Hydroxyl Radical Reaction with Phosphate Esters and the Mechanism of Phosphate Cleavage[1]

A. Samuni and P. Neta*

Radiation Research Laboratories and Center for Special Studies, Mellon Institute of Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213 (Received May 24, 1973)

The reaction of hydroxyl radicals with several phosphate esters in irradiated aqueous solutions has been followed by esr observation of the radicals and by determination of the yield of inorganic phosphate. The initial step of hydrogen abstraction takes place at all available C–H positions and, as expected, the site of reaction was found to be affected by the state of protonation of an amino group. In most cases the radicals produced by abstraction were observed by esr and in certain cases the radicals subsequently produced by elimination of H_2O or H_3PO_4 were also observed. The results for phospho glycols indicate that α-phospho radicals are the most stable toward phosphate elimination but that β-phospho radicals undergo elimination of H_3PO_4 very rapidly. Elimination of H_2O from these and other radicals appears to be slower than that of H_3PO_4. The results for ethanolamine and serine phosphates indicate that the elimination of H_3PO_4 from the radicals is slower than in the case of glycol phosphates and probably does not take place at all when the amino group is protonated.

Introduction

The mechanism of radiolytic phosphate ester cleavage has been the subject of numerous investigations because this process is believed to play a key role in the radiation induced damage to DNA. Potentially, phosphate esters can react with all the primary radicals of water radiolysis, e_{aq}^-, H, and OH, and partially release phosphate ions. The reaction of e_{aq}^- causing phosphate release by dissociative attachment

$$ROPO_3H_2 + e_{aq}^- \longrightarrow \dot{R} + H_2PO_4^- \qquad (1)$$
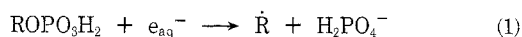
has been suggested by esr observations[2,3] on γ-irradiated alkyl phosphates at 77°K and by product analysis of irradiated aqueous solutions of trimethyl phosphate.[4] However, this reaction will be unimportant when biological systems are considered, because its rate constant is very low[4] and the electrons will be preferentially scavenged by the DNA bases which are $\sim 10^5$ times more reactive.
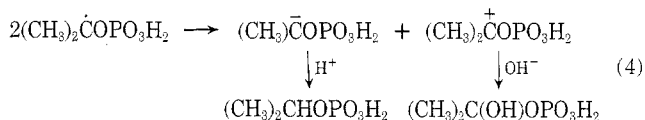
The reaction of H atoms with phosphate esters, although it can lead indirectly to partial cleavage, cannot have a major contribution because of the lower yield of H. The mechanism, however, is expected to be similar to that with OH radicals since in both cases the initial reaction is hydrogen abstraction. In fact, all the radicals that can abstract hydrogen from deoxyribose can induce single strand breaks by phosphate cleavage, as was shown for the uracilyl radical.[5]

The reaction of OH with aliphatic phosphate esters is expected to take place with a rate constant somewhat similar to that of the corresponding alcohol as was found, for example, for di- and trimethyl phosphate.[4] The initial step of reaction is also similar, i.e., abstraction of a hydrogen atom

$$RCH_2OPO_3H_2 + OH \longrightarrow \dot{R}CHOPO_3H_2 + H_2O \qquad (2)$$
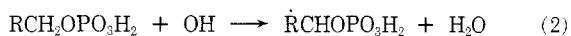
The mechanism of the subsequent release of inorganic phosphate is still controversial.[4,6-8] In previous studies a general mechanism for the phosphate release has been sought without reference to the nature of the alkyl group.

