is not definitive in respect to the relative sensitivity of the male and female pituitary to estrogens, there is suggestion that the pituitary of the adolescent male rat is somewhat less sensitive to estradiol than is the female rat pituitary. It should be pointed out that 35-40 g male rats were used in the studies reported here because of the satisfactory dose response of the testes to estrogen, in contrast to the inadequate ovarian changes induced by estrogens in intact rats of this age. Pincus and Werthessen(11) noted that the ovarian response in comparable rats was a function of duration of dosage rather than size of dose, hence we deemed immature female rats unsuitable for assay of suppressive activity.

The much lower UGSt/TGSp dose ratios obtained for several high molecular weight esters of ethinylandrostanediol and its Δ^5-analog than obtained for estradiol or ethinylestradiol suggests that these esters may be relatively more specific than the natural estrogens as inhibitors of pituitary gonadotrophic function. This possibility is being explored in a variety of appropriate follow-up experiments.

Summary and conclusions. (1) The biological activity of ethinylandrostanediol and its Δ^5-analog has been found to be predominantly estrogenic rather than androgenic. The relative testicular growth suppressing (TGSp) activity effected by these ethinyl-diols was considerably greater than observed for equivalent estrogenic doses of estradiol-17β or ethinylestradiol. (2) Low molecular weight esters (less than 6 carbon atoms) of these non-phenolic steroids were more active than their parent alcohols, but the degree of estrogenicity (UGSt) diminished more abruptly than did TGSp activity as the size of the ester group was increased. Of the compounds tested, the 3α-ethylbutyrate of ethinyl-Δ^5-androstenediol effected the greatest degree of testicular growth suppression (TGSp) per unit of estrogenicity (UGSt-ED_{50}), when 35-40 gram immature intacts rats were used.

1. Ruzicka, L., and Hoffman, K., *Helv. Chim. Acta.*, 1937, v20, 1280.

2. Masson, G., and Selye, H., *J. Pharmacol. and Exp. Therap.*, 1945, v84, 46.

3. Leger, J., and Masson, G., *Rev. Canad. Biol.*, 1946, v5, 478.

4. Overbeek, G. A., *Acta Brevia Neerland.*, 1947, v15, 68.

5. Kochakian, C. D., *Am. J. Physiol.*, 1949, v158, 51.

6. Lauson, H. D., *et al., Endocrinology,* 1939, v24, 35.

7. Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 261.

8. Wade, N. J. and Doisy, E. A., *ibid.*, 1931, v28, 714.

9. Greep, R. O., and Jones, I. C., *Recent Progress Hormone Research,* 1950, v5, 197.

10. Byrnes, W. W., and Meyer, R. K., *Endocrinology,* 1951, v48, 133.

11. Pincus, G., and Werthessen, N. T., *Am. J. Physiol.*, 1933, v103, 631.

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Relation of Gonad Hormones to X-Irradiation Sensitivity in Mice.* (22494)

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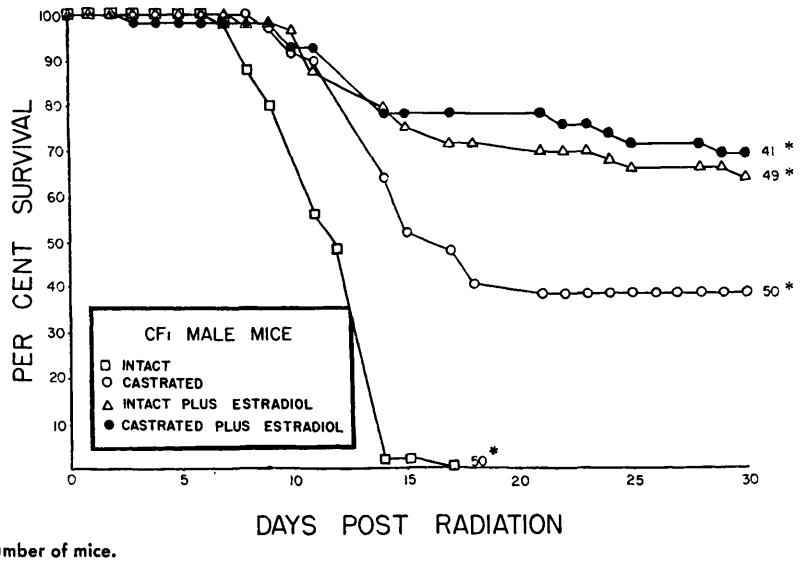
It has been reported(1-5) that a single injection of estradiol benzoate 10 days before an LD 50/30 day exposure to whole body x-rays will increase the resistance of the mouse to the exposure. The present study was designed to determine what effect estradiol benzoate would have in the intact as compared with the castrated male mouse when each is exposed to the LD 50/30 day level of whole body x-rays.

Materials and methods. Ninety-one mature CF_1 male mice were castrated by surgical excision. Aureomycin was sponged over the

* Based on work performed under contract for the Atomic Energy Commission.



* Number of mice.

