Radiolysis of Dihydrouracil and Dihydrothymine in Aqueous Solutions containing Oxygen; First- and Second-order Reactions of the Organic Peroxyl Radicals; the Role of Isopyrimidines as Intermediates

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The nature and yields of the products of radiolysis of aqueous solutions of dihydrouracil containing both N_2O and O_2 strongly depend on pH and dose rate. At a dose rate of 0.3 Gy s^{-1} and at pH 3, the major products are barbituric acid (*G* 2.4), labile material (*G* 2.8), and uracil (*G* 0.1). The labile material is converted into uracil upon treatment with acid and is largely composed of 5,6-dihydro-6-hydroxyuracil. At pH 7 barbituric acid is absent and uracil becomes the major product (*G* 4.5); some labile material is also formed (*G* 0.7). At pH 5 the pattern of the product distribution changes as a function of dose rate. With increasing dose rate *G*(uracil) decreases whereas *G*(barbituric acid) increases. The predominant radical formed in the dihydrouracil system is the 6-peroxyl radical, and it has been found, using pulse radiolysis with both optical and conductometric detection, that this peroxyl radical can eliminate $O_2^{-\cdot}$ to give uracil *via* an unstable isomeric form of the pyrimidine (isouracil). The elimination is base-catalysed so that, under alkaline conditions, uracil is the major radiolysis product. At lower pH, and also at higher dose rates, bimolecular decay of the peroxyl radical competes with the $O_2^{-\cdot}$ elimination process, barbituric acid being a specific product of the bimolecular decay route. The mechanisms of these processes are discussed.

Irradiation of dihydrothymine–N_2O–O_2 solutions gave similar results.

The radiolysis of pyrimidines and dihydropyrimidines (as models for DNA) in aqueous solutions has received considerable attention (for reviews see refs. 1 and 2), and some details are fairly well understood.

In the radiolysis of N_2O-saturated aqueous solutions, OH radicals are the predominant primary species available for reaction with solutes:

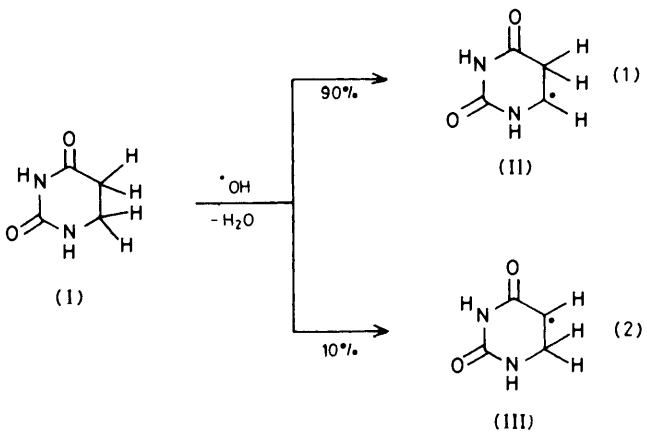
$$H_2O \xrightarrow{\text{radiolysis}} OH^{\cdot}, e^-_{aq}, H^{\cdot}, H_3O^+, H_2, H_2O_2$$
$$e^-_{aq} + N_2O \longrightarrow {}^{\cdot}OH + N_2 + OH^-$$

The OH radicals react with pyridimines by addition to the 5,6-double bond; with uracil the preferential site of attack is C(5).[3] In the case of dihydropyrimidines the OH radicals abstract H atoms at the C(5)–C(6) bond; with dihydrouracil this occurs mostly at C(6) [$\geqslant$ 90%; reaction (1)] and much less at C(5) [*ca.* 5%; reaction (2)].[4] Thus in both cases 6-yl radicals are formed predominantly.

The 6-yl radicals are readily oxidized by transition metal ions [reaction (3)].[5,6] The intermediate cation is not stable and rapidly loses the proton at N(1) [reaction (4)]. The product is an unstable isomeric form of the pyrimidine, isouracil (IV). Under alkaline and neutral conditions this species is largely converted into uracil (V) [reaction (5)], whereas in acid medium, as a result of protonation and solvolysis, 5,6-dihydro-6-hydroxyuracil (VI) becomes the major product [reaction (6)]. (Further details of the isopyrimidine–pyrimidine rearrangement will be reported.)[7]

Although in the reaction with oxygen direct electron transfer [as in reaction (3)] is a conceivable pathway, addition of oxygen takes place [reactions (7) and (8)] and the final products then arise from the decay of the peroxyl radicals (VII) and (VIII).

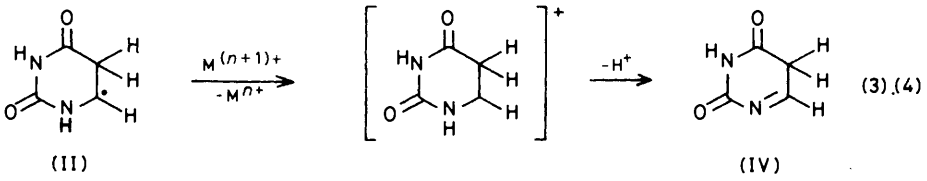
Radicals (VII) and (VIII) are secondary peroxyl radicals. Scheme 1 summarizes our present knowledge of the bimolecular decay of secondary peroxyl radicals in aqueous solutions (*cf.* ref. 8). In reaction (x) of Scheme 1 a peroxyl radical is formed whose structure is similar to that of the radical (VII) (α-OH *vs.* α-NH). The kinetic parameters of the first-order decay of α-hydroxyalkylperoxyl radicals [reactions (xi) and (xii) of Scheme 1] have been reviewed.[9]



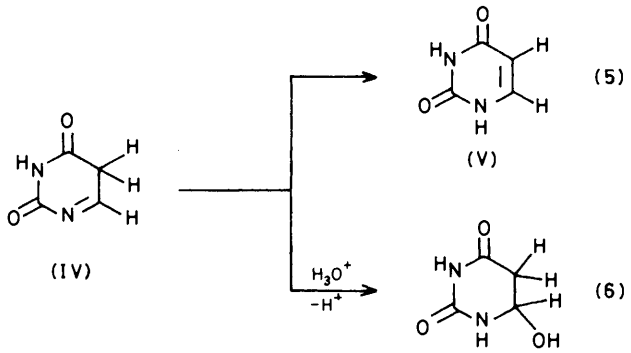
Considerable effort has been put into unravelling the products and the decay kinetics of the peroxyl radicals derived from dihydropyrimidines,[10-14] but the mechanism and often also the products have not been adequately described. Studies on peroxyl radicals derived from dihydropyrimidines were undertaken at Newcastle and at Mülheim independently some time ago. These efforts were subsequently combined and the data obtained from both steady-state and pulse radiolysis experiments now present a more detailed picture of this complicated system.

