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# Effect of ionizing radiation on cellular metabolism and virus-producing ability of cell cultures

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## ABSTRACT

The studies were conducted with the aim to investigate the possibilities of using radiation biotechnology methods for decontamination of cell culture media, stimulation of cell growth in cultures, and virus reproduction on the latter. The simulation of artificial contamination of culture media was performed by supplementing the media with bacterial agents at a dose of $1.5 \times 10^6$ CFU/mL and infectious povine rhinotracheitis virus at a dose of 0.2 $cm^3$/100 $cm^3$ (g) of a medium, with the virus titer of 6.0 lg TCD 50/$cm^3$. Both native and contaminated with the above-specified microorganisms culture media were exposed to γ radiation in the "Issledovatel" γ irradiation facility in the dose range from 0.1 to $1 \times 10^4$ Gy.

It was established that reliable decontamination of dry culture media was achieved by their exposure to γ radiation at doses of 0.5–2 × 10 Gy, whereas decontamination of liquid culture media was efficient at doses of 1.0–2 × $10^4$ Gy. Following artificial contamination of cell culture media, with microorganisms of bacterial and viral nature, reliable radiosterilization was accomplished by γ irradiation at a dose of $3 \times 10^4$ Gy.

The outcomes of cytological studies showed that a single and twice repeated exposures of Madin–Darby bovine kidney (MDBK) cell cultures to a wide dose range (0.5–10.0 Gy) of γ rays exerted divergent influence on cells: low doses (0.5–1 Gy) stimulated cell growth, development, and proliferation, whereas high doses inhibited those processes increasing cell death. Exposure of cells to a low dose (0.05 Gy) and, repeatedly, to a high dose (5.95 Gy) of γ radiation stimulated cell growth and proliferative activity in the MDBK cell line.

It was found that pre-irradiation of cells with a low dose (0.05 Gy) and a consequent re-exposure to a high dose (5.95 Gy) inhibited chromosomal aberrations in the form of bridges, fragments, and breaks.

Therefore, based on the study results, the optimal modes of decontamination of cell culture media by γ irradiation were determined. By the method of fractionated irradiation, a new MDBK-0.2 cell subline was obtained with increased proliferative and virus reproduction activity.

**Keywords:** radiation processing, contamination, decontamination, radio stimulation, chromosomal aberrations, genome instability

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## INTRODUCTION

At present, cell cultures are widely used in various areas of experimental biology and medicine for studying and solving fundamental problems of general and special virology, oncology, biochemistry, biotechnology, and so on.[1]

The level of contamination of bovine blood serum and cell cultures derived from animal organs and tissues with many *mycoplasma*, bacteria, and viruses is known to vary from 10% to 82%.[2]

Currently, the use of antibiotics and surfactants is the most common method of decontamination of growth media and cell cultures.[2] However, the rapid emergence of antibiotic-resistant types of microorganisms hampers the efficient application of existing methods of cell line decontamination with the use of antibiotics.

Therefore, a number of researchers use the resonance frequency method and radiation sterilization as alternative decontamination methods, ensuring reliable decontamination of cell culture media by their irradiation with γ rays at doses of 2.5–3.5 × $10^4$ Gy[3] and electromagnetic effect at a dose of 30 CU at a frequency of 95.56 Hz.[4]

According to the data recorded by a number of researchers,[5] the use of ionizing radiation at low doses (0.05, 5.0–10.0 Gy), as opposed to high (sterilizing) doses (1.0–3.3 × $10^4$ Gy), produces, on the contrary, a growth-stimulating effect by increasing the cell population of lymphocytes, fibroblasts, and other cells in animal and human organs and tissues by 1.5 to 2 times. Therewith, low-dose pre-irradiation of lymphocytes increases the resistance of cells to subsequent lethal irradiation and enhances their proliferative activity,[6] which is associated with the induction of radiation-induced genomic instability and may have both negative and positive consequences.[7]

## MATERIALS AND METHODS

To carry out cell dispersion, a mixture of 0.02% Versene solution and 0.25% trypsin solution was used during passage at the ratios of 9:1 and 3:1 (depending on the type of cell culture) at a temperature of 37.5±0.5°C. The proliferative activity of cell cultures was determined by

microscopic examination, and the proliferation index was calculated by a conventional method.

