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Effect of Copper(II) Ions on the γ-Radiolysis of Uracil

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Radiation sensitization of uracil (Ura) by Cu^{II} ions has been investigated using cobalt-60 γ-rays. In N_2O-saturated solution, when no additive is present, the base degradation yield, $G(-\text{Ura})$, is only *ca.* 3.3, but when Cu^{II} ions are added in either a free or complexed state to the solution before irradiation, $G(-\text{Ura})$ is found to increase to *ca.* 5.1. The enhanced degradation of the base is derived from the oxidized transient, *e.g.* $UraOH^+$, although reduction of Cu^{II} to Cu^I has been ascribed to all the primary adducts, *e.g.* UraH, UraOH and Ura^-. Glycol and isobarbituric acid constitute the major products of radiolysis, which are derived from the oxidized $UraOH^+$ species. Hydroxydihydrouracil and its dimer have also been detected in significant yields when the radiolysis is carried out at comparatively high absorbed doses. The formation of these products has been ascribed to a secondary reaction between UraOH and Cu^I which proceeds through the intermediate formation of a Cu—C bond.

The use of hyperbaric oxygen in the radiation therapy of certain tumor cells is rendered less effective because oxygen is quickly metabolised in the body tissues.[1] Recently there has therefore been an interest in finding compounds which resemble oxygen in their action but are not metabolized as oxygen. Thus various organic electrophilic compounds[1] are undergoing clinical trials of their effectiveness as radiation sensitizers. In this context the use of inorganic compounds has not been studied widely; however, recently attempts have been made to study the role of metal ions in modifying the radiosensitivity of biological targets.[2] A literature[3,4] search reveals that very few attempts have been undertaken so far to study the effects of these metal ions on the radiolysis of pyrimidine bases, even though such studies are very important in understanding the effects of metal ions in determining the radiosensitivity of nucleic acids: our studies on the effect of Fe^{III} have only recently been reported.[5] Copper(II) constitutes another easily available transition-metal ion. Holian and Garrison reported[3] earlier the oxidation of the uracilyl radical by copper(II), but their study was not sufficiently comprehensive. The object of the present investigation therefore comprises a study of the effects of Cu^{II} and some of its complexes on the radiolysis of uracil.

EXPERIMENTAL

The uracil (E. Merck) used in the investigation was recrystallized thrice from triply distilled water. [$^{14}C_2$]uracil was procured from BARC, Bombay. The Cu^{II} complexes, *e.g.* Cu^{II}EDTA, Cu^{II}NTA, Cu^{II}IDA and $Cu^{II}(IDA)_2$, were prepared by the usual methods.[6-8] EDTA, NTA and IDA represent ethylenediamine tetra-acetate, nitrotriacetate and iminodiacetate, respectively. All other chemicals and solvents were of analytical-reagent grade. Deaeration was carried out by argon saturation. Pure N_2O was used in the investigation. Irradiation was carried out with ^{60}Co γ-rays. The dose rate was determined using a Fricke dosimeter.

The products of radiolysis were separated by paper chromatography using a mixture of butan-1-ol and water (86:14) as solvent. The distribution of activity on the paper was determined by counting the radioactivity after thorough segmentation of the active chromatogram. The products were identified by comparing the retention values (with respect to uracil) with those cited in the literature[9-14] and also by qualitative chemical tests.[13-15] The base degradation yield as well as the yields of the radiolytic products were determined from the relative activity present in the respective peaks in the active chromatogram. The degradation of the base was also determined spectrophotometrically by measuring the absorbance after separation of the metal ions at 260 nm. Cu^I was determined spectrophotometrically by measuring the absorbance of its neocuproin complex[16] at 457 nm. H_2O_2 was also determined spectrophotometrically by using titanium(III) sulphate reagent.[17] Formation of formaldehyde was tested by both dinitrophenylhydrazine (DNPH)[18] and chromotropic acid,[19] and the presence of glyoxalic acid was tested using DNPH.[20]

RESULTS

When aqueous solutions of uracil containing Cu^{II}, whether in the form of simple $CuSO_4$ or in the form of Cu^{II} complexes, are irradiated with ^{60}Co γ-rays, Cu^{II} is reduced to Cu^I and uracil is degraded to give several products which can be separated by paper chromatography.

EFFECT OF $CuSO_4$

The pattern of radioactivity on paper chromatograms developed in butan-1-ol + water (86 : 14) solutions of γ-irradiated aqueous solutions of uracil in the presence of $CuSO_4$ in deaerated and N_2O-saturated solutions is shown in fig. 1. The similarities between the chromatograms obtained under both sets of conditions show that the same types of products are formed. The product at peak I is of a dimeric type and may consist of several isomeric forms.[10, 12, 14] Peaks II and III refer to *cis*- and *trans*-uracil glycols, respectively. Peak IV comprises two isomers of hydroxydihydrouracil, whereas peak V is due to isobarbituric acid. The large peak is due to undecomposed uracil.

Fig. 2 shows the increase in formation of Cu^+ species with the absorbed dose. The formation of Cu^+ is linear with dose up to a dose of *ca.* 1.5×10^{18} eV cm^{-3}, and accordingly $G(Cu^+)$ is determined from the initial slope. However, note that the product yields arising from uracil degradation were determined at relatively high doses because of the difficulty in measuring the small amounts of products formed at very low doses. Fig. 3 shows some typical plots of product formation as a function of dose [$(1-8) \times 10^{18}$ eV cm^{-3}]. Table 1 shows the effect of increasing concentration of $CuSO_4$ on the formation of Cu^+ and on the decomposition of uracil under different conditions of radiolysis. The initial $G(Cu^+)$ and $G(-\text{Ura})$ values do not depend on the initial concentration of Cu^{II}. In dilute aqueous solution the reactive primary radicals (H, OH, e^-_{aq}) derived from the radiolysis of water

$$H_2O \rightarrow H, OH, e^-_{aq}, H_2, H_2O_2, H_3O^+ \qquad (1)$$

will react with uracil depending on the relative rate constants for their reactions with uracil and Cu^{II}. From the known rate-constant data[21, 22] it is obvious that at very low concentrations of $CuSO_4$ *i.e.* 1×10^{-4} mol dm^{-3}, where [Ura] $= 10^{-3}$ mol dm^{-3}, both OH and e^-_{aq} will react with uracil but not with $CuSO_4$:

$$\text{Ura} + OH \rightarrow \text{UraOH} \qquad (2)$$

$$\text{Ura} + e^-_{aq} \rightarrow \text{Ura}^- \qquad (3)$$

$$\text{Ura}^- + H^+ \rightleftharpoons \text{UraH}. \qquad (4)$$

Hence the degradation of the base as well as the formation of Cu^+ must arise from the primary formation of UraOH and Ura^-. However, when the concentration of