It seems, however, that such a generalized approach is not valid and may well have caused some of the controversies in the literature. Based on the results with alcohols it can be assumed that primary radicals will tend to dimerize whereas secondary or tertiary radicals will tend to disproportionate, e.g.

$$2\dot{C}H_2OPO_3H_2 \longrightarrow H_2PO_3OCH_2CH_2OPO_3H_2 \qquad (3)$$

$$2(CH_3)_2\dot{C}OPO_3H_2 \longrightarrow (CH_3)\bar{C}OPO_3H_2 + (CH_3)_2\overset{+}{C}OPO_3H_2$$
$$\downarrow H^+ \qquad\qquad \downarrow OH^- \qquad (4)$$
$$(CH_3)_2CHOPO_3H_2 \quad (CH_3)_2C(OH)OPO_3H_2$$

The resulting dimers can be stable whereas the species formed by oxidation can be expected to hydrolyze rapidly, either directly or after neutralization.

$$(CH_3)_2C(OH)OPO_3H_2 \longrightarrow (CH_3)_2CO + H_3PO_4 \qquad (5)$$
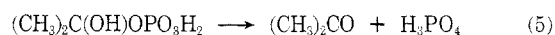
This mechanism is in line with the findings that radiosensitizers increase the yield of inorganic phosphate, most probably by oxidizing the radicals.[7]

$$R_2\dot{C}OPO_3H_2 + S \longrightarrow R_2\overset{+}{C}OPO_3H_2 + S^- \qquad (6)$$
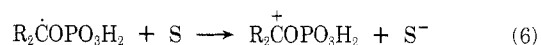
In competition with the radical reactions 3 and 4 direct hydrolysis of the radical can take place, probably catalyzed by base as suggested recently.[4]

$$R_2\dot{C}OPO_3H_2 + OH^- \longrightarrow R_2\dot{C}OH + H_2PO_4^- \qquad (7)$$
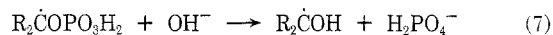
The contribution of this reaction depends on pH and dose rate indicating that it is not very rapid.

When considering phosphate cleavage in the biologically important ribose phosphate or glycerol phosphate, however, one should bear in mind that the behavior of the glycols can be quite different from that of alcohols and so can be the mechanism of phosphate release from their esters. It is known that hydrogen abstraction from glycols can be rapidly followed by elimination of water[9-11]

$$HOCH_2\dot{C}HOH \longrightarrow \dot{C}H_2CHO + H_2O \qquad (8)$$
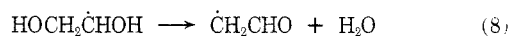
and it is reasonable to assume that a similar elimination

of phosphoric acid can also be rapid in certain radicals derived from glycol phosphates.

It is the purpose of the present study to test this assumption and to evaluate its importance with the various radicals from glycerol phosphates. Some aspects of the mechanism of phosphate cleavage have been studied both by analysis of phosphate production and by esr observation of the radicals produced in irradiated aqueous solutions.

Experimental Section

Most of the organic compounds used were obtained from Sigma Chemical Co. and were used without further purification. Monomethyl phosphate was obtained from K and K and triethyl phosphate from Aldrich. Glycerol and all the inorganic compounds were Baker Analyzed reagents. Solutions in triply distilled water were bubbled with the desired gas, irradiated in a Gammacell 220 source (5×10^{19} eV g^{-1} hr^{-1}), and analyzed for inorganic phosphate by the Molybdenum Blue method.[12] The *in situ* radiolysis esr experiments have been carried out as described by Eiben and Fessenden.[13]

In order to follow the reaction of OH radicals the solutions were saturated (2×10^{-2} M) with N$_2$O to convert the radiolytically produced e$_{aq}^-$ into OH. In strongly acid solutions the reaction of H$^+$ with e$_{aq}^-$ to produce H atoms becomes more important and one observes then the reaction of both OH and H. At pH >3 the contribution of H to the total radical reactions is only ~10%. Because most of the reactions observed involve hydrogen abstraction the organic radicals formed by either OH or H are expected to be the same.
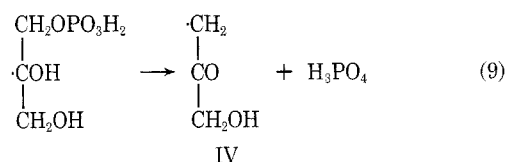
Results and Discussion

Radicals produced by the reaction of OH with phosphate esters have been studied in N$_2$O saturated aqueous solutions by the *in situ* radiolysis-esr technique. In order to facilitate the interpretation of the spectra some of the related alcohols have also been studied under similar conditions. The results of glycerol and its phosphates are summarized in Table I.

