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The Probability of Recovery in Yeast Cells does not Depend on Ionizing Radiation Quality

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Abstract

The kinetics of the liquid holding recovery was experimentally studied for diploid yeasts of wild type and some radiosensitive mutants after irradiation with gamma rays and alpha particles. A mathematical model describing the process of postirradiation recovery as a change in the effective dose was applied to experimental data obtained. The model allowed to estimate quantitatively the probability of recovery per time unit and the irreversible component, i.e. the fraction of cells incapable of recovery. It was shown that the irreversible component was enhanced for densely ionizing radiation in comparison with low-LET radiation while the probability of recovery was identical both for low- and high-LET radiations for all strains investigated. It was concluded on this basis that the recovery process itself is not damaged after densely ionizing radiation and the enhanced RBE of high-LET radiation may be caused by the increased yield of irreversible damage. Both parent strain and all radiosensitive mutants were characterised by the same probability to recover from radiation damage. It follows that the mechanism of the enhanced radiosensitivity of mutant cells is not related with the damage of repair systems themselves.

Key Words : recovery, irreversible component, probability of recovery, radiation quality, mathematical model

1. Introduction

According to classical radiobiological conceptions, the relative biological effectiveness (RBE) of densely ionizing particles is determined only by physical characteristics of ionizing radiation. But now it is widely accepted that the RBE of ionizing radiation with high linear energy transfer (LET) is dependent both on the increased probability to produce the primary radiation damage (physical events) and the reduced ability of cell to postiiradiation recovery (biological events). A relatively unexpected role of specific repair pathways in the RBE of high-LET radiation was demonstrated for bacterial [1-3] yeast [4-9] and mammalian cells [10, 11].

Studies concerning the extent of recovery from radiation damages produced with low- and high-LET radiation in cells of various origin on the macromolecular and survival level have revealed that in general at high ionization density, recovery process may be very reduced or even absent [12]. This is especially the case for recovery from potentially lethal radiation damage which can be observed in irradiated yeast and mammalian cells and which became apparent by an increase in survival when irradiated cells were kept under non-growth condition.

The reduction of cell recovery ability after high-LET radiation might be proceeded via either the damage of the mechanism of the recovery itself or via the formation of irreversible damage which could not be repaired at all. Both these processes may take place at the same time. However, the data distinguishing these possibilities are lacking in literature. This study has been undertaken to compensate this imperfection.

Yeast cells were chosen here as a test object because of some reasons. First, their recovery has been well studied both on cellular [13-15] and molecular]16-18] levels. Second, the first idea about the recovery ability in eukaryotic cells has been hypothesized for these cells [19, 20]. If the irradiated yeast cells are held in a liquid non-nutrient media at 30°C before the plating on growth medium, their survival is increased. This phenomena is known now as the liquid holding recovery (LHR). Third, a quantitative approach describing the LHR kinetics of yeast cells was described [13, 15] which enables the estimation of the probability of recovery per unit time and the fraction of irreversible damage. In the yeast *Saccharomyces cerevisiae* there are at least 8 distinct loci that control sensitivity to ionizing radiation, while the number of independent genes capable of affecting yeast sensitivity to ultraviolet radiation exceeds 20 loci; some of the mutant genes confer sensitivity to both UV and ionizing radiation [21]. It is thought that the mechanism of their enhanced radiodensitivity is related with the depressed

molecular mechanisms for DNA repair. For this case, it is also not elucidated whether this depression would be proceeded through the damage of the mechanism of the recovery itself or through the formation of irreversible damage which could not be repaired at all. However this question is stayed unresolved.

To make up these deficiencies, a quantitative approach describing cell recovery from potentially lethal damage is presented here to estimate separately the probability of cell recovery per unit time and the fraction of irreversible damage. This approach will be applied here to experimental data on the liquid-holding recovery of diploid yeast of *Saccharomyces cerevisiae* of wild type and some radiosensitive mutants retaining to some extent their ability to recover from radiation damage.