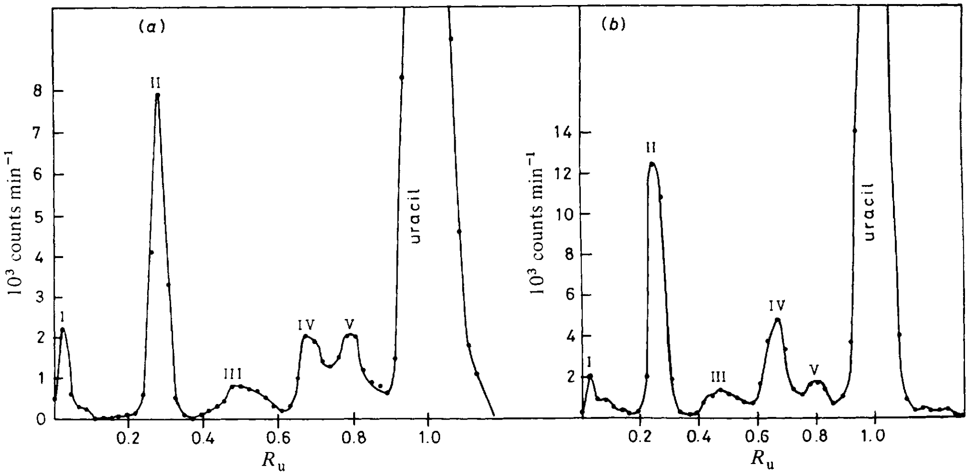

Fig. 1. Pattern of radioactivity on chromatograms of irradiated 2×10^{-3} mol dm^{-3} uracil solution in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ at pH *ca.* 5.5; dose = 8.6×10^{18} eV cm^{-3}. (*a*) Argon-saturated solution; (*b*) N_2O-saturated solution. R_u denotes the distance travelled by each product relative to the distance travelled by uracil.

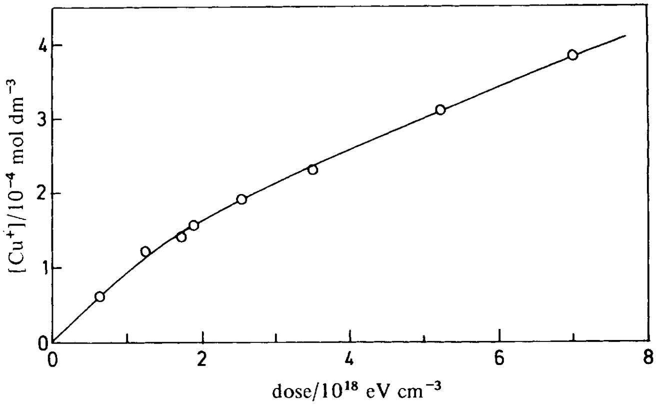

Fig. 2. Formation of Cu^+ species with absorbed dose in the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in N_2O-saturated solution.

$CuSO_4$ is raised to 2×10^{-3} mol dm^{-3} while keeping [Ura] constant, OH will react with uracil but e^-_{aq} is expected to react mostly with Cu^{II}. On the other hand, table 1 shows that there is no change in the observed yields of $G(Cu^+)$ and $G(-Ura)$.

The G values of the various uracil degradation products formed in the γ-radiolysis of uracil in the presence of $CuSO_4$ are shown in table 2, together with the yields of degradation of the base and formation of the products in the absence of a metal ion for comparison. From table 2 it is evident that when the amount of $CuSO_4$ remains the same but the concentration of uracil is doubled, the product yield distribution remains almost the same.

In absence of any metal ion, the base degradation yields in argon- and N_2O-saturated solutions of uracil were found to be *ca.* 2.7 and *ca.* 3.3, respectively. However, when $CuSO_4$ is added, $G(-Ura)$ in N_2O-saturated solution increases to as much as *ca.* 5.1,

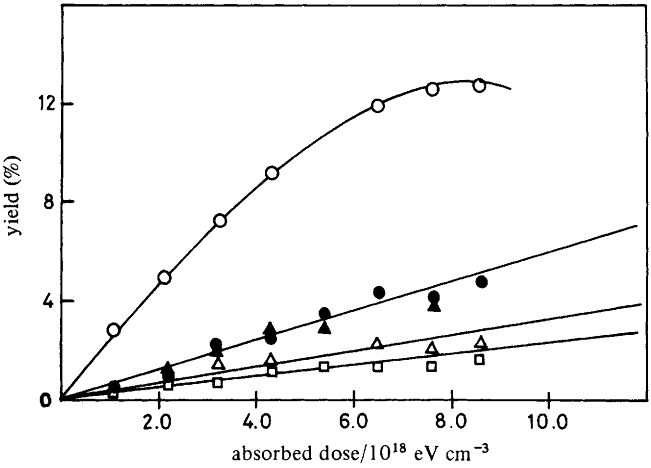

Fig. 3. Effect of absorbed dose on the formation of different base degradation products during the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in argon-saturated solution at pH *ca.* 5.5. ○, Loss of uracil; ●, *c*-uracil glycol; □, *t*-uracil glycol; △, hydroxydihydrouracil; ▲, isobarbituric acid.

Table 1. Effect of concentration of Cu^{2+} ions on initial yield of *G*(−Ura) and *G*(Cu^+) in deaerated and N_2O-saturated solution[a]

$[CuSO_4]$ /10^{-4} mol dm^{-3}	condition	*G*(Cu^+)	*G*(−Ura)
1.0	argon-saturated	5.4	2.6
5.0	argon-saturated	5.4	2.8
10.0	argon-saturated	5.6	2.6
20.0	argon-saturated	5.2	2.6
10.0	N_2O-saturated	5.3	5.6

[a] [Ura] = 10^{-3} mol dm^{-3}.

but in argon-saturated solution the value remains the same, *i.e. ca.* 2.7, which is in agreement with the value reported by Holian and Garrison.[3] Cu^{II} appears to act as a radiosensitizer in an N_2O-saturated medium, whereas no such sensitization is evident in a deaerated medium. In argon-saturated solution, although *G*(−Ura) and the observed yields of isobarbituric acid are identical to those reported by Holian and Garrison,[3] the yields of glycols determined in this investigation are much lower than those reported in ref. (3). Furthermore, in contrast to the observations of Holian and Garrison,[3] two other products (the dimer and hydroxydihydrouracil) have been detected in appreciable yields.

