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Sex Hormones as Protective Agents Against Radiation Mortality in Mice.* (21001)

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This report gives results of experiments on the protective effect of sex hormones against acute radiation mortality in dba mice. It has been known for some time(1,2) that in Swiss mice estrogens afford protection while androgens(2) do not. The dba mouse has been shown to be protected by the adrenal steroids, desoxycorticosterone acetate and cortisone(3). Moreover, this mouse strain showed a high sensitivity to head irradiation (3), which was perhaps attributable to injury of the pituitary(4). In view of the strain differences in radiation sensitivity seen in the course of these experiments, it was deemed desirable to examine the protective effect of sex hormones on dba mice. Since the hitherto reported protective action of estrogens was found only in preradiation-treated Swiss mice, the following experiments were carried out with postradiation as well as preradiation treatments.

Materials and procedures. a) *Mice.* Animals used in these experiments were all from our own inbred strain of dba mice. Males and females, 6 weeks old and weighing 20 g, were used. Purina Fox Chow and water were always available. b) *Hormones.* The two estrogenic hormones used were: diethylstilbestrol and α-estradiol benzoate. The two androgenic hormones were: testosterone propionate and depo-testosterone cyclopentylpropionate (the oil-soluble 17(beta)-cyclopentylpropionate ester of testosterone). c) *Radiation.* X-rays with a H.V.L. of 0.9 mm Cu had a dose rate of 150 r/min. at 30-cm target distance. The LD_{50} at approximately 12 days has been determined to be 500 r for whole-body radiation(1,2) of the dba mouse. This dose causes 100% mortality in about 20 days. The hormone was administered subcutaneously in 7 daily doses, with the 7th dose given immediately before radiation; in other animals it was given in 7 daily doses after irradiation, the 1st dose being given immediately after radiation. Table I gives the schedule of dose for each of the 4 hormones as well as the controls. For each hormone one control group had the radiation dose but no hormone while another control group was given hormone and no irradiation. The hormone dose was determined to be well below the limit of toxicity.

Results. Table I shows that the androgenic hormone provided no protection against the lethal effect of the 500-r x-ray dose. These hormones given before or after irradiation caused no change in the mortality rate. On the other hand, the estrogens reduced the mortality at 60 days after irradiation.

Fig. 1 shows that the mice without estrogen were all dead after 22 days. The effect of the stilbestrol treatment was to introduce a latent period before any animals died. This latent period was about 14-19 days for post-irradiation and 23-26 days for preirradiation administration of the diethylstilbestrol. The survival at 60 days is 35-40% for post- and 20-25% for pretreated mice.

The α-estradiol-benzoate-treated mice, Fig. 2, showed no latent period, except possibly

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TABLE I. Effect of Various Hormones on Mortality Rate Following Whole-Body Radiation (500 r) to DBA Mice.

Compound	Daily dose, mg	Time of administration, days*	Mortality at 60 days ♂ %	♂ No.†	♀ %	♀ No.†
Stilbestrol	.1	7 pre	80	20	75	20
"	.1	7 post	65	20	60	20
Control	.0	—	100	20	100	20
" ‡	.1	7	0	10	0	10
Estradiol benzoate	.09	7 pre	10	20	5	20
Idem	.09	7 post	30	20	15	20
Control	.00	—	100	20	100	20
" ‡	.09	7	0	10	0	10
Testosterone propionate	1.5	7 pre	100	15	100	15
Idem	1.5	7 post	100	15	100	15
Control	.0	—	100	15	100	15
" ‡	1.5	7	0	10	0	10
Depo-testosterone cyclopentylpropionate	.5	7 pre	100	15	100	15
Idem	.5	7 post	100	15	100	15
Control	.0	—	100	15	100	15
" ‡	.5	7	0	10	0	10

* *Pre* means daily dose given each of 7 days before day of irradiation. *Post* means daily dose given each of 7 days following irradiation, the 1st dose given on day of and immediately after irradiation.

† Total No. of animals employed in each experiment.

‡ No radiation.

the males treated before irradiation where the latent period may be 14 days. In all other animals deaths occurred beginning on the 3rd or 4th day. Fig. 2 shows that the survivals with estradiol dosage were significantly higher than with diethylstilbestrol.

