mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride in 2 ml of pyridine for 3.5 hr, following which 0.504 g (1.2 mmol) of 3'-O-(β-benzoylpropionyl)-N-isobutyloxycarbonyldeoxycytidine was added. The mixture was stirred to dissolve the nucleoside, approximately 1 ml of the pyridine was stripped off, and the remaining thick syrup was stirred for 72 hr. Ethanol (1 ml) was then added, the solvent was taken off, and the residue was dissolved in chloroform. The products were partially separated by chromatography on silica gel (4 × 42 cm) by elution in succession with ethyl acetate, 50% ethyl acetate–tetrahydrofuran, and 1% methanol in tetrahydrofuran. Fractions containing MTrTp(ce)Tp(ce)dC$_{\beta B}^{BOC}$ were collected and rechromatographed. At this stage the product still contained a small amount of the triisopropylbenzenesulfonic acid salt as a contaminant. Elution with tetrahydrofuran through a short column (6.5 × 3 cm) of silica gel PF$_{254}$ (Merck) yielded 0.582 g (40% based on MTrTp(ce)T) (of MTrTp(ce)pT(ce)dC$_{\beta B}^{BOC}$, mp 125–130° with softening from 120°, which was homogeneous on tlc in ethyl acetate (R_f 0.03), tetrahydrofuran (R_f 0.65), and methanol (R_f 0.65). The infrared spectrum was identical with that for the product isolated from experiment a.

Doubling Experiments. The standard reaction sequence was used to convert two blocks containing two thymidine units to TpTpTpT and two blocks containing three thymidine units to TpTpTpTpTpT on a small scale. The technique is represented by the preparation of the hexanucleoside pentaphosphate.

a. TpTpTpTpTpT. MTrTp(ce)Tp(ce)T·H$_2$O (15 mg, 0.012 mmol) and pyridinium β-cyanoethyl phosphate, after drying by distillation of pyridine *in vacuo*, were mixed with 11 mg (0.05 mmol) of mesitylenesulfonyl chloride in 1 ml of pyridine and the volume was reduced to ~0.1 ml. After 7 hr all of the nucleotide material had been phosphorylated as indicated by the fact that none of the methoxytrityl-containing material moved on tlc in tetrahydrofuran. The solution was diluted with 1 ml of pyridine and 1 ml of water, stirred for 15 hr, and evaporated. The residue was taken up in chloroform (2 ml) and washed with saturated aqueous sodium chloride. After evaporation of the chloroform, the residue was dried by distillation of pyridine (four 1-ml portions) and then dissolved in 1 ml of pyridine along with 27.5 mg (0.024 mmol) of Tp(ce)Tp(ce)T$_{\beta B}$ and 10 mg (0.024 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The solution was concentrated and stirred for 48 hr at room temperature; then the alkaline labile blocking groups were taken off by stirring with 2 ml of ammonium hydroxide and 1 ml of pyridine for 14 hr. The solvent was evaporated and the residue was warmed with 1 ml of 80% aqueous acetic acid on a steam bath for 10 min.

For characterization of the products an aliquot (0.1 ml from 1.0 ml total) was spotted on Whatman 3 MM paper and developed with solvent E. Four spots were found, corresponding to TpTpTpTpTpT (R_f^E 0.084), TpTpTp (R_f^E 0.26), TpTpT (R_f^E 0.38), and a substance at R_f^E 0.85. The products were eluted and the yields determined spectrophotometrically at 267 mμ: TpTpTpTpTpT 41% (2.62 OD units) and TpTpTp 56% (1.74 OD units); both yields were based on MTrTp(ce)Tp(ce)T. The TpTpT, which was derived from the excess Tp(ce)Tp(ce)T$_{\beta B}$, amounted to 3.61 OD units. These materials account for 90% of the total amount of MTrTp(ce)Tp(ce)T and Tp(ce)Tp(ce)T$_{\beta B}$ used in the reaction. Electrophoresis at pH 7.0 also showed four spots: R_m (relative to Tp) 0.75 for TpTpTpTpTpT, 0.97 for TpTpTp, 0.55 for TpTpT and 0.05 (unidentified).

The remainder of the reaction solution was chromatographed on paper with solvent E and the TpTpTpTpTpT fraction was eluted and rechromatographed in solvent F. The TpTpTpTpTpT was eluted and isolated by concentration and lyophilization of the extract. On enzymatic hydrolysis with snake venom phosphodiesterase a sample was completely degraded to pT (R_f^F 0.41, 7.28 OD units) and T (R_f^F 0.76, 1.55 OD units) with pT/T = 4.7.

b. TpTpTpT. This preparation was carried out in essentially the same manner used for TpTpTpTpTpT, starting with 10 mg (0.011 mmol) of MTrTp(ce)T and 34 mg (0.04 mmol) of Tp(ce)T$_{\beta B}$. When the phosphotriester condensation step was carried out for 72 hr in a concentrated solution (~0.1 ml), a 47% yield of TpTpTpT and a 51% yield of TpTp (based on MTrTp(ce)T were obtained. When the phosphotriester condensation step was carried out in 0.2 ml of pyridine for 48 hr, the yields of TpTpTpT and TpTp were 22 and 59%, respectively.

The Radiation Chemistry of Biochemical Disulfides. II. Lipoic Acid[1]

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Contribution from the Department of Chemistry, University of South Florida, Tampa, Florida 33620. *Received May 10, 1968*

Abstract: α-Lipoic acid (6-thioctic acid) (I) is destroyed in high yield upon exposure to X-rays in aerated or deaerated aqueous solution. Small amounts, only, of thiol and of oxidation products (sulfonic and sulfinic acid, etc.) and hydrogen peroxide are produced. The major product appears to be a cyclic dimer. A mechanism is presented which accommodates all of the experimental observations. Kinetic treatment of the mechanism affords it support and requires a linear increase in radiation-chemical yield with concentration such that $G_0(-\text{lipoate}) = 2(G_{OH} + G_H) + K[\text{lipoate}]$ at lipoate concentrations above 5–10 m*M*. The radiobiological significance of the work is briefly discussed.