The spectrum recorded with irradiated solutions of glycerol at pH 4.6 consisted of lines of two radicals only. Radical I showed two proton splittings of 17.43 and 11.07 G which suggest that hydrogen abstraction took place at position 1 as shown in the table. The larger hyperfine constant is assigned to the α proton and the smaller to the β proton by comparison with previous findings with other glycols.[10] Splittings by the two equivalent γ protons and one hydroxyl proton were also observed. Radical II showed a 10.40-G splitting by four equivalent protons and must, therefore, be that produced by abstraction of the central hydrogen atom. Splittings by the central hydroxyl proton and by the two equivalent terminal OH protons were also observed and support the assignment in Table I. A previous study[14] of these radicals resulted in a misinterpretation owing to poor resolution.

Radicals I and II are expected to undergo elimination of water (reaction 8) as was previously found with acid solutions of glycerol[9] and similarly with other glycols.[10] Radicals III and IV are expected to be produced from I and II, respectively. The spectrum recorded with irradiated solutions of glycerol at pH 12.5 consisted of lines which are interpreted as those of radicals III and IV while no trace of I and II was found. The interpretation shown in Table I is based on the previous assignment.[9] The present esr pa-

rameters are in good agreement with the reported values[9] within the accuracy of those values. The assignment is also supported by the higher g factors which are characteristic of β-keto radicals. In the case of radical IV the central group of lines of the 19.73-G triplet was not observed, most probably as a result of line broadening caused by slow rotation around the H$_2$C–CO bond. Some broadening has also been observed with $\dot{C}H_2COCH_3$.[10]
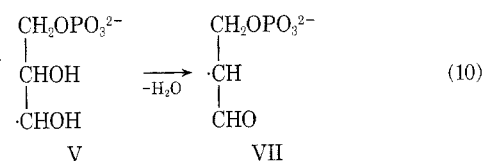
The esr spectra recorded with irradiated solutions of glycerol 1-phosphate at various pH values between 1 and 13 showed the presence of four different radicals under various conditions. The spectrum of radical IV was observed in all cases. This radical, the only one observed which does not contain the phosphate group, must be produced by elimination of H$_3$PO$_4$ from the radical resulting from abstraction of the central hydrogen

$$
\begin{array}{ccccc}
CH_2OPO_3H_2 & & \dot{C}H_2 & & \\
| & & | & & \\
\dot{C}OH & \longrightarrow & CO & + & H_3PO_4 \qquad (9) \\
| & & | & & \\
CH_2OH & & CH_2OH & & \\
& & IV & &
\end{array}
$$

The fact that radical IV has been observed at all pH values indicates that this type of elimination is very rapid. It should be also pointed out that the alternative elimination, *i.e.*, loss of water to produce ·CH$_2$COCH$_2$O-PO$_3$H$_2$ was not observed and is probably a slower process.

The other two radicals that can be produced by abstraction from position 1 or 3 have been observed before undergoing elimination. At pH 5.4 a radical was observed which showed esr parameters very similar, but not identical with those of radical I, and was, therefore, assigned as radical V (Table I). The third possible radical (VI) was only observed in mildly alkaline solutions. The assignment is quite straightforward by comparison to radical V with all the proton hyperfine constants very similar and the α hydroxyl proton splitting of 1.25 G replaced by a 5.55-G ^{31}P splitting. The magnitude of this phosphorus hyperfine constant is supported by the parameters for $\dot{C}H_2OPO_3^{2-}$ (see below).
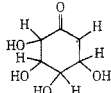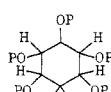
At pH 8.5–11 radical V was not observed and it was replaced by radical VII, the product of water elimination

$$
\begin{array}{ccccc}
CH_2OPO_3^{2-} & & CH_2OPO_3^{2-} & & \\
| & & | & & \\
CHOH & \xrightarrow{-H_2O} & \dot{C}H & & \qquad (10) \\
| & & | & & \\
\dot{C}HOH & & CHO & & \\
V & & VII & &
\end{array}
$$

