

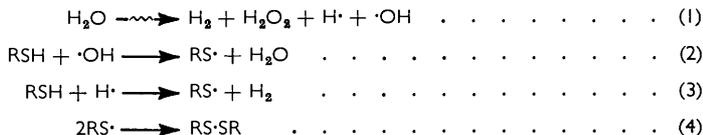
### 430. The Action of $^{60}\text{Co}$ -Gamma-rays on Aqueous Solutions of Hydrogen Sulphide and of Cysteine Hydrochloride.

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The radiolysis of dilute aqueous hydrogen sulphide and cysteine hydrochloride in the presence and in the absence of oxygen has been studied. The mechanism postulated for the short-chain reaction found only in oxygenated systems involves an intermediate sulphenylperoxy-radical,  $\text{R}\cdot\text{S}\cdot\text{O}\cdot$ , which abstracts hydrogen from a thiol in the relatively slow propagation step [ $\text{R} = \text{H}$  or  $\text{NH}_2\cdot\text{CH}(\text{CO}_2\text{H})\cdot\text{CH}_2\cdot\text{S}$ ]. It is suggested that a similar mechanism occurs in the autoxidation of hydrogen sulphide and thiol solutions.

COBALT-60  $\gamma$ -radiation interacts with water to give the reactive species  $\text{H}\cdot$  and  $\cdot\text{OH}$  and the "molecular" products  $\text{H}_2$  and  $\text{H}_2\text{O}_2$ , the primary yields being expressed as  $G_{\text{H}}$ ,  $G_{\text{OH}}$ ,  $G_{\text{H}_2}$ , and  $G_{\text{H}_2\text{O}_2}$ , where  $G_{\text{X}}$  is the number of species X formed per 100 ev of energy absorbed.<sup>1</sup> The chemistry of the radiolysis of dilute aqueous solutions is explained in terms of the reactions of these species with one another and with solutes.

The radiolysis of dilute aqueous solutions of the thiols, propane-1,3-dithiol, 2,3-dimercaptopropan-1-ol, anilinopropanedithiol, and glutathione,<sup>2</sup> cysteine,<sup>3-5</sup> cysteamine,<sup>6</sup> and mercaptoethylguanidine<sup>7,8</sup> has been studied by various workers who have shown that in oxygenated systems short-chain reactions occur, yielding the corresponding disulphides as the major products. In oxygen-free solutions the much lower yields of disulphides are interpreted in terms of non-chain reactions between the thiols and the radiolysis products of water:



<sup>1</sup> Dainton, *Radiation Res.*, 1959, *Suppl.* 1, 1.

<sup>2</sup> Barron and Flood, *J. Gen. Physiol.*, 1950, **33**, 229.

<sup>3</sup> Swallow, *J.*, 1952, 1334.

<sup>4</sup> Witcher, Rotheram, and Todd, *Nucleonics*, 1953, **11**, No. 8, 30.

<sup>5</sup> Markakis and Tappel, *J. Amer. Chem. Soc.*, 1960, **82**, 1613.

<sup>6</sup> Shapiro and Eldjam, *Radiation Res.*, 1955, **3**, 255.

<sup>7</sup> Shapiro and Dickens, *Radiation Res.*, 1960, **13**, 857.

<sup>8</sup> Anderson and Joseph, *Radiation Res.*, 1959, **10**, 507.

Swallow suggested the following modified mechanism for oxygenated systems:<sup>3</sup>



Reactions (5), (6), and (7) propagate the chain. By producing hydrogen atoms by electrical discharge in a stream of gaseous hydrogen Littman, Carr, and Brady<sup>9</sup> have confirmed that reaction (3) occurs readily. Consequently if reaction (7) is the important propagation step a chain reaction should proceed in the oxygen-free system. The fact that it does not and that reaction (7) would be ~17 kcal./mole endothermic make this mechanism improbable.

The radiation chemistry of hydrogen sulphide, which may be regarded as the simplest thiol, has not been studied in detail. Loiseleur found that an increase in pressure of hydrogen sulphide quickly repressed the formation of hydrogen peroxide and that sulphur was precipitated;<sup>10</sup> Markakis and Tappel found that sulphur and sulphate were formed in oxygenated systems.<sup>5</sup> In both cases a large proportion of the hydrogen sulphide was oxidised and consequently radiolytic oxidation of initial products would have occurred.

In this work the early stages of the radiolysis of dilute aqueous hydrogen sulphide have been studied in some detail for both oxygen-free and oxygenated systems. Measurements of cysteine lost and hydrogen peroxide formed in the radiolysis of dilute aqueous cysteine hydrochloride have also been made and from the results a new mechanism for the chain reaction in oxygenated systems is postulated.