Exposure of dilute, air-saturated solutions of the amino and peptide disulfides, cystamine,[2] cystine,[3] and oxidized glutathione,[4] to moderate doses of X-rays gives the corresponding sulfonic acids almost exclusively.

A mechanism and a kinetic treatment have been proposed.[3] These account for sulfonic acid production and for the dependence of sulfonic acid yield upon disulfide concentration and radiation dose rate among other variables. Key steps in the proposed mechanism are the formation and heterolysis of a disulfide radical cation.

It seemed likely that either the thiyl radical or the radical cation might cause disproportionation or poly-

(1) Research supported by U. S. Public Health Service Research Grant RH 379, National Center for Radiological Health. Reported in part before the Division of Nuclear Chemistry at the 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 13, 1967.

(2) G. G. Jayson, T. C. Owen, and A. C. Wilbraham, *J. Chem. Soc., B*, 944 (1967).

(3) T. C. Owen, M. Rodriguez, B. G. Johnson, and J. A. G. Roach, *J. Amer. Chem. Soc.*, **90**, 196 (1968).

(4) Paper to be published.

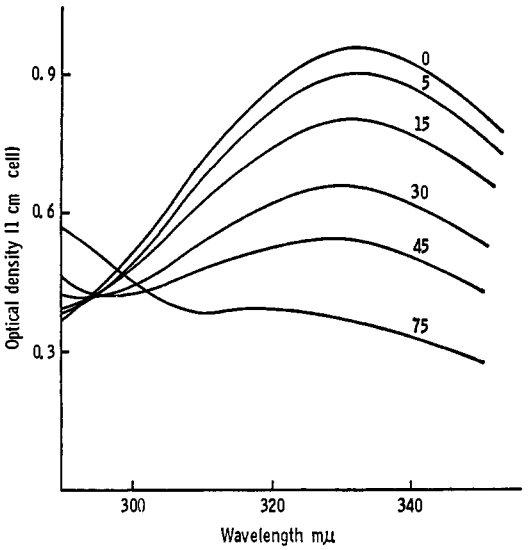
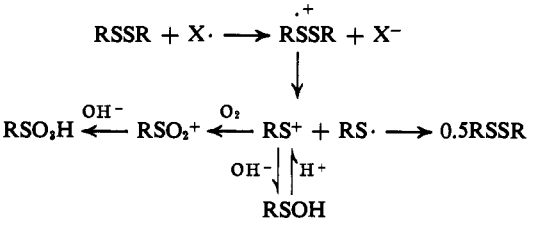


Figure 1. Changes in the uv spectrum of lipoic acid upon exposure to X-rays. Initial concentration, 8.29 mM; pH ~4. Numbers are minutes of irradiation at 3.56×10^3 rads min.$^{-1}$

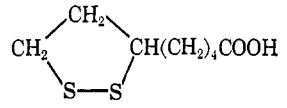


merization of an unsymmetrical or cyclic disulfide

$$RS\cdot + RSSR' \longrightarrow RSSR + \cdot SR'$$

$$RS\cdot + \overset{\frown}{S{-}S} \longrightarrow RS\overset{\frown}{S}\cdot \longrightarrow \text{polymer}$$

and we have reported[5] briefly that the cyclic disulfide



I

α-lipoic acid (I) does indeed suffer such disproportionation. The yield is concentration dependent and quite high (G_0(−lipoate) up to 25 at [lipoate] ~ 8 mM); a chain reaction is indicated. We now report this work fully.

Experimental Section

General Procedures. Irradiations were effected using a General Electric Co. Maximar II X-ray apparatus, unfiltered, operated at 125 kV and 15 MA. Dose rate was 3.56×10^3 rads min^{-1} as determined by ferrous (Fricke[6]) dosimetry corrected to pure-water values.[7] Water for solutions to be irradiated was prepared, and vessels preirradiated, as previously described.[8] Solutions to be deaerated were allowed to boil at room temperature under reduced pressure, flushed with argon, boiled again, and transferred and irradiated under a flowing blanket of argon. Optical densities were measured using a Bausch and Lomb "Precision" spectrophotometer. Molecular weights were determined by Galbraith Laboratories, Knoxville, Tenn.

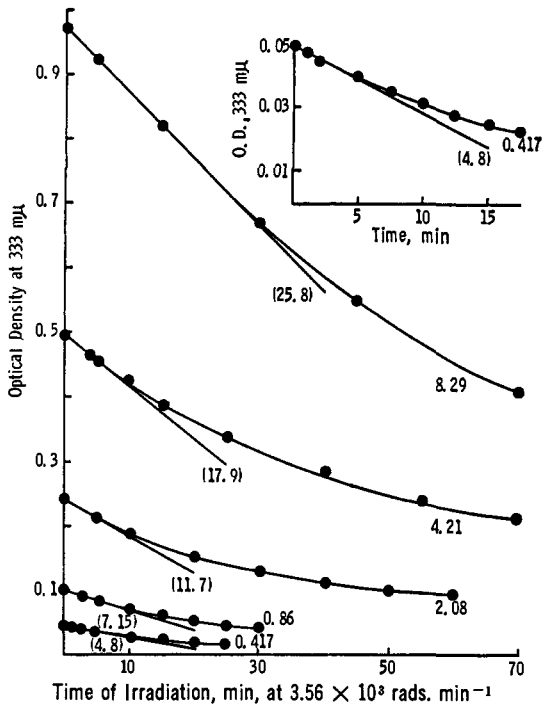


Figure 2. Changes in optical density at 333 mμ during irradiation. Numbers in parentheses are G_0(−lipoate) values (molecules/100 eV) calculated from slopes of tangents at zero time. Other numbers are initial concentrations, mM.

