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INFLUENCE OF PRE- AND POSTNATAL THIOURACIL ADMINISTRATION ON PITUITARY GROWTH HORMONE CONTENT IN MICE[1]

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Abstract. We examined the influence of thiouracil-induced hypothyroidism imposed at various stages of development upon pituitary growth hormone (GH) concentration. Balb/c-derived mice were mated and fed thiouracil, 0.25% in Purina Lab Chow, from the first day of pregnancy until 25 days postpartum, or for shorter periods of time (last ⅔ of pregnancy and postpartum, last ⅓ of pregnancy and postpartum, or postpartum only). Using disc gel electrophoresis, pituitary GH concentration of 25 day old pups exposed to thiouracil was compared with that of unexposed animals. Concentration of pituitary GH was depressed significantly in animals whose mothers were first given thiouracil at the initiation of pregnancy or ⅓ of the way through pregnancy. Young of mothers first given thiouracil ⅔ of the way through gestation or on the first day postpartum did not have significantly lower pituitary GH concentrations than controls. The factor determining the presence or absence of thiouracil-induced depression of pituitary GH concentration may be the initiation of fetal thyroid function that occurs between days 15–17, approximately ⅔ of the way through gestation.

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Hypothyroidism induced around the time of birth has long been known to retard skeletal development and growth in rats (Salmon 1938). Mice born of euthyroid mothers were reported to grow faster than those of thyroidectomized dams (Davenport and Swingle 1927). More recent studies have suggested that growth is depressed because of reduced synthesis or secretion of growth hormone (GH) induced by the hypothyroid condition. For example, Peake *et al* (1973), using radioimmunoassay, and Wilkins *et al* (1974) using disc gel column electrophoresis have shown altered pituitary GH concentration in pituitaries of rats made hypothyroid as young adults. Furthermore, pituitaries of rats thyroidectomized at birth are virtually devoid of GH at 25 days of age (Geel and Timiras 1970).

The length of time necessary for hypothyroidism to alter GH status has not been extensively studied. Hamburgh (1968) suggested that withholding thyroid hormone from developing rats *in utero* does not appreciably alter maturation of the nervous and skeletal systems. Our study was carried out to determine the importance of thyroid status during development as a factor in establishing postnatal pituitary growth hormone concentration. Hypothyroidism was induced by exposing mice to thiouracil, a drug that prevents thyroid hormone formation, during a portion of gestation and postnatally.

MATERIALS AND METHODS

Female Balb/c-derived mice were mated to males of the same strain. First day of pregnancy was determined by presence of a vaginal plug. Pregnant females were isolated and assigned to one of the following feeding schedules (see fig. 1):

(A) Thiouracil diet from the first day of pregnancy to 25 days postpartum (46 days of thiouracil exposure).

(B) Control diet during the first ⅓ of pregnancy, and thiouracil diet during the last ⅔ of pregnancy to 25 days postpartum (39 days).

(C) Control diet for the first ⅔ of pregnancy, and thiouracil diet during the last ⅓ of pregnancy to 25 days postpartum (32 days).

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(D) Control diet during pregnancy and thiouracil diet from day 1 to day 25 postpartum (25 days).
(E) Control diet from the first day of pregnancy to 25 days postpartum (0 days).

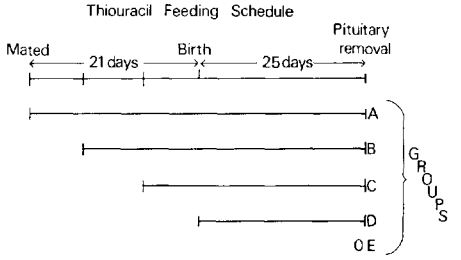

FIGURE 1. Digramatic representation of feeding schedule. Bar represents time of thiouracil incorporation in maternal diet. Groups correspond to those in table 1: A=46 days; B=39 days; C=32 days; D=25 days; E=0 days.

The control diet consisted of Purina Lab Chow *ad libitum*, and the test diet had 0.25% thiouracil added to the ground Lab Chow. Each group consisted of 5 litters except A and C, which had 4 and 6 respectively.

At birth, litters were standardized to 5 pups. At 25 days of age, all pups were killed by decapitation and body weights were determined. Thyroid and pituitary glands were rapidly excised, and the weights of individual thyroids and pooled pituitaries (5 glands per pool) were obtained to the nearest 0.1 mg. Pooled pituitaries were stored frozen at −20 °C for less than 30 days after which they were homogenized in 40% sucrose at a concentration of 4 mg pituitary tissue per ml.