Thus, the main goals of these study were (a) to clear up whether high-LET radiation affects the recovery process itself or it produces more number of severe irreversible damage which couldn't be repaired at all; (b) to elucidate the role of irreversible damages and the probability of recovery in the enhanced sensitivity of *rad* mutants in yeasts. In this paper, the liquid-holding recovery would be served as an indicator of cell repair activity. One can believe that a better insight of the relationship between radiation quality and cell recovery from potentially lethal radiation damage would be of interest both from fundamental stands and with respect to the practical applications of densely ionizing radiation in tumour therapy.

2. Material and Methods

The diploid yeast cells of *Saccharomyces ellipsoideus (vini)*, strain Megri 139-B (*RAD/RAD*), and the following diploid strains of the yeast *Saccharomyces cerevisiae* were used for these experiments: XS800 (*RAD/RAD*), XS1924 (*rad18/rad18*), XS1889 (*rad53/rad53*), XS1935 (*rad55/rad55*), XS1943 (*rad56/rad56*), XS1988 (*rad57/rad57*). In the experiments described here yeast cells were used as s model system for eukaryotic cells. They can be maintained over a long period in a certain phase of the cell cycle. In addition, it is possible to vary the irradiation conditions and environmental parameters over a wide range for testing mathematical models of radiation responses.

Cultures in stationary state were prepared for experiments in the following way: from a liquid culture of these cells a sample with 10^6 cells/ml was distributed homogeneously on solid nutrient agar (20 g/liter glucose, 20 g/liter Bacto agar, 5 g/liter yeast extract; thickness of agar plate, 6 mm). When incubated at 30^oC, cell proliferation ceased due to the reduced glucose flux limited by diffusion [22]. Maintenance energy metabolism was guaranteed for

several weeks without loss of proliferation and of repair capabilities, if the cells were kept at 6°C [23]. Before each experiment, the attainment of stationary state was checked by a number of budding cells which was usually less than 1-2 %. After attaining stationary phase of growth, cells were washed with distilled water and resuspended to make a stock solution (10^6 cells/ml).

Cells from the same stock solution were irradiated with ^{60}Co gamma-rays (10 Gy/min) and with ^{239}Pu alpha-particles (17 Gy/min). The gamma-rays dose rate was measured with a Siemens ionization chamber. The alpha-particle dose rate was determined by measuring the intensity and energy of the particles with a semiconductor silicon surface barrier detector at a distance corresponding to the cells. The LET of the particles reaching the cell monolayer was estimated to be 134 keV/μm. Exactly at about this LET value the maximum in RBE-LET relationship was observed for most eukaryotic and some prokaryotic unicellular organisms. The gamma contamination of the alpha source used was negligible, as shown by a control experiment in which the cells were shielded from the alpha particles by a thin foil.

All irradiations were carried out in air at room temperature (20 ± 2°C). Immediately after irradiation, a part of the samples was plated on nutrient agar plates for the assay of colony-forming ability. Another part of the irradiated cell suspension was placed on conditions suitable for the LHR, and in various periods of the LHR (delayed plating) their colony-forming ability was checked again. LHR was carried out in water suspension at 30°C without constant agitation. Survival response on immediate and delayed plating was determined on the basis of the colony counts obtained at the end of 5-7 days of incubation at 30°C and the counts checked again after a further period to ensure that the final score had been reached. Each data point represents average survival for three to six Petri dishes, each containing 50-300 clones. Standard errors of the mean from all experiments are indicated in Figures by bars.

3. Liquid Holding Recovery Model

During the LHR process a number of the primary radiation damage is eliminated resulting in an increased cell survival. It can be considered as the reduction in the initial dose D_1 to a certain effective dose $D_{eff}(t)$ which is proportional to the mean number of residual damages, both reparable and irreversible, after recovery during t hours. Examples of the effective dose estimation are shown in Fig. 1 by the arrows. It was demonstrated for yeast cells [13, 15] that the decrease in the effective dose $D_{eff}(t)$ with the recovery time t was fitted to an equation of the form:

$$D_{eff}(t) = D_1[K + (1 - K)\, e^{-\beta t}], \quad (1)$$

where K is an irreversible component of radiation damage, and β is the recovery constant characterizing the probability of recovery per time unit. In other words, the recovery constant is equal to a fraction of radiation damage recovering per time unit. Parameters K and β were shown [13, 15] to be constant for yeast cell tested over several decade of survival. This model was firstly suggested and its validity was tested for ionizing radiation applied alone in Baltimore [24]. This equation was never tested for high-LET radiation.