Lipoic Acid. Commercially available racemic 6-thioctic acid (Nutritional Biochemical Corp., Cleveland, Ohio), although of good chemical purity, showed variable radiation-chemical properties owing, presumably, to traces of inhibiting impurities such as thiols. Occasional samples could be used directly but even these were unreliable some days after opening. Crystallization afforded some improvement but reliably reproducible results were best obtained with solutions prepared as follows. Sodium hydroxide solution (0.1 N) was added slowly to a stirred suspension of the acid in water to give a neutral (pH 6–8) solution approximately 0.1 M in lipoate. The solution was exposed to a dose of X-rays sufficient to destroy the inhibiting impurities and 10–15% of the lipoate, absorbed on a column of Sephadex G10 or G15 gel-filtration medium, and eluted with water. A middle cut of the later fractions exhibiting maximal uv absorption at 330 mμ and the correct 330:280:250 ratio (concentration ~10 mM) were suitably diluted and adjusted to the required pH with water and very dilute sodium hydroxide or sulfuric acid, and used immediately.

Irradiations. 1. Determination of Lipoate Destruction. The uv absorption (240–350 mμ) of a sample (5–10 ml) of lipoate solution to be irradiated was determined. The solution was then transferred to a well-browned radiolysis vessel, irradiated (1–5 min: 3.56×10^3 rads min^{-1}), the uv spectrum redetermined, and the solution further irradiated. This sequence was repeated as desired, usually with steadily increasing periods of irradiation. The changes observed in a typical experiment are shown in Figure 1. Initial rates (G_0) of disappearance of lipoate were calculated from the slopes at zero time of graphs of optical density at 333 mμ *vs.* time of irradiation (Figure 2). Deaerated solutions were compared with blanks, similarly deaerated, and were irradiated only once, fresh solutions being used for each period of irradiation (Figure 3).

2. Hydrogen Peroxide and Thiol Production. Hydrogen peroxide (Figure 4) was determined using Eisenberg's[8] titanium sulfate procedure, and thiol by a modified phosphotungstate method.[9]

3. Isolation or Radiolysis Products. A portion (1 ml) of irradiated (2.5×10^5 rads) neutral lipoate solution (7.2 mM) was absorbed on a column (40 × 1 cm) of Sephadex G10 gel filtration medium (exclusion limit ~700) and eluted with water. Fractions (0.5 ml) of eluate were collected, diluted to 2.0 ml, and evaluated

(5) T. C. Owen and A. C. Wilbraham, *Chem. Commun.*, 624 (1967).
(6) H. Fricke and S. Morse, *Amer. J. Roentgenol. Radium Therapy Nucl. Med.*, **18**, 430 (1927); T. S. Hardwick, *Can. J. Chem.*, **30**, 17 (1952).
(7) A. J. Swallow, *J. Chem. Soc.*, 334 (1952).
(8) G. M. Eisenberg, *Ind. Eng. Chem. Anal. Ed.*, **15**, 327 (1943).
(9) Modified from O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 421 (1922); K. Shinohara, *ibid.*, **109**, 665 (1935).

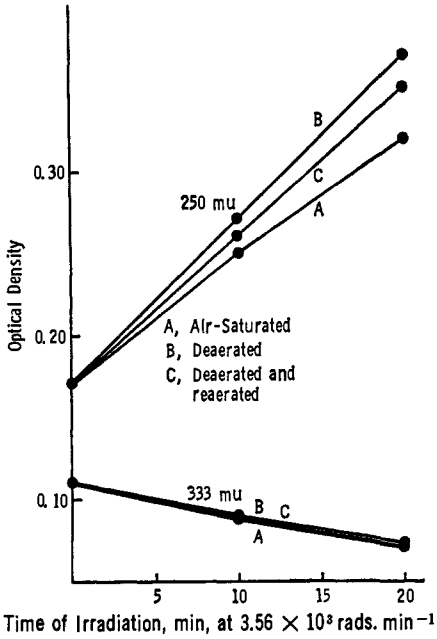


Figure 3. Effects of aeration and deaeration upon optical density changes induced by irradiation.

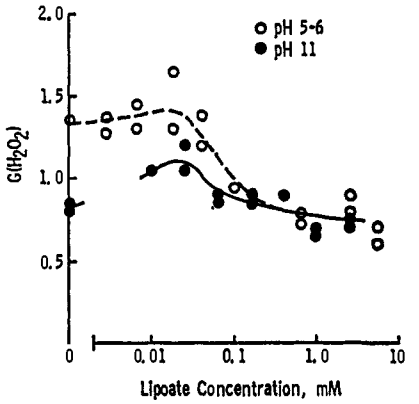


Figure 4. Hydrogen peroxide yields in irradiated lipoic acid solutions.

spectroscopically at 240, 250, and 330 mμ. The elution diagram is shown in Figure 5. A middle cut of the fractions showing high absorbance at 240–250 mμ obtained from similar fractionation of a larger sample (10 ml, 10 m*M*, 3.2 × 10⁵ rads) was acidified and agitated with a drop of benzene, when a sticky semisolid product separated at the benzene–water interface. This was isolated, washed with a little benzene, and dried at 35° in air to give a light brown somewhat rubbery substance (~5 mg, 25%), slightly soluble in benzene or chloroform, more soluble in acetone or ethanol, and soluble in dimethylformamide or dilute alkali, molecular weight (acetone) 320; see Figure 6 for the uv spectrum.

