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Laboratory of Radiation Biology

Department of Zoology

University of Texas

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Progress Report [redacted]

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Original Signed By

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Vice-President for Research

Submitted by: E L Powers

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Reprints & Preprints Removed

APPENDIX INDEX

(August 15, 1975 to August 14, 1976)

PUBLICATIONS

1. Nokes, M. A. and M. Simic. X-Ray Sensitivity of Photosynthetic and Reproductive Systems in Chlorella. Photochem. Photobiol. 21:265-268 (1975).

2. Goff, H. and M. Simic. Free Radical Reduction of Hemin c. Biophys. Biochem. Acta. 392:201-206 (1975).

3. Stevens, S. E. Jr., M. Simic and V. S. K. Rao. X-Ray Induced Inactivation of the Capacity of Photosynthetic Oxygen Evolution and Nitrate Reduction in Blue-Green Algae. Radiat. Res. 63:395-402 (1975).

4. Richmond, R. C., M. Simic and E. L. Powers. Radiation Sensitivity of Bacillus megaterium Spores in the Presence of Co(III) Complexes. Radiat. Res. 63:140-148 (1975).

5. Goff, Harold and E. L. Powers. Effects of X-Rays on Ag-DNA Complexes. Int. J. Radiat. Biol. 27:503-507 (1975).

6. Ewing, David. Two Components in the Radiation Sensitization of Bacterial Spores by p-Nitroacetophenone: The $^{\cdot}OH$ Component. Int. J. Radiat. Biol. 28:165-176 (1975).

7. Powers, E. L. Remarks on the Radiation Chemistry of Radiation Damage in Cells. Fifth Symposium on Microdosimetry, Verbania-Pallanza, Italy, 22-26 September, 1975. In press.

SUBMITTED

8. Nokes, M. A. and E. L. Powers. Sensitivity of Bacterial Spores to U. V. during Germination and Outgrowth. Photochem. Photobiol.

9. Ewing, David. Anoxic Radiation Protection of Bacterial Spores in Suspension. Radiat. Res.

10. Ewing, David and E. L. Powers. Irradiation of Bacterial Spores in Water: Three Classes of Oxygen-Dependent Damage. Science.

11. Richmond, R. C. and E. L. Powers. Radiation Sensitization of Bacterial Spores by cis-Dichlorodiammineplatinum(II). Radiat. Res.

12. Ewing, David. Oxygen Depletion during the Irradiation of Bacterial Spores and the Measurement of the Lifetime of Oxygen-Dependent Damage. Radiat. Res.

13. Ewing, David. Effects of Some ·OH Scavengers on the Radiation Sensitization of Bacterial Spores by p-Nitroacetophenone and O_2 in Suspension. Int. J. Radiat. Biol.

ABSTRACTS

14. Ewing, David. Mechanisms of Anoxic Radiation Protection of Bacterial Spores. Radiat. Res. 62:569 (1975).

15. Simic, M., S. E. Stevens, Jr., V. S. K. Rao and C. Munday. Light Induced Repair of X-Irradiated Blue-Green Algae in Aqueous Suspensions. Radiat. Res. 62:534 (1975).

16. Ewing, David and E. L. Powers. Three Components of Oxygen-Dependent Sensitization in Bacterial Spores Irradiated in Suspension. Radiat. Res. Submitted.

I. The Laboratory

Approval has been received for funds for another 5 year period on PHS NIGMS (13557) beginning April 1, 1976. The first year's (-10) funding is in the amount of $60,000 direct costs.

The Center for Fast Kinetics Research will begin its second year June 1, 1976. Funding for the center's second year has been approved in the amount of $164,238 from the Biotechnology Resources Program, NIH.

Acknowledgement to both ERDA and NIH is made for all work in this 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 PHS), one full-time secretary (funded jointly by CFKR and PHS), one post-doc funded by PHS, a graduate student funded for the summer by ERDA and two part-time undergraduate assistants (one funded at 50% time by ERDA and one at 50% time by PHS). These do not include personnel at the Center for Fast Kinetics Research which is geographically and program-wise an entity separate from the Laboratory of Radiation Biology.