However, table 2 shows that in general there are marked differences in the relative yields of the various products when radiolysis is carried out in the presence and absence of $CuSO_4$. Thus when $CuSO_4$ is present in the system, the yield of the dimeric product decreases drastically with a simultaneous increase in the yields of glycols and

Table 2. *G* values of products of the γ-radiolysis of uracil in the presence or absence of $CuSO_4$ under different conditions at pH *ca.* 5.5

products	conditions						
	A	B	C	D	E	F	G
dimer	0.36 (13.8%)	0.30 (12.2%)	0.45 (9.7%)	0.50 (10.6%)	0.40 (15.2%)	0.33 (12.2%)	1.50 (48.3%)
c-uracil glycol	0.70 (27.0%)	0.80 (32.6%)	1.70 (36.5%)	1.70 (36.2%)	0.20 (7.6%)	0.44 (16.3%)	0.40 (12.9%)
t-uracil glycol	0.30 (11.5%)	0.30 (12.2%)	0.70 (15.0%)	0.80 (17.0%)	0.30 (11.4%)	0.61 (22.6%)	0.50 (16.1%)
hydroxydihydrouracil	0.42 (16.2%)	0.40 (16.3%)	0.80 (17.2%)	0.50 (10.6%)	0.12 (4.5%)	0.27 (10.0%)	0.40 (12.9%)
isobarbituric acid	0.66 (25.4%)	0.50 (20.4%)	0.80 (17.2%)	0.90 (19.1%)	1.30 (49.6%)	0.72 (26.7%)	0.30 (9.6%)
dialuric acid	—	—	0.30 (4.3%)	0.30 (6.3%)	0.30 (11.4%)	0.32 (11.9%)	0.00
uracil degraded	2.80	2.70	5.10	5.10	2.6	2.80	3.10 (3.3)[a]

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in argon-saturated solution. (B) 1×10^{-3} mol dm^{-3} uracil in presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in argon-saturated solution. (C) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in N_2O-saturated solution. (D) 1×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in N_2O-saturated solution. (E) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in aerated solution. (F) 2×10^{-3} mol dm^{-3} uracil in oxygen-saturated solution. (G) 2×10^{-3} mol dm^{-3} uracil in N_2O-saturated solution. [a] Obtained spectrophotometrically.

isobarbituric acid. Note that in the presence of $CuSO_4$ *cis*-uracil glycol constitutes the major product.

EFFECT OF Cu^{II} COMPLEXES

The radiosensitivity of uracil in the presence of different Cu^{II} complexes, *e.g.* Cu^{II}EDTA, Cu^{II}NTA, Cu^{II}IDA and $Cu^{II}(IDA)_2$, was studied to ascertain whether the role of the Cu^{II} ion is modified due to its complexation. The specific rate constants for the reactions of OH with these Cu^{II} aminopolycarboxylates are *ca.* 10^8–10^9 $dm^3\ mol^{-1}\ s^{-1}$[6-8, 23, 24] and that with uracil is *ca.* 10^{10} $dm^3\ mol^{-1}\ s^{-1}$.[21] The concentrations of the complexes were chosen so that the OH radicals react with uracil but not with the complexes. Thus the concentrations of the complexes were chosen to be 5×10^{-4} mol dm^{-3}, whereas that of uracil was taken as *ca.* 2×10^{-3} mol dm^{-3}. Hence the complexes are expected to react with transient uracil adducts such as UraOH, Ura^- and UraH.

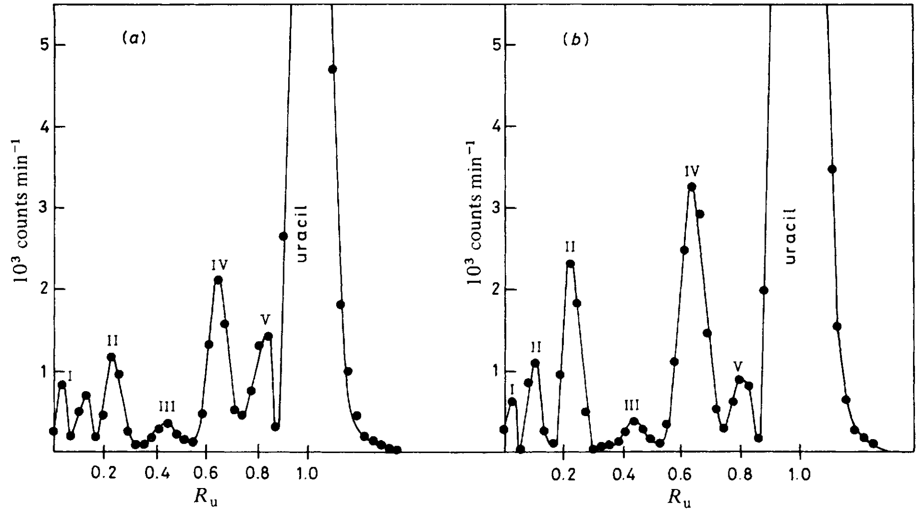

Fig. 4. Pattern of radioactivity on chromatograms of 2×10^{-3} mol dm^{-3} uracil solution irradiated in the presence of 5×10^{-4} mol dm^{-3} Cu^{II}EDTA at pH *ca.* 5.5. (*a*) Argon-saturated solution; (*b*) N_2O-saturated solution; absorbed dose *ca.* 5.9×10^{18} eV cm^{-3}.

The products formed during radiolysis in the presence of Cu^{II} complexes were separated in a similar way to that described above. The products formed in the presence of the complexes were the same as those observed in the presence of $CuSO_4$. Fig. 4 shows a typical pattern of radioactivity observed in the chromatograms of irradiated uracil in the presence of Cu^{II}EDTA. In some instances the first peak (peak I), which has been assigned to isomers of the dimer, has been resolved into two components. However, for convenience, the total dimer yield only is reported in each case.

