616. *Chemical Action of Ionising Radiations in Solution. Part XI.** *The Action of Free Radicals, produced by X-Rays, on Lactic Acid in Aqueous Solution with Particular Reference to the Effect of Molecular Oxygen.*

By G. R. A. Johnson, G. Scholes, and J. Weiss.

The reactions of lactic acid in aqueous solution with the free radicals produced by the action of X-rays on the water have been investigated with particular attention to the processes occurring in the presence of molecular oxygen. Under these conditions, pyruvic acid is formed as the main product, together with hydrogen peroxide and some acetaldehyde.

A quantitative study under different experimental conditions has led to the formulation of a mechanism which accounts for all the main experimental results. The rôle of molecular oxygen is explained in terms of reactions involving (i) HO_2 radicals, formed by the addition of oxygen to the primarily formed H atoms, and (ii) organic peroxy-radicals of the type $HO_2C\cdot CMe(OH)\cdot O_2\cdot$ produced by the addition of molecular oxygen to lactate radicals formed by a one-step dehydrogenation of lactic acid by OH radicals.

EARLIER papers of this series (cf. Part X*) have reported reactions of free OH radicals in aqueous systems, the radicals being produced from the water by ionising radiations (*e.g.*, X-rays, γ-rays). From a biochemical point of view, reactions in the presence of molecular oxygen are of particular interest, since a marked effect of oxygen on the sensitivity of certain biological systems to ionising radiations has been observed by several workers.

We have previously reported that the presence of oxygen markedly increases the yields of pyruvic acid from α-alanine in aqueous solution (Johnson, Scholes, and Weiss, *Science*, 1951, **114**, 412). We have now studied the X-ray-induced oxidation of lactic acid in some detail. The only previous investigation of this system is that of Di Bella (*Boll. Soc. ital. Biol. sperim.*, 1949, **25**, 1268) who reported the formation of pyruvic acid which he identified by a colour reaction.

It is now well-established that the chemical effects of ionising radiations in dilute aqueous solutions are due to the free radicals produced by the splitting of the water molecules following absorption of the radiations, according to the net process (Weiss, *Nature*, 1944, **153**, 748; *Brit. J. Radiol.*, 1947, Suppl. **1**, 56) :

$$H_2O \longrightarrow\hspace{-1.2em}\rightsquigarrow\ H + OH \ . \qquad \ldots \ldots \ldots \quad (1a)$$

More recently, it has been pointed out (cf. Allen, *Ann. Rev. Phys. Chem.*, 1952, **3**, 57) that a small part of the radiation energy absorbed by the water results in the direct formation of molecular hydrogen and hydrogen peroxide, which, according to Rigg and Weiss (cf. Weiss, *ibid.*, 1953, in the press), is due to the interaction of the primarily formed, excited, water molecules (H_2O^*) according to :

$$2H_2O^* \longrightarrow H_2 + H_2O_2 \quad . \qquad \ldots \ldots \ldots \quad (1b).$$

This " molecular " yield must also be taken into account in the interpretation of the experimental results.

In the results presented below, the yields are expressed in molar quantities; for convenience, therefore, the energy absorption is given in terms of ev/N where $N = 6\cdot02 \times 10^{23}$. This is related to the Roentgen unit (r), which corresponds to an energy absorption of 83 ergs/g.) by the relation : $(ev/N)/g. = 1\cdot162 \times 10^{10}$ r. The " G-value " in radiation chemistry is defined as the number of molecules of products formed (or of substance decomposed) per 100 ev of energy absorbed. G for the formation of radicals, according to process (1a) is $G_R \simeq 4\cdot1$, and for the molecular yield of hydrogen peroxide and hydrogen according to reaction (1b) is $G_M \simeq 0\cdot7$ (cf. Weiss, *loc. cit.*, 1953).

When lactic acid is oxidised by free radicals produced by radiation, pyruvic acid and acetaldehyde are formed (they have been identified as 2 : 4-dinitrophenylhydrazones),

together with hydrogen peroxide. Throughout the experiments 0·02M-aqueous lactic acid was used.

The dependence of the yield of pyruvic acid and hydrogen peroxide on total dose of radiation, at different pH values, in oxygen (1 atm.) saturated solutions, is shown in Figs. 1 and 2, the initial G-values being plotted in Fig. 3. Whereas the initial yield of hydrogen peroxide is independent of pH, that of pyruvic acid is independent only within the pH

FIG. 1. *Formation of pyruvic acid in the presence of oxygen (1 atm.). This and all other Figures refer to aqueous lactic acid (0·02M), irradiated with X-rays (200 kv).*
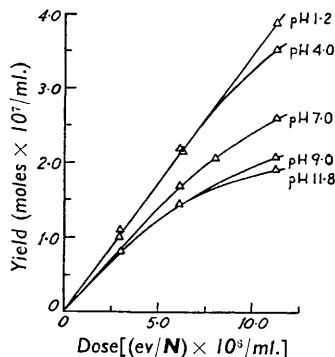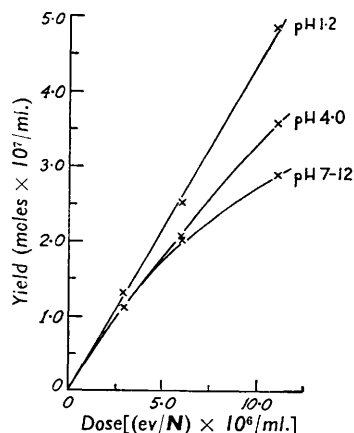