#### EXPERIMENTAL AND RESULTS

*Materials.*—Distilled water was redistilled first from acid permanganate and then from alkaline permanganate. Hydrogen sulphide (Matheson Company, U.S.A.; purified) was used as supplied, so was oxygen (New Zealand Industrial Gases). Cysteine hydrochloride was prepared by the method of Du Vigneaud *et al.* by reducing cystine with sodium in liquid ammonia.<sup>11</sup>

*Irradiations.*—Samples (10 ml.) were irradiated with gamma-rays from a 180-c cobalt-60 source in 20-ml. tubes with B24 cones, the caps consisting of a B24 socket, a vacuum-stopcock, and a B14 cone by which the tube could be attached to a vacuum-line or sample-preparation line. For temperature-controlled measurements tubes were used with outer jackets through which water from a thermostat-bath was circulated.

*Dosimetry.*—The ferrous sulphate dosimeter was used, doses being calculated on the basis of  $G(\text{Fe}^{3+}) = 15.5$ .

*Preparation of Solutions.*—Hydrogen sulphide solutions were prepared by first deaerating water (10 ml.) in the irradiation vessel by reducing the pressure periodically, with magnetical stirring, and then bringing it to equilibrium with the required partial pressure of hydrogen sulphide. For oxygenated systems, stirring was stopped and the apparatus momentarily evacuated, then the solution was brought to equilibrium at 25° with the required partial pressures of hydrogen sulphide and oxygen which had been premixed in the appropriate proportions. The total pressure of the system was controlled by the height of the mercury reservoir of the gas burette.

Cysteine hydrochloride solutions were prepared by deaerating a  $4.4 \times 10^{-3}\text{M}$ -solution for the oxygen-free irradiations and bringing the deaerated solution to equilibrium with the required partial pressure of oxygen for the oxygenated measurements; the same apparatus was used as for experiments with hydrogen sulphide.

*Analysis.*—*Hydrogen sulphide solutions.* After irradiation, hydrogen sulphide was removed by bubbling nitrogen through the solution which was then filtered through a group 6 "Membran-filter" in a metal filter-tower apparatus. Sulphur was determined spectrophotometrically

<sup>9</sup> Littman, Carr, and Brady, *Radiation Res.*, 1957, **7**, 107.

<sup>10</sup> Loiseleur, *Compt. rend.*, 1942, **215**, 536.

<sup>11</sup> Du Vigneaud, Audrieth, and Loring, *J. Amer. Chem. Soc.*, 1930, **52**, 4500.

as ferric thiocyanate at  $456\text{ m}\mu$  by Bartlett and Skoog's method.<sup>12</sup> The "Membranfilter" and sulphur were dissolved in an acetone solution of sodium cyanide, the filter having no effect on the optical density of the solution. Hydrogen was pumped into a McLeod gauge on a vacuum-line through a small mercury diffusion pump. Hydrogen peroxide was determined by formation of  $\text{I}_3^-$ .<sup>13</sup> Sulphate was determined turbidometrically by modifying the method of Sheen *et al.*<sup>14</sup> An aliquot part of the filtrate was made up to 11 ml., 2 ml. of a solution containing 240 g. of sodium chloride and 20 ml. of hydrochloric acid ( $d\ 1.18$ ) per l. were added, and the solution was stirred for 1 min. after addition of 0.058 g. of barium chloride dihydrate, then transferred to a 4-cm. spectrophotometer cell; and the optical density was measured against water after 5 min.

*Cysteine hydrochloride solution.* Cysteine was determined spectrophotometrically at  $900\text{ m}\mu$  as the complex with phosphotungstic acid by a modified Shinohara technique.<sup>15</sup> Hydrogen peroxide was determined by the titanium sulphate method.<sup>16</sup>

*Results.*—The initial yields of products from the radiolysis of hydrogen sulphide solutions are shown in the Table.

Initial  $G$  values for products from aqueous  $\text{H}_2\text{S}$ .

