Laboratory of Radiation Biology

Department of Zoology

University of Texas

Austin

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Annual

Progress Report

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PUBLICATIONS (November 15, 1978 - November 15, 1979)

(Attached as appendices carrying corresponding numbers)

In Press

1. Powers, E. L. and K. D. Held. The oxygen effects in radiation biology and radiation chemistry. Presented at the Association of Radiation Research (Great Britain), North E. Wales Institute, Wrexham, Clwyd, Wales. Elsevier, Amsterdam.

2. Held, Kathryn D. and E. L. Powers. Effects of varying O_2 concentration on the x-ray sensitivity of transforming DNA. Int. J. Radiat. Biol.

3. Held, Kathryn D. and E. L. Powers. Protection of transforming DNA from x-irradiation-induced damage by $^{\cdot}OH$ scavengers. Int. J. Radiat Biol.

4. Ewing, David and E. L. Powers. Oxygen-dependent sensitization of irradiated cells. 32nd Annual M. D. Anderson Symposium, Radiation Biology in Cancer Research, Houston, Texas

5. Powers, E. L., The spore as a model system in radiation Biology. Int. J. Radiat. Biol.

Submitted

6. Ewing, David. Breaking survival curves as a measure of oxygen-removal time in irradiated bacterial spores. Int. J. Radiat. Biol.

7. Held, K. D. and E. L. Powers. Sensitization to x-rays of transforming DNA by Ag^{+}. Int. J. Radiat. Biol.

Abstracts

8. Held, K. D. and E. L. Powers. X-irradiation induced changes in the biological activity of transforming DNA. Radiat. Res. 74:490 (1978).

9. Powers, E. L., David W. Barber, Margaret A. Centilli and Marian H. Cross. The effect of pulse length and dose rate on radiation sensitivity of cells. Radiat. Res. 74:576-577 (1978).

10. Richmond, R. C. and E. L. Powers. Radiation sensitization of bacterial spores by cis-dichlorodiammineplatinum(II). The Year Book of Cancer 1978, R. L. Clark, R. W. Crumley and R. C. Hickey, eds. Year Book Medical Publishers, Inc., Chicago, London, pp. 394-395.

11. Held, Kathryn D. and E. L. Powers. Modification of the x-ray sensitivity of transforming DNA by varying O_2 concentration. Radiat. Res. In press.

12. Powers, E. L., D. L. Ewing and Kathryn Held. The space as a model system in radiation biology. Space Radiobiology Meeting, Tokyo, Japan, May 20, 1979

I. The Laboratory

PHS NIGMS (13557) is now in its fourth year of a five year period having begun this fourth year on April 1, 1978. The fourth year's (-14) funding is in the amount of $54,944 direct costs.

The Center for Fast Kinetics Research began its fifth year June 1, 1979. Funding for the center's fifth year has been approved in the amount of $150,769 direct costs, from the Biotechnology Resources Program, NIH (RR00886).

The current staff consists of the director (Thomas 1/4 time by DOE), two full-time technicians (one funded by DOE and one by NIH), one full time secretary (funded jointly by CFKR and NIH), a part-time laboratory assistant and a part-time typist funded by NIH. 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

Several undergraduates have been employed on a part-time basis and one pre-med undergraduate currently is working in the laboratory 2 afternoons a week for experience. One graduate student completed a Ph.D. program this spring and is currently on an NSF Post-Doctoral fellowship at Gray Laboratory in Northwood, Middlesex, England. We also have working in our laboratory Ms. Eida Borrero de Saiz from Panama supported by an IAEA fellowship. She will gain experience here in our laboratory in radiation techniques under this one year fellowship enabling her to aid her country's growth in this area.

As was the case last year, the x-ray machines have been in demand by various departments with over 30 service irradiations being provided this past year.

III. Conferences and Formal Talks (November 1, 1978 - November 14, 1979)

November 3-4, 1978 — 11th Annual Meeting of the Texas Association for Radiation Research, Denton, Texas. "Effects of Varying O_2 Concentrations on the X-ray Sensitivity of Transforming DNA" K. D. Held and E. L. Powers

Margaret A. Centilli, attending

January 3-5, 1979	Association of Radiation Research (Great Britain), North East Wales Institute, Wrexham, Clwyd, Wales "The Oxygen Effects in Radiation Biology and Chemistry" E. L. Powers and K. D. Held
February 27 - March 2, 1979	32nd Annual M. D. Anderson Symposium on Radiation Biology in Cancer Research, Houston, Texas "Oxygen-Dependent Sensitization of Irradiated Cells" David Ewing and E. L. Powers Kathryn D. Held and Margaret A. Centilli attending
April 27, 1979	Seminar. "Radiolysis of Transforming DNA" K. D. Held. Laboratory of Nuclear Medicine and Radiation Biology, University of California at Los Angeles, Los Angeles, California
April 27, 1979	Seminar. "Oxygen Effects in Radiation Biology and Radiation Chemistry: Cells, Spores and DNA" K. D. Held. Laboratory of Nuclear Medicine and Radiation Biology, University of California at Los Angeles, Los Angeles, California
May 13-19, 1979	6th International Congress of Radiation Research, Tokyo, Japan "Modification of the X-ray Sensitivity of Transforming DNA by Varying O_2 Concentration" Kathryn D. Held and E. L. Powers
May 20, 1979	Spore Radiobiology Meeting, Tokyo Japan "The Spore as a Model System in Radiation Biology" E. L. Powers
July 12-13, 1979	National Bureau of Standards, Washington, D. C. "The Multi-User Laboratory" E. L. Powers

IV. Progress Report. Introduction

The report for this year is an extension of that submitted in the previous two years. This introductory statement is a revised version of the one presented last year. The progress report consists of the summary of the recent experiments done in the laboratory on the various modifiers of radiation sensitivity to which attention is currently being given. The appendices are presentations of the material put into the publication schedule this year. These consist of 5 papers accepted for publication, 2 papers submitted and 5 abstracts which represent the information given at various conferences. We shall not summarize in detail the information given in these papers.

As we said last year, after the rapid advance in knowledge of chemical kinetics during the past 15 years, especially the chemistry of e^-_{aq} and $^{\cdot}OH$ generated by high energy radiations in water, there has been a clear recognition that finally the chemistry of the irradiated cell might be investigated to recognize the mechanisms by which the strange effects induced by radiation could be caused. This laboratory has made a positive, deliberate attempt to apply radiation chemistry as it is known to the irradiated cell. And in addition, it has attempted to initiate studies in radiation chemistry that as a consequence of the radiation biological results have proved to be insufficient to explain the biology. Up to now the standard scavenging techniques have been used; removal of e^-_{aq} by certain additives, the removal of $^{\cdot}OH$ by other additives, the variations in the concentrations of these two primary species and the addition of other chemical additives. From these recognition of the relative unimportance of direct action of e^-_{aq} in the induction of radiation biological phenomena and the strong dependence of some of these effects on $^{\cdot}OH$ production has emerged. The roles of free radicals and their reactions with oxygen have been described and the oxygen effect has been dissected into at least three different characterizable identities, with a fourth being a possibility.

