

Thyroxine and Mammary Gland Growth in Rat.* (26506)

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Previous studies in this laboratory have employed desoxyribonucleic acid (DNA) as a quantitative index of mammary gland cellular proliferation in rats and mice(1,2,3). By this method, it is possible to determine the synergistic influence of various hormones on normal and experimental mammary gland growth.

Grovesnor and Turner(4,5) reported administration of hormones concerned with lactation in optimal amounts increased milk yield and decreased variation among animals. Similarly, suboptimal secretion of hormones concerned with mammary gland cellular proliferation may also limit maximum growth of gland.

With elaboration of quantitative measures of mammary gland growth and thyroxine secretion rate (TSR)(5,6), it seemed desirable to investigate influence of physiological levels of exogenous thyroxine on mammary gland proliferation of normal pregnant rats.

Methods. Groups of pregnant rats of Sprague-Dawley-Rolfsmeier strain were used. Control animals were sacrificed on days 18-20 of pregnancy and experimental groups 20 days after detection of sperm within vagina. Experimental animals were injected with 2.5, 3.0, and 3.5 μg of thyroxine (T_4)/100 g body weight (BW) respectively from days 3 to 19 of gestation. Six abdominal-inguinal mammary glands were removed from each rat and DNA determined as described previously(3).

Results. Mean total DNA of normal pregnant rats was 7.63 mg/100 g BW; whereas experimental groups were 9.38, 8.09, and 9.6 mg/100 g BW respectively for 2.5, 3.0, and 3.5 μg of T_4 /100 g BW (Table I). DNA/mg followed a similar pattern: normal (33.2 μg), 2.5 μg T_4 (38.3 μg), 3.0 T_4 (31.8 μg),

and 3.5 μg T_4 (38.3 μg). DNA/100 g BW was significantly increased at 2.5 and 3.5 T_4 levels ($P>.01$) when compared with normal pregnant values. There was a slight but non-significant increase in DNA/100 g at 3.0 μg T_4 level and also a slight decline in DNA/mg. Combined T_4 total DNA was also increased significantly ($P>.01$) compared to control pregnant animals. Calculation of frequency distribution of normal and T_4 injected pregnant animals (Fig. 1) indicated that T_4 increased mean total DNA; whereas range and variability of total DNA remained essentially the same between the 2 groups. DFFT of all groups of animals remained constant, and increase in total DNA was due to an increase in DNA/mg DFFT.

Discussion. Past studies of thyroid-mammary gland relationship were complicated by administration of unphysiological levels of T_4 . It is now possible to measure thyroid activity of individual animals and variation in secretion rate by TSR method(5,6). This procedure enables one to determine effect of physiological levels of T_4 on mammary gland development.

Grovesnor and Turner(5) reported mean daily TSR of non-lactating rats was 1.3 μg /100 g/day, while mean value of lactating animals was 2.2 μg /100 g/day. The highest observed value was 3.0 μg /100 g/day.

Studies(7,8) have indicated T_4 is not essential for experimental development of rat mammary glands. Chen *et al.*(7) reported triply-operated rats appeared to have extensive mammary gland development with evidence of lactation without addition of T_4 to the injected hormonal regime. In this laboratory(8), addition of 3 μg T_4 /100 g BW with 2 μg estradiol benzoate (EB) and 6 mg progesterone (P) had little beneficial effect in increasing total DNA of rat mammary glands in thyro-parathyroidectomized animals. Although DNA/mg DFFT was increased, total DNA remained the same due

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TABLE I. Thyroxine and Mammary Gland Growth in Pregnant Rats.

No. of animals	Stage of gestation (days)	Thyroxine level*	Body wt† (mean)	DFFT (mg) mean	DNA ($\mu\text{g}/\text{mg}$ DFFT) mean $\pm$ S.E.	Total DNA (mg/100 g B.W.) mean $\pm$ S.E.
19	18-20	—	324	749	33.24 $\pm$.8	7.63 $\pm$.39
17	20	2.5	280	724	38.3 $\pm$ 2.28	9.38 $\pm$.32 ^{1,2}
13	20	3.0	287	745	31.8 $\pm$ 4.9	8.09 $\pm$ 1.29
15	20	3.5	283	740	37.7 $\pm$ 1.43	9.66 $\pm$.46 ^{1,2}
45†	20	2.5, 3.0 & 3.5	283	736	35.9 $\pm$ 1.44	9.07 $\pm$.25 ^{1,2}

* Corrected for wt of fetuses and uterus.

† μg T_4 /100 g/day from day 3-19 of pregnancy.‡ Aggregate T_4 animals.¹ Significant to normal pregnant DNA values at 1% level.² Significant to 3.0 T_4 DNA at 2% level.

to a significant reduction in DFFT/100 g.

Moon and Turner(9) determined effect of mild hyperthyroidism upon experimental development of rat mammary glands using DNA as an index of growth. Injection of 3 or 6 μg T_4 /100 g BW with 1 μg EB and 3 mg P/day did not significantly increase mammary gland growth above animals which received EB and P alone. When amount of EB and P was doubled, a significant increase in total DNA with this level of T_4 was observed (26%). It was suggested by the authors suboptimal secretion of T_4 may be one of the limiting factors in obtaining maximum mammary gland proliferation.

In the present study, levels of T_4 were employed which approximated average TSR of lactating animals (2.5 μg /100 g/day), the highest TSR obtained during lactation (3.0 μg /100 g/day) and a level slightly greater than observed (3.5 μg /100 g/day).

Data obtained indicate that mild hyperthyroidism is beneficial in stimulating increased growth of rat mammary glands during pregnancy. The group of 45 pregnant animals injected with T_4 showed a mean

DNA of $9.07 \pm .25$, an increase of 22% above normal pregnant rats. Significantly greater DNA obtained with T_4 was not due to an increase in DFFT. As indicated by DNA/mg, cell concentration/mg tissue accounted for the increase in total DNA at the 2.5 and 3.5 μg level of T_4 .

Summary. The effect of mild hyperthyroidism upon mammary gland growth has been studied by injection of exogenous T_4 into normal pregnant rats at levels of 2.5, 3.0, and 3.5 μg /100 g BW/day. Normal pregnant rats showed mean total of DNA of $7.63 \pm .39$ mg/100 g BW. Group of 45 pregnant rats injected with T_4 showed a mean DNA of $9.07 \pm .25$ mg/100 g BW, an increase of 22%. It is suggested sub-optimal T_4 secretion may be one of the limiting factors in attainment of maximum mammary gland proliferation.

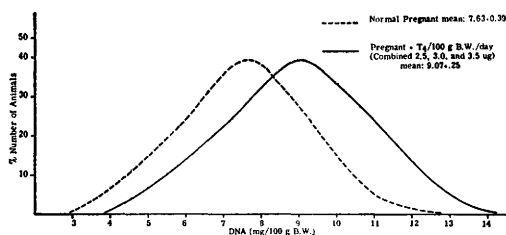


FIG. 1. Frequency distribution of DNA of mammary glands of normal rats at end of pregnancy compared with similar rats inj. with thyroxine during pregnancy. Thyroxine increased gland growth 22%.

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