Expt. no.	$[\text{H}_2\text{S}]$ ( $10^{-2}$ mole l. <sup>-1</sup> )	$P_{\text{O}_2}$ (mm. Hg)	Temp.	Dose rate: $8.1 - 8.8 \times 10^{17}$ ev min. <sup>-1</sup> .			
				$G(\text{S})$	$G(\text{H}_2)$ (molecules per 100 ev)	$G(\text{H}_2\text{O}_2)$	$G(\text{SO}_4^{2-})$
1	0.17	0	Ambient	2.7	—	0.6	—
2	1.7	0	"	3.4	3.9	0.5	—
3	7.0	0	"	3.8	—	0.5	—
4	7.0*	0	"	3.4	—	0.8	—
5	0.35	200	"	8.2	—	—	—
6	1.7	200	"	13.8	—	0.0	—
7	5.2	200	"	16.8	—	—	—
8	8.7	200	"	18.9	—	—	$6 \pm 1.5$
9	1.7	100	"	14.9	—	—	—
10	1.7	600	"	11.5	—	—	—
11	1.7	116 †	0°	12.3	—	—	—
12	8.7	200	25	19.5	—	—	—
13	8.7	116 †	0	16.6	—	—	—
14	8.7 ‡	224 †	25	19.0	—	—	—
15	8.7 §	200	Ambient	23.3	—	—	—

\*  $0.1\text{N-H}_2\text{SO}_4$ . †  $P_{\text{O}_2}$  required to make  $[\text{O}_2]$  equivalent to sulphuric acid-free solutions made up at  $25^\circ$  with  $P_{\text{O}_2} = 200$ . ‡  $1.0\text{N-H}_2\text{SO}_4$ . § Dose rate:  $0.136 \times 10^{17}$  ev min.<sup>-1</sup>.

Fig. 1 gives yield-dose plots for oxygen-free solutions. The products do not react in the absence of added acid, but in  $0.1\text{N}$ -sulphuric acid a slow reaction between hydrogen sulphide and peroxide occurs.

Fig. 2 shows the yield-dose plot for sulphur in an air-saturated solution. The decrease in oxygen concentration as it reacts at first increases  $G(\text{S})$  (see runs 6, 9, 10); then, as the oxygen supply is depleted,  $G(\text{S})$  falls to the oxygen-free value.

Fig. 3 shows yield-dose plots for sulphur at different concentrations of hydrogen sulphide, the yield increasing with increasing concentration.

Increasing the temperature (runs 11/6, 13/8/12) and decreasing the dose rate runs (8, 15) in presence of oxygen increase  $G(\text{S})$ . No hydrogen peroxide was formed while oxygen was present, but on depletion of oxygen it was formed in the same yield as in the oxygen-free system. Sulphate was formed only in the presence of oxygen, none being formed after oxygen depletion. The yield of sulphur was little changed in  $1.0\text{N}$ -sulphuric acid solution (runs 12, 14).

Fig. 4 shows the loss of cysteine, and Fig. 5 the formation of hydrogen peroxide as a function of dose for  $4.4 \times 10^{-3}\text{M}$ -cysteine hydrochloride. From the initial slope of the plots the values of  $G(-\text{RSH})_{\text{O}_2}$ ,  $G(-\text{RSH})^{\text{vac}}$ ,  $G(\text{H}_2\text{O}_2)_{\text{O}_2}$ , and  $G(\text{H}_2\text{O}_2)^{\text{vac}}$  are 13.3, 6.2, 4.4, and 0.6, respectively.

<sup>12</sup> Bartlett and Skoog, *Analyt. Chem.*, 1954, **26**, 1008.

<sup>13</sup> Allen, Hochanadel, Ghormley, and Davis, *J. Phys. Chem.*, 1952, **56**, 585.

<sup>14</sup> Sheen, Kahler, and Ross, *Ind. Eng. Chem. Analyt.*, 1935, **7**, 262.

<sup>15</sup> W. S. Simpson, personal communication.

<sup>16</sup> Eisenberg, *Ind. Eng. Chem., Analyt.*, 1943, **15**, 327.

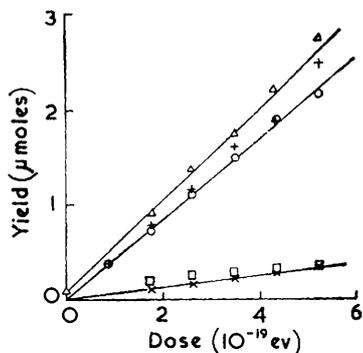


FIG. 1. Yield-dose plot for S, H<sub>2</sub>, and H<sub>2</sub>O<sub>2</sub> in oxygen-free H<sub>2</sub>S solutions. × H<sub>2</sub>O<sub>2</sub> (run 2), □ H<sub>2</sub>O<sub>2</sub> (run 4), ○ S (run 2), + S (run 4), Δ H<sub>2</sub> (run 2).

