Interaction of Triplet-state Nucleic Acid Bases with Electroaffinic Molecules in Solution by Laser Flash Photolysis

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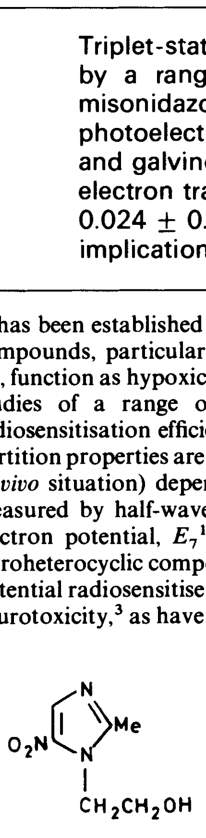
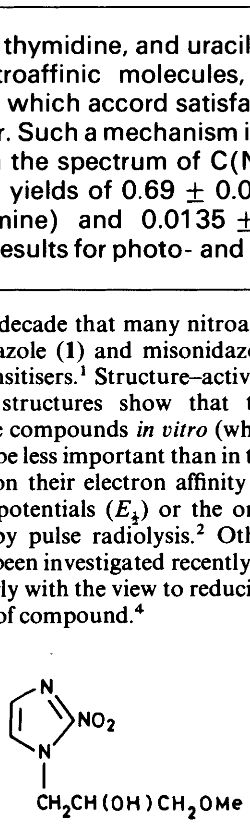
Triplet-state thymine, thymidine, and uracil, generated by 249.1 nm laser flash photolysis, are quenched by a range of electroaffinic molecules, including the radiosensitising drugs metronidazole and misonidazole, at rates which accord satisfactorily with the predictions of the Rehm–Weller equation for photoelectron transfer. Such a mechanism is confirmed for two of the selected solutes, tetranitromethane and galvinoxyl, when the spectrum of $C(NO_2)_3^-$ and of galvinoxyl anion are observed, indicating net electron transfer with yields of 0.69 ± 0.03 (thymine) and 0.40 ± 0.03 (uracil) for $C(NO_2)_3^-$ and of 0.024 ± 0.002 (thymine) and 0.0135 ± 0.0005 (uracil) for galvinoxyl anion. The mechanistic implications of these results for photo- and radiation-damage to DNA and cellular systems are discussed.

It has been established for about a decade that many nitroaryl compounds, particularly metronidazole (**1**) and misonidazole (**2**), function as hypoxic cell radiosensitisers.[1] Structure–activity studies of a range of nitroaryl structures show that the radiosensitisation efficiency of these compounds *in vitro* (when partition properties are expected to be less important than in the *in vivo* situation) depends largely on their electron affinity as measured by half-wave reduction potentials ($E_{\frac{1}{2}}$) or the one-electron potential, E_7^1, obtained by pulse radiolysis.[2] Other nitroheterocyclic compounds have been investigated recently as potential radiosensitisers, particularly with the view to reducing neurotoxicity,[3] as have other types of compound.[4]



(**1**)



(**2**)

Discussion of the mechanism of action of the radiosensitisers has centred around a few models. In the so-called charge sequestration model,[5] the lethality of ionising radiation is associated with *direct* damage to DNA *via* an ionisation mechanism to give charge separation localised at guanine centres (as positive holes) and thymine centres (as electron adducts): the action of the radiosensitising drug, for example metronidazole (Met), is to accept the negative charge and localise it as $Met^{-\bullet}$, *i.e.* to prevent charge recombination and restitution. These paramagnetic centres undergo secondary reactions with sugar residues culminating in single-strand breakage of DNA. The target site in DNA is often taken to be the base residues, particularly guanine,[6] but Greenstock and Whitehouse believe the damage is inflicted at the sugar–phosphate backbone.[7] However, biphotonic photoionisation of polyuridylic acid in anoxic aqueous solution leads to single-strand breakage implying that the uracil cation centre is the essential primary intermediate in this process.[8]

An alternative mechanism for the action of misonidazole, which is possibly more relevant to the cytotoxic effects of nitroaryl compounds, is based on the observation that electrolytically reduced misonidazole reduces both the relative viscosity and the hyperchromicity of DNA, mainly by single-strand breakage, *i.e.* the reduced form of misonidazole is viewed as the active agent.[9] Finally, a misonidazole metabolite capable of reacting with guanosine has been detected in extracts of Chinese hamster ovarian cells exposed to misonidazole under hypoxic conditions.[10]

Considerable attention has been given to the problem of demonstrating the effects of nitroimidazoles upon radical yields in the radiolysis of DNA. The claim that the presence of electroaffinic compounds enhances the yield of guanine cations determined by e.s.r. on radiolysis of oriented DNA films[6] has been refuted subsequently on the basis of experiments using frozen aqueous solutions.[11] Other model experiments have shown that flash excitation with white light of guanosine monophosphate (GMP) in the presence of *p*-nitroacetophenone (PNAP) results in formation of $PNAP^{-\bullet}$ *via* a process of electron transfer from GMP to triplet-state PNAP.[12] From this it may be deduced on the basis of the Weller equation (5) that ^3GMP is capable of transferring an electron to PNAP. Similarly, triplet-state 2-methylnaphthoquinone has been shown to attack pyrimidines *via* electron-transfer,[13a] while various DNA constituents quench triplet 4-nitroquinoline 1-oxide by electron transfer.[13b]

