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Effect of Fe(III) Ions on the γ-Radiolysis of Thymine

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Aqueous solutions of thymine (T) in the presence of Fe(III) were irradiated under deaerated conditions at pH $\approx$2. The products of radiolysis were separated by two dimensional thin-layer chromatography. In the absence of any metal ion, the G(-T) is 2.6 but when Fe(III) is present during radiolysis, the G(-T) increases significantly and reaches a limiting value of 3.2. The yields of the hydroxylated products of thymine also increase in the presence of Fe(III). The results were explained by a mechanism which involves the interactions of Fe(III) with the transient adducts of thymine.

Use of transition metal ions and their complexes as radiosensitizers in the treatment of cancer cells by radiotherapy is of recent interest.[1-4] However, the mechanism of action of these compounds is not well-understood. Radiation chemical studies of the effects of these compounds on model target molecules would enable one to understand the role of these compounds in determining their radiosensitizing efficiency at the molecular level. Studies on the role of Cu(II) ions in increasing the radiosensitivity of thymine, a model DNA constituent has been reported recently.[5] It has been observed that Cu(II) ions are quite effective in increasing the radiosensitivity of thymine in dilute aqueous solution. Here an attempt has been made to study the effect of Fe(III) on the radiolysis of thymine in dilute aqueous solution at pH $\approx$2 using ^{60}Co γ-rays.

Experimental

Materials: Thymine (Koch Light) was used after recrystallization from triply distilled water. The labelled [2-^{14}C] thymine (1.98×10^9 Bq mmol^{-1}) was procured from BARC, Bombay. All the reagents and solvents used were of analytical reagent grade. Triply distilled water was used for preparing solutions for irradiation. Deaeration was carried out by bubbling pure argon gas for 30 minutes.

Irradiation: Irradiation was carried out with ^{60}Co γ-rays. The dose rate (12 Gy min^{-1}) was determined with the help of Fricke dosimeter, taking G(Fe^{3+}) as 15.6.

Analysis: Samples of 10^{-3} mol dm^{-3} thymine containing [2-^{14}C] thymine were irradiated in the presence and in the absence of Fe(III) ions. A known volume (100 µl) of the irradiated solution was spotted on precoated silica gel 60 F$_{254}$ plates (E. Merck). The products of radiolysis were separated by two-dimensional thin-layer chromatography with the two following solvent systems:[6] (I) lower phase of chloroform : methanol : water (4 : 2 : 1) to which was added 5% of methanol; and (2) ethyl acetate : 2-propanol : water (75 : 16 : 9). For better separation of the products the first dimension was eluted twice with solvent system 1.

The ^{14}C-labelled compounds were detected on thin-layer silica gel plates by autoradiography using X-ray sensitive films (Orwo, HS90). Detection of radioactivity was made after 15 days of exposure. The products under each radioactive spot in the chromatogram were identified by comparing the retention values (with respect to thymine) with those cited in the literature.[6,7] For quantitative analysis of the products, the silica gel at each spot was scraped off and suspended in 1 ml of water. The radioactivity was measured by ^{14}C β-scintillation counting using a liquid scintillation counter (LKB, 1211 Rackbeta). The yields of the products were calculated from the relative amount of radioactivity under the various product spots compared to that under the thymine spot in unirradiated control. The base decomposition yields, G(-T), were determined both spectrophotometrically by measuring the loss of absorption at 264 nm after separating the iron through a cation exchanger and by measurements of radioactivity of thymine spot in the plate. Fe(II) was also determined spectrophotometrically at 510 nm through its complexation with 1,10-phenanthroline.[8]

