Effect of Thyroid States on Uterine Sensitivity to Estrogen[1] (34918)

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Hyperthyroidism generally produces prolonged diestrus, and hypothyroidism a prolonged estrus cycle (1–3); however, the direct effect of various thyroid states on the biochemical changes in the uterus is not well known. Brogi (4) observed that thyroparathyroidectomy of ovariectomized immature rats decreased the uterine sensitivity to estrogens whereas Liu (5) reported an increase in uterine wet weight in estrogen-treated thyroparathyroidectomized rats when compared with estrogen-treated controls. A few uterine enzyme systems have been studied in altered thyroid states (6, 7), but there has been little investigation to correlate the uterine sensitivity changes with biochemical or metabolic changes.

It was the purpose of this investigation to study the effect of various thyroid states on uterine sensitivity to estrogen. Uterine wet weight, protein concentration and succinic dehydrogenase (SDH) activity, an enzyme known to be altered by hyper- and hypothyroidism, were investigated in uteri from estrogen- and nonestrogen-treated rats in various metabolic states.

Materials and Methods. Ovariectomized Sprague-Dawley rats, weighing 80–100 g, were used in this study. Nine rats were sham thyroidectomized and 26 rats were thyroparathyroidectomized. The experimental animals received 1% calcium chloride as drinking water for 3 days after surgery; thereafter they received tap water. All animals were fed Rockland diet *ad libitum*. Three weeks after surgery, basal oxygen consumptions were determined using a multichanneled closed-circuit metabolic apparatus (8) to ascertain the level of hypothyroidism. Radioactive ^{131}I uptakes (0.5 μCi/rat) were utilized to verify the completeness of the thyroidectomies.

Nine thyroparathyroidectomized rats received daily subcutaneous injections of 10 μg/kg of body weight L-thyroxine in 0.01 *N* NaOH for 7 days prior to sacrifice. Eight thyroparathyroidectomized rats received 100 μg/kg of body weight L-thyroxine as above. Three days before sacrifice approximately one-half the animals in each group received daily subcutaneous injections of 2.0 μg/kg of body weight of estradiol 17β in sesame oil for 3 days. Animals were fasted 16 hr and BMR's were determined to demonstrate the degree of hypo- and hyperthyroidism prior to sacrifice by decapitation. A decrease of 20% in oxygen consumption following thyroparathyroidectomy was considered minimal acceptable to be classed as hypometabolic. Thyroxine treatment (100 μg/kg of body wt) increased BMR 73%. The thyroparathyroidectomized rats given a replacement dose of thyroxine (10 μg/kg of body wt) were not significantly different from euthyroid.

The uteri were freed from fat, slit lengthwise, and weighed on a torsion balance. Protein was determined according to the method of Lowry *et al.* (9). Succinic dehydrogenase activity was determined on 0.5 ml of a 5% homogenate using the reaction mixture of Schneider and Potter (10). Enzyme activity was measured using a Yellow Springs Instrument Model 53 biological oxygen monitor employing a Clark type polarographic oxygen electrode which provided "Warburg" type oxygen uptake curves. A 5-min record of percentage oxygen saturation was obtained using a Texas Instrument recorder which had been

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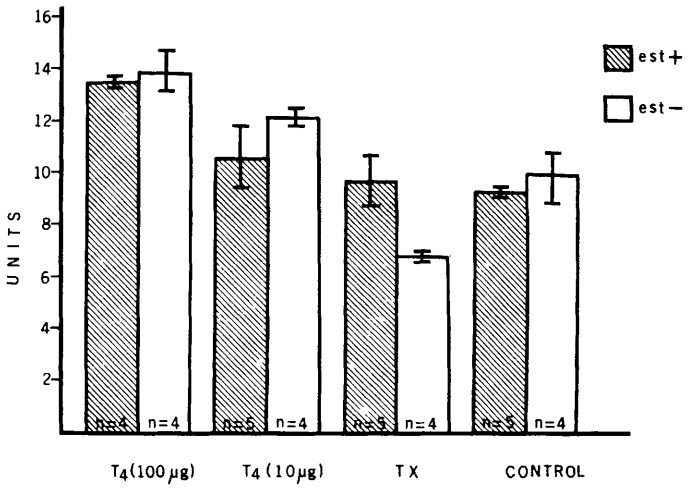

FIG. 1. SDH activity in the liver (mean ± SE): 1 unit = 1% decrease oxygen saturation in the assay medium/min/mg of protein. T_4 (100 μg) = 100 μg/kg of body weight of L-thyroxine daily for 7 days; T_4 (10 μg) = 10 μg/kg of body weight of L-thyroxine daily for 7 days; Tx = thyroparathyroidectomized; C = control; est+ = estrogen treated; est− = nonestrogen treated.

calibrated in air at 38°. The drift in the system was less than 2% full scale per hr.

