C00-3221-56

MASTER

RADIATION-INDUCED DAMAGE IN *E. COLI* B:
THE EFFECT OF SUPEROXIDE RADICALS AND MOLECULAR OXYGEN

PROGRESS REPORT

Amram Samuni and Gidon Czapski
Departments of Molecular Biology and Physical Chemistry
The Hebrew University of Jerusalem, Israel

Work carried out during the period:
December 1st 1978 to November 30th 1979

Prepared for the U.S. Energy Research & Development Administration
Under Contract No. EY-76-C-02-3221

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

6
C00-3221-55

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

RADIATION-INDUCED DAMAGE IN *E. COLI* B:
THE EFFECT OF SUPEROXIDE RADICALS AND MOLECULAR OXYGEN

PROGRESS REPORT

Amram Samuni and Gidon Czapski
Departments of Molecular Biology and Physical Chemistry
The Hebrew University of Jerusalem, Israel

296 5000
(parent exp)

Work carried out during the period:
December 1st 1978 to November 30th 1979

Prepared for the U.S. Energy Research & Development Administration
Under Contract No. EY-76-C-02-3221

86
DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

RADIATION-INDUCED DAMAGE IN *E. COLI* B:
THE EFFECT OF SUPEROXIDE RADICALS AND MOLECULAR OXYGEN

Amram Samuni and Gidon Czapski

From the Departments of Molecular Biology and Physical Chemistry,
Hebrew University, Jerusalem.

copies: 3

Pages: 18 including 1 table

figures: 2

Running Title: γ -Irradiated *E. coli* B: Effects of O_2 and $\dot{O}_2^-$.

Please address all correspondence to: Prof. G. Czapski, Office of
the Rector, Hebrew University, Jerusalem, Israel.

Samuni A., Czapski G., Radiation-Induced Damage in *E. coli* B: The Effect of Superoxide Radicals and Molecular Oxygen. *Radiat. Res.*

Abstract

The roles of superoxide radicals and of molecular oxygen in the radiodamage of *E. coli* B suspended in dilute phosphate buffer have been studied. The presence of high concentrations of polyethylene glycol in the γ -irradiated cell suspensions, had no effect on bacterial radiosensitivity. This indicates that the damage was primarily *endogenous*, i.e. originated intracellularly.

Saturation of the cell suspensions with N_2O doubles the radiosensitivity, thus indicating that OH radicals are responsible for the majority of the damage (*indirect* radiation effect).

The presence of oxygen either in the absence or presence of N_2O brings about roughly a three-fold increase in the radiosensitivity. Since in the presence of N_2O all e^-_{aq} are scavenged by the nitrous oxide rather than by oxygen, this shows that superoxide radicals play no role in the bacterial radiodamage. Our results substantiate the attribution of the oxygen effect to a direct interaction of O_2 with the OH-damaged sites on vital biomolecules, and exclude any significant contribution of e^-_{aq} and $\dot{O}_2^-$ to the cellular radiodamage.

key words: superoxide radicals; γ -irradiation; oxygen effect; *E. coli* B; *endogenous* radiodamage.

Introduction

The effect of oxygen on radiation-induced damage in biological systems has been extensively studied and reported (1-2). The enhancement of the radiodamage in the presence of oxygen, generally expressed in terms of an oxygen enhancement ratio (OER), is observed mainly, though not exclusively, in systems where mechanisms of damage repair and restitution are active. In the irradiation of dissolved enzymes, for instance, the effect of oxygen is found to be protective rather than sensitizing (3).

The manner in which oxygen affects the radiodamage has been related to, at least, two distinct characteristics. Oxygen is known to be an efficient scavenger of e^-_{aq} yielding $\dot{O}_2^-$ radicals (4), and to have a high affinity for secondary radical-sites formed in the irradiated biomolecules (5). According to this description oxygen alters the biological radiosensitivity indirectly through the action of the superoxide radicals and directly, presumably by reducing the repair.

The possible biological damage due to $\dot{O}_2^-$ radicals has been ascribed to their direct attack on vital biomolecules and/or to the formation of secondary OH radicals (6-9), possibly through the Haber-Weiss reaction (10).