Acidification of an unfractionated irradiated solution gave a material which, after benzene extraction to remove unchanged monomer and, presumably, most of the above-described major product, gave a much smaller amount of a rubbery substance, molecular weight (DMF) 1400. A product isolated in similar fashion from a lipoate solution exposed to a very high dose of X-rays (1.7 × 10⁶ rads) was deliquescent. This is probably sulfonic acid resulting from radiolytic oxidation of the initial products.

Results

α-Lipoic acid is destroyed rather rapidly in aqueous solution upon exposure to X-rays. The decomposition is characterized (Figures 1,2) by a progressive decrease in absorbance at 333 mμ (dithiacyclopentane chromophore), a concomitant increase below 300, and an isosbestic point at ~295 which is sharp at least up to 50% of the attainable diminution at 333 mμ. The initial rate of destruction, G_0(−lipoate), is markedly affected by concentration of lipoate, ranging (Figures 2 and 7) from 4–5 at 0.4 m*M* to 20–25 molecules/100 eV at 8 m*M*. The latter yield exceeds fourfold the total yield ($G_H + G_{OH}$) of water-radiolysis radicals. A chain reaction is indicated.

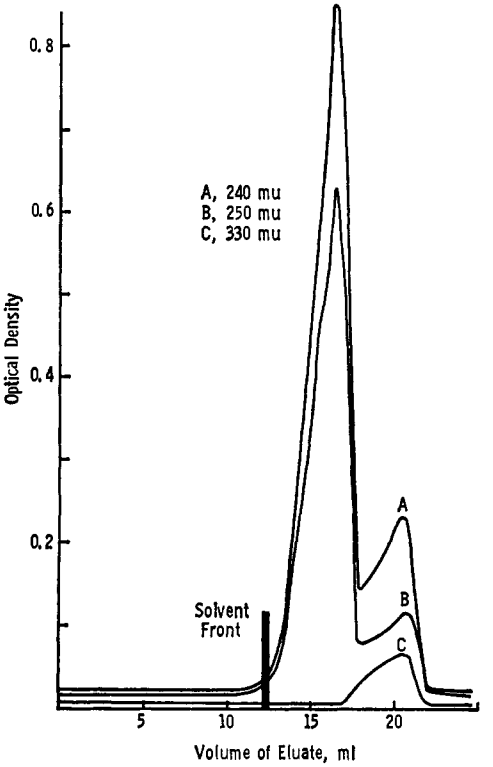


Figure 5. Eluates from irradiated lipoic acid (7.2 m*M*) separated by gel filtration on Sephadex G10.

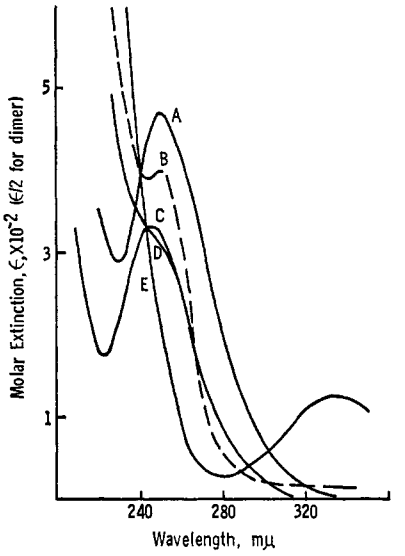


Figure 6. Absorption spectra of lipoic acid, its irradiation product, and some disulfides: A, *n*-propyl disulfide (from ref 11); B, partly purified product in EtOH; C, cystamine sulfate in water; D, cystine in dilute hydrochloric acid; E, lipoic acid in water at pH ~6.

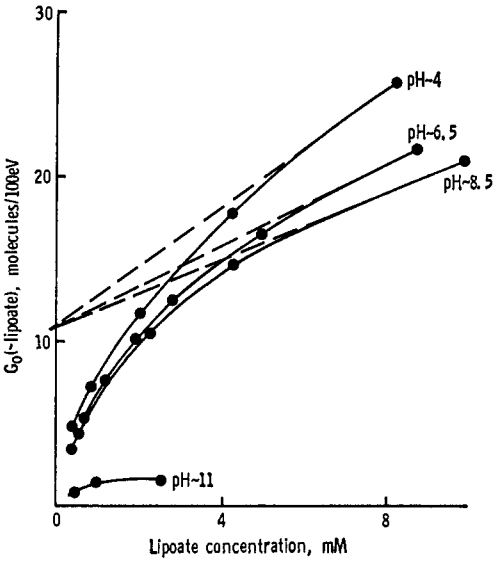


Figure 7. Effect of lipoate concentration on G_0(−lipoate).

The uv spectrum (Figure 6) of the main product separated by gel filtration shows very weak absorbance and no maximum at 333, and a shoulder at 240–254 mμ in the rising end absorbance. It is very similar to the spectrum of cystine, to that of an equimolar solution of a simple disulfide (cystamine, di-*n*-propyl disulfide) and an aliphatic acid, and to that of a photopolymer[10] of trimethylene disulfide. Ring opening to form a polymeric disulfide is clearly suggested.

The decomposition is almost unaffected by aeration or complete deaeration of the solutions (Figure 3). The product cannot, therefore, be a simple oxidation product such as β-lipoic acid or the monosulfoxide suggested[10] as the product of reaction of trimethylene disulfide or of lipoic acid with ammonium persulfate.

