Radiation-Induced Degradation of Purine and Pyrimidine 2'-Deoxyribonucleosides in Aqueous KBr Solutions

J. Cadet*, L. Voituriez*, M. Berger*, and L. S. Myers (Jr)*

a Laboratoires de Chimie, Département de Recherche Fondamentale Centre d'Études Nucléaires de Grenoble 85 X, 38041 Grenoble Cedex, France
b Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, USA


2'-Deoxyribonucleosides, Inorganic Radical, γ-Irradiation, Radical Reactions, Thymidine Oxidation

Steady-state γ-radiolysis of 5 × 10⁻⁴ M pyrimidine and purine 2'-deoxyribonucleosides in aqueous solutions saturated with N₂, N₂O and O₂, respectively, have been carried out in the presence of 0.1 M KBr. The main final degradation products have been isolated and characterised by various spectroscopic measurements including ¹H and ¹³C NMR, UV, C.D. and mass spectrometry. The radiation-induced decomposition of thymidine is mostly accounted for by an ionic mechanism involving Br₂, the decay product of Br₂⁻, as the reactive oxidizing specie. On the other hand the degradation of the purine ring of 2'-deoxyadenosine and 2'-deoxyguanosine may be accounted for by the action of Br₂⁻ or Br₂⁻⁻.

Introduction

Bromide anions have been shown to sensitize various microorganisms including B. megaterium spores [1], bacteriophage T7 [1], E. coli [2] and S. faecalis [3] bacterial cells to the lethal action of ionising radiations. The inorganic anion radical Br₂⁻ which is produced by the reaction of radiation-induced hydroxyl radical with Br⁻ [4] shows milder oxidizing ability than the very reactive OH· precursor. As a result a higher selectivity in the reaction of the secondary radical Br₂⁻ or other related inorganic radicals such as (CN)₂⁻, CO₂⁻, SO₂⁻ and Cl₂⁻ with biomolecules [5, 8] may be expected. Br₂⁻ was reported to inactivate various proteins [6] and enzymes [9]. It was shown that this anion radical was likely to react with only a few amino acids on the basis of pulse radiolysis experiments [6]. Another important target to be considered for the biological effects of ionising radiations is DNA. The reactivity of Br₂⁻ with most of the purine and pyrimidine nucleic acid constituents investigated appears at the rather low [1]. The rate constant for the reaction of 2'-deoxyguanosine-5'-monophosphate with Br₂⁻ was found to be 4 × 10⁻¹ mol⁻¹ s⁻¹. Moreover, preliminary steady-state irradiation investigations have shown that the chromophore of uracil and thymine was degraded to a significant extend by the action of Br₂⁻ or its decay products [1, 2]. However, the exact mechanisms of these reactions were not elucidated and remain open to debate.

This paper describes the results of steady-state γ-radiolysis experiments of 5 × 10⁻⁴ M purine and pyrimidine 2'-deoxyribonucleoside aqueous solutions in the presence of 0.1 M KBr. The main final degradation products of thymidine, 2'-deoxyadenosine and 2'-deoxyguanosine have been isolated by thin-layer chromatography (TLC) and/or high-performance liquid chromatography (HPLC) and characterised by various spectroscopic measurements. The effect of three saturating gases, N₂, O₂ or N₂O on the radiation-induced degradation processes of the nucleosides has been investigated as it was previously shown that the nature of the gas played a major role in KBr sensitization at the cellular level. Radical or/and ionic mechanisms are involved in the decomposition of the aglycone of the purine and pyrimidine 2'-deoxyribonucleosides. The inorganic radical Br₂⁻ appears to degrade selectively 2'-deoxyadenosine and 2'-deoxyguanosine.