Chromosomal preparations for cytological (karyological) analysis were made by standard colchicine method followed by staining with 2% Giemsa aqueous solution. The number of chromosomes in metaphase plates was counted in 50–100 cells of each line.

Cell cultures grown in the used nutrient media were exposed to $^{137}$Cs γ rays at a dose of 0.05; 1; 2; 3; 4; 5; 6; and 7 Gy (depending on the experimental conditions) with the absorption dose rate of $3.43 \times 10^{-3}$ Gy/s. In addition to the chromosome analysis in irradiated cells, the degree of DNA fragmentation was determined by the number of double-stranded DNA, using the modified method of electrophoresis of DNA of single cells immobilized in agarose.[8] The irradiation of artificially and spontaneously contaminated culture media was performed in a γ irradiation facility "Issledovatel" with $^{60}$Co radiation source at doses of $1.0 \times 10^3$ to $2.5 \times 10^4$ Gy.

Conventional methods were applied to determine total protein content, total lipids, and pH of the intact and irradiated blood sera; the concentrations of immunoglobulins A and G were defined by Mancini method; antigenic properties were evaluated by means of electrophoresis.

The virus titer was calculated using the Reed–Muench method and expressed as logarithms $TCD_{50}/cm^3$. The immunological competence of viruses grown on irradiated culture media and reproduced on radio-stimulated cell cultures was determined by the serological method involving a hemagglutination inhibition test set according to a conventional technique.[8]

The sensitivity of cell cultures grown in radio-decontaminated media to viruses was defined by contaminating the cell cultures with virus material followed by incubation of virus-infected cells and consequent virus titration in compliance with methods applied in industrial biotechnology.[9]

The statistical analysis of the data obtained was carried out using Microsoft Excel computer application. The results of the study are reported as the mean ± standard error of the mean. To evaluate the differences, a Student's *t*-test and links to the website were used.

**Table 1** **γ Ray doses ensuring decontamination of liquid culture media artificially contaminated with test strains**

| Culture medium | Doses of γ rays, $\times 10^4$ Gy, contaminated | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | | 2.0 | | 3.0 | |
| | *S aureus* | *B subtilis* | *S aureus* | *B subtilis* | *S aureus* | *B subtilis* |
| Bovine serum | – | – | + | – | + | + |
| Medium 199 | + | – | + | + | + | + |
| MEM | + | – | + | + | + | + |
| LAH | – | – | + | + | + | + |

+, positive result; –, negative result; *B subtilis*, *Bacillus subtilis*; *S aureus*, *Staphylococcus aureus*.

## RESULTS AND DISCUSSION

The first set of experiments aimed at determining the optimum γ radiation doses for decontamination of dry and liquid culture media naturally contaminated with microorganisms.

According to the results of conducted microbiological and virological research on the assessment of decontamination efficiency of γ rays in regard to native, dry, and liquid culture media, reliable decontamination of dry culture media was achieved by irradiation at doses of $0.5–1.0 \times 10^4$ Gy.

Taking into account the fact that the degree of decontamination of objects depends on contaminant concentrations, the second set of our experiments was focused on decontamination of culture media artificially contaminated with asporogenous and sporogenous microorganisms. The results of the studies are presented in Table 1.

Table 1 shows that reliable decontamination of culture media, artificially contaminated with all kinds of asporogenous and sporogenous microorganisms, was achieved by exposure to γ irradiation at a dose of $3.0 \times 10^4$ Gy.

Considering the literature data suggesting that ionizing radiation induces the formation of toxic products of radiolysis thus causing changes of physical, chemical, and biochemical properties of irradiated objects, the following sets of experiments were conducted to investigate the stability of the basic biological parameters of irradiated media.

The results of the indication of radiolysis toxic products in a γ-irradiated culture medium (bovine blood serum), depending on the dose of ionizing radiation used in the Indirect hematologic agglutination test system, showed that an increase in the radiation dose applied to the culture medium led to a more intensive formation of radiolysis toxic products—hemagglutinating quinoid radiotoxic.

The findings of parallel biochemical studies indicated that irradiation of blood serum within the dose range of $0.1–3.0 \times 10^4$ Gy did not lead to any significant change in the basic parameters: both irradiated and non-irradiated sera had almost identical values of transparency, pH, total protein, total lipids, and fractional composition of proteins (globulins and albumin).

