THYROID HORMONE SECRETION RATE IN THE LACTATING RAT*

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Summary. Thyroid secretion rate (TSR) was determined in lactating and non-lactating rats by the thyroxine substitution and 'direct output' methods. In lactating rats, apparent TSR as determined by the substitution method was significantly greater than that of controls when dietary iodine was deficient. By the direct output method, TSR was significantly reduced even when dietary iodine was adequate. It is concluded that the substitution method measures the physiological demand for thyroid hormone, which is greater during lactation, but represents true TSR only if iodine supplies are adequate. Results obtained by the direct output method show the actual TSR to be significantly reduced both during lactation and when dietary iodine is limited. Mean thyroid acinar cell height significantly increased as thyroidal iodine was depleted. It is concluded that, during lactation, TSH was elevated but iodine was insufficient to support the demand for thyroid hormone. The low serum T^4 level observed in lactating rats, as determined by a competitive binding method, supports these conclusions.

Analysis of the methods of computation of TSR by direct output showed that (1) as thyroidal iodine supplies were depleted, thyroidal iodide accumulation became more efficient, (2) the iodine-deficient thyroid recycled a greater percentage of the available iodine, (3) thyroidal ^{131}I uptake was reduced in lactating rats, (4) total thyroidal iodine was inversely correlated to its release rate (K_4) from the thyroid, and (5) thyroidal iodine, and not K_4 , was the major factor influencing TSR under these experimental conditions.

INTRODUCTION

Several authors, beginning with Courrier, Roche, Deltour, Marois, Michel & Morel (1949), have reported that a considerable amount of iodine is secreted in milk. In man, dogs, rabbits, guinea-pigs, rats and mice, the milk/plasma ratio of ^{131}I reaches 20 to 40 within a few hours of the administration of the radioisotope and in the cow, goat and sheep the ratio is 2 to 3 (see review by Brown-Grant, 1961).

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Upon noting substantial losses of iodine in the milk of goats, Flamboe & Reineke (1959) proposed that under conditions of limited iodine supply, a functional thyroid deficiency could result during lactation. As a further test of this hypothesis, the thyroid hormone secretion rate (TSR) was determined in both lactating and non-lactating rats by two methods. Thyroidal ^{131}I turnover constants, thyroidal iodine, thyroid acinar cell height, and serum thyroxine were also determined.

MATERIALS AND METHODS

In the experiments to be reported, 200-g female rats of the Carworth Farms (CFN) strain were allotted to twelve groups (A to L). Animal laboratory temperature was maintained at $76 \pm 2^\circ \text{F}$ and lights were on for 14 hr daily.

The diet comprised two-thirds ground corn and one-third soybean oil meal (50% protein) with complete vitamin and mineral supplements, except that KI was added only for certain groups (C, D, E, F, I, J, K and L) in the amount of 0.5 or 1.0 $\mu\text{g I}^-/\text{g feed}$. Rats receiving this diet without iodine supplementation develop enlarged thyroids within 2 weeks.

After 16 days on a prescribed diet, carrier-free ^{131}I was injected subcutaneously 2 days after parturition. To approximate comparable labelling of the thyroid in all groups, it was necessary to administer 3 $\mu\text{Ci } ^{131}\text{I}$ to non-lactating rats and 8 μCi to lactating rats.

The TSR was evaluated from 5 to 15 days after parturition by the thyroxine substitution method (Reineke & Singh, 1955) and direct output method of Reineke & Lorscheider (1967) in lactating rats. Body background counts were obtained from the thigh instead of the epigastric region to avoid picking up interference from the mammary glands of lactating rats. Background counts ranged from less than 1% to about 2% of thyroid count in both lactating and control rats. Non-pregnant non-lactating rats were used as controls. The thyroidal output constants were calculated by the equations of Brownell (1951), Robertson & Falconer (1961) and applied in the calculation of TSR by direct output (Reineke & Lorscheider, 1967) as follows:

