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INSTITUTE OF NUCLEAR SCIENCES "BORIS KIDRIČ"  
Laboratory of radiobiology

# AGROSTEMIN<sup>®</sup>

## RESEARCH OF BIOREGULATOR'S EFFECT IN MAMMALS

– *Resistance to radiation* –



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**INSTITUTE OF NUCLEAR SCIENCES "BORIS KIDRIČ"**  
**LABORATORY OF RADIOBIOLOGY**  
**Belgrade - Vinča**

**REALIZED PROJECTS**

**RESEARCH OF BIOSTIMULANT'S EFFECT IN ANIMALS**

In phase I of the programme, the following research is anticipated:

- a. Mitogenic effect of the biostimulant (Bioregulator –Br-) in the system of cells isolated from peripheral blood;
- b. Biostimulant's effect on regeneration and kinetics of haematopoiesis in rats

The research anticipated by the programme has been completed in full and the next stage according to the programme will continue.

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**RESEARCH OF BIOREGULATOR'S EFFECT IN MAMMALS**

The research took place according to the plan in two phases:

- a. Phase – assessment of Bioregulator's (Br) protective effect in sublethally irradiated animals and assessment of optimum time of Br application.
- b. Phase – testing possibilities of biological information transfer from Br treated animals to untreated ones.

The planned research has been completed in full and partially extended in order to give better explanation of the results obtained.

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INSTITUTE OF NUCLEAR SCIENCES "BORIS KIDRIČ"  
LABORATORY OF RADIOBIOLOGY  
Belgrade - Vinča

Report on realization of Project Phase I:

**RESEARCH OF BIOSTIMULANT'S EFFECT IN ANIMALS**

In phase I of the programme, the following research is anticipated:

- a) Mitogenic effect of biostimulant (Bioregulator –Br-) in the system of cells isolated from peripheral blood;
- b) Biostimulant's effect on regeneration and kinetics of haematopoiesis in rats

The research anticipated by the programme has been completed in full and the next phase will continue according to the programme.

*The research results were presented at XII Yugoslav symposium on protection from radiation held in Ohrid from May 31 to June 3 under the name: "Stimulating Effect of Bioregulator on Post-Irradiation Processes of Haematopoiesis". The full version of the paper was printed in the Proceedings of the Symposium, the copy of which is enclosed to this Report ..... 7*

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R E P R I N T

"Research of biostimulant's effect in animals", Institute of Nuclear Sciences "Boris Kidrič", Belgrade-Vinča, 1983.

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Report on realization of Project Phase:

## RESEARCH OF BIOSTIMULANT'S EFFECT IN ANIMALS

In this stage the studies were carried out on:

a) Peripheral blood lymphocytes within *in vitro* system. Lymphocytes with extremely simple morphology possess incredible biological potential and represent an ideal model for research of the most complex physiological processes. As it is well known that phytohaemagglutinin – PHA (non-specific mitogen) under *in vitro* conditions stimulates lymphocytes into blast transformation, i.e. influences the faster synthesis of nucleic acids and proteins as well as cell division, “lymphocyte model system” in the culture was used for assessment of mitogenic effect of the Bioregulator.

Lymphocytes isolated from peripheral blood of rats were cultivated in the basic medium adding Br in the dosage of: 60, 6, 3, 0.6, 0.006 micrograms per millilitre of the culture. In 72-hour culture the percentage was determined of blast-transformed cells, and the results were compared with the results obtained from the culture without mitogen (spontaneous transformation), as well as the cultures with known PHA mitogen. The best effect has been found in the culture with 0.6 micrograms/ml of Br where the percentage of blast-transformed lymphocytes was 50% higher than the spontaneous blast transformation. When the number of blasts from the culture with Br is compared with the height of stimulation after PHA, it can be seen that the percentage of blast-transformed lymphocytes with Br is for 28% lower in comparison with the results with PHA.