The gel-elution characteristics of the irradiated solution suggest one major product, a dimer, with little or no higher polymer. The molecular weight of the product isolated is in tolerable agreement with this. This product is insoluble in water and so is unlikely to contain sulfonic or sulfinic acid groups (contrast other disulfides, where sulfonic acid is the main product). Its infrared spectrum shows no S–O absorption. Yields of thiol are small (G = 0–0.4). Thus, since no recognizable end groups are present and oxygen is not involved, we suggest that the main product is a cyclic dimer of the dithiacyclopentane. In accordance with this suggestion, the product is converted to lipoic acid upon treatment with alkali, as are thermal and photopolymers of this substance.

Track yields ($G_{H_2O_2}$) only of hydrogen peroxide are produced at lipoate concentrations above 0.1 mM even in aerated solutions (contrast cystamine[2] and cystine[3]) so that the sequences

$$\cdot H + O_2 \longrightarrow \cdot O_2H \longrightarrow 0.5H_2O_2 + O_2$$

$$\cdot O_2H + M \longrightarrow O_2H^- + M\cdot^+$$

must not occur. Control experiments show that hydrogen peroxide in concentrations up to 1 mM does not react significantly with lipoic acid (~1 mM), pH 4 or 9 during 1 hr.

(10) J. A. Barltrop, P. M. Hayes, and M. Calvin, *J. Amer. Chem. Soc.*, 76, 4348 (1954).

Increasing the pH of the solution from ~4 to ~6.5 produces a moderate decrease in the rate of destruction of lipoic acid (Figure 7) but no change in the shapes of the yield *vs.* dose or yield *vs.* concentration curves. Little further change occurs when the pH is increased to ~8.5 but above pH 10 lipoic acid appears to be highly resistant to radiolysis. However, Thomas and Reed[11] have shown that thermal polymers of lipoic acid are depolymerized to the monomer upon dissolution in alkali. The apparent radiation resistance at high pH is, therefore, as would be expected if radiolytic decomposition involved polymerization. In addition, both a solution irradiated at pH 6 and the product isolated by gel filtration showed recovery of absorbance at 333 and disappearance of the shoulder at 240–254 mμ when made alkaline.

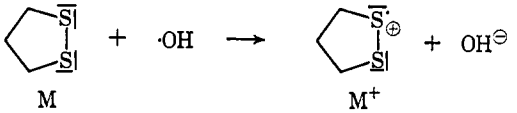
Discussion

Radiolytic degradation of solutes in water at low concentrations (<10 mM) is commonly attributed to "indirect effects" of radiation; that is, to reaction of the solute with radicals (·OH, e_{aq}^- and its conjugate acids ·H and H_2^+, and $\cdot O_2H$)[12] resulting from initial degradation of water. The present observations are so interpreted.

Simple disulfides even at very low concentrations scavenge hydroxyl radicals rapidly to give intermediates which, with dissolved oxygen, give sulfonic acids.[2-4] Solvated electrons are less reactive. At high disulfide concentrations they react with disulfides to give thiols (G(thiol) from *aerated* cystamine solutions increases from 0.2 to 2 over the concentration range 0.1–10 mM), but at lower concentrations they react with oxygen to give hydroperoxy radicals and thence give hydrogen peroxide and sulfonic acid. All of these reactions are markedly affected by dissolved oxygen.

In contrast, no significant amount of sulfonic acid results upon exposure of lipoate solutions to moderate doses of X-rays, the yields of peroxide and thiol are very much smaller than would be expected, and the disappearance of lipoate is not affected by the presence or absence of oxygen. These observations are as unique and significant as the high and concentration-dependent G(−lipoate), the apparent chain reaction and the probable nature of the product (cyclic dimer); any proposed mechanism must accommodate all of them.

We suggest that lipoic acid (M) scavenges hydroxyl radicals completely at the concentrations studied (as do other disulfides) but that the resulting *cyclic* di-

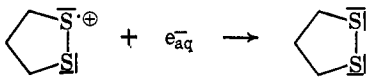


sulfide radical cation (M^+) heterolyses (and thence reacts with oxygen) significantly more slowly than does an acyclic congener. This is quite reasonable in view of the well-known stability and ease of formation of five-membered rings. The radical cation may then react with another solute molecule or with an electron-donor radical.

At low disulfide concentrations it is most likely to react with e_{aq}^- or ·H, reforming lipoate

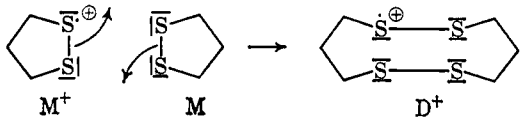
(11) R. C. Thomas and L. J. Reed, *ibid.*, 78, 6148 (1956).

(12) e_{aq}^- and ·H, and $G_{e_{aq}^-}$ and G_H are considered synonymous for our purposes.

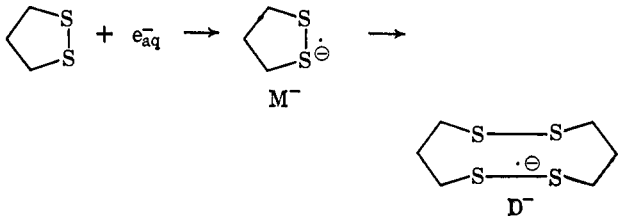


so that the net destruction is small (*viz.*, Figure 7 below 1 m*M*). The situation is analogous to the radiolysis of pure water in which the initial products react with further radicals to reform water, so that the net change is slight.