The ratio $K(t) = D_{eff}(t) / D_1$ reflects the relative part of the primary radiation damage which has not been repaired during t hours of recovery. If t is sufficiently large (for yeast cells it is about 2-3 days), the recovery curves reach a plateau when the capability of cells to recover is saturated or exhausted. For this moment, we can write

$$K = K(plateau) = D_{eff}(plateau) / D_1. \quad (2)$$

In this expression, $D_{eff}(plateau)$ - the effective dose corresponding to the plateau of the recovery curve and which is proportional to the mean number of irreversible damage. It can be easily shown that

$$e^{-\beta t} = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]. \quad (3)$$

Putting $A(t) = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]$, we have

$$\beta = -[\ln A(t)] / t. \quad (4)$$

In biological terms, $A(t)$ reflects the relative part of the reparable damage that has not been repaired yet after t hours of recovery. Thus, knowing the survival and recovery curves after cell exposure with low- and high-LET radiation, one can calculate the corresponding values of $D_{eff}(t)$, $D_{eff}(plateau)$, $K(t)$, K, and β.

4. Results

Fig. 1 exhibits survival curves (A) and LHR recovery kinetics (B) of diploid yeast cells *Saccharomyces ellipsoideus (vini)*, strain Megry 139-B. Cells were exposed to graded doses of gamma rays (curve 1) or alpha particles (curves 2). Using the results presented in Fig. 1, we estimated the decrement of the relative part of unrecovered radiation damage $K(t)$ as a

function of recovery time for low- (Fig. 2, curve 1) and high-LET (Fig. 2, curve 2) radiation. These data demonstrate that the values of $K(t)$ fluently decreased reaching a plateau after 2-3 days of recovery both for sparsely and densely ionizing radiation. It also appeared from these data that the limited quantities of $K(t)$, i.e. the values of irreversible damage $K(plateau)$, increased with ionizing density.

