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April 1969

AEC Contract No. W-7405-eng-48

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IRRADIATION OF DIHYDROTHYMINE IN FROZEN SULFURIC ACID SOLUTIONS[1]

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[1]This work was supported by AEC Contract No. W-7405-eng-48 and NASA's Bioscience Program.

No. of copies submitted: 3

No. of manuscript pages: 13

No. of figures: 8

Henriksen, T., and Snipes, W., Irradiation of Dihydrothymine in Frozen Sulfuric Acid Solutions. Radiation Research pp. __ to __ (1969).

ABSTRACT

Frozen solutions of dihydrothymine in H_2SO_4, irradiated at 77°K, contain large amounts of thymine after melting. Concentration studies show that the thymine must be produced mainly by indirect mechanisms. Low-temperature UV absorption measurements reveal that thymine is not present after irradiation at 77°K, but is formed upon warming of the samples to about 160° to 200°K.

ESR experiments suggest that the 5-thymyl radical is an intermediate in the production of thymine. At 77°K the irradiated samples contain only H atoms and some other unidentified H_2SO_4 radicals. The 5-thymyl radicals are formed upon warming to temperatures above 105°K, and they disappear in the temperature range at which thymine is formed.

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KEY WORDS:

thymine, frozen solutions, UV measurements, ESR spectroscopy

INTRODUCTION

It is well known that most radiation-induced reactions are slowed down or stopped entirely in frozen solutions at low temperatures. The freezing technique has even been used to distinguish between the direct and indirect actions of radiation, since in the frozen state the diffusion of water radicals seems to be very limited. The reduced ability of the water radicals to react with solute molecules in frozen solutions was demonstrated in some early electron spin resonance (ESR) experiments on sulfur compounds (1). Thus, it was shown that the OH radicals trapped during irradiation at low temperatures disappeared, upon warming, mainly in combination reactions. However, in more recent experiments on frozen acid and alkaline solutions, evidence has been presented for a much larger effect of the solvent radicals (2). It has been reported that both H atoms (3) and electrons (4) react with the solvent molecules.

In this paper experiments are presented which show an extremely large indirect radiation effect in frozen sulfuric acid solutions of dihydrothymine, the end product observed being thymine. An attempt has been made, by a combination of ESR technique and low-temperature ultraviolet (UV) absorption measurements, to follow the sequence of reactions giving rise to the large indirect effect. In particular, the experiments were designed to establish whether the indirect effect occurs at the irradiation temperature (77°K) or during the subsequent warming and melting of the solutions.

EXPERIMENTAL PROCEDURE

Sample Preparation and Irradiation

Sulfuric acid solutions ranging from 0.2 to 9.0 mole/liter were used. The dihydrothymine concentration varied from 0.025 to 4.0 mg/ml (i.e., 1.95×10^{-4} to 3.12×10^{-2} mole/liter). Most experiments were carried out with a dihydrothymine concentration of 1.0 mg/ml (7.8×10^{-3} mole/liter).

Samples of approximately 3 ml were rapidly frozen to 77°K in glass test tubes. In the concentration range from approximately 4 to 8 M H_2SO_4, rapid freezing usually results in glassy samples. The glassy samples turn polycrystalline if kept for some time at a higher temperature (see below).

The samples were irradiated at 77°K with 6.5-MeV electrons from a linear accelerator. Doses ranging from 0.05 to 40 MR were delivered at a rate of approximately 1 MR/min.

After irradiation, the samples were melted and the UV absorption was measured with a Cary 14 recording spectrophotometer. The intensity of the absorption peak at 265 nm was used for a quantitative determination of the amount of thymine produced. Corrections were made for the small absorption of dihydrothymine at this wavelength.

Low-Temperature UV Absorption Measurements

Whereas the quantitative determination of the thymine formation was carried out on melted samples, efforts were made to observe directly the UV absorption of frozen samples at different temperatures. For this purpose a Cary Low-Temperature Dewar, model 1440650, was used. Solutions of 5 M H_2SO_4, with or without dihydrothymine, were

carefully frozen in quartz tubes to give clear, glassy samples. The solutions containing dihydrothymine showed less tendency to crack than the pure solvent. Only those samples with a minimum of cracks were used, since any imperfection or crystallinity interferes with the UV absorption measurements.

The samples were irradiated through a quartz window in the Dewar, oriented at 90 deg from the light beam of the spectrophotometer. This arrangement prevented radiation-induced absorption in the surfaces of the sample holder through which the light beam passed.

A copper-constantan thermocouple placed on the outside of the sample holder gave an indication of the approximate sample temperature. The irradiation temperature was about 88°K, and the subsequent absorption measurements started at that temperature. By blowing nitrogen gas through the Dewar the sample temperature could be gradually increased. In most experiments the temperature increased by approximately 2.5°/min, and absorption spectra were taken continually while the sample was warming. At a temperature of about 185° to 190°K the glassy samples started to turn polycrystalline, thus preventing further absorption measurements.

ESR Measurements

ESR samples were made from 5 M H_2SO_4 solutions. Small drops were rapidly frozen at 77°K to give glassy samples. These were evacuated and sealed in quartz tubes. After irradiation, ESR spectra were recorded at 77°K with a Varian 4502 X-band spectrometer. The samples were heat-treated in a Varian Variable Temperature Accessory outside the cavity, then returned to 77°K for further measurements.

The relative quantitative data are taken from the amplitudes of certain peaks in the first- or second-derivative spectra. No attempts were made to obtain absolute yield data.

RESULTS AND DISCUSSION

Results on Melted Samples

It has been reported earlier that irradiation of dihydrothymine both in aqueous solutions and in the solid state yields thymine (5, 6). In the experiments described here, the UV-absorbing substance produced upon irradiation in the frozen solutions had an absorption spectrum identical to that of thymine, and in all subsequent calculations we assume that the product is thymine.