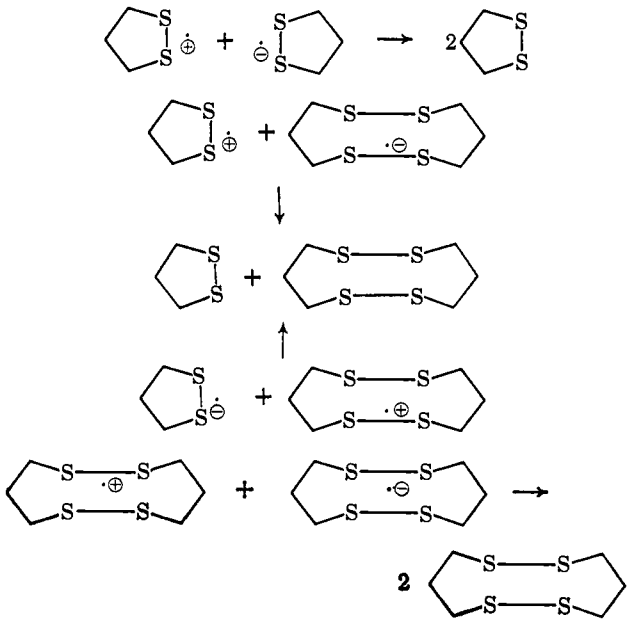
At higher solute concentrations, the cation may react with lipoate to give, we suggest, a dimer cation (D^+).



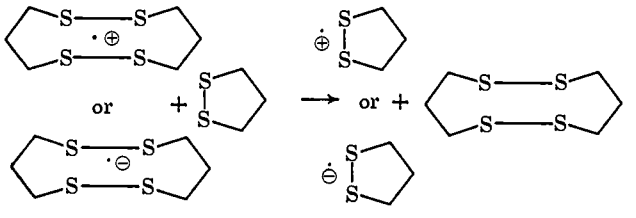
Simultaneously, reaction of e_{aq}^- with lipoate to give radical anion (M^-) and dimer anion (D^-) will become significant



and stabilization of either cation may now be by reaction with e_{aq}^-, M^-, or D^-, the latter alternatives being increasingly probable at higher concentrations.



A chain reaction is accounted for by further reaction of dimer cation or anion with lipoate in either a chain-transfer



or a polymerization reaction.

$$D^+ \text{ or } D^- + M \longrightarrow T^+ \text{ or } T^- \longrightarrow \text{polymer}$$

Since the main product is dimer, and little or no higher polymer seems to be formed, the transfer reaction must predominate. The reaction scheme is fully consistent with all of the experimental observations. It rationalizes a chain reaction producing dimer but no sulfonic acid. The termination steps require consumption of equal numbers of $\cdot OH$ and e_{aq}^- (or their progeny, M^+ and D^+, M^- and D^-, respectively) and, since G_H exceeds $G_{\cdot OH}$ only slightly, little peroxide (except the track yield) or thiol is to be expected. At pH >3, $G_{OH} = 2.4$ and $G_H = 2.9$. The surplus $\cdot H$ should appear as thiol ($G_{max} = 0.5$) at high concentration and as hydrogen peroxide ($G_{max} = G_{H_2O_2} + 0.25$) at low concentration of disulfide. This is as observed.

The scheme is amenable to steady-state kinetic treatment (see below) and the result is in excellent agreement with the characteristics of the chain reaction. It requires $G(-M)$ to be small at low concentration, and to increase nonlinearly until, at high concentration, $G(-M) = 2(G_{OH} + G_H) + 2K[M]/\sqrt{R}$ (expression 41 below).

Thus the G *vs.* [M] curve (at constant dose rate) should become linear and, upon extrapolation, should intersect the G axis at $G(-M) = 2(G_{OH} + G_H)$. Figure 7 shows that linear monomer dependence at lipoate concentrations above 8 m*M* is reasonable[13] and a tangent to *each* of the three curves at this concentration intersects the ordinate at $G(-\text{lipoate}) = 10.8$, a value very close to $2(G_{OH} + G_H)$. This agreement lends further support to the plausibility of the proposed mechanism.

The present results may have significant radiobiological implications. The secondary–tertiary structure of very many proteins and enzymes involves multiple specifically located disulfide linkages. While the average concentration of disulfide within a living cell may be low, the effective concentration within a globular protein molecule or other substructure may be as high as 0.4–0.5 *M*.[4] Simple extrapolation would require G values of about 600 for scrambling of lipoate at such concentrations. While any proposed analogy between a lipoate solution and the inner structure of a protein molecule must necessarily be tentative, we cannot but suggest that a single radiolytic radical or solvated electron, generated in or near such a molecule might cause considerable intramolecular rearrangement, denaturation, and inactivation. We are investigating this possibility. Pertinently, the nonhistone proteins which form a considerable proportion of the chromosomal shearth[14] are reported to be rich in disulfide.[15] Distribution and diffusion considerations indicate that reaction of radicals with the disulfide linkages of chromosomal sheath protein is much more probable than reaction with the nucleic acid. The resulting intramolecular rearrangement of the protein could cause

(13) Purely practical considerations, especially the low solubility of lipoic acid and the probability of "direct" effects of radiation precluded studies at high concentrations. The relationship requires the monomer-dependent term to vary inversely as the square root of the dose rate; again, experimental limitations prevented proper evaluation. Such data as could be obtained correlated roughly with this requirement.

(14) H. Busch, E. J. Starbuck, E. J. Singh, and T. S. Ro, "The Role of Chromosomes in Development," M. Lauke, Ed., Academic Press, New York, N. Y., 1964, p 51.

(15) W. Sandritter and A. Krygier, *Z. Krebsforsch.*, 62, 596 (1959); B. B. Hyde, *J. Histochem. Cytochem.*, 9, 640 (1961).

genetic and biosynthetic malfunction by preventing proper unfolding and exposure of parts of the polynucleotide chain.

