Radiolysis of Di- and Tri-methyl Phosphates in Oxygenated Aqueous Solution: a Model System for DNA Strand Breakage

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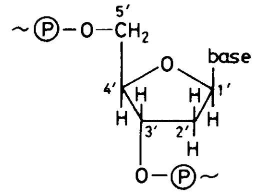
In the radiolysis of aqueous solutions of trimethyl phosphate saturated with N_2O–O_2 the major products (*G* values in parentheses) are dimethyl hydrogen phosphate (6.4), formic acid (3.1), formaldehyde (1.7), hydrogen peroxide (2.1), organic peroxide (0.5), and carbon monoxide (1.0). Oxygen is consumed (*G* 4.1). In the dimethyl phosphate (sodium salt) system the products and their *G* values are similar to those of the trimethyl phosphate system. Pulse radiolytic studies have shown that in the trimethyl phosphate system the first formed peroxyl radicals react with each other to give a short lived tetraoxide ($2k = 9 \times 10^8$ dm^3 mol^{-1} s^{-1}). This intermediate decays either by concerted mechanisms or by fragmentation to form O_2 and two oxyl radicals. Among other reactions these oxyl radicals undergo a 1,2-hydrogen shift, followed by a complex series of reactions. There are two processes that yield acids at longer reaction times. The faster process has been identified as the hydrolysis of formic dimethylphosphoric anhydride ($k = 0.3 + 2.3 \times 10^4 [OH^-]$ s^{-1}). The slower process ($k = 2\,600[OH^-]$ s^{-1}) is only observed in basic solutions and has not yet been identified with certainty. The present findings are used as a model to assist in the interpretation of some aspects of DNA radiolysis.

Ionising radiation causes strand breaks in DNA. The radiolysis of DNA in aqueous solution (indirect effect) has already been studied in some detail and a number of products formed during the strand breaking processes have been identified. Some aspects of the mechanisms leading to these products have been elucidated (for a review see ref. 1). In the radiolysis of the model system ribose-5-phosphate in oxygenated aqueous solution, two major processes are found to lead to phosphate release (in DNA: strand breaks). The first process is cleavage of the C(4)–C(5) bond, the second is oxidation at C(5) to produce pentodialdose.[2] The former process is also observed with DNA;[3,4] the latter, however, has so far escaped detection. To elucidate further the mechanistic pathways involving the C(5′) radical of DNA in oxygenated aqueous solution a study was made of the radiolysis of oxygenated aqueous solutions of both di- and tri-methyl phosphates.



Nucleotide unit in DNA

The radiolysis of dimethyl phosphate in aqueous solutions, to which some attention has already been given,[5] appears to be very similar to that of trimethyl phosphate. In this work the more detailed studies were performed with trimethyl phosphate, which is experimentally more amenable.

