Studies with Cysteinamine and Cysteine in X-irradiated Animals. (20758)

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Maisin *et al.*(1), have observed that post-irradiation cysteinamine (β-mercaptoethylamine) affords protection to rats if the liver has been shielded by lead. The role of the liver in this connection is not readily apparent. This phenomenon, however, is of considerable interest, particularly since one is prone to interpret pre-irradiation protection with chemicals of this sort in terms of the more or less immediate reactions of irradiated water. It has been shown recently, for example, that there is an inverse relationship between the protective efficiency of cysteine and ionization density which is comparable to that noted for the oxygen effect(2). Under the circumstances, it seemed worthwhile to attempt confirmation of the postirradiation action of cysteinamine and to include ancillary observations of the protective capacity and synergism of cysteinamine and cysteine.

Methods. 1) Cysteinamine and liver shielding in the rat. Male rats of the Holtzman and Sprague-Dawley strains weighing 190-225 g were used. The experimental procedure of Maisin *et al.*(3) was duplicated insofar as possible, except that the x-ray dose was 1000 r instead of 1130 r and that several dosages of cysteinamine were evaluated. The rats were divided into 4 groups. Group 1 received 1000 r total body radiation; Group 2 received 1000 r total body radiation plus cysteinamine* (17.5, 35 or 70 mg kg, I.P.) immediately, 6 and 12 hours after irradiation; Group 3 had the hepatic region shielded by a 0.5 x 3.2 x 5.0 cm lead plate during irradiation; Group 4 combined liver shielding with the 3 postirradiation injections of cysteinamine. The lead shields were placed so as not to protect the splenic region. Radiation factors were 180 kv, 15 ma, 0.225 mm Cu HVL, 55 cm target distance, and 84 r/ min. dose rate. 2) Protective effects of cysteinamine and cysteine in mice. CF_1 female mice weighing 19-24 g were divided into groups of 25 animals each and irradiated in pairs with 1000 r. Immediately before irradiation, individual animals received intravenous injections of either 75 or 150 mg/kg of body weight of cysteinamine or 600 or 1200 mg/kg of cysteine. In other studies 75 mg/kg of cysteinamine and 600 mg/kg of cysteine were combined and injected immediately before irradiation. Radiation factors were 250 kv, 15 ma, 0.5 mm Cu and 3.0 mm Bakelite filters, 29.5 cm target distance and 200 r/min. dose rate. The supralethal dose of 1000 r (30 day LD_{95} for CF_1 mice = 800 r) facilitates comparison of the protective effect with minimal numbers of animals.

Results. About 60% of rats with the liver area shielded survived exposure to 1000 r, a dose that is lethal to over 95% of unshielded animals within 30 days. Postirradiation injections of cysteinamine alone did not afford any protection. Differences in lethality of irradiated animals treated with cysteinamine in combination with liver shielding and animals with shielding alone were not statistically significant (Table I). Thus, these experiments do not verify a postirradiation action of cysteinamine in liver shielded rats (1,3). The reason for this is not obvious. Animal strain, age, and weight were not specified in the report of a postirradiation effect (1,3). Dosage was stated as 7 mg per adult rat. Three dosages of cysteinamine were employed in the present experiments. These correspond to 7 mg per 100, 200, and 400 g rat and span the body weight range for adult animals generally. Subtle differences between animal strains may be contributory. It may be noted, however, that neither Holtzman nor Sprague-Dawley rats were affected by the treatment in question.

Turning to the protective effects of cysteinamine and cysteine when given prior to irradiation we may note that the former is more efficient than the latter. Maximally tolerated

* Kindly provided by Dr. Harvey Blank, E. R. Squibb and Sons.

TABLE I. Effect of Cysteinamine on Survival of Rats Exposed to 1000 r X-rays with and without Liver Shielding.

Group	Dose (mg/kg)	Strain	No. of animals	% survival 1 wk	2 wk	3 wk	4 wk
Irradiated controls*	—	S.D. + H	66	23	4	4	4
Cysteinamine after irrad.†	17.5	S.D.	25	12	4	4	4
	35	H	41	32	7	7	7
	70	S.D.	24	17	4	4	4
Liver shielded*	—	S.D. + H	66	80	61	61	61
Idem.; cysteinamine after irrad.†	17.5	S.D.	22	91	73	73	68
	35	H	41	68	68	68	68
	70	S.D.	23	74	65	65	65

* Because survival patterns at 1000 r are similar in both Holtzman and Sprague-Dawley rats, controls are combined.

† Injected IP immediately after, 6 and 12 hr postirradiation.

doses, 150 mg/kg of cysteinamine and 1200 mg/kg of cysteine *I.V.*, afford comparable protection to mice against acute radiation lethality (Fig. 1). Cysteinamine is thus 5 times as efficient as cysteine on a molar basis. Since mice will not tolerate a combined injection of these dosages, 75 mg/kg of cysteinamine and 600 mg/kg of cysteine were employed in the additivity studies. Neither dose alone alters appreciably the absolute mortality resulting from exposure to 1000 r, although mean survival times are slightly prolonged. When given together, however, there is definite protection equivalent to that afforded by the full dosage of either substance alone.

