

COMPARATIVE DEPRESSION OF GROWTH IN MICE BY DIETHYLSTILBESTROL, ESTRADIOL AND ESTRONE

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ABSTRACT.—High estrogen concentrations in the diets of mice greatly inhibit their growth. The synthetic estrogen, diethylstilbestrol, is 5-10 times more potent than either natural estrogen (estradiol or estrone) in this respect and may act chiefly by reducing food intake. Testis and seminal vesicle weights also are more effectively reduced by diethylstilbestrol than by the natural estrogens.

Dietary estrogen administration is a convenient method of inducing mammary tumors in mice. High concentrations of a synthetic estrogen, diethylstilbestrol (DES), however, have been noted to inhibit growth and cause subsequent death of experimental animals due to inanition (Gass *et al.*, 1964). Inanition *per se*, as well as general debilitation of experimental animals, tends to reduce the frequency with which tumors develop — thus complicating quantitative studies on tumorigenesis (Tanenbaum, 1959).

Several authors (Richards and Kueter, 1941; Meites, 1949; Hartsook and Magruder, 1957) have reported that injection of both natural and synthetic estrogens inhibits body-weight gain in rats. The effects of estrogens on weight gains in rodents, then, are opposite those observed in beef cattle (where DES has been widely used as a "growth stimulant").

The present investigation was undertaken to compare the degree of growth depression in mice elicited by

oral administration of the natural estrogens, estradiol and estrone, with that produced by the synthetic, DES.

MATERIALS AND METHODS

Estradiol (as estradiol-17 β) was obtained from the California Corp. for Biochemical Research, Los Angeles; estrone from Nutritional Biochemicals Corp., Cleveland, O.; DES (α , α' -diethyl-4, 4'-stilbenediol) from Matheson, Coleman & Bell, Cincinnati, O.

One-hundred male Swiss mice (Cumberland View Farms, Clinton, Tenn.), 25 days old at the start of the diet, were randomly assorted into 10 groups of 10 mice each; 9 groups received estrogens admixed to Purina Laboratory Chow as shown in Table 1. The concentrations were selected on the basis of preliminary experiments which indicated that DES was approximately 5 times as effective as estradiol and 10 times as active as estrone in reducing body-weight gains. The control group received Chow alone. Food and water were available *ad libitum* throughout the experimental dietary period of 4 weeks, following which the mice were killed with chloroform, the testes and seminal vesicles removed, cleaned and weighed. (Seminal vesicles were severed near the junction with the urethra; fluid contents were expressed before weighing.) Body weight was measured in the early afternoon when mice are in a relative post-absorptive state since most feeding occurs at night (Anliker and Mayer, 1956).

RESULTS

The effects of estrogens on growth are summarized in Table 1. No group of mice receiving estrogen was able to approach the body weight gains made by the control group. A rep-

TABLE 1.—Effects of Estrogens on Body Weight of Maturing Male Swiss Mice.

Diet—ppb	Body Weight in Grams				Grams Gained in 4 Weeks	Gain as % of Original Weight	Grams of Food Consumed/Day/Mouse
	Initial	1 Week	2 Weeks	3 Weeks	Final		
Control.....	16.2 ¹ ± 0.75	23.7	25.0	27.1	30.0 ¹ ± 0.84	85.2	4.6
DES.....	17.1 ± 0.93	21.7	23.1	23.7	24.7 ± 1.17	44.4	2.7
DES.....	16.6 ± 1.06	19.2	21.1	22.5	23.1 ± 0.77	39.2	3.1
DES.....	15.6 ± 0.70	17.5	19.0	20.8	20.6 ± 1.01	32.1	3.1
Estradiol.....	16.7 ± 0.95	20.3	21.9	24.6	25.4 ± 0.71	52.1	3.4
Estradiol.....	16.1 ± 1.11	18.3	20.3	22.3	22.5 ± 0.85	39.8	3.5
Estradiol.....	16.8 ± 1.01	17.7	19.3	21.2	21.7 ± 0.92	29.2	3.5
Estrone.....	15.8 ± 0.84	21.2	22.0	25.3	25.7 ± 0.86	62.7	4.2
Estrone.....	16.3 ± 1.40	18.8	20.5	23.5	23.9 ± 1.02	46.6	3.8
Estrone.....	16.1 ± 0.68	18.2	18.8	20.0	20.7 ± 0.72	28.6	3.4

