Uptake and Retention of Estrogens by Uteri from Rats in Various Thyroid States[1] (34834)

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Van Horn (1) observed that hyperthyroidism produced prolonged diestrus; and hypothyroidism, prolonged estrus in rats. He further found that more estrogen was needed to produce positive estrus smears in hyperthyroid ovariectomized rats than control ovariectomized rats. A specific effect of thyroxine on the estrus cycle has been suggested since dinitrophenol did not inhibit estrus in the normal rat (2). Investigators (3) have studied the effects of metabolic disorders on different aspects of reproduction, but there is no agreement as to mechanism whereby the thyroid influences uterine sensitivity to estrogens. Most work has been concerned with thyroid–ovarian–uterine effects with little concern for the direct effects of thyroid hormones on the uterus. This investigation evaluated the *in vivo* retention and *in vitro* uptake of estrogens by the uteri from thyroidectomized and thyroxine-treated rats in view of the recent finding of a specific uterine binding protein (4, 5).

Materials and Methods. Female Sprague-Dawley rats, 21 days of age, were divided into three groups and ovariectomized. Group 1 was sham thyroidectomized, and Groups 2 and 3 were thyroidectomized. Groups 2 and 3 received 1% calcium gluconate as drinking water for the first 2 days postoperative; thereafter, they received tap water. Surgery was performed under ether anesthesia, and all groups were fed Rockland Diet *ad libitum*. At 23 days of age, Group 3 began receiving daily injections of L-thyroxine (100 μg/kg body weight) for 7 days.

6,7-^{3}H-estradiol-17β (New England Nuclear, SA 48.0 Ci/mmole in benzene–ethanol) was evaporated to dryness in air before being prepared for injection or incubation medium. The ^{3}H-estradiol was 97% pure by chromatography on formamide-saturated paper in a benzene-solvent system.

In vivo. At 31 days of age, all groups of rats were injected subcutaneously with 0.1 μg 6,7-^{3}H-estradiol-17β in 0.5 ml of 0.9% NaCl–1% ethanol and sacrificed by decapitation at ½, 1, 6, 10, and 16 hr after injection. The uteri were excised, freed of fat, blotted on filter paper, and weighed. A 5% homogenate was prepared in a buffered medium (6), and protein concentrations were determined by the method of Lowry *et al.* (7). The estradiol was extracted from the remainder of the homogenate (8), and evaporated to dryness in 20-ml glass vials. Seventeen milliliters of a scintillation counting solution (9) were added to each vial and radioactivity was determined in a Model 3380 Packard Tri-Carb scintillation counter having a counting efficiency of 46%. Extraction studies demonstrated that all of the radioactivity present in the samples was extracted.

Transformed data (reciprocals) were statistically analyzed using a $p \times q$ factorial design analysis of variance for unequal cell frequency (10). Data were transformed to obtain better homogeneity of variance. Values for p (obtained from t values related to the F statistic) of 0.05 or less were considered significant.

In vitro. A second series of three groups of rats were sacrificed at 31 days of age. The uterine horns were removed, freed of fat, slit, blotted, and placed in 25-ml Erlenmeyer flasks containing 6 ml of 10^{-10} M 6,7-^{3}H-estradiol-17β in freshly prepared Krebs-Ringer-Henseleit-glucose (KRHG) buffer

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TABLE I. *In vivo* Retention of Tritiated Estradiol in Rat Uteri[a] [1/(cpm/mg protein)] × 10^{-4}.

Time (hr)	Control	Thyroidectomized	Thyroxine-treated
½	9.25 ± 0.79 (3)[b]	4.99 ± 0.83 (3)	9.22 ± 0.89 (4)
1	5.64 ± 1.07 (5)	5.22 ± 0.84 (6)	5.68 ± 0.38 (5)
6	14.64 ± 3.38 (4)	10.70 ± 0.21 (3)	17.23 ± 1.63 (4)
10	14.37 ± 1.17 (5)	8.55 ± 0.49 (5)	14.98 ± 1.42 (5)
16	18.88 ± 2.05 (4)	14.22 ± 1.75 (4)	20.26 ± 1.80 (3)

[a] Mean ± SE (standard error of the mean).
[b] Number of uteri.

(11). The flasks were placed in a Dubnoff shaker at 38° operating at 100 cycles/min. After periods of 10, 20, 30, 60, 120, and 240 min, the uterine horns were removed and immediately rinsed three times with ice cold KRH buffer, blotted, and weighed. Protein concentrations, extractions, and radioactive determinations were done as described in the *in vivo* methods.

Data were statistically analyzed using a single-factor analysis of variance for unequal cell frequency (10). Values for p of 0.05 or less were considered significant.

Results. The concentration of radioactivity in rat uteri after single subcutaneous injections of 0.1 μg tritiated estradiol is shown in Table I and Fig. 1. The resulting curve in the control animals (Group 1) is similar in shape to that obtained by Jensen and Jacobson (12, 13). The values for the thyroidectomized animals (Group 2) were above whereas the thyroxine-treated (Group 3) values were below that of the control at all time periods studied. Analysis of variance gave F ratios which show a significant effect of treatment and time. Individual comparison of reciprocal means from the thyroidectomized and thyroxine-treated groups were significant at 6, 10, and 16 hr suggesting a greater retention of estradiol by the thyroidectomized group. It has been shown that the only radioactive compound taken up and retained by the uterus is estradiol-17β; this steroid is not metabolized in the uterus (13).