These results suggest that Br possesses mitogen characteristic which reflects in significant stimulation of lymphocytes to transform into blasts in comparison with spontaneous transformations.

b) The second part of research was performed within *in vitro* system in order to monitor biological mitogen activity of Br on constantly dividing haematopoietic tissue, which is very suitable for this kind of experiment, and which would represent a confirmation of mitogenic activity of this preparation in an organism as a whole.

The effect of Br was tested for speed and manner of regeneration of haematopoietic tissue of rats after sublethal doses of radiation. Co<sub>60</sub> was used as a source of radiation, and Br was applied to the animals directly and on the 10<sup>th</sup> day after irradiation in the dosage of 0.1 mg/kg of body weight. The changes were monitored in blood, bone marrow and spleen, and the samples were taken from the 3<sup>rd</sup> to 28<sup>th</sup> day. Both quantitative and qualitative analyses were carried out and the kinetics of proliferation of haematopoietic tissue.

The number of animals sacrificed in certain periods meets the conditions for statistical processing of the results. T-test was used to calculate significance of difference.

R E P R I N T

The animals that were irradiated and treated with Br showed faster regeneration of constantly dividing hematopoietic tissue in comparison with the control ones. Faster regeneration can be seen in: considerable increase of the number of bone marrow cells, intensive erythrocyte proliferation, considerably shorter time of regeneration of cell cycle of erythrocyte and granulocyte lineage, significant increase of proliferation capacity of both lineages, increase of reticulocytes in peripheral blood, early compensatory splenic erythropoiesis, as well as in the increase of body weight in all treated animals in comparison with the irradiated control ones.

The results obtained in Phase I of the research suggest that BIOREGULATOR has mitogen characteristics, which reflects both under *in vivo* and *in vitro* conditions. Applied in the conditions of sublethal irradiation it shows protective effect, i.e. it enables faster regeneration of hematopoietic tissue.

These significant preliminary results suggest the necessity of further research. The results of the research were presented at *XII Yugoslav Symposium on Protection from Radiation* held in Ohrid from May 31 to June 3 under the name: "Stimulating Effect of Bioregulator on Post-Irradiation Processes of Haematopoiesis". The full version of the paper was printed in the Proceedings of Symposium, **the copy of which is enclosed to this REPORT.**

Vinča, May 25, 1983

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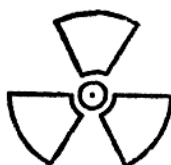
R E P R I N T

ЈУГОСЛОВЕНСКО ДРУШТВО ЗА ЗАШТИТУ ОД ЗРАЧЕЊА  
JUGOSLAVENSKO DRUŠTVO ZA ZAŠTITU OD ZRAČENJA  
JUGOSLOVANSKO DRUŠTVO ZA ZAŠČITO PRED SEVANJI  
ЈУГОСЛОВЕНСКО ДРУШТВО ЗА ЗАШТИТА ОД ЗРАЧЕЊЕ

# ЗБОРНИК НА ТРУДОВИ

XII ЈУГОСЛОВЕНСКИ СИМПОЗИЈУМ  
ЗА ЗАШТИТА ОД ЗРАЧЕЊА

КНИГА I



ОХРИД,  
31 мај - 3 јуни 1983 год.

R E P R I N T

## XII YUGOSLAV SYMPOSIUM ON PROTECTION FROM RADIATION

Ohrid, May 31 – June 3, 1983

*Ninkov Vukosava, Karanović Dobrila, Milić Olivera, Savovski Kiril, Pujić Natalija*

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**STIMULATING EFFECT OF BIOREGULATOR ON POST-IRRADIATION  
HAEMATOPOIESIS**

