stirred at room temperature for 4 hours, the yellow solid collected and washed several times with water; wt. 2.3 g. (85%), m.p. 185–186° dec. A single recrystallization from 95% ethanol provided an analytical sample, m.p. 187–189° dec. (lit.[16] 185–186° dec.), λ_{max} 4.54 and 4.66 μ.

Anal. Calcd. for $C_7H_6N_6O_4S$: C, 31.1; H, 2.2; N, 31.1. Found: C, 31.2; H, 2.5; N, 31.3.

Conversion of Arenesulfonylguanyl Azides (IV) to V.—(a) A suspension of 1.6 g. (5.6 mmoles) of IVa in 40 ml. of 10% sodium carbonate was heated on a water-bath until a clear solution had been obtained. The cooled reaction mixture was acidified with hydrochloric acid and the product collected; wt. 1.5 g. (93%), m.p. 207–212° dec.[10] (lit.[8] 207° dec.), λ_{max} 9.54 μ.

The same transformation, effected at room temperature in 0.2 *N* sodium hydroxide, gave Va in 75% yield. The infrared spectrum of Va as obtained by either of these procedures were indistinguishable from the corresponding spectra of samples of Va derived from X, XI or from the reaction of I and IIa in the presence of excess sodium carbonate.

(b) **5-(*p*-Nitrobenzenesulfonamido)-tetrazole (Vb).**—A suspension of 2.0 g. (7.7 mmoles) of the guanyl azide (IVb) 150 ml. of 1% sodium hydroxide was stirred at room temperature for *ca.* 5 minutes. The mixture was filtered, the in filtrate acidified with hydrochloric acid and the product collected; wt. 1.0 g. (50%), m.p. 203–204° dec. A single recrystallization from aqueous ethanol provided an analytical sample, m.p. 208–209° dec., λ_{max} 9.48 μ.

Anal. Calcd. for $C_7H_6N_6O_4S$: C, 31.1; H, 2.2; N, 31.1. Found: C, 31.1; H, 2.3; N, 31.2.

A sample (200 mg.) of Vb in 10 ml. of methanol containing 50 mg. of platinum oxide was shaken with hydrogen until the theoretical uptake was realized (20 minutes). The catalyst was removed and the filtrate evaporated to dryness and the residue crystallized from water; wt. 156 mg. (88%), m.p. 202–203° dec., λ_{max} 9.58 μ. An infrared spectrum of this product proved to be identical with VI.

Alternative Synthesis of Va.—To a solution of 2.06 g. (0.02 mole) of 5-aminotetrazole hydrate in 35 ml. of 10% sodium carbonate was added, portionwise with stirring, 4.9 g. (0.021 mole) of IIa. Sodium carbonate (3.5 g.) was added, portionwise, to the reaction mixture and the stirring was continued for 18 hours. The solution was then filtered, the clear solution acidified with hydrochloric acid and the solid collected; wt. 5.0 g. (88%), m.p. 202–207° dec. The crude product was dissolved in a saturated solution of sodium bicarbonate, treated with Norit, and the solid reprecipitated with hydrochloric acid; wt. 4.0 g., m.p. 207–210° dec. A single recrystallization from aqueous ethanol provided an analytical sample, m.p. 217–219° dec. (lit.[8] 207° dec.), λ_{max} 9.55 μ.

Anal. Calcd. for $C_9H_{10}N_6O_3S \cdot H_2O$: C, 36.0; H, 4.0; N, 28.0. Found: C, 36.2; H, 4.2; N, 27.5.

Reduction of IV. (a) *p*-Acetamidobenzenesulfonylguanidine (VIIa).—A solution of 2.0 g. (7 mmoles) of IVa in 150 ml. of methanol containing 0.3 g. of platinum oxide was shaken under a pressure of 3 atm. of hydrogen for two hours. The filtered solution was concentrated to a small volume and the product collected; wt. 1.4 g. (77%), m.p. 265–266° (lit.[28] 266°).

