THE INFLUENCE OF LACTATION ON THYROID SECRETION RATE AS EVALUATED BY A DIRECT OUTPUT METHOD

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

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1967

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By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1967

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. E. P. Reineke for his guidance and understanding in all phases of my laboratory and curricular endeavors while at Michigan State University.

I am also indebted to Mrs. Linda Allison and Miss Barbara Brace for their assistance in the many tedious laboratory analyses required of this research.

Special appreciation is due Drs. H. M. Klitgaard and R. C. Meade and my undergraduate professors at the University of Wisconsin for their tutelage, discipline and encouragement during my formative academic undertakings which helped me realize opportunities for graduate study.

This work was supported by the National Institutes of Health grant No. AM-08513-02 and the Michigan State University Agricultural Experiment Station.

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INTRODUCTION

Studies over the past seventeen years have shown significant secretion of iodide in the milk of many species, including man. More recently it has been suggested that lactation may tend to become self-limiting due to reduced thyroid function resulting from a thyroid-mammary competition for limited available iodine. Despite efforts to substantiate this theory conclusively, little evidence has been obtained in the lactating animal which is amenable to statistical analysis.

In studies reported to date, both a thyroxine substitution and a direct output method have been used as indices of thyroid function. Employment of the substitution method in rats has indicated an elevated thyroid secretion rate during lactation. But when evaluated by the direct output method, preliminary studies of nonlactating rats indicated a correlation between iodine supply and thyroid secretion rate. Thus it was apparent that application of the latter method might actually prove a decreased thyroid secretion rate during lactation.

Therefore, in view of these findings, the work reported herein attempts to compare the two methods for assessment of thyroid function in lactating rats maintained on various levels of dietary iodine.

LITERATURE REVIEW

Several studies, beginning with work by Courrier et al. (1949), have revealed that a considerable amount of labeled iodide is secreted in the milk. Subsequent research has shown this to occur in many species including man (Honour et al., 1952; Noble and Rowlands, 1953), cows (Lengeman et al., 1955; Lengeman and Swanson, 1957), goats (Wright et al., 1955; Flamboe and Reineke, 1959; Reineke, 1961), dogs (Van Middlesworth et al., 1953; Van Middlesworth et al., 1954), rabbits (Brown-Grant, 1956; Brown-Grant, 1957; Brown-Grant, 1958), rats (Potter and Chaikoff, 1956; Potter et al., 1959; Grosvenor, 1960), and mice (Rugh, 1951). Protein bound iodide in milk was reported to be 30-60% in the dog (Van Middlesworth, 1953; Van Middlesworth, 1954) and 85% in the rat (Potter and Chaikoff, 1956; Potter et al., 1959), where up to 50% of I^{131} was recovered in total mammary tissue plus milk twenty-four hours post administration (Potter et al., 1959).

Secretion of I^{131} by the mammae is of such magnitude that thyroidal accumulation of administered I^{131} is markedly decreased (Brown-Grant, 1956; Potter and Chaikoff, 1956; Rugh, 1951). That this is an active secretion is evidenced by the high in vivo milk to serum ratios of I^{131} (Honour et al., 1952; Flamboe and Reineke, 1959; Brown-Grant, 1957; Potter

and Chaikoff, 1956; Grosvenor, 1960), and the same active concentration of I^{131} by lactating rat mammae has also been demonstrated *in vitro* (Freinkel and Ingbar, 1956; Maqsood and Reineke, 1960a). The similarity of mammae to the thyroid in response to goitrogens has also been shown both *in vivo* (Brown-Grant, 1957; Potter *et al.*, 1959) and *in vitro* (Maqsood and Reineke, 1960a). Neither concentration of I^{131} nor goitrogen response was noted in other tissues to the degree shown by the mammae (Maqsood and Reineke, 1960b).