**ABSTRACT** *The effect of Bioregulator was examined for speed and manner of regeneration of haematopoietic tissue after irradiation. Experimental animals are Wistar rats radiated at the source of  $\text{Co}^{60}$  with the dosage of 5 Gy. Bioregulator was applied directly on the 10<sup>th</sup> day after radiation in the dosage of 0.1 mg/kg. The samples of blood, bone marrow and spleen were taken from the third until the 28<sup>th</sup> day, and both quantitative and qualitative analyses were carried out, while kinetics of proliferation process was also monitored. The results show that Bioregulator has influence on the regeneration of haematopoietic tissue and stimulates kinetics of regeneration process.*

R E P R I N T

**STIMULATIVE EFFECT OF THE BIOREGULATOR  
ON THE POSTIRRADIATION PROCESSES OF THE HAEMATOPOIESIS**

*The effect of Bioregulator on the rapidity and the mode of regeneration of the haematopoietic tissue after irradiation were studied. The animals - rats, Wistar strain, irradiated on Co<sup>60</sup> source, with dose of 5 G<sub>ee</sub> were used. Bioregulator was injected (0,1 mg/kg of the rat) immediately and 10th day after irradiation. Samples of blood, bone marrow and spleen were taken 3rd to 28th day. Quantitative and qualitative analyses were used and the kinetic of the proliferative process was followed. The results were showed that the Bioregulator influence on the regeneration of the haematopoietic tissue and stimulate the kinetic and the turnover time of the cells.*

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We would like to thank Dušanka Stepanović, technical assistant, for her expert and conscientious help.

R E P R I N T



INSTITUTE OF NUCLEAR SCIENCES "BORIS KIDRIČ"  
LABORATORY OF RADIOBIOLOGY  
Vinča

Report on project implementation:

### RESEARCH OF BIOREGULATOR'S EFFECT IN MAMMALS

The research took place according to the plan in two phases:

- a) Phase – assessment of Bioregulator's (Br) protective effect in sublethally irradiated animals and assessment of optimum time of Br application.
- b) Phase – examining possibilities of biological information transfer from Br treated animals to untreated ones.

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Vinča  
1984

R E P R I N T

*"Research of biostimulant's effect in mammals", Institute of Nuclear Sciences "Boris Kidrič", Belgrade-Vinča, 1984.*

## Discussion

It was as early as in 1950s that the research was conducted in order to find a “pill” against atomic bomb, i.e. to find radiation protectors that would be more effective than the chemical protective agents studied until that time.

In addition to possible applications in civilian and military protection protective substances can be useful as a “drug” in radiation therapy and this is why many laboratories that did research on cancer carried out also this kind of research. All tested substances had protective effect only if applied directly before radiation. They were the most efficient when injected a few minutes before radiation, and the effect declines and vanishes after one hour (5).

Our research refers to the research of the possibility to use BIOREGULATOR – isolated from the seeds of weed and cultured plants – “natural protector”, as protective substance which shows the effect when injected 24 to 72 hours before radiation and when it is administered immediately after radiation on haematopoietic system and general recuperation after radiation. The assessment of Br efficiency in radiation conditions was carried out by monitoring regenerative processes of haematopoietic tissue. In all experiments the positive effect of Br on these processes has been found.

In our earlier work (13) the effect was tested of Br which was applied immediately after sublethal dosage of 5 Gy and on 10<sup>th</sup> day afterwards, in the stage of reparation, on behaviour of kinetics, regeneration of haematopoiesis. The results show that in addition to positive effect in comparison with the irradiated control animals, in animals treated with Br immediately before the first and second application there is small inhibitory effect on haematopoiesis.

These results imposed a number of questions: should it use two Br applications or one is sufficient to cause complete regeneration in comparison with the irradiated control animals; and would the inhibitory effect show and how much it would show in non-irradiated normal animals?

One-off Br application immediately after radiation resulted in faster recuperation and the effect of stimulation was expressed all until the 28<sup>th</sup> day of observation. Inhibition between the 10<sup>th</sup> and 14<sup>th</sup> day was missing, which appeared at double injection. In our previous work when Br was injected also on the 10<sup>th</sup> day in the course of already intensive reparation, there was a stagnation in repopulation processes on the 14<sup>th</sup> day.

