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EFFECT OF Co^{60} GAMMA IRRADIATION ON STAPHYLOCOCCOS AUREUS

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ABSTRACT: The sensitivity of staphylococcus aureus to gamma radiation of varying dose rates in a nutrient broth and in a phosphate buffer solution, with and without glucose, was studied. Twenty-four-hour-old microorganisms were irradiated with doses of 20,40,60, 80, 100,200,300,400 and 500 krad at dose rates of 4 and 400 rad/sec. An increase in dose rate within the limits studied was found to produce a more effective reduction in number of germs. A dissimilar biological effect of gamma rays of varying dose rates at different integral dose levels was noted. In a nutrient broth and a phosphate buffer solution, sensitivity of staphylococcus to gamma radiation depends on dose rate, the presence of radioprotective substances in the medium and on the concentration of microbial cells.

NOTICE

At present, the most promising pathways of use of ionizing radiation for treating foodstuffs for the purpose of prolonging their storage life, have already been revealed [1,2]. However, development of this comparatively new and complicated problem for practical introduction on an industrial scale requires detailed explanation of many problems. Ascertaining the effect of Co^{60} γ-radiation dose rate on various microorganisms found in foodstuffs is of great theoretical and practical importance. An increased interest in study of the biological effects of radioactive radiations of various intensities has appeared among investigators, with the creation of powerful units for irradiation of various items [3-5]. Data in the literature on this question are controversial and insufficient for drawing an objective conclusion. Thus, some authors [6-8] assert that dose rate does not have a noticeable effect on the degree of inactivation of microorganisms. In the opinions of other investigators [9-11], the radiobiological effect is increased with decrease in dose rate. Moreover, the opinion has been expressed, according to which an increase in dose rate leads to more severe damaging action of ionizing radiation on microorganisms [12-14].

In study of the effect of dose rate on various types of bacteria, yeasts and molds causing spoiling of foodstuffs, one of the authors of this article demonstrated earlier that microorganisms react differently to change in the dose rate. Depending on the reaction of microorganisms to the effects of γ-rays of various intensities, they can be divided into three groups: 1) microorganisms not reacting to change in dose rates; 2) microorganisms, during irradiation of which, a decrease in dose rate leads to a sharp weakening of the biological effect of γ-rays; 3) microorganisms, for which the degree of injury by γ-rays decreases noticeably with increase in dose rate.

To a certain extent, these data explain the contradictory nature of the published information, and they indicate the necessity for carrying out special studies with various types of microorganisms as well, to establish more efficient conditions for irradiation of foodstuffs, the more so that man's diet now includes the most diverse products, containing extremely diverse types of microorganisms.

The task of this work included determination of the effect of dose rate on the survival of Staphylococcus aureus No. 75 in a phosphate buffer solution with and without glucose, as well as in a meat-peptone broth.

We selected Staphylococcus aureus as the object of the study, because it is widespread in nature, is frequently encountered in foodstuffs and can cause food poisoning and other diseases [15,16].

Staphylococcus strain No. 75 was obtained in the dry state from the L. A. Tarasevich Control Institute of Medical and Biological Preparations. To restore viability after freeze drying before the tests, Staphylococcus strain No. 75 was successively cultured ten times on various nutrient media.

Microorganisms for the experiments were grown on a meat-peptone nutrient agar for 24 hours, and then, in case of necessity, were washed from the nutrient medium by three-fold centrifugation in a phosphate buffer solution (pH 7.0). A twenty-four-hour-old culture was irradiated in meat-peptone broth and phosphate buffer solution, both with and without glucose, with doses of 20,40,60,80,100, 200,300,400 and 500 krad, at dose rates of 4 and 400 rad/sec, in an experimental-production γ-unit (K-300) of a branch of the All-Union Scientific Research Institute of the Canning and Dehydrated Vegetable Industry. Initial microorganism concentration (1.86×10^{6}-1.67×10^{8} cell/ml) was determined at the time of starting irradiation of the samples. Irradiated suspensions were immediately cultured on a nutrient medium, prepared from dry nutrient agar, after γ-irradiation. At least three samples for each condition were cultured in each series of tests. Cultures were incubated for two days at 37°. A conclusion was drawn as to the radiobiological effect of γ-rays at different dose rates, on the basis of the number of colonies growing in cultures of microorganisms irradiated at identical integral γ-radiation doses.

It should be noted that Staphylococcus aureus No. 75 is a very convenient object for such experiments, since it is easily counted quantitatively, owing to the capacity for uniformly distributing itself in a liquid medium, and forming distinctly isolated colonies on a solid medium.

The experiments conducted showed that the dose rate has a noticeable effect on survival of the microorganisms studied. Average data, characterizing the number of surviving cells as a function of dose rate, in tests with initial minimum concentrations (1.86-5.18×10^{6} cells per mℓ) are presented in Table 1.

