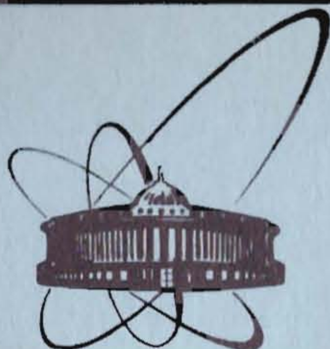

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CELL SENSITIVITY TO IRRADIATION AND DNA REPAIR PROCESSES

Oxygen Enhancement Ratio in Different Mutants of Escherichia Coli

Submitted to "Neoplasma"

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Anoxic protection of tumour cells represents one of the most serious problems in radiation therapy. The dose corresponding to given tissue reaction increases in anoxic conditions approximately 3 times. The nature of the oxygen effect remains, however, unclear as well as the possibilities of oxygen enhancement ratio (OER) modification by various agents. Two main hypotheses have been proposed to explain decreasing OER for high-LET radiation [1,2]. It will be shown that neither of them conforms to recent experimental findings. In order to explain different nature of radiation cell injuries in oxygenated and anoxic conditions, T.Alper introduced the concept of two targets for cell killing [1]. This so-called "type N, type O damage" model explained to some extent differencies in OER between various mutants under the assumption that DNA in cells is protected by oxygen. This assumption is, however, in contradiction with a number of experiments showing that DNA injuries are more frequent in oxygen, particularly DNA strand breaks. The models of the oxygen effect realisation including repair [9,11] explained decreasing OER in the case of repair deficient mutants. The reverse effect (increased OER) observed in $polA^-$ mutants of E.coli could not be explained. Molecular injuries have not been specified in these models.

We propose a new model of oxygen effect realisation in E.coli cells based on the model of cell killing developed in our previous papers [16,17]. The following aspects of oxygen effect will be analysed: 1) the values of OER in different mutants of E.coli K-12, 2) the dependence of OER on the conditions of cultivation, 3) the influence of some protectors and sensitizers on the values of OER, 4) the dependence of OER on linear energy transfer (LET) of the radiation, 5) the concentration dependence of oxygen effect. Mutual relations of the aspects mentioned above are considered, too.

METHODS

DNA Injuries, their Repair, and the Scheme of the Model

DNA injuries and their repair pathways have been described in our previous paper [16]. A simple scheme of repair kinetics has been put forward. The scheme is worked out in Fig.1 in order to stress some details which are important for the analysis of OER realisation.

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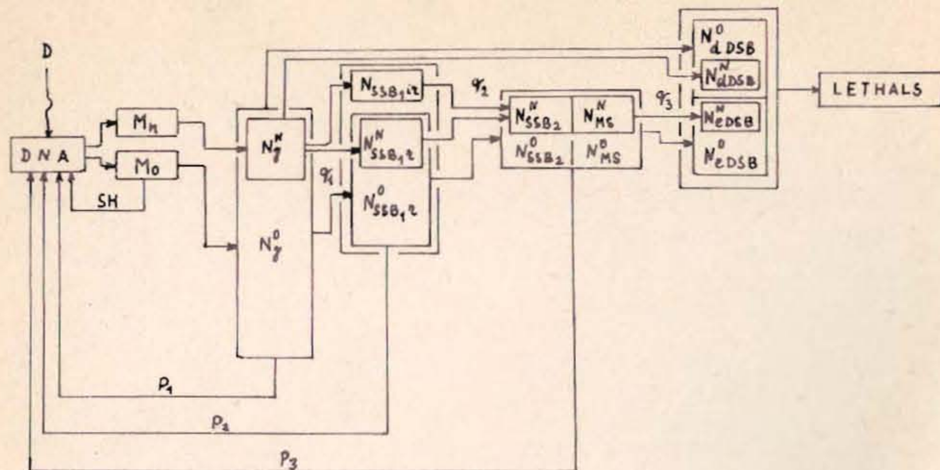

Fig.1. The scheme of DNA damage realisation and its repair by various repair processes. See text for detailed explanation.