FIG. 2. *Formation of hydrogen peroxide in the presence of oxygen (1 atm.).*

ranges 1—4 and 7—12. The marked dependence of the yield on pH at higher doses, observed both for pyruvic acid and hydrogen peroxide formation, can be explained in terms of pH-dependent secondary processes discussed below, *i.e.*, processes involving competition between the primary reaction products and lactic acid for the free radicals. The dependence of the initial pyruvic acid yield on pH (Fig. 3) cannot however be explained in this way.

FIG. 3. pH-*Dependence of the initial G-values for the formation of pyruvic acid (Δ) and hydrogen peroxide (×) in the presence of oxygen.*
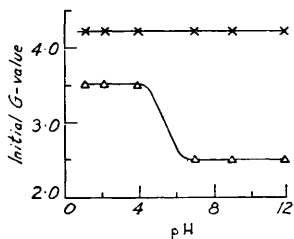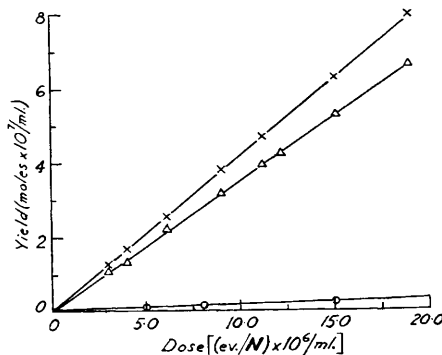

FIG. 4. *Formation of hydrogen peroxide (×), pyruvic acid (Δ), and acetaldehyde (o) in the presence of oxygen (1 atm.), at pH ca. 1·2.*

At pH 1·2 the yields of hydrogen peroxide and pyruvic acid are directly proportional to dose up to the highest doses used (Fig. 4) ; these conditions, therefore, are particularly suitable for studying the stoicheiometry of the primary reaction. From Fig. 4 it can be seen that the G-values for pyruvic acid and hydrogen peroxide formation are 3·5 and 4·2 respectively. The yield of hydrogen peroxide is, of course, made up of two parts : that produced in the actual oxidation process, and that produced directly from the water according to eqn. (1b). $G_{H_2O_2}$ of the oxidation reaction is, therefore, 3·5 (= 4·2 — 0·7), *i.e.*, equal to G_{PA} (where the suffix PA refers to pyruvic acid).

Further information has been obtained by the irradiation of air-saturated solutions at pH 1·2 (Fig. 5). The rate of formation of hydrogen peroxide per unit dose changed sharply at a dose of 9×10^{-6} ev/N, presumably owing to exhaustion of the oxygen present in solution. The total amount of hydrogen peroxide present at the " break point " is $3\cdot6 \times 10^{-7}$ ($\pm 5\%$) mole/ml. The amount of hydrogen peroxide formed directly from water (eqn. 1b) after this dose is $0\cdot6 \times 10^{-7}$ mole/ml., so that the amount produced in the oxidation reaction is $3\cdot0 \times 10^{-7}$ mole/ml., *i.e.* it corresponds closely to the amount of oxygen in an air-saturated aqueous solution ($2\cdot9 \times 10^{-7}$ mole/ml.) Therefore, nearly all the oxygen originally present in solution can be converted into hydrogen peroxide. After the " break point," when no oxygen is present in the solution, the rate of formation of hydrogen peroxide corresponds to that produced by the molecular process (1b) ($G_{M} \simeq 0\cdot7$).

Another point of interest arising from the air irradiations at pH 1·2, is that the rate of formation of pyruvic acid, within the range immediately before the oxygen is used up, exceeds the rate of formation in similar solutions saturated with oxygen (1 atm.) (Fig. 6).

In air-saturated solutions the maximum rate of formation of pyruvic acid corresponds to $G \simeq 8$.

From these results a mechanism can be derived for the reaction in acid solution.

FIG. 5. *Formation of hydrogen peroxide* ($\times$), *pyruvic acid* ($\triangle$), *and acetaldehyde* (o) *in the presence of air, at* pH *ca.* 1·2.
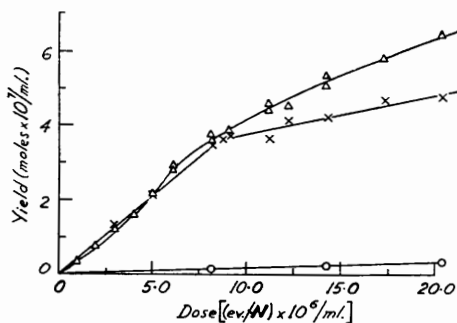
FIG. 6. *Formation of pyruvic acid at* pH *ca.* 1·2, $\triangle$ *in air,* $\blacktriangle$ *in oxygen* (1 *atm.*).
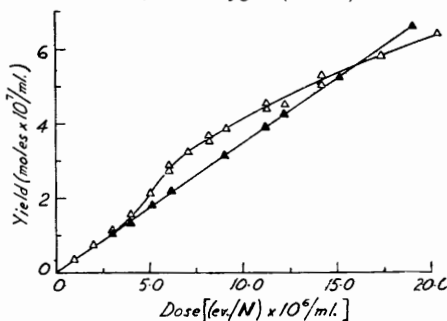
In general, the initial process in radiation-induced oxidations of organic compounds RH of this type may be assumed to be dehydrogenation by the hydroxyl radicals, produced according to eqn. (1a) :