The assignment of radical VII is easily made by comparing its esr parameters with those of radical III. The g factors are identical, all the proton splittings are very similar, and the OH proton which was not observed (*i.e.*, $a^H < 0.1$ G) in radical III is replaced in radical VII by $a^P = 0.52$ G.
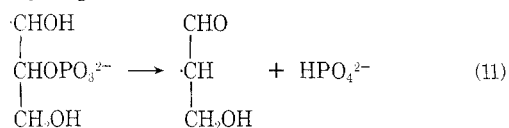
In summary of the results with glycerol 1-phosphate it is seen that the β-phospho radical undergoes rapid elimination of phosphate (compared with the time scale of the esr observation, *i.e.*, 100 μsec) even in neutral solution and was not observed. Radical IV produced from it was observed at all pH values. The γ-phospho radical V was observed at pH 5.4 and found to undergo water elimination in base to produce VII. And finally, the α-phospho radical VI which was observed at pH 8.5–11 does not undergo elimination of phosphate in the time scale of the esr measurements.

TABLE I: Esr Parameters and Suggested Structures of Radicals Produced by Reaction of OH with Phosphate Esters and Some Corresponding Alcohols

Solute	Radical	Structure	g factor[a]	Hyperfine constants[b]
Glycerol pH 4.6	I	·CHOH | CHOH | CH₂OH	2.00301	a^H_α = 17.43 a^H_β = 11.07 a^H_γ = 0.34 (2) $a^H_{OH(\alpha)}$ = 0.81
	II	CH₂OH | ·COH | CH₂OH	2.00279	a^H_β = 10.40 (4) $a^H_{OH(\alpha)}$ = 0.44 $a^H_{OH(\beta)}$ = 0.19 (2)
pH 12.5	III	CHO | ·CH | CH₂OH	2.00414	a^H_α = 18.18 $a^H_{CH_2}$ = 25.98 (2) a^H_{CHO} = 1.50
	IV	·CH₂ | CO | CH₂OH	2.00406	a^H_α = 19.73 (2)[c] a^H_γ = 2.33 (2)
Glycerol 1-phosphate pH 5.4	V	CH₂OPO₂H⁻ | CHOH | ·CHOH	2.00303	a^H_α = 17.42 a^H_β = 11.66 a^H_γ = 0.24 (2) $a^H_{OH(\alpha)}$ = 1.25 $a^H_{OH(\beta)}$ = 0.49
pH 8.5–11	VI	·CHOPO₃²⁻ | CHOH | CH₂OH	2.00293	a^H_α = 18.25 a^H_β = 11.33 a^H_γ = 0.30 (2) $a^H_{OH(\beta)}$ = 0.60 a^P = 5.55
	VII	CH₂OPO₃²⁻ | ·CH | CHO	2.00414	a^H_α = 18.34 $a^H_{CH_2}$ = 27.17 (2) a^H_{CHO} = 1.58 a^P = 0.52
pH 1–13	IV	d		
Glycerol 2-phosphate pH 11	III	d		
3-Phosphoglyceric acid pH 6–13	VIII	·CHOPO₃²⁻ | CHOH | CO₂⁻	2.00301	a^H_α = 18.20 a^H_β = 11.32 $a^H_{OH(\beta)}$ = 0.32 a^P = 4.37
	IX	·CH₂ | CO | CO₂⁻	2.00435	a^H_α = 18.86 (2)[c]
Inositol (hexahydroxycyclohexane) pH 11.4	X		2.00428	a^H_α = 17.59 a^H_β = 37.21 a^H = 2.90[e] a^H = 0.73[f] a^H = 0.16[f]
Phytic acid (inositol hexaphosphate) pH 10	XI[h]		2.00322	$a^H_{\beta 1}$ = 14.35 $a^H_{\beta 2}$ = 11.41 a^P_α = 2.29 a^H_γ or a^P_β = $\begin{cases} 1.78^g \\ 0.88^g \\ 0.88^g \end{cases}$
Ethanolamine phosphate pH 7.6	XII	·CHOPO₃²⁻ | CH₂NH₃⁺	2.00306	a^H_α = 19.00 a^H_β = 13.11 (2) a^P = 4.90 a^N = 9.51 a^H_{NH3} = 0.30 (3)
Serine phosphate pH 7.3	XIII	·CHOPO₃²⁻ | CHNH₃⁺ | CO₂⁻	2.00307	a^H_α = 18.29 a^H_β = 13.51 a^P = 4.69 a^N = 7.15 a^H_{NH3} = 0.14 (3)
pH 10.8, 14	XIV	CH₂OPO₃²⁻ | ·CNH₂ | CO₂⁻	2.00326	$a^H_{CH_2}$ = 10.85 (2) a^N = 5.69 $a^{H1}_{NH_2}$ = 2.85 $a^{H2}_{NH_2}$ = 1.87 a^P = 0.97
Methyl phosphate pH 6–13	XV	·CH₂OPO₃²⁻	2.00311	a^H = 18.62 (2) a^P = 5.70