Figure 1 shows the dose-effect curve for the formation of thymine in a 3 M H_2SO_4 solution containing 1 mg/ml dihydrothymine. The solutions were irradiated at 77°K and the measurements carried out after melting. The thymine formation appears surprisingly large for irradiation in the frozen state. Thus, from the initial portion of the dose-effect curve (see inset of Fig. 1) it is seen that 0.07 mg/ml thymine is formed per MR. If it were assumed that only the energy absorbed by the dihydrothymine could lead to the production of thymine, this corresponds to a G-value (molecules thymine per 100 eV absorbed in dihydrothymine) of 535. This large value indicates some indirect effect, and the corresponding G-value based on the amount of energy absorbed in the entire solution is 0.47.

Similar results for some other H_2SO_4 concentrations are presented in Fig. 2. All dose-effect curves were found to be linear in the low-dose region. The G-values calculated on the basis of direct action

alone are 33, 125, and 960 for 0.5, 1.0, and 6.0 M H_2SO_4 solutions respectively. Consequently, the conclusion is inescapable that in the frozen solutions a large indirect effect operates whereby radiation energy absorbed in the solvent can cause the production of thymine from dihydrothymine. Thus, subsequent G-value calculations will be based on the amount of energy absorbed by the entire solution.

Figure 3 shows the variation of G-value with H_2SO_4 concentration in the range 0.02 to 9.0 M. The H_2SO_4 concentration is plotted logarithmically, giving a linear pH dependence for this plot. In experiments with dihydrothymine in pure aqueous solution, irradiation at 77°K with doses up to 25 MR gave no detectable amount of thymine.

Further evidence for the indirect action of radiation in the frozen solutions was obtained by investigating the dependence of the thymine formation on dihydrothymine concentration. The shape of the curve shown in Fig. 4 clearly demonstrates the indirect effect. Each point in this figure is based on a total dose-effect curve. In the sections that follow, experiments are described which give some information concerning the mechanism for the production of thymine by the indirect action of radiation.

UV Absorption Experiments at Low Temperatures

The above results show that large amounts of thymine are formed by indirect action when H_2SO_4 solutions of dihydrothymine are irradiated at 77°K. However, experiments in which the assay is carried out after melting cannot yield information as to whether thymine is formed during the exposure at 77°K or during the subsequent warming and melting of the samples. In order to determine which is the case,

low-temperature UV absorption measurements were carried out on glassy 5 M H_2SO_4 solutions.

Figure 5 shows the UV absorption spectra of irradiated frozen 5 M H_2SO_4 solutions with and without dihydrothymine present. The spectrum of the unirradiated frozen solution has been subtracted from all the spectra in Fig. 5 to correct for the absorbance of the sample cell and the dihydrothymine at short wavelengths. During irradiation, the solutions turn yellow and exhibit a rather uniform absorption throughout the UV region. Both the color and UV absorption start to disappear at about 160°K and have vanished completely at about 185°K (Fig. 5b). The spectra of the dihydrothymine solutions show clearly that no thymine is formed during the exposure at approximately 88°K. Furthermore, the spectra in Fig. 5(a) demonstrate that thymine is indeed formed in the frozen solutions upon warming.

It is possible to obtain from the UV absorption spectra an estimate of the thymine formation at the different temperatures. This may be expressed as percent of the final amount of thymine present after melting, according to the formula

$$\frac{A_{sol}(T) - A_S(T)}{A_{sol}(295°K)} \times 100,$$

where $A_{sol}(T)$ and $A_S(T)$ are the 265-nm absorptions of the dihydrothymine solution and the pure solvent, respectively, at temperature T, and $A_{sol}(295°K)$ is the absorption of the dihydrothymine solution after melting. The results for three different experiments using 5 M H_2SO_4 and 1 mg/ml dihydrothymine are presented in the right-hand portion of

Fig. 6. The three curves do not coincide exactly, probably due to small variations in the warming rate and errors in temperature measurement (the thermocouple was on the outside of the sample tube). However, the curves show that in these solutions thymine is formed in a rather narrow temperature range beginning at about 160° to 170°K and extending up to about 200°K. It should be emphasized that the samples turned polycrystalline at about 190°K in these experiments and no absorption measurements could be made between this temperature and the temperature at which the samples melted.

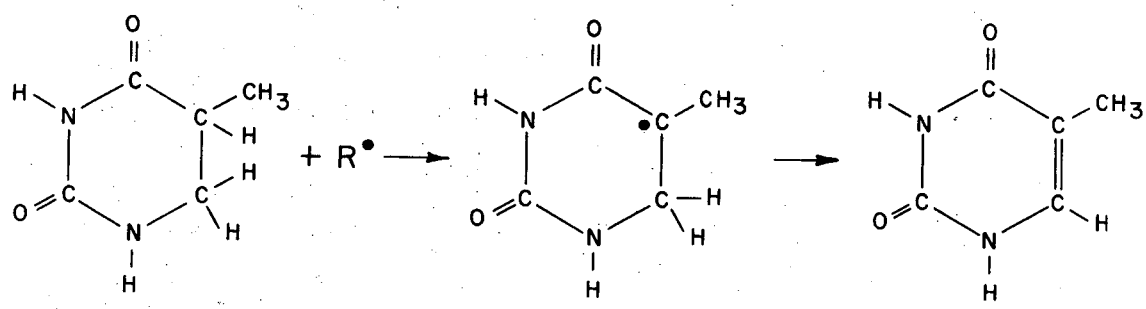
ESR Measurements

All the experiments presented above have concentrated on the formation of thymine as measured by its absorption at 265 nm. It is evident that a large indirect effect operates and that the secondary reactions take place between 77° and 200°K. In the radiation chemistry of pyrimidines it appears that the indirect action is mediated through free-radical reactions at the 5, 6 double bond (for a review see Ref. 5), but the intermediates are too transient to be observed by ESR without some trapping technique. However, in these experiments on frozen solutions free radicals are trapped and may therefore be studied by ESR.