$$RH + OH \longrightarrow R\cdot + H_2O \qquad \qquad \qquad (2)$$

The rôle of oxygen in enhancing the overall oxidation process can be ascribed to the following reactions : (*a*) Reaction of the H atoms produced in (1a), according to :
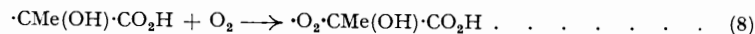
$$H + O_2 \longrightarrow HO_2 \qquad \qquad \qquad (3)$$

This process can effectively inhibit the back-reactions :

$$H + OH \longrightarrow H_2O \qquad \qquad \qquad (4)$$

$$R\cdot + H \longrightarrow RH \qquad \qquad \qquad (5)$$

(*b*) Direct addition of molecular oxygen to the organic radicals initially formed :

$$R\cdot + O_2 \longrightarrow RO_2\cdot \qquad \qquad \qquad (6)$$

In the particular case of lactic acid oxidation, the results can be accounted for on the basis of the following reactions :

$$CHMe(OH)\cdot CO_2H + OH \longrightarrow \cdot CMe(OH)\cdot CO_2H \qquad \qquad (7)$$

$$\cdot CMe(OH)\cdot CO_2H + O_2 \longrightarrow \cdot O_2\cdot CMe(OH)\cdot CO_2H \qquad \qquad (8)$$

$$\cdot O_2\cdot CMe(OH)\cdot CO_2H + HO_2 \longrightarrow Me\cdot CO\cdot CO_2H + H_2O_2 + O_2 \qquad (9)$$

Reaction (9) can be regarded as a simple electron transfer from the organic peroxy-radical to the HO_2 radical, followed by the loss of a proton and an O_2 molecule according to :

$$\cdot O_2 \cdot CMe(OH) \cdot CO_2H + HO_2 \longrightarrow \cdot O_2 \cdot CMe(OH) \cdot CO_2H\}^+ + HO_2^- \quad . \quad . \quad . \quad (9a)$$

$$\cdot O_2 \cdot CMe(OH) \cdot CO_2H\}^+ + HO_2^- \longrightarrow \cdot O_2 \cdot CMe(\cdot O) \cdot CO_2H + H_2O_2 \quad . \quad . \quad . \quad (9b)$$

$$\cdot O_2 \cdot CMe(\cdot O) \cdot CO_2H \longrightarrow Me \cdot CO \cdot CO_2H + O_2 \quad . \quad . \quad . \quad . \quad . \quad (9c)$$
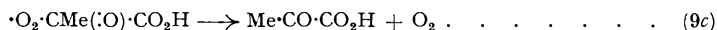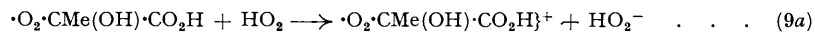
A possible alternative mechanism involves decomposition of the peroxy-radical according to :

$$\cdot O_2 \cdot CMe(OH) \cdot CO_2H \longrightarrow Me \cdot CO \cdot CO_2H + HO_2 \quad . \quad . \quad . \quad . \quad (10)$$
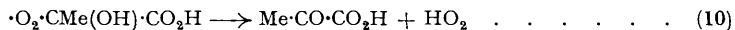
followed by the interaction of the HO_2 radicals to form hydrogen peroxide :

$$2HO_2 \longrightarrow H_2O_2 + O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$
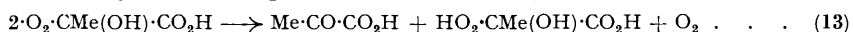
Reactions (10) and (11) would also account for the above results in acid solutions.

These two schemes would explain (i) the observed G-values for pyruvic acid and hydrogen peroxide formation, (ii) the equal yields of pyruvic acid and hydrogen peroxide, and (iii) the fact that all the molecular oxygen present in solution can be converted into hydrogen peroxide.

Another possible reaction of the organic peroxy-radicals is a dismutation; the results could be explained if this occurred thus :

$$2 \cdot O_2 \cdot CMe(OH) \cdot CO_2H \longrightarrow 2Me \cdot CO \cdot CO_2H + H_2O_2 + O_2 \quad . \quad . \quad . \quad . \quad (12)$$
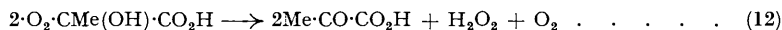
However, it is unlikely that interaction of the organic peroxy-radicals, if it occurred, would result in these products. A simple electron transfer between two of the organic peroxy-radicals should ultimately lead to the products as indicated by the following reaction :

$$2 \cdot O_2 \cdot CMe(OH) \cdot CO_2H \longrightarrow Me \cdot CO \cdot CO_2H + HO_2 \cdot CMe(OH) \cdot CO_2H + O_2 \quad . \quad . \quad . \quad (13)$$
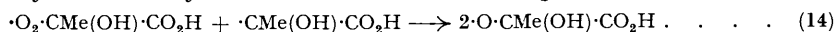
and the organic hydroperoxide thus formed would probably decompose to acetic acid, carbon dioxide, and water (cf. Bunton *et al.*, *Nature*, 1949, **163**, 444). Since, in this case, oxygen would be utilised without the production of hydrogen peroxide, a process such as reaction (13) could not explain our results.