In the present study we report the rates of quenching of triplet-state thymine and uracil, and also thymidine, by electroaffinic molecules, and analyse the results in terms of an electron-transfer mechanism. MeCN was used as the solvent, as it gives the highest absorbance of the triplet bases on flash photolysis, owing mainly to greater inter-system crossing than with water.[14] A preliminary account of this work has appeared.[15]

Experimental

Acetonitrile (spectroscopic grade) was obtained from Fluka. Water was refluxed over alkaline potassium permanganate and doubly distilled. Thymine, uracil, and thymidine were obtained from Sigma Chemical Co. and recrystallised once from purified water. Other chemicals were obtained in the highest purity available (*i.e.* either as spectroscopic or AnalaR grades): if the quoted purity was $\leqslant 99\%$ then literature purification procedures were followed.[16]

Laser Flash Photolysis.—This was performed using 15 ns pulses of 249.1 nm radiation and of *ca.* 200 mJ energy obtained

Table 1. Quenching of triplet-state thymine by electroaffinic molecules in MeCN solution (*T* 293 K)

Quencher	(No.)	$-E_{\frac{1}{2}}(S/S^{-\bullet})$/V *vs.* s.c.e.[a,b]	ΔG°_{23}/kJ mol^{-1} [c]	$k_2/10^9$ dm^3 mol^{-1}
1,4-Dinitrobenzene	**(3)**	0.71	−81.46	4.82 ± 0.35
2-Methylnaphthoquinone	**(4)**	0.77	−75.65	5.15 ± 0.36
Misonidazole	**(2)**	0.97[d]	−56.36	3.60 ± 0.31
Metronidazole	**(1)**	1.10[d]	−43.81	4.26 ± 0.36
4-Nitrotoluene	**(5)**	1.20	−33.89	1.38 ± 0.15
Nitroethane	**(6)**	1.42	−12.55	0.148 ± 0.008
1-Nitropropane	**(7)**	1.458	−9.29	0.255 ± 0.009
Nitromethane	**(8)**	1.46	−9.08	0.299 ± 0.015
1-Nitrobutane	**(9)**	1.48	−7.15	0.115 ± 0.008
1-Nitropentane	**(10)**	1.514	−3.89	0.165 ± 0.008
$[Fe(CN)_6]^{3-}$	**(11)**	−0.45[e]		9.38 ± 0.46
Tetranitromethane	**(12)**	1.4		23.0 ± 0.3
5-Bromouracil	**(13)**	1.62[f]		2.19 ± 0.21

[a] Measured in MeCN using NR_4^+ ClO_4^- (R = Et or ^{n}Pr) as supporting electrolyte at 0.1 mol dm^{-3} concentration. [b] Except where indicated otherwise, values are taken from L. Meites and P. Zuman, 'Handbook Series in Organic Electrochemistry,' vols. I—IV, CRC Press, Cleveland, Ohio, 1977—1979, and 'Techniques of Electroorganic Synthesis,' ed. N. Weinberg, Wiley Interscience, New York, 1975. [c] Calculated from equation (6) setting $E(D/D^{+\bullet})$ 1.75 V, $^3\Delta E_{0.0}$ 313 kJ mol^{-1}, $e^2/\varepsilon r$ 0.06 eV. [d] This work. [e] 'Handbook of Chemistry and Physics,' ed. R. C. Weast, CRC Press, Cleveland, 56th edn., 1975, p. D141. [f] G. Dryhurst, 'Electrochemistry of Biological Molecules', Academic Press, New York, 1977.

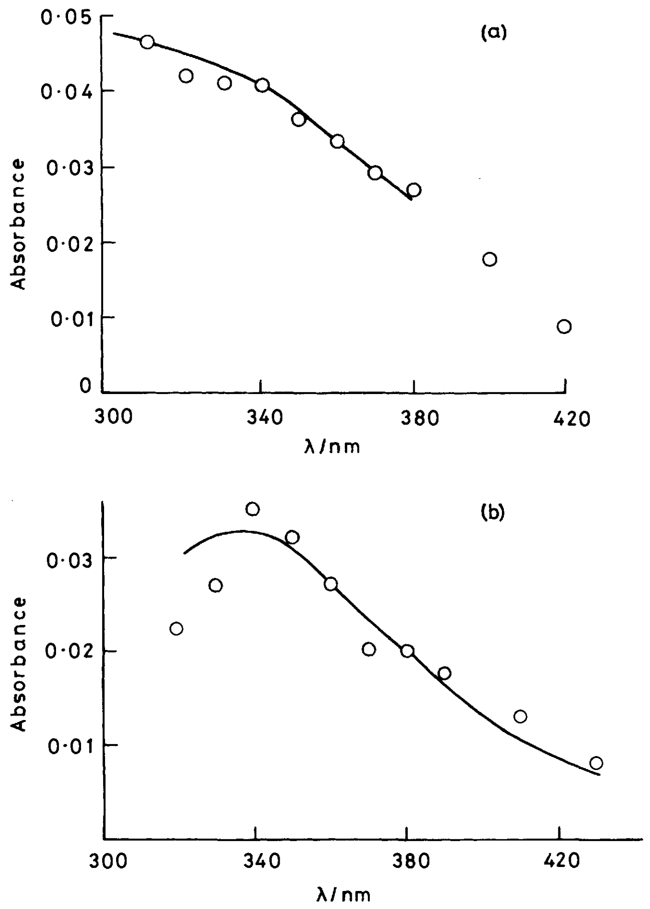


Figure 1. 249-nm Laser flash photolysis spectra of deoxygenated solutions in MeCN of (a) thymine (2.4 × 10^{-4} mol dm^{-3}) and (b) uracil (3.4 × 10^{-4} mol dm^{-3}). The full lines in both cases represent the triplet–triplet absorption spectra reported by Salet and Bensasson[14]