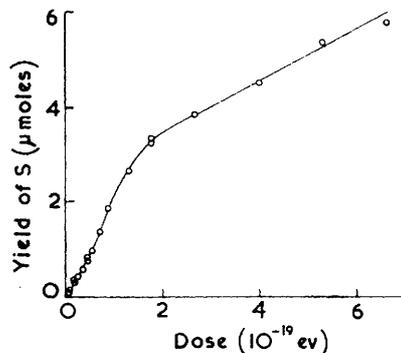


FIG. 2. Yield-dose plot for sulphur in air-saturated 0.013M-H<sub>2</sub>S.

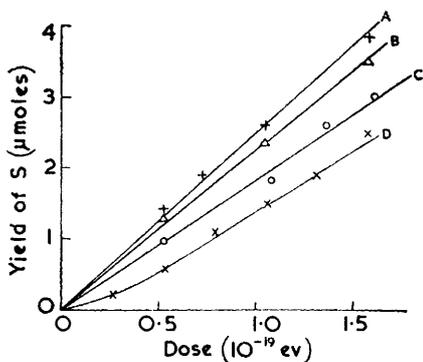


FIG. 3. Yield-dose plot for S at various [H<sub>2</sub>S] with P<sub>02</sub> 200 mm. [H<sub>2</sub>S] (10<sup>-2</sup>M): (A) 8.7 (run 8); (B) 5.2 (run 7); (C) 1.7 (run 6); (D) 0.35 (run 5).

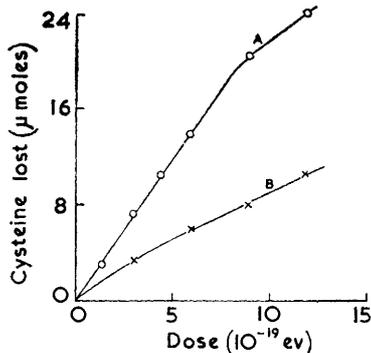


FIG. 4. Yield-dose plot for loss of cysteine in 4.4 × 10<sup>-3</sup>M-cysteine hydrochloride. P<sub>02</sub>, (A) 400, (B) 0 mm.

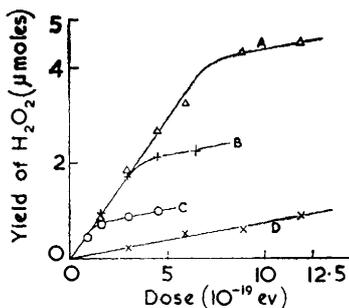


FIG. 5. Yield-dose plot for H<sub>2</sub>O<sub>2</sub> in 4.4 × 10<sup>-3</sup>M-cysteine hydrochloride at P<sub>02</sub>. (A) 400, (B) 200, (C) 100, (D) 0 mm.

### DISCUSSION

The values of  $G_H$ ,  $G_{OH}$ ,  $G_{H_2}$ , and  $G_{H_2O_2}$ , which are involved in the following discussion, are to some extent pH-dependent. In 0.8N-sulphuric acid the values are 3.68, 2.96, 0.45, and 0.81 respectively,<sup>17</sup> and  $G_H$  and  $G_{H_2O_2}$  decrease slowly as the pH is increased from 1

<sup>17</sup> Mahlman and Boyle, *J. Amer. Chem. Soc.*, 1958, **80**, 773.

to 4.<sup>1</sup> For the present purposes the different species allied to  $\text{H}\cdot$  are ignored, and reference is made to the hydroperoxy-radical although at a pH of 4 it probably exists mainly in its basic form  $\text{O}_2^-$ . The role of chloride ions in the radiolysis of cysteine hydrochloride solutions is ignored as it does not effect the overall course of the reaction.

The radiolysis of oxygen-free aqueous hydrogen sulphide can be explained by the scavenging of both radical species by the solute:



For complete scavenging the following equations hold:

$$G(\text{S})^{\text{vac}} = \frac{1}{2}G_{\text{OH}} + \frac{1}{2}G_{\text{H}}$$

$$G(\text{H}_2)^{\text{vac}} = G_{\text{H}_2} + G_{\text{H}}$$

$$G(\text{H}_2\text{O}_2)^{\text{vac}} = G_{\text{H}_2\text{O}_2}$$

It is well established for  $^{60}\text{Co}$   $\gamma$ -radiation that

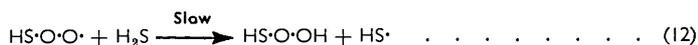
$$2G_{\text{H}_2} + G_{\text{H}} = 2G_{\text{H}_2\text{O}_2} + G_{\text{OH}}$$

and hence it follows that

$$G(\text{H}_2)^{\text{vac}} = G(\text{S})^{\text{vac}} + G(\text{H}_2\text{O}_2)^{\text{vac}}$$

