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Development of Thyroidal Responsiveness to Estrogen in the Maturing Rat (38471)

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Much controversy exists in the literature concerning the influence of estrogens on thyroid function. Several investigators have found that estrogens increase thyroidal ^{131}I uptake, whereas others have failed to demonstrate an effect. Unfortunately, experimental conditions in these studies have not been consistent, and it is difficult to make comparisons. Bogdanove and Horn (1), Noach (2), Soliman and Reineke (3, 4), and Sato and Matsumoto (5), have all failed to demonstrate significant effects of estrogen on various aspects of thyroid function in hypophysectomized rats, whereas Feldman (6), Donato *et al.* (7), and Boccabella and Alger (8), have found estrogen stimulation of ^{131}I uptake or increased thyroid/serum iodine ratios. In the rat, thyroidal ^{131}I uptake has been shown to be highest at estrus and lowest during proestrus (4, 6).

The present study was undertaken to ascertain the following: The influence of chronologic age on the hypothalamic-pituitary-thyroid axis responsiveness to exogenous estrogen stimulation; the nature of ^{131}I localized in thyroid tissue following such stimulation; and the role of age and exogenous stimulation on excretory patterns of injected ^{131}I. Solutions to these questions would be helpful in resolving the existing lack of agreement over estrogen potentiation of thyroid function in the rat.

Materials and Methods. Female Sprague–Dawley rats, hypophysectomized at 21, 30 or 50 days by Hormone Assay Laboratory, Chicago, IL, were maintained in vivarium facilities under a 14-hr light and 10-hr dark photo period at a constant temperature of 80°F. Purina rat chow and 1% NaCl drinking water were provided *ad libitum*. An acclimatization period of 8 days was allowed prior to beginning treatment. Rats were randomly divided into four treatment groups: Estradiol, TSH, estradiol-TSH, and control, and were subcutaneously injected daily at 3 PM for 5 days. Estradiol (Mann Research Laboratory) injections consisted of either 1 or 50 μg 17β-estradiol in 0.2 ml corn oil. TSH (NIH B-6) in a dosage of 0.2 mg was administered in 0.2 ml of 0.9% NaCl. Estradiol-TSH animals received 1 μg of estradiol with concomitant 0.2 mg TSH. Controls received corresponding volumes of carrier.

On day 6, each animal was given a single intraperitoneal injection of 127,000 cpm ^{131}I in 0.1 ml of 0.05 *M* phosphate buffer, pH 7.2, placed in metabolic cages, deprived of food, and sacrificed at intervals thereafter. Animals were ether anesthetized and blood samples were taken by cardiac puncture. Thyroids were dissected out, weighed and sellae turcicae inspected for completeness of hypophysectomy. Animals with incomplete operations were rejected.

Whole thyroids and samples of blood sera, feces, and urine were analyzed for radioactivity in a Packard Auto Gamma Spectrometer. Organic iodine was determined by the method of Nagataki and Ingbar (9). The activity (cpm) at injection and sacrifice were measured in order to correct for radioactive decay. ^{131}I uptake percentage was calculated as:

$$\%\ ^{131}I \text{ uptake} = \frac{(\text{radioactivity in thyroid} - \text{background}) \times 100}{\text{radioactivity injected} - \text{background}}$$

Experiments were performed to determine the optimal time period of sacrifice following Na ^{131}I administration. As shown in Fig. 1, accumulation of ^{131}I is significantly greater at 24 hr postinjection than at either two or 6 hr. As a result of this finding, all subsequent experiments were performed using a 24-hr postinjection time of sacrifice.

Results. Estradiol in doses of either 1 or 50 μg per day failed to influence accumulation of ^{131}I by thyroid glands from rats hypophysectomized on day 21 of age (Table I). Thyroids

from these animals, however, responded to both TSH and TSH-estradiol combinations with significantly increased ^{131}iodine uptake. When these studies were performed with rats hypophysectomized at 30 days of age, estradiol alone as well as TSH and TSH-extradiol combinations again significantly increased accumulation of ^{131}I over that of corn oil injected controls. The combined effect of the two hormones was always greater in both groups of animals than that induced by separate injection of either hormone. The stimulatory effect of estradiol alone on ^{131}I uptake by the thyroid in 30-day hypophysectomized animals was observed only 24 hr after ^{131}I administration and not at 2 and 6 hr (Fig. 1). Rats hypophysectomized at 50 days of age also responded to estrogen treatment with significantly increased thyroidal uptake of ^{131}iodine.