It is widely accepted at present time that DNA damages have decisive role in cell inactivation by ionizing radiation [22,7]. We shall, therefore assume in our model that DNA is the only sensitive target in the cell.

The initial DNA radicals can be divided into two classes: modifiable (M_0) and unmodifiable (M_n) by oxygen. Modifiable radicals can interact with SH compounds being recovered or they can interact with oxygen being fixed [14,18]. The main SH compound competitive with oxygen is glutathione [33,32,10]. The molecular structure of interacting SH compounds can be of two types: RSH and RS^-. Some experiments have given evidence that glutathione reacts with target radicals as an electron donor [33,10], others have shown, however, that glutathione is a hydrogen donor [14,18,4]. The main and probably the only acceptor of electrons in a cell is oxygen.

M_0 type of DNA damage is represented mainly by initial single strand breaks (SSBs) induced directly by radiation (without any action of repair enzymes) and alcali-labile sites [31,27]. The value of OER for these injuries is about 4-5 [31,25,24,27,15]. M_n type of DNA damage is formed by some base injuries: t' products [8,23], alcali-labile sites arising from O_2-independent DNA radicals - e.g., C4'- radicals of deoxyribose [34,36], pyrimidine dimers [37], crosses DNA-DNA and DNA-protein. All the mentioned DNA injuries have been called γ-sites [16]. Their fate in the repair process is different. Some part of the initial γ-sites can be repaired (probability p_1 in Fig.1). SSBs with 3'OH - 5'PO_4 ends can be repaired by DNA-ligase in adenylate complex [13,12]. In contrast to initial SSBs, base damages are transformed to SSBs by specific endonucleases. Alcali labile and alcali stable sites are transformed to SSBs by AP-endonucleases, DNA-N-glycosilases and γ-endonucleases in a short time (~1 minute [27,40]). Uvr^+ strains of E.coli are able to cut out pyrimidine dimers by UV-endonucleases. A small number of pyrimidine dimers is also induced by γ-radiation [21]. The probability per unit time of the induction of SSB from base damages or alcali labile sites have been denoted q_1 in Fig.1.

SSBs repaired according to the second DNA strand (a part of SSBs induced directly by radiation or arising from enzyme labile sites) has been calles "Type I SSBs" ($SSBs_1$) [16]. A substantial part of $SSBs_1$ is repaired by polymerase I (polA-dependent repair). This repair includes type II repair of SSBs [31] and excision repair by short fragments [42,20].

It is well known that in wild type cells there exists also repair by long fragments of DNA [42,16] which is ATP-dependent. This repair includes action of a great number of enzymes (recA protein, exonuclease V, DNA-polymerase III, etc.). The fact that SSBs are not fully repaired by short fragments of DNA is the consequence of the following reasons: 1) $SSBs_1$ when repaired by polA-dependent repair, can be randomly attacked by exonucleases [7,16], 2) the access to some $SSBs_1$ can be impeded for polymerase I [42], 3) some $SSBs_1$ can be irrepairable by polA-dependent repair [19,34]. The irrepairable $SSBs_1$ can be the breaks with the most complex structure of damaged ends [42]. The fraction of the irrepairable $SSBs_1$ has been estimated to roughly 20% of the total amount of $SSBs_1$ [12]. The 3' ends of these $SSBs_1$ are not specific to polymerase I, DNA-ligase, phosphatase, and exonuclease III. These injuries contain, possibly, remainders of deoxyribose [12].

Combined injuries of the base and deoxyribose or the break of phosphodiester bond combined with the injury of deoxyribose are probably irrepairable by polA-dependent repair, too. Such damages can arise from low energy electron clusters of ions. In the case of γ-rays approximately 5% of energy is departed to water by these low energy electrons [38]. The number of these injuries should depend on the oxygen tension to smaller extent than the number of other $SSBs_1$. We shall denote $SSBs_1^r$ the repairable by polA-dependent repair $SSBs_1$ and $SSBs_1^{ir}$ the irrepairable in this way $SSBs_1$. The main part of $SSBs_1$ is repaired by polA-dependent repair (fast repair). The probability of SSB_1^r repair per unit time will be donoted p_2.