II. Education and Training

One undergraduate has received credit this year for research work performed in this laboratory. Several undergraduates have been employed on a part-time basis as noted above. One graduate student began a Ph.D. program last summer.

As was the case last year, the x-ray machines have been in great demand by various departments with over 60 service irradiations being provided this past year.

The radiation biology course, taught this spring, had around 30 pre-medical, pre-dental,and graduate students in attendance.

III. Conferences and Formal Talks

Sept. 15-19, 1975	Miller Conference of Radiation Chemistry Bürgenstock, Switzerland E. L. Powers attending
Sept. 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
Mar. 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
Mar. 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	Radiation Research Society Meeting, San Francisco, Calif. E. L. Powers, D. L. Ewing, T. Zimek, and Catherine Held attending "Three Components of Oxygen-Dependent Sensitization in Bacterial Spores Irradiated in Suspension" D. L. Ewing and E. L. Powers

IV. Progress Report

IV-A Action of Inorganic Sensitizers

IV-A-1 Roles of e^-_{aq}, ·OH and H_2O_2.

The theory for radiation sensitization developed in the past few years involving the OH radical and in some instances H_2O_2 still stands. While no further direct supporting evidence is on hand beyond the type already reported at this time, much of the information in this report supports this theory.

Directly pertinent to this theory is the problem of accurate measurement of small amounts of H_2O_2 ($<10^{-4}$ M), which was listed among our goals for this period. Little consideration has been given to this difficult problem during this period.

IV-A-2 Metal ions and biological radiation sensitivity

Two papers are included on sensitization by metal ions. The first phase of the work on Co(III) complexes has been published and is presented here as App. 4. Similar work on a platinum complex, begun last year, is completed and a manuscript has been submitted (App. 11). This work is noteworthy in that results are reported from the spore system for a drug currently in use in antitumor therapy.

The large background of information on metal ions continues to expand but as yet this wealth of information cannot be reduced to a generalization unless it is that many metals do sensitize and probably do so by several mechanisms, some of them deducible from known radiation chemistry. A paper is being prepared to give a very general overview of these metal ion results. Table I summarizes a great deal of the data to be included in this paper. It should be noted that while this table deals mostly with results of metal ions, in some instances the anion has radiation effects per se. For instance, in the case of Ag salts, NO_3^- is a known sensitizer while no effect has been seen biologically or chemically for the $SO_4^=$ anion.

IV-A-3 Iron as a sensitizer

As has been reported previously, considerable effort has been put on Fe^{++} and Fe^{+++}. Table II summarizes these results. The conclusions from this study must be that Fe does sensitize, with the $FeSO_4$ salt being by far the most effective of the compounds studied. Work on the sulfate salts was detailed previously but is included here for comparison with oxalate and CN complex results.

The very interesting reduction in N_2 of sensitization by $Fe_2(SO_4)_3$ by ethanol, t-butanol and formate all to approximately the same level ($\approx N_2$ levels) compared to the reduction of $Fe_2(SO_4)_3$ sensitization in

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}				
	Ag_2SO_4	2.4	2.5	2×10^{-3}	2×10^{-3}				
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	2.8-1.0
	$Co(NH_3)_6Cl_3$	2.1	2.9	2×10^{-2}	2×10^{-2}	2.0-1.0	2.2-1.4	3.0	
Fe^{2+}	$FeSO_4$	2.7	5.8	2.5×10^{-4}	2.5×10^{-3}	1.3	1.4	5.8-4.4	3.0
Fe^{3+}	$Fe_2(SO_4)_3$	2.1	3.6-0.6(180)	2.5×10^{-4}	2.5×10^{-4}	1.2	1.3	1.8	1.6
Fe^{2+}	FeC_2O_4	2.2	3.6	1×10^{-4}	1×10^{-3}	1.3	1.5	2.6	2.7
Fe^{3+}	$Fe_2(C_2O_4)_3$	3.6	4.0	1×10^{-4}	1×10^{-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	1.4	2.3	2.2
	$Pt(NH_3)_4Cl_2$	2.3-? (350)	2.8-0.8(300)	2×10^{-4}	2×10^{-4}				
Tl^+	Tl_2SO_4	3.1 - 1.3(150)	3.1	5×10^{-3}	5×10^{-3}				
	Tl_2CO_3	4.2	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	2.6

* 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+}

O_2 by t-butanol to O_2^- alone levels, but reduction by ethanol and formate to N_2 levels (approximately) remains a puzzle that will be approachable only when the radiation chemistry facility of the CFKR becomes available to us. Current knowledge in radiation chemistry of iron salts as known by us cannot account for this behavior. There is also the interesting effect of the anion. It is to be noted that the minimal amount of sensitivity is achieved by the cyanide complexes. Since these effects were so small, scavenger studies on the cyanide complexes at this time seemed unwarranted.