Experimental

Chemicals

Thymidine (dThd), 2'-deoxyadenosine (dAdo), 2-D-erythro pentose and cysteine were from Sigma Chemical Company and were used without further purification. 2'-deoxyguanosine (dGuo) was purchased from Boehringer, Mannheim. Analytical grade KBr was from Merck. [¹⁴CH₃] thymidine was obtained from Département des Radiocélements, Commissariat à l'Énergie Atomique, France, and was purified prior to use by high performance liquid
chromatography on a Whatman octadeclysilil sili-
cagel ODS-3 column (25 cm x 0.47 cm I.D.) with
water-methanol (9:1 V/V) as the isocratic eluent.
The various diastereoisomers of trans 5-bromo-6-
hydroxy-5,6-dihydrothymidine [11], cis and trans
5,6-dihydroxy-5,6-dihydrothymidine [12], 5-hy-
droxy-5,6-dihydrothymidine [13], cis and trans
6-hydroxy-5,6-dihydrothymidine [14] and 5,6-di-
hydrothymidine [15] were prepared according to
literature procedures. N-(2-deoxy-\beta-D-erythro-
furanosyl)formamide [16] was synthesised by peri-
dricular oxidation of the cis diastereoisomers of
5,6-dihydroxy-5,6-dihydrothymidine.

Thin-layer chromatography
Two-dimensional thin-layer chromatographic analyses of the radiation-induced nucleoside degra-
dation products were carried out on Merck F254
precoated silicagel plates using solvent system 1:
lower phase of chloroform-methanol-water [4:2:1]
to which were added 5% of methanol and eluent 2:
ethyl acetate-2-propanol-water (75:16:9) as the
developers. Detection of UV absorbing compounds
around 260 nm was made by fluorescence quenching
with a 254 nm Desaga mineral lamp. 2'-Deoxy-
ribonucleosides were visualised as pink spots by
spraying the chromatoplates with the cysteine-
sulfuric acid reagent and subsequent heating at
100 °C for two minutes [17]. [14C] radiolabeled
compounds were located on the developed plates
by autoradiography using Kodak N5-2T X-ray
film. Overnight exposure allowed detection of radio-
activity as low as 0.01 μCi per cm². After location
the various radioactive compounds were scraped
from the silicagel plates and quantitated by β
scintillation counting using Packard model 2425
TriCarb spectrometer.

High-performance liquid chromatography
High performance liquid chromatography (HPLC)
was carried out with a Waters liquid chromatograph
consisting of a Model M 6000 dual pump and the
U6K universal injector. Eluents were made either
at 220 nm or at 260 nm using Cecil Model 212
variable wavelength detector. Bi-distilled water was
used for the mobile phase.

Electronic spectra were recorded on a Beckman
Model UV Spectrophotometer. Infrared spectra
were obtained from micro KBr pellets on a Perkin-
Elmer Model 257 spectrometer. 250 MHz 1H nuclear
magnetic resonance (NMR) spectra were recorded
either in the continuous wave or in the Fourier
transform mode using a Cameca TSN 250 apparatus.
13C NMR spectra were registered on the same
spectrometer operating at 62.87 MHz. Calibration
of the spectra in D2O was made with internal 3-(trinemethylsilyl)propionate-2,2,3,3-d4 (TSP). Elec-
tron-impact (EI) and fast atom bombardment
(FAB) mass spectra were obtained on a Model
MS50 AEI spectrometer.

Irradiation procedures
Steady-state radiolysis experiments were performed
in a 60Co-source with a dose rate of 80 Gy
mm⁻². Solutions were prepared with bi-distilled
water and were bubbled either by N2, O2 or N2O
for 15 min prior the irradiations. The flow of gas
was maintained during the steady-state γ-irradia-
tions.

Results and Discussion
1) Gamma radiolysis of thymidine aqueous solutions
in the absence of KBr
Aqueous solutions of 5 × 10⁻⁴ M dThd
saturated with either N2, O2 or N2O were exposed
to the γ-rays of 60Co in the absence of KBr. The
main purpose of these experiments was to further
facilitate the comparison of the mode of action of
Br²⁻, or its decay product with those of water
radioisolation species i.e. OH·, H atoms and solvated
electrons on thymidine in aqueous solutions. The
G values for the formation of the radiation-induced
derivatives have been previously isolated and
described in the three different experiments
described here.

