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Laboratory of Radiation Biology

Department of Zoology

University of Texas

Austin

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Three Year

Progress Report ~~and Renewal Request~~

Original Signed By

H. E. Sutton
Vice-President for Research

Submitted by: E L Powers

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PUBLICATIONS (August 15, 1974 - November 14, 1977)

(attached as appendices carrying corresponding numbers)

1. Powers, E. L. Is the water shell about the "target" involved in radiation effects in cells. Proceedings of the Fourth Symposium on Microdosimetry, Verbania-Pallanza, Italy, J. Booz et al. eds., Commission of the European Communities, Brussels, Publication #EVR 5122 d-e-f, pp. 607-624 (1973).

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28. Simic, M. and E. L. Powers. Mechanisms of oxidative radiation sensitization of cells in aqueous suspensions. Radiat. Res. 59:155-156 (1974).

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Other

36. Information Bulletin (CFKR)

I. The Laboratory

In addition to ERDA's support, the Laboratory of Radiation Biology receives support from NIH. It is now on the eleventh year of PHS NIGMS (13557) (the second year of the third five year cycle), this year having begun April 1, 1977 and being funded in the amount of $52,400 direct costs.

Acknowledgement to both ERDA and NIH is made for all work in the laboratory since all the work is closely interrelated.

The current staff consists of the director (funded 1/4 time by ERDA), 2 full-time technicians (one funded by ERDA and one by NIH), one full-time secretary (funded by two grants from NIH), 1 part-time typist (funded by ERDA), 1 post-doctoral student (funded by NIH), 1 graduate student (funded in the summers by ERDA) and 2 half-time undergraduate assistants (one funded by ERDA and one by NIH).

Closely associated with the Laboratory of Radiation Biology is the Center for Fast Kinetics Research (CFKR) funded by the Biotechnology Program of the Division of Research Resources of NIH in the amount of $143,000 for the year #3 of five. The Center is directed by E. L. Powers. It is located in a building adjacent to the Laboratory of Radiation Biology. As briefly described in Appendix 36, it exists to help scientists with problems involving radiation-induced, short-lived transients and many of the chemical problems arising from the studies described below. A detailed description of the capabilities of this Center is available on request.

II. Education and Training

Over the past three years, 5 undergraduate students have received credit for research work performed in this laboratory. Several undergraduates have been employed on a part-time basis as noted above. One graduate student is approximately mid-way in a doctoral program at this time.

As has been the case over a number of years, the x-ray machines have been used by various departments of the Univeristy, free, with over 50 service irradiations being provided this past year, about the same number as in previous years. The service includes advice on experimental set-up, in place chemical dosimetry, and consultants on experimental design and analysis. From time to time, there are also services provided in the area of UV-irradiation techniques including chemical actinometry.

The radiation biology course, taught each spring, continues to have around 30 pre-medical, pre-dental and graduate students in attendance.

III. Conferences and Formal Talks (August 15, 1974 - November 14, 1977)

June 6, 1974	Chemistry Seminar, Wayne State University, Detroit, Michigan. "Reactions of Oxygen with Free Radicals and Unstable States of Metals" M. Simic.
July 22-27, 1974	Second Annual Meeting of the American Society for Photobiology, Vancouver, B.C. "Radiation Sensitivity of the Photosynthetic System and Nitrate Reductase in Blue Green Algae" M. Simic. Attended by E. L. Powers.
August 30, 1974	Chemistry Seminar, University of Notre Dame, South Bend, Indiana. "Pulse Radiolysis and Metal Complexes" M. Simic.
October 11-12, 1974	6th Annual Meeting of South Central Photobiology Group, Austin, Texas. Chaired locally by E. L. Powers, attended by M. Centilli.
October 25-26, 1974	Texas Branch of American Society of Microbiology Meeting, Galveston, Texas. "Anoxic Radiation Sensitization of Bacillus megaterium Spores by p-Nitroacetophenone" David Ewing. Attended by E. L. Powers.
November 8-9, 1974	7th Annual Meeting of the Texas Association for Radiation Research, Dallas, Texas. "Mechanisms in the Radiation Sensitization of Spores" David Ewing. "Radiation Sensitization of Bacterial Spores by Metals" E. L. Powers.
March 18, 1975	Seminar, Ramsay Wright Zoological Laboratories, Toronto, Canada. "The Chemistry of Radiation Effects in Cells" E. L. Powers.
May 11, 1975	23rd Annual Meeting of the Radiation Research Society, Miami Beach, Florida. "Mechanisms of Anoxic Radiation Protection of Bacterial Spores" David Ewing. Attended by E. L. Powers.
Sept. 15-19, 1975	Miller Conference of Radiation Chemistry, Bürgenstock, Switzerland. E. L. Powers attending.

September 22-26, 1975	Fifth Symposium on Microdosimetry, Verbania-Pallanza, Italy. "Remarks on the Radiation Chemistry of Radiation Damage in Cells" E. L. Powers. Session Chairman, E. L. Powers.
Oct. 31-Nov. 1, 1975	Joint Meeting of the Texas Association for Radiation Research and the South Central Photobiology Group at Lakeway Inn, Austin, Texas. "Irradiated Spores in Aqueous Suspension: Three Components of Oxygen-Dependent Damage" D. L. Ewing and E. L. Powers. Session chaired by D. L. Ewing. Meeting organized by E. L. Powers.
March 10-12, 1976	29th Annual Symposium on Fundamental Cancer Research. "Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells" M. D. Anderson, Houston, Texas. E. L. Powers and D. L. Ewing attending.
March 26-27, 1976	The Third Annual Texas Genetics Society Meeting, University of Texas, Austin, Texas. E. L. Powers attending.
April 12, 1976	Seminar: Biology Department, Southwest Texas State University, San Marcos, Texas. "Oxygen Dependent Sensitization of Bacterial Spores" D. L. Ewing.
June 27-July 2, 1976	24th Annual Meeting of the Radiation Research Society, San Francisco, California. "Invitation to Potential Users of the Center for Fast Kinetics Research" E. L. Powers. "Three Components of Oxygen-Dependent Sensitization in Bacterial Spores Irradiated in Suspension" D. L. Ewing and E. L. Powers. E. L. Powers, D. L. Ewing, Z. Zimek attending.
August 18, 1976	Seminar: Strahlenzentum der Justus, Liebig-Universitat, Geissen, Germany. "Free Radicals in Radiation Damage in Cells" E. L. Powers.
October 18-19, 1976	Third International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy, Dallas, Texas. "Sensitization of Cells to X-irradiation by Metals" R. C. Richmond and E. L. Powers.

November 5-6, 1976	Ninth Annual Meeting of the Texas Association for Radiation Research, San Antonio, Texas. "The Current Status of the Center for Fast Kinetics Research" E. L. Powers, M. A. J. Rodgers, and Z. Zimek. "The Radiation Chemistry of the Loss of Biological Activity of Transforming DNA Induced by X-rays" Kathryn Held.
April 20, 1977	Seminar: Institute of Nuclear Research, Warsaw, Poland. "Chemical Mechanisms in Radiation Damage in Cells" E. L. Powers.
April 25-27, 1977	Second International Conference on Radiation Sterilization of Medical Products, Vienna, Austria. "Water as a Modulator of Radiation Damage to Microorganisms" E. L. Powers.
May 7-12, 1977	25th Annual Scientific Meeting of the Radiation Research Society, San Juan, Puerto Rico. Symposium: "Attempts at Reconciliation of Radiation Chemistry with Radiation Biology" E. L. Powers.

IV. Progress Report

The research goals of this laboratory have not changed in principle since its founding eleven years ago. We are interested in the recognition of physical and chemical intermediates between the absorption of energy and the appearance of biological evidence of radiation injury. In describing the roles of water in these effects with the use of dry spore systems, we were able to isolate at least six separate characterizable kinds of radiation damage, including two O_2 effects. More recently we have been studying the response of these cells in aqueous suspensions to recognize the extent to which free radicals and other short-lived species induced by high energy radiation contribute to biological change.

In 1970 we described the first clear evidence based on sound radiation chemistry that showed the hydroxyl radical is under certain circumstances an important free radical species in bringing about damage. The research since then has fully supported the role of the OH radical in accomplishing, under certain conditions, part of the effects observed. A large amount of work resulted in several general propositions concerning the way in which chemical processes induced by radiation change the structure of target molecules in these cells. In particular, we have proposed the electron sequestration hypothesis for the action of chemical sensitizers: this involves a subtraction of free electrons which otherwise would react with primarily produced OH radicals to form harmless OH ion. The sensitizer acts by reacting with the electron thus sparing the OH radical and increasing its effective concentration. Over the most recent period, this proposal has been refined to the point that we can argue that this kind of action must take place within tens of nanometers of the target molecule.

In seeking our general goals, we have been able to become more specific in the chemical questions asked and, as a consequence, have been able to demonstrate: a third O_2 effect which is different from the other two O_2 effects in being associated with the OH radical action of some sort; and the chemical details of the action of some organic sensitizers that demonstrate that there may be two kinds of chemically distinct actions of these sensitizers neither of which perhaps mimcs the action of O_2 as proposed by other workers in this field; that the protective action of certain compounds that interfere with OH radical action may not be the consequence of the removal per se of the radical, but rather in some instances, the production of a secondary reducing radical on the scavenger itself; that many metals are very strong sensitizers and a methodical study of the action of metals in effecting radiation sensitivity is required. In addition there are a number of other studies, some of which are in preliminary form, such as the action of singlet oxygen in radiation effects and the sensitivity of the biological activity of transforming DNA as a molecular model.

We believe that the program has been consistent in its step-wise and systematic inquiry into the chemistry and, perhaps, eventually, the physics of radiation injury. We believe further that many of these studies could have important significance, such as the understanding of

the mechanisms of the actions of radiation sensitizers with reference to radiation therapy practice, and the understanding of the significance of changes in radiation sensitivity induced by metals in this increasingly contaminated planet.

