Post-Irradiation Castration of the Male and Enhanced Survival.* (29466)

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It has been reported that in the CF1 strain of mice males are more radiosensitive than females, but if castrated the males then approximate the LD/50/30 for acute X-irradiation of the females, suggesting the adverse effect of the presence of androgens(1). In contrast, Hamilton *et al*(2) using the LAF strain of mice exposed to 145 r/daily until death observed that males survived longer than females. While this was due to the daily accumulated damage from X-irradiations and may not be comparable to the single acute exposure, it points up the fact that the irradiation conditions are important in evaluating the lethality response of mice. Further, Kohn and Kallman(3) using CAF_1 mice, and Crosfill *et al* using SAS/4 albino mice(4) showed that over the entire mouse life span there is little or no difference in lethality of the two sexes after a single acute exposure. Thus there may well be strain or species differences in the dimorphic sex response to whole body X-irradiation.

The CF1 strain used here consistently shows the female to be the more radioresistant by 50 roentgens at the LD/50/30 range. But lethality is affected by a number of extraneous or environmental variables including sexual activity(5), so that it is imperative that all variables are eliminated with the exception of the one being tested.

Materials and method. The 2 strains of male mice used were the ICR and CF1. Recently weaned males of 2 months of age, and fully matured males of 4+ months of age were used in order to determine what effect sexual maturity might have. The radiation facilities included dual X-ray tubes set for crossfire of the animals at 28.5 cm from the center of the bodies of the mice so that the air dose at that region was 300 rads/minute. Filtration included 0.28 mm Cu. and 0.50 mm Al. with an HVL of 0.6 mm Cu. The exposure was consistently for 2 minutes at 30 MA and 184 KVP. All exposures were made in the morning(6), in a 2-3 hour period during which there were no fluctuations in the radiation output. Following exposure some of the mice were castrated and all were returned to their stainless steel cages, given food and water *ad libitum,* and placed together in the animal room kept at 25°C.

The records consisted of daily checking of all mice for deaths. Fig. 1 and 2 present the daily changes in survival percentage under the different conditions imposed: X-irradiated, castrated, and X-irradiated followed by castration. Five sets of data involving 1,000 mice were collected. The two sets of data from the young and the two sets of data from the old CF1 mice were combined (Fig. 2), since they represented identical sets of variables.

Experimental findings. Since there are strain differences in mice the initial series concerned the ICR male mice (Fig. 1). Castration alone accounted for 10% mortality by 30 days in this initial series, all occurring by the 10th day. In the 4 following series with the CF1 strain only 1 castrated mouse in 200 died as a result of the operation, the difference being due to technical improvement and better asepsis, not the strain of the mouse. The ICR mice that were exposed to 600 r X-rays showed 53% survival (close to the LD/50/30 level) while similar males, also X-irradiated to 600 r but castrated within 30 minutes thereafter, showed 72% survival (54 out of 75) which is statistically significant. However, since 10% of the castrated mice might have died from the operation alone (see upper curve), this figure, corrected, would give 82% survival. Even without this correction to 29% improvement, the 19% improved survival indicated the advantage of post-irradiation castration. It will be

* Based on work performed under Contract AT-(30-1)-2740 for U.S. Atomic Energy Commission and aided by Grant RH 81 from Division of Radiological Health, Bureau of State Services, U.S.P.H.S.