As is seen in the table, an increase in dose rate from 4 to 400 rad/sec facilitates an increase in the radiobiological effect of γ-rays. Data obtained in the 20-80 krad range are evidence that, at the lowest dose rate used (4 rad/sec), 1.97-25.1% of the microbe cells survive, with respect to their initial population, while, at the maximum dose rate (400 rad/sec), the number of viable organisms is not over 0.07-7.0% (comparison with unirradiated microorganisms).

TABLE 1: EFFECT OF DOSE RATE ON SURVIVAL OF STAPHYLOCOCCUS AUREUS 75 IN PHOSPHATE BUFFER SOLUTION

a [illegible]	b [illegible]	
	c [illegible]	
	4	400
0 (контрол.) d	100	100
20	25.1	7,0
40	10,4	2,69
[illegible]	4.34	[illegible]
80	[illegible]	0,07

Note: Number of microbe cells in control is taken as 100%.

Key:
- a. Dose in krad
- b. Number of microorganisms in % of control
- c. Dose rate in rad/sec
- d. Control

Similar patterns were recorded in experiments at higher microbe cell concentrations (Table 2). These tests also permitted determination that a considerably greater number of microorganisms survive at the lower dose rate (4 rad/sec). It was found in addition that, with increase in integral dose, a distinct tendency toward increase in the difference in number of bacterial cells capable of proliferation is observed, dependent on dose rate.

We found a nonuniform effect of dose rate on the biological effect at various levels of Co^{60} γ-irradiation integral doses, within the concentration ranges studied ($1.86x10^{6}$-1.09-$1.67x10^{8}$).

TABLE 2: EFFECT OF DOSE RATE AND INITIAL CELL CONCENTRATION ON SURVIVAL OF STAPHYLOCOCCUS AUREUS IN PHOSPHATE BUFFER SOLUTION

a [illegible]	b [illegible]	c [illegible]	
		d [illegible]	
		2.25·10[illegible]	1.09—1.6·10[illegible]
0 ([illegible]) e	0 ([illegible]) e	100	100
20	4	72	82
	400	30,1	[illegible]
40	4	64,2	80,[illegible]
	400	5,58	22,9
60	4	[illegible]	[illegible]
	400	1,77	3,14
80	4	41,0	29,1[illegible]
	400	0,47	1,15
100	4	0,84	[illegible]
	400	0,001	0,31
200	4	0,0[illegible]	0,[illegible]
	400	0	0,00 [illegible]
300	4	0,00 001	0,[illegible]
	400	0	0,00 001
400	4	0	0,00 001
	400	0	0
500	4	0	0,00 001
	400	0	0

Key:
a. Dose in krad
b. Dose rate in rad/sec
c. Number of microorganisms per mℓ in % of initial concentration
d. Initial cell concentration per mℓ
e. Control

A quantitative analysis of the degree of injury to the reproductive function of Staphylococcus No. 75 cells vs. dose rate is convincing evidence that increase of dose rate from 4 to 400 rad/sec facilitates more effective suppression of viability of this microorganism, regardless of the initial cell concentration. The comparative survival characteristics of the microorganisms vs. initial concentration made it possible to determine that, with increase in cell population in a suspension, the dose necessary for inactivation of them increases noticeably (see Table 2). In tests with meat-peptone broth, it was determined that suspensions irradiated with doses of 20-300 krad, contained approximately identical numbers of proliferating individuals, regardless of change in dose rate within the ranges studied.

A difference in survivability was noted only after irradiation of staphylococcus with the maximum doses (400 and 500 krad). Using a dose of 400 krad, in the case of irradiation at the minimum dose rate of γ-radiation, several hundred cells survived, while the microorganisms were completely killed at maximum dose rate. After the action of γ-radiation at a dose of 500 krad and dose rate of 4 rad/sec, dozens of cells were found per mℓ of the suspension studied and, with a 100-fold increase in dose rate, as at 400 krad, absolutely all the microorganisms lost the reproductive function. We obtained similar results in experiments with a meat-peptone broth to which 2% glucose was added.

In comparing the radiobiological effect induced by the action of γ-rays at various dose rates on staphylococci in a meat-peptone broth, with and without 2% glucose, as well as in a phosphate buffer solution (pH 7.0), it was determined that the difference in number of cells irradiated with identical doses in the phosphate buffer was recorded at the minimum integral dose (20 krad), while, in irradiation of staphylococci in the nutrient broth, a similar pattern appeared only at the maximum radiation doses (400 and 500 krad). Moreover, it must be noted that nutrient substances contained in the meat-peptone broth have a very significant protective effect on the staphylococci. Thus, for example, at identical initial cell concentrations (10^7 per mℓ), doses of 200-400 krad are required for complete suppression of the reproductive function in the phosphate buffer solution (depending on the dose rate), while a similar effect is achieved by irradiation at doses of 400 and more than 500 krad in nutrient broth. A decrease in sensitivity of Staphylococcus aureus 75 (10^8 per mℓ) to the action of Y-rays was revealed in tests using a phosphate buffer solution with 2% glucose. A comparative evaluation of survival of microorganisms under metabolic rest conditions and in the phosphate buffer solution with glucose, in which metabolism can be carried on in the cells, revealed noticeable differences in the numbers of cells capable of proliferation after irradiation.