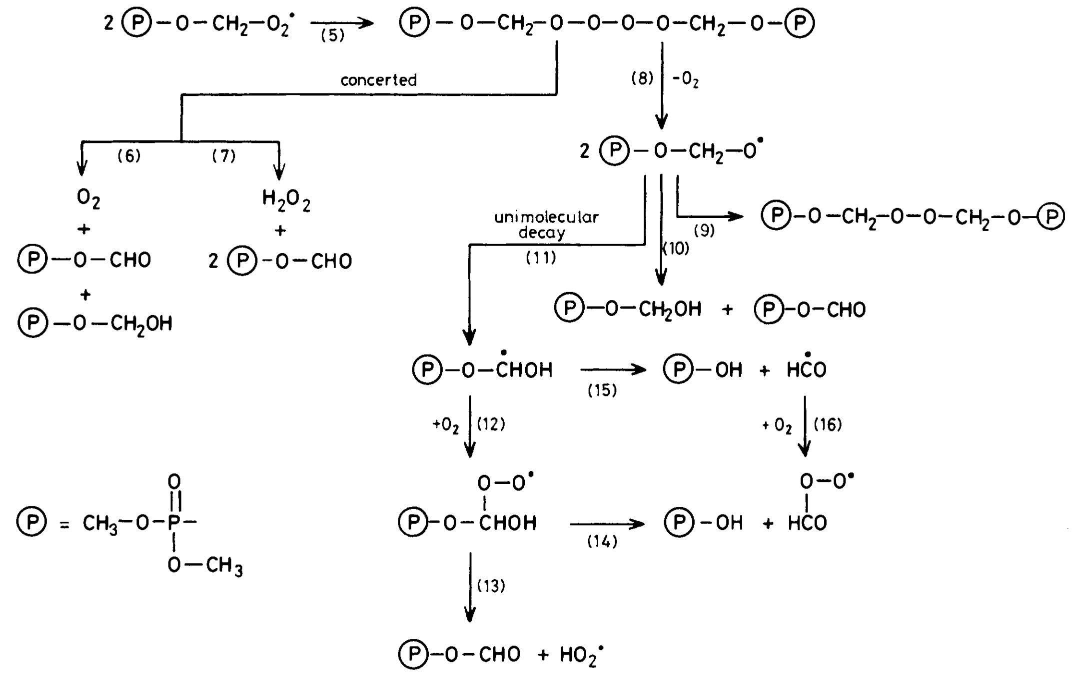
Experimental

Trimethyl phosphate (p.A., Schuchardt) was purified by fractional distillation and was pure by g.l.c. Sodium dimethyl phosphate was prepared from trimethyl phosphate with NaOH and recrystallised from water.[5]

Aqueous solutions of sodium dimethyl phosphate (0.01M) and trimethyl phosphate (0.005—0.1M) were saturated either with N_2O-O_2 (4 : 1 v/v) or with O_2 and were irradiated at room temperature (20 ± 2 °C). For product analysis irradiations were carried out with a ^{60}Co γ-source at a dose rate of 0.3 Gy s^{-1} as determined by Fricke dosimetry. Absorbed doses were between 170 and 700 Gy.

Pulse radiolysis was carried out with a 2.8 MeV van de Graaff electron accelerator. The pulse duration was between 0.4 and 2 μs and the absorbed doses were between 2 and 30 Gy. The electron accelerator and the optical detection system have been described previously.[6] For optical measurements dosimetry was performed with N_2O-saturated 0.01M-KSCN solutions taking the value of 46 000 (molecules/100 eV) dm^3 mol^{-1} cm^{-1} for the product $G \times \varepsilon$ of $(SCN)_2^{-\cdot}$ at 472 nm.[7] For the detection of charged species an a.c.-conductivity method was used[8] in which an a.c. voltage (10—40 V) of 10 MHz frequency was applied over a two-cell bridge. This set-up was supplied by the Hahn-Meitner Institut für Kernforschung, Berlin. For dosimetry, a CH_3Cl-saturated solution (0.1M) containing 0.1M-t-butyl alcohol at pH 4 was used;[9] *G*(HCl) was taken as 3.1. The experimental data were processed with a VAX 780 computer.

Formaldehyde was determined colorimetrically using the reagent chromotropic acid.[10] Total peroxide was determined iodometrically.[11] Organic peroxide was determined by the same method after H_2O_2 had been removed by catalase. Total acids were determined by potentiometric titration against 0.01M-NaOH. Formic acid was separated from formaldehyde by fractional distillation of the irradiated solutions to which acidified 2,4-dinitrophenylhydrazine had been added. Formic acid in the distillate was then determined as formaldehyde after reduction with Mg in concentrated HCl.[12] This method produced rather large errors (±30%), primarily at the reduction step.[12] Formic acid and dimethyl hydrogen phosphate were separated and determined quantitatively by high-performance ion chromatography (h.p.i.c.) using a Dionex ion chromatograph model 2000 i (eluant 1mM-$NaHCO_3$; flow rate 2 ml min^{-1}; column HPIC-AS4 with hollow-fibre suppressor; retention times dimethyl hydrogen phosphate 2.2; formic acid 3.9 min). These measurements were kindly carried out by Dr. J. Weiss, Dionex GmbH, Weiterstadt. Carbon oxides were determined by g.l.c. After separation CO and CO_2 were catalytically reduced to methane and the latter was detected by flame ionization. Nitrous oxide interferes in the determination of CO_2, and hence only CO could be measured in N_2O-O_2-saturated solutions. Oxygen



Scheme 1.

Table. Products (and their *G* values) of the γ-radiolysis of trimethyl phosphate (0.1M) and sodium dimethyl phosphate (0.01M) solutions; dose rate 0.3 W kg^{-1}; starting pH *ca.* 6.0

	Trimethyl phosphate			Sodium dimethyl phosphate
Products	N_2O–O_2	O_2	O_2 + SOD	N_2O–O_2
Formaldehyde	1.7	1.2	1.0	1.8
Formic acid	3.1;[a] 3.6[b]	3.3[b]		3.3[b]
Dimethyl hydrogen phosphate	6.4[a]			
Total acid	9.3	5.8	4.7	8.7
Hydrogen peroxide	2.1	1.9		1.8
Organic peroxide	0.5	0.6		1.1
Carbon monoxide	1.0	0.9		
Carbon dioxide		0.3		
Oxygen consumption	4.1	3.3		
Inorganic phosphate	Absent	Absent		Absent

[a] Ion chromatography. [b] Photometric method; error ±30%, see text.

consumption was determined with an oxygen-sensitive electrode (Wiss. Techn. Werkst., Weilheim, Germany). Assays for inorganic phosphate were made according to the method of Lowry and Lopez.[13]

To remove $O_2^{-\cdot}$ formed during the irradiation in some experiments superoxide dismutase (SOD) (Sigma) was added to the trimethyl phosphate solutions (20 units per ml; 3 × 10^{-7}M) prior to irradiation.

