This dilemma becomes even more obvious when applied to children. Of necessity, a child's diet is higher in calories than an adult's because the requirements for specific nutrients are higher, and decreasing the dietary even to 1200–1500 calories/day would mean going below the National Research Council requirements. Children cannot afford a drastic reduction in calories because of their need of these foods for growth and development.

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Protection Against X-rays and Therapy of Radiation Sickness with β-Mercaptoethylamine[1]

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Many substances *in vitro* partially inhibit the action of ionizing radiations on substances in aqueous solution. This fact has been correlated with the theory of indirect action through free radicals (*1–3*). The substances that were first shown to have a protective action against x-rays in animals, were toxic or had too weak an action; such is the case with thiourea, cyanide, malononitrile and azide (*4, 5*), nitrite (*6*), estradiol (*7*), cysteine and glutathione (*8*), and pyruvate (*9, 10*).

During a systematic search for a substance suitable for use in human beings, we encountered many protective substances in the amine group. Very active, but also toxic, are tryptamine, 5-hydroxytryptamine (= serotonine), para- and meta-tyramine, and dopamine (*10, 11*). The amino acids have, in general, a weak protective action in mice, a confirmation of the results of Hollaender and his group with microorganisms (*12*); the corresponding amines are generally better protectors (*10, 11*). Cysteamine $HS—CH_2—CH_2—NH_2$ (= β-mercaptoethylamine = 1573 L. = Becaptan R.N.) and the corresponding disulfide (= cystinamine), are in our opinion, the most interesting substances so far discovered, because of the power of their action and their low toxicity.

Cysteamine is a fragment of coenzyme A. Cystinamine, $\begin{matrix} S—CH_2—CH_2—NH_2 \\ | \\ S—CH_2—CH_2—NH_2 \end{matrix}$, like many diamines, is a liberator of histamine (*13*) and for this reason cannot be advocated for internal use in man. Thus our attention had been focused on cysteamine.

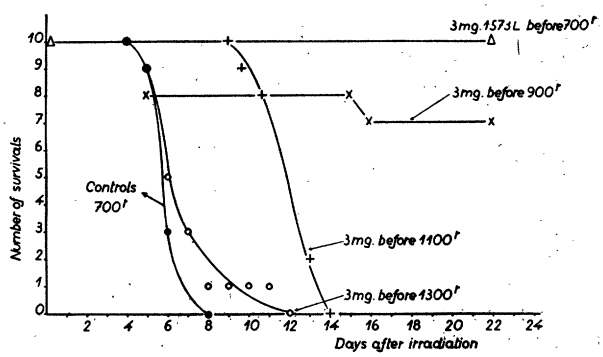

FIG. 1. Ordinates, number of mice (out of groups of 10) surviving after irradiation at day 0. Abscissae, days after irradiation. Various doses of x-rays (700 r–1300 r) are given to mice injected with 3 mg cysteamine, and the mortality compared with that of controls which receive 700 r.

Protection of mice against x-radiation. Mice of pure breed (C 57 black), about 4 months old, weighing 19 to 21 g, are irradiated by groups of 10 in a pasteboard box: 250 kv, Cu 2.5 mm, focal distance 50 cm, field 100 cm², 90 r per min. A single dose of 700 r kills practically all our controls in 5–15 days. Out of 38 series of 10 control mice, only 7 animals were alive 25 days after irradiation.

If a dose of 3 mg of cysteamine (base, neutralized with HCl to pH 6.5) is injected intraperitoneally 1–3 min before irradiation (700 r), a permanent survival of 97% is observed; it is necessary to give about 1300 r in order to obtain, in mice protected by cysteamine, the same mortality curve (Fig. 1). If it is injected 0.5–3 min after the end of irradiation, it does not affect the mortality. Cysteamine is rapidly metabolized; the mice irradiated 1 hr after the injection of cysteamine behave about like the controls. Cysteine (HCl) injected in doses equimolecular to 3 mg of cysteamine allows only 3 animals out of 10 to survive a dose of 700 r. The white cell count of irradiated mice protected with cysteamine drops just like those of the controls during the 2 or 3 days following irradiation, but recovery starts earlier in the injected animals. Similarly, the weight of protected mice, like that of the controls, decreases for 2 days after irradiation, but on the third or fourth day, the weight of the injected animals begins to increase (Fig. 2), whereas the weight of the controls drops until death.