Results and Discussion

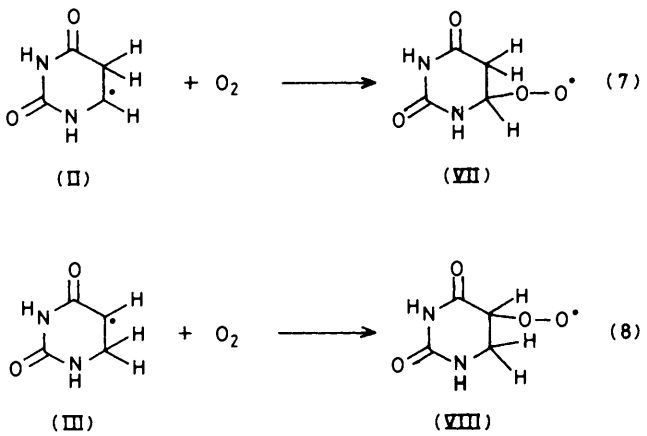
Products found in the radiolysis of solutions of dihydrouracil saturated with N_2O–O_2 were uracil (V), barbituric acid (X),



(II) (IV)



(IV)

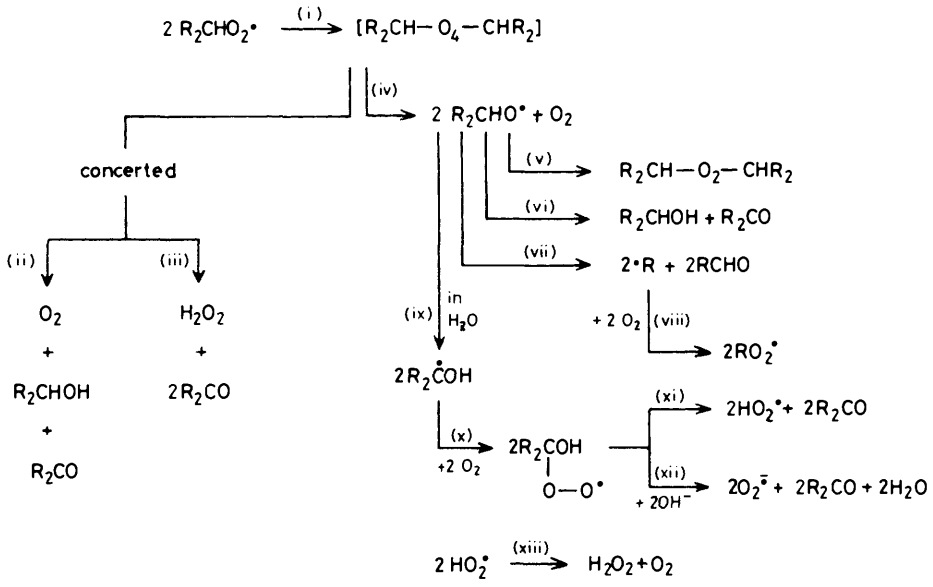


5,6-dihydro-6-hydroxyuracil (VI), 5,6-dihydro-5-hydroxyuracil, and an organic peroxidic compound; no isobarbituric acid was detected ($G \leqslant 0.1$). The yields are strongly pH-dependent (Tables 1 and 2 and Figure 1). In the pH range around neutrality the yields were found to be also dose-rate-dependent (Figure 2). At pH 7 and above, uracil is formed in high yields (Tables 1 and 2). Since uracil reacts about six times faster with OH radicals than dihydrouracil, yield–dose plots start to deviate from linearity at conversions $<10\%$. The use of ^{14}C-labelled dihydrouracil solutions for the determination of product yields requires conversions of $>10\%$ and therefore cannot be used for the determination of the G values under these conditions. The spectroscopic method, however, is sufficiently sensitive in the required conversion range. The quoted yields of labile material correspond to those of uracil produced on treatment of the irradiated solutions with acid; 5,6-dihydro-6-hydroxyuracil is dehydrated in acid solution and it was found that decay of the organic peroxidic material to uracil also occurs under these conditions.

Hydrogen peroxide is also formed. Attempts were made to determine the separate yields of hydrogen peroxide and of organic peroxidic material using either the iodide reagent [15] or the Fe^{2+}–Xylenol Orange reagent,[16] both before and after treatment with catalase. Neither of these methods proved satisfactory in the irradiated dihydrouracil system. In particular, it was found that I_3^- reacts with barbituric acid in an ill-defined manner which does not permit exact corrections. The correlation between organic peroxide and Fe^{3+} ion in the Fe^{2+}–Xylenol Orange method is unknown but is higher than for H_2O_2. Besides it appears that catalase might also react with some organic hydroperoxides. However, very recently [17] it has been found that the phenolphthalein reagent [18] is probably more reliable in this system and values for the initial yields of hydrogen peroxide and organic peroxidic material obtained by this method are 1.4 (in both cases) at pH 3, and 2.1 and 1.0, respectively, at pH 7. At pH 7, where there should be no interference from barbituric acid, the iodide method yielded values of 3.2 and 0.2, respectively. The discrepancies between the two methods clearly indicate the difficulties involved in these peroxide determinations. A detailed investigation of experimental methods which may be suitable for differentiating between hydrogen peroxide and organic peroxide material is being undertaken.