Plots of the observed yield as a function of dose for the formation of different radiolytic products as well as the decomposition of uracil are shown in fig. 5. Accordingly, the *G* values were determined from the slope of the initial linear portion of each curve. The *G* values are summarised in tables 3–6. The relative percentages of each product are indicated in parentheses. From tables 3–6 it is evident that the degradation of the base is not influenced significantly by the metal complex present.

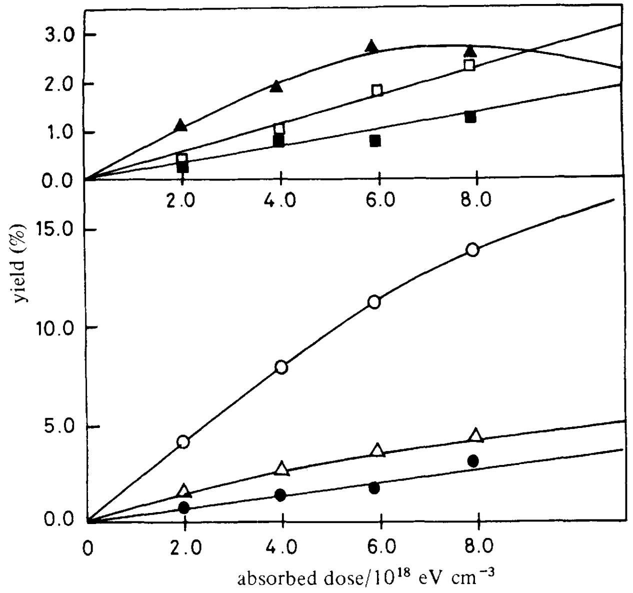

Fig. 5. Radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu^{II}EDTA$ at pH *ca.* 5.5. ○, −Uracil; □, dimer; ●, *c*-uracil glycol; △, hydroxydihydrouracil; ▲, isobarbituric acid; ■, *t*-uracil glycol.

Table 3. *G* values of products of the γ-radiolysis of uracil in the presence of CuEDTA at neutral pH (*ca.* 5.5) under different conditions

products	conditions A	conditions B	conditions C
dimer	0.45 (17.9%)	1.03 (23.4%))	0.55 (21.3%)
c-uracil glycol	0.40 (15.9%)	0.60 (13.6%)	0.15 (5.8%)
t-uracil glycol	0.20 (7.9%)	0.18 (4.1%)	0.36 (13.9%)
hydroxydihydrouracil	0.85 (33.9%)	1.4 (31.7%)	0.12 (4.7%)
isobarbituric acid	0.60 (24.3%)	1.2 (27.2%)	1.00 (38.8%)
dialuric acid	—	—	0.40 (15.5%)
uracil degraded	2.5	4.9	2.60

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuEDTA under deaerated conditions. (B) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuEDTA in N_2O-saturated solution. (C) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuEDTA in aerated solution.

G(−Ura) values for the complexes vary between 2.4 and 2.7 in deaerated solution, whereas in N_2O-saturated solution they vary in the range 4.2–5.0. The yields of the products are in general comparable for the various complexes, with certain exceptions. In general, when Cu^{II} is present as the complex rather than the free ion, hydroxydihydrouracil constitutes the major product under both argon-saturated and N_2O-saturated conditions of radiolysis.

Table 4. *G* values of products of the γ-radiolysis of uracil in the presence of CuNTA under different conditions at pH *ca.* 5.5

	conditions		
products	A	B	C
dimer	0.22 (10.3%)	0.40 (10.2%)	0.55 (18.4%)
c-uracil glycol	0.60 (28.3%)	1.10 (18.2%)	0.15 (5.0%)
t-uracil glycol	0.17 (8.0%)	0.30 (7.7%)	0.28 (9.4%)
hydroxydihyrouracil	0.80 (37.7%)	1.50 (38.5%)	0.15 (5.0%)
isobarbituric acid	0.33 (15.6%)	0.60 (15.4%)	1.50 (50.2%)
dialuric acid	0.00	0.00	0.36 (12.0%)
uracil degraded	2.44	4.2	3.0

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuNTA in deaerated solution. (B) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuNTA in N_2O-saturated solution. (C) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuNTA in aerated solution.

Table 5. *G* values of products of the γ-radiolysis of uracil in the presence of CuIDA under different conditions at pH *ca.* 5.5

	conditions		
products	A	B	C
dimer	0.28 (10.3%)	0.60 (13.3%)	0.33 (13.3%)
c-uracil glycol	0.83 (30.7%)	1.50 (33.4%)	0.20 (8.1%)
t-uracil glycol	0.16 (5.9%)	0.23 (5.1%)	0.42 (16.9%)
hydroxydihydrouracil	0.98 (36.3%)	1.50 (33.4%)	0.11 (4.4%)
isobarbituric acid	0.45 (16.7%)	0.60 (13.4%)	1.00 (40.3%)
dialuric acid	0.00	—	0.42 (16.9%)
uracil degraded	2.7	5.0	2.6

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuIDA in deaerated solution. (B) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuIDA in N_2O-saturated solution. (C) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} CuIDA in aerated solution.

DISCUSSION

Initial $G(Cu^+)$ values measured in an appreciably lower dose range during the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of $CuSO_4$ and Cu^{II} complexes are summarised in table 7. When the metal ion is free Cu^{II}, $G(Cu^+)$ is 5.4 ± 0.5 in argon-saturated solution. Under those conditions, H, OH and e^-_{aq} react with uracil[21, 22] but not with Cu^{II}. Hence the reduction of Cu^{II} must be ascribed to the reactions of metal ions with the transient uracil radicals UraOH, UraH and Ura^-.

In N_2O-saturated solution, where e^-_{aq} is converted to an equivalent amount of OH,

$$e^-_{aq} + N_2O \rightarrow OH + N_2 + OH^- \qquad (5)$$

Table 6. *G* values of products of the γ-radiolysis of uracil in the presence of $Cu(IDA)_2$ under different conditions at pH *ca.* 5.5

	conditions		
products	A	B	C
dimer	0.24 (11.2%)	0.66 (15.9%)	0.40 (15.7%)
c-uracil glycol	0.60 (27.9%)	0.90 (21.6%)	0.27 (10.6%)
t-uracil glycol	0.30 (13.9%)	0.30 (7.2%)	0.36 (14.2%)
hydroxydihydrouracil	0.83 (38.6%)	1.70 (40.9%)	0.21 (8.3%)
isobarbituric acid	0.18 (8.4%)	0.60 (14.4%)	1.10 (43.3%)
dialuric acid	—	—	0.20 (7.9%)
uracil degraded	2.4	4.3	2.5

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu(IDA)_2$ in deaerated solution. (B) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu(IDA)_2$ in N_2O-saturated solution. (C) 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu(IDA)_2$ in aerated solution.