Both repairable $SSBs_1^r$ having been attacked by exonucleases and irrepairable $SSBs_1^{ir}$ are transformed by exonucleases to long gaps ($SSBs_2$). The exonucleases desintegrate long regions of DNA strands (see [16]). The most powerfull exonuclease is

exonuclease V. The exonuclease attack probability per unit time will be denoted q_2. The nature of $SSBs_2$ is such that they lead to the formation of metastable sites [16]. Metastable sites (MS) are the DNA injuries which are measured by sedimentation techniques as double strand breaks (DSBs), but they are nevertheless repaired according to the second DNA strand by recA-lexA dependent repair (slow repair). MS does not require the second DNA copy and so they are not repaired by recombination repair [16]. MS can be fixed to enzymatic DSB (eDSB) not repairable by E.coli cells. The probabilities of MS repair or fixation per unit time are p_3 and q_3 in Fig.1. The relation between $SSBs_2$ and MS depends on the particular mechanism of MS realisation [16]. We shall assume that one SSB_2 leads to the induction of one MS. Another type of linear relation between $SSBs_2$ and MS could be assumed. This does not influence the results of our paper.

The rate of DSBs directly induced by radiation is probably markedly smaller than the rate of MS induction or enzymatic DSBs induction [7,16]. Enzymatic or direct DSBs (eDSBs or dDSBs) are lethal events in E.coli cells. They cannot be repaired.

The following denotations will be used throughout the paper: $N^{c,d}_{a,b}$ is the number of injuries induced after irradiation per unit dose per genome (dalton). $OER^{d}_{a,b}$ is the oxygen enhancement ratio. $D_0^{c,d}$ is the mean lethal dose determined from the exponential part of a survival curve. These quantities can have several indices:
left and lower (a) is the type of damage (γ = initial sites, SSB_1, SSB_2, MS, eDSB, dDSB, DSB = the sum of eDSB and dDSB, the absence of the index is concerned to lethal events),
right and lower (b) can be r = repairable or ir = irrepairable (in the case of γ-sites or $SSBs_1$),
left and upper (c) represents the conditions of irradiation O= = saturated by oxygen, N = anoxic conditions,
right and upper (d) is the type of E.coli mutant (pol = $polA^-$, rec = $recA^-$, wt = wild type, uvr = $uvrA^-$ or $uvrB^-$, ru = $recA^-uvrA^-$, and pl = $polA^-lexA^-$).
If the index is absent, it means that the quantity is concerned to all possible indiced or has general meaning. The values of radiosensitivity $1/D_0$ can be used with the c,d indices, too. Repair parameters F and S (introduced later on) can be used with the index d.

We have shown that $OER_{SSB_1 ir} < OER_{SSB_1 r}$ owing to the fact that SSB_1^{ir} include multiple demaged sites. To give clear picture of the OER realisation in different mutants of E.coli, we shall assume that oxygen does not influence SSB_1^{ir} at all. This abstraction is necessary for understanding the model and it does not change most of the results. Some differencies bet-

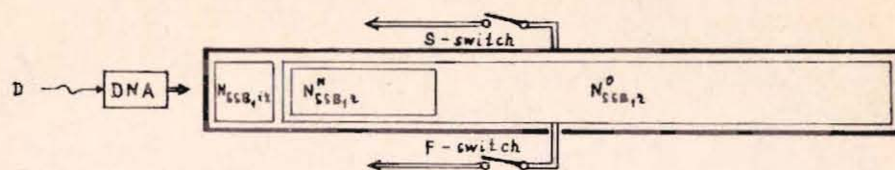

Fig.2. The realisation of oxygen effect through DNA repair. F - switch triggers fast repair, S - switch triggers slow repair. $N_{SSB_1 ir}$ *is the number of single strand breaks irrepairable by fast repair. This number does not depend on oxygen tension.* $N^{0(N)}_{SSB_1 r}$ *is the number of repairable by fast repair* $SSBs_1$ *in oxygenated (anoxic) conditions.*

ween the assumption $OER_{SSB_1 ir} = 1$ and $OER_{SSB_1 ir} > 1$ will be discussed later on.