Pituitary GH was separated from other proteins in the homogenate by the polyacrylamide disc electrophoresis methods developed by Davis (1964) and Ornstein (1964) as modified by Lewis *et al* (1965). Electrophoresis was done at room temperature using a 7% acrylamide separating gel (pH 9.5). Duplicate or triplicate analyses were made of each homogenate. After electrophoresis, gels were stained with aniline blue black (1% in 7% acetic acid) and were destained by diffusion in 7% acetic acid. Pituitary GH was quantified by densitometric comparison of gels containing GH from pituitary homogenates to those containing standard rat GH (NIAMDD Rat GH-3) supplied by Dr. A. F. Parlow as agent for the NIAMDD Rat Pituitary Hormone Distribution Program.

Differences in group means for body and organ weights and growth hormone concentrations were determined using two-way analysis of variance, and Scheffé's method of multiple comparisons was used to test for significant differences between litters within treatment groups (Scheffé 1959).

RESULTS

Multiple comparisons showed no differences between litters within treatment groups for any of the parameters studied. An analysis of variance, however, demonstrated a significant effect of thiouracil exposure on body and organ weights. Body weights indicated a depression of growth in all thiouracil-exposed pups. This influence was most pronounced in young exposed to thiouracil after birth only (25 days, table 1), but subnormal maternal food intake may have been a confounding unmeasured variable in this group. Thyroid weight gain by all thiouracil-exposed pups (overall mean 10.9 mg vs. 2.2 mg for controls) demonstrated the influence of the drug as an inducer of thyroid hypertrophy through inhibition of hormone secretion. Pooled pituitary weights showed the influence of induced hypothyroidism with a linear depression in gland mass from pups exposed to thiouracil for 0 days (3.6 mg) to those exposed for 46 days (2.4 mg) (table 1).

Growth hormone concentration of the pituitary gland was also affected by thiouracil, showing a significant linear depression from 25 days (28.1 μg/mg pituitary tissue) through 46 days (11.5 μg/mg) of exposure ($P<0.05$). No statistically significant difference existed, however, between GH concentration of pups exposed to thiouracil for 39 or 46 days and those exposed for 0, 25, or 32 days (table 1). Indeed, when analyzed as single treatment periods, pups from mothers fed thiouracil before the last ⅓ of pregnancy had a significantly depressed pituitary GH concentration (39 and 46 day groups; 14.3 μg/mg) when compared with offspring of mothers given thiouracil from the last ⅓ of pregnancy or later (25 and 32 day groups; 26.5 μg/mg). The latter animals did not differ significantly from controls (0 day group; 30.3 μg/mg).

DISCUSSION

Published values for pituitary GH concentration in untreated animals vary somewhat with species, strain, age, sex, and method of measurement. For example, Geel and Timiras (1970) reported a concentration of 17.7 μg GH/mg pituitary tissue in 25 day old rats as assayed by disc gel electrophoresis, whereas

TABLE 1

Body and organ weights and pituitary growth hormone concentrations of control and thiouracil-exposed 25 day old mice.

Thiouracil Exposure (days)	Body Weight (g)	Thyroid Weight (mg)	Pituitary Weight*** (mg)	GH Conc. (μg/mg tissue)
0	15.9*	2.2	3.6	30.3
	±0.4	±0.1	±0.2	±2.1
	(25)	(25)	(5)	(11)
25	9.6**	8.5**	3.4	28.1
	±0.5	±0.8	±0.2	±1.5
	(25)	(25)	(5)	(13)
32	11.0**	13.8**	3.0**	24.8
	±0.3	±1.2	±0.1	±2.4
	(30)	(30)	(6)	(12)
39	13.2**	10.6**	2.6**	16.5**
	±0.6	±1.0	±0.4	±1.7
	(25)	(25)	(5)	(14)
46	10.8**	9.8**	2.4**	11.5**
	±0.1	±0.8	±0.1	±2.2
	(20)	(20)	(4)	(11)

*Mean ± Standard Error.
Number in parentheses indicate mice in each group.
**Significantly different from 0 days ($P<0.05$).
***Pituitary weights are based on pooled samples.