Table 7. Initial *G* values of Cu^+ and H_2O_2 formed in the γ-radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of different Cu^{II} compounds under different conditions at neutral pH

Cu^{II} compound[a]	condition	$G(Cu^+)$	$G(H_2O_2)$
$CuSO_4$	argon-saturated	5.4 ± 0.5	0.7
$CuSO_4$	N_2O-saturated	5.3 ± 0.5	0.8
$CuSO_4$	aerated	1.1 ± 0.2	1.1
$Cu^{II}EDTA$	argon-saturated	5.1 ± 0.4	0.4
$Cu^{II}EDTA$	N_2O-saturated	5.0 ± 0.5	0.5
$Cu^{II}NTA$	argon-saturated	4.3 ± 0.3	0.7
$Cu^{II}NTA$	N_2O-saturated	4.5 ± 0.3	0.7
$Cu^{II}NTA$	aerated	2.0 ± 0.2	2.9
$Cu^{II}IDA$	argon-saturated	5.1 ± 0.5	0.6
$Cu^{II}IDA$	N_2O-saturated	5.5 ± 0.5	0.6
$Cu^{II}(IDA)_2$	argon-saturated	4.0 ± 0.5	0.8
$Cu^{II}(IDA)_2$	N_2O-saturated	4.6 ± 0.5	0.9
$Cu^{II}(IDA)_2$	aerated	1.5 ± 0.2	0.8

[a] Concentration of Cu^{II} in all the compounds is 5.0×10^{-4} mol dm^{-3}.

the initial yield of Cu^+, $G(Cu^+)$, is 5.3 ± 0.5. Thus, under both these sets of conditions, the $G(Cu^+)$ values appear to be closer to the total radical yield, $G(e^-_{aq}) + G(OH) + G(H) = 6.0$. When Cu^{II} is complexed there is no significant change in the total yield. Only when the Cu^{II} complexes are $Cu^{II}NTA$ or $Cu^{II}(IDA)_2$ are the Cu^+ yields lower in comparison with those obtained with other Cu^{II} compounds. This is probably due to the fact that some portions of $Cu^{I}NTA$ and $Cu^{I}(IDA)_2$ decompose[7] to give Cu_2O.

From table 2(A)–(D) it is evident that in the presence of $CuSO_4$, the $G(-Ura)$ value observed in N_2O-saturated solution is nearly double that observed in argon-saturated solution. Hence it may be inferred that the base degradation is initiated mainly by the UraOH radical. It has been mentioned earlier that the reduction of Cu^{II} appears to be equivalent to the total yield of all the transient radicals, UraOH, UraH and Ura^-; it may then be presumed that the UraH and Ura^- species that are formed in Ar-saturated solution reduce Cu^{II} to Cu^I and do not produce an intermediate compound that results in product formation but reverts back to the parent uracil. Thus, when a 2×10^{-3} mol dm^{-3} solution of uracil containing 5×10^{-4} mol dm^{-3} $CuSO_4$ is irradiated

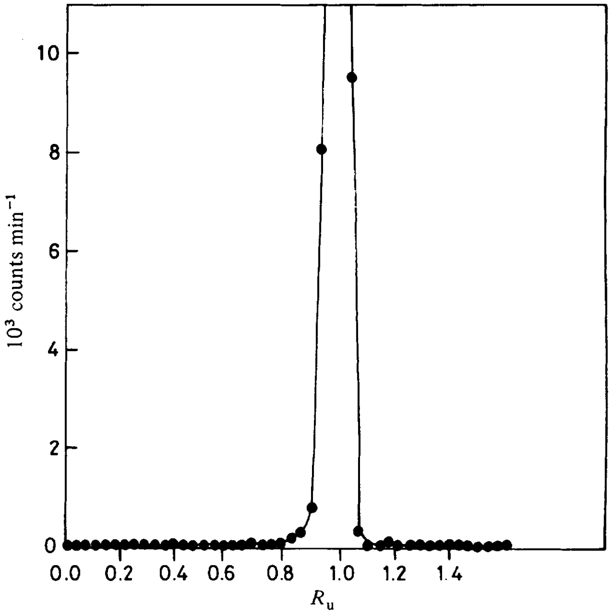

Fig. 6. Chromatogram of γ-irradiated 2×10^{-3} mol dm^{-3} uracil solution in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ and 0.4 mol dm^{-3} Bu^tOH in argon-saturated solution; pH *ca.* 5.5; absorbed dose = 4.0×10^{18} eV cm^{-3}. The peak is due to unchanged uracil.

in the presence of 0.4 mol dm^{-3} Bu^tOH in argon-saturated solution, no product formation has been identified (fig. 6). Since Bu^tOH is a good scavenger[22] of OH

$$(CH_3)_3\,COH + OH \rightarrow CH_2C(CH_3)_2\,OH + H_2O \qquad (6)$$

it leaves e^-_{aq} in the system, which consequently forms Ura^- with uracil. Hence it may be concluded that e^-_{aq} or Ura^- does not induce degradation but regenerates uracil *via* reaction (7):

$$Ura^- + Cu^{II} \rightarrow Cu^I + Ura. \qquad (7)$$

However, under these conditions no Cu^+ species are detected in the system whether the Cu^{II} is simple $CuSO_4$ or a Cu^{II} complex. Hence it transpires that either reaction (7) is absent or Cu^+ somehow disappears under these conditions. The observed yield of formation of Cu^+ in argon-saturated solution clearly indicates that Ura^- reduces Cu^{II} to Cu^I. The failure to detect Cu^I in the presence of Bu^tOH may then result from the oxidation of Cu^I by $CH_2C(CH_3)_2OH$ radicals.[25, 26]