Reports implicating the $\dot{O}_2^-$ radicals as responsible for biological damage *in vivo* in unirradiated systems and under radiation, have been published (6-9, 11-13), along with evidence contradicting it (14-16). Recently it has been proved, upon kinetic considerations, that the Haber-Weiss reaction cannot proceed as a simple elementary reaction in biological systems (17). Yet, the possibility for the occurrence of this reaction through some catalysis of transition metal ions has been suggested and substantiated (9, 17-18).

The present work further examines the oxygen effect in γ -irradiated *E.coli B*. The simplistic view that superoxide radicals formed on the irradiation of aerated solutions are responsible for the OER seems to be incorrect. Therefore, an attempt has been made to distinguish between the role of O_2 as a radiosensitizer and its role in the conversion of e^-_{aq} into $\dot{O}_2^-$ radicals.

Materials and Methods

All chemicals used were of the highest analytical grade available and were used without further purification. Bacto-Agar and Nutrient Broth were obtained from Difco; sodium formate and sodium chloride from B.D.H; poly-ethylene glycol (PEG), practical grade, was purchased from Fluka. All the solutions were made up with triply-distilled water. The test organism used was *Escherichia coli B*. Superoxide dismutase (SOD) was a product of Sigma isolated from bovine blood (2000 units/mg protein). Thermally-inactivated enzyme was prepared by boiling SOD for 30 min. Cultures of *E. coli B* were grown to stationary phase in nutrient broth containing 0.5% glucose. The cell suspensions were irradiated in glass bottles closed with rubber caps (~9ml) within a ^{137}Cs gamma source (M Gamma Meter Radiation Machinery Co. Model M38/3, dose-rate $96 \text{ krad} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). The radiation dose was measured using Fricke dosimetry. Irradiations of the suspensions ($1 \cdot 10^5 \text{ cells} \cdot \text{ml}^{-1}$) were carried out at room temperature in 0.05M phosphate buffer, containing MgSO_4 2mM (pH 7.0). The cell suspensions were appropriately diluted in buffer, then plated on nutrient agar in triplicates. The plates were incubated overnight and the colonies scored.

Oxygenation was carried out by bubbling oxygen through the suspension for 5 min. Deoxygenation, when required, was carried out by bubbling either argon (Matheson) or nitrous oxide (Matheson) through the cell suspensions for 15 min prior to irradiation.

To ensure removal of traces of oxygen in the displacing gases, they were first passed through a series of oxygen-traps (V^{2+} , HCl, Hg/Zn). In experiments where the presence of both O_2 and N_2O in cell suspensions was desired, an N_2O/O_2 9:1 gas mixture (Matheson) was used. Control experiments demonstrated that the bubbling by itself (not followed by radiation) did not affect the radiosensitivity of the cells. Also, irradiation of the buffer solution (30min) before suspending the cells had no effect on bacterial survival, thus indicating that no stable radiation products are responsible for the radiodamage.

Results

Dilute suspensions, 10^5 cells/ml, of *E. coli* B were γ -irradiated both under anoxic and oxic conditions. The respective survival data have been presented as dose-response curves in Fig. 1 traces a and b. Both plots departed, for low doses, from exponential dependence and exhibited characteristic shoulders. The experimental points, not conforming to a single-hit single-target behavior have been fitted to a multi-event (1) expression.

$$S = 1 - (1 - e^{-kD})^n \quad (2)$$

which describes the dependence of the surviving fraction (S) on the dose (D) in krads. Here n does not necessarily relate to any number of "hits" or sites and may be looked upon as a non-interpretive term-"extrapolation number" (19).

The inactivation constant k has been taken as a measure of the radiation sensitivity (19). The parameters n and k have been evaluated by a computer best fit using a non-linear least square regression program. The best estimates, according to which the solid lines in Fig. 1 have been drawn, are presented in Table I.

The major difference between the survival curves, determined under anoxic and oxic conditions, is the slope of the exponentially dependent part.

As can be seen in Table I, k for oxygenated system was roughly three-fold higher than that obtained in anoxic system.

Effect of N_2O

In the absence of oxygen the introduction of N_2O roughly doubled the radiosensitivity of bacteria in deaerated suspension. The respective survival curve is shown in Fig 2 trace a. The introduction of nitrous oxide into the deaerated suspension resulted in doubling the slope of the exponentially dependent part of the curve. The solid line in Fig. 2 trace a represents the calculated plot, evaluated using eq. 2 as explained above, with the best estimates for n and k shown in Table I.