Figure 7 shows the ESR spectra for a glassy 5 M H_2SO_4 solution containing 3 mg/ml dihydrothymine. Following irradiation at 77°K, the characteristic 506-G hyperfine doublet of hydrogen atoms is seen. The central part of the resonance, which here is entirely due to radicals induced in the solvent, has tentatively been ascribed to SO_4^- and SO_3^- radicals (7, 8), but so far no ESR evidence has been presented for this interpretation. Upon heat treatment the hydrogen lines disappear and

additional absorption lines are seen on either side of the central resonance. These lines were found to be due to the 5-thymyl radical, which has a characteristic eight-line hyperfine pattern spreading over approximately 140 G when randomly oriented. The outer components of this pattern are sufficiently separated from the central H_2SO_4 resonance that the formation of the 5-thymyl radical at different temperatures can be studied. In Fig. 7 (lower part) are presented spectra taken after heat treatments for 4 min at increasing temperatures. The sweep rate and the sensitivity are different from those for the top spectrum. In the first place, it can be concluded that very few if any 5-thymyl radicals are formed at 77°K. Secondly, the 5-thymyl radicals, which appear after heat treatment, are stable up to about 160°K, at which the remaining H_2SO_4 radicals have disappeared. Upon further heat treatment at 175°K, more than 90% of the 5-thymyl radicals have disappeared.

Some quantitative data for the appearance and disappearance of the 5-thymyl radicals are given in Figs. 6 and 8. For comparison, the disappearance of the hydrogen atoms is also given. It appears that the 5-thymyl radical disappears in the same temperature region where thymine is formed, suggesting that the radical is an intermediate in the sequence of events whereby dihydrothymine is converted into thymine in the irradiated frozen H_2SO_4 solutions. The sequence of events may therefore be



Here R· is a radical from the solvent. It is not clear whether some additional species are involved in the second reaction or whether it occurs spontaneously. If hydrogen atoms are released from the 5-thymyl radical when thymine is formed these could undergo combination reactions to produce H_2.

The data in Figs. 6 and 8 also suggest that the radical R· may in part consist of a hydrogen atom. Thus, a certain fraction of the 5-thymyl radicals (approximately 50% according to Fig. 8) are formed concomitantly with the disappearance of the hydrogen atoms. This indicates that hydrogen atoms may react with dihydrothymine when they become released from their trapping sites. This mechanism is supported by the results on neutral solutions. Thus, no hydrogen atoms and few if any 5-thymyl radicals were trapped when pure aqueous solutions were irradiated at 77°K and heat treated. Likewise, no thymine was produced even after 25-MR irradiation. The hydrogen atoms are in turn formed by reactions of H^+ ions with electrons when they become thermalized as proposed previously (7, 9). It should, however, be pointed out that the H atoms could not be the only precursor radicals for the 5-thymyl radical, since a large fraction of the latter is formed subsequent to the disappearance of the hydrogen atom doublet.

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FIGURE LEGENDS

Fig. 1. Dose-response curve for the production of thymine in frozen solutions of dihydrothymine (1 mg/ml) in H_2SO_4 (3 M). The samples were irradiated at 77°K, then melted for assay of thymine. The thymine concentration was determined by the absorbance at 265 nm. The results for the low-dose region are shown by the inset.

Fig. 2. Dose-response curves for the low-dose region for three different H_2SO_4 solutions containing 1 mg/ml dihydrothymine. Otherwise as in Fig. 1.

Fig. 3. Formation of thymine in H_2SO_4 solutions containing 1 mg/ml dihydrothymine. The yield of thymine (given as the G-value) has been calculated on the basis of the total energy absorbed by the solution. Each point is based on a full dose-response curve. Sample treatment is the same as for Fig. 1.

Fig. 4. Variation in the yield of thymine (given as the G-value) with dihydrothymine concentration in solutions containing 3 M H_2SO_4. Sample treatment is the same as for Fig. 1. Notations as for Fig. 3.

Fig. 5. UV absorption spectra taken at low temperatures on clear, glassy 5 M H_2SO_4 solutions (a) with and (b) without dihydrothymine (1 mg/ml). The samples were irradiated (0.2 MR) at 88°K and then observed before and during warming. The spectra are corrected for the absorbance of the respective unirradiated solutions at 88°K.

Fig. 6. The appearance and disappearance of some of the species induced in 5 M H_2SO_4 solutions of dihydrothymine versus the sample temperature. For the free-radical measurements (ordinate to the left), the samples were irradiated at 77°K and then stepwise heat treated for 4 min at the temperatures indicated. All observations were made at 77°K.

For the UV-absorption measurements of thymine (ordinate to the right) the samples were irradiated at 88°K and subsequently warmed at a rate of approximately 2.5°/min. The 265 nm absorption was measured at the temperatures indicated. All quantities in this figure are relative and given as % of the maximum value obtained for the particular species (i.e., the absolute values of H atoms and 5-thymyl radicals are different).

Fig. 7. Second-derivative ESR spectra of a glassy frozen solution of dihydrothymine (3 mg/ml) in 5 M H_2SO_4. The sample was irradiated (2 MR) at 77°K and all spectra were recorded at that temperature. After the first measurements (the two top spectra) the sample was heat treated for 4 min at temperatures which increased in steps of approximately 10°K. The spectra after heat treatments at 125°K and 165°K are presented here. The upper curve reveals the total resonance, including the H atom lines, before any heat treatment. The lower spectra have been recorded at a smaller sweep rate and an increased sensitivity in order to show the formation of the 5-thymyl radical. The arrows indicate the positions of the two outermost lines on each side of the 5-thymyl radical spectrum.

Fig. 8. Decay of hydrogen atoms and formation of 5-thymyl radicals at 105°K in a glassy 5 M H_2SO_4 solution of dihydrothymine. The sample was irradiated at 77°K and then heat treated for increasing lengths of time at 105°K. After each heat treatment the sample was brought back to 77°K and measured. As in Fig. 6 the data are given as % of the maximum concentration observed for the hydrogen atoms and the 5-thymyl radicals respectively.

