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TRAPPING AND REACTIONS OF HYDROGEN ATOMS  
IN GAMMA-IRRADIATED FROZEN SOLUTIONS<sup>1</sup>

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ABSTRACT--Electron spin resonance spectra of frozen acidic solutions containing thymine gamma-irradiated at 77°K and annealed to 120°K indicate that the resulting organic free radicals are formed in the pyrimidine ring of thymine by hydrogen atom addition in ices containing oxy-acids or oxy-acid anions, and by hydrogen and possibly halide atom addition in acidic ices containing halides. The relative, thymine hydrogen-atom adduct radical yields parallel the relative yields of trapped hydrogen atoms in the oxy-acid and oxy-acid anion ices, but not in the acidic halide ice. Thus, to explain the unexpectedly high yields of the thymine adduct radical in acidic halide ices, it is suggested that hydrogen atoms are formed, but are not trapped and react directly with thymine.

## INTRODUCTION

The formation and stabilization of hydrogen atoms in gamma-irradiated, frozen solutions (ices) have been extensively investigated. Livingston and Weinberger<sup>2</sup> suggested that the hydrogen atoms may be stabilized by a physical trapping mechanism in oxy-acid ices. The mechanism of formation and trapping of hydrogen atoms in gamma-irradiated ices containing oxy-acids or salts of oxy-acids has received considerable attention from Weiss and co-workers<sup>3</sup>. However, few studies have been made on subsequent reactions of radiation-induced hydrogen atoms with organic solutes in frozen solutions<sup>4-6</sup>. One study has revealed the formation of organic free radicals in irradiated oxy-acid and alkaline ices containing pyrimidine solutes<sup>5</sup>. Another study suggested that the 5,6-dihydrothymine-5-yl free radicals induced in irradiated deoxyribonucleic acid gels and oxy-anion ices containing thymine or thymidine are formed by the addition of radiation-induced hydrogen atoms to the thymine moiety<sup>6</sup>.

The work reported here will show that the formation and reaction of hydrogen atoms in gamma-irradiated ices of halide acids, oxy-acids and oxy-acid anions containing thymine are responsible for the formation of thymine radicals identifiable by the resulting electron spin resonance (ESR) spectra.

#### EXPERIMENTAL

Analar reagents and commercial samples of thymine (th) were dissolved without further purification in appropriate  $N_2$ -purged solutions. Cylinders of crystalline ice were prepared as previously described.<sup>3</sup> The ice cylinders were irradiated at  $77^\circ K$  in pyrex tubes to <sup>6000</sup> non-saturation dose of 2.5-3.5 megarads with a <sup>A</sup> gamma source at a dose rate of approximately 0.1 megarads/hr.

After irradiation, the ice-cylinders were transferred directly into a standard, quartz-tipped Dewar containing liquid nitrogen. The Dewar was placed into the ESR cavity and the spectrum recorded. The sample was then annealed to approximately  $120^\circ K$  where trapped hydrogen atoms ( $H_t$ ) and OH have disappeared<sup>e</sup>, but the solute radicals remain. After annealing to  $120^\circ K$ , the temperature was reduced back to  $77^\circ K$  and the spectrum re-recorded. The solute radical spectrum was then readily visible. Annealing to  $220^\circ K$  caused an irreversible disappearance of the solute radicals.

A Microspin ESR spectrometer operating at 9.4 KMc/sec, 100 Kc/sec modulation and a maximum microwave power of approximately 5mW was used for most of the measurements. However, a power of less than  $5 \mu\text{W}$  was used to observe  $H_t$  thereby ~~reducing~~ <sup>eliminating</sup> power saturation effects. The magnetic field was current regulated and calibrated with a proton-resonance magnetometer. Calibrations of g-factors were made by recording the spectrum when a small amount of diphenylpicrylhydrazyl (DPPH) was placed into the cavity with the sample.