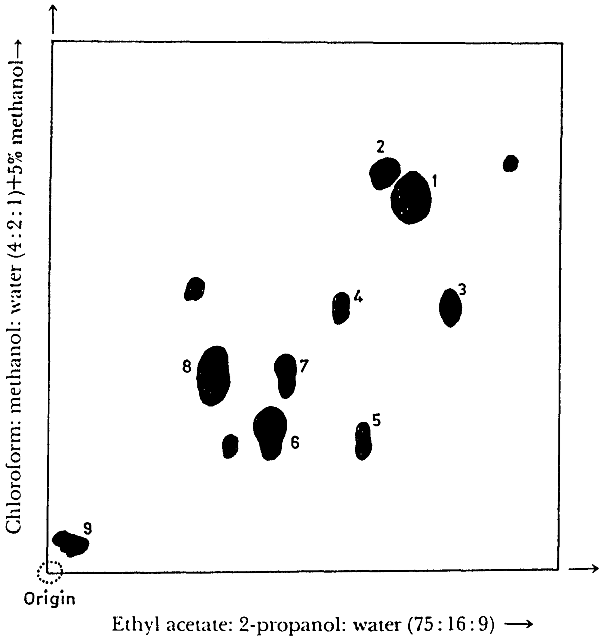


Fig. 1. Autoradiogram of the two-dimensional TLC separation on pre-coated silica gel 60 F$_{254}$ plates of the γ-radiolysis products of 10^{-3} mol dm^{-3} thymine in the presence of 5×10^{-4} mol dm^{-3} NH$_4$Fe(SO$_4$)$_2$ under argon saturated solution at pH$\approx$2. Absorbed dose=720 Gy. spot 1: thymine; spot 2: dihydrothymine; spot 3: 6-hydroxydihydrothymine; spot 4: 5-hydroxydihydrothymine; spot 5: *trans*-thymineglycol; spot 6: *cis*-thymineglycol; spot 7: 5-methylbarbituric acid; spot 8: 5-(hydroxymethyl)-uracil; and spot 9: dimeric products(?).

Results

The steady state γ-radiolysis of deaerated aqueous solution of 10^{-3} mol dm^{-3} thymine (T) containing 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$ leads to the formation of a number of modified nucleobases. These radiolysis products, however, have been separated by two-dimensional thin-layer chromatography on sillca-gel-precoated plates. A typical autoradiogram is shown in Fig. 1. Similar products were formed when the radiolysis is carried out in the absence of metal ions. Radiolytic yields of degradation of thymine at pH $\approx$2 were determined in the dose range 480 to 1800 Gy. Within this dose range the degradation of thymine is linear with dose as shown in Fig. 2. Hence the degradation yields of thymine, G(-T), were calculated from the slopes of the straight lines. The change in G(-T) values with increase in $NH_4Fe(SO_4)_2$ concentration was shown in Fig. 3. It is observed that with increase in [Fe(III)], the G(-T) value increases initially and reaches a steady value $\approx$3.2 at [Fe(III)]=4×10^{-4} mol dm^{-3}. The yields of the products could not be measured at low doses due to the large experimental errors in quantitative estimation of the products by thin-layer chromatography. However, they were measured in the dose range 720 Gy to 1800 Gy and is shown in Fig. 4. It is to be noted that upto the dose of 1450 Gy, the formations of all the products are

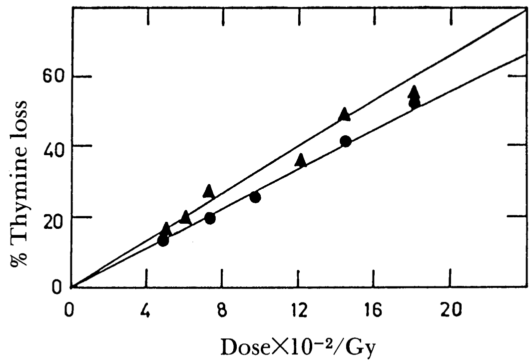


Fig. 2. Effect of absorbed dose on the degradation of thymine in the presence and absence of Fe(III) at PH$\approx$2. (●), in the absence of Fe(III) and (▲), in the presence of 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$.

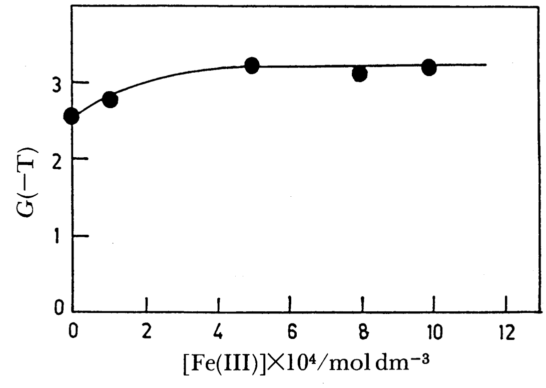


Fig. 3. Effect of Fe(III) on the G(−T) values in γ-radiolysis of 10^{-3} mol dm^{-3} thymine in deaerated solution at pH$\approx$2.

