. 3)

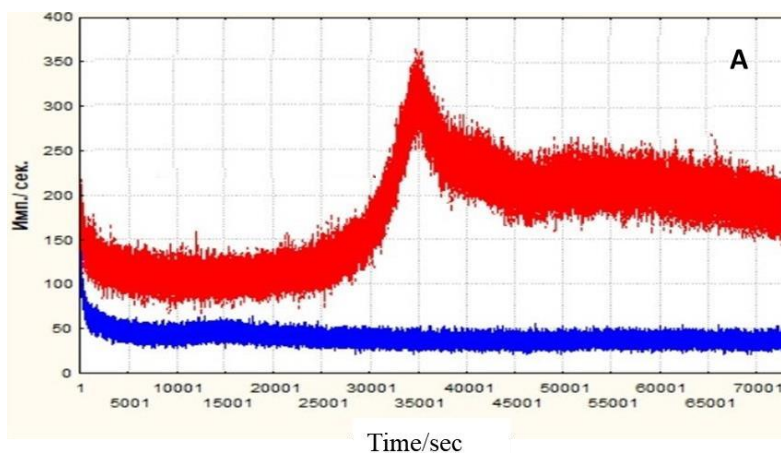


Figure 3

We interpret them as a concentration of ROS. On the graph of irradiated planaria, an increase in pulses is noticeable, that is, an increase in the concentration of ROS.

After analyzing the graph that shows the cellular processes of planarians during regeneration and which we found in articles on the study of these processes, we can assume that this peak is caused by an increased number of mitotic divisions in the cell. That is, perhaps, EHF radiation accelerates the number of cell divisions. This fact can be used in ecology and medicine. On its basis, it is possible to improve production by increasing the volume of products where it is conditioned by any biological objects. By studying the quality of this influence and which cells it spreads to, it is possible either to prevent the spread of oncological diseases, slow down their development, or, conversely, dispel myths about their dependence on this type of exposure.

Results

EHF radiation causes a violation of the cell cycle and the zygote begins to divide asynchronously, but its effect does not cause mortality.

EHF radiation effect slows down regeneration processes.

This type of radiation increases the concentration of ROS in cells.

Conclusions

As a result of a review of literary sources and conducting experimental work, we found that the action of EMP EHF:

The action of EHF EMR leads to an increase in the release of reactive oxygen species during regeneration and embryonic development.

【評語】 050019

1. In order to explore the influence of extremely high-frequency electromagnetic waves on aquatic organisms , this study uses *Xenopus laevis* , newts , and planarians as research materials to explore the effects of extremely high-frequency electromagnetic waves on regeneration and development. The aim of the project is to study the impact of electromagnetic environmental factors on model objects and processes by biotesting with the possibility of subsequent application of our results and developments in biotechnology , medicine and ecology. The study is interesting and important. However the experimental design is too simple to draw the conclusion.
2. Regarding the results of *Xenopus laevis* , it is stated in a description way that it will lead to cell cycle disorder , resulting in the asynchronous division of fertilized eggs , but will not lead to death. However , there is no relevant graphic data in the report to support it , which is relatively insufficient.

The newts experiment is supported by relevant chart data ,

but there is no statistically significant support between the experimental group and the control group.

3. The results of the planarian experiment are the same as those of the *Xenopus laevis* , but there are no relevant charts and data to support it in the report , which is relatively insufficient.