The coming of age for the Center for Fast Kinetics Research, characterized by the enabling of the electron pulse generator (the Van de Graaff accelerator), and the laser flash photolysis system, the development of the absorption and emission spectroscopic analytical instrumentation, and the bringing on-line of the PDP-11T34 Computer system that collects, analyzes, prints and plots out data within minutes after its acquisition, opens to us a marvelous opportunity to run parallel studies on the radiation chemistry of the particular biological phenomena that are described. The Laboratory of Radiation Biology supported in part first by AEC, then ERDA, has played a significant role in the development of the Center for Fast Kinetics Research, for it is this laboratory that was the operating scientific establishment during its development. I propose that the Center could not have developed as quickly as it did had it not been for the close association of this Laboratory with that entity as it was being designed and then constructed. In a real sense, ERDA support of the Laboratory of Radiation Biology has helped significantly in the development of the CFKR to its present effective form.

The report begins with specific statements describing briefly each element of the work that has been completed in the past three years and, later, sections expand on these individual statements.

N. B. Two methods of citation are used in this report. Numerical citations refer to publications copies of which are attached as appendices. These represent work of the current three-year period that has been reported in the literature. Nominal citations refer to other publications that are listed in full at the end of each section.

IV. A. General summary statements describing some of the work.

IV. A. 1. Published work

The following are general statements covering published work.

1) The hydrated electron sequestration hypothesis for the action of certain inorganic compounds which sensitize spores to ionizing radiation (Powers, 1972) has been supported by very much more work and has become proportionately more solid. A large number of papers reported below support in general the basic proposition.

2) This hypothesis supports the notion that ordinarily the solvated electron is not an active participant in radiation damage in cells. After these 10 or 15 years of knowledge of the hydrated electron, no direct evidence of its having any biological importance in a living system has been demonstrated.

3) In one exposition we have been able to argue that the action of the hydrated electron in reducing effective concentration of hydroxyl radicals must take place within two water molecules distance of the target molecule (1). This means that the effective diffusion distance of hydroxyl radicals in living cells is quite short.

4) The effectiveness of many organic and inorganic compounds in sensitizing bacterial spores to high energy radiation can be correlated very well to their redox potentials (5). While there are some deviations, the generalization holds for many of the sensitizers. This furnishes us with a handle for predictability as well as an entry into mechanisms of action.

5) Certain classes of protective agents, such as alcohols, operate only if they form strongly reducing radicals following each abstraction of $^{\cdot}H$ by hydroxyl radicals. This indicates that the protective action, being a reducing one, is directed toward some oxidizing species which is a "damaged intermediate".

6) A large number of metals are strong sensitizers (4, 13, 17, 22). These act mostly via an OH radical mechanism and in this sense they act alike. However, in regard to many details each of these sensitizers seems to be a unique substance, indicating that there is chemistry associated with each metal separate from the hydroxyl radical mechanism. That requires study.

7) The platinum series is notable and deserves special mention in this summary because of the demonstrated anti-tumor activity of some of them (17, 22). These are strong radiation sensitizers and, in another laboratory, combination radiation and platinum treatments have been tried successfully against some resistant tumors.

8) The two O_2 effects described many years ago for dry spores have been demonstrated now in aqueous suspension in this laboratory following the demonstration by Tallentire and his group in Manchester a little earlier.

9) In conjunction with this demonstration, a third O_2 effect has been discovered which is different in its properties from the other two (18). Only in the lower O_2 concentrations can this been seen; and it is associated with an action of hydroxyl radicals. It is of interest that two O_2 effects have now been discovered in mammalian cells.

10) With respect to our studies on organic sensitizers and the mechanisms of their actions, the following general comments are of interest:

a) The sensitizer para-nitroacetophenone (PNAP) can be demonstrated on the basis of concentration studies to be active in two ways (15). That is, it demonstrates two components of action. One of these is associated with hydroxyl radical activity.

b) The action of PNAP appears to be different from that of O_2, contradicting the proposals of others that PNAP and compounds like it mimic the actions of O_2 (20). This conclusion of ours is a consequence of a study of the action of a series of alcohols that reduce sensitization of both PNAP and O_2.

c) In fact, PNAP can reduce the O_2 effect by reacting with the hydroxyl radical dependent O_2 component (the third O_2 effect described above) if the O_2 and PNAP are in proper concentrations (25).

11) In Chlorella the radiation sensitivity in several complex enzymatic systems were studied (10). We have shown that photosystem II is more sensitive to x-rays than photosystem I, and that the nitrate reductase system is, in turn, more sensitive than the photosystem II, indicating a variety of radiation sensitivities in the enzymatic systems of these cells.

12) As measured by untraviolet sensitivity during the transformation of the spore to the vegetative cell, there may be two structural confirmations of DNA represented by two resistant peaks which appear at different times during the germination process (21).

IV. A. 2. Unpublished work

The following are general statements covering unpublished work.

1) The biological activity of transforming DNA is x-radiation sensitive and this is largely due to the action of $^{\cdot}OH$ radicals. The action of alcohols reveals also that other chemical mechanisms are involved. Very small concentrations of O_2 sensitize transforming DNA and large concentrations protect it, indicating unexplainable chemical actions of O_2 that must be studied.

2) Singlet O_2 quenchers can reduce the O_2 effect in spores.

3) The $G(^{\cdot}OH)$ at different $[O_2]$ in water was measured by $(SCN)_2^-$ formation with no change being seen; an inconclusive test of the $^{\cdot}OH$ explanation of part of the O_2 effect at low $[O_2]$.

4) Hydroxylalkyl radicals may react with small amounts of O_2 to form organic peroxy radicals. The yields of the three primary products of water radiolysis (e_{aq}^-, $^{\cdot}OH$ and $^{\cdot}H$) are shown to depend strongly on the effective λ of the x-rays used in this laboratory (mainly 50 kVp and 300 kVp).

5) An unexpected effect of very high dose rate correlated with pulse length on radiation sensitivity of spores in aqueous suspension has been observed.

6) It has been shown by studies earlier on EtOH and currently by a different method on Fe compounds that solutes tend to concentrate within or on the surfaces of bacterial spores. This concentration effect alters the numerical values to be considered in chemical reaction rate calculations.

7) Pre-irradiation of solutions to induce chemical changes of solute prior to irradiation of the spores has demonstrated that in some cases chemical changes of the solutions by irradiation produce or inhibit products that may be damaging. In other cases, pre-irradiation results indicate no stable products of irradiation of the solutions alone that can be the causes of sensitization.

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IV. B. Sensitization by metal ions

IV. B. 1. General summary statements

Over the past several years a number of metals have been tested in this laboratory for sensitizing effects. Appendices 1, 4, 13, 17, 22 and 34 represent publications in this area dealing with sensitization of bacterial spores to x-rays by Ag, Co and Pt compounds. It should perhaps be noted here, that all metals are tested over a concentration range to establish peak sensitization; these peak values are reported here. The bacterial spore system is a very sturdy one and, therefore, very suitable for these studies. The maximum concentrations of chemicals tested is limited in some cases by the solubility of the compound, and in a few cases by slight toxicity at very high concentrations of chemical, but, in general, this system can be used to study metals over a greater range and with more certainty than most other systems. As noted below we are certain that the effects have meaning for the eucaryotic cell.

A summary table (Table I - see page 13) has been prepared covering some of the more interesting data we have accumulated. It is apparent from this table that the anion or attached ligand, in the case of complexes, affects the sensitizing ability of the metal. A case in point is further detailed in Table II (see page 14). The $FeSO_4$ $\underline{k}$ value in O_2 is 5.8×10^{-2} $krad^{-1}$ while the other iron results do not exceed a $\underline{k}$ value of 4.0×10^{-2} $krad^{-1}$. There are known chemical effects of some anions (for instance Cl^- scavenges OH radicals, NO_3^- scavenges e_{aq}^- etc.) but in other cases no effects of the anion or ligand would be expected. In this instance SO_4 is not expected to have an effect, yet it does.

Table I

Summary of some Metal Ion Sensitization Data

Ion	Cpd. Used	Max. $\underline{k}$* in N_2	Max. $\underline{k}$ in O_2	Ion Conc. (M) @ Max. $\underline{k}$ in N_2	Ion Conc. (M) @ Max. $\underline{k}$ in O_2	$\underline{k}$ in N_2 with: EtOH	$\underline{k}$ in N_2 with: Formate	$\underline{k}$ in O_2 with: EtOH	$\underline{k}$ in O_2 with: Formate
None	None	1.3	2.5						
[1] Ag^+	$AgNO_3$	2.3	2.6	4×10^{-3}	2×10^{-3}	2.6-1.0	2.8-1.2	2.7	
	Ag_2SO_4	2.4-1.1(250)	2.6	2×10^{-3}	2×10^{-3}	2.1-1.0		2.7	
Cd^{2+}	$CdCl_2$	1.9-0.7(270)**	3.3-1.4(180)	1×10^{-4}	1×10^{-5}	1.4	1.4	2.5	2.5
	$CdSO_4$	1.6	4.1-1.2(140)	1×10^{-4}	1×10^{-4}	1.3	1.3	2.5	2.4
[2] Co^{2+}	$CoSO_4$	2.4-1.4(180)	3.1-2.1(150)	5×10^{-4}	1×10^{-4}	1.4	1.8-1.0	2.3	2.8-1.6
	$Co(NH_3)_6Cl_3$	2.1	2.9	2×10^{-2}	2×10^{-2}	2.0-1.0	2.2-1.2	3.0	
Fe^{2+}	$FeSO_4$	2.7	5.8	2.5×10^{-4}	2.5×10^{-3}	1.3	1.4	5.8-2.9	3.0
Fe^{3+}	$Fe_2(SO_4)_3$	2.1	3.6-0.6(180)	2.5×10^{-4}	2.5×10^{-4}	1.3	1.3	1.8	1.6
Fe^{2+}	FeC_2O_4	2.2	3.6	2.5×10^{-4}	1×10^{-3}	1.3	1.5	2.6	2.7
Fe^{3+}	$Fe_2(C_2O_4)_3$	2.1	4.0	2.5×10^{-4}	2.5×10^{-2}	1.4	1.4	1.8	2.0
Fe^{2+}	$K_4Fe(CN)_6$	1.8	2.6	1×10^{-4}	1×10^{-4}				
Fe^{3+}	$K_3Fe(CN)_6$	1.7	2.9	1×10^{-2}	1×10^{-5}				
Fe^{2+}	$Na_4Fe(CN)_6$	1.7	2.7	1×10^{-2}	1×10^{-5}				
Pt^+	$Pt(NH_3)_2Cl_2$	2.5-1.0(170)	3.1-1.7(190)	2×10^{-4}	5×10^{-5}	1.8-1.1	1.5	2.3	2.2
	$Pt(NH_3)_4Cl_2$	2.3-? (350)	2.8-0.7(300)	2×10^{-4}	2×10^{-4}				
Tl^+	Tl_2SO_4	3.1 - 1.3(150)	3.1	5×10^{-3}	5×10^{-3}	1.6	2.4-1.3	2.8	3.0
	Tl_2CO_3	4.2-2.2(170)	4.3	1×10^{-1}	1×10^{-1}	3.5	3.3	4.3	4.1
Zn^{2+}	$ZnSO_4$	1.5	2.8	2.5×10^{-2}	2.5×10^{-3}	1.3	1.5	2.5	2.7

