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The Radiation Chemistry of Biochemical Disulfides. I. The Low-Dose X-Radiolysis of Cystine[1]

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Abstract: All ninhydrin-positive products resulting from exposure of dilute solutions of cystine in sulfuric acid to moderate doses (1,000–80,000 rads) of X-rays have been separated, identified, and determined quantitatively. Cysteic acid is the major product under all conditions. Yields vary with cystine concentration, dose rate, photon energy, and slightly with acidity, at low cystine concentrations. A mechanism for cysteic acid production is presented and a kinetic interpretation is given. These accommodate the concentration and dose-rate effects and are consistent with the photon energy effect and the amounts of hydrogen peroxide formed along with the cysteic acid.

Organic sulfur compounds are of considerable importance in radiation biochemistry. The activity of many biochemical intermediates (sulfhydryl enzymes, coenzyme A, lipoic acid, methionine, thiamin, etc.) depends upon the chemical integrity of sulfur groups and such groups are known to be very radiation sensitive. Disulfide groups of cystine units are essential to the secondary–tertiary structure of many enzymes and proteins. Radiolytic disturbance of such groups may well be more important in radiation inactivation of enzymes than direct destruction of active sites. Many sulfur compounds show some ability to protect living systems against radiation damage and lethality. It is noteworthy that, while cysteamine, cysteine, and cystamine are among the most effective of such compounds, cystine has no radiation-protective properties.

The radiation chemistry of cystine, cystine peptides, and cystamine has received some study, but little comprehensive quantitative determination of all the organic products has been reported. Mechanisms have been proposed for processes leading from initial radicals to final products but these have not been tested and most of them show little analogy with known reactions. Early work[2] with cystine using heavy radiation doses (10^5–10^6 r) gave simple inorganic compounds (SO_4^{2-}, S, NH_3, etc.) as the major products. More recent and sophisticated studies[3] with somewhat lower radiation doses (8×10^4 r) under conditions minimizing radiolysis of initial products gave mainly cysteic acid along with some cysteinesulfinic acid and cystine dioxide[4] and a little cysteine and alanine. Much radiobiological and radiation biochemical interpretation has been based on the above results.

This complete change in products as a result of a moderate change in radiation dose indicates the need for direct determination of products at typical radiobiological lethal doses (500–1000 r). We now report the quantitative determination of all the nonvolatile ninhydrin-positive products and hydrogen peroxide produced in dilute solutions of cystine upon X-irradiation with doses as low as 1000 rads.

Experimental Section

Radiation-chemical yields are rather small. At $G \sim 1$, deposition of 1000 rads produces about 10^{-9} mole of product per milliliter of solution. Yields of minor products are correspondingly smaller. It is very difficult to detect, identify, and assay all products over a wide range of independent variation of such parameters as disulfide concentration and excess acidity, dose, dose rate, etc. Accordingly, we have used two approaches. In the first, all ninhydrin-positive products were identified and assayed over a wide total-dose range while concentration, acidity, and dose rate were varied interdependently so as to permit such assay. In the second, a limited number of products was assayed over a wide range of independent variation of irradiation conditions.

Ninhydrin-Positive Products (Table I). Preliminary experiments showed that the expected[3] radiolysis products cysteic acid, cysteinesulfinic acid, cystine dioxide, alanine, and unchanged cystine[5] are well separated by electrophoresis on cellulose acetate[6] in formic acid (40 g/l., pH 2) during 12–16 min at 25 v/cm. Color development utilized a ninhydrin reagent similar to that of Moore and Stein[7] but more concentrated. The cellulose acetate strips were dried under a warm air stream on glass plates, saturated with the reagent, sandwiched *in vacuo* between glass plates, and heated for 4–5 min at 90–95°. Reproducible color development was authenticated with specimens of pure materials and relative color yields

(1) Research supported by the Research Corporation and by U. S. Public Health Service Research Grant RH 379, Division of 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) P. Markakis and A. L. Tappel, *J. Am. Chem. Soc.*, **82**, 1613 (1960).

(3) D. W. Grant, S. N. Mason, and M. A. Link, *Nature*, **192**, 352 (1961).

(4) This compound, earlier commonly called cystine disulfoxide, almost certainly is a thiosulfonate (*cf.* J. Cymerman and J. B. Willis, *J. Chem. Soc.*, 1332 (1951); T. C. Owen and R. R. Crenshaw, *Proc. Chem. Soc.*, 250 (1961). However, the structure is not completely proved and the name cystine dioxide used here is brief, self-explanatory, and at least not incorrect.