As it turned out, addition of glucose to the phosphate buffer solution noticeably reduces the reaction of the organisms studied to the effect of ionizing radiations. With one and the same initial concentration of microbe cells, significant differences in their survival are observed with the presence of sugar in the medium. In the 20-80 krad dose range in the phosphate buffer solution with glucose, depending on dose rate, 5.7-96.1% of the microorganisms survive, with respect to their initial population, while, in the same medium without glucose, 1.45-82% survive. Similar results were recorded at higher integral doses (100-500 krad).

Figs. 1-3 illustrate what has been reported above.

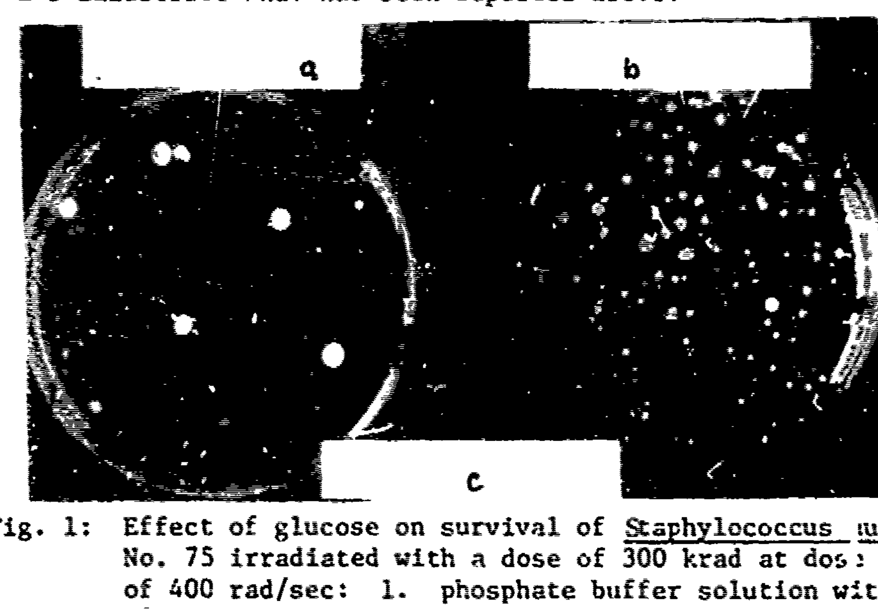

Fig. 1: Effect of glucose on survival of Staphylococcus aureus No. 75 irradiated with a dose of 300 krad at dose rate of 400 rad/sec: 1. phosphate buffer solution without glucose, 2. phosphate buffer solution with glucose.

Key: a. Phosphate buffer pH; 4% b. Glucose 2%
c. 300 krad, 400 rad/sec.

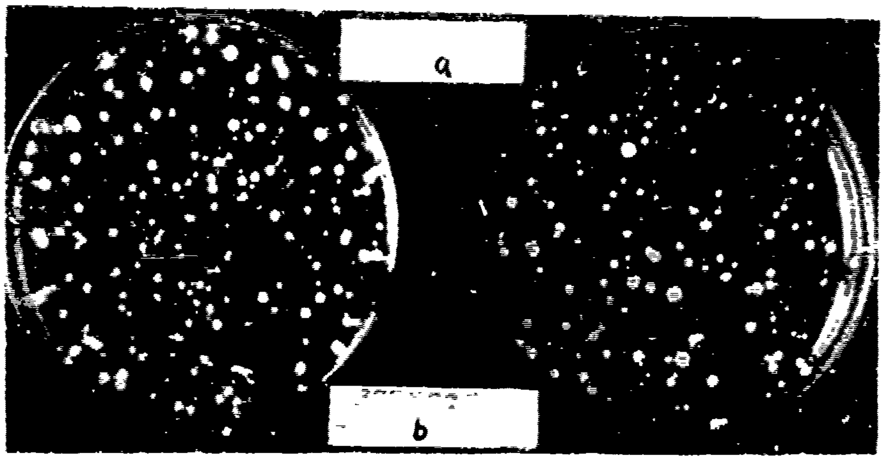

Fig. 2: Effect of dose rate on survival of Staphylococcus aureus No. 75 irradiated at a dose of 300 krad in phosphate buffer solution with 2% glucose:

1. dose rate 4 rad/sec; 2. dose rate 400 rad/sec.

Key: a. Glucose 2% b. 300 krad, 4 rad/sec, 400 rad/sec.

Fig. 3: Effect of dose rate on survival of Staphylococcus aureus No. 75 irradiated with a dose of 400 krad in phosphate buffer solution with 2% glucose;
1. dose rate 4 rad/sec; 2. dose rate 400 rad/sec.

Key: a. Glucose 2% b. 400 krad, 4 rad/sec, 400 rad/sec

Based on the resulting experimental data, it can be concluded that, in developing conditions for irradiation of foodstuffs, the dose rate, chemical composition of the medium and quantitative microorganism content must be taken into account.

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