mole) of 2-methyl-2-thiazoline in 100 ml of chlorobenzene was refluxed for 4 hr. The solvent was then removed under reduced pressure, leaving a solid residue. After one recrystallization from acetone, 39.8 g (53%) of product was obtained, mp 122.5–127°. Two additional recrystallizations from acetone gave an analytical sample, mp 126.5–128.5°. See Table I for data on other thiazolinium salts.

Anal. Calcd for $C_{19}H_{33}NO_3S_2$: C, 60.45; H, 6.14; N, 3.71; S, 16.99. Found: C, 60.47; H, 6.16; N, 3.72; S, 16.76.

N-*n*-Decyl-N-(2-mercaptoethyl)acetamide.—Nitrogen was bubbled slowly through a solution of 24.0 g (0.6 mole) of NaOH in 250 ml of water for 10–15 min. All the following steps were carried out under nitrogen, whenever possible. With the solution at 50° and while stirring (magnetically), 2-methyl-3-*n*-decylthiazolinium tosylate (62.4 g, 0.2 mole) was added. The solution was allowed to stir for 1 hr, then chilled and made strongly acid with concentrated HCl. An oil separated which was extracted into three 50-ml portions of $CHCl_3$. The extracts were combined and washed with 10% Na_2CO_3 solution and water. The chloroform extracts were dried ($MgSO_4$); concentration under reduced pressure gave 10.1 g (39%) of product. A distillation *in vacuo* gave an analytical sample, bp 145–150° (0.04 mm), $n^{25}D$ 1.4815. See Table II for data on other purified amides.

Anal. Calcd for $C_{14}H_{29}NOS$: C, 64.81; H, 11.27; N, 5.40; S, 12.36. Found: C, 64.91; H, 11.25; N, 5.46; S, 12.15.

N-(2-Mercaptoethyl)-2-phenethylamine.—A solution of 16.6 g (0.074 mole) of crude N-(2-mercaptoethyl)phenethylacetamide (prepared as described above) in a mixture of 50 ml of concentrated HCl and 50 ml of glacial acetic acid was heated under reflux in a nitrogen atmosphere for 24 hr; then the solvent was evaporated under reduced pressure. The residue was taken up in 10% Na_2CO_3 solution with stirring and warming until the pH of the water layer was 8–9. An oil separated and, on cooling, was extracted into three 50-ml portions of ether. After drying the combined extracts ($MgSO_4$), the solvent was evaporated under reduced pressure. The remaining oil was distilled *in vacuo* and gave 8.1 g (60%) of product, bp 80–84° (0.2 mm). Another distillation *in vacuo* gave an analytical sample, bp 81–82° (0.2 mm), $n^{25}D$ 1.5512.

Anal. Calcd for $C_{10}H_{23}NS$: C, 66.25; H, 8.34; N, 7.73; S, 17.69. Found: C, 66.47; H, 8.34; N, 7.72; S, 17.87.

N-(2-Mercaptoethyl)(2-methoxyethyl)amine.—A solution of 52.8 g (0.16 mole) of 2-methyl-3-(2-methoxyethyl)thiazolinium tosylate in 150 ml of water was heated under reflux for 25 hr, with nitrogen bubbling through the solution. At this time, 150 ml of concentrated HCl was added, and the solution was heated under reflux for an additional 3 hr. The acid solution was evaporated *in vacuo*, and the residue was made alkaline with 180 ml of 10% Na_2CO_3. The solution was extracted into five 40-ml portions of chloroform, dried ($MgSO_4$), then concentrated *in vacuo*. The oily residue obtained in this way was distilled under reduced pressure. There was collected 3.7 g (15%) of amine, bp 70–72° (4.5 mm), $n^{25}D$ 1.4744.

Anal. Calcd for $C_5H_{13}NOS$: C, 44.41; H, 9.69; N, 10.36; S, 23.71. Found: C, 44.20; H, 9.69; N, 10.18; S, 23.90.