Since this relation holds for run 2, and the individual yields agree with those predicted from accepted values of  $G_{\text{H}}$ ,  $G_{\text{OH}}$ ,  $G_{\text{H}_2}$ , and  $G_{\text{H}_2\text{O}_2}$ , it is probable that  $1.7 \times 10^{-2}\text{M}$ -hydrogen sulphide is a complete scavenger and that reactions (8–10) describe the course of the reaction. Acid apparently catalyses the reaction between hydrogen sulphide and hydrogen peroxide.

The great increase in yield of sulphur formed in the presence of oxygen can only be interpreted in terms of a short-chain mechanism. The fact that increase in temperature and in concentration of hydrogen sulphide both increase  $G(\text{S})^{\text{O}_2}$ , and hence the chain length, indicates that hydrogen sulphide molecules participate in a propagation step with a finite energy of activation. A possible mechanism would be a fast radical-combination reaction between oxygen and the  $\text{HS}\cdot$  radical, followed by the slower abstraction of hydrogen from hydrogen sulphide by the peroxy-radical:



The bond dissociation energies for  $\text{HS}-\text{H}$ ,  $\text{HO}-\text{H}$ , and  $\text{HO}\cdot\text{O}-\text{H}$  are 92.2, 116, and 89.5 kcal. mole<sup>-1</sup>, respectively, indicating that reaction (12) is likely to have a small enthalpy.

The dose-rate effect is that expected for a short-chain reaction.

The zero yield of hydrogen peroxide and the formation of sulphate in oxygenated systems show that the intermediate species, radical and molecular, react rapidly with one another and with hydrogen peroxide to form sulphate. The oxidation of intermediates to sulphate probably involves competition with their reduction to sulphur by hydrogen sulphide. The reason for the decrease in yield of sulphur at higher concentrations of oxygen may be that oxygen oxidises intermediate radical species, competing successfully against hydrogen sulphide. The size of the experimental error in the measurement of the small quantities of sulphate produced made it impossible to look for an increase in

$G(\text{SO}_4^{2-})$  under these conditions. No other sulphur-oxygen anions could be detected amongst the products, the dithionate ion being the only one expected to be stable in the presence of an excess of hydrogen sulphide.

The postulated chain mechanism may also be that by which neutral and alkaline solutions of hydrogen sulphide are autoxidised. Yellow polysulphides are formed, and on acidification they decompose to sulphur and hydrogen sulphide. Preliminary work by Ellis and others on dilute hydrogen sulphide solutions showed that loss of the  $\text{HS}^-$  ion did not follow any simple law with respect to oxygen concentration,<sup>18</sup> suggesting a chain-radical reaction. The initial formation of radicals may involve the electron-transfer:



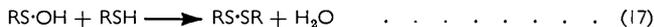
This would be in line with the effect of pH on the stability of solutions of hydrogen sulphide.

A mechanism involving analogous sulphenylperoxy-intermediates could also operate in radiolytic thiol oxidation:



The yields of hydrogen peroxide on radiolysis of  $4.4 \times 10^{-3}\text{M}$ -cysteine hydrochloride are compatible with reactions (14) and (15) and at the same time prove Swallow's mechanism to be untenable.  $G(-\text{RSH})^{\text{vac}}$  and  $G(\text{H}_2\text{O}_2)^{\text{vac}}$  show that both  $\text{H}^\cdot$  and  $\cdot\text{OH}$  are scavenged, the sulphenyl radicals presumably combining to form the disulphide. The value of  $G(\text{H}_2\text{O}_2)^{\text{O}_2}$  is approximately  $G_{\text{H}} + G_{\text{H}_2\text{O}_2}$ , showing reaction (6) to occur in the oxygenated system and also showing that hydrogen peroxide and cysteine do not react.  $G(-\text{RSH})^{\text{O}_2} > G_{\text{H}} + G_{\text{OH}}$  and therefore oxygen must play a part in the oxidation through some species other than the hydroperoxy-radical. On Swallow's mechanism all the oxygen consumed would appear as hydrogen peroxide.

The intermediate hydroperoxide is probably reduced by cysteine to the disulphide in two stages:



Again it is suggested that a comparable mechanism operates in the autoxidation of thiols.

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<sup>18</sup> A. J. Ellis, personal communication.