The protective action of cysteamine against x-rays

[1] Under the auspices of the "Conseil Supérieur de la Sécurité Civile Ministère de l'Intérieur," Brussels.

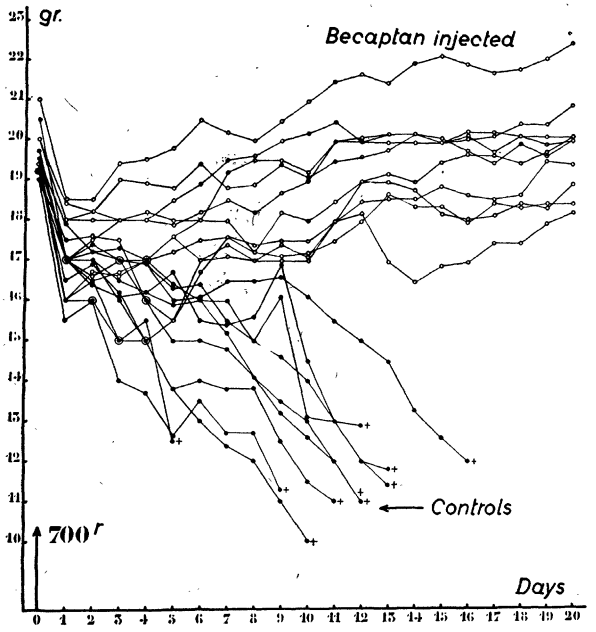

FIG. 2. Ordinates, weight in grams of 20 mice, 10 of which have been injected with cysteamine (3 mg intraperitoneally) before irradiation (700 r), 10 others serving as controls. Abscissae, days after irradiation. All the animals lose weight during the first day; there is progressive recovery of the injected animals (which survive), whereas the weight of the controls drops until death.

is a general one: it protects ciliates (*14*), reticulocytes of dog blood *in vitro* (*15*), and growing roots of peas (*10*), as well as mice or rats.

Preliminary histological examinations confirm that the primary radio-lesions are similar in both the irradiated-protected and the control mice, but regeneration is much more rapid and vigorous in the protected animals. Female mice surviving a dose of 700–1000 r, when protected by cysteamine or cystinamine, are permanently sterile. The ovaries are destroyed, and it is difficult to find a single follicle. The hair also shows progressive graying (*16*).

For cysteamine, we are inclined to accept the interpretation given by Cronkite, Brecher, and Chapman (*17*) for the protective action of glutathione. Regeneration mechanisms or factors (possibly we should add coenzyme A) would not be so heavily damaged when the organisms are irradiated in the presence of a molecule that competes for the free radicals liberated during irradiation. But cysteamine has other actions that are not explainable by the simple hypothesis of the *protection* of a regeneration factor.

Therapeutic action in radiation sickness. A single intravenous injection of 200 mg cysteamine (base, neutralized by enough HCl to obtain pH 6–7) is often sufficient to eliminate, in 24 hr, the symptoms of radiation sickness (nausea, vomiting, diarrhea, general weakness, etc.) observed in cancerous patients treated with ionizing radiations. Sometimes 2—rarely 3—injections are needed to obtain a full therapeutic effect. Many observations in Liége, and the various cancer centers in Belgium have confirmed the preliminary report of Herve (*18*) and have shown that there is no danger of tumor cells becoming refractory to ionizing radiations.