The relative yields of  $H_t$  were assumed to be directly proportional to  $hw^2$  where  $h$  is the signal height and  $w$  is the line width of the main downfield line of the  $H_t$  doublet spectrum. Relative yields of the 5,6-dihydrothymine-5-yl radical (thH) were similarly compared by recording  $hw^2$  of line 7 or 8 of the 8-lined spectrum.

#### RESULTS AND DISCUSSION

Trapped hydrogen atom yields in irradiated oxy-anion acid<sup>2</sup> and in oxy-anion salt<sup>3</sup> solutions at 77°K generally increase with radiation dose. If an  $H_2SO_4$  <sup>ice</sup> is made  $10^{-2}$ M in thymine, irradiated and annealed to above 120°K, the thH radical spectrum of figure 1 is observed<sup>5,6</sup>. This thH spectrum is almost identical with that spectrum observed during the reaction of gaseous hydrogen atoms with thymine<sup>7</sup>.

Table I gives the thH yields in 1M ices relative to a yield of unity in 1M  $H_2SO_4$  ice. If the same normalization is applied to our observed  $H_t$  yields or applied to the  $H_t$  yields as obtained by Moorthy and Weiss<sup>3</sup> (Table I), a relationship between the trapping of hydrogen atoms and observation of thH radicals might possibly be seen.

Table I: Yields of thH and  $H_t$  free radicals in irradiated IM ices relative to  $\frac{thH}{H_t}$  yield in 1M  $H_2SO_4$  ice.

IM ice	$H_t^a$	$H_t^b$	thH <sup>c</sup>
$H_2SO_4$	1.00	1.00	1.00
$H_3PO_4$	0.58	0.55	0.50
$HClO_4$	4.80	4.75	4.90
HCl	0.07	0.06	0.65
HBr	0.00	0.00	0.11
NaHSO <sub>4</sub>	0.33	0.30	0.30
Na <sub>2</sub> SO <sub>4</sub>	0.09	0.07	0.00

a) from Ref. 3, no thymine present.

b) at 77°K, no thymine present.

c) at 77°K after annealing the  $10^{-2}M$  th-ices to 120°K.

Table I shows that the relative thH yields follow the relative  $H_t$  yields in the case of oxy-acids and salts of the oxy-acids as solutes. However, the same correlation does not hold for halide acids. Thus, the trapping efficiency of halide ices for  $H_t$  may not be directly related to radiation induction of hydrogen atoms in ices. It is assumed here that the major production of thH is through addition of hydrogen atoms to thymine.  $\frac{thH}{H_t}$  relative increase of thH and  $H_t$  with oxy-anion concentration supports this assumption.

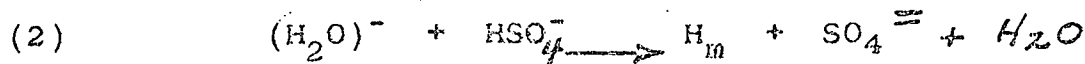
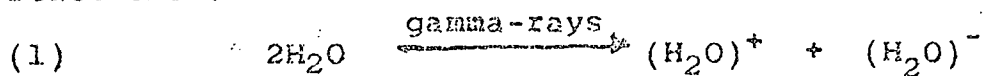
Although  $H_t$  in oxy-anion acids containing no dissolved



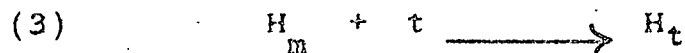
organic solute is stable with storage time at 77°K<sup>2,3</sup>, there is competition with the traps for hydrogen atoms when solutes such as thymine are present. Figure 2 shows the decrease of  $H_t$  and the corresponding increase of thH with storage time at 77°K in 1M  $H_2SO_4$  with  $10^{-2}M$  thymine. Thus, at 77°K hydrogen atoms escape their traps and react only when a solute such as thymine is present.

