Protective Action of Cysteinamine (β-Mercaptoethylamine) Against X-Irradiation-Induced Sterility in CF_1 Male Mice.* (24888)

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It has been demonstrated(1,2) that cysteinamine (β-mercaptoethylamine), an amine containing an –SH radical, is efficacious in affording protection to mice exposed to otherwise lethal doses of x-rays. Rugh and Wang (2) showed that in a group of male CF_1 mice given cysteinamine intraperitoneally prior to a single 100% lethal dose of 700 r whole-body exposure of x-rays, 67% survived for the 30-day observation period. It is the purpose of this investigation to determine whether cysteinamine has an equally protective effect against x-ray induced sterility in CF_1 male mice.

Materials and method. The albino mice used were of the CF_1 strain, 2 months of age, and weighed approximately 25 g each at time of x-irradiation. They were randomly divided into groups and treated as shown in Table I. The groups in Table I consisted of: (a) normal male controls; (b) males receiving whole-body exposures of 625 r x-irradiation; (c) males receiving exposures of 700 r x-irradiation limited to the testes; and (d) males receiving pre-treatment of cysteinamine and whole-body exposures of 700 r x-irradiation. Cysteinamine was given *via* the intraperitoneal route in a dose of 3 mg (in 1 ml physiological saline solution), 5 to 15 min. prior to irradiation. Those mice which received whole-body exposure to x-rays were irradiated 5 at a time in a perforated, cylindrical plastic cage measuring 13.25 cm inside diameter and 4 cm inside height. Calibration of the x-irradiation was made in air with the ionization chamber in the center of a closed cage. Constant movement of the mice within the cage tended to minimize the effect of any small difference in concentration of x-irradiation that might exist between the center and the periphery of the cage, all of which was well within the exposure field. The mice receiving 700 r to the testes only, were anesthetized with 0.25 ml of 0.3% pentobarbital sodium given intraperitoneally. These mice were then pinned to a board, covered with a lead shield that protected all of the body except the testes and posterior extremities, and exposed to x-rays 5 at a time. In all cases the distance from the target to the center of the mouse's body was 30 cm. Other physical constants of x-irradiation were: Westinghouse Quadrocondex constant-potential x-ray machine run at 210 KV, 15 MA, filtration 0.28 cu and 0.50 Al, and a dose rate of 265 r per min. in air. Exposure time for mice receiving a total exposure of 625 r was 2′ 22″, and for those receiving 700 r, 2′ 39″. Following x-irradiation, the animals were placed 5 in a cage, then returned to the animal rooms where they received the usual care, with food and water *ad libitum*. A daily record of deaths recorded for a period of 30 days. Then, the surviving mice were mated as follows: one x-irradiated male with 2 normal females per cage, and similar pairing for the normal controls. After mating, the mice were checked daily for fertility for a period of 9 months. The criterion for fertility was the production of litters by one of female mice in the cage for each of the 9 successive 30-day periods. Each litter was counted, weighed, recorded, then removed from the mother. The experiment was terminated when the mice reached 12 months of age, in order that natural reproductive senility should not influence the fertility records.

Results. Fertility records of the males are summarized in Table I. At no time were any of the experimental groups completely sterile, although there were differences in per cent fertility among the 4 groups. During the first 30-day mating period, fertility rate was 71.4% among mice pretreated with cystein-

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TABLE I. Mortality and Fertility Rates in Male Mice X-irradiated under a Variety of Conditions.

Period	Normal controls		625 r whole-body irrad.		700 r to testes		Cysteinamine + 700 r whole-body irrad.	
Mortality	No. surv. / No. orig.	% surv.	No. surv. / No. orig.	% surv.	No. surv. / No. orig.	% surv.	No. surv. / No. orig.	% surv.
30 days	38/40	95	60/279	21.5	80/90	88.9	46/70	65.7
Fertility (30 day periods)	No. fert. / No. surv.	% fertile	No. fert. / No. surv.	% fertile	No. fert. / No. surv.	% fertile	No. fert. / No. surv.	% fertile
1	34/38	89.5	27/59	45.8	30/78	38.5	15/21*	71.4
2	36/37	97.3	50/57	87.7	26/77	33.8	18/20	90.0
3	34/37	91.9	43/54	79.6	60/77	77.9	16/20	80.0
4	"	"	34/51	66.7	66/74	89.2	13/18	72.2
5	31/37	83.8	29/41	70.7	66/74	89.2	11/18	68.8
6	27/37	73.0	25/35	71.4	57/73	78.1	8/13	61.5
7	19/20*	95.0	18/28	64.3	43/47*	91.5	8/ 9	88.9
8	16/19	84.2	17/25	68.0	39/46	84.8	7/ 9	77.8
9	13/19	68.4	12/22	54.5	37/45	82.4	7/ 9	77.8

