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Protection Against Radiation Lethality: Effect of β-Mercaptoethylamine.* (20375)

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The search for some agent which will protect man and animals against, or alleviate them from, the effects of accidental or overexposure of ionizing radiations has been the incentive for many investigations. The most promising of the many chemical compounds used has been *l*-cysteine hydrochloride. Patt and his coworkers(1) found that if mice were injected with cysteine before exposure to x-irradiations, their tolerance would be greatly increased and many would survive doses which would normally prove fatal to 100% of the untreated controls. This led others to investigate the amines, particularly those containing the —SH radical because of the suggestion that an extra supply of this radical might temporarily neutralize certain enzymes with respect to their normally high radiosensitivity(2). Recently, Bacq and his coworkers in Belgium investigated many such compounds. One of these was related to Patt's cysteine, namely cysteinamine or β-mercaptoethylamine. By injecting this compound into C_{57} black mice, they raised the minimum 100% lethal dose of x-rays from 700 r to 1300 r(3). They also found that this compound gave complete protection to mice exposed to 100% lethal dose of x-rays. In this paper the term "cysteinamine" is used instead of β-mercaptoethylamine in order to emphasize its similarity to cysteine which has been so effective in radiation protection studies.

The reasons for the present further investigation of cysteinamine as a protective agent against ionizing radiations are: (a) Variations in sensitivity make it impossible to interpolate results with C_{57} mice for the mice more commonly used in this country, namely the Swiss albino and CF_1 strains, and (b) Radiation studies using male mice exclusively are more reliable than those using females, or both sexes.

Materials and method. The Swiss albino mice were raised in our laboratories and were 6 months of age. The CF_1 were 2 months of age when purchased from the Carworth Farms. They were brought to a uniform weight of approximately 25 g at the time of irradiation. Some 659 mice were used in all. The cysteinamine was acquired in ampoules, labelled 1573 L and produced by the Labaz Laboratory in Belgium. Each ampoule consisted of 10 cc of a 10% solution. This was refrigerated until opened, and was used within a few minutes after dilution with physiological saline. The dilution was such that each cubic centimeter contained 3 mg of cysteinamine. Several parallel experiments were run with

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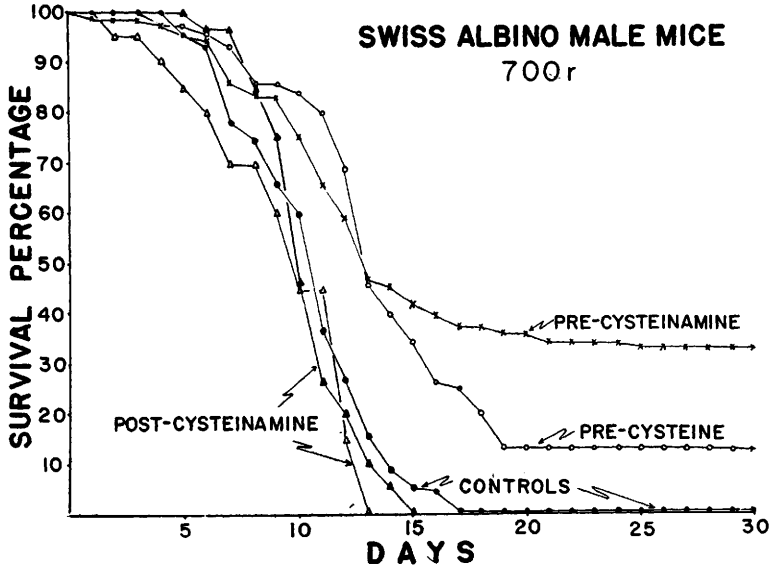

FIG. 1.