* All $\underline{k}$ values shown are in $Krad^{-1}$ ($\times 10^2$)

** (270)=Break point in survival curve occurs at 270 Krads.(Low dose $\underline{k}$ value given first.)

[1] Richmond and Powers, 1974, Radiat. Res. 58: 470-480.

[2] Richmond, Simic and Powers, 1975, Radiat. Res. 63: 140-148.

Table II

Table of Fe Sensitization

(Minimum conc. of Fe shown at which maximum sensitization occurs)

	Sulfate		Oxalate		K or Na CN complexes		
Additive	Fe^{2+}	*Fe^{3+}	**Fe^{2+}	Fe^{3+}	Fe^{2+}(K)	Fe^{3+}(K)	Fe^{2+}(Na)
In Nitrogen							
None	1.6		1.6			1.38	
Fe only	2.6	2.2	2.2	3.6	1.8	1.7	1.7
(Molarity)	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-4}	10^{-2}	10^{-2}
Fe + EtOH	1.3	1.2	1.3	1.4			
Fe + t-BuOH (5×10^{-1} M)	1.4	1.4	1.4	1.3			
Fe + Formate	1.4	1.3	1.6	1.4			
Fe + Catalase	1.8	1.6	1.6	1.7			
In Oxygen							
None	2.5		2.5			2.4	
Fe Only	5.8	3.8	3.6	4.0	2.6	2.9	2.7
(Molarity)	10^{-3}	10^{-4}	10^{-3}	$^{1}10^{-2}$	10^{-4}	10^{-5}	10^{-5}
Fe + EtOH	3.2	1.8	2.6	1.8			
Fe +t-BuOH (5×10^{-1} M)	4.8	2.6	2.5	2.5			
Fe + Formate	2.7	1.6	2.7	2.0			
Fe + Catalase	2.4	2.0	2.6	--			

* - Max conc. tested due to sol. limits, 2×10^{-3}M

** - Max conc. tested due to sol. limits, 10^{-3}M

1 - Maximum uptake measured (all 10^{-4}M conc. would be minimal uptake)

Generalizations: CN < Oxalate < SO_4 in sensitization based on $\underline{k}$ values only.

No generalization apparent as to Fe^{3+} vs Fe^{2+}

Table I also notes the presence of break point lines, common throughout the studies of metal ions, particularly at the peak sensitizing concentration. In every case, the "break" is from higher to lower sensitivity, i.e., the low dose portion has a high sensitivity; then an abrupt reduction in sensitivity occurs reducing the k value, in many cases to one much below that seen in N_2 baselines. To date, no correlation between this sharp break point and calculations based on exhaustion or formation of compounds predicted by radiation chemistry can be found.

Also listed in Table I are results of scavenging OH radicals (by means of EtOH) and scavenging OH radicals and H_2O_2 (by means of formate). Previous work by this laboratory (Powers, 1972)[4] has demonstrated a mechanism of radiation sensitization involving these two factors. In most cases these scavengers reduce the sensitization, indicating some involvement of this mechanism in metal ion sensitization. In many cases this reduction of sensitization is not complete, and in a few instances there is no reduction. Where there were break points, scavengers have erased them and in some instances the scavengers have created break points where there were none. The scavenger results in general indicate $^{\cdot}OH$ is operating in the sensitization, but the variety of kind also indicate other mechanisms are involved.

A general uncertainty obtained in all experiments like these concerns changes induced by irradiation in the added solute and during this past year "pre-irradiation" tests were completed on these several compounds to attempt to measure the importance of this. This procedure involves irradiating the solutions prior to addition of the spores, then irradiating in the normal fashion. Results of these experiments are presented in Table III (see page16) If long-lived products of irradiation of the chemical itself played a role in sensitization by these compounds, it should become apparent from results of this procedure.

Results varied a great deal as is shown in Table III. Both Ag salts and both Pt compounds showed small increases in sensitization when the solutions were pre-irradiated. A remarkable increase in sensitization occurred when $ZnSO_4$ was pre-irradiated. The k value in N_2 jumped from 1.5×10^{-2} krad^{-1} to 7.4×10^{-2} krad^{-1}. An equally dramatic increase in k value was seen in O_2. The Fe salts, on the other hand showed small decreases in sensitivity except for the oxalate salts which did not change significantly. The large k of 5.8×10^{-2} krad^{-1} produced by $FeSO_4$ in O_2, with pre-irradiation of the solutions dropped to a 3.6×10^{-2} krad^{-1}, this being approximately the k value seen in the other three Fe salts without pre-irradiation. This indicates some effect of $FeSO_4$ in O_2 particular to that compound and requiring that the spores be present during that reaction to cause the maximum sensitizing effect. One could speculate on the basis of this that some intermediate product of irradiation of the solutions or the effects of some process during irradiation causes the extra sensitization seen in $FeSO_4$ in O_2 over the other Fe compounds.

The general conclusions at this point must be that the sensitization by metal compounds is 1) very complex, 2) very effective in some cases, and 3) characteristic of the compound itself--i.e. some resemblances may exist from one compound of a metal ion to another but extreme differences also exist within compounds of the same metal ion. For these reasons, we must continue to consider each compound individually,

Table III

Pre-irradiation Data

Additive (Concentration)	Gas	Without pre-irradiation k_L[1]	k_H[2]	BP[3]	With pre-irradiation k_L	k_H	BP	DOSE[4]
$AgNO_3$ (4×10^{-3} M)	N_2	2.3			2.8	1.1	210	330
(2×10^{-3} M)	O_2	2.6			3.0			300
Ag_2SO_4 (2×10^{-3} M)	N_2	2.4	1.1	250	4.1	1.0	130	350
(2×10^{-3} M)	O_2	2.6			3.2			280
$CdCl_2$ (1×10^{-4} M)	N_2	1.9	0.7	270	2.0	0.6	250	330
(1×10^{-5} M)	O_2	3.3	1.4	180	3.3	1.5	170	200
$CdSO_4$ (1×10^{-4} M)	N_2	1.6						
(1×10^{-4} M)	O_2	4.1	1.2	140	2.3			200
$CoSO_4$ (5×10^{-4} M)	N_2	2.4	1.4	180	3.0	1.2	100	300
(1×10^{-4} M)	O_2	3.1	2.1	150	3.2	1.4	200	250
$Co(NH_3)_6Cl_3$ (2×10^{-2} M)	N_2	2.1			2.5	0.7	250	400
(2×10^{-2} M)	O_2	2.9			3.5	0.6	200	280
$FeSO_4$ (2.5×10^{-4} M)	N_2	2.7			2.0			500
(2.5×10^{-3} M)	O_2	5.8			3.6			180
$Fe_2(SO_4)_3$ (2.5×10^{-4} M)	N_2	2.1			1.9			500
(2.5×10^{-4} M)	O_2	3.6	0.6	180	3.0			500
FeC_2O_4 (1×10^{-4} M)	N_2	2.2			1.8			380
(1×10^{-3} M)	O_2	3.6			3.1	0.8	210	230
$Fe(C_2O_4)_3$ (1×10^{-4} M)	N_2	2.1			2.2	0.9	250	430
(1×10^{-2} M)	O_2	4.0			3.8	0.7	190	200
$Pt(NH_3)_2Cl_2$ (2×10^{-4} M)	N_2	2.5	1.0	170	3.1	1.4	140	250
(5×10^{-5} M)	O_2	3.1	1.7	190	3.7	0.4	210	250
$Pt(NH_3)_4Cl_2$ (2×10^{-4} M)	N_2	2.3	?	350	3.4	1.0	130	350
(2×10^{-4} M)	O_2	2.8	0.7	300	3.3			400
Tl_2SO_4 (5×10^{-3} M)	N_2	3.1	1.3	150	2.7	1.5	70	250
(5×10^{-3} M)	O_2	3.1			3.9	2.1	80	250
Tl_2CO_3 (1×10^{-1} M)	N_2	4.2	2.2	170	4.4	2.3	70	230
(1×10^{-1} M)	O_2	4.3			4.0			200
$ZnSO_4$ (2.5×10^{-2} M)	N_2	1.5			7.4	1.1	70	480
(2.5×10^{-3} M)	O_2	2.8			8.6	1.7	50	280

[1] k value for low dose portion of curve or full curve if no break point is evident ($\times 10^2$) ($krad^{-1}$)

[2] k value for high dose portion of curve when break point is evident ($\times 10^2$) ($krad^{-1}$)

[3] dose at which breakpoint occurs

[4] total dose given as pre-irradiation

while seeking sufficient understandings of the mechanisms to allow grouping and sorting of metal results by some common factors that must exist. Most importantly, serious study of the radiation chemistry of these substances under conditions obtained in these experiments must be done.

