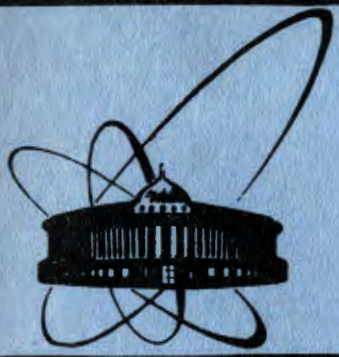

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CELL SENSITIVITY TO IRRADIATION AND DNA REPAIR PROCESSES.

DNA Damage and Its Repair in Escherichia Coli

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INTRODUCTION

It is generally accepted that macroscopic cell tissue reaction to radiation therapy is the consequence of stem cell depletition to some critical level. The new cell tissue kinetic model has enabled us to relate quantitatively the level of stem cell survival to the observed tissue reaction [20.21]. However, the utilization of the tissue kinetic models depends on the quality of the underlying cell survival model. Such a model would be of great importance in the case of high-LET therapy as well as for non-standard regimes of gamma-rays therapy.

The development of a realistic cell survival model is not a trivial problem. A lot of experimental results from molecular and cellular biology including repair processes as well as radiation physics should be considered and adequate mathematical apparatus should be derived. That is why we have tried to start from bacterial cells E.coli where the most complete information exists concerning the structure of genetic apparatus and the repair mechanisms. Furthermore, the utilization of various heavy particle beams seems to provide important additional information on the types of cell injuries and its significance for cell death. Therefore, we have analysed the effectiveness of ionizing radiation as a function of cell repair capabilities as well as the dependence of the cell sensitivity on the linear energy transfer (L) and energy (E) of the given radiation.

The effectiveness of radiation in cell killing has been explaining by a number of models based on different ideas. The development of the target theory as well as microdosimetric approach lead to formal models which considered only physical laws of the interaction of various ionizing radiations with hypothetical targets. Similarly, repair models have been highly hypothetical and far from real molecular mechanisms. It is obvious at present time, that the effectiveness of different radiations is determined by both physical distributions of energy deposits in genetic structures and biological repair processes.

Basic molecular events leading to cell death are single and double strand breaks (SSB and DSB). There are, however, several types of SSB and DSBs [22]. The main part of gamma-induced DSBs are in the case of E.coli enzymatic DSBs [5] arising as an intermediate product during the repair process. High-LET radiation will induce greater part of direct DSBs (dDSB) unrepairable by E.coli cells.

Earlier attempts to explain the effectiveness of ionizing radiation on bacterial cells [26,15] did not consider repair processes and did not distinguish different types of SSBs and DSBs.

BASIC TYPES OF DNA INJURIES AND THEIR REPAIR

SSBs and DSBs have been shown to be the most important DNA damage directly induced in DNA by ionizing radiation [5,19,3]. True SSBs represent, however, only a part of the whole SSBs number induced further by repair processes from gamma-sites (e.g., thymine-methyl groups [8,17,32], apurine and apyrimidine sites [10], base and sugar damage [24]). These gamma-sites are recognized by specific endonucleases, which transfer them to SSBs [7,27,33]. The endonucleases and polymerases activities determine the number of SSBs during and after irradiation. The production of SSBs by γ-radiation measured by various authors actually varies from 10^{-11} cGy^{-1}.D^{-1} to $2 \cdot 10^{-13}$ cGy^{-1}.D^{-1} [38,18,6]. This can be explained by the fact that different parts of SSBs are repaired by superfast and fast repair under different experimental conditions.

Three levels of repair processes have been identified [35]. Later on the superfast repair was disproved to cause the oxygen effect [18]. In spite of it, the superfast repair seems to exist [13.12] and is probably oxygen-independent. Ligase dependent repair I joins mainly 3´OH - 5´PO_4 ends. These products of γ-irradiation are measured as SSBs, nevertheless, they do not require the second strand of DNA to their recovery.

Repair II joins mainly 3´PO_4- 5´OH , 3´OH - 5´OH ends and 3´OH - 5´PO_4 ends without nucleatide [13.12]. Such SSBs can be repaired by polymerase I according to the second DNA strand during approximately 10 minutes after irradiation at 0°C.