Results and Discussion

Trimethyl Phosphate.—The products of the γ-radiolysis of aqueous solutions of trimethyl phosphate (10^{-1}M) saturated with N_2O–O_2 or O_2 are given in the Table. The yields of the measured products were linear with dose. *G* Values * calculated from such yield–dose plots are included in the Table. Water radiolysis leads to the formation of OH radicals, solvated electrons (e_{aq}^-), and H atoms as reactive species. Under oxygenated conditions the OH radicals [*G*(OH) *ca.* 2.8] will react with the substrate [reaction (1); $k_1 = 1.2 \times 10^8$ dm^3 mol^{-1} s^{-1} (ref. 14, for correction see ref. 15)] whereas the H atoms and solvated electrons are scavenged by oxygen [reactions (2) and (3)]. In solutions saturated with N_2O–O_2

$$\textcircled{P}\text{–O–CH}_3 + {}^{\bullet}\text{OH} \longrightarrow \textcircled{P}\text{–O–CH}_2^{\bullet} + \text{H}_2\text{O} \qquad (1)$$

$$\text{H}^{\bullet} + \text{O}_2 \longrightarrow \text{HO}_2^{\bullet} \rightleftharpoons \text{H}^+ + \text{O}_2^{\bar{\cdot}} \qquad (2)$$

$$e^-_{aq} + \text{O}_2 \longrightarrow \text{O}_2^{\bar{\cdot}} \qquad (3)$$

$$\textcircled{P} = (\text{MeO})_2\text{PO}$$

the solvated electrons are converted by N_2O into OH radicals [*G*(OH) *ca.* 5.6];[16] however, again the H atoms [*G*(H·) *ca.* 0.6] are still scavenged by O_2 [reaction (2)]. The substrate radicals formed in reaction (1) react with oxygen to give the

$$\textcircled{P}\text{–O–CH}_2^{\bullet} + \text{O}_2 \longrightarrow \textcircled{P}\text{–O–CH}_2\text{–O–O}^{\bullet} \qquad (4)$$

corresponding peroxyl radicals [reaction (4)]. It is the fate of these peroxyl radicals that is the major concern of this study.

(a) *Bimolecular decay of the peroxyl radicals.* Scheme 1 shows the proposed reactions subsequent to the bimolecular decay of the peroxyl radicals. It largely reflects our present knowledge of the general fate of secondary peroxyl radicals

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* The *G* value is defined as molecules formed per 100 eV of absorbed energy.

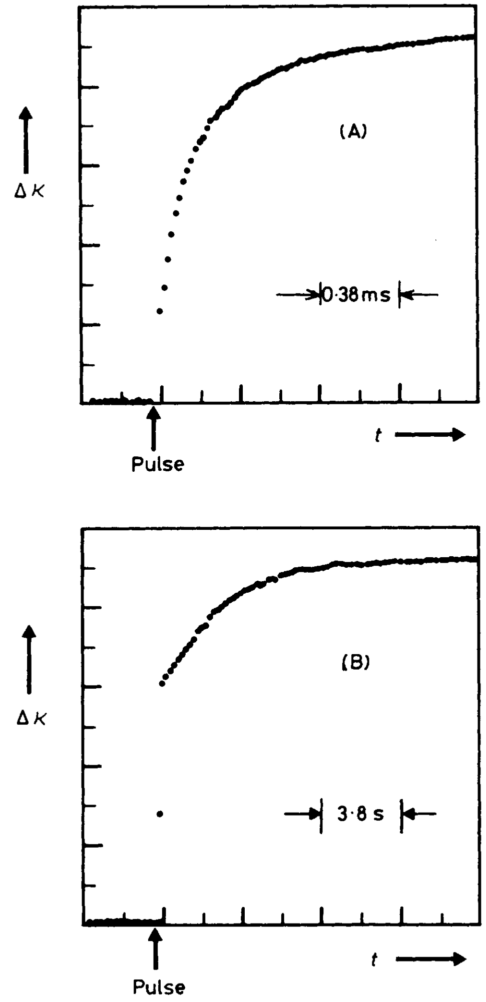


Figure 1. Conductivity change (computer print-out) of 5mM-trimethyl phosphate solution saturated with N_2O–O_2 (4 : 1 v/v), at pH 6.5, following a 1 μs electron pulse of *ca.* 16 Gy: (A) fast second-order build-up; (B) pseudo-first-order build-up identified as the hydrolysis of $(MeO)_2P(O)$–CHO [reaction (17)]

