Studies on Oxygen Poisoning: Protective Effect of β-Mercaptoethylamine.*† (20790)

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Based on theoretical considerations and analogies found in the literature, an hypothesis has been formulated(1) on a possible mechanism in common between the initial fundamental action of high oxygen pressures and the primary mechanism of x-radiation. The latter is generally considered to be mainly indirect and due to the oxidizing free radicals $OH^{\bullet}$ and $O_2H^{\bullet}$(2,3). When high oxygen pressure and x-rays were applied simultaneously to mice, a synergistic effect was revealed by a significant shortening of the survival time in the acute oxygen experiments(1).

This prompted the investigation of other possible manifestations pointing to a common path of action. Further evidence lending some support to the hypothesis is now accumulating. It was found that agents of different chemical nature known to give protective action against x-radiation lethality also prolonged significantly the survival times of our mice submitted to acute high oxygen pressure. This was the case for glutathione (4,5), cysteine(6,7), and ethanol(8) which also greatly protected against oxygen poisoning(1). Bacq *et al.*(9) showed a clear protective effect in x-irradiated mice pretreated with β-mercaptomethylamine and this was confirmed by Rugh and Wang(10). Experiments showing a similar effect in mice submitted to high oxygen pressure are presented below.

Methods. Forty "Webster" female mice of the Clara Lynch strain, weighing approximately 20 g each, were used. Two groups of 20 mice (10 controls and 10 experimentals) were tested on 2 different days. The 10 experimental mice received intraperitoneal injections of 3.33 mg of β-mercaptoethylamine in 0.2 ml of saline. (1.0 ml of Becaptan hydrochloride—S. A. Labaz—was diluted to 6.0 ml with saline.) The control mice received an equal volume of saline. Within 5 minutes after the injections the 10 control mice were put in one chamber and the 10 Becaptan-injected mice were put in the other. The 2 chambers were flushed for one-half minute with oxygen from a single cylinder fitted with a "Y" tube and then were brought directly to 6 atmospheres (75 ψ gauge) of oxygen pressure. During the experiment there was a continuous flow of oxygen sufficient to keep the carbon dioxide concentration in the chambers close to a zero level. Survival times were measured from the time that a pressure of 6 atmospheres was attained until the cessation of respirations.

Results. In the first experiment the survival times for the mice injected with β-mercaptoethylamine ranged from 51 to 84 minutes, the average being 66.1 minutes. The control mice survival times ranged from 37 to 53 minutes, the average being 44 minutes.

In the second experiment the survival times of the treated mice ranged from 70 to 102 minutes and of the controls from 37 to 54 minutes. The respective averages being 89 and 45.9 minutes.

The average survival time for both groups of treated animals was 76.9 ± 2.72 minutes and the control average was 45 ± 1.39 minutes. The actual times of death of the various animals are shown in Fig. 1.

Discussion. In the extensive work of Bacq *et al.* on the effect of β-mercaptoethylamine ($SH–Ch_2–CH_2–NH_2$ = Cysteamine = Becaptan R.N.), evidence was produced show-

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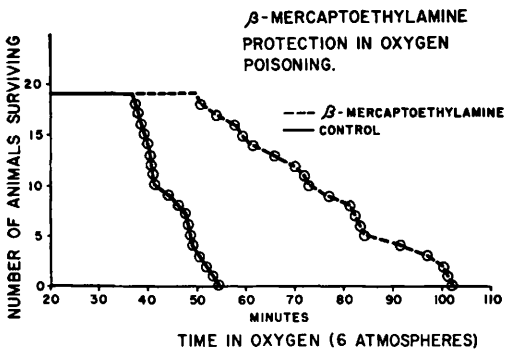

FIG. 1.