The formation of thH could also occur through electron scavenging by thymine and subsequent proton transfer to the thymine anion radical. However, the increase of the thH yields with oxy-acid concentration, and with decreasing  $H_t$  yields during storage of oxy-acid ices at 77°K while  $H_t$  is stable in ices without thymine, and the fact that low concentrations of thymine do not affect the yields of  $H_t$  in oxy-acid ices while thH is formed do not support the thymine anion radical mechanism of thH formation in acidic solutions.

The mobile hydrogen atom ( $H_m$ ) results from the following reactions<sup>3</sup>.

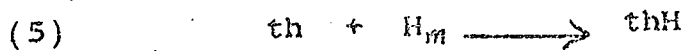
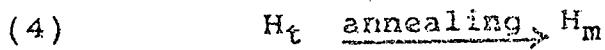


The mobile hydrogen atoms can be trapped at trapping sites (t) to yield trapped hydrogen atoms ( $H_t$ )



Kevan, Moorthy and Weiss<sup>3</sup> have shown that in the oxy-acid ices the sites of formation and trapping of hydrogen atoms are equivalent and presumably are the oxy-acid anions.

Table I and figure 2 indicate that upon annealing or storage after irradiation the following reactions occur.



In the study with the oxy-acid and oxy-acid anion ices, a small amount of thH could usually be detected before annealing.

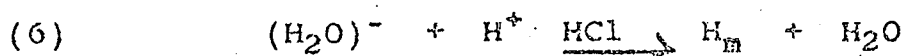
However, if a high dose rate is used so that the time of radiation delivery is short (essentially zero storage time), no thH can be observed until after annealing or storage (this experiment utilized dose rates of about 55 Krad/min from <sup>60</sup>Co source at the UCLA Laboratory of Nuclear Medicine and Radiation Biology). Thus, at essentially zero storage time at 77°K and no annealing, reactions 4 and 5 are relatively unimportant. However, with ices containing 1M HCl and thymine, thH signals are observed at 77°K along with small yield of H<sub>t</sub>.

In contrast to the H<sub>2</sub>SO<sub>4</sub> ices, annealing the HCl ices increases the thH yield only slightly. This increase probably results from the reaction of the small number of hydrogen atoms seen trapped in the unannealed sample. Moorthy et al.<sup>8</sup> did not observe H<sub>t</sub> in HCl ices; however, their use of a 1 mw rf power

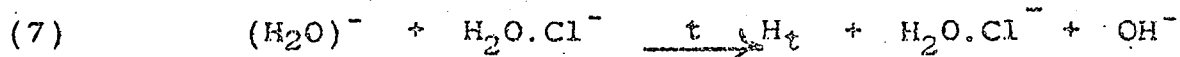
level, *a 0.84 Mrad dose and an observation after storage at 77°K may have* ~~may have eliminated the H<sub>t</sub> signal through power saturation.~~

*reduced the H<sub>t</sub> signal below detection.* Our results with HCl ices suggest that all the hydrogen atoms formed in frozen solutions at 77°K may not be trapped.

Table I shows differences between relative  $H_t$  and thH yields in acidic halide-ices. Two possible explanations for these differences between the yields are that (a) thH is formed by reactions other than reaction 5, or (b) thymine can react with  $H_m$  which are formed, but not necessarily trapped in some ices at 77°K as the following reaction illustrates.



The small  $H_t$  yield in HCl ices may result from the reaction



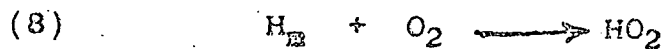
Observation of  $H_t$  formed in various ices at 4°K may allow the direct determination of the hydrogen-atom formation efficiency, since all hydrogen atoms formed should be trapped as previously observed in irradiated ice <sup>10</sup>.

Hydrogen atom spectra from irradiated  $H_2SO_4$  ices indicate that  $H_t$  has neighboring protons from which satellite lines arise <sup>11</sup>. Frozen solutions for 1M  $H_2SO_4$  are similar to 6M  $H_2SO_4$  in that each line of the  $H_t$  doublet has four satellite lines covering a total width of approximately 20 gauss.