IV-A-4 Cellular uptake of solutes

As pointed out last year, this is a general problem for all sensitizers but is now being presented with the metal ion material since our studies of uptake, for the present, are confined to the uptake of metal ions.

The general procedure and preliminary data on uptake of sulfate and oxalate salts of Fe was presented in last year's report. We note from Table II that while sensitivities differ within these four salts, the most effective sensitizer, $FeSO_4$, demonstrated maximum sensitization at 10^{-3}M where both oxalate salts were shown, by current uptake data, to be concentrating 4 as much Fe. However, we have confirmed that the uptake data is, in some cases, totally different when measurements are made after irradiation, and the saturating gas during irradiation has been shown also to effect the uptake.

Because of these results, and because of the press of other projects, the atomic absorption uptake studies were somewhat delayed. The results obtained after irradiation dictated that the uptake studies, to be pertinent to radiation sensitization data, would have to be on irradiated as well as unirradiated samples, and in the presence and in the absence of O_2 and OH scavengers. This required some modifications in procedure that greatly increases the time required for each determination. Also, centrifugation should be done at a higher speed with more carefully defined centrifugation conditions. This is now possible and work is underway to briefly recheck the unirradiated data obtained, using these modifications to make determinations of uptake after irradiation in both N_2 and O_2 and in the presence of some scavengers for all the Fe compounds.

The results from the new procedures with $FeSO_4$ are presented in Fig. 1. While the variability still appears to be large (up to 60% in the higher molarities), the pattern of uptake (unirradiated) shown previously is almost identical (solide line-X's). Irradiation in N_2 (dashed line-open squares) appears to reduce this uptake slightly . Irradiation in O_2 (dotted line-open circles) reduces to ≈0 the uptake at 10^{-4}