From these studies a general model of early events for explaining part of the radiobiological phenomena has emerged as the "electron sequestration" model in which the electron acceptors by removal of e^-_{aq} formed in the initial period after absorption of radiant energy disturbs the equilibrium reaction $e^-_{aq} + {}^{\cdot}OH$ to yield OH^-, effectively increasing $^{\cdot}OH$ yield in a sensitive area. Many experiments support this general model. It is to be underlined here that neither this nor any other model explains the entire response of the cell to high energy radiation. As a matter of fact, we have stressed that the overall radiation response must be a consequence of many physico-chemical processes, each one probably having properties different from all of the others.

In this last period, the metal studies were extended to include work on CuCl to compare with previous work on $CuSO_4$ and $CuCl_2$ and pre-irradiation studies of all 3 copper compounds, now almost complete.

With respect to the uptake of metals by the spore and our attempts to correlate this with changes in radiation sensitization, the position is that material is being summarized and prepared for publication. The studies now include uptake by the spore of $FeSO_4$, $FeCl_2$, $CdSO_4$ and Ag_2SO_4. At the present time there is little hope of rationalizing changes in radiation sensitivity with these numbers, although it is clear that this kind of information is necessary if the effects of the metals on radiation sensitivity are to be understood eventually. It is very strange that the uptake of the metal appears to be a continuous function of concentration, whereas the increase in radiation sensitivity is not. This undoubtedly has to do with the saturation of some compartments in the cell by the metal related to radiation sensitivity. This will be discussed, as it has been in a preliminary way already, in detail in the publication being prepared. Related to this general concept are the propositions given in the Ag^+-DNA study (Appendix 7) for the relation of the changes in radiation sensitivity seen in that system to the type of complex formed by the metal at different concentrations of the added cation.

As we noted last year, the second model system introduced, transforming DNA, is being studied to relate the large body of information existing in the literature on radiation chemistry of DNA to the actual changes in the biological integrity of the DNA. Some studies like this were done 15 to 20 years ago, but none has been done sufficiently recently to allow application of current radiation chemical thought. Our current studies are directed toward understanding oxidation and reduction forces or species and their effect on transforming ability. In addition to Ag^+ (described now formally in Appendix 7), Cd^{++} has been described. We expect to be able to make suggestions concerning the interrelationships among metals, DNA and the biological effects of radiations with some hope of explaining what chemistry is involved.

The relationship between the Lab of Radiation Biology and the Center for Fast Kinetics Research is developing. Here are available electron pulses of various energies, dose rates, lengths, and laser beams of varying physical properties. Currently the initial studies have been with the electron pulse beam. One of the large difficulties when applying radiation chemical information current in the literature is that most of the yields and the rate constants and chemical reaction kinetics stated are "instantaneous." That is, the information is based on observation of the chemical solutions following single pulses and at very short times after the pulse. It is obvious that if radicals and other species have even slightly extended lifetimes a succession of pulses might generate a different radiation chemistry, if there are interactions amongst the products of the successive pulses. Most radiation biology is done in a manner that would allow the second large condition to obtain should this have any meaning at all. Accordingly, we are attempting to build an experimental experience on not only single pulses in systems of interest to our biology, but also to recognize the importance of the differences induced by series of pulses.

Our attention at the CFKR has been directed toward the study of one of the nucleic acid bases as introductory to our entrance into this complex area. The experiments reported last year done partly with Dr. R. C. Richmond of Boston University have not progressed beyond the point of last year's description because of travel difficulties. Our recent experiments were done on thymidylic acid and deoxyadenylic acid. The difficulties currently being experienced are those associated with the complexity of the very early events, and the brevity of the description in the following section concerning results is not a measure of the effort that has been put into attempts to study this system.

IV.1. Sensitization of bacterial spores by Cu. Last year we reported results using $CuCl_2$ as a sensitizer of bacterial spores. A similar study was completed this year on $CuSO_4$ as a sensitizer and results using CuCl are partially complete. Fig. 1, which was included last year, shows a multiplicity of peaks of sensitivity in both O_2 and N_2 for $CuCl_2$. This figure is included again this year for comparison with the new data presented below. Regarding these results we stress that almost all of the points in these figures are from breakpoint responses with the k values plotted being k_1, i.e., the k from the low dose portion of the survival curve. In many instances (particluarly around the very high peaks) this portion of the survival curve is less than 2 log cycles in length so that while we usually consider k values as being accurate to less than ±10%, we must allow that the values in this case may not be that well-defined. Since, however, these responses are so very large, the accuracy of the k values themselves is of lesser importance at this time. Nevertheless, critical evaluations are required and will be obtained.

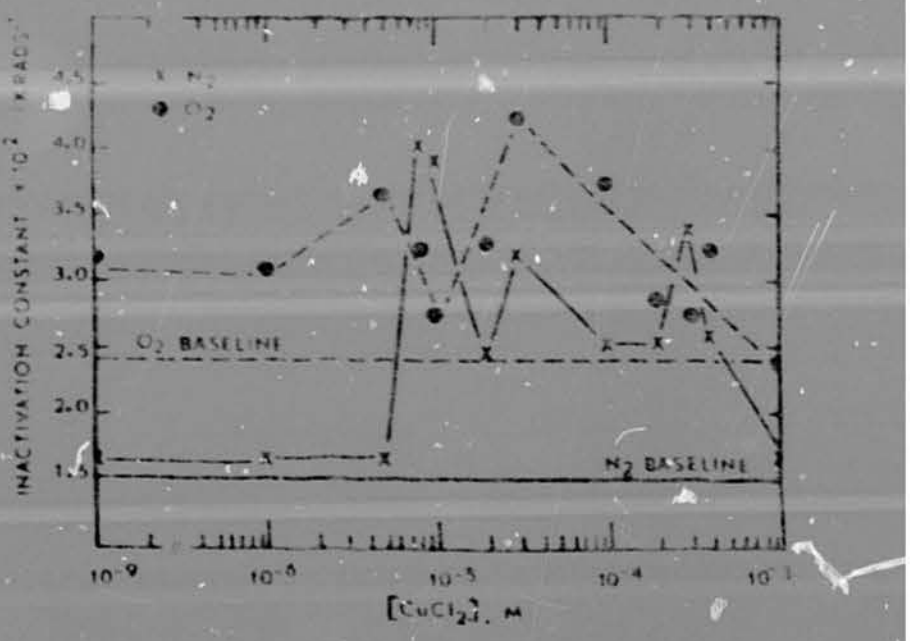

Fig. 1 Radiation sensitivty of bacterial spores vs concentration of $CuCl_2$ in O_2 (solid circles) and in N_2 (X's)