No  $\#$  The number of satellite lines in irradiated  $H_2SO_4$  ices depends on the rf power applied. At higher rf powers, the main line decreases in intensity with increasing microwave power levels while the satellite lines increase in intensity. However, in 1M HCl ice no satellite lines are observed, and depending on the applied rf power each line of the  $H_t$  doublet is split into <sup>a</sup> broad doublet covering about 27 gauss (figure 3).

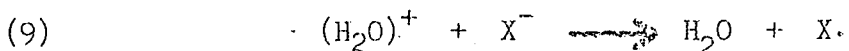
This broad doublet increases in intensity to a small extent with moderate power increases and then decreases at higher powers. Apparently,  $H_t$  in HCl ice have different rf power saturation characteristics than  $H_t$  in  $H_2SO_4$  ice<sup>9</sup>. These results indicate that the trapping sites in halide ices are different from those sites in the oxy-acid anion ices. These differences might be expected considering the hydrogen atom trapping model of Kevan, Moorthy and Weiss<sup>3</sup>.

Although dissolved oxygen does not drastically affect  $H_t$ , oxy-acid ices which are saturated with oxygen before freezing and irradiation show spectra indicating that the tHM yields are considerably reduced and that the  $HO_2$  radical does not appear to be formed until the sample is annealed to at least  $100^\circ K$ <sup>9</sup>. However in HCl ices containing oxygen most of the  $HO_2$  radicals are observed at  $77^\circ K$  without annealing. This leads to the suggestion<sup>that</sup> in oxy-acid ices reactions 1 through 3 are followed upon annealing by reactions 4 and 5, but if considerable oxygen is present reaction 5 is partially replaced by



In ices containing HCl, reactions 1, 6, and 7 are followed by reaction 5. If oxygen is present, reaction 8 competes with reaction 5. Thus, in contrast to the oxy-acid ices few of the hydrogen atoms that are formed are trapped and no annealing is required of the HCl ices to form  $HO_2$ .

The  $(\text{H}_2\text{O})^+$  react with halide ions ( $\text{X}^-$ ) in the halide ices to yield<sup>3</sup>



Upon annealing the acidic halide ices, the halide atoms ( $\text{X}.$ ) will react with halide ions and possibly with thymine in the th-ice. A small, downfield signal with the g-factor dependent on the halide ion is observed from the halide ices containing thymine. The ESR spectra of gamma-irradiated 5-bromouracil also contain absorption lines downfield of the main radical line<sup>12</sup>. A halide-containing radical has been proposed as an intermediate during the formation of uracil radicals by exposing 5-bromouracil<sup>13,14</sup> and 5-chlorouracil<sup>14,15</sup> to gaseous hydrogen atoms. However, Ward and Kuo<sup>16</sup> found that  $\text{Cl}_2^-$  destroyed thymine in solution without forming a thymine product containing chloride. Therefore, a detailed investigation of the ESR spectra and products from the proposed thymine-halide adducts in frozen solution must be made to determine the origin of the signals downfield from the main line.

It is concluded that hydrogen atoms are formed in acidic halide and oxy-anion ices upon irradiation, but are trapped predominately in the oxy-anion ices. Upon annealing the oxy-anion ices, the trapped hydrogen atoms react with solutes such as thymine and oxygen while in the halide ices the reactions with these solutes do not proceed through an intermediate trapping step. Thus, the tH radical yields can be used as an indicator of total hydrogen-atom yields in frozen acidic solutions.