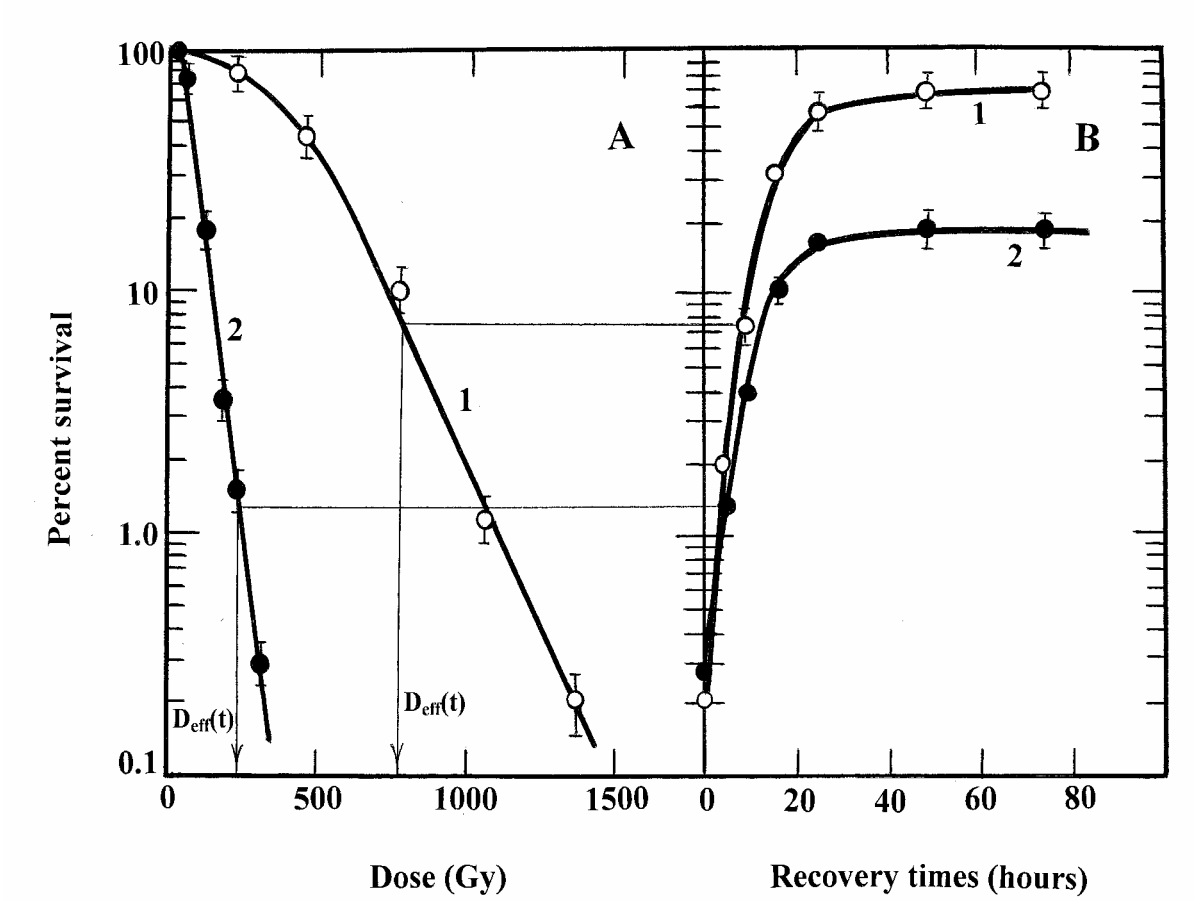

Fig. 1. Survival curves (A) and LHR recovery kinetics (B) of diploid yeast cells *Saccharomyces ellipsoideus (vini)*, strain Megry 139-B. Cells were exposed to graded doses of gamma rays (curve 1) or alpha particles (curves 2). Cells were plated on nutrient agar immediately after irradiation (A) or after various recovery times (B). The arrows indicate examples of the effective dose $D_{eff}(t)$ determination following 5 (alpha irradiation) and 9 (gamma irradiation) hours of recovery. Data are the mean values calculated from at least three independent experiments. Error bars indicate standard error of the mean. Curves were fitted to the data points by eye.