(5) Cysteine is oxidized to cystine during electrophoresis. However, cysteine yields were extremely small up to 100 μM cystine and were determined spectrophotometrically using a modified phosphotungstate reagent (*cf.* O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 421 (1922); K. Shinohara, *ibid.*, **109**, 665 (1935).

(6) "Sepraphore III" cellulose acetate, Gelman Instrument Co., Ann Arbor, Mich.

(7) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

Table I. Ninhydrin-Positive Products from X-Irradiation of Cystine[a]

Radiation dose, rads[c] × 10^{-3}	Dose rate, rads min^{-1}	Vol of soln irradiated, ml	Cystine concn, μM	H_2SO_4 concn, mM	Moles of products found, × 10^8 — Cysteic acid	Cysteine-sulfinic acid	Cystine dioxide	Cystine	G(cysteic acid)[b]	Material balance[d]
1	111	240	1.25	0.05	2.9	Trace	Trace	28.8	0.12	101
2	222	120	2.5	0.1	10.0	1.2	0.5	24.0	0.38	99
5	555	48	6.25	0.25	14.8	1.4	0.5	21.0	0.59	98
10	1111	24	12.5	0.5	19.2	2.1	None	19.2	0.77	99
20	2222	12	25	1	28.2	1.8	None	15.0	1.15	100
40	4444	6	50	2	30.0	0.4	None	15.0	1.2	100
80	8888	3	100	4	27.6	1.8	None	16.2	1.1	103

[a] Total energy deposited in each solution, 1.5×10^{16} ev; total cystine originally present in each solution, 3×10^{-7} mole; irradiation time, 9 min; photon energy, 40 kv_{max}. [b] The G value represents the number of molecules of a product produced or reactant destroyed upon deposition of 100 ev of energy from the incident radiation. [c] One rad corresponds to deposition of 6.24×10^{13} ev of energy in 1 g of material or solution irradiated. [d] Per cent of cystine accounted for.

were determined densitometrically[8] at 570 mμ.[9] For irradiation, portions (30 μl) of cystine solution (10 mM cystine, 0.2 M H_2SO_4) were diluted with triply distilled water (3–240 ml, Table I) in flat shallow dishes (petri dishes) of diameters (2–18 cm) such that the sample thicknesses were 1 cm. These were placed vertically beneath the focal spot of the X-ray tube at distances such that a constant angle (20°) was subtended at the focus by any dish diameter. Since the radiation flux varies inversely as the square of the distance of the sample from the focus while the area (and hence the volume) of sample increased as this square, the total energy deposited in the sample remained constant while the dose in rads varied inversely with the square of the distance. However, change in radiation dose is accompanied by changes in cystine and sulfuric acid concentration and in dose rate. To each irradiated sample was added 30 μl of 10 mM alanine or lysine solution as an internal quantitation standard. Each was evaporated (all-glass rotary evaporator, reduced pressure, 30–35°) to ~3 ml, transferred to a small (10 ml) conical vessel with a fine (3 × 15 mm) conical tip, and further evaporated to about 30 μl. Solutes were then separated, identified by electrophoresis, and assayed *via* the ninhydrin color reaction. Control experiments showed excellent material recovery as long as splashing, crystallization, and complete evaporation were carefully avoided.

Cysteic Acid (Table II). Cysteic acid was determined by dinitrophenylation[10] (fluorodinitrobenzene, bicarbonate), removal of DNP-cystine, cysteine, and cysteinesulfinic acid by extraction with chloroform, and spectrophotometric assay of DNP-cysteic acid in the aqueous solution. This procedure provides greater sensitivity, precision, and accuracy than does electrophoresis and permits independent variation of cystine and sulfuric acid concentrations. Irradiations at pH 6–7 were carried out using supersaturated solutions obtained by neutralization (sodium hydroxide) of solutions in dilute sulfuric acid.

Competitive Radiolysis of Cystamine and Cystine. A solution of cystamine and cystine (5 mM in each disulfide, 0.5 N in H_2SO_4) was irradiated (25,000 rads), dinitrophenylated, and extracted as above, and the aqueous layer evaporated to ~100 μl. Electrophoresis (cellulose acetate, borate buffer pH ~8, 15 min, 350 v) showed only one yellow band, DNP-taurine.