The rôle of the HO_2 radicals in this system must be as outlined above, *i.e.*, either they react with the intermediate RO_2 radicals (eqn. 9) or they recombine to form hydrogen peroxide (eqn. 11), since the dehydrogenation of lactic acid according to the reaction $RH + HO_2 \longrightarrow R \cdot + H_2O_2$ would be incompatible with the observed stoicheiometry.

Formation of radicals of the type RO_2 has previously been assumed by Kolthoff and Medalia (*J. Amer. Chem. Soc.*, 1949, **71**, 3777) to account for the influence of oxygen in the action of Fenton's reagent on various organic compounds, and also by Dewhurst (*Trans. Faraday Soc.*, 1952, **48**, 905) to explain the yields of ferric salt produced on irradiation of aerated aqueous-alcoholic ferrous sulphate solutions with γ-rays. Similarly, we have observed that the presence of Fe^{2+} ions during the irradiation of aerated lactic acid solutions greatly increases the yields of pyruvic acid. For example, from 0·02M-lactic acid containing 0·001M-ferrous sulphate (pH $\sim$ 1·0) pyruvic acid was produced in yields corresponding to $G = 90$ at a total dose of about 2×10^{-7} (ev/N)/ml. No comparable increase in the pyruvate yields was observed if lactic acid–ferrous sulphate solutions of similar concentrations were irradiated *in vacuo*. These results can be explained by a chain mechanism involving Fe^{2+} and RO_2 radicals, which will be dealt with in a later publication.

Another interesting feature is the relatively high G-value (*ca.* 8) for pyruvic acid formation attained in the irradiation of air-saturated solutions just before exhaustion of the dissolved oxygen (Fig. 5). Here it is reasonable to suppose that there is insufficient oxygen present to react with all the lactate radicals produced according to eqn. (7), so that organic peroxy-radicals may react with lactate radicals, *e.g.*, as follows :

$$\cdot O_2 \cdot CMe(OH) \cdot CO_2H + \cdot CMe(OH) \cdot CO_2H \longrightarrow 2 \cdot O \cdot CMe(OH) \cdot CO_2H \quad . \quad . \quad . \quad (14)$$
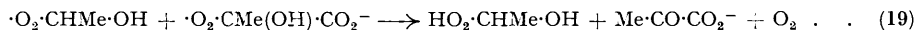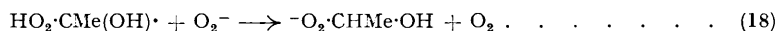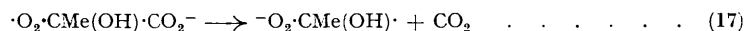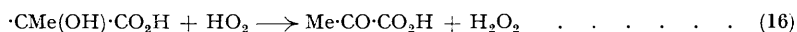
If the species produced in reaction (14) were capable of dehydrogenating lactic acid according to reaction (15), then the sequence (14), (15), (16) could lead to an increased yield of pyruvic acid as long as any oxygen is present in the solution. The relatively high G_{PA}

(*ca.* 8) in aerated solutions can only be explained on the basis of a process involving short chains such as that outlined above. Reactions (14), (15), and (16) would not alter the rate of hydrogen peroxide formation, and in fact, this remains linear with dose up to the point where the oxygen is completely exhausted (cf. Fig. 5). The amount of oxygen " lost " (*i.e.*, not converted into hydrogen peroxide) by reactions (14) and (15) is, at most, 0.35×10^{-7} mole; this is within the experimental error of determination of the " break " in the hydrogen peroxide yield–dose curve (Fig. 5).

In considering the reaction mechanism in solutions of pH >4 we are confronted with two new factors : a change in the initial G-value for pyruvate formation from 3·5 (in acid solution) to 2·5 (Fig. 3); and the occurrence of marked secondary reactions, involving the (accumulated) primary products (cf. Figs. 1 and 2).

