Obtaining radioactivated strains of microorganisms and studying their antiradiation efficiency

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Abstract. The preparations of microbial origin inactivated by irradiation on the "Researcher" gamma device were used as potential antiradiation drugs: E. coli strain "KV-1", "PL-6". The preparations were obtained by growing cultures in mesopatamia broth in a thermostat at 37 °C for 3 days, then centrifuged at 3000 rpm for 40-50 min, the supernatant was decanted, the precipitate was diluted with distilled water according to the L.A. turbidity standard. ... Tarasevich up to 1 billion / ml. From the grown cultures, smears were prepared and stained according to Gram to determine the purity and species of the grown culture. The prepared suspension was poured into sterile vials of 10, 50, and 100 ml each, sealed with rubber stoppers and rolled in with aluminium caps, marking with the indication of the strain, radiation dose and date. Irradiation of microbial material was carried out on a gamma device "Researcher", a source of $^{60}$Co, exposure dose rate 3.7 kGy / h, in the range of absorbed doses from 7.5 to 30 kGy with inter-dose intervals of 2.5 and 5 kGy. Studies to determine the radioprotective effectiveness of strains of microorganisms killed by gamma-irradiation were carried out on outbred sexually mature white mice with a live weight of 18-20 g, divided according to the principle of analogues into groups of 5 animals each according to the following scheme: irradiation + E. coli strain "KB- 1", irradiation + E. coli strain "PL-6", control of irradiation, biological control. Acute radiation sickness was simulated using the Puma gamma device with a 137Cs radioactive source at a dose of LD80-100 / 30. The test preparations were injected subcutaneously in a volume of 0.2 cm$^3$ three days after radiation exposure. It was found that the introduction of cultures of microorganisms inactivated by gamma-irradiation E. coli strain "KV-1", E. coli strain "PL-6" 3 days after external radiation exposure contributed to the preservation of 60 to 80% of irradiated white mice.

1. Introduction
In nuclear explosions and radiation accidents, there is a simultaneous or sequential effect on the body of external and local irradiation, which results in radiation sickness of varying degrees, leading to irreversible pathological changes in living organisms [1-2].

As a result of radiation exposure to the body, bone marrow hematopoiesis and lymphopoiesis are suppressed, which, in turn, leads to the devastation of the central and peripheral immunocompetent organs, the cellular and humoral links of the anti-infectious defense are affected. Dynamic disturbances in the immune system after irradiation cause the development of autoimmune processes. All this determined the intensive development of such a direction in radiation immunology as immunotherapy and immunoprophylaxis of acute radiation sickness. To date, many antiradiation drugs
from different classes have been investigated, but only a few of them reduce the lethal effects of ionizing radiation without causing side effects in the body [3-4]. Among the known radioprotective agents, chemical substances prevail [5-6], however, numerous studies have revealed the presence of antiradiation properties in representatives of other organic compounds, in particular, of biological origin: vaccines, serums and other microbial or viral preparations [7-8].

In practice, for the treatment of radiation sickness, the means of early pathogenetic therapy, means of stopping the primary reaction to radiation, antibacterial drugs, hematopoietic stimulants, antihemorrhagic drugs, detoxification drugs, treatment of intestinal syndrome, platelet and erythrocyte mass transfusion, bone marrow transplantation, plasmapheresis are used hemosorption, chelating agents, etc. [9-10].

Many researchers use a wide range of compounds containing bacterial preparations to protect animals from radiation and treat experimental radiation sickness. There is evidence of an increase in the body's resistance to the damaging effects of ionizing radiation with the help of vaccine preparations. It has been established that the antiradiation effect is observed with the use of bacterial and antiviral agents, both for prophylactic and therapeutic use [11-12].

The mechanism of the antiradiation action of most substances of a biological nature (vaccines, seras, etc.) in the treatment of acute radiation sickness is based on their ability to be fixed on the surface of immunocompetent cells, which leads to an increase in phagocytosis and the immune response, suppressed in the irradiated organism [13-14].

Thus, the search and development of new, more effective means and methods for treating radiation injuries to animals have not lost their relevance.