(b) **Sulfaguanidine (VIIb).**—A suspension of 0.6 g. of IVb (2.2 mmoles) in 125 ml. of methanol containing 0.2 g. of platinum oxide was shaken under 3 atm. of hydrogen for 2 hours. The filtered solution was concentrated to *ca.* 0.1 of the original volume and the product collected; 0.4 g. (84%), m.p. 180–183°. Three recrystallizations from water provided a colorless solid, m.p. 186–188° (lit.[16] 189–190°).

A sample (0.18 g.) of VIIb was treated with 0.1 ml. of acetic anhydride and 4 ml. of pyridine at 60° for 5 minutes and then allowed to stand at room temperature for 16 hours. The product that was deposited was collected and dried; wt. 0.15 g. (71%), m.p. 266–267° alone or when admixed with VIIa.

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[CONTRIBUTION FROM THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA]

Products of γ-Irradiation of Cysteine and Cystine[1]

BY PERICLES MARKAKIS[2] AND A. L. TAPPEL

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Cysteine and cystine in aqueous acidic solutions were exposed, in the absence and presence of oxygen, to γ-irradiation, at doses varying from 10^4 to 8×10^7 rad. Cystine, hydrogen sulfide, free sulfur, sulfate ion, ammonia and alanine were identified and determined among the irradiation products of cysteine; more than 80% of the sulfur and more than 90% of the nitrogen of cysteine could be accounted for. Qualitatively, the irradiated cystine solutions did not differ essentially from the irradiated cysteine solution; however, more free sulfur, sulfate ion and ammonia, less hydrogen sulfide, and very little cysteine were produced from cystine. Mechanisms for the radiolysis of these amino acids are discussed.

The sulfhydryl and disulfide groups which are present in amino acids, peptides, proteins and enzymes are known to play important biochemical and physiological roles.[3] The effect of ionizing radiations upon these groups is of primary concern in radiation biochemistry, and has been reviewed by Barron.[4] Malodorous sulfur compounds are produced from irradiated proteins, and have been associated with the undesirable flavors which develop during the radiation preservation of food, especially meat and fish.[5–7]

Cysteine and cystine have been the object of radiochemical investigations mainly because information from such studies will help in understanding the radiation chemistry of more complex sulfhydryl and disulfide compounds. In addition, cysteine is interesting because its injection into an animal provides some protection from lethal radiations.[8,9] Cysteine has also been used as a prototype in the search for more efficient protectors.[10]

Dale and Davies irradiated cysteine solutions with X-ray doses of up to 100,000 r., and determined the amount of H_2S liberated.[11] Swallow measured

(1) Supported in part by the U. S. Fish and Wildlife Service.

(2) Food Science Laboratory, Michigan State University, E. Lansing, Mich.

(3) E. S. G. Barron, *Advances in Enzymol.*, **11**, 201 (1951).

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(6) E. P. Marbach and D. M. Doty, *ibid.*, **4**, 881 (1956).

(7) R. A. Sliwinski and D. M. Doty, *ibid.*, **6**, 41 (1958).

(8) H. M. Pratt, E. B. Tyree, R. L. Straube and D. E. Smith, *Science*, **110**, 213 (1949).

(9) D. E. Smith, *Rad. Research*, **10**, 335 (1959).