in aqueous solutions,[17,18] but contains in addition the novel reaction (14). Some insight into the mechanistic details of the present system has been gained by comparing the measured yields of the products with data from pulse radiolysis. The pulse conductometric method in particular can be applied with some advantage. Trimethyl phosphate is a non-electrolyte but $HO_2\cdot$ (pK_a = 4.7) [19] and dimethyl hydrogen phosphate (p$K_a \leqslant 2.5$) are fully dissociated in neutral and in alkaline solutions. Consequently, the formation of $H^+ + O_2^{-\cdot}$ from reaction (13) and H^+ + dimethyl phosphate anion from reaction (14) or its equivalent (15) + (16) can be monitored [for a possible contribution of formic acid see reaction (19)]. In neutral solutions this leads to an increase in conductivity, whereas in alkaline solutions a decrease in conductivity is observed, because as a result of the neutralisation of H^+ by OH^-, OH^- (λ° = 178 Ω^{-1} cm^{-1}) is replaced by $O_2^{-\cdot}$ and dimethyl phosphate ions (λ° *ca.* 60 Ω^{-1} cm^{-1}).

The typical conductivity change of a 5mM-trimethyl phosphate solution saturated with N_2O–O_2 (4 : 1 v/v) at pH 6.5 following an electron pulse of 1 μs duration is shown in Figure 1(A). After the neutralisation of H^+ and OH^- formed during the pulse a small residual increase in conductivity [$G(H^+$ + anion) = 0.5] due to $H^+ + O_2^{-\cdot}$ [$HO_2\cdot$ from reaction (2)] was observed. This was followed by a second-order build-up of conductivity with net $G(H^+$ + anion) *ca.* 4 (at 30 Gy) to 5 (at 2 Gy). This build-up followed second-order kinetics with the reciprocal of its first half-life depending linearly on the dose rate. The second-order rate constant of the conductivity build-up was calculated to be $2k = (9 \pm 2) \times 10^8$ dm^3 mol^{-1} s^{-1}.

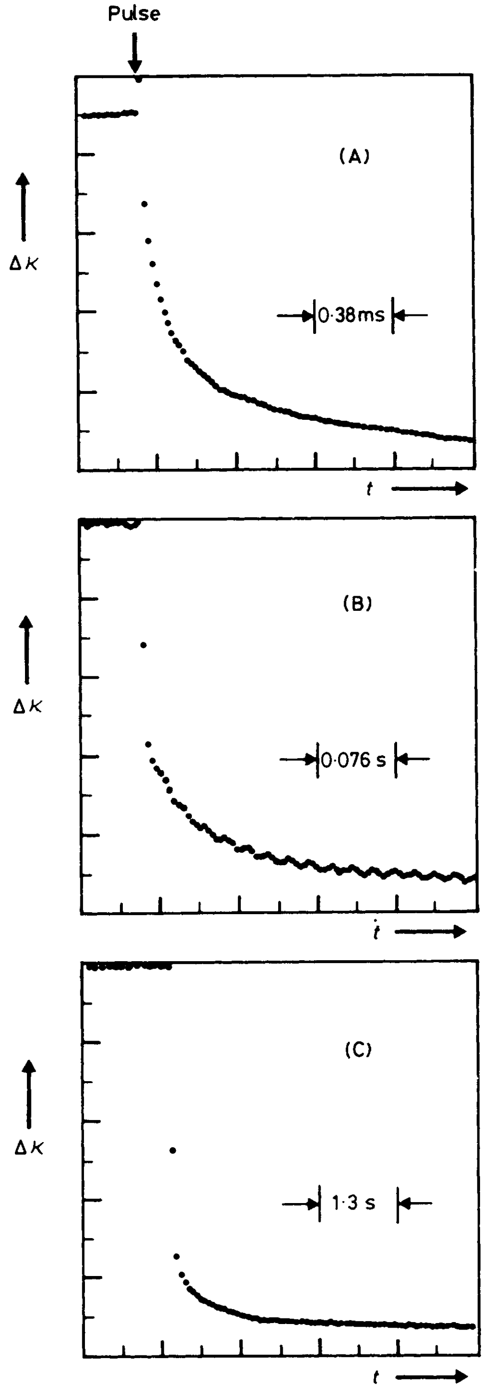
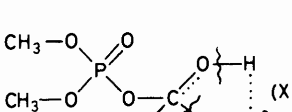
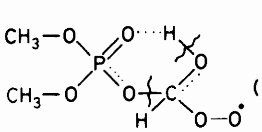