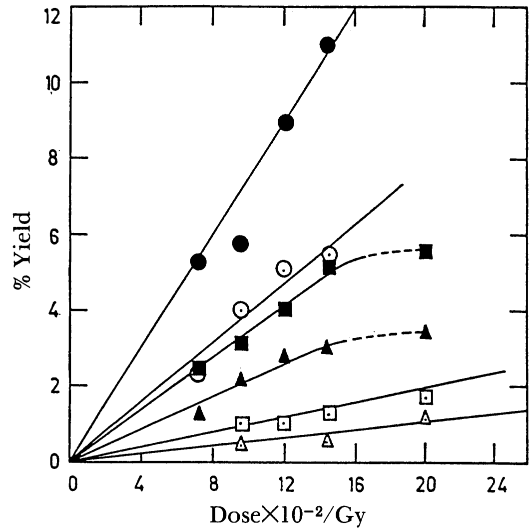


Fig. 4. Effect of absorbed dose on the formation of different base degradation products during γ-radiolysis of 10^{-3} mol dm^{-3} thymine in deaerated solutions at pH$\approx$2. Open symbols represent the yields in the absence of Fe(III) while the close symbols represent those in the presence of 5×10^{-4} mol dm^{-3} Fe(III). (circles): *cis*-thymineglycol, (triangles): 5-hydroxydihydrothymine, and (squares): 6-hydroxydihydrothymine.

Table 1. G-Values of Different Products in the γ-Radiolysis of 10^{-3} mol dm^{-3} Thymine in Argon Saturated Solution at pH$\approx$2.0 Determined in the Dose Range 700—1800 Gy

G(Products)	Experimental conditions		
	A	B	C
G(*cis*-Thymineglycol) (Spot 6)	0.40	0.72	0.0
G(*trans*-Thymineglycol) (Spot 5)	0.00	0.08	0.0
G(5-Methylbarbituric acid) (Spot 7)	0.24	0.44	0.0
G(5-(Hydroxymethyl)uracil) (Spot 8)	0.27	0.35	0.0
G(5-Hydroxydihydrothymine) (Spot 4)	0.05	0.20	0.0
G(6-Hydroxydihydrothymine) (Spot 3)	0.12	0.34	0.0
G(Dihydrothymine) (Spot 2)	0.38	0.40	0.3
G(Dimer) (Spot 9)	0.46	0.34	0.0
G(Unknown) (Spot 10)	—	—	0.3
G(−Thymine) (Spot 1)	2.6	3.2	0.6

A: in the absence of $NH_4Fe(SO_4)_2$; B: in the presence of 5×10^{-4}mol dm^{-3} $NH_4Fe(SO_4)_2$ and C: in the presence of 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$+0.4 mol dm^{-3} *t*-butyl alcohol.

linear with absorbed dose and the *G*-values of each product were calculated from the slopes of the respective straight lines. The results are tabulated in Table 1. The formation of Fe^{2+} was followed spectrophotometrically in the dose range of 6 to 600 Gy. Figure 5 shows the dependence of $[Fe^{2+}]$, formed in the radiolysis of 10^{-3} mol dm^{-3} thymine in the presence of 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$ in deaerated solutions at pH ≈2, on absorbed dose. It is found that in the very low dose range (e.g., 6 to 30 Gy), the formation of Fe^{2+} is much higher than that observed at higher absorbed doses. The initial *G* value for the formation of Fe^{2+}, $G(Fe^{2+})$ was determined from the initial slope and was found to be ≈3.6.

Discussion

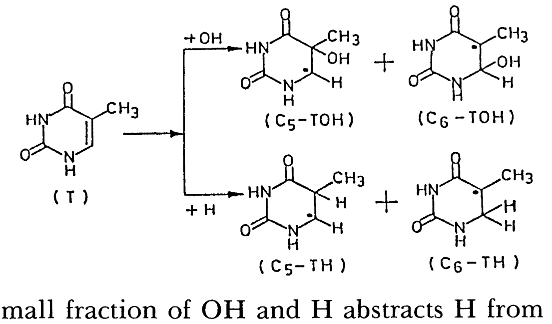
During γ-radiolysis in dilute aqueous solutions, the reactions are initiated by water-borne radicals, e.g., H, OH, and e_{aq}^-.[9)]

$$H_2O \rightsquigarrow\!\!\longrightarrow H,\ OH,\ e_{aq}^-,\ H_2O_2,\ H_2. \qquad (1)$$