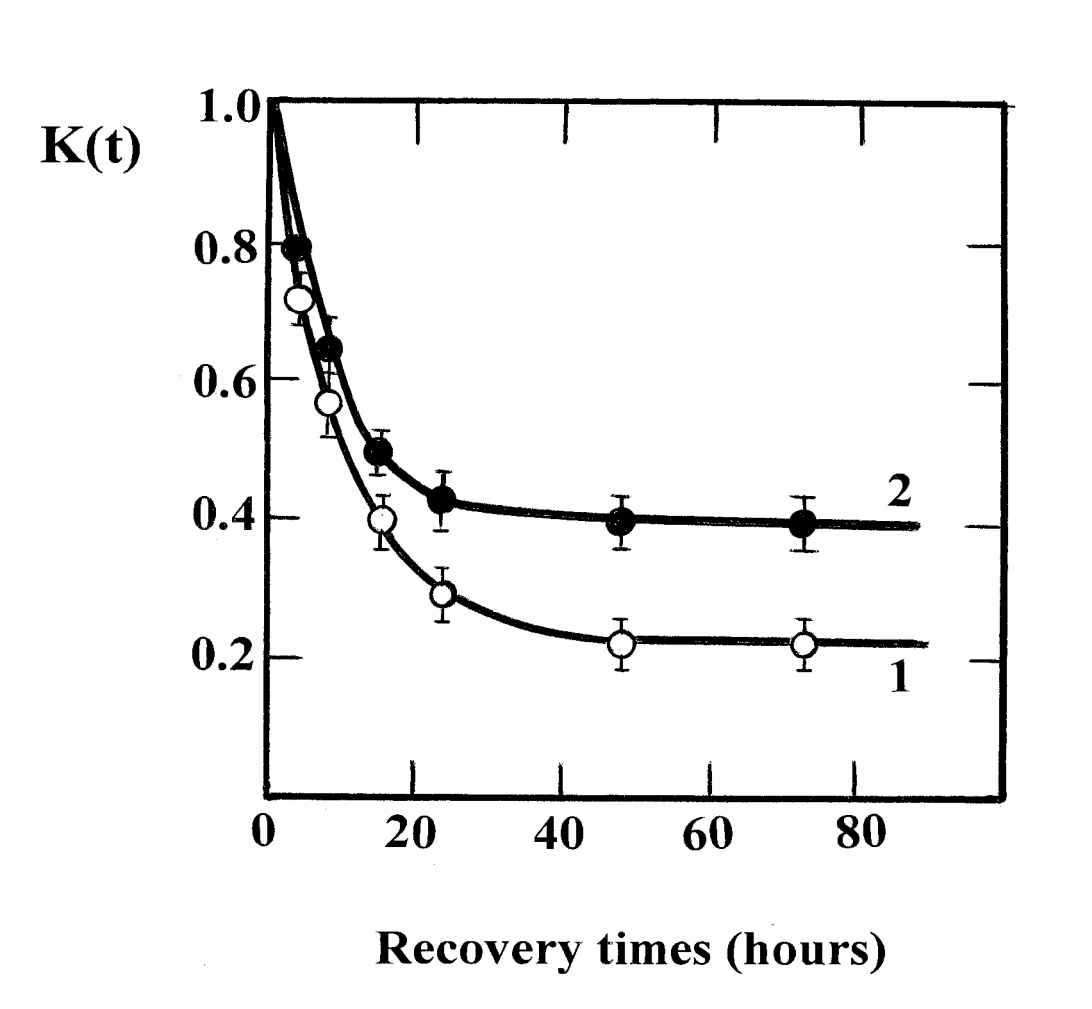

Fig. 2. The decrement of the relative part of unrecovered radiation damage $K(t) = D_{eff}(t) / D_1$ as a function of recovery time of diploid yeast cells *Saccharomyces ellipsoideus (vini)* strain Megry 139-B after treatment with gamma rays (curve 1) and alpha particles (curve 2). Data are the mean values calculated from at least three independent experiments. Error bars indicate standard error of the mean. Curves were fitted to the data points by eye.

The experimental data presented make possible calculation of the relative part of the reparable damage $A(t)$, defined by Eqn. 4. The outcome is shown in Fig. 3 both for low-LET (open circles) and high-LET (closed circles) radiation. It is evident that this function decreases exponentially with the recovery time and does not depend on the quality of radiation. Using Eqn. 4 and the results shown in Fig. 3, we obtained that the recovery constant

β = 0.1 hour^{-1} both for low- and high-LET radiation. It means that about 10% of the residual reparable damage are recovered every hour independently of radiation quality.

The same experimental data have been obtained for all other yeast strains tested in this study. The final results are collected in Table 1.