$$x = \frac{0.693}{T_{\frac{1}{2}} \text{ (days)}} \quad (1)$$

$$K'_4 = 1 - e^{-xt} \quad (2)$$

$$K_4 = \frac{K'_4}{1 - (U/100)} \quad (3)$$

where $t = 1$ day, e is the base of natural logarithms, and U is the percentage of ^{131}I dose taken up by the thyroid and extrapolated to zero time, then K_4 is the fractional ^{131}I -output rate/day corrected for recycling of metabolized ^{131}I . As previously described (Reineke & Lorscheider, 1967):

$$\text{TSR } (\mu\text{g T}_4/\text{day}) = K_4 \times \text{thyroidal I } (\mu\text{g}) \times 2.33 \quad (4)$$

Litters were equally distributed to establish a similar intensity of nursing (eight young) for each rat of all lactating groups.

For each of the three levels of dietary iodine, mean TSR values by both

methods were expressed as μg thyroxine/100 g body weight/day. In the absence of variance homogeneity, a non-parametric statistical evaluation (Mann-Whitney U-Test) was employed (Siegel, 1956) for comparison of TSR in lactating and non-lactating states.

In the direct-output rats, the weight and residual radioactivity were measured in each lobe of excised thyroid. One lobe was analysed for total iodine content as previously described (Reineke & Lorscheider, 1967); the other lobe was fixed in a modified formalin-acetic acid solution, sectioned at $8\text{ }\mu\text{m}$ and stained with haematoxylin-eosin. The total thyroidal iodine was calculated by the equation:

$$\text{Total iodine} = \frac{\text{Total thyroid count}}{\text{Specific activity in ashed lobe}} \quad (5)$$

For histological evaluation, slides were photographed at a magnification of $\times 43$ with 35 mm Ektachrome film. Film strips were projected onto a mirror set at a 45° angle that reflected the image to the underside of a translucent table top. The projection system was calibrated by photographing a stage micrometer at the same magnification used for the photomicrographs. With the geometry employed, 0.102 cm on the projected image equalled $1.000\text{ }\mu\text{m}$ on the original histological section. All measurements were made at the same geometry employed in the calibration. Both the area within the outer perimeter of the follicle (A_o) and the area within the perimeter of the colloid space (A_i) were measured in cm^2 with an area planimeter. Mean acinar cell height was calculated from the equation:

$$\text{Mean cell height } (\mu\text{m}) = \frac{\sqrt{\frac{A_o}{\pi}} - \sqrt{\frac{A_i}{\pi}}}{0.102} \quad (6)$$

Ten follicles were measured in each thyroid, the only criterion for selection being that the follicle must have been sectioned transversely.

In an additional study, serum T_4 was assayed in ten non-lactating and ten lactating rats (twelve young/litter). The rats received $1.0\text{ }\mu\text{g}$ added I/g feed and blood serum samples were taken 16 days after parturition.

Serum T_4 was measured by our modification of the Tetrasorb-125 method (Abbot Radiopharmaceuticals, North Chicago, Illinois). This method is based on a competitive protein-binding principle (Ekins, 1960; Murphy & Jachan, 1965). The analysis employs an alcoholic extract of serum T_4 which will quantitatively displace labelled T_4 from a standard thyroxine-binding globulin (TBG) solution. The displaced T_4 is subsequently bound to a resin-impregnated polyurethane sponge, and the radioactivity displaced can be expressed as the percentage of the total count that is retained in the sponge.