Walker *et al* (1977), using radioimmunoassay, found about 100 ng GH/μg pituitary protein in rats the same age. This value converts to approximately 12.6 μg GH/mg pituitary tissue if the pituitary is considered to be about 12.6% protein as reported by Treloar and Leathem (1969) in 50 day old rats. Slightly older animals appear to have higher pituitary GH concentrations as measured by radioimmunoasay. Mosier *et al* (1977) showed 150 μg GH/mg in 56 day old rats and Sinha *et al* (1977) reported levels of 60 μg/mg in female and 120 μg/mg in male mice 76–96 days old. The control GH concentration found by disc gel electrophoresis in the pituitaries of 25 day old mice in the present study (30.3 μg/mg) was slightly greater than that reported for 25 day old rats. The difference observed may be the result of using rat GH as a standard to quantify mouse GH, or perhaps the mouse actually has a slightly greater pituitary GH concentration than the rat.

Thiouracil belongs to the class of compounds that induce hypothyroidism by preventing thyroid hormone synthesis (Astwood *et al* 1943, MacKenzie and MacKenzie 1943). Our data indicate that the length of time mice are exposed to this drug *in utero* is important in determining pituitary GH concentration at 25 days of age. One explanation for the presence of an influence on pituitary GH in mice first exposed to thiouracil before the last ⅓ of gestation and the absence of effect in mice first exposed later in development can be based on the time of onset of fetal thyroid function. Functional activity of the fetal mouse thyroid gland develops between days 15 and 17 of gestation (van Heyningen 1961). Since mothers of pups exposed to thiouracil during the last ⅓ of gestation first receive thiouracil in their diet on the 15th day of pregnancy, the fetal thyroid gland probably has developed some minimal functional capability before the effect of thiouracil becomes pronounced. Therefore, pituitary GH content may be less drastically altered than it would be in pups whose mothers have been fed thiouracil from day 1 or day 8 of pregnancy. Since thiouracil can pass through the placenta (Hayashi and Gilling 1967), administration of the drug early in development could result in its presence in the fetus well before the onset of thyroid

function, bringing about significant alteration of pituitary GH concentration.

The present study does not corroborate the finding of Hamburgh (1968) that prenatal thyroid restriction does not significantly affect the developmental processes of young rodents. It may be possible to explain the discrepancy between our results and those of Hamburgh by consideration of procedural differences or differences in experimental animals. Hamburgh used the drug propylthiouracil to induce hypothyroidism but did not feed it until day 15 of gestation, hypothesizing that it was unimportant to begin treatment before the onset of fetal thyroid function. Our study shows that it is necessary to give the drug earlier to influence pituitary GH concentration. In addition, physiological differences between rats and mice that may result in dissimilar responses to thiouracil exposure are facts that cannot be ignored.

It is important to note that the depressed pituitary GH level in thiouracil-exposed mice does not warrant conclusions regarding its synthesis or secretion. As mentioned by Kraicer *et al* (1977), hormone content of a gland is the result of dynamic hormone synthesis, release, activation, and inactivation. An alteration in one component brought about by experimental manipulation (e.g., thiouracil exposure) indicates an altered balance among these 4 factors. The importance of this alteration is exemplified by the absence of a direct correlation between body weight and pituitary GH concentration in the present study. Lack of correlation is presumably the result of unmeasured metabolic alterations present in thiouracil-exposed mice. For example, in the neonatal rat Stuart *et al* (1976) found a paradoxical relationship between circulating levels of GH and somatomedin, a peptide that mediates GH action on skeletal tissues. GH levels in plasma were high, but somatomedin levels were low during a period of rapid growth. These investigators hypothesized the importance of insulin, abundant in the circulation of neonatal rats, as a growth factor in early life. Neither insulin nor somatomedin was measured in the present study. As mentioned earlier, the pups exposed to thiouracil during lactation only (25 days) may have had their growth stunted by depressed maternal food intake that probably occurred when thiouracil was first encountered at parturition. Besides the possible thiouracil-induced metabolic alterations, there appears to be an absence of direct correlation between pituitary GH content and growth in some species examined under normal physiological conditions. Borer and Kelch (1978) have reported this to be the case in the hamster, but this species is well known for endocrine responses unlike those of other experimental rodents. Our present study shows that in mice exposed to thiouracil before the last $\frac{1}{3}$ of gestation, pituitary GH concentrations were depressed whereas in those first exposed to thiouracil during the last $\frac{1}{3}$ of gestation or later, the GH content was not significantly altered. The importance of this finding in the overall metabolic scheme remains to be determined.

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