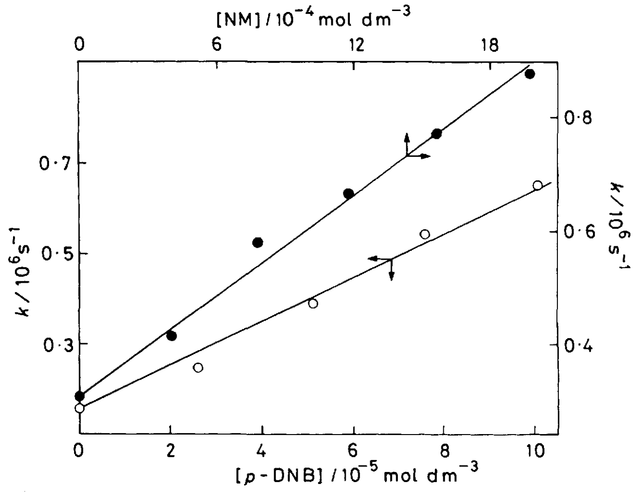


Figure 2. Kinetic quenching of the triplet state of thymine, measured at 315 nm, by nitromethane (NM) and 1,4-dinitrobenzene (*p*-DNB)

from an Oxford Lasers Model KX2 excimer laser filled with Kr and F_2 (with He as the support gas). The detection equipment was as described before[17a] except that the storage oscilloscope was triggered *via* a pulse fed directly from the laser console. The stored image of the transient decay was fed *via* a videocamera and an interface (Data Harvest, U.K.) to a BBC microcomputer where it was displayed on the screen and analysed by selecting a series of points with the cursor. The selected points, after storage, were subjected to a linear regression analysis to yield the first-order decay constants. Full details are given in ref. 17*b*.

Curve-fitting Routines.—Fitting of experimental rate constants for electron transfer to the free-energy changes of the reactions concerned was *via* the Weller equation (see Results and Discussion). A program written in FORTRAN 77 entitled WELLEREKQ enabled the parameters $\Delta G^{\ddagger}_{23}(0)$ and $E(D^{+\bullet}/D)$ to be treated as adjustable parameters.[17b]

Electrochemical Measurements.—Oxidation potentials of thymine and uracil in MeCN were determined by cyclic voltammetry at the University of Bristol by Dr. N. G. Connelly. Reduction potentials of the nitroimidazoles in MeCN were determined at Warwick by cyclic voltammetry using a stationary Pt disc, standard three-electrode cell, and purpose-built potentiostat. The counter-electrode was a large-area Pt gauze and the reference electrode was saturated calomel (s.c.e.); sweep rates were varied between 50 and 200 mV s^{-1}.

Table 2. Quenching of triplet-state uracil by electroaffinic molecules in MeCN solution (T 293 K)

Quencher	(No.)	$E_{\frac{1}{2}}(S/S^{-\cdot})/V$ [a]	ΔG°_{23}/kJ mol^{-1} [b]	$k_2/10^9$ dm^3 mol^{-1} s^{-1}
(2)			−42.56	6.24 ± 0.59
(1)			−30.02	13.1 ± 1.1
(5)			−20.08	3.91 ± 0.45
(6)			1.24	0.75 ± 0.04
(7)			4.72	0.41 ± 0.02
(8)			6.64	0.36 ± 0.02
(10)			9.92	0.50 ± 0.06
1,2-Dinitrobenzene	**(14)**	0.81	−57.99	4.45 ± 0.33
1-Chloro-4-nitrobenzene	**(15)**	1.06	−35.59	6.30 ± 0.46
Fumaronitrile	**(16)**	1.3	−10.72	5.27 ± 0.42
2,6-Dimethyl-1-nitrobenzene	**(17)**	1.402	−0.88	2.85 ± 0.34
2-Methyl-2-nitropropane	**(18)**	1.7	27.87	0.46 ± 0.04

[a] For general remarks and data for compounds **(1)**—**(10)** and **(14)**—**(18)**, see footnotes to Table 1. [b] Calculated from equation (6) setting $E(D/D^{+\cdot})$ 2.05 V, $^3\Delta E_{0,0}$ 328 kJ mol^{-1}.

