EFFECT OF TEMPERATURE ON RADIOSENSITIVITY OF Staphylococcus aureus

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Changes in the temperature of postradiation cultivation did not cause significant changes in the the survival rate of the original staphylococcal cultures, whereas the survival rate of radioresistant mutants was lower at 19 and 45° than at 37°C. Simultaneous exposure to irradiation and heat (50° for 15 min) gave the least sterilizing effect in the studied staphylococcal strains.

The study of the combined action of γ irradiation and heat on microorganisms is of considerable theoretical and practical interest because it points the way to a method of reducing the maximal effectiveness of the sterilizing dose. Facts indicating that the survival rate of several strains of Escherichia coli is dependent on the temperature of postradiation incubation have been reported in the literature [1, 3, 6]. Differences in the survival rates of irradiated E. coli cells grown at different temperatures are explained by differences in the effectiveness of postradiation regeneration or by the phenomenon of filament formation [2, 3, 5].

The object of the present investigation was to study the effect of the temperature of postradiation cultivation on survival of Staphylococcus aureus. The combined action of irradiation and heat on a staphylococcal culture also was examined.

EXPERIMENTAL METHOD

Cultures of S. aureus, strains No. 73 and B-445, and radioresistant mutants obtained from them by repeated γ irradiation in subbactericidal doses [4] were used.

Cells of a washed 18-h culture in the stationary phase of growth were irradiated in phosphate buffer (pH 7.0) with Co^{60} γ rays (dose rate 8 krad/min). After irradiation, suspensions of the microorganisms were seeded on Hottinger's agar in Petri dishes and grown for 24 h at the corresponding temperature.

The survival rate of the microorganisms was determined from the number of colonies grown on the agar in the dishes.

The culture of bacteria was exposed to the simultaneous action of γ rays and heat in a specially built water thermostat maintaining a stable assigned temperature, which was placed in the chamber of the radiation source.

EXPERIMENTAL RESULTS

Despite a marked change in many characteristics (fermentation of sugars, pigment formation, deoxyribonuclease activity, plasma-coagulating ability, etc.) of the staphylococci with increased radioresistance, the base ratio of their DNA (G + C)/(ArT) was identical to that of the original bacteria (0.51 for strain No. 73

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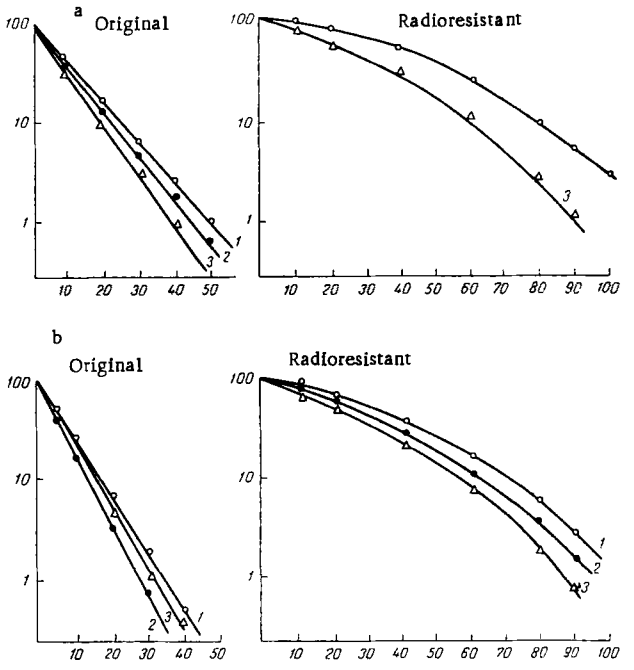

Fig. 1. Relationship between survival rate of strains of Staphylococcus aureus and temperature of postradiation cultivation of cells: a) S. aureus 73; b) S. aureus B-445. Abscissa, dose (in krad): 1) 37°, 2) 45°, 3) 19°; ordinate, survival rate (in %).

