Effect of Estrogen upon Thyroxine Secretion Rate in Intact Female Rats.* (24880)

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Estrogen administration has been reported to increase(1-5), to have little or no effect(6-8), and to depress thyroid activity(4,9). Some investigators have indicated that estrogens may stimulate or depress thyroid function, depending upon dose and period of treatment(1,4,10,11). In these studies many indices of thyroid function have been used, such as I^{131} uptake, rate of thyroidal I^{131} release, hyperplasia and thyroid cytology. In view of conflicting results and limitations of some methods used in assessing thyroid activity, it seemed desirable to reinvestigate the influence of various levels of estrogen upon thyroid activity in female rats. This was done by employing the technic of thyroxine replacement and thereby estimating daily thyroxine secretion rate.

Materials and methods. Young mature female rats of Sprague-Dawley-Rolfsmeyer strain weighing 240-260 g were housed for 3-4 weeks after purchase, under uniform temperature (78-80° F) in animal room artificially illuminated during regular daylight hours. They were given Purina Lab Chow and fresh water daily. *Exp. I.* Seventy-one rats were injected intraperitoneally with 2 μc of carrier-free I^{131}. Forty-eight hours were allowed for fixation of I^{131} by thyroid gland. External neck counts were made at this time and at 2-day intervals thereafter by first anaesthetizing each animal with ether, then placing it on lead plate with thyroid region over a scintillation probe containing a 1″ NaI crystal. Measurements of thyroidal radioactivity were made with a scintillation counter, Nuclear-Chicago (N.C. Model DS5-3) connected to rate meter (N.C. Model 1620A). Conventional corrections were made for radioactive decay and background. After initial thyroid count, subsequent neck counts were made on days 4 and 6 after I^{131} injection to establish that thyroidal I^{131} release was proceeding normally. Fifty-seven rats were injected subcutaneously with 1, 3.6, 15 or 50 μg/day estradiol benzoate (E.B.) in .1 ml olive oil commencing 5 days after injection of I^{131} and continuing until thyroid secretion rate was reached (5-9 days). Fourteen control rats were similarly treated with .1 ml olive oil/day. Each rat was injected subcutaneously with .5 μg/100 g/day l-thyroxine for 2 days commencing 6 days after I^{131}. Dose was increased at .5 μg/100 g increments, each level for 2 consecutive days with thyroid count taken the day of each increase. Thyroxine secretion rate (TSR) was estimated by plotting percentage of previous count with thyroxine dose. The dose which prevented further thyroidal I^{131} output in each rat (95-100% of previous count) was estimated as its TSR. *Exp. II.* Fifty-eight additional female rats were injected with 1, 3.6, 15 or 50 μg/day E.B. for 5 days. Eighteen served as controls of which half were injected with .1 ml olive oil/day. Body weight of each rat was recorded daily. Twenty-four hours after last injection, each animal was weighed and killed with ether. Thyroid, pituitary and adrenal glands were rapidly removed, blotted to remove surface moisture and weighed to nearest .1 mg on a Roller-Smith Torsion balance.

Results. The influence of various levels of E.B. upon average daily thyroxine secretion rate (TSR) of young mature female rats is presented in Table I. Only 3.6 μg/day resulted in significant increase of 35.5% over control value of 1.24 μg/100 g/day l-thyroxine. Body weight was not adversely affected by injection of 1 or 3.6 μg/day E.B. for 5 days. Administration of 15 or 50 μg/day for same period, however, caused a decrease in body weight of 2 and 5.7% respectively (Table II). Each rat was given the same quantity of food each day but those injected with

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TABLE I. Effect of Estradiol Benzoate upon Thyroxine Secretion Rate in Female Rats.

EB treatment (μg/day)	No. of rats	Initial body wt (g)	Thyroxine secretion rate (μg/100 g/day l-thyroxine)
Control	14	244.4	1.24 ± .10
1.0	15	253.9	1.13 ± .11
3.6	14	244.3	1.68 ± .06*
15.0	14	253.5	1.29 ± .09
50.0	14	256.0	1.25 ± .07

* Significant at 1% level.

15 or 50 μg/day apparently did not consume as much food as control animals since food progressively accumulated in their cages during period of treatment. Because of loss in body weight resulting from treatment with larger doses of E.B., weights of pituitary, thyroid and adrenal glands are expressed as mg/100 g initial body weight (Table II). Injection of 1 or 3.6 μg/day did not affect thyroid weight but significant increases of 18.6 and 26.7% occurred following administration of 15 and 50 μg/day for 5 days. Adrenal weights were lower in all groups with a significant depression occurring only in the 50 μg/day group. Pituitary weight, however, was significantly increased over controls in all but the 1 μg/day group. Maximum pituitary weight increase (42.6%) resulted from treatment with 15 μg/day level of E.B.