Blood serum samples were frozen and stored for periods of up to several months. At the time of assay, 1 ml serum was taken from the samples which had been thawed and brought to room temperature and 2 ml 95% ethanol were added. The solution was mixed immediately by using a Vortex mixer for 30 sec. To facilitate maximal T_4 extraction, the resultant denatured protein precipitate was allowed to stand at room temperature for 10 min. The mixture was then

centrifuged at 1000 *g* for 20 min. A sample (0.3 ml) of the alcoholic extract from the serum was placed in polypropylene test tubes and, while being warmed in a water bath at 35 to 40° C, the extracts were evaporated to dryness by a steady mild air stream. After complete drying, 1.0 ml ¹²⁵I-labelled TBG solution was added to each tube and allowed to equilibrate with the dried extracted T₄ for 10 min at room temperature. The tubes were then placed in an ice bath (0.5 to 2.0° C) for 5 min. At 20-sec intervals, resin-impregnated sponges were placed successively in the series of tubes (one to each tube) and saturated by lightly depressing each sponge five times, using the special Tetrasorb plunger-aspirator.

The initial radioactivity in counts/min (I) of the total labelled quantity of [¹²⁵I]TBG was obtained for each tube during the incubation period 30 min after adding each sponge. After exactly 1 hr of incubation, the reaction was stopped in successive tubes at 20-sec intervals by adding 10 ml cold (5 to 10° C) distilled water, depressing the sponge five times with the aspirator and immediately drawing off the solution. The sponge was washed three more times with 10-ml portions (approx.) of cold distilled water to remove residual labelled TBG. The final radioactivity in counts/min (F) was taken after four washes. A scintillation well-type counter (Nuclear Chicago, Model DS-5), and analyser/scaler (Nuclear Chicago, Model 8725) was employed for the radioactivity measurements. The percentage uptake of [¹²⁵I]T₄ on resin sponges was obtained using the formula:

$$\% \text{ Resin sponge uptake} = \frac{F - \text{Background}}{I - \text{Background}} \times 100 \quad (7)$$

The F and I counts were measured under constant geometric conditions. A series of standards of varying concentrations was set up for the standard curve that was prepared for each day's run and carried through the procedure already described for the alcoholic serum extracts. In our experiments, the serum T₄ concentration did not exceed 12 µg/100 ml. In the range 0 to 12 µg T₄/100 ml, there is a perfectly linear relationship between T₄ concentration and % resin sponge count. Thus, instead of reading values from a plotted standard curve, values for the T₄ standard were fitted by the method of least squares and the serum T₄ values were calculated by linear regression. The calculations are as follows:

$$X_u = \frac{Y - a}{b} \quad (8)$$

X_u = serum T₄ (µg/100 ml) uncorrected for recovery

Y = % resin-sponge uptake

a = intercept of the Y-axis

b = slope of the standard curve

In setting up this method, we found a 77.3% efficiency for extraction of T₄ from serum with 95% ethanol. The extraction efficiency was determined by mixing 1 ml rat serum with 0.02 ml [¹³¹I]T₄ (Abbot Laboratories). After 27 hr equilibration at -0.5° C, the first radioactivity measurement was obtained in the well counter, 2 ml 95% ethanol were added and the solution was treated

as described previously for serum samples. The second count was obtained from 1 ml of the alcoholic extract, and the % extraction efficiency was calculated from the difference between these counts.

To obtain true T_4 values, the results were corrected for extraction efficiency, as follows:

$$X_c = X_u \times \frac{100}{77.3} \quad (9)$$

X_c = Serum T_4 ($\mu\text{g}/100$ ml) corrected for recovery.

TEXT-FIG. 1. Thyroid secretion rates of non-lactating and lactating rats as measured by the T_4 substitution and direct output methods at three levels of iodine intake. * Number of rats per group. Significance of differences between groups by Mann-Whitney U-test: A versus B, $P \leq 0.05$; C versus D, $P \leq 0.05$; E versus F, $P \geq 0.45$; G versus H, $P \leq 0.01$; I versus J, $P \leq 0.05$; K versus L, $P \leq 0.01$; E versus K, $P \geq 0.45$.