Radioprotective Activity of Triammonium 2-Dithiocarbamoyl-3-dithiocarbonylthiopropanoate

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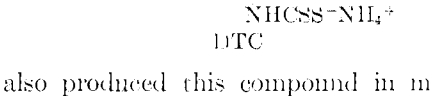
The synthesis of a water-soluble derivative of cysteine, the triammonium salt of the dithiocarbamate trithiocarbonate, is described. Toxicity studies show the compound to be two to three times less toxic than 2-aminoethylisothiouronium bromide hydrobromide (AET), and it provides good protection in mice against the lethal effect of ionizing radiation in the comparable order of activity as AET.

Trithiocarbonate zwitterions of 2-mercaptoethylamine, 2-mercaptoethylguanidine, and related compounds have shown good effectiveness as radiation protective agents.[1] These compounds show limited solubility in water and appreciable toxicity, so a compound having greater aqueous solubility and possibly lower toxicity was sought in the trithiocarbonate of cysteine. It was anticipated that both the mercapto and the amino function of cysteine would react with carbon disulfide to give either a dithiocarbamate trithiocarbonate or possibly a cyclization product. A dithiocarbamate of cystine has previously been prepared,[2] but no appreciable antiradiation properties have yet been reported for this compound.

Treatment of cysteine with carbon disulfide and ammonium hydroxide in the usual manner[1,2] gave only products of indefinite composition. Use of reaction conditions that avoided an excess of base did, however, provide the dithiocarbamate trithiocarbonate of cysteine as the triammonium salt, abbreviated hereafter as DTC. Use of ethanol as solvent with dry ammonia also produced this compound in much better yield. Infrared absorption at 1055 and 1105 cm^{-1} may be attributed to the C=S of the trithiocarbonate group,[3,4] and absorption at 995 cm^{-1} to the C=S of the dithiocarbamate group.[3] A strong absorption band at 1555 cm^{-1} may also be due to thioamide.[5]



DTC

The radiation protective ability and toxicity of DTC in mice have been determined and are reported herein. The comparative value of this compound with that of 2-aminoethylisothiouronium bromide hydrobromide (AET) is also presented.

Experimental Section

Analyses for carbon, hydrogen, and nitrogen were done by Weiler and Strauss, Oxford, England. Sulfur analysis was done

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by Parr bomb peroxide fusion, and ammonium ion was determined by a modified Kjeldahl procedure for ammonium salts. Melting points were taken on a Mel-Temp block and are corrected.

Triammonium 2-Dithiocarbamoyl-3-dithiocarbonylthiopropanoate. A.—Cysteine (6.06 g, 0.05 mole) was dissolved in 30 ml of water and 20 ml of concentrated NH_4OH. The solution was added dropwise with stirring to 7 ml (0.1 mole) of CS_2 at ice-bath temperature. The mixture was stirred for 1 hr, and 250 ml of absolute ethanol was added. After 30 min, a bright yellow precipitate appeared, which was isolated after further stirring and washed with chilled ethanol. After drying *in vacuo*, a yield of 8.4 g (49%) of finely divided yellow product was obtained which melted at 153–154°; ν_{max}^{Nujol} 730, 820, 870, 955, 1055, 1105, 1180, 1280, 1300, 1555, 1675 cm^{-1}.

Anal. Calcd for $C_5H_{16}N_4O_2S_5$: C, 18.50; H, 4.97; N, 17.32; S, 49.40; NH_4^+, 16.59. Found: C, 18.67; H, 4.94; N, 17.49; S, 50.15; NH_4^+, 16.68.

B.—Cysteine (6.06 g, 0.05 mole), suspended in a small volume of 95% ethanol, was added to a mixture of CS_2 (7 ml, 0.1 mole), 95% ethanol (75 ml), and absolute ethanol (75 ml) and cooled in an ice bath. Ammonia gas was bubbled through the solution with constant stirring for 1 hr. The yellow product was isolated and purified as above. A yield of 15.4 g (95%) was obtained.

The compound is extremely water-soluble and showed evidence of some hydrolysis in water after several minutes at pH 7.4. At pH 8.7, greater stability was observed. It is probable that the trithiocarbonate function is the least stable group present.[6]

Procedure for Radiation Protection.—The compounds tested were dissolved in sterile saline solution and 0.4 ml of this solution was administered intraperitoneally at approximately 10 min preceding radiation exposure. The pH of the solution was about 8.2, which was considered not irritating to the peritoneal surface. The dose was calculated on the basis of micromoles per mouse so that meaningful comparison between relative toxicities and radioprotective capabilities could be made with AET.