REFERENCES

Powers, E. L. (1972) The hydrated electron, the hydroxyl radical and hydrogen peroxide in radiation damage in cells. Symposium on Molecular Basis of Radiation Biology, Hebrew University of Jerusalem, Stein, G. ed., Isr. J. of Chem. (No. 6) 10:1199-1211.

IV. B. 2. Additional studies on Pt complexes

The Pt series is of special interest because some Pt compounds have been described as effective anti-tumor agents (Rosenberg, 1977). One serious problem is that they are very toxic in humans at effective anti-tumor levels. Our Co-complex results indicated the Pt-complex sensitization study. The outside hope is that combined irradiation-Pt therapy might allow low doses of both agents in tumor therapy.

Two new complexes of Pt are being tested, sulfato (1,2-diammino-cyclohexane)Pt(II) and malonato (1,2-diammino-cyclohexane)Pt(II). These tests are as yet incomplete but results to date as compared to those previously achieved with cis-dichlorodiamminePt(II) (17,22) are shown in Fig. 1. In N_2 the dichlorodiammine and the dichlorotetrammine are very effective sensitizers over a wide low range of concentrations.

Since publications of (17) tests by Douple (1977) using combined Pt-x-irradiation therapy on a rat brain tumor resulted in complete tumor control in 5 of 12 animals. This tumor had not responded to any treatment before.

REFERENCES

Rosenberg, B. and Van Camp, L. (1970) Cancer Res. 30:1799-1802.

Douple, E. B., Richmond, R. C. and Logan, M. E. (1977) J. Clin. Hematol. and Oncology 7:585-603.

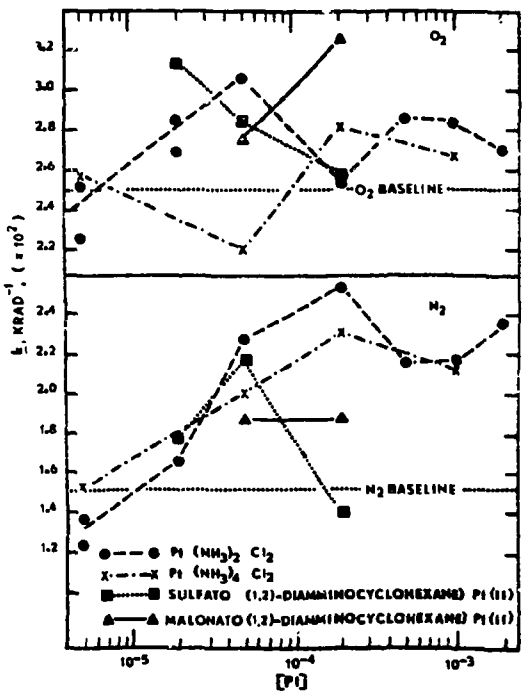

Fig. 1 Radiation sensitivity of bacterial spores vs concentration of Pt(II) complexes in O_2 (top) and in N_2 (bottom).

IV. B. 3 Additional studies on Ag_2SO_4

In the study of Ag^+ (4), the curious result in O_2, namely, 20% O_2 + Ag^+ increased sensitivity to more than 100% O_2 + Ag^+, led to a series of experiments to test 2×10^{-3} M Ag^+ (Ag_2SO_4) against various concentrations of O_2 in N_2. The results of these experiments are shown in Fig. 2. It will be noted when comparing this figure with the original work, there is some slight difference in baseline k values, both the N_2 and O_2 baseline currently being slightly higher than previously.

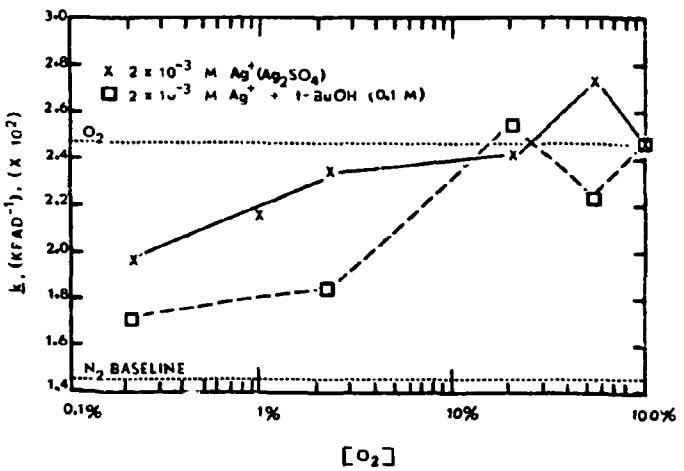

Fig. 2 Radiation sensitivity of bacterial spores in the presence of 2×10^{-3} M Ag^+ (Ag_2SO_4) with and without t-BuOH vs O_2 concentration in N_2

There is a sharp peak effect at 55% O_2 in N_2. If one calculates reactions with e_{aq}^- assuming that Ag^+ and O_2 are competing for the electron, and that the reactions with Ag^+ therefore subtract from the possible reactions with O_2, the normal requirement of over 5% O_2 needed for a full O_2 effect then increases to approximately 55%. Therefore, it could be that this peak is due to the maximum O_2 effect being achieved and being at least partially additive to the Ag^+ effect.

The effects of t-BuOH, which scavenges $^{\cdot}OH$, indicate some involvement of the $^{\cdot}OH$ mechanism of sensitization in the Ag-O_2 sensitization. Since, however, the t-BuOH only affords partial protection, this would not appear to be the only mechanism involved. It is also contradictory that the t-BuOH would sensitize at 21% O_2 in N_2. We have however, seen other cases indicating that t-BuOH itself, under very special circumstances seems to be a sensitizer. At 100% O_2, there is no effect of t-BuOH--as would be the case if there were no Ag^+ present. This seems to infer some action of O_2 which interferes with an action of Ag^+ that relates to $^{\cdot}OH$. Or, in other words, some portion of Ag^+ sensitization is due to $^{\cdot}OH$ and this portion is either not present or interfered with when 100% O_2 is present.

IV. B. 4. Studies related to metal ion sensitization

IV. B. 4. 1. Ag^+-DNA studies

The known complexing of Ag^+ with DNA prompted a series of in vitro experiments with this cation and calf thymus DNA (14). DNA loses its hypochromicity (i.e. the extinction coefficient, ε, increases) with increasing doses of radiation delivered anoxically; this kind of damage probably indicates DNA strand breakage and subsequent unwinding of the molecule. As the dose is increased further, ε peaks and then declines, showing DNA base damage. $^{\cdot}OH$ are involved in both these kinds of damage; EtOH or t-BuOH prevented these changes of optical density. When N_2O was present during irradiation, increased amounts of both kinds of damage were observed. Complementary experiments were also run which used

sedimentation gradients to separate degraded fragments of irradiated DNA. The presence of Ag^+ plays no apparent role in altering the rates of DNA degradation. Although all the results are not entirely straightforward, the expected complexing of Ag^+ with DNA seems to have little significance regarding the actions of this cation as a biological radiation sensitizer; the responsible sensitizing mechanisms seem to be wholly 'OH reactions.

IV. B. 4. 2. Cellular uptake of solutes

While the problem of the concentration of sensitizers in the cell is not peculiar to metal ions, it is reported here since, at this time, a large portion of our studies of uptake are by Atomic Absorption Spectrophotometry, applicable to all the metal ions. These past 3 years, techniques have been developed for analysis for Fe ions. This technique appears to be useable and fit for the other metal ions of interest to us, with the possible exception of Hg. This method has shown that the concentration of the solution and the number of spores present both affect the amount of metal ion taken up by the spore. The technique involves suspending the spores in metal ion solutions, centrifuging and measuring the loss of metal ion from the supernatant, as well as the gain of metal ion in the pellet. At lower concentrations of metal ion, the two measurements agree quite well; but at high concentrations, the supernatant loss is somewhat greater than the pellet gain. This discrepancy is, as yet, unresolved; therefore, averages of the two determinations were used to obtain the estimates of concentrations.

As can be seen in Fig. 3, there can be a considerable concentration effect of the metal in the spore. The amount of this "uptake" is affected by radiation and the gas present during irradiation as well as the presence of alcohols. Also observed in these studies is the tightness with which the $FeSO_4$ is bound or absorbed by the bacterial spore. Efforts to "wash out" this compound are not completely successful even using 1 M acetic acid as a wash. An alternative wash material, o-phenanthroline (known for its ability to complex Fe) did not remove any of the Fe "taken up" by the spore.

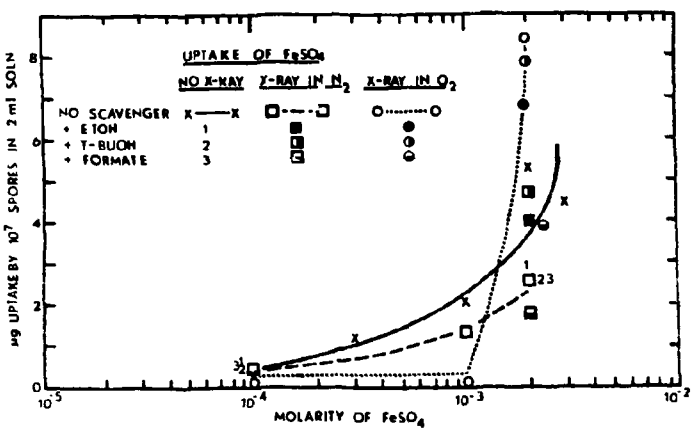

Fig. 3. Effects of irradiation and the presence of scavengers and gaseous atmosphere during irradiation on the uptake of $FeSO_4$ by bacterial spores.

Results obtained so far on other salts of Fe indicate that as in the radiation sensitization, each compound will exhibit individual characteristics. These results do emphasize the need for this information on all the sensitizers since actual concentrations of the sensitzer in (or on) the spore must be known to make accurate calculations of chemical reaction rates.

An alternative measurement technique available to us on some metal ions is by electrode measurements. This technique, when possible, will augment and confirm the data obtained by atomic absorption.