Effect of formate.

In order to study the contribution of superoxide radicals to the radiodamage, formate has been added to oxygenated cell suspensions prior to irradiation. In the presence of formate and oxygen practically all the water radicals are converted into $\dot{O}_2^-$. The addition of formate, up to 40mM, decreased the cell radiosensitivity as can be seen in Fig. 1 trace c.

O_2 effect in the presence of N_2O .

Suspensions of *E. coli* B had been saturated with a mixture of N_2O and O_2 (9:1) before irradiation and the surviving fractions of the cells determined. The dose-response curve is shown in Fig. 2 trace b and the respective n and k values are presented in Table I. Due to the different gas solubilities, the concentrations ratio $[N_2O] / [O_2]$ in the solution was ~ 100 . Taking into account the respective rate constants of these two gases with e^-_{aq} (20) indicates that $k_{N_2O+e^-} \cdot [N_2O] / k_{O_2+e^-} \cdot [O_2] \sim 10$, thus implying that under such experimental conditions the hydrated electrons reacted ^{predominantly} with N_2O , yielding $\dot{OH}$ radicals.

Comparison of Figs. 1 and 2 indicates that cell radiosensitivity is roughly 3-fold higher in the presence of N_2O and oxygen than in the presence of N_2O alone, and about 6-fold higher than in the absence of N_2O and oxygen. In other words,

the relative radiosensitivities of the systems saturated with: Ar; O_2 ; N_2O ; $N_2O + O_2$, were roughly: 1:3:2:6 respectively (see also Table I).

Effect of superoxide dismutase.

In an attempt to check the radiodamage caused by O_2^- radicals, superoxide dismutase (SOD) has been added, up to 40mg/ml to the cell suspensions. This enzyme is known to catalyze efficiently the dismutation of the superoxide radicals (21). Irradiations were carried out in the presence of formate and O_2 , and in the presence of $N_2O + O_2$ mixture (9:1) with SOD. In both cases SOD (21), whether native or thermally-inactivated did not alter the radiation response.

Effect of polyethylene glycol.

To estimate the relative contributions of *exogenous* and *endogenous* radiation effects to the total radiodamage, the influence of polyethylene glycol (PEG) on cell survival has been examined. This polymeric alcohol (M.W. ~ 4000) effectively scavenges the *exogenous* OH radicals (22) but it does not penetrate the cell. The use of PEG as a scavenger for *exogenous* OH radicals has been recently demonstrated (16). Cell suspensions, either deaerated or oxygenated or saturated with N_2O , have been irradiated in the absence and in the presence of PEG. But, PEG up to concentration of 10% w/V, (the maximum tested), did not modify the cell radiosensitivity. Although no bubbling was carried out during irradiations no significant depletion of the saturating gases would be expected. This is true also for the oxygen in the $N_2O + O_2$ gas mixture. There, the hydrated electrons reacted with the nitrous oxide rather than with O_2 , and $[O_2]$ might have been decreased through reactions with the OH-damaged sites in cellular components only. As the concentration of these sites in dilute cell suspensions is very small, their reaction with O_2 would hardly affect its concentration.

DISCUSSION

In many biological systems $\dot{O}_2^-$ radicals have been implicated to be very toxic (6-9, 11-12). Since, during irradiation in oxic conditions, e_{aq}^- are converted into $\dot{O}_2^-$, the OER has been attributed, by some authors, to this radical species (6, 9). It has also been reported that SOD provided radioprotection to *E. coli* cells irradiated in the presence of oxygen (9). This description agrees neither with previous observations (14-16) nor with the results of the present study.

Role of OH. The introduction of N_2O into deaerated cell suspensions has approximately doubled the radiosensitivity as compared with that observed under anoxia (see Table I). This implies that $\dot{OH}$ causes the main damage within the cell. In the presence of N_2O the e_{aq}^- are converted into $\dot{OH}$ whose concentration is doubled. In fact, the two-fold increase in k indicates that the contribution to the damage by other water-derived radicals (e_{aq}^- , $\dot{O}_2^-$) and of *direct* radiation effects are negligible or at least minor. With regard to the role of $\dot{OH}$ in radiodamage in *E. coli* B, the above conclusion is now well established (23) and also agrees with our study with bacteriophage (16).