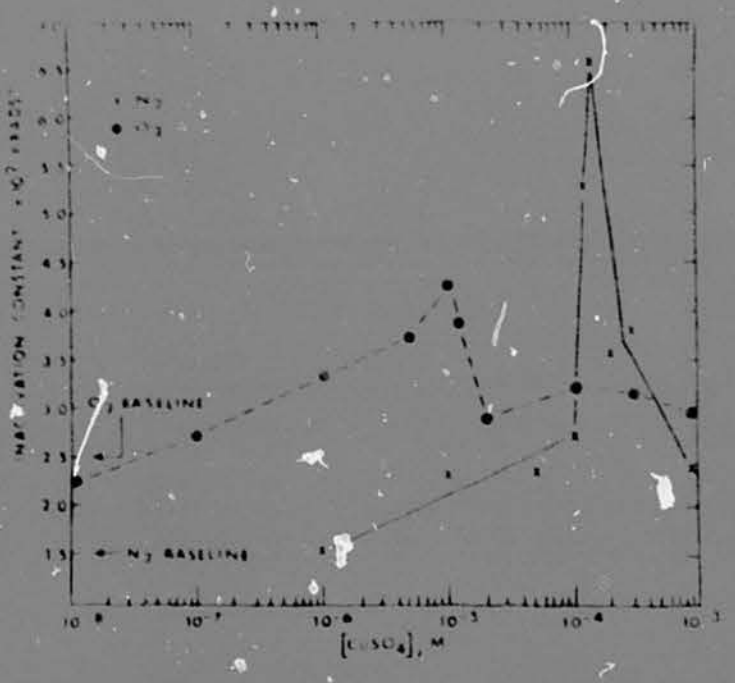

Fig. 2 Sensitization of bacterial spores to x-rays by $CuSO_4$ in N_2 and O_2.

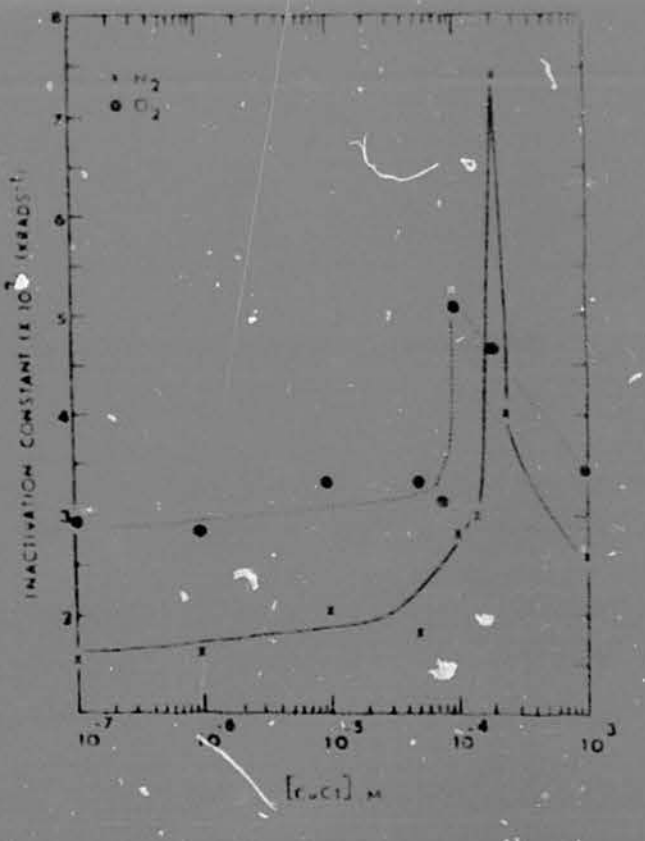

Fig. 3. Sensitization of bacterial spores to x-rays by CuCl in N_2 and O_2.

Figures 2 and 3 show results of studies of $CuSO_4$ and CuCl respectively. These two compounds are very similar in their responses sensitizing in N_2 to a much greater extent (4 to 5 x the N_2 baseline) than $CuCl_2$ (2.7 x the N_2 baseline). This large sensitizing effect occurs at a higher concentration of Cu, namely 10^{-3} M for $CuSO_4$ and CuCl vs 10^{-5} M for $CuCl_2$. The results in Figs. 3 and 4 also clearly show only one peak of sensitivity in N_2 and in O_2 in contrast to the multiple peaks of sensitivity resulting from use of $CuCl_2$ as a sensitizer (Fig. 1).

The k values for the peak sensitization by $CuSO_4$ and CuCl are 6.5×10^{-2} $krads^{-1}$ and 7.4×10^{-2} $krads^{-1}$ respectively. These values are almost twice as high as seen previously in N_2 in this laboratory whose experience now encompasses some 30 metal compounds. In studies previously reported on Fe and Zn we noted very high sensitization by $SO_4^=$ compounds relative to other salts of these two metals. These effects were however, in O_2 not in N_2.

The remarkably high k values and single peak characteristics shown by $CuSO_4$ and CuCl as contrasted to the response for $CuCl_2$ provide very clear support for our previous position that there are very different responses for any particular metal ion depending upon the compound or complex in which it is presented and the valence state of the metal ion itself. In other words, the metal itself may have intrinsic sensitizing characteristics but if so, they are so greatly modified depending upon the anion or complexing portion of the compound used, the valence state of the metal ion, etc. that the response due to the action of the metal ion itself is not evident. In fact, from our experience the very great similarity seen in the results of $CuSO_4$ and CuCl are very remarkable. No two responses of almost 30 metal compounds have been as closely alike as those in Figs. 2 and 3. Yet it must be noted that in these two very similar responses, the anion differs as well as the valence state of the Cu. Where only valence state differs (i.e., CuCl vs $CuCl_2$ -- Fig. 3 vs Fig. 1) or where only anion differs ($CuCl_2$ vs $CuSO_4$ -- Fig. 1 vs Fig. 2) the responses are different.

Results of scavenger and pre-irradiation studies on two of these compounds of Cu are presented in Table 1. These same studies are now in progress on CuCl so comparisons regarding sensitization based on this sort of study will be limited at this time. It seems important to note however in Table 1 that in every case to date, EtOH, which scavenges $^{\cdot}OH$, reduces the sensitization of the peaks to about the baseline k value for the gas present during irradiation. This would indicate some involvement of $^{\cdot}OH$ in all the sensitization we are seeing. The formate results would however, be expected to be similar since formate also scavenges $^{\cdot}OH$ as well as H_2O_2. In every case the formate does reduce the sensitization but not to the baseline level as does the EtOH. There are some known instances of formate reacting directly with metal ions to form organic-metal compounds (Kelm, et al 1973) and that may be the reason for the discrepancy of these two columns in Table 1 with the EtOH