Adult female Ha/ICR (Swiss) mice weighing 20–26 g and with ages between 10 and 12 weeks were used; a detailed description of the handling of animals and conditions of radiation exposure have been described previously.[7] All chemicals were weighed as their salts.

The radiation was administered by using a General Electric maxitron unit at 300-kv peak, 20 ma, with 1.15 mm of copper and 1.0 mm of aluminum added filtration. The half-value layer for these conditions was 1.92 mm of copper. The mice were irradiated in a rotating cage at a target-to-source distance of 45 cm. The average rate of radiation was 168 r/min.

The lethal radiation dose for these conditions in mice was determined. Varying concentrations of DTC or AET in protecting mice against the lethal effects of such radiation were studied. Other established radioprotective chemicals used in this study were 5-hydroxytryptamine (serotonin) creatinine sulfate and mercaptoethylamine hydrochloride (MEA).

Toxicities.—In this study, the LD_{50} for AET is approximately 25 μmoles/mouse while the LD_{50} for DTC is over 75 μmoles/mouse (see Table I). Thus, DTC is three times less toxic than AET. Intraperitoneal toxicities of different concentrations of either DTC of AET were also compared when admixed with 2 μmoles of serotonin and 25 μmoles of MEA. The results (Table II) indicate that DTC also appears to be less toxic than AET when combined with 2 μmoles of serotonin and 25 μmoles of MEA.

Radiation Protection Studies.—The LD_{100} for these mice under the present conditions of total body irradiation is about 775 r. For comparing the effectiveness of AET and DTC as radioprotective compounds, the mice were exposed to a supralethal dose of 800 r. The results (Table III) illustrated that 20 μmoles of AET provided 68.6% survival, while 20 μmoles of DTC gave only 50% survival. The difference in survival, however, is statistically not significant (χ^2 = 2.72, at >0.05). The decreased radioprotective activity at concentrations above and below 20 μ*M* AET or DTC can only be explained by the bell type of dose–response curve. The total lack of radioprotective activity of DTC at higher but not chemically toxic concentrations cannot be explained until further data is available. Neither AET nor DTC was capable of providing any protection to mice when the dose of radiation exposure was increased to 900 r. As reported earlier[8] radiation protection from the use of a single radioprotective chemical is limited. The result of use of two chemicals was also not as favorable as the optimal concentration of three chemicals.[9]

It appeared logical, therefore, to utilize previously published findings and administer a mixture containing 2 μmoles of serotonin and 25 μmoles of MEA with either AET or DTC preceding exposure to 800, 900, and 1100 r of total body radiation. The results of this study (Table IV) show that at equimolar concentrations almost similar protection to mice was provided at 800 r by AET (85–90% survival) and DTC (80% survival). At 900 r, a chemical mixture containing 10 μmoles of AET offered 100% protection, while that containing 10 μmoles of DTC provided 70% protection. The difference, however, is statistically not significant (χ^2 = 1.57, at >0.10). At 1100 r, chemical mixtures containing 5 or 10 μmoles of AET offered 45 or 86% protection, respectively, while those containing equimolar concentrations of DTC gave no protection. The differences are statistically significant (χ^2 = 3.27 for 5 μmoles at <0.05; and

TABLE I

RELATIVE INTRAPERITONEAL TOXICITIES OF DTC AND AET

Chemical	Dose, μmoles/mouse	No. of mice used	No. of mice died	% mortality
AET	10	30	0	0
	20	70	0	0
	30	5	4	80
	40	5	5	100
	50	5	5	100
	60	5	5	100
DTC	10	20	0	0
	20	40	0	0
	30	10	0	0
	40	15	0	0
	50	20	0	0
	60	20	0	0
	70	10	0	0
	75	4	1	25
	85	4	4	100
	95	4	4	100

TABLE II

RELATIVE INTRAPERITONEAL TOXICITIES OF MIXTURES

Chemical, μmoles/mouse				No. of mice used	No. of mice died	% mortality
AET	DTC	Serotonin	MEA			
5	..	2	25	20	0	0
10	..	2	25	50	3	6
..	5	2	25	20	0	0
..	10	2	25	40	0	0
..	20	2	25	22	1	5
..	30	2	25	10	1	10

