

# The Influence of Estrogen on Cancer Incidence and Adrenal Changes in Ovariectomized Mice on Calorie Restriction\*

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The occurrence of mammary carcinoma (1, 2, 3, 4) following ovariectomy in certain strains of mice and the delay of mammary carcinogenesis in such mice by calorie underfeeding seen in preliminary experiments (5) suggested the testing of the effects of exogenous estrogen administration, particularly in the calorie restricted animal, on cancer production. Studies were therefore made on full-fed and calorie restricted ovariectomized mice, both with and without exogenous estrogen, observing effects upon the reproductive apparatus, the adrenal cortex, and upon mammary cancer incidence.

## METHODS

C3H strain mice were ovariectomized at weaning (21 to 23 days) and caged individually in a room held at  $78^{\circ} \pm 4^{\circ}$  F. and  $45 \pm 10$  per cent

some instances the animals were sacrificed for morphologic study when a mammary tumor was noted and in other instances the tumor was removed surgically under anesthesia and the mouse studied further as will be noted under results. The adrenal glands, the uteri, and the mammary tumors were sectioned and studied microscopically. In the estrogen treated animals 0.5  $\gamma$  of diethylstilbestrol per day per mouse was administered in the food.

## RESULTS

Table 1 presents a summary of the major findings in the study. It will be seen that the diethylstilbestrol-treated C3H castrates on full feeding show a markedly lower average cancer age than their controls without estrogen. This occurs with a total suppression of adrenal adenomatous change

TABLE 1  
THE INCIDENCE OF MAMMARY CARCINOMA ADRENAL ADENOMA IN OVARIECTOMIZED C3H MICE WITH AND WITHOUT EXOGENOUS ESTROGEN ON FULL FEEDING AND 33 PER CENT CALORIE RESTRICTION

No. of MICE	FEEDING	DIETHYL-STILBESTROL $\gamma$ PER MOUSE PER DAY	BODY WT. AT 35 Wks. IN Gms.	PER CENT WITH MAMMARY TUMORS		AVERAGE CANCER AGE (MONTHS)	PER CENT WITH ADRENAL ADENOMATA
				at 35 Wks.	at Death		
18	Full	0.0	29	28	94	11.1	100
15	Full	0.5	26	89	100	7.6	0
30	Restricted	0.0	18	0	0		100
8	Restricted	0.5	16	13	100	13.4	0

relative humidity. Groups as indicated were placed on full feeding and on 33 per cent calorie restriction, the diets being those described previously (6). In these diets the calorie restricted mice receive the same average daily intake of protein, vitamins, and minerals as their controls, but smaller amounts of carbohydrate and fat. Vaginal smears were studied in intervals; body weights were recorded and the mice were examined for tumors weekly. In

as would be expected from the report of Woolley and Little (7). The mice showed enlarged uteri and the vaginal wall and smears showed a high degree of estrogenic stimulation.

The calorie restricted ovariectomized C3H mouse showed a zero incidence of mammary cancer, while the adrenal adenomatous change was invariable in its occurrence. On the same diet with diethylstilbestrol the mammary cancer incidence became 100 per cent. However, the average cancer age was nearly double that in the fully fed estrogenized mice. The adrenal cortices in the calorie

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restricted estrogenized mice did not show adenomata. The accessory sex apparatus was similar to that in the full-fed mice.

In six cases the mammary tumors were surgically removed from calorie restricted estrogenized C3H mice and the administration of diethylstilbestrol stopped after 6 to 10 months of administration. The calorie restriction was continued. The animals were sacrificed after 1 month or more without estrogen. In each case adenoma of the adrenal cortex was found. Therefore it can be concluded that even after 10 months of diethylstilbestrol treatment the suppression of adrenal cortex adenoma by exogenous estrogen is capable of prompt reversal.

#### DISCUSSION

The observation that mammary cancer incidence in the calorie restricted ovariectomized C3H mice given exogenous estrogen may reach 100 per cent, as compared with zero without estrogen provides additional evidence that the pertinent end-organs are by no means insensitive to estrogen in the calorie restricted state. The sensitivity may not be as great as in the full-fed mouse, since there is a large difference in average cancer age.

It is significant in these results that calorie underfed ovariectomized C3H mice develop adrenal cortical adenomata indistinguishable histologically from those in fully fed animals. Nevertheless in the calorie underfed state the adrenal adenoma does not produce estrogen, which it is assumed to do in the fully fed castrate, as evidenced by the maintenance of a continuous subestrus state and by mammary gland and carcinoma development. The calorie underfed castrate does not show any of the latter changes except when exogenous estrogen is administered. It has been pointed out previously (8, 9) that calorie restriction appears to reduce the sensitivity of the mammary gland to estrogen. The lengthened average cancer age in these experiments may be due to this effect. There is no evidence as to how this effect is mediated but it might be through suppression of a pituitary hormone other than gonadotropin in calorie underfeeding.

The most significant point in these studies seems to be the confirmation they give to the hypothesis that calorie restriction in mice lowers mammary cancer incidence by reducing estrogen production (6, 8). There seems to be no other acceptable interpretation of the results as they appear. Any other interpretation would involve complicated postulates as to direct or remote (by way of the pituitary) effects of exogenous estrogen upon the adrenal cortex and these effects would have to be different in the full-fed and the calorie restricted animals. It therefore seems reasonably well estab-

lished that the primary reason for suppression of mammary cancer in mice by calorie underfeeding is the suppression of estrogenic hormone production which it causes.

#### CONCLUSIONS

1. Ovariectomized C3H mice given 0.5  $\gamma$  of diethylstilbestrol per day show an eventual 100 per cent incidence of mammary carcinoma regardless of whether the animals are fully fed or are restricted in calories to the extent that in the absence of exogenous estrogen the cancer incidence would have been zero.

2. The average cancer age in estrogen treated ovariectomized C3H mice is greatly increased by calorie restriction.

3. The observation that calorie restriction does not prevent estrogen induced mammary carcinoma in cancer susceptible mice lends added support to the hypothesis that calorie restriction reduces mammary cancer incidence in the intact or ovariectomized non-estrogenized mouse mainly by suppression of endogenous estrogen production.

4. The suppression of adrenal adenoma formation in ovariectomized C3H mice produced by exogenous estrogen is quickly reversible after 6 to 10 months of administration.

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