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TABLE I

ANALYSIS OF γ-IRRADIATED SOLUTIONS OF 0.1 *M* CYSTEINE HYDROCHLORIDE

Dose, rad	Cysteine, $M \times 10^3$	Cystine, $M \times 10^3$	H_2S, $M \times 10^3$	S, $M \times 10^{3a}$	SO_4^{--}, $M \times 10^3$	Ammonia, $M \times 10^3$	Alanine, $M \times 10^3$	pH	Sulfur balance, %	Nitrogen balance, %	Carbon balance, %
					Oxygen-free solutions						
0	94.6	2.7	0.00	0.0	0.0	0.0	0.0	1.55	100.0	100.0	100.0
10^4	94.0	2.9	.03	.0	.0	.0	.0	1.55	99.8	99.8	99.8
10^5	91.7	3.7	.17	.0	.0	.0	.0	1.55	99.3	99.1	99.1
10^6	86.5	4.6	3.03	.0	.0	.5	3.0	1.55	98.7	99.2	98.7
10^7	31.0	26.6	5.88	2.2	.4	5.4	6.8	1.50	92.7	96.4	91.0
2×10^7	13.9	28.5	3.68	12.4	1.1	11.0	16.5	1.45	88.1	98.4	87.4
4×10^7	5.3	21.8	1.53	24.7	4.5	21.1	22.5	1.40	79.6	92.5	71.4
8×10^7	5.6	13.0	2.19	38.4	7.4	23.5	...	...	79.6	...	...
					Oxygen-saturated solutions						
0	78.6	11.4	0.02	0.0	0.0	0.0	0.0	1.55	100.0	100.0	100.0
10^4	59.4	21.0	.04	.0	.0	.0	.0	1.55	100.0	100.0	100.0
10^5	58.2	19.7	.16	.0	.1	.2	.2	1.55	96.5	96.6	96.4
10^6	65.3	13.5	2.62	1.0	2.4	2.0	3.9	1.50	96.9	96.8	94.8
10^7	24.2	26.5	4.41	3.7	7.8	8.0	7.9	1.45	91.8	91.8	83.9
2×10^7	5.0	29.0	3.21	7.2	9.4	15.8	13.9	1.40	81.6	91.4	75.8
4×10^7	3.3	16.4	1.40	15.2	23.6	31.3	24.2	1.35	75.3	90.3	59.5
8×10^7	2.4	11.6	1.80	30.2	32.7	47.4	19.3	1.20	89.0	91.0	44.3

[a] Calculated as $M = 32$.

the disappearance of cysteine in solution upon exposure to X-ray doses up to 250,000 r.; in addition he irradiated aqueous cystine in a hydrogen atmosphere and measured the cysteine formed.[12] Whitcher, Rotheram and Todd exposed solutions of cysteine to X-rays of up to 20,000 r. and quantitatively determined the disappearance of the thiol group and the formation of H_2O_2, H_2S and cystine[13]; the same authors irradiated solutions of cystine and measured its destruction.[14] Littman, Carr and Brady studied the effect of atomic hydrogen on cysteine in solution and quantitatively determined the H_2S and cystine formed.[15] Scott and Livermore reported the formation of alanine upon cysteine irradiation.[16]

This paper reports a quantitative study of the products resulting from γ-irradiation of aqueous solutions of cysteine and cystine over a wide dose range.

Experimental

Materials and Sample Preparation.—Concentrations of cysteine and cystine were chosen on the basis of solubility and to facilitate comparison with previous studies. Acid solutions were used to minimize autoxidative reactions of the reactants and products during shipment and storage.

Cysteine hydrochloride monohydrate (Fisher Co., reagent grade) was dissolved in triple-distilled water at the concentration of 0.1 *M*. The *p*H of the solution was 1.55. Triple-distilled water was prepared from water distilled first in a commercial tinned still, secondly in an all-glass apparatus from alkaline permanganate solution, and thirdly from dilute sulfuric acid.

Cystine (Nutritional Biochemicals Corp.) was dissolved in triple-distilled water at the concentration of 0.01 *M*; hydrochloric acid was used to dissolve the cystine, and the *p*H of the solution was 1.10.

The solutions were transferred to Pyrex glass tubes (20 × 150 mm.), holding 5 or 10 ml. each. Also 7 × 120 mm. tubes, containing 1 or 2 ml. of solution, were used in cases in which small quantities of sample were more convenient for analysis. After filling, the tubes were drawn to a capillary constriction about 4 cm. above the liquid surface, and the air in the tube was replaced by pure nitrogen or oxygen. For this replacement, the tubes were placed in a gas exchange chamber and subjected to gas evacuation (5 mm.) and refilling with the proper gas five times, after which the capillary was closed by fusion. The tubes were sealed in no. 2 metal cans and transported to and from the irradiation site by Air Express, at ambient temperature.