Table 1

Compound	Peak Sensitization		EtOH	Formate	Pre-Irradiation
	Conc., M	$^{*}k_L - k_H$ (BP)	$k_L - k_H$ (BP)	$k_L - k_H$ (BP)	$k_L - k_H$ (BP)
$CuCl_2$ (in O_2)	3×10^{-5}	4.24-1.92(80)	2.56-1.35(190)	2.56-1.21(150)	3.46-1.64(130)
$CuCl_2$ (in N_2)	3×10^{-5}	3.21-1.20(100)	1.31	2.09-.93(140)	3.29-1.84(60)
$CuCl_2$ (in O_2)	5×10^{-6}	3.64-2.06(80)	2.44	3.06	2.63
$CuCl_2$ (in N_2)	8×10^{-6}	4.06-1.50(70)	1.42	1.35	2.76-1.69(140)
$CuSO_4$ (in O_2)	1×10^{-5}	4.27-1.55(75)	2.48	2.73	6.58-2.34(50)
$CuSO_4$ (in N_2)	1×10^{-4}	6.58-1.47(63)	1.69	3.46-1.61(90)	2.72-1.43(170)

$^{*}k$ is $\times 10^{-2}$ in $krads^{-1}$. k_L refers to the low dose portion of the survival curve; k_H refers to the high dose portion of the survival curve. (BP) refers to the dose (krads) at which the breakpoint occurs.

result probably being the more clear result at this time. Pre-irradiation of the solutions of $CuCl_2$ resulted in the same or lower levels of sensitization. The pre-irradiation result in $CuSO_4$ cannot be explained but is a very clear indication of some large effect of pre-irradiation. Pre-irradiation of $CuSO_4$ solutions in O_2 results in sensitization of the spores to a k of 6.58×10^{-2} $krads^{-1}$ while pre-irradiation of the solutions in N_2 reduces the sensitization from 6.58×10^{-2} $krads^{-1}$ to 2.73×10^{-2} $krads^{-1}$. This surely indicates some chemistry of the solutions themselves that has a large effect on sensitization of the spores.

Kirschner, et al (1970) using $CuSO_4$ showed a sensitizing effect on B. cereus. They suggested that this effect was due to the formation of Cu(III) species by $^{\cdot}OH$ oxidation of Cu(II). While this should be considered, previous studies in this laboratory in water alone and with alcohol scavengers (Powers, 1972) indicate the action of $^{\cdot}OH$ on the cell itself rather than the action of the metal is of more importance.

Winstead and McNees (1974) also using $CuSO_4$, showed radiation sensitization of lactate dehydrogenase by Cu. Their conclusions were that the Cu^{++} bound to the sulfhydryl groups of the enzyme and then was attacked by the OH radicals causing ultimately the damaging oxidation of the sulfhydryl groups, with Cu(III) being intermediate in this process. This type of sequence seems more consistent with results from this laboratory.

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Kelm, M., Lilie, J., Henglein, A. and Janata, E. (1974) J. Phys. Chem. 78:882-887.

Kirschner, I., Citri, N., Levitzki, A. and Anbar, M. (1970) Int. J. Radiat. Biol. 17:81-85.

Powers, E. L. (1972) Isr. J. Chem. 10:1199-1211.

Winstead, J. A. and McNees, D. E. (1974) Radiat. Res. 59:466-474.

IV.2. DNA Studies

Appendices 2 and 3 present data on the protection of transforming DNA from x-rays by $^{\cdot}OH$ scavengers and on the effects of various O_2 concentrations on the activity of irradiated transforming DNA. Some of these O_2 results are also presented in appendix 1. Data on the effects of Ag^+ on the radiation sensitivity of transforming DNA are presented in appendix 7.

IV.2.1. Effects of Cd^{++} on the radiation sensitivity of transforming DNA

In recent years Cd^{++} has become a concern as an environmental contaminant. Cd^{++} reacts rapidly with e^-_{aq} like Ag^+, and thus should be another test of the e^-_{aq} sequestration theory. Because of these two facts, the effects of Cd^{++} on

the radiation sensitivity of transforming DNA were studied in Fig. 4 where the inactivation rate constant for transforming DNA is shown as a function of concentration of $CdSO_4$ when the irradiation is performed with N_2 or O_2 as equilibrating gases. In N_2 there is a steady increase in sensitivity of the transforming DNA from 10^{-9} to 10^{-3} M $CdSO_4$. In O_2 the sensitivity is constant from 10^{-9} to about 10^{-4} M $CdSO_4$, then starts to increase with increasing $CdSO_4$ concentration. Concentrations above 10^{-3} M $CdSO_4$ could not be investigated due to control losses. It is significant to note that even at very low concentrations of Cd^{++} (10^{-9} M), the sensitivity in both N_2 and O_2 is over three times that of the respective baselines. At the highest sensitivity in Cd^{++} the DNA is sensitized by over a factor of four by Cd^{++}. These patterns of sensitization differ from that seen in Ag^+ sensitization of DNA (appendix 7) and the differences cannot now be explained.

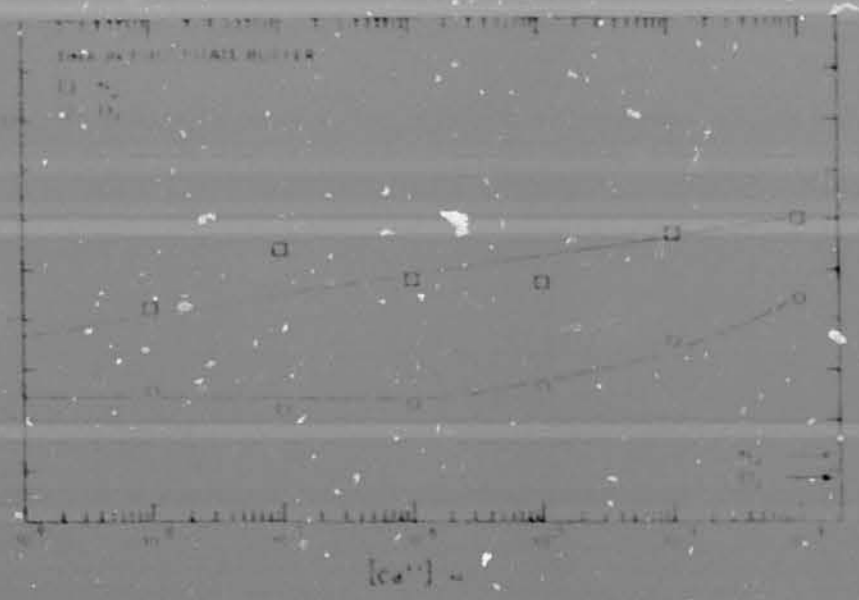

Fig. 4 Inactivation rate constant for transforming DNA vs Cd^{++} concentration when x-irradiated in N_2 or O_2.

IV.2.2. Uptake of Cd^{++} by DNA

Cd^{++} is known to bind to both bases and phosphate groups in DNA. It binds less strongly to the bases and more strongly to the phosphate group than does Ag^+. Studies paralleling those done on binding of Ag^+ to DNA have also been done on the binding of Cd^{++} to DNA. The uptake of Cd^{++} by calf thymus DNA was measured by use of a Cd^{++} specific ion electrode. Typical results for 3×10^{-5} M DNA (the concentration used in the transformation experiments) are given in Fig. 5. Although there is considerable scatter in the data presented here, especially at high $[Cd^{++}]$, it is evident that as Cd^{++} concentration increases there is an increase in the ratio of Cd^{++} bound per DNA nucleotide from 10^{-6} M Cd^{++} to the highest level studied.