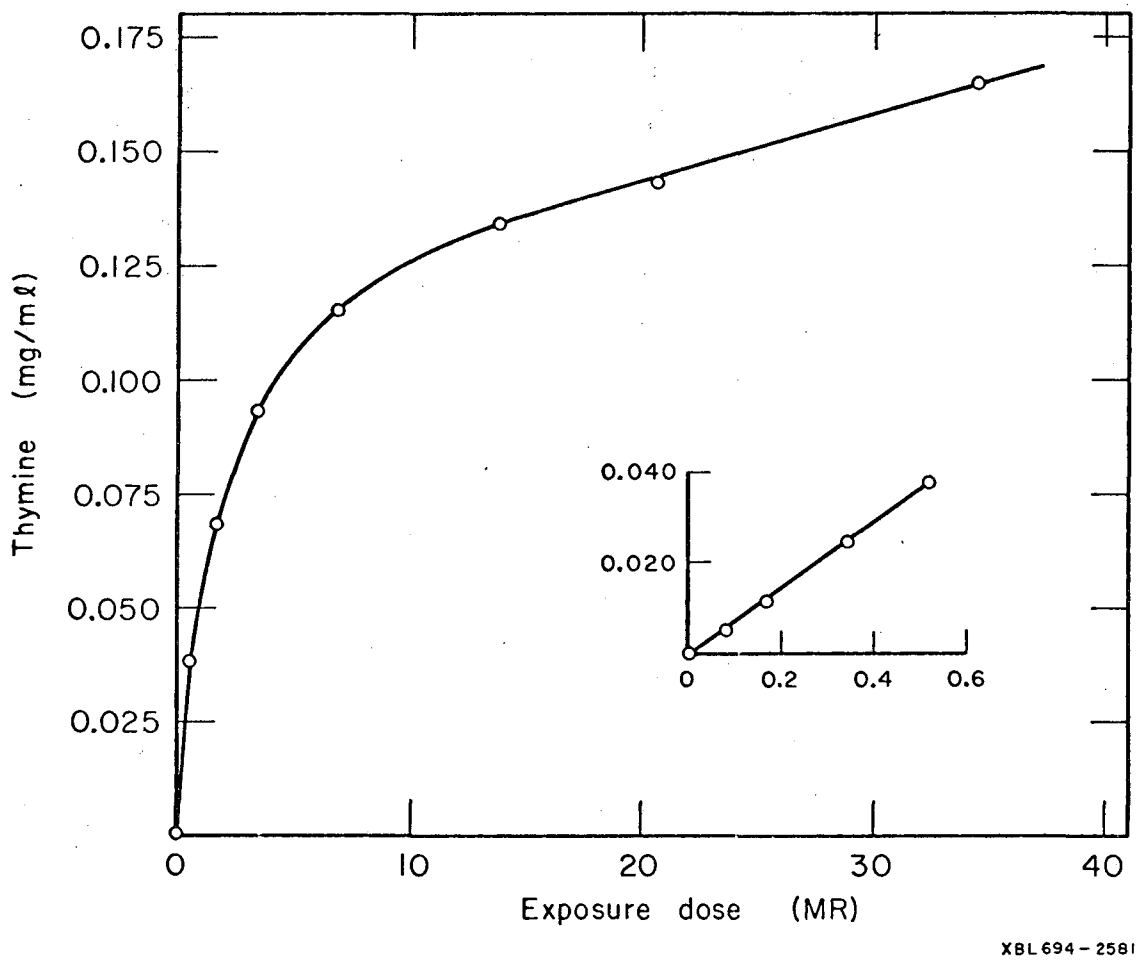
0.175
0.150
0.125
0.100
0.075
0.050
0.025
Thymine (mg/mℓ)
0
10
20
30
40
Exposure dose (MR)
0.040
0.020
0
0.2
0.4
0.6
XBL 694-2581

Fig. 1

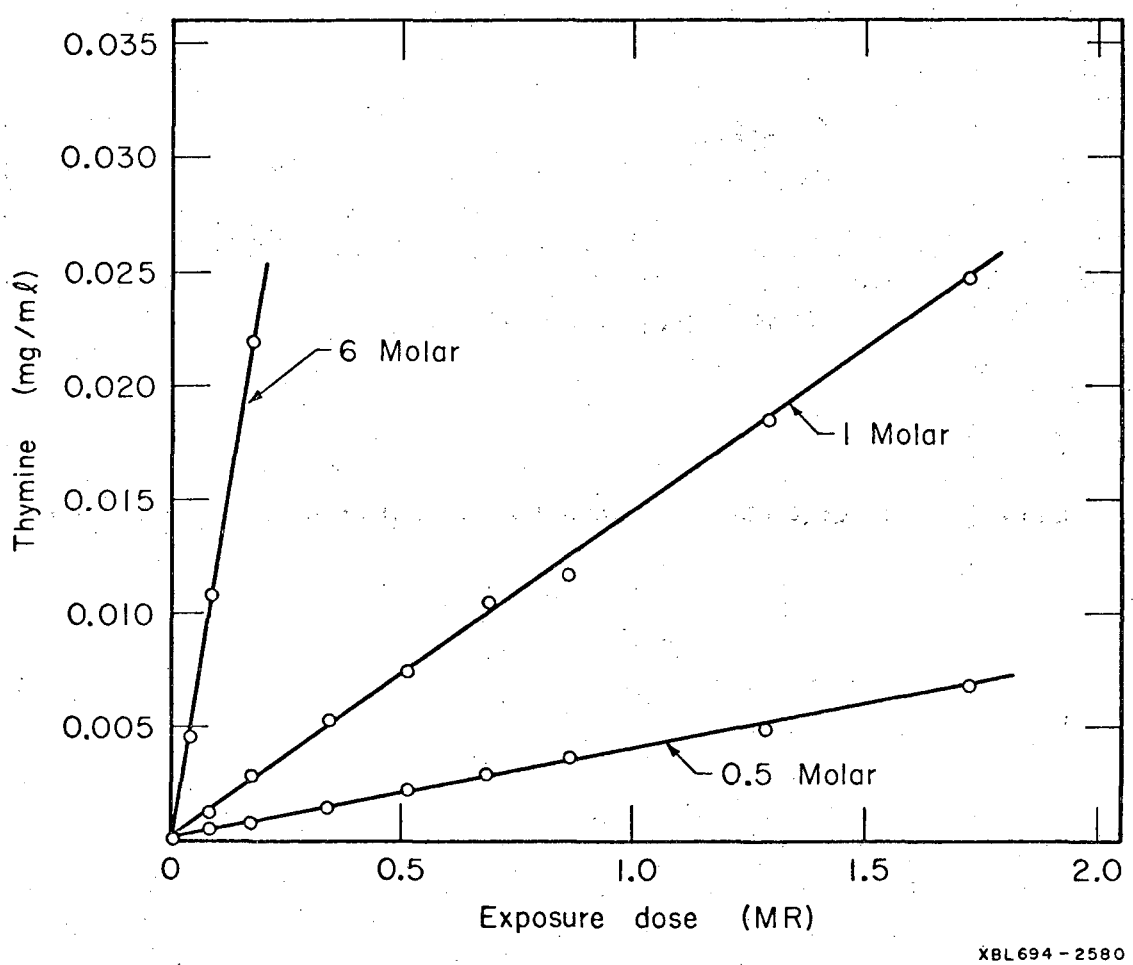
0.035
0.030
0.025
0.020
0.015
0.010
0.005
Thymine (mg/mℓ)
6 Molar
1 Molar
0.5 Molar
0
0.5
1.0
1.5
2.0
Exposure dose (MR)
XBL694-2580