Based on the foregoing, the purpose of this research was to test the protective efficacy of radioinactivated strains of microorganisms in experimental acute radiation sickness.

2. Materials and methods
In the first series of experiments, a preparation of microbial origin was obtained using the production strain of E. coli "PL-6" and the pathogenic strain of E. coli "KV-1" obtained by us in the laboratory of the Museum of Strains FGBNU "FCTR-B-VNIVI". The cultures were grown in Mesopotamia broth in a thermostat at 37 °C for 3 days. The grown suspension was centrifuged at 3000 rpm for 40-50 min, the supernatant was decanted, and the precipitate was adjusted with distilled water according to the L.A. turbidity standard. Tarasevich up to 10 units (1 billion / ml) and calorimetrically using KFK-2 (light filter with a wavelength of 540 nm.). From the grown cultures, smears were prepared and stained according to Gram to determine the purity and species of the grown culture.

The prepared suspension was poured into sterile 10, 50, or 100 ml vials, sealed with rubber stoppers and rolled with aluminum caps, marking with the strain, radiation dose, and date.

Irradiation of microbial material was carried out on a gamma device "Researcher", a source of $^{60}$Co, exposure dose rate 3.7 kGy / h, in the range of absorbed doses from 7.5 to 30 kGy with inter-dose intervals of 2.5 and 5 kGy.

The degree of inactivation of gamma-irradiated E. coli cultures was determined by plating them on mesopatamia agar and thermo stated for 168 hours, registering the presence or absence of microorganism growth.

From the grown cultures, smears were made, stained according to Gram, and microscoped under immersion with 90 magnifications.

In the second series of experiments, the radioprotective effectiveness of inactivated strains of microorganisms was determined. The experiments were carried out on outbreed sexually mature white mice with a live body weight of 18-20 g, divided according to the principle of analogues into groups of 5 animals each. White mice of the 1st group irradiated at a dose of 7.7 Gy were injected with a 30 kGy culture of E. coli, strain "KV-1", the irradiated animals of the 2nd group were injected with a microorganism E. coli 6, the irradiated animals of the 3rd group were not treated, and the mice of the
4th group were not treated or irradiated - they served as a radiation control and biological control, respectively.

Acute radiation sickness was simulated using the Puma gamma device with a radioactive source of cesium-137 at a dose of 7.7 Gy with an exposure dose rate of 5.38 R/min.

The preparations of microbial origin obtained by inactivation on a gamma device "Researcher" were used as antiradiation drugs: Escherichia coli strains "KV-1" and "PL-6", exposed to ionizing radiation at a dose of 30 kGy.

The test preparations were injected subcutaneously in white mice in a volume of 0.2 cm³ 3 days after radiation exposure.

The animals of the experimental and control groups were monitored daily, taking into account the general condition, behavioral reactions, food intake and water consumption, the state of visible mucous membranes, recorded the death of animals, on the basis of which the survival rate and average life expectancy (ALE) of the dead animals were calculated.

3. Results
The studies carried out have established that the timing and degree of growth of irradiated cultures of E. coli strain "PL-6" and "KV-1" are in direct proportion to the dose of radiation exposure, the complete inactivation of which occurs when they are irradiated at a dose of 25 kGy (figure 1).

![Figure 1](image1.png)

**Figure 1.** Growth of culture of E. coli strain "PL-6", irradiated with γ-rays in the dose range from 7.5 to 30 kGy.

The data in figure 1 show that the culture of E. coli strain "PL-6" is resistant to the effects of ionizing radiation. Gamma irradiation at a dose of 7.5 to 15 kGy inhibits growth for 24 hours after sowing. Irradiation of them at a dose of 17.5 and 20 kGy inhibits the development of the culture, which manifests itself in weak growth in the first 4 days after sowing. On the 6th day after sowing, abundant growth was observed in samples irradiated at doses of 7.5 and 15 kGy, moderate at doses of 17.5 and 20 kGy, and no growth at doses of 25 and 30 kGy.