RESULTS

Determination of Oxygen Effect and Cell Sensitivity by DNA Repair Processes

A principal scheme of the model is shown in Fig.2. The initial steps of physical, chemical and fast enzymatic processes lead to short single strand breaks ($SSBs_1$). The number of these DNA damages should be equal for wild types and some mutants which do not differ in ligase and endonucleases activities. Each single strand break can be repaired by fast (F) or slow (S) repair except $SSBs_1^{ir}$ which are irrepairable by fast repair (see Fig.2). The F-repairable $SSBs_1^{r}$ are mostly repaired by fast repair in wild type cells as the fast repair operates more quickly than the slow repair. According to our assumption discussed above the irrepairable injuries are also unmodifiable by oxygen. The F-repairable $SSBs_1^{r}$ are mostly modifiable by oxygen except for some fraction of them remaining in anoxic condition $N^{N}_{SSB_1 r}$ (see Fig.2). Oxygen enhancement ratio for $SSBs_1$ is $OER_{SSB_1} = (N^{0}_{SSB_1 r} + N_{SSB_1 ir})/(N^{N}_{SSB_1 r} + N_{SSB_1 ir})$. The experimentally determined value for E.coli is about $OER_{SSB_1} = 4-5$ (see the Table).

Two repair systems (fast and slow) operate in wild type cells. They can operate with different efficiency in different conditions or mutants. The unrepaired DNA injuries (eDSBs in the case of wild type cells) are lethal events in E.coli cells.

Table

Oxygen enhancement ratios of the initial DNA single strand breaks

Strain (authors)	OER
K-12 (Johansen et al.)	4
PolA⁻(Johansen et al.)	4
K-12 (Town et al.)	5.5
PolA⁻ (Town et al.)	3.2
B/r (Sapora et al.)	3.7
B/r (Sapora et al.)	3.6
K-12 (Webb et al.)	4.2
PolA⁻ (Sapora et al.)	5.6

We shall consider two extreme situations when one of the repair pathways is switched off.

I. Block of Slow Repair

If the S switch is off, the fast repair is the only remaining repair of SSBs$_1$. There is no competition between the two repair systems. All F-repairable SSBs$_1^r$ will be fully repaired by fast repair (switch F is on) after irradiation in both oxygenated and anoxic conditions. The remaining SSBs$_1^{ir}$ are lethal events as they cannot be repaired at all. The number of SSBs$_1^{ir}$ does not depend on oxygen concentration and therefore, oxygen does not influence cell sensitivity (OER = 1). The number of SSBs$_1^{ir}$ is markedly higher than the number of lethal events in wild type cells and therefore the sensitivity is increased.

II. Block of Fast Repair

If the F switch is off, the slow repair is the only remaining repair of SSBs$_1$. There is no competition of the two repair systems again. All SSBs$_1$ (both SSBs$_1^r$ and SSBs$_1^{ir}$) will be partly repaired by slow repair (switch S is on). The fraction of unrepaired injuries form lethal events. The OER of lethal events is equal to the OER of the initial SSBs$_1$(OER$_{SSB_1}$ = 4) as the fraction of S-repaired SSBs$_1$ is the same in both oxygenated and anoxic conditions of irradiation. The OER of the sensitivities OER = D_0^N/D_0^0 has the greatest possible value.

If any of the two repair systems is switched off, the sensitivity increases.

We have come to the following conclusions:

1) the *block of slow repair* leads to *increased sensitivity* and *decreased oxygen enhancement ratio* of the sensitivities,
2) the *block of fast repair* leads to *increased sensitivity* and *increased oxygen enhancement ratio* of the sensitivities.