Fig. 2

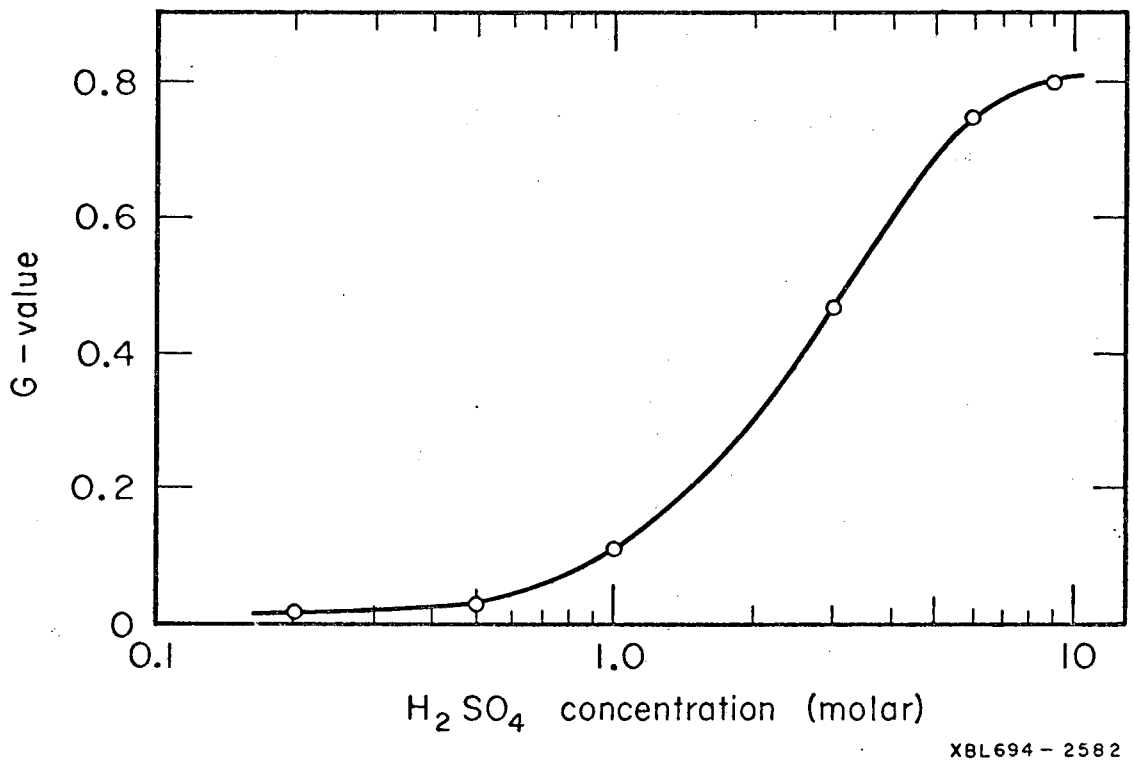
0.8
0.6
0.4
0.2
0
G - value
0.1
1.0
10
H_2SO_4 concentration (molar)
XBL694 - 2582