FIG. 1.

wound to prevent subsequent infection. The mice were then allowed one week to recover fully from the operation at which time 41 castrates and 49 normal, intact males of the same age were each injected intraperitoneally with 0.166 mg of estradiol benzoate in 0.1 cc of sesame oil. Fifty other castrates and 50 intact mice, all of the same age, received no injections. Ten days after hormone injection all of the mice were exposed to 625 r whole body x-irradiation delivered by a Quadrocondex constant potential x-ray machine run at 210 KVP and 15 ma. The rays were selectively hardened by filtration with 0.28 mm Cu and 0.50 mm Al. The distance to the center of the body of each mouse was 30 cm and the output of the machine 271 r/min in air at that position. The mice were irradiated in a plastic cage measuring 14.5 cm in diameter and 4 cm in depth. Lethality data were collected for a period of 30 days.

Experimental. When the percentage survival is plotted against days post-irradiation it is found that the intact male controls, which received no hormone injection, all died by the 17th day. This is not surprising because the dose chosen, 625 r, is LD 50/30 for this species but includes the females which are more radioresistant than the males(6). This dose must then be considered as minimal LD/100/30 days for the adult male CF_1 mouse. At 30 days post-irradiation some 38% of the castrated males were still alive indicating an adverse effect of the presence of the male hormone, testosterone. Maximum survival was found in the castrated males which had also been injected with estradiol benzoate. Of these, 68.3% were still alive. The normal, intact males which had been injected with this hormone showed a survival which was almost as good, namely 63.3%. Thus, the injected female hormone must have counteracted almost entirely the adverse effects of the male hormone.

Discussion. It has been reported that in order to have any protective effect this female hormone, estradiol benzoate, must be injected a number of days before the irradiation(1-5). According to Patt(2) estradiol exerts the maximum protective effect when it is given 10 days before x-irradiation. Patt has also stated that testosterone is ineffectual in protecting mice against irradiation. The above data, relative to the intact mouse, indicate that testosterone is probably deleterious in so far as resistance to x-irradiation is concerned, since all such animals died by the 17th day whereas some 38% of the castrated males, without the presence of the male hormone, survived the 30 day period. In this study

the estradiol exerted its maximal protection when coupled with castration of the male and the consequent removal of the male hormone. Estradiol in the body of the male appears to overcome the deleterious effect of testosterone to a large extent. The intact males which received estradiol showed survival of only 5% less than the castrates plus estradiol. This implies the greater potency of the female as compared with the male hormone, at least in the concentrations available.

Graham and Graham(3) suggested that the resistance to radiation is not related to a simple sex hormone activity. They feel that some extra-gonadal system may be stimulated and protection results. Patt *et al.*(2) suggest that the protective action of estrogens is mediated through the adrenals whereas Ellinger(5) expands this hypothesis stating that "the effect of estrogens may well be mediated by the spleen which is recognized to be under the influence of the adrenal cortex and the functional status of which appears as of great importance for the radiosensitivity in total body irradiation." Bethard, Simmons, and Jacobson(4) report that the survival of mice given estrogens, followed by irradiation with spleen shielding, was greater than when either method was used alone.

Summary and Conclusions. The differential in survival of male and female mice, when exposed to whole body x-irradiation, seems to be due in part to the gonadal hormone present. Intact males, with the normal quota of testosterone, have less survival value than do castrate males. Further, castrate males injected with the female hormone, estradiol benzoate, exhibit maximum survival, approaching that of the intact female.

1. Treadwell, A. de G., Gardner, W. W., and Lawrence, J. H., *Endocrinology,* 1943, v32, 161.

2. Patt, H. M., Straube, R. L., Tyree, R. B., Swift, M. N., and Smith, D. E., *Am. J. Physiol.,* 1949, v159, 269.

3. Graham, J. B., and Graham, R. M., *Cancer,* 1950, v3, 709.

4. Bethard, W. F., Simmons, E. L., and Jacobson, L. O., 1951, ANL 4265, 48.

5. Ellinger, F., *Radiol. clin.,* 1954, v23, 182.

6. Rugh, R. and Clugston, H., *Radiation Res.,* 1955, v2, 227.

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Development of Heterotypic Combinations of Dissociated Embryonic Chick Cells.* (22495)

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Some time ago, attempts were initiated to study the behavior *in vitro* of dissociated embryonic chick cells(1-3). Suspensions of viable cells were obtained by disintegrating organ rudiments of the early chick embryo into their constituent cells, using a procedure based on treatment with trypsin. It was found that, under suitable conditions of cultivation, the discrete cells re-aggregated into clusters, re-established a tissue-like association, and subsequently resumed their characteristic histotypical development. Thus, aggregates of limb-bud mesoblastic cells gave rise to cartilage and muscle, and aggregates of mesonephric cells to characteristic nephric tubules and vesicles. These observations were in line with the results of investigations by Weiss and coworkers(4-6) on the fate of embryonic cells in suspension introduced into embryos or grown *in vitro*. The combined evidence strongly indicated that, under the conditions explored, the dissociated cells retained their ability to restore the special histogenetic pattern of their erstwhile associa-

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