Oxygen Effect in Repair Deficient Mutants of E.coli

The described model situations are not fully realised, of course. There are, however, some repair deficient mutants of E.coli K-12 with different deffects in their repair systems. We shall try to identify their repair defects with our model situations. At first the recA⁻ and polA⁻ mutants will be discussed. The two mutants are markedly more sensitive to γ-radiation than their wild type cells, while their oxygen effects differ from wild type cells (OER = 3) in the opposite directions: OER = 2 in the case of recA⁻ mutant cells and OER = 4 in the case of polA⁻ mutant cells [/27,3,41/]. These facts could not be explained so far.

According to our model these differencies are a logical consequence of defects in the repair systems of E.coli mutant cells: the slow repair is absent in recA⁻ cells and the fast repair is absent in polA⁻ cells. We can estimate the volumes of the fast (F) and slow (S) repair processes in these mutants. F(S) is the ratio of the number of breaks entering the repair procedure and the number of breaks unrepaired by this repair. F = 1 (S = 1) means that the repair is absent. The sensitivity of E.coli cells can be calculated according to the formula (dDSB are neglected):

$$1/D_0 = \frac{N_{SSB_1 r}}{F.S} + \frac{N_{SSB_1 ir}}{S} \qquad (1)$$

and OER$_0$ then reads

$$OER = \frac{N_{SSB_1 r}/F + N_{SSB_1 ir}}{N^N_{SSB_1 r}/F + N_{SSB_1 ir}}. \qquad (2)$$

OER is, therefore, determined by the value of F and the sensitivity depends on both F and S. The dependence of the sensitivity on S is more pronounced (the sensitivity is inversely proportional to S) than its dependence on F. We have assumed that the number of SSBs$_1$ (both F-repairable and irrepairable) is the same for recA⁻, polA⁻ mutants, and for wild type cells (the mutations does not influence ligase or endonucleases activities). Therefore, the only differencies between these strains of E.coli are the volumes of fast and slow repair (F,S).

Such differencies should explain the values of oxygen effect and sensitivities to irradiation. We have obtain the following experimental values[3]:

Strain	$1/D_0$	OER
recA$^-$	0.062	1.8
wild	0.010	3.4
polA$^-$	0.049	4.6

The analysis of these data can be done in terms of our model. In the case of the polA$^-$ mutant we can write Eq.(2) (F=1):

$$OER^{pol} = 4.6 = \frac{N^0_{SSB_1 r} + N_{SSB_1 ir}}{N^N_{SSB_1 r} + N_{SSB_1 ir}}. \qquad (3)$$

For the recA$^-$ mutant we can write Eqs.(1,2):

$$1/D_0^{rec} = 0.062 = N^0_{SSB_1 r}/F^{rec} + N_{SSB_1 ir}, \qquad (4)$$

$$OER^{rec} = 1.8 = \frac{N^0_{SSB_1 r}/F^{rec} + N_{SSB_1 ir}}{N^N_{SSB_1 r}/F^{rec} + N_{SSB_1 ir}}. \qquad (5)$$

The relation between $N_{SSB_1 ir}$ and F^{rec} can be derived from the three equations (3-5):

$$N_{SSB_1 ir} = 0.026 \cdot \frac{F^{rec}}{F^{rec} - 1}. \qquad (6)$$

The value of $N_{SSB_1 ir}$ is in Gy^{-1} $genome^{-1}$. Owing to the fact that the fast repair is not affected in the recA$^-$ mutant[29] and it eliminates the number of DNA breaks approximately by one order, the value of F^{rec} is fairly greater than 1. The ratio $F^{rec}/(F^{rec} - 1)$ should be very near to 1.1. Therefore, the induction of irrepairable single strand breaks should be very near to 0.029 Gy^{-1} $genome^{-1}$ ($10^{-13} cGy^{-1} \cdot dalton^{-1}$).