Fig. 3

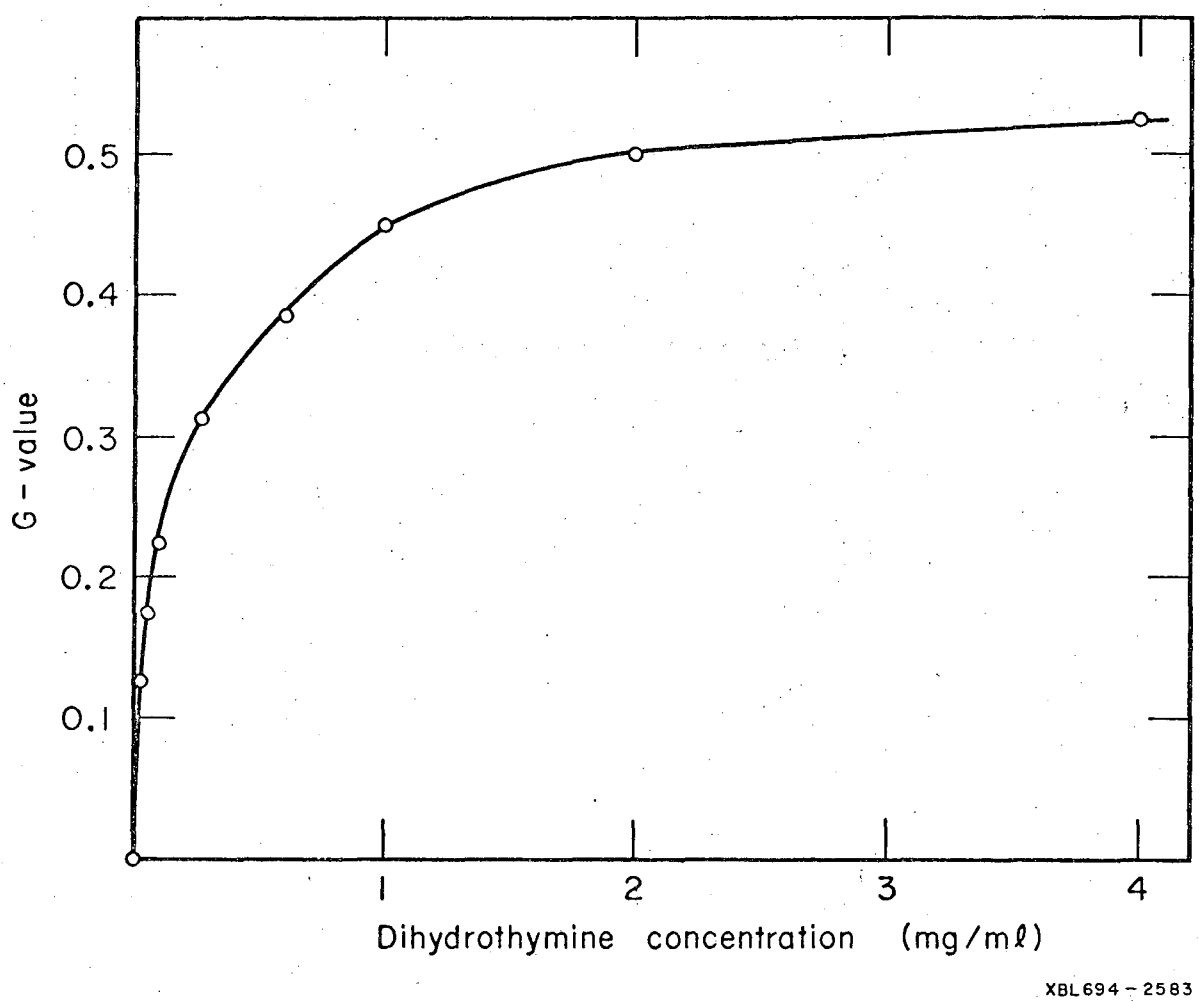
0.5
0.4
0.3
0.2
0.1
G - value
1
2
3
4
Dihydrothymine concentration (mg/mℓ)
XBL694-2583

Fig. 4

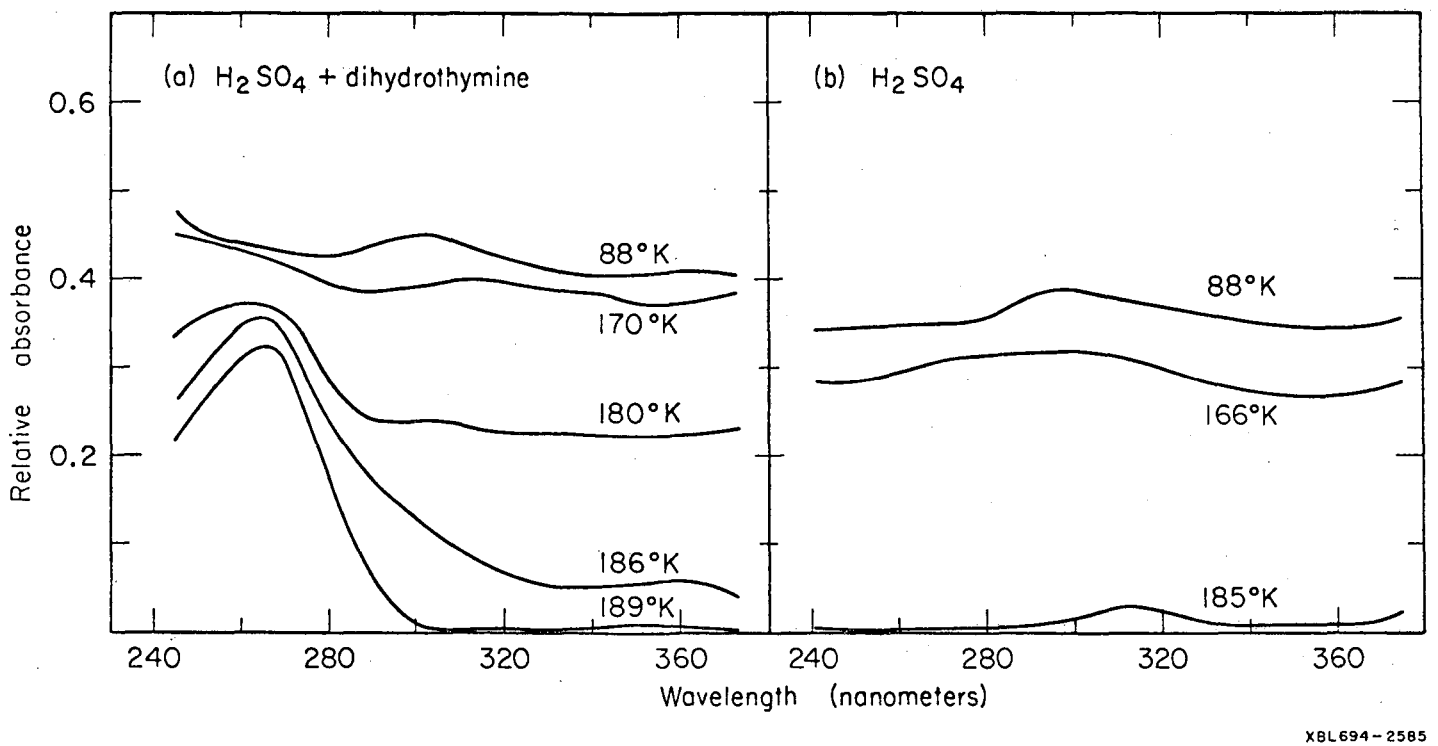
(a) H_2SO_4 + dihydrothymine
(b) H_2SO_4
Relative absorbance
0.6
0.4
0.2
88°K
170°K
180°K
186°K
189°K
88°K
166°K
185°K
240
280
320
360
240
280
320
360
Wavelength (nanometers)
XBL694-2585

