Further Studies on Modification of Sensitivity to X-rays by Cysteine. (17561)

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In a previous communication(1) it was shown that cysteine greatly reduced the sensitivity of rats to lethal amounts of x-rays delivered at high dose rates, provided that the amino acid was given before the exposure. In contrast to the protective influence of cysteine was the finding that cystine did not alter survival of the irradiated animal. We should like to present in this paper further data concerning the time course of the cysteine protection as well as a comparison of the influence of certain substances on radio sensitivity.

Methods. The methods were the same as those reported earlier.(1) Control and experimental animals were irradiated simultaneously, caged together, and otherwise similarly handled. Male and female rats (Sprague-Dawley) weighing 150-250 g and female mice (CF-1) weighing 20-25 g were employed. Cysteine was administered as a 10% solution of the hydrochloride neutralized to pH 7-8 with 2N sodium hydroxide. The cysteine dose was 950 mg/kg intravenously or 1900 mg/kg orally. Although the oral dose is well tolerated, the smaller intravenous dose is near the maximal tolerated amount and must be injected slowly to prevent acute death in some of the animals.* Cysteine was injected into a tail vein or given by stomach tube at one of the following times relative to irradiation: 5 minutes, 30 minutes, 1 hour, 6 hours, or 24 hours before or 5 minutes after. Irradiated controls received comparable volumes of 5% sodium chloride intravenously or orally.

Comparison was also made of the influence on sensitivity to total-body X radiation of cystine (I.V. and oral), glutathione (I.V. and oral), ascorbic acid, sodium sulfide, and colloidal sulfur (all I.V.). These substances were administered within an hour before the exposure. In most instances, two doses of each substance were used, the maximal tolerated dose and one-half of this. In the case of cystine and methionine, dosage was determined largely on the basis of solubility. Colloidal sulfur was prepared by dissolving recrystallized sulfur in hot 95% ethyl alcohol, addition of cold distilled water, and subsequent removal of the alcohol by heating. Rats received 800 r total-body X radiation in a single exposure, mice 600 r. The radiation factors were 250 kv, 15 ma, 0.5 mm Cu, and 3.0 mm Bakelite filters, 26.5 cm target distance, 1.5 mm Cu half-value layer, and 210-220 r/minute dose rate. In a few experiments rats and mice were given 800 r and 600 r, respectively, at 15-20 r/minute in order to compare cysteine protection against x-rays delivered at high and low intensities. Survival at 28 days post irradiation was the criterion selected to evaluate protection.

Results. Rather comparable protection is observed when cysteine is administered intravenously to rats 5 minutes or 1 hour before X irradiation (90% survival of 114 cysteine irradiated, 10% survival of 117 irradiated controls). Injection 5 minutes after or 6 or 24 hours before is without influence on survival of the irradiated rat. These findings are presented in Fig. 1. Acute radiation deaths occur largely from 1 to 3 weeks after the exposure with a peak in the second week. The few deaths occurring in the groups given cysteine within an hour prior to irradiation are scattered over this interval. Cysteine administered orally also confers significant protection to rats and mice when it is given before the exposure. However, oral administration is not as efficient as intravenous even

1. Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science*, 1949, v110, 213.

* Recent experiments on x-ray-cysteine dosage relationships have revealed that lower doses of cysteine can afford rather equivalent protection to rats against 800 r. (Smith, D. E., Patt, H. M., Tyree, E. B., and Straube, R. L., American Physiological Society Fall Meeting, Augusta, Georgia, September 14-16, 1949).

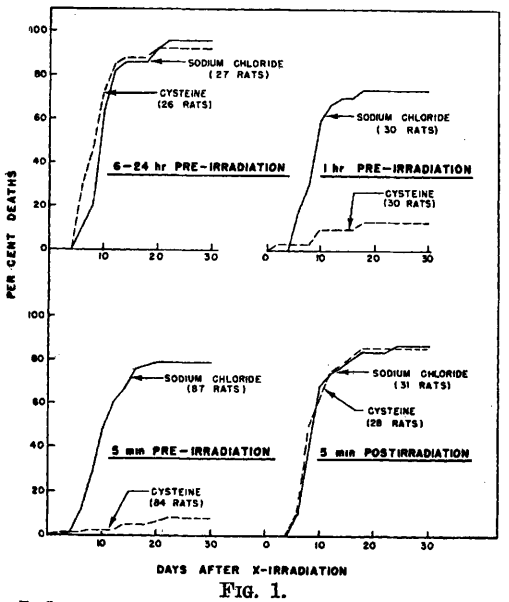

FIG. 1.

Influence of time of injection of cysteine (950 mg/kg I.V.) on sensitivity of rats to total-body X irradiation with 800 r.