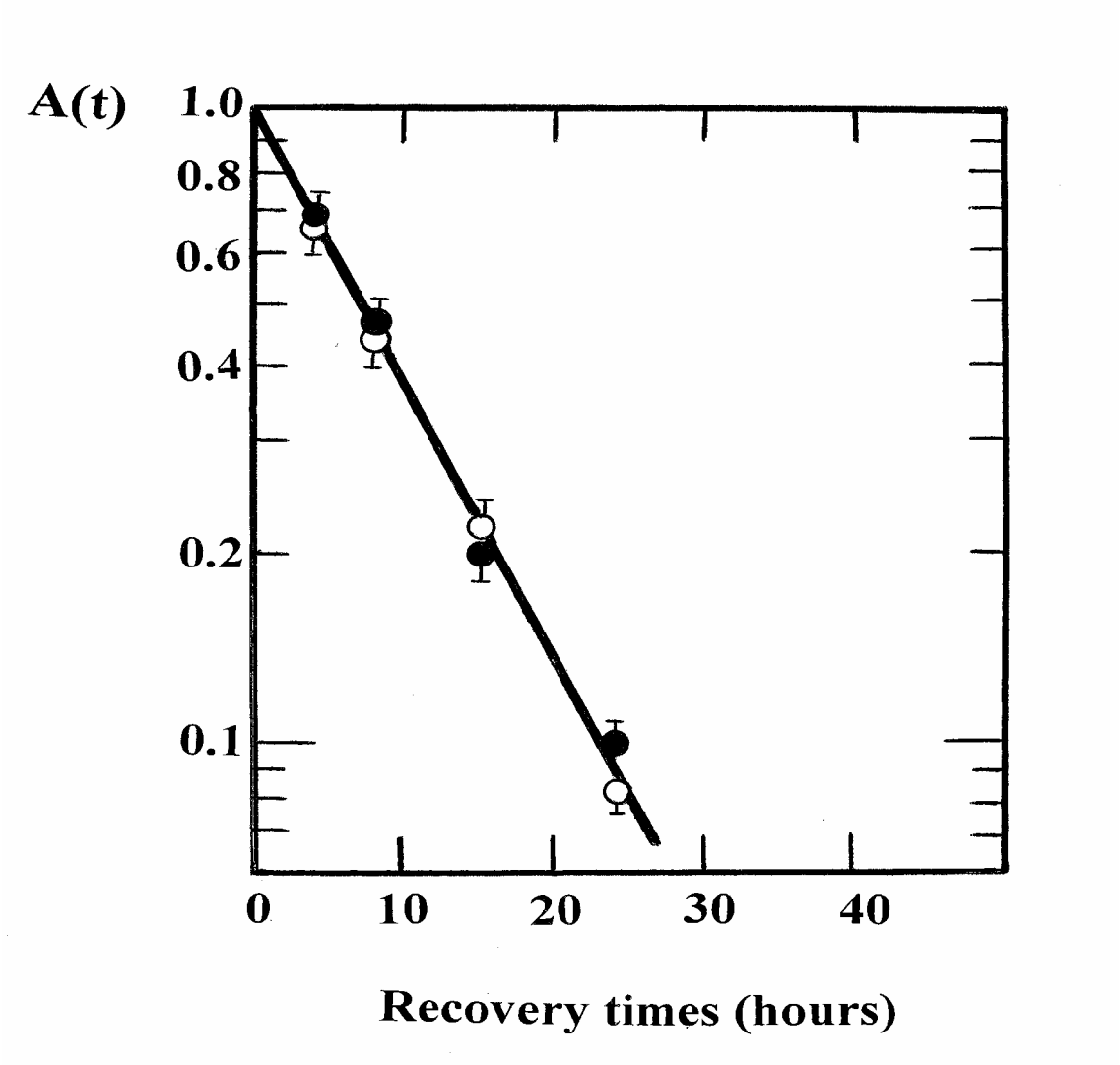

Fig. 3. The decrement of the relative part of recovered radiation damage $A(t) = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]$ as a function of recovery time of diploid yeast cells *Saccharomyces ellipsoideus (vini)* strain Megry 139-B after after treatment with gamma rays (open circles) and alpha particles (closed circles) Data are the mean values calculated from at least three independent experiments. Error bars indicate standard error of the mean. Curves were fitted to the data points by the least-squares method.

Table 1. Radiobiological parameters of diploid yeast cells of different species and strains exposed to γ-rays and α-particles

Strains	Genetict type	$D_o(\gamma)$, Gy	$D_o(\alpha)$, Gy	RBE	K(γ)	K(α)	β(γ)	β(α)
Megry 139-B	RAD RAD	150 ± 12	41 ± 5	3.7 ± 0.3	0.22 ± 0.02	0.40 ± 0.03	0.1 ± 0.02	0.1 ± 0.02
XS800	RAD RAD	265 ± 28	52 ± 7	5.1 ± 0.6	0.32 ± 0.03	0.50 ± 0.04	0.05 ± 0.007	0.05 ± 0.007
XS1924	*rad18* *rad18*	120 ± 14	46 ± 6	3.3 ± 0.2	0.30 ± 0.02	0.52 ± 0.05	0.05 ± 0.007	0.05 ± 0.007
XS1889	*rad53* *rad53*	130 ± 15	30 ± 4	4.3 ± 0.3	0.50 ± 0.04	0.68 ± 0.06	0.05 ± 0.007	0.05 ± 0.007
XS1935	*rad55* *rad55*	130 ± 16	34 ± 3	3.9 ± 0.3	0.37 ± 0.04	0.54 ± 0.04	0.05 ± 0.007	0.05 ± 0.007
XS1943	*rad56* *rad56*	200 ± 21	45 ± 5	4.4 ± 0.3	0.43 ± 0.04	0.45 ± 0.05	0.05 ± 0.007	0.05 ± 0.007
XS1988	*rad57* *rad57*	186 ± 15	43 ± 5	4.3 ± 0.4	0.36 ± 0.04	0.44 ± 0.04	0.05 ± 0.007	0.05 ± 0.007

