Uterine Concentration of Thyroxine During the Estrous Cycle[1] (37547)

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(Introduced by Monte A. Greer)
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The results of several investigations have indicated a functional relationship exists between the thyroid gland and ovary (1–3), but as yet this complex interaction is not well understood. More studies have been concerned with modification induced in thyroid activity as a result of manipulating estrogen levels than vice versa. However, various levels of thyroid activity can affect some aspects of uterine protein metabolism. Miura and Koide (4) have reported that thyroxine influences uterine RNA synthesis and potentiates the stimulatory effect of 17β-estradiol. Ruh, Ruh and Klitgaard (5, 6), on the other hand, have found that euthyroid and hypothyroid but not hyperthyroid states are compatible with increased uterine sensitivity to estrogen treatment.

One method used to search for hormone sites of action has been the localization of labeled hormones in peripheral tissues. This method has provided strong evidence for specific estrogen receptor sites in brain, anterior pituitary, and uterus which preferentially accumulate and retain radio-labeled estradiol (7, 8). In unpublished experiments concerned with uptake and retention of thyroid hormone by various tissues, we found that uteri from intact and thyroidectomized rats accumulated notably larger amounts of thyroxine than other tissues felt to be specific concentrating sites for the hormone (*e.g.*, pituitary and median eminence). In light of possible thyroid–ovary interaction, we examined the relationship between uterine uptake of thyroxine and various stages of the reproductive cycle.

Materials and Methods. Young adult female Sprague-Dawley rats weighing 200 ± 20 g were maintained in a light-controlled (12 hr light beginning at 6 AM), air-conditioned (72.0 ± 2.0°F) room with free access to food and water. The animals were used at known stages of the estrous cycle as determined by vaginal smears taken daily by lavage between 9:00 and 11:00 AM for at least 2 wk prior to use. The rats were injected ip with 20 μCi (0.38 μg) ^{131}I-thyroxine (^{131}I-T_4) (Tetramet-131, Abbott) at 9:00 AM. Three hours later, they were killed by ether inhalation. Three hours was chosen as a suitable time period since we found (unpublished data) maximum tissue concentrations of radio-labeled hormone at 2 to 3 hr, even though as also observed by others (9) high plasma levels persist. Segments of uteri were slit lengthwise, washed, pressed dry between filter paper and weighed. The concentration of radioactivity in all tissue samples was determined by counting in a well-type scintillation counter equipped with a gamma spectrometer. In one experiment dry weights were also obtained after vacuum desiccation for 48 hr. Results are expressed as counts per minute (cpm) per wet weight of tissue and in one experiment, as cpm per dry weight of tissue. In a third experiment whole uteri were weighed wet as described above and the entire uterine radioactivity was determined.

The nature of the uterine radioactivity was chromatographically analyzed in a butanol–ethanol–0.3 *M* NH_4OH (5:1:2) (BEA) ascending system (10). This permitted an assessment of the relative amounts of radioactivity present as iodide (I^-), thyroxine

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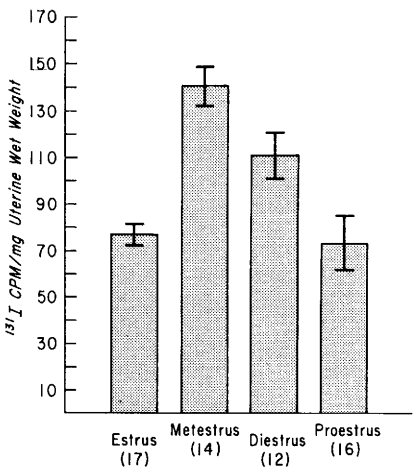

FIG. 1. ^{131}I-Thyroxine uptake in uterus during stages of the estrous cycle: results expressed as mean ± SEM; number of animals given in parentheses.

(T_4), triiodothyronine (T_3), and as iodotyrosines (MIT, DIT).

Results. The 3-hr concentrations of radioactivity in uteri examined at various times of the estrous cycle are summarized in Fig. 1. The concentration during metestrus was significantly greater than at any other phase of the estrous cycle and was twice that during estrus or proestrus ($p < 0.001$). The concentration during diestrus was greater ($p < 0.02$) than during either estrus or proestrus. There was no difference between estrus and proestrus.

The results of a second experiment of similar design are shown in Fig. 2A. The metestrus and diestrus values were combined as were the estrus and proestrus since a small group of rats was used. The results were similar to those of the first experiment with the concentration of label in the metestrus–diestrus group being notably different ($p <$ 0.001) from the estrus–proestrus group.

It was also considered possible that since the uterus is slightly larger in proestrus than in metestrus the changes in concentration of radioactivity were simply a reflection of total weight changes in the uterus. Accordingly, a third experiment was performed in which uterine uptake was calculated as a function of total uterine weight. The uterine weight of rats in proestrus–estrus was 388 ± 22 g (mean ± SE; 10 rats) while those in metestrus–diestrus was 322 ± 23 g (14 rats). The total 3-hr uterine uptake of radioactivity of the proestrus rats was 21,840 ± 560 cpm, while that of metestrus–diestrus rats was 28,980 ± 2735 cpm. This difference was statistically significant ($p < 0.01$). Thus, both the total uptake and concentration of radioactivity were greater during metestrus–diestrus than during proestrus–estrus.