Irradiation.—The samples were irradiated in the cans by exposure to 0.6 to 2 Mev. γ-radiation from spent radioactive fuel rods at the Materials Testing Reactor, Idaho Falls, Idaho. Doses of 10^4, 10^5, 10^6, 10^7, 2×10^7, 4×10^7 and 8×10^7 rad were applied, at dose rates varying from 3×10^6 to 7×10^6 rad per hour. The measurement of doses and dose rates were performed by the operators of the Materials Testing Reactor.

Non-irradiated control samples also were shipped to and from the reactor. Because of its great oxygen lability cysteine was partially oxidized to cystine during laboratory and reactor handling and transportation as is shown in analysis of the control samples in Table I. All samples were stored at −20° after irradiation and prior to analysis.

Methods of Analysis.—For identification of the degradation products a variety of tests was used. Descending paper chromatography was employed to verify cysteine and cystine and to identify alanine. Whatman No. 1 paper, two different solvent mixtures, *i.e.*, 72% phenol (Mallinckrodt Co., special), and 1-butanol:acetic acid:water 40:10:25 v./v., and a spray of 0.5% ninhydrin in absolute ethanol were used for chromatography. Cysteine was coupled with N-ethylmaleimide before chromatography. For this purpose, a proper aliquot of the sample was dried under a stream of nitrogen, and the residue was redissolved in 0.13 *M* solution of the coupling agent. Sulfur was identified by its solubility and melting point (120°). Soluble sulfate was detected by means of the permanganate test.[17] The identity of hydrogen sulfide was established by its odor, the lead acetate paper test, and the methylene blue reaction. Ammonia was detected by the Nessler test.

Quantitatively, cysteine and cystine were determined by the method of Kolb and Toennies[18] adapted to an Evelyn colorimeter. Hydrogen sulfide was measured by the method of Marbach and Doty[6] modified as follows. The

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TABLE II

ANALYSIS OF GAMMA-IRRADIATED SOLUTIONS OF 0.01 *M* CYSTINE HYDROCHLORIDE

Dose, rad	Cystine, $M \times 10^4$	Cysteine, $M \times 10^4$	H_2S, $M \times 10^4$	S, $M \times 10^{4a}$	$SO_4^=$, $M \times 10^4$	Ammonia, $M \times 10^4$	Alanine, $M \times 10^4$	Sulfur balance, %	Nitrogen balance, %	Carbon balance, %
				Oxygen-free solutions						
0	100	0	0.0	0	0	0	0	100	100	100
10^4	96	0	.2	0	0	2	0	96	97	96
10^5	86	4	.5	0	0	4	0	88	90	88
10^6	74	11	1.0	20	2	21	3	91	92	81
10^7	38	6	0.9	99	5	84	22	93	94	52
2×10^7	8	0	.4	140	32	142	26	94	92	21
4×10^7	0	0	.2	138	50	168	14	94	91	7
8×10^7	0	0	.4	140	56	192	1	98	97	1
				Oxygen-saturated solutions						
0	100	0	0.0	0	0	0	0	100	100	100
10^4	95	0	.2	0	0	2	0	95	96	95
10^5	88	2	.3	0	0	9	0	89	94	89
10^6	70	8	1.2	14	6	28	6	85	91	77
10^7	38	6	1.8	82	8	82	18	87	91	50
2×10^7	9	0	0.6	120	44	144	22	91	92	20
4×10^7	0	0	.2	115	66	174	8	91	91	4
8×10^7	0	0	.2	100	85	188	1	93	95	1

[a] Calculated as $M = 32$.