Fig. 5

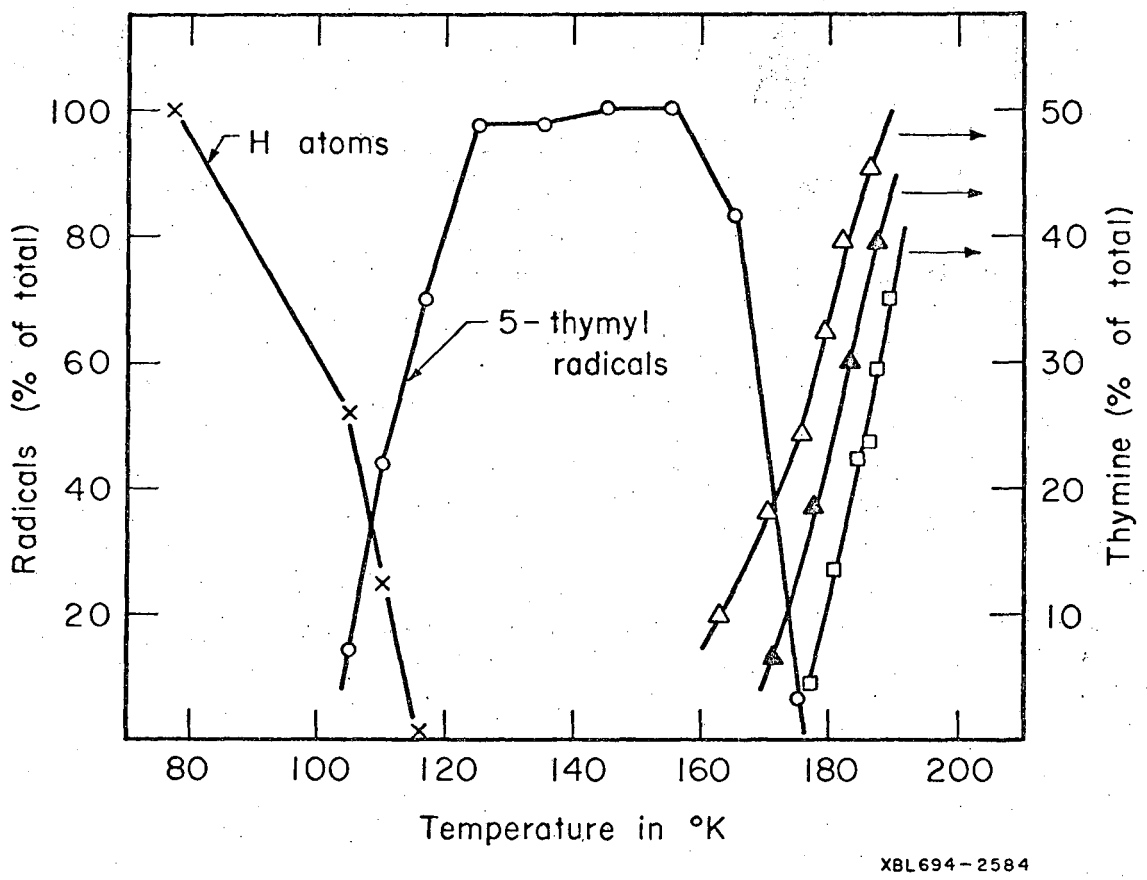
H atoms
5-thymyl radicals
Radicals (% of total)
Thymine (% of total)
Temperature in °K
100
80
60
40
20
50
40
30
20
10
80
100
120
140
160
180
200
XBL694-2584