Analogous results were obtained in the radiolysis of dihydrothymine–N_2O–O_2 solutions. Figure 3, for example, shows the pH dependence of the yields of thymine, 5-methylbarbituric acid, and acid-labile material. From a mechanistic point of view dihydrothymine differs from dihydrouracil only in a somewhat different ratio of the 5-yl (*ca.* 4%) and 6-yl (*ca.* 80%) radicals upon ·OH attack and the fact that OH radicals also attack the methyl group ($\leqslant 10\%$).[4] Only the dihydrouracil system will be discussed in detail here.

Neutral and Alkaline Solutions; Unimolecular Decay of the Peroxyl Radical (VII).—In buffered neutral and in alkaline solution the major stable product from dihydrouracil is uracil (Tables 1 and 2). Given that G(OH) in the present system is *ca.* 5.4 it is clear that the uracil yield (G 4.5) is close to the yield of OH radicals which abstract a hydrogen atom from the 6-position. It is therefore concluded that the precursor of uracil is the peroxyl radical (VII). The formation of uracil can be described by reactions (9) and (10) in which



Scheme 1.

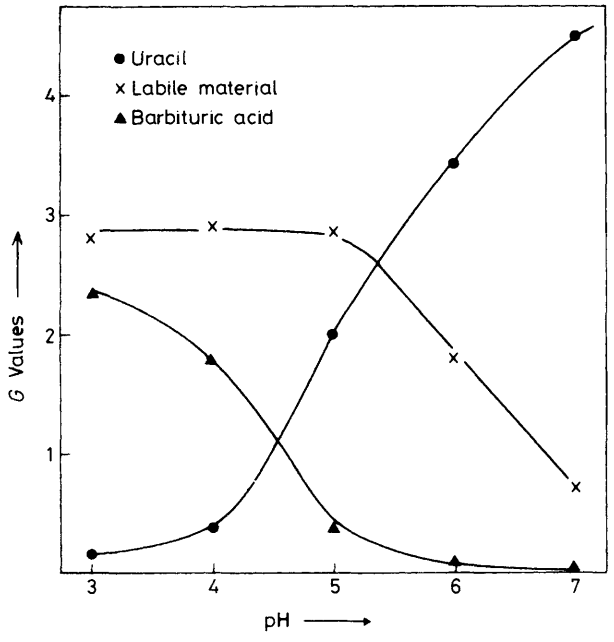


Figure 1. Effect of pH on the G values of products in the γ-radiolysis of solutions of dihydrouracil (1mM) saturated with N_2O–O_2 (7 : 3 v/v); dose rate 0.3 Gy s $^{-1}$; dose range 70—290 Gy

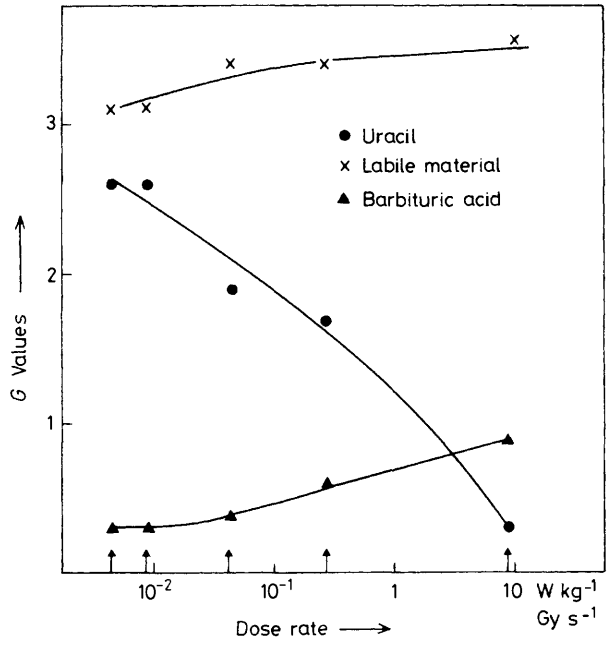


Figure 2. Effect of dose rate on the G values of products in the γ-radiolysis of solutions of dihydrouracil (1mM) saturated with N_2O–O_2 (4 : 1 v/v), at pH 5 (acetate buffer 0.5mM); dose range 70—290 Gy

isouracil (IV) is formed, followed by the rearrangement of the isouracil (IV) into uracil (V) [reaction (5)]. The reaction sequence (9), (10) is analogous to the elimination of $O_2^{-\bullet}$ from α-hydroxyalkylperoxyl radicals [reaction (xii) of Scheme 1].

Elimination of $O_2^{-\bullet}$ can be observed by using pulse radiolysis with conductivity detection. In reaction (9) followed by reaction (10), OH^- (182 Ω^{-1} cm^2 equiv^{-1} at 21 °C) is replaced by $O_2^{-\bullet}$ (65 Ω^{-1} cm^2 equiv^{-1}). The $O_2^{-\bullet}$ disappears very slowly [$2k(O_2^{-\bullet} + O_2^{-\bullet}) < 0.3$ dm^3 mol^{-1} s^{-1}].[19] In alkaline 1 mM-solutions of dihydrouracil saturated with N_2O–O_2 following an electron pulse of 1—3 Gy, the conductivity of the solution was found to decrease by a first-order rate process to a lower and constant level. At low [OH^-] the extent of this decrease can be attributed to reactions (9) and (10) plus the formation of $O_2^{-\bullet}$ from the primary H atoms (G *ca.* 0.5) and leads to $G(O_2^{-\bullet}) = 4.8$ at pH 8.3 (Figure 4). Thus the G value of $O_2^{-\bullet}$ elimination from reaction (10) is *ca.* 4.3, which approaches the expected yield of the peroxyl radical (VII). At higher [OH^-] the conductivity decreases further and gives a limiting value of G *ca.* 10 (anions formed, OH^- consumed) (Figure 4). This further change in conductivity is thought to be the result of deprotonation of the

Table 1. Products and G values in the γ-radiolysis of dihydrouracil (10^{-3}M) in aqueous solution saturated with N_2O–O_2; dose rate 0.3 Gy s^{-1}

	pH 3	pH 3	pH 7 [a]
Uracil (V)	<0.1 [c]	0.1 [d]	4.5 [d]
Barbituric acid (X)	2.4 [c]	2.4 [d]	Absent [d]
Labile material [b]		2.8 [d]	0.7 [d]
5,6-Dihydro-6-hydroxyuracil (VI)	1.7 [c]		
5,6-Dihydro-5-hydroxyuracil	0.2 [c]		
Organic peroxidic material	0.8 [c]		
Unknown product	0.15 [c]		

[a] 5×10^{-4}M phosphate buffer. [b] Includes 5,6-dihydro-6-hydroxyuracil and labile organic peroxidic material. [c] By using [^{14}C]dihydrouracil; dose 500 Gy. [d] Spectrophotometrically, dose range 70—290 Gy.