In reducing Cu^{II} to Cu^I the UraH and UraOH radicals presumably give rise to $UraH^+$ and $UraOH^+$:

$$UraH + Cu^{II} \rightarrow Cu^I + UraH^+ \qquad (8)$$

$$UraOH + Cu^{II} \rightarrow Cu^I + UraOH^+. \qquad (9)$$

However, the carbonium ion, $UraH^+$, is known to give back uracil[5, 13, 27]

$$UraH^+ + H_2O \rightarrow Ura + H_3O^+. \qquad (10)$$

Thus the products may be thought of as being formed from $UraOH^+$ only, since $UraOH^+$ is known[10] to give rise to glycols and isobarbituric acid by reaction (11)

$$UraOH^+ \begin{array}{l} \xrightarrow{H_2O} Ura(OH)_2 \\ \xrightarrow[H_2O]{} UraO \text{ (isobarbituric acid).} \end{array} \qquad (11)$$

Cis-uracil glycol constitutes the major product of radiolysis under these conditions, and other products comprise *trans*-uracil glycol and isobarbituric acid. Kochi *et al.*,[28] however, suggested a one-step process for the electron transfer [reaction (9)] without the formation of discrete carbonium ion

$$UraOH + Cu^{II} \rightarrow Cu^{I} + Ura(OH)_2 + UraO. \qquad (12)$$

The question may arise as to whether Cu^I formed in the aforementioned reactions undergoes Fenton's reaction with molecular H_2O_2. This possibility can be eliminated for two reasons; first, the observed H_2O_2 yield corresponds to that due to the molecular product (table 7), which means that there is no loss of H_2O_2; secondly, if such a reaction were to occur, more OH radicals would be produced, which would lead to an increase in $G(-Ura)$ and this is not compatible with experimental observations (table 2).

The initial $G(Cu^+)$ values (tables 1 and 7), as computed from the low-dose region, do not have any significant contribution arising from the reduction of Cu^{II} to Cu^I by H_2O_2 in the presence of neo-cuproin. This is because this reduction is dependent on the concentration of H_2O_2, and at this low dose the concentration of H_2O_2 formed as molecular product does not exceed *ca.* 10^{-5} mol dm^{-3}. Such low concentrations of H_2O_2 have been found to have every little effect on the observed yield of Cu^I.

Product-analysis studies show that besides the formation of glycols and isobarbituric acid, as discussed above, two other products, *i.e.* dimers and hydroxydihydrouracil (UraHOH), have been detected in these systems (tables 2–6). When Cu^{II} ion is present as a Cu^{II} complex, the amounts of these two products increase at the expense of the glycols and isobarbituric acid. Obviously hydroxydihyrouracil and the dimer cannot both be formed from $UraOH^+$. Although the dimer can be formed by the combination of ion pairs,[12] $UraOH^+$ and $UraOH^-$, the only possible precursor for the formation of hydroxydihydrouracil is $UraOH^-$.[5, 10, 13] Hence a possible mode of reaction that may lead to the formation of $UraOH^-$ must be sought.

Recent pulse-radiolysis studies[29] indicate that the C_5—OH adduct of uracil reduces tetranitromethane whereas C_6—OH adduct of uracil oxidizes *N*,*N*, *N*′,*N*′-tetramethyl-*p*-phenylene diamine. Then it might be argued that the C_6—OH adduct of uracil oxidizes the Cu^{II} complex from which $UraOH^-$ and UraHOH should be formed. However, in that case the oxidized product should be either Cu^{III}[7, 8, 30, 31] or a dehydrogenated radical[6, 8] species which may ultimately give rise to Cu^I and carbonyl compounds such as formaldehyde or glyoxalic acid. However, the absence of carbonyl compounds excludes the proposed oxidation of Cu^{II} by UraOH.

From fig. 2 it is evident that the rate of formation of Cu^+ is not the same at low and high doses. The observed results of the formation of Cu^+ at higher doses also include the contribution arising from the reduction of Cu^{II} to Cu^I by H_2O_2 in the presence of neo-cuproin. It may be concluded that the rate of formation of Cu^+ is significantly lower at higher doses than that at low doses. This change is more marked in the case of Cu^{II}EDTA, as shown in fig. 7. This depletion of Cu^I at high doses must arise from secondary reactions involving Cu^I. One possibility is that Cu^I disproportionates into

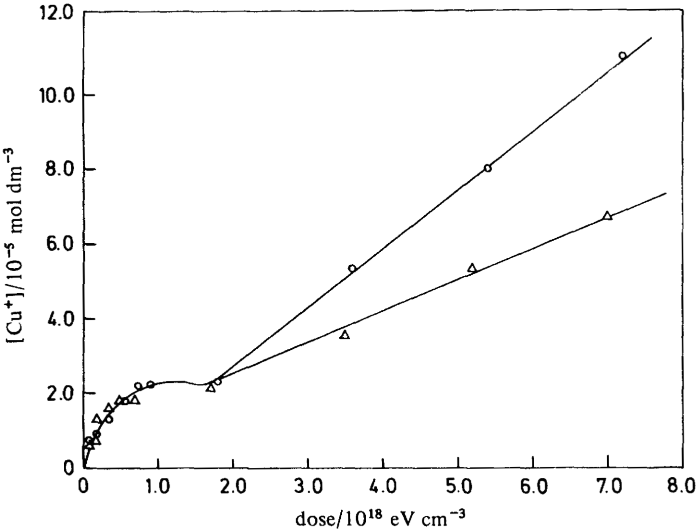

Fig. 7. Effect of absorbed dose on the formation of Cu^+ during the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu^{II}EDTA$ at pH *ca.* 5.5. ○, Argon-saturated solution; △, N_2O-saturated solution.

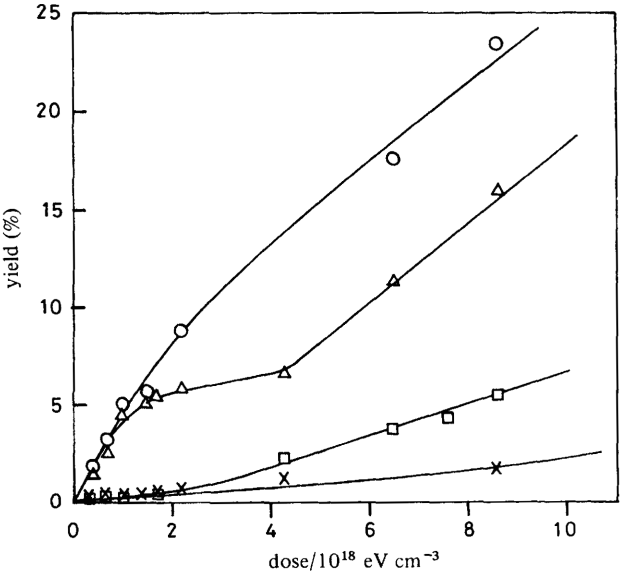

Fig. 8. Effect of absorbed dose on the decomposition of uracil and the formation of different products in the γ-radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in N_2O-saturated solution. ○, Decomposition of uracil; △, *c*-uracil glycol + *t*-uracil glycol + isobarbituric acid; □, hydroxydihydrouracil; ×, dimer. The product formation as well as base decomposition were followed from very low absorbed dose.