Rat liver SDH was also determined to validate the use of the oxygen electrode system to measure this enzyme. Since extensive SDH studies using the Warburg method have been reported for liver from animals in various thyroid states, this tissue served as a basis for comparing the two techniques. Optimal amounts of uterine and liver tissues for assay were determined and activity of enzyme versus tissue concentration was linear in the range studied.

The data were statistically analyzed using a factorial design analysis of variance for unequal cell frequency. The F_{max} statistic was used to test homogeneity of variance; p values (obtained from t test related to F statistic) of 0.05 or less were considered significant (11).

Twenty-one-day-old female Sprague-Dawley rats were ovariectomized. Of these, 9 rats were sham thyroidectomized and the others were thyroparathyroidectomized. All animals were maintained on Purina laboratory chow *ad libitum*. For the first 2 days postoperative, 1% calcium gluconate was supplied as drinking water to the experimental rats; thereafter, tap water was administered. The rats were kept to 97 days of age, and then were sacrificed (5). Six thyroparathyroidectomized rats received daily subcutaneous injections of 100 μg/kg of body weight of L-thyroxine in 0.01 N NaOH for 7 days prior to sacrifice. All rats received a total dose of 0.5 μg of estradiol 17β in 0.1 ml of sesame oil, divided into 2 equal subcutaneous injections, at 24 and 16 hr before sacrifice. The accuracy of the injections used was assured by the use of a 50-μl syringe (Hamilton). Uterine wet weights and protein content were determined.

Results. Hyperthyroidism significantly elevates SDH in the liver in estrogen- and nonestrogen-treated animals when compared with controls (Fig. 1). Succinic dehydrogenase in thyroidectomized rat livers is reduced significantly in nonestrogen-treated animals but not in estrogen-treated animals. SDH is significantly elevated in uteri from estrogen-treated rats when compared with nonestrogen-treated rats (Fig. 2). Hyperthyroidism significantly elevates uterine SDH in estrogen- and nonestrogen-treated animals when compared with euthyroid.

Estrogen treatment significantly increased uterine wet weights above those of nonestrogen-treated rats except in the hyperthyroid animals (Fig. 3). There are no significant differences in uterine wet weights within the

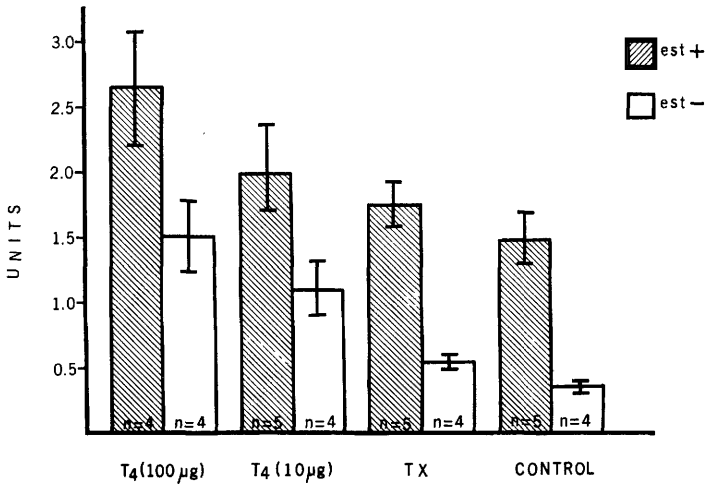

FIG. 2. SDH activity in the uterus (mean ± SE): 1 unit = 1% decrease oxygen saturation in the assay medium/min/mg of protein. T_4 (100 μg) = 100 μg/kg of body weight of L-thyroxine daily for 7 days; T_4 (10 μg) = 10 μg/kg of body weight of L-thyroxine daily for 7 days; Tx = thyroparathyroidectomized; C = control; est+ = estrogen treated; est− = nonestrogen treated.

estrogen-treated or the nonestrogen-treated groups.

There is no significant difference in uterine protein concentrations (μg of protein/mg of uterine tissue), however, estrogen significantly increases the total uterine protein in all animals except those which were hypermetabolic (Fig. 4).

There were no significant differences in protein concentration (μg/mg of tissue) and uterine wet weight of the control, thyroparathyroidectomized, or thyroxine-treated rats that received 0.5 μg of estradiol 17β in 24 hr.

Discussion. The SDH data from nonestrogen-treated rat liver verifies that the oxygen electrode technique can measure fluctuations in enzyme activity since SDH activity was directly related to the oxygen consumption of the animal. Although these findings are well established, the explanation for no decrease in SDH activity in liver from estrogen-treated thyroparathyroidectomized rats is not known.