In acidic pH ≈2, the e_{aq}^- is converted to H

$$e_{aq}^- + H^+ \longrightarrow H. \qquad (2)$$

Hence when thymine is radiolyzed in deaerated medium at this acidic pH range, the radicals present, e.g., H and OH react with thymine to give respective adducts. It is well-known that both these primary radical species add on C_5—C_6 double bond of thymine to give C_5 and C_6 adducts of OH and H.[10,11)]

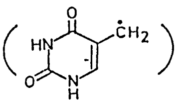


(3)

(4)

A small fraction of OH and H abstracts H from C_5-methyl of thymine giving UCH_2.

$$T + H/OH \longrightarrow U\dot{C}H_2 \qquad (5)$$



In the absence of any additive, the *G*(-T) was ≈2.6 (Table 1,A) which is much less in comparison with the total yield of the primary radicals (G_{OH} ≈2.7, G_H ≈3.4).[9)] This indicates that the transient species TOH and TH undergo disproportionation to give parent thymine back.[11)] However, under the similar conditions when Fe(III) is present during radiolysis, the *G*(-T) increases significantly and reached a limiting value of ≈3.2 (Fig. 3). It is also evident from the Fig. 4 and Table 1, that in the presence of Fe(III), the hydroxylated products of thymine (products at the spots 3 to 8) arising from the oxidation of thymine increase in comparison with that formed in the absence of Fe(III). From a consideration of the rate constant data,[12)] it is evident that under our experimental conditions, the OH and H radicals would react with thymine to give TOH, TH and UCH_2 through reactions (3)—(5). Hence the increase in the *G*(-T) as well as in the *G*-values of the hydroxylated products arise from the interaction of the Fe(III) ions with TH and TOH. It is well-documented[11,13)] that the electron affinic compounds oxidize the pyrimidine adducts of H and OH through electron-transfer reactions. Fe(III) being highly oxidizing in nature, it might be expected that it would also oxidize the transient TOH and TH radicals.

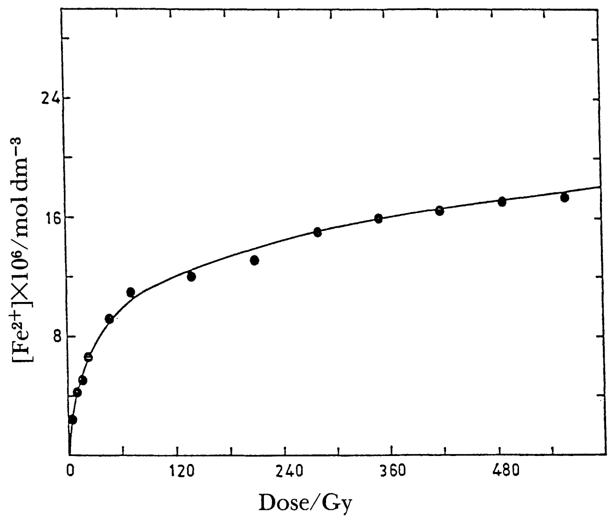
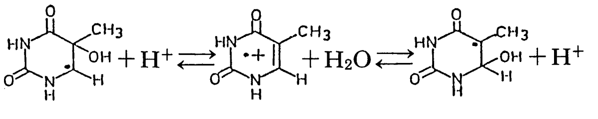


Fig. 5. Formation of Fe^{2+} species with absorbed dose in the radiolysis of 10^{-3} mol dm^{-3} $NH_4Fe(SO_4)_2$ in deaerated solution at pH≈2.

$$TOH + Fe(III) \longrightarrow TOH^+ + Fe(II) \qquad (6)$$

$$TH + Fe(III) \longrightarrow TH^+ + Fe(II) \qquad (7)$$

Under the conditions, the formation of Fe^{2+} was followed and from the observed $G(Fe^{2+})$ ≈3.6 calculated from the initial slope of the yield/dose plot (Fig. 5) is much less in comparison with the total yields of TH and TOH radicals. The results suggest that a part of TH and TOH does not reduce Fe(III) but undergoes such reactions which are taking place in the absence of metal ions. In this context it is worthful to consider the redox behavior of the TH and TOH radicals. The relative steady state abundance of the C_5-TOH and C_6-TOH was reported to be 60% and 30% of total TOH.[11)] Among these two types of TOH radicals, the C_5-TOH is reducing while the other is oxidizing in nature.[10,11)] However, the two OH adducts of thymine undergo acid-catalyzed isomerisation (reaction 8).[11)]