Discussion. The effect of various estrogens upon thyroid function has interested many investigators. Results however, have been conflicting due in part to varying time-dose relationships, species or strain differences and in technics used in evaluating thyroid function. Since I^{131} became available, more sensitive methods have replaced those based on morphological changes of thyroid gland. The 2 most widely used, I^{131} uptake and rate of release of thyroidal I^{131}, are, however, qualitative, and values obtained cannot be translated into quantitative terms(12). The technic of measuring daily thyroxine secretion rate (TSR) is a quantitative measure of thyroid activity based upon the classical procedure in physiology of hormonal replacement. Results of our study based on this method clearly indicate that a relatively low level of estradiol benzoate (3.6 μg/day) administered for a short period markedly increased daily thyroid hormone output, whereas higher levels had no effect. Increase in TSR was not, however, associated with increase in weight of thyroid gland. It has been reported that estrogen depresses appetite and body weight in laboratory animals(13,14) which may lead to diminished thyrotropin secretion by the pituitary gland(5,13). The failure of 15 and 50 μg/day E.B. in our study to increase significantly TSR, may perhaps be explained on this basis since a decrease in appetite and body weight occurred. These levels, however, elicited marked increase in thyroid weight which may indicate a compensatory enlargement has occurred as a result of partial inanition(5). It is also possible that high levels of E.B. stimulate the thyroid directly(1) or that thyroid weight increase may be due in part to the well known vasodilating action of estrogen with resulting hyperemia.

It is thought that the increasing level of estrogen secreted during late pregnancy is responsible for increased secretion of lactogenic hormone by the pituitary(15). The significant increase in TSR obtained in our study with a dose of E.B. (3.6 μg/day), slightly higher than the accepted physiological dose in rats, suggests that increased secretion of estrogen in late pregnancy may also provide the stimulus for secretion of increased amounts of thyrotropin and, subsequently, more thy-

TABLE II. Some Effects of Estradiol Benzoate in Female Rats. (E.B. inj. daily for 5 days.)

EB treatment (μg/day)	No. of rats	Body wt (g) Initial	Final	% change	Avg- Pituitary	Adrenals	Thyroid
					mg/100 g initial body wt		
Control	18	254.3	257.7	+1.3	4.79 ± .15	27.3 ± .81	4.68 ± .15
1.0	15	256.2	258.6	+ .9	5.08 ± .13	25.7 ± .63	4.50 ± .21
3.6	14	246.4	248.0	+1.1	5.83 ± .27*	25.3 ±1.16	4.61 ± .16
15.0	15	250.4	245.4	−2.0	6.83 ± .22*	26.6 ±1.09	5.55 ± .26*
50.0	14	253.1	238.7	−5.7	6.55 ± .26*	24.5 ± .98†	5.93 ± .30*

* Significant at 1% level from control. † Significant at 5% level from control.

roxine for lactation. This would provide an explanation for the significant increase in thyroid hormone output which occurs during early lactation in rats(16) and sheep(17).

Summary. The effect of various levels of estradiol benzoate (E.B.) upon thyroxine secretion rate (TSR) has been studied in young mature female rats. A dose of 3.6 μg/day E.B. caused significant increase of 35.5% in TSR over average control value of 1.24 μg/100 g/day l-thyroxine while 1, 15 or 50 μg/day had no effect. In another experiment, body weight and thyroid weight/100 g were not affected by injecting 1 or 3.6 μg/day E.B. for 5 days. Thyroid weight/100 g was significantly increased while appetite and body weight were markedly reduced following 15 or 50 μg/day for same period. Pituitary weight/100 g was significantly increased in all but the 1 μg/day group. Adrenal weights/100 g were lower in all groups with significant depression occurring in 50 μg/day group. These data are discussed in relation to stimulation or depression of thyroid activity by estrogen.

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Effect of 2-PAM on Neuromuscular Blockade Induced by Certain Chemicals. (24881)

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Holmes and Robins(1) found that 2-formyl-1-methylpyridinium iodide oxime (2-PAM) induces paralysis of isolated phrenic nerve-diaphragm preparation of the rat; the curaremimetic action of this compound has been corroborated for calf muscles of the cat, excited *in situ* through sciatic nerve stimulation by ourselves and for adductor muscle of human little finger, excited by stimulation of ulnar nerve(2). In addition, 2-PAM (5 x 10^{-3} M) decreases rate of shortening of isolated rectus abdominis of the frog in response to acetylcholine, but smaller concentrations of 2-PAM increase this rate(3). Grob and Johns reported also(2) that some patients given 2-PAM intravenously experienced thereafter orthostatic tachycardia and hypotension. These findings, and those that 2-PAM is particularly active in promoting reactivation of inhibited cholinesterase in cholinergic neurons of ganglia(4) and in motor end-plates of skeletal muscle(4,5), suggest that the oxime has a special affinity for nicotinic effectors, where it has a depolarising action of its own and also competes with other quaternary molecules (acetylcholine, for example) for the receptor site. Wagley(6) found, indeed, a reversible increase of about 40% in end-plate potential of frog muscle treated with 2-PAM *in vitro;* this is the type of change expected from a de-