General cellularity of the bone marrow and the behaviour of erythrocyte and granulocyte kinetics in normal Br treated rats suggests that immediately after application the inhibition disappears. On the first day the number of bone marrow cells drops to around 50%, on the third day it drops to 28%, which suggests that inhibition recedes gradually and that the condition is normalized. It follows from this that in normal conditions also Br disturbs feed-back relations and that normalization oc-

curs only after the third day, which at the same time explains the positive effect of Br application before radiation on the first, and probably until the third day.

In the experiments where Br is applied before radiation (24 hours) there is statistically significant protective effect achieved (Figure 3), which could have been expected. However, when kinetics is observed of marrow cell regeneration, it can be seen that the time of regeneration of erythrocyte and granulocyte population is the same, whether the Br was applied before or after radiation. This suggests that process stimulation is the same regardless of the time of application. At the same time the number of marrow cells varies significantly in favour of pre-radiation treatment. Increased marrow cellularity is probably the consequence of maintaining stem cell pool in the stage of inactivity ( $G_0$ ), i.e. in the stage less sensitive to radiation. Thus, in this case, the larger number of cells enter the phase of division and then the phase of differentiation, which reflects also in a larger number of cells, regardless of the same speed of proliferation in the group of animals treated with Br after radiation.

As after the dosage of 5 Gy radiation all animals survive the period up to 30<sup>th</sup> day, we used the dosage of 7 Gy the lethality of which is 30% in our conditions and we used Br after radiation for protection. Haematopoietic regeneration even after this dosage of radiation is significantly increased in Br treated rats.

If we follow the behaviour of kinetic proliferation in bone marrow of both erythrocyte and granulocyte lineage, it can be seen that regardless of the strength of dosage, the number of application and the manner of application, Br stimulates both lineages. The results of other authors so far (10, 12, 13) with plant and animal material, as well as our results in both *in vitro* and *in vivo* conditions, show that Br has significant mitogenic capacity. Considering the wide range of its effects, i.e. since it has effect not on certain cells but on mitotic activity of "every cell", it can be considered that Br belongs to non-specific mitogens.

It has been found in our experiments that the time of cell regeneration cycle is shorter in all animals treated with Br which suggests that it is primarily the consequence of shortening of  $S$  phase. It is significant that shorter time of erythrocyte pool regeneration in these experiments is not below critical value, so it provides for creation and growth of normal physiologically active cell. According to Lajtha (9), if the time of erythroid lineage regeneration is less than 9 hours, deficient cells are created, i.e. deficient erythropoiesis occurs.

In the second part of the research the possibility was examined to transfer information for stimulation of division activity from one individual after treatment with Br to another. The research on plant material gives a lot of data on indirect positive influence of Agrostemin on the growth speed, better quality and yield in arable crops (3, 4), as well as on allelopathic effect of Agrostemin in certain ecological units (10).

In order to study indirect effect of Bioregulator the transplantation of bone marrow cells served as a model-system in our experiments. Immediately after expo-

sure the irradiated animals received the optimum quantity of bone marrow cells which is  $5 \times 10^7$  cells taken from normal rat donors or those previously treated with Br. It is common to transplant bone marrow cells after lethal radiation in order to research the protective effect of transplantation. However, in our case we did not wish to examine that effect but to get an answer if the information from the animal previously treated with Bioregulator is transferred via bone marrow cell to the recipient. From the previous results it is seen that normal animals who had Br applied on the 24<sup>th</sup> hour show stagnation of proliferative processes and that inhibition stops on the 72<sup>nd</sup> hour. This was the reason that the animals treated with Br on the 24<sup>th</sup> and 72<sup>nd</sup> hour respectively prior to sacrificing and non-treated normal animals were used as bone marrow donors. The results showed that highly significant difference occurs in the recipients of Br donor treated on the 24<sup>th</sup> hour. The effect exists also in the recipients of the marrow of the donors pre-treated on the 72<sup>nd</sup> hour, however this difference in comparison with the effect of normal transplants is only 15% for general cellularity. Since in all three experimental groups the same number of cells is given in suspension, it is obvious that the difference is ascribed first of all to the difference in the number of input stem cells of various donors.