The synergism between minimally effective doses of cysteinamine and cysteine may be taken as presumptive evidence that similar reactions are responsible for the protective effects. The greater efficiency of cysteinamine, when compared to cysteine, may be attributed to differences in the distribution and biological life of these substances. Cysteinamine, for example, may be oxidized in transit less rapidly than cysteine, which could account for its greater toxicity. The importance of the pharmacological attributes

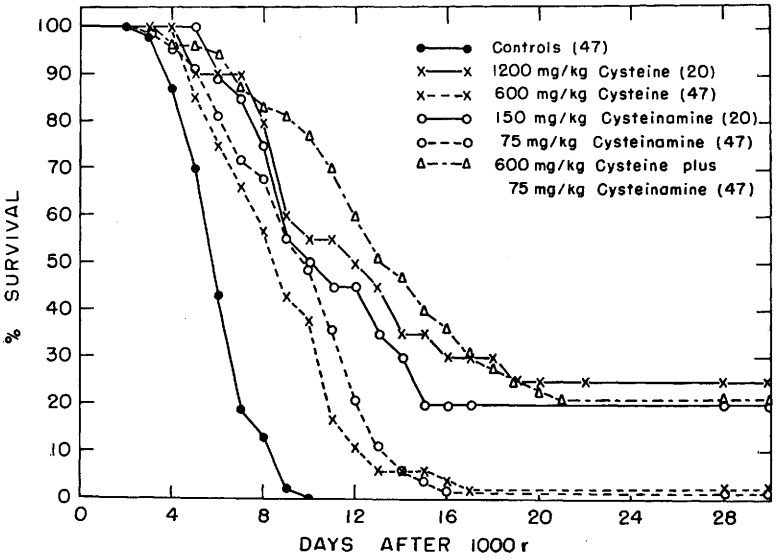

FIG. 1. Synergistic effect of cysteinamine and cysteine upon survival of mice following exposure to 1000 r total-body irradiation. Figures in parentheses indicate No. of animals in each group.

of the various substances is illustrated further by the fact that mercaptosuccinate is not protective, in contrast to cysteine, cysteinamine, glutathione and BAL(4). In perhaps the the same sense, it may be recalled that cystinamine, $(S\text{–}CH_2\text{–}CH_2\text{–}NH_2)_2$, is an effective protective agent under certain circumstances but that cystine is inactive. The effect of cystinamine, unlike cysteinamine or cysteine, is restricted to the intact animal. This suggests that an *in vivo* interconversion to an active form is necessary. Cystinamine, for example, does not modify the radiosensitivity of pea roots(7) or reticulocytes of dog's blood *in vitro*(8). Protection of the whole animal may, perhaps, be attributed to its reduction to cysteinamine(6) or to the rapid formation of histamine(7). Large doses of histamine and other amines have been shown to enhance the survival of irradiated mice perhaps because of altered tissue oxygen tension resulting from vascular effects(5,7). Cysteinamine, or the other hand, does not liberate histamine(7) and presumably exerts a more direct action as a consequence of its sulfhydryl group, an action comparable to that of cysteine. This is, in fact, suggested by the apparent additivity of the 2 compounds.

Summary. 1. The observation of a post-irradiation effect of cysteinamine in rats with the liver area shielded has not been verified. 2. Cysteinamine is about 5 times as effective as cysteine when given to mice intravenously in equimolar amounts prior to x irradiation. However, maximally tolerated doses of each give equivalent protection. 3. Cysteinamine and cysteine appear to be additive in their protective effect against acute radiation lethality. This is suggestive of a similar mechanism of action.

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Rapid Colorimetric Method for Determination of Isonicotinic Acid Hydrazide in Blood Plasma. (20759)

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The observation that isonicotinic acid hydrazide (isoniazid*) and its isopropyl and glucose derivatives have activity against tuberculosis in experimentally infected laboratory animals and against human tuberculosis (1) has raised the need for methods of assay of these compounds in body fluids. Reports of 3 methods appeared simultaneously as follows: S. Rubin and his coworkers(2) described an oxidation procedure and assay of the resulting isonicotinic acid with cyanogen bromide and ammonia. The degree of sensitivity was not stated. B. Rubin and collaborators (3) employed a rapid extraction method, sensitive to 5 $\mu g \pm 10\%$ per ml of blood plasma, which involved estimation of the ultraviolet absorption of an isoamyl alcohol extract. Kelly and Poet(4) extracted hydrazide from alkalinized plasma or urine by the addition of ammonium sulfate and an isoamyl alcohol-

* See footnote 5, *Am. Rev. Tuberc.,* 1952, v65, 754.