#### REFERENCES

- 1) The data, ideas and conclusions presented in this manuscript are a direct result of the authors' association and collaboration with Professor J. J. Weiss in the Laboratory of Radiation Chemistry during the periods of 1966-1967 (DEH, Fellow in Radiological Research of the James Picker Foundation) and 1966-1970 (NBN, supported by the Nuffield Foundation).
- 2) R. Livingston and A. J. Weinberger, 1960, *J. Chem. Phys.*, 33, 499.
- 3) L. Kevan, P. N. Moorthy and J. J. Weiss, *J. Am. Chem. Soc.*, 1964, 86, 771; P. N. Moorthy and J. J. Weiss, *Solvated Electron, Adv. in Chem., Series 50*, *Am. Chem. Soc.*, 1965, 180.
- 4) N. M. Bazhin, E. V. Kuznetsov, N. N. Rubnov and V. V. Voevodskii, *Kinetics and Catalysis (USSR) (English translation)*, 1966, 7, 140, 643; N. M. Bazhin, *ibid*, 1967, 8, 459; V. V. Voevodskii, N. M. Bazhin and N. M. Katkova, *ibid*, 1968, 9, 432; K. Vacek and D. Schulte-Frohlinde, *J. Phys. Chem.*, 1968, 72, 2686; W. Kühnlein and D. Schulte-Frohlinde, *Radiat. Research*, 1969, 38, 173.
- 5) C. Elston, Ph. D. Thesis, 1967, Chemistry, The University, Newcastle upon Tyne, England.
- 6) D. E. Holmes and J. J. Weiss, *Int. J. Radiat. Biol.*, 1968, 14, 187.
- 7) H. C. Heller and T. Cole, *Proc. Natl. Acad. Sci., U. S.*, 1965, 54, 1486; J. N. Herak and W. Gordy, *Proc. Natl. Acad. Sci., U. S.*, 1965, 54, 1287; D. E. Holmes, L. S. Myers, Jr. and R. B. Ingalls, *Nature*, 1966, 209, 1017.

- 8) P. N. Moorthy, C. Gopinathan and K. N. Rao, Radiat. Effects, 1970, 2, 175.
- 9) D. E. Holmes, N. B. Nazhat and J. J. Weiss, J. Phys. Chem., 1970, 74, 1622.
- 10) L. H. Piette, R. C. Rempel, H. E. Weaver and J. M. Flournoy, J. Chem. Phys., 1959, 30, 1623.
- 11) H. Zeldes and R. Livingston, Phys. Rev., 1954, 96, 1702; W. Kühnlein and J. H. Venable, Jr., Nature, 1967, 215, 618.
- 12) W. Kühnlein, Strahlenther, 1963, 122, 437.
- 13) D. E. Holmes, Ph. D. Dissertation, 1966, Univ. of Calif., Los Angeles.
- 14) R. B. Ingalls, D. E. Holmes, L. S. Myers, Jr. and J. W. Glass, Radiat. Research, 1967, 31, 545.
- 15) J. N. Herak and W. Gordy, Proc. Natl. Acad. Sci., U. S., 1966, 56, 1354.
- 16) J. F. Ward and I. Kuo, Adv. in Chem., Series 81, Am. Chem. Soc., 1968, 368; Int. J. Radiat. Biol., 1969, 15, 293.

Figure 1: First derivative ESR spectrum of the thH radical observed at 77°K in irradiated 1M H<sub>2</sub>SO<sub>4</sub> ice containing thymine after annealing to 175°K.