Mercaptoethylamine salicylate is the only nonhygroscopic salt thus far obtained that is stable in air. It is a white crystalline substance, melting point 104–105°, highly soluble in water and alcohol, insoluble in ether and petroleum, slightly soluble in chloroform. It is very effective when given by mouth in gelatin capsules (300 mg 3 times a day) to irradiated cancerous patients experiencing typical radiation sickness. At first glance there seems to be a contradiction between this fact and the observations summarized above. Obviously, the action of cysteamine on radiation sickness is not explainable on a simple physicochemical basis (competition for free radicals), because in this case the substance is injected *after* irradiation and changes a general syndrome affecting tissues that have not been irradiated (actions *à distance*). Preliminary observations on the metabolism of mercaptoethylamine, and on the reactions of the body to that substance, seem to show the way to a possible interpretation.

Metabolism. Both cysteamine (SH) and cystinamine (S—S) disappear from the blood in 20–30 min when injected intravenously in large amounts (up to 75 mg/kg) in dogs and rabbits. Cystinamine is reduced *in vivo* to cysteamine, as shown by the appearance of titrable —SH functions in plasma and urine, and by paper chromatography and polarographic analysis. *In vitro,* when cystinamine is put in contact with cysteine at neutral pH and room temperature, a precipitate of cystine appears in a few minutes (*19*). Therefore, we think that the main function of intracellular thiol groups of glutathione and proteins, may be to keep coenzyme A in reduced condition.

About 35% of intravenously injected cysteamine is excreted unchanged by the kidneys. After cysteamine injection large quantities of ascorbic acid and other, unidentified reducing substances not bearing thiol functions, appear in the plasma of rabbits and in the urine of dogs and man. Table 1 summarizes some observations (see also *19*). It must be clearly understood that by ascorbic acid we mean a substance without —SH, reacting with dichlorophenolindophenol. In some cases, polarographic and spectrographic analysis have given clear indications that ascorbic acid is the main substance involved. The ascorbic acid content of the suprarenal glands of rats drops to about 35% of normal 4 hr after intraperitoneal injection of 10 mg per 100 g cysteamine. There is no change in the cholesterol content of these glands, the number of circulating eosinophiles does not drop, and there is no increased urinary excretion of corticoids. Thus, cysteamine has no ACTH-like action, although large quantities of ascorbic acid disappear from the suprarenal glands (*20, 21*).

Cysteine in equimolecular doses does not liberate reducing substances in the plasma of rabbits.

TABLE 1

ASCORBIC ACID IN PLASMA AND URINE OF ANIMALS AND MAN AFTER INTRAVENOUS INJECTION OF CYSTEAMINE

Animal	Weight in kg	Dose injected in mg of base	Fluid examined	Time after injection	Concentration of ascorbic acid μg/ml	Total ascorbic acid excreted, mg
Rabbit	1.9	200	Plasma	0	3.6	
				1 min	2.2	
				5 "	3.8	
				15 "	5.4	
				45 "	10.4	
				55 "	9.8	
Rabbit	2	200	Plasma	0	4.4	
				1 min	4	
				5 "	4.2	
				15 "	8.2	
				45 "	9.4	
				75 "	10.5	
				90 "	4.2	
Dog	9.5	300	Urine	1 hr before		0.80
				1 hr after		2.88
				2 hr "		2.73
				3 hr "		4.38
				4 hr "		4.92
				5 hr "		3.27
				6¼ hr after		3.71
				7¼ hr "		3.10
Man	65	500	Urine	0	5.7	
				15 min	35.2	
				2 hr	32.6	
Man	68	200	Urine	1 hr before		0.058 μg
				1 hr after		0.098 "
				2 hr "		0.297 "
				3 hr "		0.070 "
				4 hr "		0.081 "

Actions on the liver. Many facts indicate that the liver is the site of important reactions to ionizing radiations and cysteamine:

1) Chevallier (*22*) has observed that, if 25–50 r are given daily to rats, they die, when they have received a total dose of 2500–3000 r, from fatty degeneration of the liver.