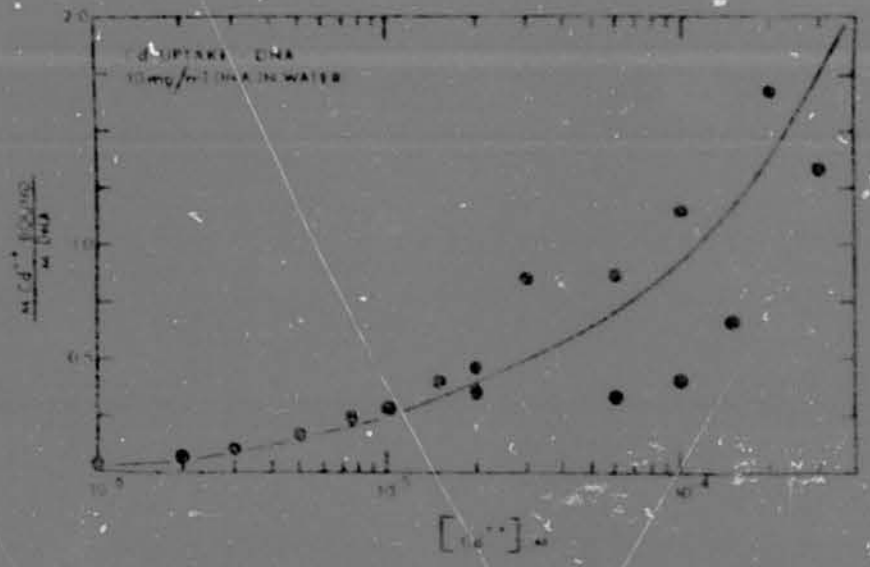

Fig. 5 Uptake of Cd^{++} by calf thymus DNA in water.

Since most transforming DNA studies have been performed in the presence of phosphate buffer, the effect of phosphate on Cd^{++} uptake by DNA was investigated. As seen in Fig. 6 the uptake pattern in phosphate buffer is the same shape as that of DNA in water although much more Cd^{++} appears to be bound per mole DNA. Presumably this reflects the reaction of Cd^{++} with $PO_4^{=}$ which would remove free Cd^{++} from solution, not the actual binding of additional Cd^{++} to DNA.

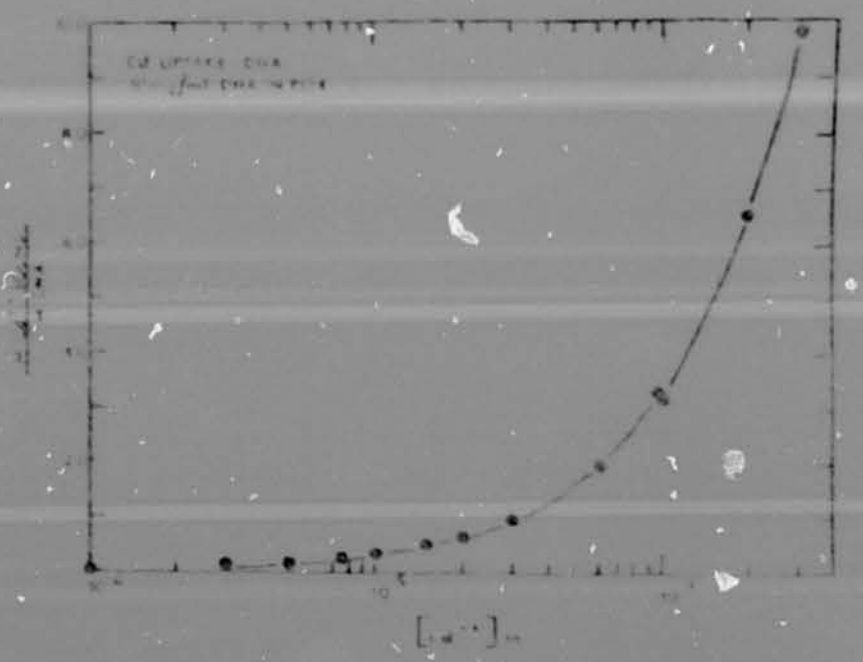

Fig. 6 Uptake of Cd^{++} by calf thymus DNA in phosphate buffer.

Because EtOH and t-BuOH are used as $\cdot OH$ scavengers in our studies we investigated the possible interference of these compounds with uptake of Cd^{++} by DNA. Neither of these compounds interacts with Cd^{++} alone. As can be seen in Figs. 7 and 8, where the dashed line represents the uptake of Cd^{++} by DNA from Fig. 5, the addition of quite high concentrations of these scavengers may alter the uptake pattern slightly, but it is uncertain if this is significantly different from the result in the absence of scavengers because of the considerable scatter in the curves. When Cd^{++}-DNA solutions (10^{-4} or 5×10^{-5} M Cd^{++}) were irradiated in N_2 or in O_2 (4 or 6 krads respectively) there was no detectable change in the uptake of the Cd^{++} by DNA as compared to the binding in unirradiated solutions. This effect may differ from that seen when Ag^{+}-DNA solutions are irradiated but no further discussion is possible at this time.