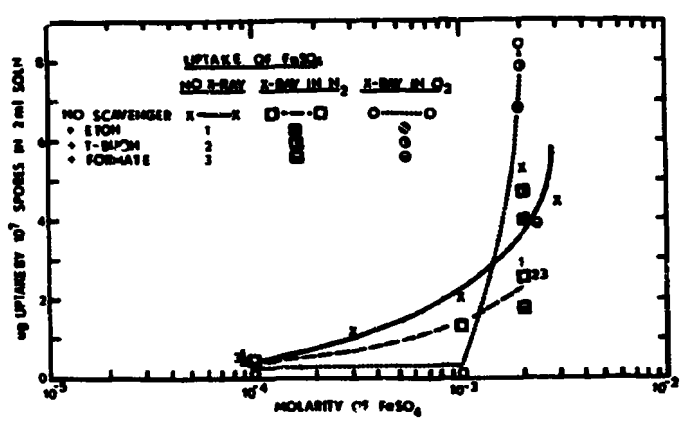

Fig. 1

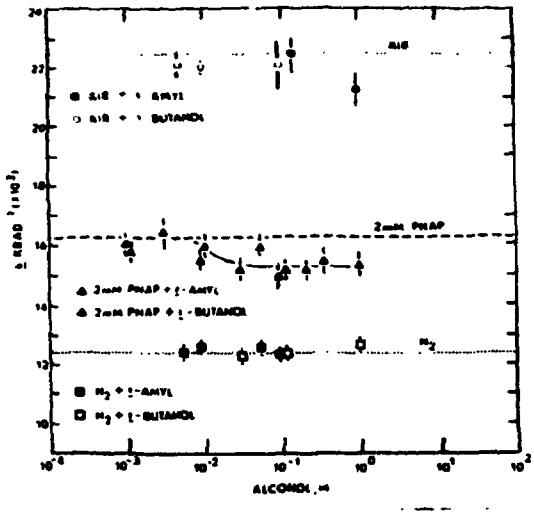

FIG. 2

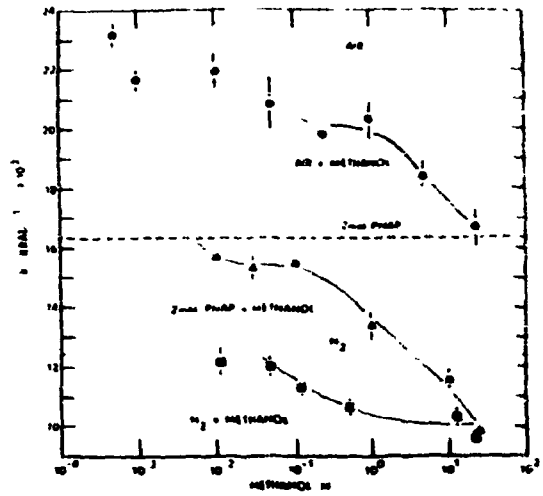

FIG. 3

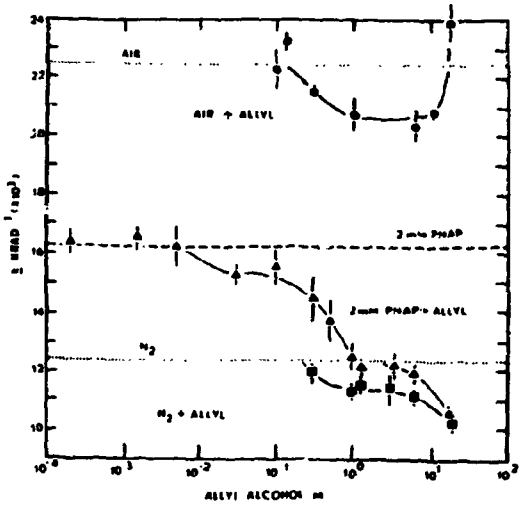

FIG. 4

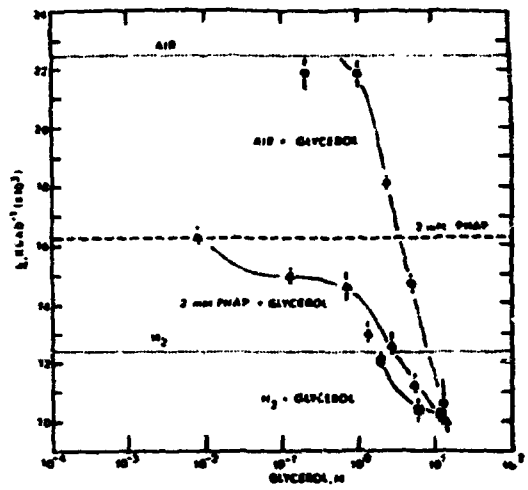

FIG. 5

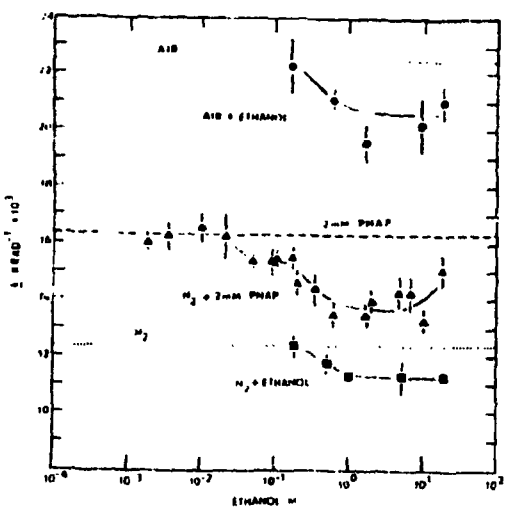

FIG. 6

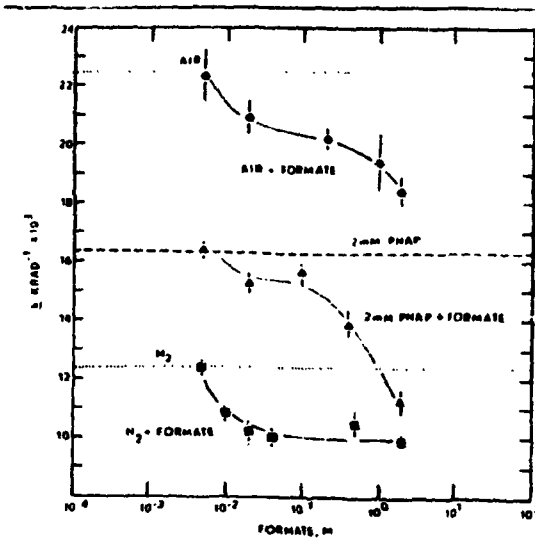

FIG. 7

and 10^{-3}M $FeSO_4$, but at 2 x 10^{3}M $FeSO_4$ the uptake appears to almost double. Addition of EtOH, t-butanol and formate to these $FeSO_4$ solutions results in uptake almost identical to results seen without these OH scavengers when unirradiated. The presence of the OH scavengers does, however, seem to affect to some degree, the amount of $FeSO_4$ taken up by the spores when measurements were made on samples irradiated in O_2 or in N_2. The uptake by spores irradiated in O_2 seems somewhat less with scavengers present while that in N_2 seems somewhat greater, with a possible pattern developing with uptake being T-BuOH >EtOH > Formate on irridated samples (O_2 or N_2 during irradiation).

These results indicate that considerable caution is required at this time before critical interpretations are possible. It will be nedessary for us to focus our attention on one or two of these systems to test the analytical procedure critically for we cannot understand at this time why the radiation of spores should have such an effect and why the presence of scavengers should alter the effects.

Unresolved at the present time is the possibility that we are measuring, in most of these instances, a cation thatis adsorbed to the surface of these spores rather than absorbed into the spore. We have seen, in some instances, that washing the spores with up to 0.1M acetic acid does not alter the uptake and that increasing concentrations of the acid wash almost to the point of cell wall destruction, while reducing the measured uptake, do not reduce the uptake to zero.

IV-B Organic Sensitizers and Protectors