* Some mice in these groups were discarded by mistake, and were not tested for fertility.
Surv. = survival; orig. = original; fert. = fertile.

amine which survived 700 r whole-body exposure. This high fertility rate is not significantly different from that for the normal controls (P greater than 10% using χ^2 test corrected for continuity). On the other hand, during the same mating period, there is a significant difference between normal controls and mice which received only 625 r x-irradiation without cysteinamine pretreatment as well as those which received 700 r to the testes only (P less than 1%). There is also a significant difference between the cysteinamine pretreated group receiving 700 r whole-body irradiation and the untreated group with irradiation only to the testes (P less than 5%).

By the end of 90 days post-irradiation, the untreated group which received 625 r whole-body irradiation had fertility rates not essentially different from those observed in the group pretreated with cysteinamine and irradiated with 700 r over the whole body. On the other hand, the untreated group which received 700 r to the testes only showed recovery in fertility during the third period, or 120 days post-irradiation. During these periods, there is no significant difference in fertility rates among all groups.

Discussion. When administered prior to x-irradiation, cysteinamine has been shown to be very effective in increasing percentage of survival of mice given otherwise lethal doses of x-rays(1,2). Our data (Table I) on percentage of survival is well in accord with that reported by the above authors; 65.7% of the males pretreated with cysteinamine survived 30 days after whole-body x-ray exposure of 700 r. Of the males pretreated with cysteinamine which survived exposure to 700 r, 71.4% proved to be fertile 60 days after irradiation; the difference in per cent of fertile animals between this group and the normal controls is not significant. At the same time, the per cent of fertile males was significantly below normal (P less than 1% for both groups) in those groups which were not pretreated with cysteinamine and received, respectively, dose of 625 r total-body exposure and 700 r directly to the testes. The former of these 2 groups returned to normal 90 days after being x-rayed, while the latter did not return to normal until 120 days after x-irradiation (Fig. 1). Thus, the most important finding in this investigation is that cysteinamine is capable of preventing the transient sterility in the first 2-3 months following x-irradiation given to the whole body or to the testes alone.

Rugh and Wolff(3) found that whole-body x-irradiation of 50 r will sterilize all CF_1 female mice within a period of 30 weeks, and that 100 r will sterilize all the mice within a period of 8 weeks. They(4) also found that cysteinamine injected prior to x-irradiation with 50 r, prolonged average fertility expectancy by 11.42 weeks, but did not prevent

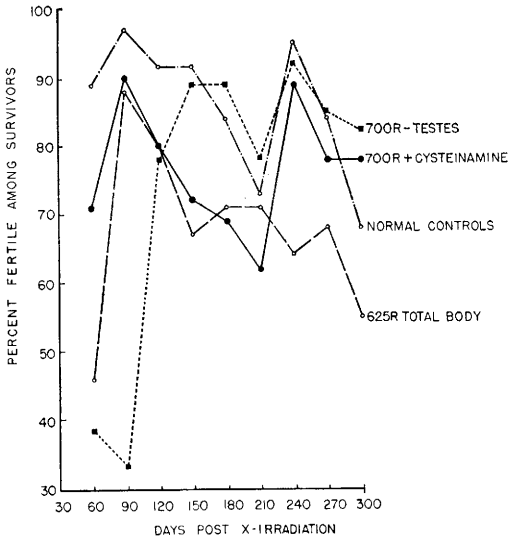

FIG. 1. Fertility records for males.

eventual sterilization even at this low dosage. Their preliminary experiments using 100 r indicated that this level of exposure was so high, and onset of sterilization so rapid, that it would be difficult to demonstrate the protective effect of any drug.

In male mice, there ensues following x-irradiation a brief period of fertility attributable to the presence at time of exposure of resistant mature sperm(5). Since our males were not mated until 30 days post-irradiation, we could not observe whether cysteinamine had any effect on this period of fertility. Following x-irradiation, there occurs a maturation depletion period(6), *i.e.*, progressive loss of spermatogenic cells, beginning with spermatogonia and progressing in order of developmental sequence until reduction in number of spermatozoa occurs(7). This "maturation depletion" leads to a period of sterility owing to the fact that the maturation process has been interrupted.