RESULTS

The mean TSR of non-lactating and lactating rats as measured by the T_4 substitution and direct output methods at three levels of iodine intake, ranging from deficient to adequate, is shown in Text-fig. 1. In experiments employing the substitution method, apparent TSR was significantly elevated in lactating

TABLE 1
THYROID WEIGHTS AND ACINAR CELL HEIGHTS OF NON-LACTATING AND LACTATING RATS ON THREE LEVELS OF IODINE INTAKE

<i>Diet ($\mu\text{g I added/g feed}$):</i>	0.00		0.50		1.00	
	<i>Non-lactating</i>	<i>Lactating</i>	<i>Non-lactating</i>	<i>Lactating</i>	<i>Non-lactating</i>	<i>Lactating</i>
Mean thyroid weight $\text{mg} \pm \text{S.E.}$ <i>P</i>	25.53 $\pm$ 0.86 <0.01	31.22 $\pm$ 1.50	19.77 $\pm$ 0.92 > 0.05	22.17 $\pm$ 1.13	16.90 $\pm$ 1.51 > 0.05	18.80 $\pm$ 1.06
Mean cell height $\mu\text{m} \pm \text{S.E.}$ $\left(\sqrt{\frac{A_2}{\pi}} - \sqrt{\frac{A_1}{\pi}}\right)$ <i>P</i>	18.06 $\pm$ 0.39 <0.01	20.69 $\pm$ 0.40	13.46 $\pm$ 0.25 <0.001	20.04 $\pm$ 0.72	12.57 $\pm$ 0.24 <0.001	16.23 $\pm$ 0.19

TABLE 2
COMPONENTS OF THE EQUATION FOR CALCULATING THYROID SECRETION RATE BY DIRECT OUTPUT IN NON-LACTATING AND LACTATING RATS ON THREE LEVELS OF IODINE INTAKE

Group	Diet (μg I added/ g feed)	No. of rats	K'	$\frac{U}{\% \text{ inj. dose}}$ 0 time uptake	K_4	Total thyroidal I (μg)	K_4/K'
Non-lactating	0.00	5	0.089 $\pm$ 0.0006*	49.90 $\pm$ 3.75*	0.178 $\pm$ 0.023*	3.89 $\pm$ 0.81*	2.00
	0.50	10	0.110 $\pm$ 0.004	50.40 $\pm$ 2.35	0.228 $\pm$ 0.018	5.42 $\pm$ 0.36	2.07
	1.00	13	0.101 $\pm$ 0.003	28.16 $\pm$ 2.47	0.144 $\pm$ 0.009	19.64 $\pm$ 2.38	1.43
Lactating	0.00	9	0.159 $\pm$ 0.013	13.70 $\pm$ 2.13	0.187 $\pm$ 0.018	1.11 $\pm$ 0.14	1.18
	0.50	9	0.083 $\pm$ 0.011	10.61 $\pm$ 1.52	0.095 $\pm$ 0.015	3.55 $\pm$ 0.65	1.14
	1.00	16	0.080 $\pm$ 0.005	4.12 $\pm$ 0.37	0.084 $\pm$ 0.005	14.33 $\pm$ 1.48	1.05

For abbreviations, see text.

* Values expressed as mean $\pm$ S.E.

TABLE 3
CORRELATION OF COMPONENTS OF THE DIRECT OUTPUT EQUATION IN NON-LACTATING AND LACTATING RATS

Group	K_4 versus Total thyroidal I	Total thyroidal I	Total thyroidal I versus TSR	U versus Total thyroidal I	K_4 versus TSR
Non-lactating rats No. = 28	$Y = 29.010 - 0.090X$ $r = -0.580$ $P < 0.01$		$Y = 0.456 + 0.108X$ $r = 0.891$ $P < 0.001$	$Y = 30.175 - 0.483X$ $r = -0.652$ $P < 0.01$	$Y = 2.919 - 7.033X$ $r = -0.337$ $P > 0.05$
Lactating rats No. = 34	$Y = 17.830 - 0.080X$ $r = -0.640$ $P < 0.01$		$Y = 0.172 + 0.052X$ $r = 0.873$ $P < 0.001$	$Y = 14.911 - 0.787X$ $r = -0.628$ $P < 0.01$	$Y = 1.018 - 3.541X$ $r = -0.308$ $P > 0.05$