Table II and Fig. 2 show the radioactivity concentration in uterine horns after incubation in 10^{-10} M tritiated estradiol. An increased incorporation of radioactive estro-

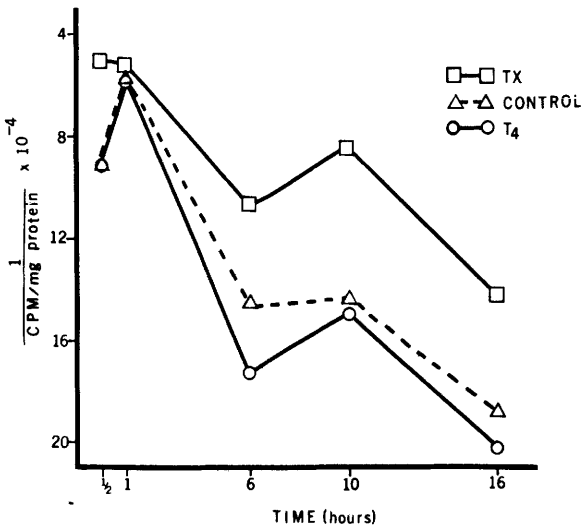

FIG. 1. The concentration of radioactivity in uteri after single subcutaneous injections of 0.1 μg 6, 7-^{3}H-estradiol-17β in 0.5 ml 0.9% NaCl-1% ethanol. Each point is the mean of three to six uteri. Tx = thyroidectomized. T_4 = thyroxine-treated.

TABLE II. *In vitro* Uptake of Tritiated Estradiol in Rat Uterine Horns[a] (cpm/mg protein).

Time (min)	Control	Thyroidectomized	Thyroxine-treated
10	28424 ± 3194 (9)[b]	21963 ± 2137 (10)	22495 ± 1098 (10)
20	35462 ± 1264 (7)	32681 ± 1494 (10)	32083 ± 1950 (9)
30	32813 ± 1685 (15)	38890 ± 2064 (16)	32796 ± 1756 (17)
60	34465 ± 2261 (7)	40641 ± 2568 (7)	35980 ± 2555 (8)
120	48642 ± 5051 (7)	52182 ± 5362 (8)	37435 ± 2510 (8)
240	45345 ± 6469 (7)	46738 ± 5110 (8)	39568 ± 2470 (8)

[a] Mean ± SE (standard error of the mean).
[b] Number of uterine horns.

gen in control uteri was similar to that obtained by Jensen *et al.* (11). The thyroxine-treated group had a maximal uptake of estrogens at 30 min, while the thyroidectomized group had its greatest uptake at 240 min. There was a significant difference when comparing thyroidectomy and thyroxine treatment at 30 and 120 min.

Discussion. Since binding of estrogens to a specific uterine protein is necessary for physiological action (11), a decreased estrogen retention by uteri from thyroxine-treated rats and an increased retention in thyroidectomized rats could result in impaired uterine function. This study suggests that the prolonged estrus in hypothyroid rats and continued diestrus in hyperthyroid rats may result from altered estrogen uptake and retention in the uterus. There is also the possibility that thyroxine may influence the cell membrane and consequently effect the permeability or transport of estradiol into uterine cells. Ovarian–hypophyseal function and estrogen metabolism in the liver must be considered when evaluating the effects of thyroxine on the estrus cycle and reproduction. This study, however, suggests that there may be a direct effect of the thyroid on the uterus.

Summary. Uterine uptake and retention of ^{3}H-estradiol-17β in rats subjected to varying levels of thyroid hormones were studied *in vivo* and *in vitro*. Uteri from thyroxine-treated rats had a lesser *in vivo* retention and *in vitro* uptake of estrogens whereas thyroidectomized rat uteri had greater *in vivo* retention and *in vitro* uptake compared with controls.

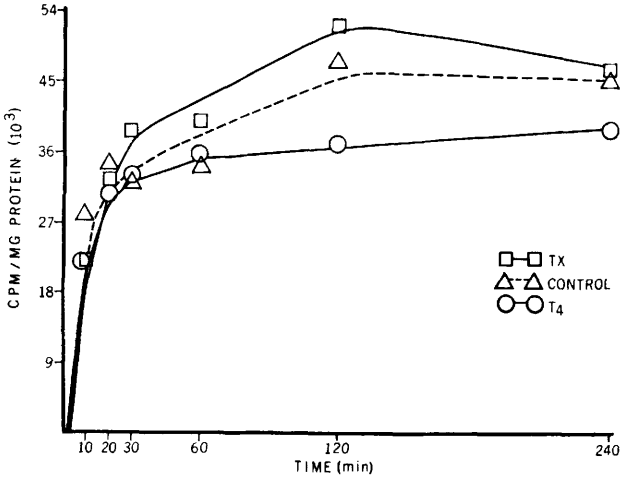

FIG. 2. The concentration of radioactivity in uterine horns after incubation in 10^{-10} *M* 6, 7-^{3}H-estradiol-17β. Each point is the mean of 7–17 uterine horns. Tx = thyroidectomized. T_4 = thyroxine-treated.

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