There is no evidence to suggest that the primary effect of the ionising radiation on water changes over the pH range considered here. Furthermore, in view of the constancy of the initial $G_{H_2O_2}$, it appears that the ratio of the number of free radicals attacking lactic acid to the number of free radicals lost in other ways (*e.g.*, by recombination) remains constant over the whole pH range. The observed initial G-values can, however, be explained tentatively in terms of the stability of the organic peroxy-radical. At pH >4 this species might exist predominantly in the anionic form $\cdot O_2 \cdot CMe(OH) \cdot CO_2^-$. Since the hydroperoxy-radical (HO_2) would exist here largely as the anion O_2^-, reaction (9) might be inhibited by electrostatic forces. For the same reason, any dismutation reaction between these anionic organic peroxy-radicals could be minimised. As these radicals might be expected to have only a limited life they may undergo spontaneous decarboxylation, so that the reactions (17) and (18), or (19) may occur

$$\cdot O \cdot CMe(OH) \cdot CO_2H + CHMe(OH) \cdot CO_2H \longrightarrow Me \cdot CO \cdot CO_2H + \cdot CMe(OH) \cdot CO_2H + H_2O \quad (15)$$

$$\cdot CMe(OH) \cdot CO_2H + HO_2 \longrightarrow Me \cdot CO \cdot CO_2H + H_2O_2 \quad \ldots \ldots (16)$$

$$\cdot O_2 \cdot CMe(OH) \cdot CO_2^- \longrightarrow {}^- O_2 \cdot CMe(OH) \cdot + CO_2 \quad \ldots \ldots (17)$$

$$HO_2 \cdot CMe(OH) \cdot + O_2^- \longrightarrow {}^- O_2 \cdot CHMe \cdot OH + O_2 \quad \ldots \ldots (18)$$

$$\cdot O_2 \cdot CHMe \cdot OH + \cdot O_2 \cdot CMe(OH) \cdot CO_2^- \longrightarrow HO_2 \cdot CHMe \cdot OH + Me \cdot CO \cdot CO_2^- + O_2 \quad (19)$$

These reactions would thus lower the initial yield of pyruvic acid. The apparent yield of peroxide might not be altered, however, since the 1-hydroxyethyl hydroperoxide (Rieche, *Ber.*, 1931, **64**, 2378) formed in reaction (19) should behave similarly to hydrogen peroxide in the reaction used for determination of the latter (see Experimental section).
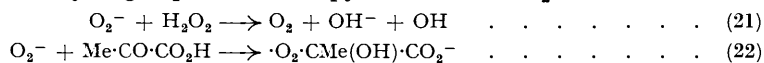
The above explanation of the effect of pH on the initial G-values is necessarily tentative; it does, however, account for the production of acetaldehyde and qualitatively also for the pH-dependence of the yield of this substance (cf. Figs. 4 and 7), since the aldehyde could arise from a dismutation process, *viz.* :

$$2 \cdot O_2 \cdot CHMe \cdot OH \longrightarrow HO_2 \cdot CHMe \cdot OH + Me \cdot CHO + O_2 \quad \ldots \ldots (20)$$
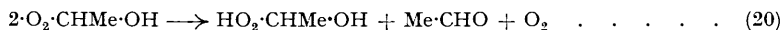
The explanation of the non-linearity of the yield–dose curves for pyruvic acid and hydrogen peroxide in neutral and alkaline solutions (Figs. 1 and 2) cannot be attributed solely to an oxidation of pyruvic acid by hydrogen peroxide, produced during the irradiation. Although this oxidation is known to take place in neutral and alkaline solution (cf. Everett and Sheppard, *Univ. Oklahoma Med. School, Dept. Biochem.*, 1944), it can be shown that it is insufficient to account for the shape of the curves in Figs. 1 and 2. For instance, in solutions containing 2×10^{-4}M-pyruvic acid and 2×10^{-4}M-hydrogen peroxide at pH 7, the loss of pyruvic acid and hydrogen peroxide was found to be less that 10^{-7} mole/min. The alternative explanation is that pyruvic acid and/or hydrogen peroxide, when formed in sufficient amounts, can compete with lactic acid for the primary radicals. That such secondary processes do, in fact, take place, has been shown by experiments in which pyruvic acid or hydrogen peroxide was added to lactate solutions before irradiation; the addition of either of these substances in concentrations of about 10^{-4}M can effect a marked lowering of the oxidation yields.

Secondary processes might be expected since it is well-known that the HO_2 radical is ionised $(HO_2 \rightleftharpoons H^+ + O_2^-)$, and as the hydrogen peroxide and pyruvate concentrations

increase, reactions (21) and (22) could become significant.   Reaction (21) would lower the yield of hydrogen peroxide but not necessarily that of pyruvic acid, as here a further OH radical is generated.   Reaction (22) would lower the yields of both hydrogen peroxide and pyruvic acid.   The experimental results can be accounted for qualitatively by a pH-dependent competition of hydrogen peroxide and pyruvic acid for O_2^-.

$$O_2^- + H_2O_2 \longrightarrow O_2 + OH^- + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

$$O_2^- + Me\cdot CO\cdot CO_2H \longrightarrow \cdot O_2\cdot CMe(OH)\cdot CO_2^- \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

A further feature of the results obtained on irradiation of aerated solutions is the rate of formation of pyruvic acid after the oxygen initially present in the solution has been used up, which exceeds that observed in evacuated solutions (cf. Figs. 5, 7, and 8).   This effect can be attributed, in part, to the hydrogen peroxide present in the solution, since

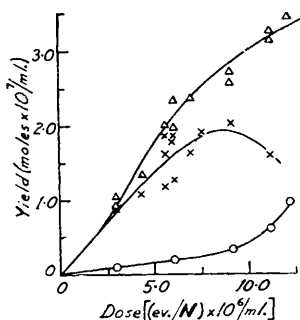FIG. 7.   *Formation of hydrogen peroxide* ($\times$), *pyruvic acid* ($\triangle$), *and acetaldehyde* (o) *in the presence of air at pH ca. 7·0.*