The damage that cannot be repaired by the two mentioned steps is the substrate for repair III, controlled mainly by rec genes. This repair needs synergic action of several enzymes [5,3].

Initial γ-sites, when repaired, can be incised by endonucleases [13,12] as well as irreparable sites. The short gaps (SSB_1), when repaired by means of repair II, can be excised by exonucleases as well as $SSBs_1$ irreparable by repair II. Exonucleases (mainly exonuclease V) form wide gaps in DNA [5,3]. Extensive degradation of DNA (several tens of percentage) leads to the destruction of the whole genome in some cells [28-30] others can restrict the degradation by means of lexA-recA genes controlling recA-protein.

According to the latest data on the induction and repair of DNA DSBs they are induced mainly by enzymatic repair [5,1]. It was reasoned that the first DSB fixed in E.coli leads to the

cell death[5] because of the correlation between the dose forming in average one fixed DSB (D_{DSB}) and the mean lethal dose D_0 under various conditions. Enzymatically induced DSBs (eDSBs) are measured as DSBs by both sedimentation and elastoviscosimetric methods although they are repaired as SSBs by repair enzymes. The numbers of SSBs repaired by slow repair has been measured as absolute numbers (3-5 breaks per cell) in some experiments[35] or as relative - dose dependent - numbers[6]. The dose-effect curves for DSBs have been straight lines for both mammalian and E.coli cells[2]. The hypothesis explaining these facts will be put forward.

There is no correlation between D_{DSB} and D_0 dose for sensitive mutants, e.g., for rec ABC mutant cells D_{DSB} = 100 Gy and D_0 = 10 Gy. It means that there are other lethal events in the case of sensitive mutants. The first fixed DSB in the genome of superresistant Gamm 444 mutant arises when the dose corresponds to the mean lethal dose D_0. This fact suggests that there could be no repair of DSBs in the case of Gamm 444 mutants.

We can conclude that the initial DNA sites could be SSBs or can be transformed to SSBs by repair processes. The repair is controlled by a number of structural and regulation genes. Three levels of the repair could be established. The degradation is a necessary step of repair processes and leads to enzymatic DSBs. The degradation can be restricted but cannot be avoided. The mechanisms of DNA DSBs formation will be discussed laler.

Current experimental evidence enabled us to perform theoretical analysis of DNA repair processes after irradiation, cell lethality and the effectiveness of various types of radiations on E.coli.

THE SCHEME OF THE MODEL, METASTABLE SITES

The described repair processes can be illustrated by the following scheme (Fig.1). The dose D induces the initial γ-sites (N_γ) and direct DSBs. There are γ-sites repairable by level I of repair processes (N_γ^r) and irrepairable in this way dites (N_γ^{ir}). The repairable sites may be repaired or incised by endonucleases.

Let the probabilities of the induction and the repair of various injuries be q_1, q_2, q_3; p_1, p_2, p_3 per time unit. The repairable γ-sites are repaired with the probability p_1 and incised with the probability q_1 similarly, the irrepairable γ-sites are incised with the same probability. SSBs arised after this step of repair process are short (up to some tens of nucleotides). We shall denote them SSB_1. These injuries can be repaired by polymerase I. It is reasonable to assume that $SSBs_1$ can be both repairable and irrepairable by fast repair II. Cor-

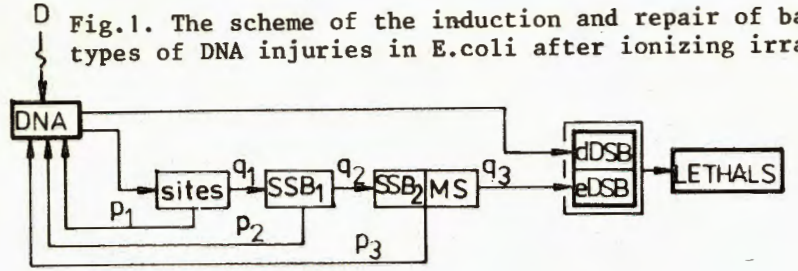

Fig.1. The scheme of the induction and repair of basic types of DNA injuries in E.coli after ionizing irradiation.

responding probabilities of the repair and exonuclease excision are p_2 and q_2, respectively. Extensive degradation and resynthesis may shift the gaps close together and form metastable sites (MS). They can be formed also by S1-endonucleases which can transfer SSBs to DSBs. MS can be repaired as SSBs (SSB_2) though they are measured as DSBs. They are repaired with the probability p_3 and fixed with q_3.