We have studied the effects of t-butanol, t-amyl alcohol, benzyl alcohol, ethanol, glycerol, allyl alcohol, methanol, CO_2, and sodium formate both in the presence and absence of p-nitroacetophenone (App. 6, 9). (PNAP is an aromatic Ketone which increases the anoxic radiation sensitivity of Bacillus megaterium spores by about 35%). This series of tests has now been expanded to study the effects some of these additives have on the response in air (App. 13). Ethanol, methanol, t-butanol, t-amyl alcohol, formate, blycerol and allyl alcohol were used. A comparison of the effects of these additives under the three conditions (anoxia with no sensitizer, in anoxia with 2mM PNAP and in O_2,(Fig. 2 through 7), indirectly reveals important differences between the sensitizing actions of PNAP and O_2.

IV-B-1 Anoxic protection (no sensitizer present)

Most of these results were discussed in last year's report. t-Butanol and t-amyl alcohol do not protect; the remaining additives can reduce the anoxic radiation sensitivity, although they are not equally effective on a concentration basis (Figs. 2 - 7). Protection here does not seem to be related to a simple removal of ·OH; however, the reaction between the additive and a water-derived radical may be necessary before protection can occur. As previously noted among these additives, protection is observed only with those agents that react with a water-derived radical (usually ·OH) and form an α-hydroxy radical (App. 9).

IV-B-2 Anoxic desensitization when PNAP is present

In 2 mM PNAP, all the additives that were tested can reduce the response. t-Butanol and t-amyl alcohol remove only the small component of sensitization identified as an "·OH component" (about half the sensitizing action of PNAP), although the remaining additives not only completely eliminate the sensitization from PNAP (except for ethanol and perhaps formate), but reduce the response to the same protected level seen without PNAP (Fig. 2 - 7).

A comparison of data in Fig. 2 - 7 reveals a correlation between the concentrations of additives that protect in anoxic water and those concentrations that remove this second "non-·OH component" of sensitization from PNAP. Although the specific mechanisms are not clear, one interpretation of these results is that whatever these additives do that results in protection in water also prevents the sensitization by PNAP.