O2 and N2 saturated aqueous solutions
The decomposition of 0.5 mM thymidine induced
by gamma rays in oxygenated aqueous solution is
quite comparable to those observed previously in
more concentrated solution [19]. The formation of
N-(2-deoxy-\beta-D-erythro-pentofuranosyl) formamide
16] and of the various diastereoisomers of 5,6-di-
hydroxy-5,6-dihydrothymidine results from the
hydrolytic degradation of rather unstable transient
thymidine hydroxyhydroperoxides [20]. The release
of free thymine and related radiation-induced
derivatives may be accounted for by initial hy-
drogen abstraction by hydroxyl radicals at various
carbons of the osidic moiety [21].

In the absence of oxygen, the bulk of the water
radiolysis products react with thymidine and par-
Table I. G values of the radiation-induced\(^a\) degradation products of 5 \(\times\) 10\(^{-4}\) M thymidine\(^b\) in various aqueous solutions\(^c\).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(O_2)</th>
<th>(N_2)</th>
<th>(N_2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-cis((5S, 6R))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.26</td>
<td>0.12</td>
<td>0.85</td>
</tr>
<tr>
<td>(−)-cis((5R, 6S))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.21</td>
<td>0.11</td>
<td>0.92</td>
</tr>
<tr>
<td>(−)-trans((5S, 6S))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.25</td>
<td>−</td>
<td>0.12</td>
</tr>
<tr>
<td>(+)-trans((5R, 6R))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.24</td>
<td>−</td>
<td>0.10</td>
</tr>
<tr>
<td>(5R) and (5S) 5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>−</td>
<td>0.12</td>
<td>−</td>
</tr>
<tr>
<td>(5R, 6R) and (5S, 6R) 6-Hydroxy-5,6-dihydrotimidine</td>
<td>−</td>
<td>0.06</td>
<td>−</td>
</tr>
<tr>
<td>(5S, 6S) and (5R, 6S) 6-Hydroxy-5,6-dihydrotimidine</td>
<td>−</td>
<td>0.05</td>
<td>−</td>
</tr>
<tr>
<td>(5R) and (5S) 5,6-Dihydrotimidine</td>
<td>−</td>
<td>0.16</td>
<td>−</td>
</tr>
<tr>
<td>N(2-Deoxy-β-D-erythro pentofuranosyl) formamide</td>
<td>0.22</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(5R)(2-Deoxy-β-D-erythro pentofuranosyl)-5-hydroxy-5-methyl hydantoin</td>
<td>0.06</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(5S)(2-Deoxy-β-D-erythro pentofuranosyl)-5-hydroxy-5-methyl hydantoin</td>
<td>0.07</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5-Hydroxymethyl-2'-deoxyuridine</td>
<td>0.11</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Thymidine dimers (non cyclobutane type)</td>
<td>−</td>
<td>0.58</td>
<td>1.05</td>
</tr>
<tr>
<td>Thymidine(^d)</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>cis((5R, 5S))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.17</td>
<td>0.11</td>
<td>0.89</td>
</tr>
<tr>
<td>trans((5R, 5S))-5,6-Dihydroxy-5,6-dihydrotimidine</td>
<td>0.16</td>
<td>0.07</td>
<td>0.19</td>
</tr>
<tr>
<td>5,6-Dihydrotimidine</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5-Hydroxy-5-methyl barbituric acid)</td>
<td>0.29</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5-Hydroxy-5-methyl hydantoin }</td>
<td>0.04</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>N-Acetyl urea</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

\(^a\) Exposure to gamma rays from \(^{60}\)Co for 20 min (except in \(N_2O\) solutions for which exposure was reduced to 10 min) at a dose rate of 80 Gy/min; \(^b\) quantitation was made by scintillation counting of the various (\(^14\)C) radioactive compounds which were separated by two-dimensional thin layer chromatography; \(^c\) the solutions were equilibrated with the various gases for 15 min prior the irradiations; \(^d\) degradation values.

particularly with the pyrimidine ring generating the different possible 5-(6)-hydroxy-5,6-dihydrotimidyl-6-(5)yl and 5,6-dihydrotimidyl-5-(6)yl radicals. The formation of the various 5,6-dihydrotimidine derivatives may be explained by electron transfer reactions between pyrimidinyl radicals [22]. Another important reaction to be considered is the recombination between mostly 5-(6)-hydroxy-5,6-dihydrotimidyl-6-(5)yl radicals which lead to the formation of non cyclobutane type dimers [23].