glass ampoule containing the sample was opened by breaking its tip within a sleeve of Tygon tubing clamped at both ends and containing 0.3 *N* ammonium hydroxide in excess of the acids of the sample; thorough mixing of the liquids followed before the sleeve was unclamped. With the large diameter ampoules, it was found practical to make one end of the Tygon sleeve gas tight to the glass ampoule, while the other end was clamped before breaking the tip. A suitable aliquot of the mixture was transferred to the distillation flask and the apparatus assembled. For the release of the H_2S, 1 *N* HCl, in excess of the alkali in the flask, was added through the three-way stop-cock of the apparatus. The H_2S was led into a 12 × 150 mm. tube containing a trapping liquid composed of 2.5 ml. of 0.1 *N* $Cd(OH)_2$ and 0.5 ml. of 0.1 *N* NaOH. After 30 min. of bubbling alkaline pyrogallol-washed nitrogen through the sample in the flask, which was kept at 65°, the color reagents were added to the trap by means of a side arm. Half of the volume of the color reagents described in the original method was used and the volume of the developed methylene blue solution was made up to 12.5 ml. A Beckman DU spectrophotometer and 1-cm. cuvettes were employed in measuring the color. A straight reference line could be obtained for the range of 1.5 to 13.0 micrograms of H_2S per 12.5 ml. of colored solution. In this range, the optical density corresponding to H_2S liberated from Na_2S and measured by this technique was 95% of that corresponding to equivalent amount of Na_2S added directly to the color reagents.

Sulfur was measured by direct weighing on a semi-micro balance, after first washing the precipitate from irradiated cysteine or cystine with 1 *N* HCl, and water, then drying, dissolving the sulfur in a carbon disulfide–pyridine mixture (50:50 v./v.), evaporating off the solvent in an air draft, and drying *in vacuo* at 68°. Soluble sulfate was determined gravimetrically as barium sulfate, after first filtering the irradiated samples, bubbling N_2 through them, then adding 10% $BaCl_2$ solution, and heating the precipitate to 500°. Ammonia was measured by micro-Kjeldahl distillation, and titration, using 0.5 g. of MgO per sample to release the ammonia. Alanine was determined by the paper chromatographic method of Porter, *et al.*[19] The cysteine was coupled with N-ethylmaleimide before chromatographing. A standard curve for alanine was prepared with each chromatogram. The *p*H was measured by means of a Beckman model G *p*H meter.

Results and Discussion

The compounds which could be identified in the irradiated solutions of cysteine and cystine are listed in Tables I and II. In the same tables are presented the concentrations of these products of irradiation. Zero values indicate that the concentration of product, if present, was below the sensitivity limits of the methods employed. All values are averages of at least two determinations performed on two separate samples.

Cysteine in Oxygen-free Solutions.—From the data obtained, the over-all pattern of radiolysis of cysteine in oxygen-free solution is shown to involve oxidation to cysteine, detachment of the –SH group with concomitant formation of H_2S and alanine, and deamination. Although decarboxylation was not followed quantitatively in this research, a large amount of CO_2 was found at high radiation doses using a $Ba(OH)_2$ trap. Therefore, decarboxylation is also important in the over-all pattern of cysteine radiolysis.

The high percentages of the total sulfur and nitrogen accounted for by chemical analysis indicates that no major degradation products containing sulfur or nitrogen have been missed. However, by paper chromatography it was found that two more ninhydrin-positive products are formed at the higher radiation doses. These products were found not to be cysteine sulfinic acid, cystine disulfoxide, cysteic acid or taurine. A second fraction containing unidentified degradation products is the yellow-brown precipitate left after dissolving away the precipitated cystine and sulfur of the solutions receiving doses higher than 10^7 rad A third group of unidentified minor products is in the gases of heavily irradiated solutions, the cooked cabbage-like odor of which suggests the presence of products in addition to hydrogen sulfide.

The production of H_2S and alanine in similar quantities at 10^5 to 10^7 rad indicates several possible reactions involving H· from the radiolysis of water (eq. 1–5).