IV. C. Correlation of sensitization and redox potential

Appendix 5 describes an area of our research which is still active. Four compounds have been tested this past year which were suggested for biological testing by their redox potentials. One of these, diamide, showed a maximum k value of 2.3×10^{-2} krad^{-1} in N_2 at 1×10^{-4} M. Since it was reported to have a redox potential of -0.2, it was expected to have maximum sensitization at 10^{-3} M rather than 10^{-4} M.

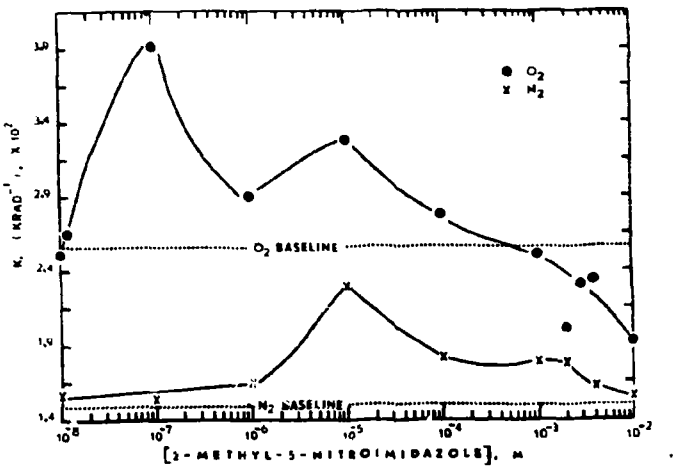

Fig. 4. Radiation sensitivity of bacterial spores vs concentration of 2-methyl-5-nitroimidazole in O_2 and N_2.

Chloramine T, which was expected to peak at 4×10^{-4} M demonstrated no large sensitizing effects when tested from 10^{-2} to 10^{-6} M. A very slight sensitization was observed from 1×10^{-2} to 4×10^{-4} in N_2--the area predicted by redox potential.

Another compound, 2-methyl-5-nitroimidazole showed remarkable ability to sensitize at a very low concentration as shown in Fig. 4. Since the E^o for this compound is -.542, it was expected that it would show peak sensitizing effects in N_2 at 5×10^{-3} M. This, however, was not the case but the results seem worth reporting at this time because of the ability of this compound to sensitize so effectively at such a low concentration. In O_2, 10^{-7} M produced a k value of 3.9×10^{-2} krad^{-1} and in N_2 the k value at 10^{-5} M was 2.3×10^{-2} krad^{-1}. There is a small hump of sensitization at 5×10^{-3} M in N_2 which may be due to the redox potential correlation with sensitivity but if so it is abundantly clear for this particular compound that other factors, in lower concentrations, are far more significant in the total sensitizing effects.

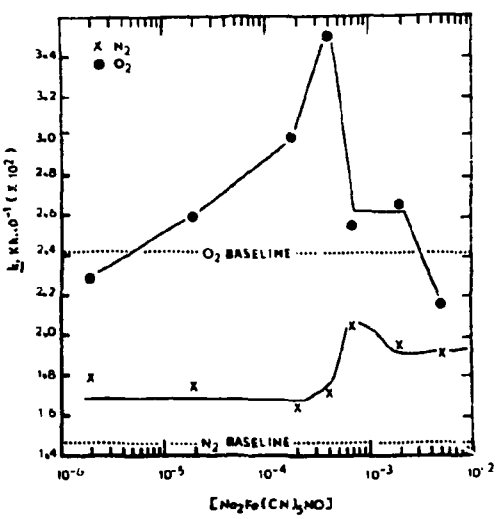

Fig. 5. Radiation sensitivity of bacterial spores vs concentration of a nitroprusside compound in O_2 and N_2.

The fourth compound tested was a nitroprusside ($Na_2Fe(CN)_5NO$) with an E^o of -0.1. This redox potential predicted peak sensitization in N_2 at 7×10^{-4} M. This, as shown in Fig. 5 was the case.

Again other sensitizing features in O_2 at somewhat lower molarities seemed of importance and therefore are included.

As was stated in the original publication on redox potential vs sensitization (5) there is a good correlation in many compounds between redox potential and sensitization in N_2, allowing, as we saw in the prusside compound, a prediction of the concentration at which peak sensitization will occur. The precision and pertinence of the E^o values as related to sensitizing reactions may be a factor limiting the number of compounds to which this correlation applies. And, as can be seen by the examples above and those in the publication, other factors contribute to sensitization, sometimes at other molarities and sometimes at the same molarities. When the redox correlation is better understood, it can then be sorted from the other factors allowing further understanding of the mechanism of sensitization as a whole.

IV. D. Organic sensitizers

As noted previously, this portion of the research work has been placed somewhat in the background with the departure from our staff of Dr. David Ewing. Publications covering this research are presently in various stages and may be noted as Appendices 15, 18, 19, 20, 24-27, 30, 31, 33, and 35.

IV. E. X-ray effects in photosynthetic organisms

Parallel studies have been run with a procaryotic blue-green alga and an eucaryotic alga, Chlorella, (10, 12, and 32). In the blue-green algae, survival, loss of photosynthetic ability (as measured by a decrease in oxygen evolution), and the radiation sensitivity of an essential enzyme were examined. With Chlorella, lethality (loss of reproductive ability) and loss of oxygen-evolving ability were studied. With either organism, the photosynthetic apparatus was relatively more resistant to irradiation than reproductive ability. In Chlorella, the sensitivity of photosystem II is greater than that of photosystem I.

IV. F. UV effects

Appendix 21 is the result of a small project studying effects of UV on sensitivity during germination, and represents the only UV work done during this period.

IV. G. Transformation studies

IV. G. 1. Preliminary studies

The transformation studies were begun two years ago as a means of studying the chemical mechanisms involved in the x-ray induced loss of biological activity of DNA. Extraction of DNA from Bacillus subtilis W23, wild type, is according to the procedure of Marmur (1961), with modifications by Synek (1967).

The biological activity of this DNA is determined by its ability to transform a tryptophan -requiring mutant, B. subtilis 168M, to tryptophan independence, as measured by ability to form colonies on minimal media. This procedure is from Anagnostopoulis and Spizizen (1961) with modifi-

cations by Syrek (1967) and further adaptations to increase efficiency in our laboratory. The DNA is stored and irradiated in 29 mM phosphate buffer, pH 7.3. No loss of activity is seen in samples stored up to nine months.

1 ml samples of DNA of appropriate concentration are exposed for varying times to 45kVp x-rays in chambers through which the desired saturating gas is flowing, as in our standard method (Powers, 1970). Two dose rates are used, 2.13 krads/min. and 11.73 krads/min. These irradiated samples are then checked for their ability to transform cells to tryptophan independence. For each experiment, two controls are used--one is an untreated DNA sample and the other is a 1 ml DNA sample exposed to stirring and gaseous equilibration as are the irradiated samples. The average of these two controls is taken to be 100% relative transforming ability, and irradiated samples are expressed as a percent relative transforming ability. This relative activity is plotted vs dose to give a typical survival curve with an inactivation rate constant, $\underline{k}$, from the expression $N/N_o = e^{-\underline{k}D}$.

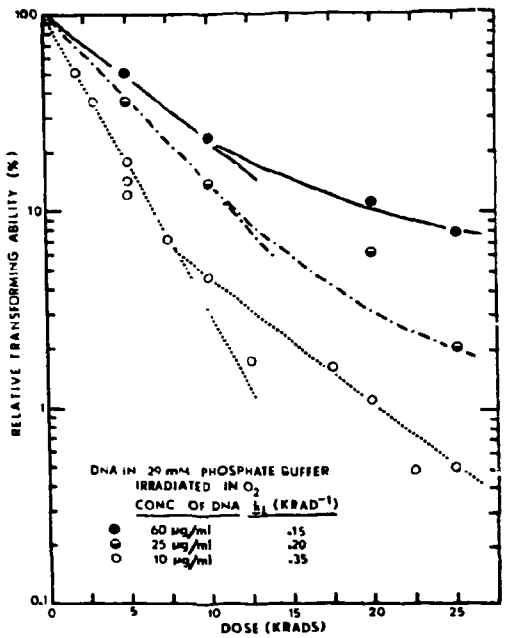

Fig. 6 Effects of DNA concentration on radiation sensitivity of the transforming ability of DNA.

Figure 6 shows survival curves for DNA of various concentrations irradiated in O_2. Most notably these curves are at least biphasic, showing an initial radiation sensitive portion at low doses and a more resistant portion at higher doses. This set of curves clearly demonstrates the existence of a concentration effect in transforming DNA. Therefore a DNA concentration of 10 µg/ml was used in all other experiments.

The effects on transformation of exposing DNA during irradiation to various saturating gases is seen in Fig. 7. These curves are at least triphasic showing an initial radiation sensitive portion inaccessable to study by our present methods, a middle portion with which we will deal primarily, and a radiation resistant region at higher doses which often shows considerable scatter and low colony counts. As noted by the $\underline{k}$ values shown, DNA exposed to x-rays

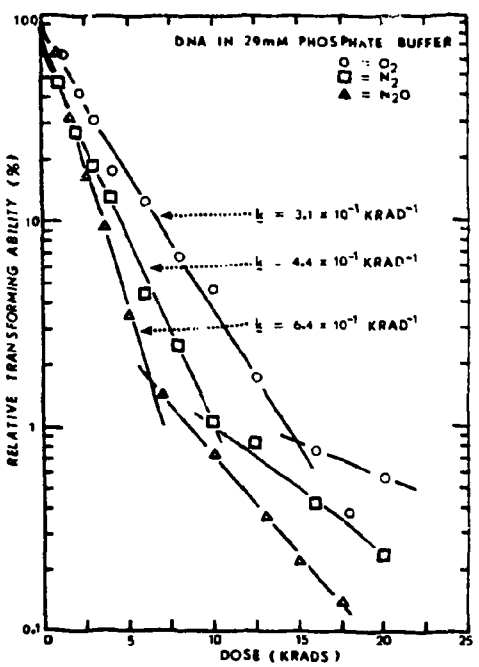

Fig. 7 Effects of O_2, N_2 and N_2O on radiation sensitivity of the transforming ability of DNA.

during gassing with O_2 is least sensitive, in N_2 it is more sensitive, and in N_2O the DNA is most sensitive to irradiation. Although this order of sensitivities differs from that seen in cellular systems, it is qualitatively the same as seen in studies with other biological molecules and phages. This O_2 "protective" effect was seen by others in our laboratory (Gampel, 1972) when using T-7 phage irradiated in media. However, when the phage was purified of organic materials, the k values for exposure in N_2 and O_2 were the same. Perhaps the contamination present in the DNA preparations can account for the O_2 result.