Following this period of sterility, if the animal does recover fertility, the time of its return depends on dose and species used. Eschenbrenner and Miller(8) showed that in mice given a whole-body exposure of 400 r, one finds spermatozonia, spermatocytes and a very few spermatozoa 4 weeks after x-irradiation; and at 6 weeks after exposure the tubules of the testes contain all spermatogenic elements from spermatogonia to mature spermatozoa, although the population is not yet normal in numbers. Snell(9) demonstrated that in 2 males x-rayed with 800 r, motile sperm had reappeared in the epididymides by 20 weeks after treatment, and then when one of these males mated with 2 normal females, each female gave birth to a litter 16 weeks after treatment. The similar mating of a male x-rayed with 600 r resulted in litters at 26 and 28 weeks after treatment. It can be calculated from our data that for CF_1 males, it takes up to 70 days for total recovery to occur after a whole-body exposure of 625 r, and 100 days after 700 r irradiation given to the testes. The time span accords with that reported in the literature.

Investigators agree that the spermatogonia are the most radiosensitive of the spermatogenic elements, and that owing either to the effect of x-rays in inhibiting mitosis in the spermatogonia(8,10,11) or to the fact that irradiation causes their death(7), there is no restoration of the spermatogenic elements until the spermatogonia are replenished. It would appear from our observations on the mice x-irradiated with 700 r and pretreated with cysteinamine, that cysteinamine is capable of protecting the pre-spermatogenic elements from x-ray damage. It is thought possible that the effective –SH carrying compounds may combine temporarily with some radio-sensitive enzymes, thus making them radio-resistant(4).

Summary and conclusions. Cysteinamine, when administered prior to x-irradiation, has been shown to be very effective in increasing percentage of survival of CF_1 mice given otherwise lethal doses of x-rays. It is also capable of preventing transient sterility in male mice during first 2 or 3 months following x-irradiation given to whole body or to testes alone.

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A Method for Investigating Net Water Fluxes Across Individual Proximal Tubules.* (24889)

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In attempting to evaluate possible mechanisms involved in transport of materials by the kidney, it would be useful to know the kinetics of the transport processes. Experiments estimating rates of water movement across the tubules of Necturus have been carried out by Schatzmann *et al.*(1), using a perfusion system. It is the purpose of this report to describe a simple method for determining net water fluxes across tubules of rat kidneys.

Methods. Micropipettes were drawn with a Brinkman Micro-puller. They were half filled with a test solution by gentle heating of the broad end and plunging the still hot pipette into a beaker containing the test solution. As the air within the pipette cooled and contracted, the solution was drawn into the pipette. The pipette was then mounted in the pipette holder of an Aloe microinjection apparatus. Oil was then forced in the open end of the pipette, expelling the remaining air through the micro-orifice, thereby filling the remainder of the pipette. The microinfusion apparatus is shown in Fig. 1. A one cubic centimeter syringe was mounted on a brass rack which held a threaded rod for adjusting delivery rate. A short length of rubber pressure tubing was wired to the syringe and the other end of the pressure tubing was wired to an adapter which was in turn connected to the pipette holder by means of plastic tubing. The whole system was filled with mineral oil. Upon pushing in the syringe plunger, the pressure tubing inflated and thereby acted as a pressure reservoir providing a relatively constant rate of infusion. Some pressure drop undoubtedly takes place as delivery proceeds but since the volume delivered is very small compared to total volume of the system (less than one part in 2000), pressure falls caused by fluid infusion can be neglected. To avoid leakage around the syringe plunger, tight fitting tuberculin syringes were selected, and no effect of leakage on infusion rate has been detected. A control experiment whereby delivery rate of solution was determined as a function of time is shown in Fig. 2. In this experiment, time required for the oil-solution meniscus to move between the major divisions of an ocular micrometer was determined as the infusion apparatus delivered solution into a pool of fluid held in a planchet. The volume delivered was estimated from measurements of the pipette dimensions. Rate of delivery was purposely made higher than that to be used in biological experiments since it was thought that at high delivery rates the chance of detecting inadequacies in the system would be enhanced. To determine infusion rate, movement of the oil-solution interface was monitored with a telescope mounted on a microscope. The fine adjustment knob on

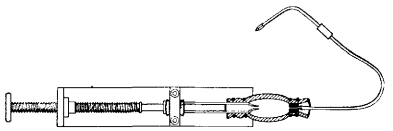

FIG. 1. Schematic drawing of infusion apparatus.

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