Cu^{II} and metallic copper. However, this appears not to be the case because no metallic copper could be identified in the system. The question then arises as to whether Cu^{I} is involved in other oxidation–reduction reactions involving radicals. In this context the C_6—OH radical is known to be oxidizing;[29] such an UraOH adduct may oxidize the Cu^{I} species to Cu^{II}. This postulate can be verified if one follows the degradation of uracil and the formation of products at very low doses. Although in the low dose range the quantitative measurement of products becomes difficult because their concentrations are low, attempts were made to measure the products at these low doses. The irradiated solution was evaporated to dryness at 35 °C and then dissolved in a minimum of water. A known volume of this concentrated solution was spotted and developed by the same chromatographic procedure as described before. However, during the process of evaporation *cis*- and *trans*-glycols may isomerize and the glycols may be converted to isobarbituric acid;[14] hence instead of considering these products separately their total yields were only evaluated at low doses. Fig. 8 shows the formation of products in both low- and high-dose ranges in the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of $CuSO_4$ in an N_2O-saturated medium. The radiation-chemical yields for the degradation of uracil, whether determined at low or high doses, do not change, but the yields of glycols and isobarbituric acid when determined at low doses are much higher than those estimated at higher doses. Initially the accumulation of both dimer and hydroxydihydrouracil is negligibly small, but at higher doses it becomes appreciable and increases linearly with dose. Similar behaviour is also observed in the radiolysis of 2×10^{-3} mol dm^{-3} uracil in presence of 5×10^{-4} mol dm^{-3} Cu^{II}EDTA as shown in fig. 9. Hence from such product analysis as well as from following the fate of Cu^{I} species it may be inferred that hydroxydihydrouracil and its dimer are secondary products which may arise from the reaction between UraOH and Cu^{I}:

$$Cu^{I}+UraOH \xrightarrow{H_2O} Cu^{II}+UraHOH+OH^- \tag{13}$$

$$Cu^{I}+UraOH \xrightarrow{UraOH^+} Cu^{II}+UraOH\cdot UraOH. \tag{14}$$

One consequence of reactions (13) and (14) is that the formation of Cu^{I} should be decelerated at higher doses. On the contrary, when uracil is radiolysed in the presence of Cu^{II} or its complexes (except that with EDTA) the formation of Cu^{I} is not reduced significantly at higher doses. Moreover, since $UraOH^-$ is the plausible intermediate in such reactions [reactions (13) and (14)] the reversion of $UraOH^-$ to uracil also constitutes a distinct possibility:[10, 13]

$$UraOH^- \xrightarrow{H_2O} Ura+OH^- \tag{15}$$

whence the base degradation yield should decrease. However, this is not reflected in the results shown in the tables 2–6.

The observed results may find an explanation if an intermediate is formed between Cu^{I} and UraOH which will lead to the formation of Cu^{II} in the presence of O_2 but will give evidence of Cu^{I} when reacted with neo-cuproin. Such a situation has been reported[7] earlier in the radiolysis of Cu^{II}NTA in presence of alcohol. When Cu^{II}NTA is radiolysed in the presence of alcohols in argon-saturated solution the proposed intermediate was shown to contain a copper–carbon bond:

$$Cu^{I}NTA+R_1R_2COH \rightarrow NTACu^{II}\text{—}CR_1R_2OH. \tag{16}$$

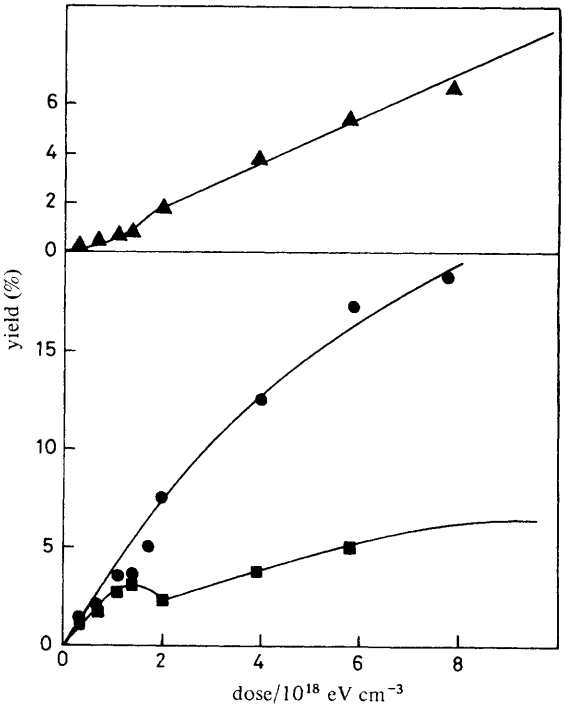

Fig. 9. Effect of absorbed dose in the radiolysis of uracil (2×10^{-3} mol dm^{-3}) in the present of 5×10^{-4} mol dm^{-3} $Cu^{II}EDTA$ in N_2O-saturated solution; ●, decomposition of uracil; ■, formation of glycols (*cis*- and *trans*-)+isobarbituric acid; ▲, formation of dimer+hydroxydihydrouracil.

The proposed intermediate was found to absorb in the region 270–620 nm, having two peaks: one in the region 340–350 nm and the other in the region 560–565 nm.

