

# RELATIVISTIC ELECTRON INFLUENCE ON SANITARY-MODEL MICROORGANISMS AND ANTIBIOTICS IN MODEL SAMPLES

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A series of the investigations of the electron beam influence on sanitary-model test cultures and antibiotics in model solutions has been carried out. For each of the test objects, the authors have found the boundary doses of the absorbed radiation. The higher doses cause the sharp increase in the bactericidal influence, which becomes complete. The sanitary-bactericidal indices of the water samples remain stable during 6 days. The samples of antibiotics in various concentrations (from 100 UA) have been irradiated. It is proved that the substratum processing by the beam (in the regimes 30 kGy) causes diminution and complete neutralization of the antibacterial activity in all probes of the samples.

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## 1. INTRODUCTION

Lately a new prospective approach to the problem of the water disinfection takes shape. The approach includes the application of physical methods (processing with  $\gamma$ -irradiation, electron beams, ultraviolet and ultrasound). The use of electron beams is especially prospective in the branch of disinfection and purification of waste-waters. Processing of industrial and domestic effluents with electron beams can provide not only annihilation of pathogenic bacteria but also purification of effluents from biological and synthetic admixtures. The advantage of this method of the water purification is that its effectiveness is independent of the water clarity - i.e., the efficiency of beam energy expenditure makes 100%.

## 2. TECHNICAL APPROACH

Microbiological objects have been chosen among sanitary-model microflora of taxonomic groups - such as the cultures *E.coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citobacter freundii*, *Serratia marcescens* as well as the plasma-coagulating staphylococcus *S.aureus* and enterococcus *E.faecalis*.

Model samples of the test cultures had been prepared and sowed. Their biological characteristics were studied before and after processing of the model solutions with physical and chemical agents in accordance with the methodical guidelines.

The beam influence on antibiotics (penicillin, streptomycin and tetracycline) is investigated by the express-method. In this method, one applies antimicrobial medications, able to suppress dehydrogenation activity of test cultures in a rare nutrient medium.

The processes of water disinfection and purification have been investigated with a laboratory installation, elaborated in the NSC KIPT. Its principal functional units are the following: a linear resonance accelerator of electrons with a system measuring the beam parameters; a device for the beam extraction into the atmosphere; contact reservoirs for the water sample processing by irradiation and a measurer of the absorbed irradiation dose.

For the water sample irradiation with various portions of relativistic electrons, a linear resonance accel-

erator of electrons has been used. The parameters of the beam, generated in the accelerator, are the following: the electron energy is 3...4 MeV; the electron current is 0.5 A; the current pulse duration is  $2 \mu s$ . If the electron energy is 4 MeV, the track length of electrons in water amounts to 2.5 cm.

In the experiments, the mode of the accelerator operation is chosen so that to provide a broad distribution of the electrons at the accelerator output. The electron energy losses in the atmosphere diminish, not exceeding 100...150 keV at the distance up to 30 cm from the accelerator output window. The irradiation dose absorbed by the sample is measured with the help of dacryl sensors.

Water samples have been irradiated in test tubes of the volume (2...3) ml and specially designed 500-ml volume pans of stainless steel (the height is 50 cm; the width is 5 cm; the depth is 2 cm). For the pan pressurization, a 50- $\mu m$  thick mayoral film is used, which minimizes the beam energy losses. For irradiation of samples of large volumes (in the pans), we have scanned the beam with an electromagnet, placed at the accelerator output after the extraction window.

## 3. THE EXPERIMENTAL RESULTS

The study of the beam influence has indicated that, among the test cultures examined, the following representatives of enterobacteria have to be considered as the most vulnerable to the effect of the given physical agent: *E.coli*, *E. cloacae*, *C. Freundii* and *S. marcescens*.

Low-intensive irradiation of model samples of the given test-cultures (0.8...3.0) kGy has caused the total annihilation of the bacteria in 54 of the 270 tests ( $19.6 \pm 2.4\%$ ) during 6 days. In 129 tests ( $47.0 \pm 2.4\%$ ), we have observed a partial delay in the microbe multiplication, an amount of the viable cells being substantially diminished. In other cases (33.4%), the colony-forming subpopulations have survived in the initial concentration. These data indicate that, under the given regimes of irradiation, there takes place the microbial suspension disinfection mainly due to getting of the biological objects into the temporally inactive state. The beam bacteriostatic aftereffect is characterized by a steep diminution in the number of the colony-forming units of enterobacteria ( $10^3$ ... $10^7$  times as small). Such delay in the

test-culture development has been observed mainly during the first hours after the experiment ( $(62.8 \pm 4.3)\%$ ). At the third and sixth days, the number of the model samples with this specificity has diminished ( $(2...7)$  times as little).