Of the two reactions involving RSH and H·, (1) should occur with a higher frequency than (4). These reactions are in accord with the studies of

(19) C. A. Porter, D. Margolis and P. Sharp, *Contrib. Boyce Thompson Inst.*, **18**, 465 (1957).

$$RSH + H\cdot \longrightarrow R\cdot + H_2S \quad (1)$$
$$R\cdot + H\cdot \longrightarrow RH \quad (2)$$
$$R\cdot + RSH \longrightarrow RH + RS\cdot \quad (3)$$
$$RSH + H\cdot \longrightarrow RH + \cdot SH \quad (4)$$
$$\cdot SH + H\cdot \longrightarrow H_2S \quad (5)$$

$R = -CH_2CHNH_2COOH$

Littman, *et al.*,[15] on H_2S production from the reaction of atomic hydrogen and cysteine.

The major primary reaction is the oxidation of cysteine to cystine. Probable reactions involve the major products from the radiolysis of water, H· and ·OH, and also some of the minor products, ·OOH and H_2O_2.

$$RSH + \cdot OH \longrightarrow RS\cdot + H_2O \quad (6)$$
$$RSH + H\cdot \longrightarrow RS\cdot + H_2 \quad (7)$$
$$RSH + \cdot OOH \longrightarrow RS\cdot + H_2O_2 \quad (8)$$
$$2RSH + H_2O_2 \longrightarrow 2RS\cdot + 2H_2O \quad (9)$$
$$RS\cdot + RS\cdot \longrightarrow RSSR \quad (10)$$

At the dose of 10^6 rad the ionic yields of 1.2 (the *G*-value calculated as molecules reacting per 100 e.v. of energy absorbed = 3.7) for cystine production and 0.95 (*G* 2.9) for alanine and H_2S production indicate non-chain reactions; at lower doses, however, higher ionic yields, suggesting short chain reactions, are found.

As the radiation dose increases, secondary products of radiolysis begin to accumulate. Some of the free sulfur originates from H_2S probably by a mechanism similar to that for the photochemical dissociation of H_2S.[20]

$$H_2S + \gamma\text{-radiation} \longrightarrow H\cdot + HS\cdot \quad (11)$$
$$H\cdot + H_2S \longrightarrow H_2 + HS\cdot \quad (12)$$
$$HS\cdot + HS\cdot \longrightarrow H_2S + S \quad (13)$$

The atomic sulfur apparently forms S_8 readily because this product precipitates out. Formation of free sulfur by irradiation of H_2S was first described by Loiseleur.[21] In a confirming experiment, we irradiated 6×10^{-3} *M* H_2S at 10^7 rad and recovered 60% of the sulfur as S_8 and 5% as $SO_4^=$. Free sulfur may also originate, again as a secondary product, from the primarily produced cystine. The irradiation of cystine, which will be discussed later, readily produces free sulfur.

Sulfate production becomes appreciable when cysteine is irradiated in the range 10^7 to 8×10^7 rad. The most likely pathway for sulfate production is the progressive oxidation of primarily formed cystine by ·OH, ·OOH and H_2O_2. Among the probable intermediates are sulfinic and sulfonic acids, as Shapiro and Eldjarn[22] found in their study of the mechanism for the degradation of cystamine by ionizing radiation. In our study only the more stable products accumulated, and no oxidation intermediates of cystine were found.

Possible precursors of NH_3 are cysteine and the primary products cystine and alanine. Dale and Davies found only very small amounts of NH_3 when they irradiated cysteine to 10^5 rad with X-rays. We get small amounts of NH_3 in the dose range of 10^6 rad. Sharpless, *et al.*,[23] showed that ammonia is produced from 1 *M* alanine solutions receiving doses as low as 10^5 rad. Some of the NH_3 produced in our study may have come from the primarily produced alanine. Also, some of the NH_3 must have come from the primarily produced cystine; this will be more clearly shown when cystine radiation is discussed. This primarily produced cystine appears to be degraded by three main pathways: deamination, loss of sulfur with the formation of free sulfur, and disulfide oxidation to sulfate. It is not apparent from our data whether NH_3 is produced by direct deamination of cysteine at doses of 10^6 rad and above. Although cysteine is unique among the amino acids in its resistance to deamination, presumably because its -SH competes successfully for free radicals, there is no theoretical reason why the large amount of radiation used here would not bring some direct deamination.