Fig. 10 shows the difference spectrum of the products formed in the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $Cu^{II}NTA$ in N_2O-saturated solution at different absorbed doses. At low absorbed doses no appreciable absorption was observed in the range 320–700 nm. However, if the dose exceeds *ca.* 3.5×10^{18} eV cm^{-3} the radiolysed solution has an appreciable absorption in that range. Further, the observed spectrum consists of two absorption bands in the regions 320–460 and 540–680 nm. Under the experimental conditions employed, the OH radical attacks uracil, but not $Cu^{II}NTA$, to form UraOH. $Cu^{II}NTA$ is not oxidized by UraOH as observed earlier, but is readily reduced by it as a result of which some $Cu^{I}NTA$ is formed. When sufficient $Cu^{I}NTA$ is formed at higher doses it may form a similar copper–carbon linkage with UraOH:

$$Cu^{I}NTA + UraOH \rightarrow NTACu^{II}\text{—}UraOH. \qquad (17)$$

Similar spectral characteristics leading to the formation of a copper–carbon bond have also been observed when uracil is irradiated in the presence of $CuSO_4$ or other Cu^{II} complexes under study. Such an intermediate, when treated with neo-cuproin, will respond to the test as Cu^{I} (table 8), but when allowed to stand in air or O_2 will slowly decompose to give Cu^{II} species, as is also evident from the observed post-radiolytic decrease in the absorbance in the region 320–680 nm (fig. 11).

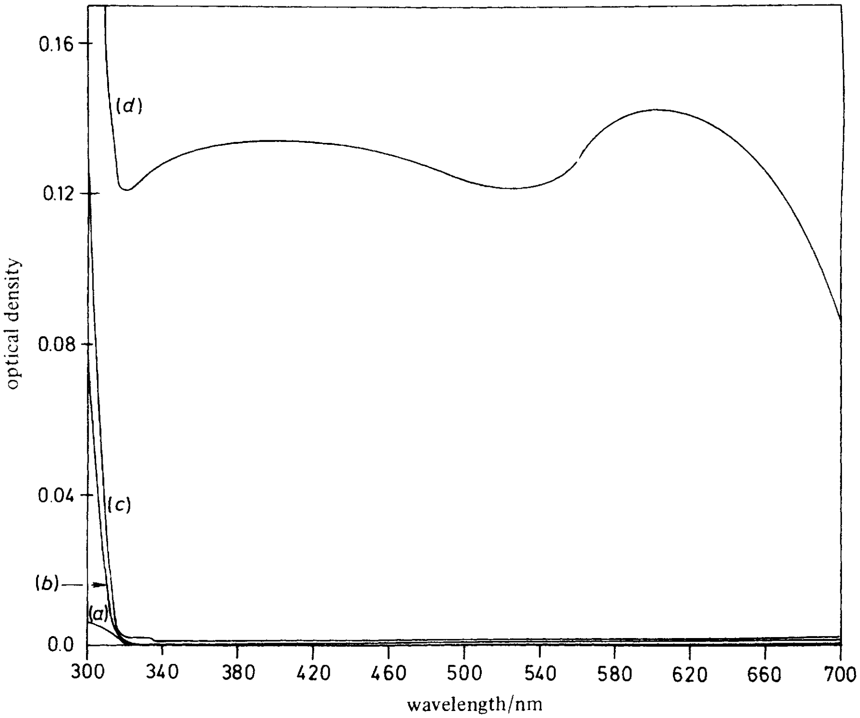

Fig. 10. Difference spectrum of the products formed in the γ-radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} Cu^{II}NTA in N_2O-saturated solution at absorbed doses of (*a*) 0.8×10^{18}, (*b*) 1.7×10^{18}, (*c*) 2.6×10^{18} and (*d*) 3.4×10^{18} eV cm^{-3}. pH *ca.* 5.5. Time required for recording each spectra was *ca.* 5 min.

Table 8. Post-radiolytic decrease in the concentration of Cu^I formed during the radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} $CuSO_4$ in N_2O-saturated solution. Absorbed dose = 1.74×10^{18} eV cm^{-3}.

post-radiolytic time/h	[Cu^I]/10^{-5} mol dm^{-3}
0	13.0
1.5	6.7
23	4.5
24	4.4
25	4.4

EFFECT OF OXYGEN

When aerated solutions of uracil are radiolysed in presence of Cu^{II} ions, the concentration of hydroxydihydrouracil becomes negligibly small with a concomitant increase in the yields of *trans*-glycol and isobarbituric acid. In aerated solutions some amount of dialuric acid ($R_u = 1.3$ in the paper chromatogram) has also been

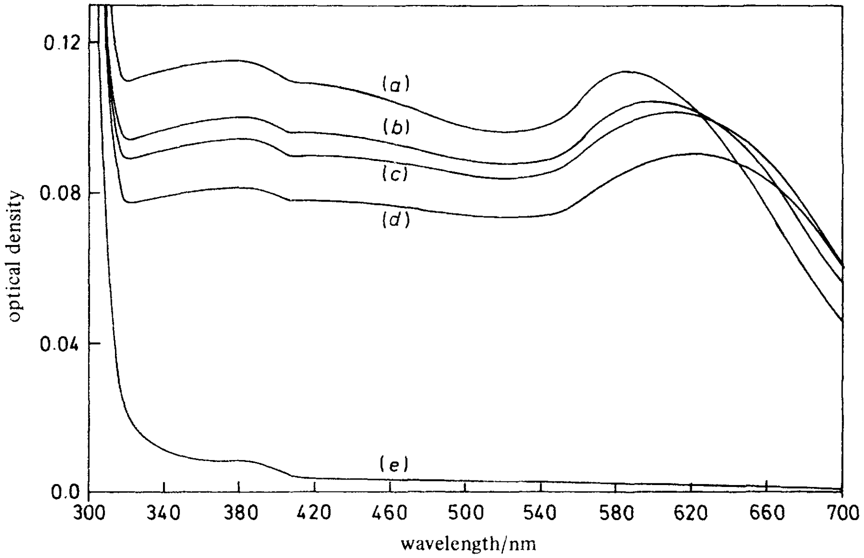

Fig. 11. Radiolysis of 2×10^{-3} mol dm^{-3} uracil in the presence of 5×10^{-4} mol dm^{-3} Cu^{II}NTA in N_2O-saturated solution. Post-radiolytic effect on the absorbtion spectra of the Cu^{I}NTA and UraOH adduct. Spectrum recorded (*a*) 5 min, (*b*) 30 min, (*c*) 60 min and (*d*) 90 min after irradiation; (*e*) spectrum recorded 30 min after oxygen diffusion into the radiolysed solution.

identified. This can be readily explained by assuming that UraOH has no other alternative but to react with O_2, leading to the formation of peroxides which are known to lead to the formation of glycols, dialuric acid and isobarbituric acid:[9, 21]

$$\mathrm{UraOH} + O_2 \rightarrow \mathrm{UraOH}(O_2) \rightarrow \mathrm{UraOH} \cdot O_2H \rightarrow \text{products.} \qquad (18)$$

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