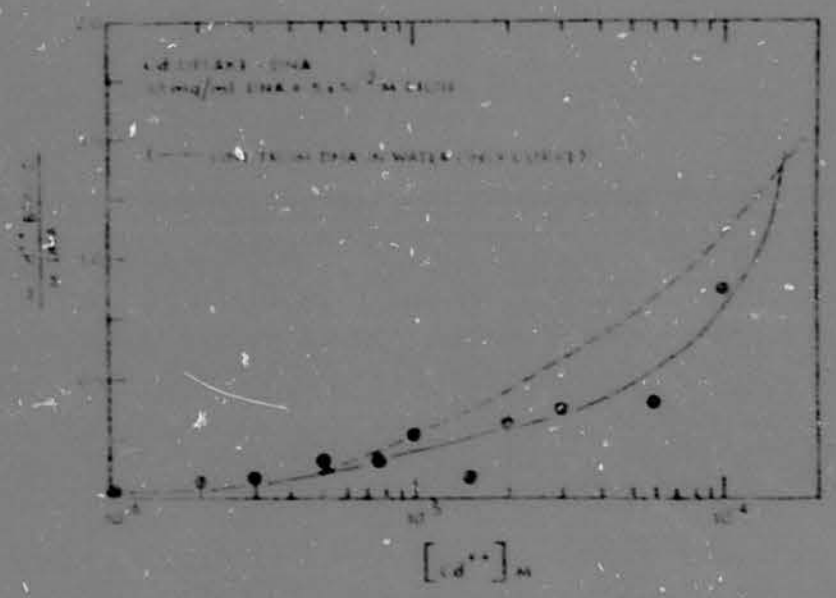

Fig. 7 Uptake of Cd^{++} by calf thymus DNA in EtOH

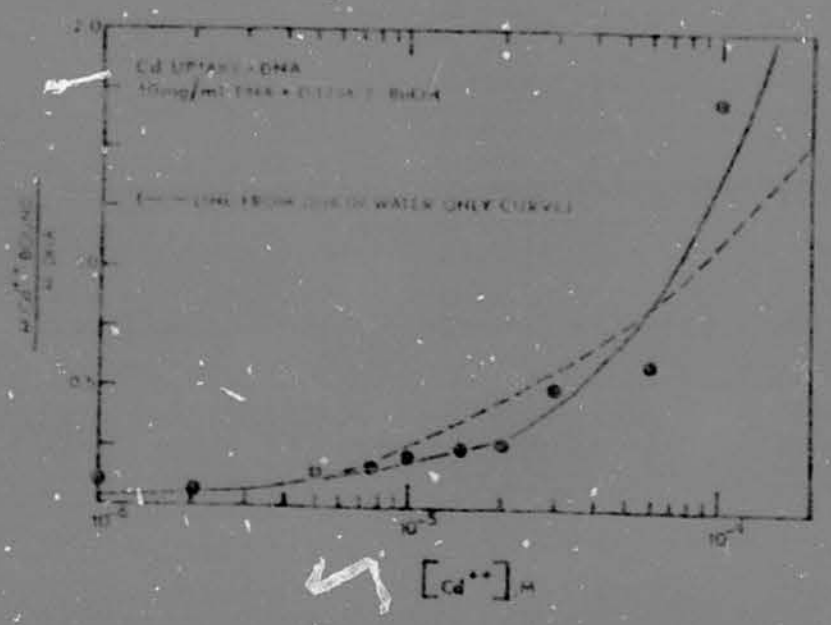

Fig. 8 Uptake of Cd^{++} by calf thymus DNA in t-BuOH

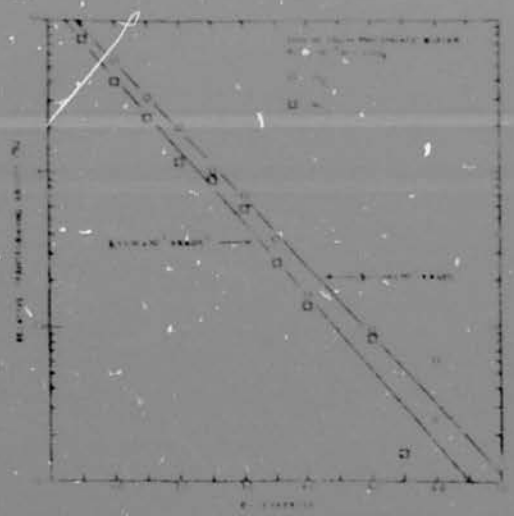

Fig. 9 Survival of transforming DNA in the presence of 10^{-4} M $CuSO_4$ when x-irradiated in O_2 or N_2.

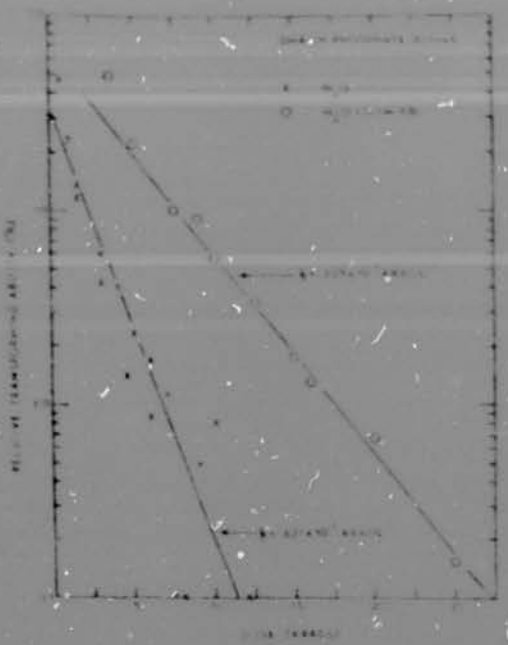

Fig. 10 Survival of transforming DNA when x-irradiated in N_2O with and without KBr.

IV.2.3. Effects of $CuSO_4$ on the radiation sensitivity of transforming DNA

Another metal which causes radiation sensitization of bacterial spores and other cells (Hesslewood et al 1978, Cramp 1967) is copper. Cu^{++} is also known to bind to DNA, particularly to DNA bases (Zimmer et al 1974). Preliminary studies have been done on the effects of $CuSO_4$ on the radiation sensitization of transforming DNA and they indicate that, at least at fairly high concentrations of $CuSO_4$ (10^{-4} M) (for example, Fig. 9), DNA is sensitized in both O_2 and N_2 to about the same level. This high degree of sensitization of DNA in O_2 compared to N_2 differs from that seen in the other two metals on which data has been presented here. More study along this line is underway.

IV.2.4. Effects of KBr on the radiation sensitivity of transforming DNA

Some recent work by Dr. L. S. Myers and his colleagues at the Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los

Angeles indicates that bromide enhances the radiation sensitivity of DNA to base damage and this enhancement may be due to a mechanism other than $^{\cdot}OH$ attack. (Myers et al 1979). At the request of Dr. Myers an experiment on the effects of KBr on the radiation sensitivity of transforming DNA was performed. These results are given in Fig. 10. KBr reduces the sensitivity of transforming DNA irradiated in N_2O by a factor of about three. This protection may be due to the reaction of KBr with $^{\cdot}OH$ radicals, thus removing $^{\cdot}OH$ radicals which have been shown in this laboratory to be damaging to transforming DNA. However this protection is not nearly as large as that afforded transforming DNA by a comparable concentration of EtOH. For example in 6×10^{-3} M EtOH, a concentration which gives the same k_t for reaction with $^{\cdot}OH$ as does 0.1 M KBr, the inactivation rate constant, $\bar{k}$, is 0.0497 krads^{-1}, a much larger protection. This suggests that the secondary bromide radical causes some sort of radiation-induced sensitization of transforming DNA. This radical, an oxidizing species similar to $^{\cdot}OH$ is formed by:

$$^{\cdot}OH + Br^{-} \longrightarrow {}^{\cdot}Br + OH^{-}$$

$$^{\cdot}Br + Br^{-} \longrightarrow {}^{\cdot}Br_2^{-}$$

Previous work using the bromide radical as a sensitizing agent on spores and bacteriophage was reported by this laboratory in 1974 (Simic et al 1974).