Figure 2. Conductivity change of a 5mM-trimethyl phosphate solution saturated with N_2O–O_2 (4 : 1 v/v), at pH 10.8, following a 1 μs electron pulse of *ca.* 19 Gy: (A) fast second-order rate process; (B) subsequent fast pseudo-first-order rate process identified as the hydrolysis of $(MeO)_2P(O)O$–CHO [reaction (17)]; (C) subsequent slow pseudo-first-order rate process not yet identified

At pH 10—11 a decrease in conductivity was observed [*e.g.* Figure 2(A)] which again can be attributed to the formation of acid(s). This decrease in conductivity also followed second-order kinetics with a rate constant identical with the value found at pH 6.5, namely, $2k = (9 \pm 2) \times 10^8$ dm^3 mol^{-1} s^{-1}. The value of G(acid) calculated from the consumption of OH^- and the formation of an anion was 6.0, which includes a contribution from reaction (2). This second-order process is attributed to the bimolecular self-termination (dimerisation) of the peroxyl radicals derived from trimethyl phosphate [reaction (5)], which is the rate-determining step. The resulting tetraoxide then rapidly decomposes by a number of different pathways, one of which [reaction (11)] leads to the formation of H^+ by a non-rate-limiting step. To test whether this conductivity increase was due to the formation of $H^+ + O_2^{-\cdot}$ [reaction (13)] or H^+ + dimethyl phosphate anion [reaction (14)], or a mixture of the two, tetranitromethane (0.04—0.09mM) was added to the N_2O–O_2-saturated trimethyl phosphate solution to scavenge $O_2^{-\cdot}$. Tetranitromethane (TNM) reacts at near diffusion-controlled rates with $O_2^{-\cdot}$, nitroform anion (NF^-), O_2, and $NO_2^\cdot$ being the products,[20] but it reacts with the primary radical derived from trimethyl phosphate only slowly (k *ca.* 10^8 dm^3 mol^{-1} s^{-1}). The only other species with possible reducing property in our system are the radicals $(MeO)_2P(O)O$–$\dot{C}HOH$ and $H\dot{C}{=}O$. These radicals, however, are expected to add O_2 which is present in large excess in comparison with tetranitromethane. The peroxyl radicals thus formed will no longer have reducing properties. Nitroform anion is detected at 350 nm [ε (350 nm) $= 1.46 \times 10^4$ l mol^{-1} cm^{-1}]. After an electron pulse (2—17 Gy) a prompt absorption increase (<20 μs) at 350 nm was observed in the TNM-containing trimethyl phosphate solution (5mM, pH *ca.* 6.5) with $G(NF^-) = 0.6$ due to $O_2^{-\cdot}$ from reaction (2), which was followed by a second-order absorption build-up with net $G(NF^-) = G(O_2^{-\cdot}) = 0.9$ at the plateau, [$2k = (9 \pm 2) \times 10^8$ dm^3 mol^{-1} s^{-1}]. Both the rate constant and the G value of this NF^- formation were independent of tetranitromethane concentration. Thus only a part of the bimolecular decay of the trimethyl phosphate-derived peroxyl radicals that leads to conductivity is due to $HO_2^\cdot/(H^+ + O_2^{-\cdot})$ formation ($G = 0.9$) according to reaction (13). The remainder is attributed to reaction (14).

The type of reaction exemplified by (13) is a well documented process that involves a five-membered transition state (X) (*cf.* ref. 21). By using a six-membered transition state reaction (14) can be written in a similar fashion (Y).


(X)


(Y)

It is known that α-halogenohydroxyalkyl radicals rapidly ($t_{\frac{1}{2}} \leqslant 1$ μs) eliminate hydrogen halides (*cf.* ref. 22). In a similar way the α-phosphatohydroxyalkyl radical formed in reaction (11) could eliminate dimethyl hydrogen phosphate. This reaction might be so fast that it competes with the oxygen addition [reaction (12)]. Reactions (15) and (16) are then alternatives to reactions (12) and (14). A distinction between these two routes cannot be made at present. The products would be the same in both cases and the oxygen concentration cannot be varied over a sufficiently large range in order for the two processes to be distinguished by kinetic measurements. Although reaction (11) is the major decay route (*ca.* 70%), other processes must also be considered, such as reactions (6), (7), (9), and (10). These reactions are analogous to well documented reactions of peroxyl radicals.