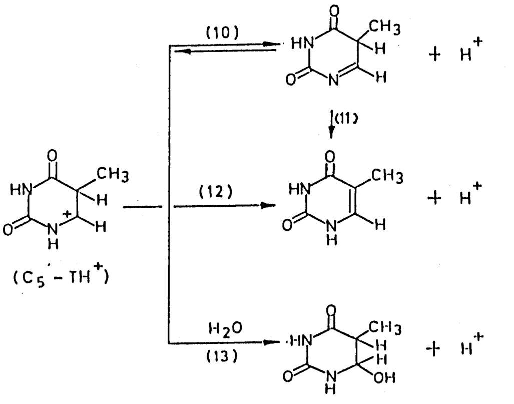
(8)

Thus the TOH radicals can be oxidized or reduced depending upon their reaction partner. Hence it may be presumed that in the presence of strong oxidizing agent, Fe(III), the oxidizing C_6-TOH will be isomerised to reducing C_5-TOH and thereby increase the steady state concentration of the reducing C_5-TOH radical. On the other hand, due to the presence of methyl group in C_5 position of thymine, major portion (≈50%) of H atom adds on C_6 position of thymine giving C_6-TH which is in no way reducing.[11] Only 37% of H atom gives rise to reducing C_5-TH radical and this C_5-TH reduces Fe(III) to Fe^{2+}. However, the weakly reducing UCH_2 radical arising from H abstraction from C_5-methyl group of thymine by OH and H radicals may reduce strongly oxidizing Fe(III) ions.

$$UCH_2 + Fe(III) \longrightarrow UCH_2^+ + Fe(II) \qquad (9)$$

Hence, the yield of $G(Fe^{2+})$ should correspond to $G(TOH)+G(C_5\text{-}TH)+G(UCH_2)$. Under the experimental conditions of pH ≈2, taking[9] G_{OH} ≈2.7 and G_H ≈3.4, the relative yields of TOH, C_5-TH, and UCH_2 were calculated from the fractions of OH and H giving rise to these above radical species. Thus the respective values of G(TOH), $G(C_5\text{-}TH)$, and $G(UCH_2)$ become[11] as: $G(TOH)=90\%$ of $G_{OH}=0.9\times2.7\approx2.4$, $G(C_5\text{-}TH)=37\%$ of $G_H=0.37\times3.4\approx1.3$ and $G(UCH_2)=10\%$ of $G_{OH}+3.5\%$ of $G_H=0.1\times2.7+0.035\times3.4\approx3.4$; and therefore $G(Fe^{2+})$ should correspond to $\approx2.4+1.3+0.4=4.1$ and the observed $G(Fe^{2+})$ is not far from this expected value.

The TH^+ formed in reaction (7) deprotonates to give isothymine which undergoes spontaneous rearrangement to give thymine. However, when proton concentration increases, a small amount of 6-hydroxydihydrothymine is formed.[11]



That the TH^+ gives back thymine through reactions (10—12) is also evident from the results of the γ-radiolysis of dihydrothymine (TH_2) in the presence of Fe(III) ions at pH≈2, where both H and OH abstract H from dihydrothymine (TH_2) with the formation of TH radicals.[14]

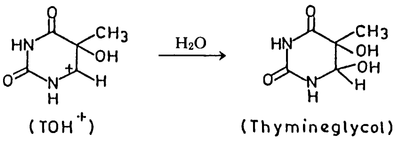
$$TH_2 + H/OH \longrightarrow TH + H_2/H_2O \qquad (14)$$

By irradiating 10^{-3} mol dm^{-3} dihydrothymine in the presence of 5×10^{-4} mol dm^{-3} Fe(III) ions, the observed yields were found to be $G(Fe^{2+})=5.2$ and $G(+T)\approx5.2$. This indicates that the TH radical quantitatively transfers electron to Fe(III) and the resulting TH^+ gives back thymine. That an appreciable fraction of TH e.g., the C_6-TH does not reduce Fe(III) but undergoes disproportionation reaction to give dihydrothymine is evident by carrying out the radiolysis in the presence of 0.4 mol dm^{-3} *t*-butyl alcohol. Figure 6 shows the autoradiogram of two-dimensional TLC separation of products formed in the radiolysis of 10^{-3} mol dm^{-3} thymine in the presence of 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$ and 0.4 mol dm^{-3} *t*-butyl alcohol. It is observed under these conditions that only dihydrothymine (Spot 2) and an unknown product (Spot 10) have been formed (Table 1). Here due to the presence of 0.4 mol dm^{-3} *t*-butyl alcohol all the OH radicals and a part of H atoms would be scavenged[12] leaving only a part of H atoms which would undergo reaction (4) to give TH. Then the dihydrothymine is formed by the disproportionation of the C_6-TH radicals

$$2TH \longrightarrow T + TH_2. \qquad (15)$$

Formation of dihydrothymine clearly indicates that a fraction of TH does not reduce Fe(III) or otherwise dihydrothymine would not have been formed.