The number of unrepaired $SSBs_1^r$ ($N_{SSB_1 r}/F^{rec}$) can be estimated from Eq.(4):

$$N^0_{SSB_1 r}/F^{rec} = 0.033. \qquad (7)$$

The parameter F_0^{rec} can be derived from the whole number of induced $SSBs_1$ ($N^0_{SSB_1}$):

$$F^{rec} = 30 \cdot (N^0_{SSB_1} - 0.029). \qquad (8)$$

Analogically for the polA$^-$ mutant Eq.(1) can be written:

$$S^{pol} = 20 \cdot N^0_{SSB_1}. \qquad (9)$$

If the value of $N^0_{SSB_1}$ is taken in accordance with[16]: $N^0_{SSB_1} = 2.2\ 10^{-12} cGy^{-1}\ dalton^{-1} = 0.66\ Gy^{-1}\ gemone^{-1}$, we obtain $F^{rec} = 18.9$ and $S^{pol} = 13.2$.

In the case of wild type cells:

$$1/D_0^{0,wt} = 0.01 = \frac{N^0_{SSB_1 r}}{F^{wt} \cdot S^{wt}} + \frac{N_{SSB_1 ir}}{S^{wt}}, \qquad (10)$$

$$OER^{wt} = 3.4 = \frac{N^0_{SSB_1 r}/F^{wt} + N_{SSB_1 ir}}{N^N_{SSB_1 r}/F^{wt} + N_{SSB_1 ir}}. \qquad (11)$$

The two equations give us the parameters of wild type cells: $F^{wt} = 3.6$ and $S^{wt} = 21.2$. The determined parameters are given in the following table

Strain	F	S
recA$^-$	18.9	1
wild	3.6	21.2
polA$^-$	1	13.2

The values of F and S for wild type cells depend on cultivation conditions as it will be shown later on. Nevertheless, it is clear that the efficiency of the fast repair in recA$^-$ mutant cells is greater than in wild type cells. The repair eliminates 19 of 20 repairable breaks in recA$^-$ cells while in wild type cells approximately 25% of the breaks is drawn to slow repair. The efficiency of slow repair in polA$^-$ cells is somewhat smaller than in the case of wild type cells grawn in the same conditions.

Oxygen Effect in uvrA$^-$ and uvrA$^-$recA$^-$ Cells

The two E.coli mutants are deficient in UV-endonucleases. This endonuclease is necessary for incision of pyrimidine dimers produced by UV radiation. The uvrA$^-$ mutant is, therefore, sensitive to UV-irradiation, but the sensitivity to γ-irradiation does not strongly differ.

The number of pyramidine dimers induced by γ-radiation is $N_{PD} = 1.4 \cdot 10^{-13}\,cGy^{-1}\,dalton^{-1} = 0.042\,Gy^{-1}\,genome^{-1}$ [35]. This value is more than one order smaller than the number of induced breaks. Pyrimidine dimers are effectively repaired by excision repair in uvr$^+$ strains. They do not contribute significantly to the lethal effect.

In the case of uvr$^-$ mutants the pyrimidine dimers cannot be repaired by fast repair and they are lethal events in the double mutant uvrA$^-$recA$^-$. In uvrA$^-$ strain pyrimidine dimers are repaired in postreplicative repair [42]. Pyrimidine dimers in uvr$^-$ mutants have the characteristics very similar to $SSBs_1^{ir}$. They are not repairable by polA-dependent repair and their oxygen enhancement ratio is 1 [37,42]. It can be, therefore, expected that the value of the OER in the case of uvr$^-$ mutants will be smaller in comparison with their uvr$^+$ strains. We shall try to explain the OERs and D_0 values of uvr$^-$ mutants on the basis of our model. Eqs.(1,2) read:

$$1/D_0^{0,uvr} = \frac{N^0_{SSB_1r}}{F^{wt} \cdot S^{wt}} + \frac{N_{SSB_1ir}}{S^{wt}} + \frac{N_{PD}}{Q^{uvr}}, \tag{12}$$

$$OER^{uvr} = \frac{N^0_{SSB_1r}/F^{wt} + N_{SSB_1ir} + N_{PD}(S^{wt}/Q^{uvr})}{N^N_{SSB_1r}/F^{wt} + N_{SSB_1ir} + N_{PD}(S^{wt}/Q^{uvr})}. \tag{13}$$

The value of $OER^{uvr} = 2$ [27] leads to $S^{wt}/Q^{uvr} = 2.1$ and $Q^{uvr} = 10$. Then the sensitivity $1/D_0^{uvr} = 0.014$ in oxygenated conditions. It is in agreement with experimental findings.