Table 2. Products and their G values [a] from dihydrouracil (10^{-3}M) in aqueous solution saturated with N_2O–O_2 after a train of 1 μs electron pulses of 3—10 Gy (repetition rate 1 Hz); dose range 200—600 Gy

	pH 3	pH 5 [c]	pH 7 [d]	pH 10.4
Uracil (V)	0.1	0.4	3.6 [e]	4.8 [e]
Barbituric acid (X)	2.1	1.3	0.3	Absent
Labile material [b]	2.8	2.7	1.1	0.1

[a] Determined spectrophotometrically (see text). [b] Contains 5,6-dihydro-6-hydroxyuracil and labile organic peroxidic material. [c] 5×10^{-4}M-Acetate buffer. [d] 10^{-2}M-Phosphate buffer. [e] Values obtained by extrapolation of G *vs.* dose plots to zero dose.

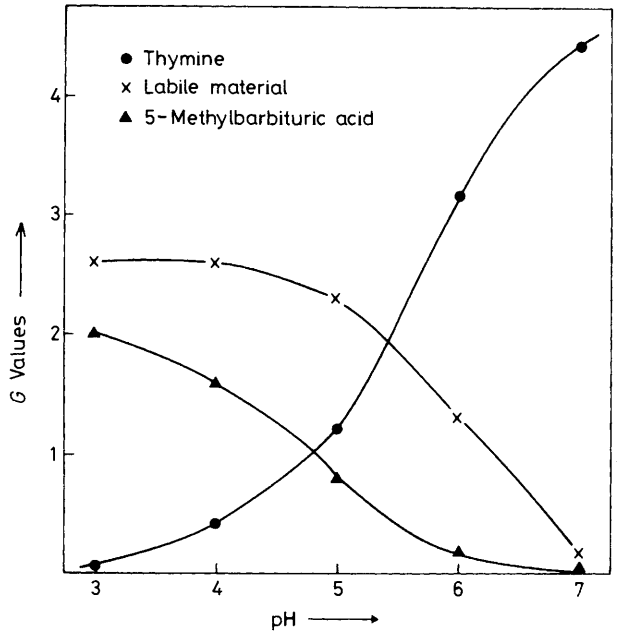
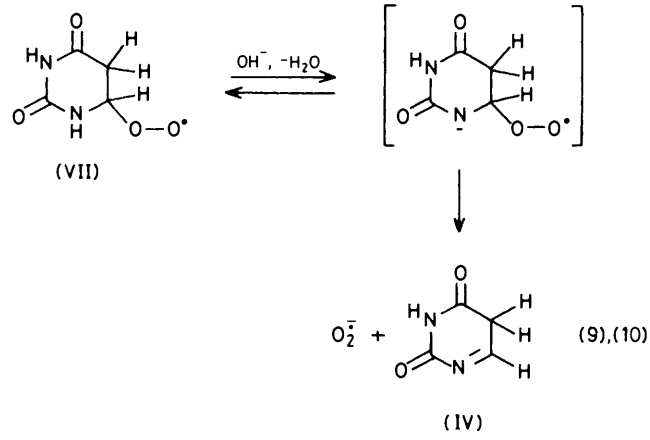


Figure 3. Effect of pH on the G values of products in the γ-radiolysis of solutions of dihydrothymine (1mM) saturated with N_2O–O_2 (7 : 3 v/v); dose rate 0.3 Gy s^{-1}; dose range 70—290 Gy



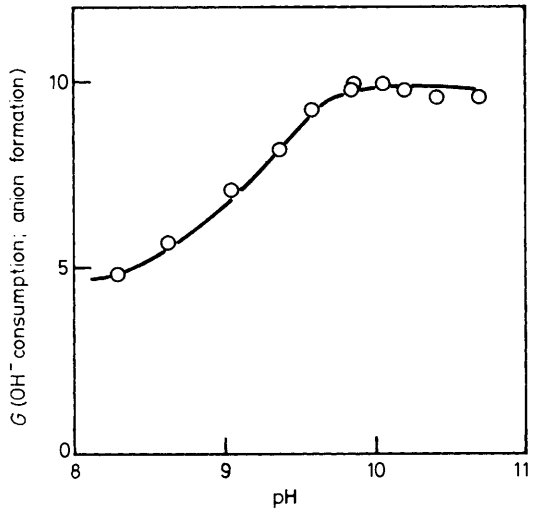


Figure 4. Effect of pH on the G values calculated from the conductivity decrease [substitution of OH^- by an anion ($O_2^{-\bullet}$ and uracilate)] observed in the pulse radiolysis of 1mM-solutions of dihydrouracil saturated with N_2O–O_2 (4 : 1 v/v); 0.4 μs electron pulses of *ca.* 1 Gy

isouracil which has pK_a *ca.* 9.4 (see later). The dissociation of isouracil to isouracilate ion involves the consumption of 1 equiv. of OH^- in addition to that due to reactions (9) and (10).