l-cysteine. This was made up so that each cubic centimeter contained 12.5 mg, and was neutralized to pH 7.25 with NaOH. The treatment consisted of intraperitoneal injections of 1 cc of either cysteinamine or cysteine. In most cases the injection was made within 5 minutes before x-irradiation, but, as noted below, in a few experiments single or multiple injections were made after irradiation. The mice were x-irradiated 5 at a time in a perforated and circular plastic cage measuring 14 cm in inside diameter and 4 cm in inside height. The calibration of the x-irradiation was made with the ionization chamber in the center of the closed cage. The distance from the target to the center of the mouse's body was 30 cm. Constant movement of the mice within the cage tended to balance out any very small difference that might exist between the center and the periphery of the cage. The physical constants of x-irradiation were: Westinghouse Quadrocondex constant-potential x-ray machine run at 2.0 KV, 15 MA, filtration 0.25 Cu and 0.50 Al, and a dose rate of 274 r/minute in air. The exposure was for 2′ 42″ for each group of mice. After x-irradiation the mice were returned to the animal room where they received the usual care, with food and water *ad libitum*. A daily record of deaths was taken for the experimental period of 30 days.

Experimental data. The injected dose of cysteinamine or cysteine was not varied. However, in some experiments the mice were injected prior to x-irradiation (pre-cysteinamine series) and in some they were injected just after x-irradiation (post-cysteinamine series). The data are presented in Fig. 1 and 2, which include all groups of animals, control as well as treated, with the survival values expressed as percentages.

Discussion. According to Bacq and Herve (3,5), and Bacq, Fischer, and Pirotte(6), cysteinamine is very quickly metabolized, disappearing from rabbit plasma within 15 minutes after injection and appearing in the urine within half an hour. It was estimated that about 1/3 of it is eliminated during the first half hour following injection, and mice x-irradiated one hour following injection received no protection thereby. This substance is relatively non-toxic to mice. The median lethal dose for C_{57} black mice has been determined as 0.35 mg/g of body weight and the minimum lethal dose for all animals studied was 0.5 mg/g of body weight(4). Mice receiving the dose of 3 mg I.P., used in these experiments, showed no ill effects whatsoever.

Bacq and his coworkers have found that the dose of x-irradiation required to kill all

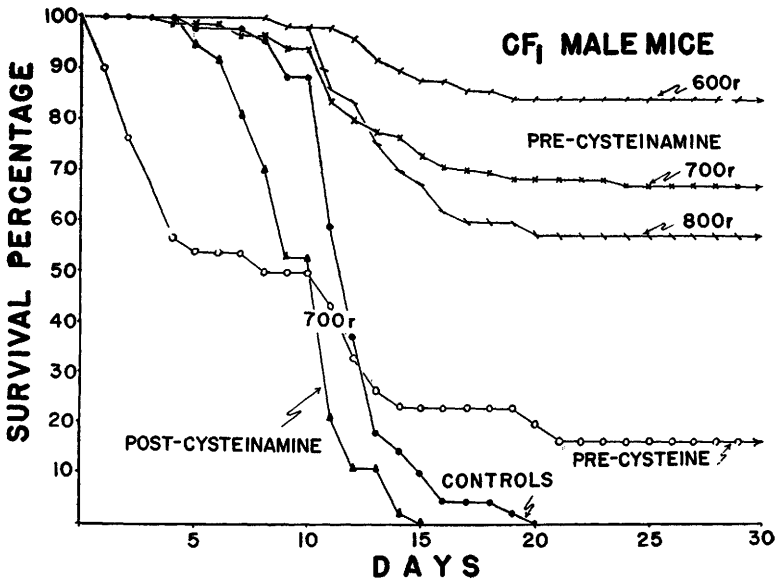

FIG. 2.