IV-B-3 Effects of the additives in air

Although all the additives except t-butanol and t-amyl alcohol can reduce the response in air (Fig. 2-7), it is possible that these agents do not act against O_2-dependent damage. Glycerol (Fig. 5) is the clear exception; as Webb and Powers showed earlier (Int. J. Radiat. Biol., 1963, 14, 313-330), glycerol clearly eliminates O_2-dependent damage. Again, there is a good correspondence between the additive concentrations that protect in N_2 and in air; equally important, the amount the response is reduced in air is the same as in N_2 (cf Fig. 7). It is reasonable to conclude that these compounds function in air only as they do in N_2; and they do not act specifically against damage from O_2 itself. This is quite different from the effects these additives have in PNAP and may indicate that the sensitizing mechanisms of PNAP and O_2 are not as similar as first believed. It might also indicate that the actions of the protectors are different in the presence of the two sensitizers.

IV-B-4 Oxygen-dependent sensitization

A simple removal of ·OH does not protect the spores in N_2 (with no other additive) or in air. However in 0.8% O_2, 0.1 M t-butanol does

reduce the response significantly (Ewing, 1974, Radiat. Res. 59:156). This result prompted a study with t-butanol and different concentrations of O_2 (App. 10, 16). Spores suspended in water or water with 0.1 M t-butanol were equilibrated with different O_2 concentrations and irradiated. An analysis of the results (Fig. 8) indicates at least three components of O_2-dependent damage that can be recognized this way. The separation of different kinds of O_2-dependent damage in suspension is a highly significant result that should be revelant to radiation therapy because now we can recognize that the chemistry of radiation damage in partially oxygenated cells is different from that in fully oxygenated cells.

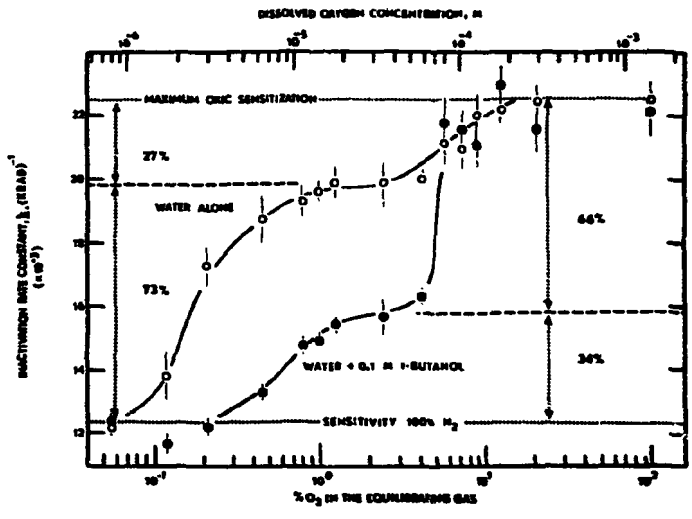

FIG. 8

IV-C Mutagenesis

Our mutation studies focus on examining rates of mutation induction in bacterial spores under different irradiation conditions. The goal is to ascertain the extent to which information we have accumulated on processes leading to cell inactivation (i.e., lethal damage) applies to the induction of mutations. During vegetative growth, Bacillus megaterium is sensitive to the presence of streptomycin; the first mutation selected for study was the change from sensitivity to resistance ($Str^s \rightarrow Str^r$) to this antibiotic. The spontaneous rate for this change is very low, $\sim 7 \times 10^{-10}$.

In last year's report we described ourinitial experiments and the difficulties in quantitating results. These difficulties are serious since we aspire to a precision like that in our lethal damage studies.

These difficulties have not been overcome. Several spore stocks (all B. megaterium grown under the same conditions but at different times) show spontaneous rates ($Str^s \rightarrow Str^r$) that differ by as much as a factor of 10. Similar differences were also observed when repeat experiments with the same spore stock were done. In addition, in the irradiation experiments, although similar qualitative relationships were found for the dose response curves (see last year's report), the same X-ray dose

under identical conditions could induce greatly variable numbers of Str^r mutants. The difficulty was with the mutation itself, not simply in handling the large number of spores; the companion survival curves were highly reproducible. It was not possible to normalize the mutation data to allow the necessary quantitative analysis.