Discussion. The foregoing data show that the acute radiation mortality due to 500-r whole-body dose is altered by the administration of estrogenic hormones either before or after the irradiation. There is a difference between the types of survival which result from the use of the two estrogens as protective agents. The synthetic compound, diethylstilbestrol, causes a delay in mortality, the delay being about 10 days longer when the hormone is given before radiation than when given after radiation.

The delay, or latent period, occurs in all animals treated with diethylstilbestrol, whereas it is seen only as a 9-day delay in males treated preradiation with estradiol. All other animals treated with estradiol showed the same immediate lethal effects of radiation as did the controls, although the ultimate survival with this estrogen was greater than with diethylstilbestrol. The data show that the natural hormone is more effective in dba mice than is the synthetic compound. This difference in estrogen protection is seen in the results of Graham and Graham(2) who worked with Swiss mice exposed to 400-r whole-body irradiation.

Fig. 1 and 2 show that estrogens afford protection when administered daily for 7 days after the radiation dose. This is in contrast with the results reported earlier by Treadwell *et al.*(1) and Graham and Graham(2) in which estrogens given simultaneously with or immediately after the radiation increased the lethal reaction of radiation. With the exception of 25 males given diethylstilbestrol in the Graham's report, the estrogens appear to be "toxic" to mice when administered immediately after the radiation exposure. The fact that the dba mice reported here were protected by estrogens given for 7 days post-radiation raises the interesting question as to when the estrogen reaction changes from one of "toxicity" to one of protection in the interval after irradiation. Since protection is found in the dba mice and had not been found in the Swiss mice, the effect is being studied with possible mouse-strain differences in mind.

Testosterone propionate administered immediately after radiation showed a marked

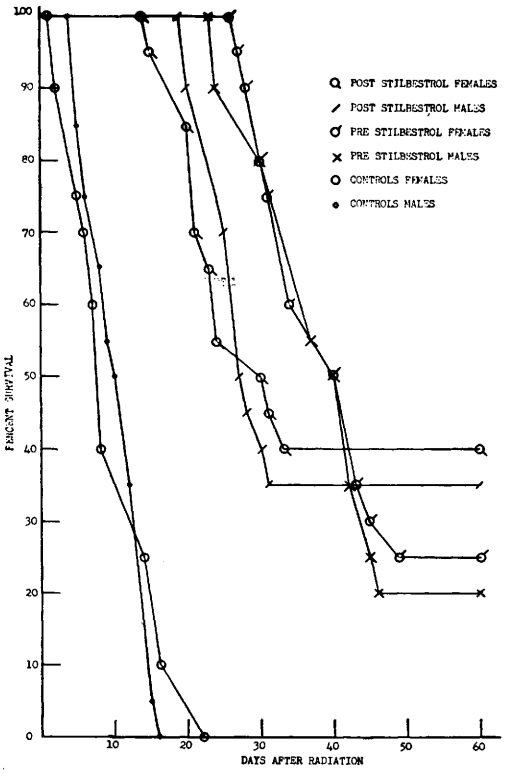

FIG. 1. Survival curves of dba mice exposed to 500-r whole-body radiation. Comparison of mice given diethyl stilbestrol for 7 days before and 7 days after radiation with controls which received no estrogen.

"toxic" effect in Swiss mice as reported by Graham and Graham(2). The same mice showed no evidence of protection when the androgen was administered 10 days before the radiation, which is corroborated by the data in Table I on dba mice. It should be pointed out, however, that the two androgens given postradiation for 7 days in our work did not increase the rate of mortality, *i.e.*, the apparent "toxic" effect reported by the Graham's was not seen in our results. However, opportunity to exhibit a toxic effect may not have been present in our experiment because of the larger radiation dose used, *i.e.*, 500 r as compared to 400 r.

With the qualification that there may be important mouse-strain differences, it appears that immediately after irradiation the mouse physiology cannot accommodate androgens and estrogens. Recovery is rapid, in the dba mouse much more rapid than the lethal processes, so that by the 7th day postradiation normal accommodation has been restored to the extent that estrogens alter the lethal processes initiated by radiation injury. Further work is in progress to determine the duration of the interval in which recovery occurs.