The results of parallel radiomicrobiological studies using the virulent strain of E. coli "KB-1" are shown in figure 2.
The data shown in the figure indicate that the E. coli strain "KV-1" is less radioresistant to the effects of γ-rays than "PL-6", which is expressed in a more distinct suppression of the growth of the culture on the first day after sowing the irradiated doses from 10 to 20 kGy of material, moderate - at a dose of 7.5 kGy and abundant in the control sample. Irradiation in dose ranges from 15 to 20 kGy is marked by weak growth of the culture in the first 6 days after sowing. On the seventh day of cultivation of the culture exposed to irradiation in the dose range from 12.5 to 20 kGy, moderate growth was observed. The absence of growth was noted in the samples irradiated at doses of 25 and 30 kGy.

Microscopic examination of smears made from non-irradiated and irradiated in different dose ranges of 7.5, 10, 12.5, 15, 17.5, 20, 25, 30 kGy of Escherichia coli cultures, strains KV-1 and PL-6 revealed gram-negative, non-spore-forming rods, located in smears singly.

In the next series of experiments, it was shown that irradiation of white mice on the Puma gamma device with a 137Cs radiation source at a dose of 7.7 Gy caused severe acute radiation sickness (LD80 / 30) in them, which manifested itself in thirst, ruffled hair, pallor of the fundus, the presence of drying crusts at the outer corners of the eyes, food intake and physical activity were reduced. These clinical signs progressed by 10-11 days, causing the death of animals.

Autopsy of the dead mice revealed changes in the internal organs characteristic of severe radiation sickness: extensive hemorrhages in the internal organs, intestines, mesenteric lymph nodes, the spleen is reduced in size (devastated); the presence of hemorrhagic syndrome is visible.

The use of the tested drugs modified the radiation sickness caused by γ-irradiation, transferring it from severe to mild. The results of the experiments are presented in table 1.

| Groups | A drug                          | Survival,% | ALE (days) |
|--------|---------------------------------|------------|------------|
| 1      | E. coli pcs. KV-1 reg. at a dose of 30 kGy | 80         | 18.0       |
| 2      | E. coli pcs. PL-6 reg. at a dose of 30 kGy | 60         | 15.0       |
| 6      | Exposure control                 | 20         | 14.5       |
| 7      | Biological control               | 100        | -          |
The data in the table indicate a high radioprotective effectiveness of microbial preparations injected into animals of the first group, the survival rate of which was 80%, in the 2nd group it was 60% with a life expectancy of 18.0 and 15.0 days, respectively, in the radiation control group - 20%, with a life expectancy of 14.5 days.

4. Discussion and conclusion
When obtaining bacterial radioprotective drugs, we were guided by the previously used methods and techniques for growing and inactivating biological material [15].

Evaluation of radioprotective efficacy was studied in laboratory animals (white mice). When choosing a dose of external gamma-irradiation that causes severe radiation sickness, we were guided by the results of our previous studies [9], as well as literature data [2].

It was found by the experiments that the subcutaneous administration of radioprotective preparations of microbial origin inactivated by gamma-irradiation is most effective if they are injected 3 days after the radiation exposure. Our data concerning the timing of the introduction of radioprotective drugs are in part consistent with the literature data [8; 11; 12].

The study of the antiradiation efficacy of the tested preparations of microbial origin inactivated by gamma-irradiation showed that they have high therapeutic properties. The introduction of radioactive cultures of E. coli strain "KB-1" prevented the death of 80% of all irradiated mice and 60% under the condition of injecting an inactivated culture of E. coli strain PL-6.

The obtained data on the survival rate are quite consistent with the results of studies to assess the radioprotective efficacy of inactivated cultures of microorganisms [11; 13].

In experiments on white mice to determine the effectiveness of the tested antiradiation drugs in radiation injury, it was shown that preparations of microbial origin E. coli strain "KV-1", E. coli strain "PL-6" have high medicinal properties, if they are parent rally administered through 3 days after radiation exposure.

Thus, radioinactivation of E. coli microorganisms "KV-1" and "PL-6" leads to a decrease in virulence, toxicity of strains and an increase in radioprotective activity. The introduction of cultures of E. coli microorganisms inactivated by gamma irradiation of the strains "KV-1", "PL-6" 3 days after external radiation exposure contributed to the preservation of 60 to 80% of irradiated white mice.

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