A second spore forming organism is now being used (B. subtilis, 168M) to study another mutational change (tryptophan-dependence to tryptophan-independence). Cells (trp^-) in vegetative growth may be inoculated into minimal medium or onto agar plates; growth occurs only in the presence of added tryptophan. However, after sporulation either in liquid culture or on potato agar plates (2 stocks have been prepared), the harvested spores will germinate and form colonies at virtually the same frequency on plates either with or without tryptophan. Since it is known that the transforming ability of spore DNA and vegetative cell DNA (at the histidine locus) are equivalent (Tanooka and Sakakibara, 1968, Biochim. Biophys. ACTA, 155, 130-142), the extremely high "reversion rate" from our data probably represent an artifact. It is possible that the sporulating bacteria enclose tryptophan from the medium and use it for germination and growth in the absence of added tryptophan, although our attempts to remove this tryptophan by washing have failed. We are continuing these studies with spores and at the same time trying to establish a workable mutational system using vegetative cells of this organism.

IV-D Transformation

Although it has been shown that DNA is a primary target of ionizing radiation in cells, the chemical mechanisms involved in the biological inactivation of DNA are uncertain; in addition, the effects of radiation sensitizing and protective agents on this inactivation are virtually untested. By use of a transformation system involving the tryptophan marker of Bacillus subtilis and the knowledge of recent advances in radiation chemistry, we hope to develop a model for the chemical mechanism(s) involved in this inactivation.

The initial eight months of work was largely preparatory. Cultures of B. subtilis W23, wild type, and B. subtilis 168M, a tryptophan requiring mutant were obtained and rejuvenated. The procedure for extraction and purification of biologically active DNA according to Marmur (J. Mol. Biol., 3, 208-218, 1961), with modifications by Synek (Ph.D. Dissertation, University of Chicago, Department of Microbiology, 1967) was accomplished in this laboratory, and a number of DNA samples was prepared. The first six samples averaged a concentration of 575 mg/ml with 5.5ml per sample and had a protein contamination of less than 0.1%. The transformation procedure according to Anagnostopoulos and Spizizen (J. Bacteriol, 81, 741-746, 1961), with modifications by Synek (l.c.) was further adapted to increase efficiency of laboratory manipulation. Although the above procedures call for storage of the DNA dissolved in

standard sodium citrate solution (SSC), we wish to irradiate the DNA in phosphate buffer in order to avoid the sensitization due to citrate. The DNA samples dissolved in this buffer (29 mM phosphate at pH 7.3) retain the same transforming activity as when in SSC, and this transforming ability remains stable for five months, to date. The procedure for irradiating 1.0 ml aliquots of the DNA solution at low (2.13 krads/min) and high (11.73 krads/min) dose rates under varying conditions of saturating gases is by our standard method (Powers and Cross, 1970, Int. J. Radiat. Biol. 17: 501-514) and subsequent transformation procedures are working smoothly.

The first experimental results obtained are summarized in Fig. 9 and 10. The survival curves, transforming activity vs. dose, are two