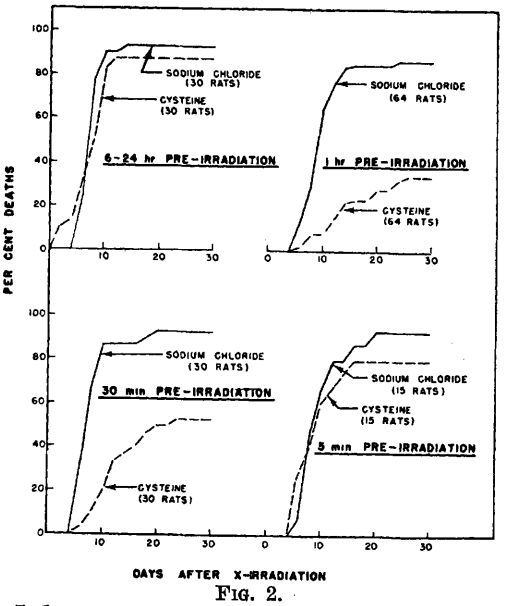

FIG. 2.

Influence of time of oral administration of cysteine (1900 mg/kg *per os*) on sensitivity of rats to total-body X irradiation with 800 r.

though the oral dose is twice the latter. The effective interval in the case of cysteine administered by stomach tube is 30 to 60 minutes before irradiation. Since cysteine protection is similar in irradiated rats and mice, only data obtained in the former are presented (Fig. 2). The cysteine effect is apparently independent of x-ray dose rate, at least within wide limits. A similar reduction in mortality is obtained when the amino acid is given to mice and rats exposed to 600 r and 800 r, respectively, at either 15 to 20 r/minute or 210 to 220 r/minute. At the former dose rate, 69% of 45 mice given cysteine orally survive as compared with 28% of 47 irradiated controls. When cysteine is administered intravenously to rats before exposure at the low dose rate, 84% of 19 cysteine irradiated survive as compared with 16% of 25 irradiated controls. This protection is of the same order as presented in Figs. 1 and 2 for high dose rate exposures.

The influence of related substances on the sensitivity of rats to x-rays is summarized in Table I. Cystine is not effective either intravenously or orally. Glutathione can reduce x-ray toxicity in rats but only when it is given intravenously before the exposure.† The reduction in sensitivity to x-rays by glutathione is roughly two-thirds that observed with cysteine. In contrast to the protective influence of cysteine by the oral route, glutathione is without effect in mice and rats when administered orally (mice, 5000 mg/kg glutathione *per os*, 50% survival of 20; irradiated controls, 40% survival of 20—rats, see Table I). Methionine, ascorbic acid, and sodium sulfide in the dosages used failed to alter survival of rats after irradiation. Colloidal sulfur appeared to reduce radiation toxicity in one experiment but not in another.

Discussion. Studies on the time course of cysteine protection against total-body X irradiation reveal that the amino acid must be given before the exposure. This suggests that the reactions leading to morbidity are complete when the irradiation is terminated and that these reactions are apparently not reversible. It is, of course, possible that these reactions could be reversed if the cysteine were given quickly enough, perhaps

† In a personal communication, E. P. Cronkite (Naval Medical Research Institute) has indicated that he has obtained similar protection in mice given glutathione subcutaneously before X irradiation.

TABLE I.
Influence of Related Substances on Sensitivity of Rats (Sprague-Dawley) to 800 r X-rays.

Treatment group	Dose, mg/kg	Route*	No. of rats	% survival, wk after irradiation			
				1	2	3	4†
Control	—		48	67	27	21	21
Glutathione	625	I.V.	30	93	73	67	*63*
Control	—		14	93	43	21	21
Glutathione	900	I.V.	14	93	64	64	*64*
Control	—		16	31	6	0	0
Glutathione	1250	I.V.	10	50	40	40	*40*
Control	—		30	30	7	7	7
Glutathione	2000	Oral	29	34	7	3	3
Control	—		27	52	22	15	15
Cystine	280	I.V.	22	68	18	18	18
Control	—		15	27	0	0	0
Cystine	2500	Oral	13	23	8	8	8
Control	—		33	58	27	21	21
Methionine	255	I.V.	16	56	31	25	25
Control	—		15	40	20	20	20
Ascorbic acid	500	I.V.	15	53	13	7	7
Control	—		15	47	20	20	20
Ascorbic acid	1000	I.V.	15	53	13	13	13
Control	—		15	87	7	7	7
Sodium sulfide	2.5	I.V.	15	73	20	7	7
Control	—		15	67	27	13	0
Sodium sulfide	5.0	I.V.	15	73	13	7	7
Control	—		15	87	27	0	0
Colloidal sulfur	17.5	I.V.	12	100	67	42	*43*
Control	—		15	93	20	20	20
Colloidal sulfur	20	I.V.	14	79	29	29	29

* All I.V. injections were made 5 minutes before irradiation; oral administration, 1 hour before.