The postradiation administration of estrogen and its protective action on mice is of interest when compared with other substances. Cole *et al.*(5) have already pointed out that cysteine, glutathione, and sodium nitrite are effective protectors when administered before but not after irradiation. They also cite anoxia during radiation, which is protective if administered during but not after irradiation. Chemicals which are effective protectors when given postirradiation appear to be: desoxycorticosterone acetate in Swiss male mice, as reported by Ellinger(6), and in dba mice, as reported by Mirand, Reinhard, and

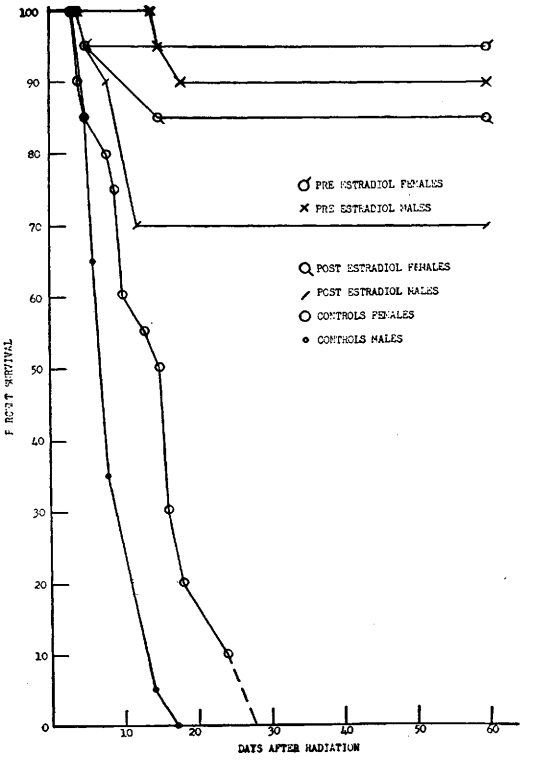

FIG. 2. Survival curves of dba mice exposed to 500-r whole-body radiation. Comparison of mice given estradiol benzoate for 7 days before and 7 days after radiation with controls which received no estrogen.

Goltz(3); the latter also reported cortisone as effective in dba mice. Jacobson *et al.*(7) found spleen transplants to be protective; this work was extended by Cole *et al.*(5) who found spleen homogenates affording protection in LAF_1 mice. Bacq *et al.*(8) mention cysteine as being a protective agent in rats if the rat liver is shielded during irradiation.

The marked ability of estrogens to ameliorate radiation injury has been noted in clinical radiotherapy. Postmenopausal patients show much more drastic symptomatic reactions than do premenopausal cases. The inference has been that endogenous levels of estrogen are sufficiently high to afford protection against radiation injury in the premenopausal state but are lacking in the postmenopause period.

Summary. 1. In dba mice diethylstilbestrol and estradiol benzoate reduce the acute radiation mortality. The androgens, testosterone propionate and depotestosterone cyclopentylpropionate, afford no protection against acute lethal radiation dose. 2. The estrogens afford protection when given for 7 days before or after the irradiation. 3. No toxic effects due to estrogens or androgens were seen in the administration of the hormones for 7 days after irradiation.

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Influence of X-Irradiation on Oxygen Poisoning in Mice.* (21002)

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The initial work on this subject already reported(1) showed a synergism between x-irradiation (8800 r) and oxygen (5-6 atmospheres) in their simultaneous effects on the survival times of mice. When intervals of 2 minutes, 30 minutes, and 2 hours were allowed between X-irradiation and oxygen poisoning, the small but significant shortening of the survival times in oxygen gradually decreased and disappeared within 5 hours. These experiments gave some support to our hypothesis on a possible common mechanism between the initial effects of X-irradiation and the effects of high oxygen tensions. When an 18-hour interval was allowed, a nonsignificant, slight reversal of this phenomenon was noticed, showing now a slight tendency to protect against O_2 poisoning. It was evident that further investigations with increasing intervals might reveal some interesting influence of the secondary phenomena of X-irradiation on oxygen poisoning.

The material presented here is a study of the effects of 8800 r (which will kill these mice in about 4 days) on the survival times of mice submitted to 6 atmospheres of oxygen

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