of the control C_{57} black mice within 8 days was 700 r whole body exposure, but that mice receiving prior injection of 3 mg of cysteinamine could tolerate as much as 1300 r or as well as did the controls at 700 r. Further, those receiving the cysteinamine and 700 r showed complete survival to the termination of the experiment in 24 days. The most plausible theory with regard to the mechanism of protection relates to the possibility that some —SH compounds partially or completely inhibit the deleterious action of free radicals, normally found in x-irradiated tissue, or they may even modify their nature in such a manner as to render them less harmful (see discussions of the possible protective mechanism of these compounds)(4,7-12). It is also thought possible that the effective —SH carrying compounds may combine temporarily with some radiosensitive enzymes, making them radioresistant(4). Cysteinamine is reported to be a powerful anti-mitotic agent in concentrations above 10^{-4} in tissue cultures(3,5). In cats and dogs it is reported to produce no change in arterial pressure and respiration (3,5). In rabbits it does show anti-inflammatory properties(13). Some physiological and cancericidal studies have been made in humans(14).

The experiments reported here indicate that cysteinamine is very effective in protection against the radiation syndrome when injected prior to exposure. Although the percentage of survival was not as striking as that reported by Bacq and his coworkers, we have observed that some 32% of the Swiss albino and some 67% of the CF_1 male mice exposed to the lethal dose of 700 r survived beyond the experimental period of 30 days. In fact, 97% of these have survived 45 days. Cysteinamine, if given after x-irradiation not only is ineffectual, but also increases the mortality rate, *i.e.*, the mice thus treated died more quickly as compared with the controls.

Over 100 male CF_1 survivors of 700 r x-rays, pre-treated with cysteinamine, are currently being tested for fertility. A goodly number have already proven to be fertile in spite of the high level of exposure. A complete blood analysis is being made and histopathological changes are being investigated. The results of these studies, to be reported in full at a later date, should cast more light on the mechanism of protection by cysteinamine. With the increasing use of ionizing radiations, both in therapy and diagnosis, the imperative aspect of standardizing the protective procedures is obvious.

Summary and conclusions. Some 32% of the Swiss albino and 67% of the CF_1 male

mice injected intraperitoneally with β-mercaptoethylamine (*i.e.*, cysteinamine) prior to a lethal exposure of 700 r whole body x-irradiation survived the 30-day observation period. Some 97% of these are still alive 60 days after irradiation. The CF_1 mice, exposed to the higher dose of 800 r after injection, showed 58% survival while the controls, receiving only 700 r, all died within 20 days. Thus prior injection of this compound definitely affords radiation protection to mice. Injection after x-irradiation, whether single or multiple, had an adverse effect on survival.

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A Filterable Agent, Recovered from Ak Leukemic Extracts, Causing Salivary Gland Carcinomas in C3H Mice.* (20376)

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In previous experiments reported from this laboratory, Seitz filters were used for the filtration of Ak leukemic extracts; the results, however, did not appear to be entirely satisfactory, since only a comparatively small number of C3H mice developed leukemia following inoculation with such filtered extracts (1). The possibility had to be considered that much of the leukemic agent might have been absorbed, and thus retained, by the asbestos pads used in Seitz filters. For that reason a series of experiments has been carried out in which, instead of Seitz, the Selas† microporous porcelain filter candles have been used for filtration of leukemic extracts. Since the leukemic extracts became more active following centrifugation at 7000 x g(2), such centrifugation was carried out, in this study, prior to filtration of the extracts. Newborn (less than 16 hours old) C3H or C3H(f) mice were inoculated with such filtered extracts, and were then observed for development of leukemia. The results were surprising. Some of the inoculated mice developed typical generalized leukemia. Others, however, developed bilateral carcinomas in the cervical region, arising probably in the salivary glands.

Materials and methods. Leukemic extracts. Mice of the Ak line that developed leukemia either spontaneously, or as a result of transplantation of Ak leukemic cells, were sacrificed by ether inhalation; the livers, spleens, mesenteric tumors and peripheral lymph glands were removed aseptically, and ground for 2 to 3 minutes in a porcelain mortar, chilled (+4°C) physiological solution of sodium chloride added in sufficient quantity to obtain cell suspensions of 20% concentration. These leukemic cell suspensions were

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