In vivo secretion of iodine into milk occurs both as free iodide and as monoiodotyrosine (MIT) in peptide linkage in milk protein (Potter and Chaikoff, 1956). It was indicated that this iodide concentrating ability of the mammae is probably associated with an oxidative enzyme system (Maqsood and Reineke, 1960a). This and an additional enzymatic system for the formation of MIT were further characterized as being associated with the lactation process and requiring cofactors of Cu^{++} and Mn^{++}. Both I^{131} concentration and MIT formation decreased with mammary involution (Reineke, 1963). Obviously iodine combined with milk protein is not available for hormone synthesis and thus measurements of thyroxine output might be expected to be altered.

Over the past twenty-five years various methods have been developed to estimate thyroxine secretion rate (TSR); these have included the goitrogen technique (Dempsey and Astwood, 1943) and the thyroxine degradation procedure

(Sterling et al., 1954; Ingbar and Freinkel, 1955). A thyroxine (T_4) substitution method for determining thyroid hormone secretion rate was first described in individual animals (Henneman et al., 1952) and subsequently applied in several other species (Reineke, 1959). This method has yielded consistent values under ordinary conditions of management.

As further application of the thyroxine degradation method has pointed out, a quantitative assessment of thyroid function necessitates a definition of the proportional metabolic effect of triiodothyronine (T_3) (Gregerman, 1963). And in addition to necessary corrections for T_3 activity, experiments have demonstrated that iodine intake may also influence thyroid secretion rate. In a preliminary report a more direct measurement of thyroxine secretion (fractional output rate times thyroidal iodine content), which included corrections for the more biologically active T_3 component, revealed a significant correlation between dietary iodine supply and thyroxine secretion rate. But the thyroxine substitution method exhibited no such correlation (Reineke, 1964).

Using the substitution method it was first proposed by Flamboe and Reineke (1959) that lactation might become a self-limiting process due to reduced thyroid function resulting from a thyroid-mammary-kidney competition for limited available iodine. However, Grosvenor and Turner (1958) had reported significantly elevated thyroxine secretion rates in

lactating rats when employing the thyroxine substitution method of Reineke and Singh (1955). Since Reineke (1964) had demonstrated with the direct output method that a correlation exists between iodine supply and thyroxine secretion, it was hypothesized that under conditions of limited available iodine, such as lactation, this direct method might actually reveal a decreased thyroid secretion rate.

In view of these findings a comparison of the thyroxine substitution and direct output methods for evaluating thyroid function was made in lactating rats on various levels of iodine intake. With the latter method corrections were made for T_3 release. It was attempted to substantiate the theory that lactation is a self-limiting process and to further define the interrelationships of thyroid-mammary function during lactation.

MATERIALS AND METHODS

I. General

Thyroid secretion rates (μg T_4/100 gm. B.W./day) of lactating and control rats maintained on three levels of dietary iodine were determined by the thyroxine substitution and direct output methods.

Three groups of female rats of the Carworth CFN strain weighing approximately 200 grams each were fed a corn-soybean diet for a thirty day period beginning two weeks prior to isotope administration until sixteen days post partum. Laboratory temperature was maintained at $24.5^{\circ}C. \pm 1^{\circ}$ and lights were on 14 hours per day. The feed (Appendix A) consisted of a finely ground corn meal, soybean oil meal, vitamin supplements, and a special mineral salt premix prepared at the Michigan State University Department of Animal Husbandry feed mixing plant. The diet of group number I was low in iodine. A 0.2% stock solution of potassium iodide was added to the diet at levels of 0.65 μg iodine and 1.30 μg iodine/gram of feed for groups II and III respectively.