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IV.3. Pulse Radiolysis Studies

Our investigations into the details of the radiation chemistry of the bacterial spore has begun with self-calibration experiments with the DNA bases. The rationale for this approach is as follows: the physical-chemical experiments that we have done over the years with the bacterial spore suggest very strongly that DNA or some other critically important molecule like it (the existence of which we cannot imagine) must be involved to explain the first order relationships that we continually have found in a large variety of experimental conditions, i.e., the effect must be brought about by single

event processes, and thus must be a lethal mutation. Recently, it has been demonstrated by several laboratories that the effects seen in the spore are undoubtedly associated with DNA damage. Al-Shalchly and Tallentire (1974) have shown in the spore of the thy⁻ mutant of Bacillus subtilis that the substitution of 5-bromouridine for thymine results in markedly different responses of the various compartments of damage to gamma radiation; all the while, first order kinetics being observed. And more recently, Tanooka (1978) has shown in the spore that the rate of induction of mutations by ionizing radiation varies with experimental conditions in just the way that lethality does. Since lethality of the spore has been the biological end-point utilized over the years, Tanooka's demonstration is very firm support for the notion that we are dealing with a DNA response to radiation in measuring lethality.

Accordingly, we now turn our attention to DNA, utilizing the facilities of the Center for Fast Kinetics Research, an unparalleled set of instruments for the analysis of very early events in radiation chemistry. It was toward this goal that we began several years ago the program on the steady-state radiation sensitivity of transforming DNA, that allows the monitoring of the "functional ability" of the DNA following various experimental treatments. This will be the bridge between the chemistry of DNA in solution and the response of DNA within the cell. The experiments begun this year deal with the constituent bases to satisfy ourselves that our experiments are consistent with the several investigations that have been reported to date. We expect also that because of improvements in technique, more detailed analyses of radiation induced change in these compounds will be possible in the CFKR.

Amongst the published studies are the studies of Willson (et al 1970) who has presented information on some of the early events observed after irradiating the four constituent bases of DNA. Using conditions closely approximating his, namely, 20 Gy/pulse of electrons with a 400 ns pulse length (he used 200 ns) we have looked into the behavior of adenylic acid (dAMP). The overall response is approximately the same, namely, there is a very fast increase in an absorption band at approximately 340 nm that decays over a long period in our experiments. Figure 11 presents the absorption spectra seen at varying times after the end of the pulse. Note that Curve A is the absorption spectrum at 5 μs and Curve I is the absorption spectrum at 622.5 μs. There is also a band present at 5 μs after irradiation at 480 nm that decays over the long interval. Our inquiry into the early processes during the growth of these species is demonstrated in Fig. 12. It reveals the growth of the 340 nm band from .5 μs up to 67 μs (points A through E). These growth curves will form the basis for analysis of the kinetics of these events in this time period. Note that at 400 nm there is growth of a distinct absorption band which decays rapidly. This is not observed on the time scale used in Fig. 11; also, in Fig. 12 the 480 nm band is present.

Our understanding of these events is impeded at the present times by several analytical difficulties associated with baseline behavior. Various numerical and experimental methods are being used to try to attempt recognition of final equilibrium conditions. However, even with this uncertainty

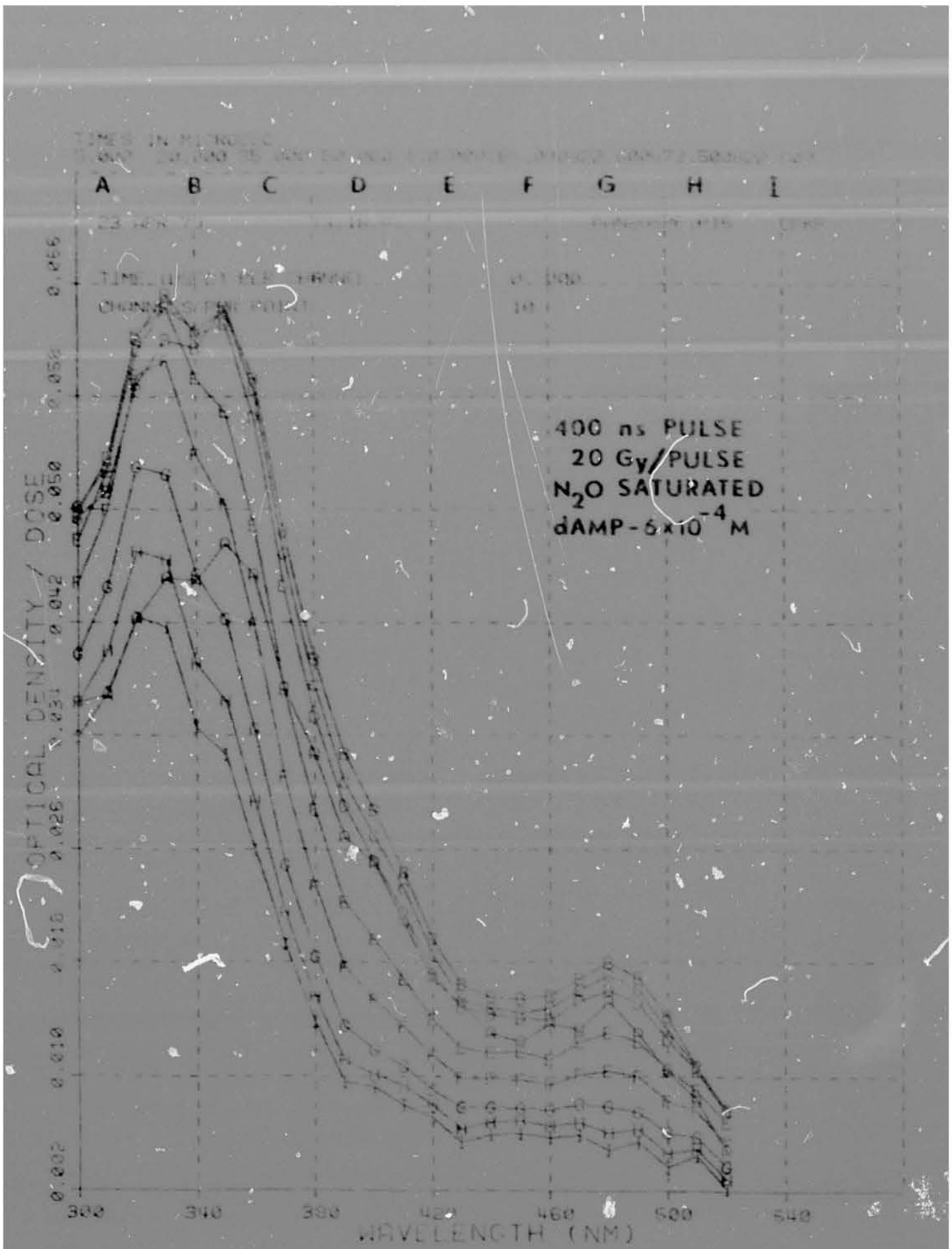

Fig. 11 Absorption spectra of dAMP (6×10^{-4} M) in N_2O from 5 μs to 622 μs after a 400 ns pulse of electrons delivering 20 Gy.