This Table involves The mean lethal doses D_o after γ- and α-irradiation, the RBE values, and both parameters of postirradiation recovery – the irreversible component and the probability of recovery per time unit both after γ- and α-irradiation. The mean lethal dose corresponds to the dose required to reduce survival to 37% in the exponential region of the survival curves. All survival curves of *rad* mutants were exponential. The RBE of α-particles was calculated as the ratio of the mean lethal doses after low- and high-LET irradiations: RBE = $D_o(\gamma)/D_o(\alpha)$. It could be seen from this Table that the irreversible component was significantly enhanced for densely ionizing radiation in comparison with low-LET radiation while the probability of recovery was identical both for low- and high-LET radiation for all strains investigated. Moreover, the probability of recovery was identical for parent strain (XS800) and radiosensitive mutatnts.

5. Discussion and Conclusions

The action of ionizing radiation on living cells is determined both by physical property of radiation and biological ability to repair potentially effective damage. The pattern of physical energy deposition leads to a certain number and a certain localization and distribution of such damages. They have been extensively studied by microdosimetric models. Data concerning

the role of recovery processes in the RBE of densely ionizing radiation are still limited and a satisfactory mathematical approach to estimate different aspects of this problem is missing.

In this study survival curves and repair kinetics were obtained for a number of diploid yeast cells with different radiosensitivity after low- and high-LET radiation. Using a mathematical model of cell recovery and experimental data obtained in this study it was shown that the irreversible component was enhanced for densely ionizing radiation in comparison with low-LET radiation for all yeast strains studied. It corresponds to a number of data obtained by others in cellular and molecular levels [4-7, 12, 23]. There are several indications from experiments using high-LET radiations which point to the possibility that the principal effect of densely ionizing particles is on the quality of the induced lesions – low-LET radiation produces a relatively large number of reparable damage while high-LET particles generate preferentially irreparable (irreversible) damage [25-27].

The major new results of this paper are related with the probability of recovery. Firstly, it was demonstrated that the probability of recovery was identical both for low- and high-LET radiation for all strains investigated. It means that an identical fraction of reversible radiation damage are recovered per time unit both for sparsely and densely ionizing radiations. It may be concluded on this basis that the recovery process itself is not damaged after densely ionizing radiation and the enhanced RBE of high-LET radiation may be caused by the increased yield of irreversible damage which cells are incapable to recover. Secondly, the probability of recovery was identical for parent strain (XS800) and radiosensitive yeast strains obtained from their parent cells due to some *rad* mutations. These data indicate that the mechanism of their enhanced radiosensitivity is not related with the damage of repair systems themselves but with the production of more severe damage which cells are incapable to recover.

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References

1. R. H. Haynes, “The interpretation of microbial inactivation and recovery phenomena,” Radiat. Res., suppl. 6, 1-29 (1966).