IV. G. 2. Effects of alcohols on transforming DNA

Although the increase in radiation sensitivity of transforming DNA when irradiated in N_2O as opposed to N_2 suggests the involvement of the OH radical in DNA inactivation, an additional test is the use of alcohols as competitors for the $^{\cdot}OH$. Figure 8 shows that the presence of 5×10^{-2} M EtOH in the DNA solution during irradiation in all three saturating gases has a very dramatic protective effect, the sensitivities in each gas plus EtOH being about one tenth that in the gas alone. Included in this figure is the N_2 baseline from Fig. 7 for comparison. The points on each line represent at least two experiments. It appears that in the presence of EtOH the order of sensitivities is changed, with exposure in O_2 being least sensitive, followed by N_2O, then N_2 showing the greatest sensitivity. From these data alone

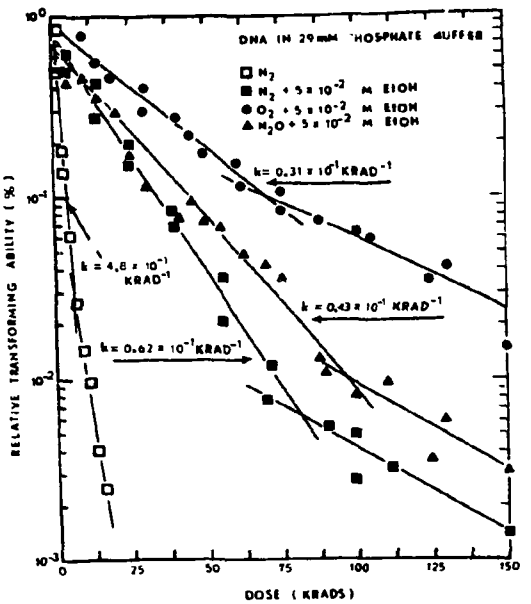

Fig. 8 Effects of EtOH on the transforming ability of DNA during irradiation in O_2, N_2 and N_2O.

it is difficult to assess the significance of this change, but the EtOH protection in all three gases is important as an indication of the affect of $^{\cdot}OH$ in causing DNA inactivation.

This EtOH protective effect was further investigated by a study of the influence of a wide range of EtOH concentrations on DNA irradiated in all three gases. These results are shown in Fig. 9. It can be seen in all three gases that the

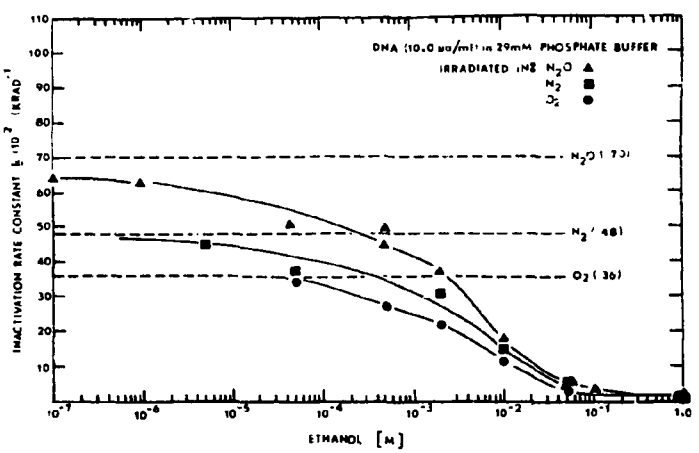

Fig. 9 Inactivation of transforming ability of DNA vs concentration of EtOH during irradiation in O_2, N_2 and N_2O.

inactivation rate constant, k decreases slowly from the baseline to approximately 10^{-3} M EtOH, after which there is a more rapid increase in protection with increasing EtOH concentration to a leveling off starting at about 5×10^{-2} M EtOH in all three gases. Based on a $G_{(CH_3 \cdot CH_2OH)} = 5.4$ for reaction of EtOH with $\cdot OH$ in N_2O-saturated solutions, it can be calculated that 8.4×10^{-4} M EtOH is sufficient to scavenge all OH radicals produced at the maximum doses used in these experiments. As noted from the graph, this level of added EtOH lowers radiation sensitivity of the DNA almost to the O_2 baseline. Addition of EtOH beyond the 10^{-3} M concentration level continues to increase protection of the DNA up to about 5×10^{-2} M EtOH where a leveling off begins in all three saturating gases with the same level of protection reached in all gases at 1.0 M EtOH. This suggests that at these high concentrations, EtOH is exerting some protective effect in addition to $\cdot OH$ removal. More experimentation in this area is needed.

The results of experiments with OH radical scavengers other than EtOH are presented in Table IV. These experiments were conducted in N_2 with the molarity of additive chosen so as to give the same kC (bimolecular rate constant for reaction of the alcohol with $\cdot OH$ times concentration) as EtOH at 5×10^{-2} M. Thus if the $\cdot OH$ scavenging by these alcohols leads to protection by a mechanism similar to that in EtOH, similar k values would be expected. It can be seen from the table that in the five alcohols tested the k values are indeed very close, with slightly more protection seen in allyl alcohol. It is interesting to note that at a concentration of 1.0 M the k in allyl alcohol is 2.0×10^{-3} krad^{-1} as compared to 2.0×10^{-2} krad^{-1} in EtOH. This additional protection may be due to scavenging of $\cdot H$ by allyl. The indication of similar protection mechanisms at the same kC in all alcohols tested is particularly interesting in light of other experiments performed in this laboratory (13 and 19). In these papers it is reported that in the B. megaterium spore system alcohols which react with $\cdot OH$ to form hydroxy radicals at the α-carbon position, e.g., ethyl, methyl and allyl alcohols, protect in anoxia, yet those alcohols which do not form such reducing species, e.g. t-BuOH and t-amyl alcohol, do not protect. Obviously this correlation does not hold up in the transforming DNA system where anoxic protection by alcohols is independent of the type of radical formed.

Anoxic Radiation Sensitivity of Transforming DNA trp^+ in Bacillus subtilis

Alcohol Added	[M]	kc	k(krad)$^{-1}$ ($\times 10^{-2}$)
0	-	-	48.0
t-Butyl*	1.7×10^{-1}	9×10^{7}	7.4
Methyl	1.0×10^{-1}	9×10^{7}	5.9
Ethyl	5.0×10^{-2}	9×10^{7}	6.3
t-Amyl*	5.0×10^{-2}	9×10^{7}	8.3
Allyl	1.5×10^{-2}	9×10^{7}	3.5
Mean of alcohols			7.0

* form β-C radicals

In addition, when 1.7×10^{-1} M t-BuOH is present in a DNA solution irradiated with O_2 as saturating gas, $k = 0.28 \times 10^{-1}$ krad^{-1}, and with N_2O as saturating gas, $k = 0.54 \times 10^{-1}$ krad^{-1}, both approximately the same as the protection seen with EtOH under these conditions.

IV. G. 3. Effects of O_2 concentration on transforming DNA

The fact that the order of sensitivity for transforming DNA inactivation varies from that seen in cellular systems and the knowledge of the effects of various concentrations of O_2 on the radiation sensitivity of spores (18) prompted a study of the effects of various concentrations of O_2 on transforming DNA. The results obtained thus far as shown in Fig. 10, indicate at least three actions of O_2. At very low concentrations of O_2 in N_2 there is an enhancement of radiation sensitivity of at least two times that sensitivity seen in 100% O_2 and 1.6 times in 100% N_2. At this time we lack sufficient data to state exactly at what concentrations this peak sensitization occurs although it appears to be at about 0.22% O_2. Further addition of O_2 at concentrations above this peak causes a partial reversal of this sensitization to about the level of the N_2 baseline over a wide range of O_2 concentrations. At concentrations greater than 86% O_2 in N_2, the O_2 baseline is seen. It is interesting to note that the survival curves for the intermediate O_2 concentrations are exponential over the three decades studied. However, at above approximately 40% O_2 the survival curves begin to break, having a region at low doses with the $\underline{k}$ value of the N_2 baseline and a region at higher doses with a $\underline{k}$ equivalent to that for the O_2 baseline. It should be noted that only those $\underline{k}$ values for the low dose region of the survival curves are given in Fig. 10. At O_2 concentrations of about 86% or above, the survival curves are again exponential, this time showing sensitivity of the O_2 baseline. At this time we can propose no explanation for these effects.

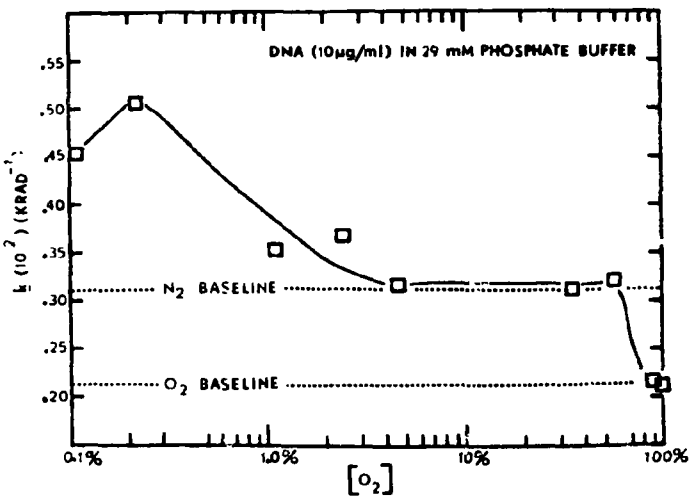

Fig. 10 Inactivation of transforming ability of DNA by x-irradiation in various concentrations of O_2 in N_2.