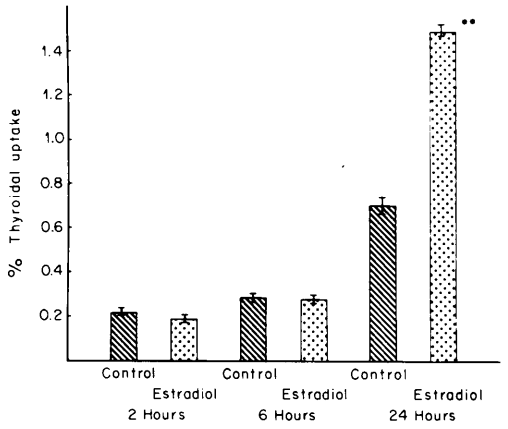

FIG. 1. Influence of estradiol (1 μg/day for 5 days) on thyroidal ^{131}I uptake in female rats hypophysectomized at 30 days of age. Values are % thyroidal ^{131}I uptake at various time intervals after ^{131}I administration. Each bar represents the mean ± S.E. based upon a minimum of four animals per group. ** Significantly different from control at $P < 0.01$.

A second series of experiments were performed to determine the nature of accumulated ^{131}I under the influence of both estrogen and TSH stimulation in 30-day hypophysectomized animals. As the data in Table II demonstrate, only a small portion of ^{131}iodine injected into control animals was retained in the thyroids in an inorganic form for 24 hr. Treatments did not significantly modify these levels. The major portion of thyroidal ^{131}I appeared to be organically bound. Both estradiol and TSH alone and in combination significantly increased organic ^{131}iodine in these animals.

The results shown in Table III indicate that in 21- and 30-day hypophysectomized rats, 17–43 % of ^{131}I injected is excreted in the urine. Excretion patterns of ^{131}iodine in urine were not altered by estradiol or TSH in 21-day or 30-day animals but were significantly decreased in the TSH-estradiol treated groups. This decrease, however, could be partially accounted for by the enhanced level of ^{131}I uptake into the thyroid in TSH-estradiol-treated animals of both groups (Table I). In addition, the known enhancement of thyroxine binding to TBG under the influence of estrogen would con-

TABLE I. INFLUENCE OF AGE ON ^{131}IODINE UPTAKE IN RESPONSE TO ESTRADIOL AND TSH IN HYPOPHYSECTOMIZED FEMALE RATS.

Experimental group	Experiment	21-Day hypophysectomized	30-Day hypophysectomized	50-Day hypophysectomized
Corn oil injected controls	1	1.01 ± 0.11[a]	.68 ± 0.03	0.35 ± .04
	2	1.00 ± 0.10	.40 ± 0.07	—
17β-estradiol (1 μg)	1	1.03 ± 0.09	1.48 ± 0.41[c]	—
17β-estradiol (50 μg)	2	1.01 ± 0.10	2.39 ± 0.56[c]	1.02 ± .05[b]
TSH (.2 mg)	1	5.32 ± 0.31[c]	2.70 ± 0.74[c]	—
TSH (.2 mg) plus 17β-estradiol (1 μg)	1	7.10 ± 0.50[c]	3.33 ± 0.66[c]	—

[a] Values are % ^{131}I thyroidal uptake as calculated by the equation in Material and Methods and represent the mean ± S.E. based upon a minimum of four animals per group.

[b] Significantly different from control at $P < 0.05$.

[c] $P < 0.01$.

TABLE II. NATURE OF THYROIDAL BOUND ^{131}IODINE IN 30-DAY HYPOPHYSECTOMIZED FEMALE RATS.

Experimental group	Inorganic ^{131}Iodine	Organic ^{131}Iodine
Corn oil injected controls	0.06 ± 0.08[a]	0.76 ± 0.12
17β-estradiol (1 μg)	0.04 ± 0.06	1.45 ± 0.16[b]
TSH (.2 mg)	0.12 ± 0.12	2.58 ± 0.41[b]
TSH (.2 mg) plus 17β-estradiol (1 μg)	0.01 ± 0.03	3.33 ± 0.32[b]

[a] Values are % organic and inorganic thyroidal ^{131}iodine as calculated by the equation in Material and Methods and represent the mean ± S.E. based upon a minimum of four animals per group.