The observed rate constant ($k_{obs.}$) for the first-order conductivity decrease after the pulse increased with increasing [OH^-], approaching a limiting value of 8×10^4 s^{-1} (Figure 5). This behaviour is consistent with reactions (9) and (10) in which a pre-equilibrium is established between the peroxyl radical (VII) and its ionized form. For this situation, kinetic analysis assuming steady-state conditions leads to the relationship (*a*). It is seen from the relationship (*a*) that at low

$$k_{obs.} = \frac{k_9 k_{10}[OH^-]}{(k_9 + k_{10}) + k_9[OH^-]} \qquad (a)$$

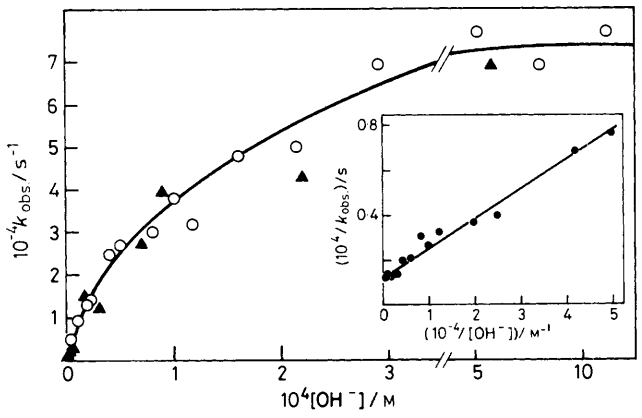


Figure 5. [OH^-] Dependence of the observed first-order rate constants ($k_{obs.}$) of conductivity decrease (open circles) and optical absorption build-up at 260 nm (filled triangles) in 1mM-solutions of dihydrouracil saturated with N_2O–O_2 (4 : 1 v/v), irradiated with an 0.4 μs electron pulse of 1—3 Gy; *inset* plot of $1/k_{obs.}$ *vs.* $1/[OH^-]$

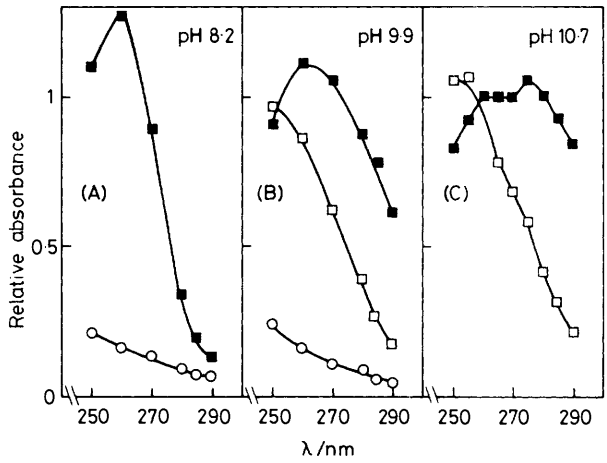


Figure 6. Absorption spectra of 1mM-dihydrouracil solutions saturated with N_2O–O_2, irradiated with a 0.4 μs electron pulse of *ca.* 3 Gy; (A) pH 8.2, (B) pH 9.9, and (C) pH 10.7; open circles, spectra taken *ca.* 2 μs after pulse; open squares *ca.* 50 μs after pulse; filled squares 4 ms after pulse

[OH^-], $k_{obs.}$ is approximately a linear function of [OH^-], as is found in Figure 5. At high [OH^-], we have $k_{obs.} = k_{10}$.

The relationship (*a*) can be rearranged as (*b*). The reciprocal

$$\frac{1}{k_{obs.}} = \frac{(k_{-9} + k_{10})}{k_9 k_{10}} \cdot \frac{1}{[OH^-]} + \frac{1}{k_{10}} \qquad (b)$$

plot ($1/[OH^-]$ *vs.* $1/k_{obs.}$) of Figure 5 (inset) gives an intercept corresponding to $k_{10} = 8.3 \times 10^4\ s^{-1}$.

The $HO_2^{\bullet}$ elimination from α-hydroxyalkylperoxyl radicals has been found[9] to be the composite of a spontaneous reaction [reaction (xi) in Scheme 1] and a base-induced reaction [reaction (xii) in Scheme 1]. The rate constant for the spontaneous reaction (k_0) depends strongly upon the nature of the flanking substituents. In the present case, spontaneous elimination of $HO_2^{\bullet}$ from (VII) appears too slow to be detected in neutral solution of dihydrouracil. Also, extrapolation of $k_{obs.}$ in Figure 5 to zero [OH^-] yields an intercept close to zero. According to our previous experience[20] we should have been able to measure k_0 if it was $\geqslant 50\ s^{-1}$.

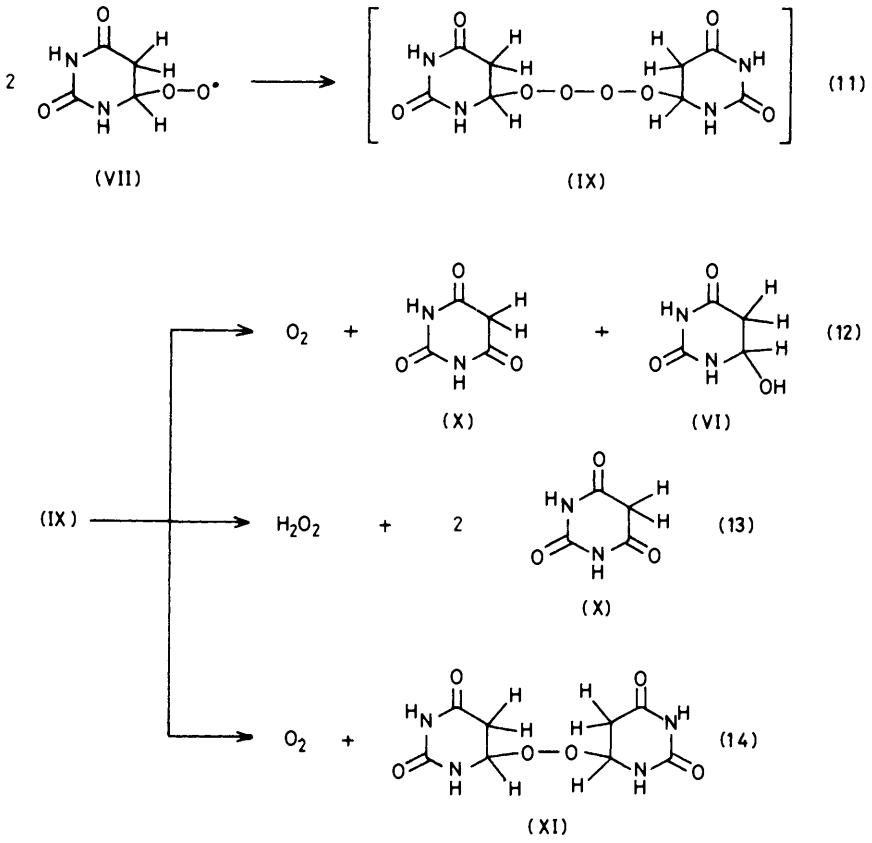
Using the optical detection technique in pulse radiolysis one can follow the formation of uracil *via* reactions (9), (10), and (5) at pH 8—9 where the rearrangement reaction (5) is relatively fast (*cf.* ref. 7), so that the rate-determining step is reaction (9). In Figure 6(A) the spectrum immediately after the pulse is attributed to the peroxyl radicals derived from dihydrouracil [mostly (VII)] and the spectrum 4 ms following the pulse is mainly that of the final product uracil (V) (plus a small contribution from $O_2^{-\bullet}$). The intermediate isouracil (IV) does not have significant absorption in this pH range and in this wavelength region.[7] The observed first-order rate constants of the absorption build-up at 260 nm were found to be dependent on [OH^-] and are similar to those obtained by conductivity measurements. These are also plotted in Figure 5.