Cysteine in Oxygen-saturated Solutions.—The radiation degradation of cysteine in oxygen is qualitatively similar to anaerobic radiation, but quantitatively there are more sulfur products of higher oxidation state. More cystine and $SO_4^=$ and less H_2S and S_8 are produced. The larger amounts of NH_3 formed in oxygen-saturated solutions may come from the larger amounts of primarily formed cystine and may also indicate the importance of mechanisms of oxidative deamination. Oxidative deamination would be one mechanism for the formation of carbonyl groups, which are known to be formed by irradiation of amino acids.[24] Carbonyl compounds were readily detected in irradiated cysteine by the 2,4-dinitrophenylhydrazine reaction but were not quantitatively measured here. Conversion of cysteine to cystine shows an irregular function of radiation dose at 10^6 rad for which no adequate explanation has been found. Decrease in *p*H both in oxygen-saturated and anaerobic solutions is an index for the formation of more acidic products, $SO_4^=$, H_2S and CO_2 than basic products like NH_3.

Cystine in Oxygen-free and Oxygen-saturated Solutions.—From the products identified in irradiated cystine and cysteine solutions, no significant qualitative differences are apparent. However, there are important quantitative differences as shown in Table II. Whereas oxidation of cysteine to cystine proceeds very readily the amount of reduction of cystine to cysteine is very small. Because the reduction of cystine to cysteine by radiation is a controversial problem,[25] in this research paper chromatography and the iodine–azide test were employed in addition to the quantitative phosphotungstic reaction to confirm the presence of cysteine. Possible reactions for cysteine production include

$$RSSR + \gamma\text{-radiation} \longrightarrow 2RS\cdot \quad (14)$$
$$RS\cdot + H\cdot \longrightarrow RSH \quad (15)$$
$$RSSR + H\cdot \longrightarrow RSH + RS\cdot \quad (16)$$

Since cysteine does not accumulate in large amounts there must be many competing primary and sec-

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(21) J. Loiseleur, *Compt. rend.*, **215**, 536 (1942).
(22) B. Shapiro and L. Eldjarn, *Rad. Research*, **3**, 393 (1955).
(23) N. E. Sharpless, A. E. Blair and C. R. Maxwell, *ibid.*, **3**, 417 (1955).
(24) L. Duran and A. L. Tappel, *ibid.*, **9**, 498 (1958).
(25) E. Collinson and A. J. Swallow, *Chem. Revs.*, **56**, 471 (1956).

ondary reactions. Competing primary reactions would include radiation breakdown of cystine to release free sulfur, oxidation of cystine to sulfate, and deamination of cystine. Secondary reactions of the primarily formed cysteine would include formation of H_2S and alanine and oxidation back to cystine.

Hydrogen sulfide formation from cystine is much smaller than from cysteine; one obvious reason is the lack of the –SH group. On the other hand, free sulfur is more readily liberated from cystine. As previously mentioned, cystine is more susceptible to radiation deamination than cysteine. The fact that NH_3 appears among the irradiation products of cystine at lower doses than alanine does shows that alanine is not a compulsory intermediate for NH_3 formation.

The amounts of cystine which have reacted and the total amounts of sulfur in the form of S and $SO_4^=$ are very similar for the same radiation doses, either in oxygen-free or oxygen-saturated solution. The large amount of $SO_4^=$ from cystine radiation, especially that from the oxygen-saturated solution, is a good indication that the $SO_4^=$ comes from the progressive oxidation of the sulfur from −1 oxidation state in cystine through a number of intermediates to the +6 oxidation state in $SO_4^=$. No significant *p*H changes were found in the solutions of irradiated cystine.