Table 3. Quenching of triplet-state thymidine by electroaffinic molecules in MeCN solution (T 293 K)[a]

Quencher	ΔG°_{23}/kJ mol^{-1} [b]	$k_2/10^9$ dm^3 mol^{-1} s^{-1}
(3)	−100.8	2.84 ± 0.13
(4)	−94.6	3.11 ± 0.21
(2)	−75.6	3.68 ± 0.28
(1)	−63.1	7.29 ± 0.49
(6)	−31.8	0.43 ± 0.005
(7)	−28.6	0.080 ± 0.001
(8)	−28.4	0.127 ± 0.004
(9)	−26.4	0.066 ± 0.005
(10)	−23.0	0.132 ± 0.0012
(11)		6.20 ± 0.29
(12)		27.7 ± 0.05
(13)[c]		2.19 ± 0.21

[a] For details concerning reduction potentials, experimental conditions, *etc.* see footnotes to Tables 1 and 2. [b] Calculated from equation (6) setting $E(D/D^{+\cdot})$ 1.55 V, $^3\Delta E_{0,0}$ 313 kJ mol^{-1}. [c] Measured in a medium of MeCN (90%)-H_2O (10%) (v/v).

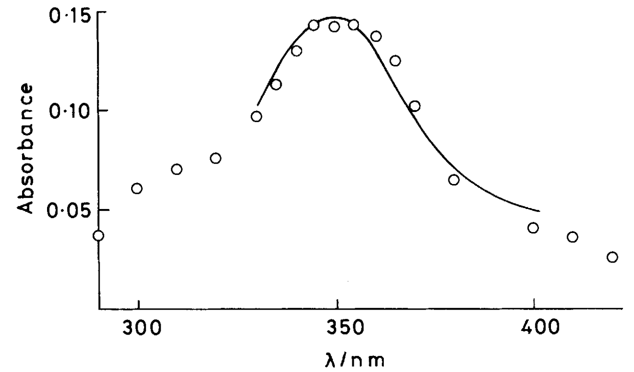


Figure 3. Transient spectrum (○) formed on 249-nm flash photolysis of thymine (2.6 × 10^{-4} mol dm^{-3}) and tetranitromethane (9.32 × 10^{-4} mol dm^{-3}) in MeCN. Full line-spectrum of $C(NO_2)_3^-$ taken from D. J. Clover, *Tetrahedron*, 1963, **19**, Suppl 1, 219

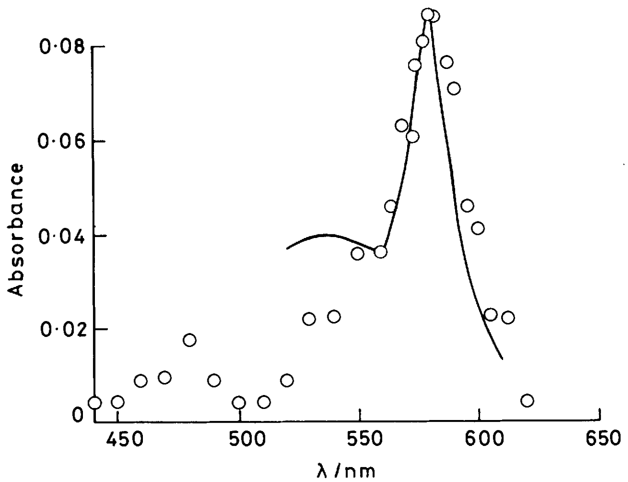


Figure 4. Transient spectrum (○) formed on 249-nm flash photolysis of thymine (2.6 × 10^{-4} mol dm^{-3}) and galvinoxyl (9.5 × 10^{-5} mol dm^{-3}) in MeCN. Full line-spectrum of galvinoxyl taken from ref. 21*b*

Results

Spectra.—Laser flash photolysis of solutions of thymine (2.6 × 10^{-4} mol dm^{-3}) and uracil (3.4 × 10^{-4} mol dm^{-3}) in argon-flushed MeCN gave the spectra presented in Figure 1 onto which are superimposed those obtained for MeCN solutions by Salet and Bensasson.[17] The agreement is good and both sets are generally similar to the triplet–triplet absorption spectrum for thymine ($\lambda_{max.}$ 340 nm) and uracil ($\lambda_{max.}$ 370 nm) obtained in water by Whillans and Johns.[18] However, all these data differ from those obtained by Hayon[19] for uracil triplet by pulse radiolysis of solutions in CH_3CN, dimethylformamide, and propan-2-ol; Hayon gives $\lambda_{max.}$ 289 ± 1 nm, $\varepsilon \geqslant 9.1 \times 10^4$ dm^3 mol^{-1} cm^{-1} and τ 5—10 ms depending on solvent.

Triplet Quenching Data.—Addition of electroaffinic molecules, S, to solutions of thymine, uracil, and thymidine shortened the lifetime of the triplet state of the base in a systematic manner according to equation (1),

$$k_{obs} = k_0 + k_2[S] \qquad (1)$$

where k_{obs} is the observed first-order decay constant of the triplet base, k_0 is the triplet decay rate in the absence of S, and k_2 is the bimolecular quenching rate constant. A typical quenching plot is depicted in Figure 2 and values for k_2 for the three triplet states are summarised in Tables 1—3.

Spectra of Electron-capture Intermediates.—A direct demonstration of electron transfer from triplet base to a ground-state electroaffinic molecule was achieved with the solutes tetranitromethane and galvinoxyl. The former reacts with electrons to yield the nitroform anion [equations (2) and (3)].