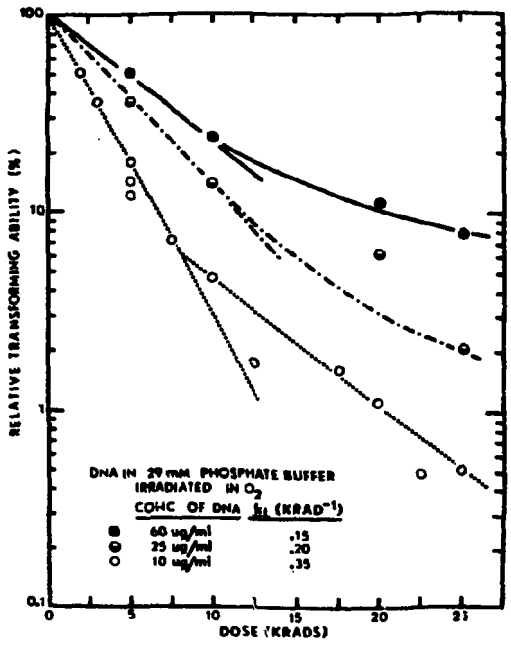

FIG. 9

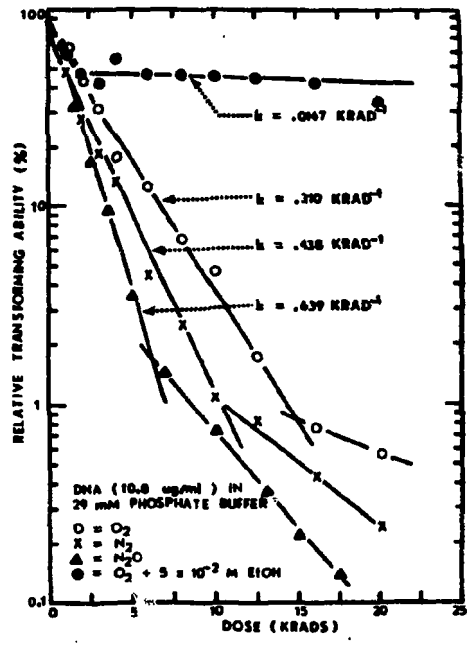

FIG. 10

component - an initial radiosensitive portion at low doses and at higher doses a more radiation resistant component - under all conditions studied thus far.. Fig. 9 demonstrates that transforming ability increases with DNA concentration; at a DNA concentration of 60 mg/ml, $k=0.15$ krad^{-1} for the initial radiation sensitive portion of the curves.

Fig. 10 shows the effects on transformation of exposure of the DNA during irradiation to various saturating gases and one experiment showing the effect of EtOH, testing presumably the role of •OH. All the experiments

shown in this figure were done using the same DNA sample, dissolved in 29 mM phosphate buffer at a concentration of 10.8 mg/ml during irradiation. These experiments, with the exception of the ethanol addition, have been performed on two other DNA preparations with results which were qualitatively the same, although quantitatively somewhat different. For the figure show, the rate constants for inactivation of the transforming ability of the radio sensitive portion of the curve are as follows: for irradiation in O_2, $k = 0.310$ krad^{-1}; in N_2, $k = 0.438$ krad^{-1}; in N_2O, $k = 0.639$ krad^{-1}; and in 5×10^{-2} M EtOH in O_2, $k = 0.0147$ krad^{-1}. Although this order of sensitivities varies from that seen in cellular systems, it is qualitatively similar to the response reported for other biomolecules and phages. In addition, a similar action of ethanol was seen in phage, although no nearly so dramatic. (Powers and Jobbagy, 1972, Int. J. Radiat. Biol., 21:353-359).

V. Proposals for continuation

The research over the next year will be a continuation of that of the past several years. There will be no abrupt change in direction nor in technique, except in the one instance mentioned below concerning the use of the pulse radiolysis techniques. Our general goal is to reveal chemical mechanisms of radiation damage. Our interest in sensitization and the chemistry of sensitization mechanisms has led us to the general problem of the sensitizing actions of certain metallic cations. Fortunately, these are convenient to handle experimentally. The cations should be amenable to direct physical chemical investigation (with respect to the production of transient intermediate states of conceivable importance in radiation biology) and are of interest currently as environmental contaminants. This will be commented on below.

Amongst the immediate urgent problems is the matter of the uptake of these solutes by the cells and their distribution within the cells after uptake. It might be that this is insoluble at the present time because of the analytical methods available to us. However, an earnest effort will be made at least to eliminate the possibility that an adsorption phenomenon on the surface of the spores is accounting for the very large uptake observed with some of these metals. It is possible to make preparations of spore coats which are relatively intact and which might retain the physico-chemical surface properties of the spore coat of the intact spore. Spore coat preparations will be measured together with, if possible, concomitant measures of the protoplasts by atomic absorption procedures. This will be done for iron at the beginning, and then cadmium and silver.

The continuing investigation of sensitization by Fe will be an important part of our activity in the next year. Several very curious relationships must be resolved. We note that solutions which presumably contain reduced iron as $FeSO_4$ sensitize the spores to a very high degree, very much higher than the oxidized iron in the same salt; and that the reduction of the sensitization by ethanol is approximately