At pH > 9 the OH^--induced reaction (9) becomes faster and the rearrangement of the isouracil becomes slower because of the formation of the slowly rearranging isouracilate ion.[7] The products isouracil (IV) (pK_a *ca.* 9.4)[7] and uracil (pK_a 9.5) exist increasingly in their deprotonated forms, which absorb differently from their protonated forms. Figure 6(B) shows the absorption spectra at various times of a dihydrouracil solution saturated with N_2O–O_2 (4 : 1 v/v) irradiated by an electron pulse at pH 9.9. Immediately following the pulse one observes the weak absorption of the peroxyl radicals. This is transformed by a fast first-order rate process ($k_{obs.}$ $4 \times 10^4\ s^{-1}$) into a species with an absorption maximum below 250 nm [Figure 6(B), middle spectrum, taken 50 μs after pulse] which is attributed to the deprotonated form of isouracil (IV). (The spectrum is slightly distorted below 260 nm owing to the presence of $O_2^{-\bullet}$; see also ref. 7.) This intermediate is then transformed by a slower process ($k_{obs.}$ *ca.* $10^3\ s^{-1}$) into the final products, exhibiting an absorption maximum at 260 nm [top spectrum, filled squares, in Figure 6(B)]. This latter spectrum is a composite of those of uracil, uracilate ion, and $O_2^{-\bullet}$. At pH > 9 the observed rate constant for the formation of the isouracilate ion as monitored at 260 nm increased with [OH^-] (Figure 5), tending to a limiting value, in agreement with the mechanism proposed here involving pre-equilibrium between peroxyl radical (VII) and its anion.

The final optical absorption of the irradiated dihydrouracil solution at pH > 10 shifts its maximum increasingly towards 280 nm, *i.e.* approaching the spectrum of the uracilate ion [Figure 6(C), filled squares]. The uracilate ion spectrum has a maximum at 284 nm and a shoulder at 260 nm. In Figure 6(C) (filled squares) the absorption is again a composite. The presence of large amounts of $O_2^{-\bullet}$ and some contribution from the strongly absorbing undissociated uracil enhance the absorbance around 260 nm. The kinetics of the rearrangement of the isouracil (IV)/isouracilate into uracil (V)/uracilate [reaction (5)] has been studied at pH > 9 where the OH^--catalysed $O_2^{-\bullet}$ elimination becomes faster than the rearrangement.[7]

Acidic Solutions; Biomolecular Decay of Peroxyl Radicals.—The chemistry in acidic solutions is much more complex. The yield of uracil (V) decreases and those of barbituric acid (X) and 5,6-dihydro-6-hydroxyuracil (VI) (uracil hydrate) become more prominent as the pH is lowered (Figure 1). Acid-labile peroxidic material is also formed. [Although this was not characterized it is probably 5,6-dihydro-6-hydroperoxyuracil (XII) and/or the corresponding peroxide (XI).] There are also considerable amounts of hydrogen peroxide and some other minor products (Tables 1 and 2).

The reason for the decrease in *G*(uracil) as the pH is lowered at a given dose rate is first of all a competition between the bimolecular decay [reaction (11)] of the peroxyl radicals (see later) and the $O_2^{-\bullet}$ elimination [reactions (9) and



(10)]. Whereas at a given dose rate the former process should be independent of pH, the latter process is retarded with decreasing pH (decreasing $[OH^-]$). Similarly, at a given pH (*e.g.* pH 5, *cf.* Figure 2), products arising from a bimolecular decay of the peroxyl radical become more prominent with increasing dose rate, whereas those stemming from the first-order decay (predominantly uracil) are observed at low dose rates. From both sets of experiments it is evident that barbituric acid (X) is the typical product of bimolecular decay. It could be formed together with uracil hydrate (VI) according to the Russell mechanism [reaction (12); *cf.* reaction (ii) of Scheme 1]. Another possible route [reaction (13)] would lead to two molecules of barbituric acid (X) and one molecule of hydrogen peroxide. Such a reaction could be a concerted process [*cf.* reaction (iii) of Scheme 1] or could have free radicals as intermediates [*cf.* reactions (iv)—(ix), (x), (xi), and (xii) of Scheme 1]. In either case, G(barbituric acid) should be equal to or larger than G(uracil hydrate). At pH 3 this has been found to be the case (Table 1). Another bimolecular process could lead to the peroxide (XII) [reaction (14)]. Peroxides are often formed, albeit in low yields, in the bimolecular decay of peroxyl radicals [reaction (v) in Scheme 1; *cf.* ref. 8].

