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A Sex Difference in Plasma Thyroxine and Thyroid Stimulating Hormone in Rats[1] (38239)

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(Introduced by L. K. Dahl)

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During the course of genetic experiments with spontaneously hypertensive rats, plasma thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations were measured in a large population of rats. The original purpose of such work was to look for correlations between blood pressure and T_4 or TSH. Although no such correlations were found, a definite difference between the sexes for these two hormones was incidently observed.

Methods. The 193 rats used were an F_2 population obtained by crossing nomotensive Wistar rats (MW-3) and rats of the spontaneously hypertensive (SH) strain (1), both obtained from Purina Laboratory Animals, Vincentown, N. J. There were 97 males and 96 females. Animal rooms were maintained at 75°F. All rats were killed at 27–28 weeks of age by rapid decapitation. Trunk blood was collected with heparin (<20 units/ml of blood) into tubes in ice and centrifuged at 4°. Plasma was stored at −20° until assayed. Rats were killed in random order in 5 blocks of approximately equal size. The TSH assays were performed in these same 5 blocks but T_4 measurements were done in 20 blocks. All determinations were done in duplicate.

Plasma T_4 assays were done by the competitive protein binding assay of Murphy and Pattee (2) as modified (3). L-Thyroxine as the free acid (Sigma) was used as standard. Plasma TSH was done by radioimmunoassay using reagents supplied by the rat pituitary hormone program of the National Institute of Arthritis and Metabolic Diseases. In this assay purified TSH was iodinated with I-125 after the method of Greenwood *et al.* (4) and purified on a Sephadex G-75 column. Free and bound iodinated TSH were separated in the radioimmunoassay using goat antibody to rabbit gamma globulin (Calbiochem). Following precipitation and centrifugation, radioactivity in an aliquot of supernatant was determined by liquid scintillation counting following solubilization in Ready Solv VI (Beckman Instruments). Results were expressed in terms of the standard preparation TSH-RP-1 (reference preparation—1) which contained .22 USP (bovine) TSH units/mg (McKenzie assay).

Results. Histograms for TSH for both males and females are given in Fig. 1 and show a marked tendency for skewness to the right. The coefficient of skewness, g_1, (5) was significantly different from zero by the appropriate *t*-test for both males and females ($P < .005$). When a logarithmic transformation was made the g_1 values were not significantly different from zero for either males or females ($.1 < P < .25$). The mean TSH value for males was about twice that for females (Fig. 1). This difference was significant ($P < .005$) when tested by *t*-tests using either the original data or the logarithmically transformed data.

Histograms for plasma T_4 are given in

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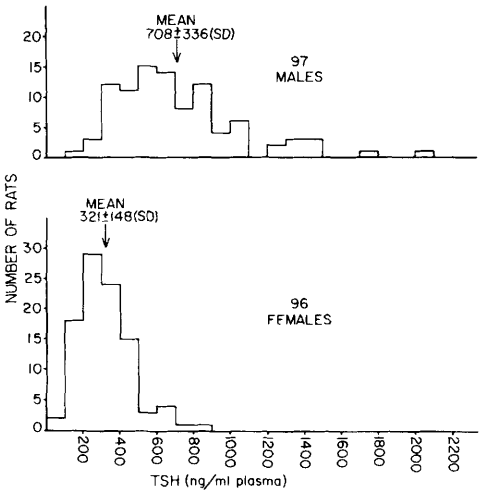

FIG. 1. Histograms for plasma thyroid stimulating hormone (TSH).

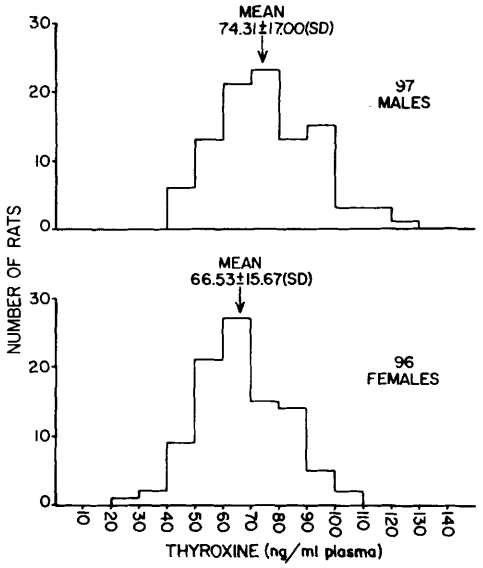

FIG. 2. Histograms for plasma thyroxine.

Fig. 2; no skewness was evident. The mean value for males was slightly higher than females and in spite of the small magnitude of difference, this difference was significant by a *t*-test ($P < .005$).

The data were examined for correlations between plasma TSH and plasma T_4. The correlation coefficients were $-.098$ and .116 for males and females, respectively. Neither value was significantly different from zero.

Discussion. It is reasonable to suppose that the higher plasma T_4 in males over females results from their higher plasma TSH levels. The origin of the higher TSH levels in males is, however, obscure. The mean plasma TSH for males in Fig. 1 of 708 ng/ml corresponds to 15.5 mU/100 ml which is lower than the 30–50 mU/100 ml as measured by radioimmunoassay by Reichlin *et al.* (6) for male rats of 200–250 g body wt. Male rats in the present experiment weighed 500–600 g (6 mo old) and this age factor might account for the difference. Plasma TSH in male Wistar rats 150–200 g has also been bioassayed and found to be lower, about 6 mU/100 ml, (7) than the radioimmunoassay values reported here or by Reichlin *et al.* This could mean that biologically inactive TSH fragments are measured in the radioimmunoassay.

A negative feedback loop between the thyroid and the hypothalamic-pituitary system is well known. This mechanism has been demonstrated in rats by thyroidectomy with T_4 injection and plasma TSH measurement (6). Superimposed on this are positive imputs from the hypothalamus. Given these facts, one is, however, at a loss to predict *de novo* if plasma TSH and plasma T_4 will be correlated, and in what direction in normal individuals of a large population. Beside an environmentally induced variable metabolic status between animals, genetic variation in the population studied here is likely to exist since it was an F_2 population derived from 2 lines (SH and Wistar) rather than a single highly inbred line. SH rats compared to Wistar are known to have greater thyroid and pituitary weight (8), thyroid hormone secretion rate (9), thyroid response to cold (10) and acid phosphatase levels in the thyroid (11). The sum action of multiple, uncorrelated, environmental and genetic variables would seem to be the essentially zero correlation found between TSH and T_4 in this population.

Summary. Plasma thyroid stimulating hormone (TSH) and thyroxine (T_4) were measured in a large population (97 males and 96 females) of rats. Both hormones were significantly higher in males than females. Mean plasma TSH was 708 ng/ml (corresponds to 15.5 mU/100 ml) in males

and 321 ng/ml (7.0 mU/100 ml) in females. Mean plasma T_4 was 74.31 ng/ml for males and 66.53 ng/ml for females. The correlation between plasma TSH and T_4 in this resting, unmanipulated, genetically heterogeneous population was essentially zero.

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