Subcutaneous injections of 3 μc and 8 μc of carrier free I^{131} were given each control and lactating rat, respectively. External thyroid and body background counts

(epigastric region) were taken under Nembutal anesthesia (3 mg/100 gm of body weight), for all subjects on alternate days starting with the third day post I^{131} administration for a series of six counts. Counting was achieved with a radiation analyzer-scaler connected to a well-type collimated NaI scintillation detector monitored with a count rate meter for optimal geometry. The 2" NaI crystal was mounted beneath a 2.5 cm aperture of a 2.5 x 25 x 35 cm. lead shield platform. A standard prepared at one-tenth of the injected dose was counted with each group. Counts were corrected for body and room background by the method of Wolff (1951). Thyroidal I^{131} concentration was expressed as a per cent of the injected dose to correct for physical isotopic decay using the formula:

$$\text{Thyroidal } I^{131}\text{, as per cent of injected dose} = \frac{\text{thyroid count} - \left(\frac{\text{body background-room background}}{2}\right)}{(\text{standard count-room background})} \times 100 \quad (1)$$

The thyroid secretion rate (TSR) of rats on three levels of iodine intake was evaluated by a comparison of the thyroxine substitution method (Reineke and Singh, 1955) and direct output method (Reineke and Lorscheider, in publication) under both lactating and nonlactating conditions (Table 1). Litters were equally distributed to establish a similar degree of nursing for all lactating subjects.

Table 1. Experimental design for comparison of thyroid secretion rates (TSR) of lactating (L) and control (C) rats by T_4 substitution and direct output methods at three levels of iodine intake.

Group	Dietary iodine level (μg I added/gm. feed)	TSR T_4 substitution		TSR Direct output	
		L	C	L	C
I	0.00				
II	0.65				
III	1.30				

II. Thyroxine Substitution Method

A 0.1% stock solution of L-thyroxine was prepared by first dissolving the T_4 pentahydrate crystals in NaOH and then neutralizing the alkaline solution to a pH of approximately 8.6 with HCl. At this point the T_4 precipitates as a fine opalescent suspension.

With the thyroxine substitution method graded dosages of L-thyroxine (Merck) are injected subcutaneously daily and increased every third day (0.75, 1.50, 2.25, 3.00, 3.75 μg T_4/100 gm. body weight) until thyroidal I^{131} output is maximally supressed (Figure 1-A). The per cent of the previous per cent of the injected dose vs. μg T_4/100 gm. body weight is then replotted linearly. The end point of maximal suppression (97.5%) yields the amount of T_4 needed to establish a static thyroid-pituitary state (Figures 1-B and 1-D). Thus TSR by the T_4 substitution method is that level of exogenous thyroid hormone that reduces further output of previously collected thyroidal I^{131} to 97.5% of the prior count.

III. Direct Output Method

By the direct output method TSR was estimated as the product of daily fractional output rate times the thyroxine equivalent of total thyroidal iodine content (Figure 1-C). The calculations used to compute fractional turnover of iodine per day are the following:

Figure 1

1-A. Thyroxine substitution method illustrating suppression of thyroidal I^{131} output with graded dosages of exogenous thyroxine.

1-B. Thyroxine substitution method illustrating the extrapolation of the suppression end point of thyroidal I^{131} output to thyroidal secretion rate.

1-C. Direct output method illustrating the procedure for determining the fractional daily output of total iodine from the thyroid.

1-D. Illustration of the static thyroid-pituitary state achieved by the T_4 substitution method.