Hydrogen Peroxide, Cysteine, and Hydrogen Sulfide. Hydrogen peroxide determinations used Eisenberg's titanium sulfate procedure.[11] The very small amounts of cysteine and hydrogen sulfide produced were determined using the phosphotungstate[5] and methylene blue[12] procedures, respectively. Cysteine was characterized occasionally by reaction with N-ethylmaleimide[3,13] before evaporation and electrophoresis.

General Procedures. Irradiations at 125 kv were effected using a General Electric Co. Maximar II X-ray apparatus; irradiations at 100 kv utilized a Keleket instrument and Machlett tube while those at 40 kv utilized a GE fluoroscope of uncertain vintage. Radiations were unfiltered. Radiation intensity was measured by ferrous (Fricke[14]) dosimetry using either a Beckman DU or a Bausch and Lomb "Precision" spectrophotometer at 305 mμ and 25° and corrected to pure water values.[15] Water for solutions to be irradiated was distilled first from phosphoric acid, then from alkaline permanganate, and finally through a 30-in. Vigreux column from dichromate solution acidified with phosphoric acid. Irradiation vessels were preirradiated until brown while filled with triply distilled water.

Results

Irradiation conditions and product yields are detailed in Tables I and II. Cysteic acid yields are also shown in Figure 1. Each yield in Table I is the mean of 25 or more determinations (five samples from each of five irradiated solutions). Each G_0 value in Table II is calculated from the initial slope of a five-point yield

Table II. Cysteic Acid and Hydrogen Peroxide Yields from X-Irradiation of Cystine Solutions

Item	Dose rate, rads min^{-1}	H_2SO_4 concn, mM	Cystine concn, μM	G_0-(cysteic acid)	G_0-(H_2O_2)	Photon energy, kv_{max}
1	1200	10	10	1.56	2.22	125
2	1200	10	50	1.96	2.14	125
3	1200	10	100	2.35	1.75	125
4	1200	10	500	2.50	1.13	125
5	1200	10	1000	2.48	1.13	125
6	1200	pH 5.9	10	0.94	1.79	125
7	1200	pH 5.9	50	1.56	1.55	125
8	1200	pH 6.6	100	1.68	1.62	125
9	1200	pH 6.5	500	1.59	1.44	125
10	1200	pH 5.8	1000	1.88	1.23	125
11	700	0.2	3.3	1.09		100
12	700	2.0	3.3	1.15		100
13	700	20	3.3	1.22		100
14	700	2.0	33	1.40		100
15	700	20	33	1.46		100
16	700	1.25	50	1.52		100
17	700	2.5	100	1.75	1.57	100
18	700	12.5	500	2.35		100

(8) Photovolt Model 542 "Densicord" densitometer, Photovolt Corporation, 1115 Broadway, New York, N. Y.

(9) The electrophoretic separation and color development procedures for these and other amino acids will be reported elsewhere. The quantitative estimations are accurate to ±20%, adequate for the present purposes.

(10) T. C. Owen, A. Wilbraham, B. G. Johnson, and J. A. G. Roach, submitted for publication.

(11) G. M. Eisenberg, *Ind. Eng. Chem. Anal. Ed.*, **15**, 327 (1943).

(12) A. E. Sands, M. A. Grafius, H. W. Wainwright, and M. Wilson. *U. S. Bur. Mines Rept. Invest.*, No. 4547 (1949); R. F. Milton and W. A. Waters, "Methods of Quantitative Microanalysis," 2nd ed, Arnold Publishers, Ltd., London, 1953, p 311.

(13) E. Friedmann, D. H. Marrian, and I. Simon-Reuss, *Brit. J. Pharmacol.*, **4**, 105 (1949); *Biochim. Biophys. Acta*, **9**, 61 (1952).

(14) H. Fricke and S. Morse, *Am. J. Roentgenol. Radium Therapy*, **18**, 430 (1927); T. S. Hardwick, *Can. J. Chem.*, **30**, 17 (1952).

(15) A. J. Swallow, *J. Chem. Soc.*, 135, 334 (1952).