[b] Significantly different from control at $P < 0.01$.

TABLE III. INFLUENCE OF AGE ON URINARY ^{131}IODINE EXCRETION PATTERNS IN HORMONE-TREATED FEMALE HYPOPHYSECTOMIZED RATS.

Experimental group	21-Day hypophysectomized	30-Day hypophysectomized
Corn oil injected controls	26.57 ± 3.66[a]	37.61 ± 3.11
17β-estradiol (1 μg)	30.15 ± 5.42	32.59 ± 2.92
TSH (.2 mg)	29.60 ± 2.67	43.63 ± 5.16
TSH (.2 mg) plus 17β-estradiol (1 μg)	17.76 ± 1.63[b]	29.26 ± 2.84[b]

[a] Values are % urinary ^{131}iodine excreted as calculated by the equation in Material and Methods and represent the mean ± S.E. based upon a minimum of four animals per group.

[b] Significantly different from control at $P < 0.05$.

tribute to the observed decrease in urinary excretion. ^{131}I excretory patterns did not significantly differ in untreated 21 and 30-day hypophysectomized groups although 30 day urinary outputs were greater.

Fecal excretion was found to be a relatively minor pathway for ^{131}iodine elimination (<1% of injected dose) with no significant variation seen among treated groups in 21- or 30-day animals.

Discussion. Our studies demonstrate that estrogen alone significantly increases thyroidal accumulation of ^{131}iodine in rats hypophysectomized on 30 or 50 days of age but not when operations are performed on 21 days of age. Thyroids from these latter animals are, however, responsive to TSH stimulation. These studies imply a development of thyroidal responsiveness to estrogen at the physiological age at which larger quantities of estrogen appear in the rat circulation.

We have further demonstrated that the majority of ^{131}iodine localized in thyroids in response to estrogen is organified, thus indicating stimulation of a natural uptake process by estrogen. In addition, differences found in these studies cannot be accounted for by differing excretory patterns in 21- and 30-day groups since neither treatment nor age substantially altered urinary output of radioactivity.

As our results suggest, age of the animals employed may ultimately influence the effect seen. Our data indicate that significant changes take place in thyroid function in the intact rat between 21 and 30 days of age. These changes may be related to the maturation of the hypothalamic-pituitary-thyroidal axis during this period. Similar observations on the sensitivity of the hypothalamic-pituitary-ovarian axis to estrogens have been reported by McCann and Ramirez (10). Although seemingly contradicted by Swerdloff *et al.* (11), these studies have been confirmed in the rat by Eldridge *et al.* (12).

We feel that the discrepancies found in the literature may be due to the following: differences in the physiological age of the animals used, use of estrone or 17α-estradiol as the stimulatory agent, differences in the timing of estrogen administration and subsequent evaluation of thyroid function, and finally criteria used for assessment of thyroid function.

Summary. The incidence of thyroid disorders around the time of puberty is frequent in human females. The influence of estrogen on thyroid function during this period has been controversial. Female rats hypophysectomized at 21, 30 or 50 days of age were treated with either 17β-estradiol (E_2) or TSH or a mixture of E_2 and TSH. Determinations

were then made of thyroidal ^{131}I-uptake, organic ^{131}I, and ^{131}I excretory patterns. In 21-day old animals, E_2 alone had no effect on thyroidal ^{131}I uptake, whereas in 30-day or older animals E_2 significantly increased ^{131}I uptake. TSH or TSH-E_2 combinations markedly increased ^{131}I uptake in both groups. Thyroidal ^{131}I was shown to be almost exclusively protein-bound in each instance. No difference was observed between 21 and 30-day old rats with respect to ^{131}I excretory patterns, except for decreased urinary output in the TSH-E_2 groups; this decrease, however, appeared to be partially attributable to an increased thyroidal uptake of iodine in these animals. The results suggest that the thyroid gland becomes responsive to estrogen only at or around the time of puberty.

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