- \(N_2O\) saturated aqueous solution

The competition for electrons between 2.2×10\(^{-2}\) M \(N_2O\) \[k(e^-_aq + N_2O) = 9.1 \pm 0.2) \times 10^9 \text{ mol}^{-1} \text{ S}^{-1}\] [25] and 5 \(\times\) 10\(^{-4}\) M thymidine \[k(e^-_aq + thymine) = 1.7 \times 10^9 \text{ mol}^{-1} \text{ S}^{-1}\] [26] \(\approx k(e^-_aq + thymidine)\) is mostly displaced towards \(N_2O\). In these conditions, more than 98% of the solvated electrons react with \(N_2O\) giving rise to OH radicals according to the following equation:

\[e^-_aq + N_2O + H_2O \rightarrow OH + N_2 + OH^-\]

As a result the reactive water radiolysis products consist of about 90% of OH radicals (\(G \approx 5.4\)) and 10% of hydrogen atoms (\(G = 0.55\)). Similar yield values have been obtained for the radiation-induced degradation of uracil in \(N_2O\) aqueous solutions at neutral pH [27, 28]. The two-fold increase in the yield of \(OH\) radicals by saturating the oxygen free aqueous solution has pronounced effects on the radiation-induced degradation of thymidine. This leads in particular to a substantial increase in the \(G\) values of both \((+)-cis\ (5S, 6R)\) and \((-)-cis\ (5R, 6S)\) diastereoisomers of 5,6-dihydroxy-5,6-dihydrotimidine (Table I), The yield of the dimeric nucleosides increases also notably as expected since it has been shown that 5- or 6-hydroxy-5,6-dihydrotimidyl-6 or -5-yl radicals appear to be mostly involved in their formation [24]. Another important aspect to be emphasised is the relatively high value of the overall degradation of thymidine \((G_\text{total} = 4.75)\) which increases by a factor of three with respect to those observed in \(N_2\) aqueous saturated solutions [24]. Under these conditions, restitution processes are quite low and it may be postulated that both 6-(5)-hydroxy-5,6-dihydrotimidyl-5-(6)-yl and 5,6-dihydrotimidyl-5-(6)-yl radicals are involved in the reactions leading to the starting nucleoside in oxygen-free solution.
2) Radiation-induced degradation of thymidine in KBr aqueous solutions

The presence of KBr in the \( \gamma \)-irradiated aqueous solution of thymidine saturated with either \( \text{O}_2 \), \( \text{N}_2 \), or \( \text{N}_2\text{O} \), respectively, leads to a notable decrease in the number of thymidine decomposition products. A typical separation of the radiation-induced degradation products of thymidine in KBr aqueous solution saturated with \( \text{N}_2\text{O} \) is illustrated in the Fig. 1. A similar decomposition pattern is observed when the solution is irradiated in the presence of oxygen or nitrogen. The major nucleoside derivatives have been characterized as the four \( \text{cis} \) and \( \text{trans} \) diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine on the basis of \( \text{H} \) and \( ^{13}\text{C} \) NMR, CD and mass spectrometry analyses. Confirmation of the assignment was provided by further comparison of these spectroscopic measurements with those of authentic samples [12, 18]. It is interesting to note that the two \( \text{cis} \) or two \( \text{trans} \) diastereoisomers of these thymidine glycols are not produced in a similar yield as it is usually observed in radiation degradation studies (\textit{vide supra}). The four other relatively minor decomposition products have been respectively assigned as the \( (5\text{R}) \) and \( (5\text{S}) \) diastereoisomers of 5-hydroxy-5,6-dihydrothymidine [13] and 5,6-dihydrothymidine [24]. It should be also noted that the presence of KBr in the irradiated thymidine solution prevents any radiation-induced splitting of the N-glycosidic bond of the substrate and subsequent release of free thymine.