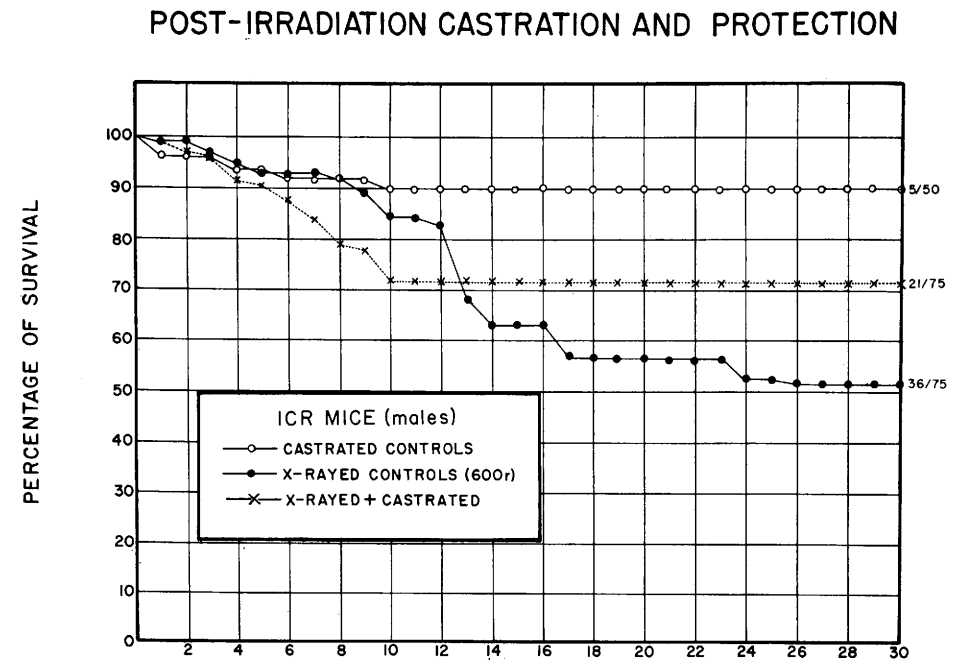
POST-IRRADIATION CASTRATION AND PROTECTION
PERCENTAGE OF SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
ICR MICE (males)
CASTRATED CONTROLS
X-RAYED CONTROLS (600r)
X-RAYED + CASTRATED
5/50
21/75
36/75
DAYS FOLLOWING WHOLE BODY X-IRRADIATION

FIG. 1.

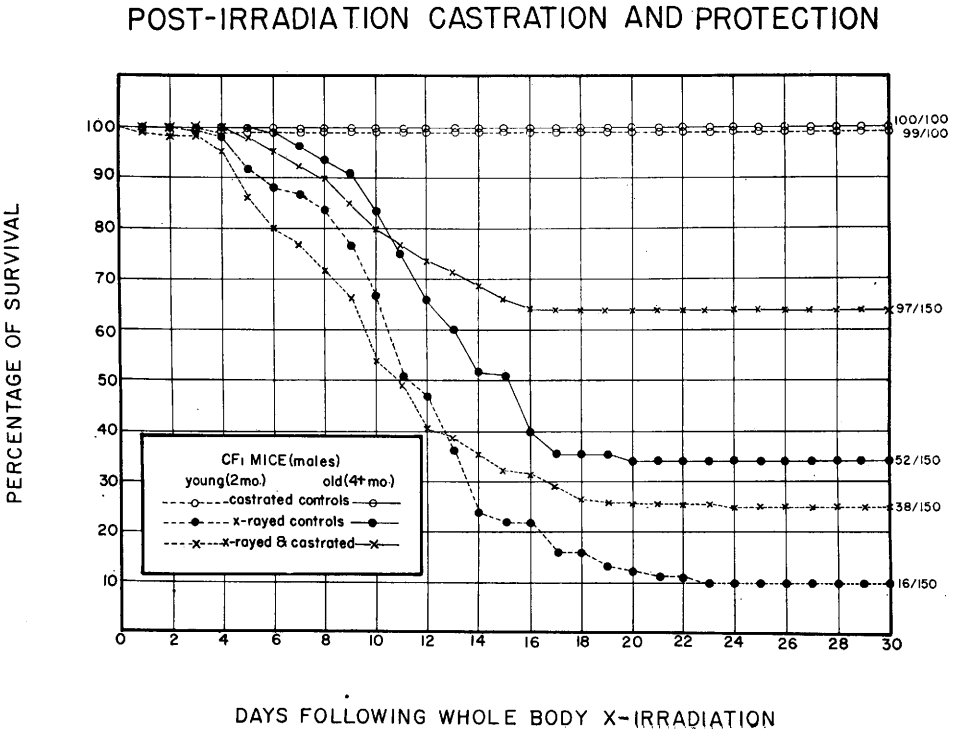
POST-IRRADIATION CASTRATION AND PROTECTION
PERCENTAGE OF SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
CF1 MICE (males)
young (2mo.) old (4+mo.)
castrated controls
x-rayed controls
x-rayed & castrated
100/100
99/100
97/150
52/150
38/150
16/150
DAYS FOLLOWING WHOLE BODY X-IRRADIATION

FIG. 2

noted that deaths among the X-irradiated (but not castrated) continued to the 26th day while among those that were both X-rayed and castrated deaths had ceased by the 10th day.