All Values Are an Average of Ten Animals

¹Mean ± Standard Error

representative growth curve for mice receiving DES (Figure 1) reveals that depression of body-weight gain occurs in the first week of estrogen administration; this also is true of the natural estrogens at the dose levels employed in this study. After 4 weeks of estrogen treatment, growth of all experimental groups reached a plateau while the control animals were still making rapid gains. The greater relative potency of DES in decreasing body weight is apparent at each of the 3 dose levels. The degree of growth inhibition is a linear function of the concentration of estrogen in the diet over the ranges tested (Figure 2).

One-thousand parts per billion of DES in the diet were sufficient to reduce testis weight to about half the control value, whereas this effect was

not equaled by 15,000 ppb of estradiol nor 30,000 ppb of estrone (Table 2). (1000 ppb in the diet are equivalent to an intake of approximately $3.5\mu\text{g}/\text{day}$, assuming that mice consume 3.5 g of Chow per day.)

Seminal vesicles of all groups receiving estrogens are significantly smaller ($p < 0.01$) than the control value (Table 2); this indicates that androgen secretion by the testes is greatly diminished or even abolished. Each dose level of DES produced the maximum observable depression of seminal vesicle development, as did the two highest concentrations of estradiol; no dose level of estrone achieved this depression.

DISCUSSION

Several mechanisms by which estrogens might depress growth in ro-

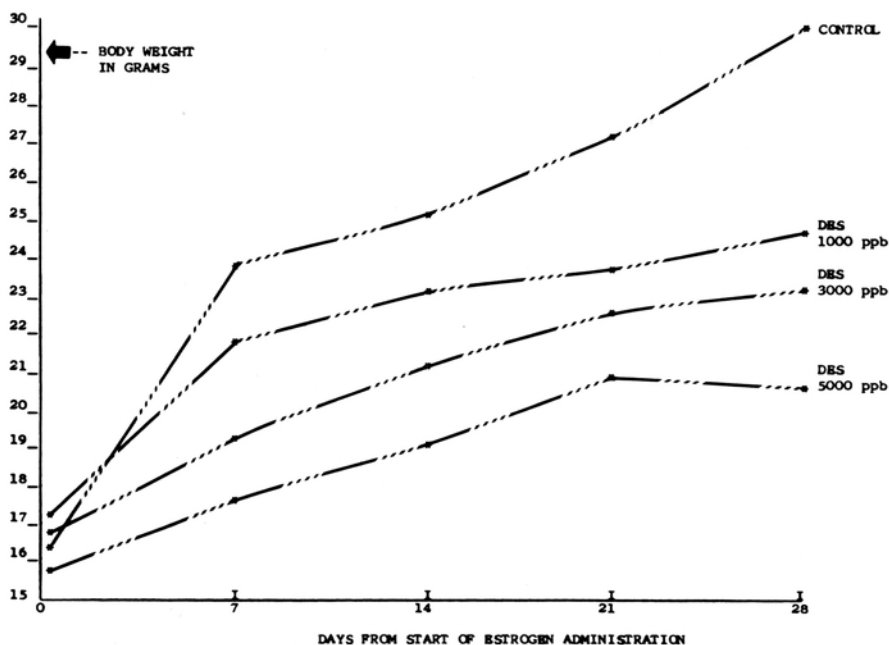


FIGURE 1.—Depressed Growth Rate of Male Swiss Mice Receiving Diethylstilbestrol in the Diet. (ppb = parts per billion of DES in the diet)