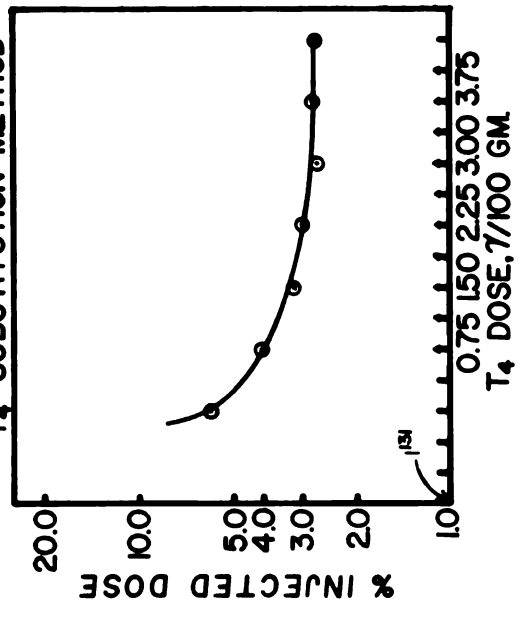


Figure 1-A

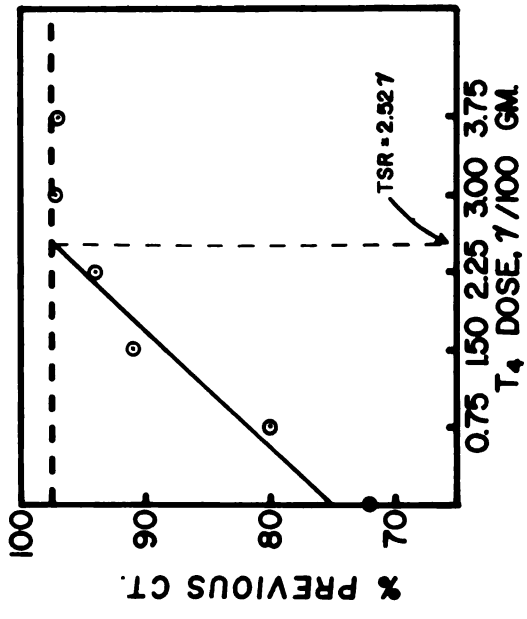


Figure 1-B

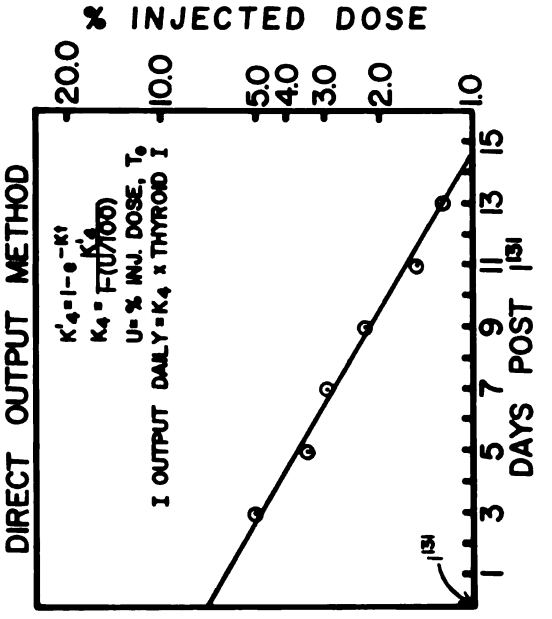


Figure 1-C

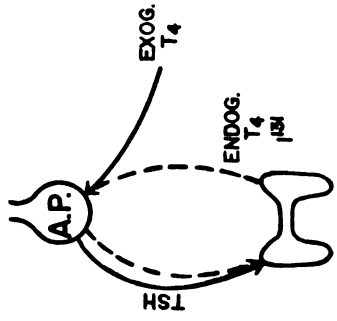


Figure 1-D

$$K'_4 = 1-e^{-kt} \qquad (2)$$

where: $k = \frac{.693}{T\ 1/2}$, $t = 1$ day

The apparent release constant for thyroidal I^{131} for a given subject (K'_4) was corrected by employing the Brownell (1951) equation, since verified by others (Sorenson, 1958; Robertson and Falconer, 1961), to account for recycling of iodide to the thyroid thus yielding K_4, the actual turnover rate. This equation is as follows:

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

where: U = extrapolated log of the injected isotope dose at zero time uptake.