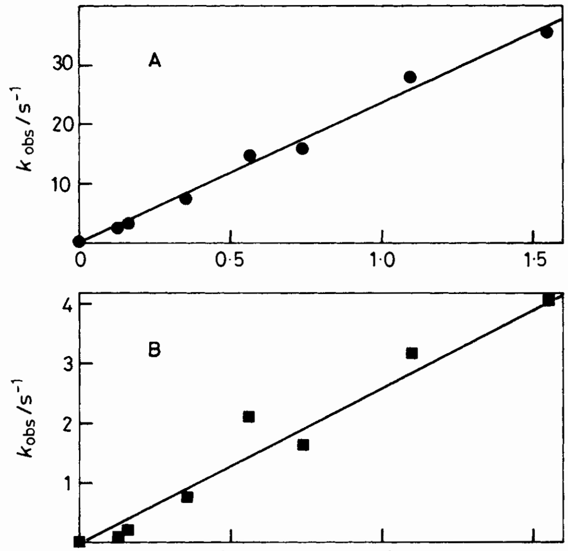


Figure 3. Pulse radiolysis of 5mM-trimethyl phosphate solutions saturated with N_2O–O_2 (4 : 1 v/v); observed first-order rate constants obtained by conductivity measurements as a function of $[OH^-]$: (A) fast process identified as the hydrolysis of $(MeO)_2P(O)OCHO$ [reaction (17)]; (B) slow process not yet identified

(b) *Hydrolysis of non-radical intermediates.* The products formed in reactions (6), (7), (10), and (13) are initially non-electrolytes but will undergo hydrolysis to give ionic species [reactions (17) and (18)]. The slow hydrolysis of one species

$$\textcircled{P}\text{–O–CHO} + H_2O \longrightarrow \textcircled{P}\text{–O}^- + HCOO^- + 2H^+ \qquad (17)$$

$$\textcircled{P}\text{–O–CH}_2OH + H_2O \longrightarrow \textcircled{P}\text{–O}^- + H_2C(OH)_2 + H^+ \qquad (18)$$

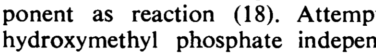
in neutral and acidic solutions and of two species in alkaline solutions has been observed at longer times after the pulse. At pH 6.5 following the second-order build-up already discussed there is a slow first-order component (k 0.2 s^{-1}) with $G(H^+ + \text{anion})$ *ca.* 3 [Figure 1(B)]. At such long times $HO_2^\cdot/O_2^{-\cdot}$ is no longer present, and should have decayed.[19] The observed yield is attributed to the net conductivity change resulting from the conductivity decrease due to $HO_2^\cdot/O_2^{-\cdot}$ decay and the increase of conductivity due to reaction (17). In reaction (17) two acids are formed, the high yield (which effectively should be higher because $HO_2^\cdot/O_2^{-\cdot}$ decays during their build-up) agrees with that expected for this process. The assignment of this conductivity build-up to reaction (17) is supported by the fact that the yield of protons decreases to G *ca.* 2 at pH 3.1 where formic acid ($pK_a = 3.7$) is no longer fully dissociated. The residual conductivity increase is now mainly due to the contribution of the dimethyl hydrogen phosphate acid ($pK_a \leqslant 2.5$).

In basic solutions $O_2^{-\cdot}$ is a rather stable species [19] and is not expected to decay within the time scale of our experiments; the corrections for its decay necessary at pH 6.5 need not be made at pH > 10. Under such conditions two distinct first-order processes of conductivity decrease have been observed [Figures 2(B) and (C)] and values of G(acid) of 4.0 for the faster process and 2.0 for the slower process have been obtained. The first process is again identified as reaction (17). The second process [Figure 2(C)] has been identified as reaction (18), owing to the approximate agreement of *G*(HCHO) (1.7) with the *G* value of acid formation (2.0).[23] The rates of both these processes increase with increasing OH^- concentrations [Figures 3(A) and (B)] and from these plots the rate constants may be calculated (error ± 30%) as $k_{17} = 0.3 + 2.3 \times 10^4\ [OH^-]\ s^{-1}$ for the fast process and $k = 2\,600\ [OH^-]\ s^{-1}$ for the slow process.

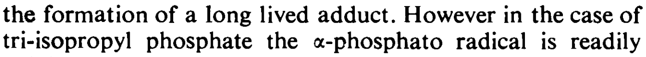
The identification of the faster process as reaction (17) appears to be on firmer grounds than that of the slower component as reaction (18). Attempts to produce dimethyl hydroxymethyl phosphate independently by oxidising the radical with *e.g.* tetranitromethane were not successful due to the formation of a long lived adduct. However in the case of tri-isopropyl phosphate the α-phosphato radical is readily oxidised and the α-phosphato-alcohol thus formed decomcomposes rapidly ($k \geqslant 3 \times 10^4\ s^{-1}$) into di-isopropyl hydrogen phosphate and acetone. This recent observation throws some doubt on the previous suggestion that dimethyl hydroxymethyl phosphate is hydrolysed so slowly. As an alternative to reaction (18) the slow process might be the reaction of CO_2 with OH^-. This possibility can, however, be excluded because of the known rate constant [k 6 900 $dm^3\ mol^{-1}\ s^{-1}$] [24,25] and the low yield of CO_2 (*cf.* Table).