The total annihilation of test cultures of non-capsular enterobacteria is correlated with the model sample irradiation in more intensive regimes - to start from 3.6 kGy.

That is, irradiation of staphylococcus test-cultures and enterococcus suspensions in the regimes (0.8...3.0) kGy has not provided for the model sample total disinfection at the first day. However, at the third day after the irradiation, the number of the test-objects, where the beam bacteriostatic effect is detected, has become twice as large. At the sixth day, microbial cells in all the probes have become sterile. Processing of staphylococcus cultures and samples of enterococci with the beam has resulted in the bactericidal effect already during their irradiation with the doses heightened up to 3.6 kGy.

For instance, in Fig.1 one can see the average indices lg KYO/ml for staphylococci as a function of the irradiation dose at different days of the observation. There exist the analogous dependences for the other test cultures (enterobacteria, enterococci and klebsiella).

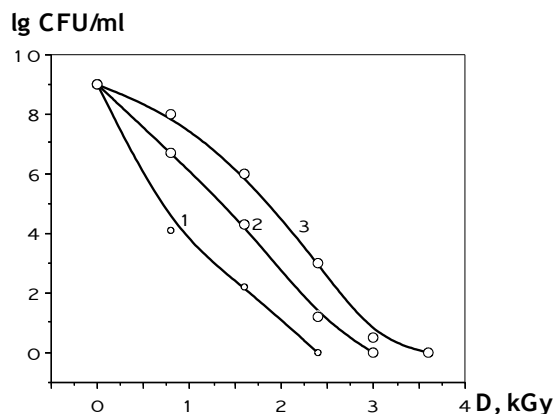


Fig. 1. Average indices lg KYO/ml for staphylococci in various regimes of the irradiation at the first (1), second (2) and third (3) days of the observation

In contrast to the bacteria mentioned above, the total restoration of the initial concentration of colony-forming units of the subpopulation ( $10^9$ CFU/ml, the CFU symbol marks the colony-forming units) at the sixth day after the processing with the beam is typical of Klebsiella model samples but not a partial destruction of viable cells. An increase in the power load ( $(3.6...4.0)$  kGy) on the given test cultures has caused a stable decrease in the number of alive bacteria in  $(88.1 \pm 3.5)\%$  of the tests. However, in the other cases  $(11.9 \pm 3.5)\%$ , the final result does not differ from the check experiments. Only the irradiation dose 7.2 kGy results in the bactericidal effect.

In our further experiments, the results of the model sample disinfection in small volumes have completely coincided with the data concerning the irradiation of the same test cultures in the volumes, substantially larger (100 times). This testifies to the same nature of the

physical agent influence both on the cultures in small and large pans.

The irradiation effect in the bacteriostatic and bactericidal regimes has been examined from the viewpoint of detecting the microbes in the association, most resistant to the beam influence. The investigations indicate that the test-object reaction depends not on the species of the bacteria in the model solutions but it is correlated with the concentration of each kind of the bacteria, taken into the investigations. The response reaction of 2-or 3-component microbial associations essentially differs from the reaction of individual test cultures only in the cases when their total concentration exceeds the concentration of a single suspension. If the initial density (9 lg CFU/ml) is preserved after the composition of the association, the beam aftereffect in compound model samples is the same as in the case of irradiating each member of the association under the given density.

The data obtained testify that, for the microbial suspension disinfection, low or medium intensities of the beam are required. For the destruction of medicaments, higher energy expenditures are necessary.

The beam absorbed doses within (3.6...7.2) kGy provide destruction of antibiotics in 19 of 36 samples ( $(52.8 \pm 8.3)\%$ ) with the antibiotic low concentration (0.31...5.0) UA, the symbol UA marks "the units of activity" and in 9 of 36 samples with higher concentration of antimicrobial means ( $(10.0...100.0)$ UA).