Endogenous and exogenous effects. It was shown in a previous study with T4 bacteriophage that the radiodamage was primarily *exogenous*, and could be protected against by the addition of PEG (16). Here the same approach has been used in order to estimate the relative contributions of the *exogenous* and *endogenous* radiation effects. The radiodamage in the presence and in the absence of PEG were compared. These results indicate, in contrast to the case of T4, that PEG, which cannot penetrate the cells, did not affect the radiosensitivity. Therefore it may be concluded that in contrast to previous suggestions (9), with *E. coli* the damage was almost exclusively *endogenous*. This situation is different

from that in bacteriophage, a result which is self-evident in view of the different sizes of the two test organisms.

In principle the relative contributions of the *endogenous* and *exogenous* radiation effects would be determined by the volume-to surface ratio of the target. This ratio is roughly 50 fold higher in *E. coli* as compared with T4 bacteriophage.

In the latter the observed *endogenous*-to *exogenous* damage ratio was about 0.1, hence it was anticipated to find in the former predominantly *endogenous* effect.

The direct and indirect radiation effects. The results given in Figs. 1 and 2 show that N_2O which doubled the $\dot{O}H$ concentration, also increased the radio-sensitivity by, roughly, the same extent. This indicates that most of the damage, primarily *endogenous*, was due to the $\dot{O}H$ radicals, *i.e.* *indirect* damage.

It has been reported that adding ethanol, up to 1M, to the cell suspension reduces the radiosensitivity of *E. coli* by approximately 50% in both oxic (23) and anoxic (24) conditions. Based on these findings it was concluded that *indirect* effects amounts to 50% only of the cell radiodamage (23-24). This assumed that 1M ethanol scavenged all the $\dot{O}H$ radicals.

In fact, the lack of full protection by the alcohol against the radiation damage might also be attributed to an unsuccessful competition of the protectant with the very high concentrations of the cellular compounds for the $\dot{O}H$ radicals.

Another possibility which might be considered is that the alcoholic radicals themselves are not completely inactive (25). The above observations do not necessarily contradict our conclusion.

Do $\dot{O}_2^-$ radicals contribute to the radiodamage?

Comparison of traces b and c in Fig. 1 shows that formate, in oxygenated suspensions, provides considerable radioprotection to the irradiated *E. coli* cells. Under

such experimental conditions $\dot{\text{O}}\text{H}$ radicals, scavenged by formate, are converted into $\dot{\text{O}}_2^-$. Thus, the reduction in the radiosensitivity observed in the presence of formate is attributable to the replacement of part of the highly reactive OH radicals by the harmless $\dot{\text{O}}_2^-$ species. Again, the lack of full radioprotection due to formate is not surprising in view of the *endogenous* nature of the radiodamage. Formate can scavenge OH radicals outside the cell, but an efficient OH scavenging inside the bacteria is not expected. Even if the concentration of formate in the cell is comparable to its extracellular concentration, it can compete only partially with the reaction of the cellular constituents with OH , due to their predominant concentrations.

It has been previously reported that the level of the *endogenous* SOD within *E. coli* cells had no effect on their radiosensitivity (13). Addition of SOD before irradiation might have an effect on *exogenous* superoxide radicals only. Under our experimental conditions, SOD had no observable effect on cell survival.

This result, which contradicts previous reports (9), supports the assumption that the *exogenous* $\dot{\text{O}}_2^-$ radicals, formed outside the bacteria, do not participate in the radiodamage. This result is in accord with the lack of any detectable effect of *exogenous* $\dot{\text{O}}_2^-$ on T4 survival as was recently reported (16). Further evidence supporting this conclusion will be discussed below.

The oxygen effect in the absence and in the presence of N_2O .

The effect of the presence of $\text{N}_2\text{O}+\text{O}_2$ gas-mixture on the radiosensitivity has been examined for bacterial spores and it was reported that O_2 and N_2O did not operate additively or synergistically (26). In other words, N_2O did not increase the radiosensitivity in oxygenated suspensions (26).

The present experimental results show that the bacterial radiosensitivity is increased roughly three-fold by oxygen. This enhancement was observed both in the absence and in the presence of N_2O . The N_2O affects the system by converting e_{aq}^- into $\dot{OH}$ radicals which in turn react with the biomolecules to form secondary radical sites $\dot{R}$. If the interaction of O_2 is with these sites, then it is anticipated to observe the same OER in the presence and the absence of N_2O .