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[CONTRIBUTION FROM THE EXPLOSIVES AND PROPELLANTS LABORATORY, PICATINNY ARSENAL]

Carbon-14 Tracer Studies of the Nitrolysis of Hexamethylenetetramine

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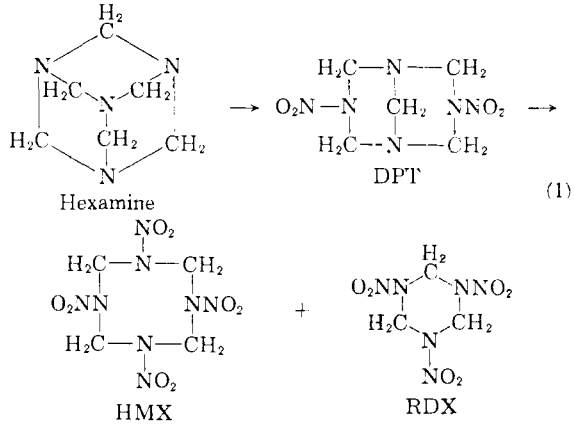
The path of carbon atoms in the nitrolysis of hexamethylenetetramine (hexamine) to a mixture of the homologous cyclic methylenenitramines,1,3,5,7-tetranitro-1,3,5,7-tetraazacycloöctane (HMX) and 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), was traced using carbon-14. Under conditions employed in this research both cyclic methylenenitramines are derived from the complete non-selective degradation of the hexamine molecule to fragments containing chemically equivalent methylene groups. When paraformaldehyde $[(CH_2O)_x]$ is included in the nitrolysis medium, its methylene groups can enter into a common pool with those from hexamine for the formation of final products. 1,5-Dinitroendomethylene-1,3,5,7-tetraazacycloöctane (DPT) is a partial nitrolysis product which can be isolated by quenching the reaction mixture at an intermediate stage. This compound, upon further nitrolysis, also degrades completely and non-selectively to species containing chemically equivalent methylene groups with subsequent recrystallization to the final insoluble cyclic methylenenitramines.

Introduction

Bachmann and Sheehan at the University of Michigan[1] developed a new method of preparing RDX containing small quantities of HMX (RDX/HMX). The method involves the nitrolysis of hexamine with ammonium nitrate–nitric acid solution and acetic anhydride. By varying parameters of temperature and acid strength, together with quantities of ammonium nitrate and acetic anhydride, it was shown[2] that the ratios of RDX to HMX could be altered. These results led Bachmann and co-workers[3] to prepare mixtures rich in HMX (HMX/RDX). The optimum yields obtained represented 82% conversion of hexamine to HMX/RDX containing 73% HMX.

The formation of RDX and/or HMX molecules from the nitrolysis of hexamine has been postulated to take place *via* two separate paths. One involves the selective cleavage of the hexamine molecule[4] and the other, the combination of methylene and amino type fragments.[5] However, direct evidence in support of either of these possible paths of nitrolysis to HMX/RDX has been lacking. Regardless of the over-all mechanism involved, the formation of DPT has been postulated[2] as a possible precursor to the final products, equation 1.



(1)

The nitrolysis of hexamine to HMX/RDX has been studied in these laboratories and using essentially the same conditions as reported by Bachmann,[3] similar yields of HMX/RDX were obtained. It was observed that by including a small quantity of paraformaldehyde in the reaction mixture, there resulted a higher yield of mixed products to the extent of approximately 10%. This observation suggested that paraformaldehyde or its methylene groups in some manner can take part in this reaction to form cyclic methylenenitramine molecules. At the same time it also sug-

(1) W. E. Bachmann and J. C. Sheehan, THIS JOURNAL, **71**, 1842 (1949).

(2) (a) W. E. Bachmann, *et al.*, unpublished work at the University of Michigan reported in Office of Scientific Research and Development (OSRD) Report No. 5186; (b) S. Epstein and C. A. Winkler, *Can. J. Chem.*, **30**, 734 (1952).

(3) W. E. Bachmann, *et al.*, unpublished work at the University of Michigan as reported in OSRD Report No. 1981.

(4) G. F. Wright, *et al.*, *Can. J. Chem.*, **27**, 520 (1949).

(5) C. A. Winkler, *et al.*, *ibid.*, **29**, 725 (1951).