An increase in the radiation dose up to $6.0 \times 10^4$ Gy led to opalescence and an increase in the optical density by 1.55 times ($p < 0.05$), acidification of culture medium (reduction of pH by 1.30 times, $p < 0.05$), a decrease in the total protein content

**Table 2 MDBK cell survival on the fourth day after single and twice-repeated irradiations at doses of 0.05–6.0 Gy**

| Doses and schedule of cell culture irradiation | No. of survived cells (%) | |
|---|---|---|
| | monolayer | suspension |
| Non-irradiated (control) | 98.8±0.2 | 97.7±0.3 |
| Irradiated once at a dose of 0.05 Gy | 99.9±0.05 | 99.8±0.1 |
| Irradiated once at dose of 5.95 Gy | 97.1±0.07 | 89.1±03* |
| Irradiated once at a dose of 6.0 Gy | 96.3±0.1* | 87.3±0.5* |
| Irradiated once at a dose of 7.0 Gy | 85.7±0.3* | 79.5±0.1* |
| Irradiated twice with a 3-minute interval: dose 1 = 0.05Gy, dose 2 = 5.9 Gy | 99.9±0.1 | 99.8±0.05 |
| Irradiated twice with a 3-minute interval: dose 1 = 0.05Gy, dose 2 = 6.0 Gy | 99.8±0.07 | 99.7±0.1 |
| Irradiated twice with a 3-minute interval: dose 1 = 0.05Gy, dose 2 = 7.0 Gy | 98.3±0.01 | 97.1±0.05 |

MDBK, Madin–Darby bovine kidney.

**Table 3 Dynamics of accumulation and proliferation activity of twice-irradiated (0.05 Gy, 3-minute interval; 5.95 Gy) cells in suspension and monolayer**

| Cultivation time (h) | CC ($\times 10^6$ cells/cm$^3$) and PI | | | | | |
|---|---|---|---|---|---|---|
| | Control | | Monolayer | | Suspension | |
| | CC | PI | CC | PI | CC | PI |
| 24 | 0.84±0.03 | 1.9 | 1.28±0.05* | 2.1 | 1.23±0.07* | 2.0 |
| 48 | 1.17±0.10 | 2.1 | 2.01±0.09* | 3.2 | 1.99±0.13* | 2.7 |
| 72 | 1.21±0.09 | 2.3 | 1.73±0.05* | 2.7 | 1.57±0.15* | 2.5 |

Seeding density: $0.4 \times 10^6$ cells/cm$^3$, *- $p < 0.05$. CC, cell concentration; h, hours; PI, proliferation index.

**Table 4 Cytogenetic characteristics of twice-irradiated MDBK cells under long-term culture conditions**

| Passage | No. cells with chromosomal aberrations (%) | | | | | |
|---|---|---|---|---|---|---|
| | Control | | | Irradiated at a dose of 0.05 Gy, 3-minute interval, 5.95 Gy | | |
| | Bridges | Fragments | DM | Bridges | Fragments | DM |
| 4 | 1.03±0.10 | - | - | 1.31±0.49 | 1.73±0.27* | 0.9±0.13 |
| 14 | 1.01±0.05 | - | - | 1.29±0.29 | 1.21±0.31* | 0.51±0.15 |
| 20 | 1.00±0.07 | - | - | 1.17±0.19 | 0.53±0.07* | 0.23±0.06 |
| 25 | 1.01±0.01 | - | - | 1.11±0.23 | 0.09±0.01* | 0.03±0.003 |
| 30 | 1.02±0.03 | - | - | 1.03±0.31 | - | - |

DM, double minute chromosome; MDBK, Madin–Darby bovine kidney.

by 1.1 times ($p < 0.05$), a decrease in globulins by 1.07 times ($p < 0.05$), a decrease in albumin by 1.19 times ($p < 0.05$), and a decrease in lipids by 1.16 times ($p < 0.05$).

The study of the proliferative activity of Madin–Darby bovine kidney (MDBK) culture grown in media exposed to different doses of γ rays showed that their irradiation in a dose range of $0.1–3.0 \times 10^4$ Gy does not have a significant effect on the growth and proliferation of MDBK cells. The cell proliferation indices were 3.58 (on media irradiated with $0.1 \times 10^4$ Gy), 3.81 ($0.5 \times 10^4$Gy), 3.79 ($1.0 \times 10^4$Gy), 3.75 ($0.3 \times 10^4$Gy), and 3.11 ($6.0 \times 10^4$Gy), respectively, which were slightly lower ($p > 0.05$) compared with control. The data indicate the absence of toxicity of the culture media irradiated with the specified doses.