It can be assumed that MS arise as the consequence of the mechanisms restricting degradation in E.coli cells. The degradation is restricted by recA-protein coded by recA genus. recA-protein is induced by the products of DNA degradation. The process is regulated by recA and lexA genes[14].

The length of degraded fractions can have some maximum value but may be shorter in the case when damaged DNA is met on the opposite strand of DNA and MS is formed from a pair of $SSBs_2$ on the opposite DNA strands separated by a short region of undamaged DNA so as they are measured as DSBs. Each SSB_2 contributes to the induction of two MS and so the dependence of N_{MS} on the dose is linear (at least for greater doses).

The given assumption is in agreement with the fact that the dependence of the amount of degraded DNA on the dose forms plateau for great doses[16]. The existence of some average length of DNA regardless of the presence of damage on the opposite DNA strand in the degraded region should lead to unlimited degradation with increased dose.

KINETIC EQUATIONS OF THE REPAIR PROCESSES

We shall denote: n_γ^r and n_γ^{ir} are the numbers of repairable and irrepairable γ-sites at the moment t after a short pulse of radiation ($n_\gamma = n_\gamma^r + n_\gamma^{ir}$); $n_{SSB_1}^r$ and $n_{SSB_1}^{ir}$ are the numbers of repairable and irrepairable $SSBs_1$; again $n_{SSB_1} = n_{SSB_1}^r + n_{SSB_2}^{ir}$ is the number of SSB_2 at the moment t after irradiation, n_{MS} is the number of metastable sites. We shall assume independent and random repair of individual sites or breaks.

If the dose is great enough, every additional SSB_2 leads to the induction of an additional MS and so $n_{MS} = n_{SSB_2}$.

The kinetics of repair processes will be approximated by the following differential equations:

$$dn_\gamma = -(q_1 + p_1)\cdot n_\gamma^r \cdot dt - q_1 \cdot n_\gamma^{ir} \cdot dt, \quad (1)$$

$$dn_{SSB_1} = -(q_2 + p_2)\cdot n_{SSB_1}^r \cdot dt - q_2 \cdot n_{SSB_1}^{ir} \cdot dt, \quad (2)$$

$$dn_{MS} = q_2 \cdot n_{SSB_1} \cdot dt - (q_3 + p_3) n_{MS} \cdot dt \quad (3)$$

with the following initial conditions: $n_\gamma^r(0) = N_\gamma^r$, $n_\gamma^{ir}(0) = N_\gamma^{ir}$, $n_{SSB_1}^r(0) = N_{SSB_1}^r$, $n_{SSB_1}^{ir}(0) = N_{SSB}^{ir}$, $n_{SSB_2}(0) = n_{MS}(0) = 0$. The repair I and II will be considered as independent pathways of DNA damage elimination. The speed of processes 2-3 corresponding to repair levels II-III differs at least by one order. The number of SSB_1 after finishing repair I is

$$N_{SSB_1} = N_\gamma^r \frac{q_1}{q_1 + p_1} + N_\gamma^{ir}. \quad (4)$$

The number of SSB_2 after finishing repair II is

$$N_{SSB_2} = N_{SSB_1}^r \frac{q_2}{q_1 + p_2} + N_{SSB_1}^{ir}. \quad (5)$$

The number of enzymatic DSBs (n_{DSB}) arising from MS with the probability q_3 can be calculated at the moment t after irradiation by solving the equation

$$dn_{DSB} = q_3 \cdot n_{MS}(t) \cdot dt \quad (6)$$

with the initial condition $n_{DSB}(t = 0) = 0$:

$$n_{DSB}(t) = \frac{q_2 \cdot N_{SSB_1}^r \cdot q_3}{p_3 + q_3 - p_2 - q_2} \cdot \left[\frac{1 - e^{-(p_2 + q_2)\cdot t}}{p_2 + q_2} - \frac{1 - e^{-(p_3 + q_3)\cdot t}}{p_3 + q_3}\right] + \frac{q_2 \cdot N_{SSB_1}^{ir} \cdot q_3}{p_3 + q_3 - q_2} \cdot \left[\frac{1 - e^{-q_2 \cdot t}}{q_2} - \frac{1 - e^{-(p_3 + q_3)\cdot t}}{p_3 + q_3}\right] + N_{dSSB}, \quad (7)$$

where N_{dSSB} represents the number of direct DSBs (formed by radiation).