Kinetic Treatment

The reactions postulated above are (r = rate, see ref 16)

Initiation

$$H_2O \rightsquigarrow n(\cdot OH) + m(e_{aq}^-) \quad (1)$$

$$d(\cdot OH)/dt = k_0R = r_0$$

$$de/dt = k_1R = r_1$$

$$M + \cdot OH \xrightarrow{k_2} M^+ + OH^- \quad (2)$$

$$r_2 = k_2M(\cdot OH) = r_0$$

$$M + e \xrightarrow{k_3} M^- \quad (3)$$

$$r_3 = k_3Me$$

Propagation

$$M^+ + M \xrightarrow{k_4} D^+ \quad (4)$$

$$r_4 = k_4MM^+$$

$$M^- + M \xrightarrow{k_5} D^- \quad (5)$$

$$r_5 = k_5MM^-$$

Transfer

$$D^+ + M \xrightarrow{k_6} D + M^+ \quad (6)$$

$$r_6 = k_6MD^+$$

$$D^- + M \xrightarrow{k_7} D + M^- \quad (7)$$

$$r_7 = k_7MD^-$$

Termination

$$M^+ + e \xrightarrow{k_8} M \quad (8)$$

$$r_8 = k_8M^+e$$

$$M^+ + M^- \xrightarrow{k_9} 2M \quad (9)$$

$$r_9 = k_9M^+M^-$$

$$M^+ + D^- \xrightarrow{k_{10}} M + D \quad (10)$$

$$r_{10} = k_{10}M^+D^-$$

$$D^+ + e \xrightarrow{k_{11}} D \quad (11)$$

$$r_{11} = k_{11}D^+e$$

$$D^+ + M^- \xrightarrow{k_{12}} D + M \quad (12)$$

$$r_{12} = k_{12}D^+M^-$$

$$D^+ + D^- \xrightarrow{k_{13}} 2D \quad (13)$$

$$r_{13} = k_{13}D^+D^-$$

The rate of consumption of monomer is given by

$$d\,-M/dt = r_2 + r_3 + r_4 + r_5 + r_6 + r_7 - (r_8 + 2r_9 + r_{10} + r_{12}) \quad (14)$$

(16) X denotes [X], mol l.$^{-1}$; e denotes the solvated electron; dX/dt (mol l.$^{-1}$ sec^{-1}) = $GR/100N$; G = molecules/100 eV; R = dose rate, eV l.$^{-1}$ sec^{-1}; N is Avogadro's number.

Steady state in M^+, D^+, e, M^{-1}, and D^- gives

$$dM^+/dt = d\,-M^+/dt$$

or (15)

$$r_2(=r_0) + k_6MD^+ = k_4M^+M + k_8M^+e + k_9M^+M^- + k_{10}M^+D^-$$

$$dD^+/dt = d\,-D^+/dt$$

or (16)

$$k_4M^+M = k_{11}D^+e + k_{12}D^+M^- + k_{13}D^+D^- + k_6MD^+$$

$$de/dt = d\,-e/dt$$

or (17)

$$r_1 = k_3Me + k_8M^+e + k_{11}D^+e$$

$$dM^-/dt = d\,-M^-/dt$$

or (18)

$$k_3Me + k_7MD^- = k_5MM^- + k_9M^+M^- + k_{12}D^+M^-$$

$$dD^-/dt = d\,-D^-/dt$$

or (19)

$$k_5MM^- = k_{10}M^+D^- + k_{13}D^+D^- + k_7MD^-$$

Summing the steady-state concentrations of cations and of anions

$$M^+ + D^+ = A^+ \quad (20a)$$

$$e + M^- + D^- = A^- \quad (20b)$$

and assuming that the rate constants for all the termination steps are approximately equal ($k_8 \sim k_9 \sim k_{10} \sim k_{11} \sim k_{12} \sim k_{13} = k$), statements 15–19 simplify to 15a–19a.

$$r_0 + k_6MD^+ = M^+(k_4M + kA^-) \quad (15a)$$

$$k_4M^+M = D^+(kA^- + k_6M) \quad (16a)$$

$$r_1 = e(k_3M + kA^+) \quad (17a)$$

$$k_3Me + k_7MD^- = M^-(k_5M + kA^+) \quad (18a)$$

$$k_5MM^- = D^-(kA^+ + k_7M) \quad (19a)$$

Summing eq 15a–19a

$$r_0 + r_1 = 2kA^+A^- \quad (21)$$

Since $r_0 \sim r_1$, let each be represented by $\bar{r} = (r_0 + r_1)/2$. Also $A^+ \sim A^-$ since they are produced and destroyed in almost equal numbers; each may be represented by $\bar{A}$, so that

$$\bar{A} = \sqrt{\bar{r}/k} \quad (22)$$

a constant at constant dose rate. Rearranging (17a) we have

$$e = \bar{r}/(k_3M + \sqrt{k\bar{r}}) \quad (23)$$

From (16a) and (20a)

$$D^+ = k_4M\bar{r}/(k_4M + k_6M + \sqrt{k\bar{r}})(\sqrt{k\bar{r}}) \quad (24)$$

and

$$M^+ = \bar{r}(k_6M + \sqrt{k\bar{r}})/(k_4M + k_6M + \sqrt{k\bar{r}})(\sqrt{k^-}) \quad (25)$$