[a] Measured relative to the peak from the silica cell and accurate to ±0.00005. Second-order corrections have been made [R. W. Fessenden, *J. Chem. Phys.*, **37**, 747 (1962)]. [b] Given in gauss and accurate to ±0.03 G. The number of nuclei displaying the splitting is given in parentheses if different than one. [c] The central line or group of lines of this triplet was not observed because of broadening. [d] See structure and parameters of same radical above. [e] This splitting can be possibly assigned to the γ proton beyond the carbonyl group. [f] This splitting can be assigned either to a γ CH or a β OH proton. [g] Assignment of these three splittings to specific nuclei cannot be made with certainty. [h] In the structure of this radical OP stands for the phosphate group.
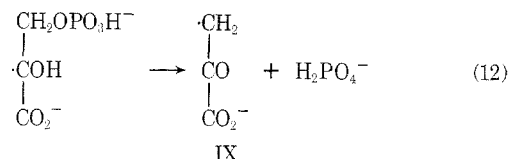
The spectrum recorded with irradiated alkaline solutions of glycerol 2-phosphate showed the presence of radical III. This radical can only be formed by elimination of phosphate from an initial radical produced by abstraction of a terminal hydrogen

$$
\begin{array}{ccc}
\cdot CHOH & & CHO \\
| & & | \\
CHOPO_3{}^{2-} & \longrightarrow & \cdot CH \quad + \quad HPO_4{}^{2-} \qquad (11) \\
| & & | \\
CH_2OH & & CH_2OH
\end{array}
$$

This reaction is in line with reaction 9 found in the case of glycerol 1-phosphate. In both cases β-phospho radicals eliminate phosphate rapidly.

The other radical expected to be formed from glycerol 2-phosphate is $HOCH_2\dot{C}(OPO_3{}^{2-})CH_2OH$, which is not expected to undergo elimination in parallel to the behavior of radical VI. However, this radical was not observed and was probably produced with a low yield.