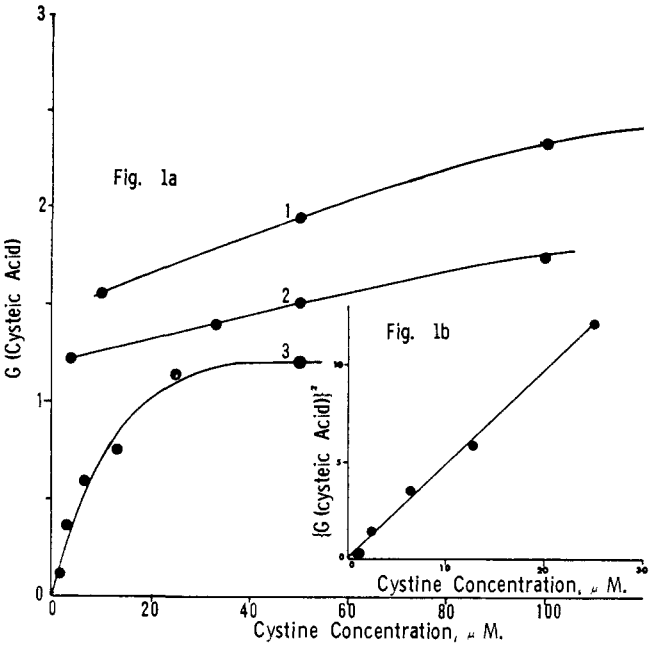


Figure 1. (a) Cysteic acid yields from irradiated cystine solutions; dependence on cystine concentration: 1, G_0(cysteic acid) at 1200 rads min^{-1}, 125 kv; 2, G_0(cysteic acid) at 700 rads min^{-1}, 100 kV, 3, G(cysteic acid), 40 kv, dose rate variable. (b) $\{G(\text{cysteic acid})\}^2$ *vs.* cystine concentration, 40 kv, dose rate α-cystine concentration.

vs. dose curve. The material balances (Table I) indicate that all significant products are accounted for. Cysteic acid is the only major product; cysteinesulfinic acid and cystine dioxide are produced in very small amounts. Yields are fairly constant at cystine concentrations above about 100 μM under any given set of conditions (the normal "indirect effect") but vary somewhat with acidity, markedly with photon energy, and, at lower concentrations, are very dependent upon both cystine concentration and rate of deposition of energy. Hydrogen peroxide yields fall as cysteic acid yields rise with increasing concentration.

Discussion

1. Production of Cysteic Acid. Radiolytic degradation of cystine occurs exclusively at the disulfide group, at least through the range of conditions recorded in Table I (material balance). Cysteic acid is the sole significant radiolysis product, even at extremely low dose rates and, hence, steady-state concentrations of reactive radicals. The probability of bimolecular interaction between radicals and reactive intermediates must be negligible at low dose rates; mechanisms involving radical–radical interaction must therefore be highly suspect. We suggest that the sulfonic acid is produced directly from cystine rather than by further radiolysis of initial products, by reaction with a single one-electron oxidizing radical and subsequent reaction only with stable molecular solutes (O_2, H_2O, cystine). The following mechanism appears to meet these requirements.

$$1.\ \text{R–S–S–R} + \cdot\text{X} \longrightarrow \text{R–S}\overset{+}{\text{–}}\overset{\cdot}{\text{S}}\text{–R} + \text{X}^-$$

$$2.\ \text{R–S}\overset{+}{\text{–}}\overset{\cdot}{\text{S}}\text{–R} \longrightarrow \text{R–S}^+ + \cdot\text{S–R}$$

$$3.\ \text{R–S}^+ + \text{O}_2 \longrightarrow \text{R–SO}_2^+$$

$$4.\ \text{R–SO}_2^+ + \text{OH}^- \longrightarrow \text{R–SO}_3\text{H}$$

$$5.\ 2\text{R–S}\cdot + \text{R–S–S–R}$$

Electron abstraction (step 1) probably is effected mainly by ·OH, at least below 100 μM disulfide. Cystamine reacts readily only with ·OH under comparable conditions[16] and cystine is less reactive than cystamine (competition experiments). The disulfide radical cation would be stabilized by resonance (charge and deficiency delocalization)[17] which may well contribute to the selectivity for attack on sulfur. It may also rationalize the lower reactivity of cystine than cystamine since the electron-attracting carboxyl groups of cystine (in acid solution) will disfavor formation of a positively charged center. Heterolysis (step 2) bears good analogy to heterolytic fission of other RSX species[18] in which X is much more electronegative than S. Analogies in radiation chemistry are afforded by breakdown of $H_2O\cdot^+$ and $CHCl_3\cdot^+$ into ·OH and ·CCl_3, respectively, and H^+. Reaction of the sulfenium cation with oxygen (step 3) is, to our knowledge, a novel suggestion. It should be observed in SN1 hydrolysis of dilute ($\sim 10^{-4}$ M) solutions of sulfenyl compounds. We are currently studying this possibility. Preliminary results show that the slow hydrolysis of cystine dioxide in dilute, aerated acidic solution at room temperature does give cysteic acid. Hydroxylation (step 4) of the sulfonium cation requires no comment. The thiyl radical (steps 2 and 5), being unreactive toward any of the molecular solutes in the system, would build up to a relatively high steady-state concentration and disappear by recombination. This radical should cause disulfide "scrambling" in solutions of mixed disulfides, and we have recently reported[19] that high and concentration-dependent yields of polymerization result upon X-irradiation of solutions of α-lipoic acid.