For abbreviations, see text.

animals when 0.00 or 0.50 μg iodine was added/g diet (Groups B and D). By the direct output method (Text-fig. 1), the TSR of lactating animals was significantly reduced below that of controls on all three levels of dietary iodine (Groups H, J and L versus Groups G, I and K). With increasing levels of dietary iodine, the TSR of lactating rats was partially restored, approaching 50% that of the controls at the highest level. When comparing the TSR values of non-lactating rats determined by both methods, it is apparent that the substitution method did not detect a decrease in thyroid hormone production during iodine deficiency whereas the direct output method did.

Thyroid weights of lactating rats in the direct output experiments were significantly increased above those of non-lactating rats only on the diet with no iodine added (Table 1). However, mean thyroid acinar cell height was significantly greater in lactating rats than in controls at all levels of iodine intake. As total thyroidal iodine (Table 2) decreased from 19.64 to 3.89 μg in non-lactating rats and from 14.33 to 1.11 μg in lactating rats, mean acinar cell height significantly increased from 12.57 to 18.06 μm and from 16.23 to 20.69 μm , respectively (Table 1).

In calculating the data in Table 2, Equation 3 was used to correct K'_4 for the recycling of endogenous I back to the thyroid. As the thyroidal iodine supplies became depleted, the increased efficiency of thyroidal iodine accumulation was reflected by higher U values. There was a high inverse correlation ($P < 0.01$) between U and total thyroidal iodine in both non-lactating and lactating rats (Table 3). As U increased (Table 2), the K_4/K'_4 ratio increased from 1.43 to 2.00 showing that, at least in the non-lactating rat, the iodine-deficient thyroid recycles a greater percentage of the available iodine, thus requiring a larger correction of K'_4 . A similar trend was found in lactating rats but U was much lower than in controls due to the competition for iodine by the functioning mammae. In both non-lactating and lactating rats (Table 3), K_4 was inversely correlated to thyroidal iodine ($P < 0.01$), indicating a more rapid turnover of iodine in iodine-deficient thyroids. However, a between-groups comparison (Table 2) indicates that the thyroidal iodine turnover rate (K_4) tended to be lower during lactation. Furthermore, K_4 showed no definitive relationship to TSR ($P > 0.05$), whereas thyroidal iodine was highly correlated with TSR ($P < 0.001$), as shown in Table 3.

In support of the results already described, the serum thyroxine (T_4) of ten rats that had suckled twelve young/rat to the 16th day of lactation and received the basal diet with 1.0 μg of iodine added/g of feed was compared to that of ten non-lactating controls receiving the same diet. The mean serum T_4 values, expressed as $\mu\text{g } T_4/100 \text{ ml serum}$, were 1.96 ± 0.29 and 3.94 ± 0.15 in lactating and control rats, respectively.

DISCUSSION

The apparent increase of TSR in lactating rats as measured by the substitution method in the present work is in accord with the results of Grosvenor & Turner (1958) who employed a similar method in another strain of rat receiving a presumably normal iodine intake. In marked contrast to these results, there was

approximately a 50% reduction in the TSR of lactating rats as determined by the direct output method together with about the same reduction in serum T_4 . The TSR values obtained by the two methods are not significantly different in non-lactating rats receiving a normal iodine intake (Reineke & Lorscheider, 1967). On an iodine-deficient diet, values obtained by the substitution method are elevated and by the direct output method they are greatly reduced.