TABLE III

RELATIVE RADIOPROTECTIVE EFFICACY OF DTC AND AET AT 800 r

Chemical, μmoles/mouse		No. of mice used	No. of chemical deaths	No. of radiation deaths	% survival
DTC	AET				
10	..	20	0	16	20
20	..	20	0	10	50
30	..	10	0	10	0
40	..	15	0	15	0
50	..	15	0	15	0
60	..	15	0	14	7
..	10	20	0	11	45
..	20	35	0	11	69
..	25	15	0	10	33

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TABLE IV

RELATIVE EFFICACY OF DRUG MIXTURES CONTAINING EITHER DTC OR AET

Chemical, μmoles/mouse				Radiation dose, r	No. of mice used	No. of chemical deaths[a]	No. of radiation deaths	% survival
DTC	AET	Serotonin	MEA					
5	..	2	25	800	10	0	2	80(8/10)
10	..	2	25	800	10	0	2	80(8/10)
20	..	2	25	800	10	0	1	90(9/10)
30	..	2	25	800	5	0	1	80(4/5)
..	5	2	25	800	10	0	1	90(9/10)
..	10	2	25	800	15	2	2	85(11/13)
10	..	2	25	900	10	0	3	70(7/10)
..	10	2	25	900	10	0	0	100(10/10)
5	..	2	25	1100	10	0	10	0(0/10)
10	..	2	25	1100	10	0	10	0(0/10)
20	..	2	25	1100	10	1	6	33(3/9)
30	..	2	25	1100	5	1	1	75(3/4)
..	5	2	25	1100	9[b]	0	5	45(4/9)
..	10	2	25	1100	15	1	2	86(12/14)

[a] When the mice died within 48 hr after injection of the chemicals and radiation exposure, they are counted as chemical deaths. The per cent survival does not include chemical deaths. [b] One mouse ran out of the cage during radiation. This happened once in the last 2 years of work. Therefore, only nine mice were included.

$\chi^2 = 13.9$ for 10 μmoles at <0.01). It was possible, however, to increase the concentration of DTC in the chemical mixture to 20 or 30 μmoles and obtain 33 or 75% protection, respectively, with minimal chemical toxicity. In other words, at 1100 r, DTC in chemical mixture can provide good protection, not at low concentrations or equimolar concentrations where AET is effective, but at high concentrations.

Discussion

Among the more promising radioprotective agents so far investigated, such as AET, serotonin, and MEA, a number have been found to offer appreciable protection but the chemical toxicity of these agents to the animal is a limiting factor even though satisfactory protection can be obtained at lethal doses of radiation. At higher doses of total body radiation no appreciable protection has been obtainable by any one of these agents. Increasing the concentration of these agents has only increased the chemical toxicity without providing significantly greater radiation protection. On the other hand, if a mixture of radioprotective agents is used, a greater degree of protection can be obtained at 900 and even 1100 r. The optimal concentration of each compound in the mixture is appreciably lower than if used alone.

It is noteworthy that DTC is two-three times less toxic to mice than AET. DTC appears to be slightly less effective than AET, although in the present study 20 μmoles of DTC or AET offers statistically similar protection when exposed to 800 r. The advantage of DTC over AET is noticeable when good protection to 800 r is obtainable at nontoxic concentrations of DTC in the chemical mixture containing 2 μmoles of serotonin and 25 μmoles of MEA. At 1100 r, a chemical mixture with AET at either 5 μmoles or 10 μmoles is distinctively more effective than that of equimolar concentration of DTC in similar mixture. However, when the concentrations of DTC in the chemical mixture were increased to 20 μmoles and 30 μmoles, good protection (33 and 75% survival, respectively) was achieved with minimal increase in chemical toxicity. From the results of this investigation it is concluded that good protection against the lethal effects of ionizing radiation can be obtained by using a compound such as DTC which is less toxic than AET. Although it appears that DTC provides comparable order of activity as AET, further research is needed, however, to ascertain the precise relative efficacy of DTC and AET as radioprotective agents.

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