From (19a), (20a), and (23)

$$D^- = k_3k_5M^2\bar{r}/(k_3M + \sqrt{k\bar{r}}) \times (k_5M + k_7M + \sqrt{k\bar{r}})(\sqrt{k\bar{r}}) \quad (26)$$

and

$$M^- = k_3M\bar{r}(k_7M + \sqrt{k\bar{r}})/(k_3M + \sqrt{k\bar{r}})(k_5M + k_7M + \sqrt{k\bar{r}})(\sqrt{k\bar{r}}) \quad (27)$$

Using expressions 23–27 we may now deduce the ten rates contributing to (14). Thus, from (3) and (23)

$$r_8 = k_3M\bar{r}/(k_3M + \sqrt{k\bar{r}}) \quad (28)$$

From (6) and (24)

$$r_6 = k_4k_6M^2\bar{r}/(k_4M + k_6M + \sqrt{k\bar{r}})(\sqrt{k\bar{r}}) \quad (29)$$

From (7) and (26)

$$r_7 = k_3k_5k_7M^3\bar{r}/(k_3M + \sqrt{k\bar{r}}) \times (k_5M + k_7M + \sqrt{k\bar{r}})(\sqrt{k\bar{r}}) \quad (30)$$

From (4), (16a), and (6)

$$r_4 = k_6MD^+ + kD^+\bar{A} = r_6 + r_4' \quad (31)$$

where, from (2) and (24)

$$r_4' = kD^+\bar{A} = k_4M\bar{r}/(k_4M + k_6M + \sqrt{k\bar{r}}) \quad (32)$$

Similarly, from (5), (19a), and (7)

$$r_5 = k_7MD^- + kD^-\bar{A} = r_7 + r_5' \quad (33)$$

where, from (2) and (26)

$$r_5' = kD^-\bar{A} = k_3k_5M^2\bar{r}/(k_3M + \sqrt{k\bar{r}}) \times (k_5M + k_7M + \sqrt{k\bar{r}}) \quad (34)$$

From (8), (23), and (25)

$$r_8 = \bar{r}(\sqrt{k\bar{r}})(k_6M + \sqrt{k\bar{r}})/(k_3M + \sqrt{k\bar{r}}) \times (k_4M + k_6M + \sqrt{k\bar{r}}) \quad (35)$$

From (9), (25), and (27)

$$r_9 = k_3M\bar{r}(k_6M + \sqrt{k\bar{r}})(k_7M + \sqrt{k\bar{r}})/(k_3M + \sqrt{k\bar{r}})(k_4M + k_6M + \sqrt{k\bar{r}}) \times (k_5M + k_7M + \sqrt{kr}) \quad (36)$$

From (10), (25), and (26)

$$r_{10} = k_3k_5M^2\bar{r}(k_6M + \sqrt{k\bar{r}})/(k_3M + \sqrt{k\bar{r}})(k_4M + k_6M + \sqrt{k\bar{r}}) \times (k_5M + k_7M + \sqrt{k\bar{r}}) \quad (37)$$

From (12), (24), and (27)

$$r_{12} = k_3k_4M^2\bar{r}(k_7M + \sqrt{k\bar{r}})/(k_3M + \sqrt{k\bar{r}}) \times (k_4M + k_6M + \sqrt{k\bar{r}})(k_5M + k_7M + \sqrt{k\bar{r}}) \quad (38)$$

The complete rate equation, from (14), (31), and (33) now becomes[17]

$$\mathrm{d}-M/\mathrm{d}t = r_0 + r_3 + r_4' + r_5' + 2r_6 + 2r_7 - (r_8 + 2r_9 + r_{10} + r_{12}) \quad (14a)$$

At low monomer concentration, ($k_nM \ll \sqrt{k\bar{r}}$), expressions 28–30, 32, and 34–38 show that all terms become very small except r_0 and r_3 which both approach $\bar{r}$, so that $\mathrm{d}-M/\mathrm{d}t$ becomes small.

The monomer dependence is complex and nonlinear at intermediate concentrations, but at higher concentrations ($k_nM \gg \sqrt{k\bar{r}}$) becomes[18]

$$\mathrm{d}-M/\mathrm{d}t = 2r_0 + 2r_1 + 2M(k_6 + k_7)\sqrt{\bar{r}/k} \quad (39)$$

which is equivalent[16] to

$$G(-\mathrm{M}) = 2(G_{\mathrm{OH}} + G_{\mathrm{H}}) + 2M100N(k_6 + k_7) \times \sqrt{k_0/kR} \quad (40)$$

or

$$G(-\mathrm{M}) = 2(G_{\mathrm{OH}} + G_{\mathrm{H}}) + 2KM/\sqrt{R} \quad (41)$$

If M is millimoles/liter and R is rads/minute, G being molecules/100 eV, then K = 54 at pH ~4, 36 at pH ~6.5, and 30 at pH ~8.5.

(17) Some variations, particularly of the termination and transfer steps are possible. For example, (9) could be $M^+ + M^- \rightarrow D$. Such changes do not significantly affect the form of the rate equations deduced for monomer consumption; nor do they affect the purpose and significance of the kinetic treatment. Variations requiring oxygen dependence, or production of hydrogen peroxide and reduced or oxidized sulfur end groups are, of course, untenable.

(18) This reduction of the rate equation at high M requires $k_6 \ll k_4$. The shortness of the chain suggests this. Furthermore, the r_6 term in (31) clearly is that part of r_4 which arises from the locked-step chain-transfer process while r_4' corresponds to the first conversion of an M^+ species arising from (2) into D^+. (This can be confirmed kinetically by considering a hypothetical case in which transfer does not occur, when it is found that $r_4 = r_4'$.) Simple logic also requires that $r_4' = \bar{r}$ at high M, and, from (32), this can only be so if $k_6 \ll k_4$. Similar considerations require $k_7 \ll k_5$.