The \( \gamma \)-radiolysis of aqueous solutions of KBr has been previously investigated in detail by pulse radiolysis [29]. In the presence of \( 5 \times 10^{-4} \) M thymidine \( (k_{\text{thymidine}+\text{OH}} = 3 \times 10^9 \text{ mol}^{-1}\text{ s}^{-1}) \) [26] the bulk of \( \cdot \text{OH} \) radicals are scavenged by the 0.1 M KBr on \( \text{F}_{254} \) pre-coated silica gel plates.

\[ \cdot \text{OH} + \text{Br}^- \rightarrow \text{OH}^- + \text{Br} \]
\[ k = 1 \times 10^{10} \text{ mol}^{-1}\text{ s}^{-1} \] [30]

\[ \text{Br} + \text{Br}^- \rightarrow \text{Br}_2^2\text{^-} \]
\[ k = 1.9 \times 10^8 \text{ mol}^{-1}\text{ s}^{-1} \] [30]

\[ \text{Br}_2^2\text{^-} + \text{Br}^- \approx \text{Br}_3^2\text{^-} \]

In the absence of any reactive substrate dismutation reaction of the inorganic radicals \( \text{Br}_3^2\text{^-} \) would give rise to the tribromide anion according to the following equation:

\[ 2 \text{Br}_3^2\text{^-} \rightarrow \text{Br}_3^- + \text{Br}^- \]

\( \text{Br}_3^- \) which is a powerful oxidising agent is in equilibrium with \( \text{Br}_3^- \) in aqueous solution:

\[ \text{Br}_3^- \approx \text{Br}_3^- + \text{Br}^- \]

Electron transfer seems to be the more likely mechanism involved in the reaction of \( \text{Br}_3^- \) with reactive organic compound [6, 10]. However, the radiation-induced formation of the four \( \text{cis} \) and \( \text{trans} \) diastereoisomers of 5,6-dihydroxy-5,6-dihydro-
thymidine which are the main degradation products of thymidine in KBr aqueous solution appears to be not mediated by radical processes. The specificity in the formation of the (5S) cis and trans diastereoisomers (ratio 2:1 with respect to the corresponding (5R) derivatives) would suggest an ionic mechanism. This receives an indirect confirmation from the consideration of the chemical reactions of the thymidine radical cation, the possible intermediate which would be generated by electron transfer process. This radical which was postulated to be the product of the reaction of pyrimidine nucleobases with Cl2 [31, 32] was shown to be formed in the photosentization reaction of thymidine by menadione [33]. Subsequent reaction of water molecules with this radical cation gives rise preferentially to the trans diastereoisomers of the thymidine glycol. It may be opposed that the cis diols are the major products of the radiation-induced degradation of thymidine in KBr aqueous solutions (vide supra). Another major difference to be outlined is the formation in a similar yield of either the two cis or the two trans diastereoisomers of the thymidine diol. A second competitive reaction of the thymidine radical cation is the deprotonation of the methyl group with subsequent formation of 5-formyl-2'-deoxyuridine [33]. This oxidised thymidine derivative is lacking in the present radiolysis experiment.

A likely ionic mechanism to be considered for the radiation-induced formation of 5,6-dihydroxy-5,6-dihydrothymidine in KBr aqueous solutions would involve Br2, the decay product of Cl2, as the reactive species. Bromine or hypobromous acid is known to react with thymidine in a quite stereo-specific way [11], giving rise to a mixture of the predominant (+) trans (5R,6R)-5-bromo-6-hydroxy-5,6-dihydrothymidine and of the minor (−) trans (5S,6S) diastereoisomer in the ratio 2:1 (Fig. 2). These bromohydrins are unstable in the presence of high salt concentration in the aqueous solution. Only a small amount of these compounds have been isolated by HPLC analysis without evaporation of the irradiated solutions. The conversion of the two thymidine bromohydrins to the corresponding glycols involves an ionic mechanism which is SN1 like [12]. The departure of the bromide ion and the intramolecular attack by the 6-hydroxyl group take place in a concerted manner giving rise to a transient bridged ion (Fig. 2). As this rearrangement shows an anti character, an inversion of the configuration at carbon C(5) is expected. Nucleophilic attack at position 6 takes place preferentially with retention of configuration. As a result, the two cis (5S,6R) and (5R,6S) diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine are mostly produced, the (5S,6R) derivative being the major product as observed in the γ-radiolysis experiments (Table II).