Bimolecular reactions of the peroxyl radicals derived from dihydrouracil have been followed by pulse radiolysis at near neutral to acidic solutions with pulses of 10—20 Gy. Figure 7(A) shows absorption spectra obtained at pH 5.1. The absorption spectrum immediately following the pulse (lower spectrum, circles) is attributed to the peroxyl radicals [mainly (VII)], which is similar to those obtained in basic solutions [Figure 6(A),(B)]. This is then transformed by a slow first-order rate process (k 22 s^{-1} at 19 °C as monitored at 260 nm) into the upper spectrum (triangles) which is attributed to the barbiturate anion (ε_{260} = 20 900 l mol^{-1} cm^{-1}). In reactions (12) and (13) barbituric acid is formed in its keto form. The pK_a value of barbituric acid is 4.0 and therefore at pH 5.1 it is *ca.* 90% dissociated. The low rate constant is then the rate constant of the ionic dissociation of barbituric acid [reaction (16)], which agrees well with literature values [21,22] (*cf.* ref. 23). The dissociation of 5-methylbarbituric acid formed in the radiolysis of the dihydrothymine system has also been followed in a similar way. The dissociation rate constant (k 24 s^{-1} at 24 °C) has been found to be quite close to that of barbituric acid. From the final absorption of the dihydrouracil solution at 260 nm [Figure 7(A)] one can calculate that the yield (G) of barbituric acid/barbiturate anion is *ca.* 2.0, which agrees well with the value obtained by product analysis at pH 3 (Table 2). The lower G value for barbituric acid obtained by pH 5 with acetate buffer (Table 2) may be caused by the catalytic effect of the buffer on the competing unimolecular $O_2^{-\cdot}$ elimination (see later).

At pH 3 barbituric acid exists essentially in its undissociated form, which only absorbs weakly in the near-u.v. region (ε_{260} = 720 l mol^{-1} cm^{-1}). Following a pulse of *ca.* 15 Gy given to a dihydrouracil solution at pH 3, one observes the absorption spectrum of the peroxyl radical immediately after the pulse [Figure 7(B), upper spectrum, circles]. After 60 ms there is no build-up of the barbiturate anion absorption as in the solution at pH 5.1 already discussed, but there is a decrease of absorption $\geqslant$ 270 nm [Figure 7(B), lower spectrum, filled circles]. There is some residual absorption increase at 260 nm due to the small fraction of the strongly absorbing barbiturate anion at the given pH. The rate of decrease of the absorption of the dihydrouracil solution as monitored at 280 nm was found to follow second-order kinetics, with $2k \simeq 2 \times 10^7$ dm^3 mol^{-1} s^{-1}, which is attributed to the bimolecular decay of the peroxyl radicals (VII) and (VIII).

The deprotonation of barbituric acid has also been followed

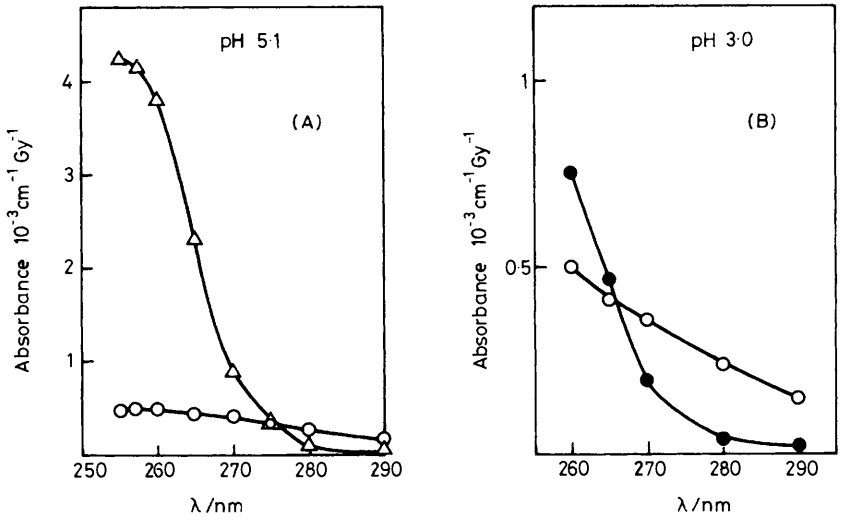
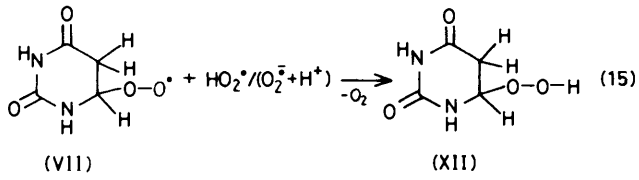
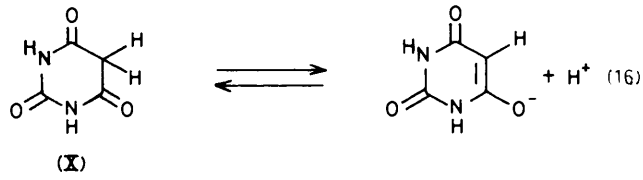


Figure 7. Absorption spectra of 1mM-dihydrouracil solution saturated with N_2O–O_2 (4 : 1 v/v), irradiated with a 1 μs pulse of 15 Gy; (A) pH 5.1, (B) pH 3.0; open circles, spectra taken *ca.* 5 μs after pulse; filled circles, spectrum taken 60 ms after pulse; triangles, spectrum taken 0.3 s after pulse





by pulse radiolysis using the conductivity detection technique. The conductivity of a dihydrouracil solution saturated with N_2O–O_2 (4 : 1 v/v) and irradiated with a pulse of 12 Gy at pH 5.4 increased slightly [$G(H^+/O_2^{-\cdot})$ *ca.* 0.3] owing to the formation of H^+ and $O_2^{-\cdot}$ from the reaction of O_2 with the radiolytically formed H atoms. This conductivity change remained constant up to *ca.* 2 ms. After longer times, a slow build-up with a half-life of *ca.* 40 ms was observed. The plateau value corresponded to $G(H^+/\text{barbiturate}) = 2.1$. The slow conductivity build-up is attributed to the deprotonation of barbituric acid [reaction (16)]. If the dihydrouracil solution was pulse-irradiated at pH 3, only a very small conductivity change [$G(H^+/\text{anion})$ *ca.* 0.2] was observed. This finding agrees with the identification of the conducting species as barbituric acid, pK_a 4.0. The low decay rate of the peroxyl radicals would allow reactions (9) and (10) to proceed at low dose rates, even in slightly acid medium. Thus, under these conditions, $HO_2\cdot/O_2^{-\cdot}$ could react with radical (VII) [reaction (15)], leading to the hydroperoxide (XII). Another feature of peroxyl radical (VII) is that its unimolecular decay is catalysed by the presence of even small amounts of buffer. For this reason one observes considerable amounts of uracil even under pulse-radiolysis conditions at pH 7 and 5 (see Table 2). A catalysis rate constant of the order of 10^5 dm^3 mol^{-1} s^{-1} could already account for this effect.