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IV. H. Free radical studies

IV. H. 1. Introduction

Studies of the application of current knowledge in radiation chemistry to radiation-induced phenomena in cells continue here in the Laboratory of Radiation Biology at the University of Texas at Austin. The advent of operational status of the Van de Graaff accelerator in the CFKR provides an opportunity for us to relate directly to the cellular system radiation chemistry as it is performed currently in many laboratories in the world, i.e., with short pulses of high energy radiation delivered at extremely high dose rates within the pulse. These conditions are different from those usually used in radiation biology: x-rays of longer wavelength producing high LET's are delivered at dose rates considerably lower than those obtained in accelerator pulses. Since these are important variables, the general question then arises as to the propriety of the application of chemistry from one condition to the other.

IV. H. 2. Oxidation and electron-transfer studies*

The ability of compounds to oxidize damaged sites on organic molecules has been studied during this three-year period. Appendices 6, 8, 9 and 11 represent publications in this area.

IV. H. 3. Oxygen and peroxy radicals*

In dry spores, there is evidence that one of the pathways for oxygen-dependent sensitization involves the formation of peroxy radicals (Powers, 1966). The formation and subsequent reactions of peroxy radicals in chemical systems have been extensively studied (3 and 7). Our understanding of these processes and their possible relevance to radiation biology is still limited. An electrophilic molecule such as O_2 might oxidize or form an adduct at a damaged cellular site. One study, reported previously, devised a method for distinguishing between the products after oxidation ($^{\cdot}O_2^-$) and after addition ($^{\cdot}RO_2$, a peroxy radical). The acid-base properties of $^{\cdot}RO_2$ were also studied (3) since this radical will protonate easily and can decompose to $^{\cdot}O_2^-$.

*This research done elsewhere previous to CFKR operational status.

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IV. H. 4. Effect of D_2O, DABCO, dimethylfuran and sodium azide on survival of spores in oxic solution

In a previous communication from this laboratory (18) it was concluded that there is a third O_2 effect different from the other two reported years ago operative in radiation-induced lethality of B. megaterium spores in aqueous suspension. It was held to be hydroxyl radical dependent, as it may be removed by t-BuOH. It has been suggested (Goscin, 1973) that the superoxide anion is largely responsible for the O_2 effect in E. coli B; evidence for this comes from the radioprotective nature of the enzyme superoxide dismutase in this organism.

Work is currently in hand which may show that either this superoxide component, or a third component of the O_2 effect in B. megaterium spores, may in fact be due to the $^1\Delta_g$ state of excited O_2 ("singlet oxygen"). DABCO (1,4-diazobicyclo(2,2,2)octane) and dimethylfuran, both well known quenchers of singlet oxygen, have been shown to reduce the O_2 enhancement ratio by approximately 25% over certain concentration ranges. The effect of sodium azide, also a quencher of singlet oxygen, on the oxic B. megaterium system, is currently under investigation. In the case of DABCO, in the presence of from 0 to ≈10% O_2 in equilibrium with the spore suspension, the protective effect appears to be additive to, and therefore different from the previously reported hydroxyl radical dependent component. D_2O enhances the O_2 effect at low O_2 concentrations; this would appear to be in accord with the prediction of Merkel et al. (1972) that the lifetime of singlet oxygen in D_2O is five times that in H_2O.

Pulse radiolysis experiments are being carried out to ascertain whether the protective effects of DABCO and dimethylfuran are due to scavenging of other species such as $^{\cdot}OH$ or $^{\cdot}O_2^-$; however, the D_2O enhancement would seem to suggest this is unlikely. Speculation has been made concerning the nature of the generation of singlet oxygen in a) pure water and b) concentrated solutions of solutes such as proteins.

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IV. H. 5. The OH radical and the O_2 effect(s)

Recently we have published evidence that the O_2 "effect" is divisible into three effects, depending upon the concentration of O_2 that surrounds the experimental organism (in this case, bacterial spore) and that one of these is associated with an action of the OH radical (18). In these experiments, sensitization of spores by O_2 commences at about 10^{-6} M (dissolved O_2), reaches a plateau at 1.5×10^{-5} M to 5×10^{-5} M, then increases to a maximum above 2×10^{-4} M. Addition of 0.1 M t-BuOH reduces the level of sensitization at the plateau but has no effect on sensitization at O_2 concentration greater than 8×10^{-5} M. This indicates that the sensitization of spores by O_2 can depend on three chemical processes. One of these processes involves reactions of OH radicals, the other two apparently do not. In terms of OH radical scavenging by alcohols, Ewing (19) following the suggestion of Richmond et al. (4) has found that a series of alcohols, those which form α-hydroxylalkyl radicals on reaction with $^{\cdot}OH$ protected under anoxic conditions, while those (e.g. t-BuOH, t-amyl alcohol and benzyl alcohol) forming other radicals, did not protect. However, both t-BuOH and t-amyl alcohol did show protection at an intermediate O_2 concentration ($[O_2] = 3.5 \times 10^{-5}$ M).

Accordingly, two pulse radiolysis experiments were carried out utilizing the CN Van de Graaff at CFKR. This machine produces 4 MeV electrons at beam current intensities of 250ma. Electron pulses of 200 or 800 ns were used in the experiments to be reported. Transients were produced in a quartz cell approximately 9cm from the accelerator exit port, and were detected by means of light from a pulsed 450W xenon arc lamp. The light, after leaving the cell, was focused on the entrance slit of a Bausch and Lomb high intensity monochromator, and from there onto the photocathode of an RCA 1P28 or Hamamatsu 928 photomultiplier tube. The signal from the photomultiplier was stored in a Biomation 8100 transient recorder and was subsequently plotted for kinetic analysis on an X-Y plotter.

First, the G value for OH radical formation (number of ·OH produced per 100eV of energy absorbed) was measured over a range of dissolved O_2 concentration from 0 to 3 x 10^{-4} M (air-saturated) using the formation of the thiocyanate diradical anion as a monitor of ·OH production:

$$SCN^- + {}^{\cdot}OH \rightarrow SCN^{\cdot} + OH^-$$

$$SCN^{\cdot} + SCN^- \rightleftarrows (SCN)_2^{\cdot -}$$

$G_{OH} \approx 2.7$ in N_2 saturated water. Powers et al. (1973) have shown that yield of ·OH may increase in the presence of high concentrations (>10^{-2} M) of nitrate ion. In similar fashion an increase in G_{OH} with increased O_2 (also an efficient electron scavenger) would correlate well with the biological observation. However, as may be seen from Fig. 11 no such effect was observed and, indeed, there is no known chemical reason to expect such an increase.

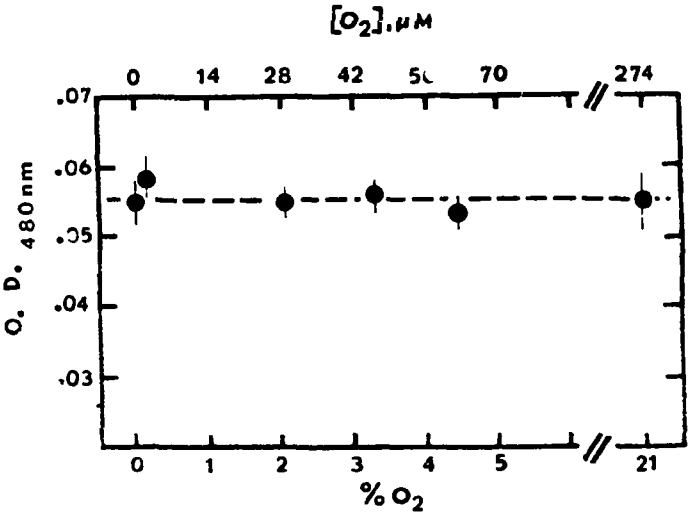

Fig. 11 Absorption of $(SCN)_2^-$ at 480 nm against $[O_2]$ during irradiation.

Secondly the rates of reaction of ·OH with EtOH and formate ion (which form α-hydroxylalkyl radicals) and benzyl alcohol and t-BuOH (which do not) are measured at a range of low O_2 concentrations by means of competition kinetics using again the thiocyanat diradical anion system (Baxendale, 1968, and Willson, 1971). In this case, the reactivity of the hydroxylalkyl radical with O_2 to form a peroxy radical could be expected to have an effect in the biological system; the reaction rates of α- or β-hydroxylalkyl radicals with O_2 may differ

$$^{\cdot}OH + HR\text{-}OH \rightleftarrows H_2O + {}^{\cdot}R\text{-}OH$$
(Hydroxylalkyl)

$$\cdot R\text{-}OH + O_2 \rightarrow \underset{\text{(peroxy)}}{\cdot OOR\text{-}OH}$$

or the nature of the peroxy radicals themselves may influence the biological system (3).

This experiment also proved inconclusive (Fig. 12). No appreciable effect of O_2 was seen on the rate of reaction of $\cdot OH$ with these alcohols.

In the literature of pulse radiolysis it is generally implied that hydroxylalkyl radicals, once formed (as they are commonly in $\cdot OH$-scavenging reactions), are subsequently unreactive. However, O_2 in its normal $^3\Sigma_g^-$ state is extremely reactive with most free radicals, and hydroxylalkyl radicals are no exception. Two processes may occur (3):

$$\cdot RH - OH + O_2 \begin{cases} \nearrow \cdot OORH\text{-}OH \text{ (addition)} \\ \searrow R\text{-}OH + H^+ + \cdot O_2^- \text{ (oxidation)} \end{cases}$$

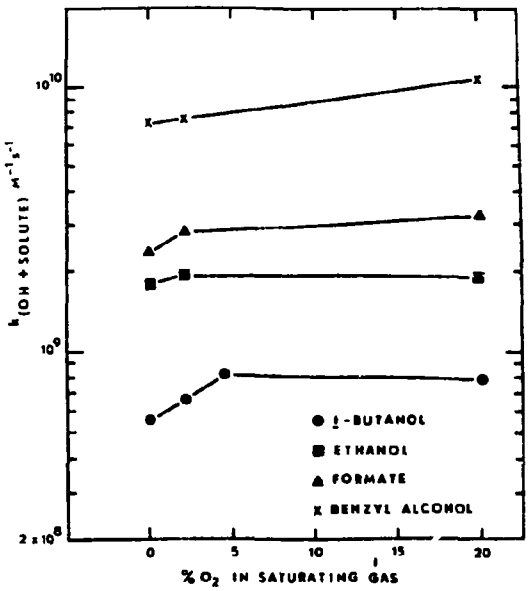

Fig. 12 Rate constant (k) of $\cdot OH$ + solute vs O_2 concentration in several $\cdot OH$ scavengers.