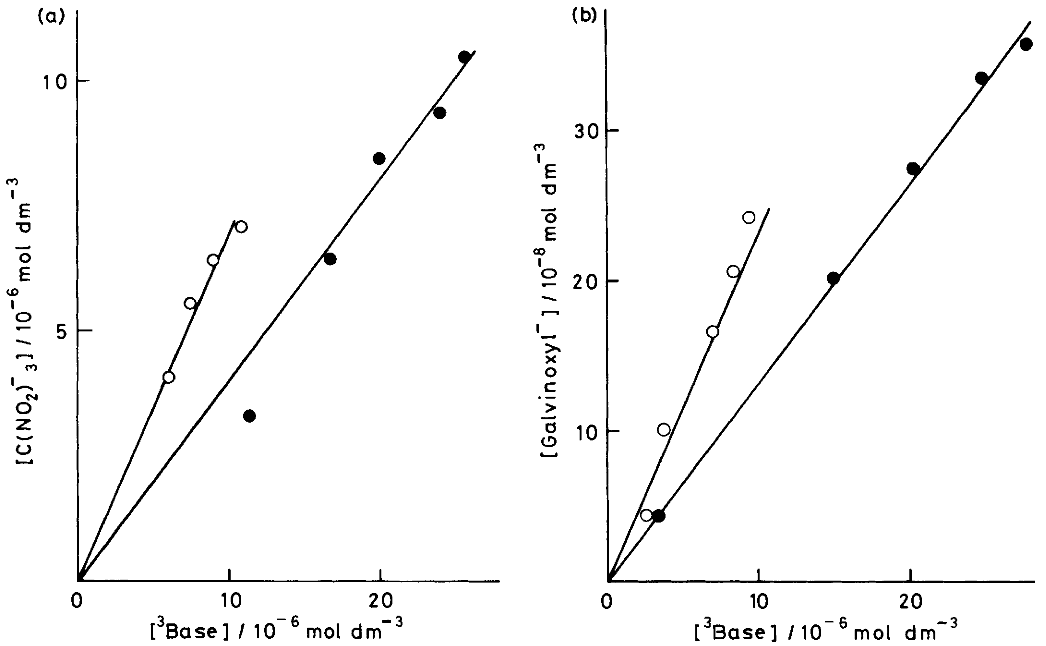


Figure 5. Relationships between triplet base concentration and electron-adduct concentration on 249-nm flash photolysis of thymine (○, 2.6 × 10^{-4} mol dm^{-3}) and uracil (●, 3.4 × 10^{-4} mol dm^{-3}) in the absence and presence of (a) tetranitromethane (9.32 × 10^{-4} mol dm^{-3}) and (b) galvinoxyl (9.5 × 10^{-5} mol dm^{-3}) with varying pulse size

Table 4. Conversion yields in the formation of acceptor anions by electron transfer from triplet thymine and uracil

Base	$[C(NO_2)_3^-]/[^3Base]$	$[Galvinoxyl^{-\cdot}]/[^3Base]$
Thymine	0.69 ± 0.03	0.023 ± 0.002
Uracil	0.402 ± 0.003	0.013 ± 0.0005

Table 5. Estimations of $E_{\frac{1}{2}}^{red}$ for electroaffinic drugs in MeCN from cyclic voltammograms

Drug	Sweep rate/mV s^{-1}	$-E_p$ (cathodic)/V	$-E_p$ (anodic)/V	$-\Sigma E/2$/V
Metronidazole (**1**)	50	1.22	1.02	1.12
	100	1.26	1.00	1.13
	200	1.31	0.94	1.125
Misonidazole (**2**)	50	1.12_2	0.85	0.98_6
	100	1.17	0.76_2	0.96_6
	200	1.20	0.71_8	0.95_9

$$C(NO_2)_4 + e^- \longrightarrow C(NO_2)_4^{-\cdot} \quad (2)$$

$$C(NO_2)_4^{-\cdot} \longrightarrow C(NO_2)_3^- + NO_2 \quad (3)$$

$C(NO_2)_3^-$ absorbs very strongly with $\lambda_{max.}$ 350 nm and $\varepsilon_{max.}$ 15 000 dm^3 mol^{-1} cm^{-1}.[20]

The free radical, galvinoxyl, accepts an electron to yield the anion with a narrow band, $\lambda_{max.}$ 580 nm,[21a] of high intensity, $\varepsilon_{max.}$ 2.2 × 10^5 dm^3 mol^{-1} cm^{-1},[21b] in agreement with Capellos and Allen.[21b]

Figures 3 and 4 depict the spectra deriving from electron transfer from triplet thymine. These experiments were performed with solute concentrations selected such that >90% of the incident 249-nm pulse was absorbed by thymine. ('Blank' experiments utilising only the electroaffinic molecules gave only *ca.* 20% of the yield of electron-adduct: after accounting for the inner-filter effect of the thymine when present we conclude that 98% of the observed adduct derives from electron-transfer.)

Yields of Electron-capture Intermediates.—The yields of the electron-adducts at a particular scavenger concentration varied with laser pulse energy in a strictly linear fashion: this indicates that the process of their production is purely monophotonic and has no biphotonic component. The yields of triplet base also depended linearly upon laser pulse energy. Plots of $O.D._{max.}$ (electron-adduct) for high scavenger concentration *versus* $O.D._{max.}$ (base) triplet for a 10-fold variation in pulse energy were accurately linear (Figure 5) and, when combined with literature data for $\varepsilon_{max.}$ for both species, gave yields of electron-adducts per molecule of triplet base. These electron-transfer yields are summarised in Table 4. The ratios of the ion yields from the two bases for different acceptors are in excellent agreement: for tetranitromethane the ratio (thymine:uracil) is 1.70 and for galvinoxyl it is 1.77.