Castration in CF1 mice resulted in but one death in 200 operations. The young mice showed better survival by 15% if, after irradiation, they were castrated. The X-irradiated older mice showed a life saving benefit of 30% by castration. Thus, in both the young and the older mice there was a statistically significant increase in the survival of mice castrated shortly after X-irradiation at the LD/50/30 level. The protective value of castration was somewhat less among the younger mice but in both groups deaths occurred among the X-rayed mice at dates later than among those that had been castrated. It will be noted that the LD/50/30 for the CF1 males must be below 600 r, since this exposure provided for 34% survival, but for the ICR strain 600 r does allow 52% survival of the older mice. The time of approximating the lethality curves of both young and older CF1 mice was at 11 days, after which the benefits of castration were noticeable. It should be noted that for the duration of the experiment (30 days) there was no significant alteration in total body weight following the various procedures, except possibly for those mice that were moribund. Those alive at 30 days were quite normal.

Certain supplementary observations are made, the percentage values given on the basis of 50 males (CF1) for each set of categories. When estradiol benzoate (0.166 mg/mouse) is given intraperitoneally after X-irradiation, there is a 50% increment in the final survival figures for the young males while the more adult males showed a 26% increment. This dose had been shown to be effective with these mice(1). For some reason the castration benefits were nullified by the subsequent injection with estradiol, and no improved survival accrued. The simple delay of castration by 24 hours improved slightly the survival of both young and old mice, but not to the significant level attained by immediate castration. Finally, if the castration was delayed 24 hours and the mice were then injected with the estradiol, there was considerable (46%) improvement in survival for the older mice. The contrast of survival data of those castrated immediately after irradiation, and injected with hormone, and those in which this was done after a 24-hour delay, may be attributable to the physiological adjustments necessary on the part of the male suddenly deprived of testes androgen and being infused with estradiol benzoate, all superimposed upon the trauma of castration.

The general results of this supplemental portion of the study suggest that the best situation for survival obtains when there is immediate castration without addition of the female hormone. If castration is necessarily delayed by 24 hours, then addition of estradiol benzoate seems indicated.

Since the impetus for this study was the idea that castration might cause leucocytosis and hence aid in survival of LD 50 levels of X-irradiation, it seemed appropriate that a series of parallel blood studies be made. The data are preliminary for the ICR mice and do not include large numbers. The data are suggestive and include castrates and sham-operated mice, all checked before the surgery. The sham operation consisted of opening the scrotal sac without removal of the testes. It was found that castrated mice showed an average increase in average total white count to 14,870 which was 5,920 above the control average of 8,950. The sham operation showed an average total white count of only 9,900. The lymphocyte count also was increased among the castrated mice, to 8,690 or 2,872 above the control average of 5,818. Likewise the neutrophil averages were higher among the castrates than among the controls or sham operated mice. In general the increments were greater toward the end of the observation period.

Both the young and the older CF1 male mice studied hematologically over the 4-week post-castration period showed a rise in absolute lymphocyte counts. There was a higher increment of lymphocytes among the young mice, while their neutrophils maintained the original level. Thus, in these studies the average lymphocyte counts of *both* ICR and

CF1 male mice increased with castration. While the data are statistically adequate, they are indeed suggestive that one of the possible reasons for the better survival of the post-irradiated and castrated males is that castration stimulates lymphopoiesis.

Discussion. Castration of the male rodent can be accomplished easily, and is well tolerated. Surgical castration results in regression of the accessory organs(7), histologically demonstrable by 20 days. In the CF1 strain, where the female is more radioresistant than the male(1), castration improved the survival of males to 50% over the intact males, receiving 625 r X-rays, all of which died within 17 days. When castrated males were pre-treated with estradiol benzoate and then X-irradiated, their survival was further improved, almost to the level of the intact females. Local tissue effects of irradiation on the jaws of rabbits were inhibited by castration or enhanced by the injection of testosterone(8). Irradiation does not endocrinologically castrate the testes because it has no appreciable effect on androgen production (9). Following irradiation the mortality and morbidity of mice were enhanced by injection with testosterone propionate, but not with testosterone cyclopentylpropionate which also has an anabolic and androgenic effect(10). In the strain used here (CF1) there is no doubt but that, with respect to the tolerance of X-irradiation, the androgens are deleterious and the estrogens are beneficial. But estrogens given 7 days or more *before* the irradiation to intact males appear to be the most protective(11,1). Over a longer period, castration of males increased the probability of breast cancer(12) so that there is evidence of a slow but direct hormonal relation to the health, resistance to irradiation, and well-being of the male rodent. The presence of androgens reduced resistance to irradiation but their removal made the mice subject to female malignant sequelae.