After several hours repair III is finished and Eq. (7) turns to

$$n_{DSB} = N_{SSB_2} \cdot \frac{q_3}{p_3 + q_3} + N_{dDSB}, \quad (8)$$

where the first term represents the number of enzymatic DSBs, the second is the number of direct DSBs.

THE DOSE DEPENDENCE OF MS OR DSBs INDUCTION

The dose dependence is an important characteristic. It has been shown that this dependence is quadratic for DNA solutions but linear for DNA in cells. According to the model of Chadwick and Leenhouts /9/ the DSBs can be formed from two SSBs and so the dose dependence should be quadratic.

Our enzymatic DSBs are also formed from SSBs. If the degradation is responsible for mechanism, the dose dependence will be quadratic for very low doses only /22/:

$$N_{DSB}(D) = \frac{q_3}{p_3 + q_3} \cdot N_{SSB_2} \cdot D \cdot \left(1 - e^{-\frac{N_{SSB_2} \cdot \ell_0 \cdot D}{M}}\right). \quad (9)$$

For doses great enough the dependence will turn to linear. If maximum length of DNA degradation ℓ_0 is great enough, the dependence will be linear even for low doses. Of S1 endonucleases induce MS, the dose dependence will be linear, too.

THE PARAMETERS OF E.COLI CELLS

The parameters of wild cells. The induction of SSB_1 immediately after irradiation is $2 \cdot 10^{-12}$ cGy^{-1} D^{-1} /2,11,36,34/. The induction of SSB_2 is of the order 10^{-13} cGy^{-1} D^{-1} /3,6,25/. The number of determined DSBs equals the sum of enzymatic DSBs, MS, and direct DSBs:

$$n_{DSB}^{measured} = N_{SSB_2} \cdot \frac{q_3}{p_3 + q_3} \cdot (1 - e^{-(p_3 + q_3)\cdot t}) + N_{SSB_2} \cdot e^{-(p_3 + q_3)\cdot t} + N_{dDSB}. \quad (10)$$

The dependence has been experimentally determined /5/ and the optimal parameters corresponding to this dependence are $N_{SSB_2} = 0.047$ Gy^{-1} per genom, $p_3 = 1.8$ h^{-1}, $q_3 = 0.225$ h^{-1} (we have assumed $N_{dDSB} = 0$), see Fig.2.

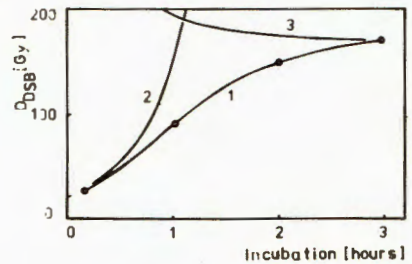

Fig.2. The dependence of the dose inducing in average one DSB per genom of E.coli D_{DSB} on the time of incubation (1). Experimental points taken from /5/. Curve 2 corresponds to metastable sites, curve 3 corresponds to fixed eDSBs.

In the case when degradation leads to MS induction, the number of lethal events per genom at the dose D will be

$$H = N_{SSB_2} \cdot D \cdot \frac{q_3}{p_3 + q_3} \cdot \left(1 - e^{-\frac{N_{SSB_2} \cdot D \cdot \ell_0}{M}}\right) + N_{dDSB} \cdot D. \qquad (11)$$

Cell survival can be then calculated as exp(–H). The shoulder on the survival curve determines the lower limit for ℓ_0 (the shoulder can be formed by further mechanisms, too). $\ell_0 \geq 5 \cdot 10^5$ nucleotides for wild type cells[3].