In other word, the total enhancement of the radiosensitivity is expected to equal the product of the sensitization factors of the two sensitizers. The results seen in Figs. 1 and 2 show that in our system this is the case. The relative

radiosensitivities of 1:3:2:6 measured in the systems: deaerated; oxygenated; N_2O saturated; $N_2O + O_2$ saturated, indicate that a) the damage is initiated by $\dot{OH}$ but not by e_{aq}^- or $\dot{O}_2^-$ radicals, and b) oxygen roughly triples the damage caused by the $\dot{OH}$. (The observation that the effect of oxygen is the same in both the presence and the absence of N_2O further excludes $\dot{O}_2^-$ as being responsible for the radiodamage).

CONCLUSIONS

The radiation induced damage in *E. coli* B was shown to be primarily an *endogenous* effect even in dilute buffered suspensions.

This *endogenous* radiodamage was predominantly due to the water radicals, *i.e.* *indirect* radiation effect.

It was the $\dot{OH}$ among the water radicals which was responsible for the majority of the damage while the e_{aq}^- was apparently inactive.

The superoxide radicals, $\dot{O}_2^-$, seemed to have no role in the radiation induced damage.

The maximal enhancement of the radiation sensitivity was observed in the

presence of $N_2O + O_2$. N_2O practically doubled the $[\dot{OH}]$, while the O_2

roughly tripled the effect of these radicals.

The OER appears to result from the direct interaction of the molecular oxygen with the secondary radical sites within the biomolecules.

Acknowledgement: This work was supported by ERDA under Contract E(11-1)-3221, and partially supported by Gesellschaft für Strahlen Forschung Neuherberg.

References

1. H. Dertinger and H. Jung, *Molecular Radiation Biology* pp. 70-113, 195-217, 1970. Springer-Verlag, New York.
2. G.E. Adams. Radiation chemistry in biology. *Adv. Radiat. Chem.* 3, 125-208 (1972).
3. T. Brustad, The effects of radicals scavengers on the radiosensitivity of lysozyme in dilute aqueous solutions of varying pH, *Radiat. Res.* 27, 456-473 (1966).
4. G. Czapski, Radiation chemistry of oxygenated aqueous solutions. *Annu. Rev. Phys. Chem.* 22, 171-208 (1971).
5. Z.M. Bacq and P. Alexander, *Fundamentals of radiobiology*, pp. 45-69, 280-292, Academic Press New York, 1961.
6. J.M. McCord, B.B. Keele Jr. and I. Fridovich. An enzyme-based theory of obligate anaerobiosis: The physiological function of superoxide dismutase. *Proc. Natl. Acad. Sci. USA* 68, 1024-1027 (1971).
7. L.W. Oberley, A.L. Lindgren, S.A. Baker and R.H. Stevens, Superoxide ion as the cause of the oxygen effect. *Radiat. Res.* 68, 320-328 (1976).
8. F. Lavelle, A.M. Michaelson and L. Dimitrijevic, Biological Protection by superoxide dismutase, *Biochem. Biophys. Res. Commun.* 55, 350-357 (1973).
9. H.P. Misra and I. Fridovich, Superoxide dismutase and the oxygen enhancement ratio of radiation lethality, *Arch. Biochem. Biophys.* 176, 577-581 (1976).
10. F. Haber and J. Weiss, Catalytic decomposition of hydrogen peroxide by iron salts, *Proc. R. Soc. London A* 147, 332-351 (1934).
11. G. Cohen and R.E. Heikkila, 6-Hydroxydopamine: Evidence for superoxide radical as an oxidative intermediate. *Science* 181, 456-457 (1973).
12. B.M. Babior, J.T. Curnutte and R.S. Kipnes, Biological defense mechanisms. Evidence for the participation of superoxide in bacterial killing by xanthine oxidase, *J. Lab. Clin. Med.* 85, 235-244 (1975).