FIG. 8.   *Formation of hydrogen* ($\square$), *hydrogen peroxide* ($\times$), *pyruvic acid* ($\triangle$), *and carbon dioxide* ($\bullet$) *in vacuo at pH ca. 1·2.*
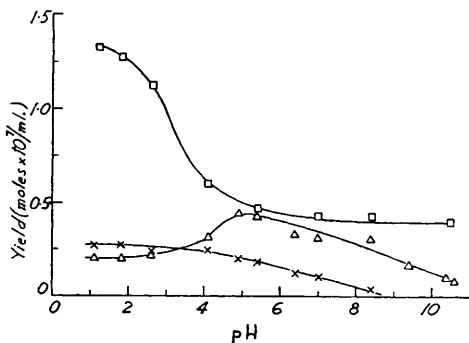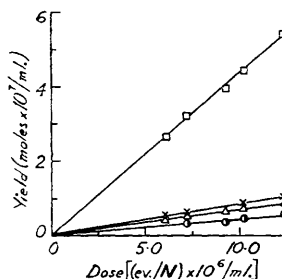

FIG. 9.   pH-*Dependence of the formation of hydrogen* ($\square$), *pyruvic acid* ($\triangle$), *and hydrogen peroxide* ($\times$), in vacuo. *Dose* $= 3·0 \times 10^{-6}$ (ev/N)/ml.

addition of hydrogen peroxide to evacuated lactic acid solutions before irradiation increased the yields of pyruvic acid.   At pH 1·2, however, hydrogen peroxide does not apparently undergo any net decomposition (cf. Fig. 5).

The explanation of this effect in acid solution is not quite clear.   Since, however, it has been suggested (Weiss, *Nature*, 1950, **167**, 728) that an equilibrium exists between hydrogen atoms and hydrogen ions :

$$H + H^+ \underset{\longleftarrow}{\overset{\longrightarrow}{\rightleftharpoons}} H_2^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$
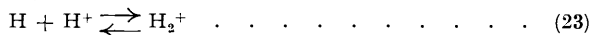
a possible rôle of hydrogen peroxide may, in this case, involve reaction (24a) or (24b), followed by (25).   Although, on general kinetic grounds, reaction (24a) is perhaps more likely than (24b), it is doubtful whether the former can play any significant part in acid solution, where the concentration of HO_2^- is very small.   However, too little is known at

$$H_2^+ + HO_2^- \longrightarrow H_2 + HO_2 . \quad . \quad . \quad . \quad . \quad . \quad . \quad (24a)$$

$$H_2^+ + H_2O_2 \longrightarrow H_2 + HO_2 + H^+ . \quad . \quad . \quad . \quad . \quad (24b)$$

$$\cdot CMe(OH)\cdot CO_2H + HO_2 \longrightarrow Me\cdot CO\cdot CO_2H + H O_2 \quad . \quad . \quad . \quad . \quad (25)$$
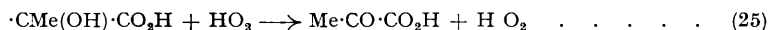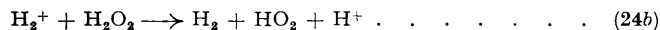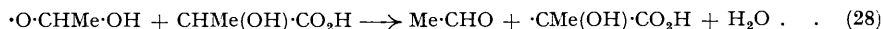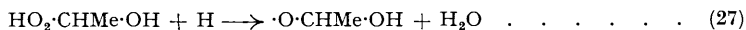
present about the relevant rate constants and about the local concentrations of H atoms and other radicals produced by the radiation to make decision possible. On the other hand, our experiments seem to indicate that a chain reaction between the organic radical ·CMe(OH)·CO$_2$H and hydrogen peroxide, as suggested by Waters (" The Chemistry of Free Radicals," Oxford, 1949, p. 251), does not take place under these conditions.

At pH 7 the results are somewhat irreproducible, but it has been established that the yield of hydrogen peroxide falls after exhaustion of molecular oxygen (Fig. 7). This can be attributed to the decomposition reaction :

$$H + H_2O_2 \longrightarrow H_2O + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$
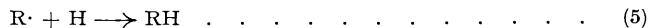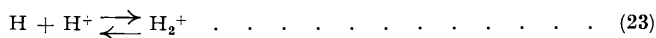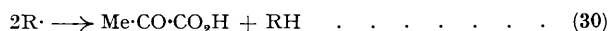
which produces another OH radical and so increases the yield of pyruvic acid. The observed increase in the yield of acetaldehyde after exhaustion of oxygen could, perhaps, be accounted for by the reactions :

$$HO_2·CHMe·OH + H \longrightarrow ·O·CHMe·OH + H_2O \quad . \quad . \quad . \quad . \quad . \quad (27)$$

$$·O·CHMe·OH + CHMe(OH)·CO_2H \longrightarrow Me·CHO + ·CMe(OH)·CO_2H + H_2O \quad . \quad . \quad (28)$$

The mechanism of the effects of X-rays on lactic acid in evacuated solutions presents a somewhat complex picture, particularly with regard to the pH-dependence of the yields. An unusual feature is the rather high yield of molecular hydrogen in acid solution, which decreases rapidly with increasing pH. Again, the initial yield appears to depend on pH. It should be noted also that even the hydrogen peroxide produced by the molecular reaction (1b) is rapidly decomposed in non-acid solutions (Fig. 9).