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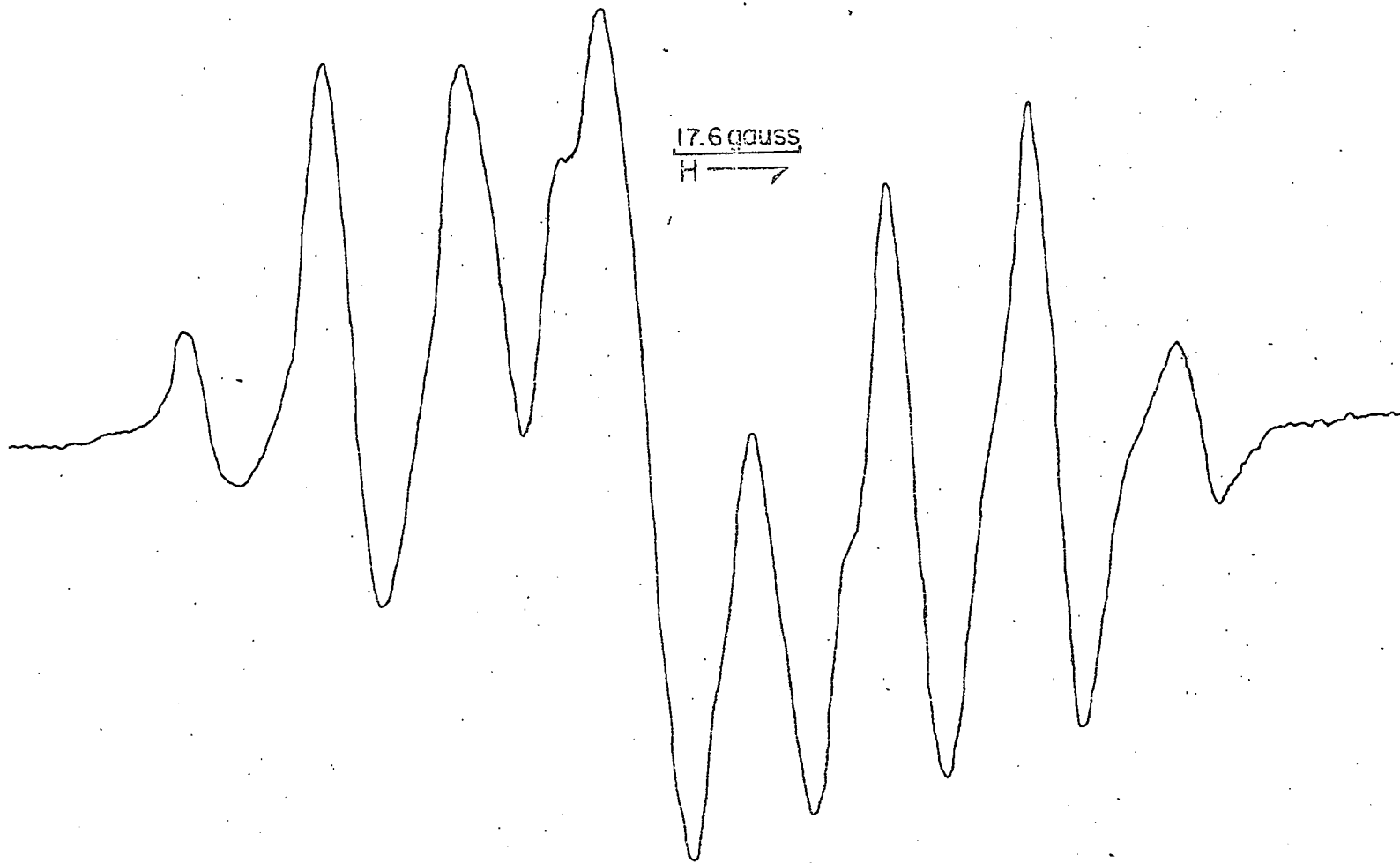
Figure 2: Relative thH  $\times$  and H<sub>t</sub>  $\circ$  radical yields in irradiated 1M H<sub>2</sub>SO<sub>4</sub> ice during storage at 77°K.

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Figure 3: First derivative ESR spectra of the downfield H<sub>t</sub> line in irradiated 1M HCl ice at different rf powers and 77°K.