13. G. Cohen and R.E. Heikkila, The generation of hydrogen peroxide, superoxide radical and hydroxyl radical by 6-hydroxydopamine, dialuric acid and related cytotoxic agents. *J. Biol. Chem.* 249, 2447-2452 (1974).
14. S.A. Goscin and I. Fridovich, Superoxide dismutase and the oxygen effect, *Radiat. Res.* 56, 565-599 (1973).
15. J. Blok and W.S.D. Verhey. The attack of free radicals on biologically active DNA in irradiated aqueous solutions. *Radiat. Res.* 34, 689-703 (1968).
16. A. Samuni, M. Chevion, Y.S. Halpern, Y.A. Ilan and G. Czapski. Radiation-induced damage in T4 bacteriophage: The effect of superoxide radicals^{and} molecular oxygen. *Radiat. Res.* (in press).
17. G. Czapski and Y.A. Ilan, On the generation of the hydroxylation agents from superoxide radical - can the Haber-Weiss reaction be the source of OH radicals? *Photochem. Photobiol.* (in press).
18. K.L. Fong, P.B. McCay, J.L. Poyer, H.P. Misra and B.B. Keele, Evidence for superoxide dependent reduction of Fe^{3+} and its role in enzyme-generated hydroxyl radical formation. *Chem. Biol. Interact.* 15, 77-89 (1976).
19. E.L. Powers, Considerations of survival curves and target theory *Phys. Med. Biol.* 7, 3-28 (1962).
20. M. Atbar, M. Bambenek and A.B. Ross. NSRDS - Nat. Stand. Ref. Data Sys. , Natl. Bur. Stand. (1973).
21. I. Fridovich, Superoxide dismutase, In *Molecular Mechanisms of Oxygen Activation* (O. Hayaishi Ed.) pp. 453-474. Academic Press, N.Y. 1974.
22. M.S. Matheson, A. Mamou, J. Silverman and J. Rabani, Reaction of OH radical with polyethylene oxide in aqueous solutions, *J. Phys. Chem.* 77, 2420-2424 (1973).
23. I. Johansen and P. Howard-Flanders, Macromolecular repair and free radical scavenging in protection of bacteria against X rays. *Radiat. Res.* 24, 184-200 (1965).

24. T. Sanner and A. Pihl. Significance and mechanism of the indirect effect in bacterial cells. The protective effect of added compounds in *E. coli* B irradiated in liquid and in frozen suspension. *Radiat. Res.* 37, 216-227 (1969).
25. H. Loman, S. Voogd and J. Blok, Indirect radioprotection by RSH compounds: A model chemical system. *Radiat. Res.* 42, 437-445 (1970).
26. E.L. Powers and M. Cross, N_2O as a sensitizer of bacterial spores to X-rays *Int. J. Radiat. Biol.* 17, 501-514 (1970).

TABLE I

Sensitivity towards ionizing radiation of *E. coli* B γ -irradiated in 50 mM phosphate pH 7. The effect of O_2 , N_2O and $N_2O + O_2$ gas mixture.

Experimental conditions	Extrapolation Number n	Inactivation rate constant k (krad ⁻¹)
Ar saturated	1.4	0.049
O_2 saturated	1.7	0.17
N_2O saturated	2.9	0.11
$N_2O + O_2$ (gas mixture 9:1) saturated	3.3	0.33
O_2 saturated, 40 mM formate	2.0	0.13

Irradiation conditions were: room temperature; 10^5 cells ml⁻¹; pH 7; dose rate; 96 krad g⁻¹ . h⁻¹.

n and k are the best estimates evaluated by fitting the "multi-event" survival curves to eq. 2

$$S = 1 - (1 - e^{-kD})^n \quad (\text{see text}).$$

Figure 1

Figure 2

LEGENDS TO FIGURES

Fig. 1. Effect of oxygen and formate on bacterial radiosensitivity.

Dose-response survival curves of *E. coli* B γ -irradiated in 50 mM phosphate buffer, pH 7.

The solid lines are the calculated survival plots using eq. 2 (see text).

Trace a: deaerated suspensions (o).

Trace b: oxygenated suspensions (Δ).

Trace c: oxygenated suspensions containing formate 40 mM ($\square$)

Fig. 2. Effect of N_2O and of $N_2O + O_2$ on cell radiosensitivity. Experimental conditions as in Fig. 1.

Trace a: N_2O saturated suspension (o).

Trace b: Suspension saturated with gas mixture (9:1) $N_2O + O_2$ (Δ).