The results obtained *in vacuo* can be explained tentatively by the following reaction mechanism [R = CMe(OH)·CO$_2$H] :

$$RH + OH \longrightarrow R· + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$$R· + RH \longrightarrow R_2 + H \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (29)$$

$$2R· \longrightarrow Me·CO·CO_2H + RH \quad . \quad . \quad . \quad . \quad . \quad . \quad (30)$$

$$H + H^+ \rightleftharpoons H_2^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

$$H + H_2^+ \longrightarrow H_2 + H^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

$$R· + H \longrightarrow RH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

In strongly acid solutions, it appears that most of the R radicals react with lactic acid [reaction (29)] since the G-value for hydrogen is quite high (4·4). Pyruvic acid is formed by the dismutation process (30).

When the pH is increased the concentration of H_2^+ decreases, thus leading to an increase in the back-reaction (5). At the same time, increased ionisation of the lactic acid may inhibit the reaction (29), thus leading to an increase in the disproportionation reaction and to a greater yield of pyruvic acid. This in turn (at pH 3—5) (Fig. 9) may be associated with an increase in the number of OH radicals from the decomposition of hydrogen peroxide by hydrogen atoms according to reaction (26). At high pH's, the yield of pyruvic acid is not fully reproducible, although there appears to be a net decomposition of this substance, probably of a reductive nature. Complete analysis of the mechanism in evacuated solutions has not yet been achieved.

The addition of oxygen to an organic radical, as postulated above, may be of importance in certain other radiation-induced oxidations, as, for example, in the formation of acetaldehyde from ethanol and from ethylamine, the formation of oxaloacetic acid from malic acid, and of α-keto-acids from α-amino-acids where it has been observed (Johnson, Scholes, and Weiss, unpublished experiments) that, as with lactic acid, oxygen enhances the yields very considerably.

It has been reported that oxygen has a marked effect on the sensitivity of certain biological systems to ionising radiations; decrease in oxygen tension during irradiation has been found to decrease the frequency of lethal mutations and chromasomal abberations (cf. Giles, Symposium on Radiobiology, New York, 1952, p. 257; Anderson, *Proc. Nat. Acad. Sci.*, 1951, **37**, 340), and to decrease the inactivation rate and duration of mitotic

inhibition (Hollaender, Stapleton, and Martin, *Nature*, 1950, **167**, 103; Gaulden and Nix, *Genetics*, 1950, **35**, 665). In view of our results, it is apparent that such enhancing effects of oxygen *in vivo* should not be attributed simply to HO_2 radicals and/or hydrogen peroxide, but also to the formation of peroxy-radicals of the type RO_2, *i.e.*, to the addition of oxygen to a primarily formed organic free radical. Although in oxygen-saturated solutions we have not observed any chain reactions in the system studied, it is conceivable that some RO_2 radicals, produced from cellular components, may undergo chain reactions and thus lead to a marked enhancement of the effects of the radiation.

Finally, the fact that pyruvate can compete very successfully for the free radicals produced by the radiation, even in the presence of relatively large concentrations of lactate, indicates that pyruvate is a very efficient radical-acceptor. This may be of some significance in view of the finding (Thomson, Mefford, and Wyss, *J. Bact.*, 1951, **62**, 39) that pyruvate can protect bacterial suspensions against the lethal and mutagenic action of *X*-rays.

EXPERIMENTAL

Irradiations.—The solutions (150 ml.) were irradiated with *X*-rays generated from a Victor Maximar therapy tube operating at 200 kv and 15 mA, the irradiation arrangements being similar to those described in Part I (Farmer, Stein, and Weiss, *J.*, 1949, 3241). The dose rate was determined by measuring the oxidation of 0·001M-ferrous sulphate in 0·1N-sulphuric acid and was 2×10^{-7} (ev/N) per ml. per min., $G_{Fe^{2+}}$ being assumed to be 18 (Rigg and Weiss, in the press).

The lactic acid solutions were prepared in triply distilled water, ordinary distilled water being redistilled in an all-glass still from alkaline permanganate and then from phosphoric acid. Where necessary, the solutions were buffered by the following systems: for pH<4, H_2SO_4; pH 5—8, KH_2PO_4—Na_2HPO_4 (Sørensen); pH 8—10, $Na_2B_4O_7$—Na_2CO_3 (Parnas); for pH>10, NaOH.

Irradiations were carried out in the presence of air or of oxygen (the solutions being saturated by passage of oxygen for *ca.* 20 min.), and also after evacuation as follows: The solution was pumped out by means of a Hyvac oil pump, shaken for a few minutes, and finally evacuated with a mercury diffusion pump. After this procedure less than 0·01 ml. of non-condensable gas remained.

