Influence of Toxic Amounts of Estrin Upon Intact and Castrated Male Marsh-Buffalo Mice*

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(Received for publication November 24, 1941)

Evidence (1, 2, 3, 4) has been produced indicating that the Marsh-Buffalo strain of mice is a stumbling block to any attempt to correlate tendency to development of cancer of the mammary gland with the amount of exogenous estrin required to enhance the incidence of this form of cancer in females or to produce it in males. Amounts of estrin which enhance formation of breast tumors in other high-cancer strains of mice fail to do so in either males or females of the Marsh-Buffalo strain. By the use of larger and toxic amounts of estrin in the female, an increased tendency to formation not only of breast tumors, but also of lymphoid tumors has been noted. The purpose of the present experiment was to ascertain whether these toxic doses could produce tumors of the mammary gland in males, whether the gonads were involved and to what extent a response, if elicited, was produced in tissues other than those of the mammary gland.

MATERIALS AND METHODS

Two treated groups of 36 mice each and one control group of 43 mice were segregated by random sampling at the age of 1 to 1½ months. At this time the mice of one treated group were castrated. Administration of the estrogen was begun 1 week later. The crystalline hormone (estradiol)[1] was dissolved in sesame oil in a concentration of 2 mgm. per cc. This was first administered parenterally in doses of 0.04 cc. per mouse twice weekly to both treated groups. The schedule of dosage, the amounts of hormone administered per mouse, and the periods of noninjection, which were interposed when toxic manifestations appeared, follow: 1.36 mgm. hormone per mouse in 60 days, 40-day rest period, 0.64 mgm. hormone in 30 days, 45-day rest period, 0.32 mgm. hormone in 10 days, 80-day rest period, 1.20 mgm. hormone in 95 days, at the end of which time dosage was discontinued.

In Table I are recorded, for the two treated and control groups, the cumulative percentage incidences of lymphoid tumors, breast tumors (adenocarcinoma), and death due to causes not related to tumor formation. The latter data also included mice sacrificed in a moribund condition. The experiment was ended when the mice had reached the age of 15 months as the numbers of surviving mice in the treated series were not large enough to produce additional data which would have significance when subjected to statistical analysis. At this time 12 mice were alive in the castrated group, 10 mice alive in the intact group, and 33 mice alive in the controls. Twenty-five per cent of the intact dosed mice developed scrotal hernias during the course of the experiment. One of the castrated mice and one control mouse developed a fibrosarcoma.

RESULTS

Toxicity.—It was originally planned to administer 3 to 4 mgm. of estrogen per mouse in a 5- to 6-month period as was done in the experiments with female Marsh-Buffalo mice, but the physical condition of the mice after a 2-month period did not warrant a course of continuous treatment. The mice received 2.3 mgm. estrogen per mouse in the first 6-month period and a total of 3.5 mgm. in a 12-month period, so that the total amount was approximately the same as that received by the females. The incidence of death due to causes not related to tumor formation in the series of treated intact mice demonstrates that the amount of estrogen administered was toxic. The high incidence of spontaneous death which occurred when the females received the massive dosage in a 6-month period was avoided. Both series of treated mice increased in weight during the course of the experiment. When first dosed the mean body weights were respectively 15.6 and 16.0 gm. At the age of 11 months the weights were 26.7 and 28.0 gm. respectively.

Tumors.—The incidence of lymphoid tumors for the intact treated male mice is not significantly above the range obtained for the controls. In contrast the high incidence for the castrated mice should be noted, the difference being significantly greater.

No adenocarcinomas of the breast occurred in any

* This investigation was aided by a grant from The International Cancer Research Foundation.

[1] The authors are indebted to Dr. Erwin Schwenk of the Schering Corporation for the supply of estradiol.

but the castrated group, in which only 2 tumors, not differing histologically from those found in females, appeared.

DISCUSSION

The control mice in the present experiment did not receive injections of sesame oil, which we (2) had previously found was slightly toxic to the female and decreased somewhat the incidence of tumors of the breast. Since the male mouse does not develop tumors of the breast spontaneously and since the incidence of lymphoid tumors in control and treated intact mice was the same, the influence of sesame oil in the present experiment cannot be measured.

In the female Marsh-Buffalo mouse, toxic doses of estrin increased the incidence of lymphoid tumors in both intact and ovariectomized animals, and the results of the present experiment are of particular interest in that the castrated male showed a significant increase in incidence of lymphoid tumors in contrast to the intact male. Since the comparison between intact and castrated mice for both sexes was made under strictly controlled experimental conditions (housing, food, injection of sesame oil), it may be concluded that the male but not the female gonad is involved in a defense mechanism. In the case of the adenocarcinoma of the breast the results are similar but less striking. In view of the fact that the occurrence of breast tumors in untreated male mice of the Marsh-Buffalo strain has not been observed in this laboratory or reported from other laboratories, the occurrence of 2 breast tumors in the castrated dosed mice would hardly be fortuitous. The appearance of only 2 tumors confirms previous deductions that the strain is highly resistant to the action of exogenous estrin; that when the action can be demonstrated, it is only after the administration of toxic amounts; that under such conditions the action is not confined to tissues of the mammary gland and the effect upon lymphoid tissue is more marked than the effect upon the breast.

Table I: Cumulative Incidence of Lymphoid Tumors, Adenocarcinoma of Breast, and Death Due to Causes Not Related to Tumor Formation in Intact and Castrated Male Marsh-Buffalo Mice Which Had Received Estrogen and in Controls

	Dosed—castrated (36 mice)			Dosed—intact (36 mice)			Control (43 mice)		
Age in months	Per cent dead of other causes	Per cent lymphoid tumors	Per cent adeno-carcinoma	Per cent dead of other causes	Per cent lymphoid tumors	Per cent adeno-carcinoma	Per cent dead of other causes	Per cent lymphoid tumors	Per cent adeno-carcinoma
5	0	0	0	8	0	0	0	0	0
6	11	3	0	11	0	0	0	0	0
7	11	11	0	20	0	0	2	0	0
8	17	19	0	22	0	0	5	0	0
9	17	22	0	28	3	0	5	0	0
10	20	29	0	28	8	0	5	0	0
11	20	29	6	28	8	0	5	0	0
12	20	31	6	33	8	0	7	2	0
13	22	31	6	44	8	0	7	5	0
14	25	34	6	56	8	0	16	5	0

To date carcinoma of the breast has not been produced in intact male Marsh-Buffalo mice by the administration of estrogens over a long period of time either in toxic or nontoxic doses.

SUMMARY AND CONCLUSION

Estradiol in sesame oil was administered in a total dose of 3.5 mgm. per mouse intermittently over a 12-month period to groups of castrated and intact male Marsh-Buffalo mice.

In the castrated mice the incidence of lymphosarcoma was increased significantly above that occurring in both estrin-treated and control intact mice.

No mammary gland tumors appeared in the series of intact males and only 6 per cent (2 tumors) developed in the castrated group, which had received estrin.

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