Fig. 6

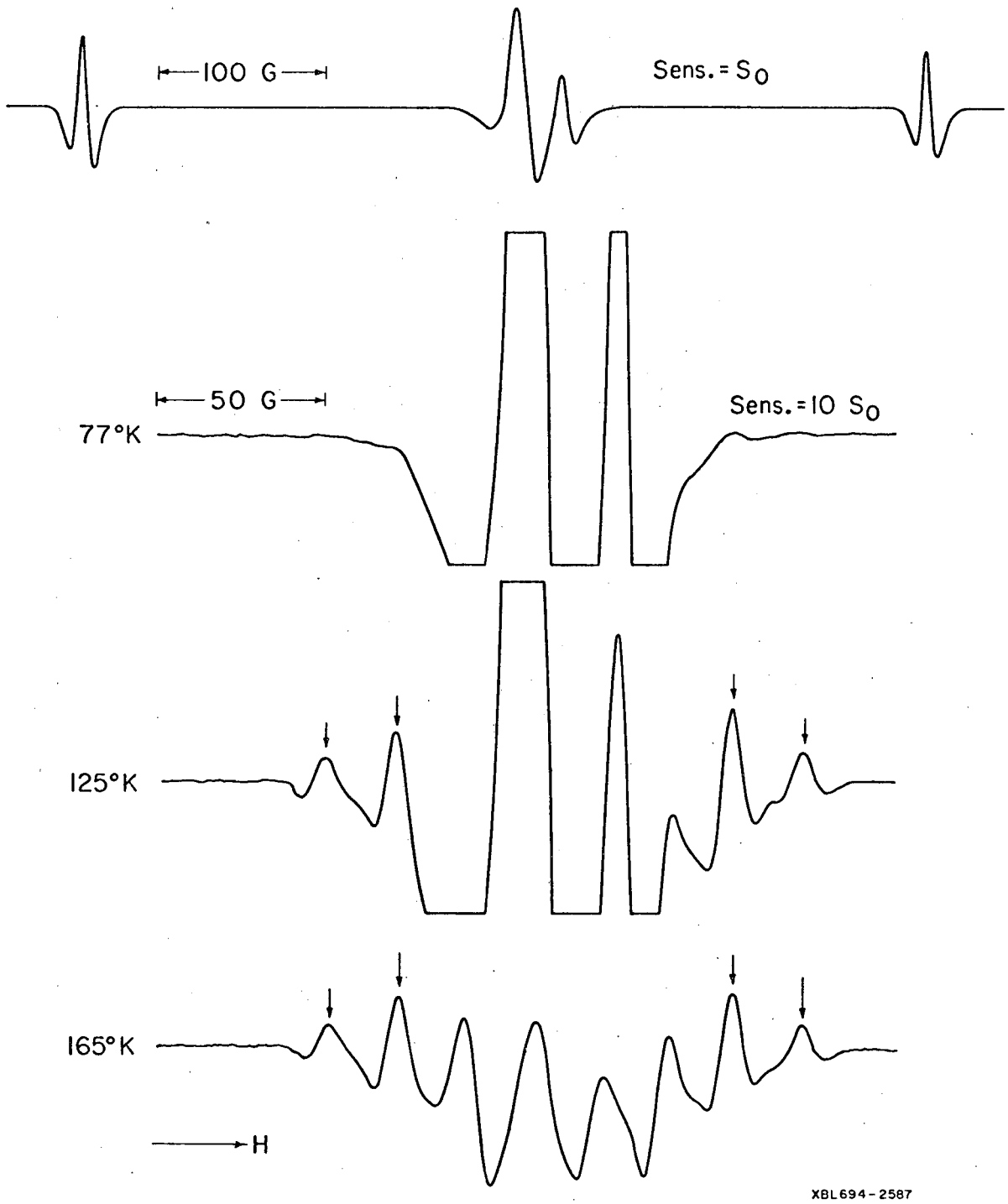

Fig. 7

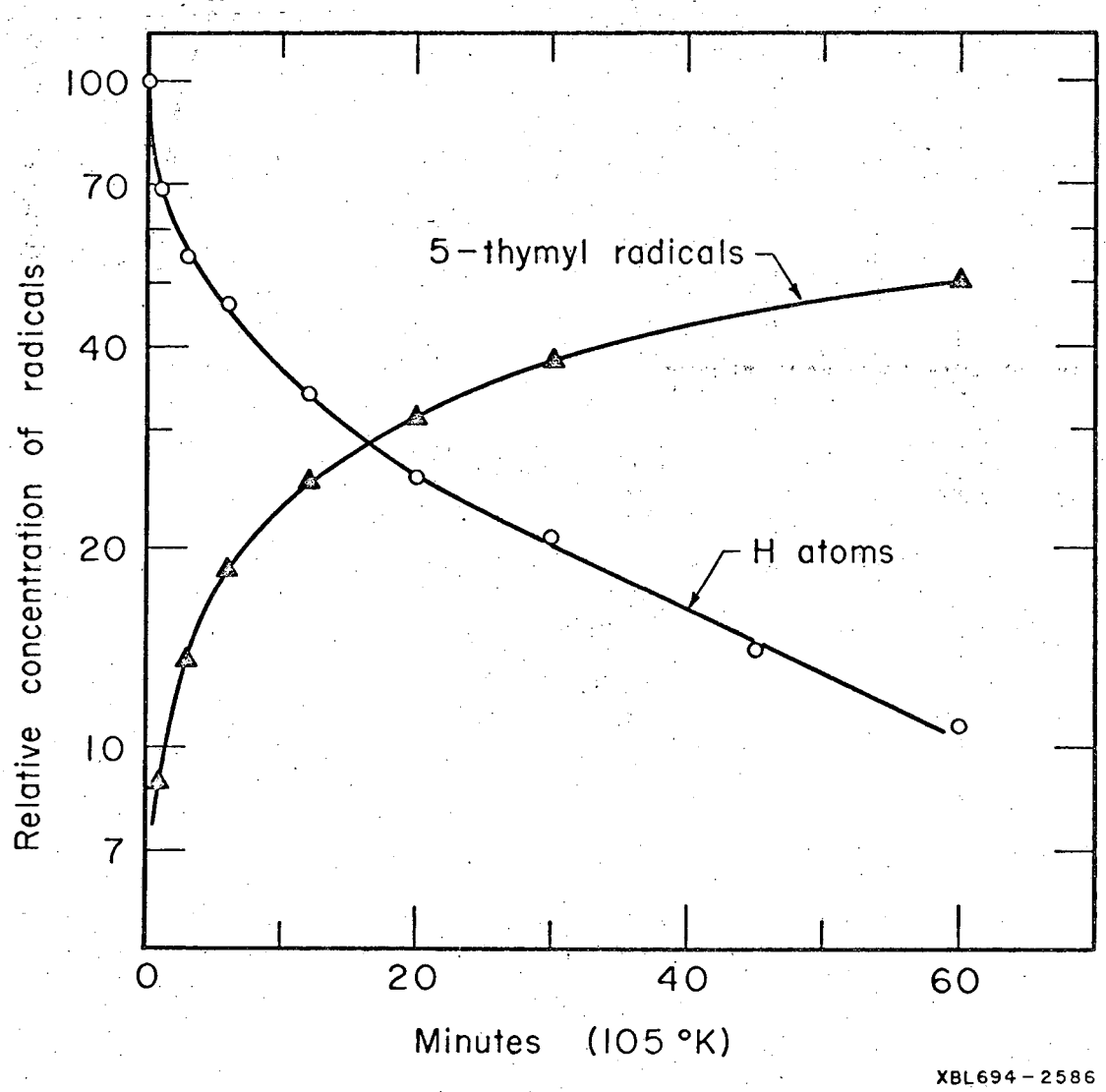
100
70
40
20
10
7
Relative concentration of radicals
5-thymyl radicals
H atoms
0
20
40
60
Minutes (105 °K)
XBL694-2586

Fig. 8

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