2. R. J. Munson, G. J. Neary, B. A. Bridges, and R. J. Preston, “The sensitivity of *Escherichia coli* to ionizing particles of different LETs,” Int. J. Radiat. Biol., 13, 205-224 (1967).
3. T. Alper, “Cell death and its modification: the role of primary lesions in membranes and DNA.” In: Biophysical Aspects of Radiation Quality, IAEA, Vienna, 171-184 (1971).
4. V. G. Petin, “Effect of gamma and alpha irradiation on survival of wild-type and sensitive mutants of yeast,” Mutat. Res. 60, 43-49 (1979).
5. V. G. Petin, and N. M. Kabakova, “RBE of densely ionizing radiation for wild-type and radiosensitive mutants of yeast”, Mutat. Res. 82, 285-294 (1981).
6. M. Frankenberg-Schwager, D. Frankenberg, and R. Harbich, “Repair of DNA double-strand breaks as a determinant of RBE of alpha-particles,” Br. J. Cancer 49, suppl. VI, 169-173 (1984).
7. M. A. Hannan, M. Paul, and R. L. Phillips, “Fast neutron r.b.e. for lethality and genotoxicity in a wild-type and repair-deficient strain of yeast,” Int. J. Radiat. Biol., 50, 811-824 (1986).
8. D. V. Petin, and V. G. Petin, “Genetic control of RBE of α-particles for yeast cells irradiated in stationary and exponential phase of growth,” Mutat. Res. 326, 211-218 (1995).
9. V. I. Korogodin, Yu. G. Kapul’tsevich, V. G. Petin, and K M. Blisnik, “Radiosensitivity of haplont yeast cells irradiated with sparsely and densely ionizing radiations.” Mutat. Res. 357, 67-74 (1996).
10. C. A. Tobias, E. A. Blakely, P. Y. Chang, L. Lommel, and R. Roots, “Response of sensitive human ataxia and resistant T-1 cell lines to accelerated heavy ions,” Br. J. Cancer 49, suppl. VI, 175-185 (1984).
11. T. M. Coquerelle, K. F. Weibezahn, and C. Lücke-Huhle, “Rejoining of double strand breaks in normal human and ataxia-telangiectasia fibroblasts after exposure to ^{60}Co gamma-rays, ^{241}Am alpha-particles or bleomycin,” Int. J. Radiat. Biol., 51, 209-218 (1987).
12. U. Bertsche, and G. Iliakis, “Radiation quality and repair of potentially lethal damage,” In: Radiation Protection (Ed. By J. Booz and H.G. Ebert), EUR 8395, Brussels, 383-395 (1983).
13. V. I. Korogodin, Yu. G. Kapultcevich, M. N. Myasnik, A. F. Mosin, and V. V. Gridnev, “Cellular repair processes: survival of irradiated yeast, bacteria, and phages under different postradiation conditions,” Adv. Biol. Med. Physics, 12, 253-274 (1968).

14. V. I. Korogodin, Yu. V. Korogodina, M. N. Myasnik, and E.N. Sokurova, “Radiation induced damages to cells,” Studia Biophysica, 15/16, 127-144 (1969).
15. V. I. Korogodin, “The study of post-irradiation recovery of yeast: the ‘premolecular period’,” Mutat. Res., 289, 17-26 (1993).
16. M. Brendel, and R.H. Haynes, “Interaction among genes controlling sensitivity to radiation and alkylation in yeast,” Mol. Gen. Gen., 125, 197-216 (1973).
17. A. N. Luchnik, V. M. Glaser, and S. V. Shestakov, “Repair of DNA double-strand breaks requires two homologous DNA duplexes,” Mol. Biol. Repts., 3, 437-442 (1977).
18. T. Saeki, I. Machida, and S. Nakai, “Genetic control of diploid recovery after γ-irradiation in the yeast *Saccharomyces cerevisiae*,” Mutat. Res., 73, 251-265 (1980).
19. V. I. Korogodin, “Some regularities of the post-irradiation changes in resting yeast cells,” Biofizika 3, 703-710 (1958).
20. M. H. Patrick, R. H. Haynes, and R. B. Uretz, “Dark recovery phenomenon in yeast. 1. Comparative effects with various inactivating agents,” Radiat. Res., 21, 144-163 (1964).
21. R. H. Haynes, “DNA repair and the genetic control of radiosensitivity in yeast,” In: Molecular Mechanism for Repair of DNA (P. C. Hanawalt, R. B. Setlow, Eds.) Part B, Academic Press, New York, 529-540 (1975).
22. W. Pohlit, and I. R. Heyder, “Growth of cells on solid culture medium,” Radiat. Environ. Biophys., 14, 213-230 (1977).
23. U. Bertsche, “The response of diploid yeast to radiations of different LET,” Radiat. Res., 76, 349-367 (1978).
24. H. O. Davidson, B*iological Effects of Whole-Body Gamma Radiation on Human Being.* The Johns Hopkins University, Baltimore, 1957.
25. W. E. Pohlit, M. Schäfer, “Recovery and repair in yeast cells after irradiation witjh densely ionizing particles,” In: B*iological Effects of Neutron Irradiation*, Vienna, IAEA-SM-179/23, 177-184 (1974).
26. W. F. Ward, H. Aceto, and M. Sandusky, “Repair of sublethal and potentially lethal radiation damage by eat embryos exposed to gamma rays or helium ions,” Radiology, 120, 695-700 (1976)/
27. M. A. Ritter, J. E. Cleaver, and C. A. Tobias, “High-LET radiations induce a large proportion of non-rejoining DNA breaks,” Nature, 266, 653-655 (1977).