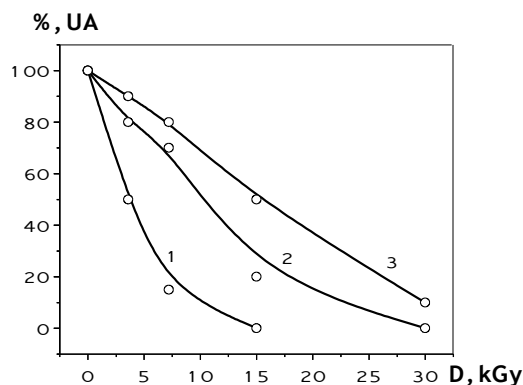


Fig. 2. The beam influence on the antibiotics in the concentrations: 5 UA (1); 10 UA (2) and 100 UA (3)

The general pattern of the dependence of the degree of antibiotic decomposition versus the irradiation dose for various values of UA is given in Fig.2.

Heightening of the power load up to 15 kGy favors neutralization of chemical therapeutic medicaments in 25 of 30 ( $(83.3 \pm 6.8)\%$ ) model samples in contrast to 44 of 72 ( $(38.9 \pm 5.7)\%$ ) samples, irradiated in the above-mentioned regimes - i.e., twice as more often. The further investigations have indicated that the irradiation optimal regimes are achievable with the power loads that provide absorbed doses from 30 kGy and higher. In these regimes, antibiotics have been destroyed in all the samples, including those with the medicaments in high concentrations ( $(50.0...100.0)$  UA).

#### 4. CONCLUSIONS

Thus, it is demonstrated that the absorbed doses (2.4...3.6) kGy make the threshold of the sanitary-model florula sensitivity to the beam influence. The bactericidal effect has been observed under the model sample irradiation with the following doses: about 3.8 kGy for enterobacteria (except *Klebsiella*, the dose for which makes 10 kGy); 4.5 kGy for staphylococci and 4.8 kGy for enterococci.

It is found out that there is no essential difference in the character of antimicrobial effect between the beam influence on small and large volumes of model samples of the test cultures examined.

In the case of the irradiation of 2- and 3-component associations of test cultures, their response reaction de-

pends on the final density of the model samples. If the total concentration of microbial association does not exceed that of a monosuspension ( $10^9$  CFU/ml), the bactericidal doses coincide with those that cause destruction of the most resistant members of the association.

The beam absorbed dose 7.2 kGy decomposes antibiotics (penicillin, streptomycin and tetracycline) in the concentrations (0.31...5.0) UA. If the medicament concentrations are higher (up to 100 UA), the chemical compound is decomposed with the doses (15...30) kGy.

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#### ВЛИЯНИЕ РЕЛЯТИВИСТСКИХ ЭЛЕКТРОНОВ НА САНИТАРНО-ПОКАЗАТЕЛЬНЫЕ МИКРООРГАНИЗМЫ И АНТИБИОТИКИ В МОДЕЛЬНЫХ ОБРАЗЦАХ

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Выполнен цикл исследований по влиянию электронного пучка на санитарно-показательные тест-культуры и антибиотики в модельных растворах. Для каждого тест-объекта установлены предельные дозы поглощенного облучения, выше которых бактерицидное действие резко усиливается и становится полным. При этом обеспечивается стабильность санитарно-бактериологических показателей образцов воды на протяжении 6 дней. Показано, что обработка электронным пучком модельных образцов антибиотиков, взятых в концентрациях до 100 ОА, в режимах до 30 кГр приводило к уменьшению и полной нейтрализации антибактериальной активности во всех пробах образцов.

#### ВПЛИВ РЕЛЯТИВІСТСЬКИХ ЕЛЕКТРОНІВ НА САНИТАРНО-ПОКАЗОВІ МИКРООРГАНІЗМИ ТА АНТИБІОТИКИ В МОДЕЛЬНИХ ЗРАЗКАХ

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Виконано цикл досліджень по впливу електронного пучка на санітарно-показові тест-культури та антибіотики в модельних розчинах. Для кожного тест-об'єкта визначено граничні дози поглинутого випромінювання, вище яких бактерицидна дія різко посилюється і стає повною. При цьому забезпечується стабільність санітарно-бактеріологічних показників зразків води протягом 6 діб. Показано, що оброблення електронним пучком модельних зразків антибіотиків, взятих в концентраціях до 100 ОА, в режимах до 30 кГр приводило до зменшення та повної нейтралізації антибактеріальної активності у всіх пробах зразків