All materials used were of "AnalaR" grade. The lactic acid contained not more than 15% of anhydrides as measured by the method of Fetzer and Jones (*Analyt. Chem.*, 1952, **24**, 835). The presence of these amounts of anhydrides during irradiation had no effect on the yields; identical results were obtained when the lactic acid solutions contained anhydrides and when the anhydrides were removed by the hydrolysis procedure of Dietzel and Krug (*Ber.*, 1925, **58**, 1307).

Identification of Pyruvic Acid.—The irradiated solution was treated with 0·25% solution of 2 : 4-dinitrophenylhydrazine in 30% perchloric acid, and after 30 min. extracted twice with chloroform. The hydrazone was removed from the chloroform by extraction with 10% aqueous sodium carbonate. Pyruvic acid 2 : 4-dinitrophenylhydrazone, precipitated on acidification of the carbonate extract with concentrated hydrochloric acid and recrystallised from ethanol, had m. p. and mixed m. p. 212—214°.

Identification of Acetaldehyde.—The irradiated solution was treated with 2 : 4-dinitrophenylhydrazine solution, as described above, and after 30 min. extracted with carbon tetrachloride. The organic layer was washed with 10% aqueous sodium carbonate, and twice with 30% perchloric acid to remove excess of the hydrazine reagent, and evaporated to dryness *in vacuo*. The residue was taken up in light petroleum (b. p. 80—100°; 1 ml.). The presence of acetaldehyde 2 : 4-dinitrophenylhydrazone in this residue was established by paper chromatography with light petroleum–methanol as solvent (Meigh, *Nature*, 1952, **170**, 579).

Quantitative Methods.—To avoid possible interaction between hydrogen peroxide and pyruvic acid formed in the solution, analyses were carried out immediately after irradiation.

(a) *Hydrogen peroxide.* An aliquot of the irradiated solution was treated with titanium sulphate reagent (5 ml.) (Eisenberg, *Ind. Eng. Chem., Anal. Edn.*, 1943, **15**, 327), then made up to 50 ml., and the extinction was measured on a "Spekker" photoelectric absorptiometer with an Ilford 601 filter.

(b) *Pyruvic acid.* Friedman and Haugen's method (*J. Biol. Chem.*, 1943, **147**, 415), which involves the determination of pyruvic acid as its 2 : 4-dinitrophenylhydrazone, was modified to

permit the use of larger samples and lower pyruvate concentrations. To an aliquot (1—20 ml.) of the test solution in a separating funnel 5 ml. of a 0·25% solution of 2 : 4-dinitrophenyl-hydrazine in 30% perchloric acid were added. After 15 min. at room temperature the mixture was extracted with chloroform (2 × 20 ml.). The extracts were extracted with 10% aqueous sodium carbonate (10 ml.), and the aqueous layer was washed into a 50-ml. flask with a further 10 ml. of 10% sodium carbonate solution. 2N-Sodium hydroxide (5 ml.) was added, and the whole then diluted to 50 ml. After a few minutes the extinction of the final cherry-red solution was measured, with an Ilford 603 filter. For each estimation a blank test was carried out.

Calibration was with pyruvic acid (three times distilled *in vacuo*) and with pyruvic acid 2 : 4-dinitrophenylhydrazone (m. p. 212—214°), which gave identical results.

The method gave good recoveries of pyruvic acid from lactic acid solutions under the conditions used in the irradiation experiments and was accurate to ±3% with pyruvate concentration of not less than 5×10^{-6}M.

(c) *Acetaldehyde.* An aliquot of the test solution (5—25 ml.) was diluted to 25 ml. and to it was added a solution (5 ml.) containing 0·25% of 2 : 4-dinitrophenylhydrazine in 30% perchloric acid. After 30 min. at room temperature the mixture was extracted with carbon tetrachloride (20 ml.) for exactly 1 min. and then again with carbon tetrachloride (5 ml.) for 30 sec. Pyruvic acid 2 : 4-dinitrophenylhydrazone was removed by shaking the combined carbon tetrachloride extracts with 10% aqueous sodium carbonate (10 ml.) for 30 sec. The carbon tetrachloride layer was made up to 50 ml. with ethanol, and the final solution treated with 2 ml. of ethanolic 0·1N-sodium hydroxide. After exactly 5 min. the intensity of the red colour was determined with an Ilford 603 filter. Blank determinations were carried out. Calibration was by acetaldehyde (purified through the ammonia derivative; cf. Snell, "Colorimetric Methods of Analysis," New York, 1937, Vol. II, p. 64) and its 2 : 4-dinitrophenylhydr-azone (m. p. 161—163°), which gave identical results.

The method was reproducible within ± 3% with aldehyde concentrations down to 5×10^{-6}M.

(d) *Gas analysis.* The apparatus was similar to that described by Stein and Weiss (*J.*, 1949, 3245). After irradiations *in vacuo*, the gases were pumped from the vessel through a liquid air trap by means of a semi-automatic Töpler pump. The hydrogen was determined manometrically after combustion in the presence of excess of oxygen. Carbon dioxide which was condensed in the liquid-air trap was determined manometrically after fractional distillation from freezing acetone (Baldwin, *J.*, 1949, 720).

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