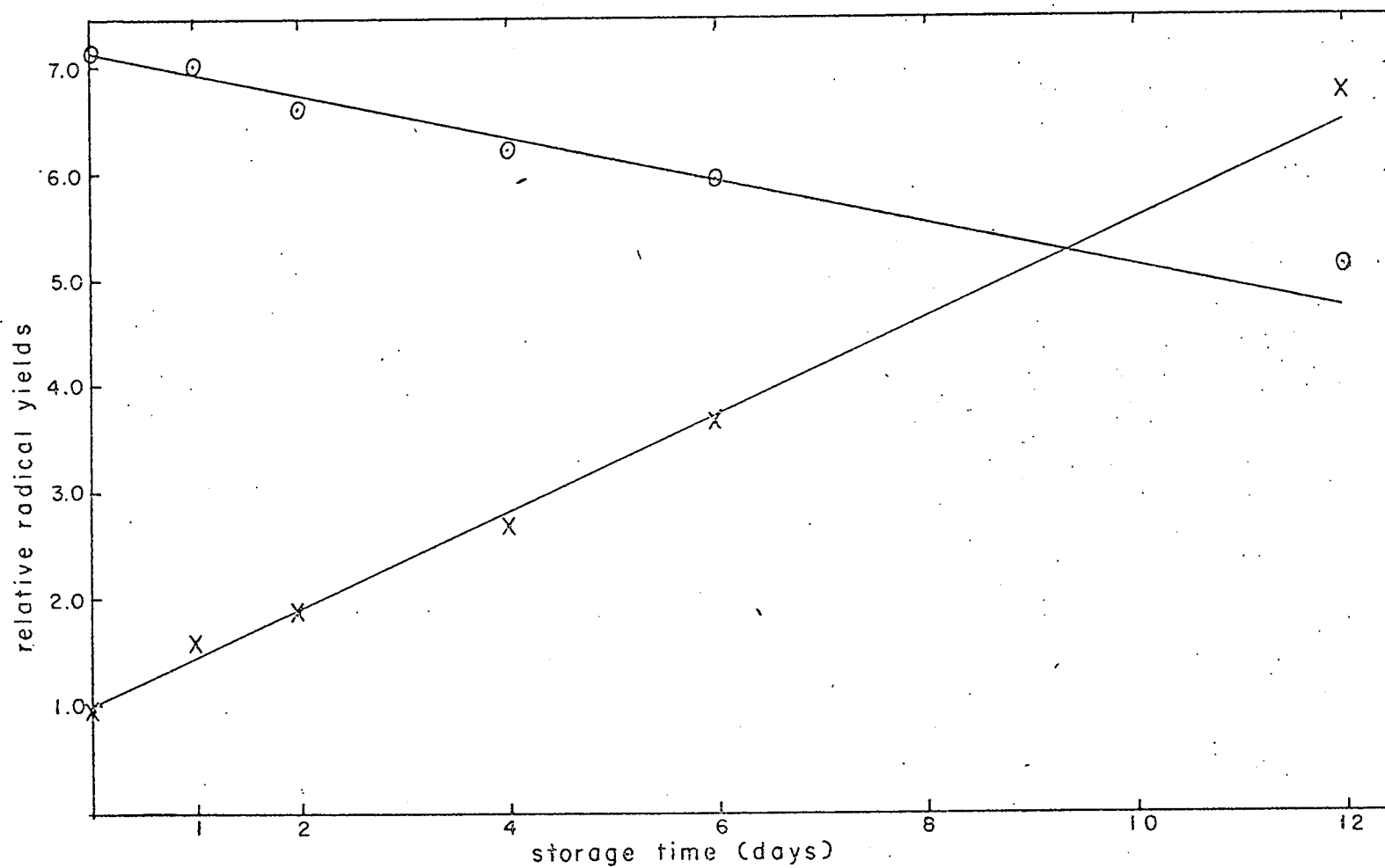
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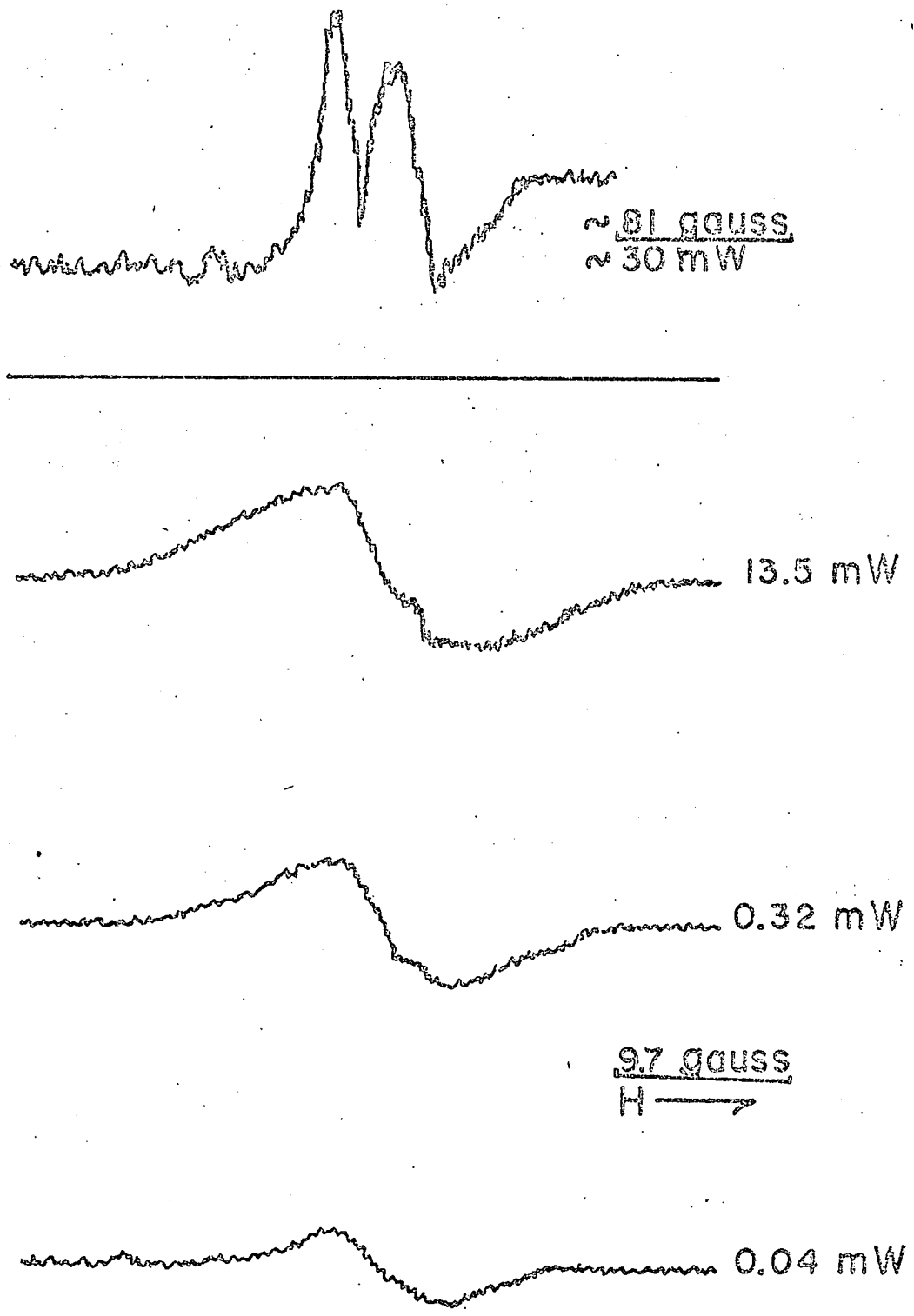
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Figure 1: First derivative ESR spectrum of the  $tH$  radical observed at  $77^{\circ}K$  in irradiated  $1M H_2SO_4$  ice containing thymine after annealing to  $175^{\circ}K$ .



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Figure 2: Relative thH (X) and H<sub>t</sub> (O) radical yields in irradiated 1M H<sub>2</sub>SO<sub>4</sub> ice during storage at 77°K.



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Figure 3: First derivative ESR spectra of the conifield H<sub>2</sub> line in irradiated 1M HCl ice at different rf powers and 77°K.