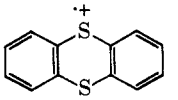
2. Effect of Acid Concentration on Cysteic Acid Yield. The changing cysteic acid yields recorded in Table I must result from variation of either cystine concentration, sulfuric acid concentration, or dose rate. Items 11 through 15 (Table II) show the effects of varying acidity at two cystine concentrations and constant dose rate. The effect of acid is much less than that of cystine concentration; a 100-fold change in acid (0.2–20 mM) produces an increase in G value only half as great as that produced by a tenfold change in cystine concentration. When $G_0(RSO_3H)$ is plotted against log [H_2SO_4] a straight line may be drawn through the three points at [RSSR] = 3.3 μM. The line is parallel to one through the two points at [RSSR] = 33 μM, which indicates that the effect of changing acid concentration is independent of disulfide concentration. It may be expressed in the form

$$G_0(\text{RSO}_3\text{H}) = A + 0.068(\log [\text{H}_2\text{SO}_4])$$

where the value of the intercept A is determined by the

(16) G. G. Jayson, T. C. Owen, and A. C. Wilbraham, *J. Chem. Soc.*, in press.

(17) Compare p-$CH_3SC_6H_4S\cdot^+CH_3$: A. Zweig, W. G. Hodgson, W. H. Jura, and D. L. Maricle, *Tetrahedron Letters*, 1821 (1963), and H. J. Shine and L. Piette, *J. Am. Chem. Soc.*, **84**, 4798 (1962).



(18) O. Foss, "Ionic Scission of the Sulfur–Sulfur Bond," and N. Kharasch, Sulfenium Ions and Sulfenyl Compounds," in "Organic Sulfur Compounds," N. Kharasch, Ed., Pergamon Press Inc., New York, N. Y., 1961.

(19) T. C. Owen and A. C. Wilbraham, *Chem. Commun.*, 624 (1967).

cystine concentration, being 1.58 and 1.32 at [RSSR] = 33 and 3.3 μM, respectively.

The logarithmic relationship suggests a dependence of yield upon pH, especially since the pH range studied centers about the pk_a for the second carboxyl group of cystine. It is possible that the singly protonated molecule is slightly more radiation sensitive than is the zwitterion. However, as Swallow[15] has shown, the linear energy transfer from X-rays into sulfuric acid solutions increases with increasing concentration so that most of the effect probably is due simply to change in energy deposited in solutions of varying stopping power. In any event, the effect is much too small to account for the yield variations in Table I.

3. Effects of Cystine Concentration and Dose Rate on Cysteic Acid Yield. Items 1–5, and 12, 14, 16–18 (Table II) show the effect of varying cystine concentrations while holding other variables (dose rate, acidity, and photon energy) essentially constant at two sets of values. The effect is considerable, almost linear up to 100 μM cystine (lines 1 and 2, Figure 1a), and decreases at higher concentrations. The data from Table I (line 3, Figure 1a) do not show a linear change in G(cysteic acid) with cystine concentration, indicating that a dose-rate effect is superimposed on the concentration effect in this case (acid effects are neglected). However, the yield does change with the square root of the disulfide concentration (Figure 1b). These characteristics are consistent with the relationship $G^2 \propto C^2/R$ (G = yield, C = disulfide concentration, R = dose rate); at constant dose rate $G \propto C$, and where dose rate varies linearly with concentration (Table I; Figure 1b) $G^2 \propto C$. Making certain simplifying assumptions, we deduce the relationship (G_r is the yield of water-radiolysis radicals)

$$C^2 = kRG^2/(G_r - G)$$

which simplifies to the required $C^2 = k'RG^2$ at low concentrations (low yields; G_r considerably exceeds G) and also requires G to reach a limiting value (the normal indirect effect) at higher concentrations. The derivation is as given by the following assumptions. (1) A steady-state situation obtains during irradiation. (2) The initial and rate-determining step is a bimolecular second-order radical–solute interaction. (3) Reaction of any radical with solute may be represented by a single rate constant (as noted above, cystine probably reacts mainly with ·OH). (4) Bimolecular radical recombinations may be represented by an average rate constant.