Electrochemical Potentials.—The reduction potentials of compounds (**1**) and (**2**) were determined in MeCN by cyclic voltammetry as set out in Table 5 and average values of −1.125 ± 0.005 V for (**1**) and −0.970 ± 0.014 V for (**2**) (both *versus* s.c.e.) are used in our calculations.

The anodic oxidations of thymine and uracil in MeCN gave only *very* weak waves at 2.0 ± 0.1 (thymine) and 2.3 ± 0.1 V (uracil): these seemed genuine features but left some uncertainty. An alternative but indirect approach to estimate the oxidation

Table 6. Quenching of the fluorescence of 9-cyanoanthracene (CNA) and 9,10-dicyanoanthracene (DCA) by electron donors[a]

Fluor	Quencher	$k_2/10^9$ dm^3 mol^{-1} s^{-1}	E_{ox}/V *vs.* s.c.e.
CNA	Durene	0.175[b]	1.62
	Thymine	0.59 ± 0.03[c]	
	1,1-Diphenylethene	1.05[b]	1.52
DCA	Biphenyl	3.06[b]	1.91
	Thymine	3.6 ± 0.2[c]	
	1,1-Diphenylethene	8.49[b]	1.52
	Thymidine	10.8 ± 0.7[c]	
	Anisole	12.2[b]	1.76

[a] Solvent MeCN except for thymine when MeOH was used to achieve adequate solubility. [b] Data from ref. 22. [c] Our data.

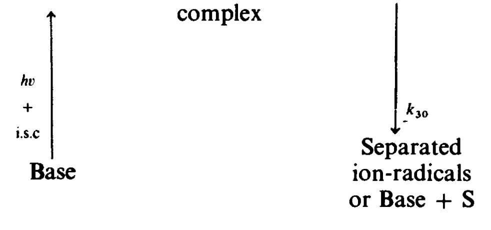
potential was that of measuring the fluorescence quenching of 9-cyanoanthracene and 9,10-dicyanoanthracene by thymine and thymidine (acting in this sense as ground-state electron donors) and interpolating the resulting rate constants into a table of quenching rates by other donors.[22] The relevant data are summarised in Table 6.

From Table 6 it may be concluded that E_{ox} (thymine) is *ca.* 1.7 ± 0.2 V *versus* s.c.e. while E_{ox} (thymidine) is *ca.* 1.6 ± 0.1 V *versus* s.c.e. A third approach is to relate the observed gas-phase ionisation energies of thymine and uracil (9.43 and 9.82 eV respectively) to oxidation potentials through one of the several empirical equations available for this purpose. The relation $E_{ox} = (0.92I) - 6.2$[23] gave E_{ox} values respectively of 2.48 and 2.83 V *versus* s.c.e.: while these are in the right sequence we regard them as unacceptably high, particularly as the bases are known[24] to be subject to facile one-electron oxidation by $Cl_2^{-\bullet}$ [$E°(Cl_2^{-\bullet}/2\ Cl^-) = 1.85$ V *versus* s.c.e.].[25] The fourth approach is to apply a continuous variation to the value of $\Delta G°_{23}$ in equation (6), see below, in order to optimise the fitting to the Weller equation (5): such a procedure involves the assumption of such a fit; however it cannot produce the highly characteristic shape, but merely adjusts the location of the 'drop' region. Such a fitting procedure yielded the following oxidation potentials *versus* s.c.e.; thymine 1.75 ± 0.05, uracil 2.05 ± 0.1, thymidine 1.55 ± 0.2 V.

Considering the various figures, all of which except those obtained from the ionisation energy, E_{ox}, correlation fall within 0.3 V for a particular base, we suggest 'best' values as follows: thymine 1.75, uracil, 2.05, and thymidine 1.60 V.

Discussion

Analysis of Kinetic Data.—Correlations between the observed rate constants k_2 of electron-transfer reactions and the free energies of such reactions are most commonly made using the scheme of Rehm and Weller.[26] The individual steps of the electron-transfer process, adapted to our case, are viewed as in equation (4).



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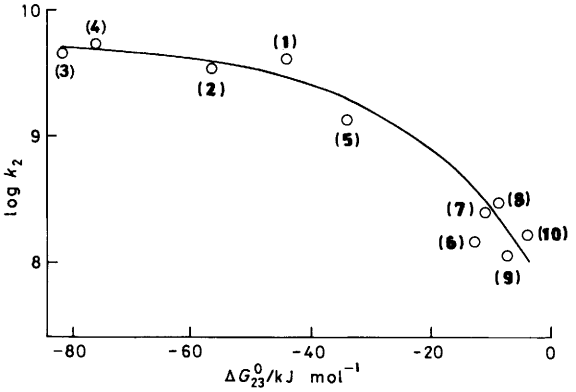

Figure 6. Weller plot for the quenching of triplet thymine by electro-affinic molecules in MeCN. Data and numbering scheme given in Table 1. Parameters optimised to the values: $E(D/D^{+\bullet})$ 1.75 V, $\Delta G^{\ddagger}_{23}(0)$ 16.5 kJ mol^{-1}