It has been reported that estrogens increase rat uterine SDH activity (12, 13), however, an effect of thyroxine on uterine

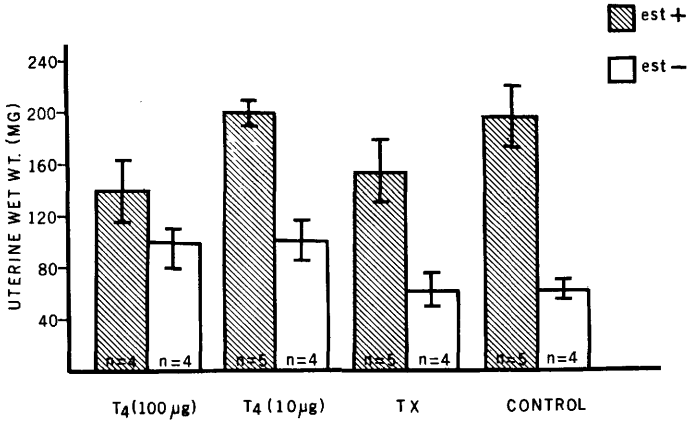

FIG. 3. Uterine wet weight (mean ± SE): T_4 (100 μg = 100 μg/kg body weight of L-thyroxine daily for 7 days; T_4 (10 μg) = 10 μg/kg of body weight of L-thyroxine daily for 7 days; Tx = thyroparathyroidectomized; C = control; est+ = estrogen treated; est− = nonestrogen treated.

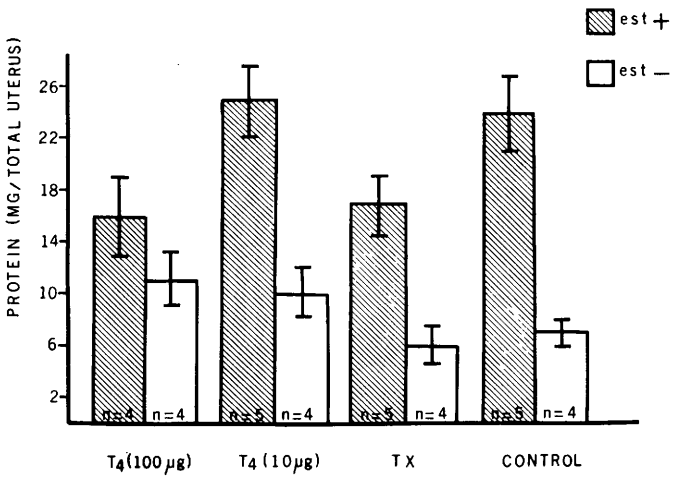

FIG. 4. Protein content in the uterus (mean ± SE): T_4 (100 μg = 100 μg/kg of body weight of L-thyroxine daily for 7 days; T_4 (10 μg) = 10 μg/kg of body weight of L-thyroxine daily for 7 days; Tx = thyroparathyroidectomized; C = control; est+ = estrogen treated; est− = nonestrogen treated.

respiration has not been established (7, 14–16). The present study suggests that hyperthyroidism may increase uterine SDH activity independent of the effects of estrogens.

Although the dose of estradiol 17β used in this study gives optimum uterine growth (17), the uterine wet weight and total protein did not increase significantly in hyperthyroid rats given estrogens. This suggests a decreased uterine sensitivity to estrogens in hyperthyroidism and gives a possible explanation for the prolonged diestrus occurring with increased circulating thyroid hormones. It has been postulated that the decreased response to estrogens in hyperthyroidism may be caused by decreased circulating estrogens through increased liver degradation (1, 18), but a specific effect of thyroxine has been suggested since a dinitrophenol-induced heightened metabolism did not cause the same effects as hyperthyroidism (19). Although estrogen metabolism in the liver may be influenced by thyroxine, recently the authors proposed that the uptake and retention of estradiol 17β in uteri from the thyroxine treated rats may decrease *in vivo* and *in vitro*. It is also possible that the thyroid may indirectly influence uterine protein deposition through alteration in corticosterone secretion by the adrenals (20).

There were no changes in uterine wet weight or total protein in hypo- or hyperthyroid rats receiving 0.5 μg of estradiol 17β in 24 hr. Liu (5) reported an increased sensitivity of ovariectomized–thyroparathyroidectomized rat uteri to estrogens as measured by uterine wet weight and weight of luminal fluid. This was in contrast to the findings of Brogi (4) who reported a decreased uterine water content in thyroparathyroidectomized rats given estrogen. These conflicting findings could possibly be related to differences in the accuracy of administering the exact dose of estrogen.

Summary. The effect of hyper- and hypothyroidism on uterine wet weight, total protein, and SDH activity was studied in estrogen (2.0 μg of estradiol 17β/kg of body wt for 3 days)- and nonestrogen-treated ovariectomized rats. Uterine SDH activity appeared to increase in hyperthyroidism independent of the effect of estrogens. Uterine wet weight and total protein were significantly increased with estrogen treatment in the hypo- and euthyroid groups but not the hyperthyroid group. Uterine wet weights and total protein were not significantly different in uteri from control, thyroparathyroidectomized, or thyroxine-treated ovariectomized rats given a total dose of 0.5 μg of estradiol 17β.

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