The radiation-chemical yield (G value) is the constant in the yield–dose relationship

$$G = \mathrm{d(yield)/d(dose)} = 100AV\mathrm{d(yield)}/RV\mathrm{d}t$$

(yield in moles; A is Avogardo's number; R is dose rate, ev sec^{-1} $l.^{-1}$; t is time, sec; V volume of solution, liters) or

$$\mathrm{d(yield)/d}t = GR/100A$$

If all radicals (r) are consumed by reaction with solute (S) or with each other

$$\mathrm{S} + \mathrm{r} \xrightarrow{k_1} \text{product (P)}$$
$$\mathrm{d(P)/d}t = k_1[\mathrm{S}][\mathrm{r}] = RG(\mathrm{P})/100A \quad (1)$$

$$\mathrm{r} + \mathrm{r} \xrightarrow{k_2} \mathrm{r\text{–}r}$$
$$\mathrm{d(r\text{–}r)/d}t = k_2[\mathrm{r}]^2 = RG(\mathrm{r\text{–}r})/100A \quad (2)$$

$$G(\mathrm{P}) + 2G(\mathrm{r\text{–}r}) = G_r \quad (3)$$

Eliminating G(r–r) between eq 2 and 3

$$G(\mathrm{P}) + 200Ak_2[\mathrm{r}]^2/R = G_r \quad (4)$$

and eliminating [r] between eq 1 and 4

$$G(\mathrm{P}) + 2Rk_2G(\mathrm{P})^2/100A(k_1)^2[\mathrm{S}]^2 = G_r$$

rearranging and combining constants ($k = 2k_2/100\mathrm{A}\cdot(k_1)^2$)

$$[\mathrm{S}]^2 = kRG(\mathrm{P})^2/[G_r - G(\mathrm{P})] \quad (5)$$

4. Effect of Radiation Quality (Photon Energy). Dose-rate considerations suggest that the cysteic yields at 700 rads min^{-1} (line 2, Figure 1) should be greater than those at 1200 rads/min^{-1} (line 1). The converse is observed. The position of line 3 similarly is anomalous. The only unaccounted variable between the three sets of conditions is the energy of the incident photons which must, therefore, cause a yield decrease with decreasing photon energy. The lower the energy the more closely spaced along the track are the ionizing events (spurs) and we suggest that the lower product yields reflect increased radical recombination *between* spurs at the expense of radical–solute interaction. The average spur–spur distance (~1000 A for 40 kv_{max}, 25–30 kv_p; ~2000 A for 125 kv_{max}, ~70 kv_p) varies exponentially with photon energy[20] and is comparable to the average distance (500–1000 A) between solute molecules in dilute ($\lesssim 100$ μM) solution. In relationship 5 above, this effect will appear as a lower effective yield, G_r, of radicals diffusing out of the track.

5. Hydrogen Peroxide Yields. The effect of cystine concentration on hydrogen peroxide yields is similar to that observed for cystamine[16] but rather less clear-cut. However, analogy with that more reactive disulfide permits a reasonable interpretation. At low concentrations of either disulfide in acidic solution, $G_0(H_2O_2)$ approximates to $G_{H_2O_2} + 0.5G_H$.[21] In the cystamine case we have proposed that all ·OH react with disulfide (G_0(taurine) ~ G_{OH}) and, at low disulfide concentration, all e^-(aq) and H disappear by reaction with oxygen followed by recombination ($\cdot H + O_2 \rightarrow \cdot O_2H \rightarrow 0.5H_2O_2 + 0.5O_2$). With cystine, at low concentrations, G_0(cysteic acid) is somewhat lower than G_{OH}. We suggest that additional H_2O_2 production from dimerization of unconsumed ·OH is balanced by reaction with ·H, O_2H, and H_2O_2. Increasing cystamine concentration (to 100 μM) in acid solution causes G_0(taurine) to increase almost to $G_{OH} + G_H$ while $G_0(H_2O_2)$ concomitantly falls to $G_{H_2O_2}$. These changes are as would be predicted if, at higher concentrations, cystamine competes with dissolved oxygen for e^-(aq) and ·H (RSSR + ·H + $H^+ \rightarrow H_2$ + RSSR·$^+ \rightarrow$ taurine). Cysteic acid yields from cystine also rise, and peroxide yields fall, with increasing cystine concentration. The changes are smaller but are in the expected 2:1 ratio up to 100 μM cystine, again in accordance with increased scaveng-