Organic peroxy radicals formed by the addition process generally absorb below 300 nm, and so their rate of formation may be observed directly by pulse radiolysis. 0.1 M solutions of methanol, t-BuOH, iso-propanol and sodium formate in water were saturated with a mixture of 7.5% O_2 in N_2O and subjected to pulse radiolysis. The N_2O scavenges electrons, converting them to OH radicals and effectively doubling the $\cdot OH$ yield, and also prevents formation of the superoxide radical by the reaction:

$$e^-_{aq} + O_2 \rightarrow \cdot O_2^-$$

$\cdot O_2^-$ absorbs at 280 nm, the monitoring wavelength, and could interfere with the peroxidation reaction. Beam conditions used were ~250 mA current with a pulse-width of 200 ns. By observing the transient growth at λ=280nm, rates of formation of the peroxy radicals of methanol and t-BuOH were measured as $1.6 \pm 0.3 \times 10^9\ M^{-1}s^{-1}$ and $2.6 \times 10^9\ M^{-1}s^{-1}$ respectively. In the case of iso-propanol the formation occurred substantially within the duration of the pulse, suggesting a rate of formation of the peroxy radical of $k \simeq 2 \times 10^{10}\ M^{-1}s^{-1}$. The transient observed when sodium formate was pulse-irradiated under these conditions developed maximum absorption within the pulse then decayed with a half-life of 2.2μs to a constant level. In this case, because of the high pH (10.7) the reaction involved was probably not a peroxidation.

We conclude that hydroxylalkyl radicals may react rapidly with quite small amounts of dissolved O_2, to form organic peroxy radicals. Little is known of the subsequent reactivity of these peroxy radicals in biological systems lacking catalase.

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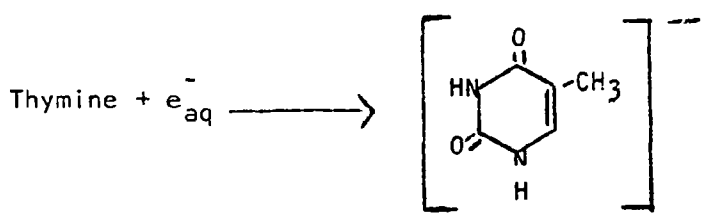
IV. H. 6. The effects of x-ray photon energy on primary yields

For chemical data, commonly obtained in experiments using accelerated electrons or ^{60}Co γ-rays, the yields, or G-values, of the primary radicals are taken as being $G_{e^-_{aq}} = 2.7$, $G_{\cdot OH} = 2.7$, $G_{\cdot H} = 0.55$ (Swallow, 1973). Many radiation biology experiments are carried out utilizing x-rays of longer wavelength and higher Linear Energy Transfer (LET), which produce different primary yields (Kupperman, 1967 and Thomas, 1969). Moreover, many of these biological experiments are carried out in buffered solutions; the radiation chemistry of phosphate salts has been reported (Grabner, 1973) and it is anticipated that presence of such a buffer would alter the effective yield of primary radical species, both by production of electrons resulting from direct energy absorption by $H_2PO_4^-$, and conversion of e^-_{aq} into H atoms.

The system chosen for measurement of primary yields of e^-_{aq}, $\cdot OH$, and H atom, in both water and phosphate buffer, was a dilute solution of thymine. Thymine has strong absorption in the UV (λ_{max} = 264nm; ε_{264nm} = 7.95×10^3 $M^{-1} cm^{-1}$) due to the 5:6 double bond in the pyrimidine ring. Hydroxyl radicals or H atoms react rapidly by addition to this double bond, resulting in loss of the 264nm absorption. Thymine reacts with hydrated electrons as follows:



In this reaction the chromophore is not destroyed. By observing the loss of absorption of the chromophore under various conditions, it is possible to calculate the number of molecules of thymine destroyed as a function of absorbed dose. The strategy used in these experiments is as follows:

1) Thymine $\xrightarrow[0.3M\ t\text{-BuOH}]{N_2O}$ $G_{-Th} = G_{\cdot H}$

2) Thymine $\xrightarrow{N_2O}$ $G_{-Th} = (G_{\cdot H} + G_{\cdot OH} + G_{e^-_{aq}})$

3) Thymine $\xrightarrow{N_2}$ $G_{-Th} = (G_{\bullet H} + G_{\bullet OH})$

This was done for x-rays produced by tube voltages of 50, 102, 148, 210, and 300 kVp. In all cases except the 50 kVp, the x-ray beams were filtered to half-intensity by addition of external Al of Cu filters, to "harden" the x-rays produced. Results are shown in Table V.

It can be seen that increasing the LET of the ionizing radiation generally reduces the primary yields of e^-_{aq} and $^{\bullet}OH$ while increasing the yield of H atoms. Addition of phosphate buffer has a similar effect, and leads to a four-fold increase in the yield of H atoms. This may be of relevance to the radiation biology of DNA, towards which H atoms are fairly reactive.

X-RAYS kVp	YIELDS, radicals per 100eV absorbed: $G_{e^-_{aq}}$	G_{OH}	G_H	FORMED IN:
50	1.17	1.40	0.91	Water
102	1.92	1.51	0.72	
148	2.08	2.24	0.68	
210	2.46	2.26	0.64	
300	3.18	2.30	0.55	
50	0.46	0.92	2.26	26.4mM phosphate
300	0.95	1.99	2.09	buffer, pH 7.3

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IV. H. 7. The effect of pulse length, frequency and dose rate on radiation sensitization of cells

We attempted recognition of the OH radical in radiation-induced effects in spores using "accelerator" conditions, and the experimental techniques that revealed its presence in the O_2 effect in the bacterial spore.

Experiments were designed first to test for the regular 100% O_2 effect in the pulsed beam. The experimental procedure was similar to that described in Powers (1970). The procedure differed in that 10^7 spores/ml was irradiated for all levels of survival and appropriate dilutions were made following irradiation to reach a number of colonies within proper counting range. The spore stock used in these experiments also was centrifuged once and the pellet suspended in sterile water to the 10^7 spores/ml concentration.

The gassing and plating are exactly as described in the reference. The dose rate in the accelerator pulse was determined by Fricke dosimetry to be 570-710 rads/pulse. Since pulse length in these experiments was 800ns, dose rate within the pulse varied from 4.3×10^7 krad/min to 5.3×10^7 krad/min. This is to be compared to approximately 14 krad/min on the 50 kVp x-ray machine.

Initially, 100% O_2 (or N_2) was flowed over the biological system and the accelerator set to deliver six pulses/s. At six pulses/s the O_2 effect is observed in preliminary experimentation. The ratio $\underline{k}_{O_2}/\underline{k}_{N_2} = 1.7$, approximately equal to that usually seen in experiments at 50 kVp at 14 krad/min. But the absolute values of the inactivation constants are considerably below those that were observed in the 50 kVp x-ray experiments indeed a factor of three below, indicating that either pulse repetition frequency or the dose rate within the pulse is having a serious modifying effect of the radiation sensitivity.

This then led to further preliminary experimentation in which the pulse rate was reduced to one pulse every two seconds, the dose rate within the pulse remaining the same as in the previous experiment. Under these conditions, again an O_2 effect was observed giving an O_2 enhancement ratio similar to that seen in the 50 kVp experiments. But again, the absolute values of the inactivation constants are considerably below those observed in the 50 kVp x-ray experiments.

These preliminary experiments indicated that a serious methodical study of the effect of high dose rate within the pulse and the response of the spores to radiation must be done. Fig. 13 shows the results to date of this study. It is apparent from the figure that there is a peak sensitivity occurring at slightly over 12,000 rads/μs dose rate within the pulse when the pulse is 400 or 800ns in length. This peak is not present with a 100ns pulse length although a slight rise in sensitivity occurs with the shorter pulse length around 8500 rads/μs within the pulse. For these experiments the frequency of the pulse remained at 6 pulses/s.

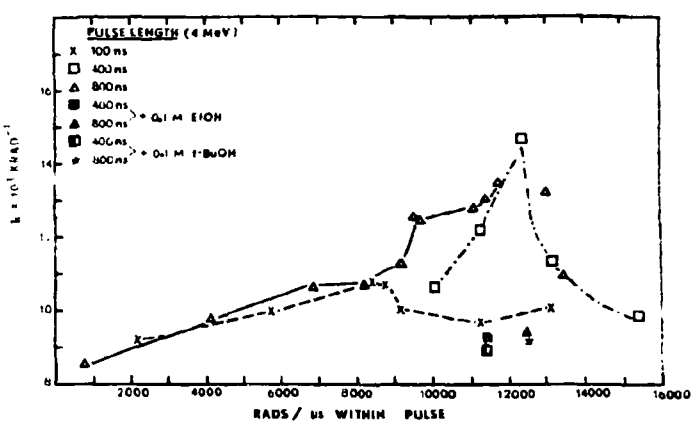

Fig. 13 Sensitivity of bacterial spores when exposed to 4 MeV electron pulses of different pulse lengths with dose rate varying within the pulses.

The peak sensitizing effects were further tested by addition of EtOH or t-BuOH ($^{\cdot}OH$ scavengers). With either of these scavengers present the sensitizing effect of dose rate within the pulse is removed, indicating strongly the involvement of OH radicals in this sensitizing effect.