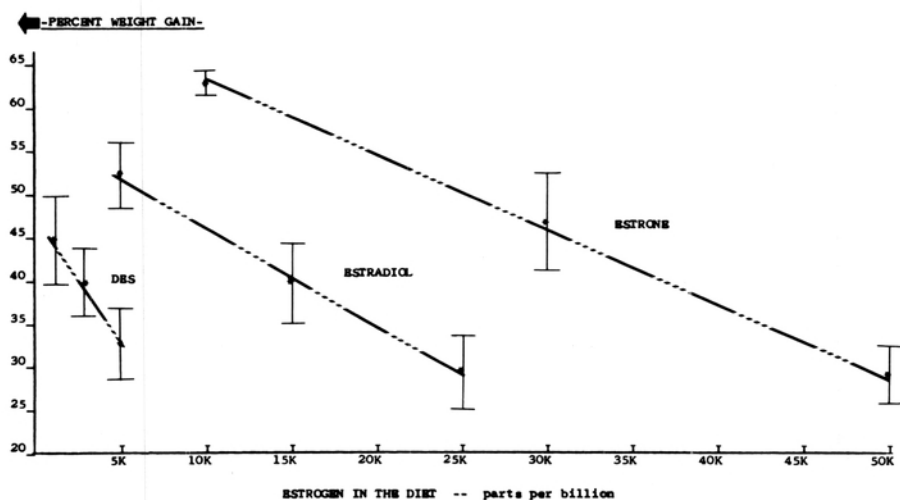


FIGURE 2.—Linear Dose-Response Relationship Between the Concentration of Estrogen in the Diet and Weight Gain in Male Swiss Mice. (Brackets indicate the standard errors of the plotted mean values.)

TABLE 2.—Effects of Estrogens on Testes and Seminal Vesicles of Maturing Male Swiss Mice.

Diet—ppb		Testes Weight in mg ¹	Seminal Vesicle Weight in mg ¹
Control.....		205.0 ² ± 13.1	42.3 ² ± 2.68
DES.....	1,000	105.8 ± 11.2	8.2 ± 0.93
DES.....	3,000	67.6 ± 7.7	9.3 ± 0.61
DES.....	5,000	59.5 ± 5.1	9.1 ± 0.64
Estradiol.....	5,000	160.0 ± 6.5	23.1 ± 3.02
Estradiol.....	15,000	115.3 ± 15.0	9.6 ± 1.01
Estradiol.....	25,000	74.7 ± 8.0	9.0 ± 1.06
Estrone.....	10,000	153.7 ± 16.1	23.9 ± 6.12
Estrone.....	30,000	145.8 ± 20.3	21.8 ± 5.56
Estrone.....	50,000	73.0 ± 5.9	11.5 ± 1.12

All Values Are an Average of Ten Animals

²Paired Organ Weights

¹Mean ± Standard Error

dents have been suggested; chief among these are that estrogens reduce anterior pituitary secretion of growth hormone (Richards and Kue-ter, 1941) or that they reduce food intake (Meites, 1949). Meites states that DES curtails growth in rats principally by decreasing appetite, while natural estrogens inhibit growth without a corresponding decrease in appetite. Measurements of food consumption of mice in this study show a marked decrease below control group intake at all dose levels of DES. Mice receiving natural estrogens exhibited a less pronounced restriction of appetite despite the much higher concentrations administered. Rats and mice seem to be similar, then, in regard to food intake when given either natural or synthetic estrogens. However, rats on natural estrogens (0.1mg/day) showed no reduction in growth rate until after 30 days (Meites, 1949) while restricted growth in mice occurs in the first week of estrogen treatment.

This investigation may provide a guideline to levels of natural and synthetic estrogens in the diet which allow some degree of growth in ex-

perimental mice, particularly those used in quantitative studies on carcinogenesis.

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