Now, from the above mentioned discussion it appears that the TOH radicals reduce Fe(III) (reaction



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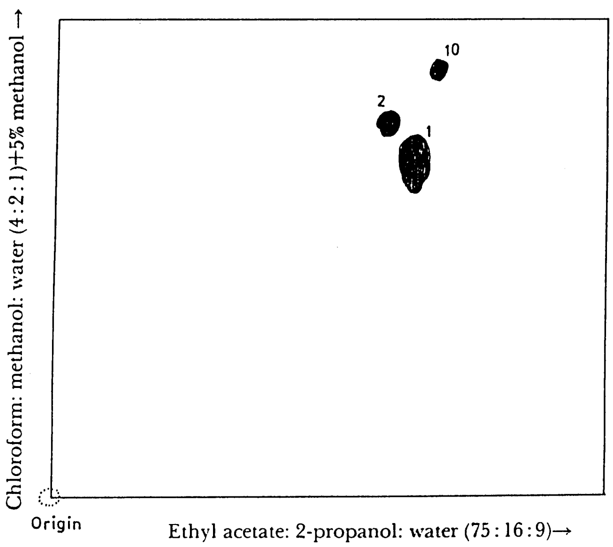
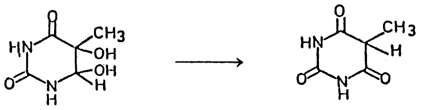


Fig. 6. Autoradiogram of the two-dimensional TLC separation on precoated silica-gel plates of the γ-radiolysis products of 10^{-3} mol dm^{-3} thymine in the presence of 5×10^{-4} mol dm^{-3} $NH_4Fe(SO_4)_2$ and 0.4 mol dm^{-3} *t*-butyl alcohol under argon saturated solution at pH≈2. Absorbed dose=720 Gy. Spot 1: thymine; spot 2: dihydrothymine and spot 10: unknown product.

6). However, the TOH^+ would react with water giving thymineglycol (reaction 16).

The thymineglycol would be converted to 5-methylbarbituric acid.

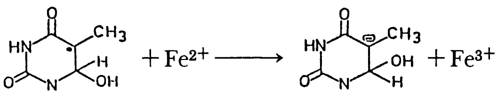


(17)

The carbonium ion UCH_2^+ would give rise to 5-(hydroxymethyl)uracil,

$$UCH_2^+ \xrightarrow{H_2O} UCH_2OH. \tag{18}$$

Hence the major amount of the thymine oxidation products resulted from the TOH radicals via electron transfer to the Fe(III). It is to be noted here that due to experimental difficulty in estimating small amounts of products, the product estimation was done by irradiating thymine at appreciably higher doses. Figure 5 shows that though the initial yield of Fe^{2+} is much higher, it rapidly falls when the absorbed dose exceeds ≈90Gy. At this higher doses, secondary reactions of initially formed Fe^{2+} must be considered. When appreciable amount of Fe^{2+} is accumulated in the system, the C_6-TOH radical which is oxidizing[11] might react with Fe^{2+} giving $C_6\text{-}TOH^-$, which would react with water to give 6-hydroxydihydrothymine



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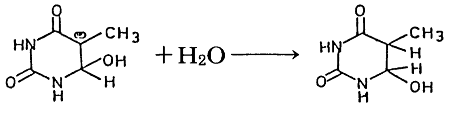

(20)

or would combine with TOH^+ to give the dimeric products.

$$TOH^- + TOH^+ \longrightarrow TOH\text{-}TOH \tag{21}$$

Thus the above discussion indicates how the identity of the observed products can be readily explained on the basis of the reaction of Fe(III) with the transient adducts derived from thymine. Moreover, since the overall degradation of thymine is increased by the presence of Fe(III), use of Fe(III) in a suitable environment may be probed with regard to its beneficial role as a radiosensitizer.

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