The bimolecular quenching rate is given by equation (5),

$$k_2 = \frac{k_{12}}{1 + (k_{21}/\kappa°)[\exp(\Delta G^{\ddagger}_{23}/RT) + \exp(\Delta G°_{23}/RT)]} \quad (5)$$

where the subscripts refer to the individual steps in equation (4) and $\kappa°$ is a transmission coefficient. The ratio $k_{21}/\kappa°$ is usually taken as 0.25[26] while $\Delta G°_{23}$ is calculated from the energetics of the electron-transfer step, equation (6).

$$\Delta G°_{23}/\text{kJ mol}^{-1} = 96.48$$
$$\{E°(\text{Base}/\text{Base}^{+\bullet}) - E°(\text{S}^{-\bullet}/\text{S}) - e^2/\varepsilon r - \Delta^3E_{0,0}\} \quad (6)$$

Here $E°$ refers to the polarographic half-wave oxidation–reduction potentials in volts; $e^2/\varepsilon r$ to the Coulombic term, and $\Delta^3E_{0,0}$ to the zero–zero spectroscopic energy of the triplet base. $\Delta G^{\ddagger}_{23}$ Is calculated from the empirical expression due to Rehm and Weller [equation (7)]. Here $\Delta G^{\ddagger}_{23}(0)$ is the free energy of

$$\Delta G^{\ddagger}_{23} = \frac{\Delta G°_{23}}{2} + \left\{\left(\frac{\Delta G°_{23}}{2}\right)^3 + [\Delta G^{\ddagger}_{23}(0)]^2\right\}^{\frac{1}{2}} \quad (7)$$

activation for an isoergonic reaction, *i.e.* when $\Delta G°_{23}$ is 0, and is known as the 'intrinsic barrier' to reaction. It is often taken as 10.04 kJ mol^{-1} following the pioneering work of Rehm and Weller on electron transfer between excited and ground-state aromatic molecules.[26] Values close to this have been used successfully in some inorganic systems,[27] but several groups have favoured higher values in a number of recent studies where more extensive reorganisation is involved in electron transfer.[28,29] Our fitting routine enabled continuous variation of both k_{12} and $\Delta G^{\ddagger}_{23}(0)$ to achieve optimisation.

Tables 1—3 list values for $E°(S^{-\bullet}/S)$ and $\Delta G°_{23}$ and Figures 6—8 illustrate computer-iterated fits to the Weller equation. The fits may be regarded as reasonable: the plateau region is achieved for all three bases and there is clear evidence for the linear sloping region as $\Delta G°_{23}$ approaches zero (for thymine and thymidine) or becomes positive (for uracil). The curves for thymine and thymidine are shallower, *i.e.* the so-called intermediate region appears more extended: this is an established feature[28] of Weller curves when the value of $\Delta G^{\ddagger}_{23}(0)$ is rather more than the 'classical' figure of 10.04 kJ mol^{-1}.[26] $\Delta G^{\ddagger}_{23}(0)$ for uracil is found to be 6.7 kJ mol^{-1} whereas the figures for thymine and thymidine are, respectively, 16.5 and 19.5 kJ mol^{-1}.

The alternative quenching mechanism for the base triplet

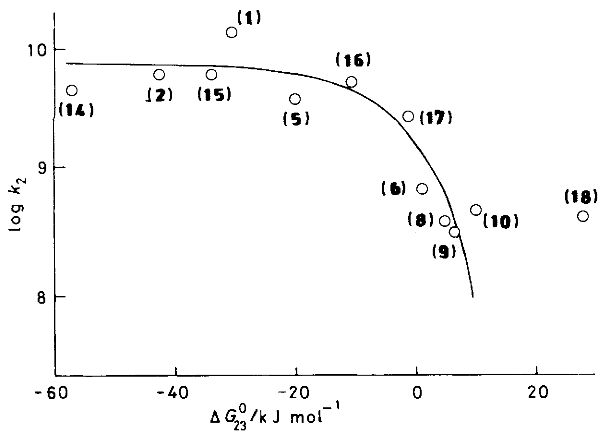

Figure 7. Weller plot for the quenching of triplet uracil by electroaffinic molecules in MeCN. Data and numbering scheme given in Table 2. Parameters optimised to the values: $E(D/D^{+\cdot})$ 2.05 V, $\Delta G^{\ddagger}{}_{23}(0)$ 6.7 kJ mol^{-1}

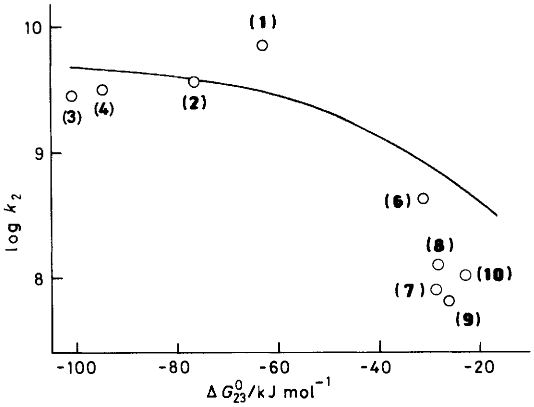

Figure 8. Weller plot for the quenching of triplet thymidine by electroaffinic molecules in MeCN. Data and numbering scheme given in Table 3. Parameters adjusted to the values: $E(D/D^{+\cdot})$ 1.55 V, $\Delta G^{\ddagger}{}_{23}$ (0) 19.5 kJ mol^{-1}