TABLE 1. Effect of Heat on Survival Rate of Irradiated Staphylococcus aureus Cells

Dose of irradiation (in krad)	Survival rate (in percent)			
	without heating (9)	of culture heated (50°, 15 min)		
		before irradiation (11)	after irradiation (10)	simultaneously with irradiation (11)
5	58,2±3,4	18,0±1,9	6,6±1,0	3,5±0,4
10	28,0±2,3	3,7±1,1	3,9±0,7	2,4±0,2
20	5,1±0,9	2,2±0,6	0,15±0,04	0,05±0,006
30	1,6±0,3	0,5±0,1	0,02±0,006	0,004±0,0001

Note. Number of experiments shown in parentheses.

and 0.47 for strain B-445). Dose–survival curves for cultures of staphylococcal strains Nos. 73 and B-445 were exponential in character for the original strains and sigmoid for staphylococci with increased radioresistance (Fig. 1).

The values of LD_{90} for the original staphylococcal cultures (strains No. 73 and B-445) when incubated after irradiation in the complete medium at 37°C were 27 and 17.5 krad, respectively, while for the radioresistant mutants they were 80 and 68 krad.

A change in the temperature of postradiation cultivation (19 and 45°) did not produce any significant change in the survival rate (LD_{90}) of the original strains compared with that of the bacteria grown on Hottinger's agar at 37°. There was only a tendency for survival to decrease if these bacteria were incubated at 19 and 45°.

The sigmoid character of the dose curves for the radioresistant mutants remained when the cultivation temperatures were 19 and 45°. The lowest survival rate was found at 19°. For instance, the values of LD_{90} for mutants of S. aureus B-445 and No. 73 when incubated at 19° were 59.6 and 60.2 krad respectively. In general, cells of the radioresistant mutant of S. aureus No. 73 did not form macrocolonies at 45°.

An increase of 2-2.5 times in the size of the cells for radioresistant staphylococcal mutants and disturbance of cell division after irradiation, discovered by electron-microscopic investigations [4], must evidently influence the differences in survival at the incubation temperatures specified above.

The increase in bactericidal effect of ionizing radiation combined with exposure to heat was studied on a culture of S. aureus B-445. The results showed that the inhibitory effect of this treatment on growth of the bacteria depended on the order of application of heat and γ rays. The greatest synergistic action was observed if γ ray irradiation and heating were applied simultaneously (Table 1), and heating after irradiation was less effective. Irradiation at room temperature after heating was the least effective of all.

This result of the combined action of γ irradiation and heat has been observed on certain other microorganisms [8-10], although this same order of sterilization was not the most effective in all cases.

The mechanism of the sterilizing action of combined irradiation and heat has not been adequately investigated. Some interesting results showing similarity between the structural damage to cellular DNA at a temperature of the order of 50°, causing inactivation of E. coli, and lethal injuries produced by ionizing radiation, have been published [7].

The results of the present experiments with strains of Staphylococcus aureus also demonstrate a correlation between the character of the injuries produced by the action of ionizing radiation and of heat.

LITERATURE CITED

1. É. Ya. Graevskii, Dokl. Akad. Nauk SSSR, 83, 565 (1952).
2. V. D. Zhestyanikov, Tsitologiya, No. 12, 1460 (1967).
3. M. N. Myasnik and V. I. Korogodin, Radiobiologiya, No. 6, 830 (1968).
4. M. A. Tumanyan, Z. G. Pershina, I. B. Pavlova, et al., Mikrobiologiya, 39, No. 1, 112 (1970).
5. H. I. Adler, W. D. Fischer, A. A. Hardigree, et al., J. Bact., 91, 737 (1966).
6. T. Alper and N. E. Gillies, J. Gen. Microbiol., 22, 113 (1960).
7. B. A. Bridges, M. J. Ashwood-Smith, and R. J. Munson, Biochem. Biophys. Res. Commun., 35, 193 (1969).
8. L. L. Kempe, Appl. Microbiol., 3, 346 (1955).
9. J. J. Licciarsello, J. Food Sci., 29, 469 (1964).
10. G. Stehlik and K. Kaindl, in: Food Irradiation, Vienna (1966), p. 299.