The number of stem cells 24 hours after injection of Br (Table 16) is increased since normal kinetics of cell regeneration process is inhibited at the moment when the block recedes, while on the 72<sup>nd</sup> hour the great number of stem cells already enters into division phase and differentiation so their general number in the suspension alternates. However, in Br-72-marrow recipients, if the absolute number of erythrocyte cells is observed, where there is the most intensive mitotic activity after radiation, it is seen that their number abruptly rises and it is 50% higher in comparison with the values after administering the suspension of normal donors.

On the other hand, the big rise in number of cells (two and a half times more than after normal suspension) and rather accelerated reparation of erythrocytes and granulocytogenesis after transplantation of Br 24<sup>th</sup> hour cell donors suggest that in addition to a larger number of input stem cells there is stimulation of divisional repairing activity of marrow.

All these results suggest that Br stimulating capacity is transferred in suspension of bone marrow cells as information for increased cell proliferation processes.

It is well known that the condition of microenvironment which accepts the transplant is of primary significance for accepting transplants of irradiated recipients, but the effect of previous information born by donor stem cells cannot be excluded. In our papers from 1978 and 1980, it has been proven that information brought from marrow cell donor influences the haemo-differentiation of stem cells and that it cannot be ignored even if the microenvironment of the recipient which accepts the transplanted cells (7, 8) is of primary significance.

### Conclusion

–Bioregulator has protective capacity in sublethally irradiated animals and can be considered a potential natural protector.

*(This means that “Agrostemin bioregulator, when applied with non-lethal dosage before and after radiation, has positive influence at the cell level on its division, i.e. it has protective effect”<sup>\*)</sup> )*

–One-off application of Bioregulator is sufficient for stimulation of post-radiation processes of haematopoietic regeneration.

*–(This means that “Agrostemin Bioregulator when treating animals immediately before and after radiation has positive effect if it is used only once in comparison with the irradiated control group.”<sup>\*)</sup> )*

Bioregulator has protective effect regardless of whether it is applied before or after radiation.

–The cells that were in contact with Bioregulator transfer information into untreated recipient.

*–(This means that “Agrostemin bioregulator acts both directly and indirectly and that its effects are transferred from a treated cell into untreated one.”<sup>\*)</sup> )*

–As for the manner of its action, Bioregulator can be classified into non-specific plant mitogens.

*(This means that “Agrostemin bioregulator does not have effect on certain cells but it has effect on mitotic activity of every cell.”<sup>\*)</sup> )*

–Examinations of inhibitory-stimulating effect within *in vivo* system are required

*(This means that “since the examinations were at the cell level the examination of a live organism – individual is required”<sup>\*)</sup> )*

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<sup>\*)</sup> this comment to the conclusions was given by the Institute of Nuclear Sciences "Boris Kidrič" Beograd – Vinča in a separate document, singled out from the original Report

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R E P R I N T

The research on this project was carried out in the Laboratory of Radiobiology at the Institute of Nuclear Sciences "Boris Kidrič" in Vinča.

The work has been realized in haematological group of this Laboratory, the head of research is dr Vukosava Ninkov, Principal Research Fellow, with the associates, dr Dobrila Karanovic, Senior Research Associate, dr Nadezda Petrovic, Senior Research Associate, mr Natalija Pujic, Assistant and researchers Kiril Savovski, *Nada Radotić* and *Olivera Milić*, and technical assistants *Dušanka Stepanović* and *Desanka Bojović*.

The paper titled "Bioregulator as a Protector" is being prepared from this material and it will be sent for publishing in Strahlentherapie Journal, and the results will be presented at a conference that could accept this topic next year.

October 31, 1984.  
Vinča

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