Solutions of 3-phosphoglyceric acid have also been irradiated and the recorded esr spectra showed the presence of a mixture of radicals. Only two radicals have been successfully characterized and their parameters are given in Table I. The unidentified radicals seem to be produced by secondary reactions. Radical VIII shows coupling constants which parallel those of radical VI, except for the small CH_2 splitting which is absent because this group is replaced by a carboxyl group. By this analogy radical VIII is easily identified as shown in Table I. The second radical observed resembles radical IV produced from glycerol and from glycerol 1-phosphate and, therefore, the structure and the mechanism of its production must also be the same

$$
\begin{array}{ccc}
CH_2OPO_3H^- & & \cdot CH_2 \\
| & & | \\
\cdot COH & \longrightarrow & CO \quad + \quad H_2PO_4{}^- \qquad (12) \\
| & & | \\
CO_2{}^- & & CO_2{}^- \\
& & IX
\end{array}
$$

Only the external lines of radical IX have been observed, similar to the case of radical IV, where the central group of lines was not detected because of broadening. The results with 3-phosphoglyceric acid support the findings with the glycerol phosphates, namely, that the β-phospho radicals eliminate phosphoric acid very rapidly whereas the α-phospho radicals do not. To further examine this behavior we chose to study the reaction of OH with phytic acid (inositol hexaphosphate) where only α-phospho radicals can be produced (although they are β-phospho as well).

The results for inositol and phytic acid are also summarized in Table I. Radical X observed with solutions of inositol at pH 11.4 has been previously observed in acid solutions using Fenton's reagent,[9] and the hyperfine constants determined in both cases are in good agreement. In the present work two additional small splittings were observed which can be assigned to a β-OH and a γ-CH protons. The initial radical formed by abstraction from inositol, which has been observed in acid solutions,[9] was not detected at pH 11.4.

Radical XI observed with solutions of phytic acid at pH 10 shows $g = 2.00322$. This value indicates the absence of a β-keto group, i.e., as expected elimination of phosphate did not take place from this α-phospho radical. The β proton hyperfine constants are much smaller than those observed with inositol itself (30.5 G)[9] indicating that the geometrical conformation is quite different. This differ-

ence is also demonstrated by the lack of symmetry in radical XI in contrast to the case of inositol.[9] It should be pointed out that the phosphorus splitting is smaller here than in all the other cases of α-phospho radicals. The three additional small splittings cannot be assigned with certainty to specific protons or phosphorus nuclei. At pH 13.5 the spectrum of radical XI was not observed. Several lines were found which could not be given a clear interpretation.

Ethanolamine and serine phosphates have also been studied by comparison with the glycol phosphates. The esr spectrum recorded with irradiated neutral solutions of ethanolamine phosphate consisted of lines of one radical only. The parameters calculated are given in Table I and their assignment to radical XII is straightforward. The magnitude of the large proton splittings and the nitrogen splitting are somewhat similar to those observed with ethanolamine itself.[10] The magnitude of a_P is similar to that observed for the other α-phospho radicals in Table I. The site of the hydrogen abstraction from ethanolamine phosphate in neutral solution is in line with the expectation that the protonated amino group will strongly deactivate its neighboring hydrogen atoms. It is expected that in alkaline solution, when the amino group is not protonated, abstraction will take place to a large extent α to this group. Unfortunately, however, no esr lines were detected in alkaline solutions, indicating that a different radical with a shorter lifetime is produced.

Similar experiments with serine phosphate support the above argument and show a change with pH in the site of abstraction. In neutral solutions radical XIII was observed and its parameters are somewhat similar to those of XII. The presence of the carboxyl group causes an appreciable decrease in a^N but smaller changes in the other coupling constants. In alkaline solutions a different spectrum was found. The assignment in Table I was easily made on the basis of results with several amino acids.[15,16] The structure of radical XIV shows, indeed, that abstraction takes place from position α to the free amino group as was also found with the amino acids.[15,16]

The magnitude of the phosphorus splitting in most of the α-phospho alkyl radicals in Table I is in the range of 4.4–5.5 G. These values are much lower than those observed previously with dialkyl and trialkyl phosphates.[17,18] Because it was previously found that a_P for $R\dot{C}HO$-$P(O)(OH)OCH_2R$ is smaller than that for $R\dot{C}HO$-$P(O)(OCH_2R)_2$ (e.g., 10.5 and 7.8 G for the radicals from tri- and dimethyl phosphate, respectively)[17] it is reasonable to assume that a^P for monoalkyl phosphate will be even lower. To substantiate this assumption we studied monomethyl phosphate. The results for radical XV in Table I show that the phosphorus splitting is lower for this radical and should be similarly low for radicals from other monoalkyl phosphates.