A third possibility would be a so far unknown long lived intermediate derived from the reactions of the formylperoxyl radicals in basic solution. This possibility still warrants investigation.

The formylperoxyl radical is a major radical species in our system (*G ca.* 3). Although reactions of this radical have not been previously encountered in pulse radiolytic studies it can reasonably be assumed to be the precursor of CO_2 and CO in neutral solutions [for example, reactions (19) and (20)].

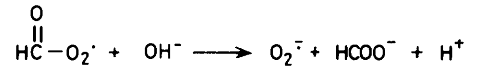


$$2\ \mathrm{H\overset{\overset{O}{\|}}{C}-O_2^{\bullet}} \longrightarrow CO_2 + O_2 + HCOOH \qquad (19)$$



$$\mathrm{H\overset{\overset{O}{\|}}{C}-O_2^{\bullet}} + \text{Ⓟ}\mathrm{-O-CH_2-O_2^{\bullet}} \longrightarrow CO + O_2 + \text{Ⓟ}\mathrm{-O-CH_2OOH} \qquad (20)$$

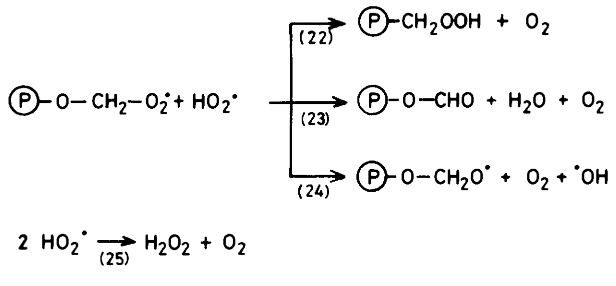
The hydrate of the formylperoxyl radical, the dihydroxymethylperoxyl radical, is known to eliminate $HO_2^{-}\cdot$ rapidly.[26] Thus in alkaline solutions the formylperoxyl radical probably eliminates $O_2^{-}\cdot$ by reacting with OH^- [reaction (21)]. In fact



$$\mathrm{H\overset{\overset{O}{\|}}{C}-O_2^{\cdot}} + OH^- \longrightarrow O_2^{-\cdot} + HCOO^- + H^+ \qquad (21)$$

G(formic acid) has been found to be higher by about 10% at pH 11.5 than at pH ⩽ 6, and no CO was detected if γ-irradiations were carried out in alkaline solutions. These two findings explain the higher yield of acid formation in alkaline solutions but strongly suggest that the proposed reaction (21) cannot be the only decay route of the formylperoxyl radical in alkaline solution.

In the pulse radiolysis experiments with trimethyl phosphate saturated with N_2O–O_2 the reactions of the peroxyl radicals are very little influenced by the small initial amounts of $HO_2\cdot/O_2^{-}\cdot$ radicals formed by H atom scavenging, and additional $HO_2\cdot/O_2^{-}\cdot$ radicals are only formed in the course of the bimolecular decay processes. Very little interaction between the organic peroxyl radicals and the $HO_2\cdot/O_2^{-}\cdot$ radicals is therefore to be expected under these conditions. In steady-state radiolysis, especially in O_2-saturated solutions, the $HO_2\cdot/O_2^{-}\cdot$ radicals will attain a rather high steady-state concentration.[19] These radicals will not simply decay by self-termination [*cf.* Scheme 2, reaction (25)], but may now react also with the substrate peroxyl radicals. Scheme 2 shows three conceivable reactions; the formation of a hydroperoxide [reaction (22)], the formation of formyl dimethyl phosphate plus water [reaction (23)], and a reaction which leads to further OH radicals [reaction (24)] (*cf.* ref. 27). The last reaction initiates a chain reaction which is, however, rather short because of both the presence of the three competing reactions (22), (23), and (25), and the low yield of $HO_2\cdot/O_2^{-}\cdot$ from the organic oxyl radical [reaction (13)]. That a chain reaction occurs in this system is indicated by the *G* values given in the Table. The value of *G*(total acid), for example, is greater than the expected yield of one half of that found under N_2O–O_2 conditions, and this observation can be extended to the other products.

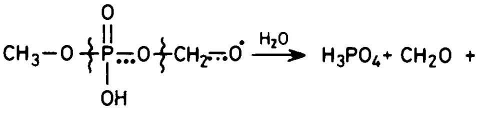


Scheme 2.