The peroxyl radical (VIII) is formed with a G value of less than 0.5. Isobarbituric acid has not been detected but some 5,6-dihydro-5-hydroxyuracil was found (Table 1) (G 0.2). Paper chromatography and t.l.c. using ^{14}C-labelled dihydrouracil, and h.p.l.c., show traces of a material other than that already described. Thus, there is no single process which can be considered typical for this radical and which would lead to one prominent product. Bimolecular reactions between peroxyl radicals (VIII) and (VII) are most likely to occur, and a series of subsequent reactions as depicted in a general way in Scheme 1 will ensue.

Experimental

Dihydrouracil (Sigma) was purified by recrystallisation three times from triply distilled water. After this procedure the substance was free of buffering material which otherwise hampers the conductivity studies in the pulse radiolysis experiments. Aqueous 10^{-3}M-dihydrouracil solutions were saturated with N_2O–O_2 (70 : 30 or 80 : 20) prior to and during irradiation. Irradiations were carried out at room temperature using a ^{60}Co γ-source for steady-state radiolysis. Irradiations were carried out at 20 ± 2 °C. Very high dose rates were applied in a sequence of 1 μs pulses from a 2.8 MeV van de Graaff generator of 1 Hz frequency and with doses of 3—10 Gy per pulse.

The G values of uracil, barbituric acid, and labile materials which upon treatment with acid yield the pyrimidine were obtained from dose–yield plots. The yields of uracil and barbituric acid were determined by measuring the optical density at 260 nm at pH 7 and pH 2. The extinction coefficient of uracil is 8 200 l mol^{-1} cm^{-1} both at pH 2 and 7, whereas that of barbituric acid is 720 l mol^{-1} cm^{-1} at pH 2 and 20 900 l mol^{-1} cm^{-1} at pH 7. From simultaneous equations the concentrations of these two components were calculated. Labile material which has only a negligible absorption at 260 nm was converted into uracil at pH 2 and 50 °C overnight.

[^{14}C]Dihydrouracil was used for paper and thin-layer chromatography (Whatman 1 and polygram GEL 400 UV-254 cellulose). [^{14}C]Uracil (Amersham) was hydrogenated over 5% rhodium–alumina. For the detection of dihydrouracil, 5,6-dihydro-6-hydroxyuracil, and barbituric acid, Fink's

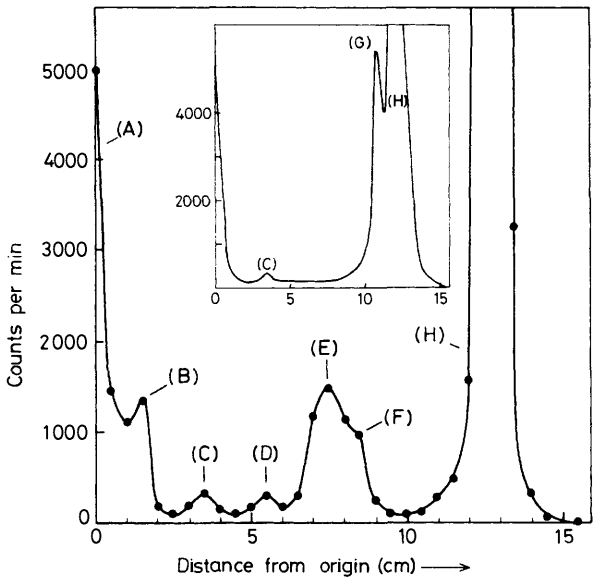


Figure 8. Typical t.l.c. strip-scanograms of the products from γ-irradiated aqueous solutions of [^{14}C]dihydrouracil at pH 3, saturated with N_2O–O_2 (7 : 3 v/v); dose rate 0.3 Gy s^{-1}; dose 500 Gy; (A) barbituric acid, (B) organic peroxidic material, (C) 5,6-dihydro-5-hydroxyuracil, (D) unknown, (E) 5,6-dihydro-6-hydroxyuracil, (F) unknown, (G) uracil, (H) dihydrouracil; *inset* products after hydrolysis; solvent chloroform–methanol–water (4 : 2 : 1), with 5% methanol added to the lower layer

reagent [24,25] was used and for detection of isobarbituric acid, the chromatograms were sprayed with a mixture of equal volumes of a 1% solution of $FeCl_3$ and a 1% solution of $K_3Fe(CN)_6$. Peroxidic material was detected with the iodide–starch and Fe^{2+}–Xylenol Orange [16] reagents. Typical t.l.c. strip-scanograms of freeze-dried samples are shown in Figure 8. In the case of paper chromatography [solvent butan-1-ol–H_2O (86 : 14)] R_f values (in parentheses) are as follows: barbituric acid (0.02), organic peroxidic material (0.04), 5,6-dihydro-5-hydroxyuracil (0.13),[26] unknown (0.22), isobarbituric acid (0.27), 5,6-dihydro-6-hydroxyuracil (0.29), dihydrouracil (0.34), uracil (0.44).

The 2.8 MeV van de Graaff electron accelerator and the optical and conductivity detection techniques have been described previously.[27,28] The solutions were irradiated at room temperature with 0.4—1 μs pulses using doses in the range 1—20 Gy. The pH values of the solutions were adjusted with $HClO_4$ and NaOH for most measurements, except product studies around pH 7 where 0.5 mM-acetate or phosphate buffers were used. Fricke dosimetry was used for the optical detection, and nitroform dosimetry [29] with N_2O-saturated 2mM-dioxane solution containing 0.1mM-tetranitromethane was performed for the conductivity detection system, taking $G[H^+/C(NO_2)_3^-] = 6.0$. Reproducibility of all the data was generally better than or equal to ±10%.

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