The formation of other, relatively minor 5,6-dihydrothymidine derivatives may be accounted for in terms of radical mechanism. The bulk of the H atoms are scavenged by thymidine, particularly in the absence of oxygen. Preferential addition of H at carbon C(6) would give rise to the transient 5,6-dihydrothymid-5-yl radical. In N2 or N2O saturated aqueous solution dismutation of these radicals would generate 5,6-dihydrothymidine [24] and thymidine according to the following scheme

\[
2 \text{dThd}(\text{H}) \rightarrow \text{dThd} + \text{h dThd}
\]

Another possibility to be considered is electron transfer which was proposed by Haysom et al. [22]. As a result ion pairs are produced which may undergo various competitive reactions:

- nucleophilic SN1 substitution at carbon C(5) of the resulting cation would lead to the formation of 5-hydroxy-5,6-dihydrothymidine (Fig. 3).
- protonation of the anion would produce 5,6-dihydrothymidine.
- elimination reaction (E2 mechanism) would result in the formation of the starting thymidine.

The presence of molecular oxygen leads to a significant decrease of the two diastereoisomers of (5R) and (5S) 5,6-dihydrothymidine. Under such conditions, a reasonable mechanism for the formation of 5-hydroxy-5,6-dihydrothymidine would involve a fast reaction of the radiation-induced 5,5,6,6-dihydrothymid-5-yl radical with O2, followed by reduction and protonation of the resulting hydroperoxyl radical and subsequent decomposition of the unstable hydroperoxide.

The formation of 5,6-dihydrothymidine and other monohydroxylated derivatives is not prevented by the presence of 2.5 × 10⁻⁴ M cysteine in N2 aqueous saturated solutions of thymidine. On the other hand, we note a complete lack of any thymidine glycol which is explained by the efficient scavenging of
Fig. 2. Formation of the four diastereoisomers of 5,6-dihydroxy-5,6-dihydrothymidine by addition of Br₂ across the 5,6-pyrimidine bond of thymidine and subsequent nucleophilic substitution of the halogen atom.

Table II. G values of the radiation-induced® degradation products of 5 × 10⁻⁴ M thymidineb in aqueous solutions of 0.1 M KBr saturated with various gases.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>N₂</th>
<th>O₂</th>
<th>N₂O</th>
<th>N₂ (RSH)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)cis(5S,6R)-5,6-Dihydroxy-5,6-dihydrothymidine</td>
<td>0.33</td>
<td>0.55</td>
<td>0.91</td>
<td>—</td>
</tr>
<tr>
<td>(--)cis(5R,6S)-5,6-Dihydroxy-5,6-dihydrothymidine</td>
<td>0.18</td>
<td>0.33</td>
<td>0.53</td>
<td>—</td>
</tr>
<tr>
<td>(--)trans(5S,6S)-5,6-Dihydroxy-5,6-dihydrothymidine</td>
<td>0.12</td>
<td>0.21</td>
<td>0.40</td>
<td>—</td>
</tr>
<tr>
<td>(+)trans(5R,6R)-5,6-Dihydroxy-5,6-dihydrothymidine</td>
<td>0.04</td>
<td>0.11</td>
<td>0.16</td>
<td>—</td>
</tr>
<tr>
<td>(5R) and (5S)-5-Hydroxy-5,6-dihydrothymidine</td>
<td>0.06</td>
<td>0.02</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>(5R,6R) and (5S,6R)-6-Hydroxy-5,6-dihydrothymidine</td>
<td>0.02</td>
<td>—</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>(5S,6S) and (5R,6S)-6-Hydroxy-5,6-dihydrothymidine</td>
<td>0.02</td>
<td>—</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>(5R) and (5S)-5,6-Dihydrothymidine</td>
<td>0.09</td>
<td>0.03</td>
<td>0.14</td>
<td>0.15</td>
</tr>
<tr>
<td>Thymidine*</td>
<td>—0.93</td>
<td>—1.35</td>
<td>—2.38</td>
<td>—0.32</td>
</tr>
</tbody>
</table>