†Italic figures are significantly different ($p < .05$) from the 4-week survival of the appropriate irradiated controls.

within seconds, after the exposure. Less than 10% of the intravenously injected dose can be accounted for in blood as cysteine and cystine within several minutes after its administration.(2) The blood level of cysteine at the time of irradiation is, therefore, probably not critical since comparable protection is obtained when cysteine is injected 5 minutes or 1 hour before the exposure. Differences in the protection afforded by the intravenous and oral routes when the amino acid is given 5 minutes before irradiation are apparently a reflection of the time required for absorption of the material from the gastro-intestinal tract and its distribution by the blood. Under any circumstances, oral administration of cysteine is not as efficient as intravenous even though the oral dose is twice the latter.

It was hoped that some insight into the mechanism of the cysteine protection might be forthcoming from a consideration of the influence of certain related substances on sensitivity to radiation. The term, related substances, is used in a broad sense and is not meant necessarily to imply either structural or pharmacological similarity. It is significant that cystine is not effective either intravenously or orally. The related amino acid, methionine, is also without influence on radiosensitivity. On the other hand, glutathione, the tripeptide containing cysteine, can reduce x-ray toxicity when it is given intravenously before the exposure. When glutathione is given by stomach tube, however, survival of irradiated rats and mice is unchanged even though the oral dose is equivalent to the effective oral dose of cysteine in sulfhydryl content. Our data do not permit comparison of the relative effectiveness of intravenously administered cysteine and glutathione on an equimolar basis. The maximum tolerated glutathione dose (1250 mg/kg I.V.)

2. Patt, H. M., Smith, D. E., Straube, R. L., and Tyree, E. B., unpublished observations, 1949.

is equivalent to approximately half of the cysteine dose used for comparison (950 mg/kg I.V.) in terms of sulfhydryl content.

Sodium sulfide and colloidal sulfur were included to test further the possible importance of sulfur in the protection afforded by cysteine against x-rays. The former was ineffectual, while the latter gave equivocal results. Ascorbic acid selected because of its reducing properties and its presumed importance in biologic oxidation-reductions failed to alter survival of the irradiated rat. A sulfhydryl group is the most evident distinguishing feature between the substances which definitely protect and those which do not. It should be borne in mind, however, that all sulfhydryl-containing materials may not necessarily protect and that other reducing substances which are properly distributed, temporally and spatially, may.

Since cysteine, but not cystine, is effective in altering sensitivity to radiation, one may speculate that the cysteine acts by protecting certain cellular components from oxidation by the products of irradiated water. This could be accomplished indirectly by the reduction of one or more of the critical links in the metabolic chain or directly by neutralization of the oxidizing agents presumably produced by radiation. Barron and co-workers(3) have reported that small doses of x-rays inactivate sulfhydryl enzymes *in vitro* by oxidation of their -SH groups (reversible inhibition) and large doses by denaturation of the protein moiety (irreversible inhibition). Non-sulfhydryl enzymes may be irreversibly inhibited by still larger amounts of x-rays, primarily by protein denaturation. If enzyme inactivation of the types reported by Barron is an important factor leading to death of the irradiated animal, it would appear from the above considerations and our data on the time course of the cysteine protection that the inactivation is of the irreversible non-specific type resulting from protein denaturation.

Summary. Cysteine greatly reduces the sensitivity of rats and mice to lethal amounts of x-rays delivered at either high or low dose rates provided that the amino acid is given before the exposure. Rather comparable protection is obtained when cysteine is injected intravenously in rats immediately or 1 hour before irradiation with 800 r (90% reduction in the 28-day mortality). Injection immediately after exposure or 6 or 24 hours before is without influence. Cysteine also improves survival significantly when it is given orally 30 to 60 minutes prior to the irradiation. Glutathione (I.V. but not oral) can also diminish radiation toxicity. Cystine, methionine, ascorbic acid, and sodium sulfide do not alter survival of the irradiated animal. Results with colloidal sulfur are equivocal. The possible significance of these observations is discussed.

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3. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *Jour. Gen. Physiol.*, 1949, v32, 537.

Vitamin B-12 in Amino Acid Metabolism.* (17562)

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McGinnis *et al.*(1) reported that blood non-protein nitrogen content was higher in chicks deprived of animal protein factor (APF) than in normal controls, and suggested that this may have been due to a function of APF in enhancing amino acid utilization. Zucker and Zucker(2) found that non-protein nitrogen, and urea values in rat blood were in-

* Scientific Journal Series No. 311, Colo. Agr. Exp. Station.

1. McGinnis, James, Hsu, P. T., and Graham, W. D., *Poultry Sci.*, 1948, v27, 674.