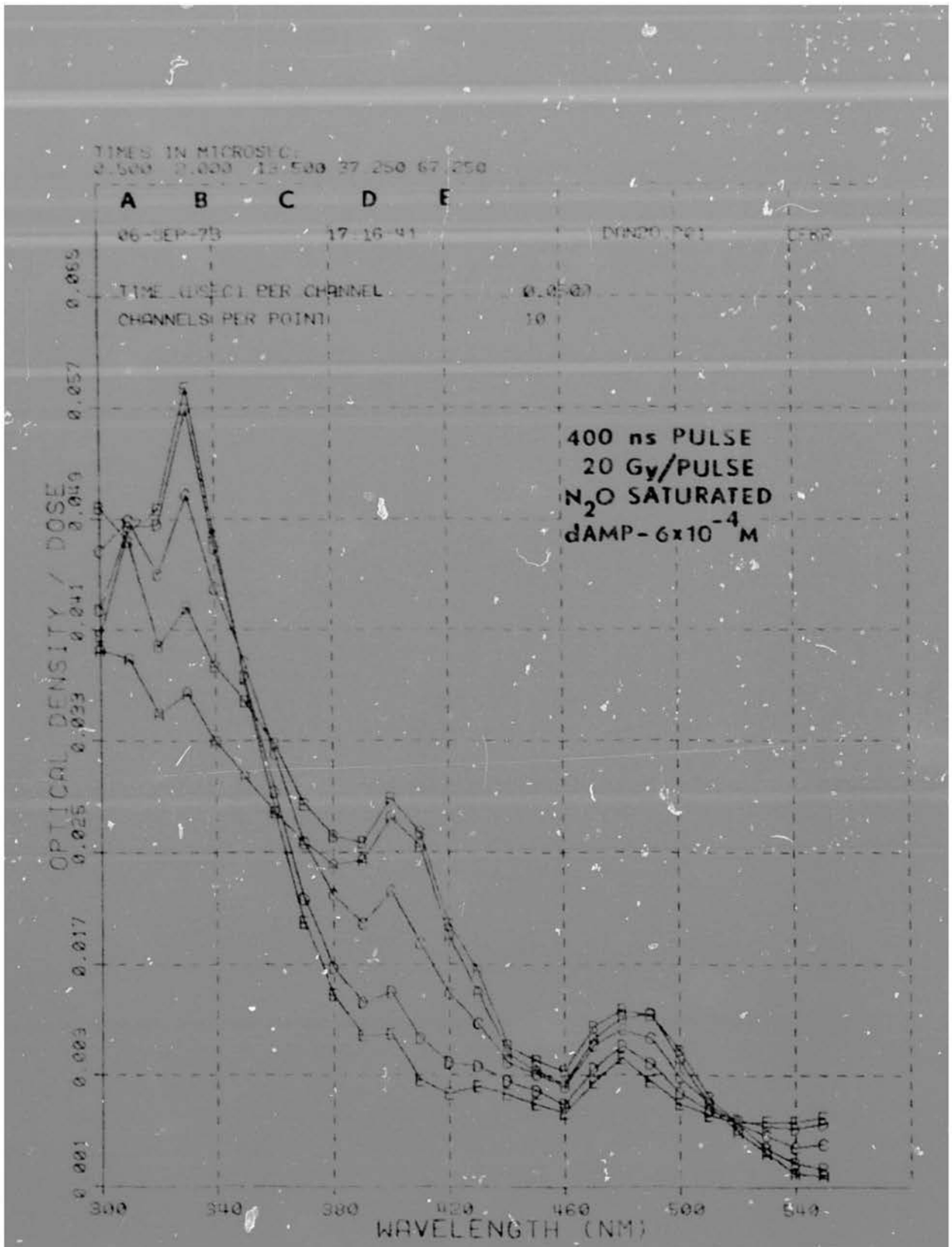

Fig. 12 Absorption spectra of dAMP (6×10^{-4} M) in N_2O from 0.5 μs to 67 μs after a 400 ns pulse of electrons delivering 20 Gy.

there is sufficient evidence that in the set of curves there is evidence for first order growth processes, first order decay processes, and perhaps a combination of first and second order decay processes.

It is also clear that these absorption bands represent different species. Figure 13 reports a competition experiment constructed by introducing t-BuOH at different concentrations into the dAMP solution and observing the behavior of the two bands, one at 340 and one at 480 nm. Here we presume that the absorption bands are caused as a consequence of hydroxyl radical reaction with the base. We note that the competition relationship

$$D_o/D = 1 + \frac{k_{(t\text{-BuOH} + \cdot OH)} \cdot [t\text{-BuOH}]}{k_{(dAMP + \cdot OH)} \cdot [dAMP]}$$

as set out by Adams (1967) says that D_o/D is linear with varying concentrations of one of the solutes (the other held constant) with the slopes being the ratio of the rate constants of each of the constituent solutes with hydroxyl radical, and has an intercept of 1. Figure 13 shows that at the 2 wavelengths the relationship is approximately linear, and the intercept is approximately one. Using the literature k for t-BuOH + $\cdot$OH of 5.2×10^8 $M^{-1}s^{-1}$ (Dorfman and Adams 1973) we calculate a rate (k) of 4.65×10^9 $M^{-1}s^{-1}$ for hydroxyl radical with dAMP as measured at 340 nm. At 480 nm we note from the figure that the rate is 3.5×10^8 $M^{-1}s^{-1}$, a number different than that observed at 340. Therefore, we are dealing with two hydroxyl radical reactions with the dAMP. The rate frequently cited is 3.5×10^9 $M^{-1}s^{-1}$ that Scholes et al (1965) determined at 500 nm, identical to the rate we measure at 480 nm.

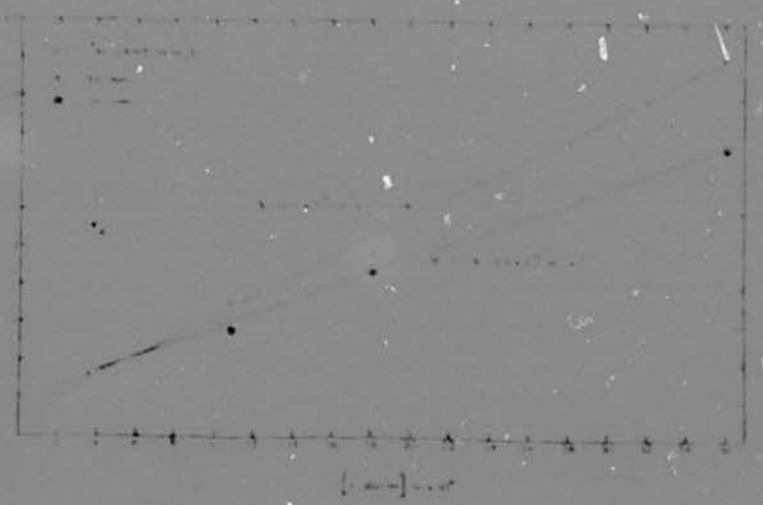

Fig. 13 Rate of formation of the OH adduct(s) of dAMP in N_2O determined by competition of dAMP and t-BuOH for OH radicals as seen at 340 nm and 480 nm.

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