According to one suggested mechanism[4] for phosphate cleavage the phosphomethyl radical XV can be expected to hydrolyze in base as shown in reaction 7. In an attempt to support this mechanism we searched for the esr lines of the hydroxymethyl radical. However, the fact that they were not observed does not indicate their absence because they are known[13] to be broad in the alkaline region and, therefore, more difficult to detect under our experimental conditions. It should be easier to detect the spectrum of the $CH_3\dot{C}HOH$ or $CH_3\dot{C}HO^-$ radical, which can be produced from triethyl phosphate by abstraction and hydrolysis. We have irradiated alkaline solutions of triethyl

TABLE II: Yield of Inorganic Phosphate in Irradiated Aqueous Solutions of Ethanolamine Phosphate[a]

Additives	G		
	pH 4.0	pH 7.3	pH 12.0
None	0.9 (0.9)[b]	1.4	2.3
$2 \times 10^{-2} M$ N_2O	1.9 (1.6)[b]	2.7	4.2
$1.5 \times 10^{-2} M$ N_2O + $5 \times 10^{-4} M$ O_2	4.0	4.5	4.4
$2 \times 10^{-2} M$ N_2O + $2.5 \times 10^{-3} M$ Cu^{2+}	5.1		

[a] Solutions containing $1 \times 10^{-2} M$ ethanolamine phosphate were deoxygenated by bubbling with nitrogen or saturated with the desired gas or gas mixture and were irradiated in a Gammacell 220 (Atomic Energy of Canada Limited) with doses of 10^{18}–10^{19} eV g^{-1}. The amount of inorganic phosphate present in solution before irradiation was determined and corrected for with each solution and was always <1% of the phosphate ester. [b] From ref 7.

phosphate at various pH values and could not detect the lines of the α-hydroxyethyl radical. It can be, therefore, concluded that if the hydrolysis shown in reaction 7 contributes to the radiolytic phosphate cleavage it must be taking place on a time scale slower than the millisecond range of the esr observation.

As a supplement to the esr findings we have measured the yield of inorganic phosphate from γ-irradiated solutions of several phosphate esters. The yield from the glycerol phosphates was found to be very close to $G(OH)$ + $G(H)$ at all pH values and over a wide range of concentrations. For example, with $10^{-2} M$ glycerol 2-phosphate solution saturated with N_2O G(inorganic phosphate) was 5.7, 6.2, and 6.7 at pH 2.3, 7.9, and 12.4, respectively. These results are in agreement with the previously reported values for deaerated solutions[6] and both indicate that most of the radicals produced by hydrogen abstraction release inorganic phosphate. Combined with the esr observations these results suggest that the α-phospho radical also releases phosphate, but on a time scale longer than that of the esr experiments.

In contrast to the full yield found for the glycerol phosphates the yield from methyl and ethyl phosphate is much lower.[4,7] We have also examined ethanolamine phosphate and the results are shown in Table II. Two of the values in this table have been reported previously[7] and both results are in agreement. It is evident from Table II that despite the similarity between ethanolamine and glycol the mechanism of phosphate release is quite different, at least when the amino group is protonated. In the latter case, i.e., at neutral or acid pH much of the abstraction takes place α to the phosphate as shown by the esr results. The resulting radicals do not eliminate phosphoric acid but instead they probably disproportionate to a certain extent to produce inorganic phosphate with a yield of $\sim\frac{1}{2}G(OH)$. When oxygen or copper ions are added to the solution the yield of phosphate is increased by about a factor of 2 as the result of oxidation of the organic radicals as discussed above. In alkaline solutions the majority of the OH radicals react at position α to the free amino group and the resulting radical appears to behave similarly with the glycols and to eliminate phosphoric acid rapidly. Therefore, the yield is found to be higher at pH 12 than at pH 4 and 7 and oxygen is found to affect the former yield. The value of $G = 4.2$ at pH 12 indicates, though, that the elimination process is not as efficient with ethanolamine phosphate as with the glycerol phosphates.