In attempting to explain the differences between methods, it must be kept in mind that the substitution method is quite indirect, the end point being obtained by determining the amount of T_4 that must be injected daily to block the output of previously injected radioactive iodine from the thyroid. It would appear that the elevated TSR values obtained by application of the substitution method in lactating rats reflect an increase in thyroid-stimulating hormone (TSH) output triggered by the deficit in thyroid hormone production. Thus, the results indicate the physiological demand for thyroid hormone but represent the actual TSR only if iodine intake is adequate to meet the demand. The direct output method is more applicable in any case where iodine is likely to be a limiting factor on TSR.

In the calculations for TSR by the direct output method, it has been assumed that the ratio of triiodothyronine (T_3) to thyroxine released by the thyroid remains constant. However, Heninger & Albright (1966) reported a shift toward greater T_3 production in the rat when iodine intake was reduced. Reineke & Lorscheider (1967) quantitatively assessed the effect of such a shift in iodine-deficient rats and concluded that the possible increase in T_3 production could only compensate to a small degree for the deficiency in total thyroid hormone output.

Inasmuch as only 30% of an administered dose of ^{131}I is excreted in the urine and 70%, mostly as T_4 iodine, in the faeces of the rat (Jones & Van Middlesworth, 1960), there is some question as to the validity of employing the 'U' correction (Equation 3) for the thyroidal recycling of iodine in lactating rats. Rats in heavy lactation eat more food and could be expected to excrete an even greater proportion of iodine in the faeces in addition to the well-documented losses of iodine in milk. With an iodine supplementation which is usually considered to be adequate for the rat (1.0 to 1.5 μg I/g feed), the mean U value for non-lactating control rats was about sevenfold greater than in lactating rats (Table 2). This resulted in a much higher correction of K'_4 to K_4 in the controls than in the lactating rats. If endogenous thyroidal iodine recycling was not considered to be a factor in lactating rats, thereby permitting K'_4 to be used instead of K_4 to compute TSR, the differences between non-lactating and lactating rats would be even greater.

The fact that K_4 shows no definitive relationship to TSR whereas thyroidal iodine is highly correlated to TSR (Table 3) would imply that the thyroidal iodine is the major determining factor on TSR even though TSR is the product of several parameters. This relationship may be a reflection of the limited iodine content of the diets employed. The TSR may become more dependent on K_4 when iodine supplies are relatively unlimited.

Interpretation of TSR data in lactating rats is complicated further by the recycling of both ^{131}I and ^{127}I back to the mother by way of the urine of the

suckling young (Capek & Jelinek, 1956; Samel, Caputa & Struharova, 1963; Samel & Caputa, 1965). If the infant-maternal iodine cycle were quantitatively significant, K_4 would decrease, resulting in an underestimation of TSR. This effect would be counterbalanced, at least in part by an increase in the thyroidal iodine component of the direct output equation.

Rawson & Starr (1938) first measured individually selected thyroid acinar cells to determine thyroid hyperplasia. The method employed in the present study eliminates cell selection and simultaneously estimates the mean height of all cells in a follicular cross section. The cell height values of non-lactating rats on the diet with $1.0 \mu\text{g}$ iodine added/g feed, reported herein, agree with normal rat thyroid cell measurements (10 to $15 \mu\text{m}$) reported by others (Wissig, 1964). When differences in thyroid weight are small, thyroid cell height appears to be the more sensitive indicator of TSH-induced hyperplasia.

Taken overall, our experiments clearly establish that, in the lactating rat, there is a reduced thyroid secretion rate that is accompanied by lowered serum T_4 levels. There is a compensatory increase in thyroid-stimulating hormone output, as reflected in the apparent increase in TSR measured by the substitution method and a significant increase in thyroid cell height. We believe that these effects are caused by the reduction in available iodine, due to losses of this element in milk. Despite the reduction in available T_4 , the rats on all levels of iodine intake successfully raised their young. Thus, these results do not demonstrate an essential need for increased thyroid hormone during lactation in the rat.

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