(20) N. Miller, *Rev. Pure Appl. Chem.*, 7, 123 (1957).
(21) G_H represents $G_{\cdot H} + G_{e^-_{aq}}$ unless otherwise specified.

ing of both ·OH and ·H by disulfide producing sulfonic acid at the expense of peroxide. Current work shows hydrogen yields considerably above G_{H_2} under these conditions. Cysteic acid (and taurine) yields are noticeably lower in neutral than in acid solutions as would be expected if one of the oxidizing species were a protonated form of $e^-(aq)$ such as H_2^+. Peroxide yields do not increase correspondingly, suggesting that $e^-(aq)$ (or ·H) are partially scavenged by *addition* to disulfide in neutral solution ($RSSR + e^-(aq) \rightarrow RS^- + RS\cdot$) producing thiol which further reduces $G(H_2O_2)$ (H_2O_2 and cysteine react readily at pH ~6) and may even scavenge some ·OH. Significant yields of thiol (G_0(cysteine) up to 0.4, G_0(cysteamine) up to 1.0) do result upon irradiation of both neutral and acidic cystine and cystamine solutions of such concentration ($\lesssim 1$ m*M*) that [RSSR] greatly exceeds [H^+] (unpublished work).

Thus the peroxide yields indicate (although somewhat less clearly for cystine than for cystamine) that the one-electron oxidizing agents ·OH and H_2^+ (possibly ·H + H^+) are the species responsible for sulfonic acid production, as required by the radical-cation mechanism.

Certain of the present results have considerable radiobiological significance. The lower yield of destruction of cystine than of cystamine and the protection of the former by the latter indicate a much lower ease of electron abstraction from the former disulfide. This is consistent with destabilization of the positively charged disulfide radical-cation grouping by the inductively electron-attracting carboxyl groups of cystine. Thus cystine is less effective as a radical trapping or repair (by electron donation) agent than cystamine, and, conversely, assuming that cystine peptides simulate cystine, cystamine may be a radiation protective agent by virtue of the low electron affinity of its disulfide group. Radical scavenging or repair by thiols undoubtedly involves electron (or H) abstraction to give thiyl radicals ($RSH + \cdot OH \rightarrow RS\cdot + H_2O$). This does not involve radical cations and is unaffected by the inductive effect of the carboxyl group. Thus cysteamine and cysteine are as effective as cystamine in radiation protection, while cystine is ineffective. Rapid fixation of sulfenium cations as mixed disulfides ($R\text{-}S^+ + R'\text{-}S^- \rightarrow RSSR'$) may also contribute to radiation protection by thiols.

Significant though the disulfide radical cation and the sulfenium cation may be, we suggest that even greater radiobiological significance may attach to the simple proposal that the thiyl radical produced along with the sulfenium cation does *not* undergo rapid irreversible reaction with oxygen. Such radicals will build up to a significant steady-state concentration; equivalently, any such radical has a long effective lifetime, during which it may "scramble" many disulfide groups ($RS\cdot + R'SSR' \rightarrow RSSR' + R'S\cdot$). In the case of α-lipoic acid this leads to a very high and concentration-dependent yield of polymerization of the cyclic disulfide with *G* values at high as 25 even in 10^{-3} *M* lipoate solution.[14] The effective concentration of disulfide linkages within globular protein molecules frequently is rather high; the ribonuclease molecule with a partial specific volume of 0.728 cm^3/g and four cystine units is effectively a 0.43 *M* solution of disulfide links. Serum albumin (partial specific volume 0.734, mol wt 65,000, 17 cystine units) similarly is about 0.35 *M* in disulfide. Although any such system probably is more akin to a semirigid gel than to a simple solution, generation of only one thiyl radical within the molecule possibly would cause considerable disulfide scrambling and denaturation. It is pertinent to note that the nonhistone proteins of the chromosomes are rich in disulfide sulfur.