* Gamma rays from ⁶⁰Co for 20 min (dose-rate = 80 Gy/mn); ⁵¹(CH₃) dThd was used as the substrate. The radiation-induced degradation products were separated by two dimensional TLC, determined by autoradiography and subsequently quantitated by scintillation counting; c the solutions were purged with the appropriate gas for 15 min prior irradiation;  the flow was maintained during the irradiation; ⁴ 0.25 mM in cysteine;  e degradation values. The yields of the decomposition products were linear functions of the dose in a dose range 400 to 3000 Gy.
Br$_5^-$ by the sulphydryl agents ($k_{\text{cysteine}} + Br_5^- = 1.8 \times 10^8 \text{ mol}^{-1} \text{ s}^{-1}$) [6]. The formation of the (5R) and (5S) diastereoisomers of 5,6-dihydrothymidine may be accounted for by a radical mechanism involving the 5,6-dihydrothymid-5-(6)-yl radicals. In these conditions hydrogen transfer from cysteine to 5- or 6-yl radicals is a likely process since the rate constant for such a reaction is about $10^6 \text{ M}^{-1} \text{ s}^{-1}$).

It may be also emphasised that these radical reactions regarding the pyrimidine moiety are still significantly important when aqueous solutions of thymidine are irradiated in the presence of purine nucleosides (vide infra). The rate for the reaction of H atoms with purines is probably lower than with pyrimidines [34]. The lack of any detectable reactivity of Br$_5^-$ of Br$_3^-^-$ with the osidic moiety of DNA components has been further substantiated by recent experiments [36]. The gamma irradiation of aqueous solutions of 0.5 mM thymidyl [3'-5'] thymidine containing 0.1 M KBr did not induce the cleavage of the N-glycosidic and of the phosphodiester bonds to any significant extend.

Gamma radiolysis of aqueous solutions of purine-2'-deoxyribonucleosides containing KBr

The major radiation-induced degradation product of $5 \times 10^{-4}$ M 2'-deoxyadenosine or 2'-deoxyguanosine in 0.1 M KBr aqueous solutions saturated either with N$_2$, O$_2$ or N$_2$O has been characterised as the 2-deoxy-D-erythro pentose (in fact, a mixture of the a and b furanoid and pyranoid isomers). The mechanism involved in the initial step of the reac-
tion appears to be of a radical nature as shown by indirect evidence (vide infra). The G value of formation of 2-deoxy-D-erythro pentose in the gamma irradiated (dose: 1600 Gy) aqueous oxygenated solutions of 2'-deoxyadenosine or 2'-deoxyguanosine was estimated to be 0.5. It is reasonable to suggest, that Br$_7$ or Br$_3$ would react with the aglycone inducing the disruption of the purine moiety. It is well established that the opening of the imidazole ring between atoms N(7) and C(8) moiety. It is well established that the opening of the imidazole ring between atoms N(7) and C(8) would react with the reactive 8-yl radical and subsequent hydrogen abstraction at the 4 and 5 positions of sugar ring [42] was not observed. These results give further support to the lack of ionic reaction of Br$_2$ with 2'-deoxyguanosine and 2'-deoxyadenosine.

As mentioned above, the presence of a purine nucleoside in the $\gamma$-irradiated solution of thymidine leads to the disappearance of the thymidine glycols. Only 5,6-dihydrothymidine and 5-hydroxy-5,6-dihydrothymidine are produced in these conditions. On the other hand, 2-deoxy-D-erythro pentose is the major radiation-induced degradation product of 2'-deoxyadenosine and 2'-deoxyguanosine. In conclusion, it may be postulated that in a DNA chain Br$_7$ or Br$_3$ would react specifically with purines, preventing the formation of the decay products Br$_7$ and Br$_3$.

---