ing that this substance exerts a marked protective action against the effects of irradiation on mice and on certain other biological systems. A discussion of the biological actions of β-mercaptoethylamine can be found in the paper by Bacq *et al.*(9). This substance is not only interesting because of its –SH group, which might account for its protective action, but also because it has been shown to be a part of Coenzyme A(11-13). In this connection Bacq and Herve(14) have found that Coenzyme A, in equimolecular concentrations, seems to be much more active than β-mercaptoethylamine as a protector against x-rays. On the other hand, Stadie *et al.*(15), Mann and Quastel(16), and Dickens(17) have studied a number of –SH enzymes showing different degrees of sensitivity to high oxygen tensions. Succinic dehydrogenase, among others, was inactivated by increased oxygen tensions, but the enzyme system showing the greatest sensitivity was the pyruvic oxidase system. Dickens(17) working with brain tissues, suggested that the primary effect of oxygen poisoning may be an inactivation of the pyruvic oxidase system, although possible effects on other enzyme systems were not excluded. The *in vitro* inactivation of –SH containing and other enzymes by ionizing radiations has been well demonstrated by Barron *et al.*(18). It has been claimed both for oxygen poisoning(19) and irradiation(20) that *in vivo* there is no appreciable change in the activity of the oxidative enzyme systems studied and no significant change in oxygen uptakes, to explain the deleterious effects produced by these agents. However, oxygen uptake may not always be a good index of a normal metabolic ability of the cell(21).

Among the many factors contributing to the sum of biochemical lesions resulting in the symptoms produced by oxygen poisoning or by x-radiation, the role of changes produced in fatty acid metabolism(22,23) may prove to deserve consideration.

If one chooses to think that the protective effect shown by β-mercaptoethylamine is due to its role as a component of Coenzyme A, (a coenzyme for the pyruvic oxidase system) and if pyruvic acid is regarded as linking the metabolism of carbohydrate, fat, and protein, then a general metabolic breakdown may be pictured when there is interference with the normal oxidative decarboxylation of pyruvic acid. In the above mentioned experiments of Bacq and Herve(14) protection against x-irradiation was obtained with 3 mg of Pabst Coenzyme A per 20 g mouse, injected intraperitoneally, this being equivalent to approximately 0.3 mg of β-mercaptoethylamine on a molecular basis. In order to find out if Coenzyme A would also protect against oxygen poisoning, we used the same dose and site of injection, and have obtained in a few experiments a highly significant protection.

With Coenzyme A (Pabst), Lot No. 402, in 2 experiments with a total of 9 treated and 11 control mice we obtained 26.4 minutes of protection which was significant at the 0.3% level. However, with Coenzyme A from Lot No. 403 in 4 experiments with 30 treated and 30 control mice, we obtained only 8.0 minutes of protection, significant at a 2.6% level. Because there was a consistent difference in the protection with each lot, we plan to do further oxygen poisoning experiments with Coenzyme A. Since Coenzyme A is a coenzyme for a number of enzymatic systems other than the pyruvic oxidase system, it would be well to keep in mind the possibility that x-rays and high oxygen pressure might affect any of these systems through a primary action on Coenzyme A.

The possible applications of this substance to deep sea diving and retrolental fibroplasia problems are now under study.

Conclusions. Mice exposed to 6 atmospheres of oxygen are protected and their

survival prolonged 70% by previous intraperitoneal injection of 3.33 mg of β-mercaptoethylamine per 20 g body weight.

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Excretion of Carbon-14 by Man after Administration of Morphine-N-Methyl-C^{14}.*† (20791)

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In contrast to the considerable information available concerning the excretion of morphine in the human addict(1-3), little attention has been directed to morphine excretion in non-addicted man following administration of a single therapeutic dose. Oberst has presented data on urinary concentration of free and bound morphine in normal man but figures for complete excretion are lacking(3). Thus most of our present knowledge regarding morphine excretion in the normal animal has been derived from data on dogs(4-6) and rats(7). In both these species, it has been shown that with the development of tolerance there is a concomitant alteration in the excretion of morphine metabolites as evidenced by a marked reduction in the recovery of bound morphine from urine. Although it is possible that a parallel alteration occurs in man following the development of addiction, nevertheless, precise data on normal man are desirable. In view of the relatively small amount of morphine contained in a therepeutic dose, it was felt that the application of tracer technics to this problem would be helpful.

Experiments on rats using N-C^{14}H$_3$ labeled morphine indicated that excretion of radio-

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