

Effectiveness of Estrogens in Stimulating Duct Growth of the Mammary Glands of Rats.* (17655)

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With the increasing number of available estrogens, we became interested in comparing their effectiveness in stimulating duct growth of the mammary glands. Although the male mouse is admirably suited for such work [Lewis and Turner(1)], the weanling male rat is not, since its mammary gland shows enlarged end buds which characterize a gland that is increasing in area. The prolonged injection of an estrogen into such a rat will increase gland area, but shorter periods of injections produce changes that are difficult to distinguish from those occurring in the non-injected rat. Myers' studies(2) on the effect of inanition on the mammary gland of the rat, from birth to 10 weeks of age, suggested a possible means of comparing the mammary-growth-stimulating properties of estrogens. These studies showed that severe inanition retarded growth of the milk ducts during the first week but did not completely inhibit their growth. In animals held at birth weight for a longer time, the ducts ceased to grow and remained in a condition only slightly more developed than at the time of birth.

In developing an assay method and in comparing various estrogens, 592 weanling male rats were used. Male rats, 21 days old, were fed various diets in different amounts until one was found that resulted in mammary glands devoid of enlarged end buds. An estrogen was then injected daily (in 0.05 cc oil) into rats on restricted diets for various periods to determine the shortest period in which enlarged end buds could be produced. It was found that the daily feeding of 2 g per rat

of a diet consisting of 50% Purina Fox Chow and 50% corn starch resulted in mammary glands free of enlarged end buds. Eight daily injections of an estrogen were found to be the minimum number that would consistently produce enlarged end buds of the mammary gland. In all subsequent work 21-day-old male rats were fed the restricted diet for 10 days and injected with an estrogen during the last 8 days of the feeding period. The rats were killed on the day after the last injection. The right abdominal mammary gland of each was removed, fixed, stained *in toto* with Mayer's hemalum, and examined for evidence of duct growth. After the initial assay, for any given estrogen, the dosage was either reduced one-half or doubled until a level was attained that produced a $50 \pm 10\%$ response in a group of 10 rats.

In 409 rats that were used to compare various estrogens, the average initial body weight was 39 g; on the first day of injection it was 34 g; and at the time of autopsy it was 31 g. Of the 9 estrogens assayed, diethylstilbestrol and hexestrol showed the greatest activity whereas their dimethyl ethers showed the least. The monomethyl ether of diethylstilbestrol was more effective than the dimethyl ether but much less active than diethylstilbestrol. Estradiol and estradiol benzoate ex-

TABLE I.
A Comparison of the Mammary Stimulating Properties of Nine Estrogens.

Estrogen	Effective daily dose in μ g
Estradiol	.06
" benzoate	.06
" dipropionate	.13
Estrone	2
Diethylstilbestrol	.03
Monomethyl ether of diethylstilbestrol	1
Dimethyl ether of diethylstilbestrol	8
Hexestrol	.03
Dimethyl ether of hexestrol	8

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1. Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1939, 310.

2. Myers, J. A., *Am. J. Dis. Child.*, 1919, v17, 311.

hibited similar activity but the dipropionate was only about one-half as effective. Estrone was found to be much less effective than estradiol. The effective daily dose of 9 estrogens is given in Table I.

It will be noted that the estrogens differ greatly in their ability to stimulate duct growth. Whether or not the restricted feeding regime decreases the mammary gland response to estrogen cannot be determined in the growing male rat. Trentin and Turner(3) however, have shown that as the food intake decreases, the amount of estradiol benzoate required to produce a minimum duct growth response of the mammary glands of male albino mice is considerably and proportionately increased. Our results contrast markedly with those of Astwood *et al.*, who reported that the daily injection of 5 μ g of estrone for 14 days into young rats on a restricted diet

caused no duct extension whatsoever.

Summary. A method was developed whereby it is possible to use the immature male rat to compare estrogens for their ability to stimulate mammary duct growth. Nine estrogens were compared and, in increasing order of effectiveness, they were: dimethyl ether of diethylstilbestrol and dimethyl ether of hexestrol; estrone; monomethyl ether of diethylstilbestrol; estradiol dipropionate; estradiol benzoate and estradiol; and, diethylstilbestrol and hexestrol.

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3. Trentin, J. J., and Turner, C. W., *Endocrinology*, 1941, v29, 984.

4. Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, v61, 373.

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Normal Discontinuity of Progression of Dental Caries.* (17656)

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When appraising the presumed efficacy of a specific agent to prevent tooth decay, it is common practice to note the length of time during which the subject remains free from caries advance, without regard for the possibility that non-advance may be encountered without the use of any imposed regimen. If tooth decay normally is an intermittent process, as determined through clinical or roentgenologic examination, then one may expect non-advance to be evident at times regardless of the agent being tested. It becomes important to set up bases for prediction as to the

likelihood of such non-advance and its probable duration.

A previous publication has reported that intermittency of caries progression had been noted among children being seen recurrently as patients in a dental clinic(1). Because dental therapy in itself may be expected to modify the progression of tooth decay, it was desirable to determine the intermittency of caries advance among a population for whom dental therapy was not being provided. We have done this in a state-controlled custodial institution, using teen-aged children as sub-

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1. Boyd, J. D., and Cheyne, V. D., *J. Pediat.*, 1946, v29, 157.