Summary and Conclusions

The esr spectra obtained with irradiated aqueous solutions of glycerol phosphates and related compounds showed the presence of two kinds of radicals: those pro-

duced directly by the initial abstraction reaction showing g factors $\sim$2.003 and those produced subsequently by elimination of H_2O or H_3PO_4 showing g factors $\sim$2.004. The initial radicals undergo phosphate elimination at different rates. The α-phospho radicals are stable in the millisecond time scale of the esr observation but they undergo elimination of phosphate at longer times as shown by the final yield of phosphate. The β-phospho radical can potentially undergo elimination of either H_2O or H_3PO_4, but the latter was found to eliminate rapidly even in neutral solutions so that the initial radicals were not observed in esr experiments. The γ-phospho radicals can eliminate H_2O only and because these radicals were observed in neutral solutions and their elimination products in alkaline solutions this process is not as rapid as the elimination of H_3PO_4.

Ethanolamine and serine phosphates react with OH radicals in neutral solutions to undergo H abstraction mainly at the position α to the phosphate because the other position is deactivated by the neighboring protonated amino group. The resulting α-phospho radicals have been observed by esr and are somewhat stable. The final yield of phosphate shows that these radicals do not lose their phosphate quantitatively by elimination but probably disproportionate and cleave half of their phosphate. In alkaline solutions the free amino group directs most of the abstraction to its α position and the β-phospho radicals produced undergo elimination of phosphate, although not as rapid as in the corresponding case with glycols.

References and Notes

(1) Supported in part by the U. S. Atomic Energy Commission.
(2) C. M. L. Kerr, K. Webster, and F. Williams, J. Phys. Chem., **76**, 2848 (1972).
(3) A. Begum, S. Subramanian, and M. C. R. Symons, J. Chem. Soc. A, 1334 (1970).
(4) C. V. Sonntag, G. Ansorge, A. Sugimori, T. Omori, G. Koltzenburg, and D. Schulte-Frohlinde, Z. Naturforsch. B, **27**, 471 (1972).
(5) J. D. Zimbrick, J. F. Ward, and L. S. Myers, Jr., Int. J. Radiat. Biol., **16**, 525 (1969).
(6) G. Scholes, W. Taylor, and J. Weiss, J. Chem. Soc., 235 (1957).
(7) J. A. Raleigh, C. L. Greenstock, and W. Kremers, Int. J. Radiat. Biol., in press.
(8) See also references cited in ref 4 and 6.
(9) R. E. Florin, F. Sicilio, and L. A. Wall, J. Res. Nat. Bur. Stand., Sect. B, **72**, 49 (1968).
(10) B. C. Gilbert, J. P. Larkin, and R. O. C. Norman, J. Chem. Soc., Perkin Trans. 2, 794 (1972).
(11) K. M. Bansal, M. Gratzel, A. Henglein, and E. Janata, J. Phys. Chem., **77**, 16 (1973).
(12) D. F. Boltz, Ed., "Colorimetric Determination of Nonmetals," Interscience, New York, N. Y., 1958, p 32.
(13) K. Eiben and R. W. Fessenden, J. Phys. Chem., **75**, 1186 (1971).
(14) P. J. Baugh, O. Hinojosa, and J. C. Arthur, Jr., J. Phys. Chem., **71**, 1135 (1967).
(15) H. Paul and H. Fischer, Ber. Bunsenges. Phys. Chem., **73**, 972 (1969).
(16) P. Neta and R. W. Fessenden, J. Phys. Chem., **75**, 738 (1971).
(17) E. A. C. Lucken, J. Chem. Soc. A, 1354 (1966).
(18) A. R. Metcalfe and W. A. Waters, J. Chem. Soc. B, 340 (1967).