Analogically Eqs.(1,2) for the double mutant recA$^-$vuvrA$^-$ read:

$$1/D_0^{0,ur} = \frac{N^0_{SSB_1r}}{F^{rec}} + N_{SSB_1ir} + N_{PD} \tag{14}$$

$$OER^{ur} = \frac{N^0_{SSB_1r}/F^{rec} + N_{SSB_1ir} + N_{PD}}{N^N_{SSB_1r}/F^{rec} + N_{SSB_1ir} + N_{PD}}. \tag{15}$$

The quantities D_0^{ur} and OER^{ur} can be calculated: $1/D_0^{ur} = 0.1\,Gy^{-1}$ and $OER^{ur} = 1.35$. They are in agreement with the experimental values [27].

On the basis of our model the values of OER in other mutants can be also understood. For example, in the case of the lexA$^-$ mutant the values of OER are also smaller than in wild type cells [41]. The sensitivity to γ-radiation increases owing to the depression of slow repair. The introduction of the polA$^-$ defect into the lexA$^-$ strain increases the values of OER again, although the sensitivity further increases. It means that the efficiency of fast repair sufficiently decreases: $F^{pl} = 3.5$. The value of F is, however, not equal to 1, as the main pathway of slow repair is switched off, too. The volume of slow repair is $S^{pl} = 1.4$ (according to data from [41]).

The Balance of Repair Systems of E.coli Wild Type Cells

Escherichia coli K-12 cells grown and plated in rich medium are more resistant to X-rays than cells grown and plated in minimal medium [28]. Similar observations have been made for E. coli B/r [1]. The values of OER differ, too [28,1]. More resistant cells have greater OER and vice versa. These experimental observations could not be explained so far. Our model gives a simple interpretation.

Slow repair is medium dependent [42]. Therefore, the balance between fast and slow repair must be shifted to fast repair in poorer medium. Fast repair cannot, however, repair $SSBs_1^{ir}$ and therefore the shift of the repair volumes from slow repair to fast one is accompanied by an increase of cell sensitivity.

If slow repair is depressed in poor medium (the valve S in Fig.2 is throttled), a greater part of F-repairable $SSBs_1^r$ is taken by fast repair (the valve F is open). The probabilities of F- or S repair can be written as follows (see Fig.1):

$$F = \frac{p_2}{q_2} + 1, \tag{16a}$$

$$S = \frac{p_3}{q_3} + 1. \tag{16b}$$

The values of p_2/q_2 can be calculated in both rich and poor conditions of cell cultivation. The polA-repair is medium independent and so the ratio q_2^{rich}/q_2^{poor} reads:

$$\frac{q_2^{rich}}{q_2^{poor}} = \frac{F^{poor} - 1}{F^{rich} - 1}. \tag{17}$$

If the value of q_3 (the probability of MS fixation) is the same in both poor and rich medium then the ratio of p_3^{rich}/p_3^{poor}

(the probabilities of resynthesis) reads:

$$\frac{p_3^{rich}}{p_3^{poor}} = \frac{S^{rich} - 1}{S^{poor} - 1}. \qquad (18)$$