states is energy transfer to the ground state of the electroaffinic molecules. The triplet energies of the pyrimidine bases are high; thus, at 80 K and at pH 7 in aqueous solution, $\Delta^3E_{0,0}$[30] is given as 314.5 and 327.7 kJ mol^{-1} for thymine and uracil, respectively. Those for the electroaffinic molecules are generally considerably lower; thus for the nitroaromatic systems $\Delta^3E_{0,0}$ is *ca.* 240 kJ mol^{-1}.[31] For the nitroalkanes, INDO calculations indicate $\Delta^3E_{0,0}$ to be dependent on the conformation of the molecule: for the lowest-energy conformations (with the C–C bond of $EtNO_2$ coplanar with the nitro group) the S_0–T_1 splitting is 149.9 (for $EtNO_2$) and 155.7 kJ mol^{-1} (for $MeNO_2$), while for the next, lowest-energy conformation (with the C–C bond at 90° to the NO_2 plane) the splittings become 283.0 and 300.8 kJ mol^{-1} respectively.[32] In either case, the energy differences between the triplet states of the pyrimidines and those of the nitroalkanes would result in a diffusion-controlled process. Discrimination between energy and electron-transfer processes, when both are exoergonic, presents difficulties, and exactly opposite conclusions have been reached in certain cases, *e.g.* the quenching of $*[Ru(bipy)_3]^{2+}$ by cobalt(III) complexes[33] and by $[Fe(CN)_6]^{3-}$.[34] The two processes occur simultaneously in the quenching of certain triplet donors such as benzophenone and $[Ru(bipy)_3]^{2+}$ by a series of bis(pyridylethene) salts and related compounds: in particular the rates adhere to the Weller equation for electron-transfer quenching of $[Ru(bipy)_3]^+$ despite the possibility of energy transfer.[35]

Wilkinson and Tsiamis have shown that whereas the quenching of a series of organic triplet states by tris(acetylacetonato)chromium(III) is due to energy transfer, that by tris(hexafluoroacetylacetonato)chromium(III) also contains a contribution from electron transfer, quenching by which is followed by fast back-electron transfer (k_{32} > 10^8 s^{-1}).[36] Our conclusion is that the close adherence of our own results to the Weller equation is good evidence for the electron-transfer mechanism and that any role of energy transfer is minor.

Observation of Semi-reduced Species.—One of the main difficulties in using 249-nm excitation is that of the near-universal absorption of light at this wavelength by other than the simplest organic molecules. With solute pyrimidines, the competitive absorption of the exciting pulse proved prohibitive except in a few cases. Nonetheless, galvinoxyl and tetranitromethane were found to have the requisite optical and electroaffinic qualities and yielded unmistakable evidence of their electron-adducts (Figures 3 and 4). The much higher ion yields for tetranitromethane are, in our view, associated with the dissociative character of the electron-capture process [equations (2) and (3)] which results in a high value for k_{30} and a low one for k_{32}.

General.—The ability of thymine and uracil triplets to function as effective electron-transfer agents to electroaffinic solutes, coupled with the previous observation on guanosine monophosphate,[11] implies that DNA itself can act as an electron source following excitation either photochemically or radiation chemically. This accords with the observations of Sevilla *et al.*[37] that photolysis of DNA in aqueous glasses ($[NaClO_4]$ 8.0 mol dm^{-3}) leads to photoionisation, evidenced by the production of guanine positive ions detected by e.s.r., a process involving ejection of a photoelectron from the triplet base.

The attractively simple picture of the electroaffinic drug operating by sequestering negative charge, thereby increasing the number of positive centres in DNA, which appeared to be supported by the observations of Gräslund,[6] needs careful re-evaluation following the claims by Symons *et al.*[11] (i) that Gräslund's observation on (dry) oriented fibres of DNA cannot be repeated for glassy solutions of DNA, and (ii) that the presence of the electroaffinic drugs actually *reduces* the number of single- and double-strand breaks in DNA on radiolysis as compared with drug-free controls. The later work[11] supports the view that the drugs act simply as electron-capture agents which reduce the yield of thymine anions. However, extrapolation of observations using fibres or glasses to cellular radiosensitisation obviously requires considerable caution. Interestingly, addition of tetranitromethane to γ-irradiated N_2O-saturated aqueous solutions of poly(uridylic) acid strongly suppresses release of uracil, probably by scavenging the major 5-hydroxyuracil-6-yl radical.[38]

The present results, whilst demonstrating an electron-affinity relationship for quenching of excited DNA bases, show that overall quenching *rates* k_2 are diffusion-controlled for compounds of reduction potentials similar to, or higher than that of, for example, metronidazole, that are of most biological interest. The experimental difficulties noted above in quantifying the *yield* of separated ion-radicals [equation (4)] with most of the compounds of interest currently preclude prediction of the likely redox relationship for the sensitisation of the formation of potentially damaging base radical-cations. Whilst further work is needed to address this important question, these studies demonstrate that redox reactions between typical radiosensitisers and excited states of DNA bases are facile. Renewed interest in the importance of the 'direct effect' of radiation damage[8] underlines the importance of quantifying these electron-transfer processes.

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