The normal uterus responds in a variety of ways to estrogen stimulation. For example, the uterine water compartment increases dramatically as it grows from the metestrus to the estrus stage of the reproductive cycle. Although each uterine segment was blotted thoroughly before being weighed and counted in the above experiments, we decided to

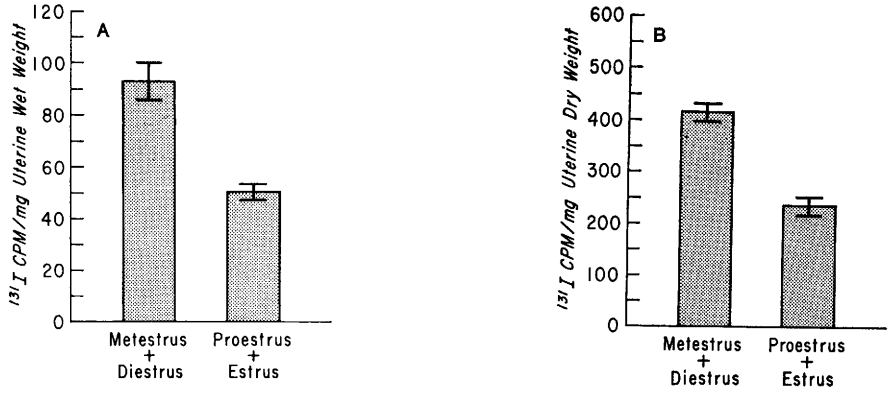

FIG. 2. ^{131}I-Thyroxine uptake in uterus during the estrous cycle. Results are given as function of uterine wet weight and dry weight in (A) and (B), respectively; results expressed as mean ± SEM.

determine concentration of label as a function of dry weight in the second experiment. There was no significant difference in the results when the uptake was measured after desiccation compared with measurement before desiccation (Fig. 2B).

Chromatography of uterine homogenates ($n = 2$) revealed the major radioactive component to have the chromatographic mobility of T_4 (59.2%). Lesser amounts corresponded to I^- (4.7%) and T_3 (7.0%). No peak corresponding to iodotyrosines was found.

Discussion. The results of this study indicate that there is a cyclic variation in the uterine concentration of thyroid hormone which can be correlated with the stages of the estrous cycle and thus with endogenous estrogen levels. Three hours following an ip injection of ^{131}I-T_4 the concentration of radioactivity in the uterus was highest in metestrus, a time when the endogenous estrogen level is low (11). Uterine concentration of label fell to intermediate values in diestrus and to the nadir in proestrus and estrus when the estrogen levels would be expected to be high.

Recently, in a preliminary report, Reilly and Schussler (12) examined T_4 uptake by the uterus during the rapid phase of uterine growth which follows exogenous estrogen administration to immature rats. They reported an increase in T_4 uptake in the stimulated uterus which they attributed to local changes in capillary permeability caused by estrogen stimulation. Assuming the uterotropic response is similar whether caused by exogenous or endogenous estrogen stimulation, our results appear to conflict with theirs. However, the discrepancy may also be due to the different ages of the rats used.

The uterus is one of the more dynamic tissues of the body, attaining a high degree of proliferation and differentiation in a short time with estrogen stimulation. It seemed reasonable for us to suspect a dilution of the radio-labeled hormone concentration in the stimulated uterus due to the increased extracellular water compartment which accompanies the normal uterotropic response. This apparently was not the case as illustrated in Fig. 2. Determination of thyroxine uptake on a dry weight basis yielded results similar to those obtained when the data were calculated per unit wet weight.

In an unpublished study, we examined the uptake of systemically administered radiolabeled thyroxine in various loci in the rat; in normal rats, the uterus exhibited a much higher concentration than leg muscle, pituitary, or brain. In the present investigation, we found that the concentration of uterine radioactivity varied inversely with estrogen concentration and with stages of the estrous cycle being greatest in metestrus and lowest in estrus and proestrus. The physiological significance of this finding is unknown. Perhaps, as suggested by Miura and Koide (4) and Ruh, Ruh and Klitgaard (5, 6), thyroid hormone is one factor which regulates uterine sensitivity to estrogen.

A considerable amount of information is available concerning the physiochemical characteristics and mode of action of the uterine binding-receptors which exhibit specific and preferential affinity for estradiol and other biologically active estrogens or antiestrogens (7, 8). Our observations of high ^{131}I-T_4 concentrations in the uterus which vary with the reproductive cycle pose a few related hypothetical problems. For example, is there a uterine thyroxine-binding receptor system similar to the one described for estrogens or do thyroid hormones compete with estrogens for the same receptor sites? Also, since in this study we examined the entire uterus as a unit (myometrium plus endometrium), additional microscopic and autoradiographic investigations may yield information regarding possible differential effects of the component parts of the uterus.

Summary. Uterine ability to concentrate ^{131}I-thyroxine in female rats during various stages of the estrous cycle was studied. Uteri from metestrous rats had the highest concentration of radioactivity. Uptake during diestrus although less than metestrus was significantly greater than during proestrus or estrus.

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