Regarding the differential sex sensitivity to whole body X-irradiation there are indeed disparities in the literature. It seems that there are strain differences or that the quality and/or quantity and/or the dose rate factors are important considerations. In our CF1 strain there has always been about a 50 r difference in the tolerance of females over males, at LD/50/30 levels and to acute exposure. In the LAF strain(2) when the 2 sexes were subjected to daily exposures of 145 r the females succumbed about 7 days prior to the males. This situation did not involve acute exposure and may be explained on the basis of the better recovery of the male from fractionated doses. Kohn and Kallman(3), and Crosfill *et al*(4) were unable to demonstrate any sex difference in response to whole body irradiation over rather long periods of the life spans of still different strains of mice. More recently Aleksandrov and Galkovskaya(13) attributed any sex difference in radiosensitivity of white mice to the superior ability of the female to repair irradiation damage. Thus, sex differences in radiosensitivity are not consistent in the different strains.

Castration imposed within minutes after irradiation can hardly have an instantaneously beneficial effect. Nevertheless, the delayed effects of castration, especially if they involve alterations in tissue metabolism(14) could be significant. But castration is said to cause an increase in liver, kidney, and brain tissue uptake of oxygen, and testosterone causes a decrease. This does not fit into the known situation of enhanced resistance to X-irradiation of anoxia, unless the increased uptake of oxygen by some tissues following castration results in the better tolerance by vitally more important tissues.

The original incentive for this study was the suggestion that castration led to lymphopoiesis, and knowing that X-irradiation caused lymphopenia, it was thought that the effects of the two conditions—castration and irradiation—might be mutually neutralizing, resulting in better survival. Thus, the supplemental study of blood cell changes, although based on relatively few animals, does suggest that castration increases absolute lymphocyte production, particularly after some delay. It should be pointed out that the curves of lethality cross at about 11 days, indicating that before that time there is no beneficial effect of castration.

The practical applications of findings such as these are limited. Since the protective value of castration is within the range of 30% or so, it might be recommended in human cases, only when the whole body exposure was believed to be close to, but not above, the LD/100/30 level, (or 600 r X- or gamma rays). In such cases a combined treatment with bone marrow (or spleen) and castration might well mean the difference between survival and death of the irradiated male.

Summary and conclusions. 1. Male mice of the ICR and CF1 strains were X-irradiated to the LD/50/30 range and some were castrated in order to determine whether the loss of testosterone might alter survival. 2. In both strains, post-irradiation castration (within 30 minutes) did indeed increase statistically the survival of the mice. 3. A delay in castration of 24 hours after irradiation (of the CF1 males) increased survival chances, and estradiol (at that time) further improved survival probability. However, estradiol benzoate given immediately after X-irradiation and castration had an unfavorable effect. 4. It is suggested that the mechanism of effect of castration may be in the encouragement of lymphocyte recovery, thus reducing the usual effects of lymphopenia attendant upon X-irradiation. 5. Since the survival improvement by post-irradiation castration is statistically significant, but amounts to only about 30%, its efficacy lies near the LD/100/30 range, and not higher. Possibly in combination with bone marrow the benefits would be additive, or even synergistic.

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Received May 5, 1964. P.S.E.B.M., 1964, v116.

Toxicity of High Levels of Calcium-45, Strontium-90 and Promethium-147 to Avian Embryos.* (29467)

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The increasing exposure of man to radioactive substances in his environment necessitates a knowledge of the biological effects of prolonged or continuous irradiation. The chick embryo is particularly suited for a study of irradiation effects as it is completely

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† Research Participant, ORINS, on leave from Dept. of Poultry Science, Texas A. & M. Univ., College Station.

‡ Operated by Tennessee Agri. Exp. Station for U.S. Atomic Energy Commission under Contract No. AT-40-1-GEN-242.