Assuming further that the degree of repair anzymes depression in poor conditions is correlated by an expression

$$q_2 = a \cdot (p_3)^b, \qquad (19)$$

where a, b are constants, we can obtain quantitative predictions from our model. Eqs. (17-19) give us

$$K = (F^{rich} - 1)(S^{rich} - 1)^b = (F^{poor} - 1)(S^{poor} - 1)^b = \text{const}, \qquad (20)$$

where the values of the constants are in the case of our wild type cells $b = 0.7$ and $K = 21.5$. The value of b is near one and so repair enzymes depression in poor conditions is correlated nearly linearly. Eqs.(1), (2) and (20) give us the following formulae:

$$1/D_0^{0,wt} = \left(\frac{N^0_{SSB_1r}}{F^{wt}} + N_{SSB_1ir}\right) / \left(\left(\frac{K}{F^{wt} - 1}\right)^{\frac{1}{b}} + 1\right), \qquad (21)$$

where

$$F^{wt} = \left(\frac{N^0_{SSB_1r} - OER^{wt} \cdot N^N_{SSB_1r}}{(OER^{wt} - 1) \cdot N_{SSB_1ir}}\right). \qquad (22)$$

For the values of b and K given above $OER^{wt} = 2$ corresponds to the sensitivity $1/D_0^{wt} = 0.025\ Gy^{-1}$.

DISCUSSION

The oxygen effect realises as the consequence of the induction of different injuries in cells with various initial oxygen enhancement ratios (OERs). The initial injuries arise from radicals modifiable by oxygen to different extent. The repair processes can eliminate the injuries but different types of the injuries have different probability of its transformation to the lethal events. The contribution of the particular damage to OER realisation can be investigated using mutant cells with specific defects in their repair pathways.

We have attempted to propose simple model explaining different values of OER in $recA^-$, $polA^-$ and $uvrA^-$ mutants and the dependence of OER on cultivation conditions in wild type cells. We have considered in our model two repair systems (fast and

slow) in E.coli and predicted decreased OER for cells with defects in slow repair (e.g., $recA^-$) and increased OER for cells with defects in fast repair ($polA^-$). The increased OER in the case of $polA^-$ strain should reflect the OER of the initial DNA injuries. Actually $OER^{pol} = OER_{SSB_1} = 4$-$5$. The injuries irrepairable by fast repair are lethal events in the mutants defective in slow repair. They have been assumed to be unmodifiable by oxygen. In fact, they can be modified by oxygen to the same extent as $recA^-$ mutant sensitivities ($OER^{rec} = 2$). It is however, unprobable as it would mean that 100% $SSBs_1r$ is repaired by fast repair in $recA^-$ strain. The production of $SSBs_1ir$ by γ-radiation is about $10^{-13} cGy^{-1}$ $dalton^{-1}$.

The model enabled us to explain and describe quantitatively the dependence of the cell sensitivity to γ-radiation on cultivation conditions as the consequence of shifted balance of the two repair systems.

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Козубек С., Красавин Е.А. E19-84-826
Чувствительность клеток к облучению и процессы репарации ДНК. Кислородный эффект у различных мутантов Escherichia Coli

Предлагается новая модель реализации кислородного эффекта у клеток E.coli. Модель объясняет различные значения величины кислородного эффекта /КЭ/ у клеток дикого типа и репарационных мутантов. Эти различия связываются с соответствующими дефектами в их репарационных системах. Проведен количественный анализ этих различий. Обсуждается зависимость величины КЭ и радиочувствительности клеток от состава среды роста клеток. Эта зависимость, выражающаяся в уменьшении КЭ и возрастании радиочувствительности клеток, объясняется снижением объема репарации медленного типа и компенсаторным возрастанием быстрой репарации.

Работа выполнена в Лаборатории ядерных проблем ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна 1984

Kozubek S., Krasavin E.A. E19-84-826
Cell Sensitivity to Irradiation and DNA Repair Processes. Oxygen Enhancement Ratio in Different Mutants of Escherichia Coli

A new model of oxygen effect realisation is proposed for E.coli cells. The model explains differencies in oxygen enhancement ratio (OER) between wild type cells and repair deficient mutants. These differencies are logically linked to corresponding defects in repair systems. A quantitative analysis has been performed. The dependence of OER and cell sensitivity on the properties of cultivation medium is considered, too. Decreasing OER and increasing sensitivity in poor conditions are explained as the consequence of the shift of repair capacity from slow to fast repair system.

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

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