2) Maisin *et al.* (*23*) have shown that mercaptoethylamine is effective in rats when given *after* irradiation, if the liver has been protected by lead. A typical series of experiments shows: (a) 24 rats given 1130 r without any protection were all dead on the 8th day. (b) 24 rats similarly irradiated, which received 3 intraperitoneal injections of 7 mg cysteamine (the first immediately after irradiation, the second 6 hours after irradiation, and the third 12 hours after irradiation), were also dead on the 8th day. (c) Out of 24 rats that received 1130 r with the liver (but not

TABLE 2

CONTENT IN ASCORBIC ACID (μg/g) OF THE LIVER OF C 57 BLACK MICE (19–21 g) INTRAPERITONEAL INJECTION OF 3 MG CYSTEAMINE (BASE)

Controls	After injection							
	15 min	45 min	2 hr	3½ hr	4 hr	5 hr	6½ hr	28 hr
197								
200	200	271	256	292	285	314	223	220
191	211	244	271	241	288	315	243	225
210	220	246	267	290	274	281	240	
204								
206								
190								
180								
192								
Mean 197	210	254	265	274	282	303	235	

the spleen) protected, 16 were still living on the 8th day, but only 5 on the 30th day. (d) Out of 24 rats that received 1130 r with the liver protected and 3 injections of becaptan as in (b) (i.e., *after irradiation*), 14 were living and in excellent condition 30 days after irradiation. If another part of the rat's body (a hind limb, for instance) is protected, cysteamine does not decrease mortality if given after irradiation.

3) Becaptan reduces the weight of the liver in mice, and increases its content by 50% in ascorbic acid (Table 2, Fischer and Bacq, unpublished).

Other actions. β-Mercaptoethylamine has a peculiar antimitotic action on tissue cultures of chick embryo. It seems to act mainly on the cytoplasm of cell in interkinesis; the nucleus divides, but the cytoplasm does not; thus binucleated cells are formed (*23*).

Large doses of cysteamine (up to 1 g daily, for 15 days) have been given intravenously in 11 cases of chronic leukemia, with good results in 4 cases. Cysteamine does not change the basal metabolic rate and has no antithyroid action (*21*). It inhibits the tuberculinic reaction in the skin of sensitized humans and increases the time necessary to observe the trypan blue color in the skin of rabbits after local chloroform application, about 500% (Ambrose and de Eds test) (*24*). It also inhibits the allergic passive Arthus reaction in the skin of rabbits.

Conclusion. At the present time β-mercaptoethylamine (= cysteamine) thus seems to be the most practical substance to protect mammals against ionizing radiations. It remains active when given after irradiation, if the liver has been protected. It has a remarkable therapeutic action against radiation sickness in cancerous patients locally irradiated. Other observations show that mercaptoethylamine is a molecule of great biological interest, the study of which is being actively pursued in our laboratories.

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Incorporation of Tritium Oxide Into Growing *Chlorella pyrenoidosa* Cells[1]

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It is well established that water is the source of hydrogen in green plants and that photosynthesis is the mechanism whereby the hydrogen is incorporated. Presumably, tritium (H^3) should follow the same pathway in plants and should be incorporated at the same rate as ordinary hydrogen, if no isotope effect exists. From the work of Reitz and Bonhoeffer (*1*) with deuterium, however, it appears that an isotope effect should be expected with tritium, for these workers found that *Scenedesmus acutis* and *Chlaymdomonas spp.* incorporated deuterium in the nonexchangeable portion of growing algae to the extent of only 30 to 70% of its concentration in the nutrient solution. Deuterium oxide concentrations of 12 to 47% were used.

In the experiments to be described algae were grown under standard conditions for varying periods of time. The cells were harvested, and determinations were made of dry weight, and of hydrogen and tritium content. The results were expressed as mc of tritium per ml of water of combustion of the dry cells. The values were then plotted against the number of weight doublings of the original cells, and comparisons were made with a theoretical curve derived on the assumption that no isotope effect exists. Details of the experimental procedure follow.

The green alga, *Chlorella pyrenoidosa,* was used. The culture, obtained from the American Type Culture Collection, was grown on agar slants enriched with sucrose, and transfers were made at monthly intervals. Algae to serve as inocula in experiments were transferred to a sucrose enrichment culture and then, after growth had taken place, to the completely

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