The parameters of the sensitive mutants. A number of repair defects can lead to the increase of the sensitivity to ionizing radiation[3,38,37]. In recA mutants the synthesis of recA-protein as well as other functions of recA genus are restricted. This leads to markedly increased value of D_0, D_0 = 10 Gy[3,35]. Extensive degradation has been observed[3,4]. The increased sensitivity can be explained by the assumption that the first SSB_2 leads to the lethal effect. However, this assumption leads to the conclusion that repair levels I and II operate with increased efficiency. Actually, these repair levels have been shown to be more effective for rec mutants[31]. The value of D_0 = 10 Gy corresponds to the production of SSB2: N_{SSB_2} = 0.1 Gy^{-1}.

RecABC mutants possess the defects mentioned above; in addition to them there is inhibited exonuclease V (this exonuclease provides degradation in wild type cells and recA mutant). The recABC mutant cells are very sensitive to radiation[5,4], D_0 = = 10 Gy although the dose D_{DSB} = 100 Gy and does not depend on the time of postirradiation incubation[5]. The lethal events could be again SSB_2 but without long gaps. The length corresponding to D_{DSB} = 100 Gy is $\ell_0 = 2 \cdot 10^4$ nucleitudes[22].

The pol^- mutant is similarly sensitive to radiation as rec mutants (D_0 = 10 Gy). The only defect in polymerase I brings about defect in repair II and probably in repair III to some extent, too. This should lead to an increased induction of MS repaired with some probability. Owing to the value of D_0 = = 10 Gy the fixation probability should be of about 0.2.

The parameters of the superresistant mutant Gamm 444. The processes of degradation and recA-protein induction are probably better coordinated in the case of Gamm 444 mutants. The characteristics are: D = 720 Gy, D_{DSB} = 150 Gy immediately after irradiation and D_{DSB} = 800 Gy after 3-hour incubation[6]. The radioresistance can be explained by greater efficiency of the repair III (lower value of q_3) with lower induction of enzymatic DSBs. If N_{SSB_2} = 0.05 Gy^{-1} per cell (as for wild

type) we obtain q_3 = 0.05 h^{-1}. The lower limit for ℓ_0 is $2 \cdot 10^5$ nucleotides. An alternative possibility is better repair of SSBs (repair II). This should lead to lower values of SSBs after short periods of incubation and to lower values of OER after γ-irradiation[23].

CONCLUSIONS

We have reviewed the main types of DNA injuries and the pathways of their repair. A mathematical model of the repair processes has been proposed and some parameters estimated in the case of both wild type cells and various mutants of E.coli. The model provides a basis for further theoretical thinking explaining main features of important radiobiological problems including the LET dependence of cell sensitivity, oxygen enhancement ratio, cell sensibilization and protection against ionizing radiation. All these factors are important for the radiation treatment planning and their understanding could markedly improve the control probability rates in many cases.

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Чувствительность клеток к облучению и процессы репарации ДНК. Повреждения ДНК и их репарация у Escherichia coli

Рассмотрены основные типы повреждений ДНК клеток, индуцируемых γ-облучением. Показано, что у E.coli разным уровням процесса репарации повреждений соответствуют различные типы повреждений ДНК. Обсуждается роль баланса активности ферментов репарации в связи с образованием энзиматических двухнитевых разрывов ДНК. Построена простая модель инактивации различных мутантов E.coli на основе имеющихся экспериментальных данных. Вводится понятие "метастабильное состояние". "Метастабильное состояние" образуется из больших деструктивных пробелов в молекуле ДНК, измеряемых как ДР и репарируемых как ОР. Приводится решение кинетических уравнений модели и ее параметры, определенные для чувствительных и резистентных мутантов.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

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Cell Sensitivity to Irradiation and DNA Repair Processes. DNA Damage and Its Repair in Escherichia Coli

Basic types of DNA damage produced by γ-radiation in cells are reviewed. Different types of DNA injuries are related to various levels of DNA repair processes, established in the case of E.coli. The role of the balance of repair enzymes activities is considered in connection with the induction of enzymatic DNA double strand breaks. A simple model of the inactivation of different mutants of E.coli cells has been constructed on the basis of available experimental data. The concept of "metastable sites" has been introduced. "Metastable sites" are formed from great nucleolytic gaps, they are measured as DSBs although they can be repaired as SSBs. Kinetic equations of the model are solved and the parameters are determined for both sensitive and resistant mutants.

The investigation has been performed at the Laboratory of Nuclear Problems, JINR.

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