To check the contribution of the $HO_2\cdot/O_2^{-}\cdot$ radicals in this system superoxide dismutase (SOD) was added prior to irradiation. The data given in Table 1 show that *G*(total acids) drops by 0.9 and *G*(formaldehyde) by 0.2 units in the presence of SOD. As might be expected, the contribution of the chain reaction is small but nevertheless measurable.

Sodium Dimethyl Phosphate.—In the γ-radiolysis of sodium dimethyl phosphate in solutions saturated with N_2O–O_2 the *G* values of corresponding products are very similar to those from the γ-radiolysis of trimethyl phosphate (Table). It is believed that the mechanisms involved are very similar, although the rates might be somewhat different. A hitherto open question, not easily resolved by the trimethyl phosphate system, could be answered here. Oxyl radicals are known to undergo β-fragmentation reactions quite readily. Oxyl radicals derived from dimethyl phosphate formed in a reaction analogous to reaction (8) might undergo β-fragmentation and the resulting radical might even fragment further [reaction (26)].

This reaction would provide an alternative route to formaldehyde. {The methoxyl radical would also eventually lead to formaldehyde [*cf.* reactions (11)—(13)].} No inorganic phosphate is formed in the radiolysis of dimethyl phosphate. This excludes route (26) as a possible pathway.



$$\mathrm{CH_3-O-\underset{OH}{\overset{\overset{O}{\|}}{P}}-O-CH_2-O^{\bullet}} \xrightarrow{H_2O} H_3PO_4 + CH_2O + CH_3O^{\bullet} \qquad (26)$$

No attempts have been made to investigate this compound by pulse radiolysis. Because of the background conductivity inherent in solutions of dimethyl phosphate results would be more difficult to obtain with this substrate than with trimethyl phosphate.

The Methyl Phosphates as Models for DNA Radiolysis.— The peroxyl radicals of trimethyl phosphate decay bimolecularly. One might ask the question whether such a bimolecular decay of peroxyl radicals in a large macromolecule such as DNA is at all possible. In the absence of unimolecular decay routes, such radicals must in fact decay bimolecularly, albeit with rate constants lower than those of the small model system. Lindenau *et al.*[28] investigated the kinetics of the radiation-induced strand breaking of single-stranded DNA in oxygenated aqueous solutions. They observed that the half-life of DNA strand breaking decreases with increasing dose rate (dose per pulse). Such behaviour is typical of a reaction which is kinetically of the second order in the radicals involved. In a variation of their interpretation of these data we conclude that the rate-determining step is the bimolecular decay of the DNA peroxyl radicals. We calculate from their data that the DNA peroxyl radicals decay with a bimolecular rate constant of *ca.* 4.5×10^7 dm^3 mol^{-1} s^{-1} (assuming $G = 3$ for DNA peroxyl radicals) or *ca.* 2.3×10^7 dm^3 mol^{-1} s^{-1} [assuming G(DNA peroxyl radicals) $= 3$ and $G(O_2^{-\cdot}) = 3$ and further assuming that $HO_2^\cdot/O_2^{-\cdot}$ also interact with the DNA peroxyl radicals]. These rate constants are 20—40 times less than those of our model system.

If there is some analogy between our present system and the DNA 5′-peroxyl radical one would expect that the decay of the latter would produce strand breaks consisting of a 3′-phosphate end group and a 5′-carbonyl or carboxy function. Neither of these latter structures has yet been observed in γ-irradiated DNA. From an earlier study of ribose-5-phosphate [2] together with the present results it would be expected that processes analogous to reactions (6) and (7) would occur in irradiated DNA systems and that these so far undetected types of damage must be present. Our results indicate the formation of oxyl radicals as intermediates. It is known that besides the 1,2-H shift [*cf.* reaction (11)], oxyl radicals can undergo β-fragmentation of C–C bonds. With oxyl radicals having an alkoxyl group in the β-position the 1,2-H shift may even no longer be detectable because of the readiness of C–C fragmentation.[18] The 5′-oxyl radical of DNA also has an alkoxy group in the β-position. One would expect therefore that the β-fragmentation [cleavage of the C(4′)–C(5′) bond] would be an important process, probably more important than the 1,2-H shift. Indeed, products that can arise from such a β-fragmentation process have already been detected.[3,4] The present results also indicate that although $HO_2^\cdot/O_2^{-\cdot}$ radicals are not quite so reactive as to attack DNA, they might interact with DNA peroxyl radicals. Since they can induce short chain reactions with the reactive OH radical as chain carrier [*cf.* reaction (24)] they might contribute substantially to DNA destruction (*cf.* ref. 17).

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