In view of the currently available radiation cytology data on the radiostimulative effect of fractionated irradiation on animal, plant, and microbial cells, we carried out the following set of experiments aimed at exploring the possible stimulating effect of γ rays on the MDBK cell culture in case of repeated exposures to high doses of ionizing radiation.

Single and contacting cells grown by a conventional method in Minimum Essential Medium containing 10% bovine serum supplemented with penicillin and streptomycin at a dose of 100 U/mL were irradiated twice according to the following schedule: first at a dose of 0.05 Gy, and then, 3 minutes later, at a dose of 5.95 Gy (total dose 6.0 Gy).

The results of the experiments on cell survival depending on the dose and schedule of irradiation are presented in Table 2.

It was found that fractionated irradiation had a radio-modifying effect both on single cells (suspension) and the contacting cells (monolayer) of the MDBK cell culture: for both culture methods, cell survival exceeded that of control. Therefore, pre-irradiation of cells with a low dose (0.05 Gy) produces a radiation-sensitizing effect which leads to the development of cell resistance to the second irradiation at larger doses (5.95, 6.0 Gy), thus increasing cell survival.

The next set of experiments was conducted to investigate the effects of twice-repeated irradiations on the dynamics of cell growth. The results of these studies are given in Table 3.

Table 3 data show that the maximum increase in the concentration of irradiated cells in a monolayer occurs after 48 hours of culturing constituting $2.01 \pm 0.09 \times 106$ cells/cm$^3$ with a proliferation index of 3.2, whereas, in suspension, this indicator was $1.99 \times 10^6$ cells/cm$^3$ with the proliferation index of 2.7.

Thus, twice-repeated irradiations of cells, first at a low dose (0.05 Gy) and then at a higher dose (5.95 Gy), had a stimulating effect on the growth and development of MDBK cell culture increasing cell concentrations by 1.77 and 1.70 times, respectively ($p < 0.05$), with a proliferation index of 3.2 and 2.7, respectively, compared with that of 2.1 in control.

Taking into consideration, the literature data suggesting that long-term effects of cell irradiation may have negative consequences, the following set of experiments was conducted to study the degree

of chromosome (genome) damage caused by exposure of MDBK cells to γ irradiation at an adapting (low) dose at different time points after stimulating cell division.

Table 4 presents the outcomes of the study of cytogenetic changes induced by γ radiation at test doses in MDBK cells under the long-term culture conditions.

Table 4 data indicate a high level of karyological stability of MDBK cell populations grown in a culture medium decontaminated by γ irradiation, and exposed to twice-repeated γ irradiations, first at an adapting dose (0.05 Gy) and then at a test dose (5.95 Gy). Based on the above data, the irradiated MDBK cell culture can be attributed to the group of cell lines with stable karyotypes.

The results of the virological studies showed that the use of a cell culture medium exposed to a sterilizing dose ($1 \times 10^4$ Gy) of γ rays led to an increase in the virus titer by 1.10 times ($p < 0.05$) compared with control, which by 9.8% exceeded that of control.

## CONCLUSION

As a result of the research conducted, we obtained a new radio-stimulated passaged subline of MDBK-02 cells, which was grown in a culture medium decontaminated by γ rays. It was found that the exposure of one of the most important components of a culture medium, blood serum obtained from bovine kidney, to $^{137}Cs$ γ rays at a dose of $1 \times 10^4$ Gy ensured reliable decontamination of the media from bacterial and viral microorganisms. Twice repeated exposures of MDBK culture cells to a low dose (0.05 Gy) and, after a 3-minute interval, to a high dose (5.95 Gy) of γ rays ensured the development of adaptive cell response to ionizing radiation, thus ensuring an increase in cell survival to lethal irradiation (radioresistance development) and an increase in the proliferation index and the final cell density. Pre-exposure of cells to an adapting dose of γ rays prevented the development of processes associated with the induction of genomic instability and accompanied by an increase in chromosomal damage (bridges, fragments, and double-minute chromosomes).

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