Thyroids were excised for iodine analysis after the thyroidal biological half-life of I^{131} was sufficiently established over a twelve day period. Glands were removed, carefully trimmed, weighed on a Roller-Smith torsion balance, and fixed in Dietrich's solution. Each lobe was counted for radioactivity in a well-type scintillation detector. One lobe was used for histological evaluation. The other lobe was analyzed for total iodine content (Appendix B) by an adaptation of the alkaline ashing method (Barker and Humphrey, 1950). Sufficient radioactivity remained in the glands to allow for corrections of total iodine loss as a result of sublimation in ashing procedures. Iodine was determined on an aliquot of the diluted digest after stopping the timed colorimetric

reaction with brucine sulfate (Faulkner et al., 1961). The color was measured with a Coleman Universal Spectrophotometer at 480 millimicrons set for 100% transmission through a water blank. Iodine content was then read from a previously prepared calibration curve in which net per cent transmission (sample) was plotted against known iodine concentrations (Lennon and Mixner, 1957).

An assay conducted for the determination of comparative potencies of L-triiodothyronine and L-thyroxine, which used the substitution method of Reineke and Singh (1955), revealed the ratio of T_3:T_4 activity to be 4.09 per unit weight, 3.42 per mole or 4.60 per unit weight of iodine (Appendix C).

Pitt-Rivers and Rall (1961) reported that thyroxine and triiodothyronine comprised 18% and 3%, respectively, of the total thyroidal iodine and suggested that the thyroid secretes them in the same ratio as they are present in the gland. Calculations for the direct output method, summarized in Table 2, are based on the assumption that substantially all of the iodine normally released from the thyroid is in hormonal form and, secondly, that T_4 and T_3 are released in the same proportions in which they are found in the thyroid.

In Table 2 (Reineke and Lorscheider, in publication), 85.7% and 14.3% are respectively assigned to T_4 and T_3 released. Since the hormone assay revealed T_3 to be 4.60 times as potent as T_4 per unit weight of iodine, the sum of the T_4 and T_3 activity ratios yields a total T_4 activity factor of

Table 2. Computations for the total thyroxine activity equivalent of iodine (Reineke and Lorscheider, in publication.)

	T_4 Iodine	T_3 Iodine
Per cent total in thyroid	18.0	3.0
Per cent released	(6/7) 85.7	(1/7) 14.3
T_4 activity ratios per cent	0.857 x 1	0.143 x 4.60 = 0.658

$T_4 + T_3$ in terms of T_4 activity = 0.857 + 0.658 = 1.52

$T_4 : T_4$ iodine = 1/ 0.6534 = 1.53

Activity of $T_3 + T_4$ in terms of T_4 iodine = 1.52 x 1.53 = 2.33

TSR (μg T_4) = K_4 x μg thyroidal iodine x 2.33

1.52. And the molecular weight of thyroxine being 65.34% iodine, gives a T_4 to T_4 iodine ratio of 1.53. Thus the product of total T_4 activity times the T_4 equivalent of iodine equals the total T_4 activity equivalent of iodine, a correction factor of 2.33. When multiplied by this T_4 iodine equivalent correction factor (2.33), the fractional daily turnover of iodine (K_4 times thyroidal iodine/100 gm. body wt.), yields the extimated TSR in μg T_4/100 gm. body wt. In summary, the equation for the direct output method is as follows:

$$\text{TSR}(\mu\text{g } T_4/100 \text{ gm. body wt./day}) = T_4 \times \mu\text{g thyroidal iodine} \times 2.33 \quad (4)$$

Histological analysis of thyroid lobes of the lactating and control rats was performed to determine the relationship of thyroid follicle cell heights to dietary iodine intake. The error involved in measuring individual cell heights with an ocular micrometer is that heights may vary within a given follicle. Thus extensive sampling becomes necessary to detect relatively small differences in cell heights.

If the in vivo form of a thyroid follicle is assumed to be essentially spherical, then the geometry of a cross section would approach two concentric circles. Since the follicle perimeter is a single layer of low cuboidal cells, the outer and inner borders of the cell shell may be designated the outer and inner circumferences, respectively, of the two concentric circles. Previous studies (Meade and Lorscheider, unpublished) have indicated that the variable parameter of a

rat thyroid follicle, stimulated with TSH, is the inner circumference. This hyperplasia in effect reduces the colloid space as the cuboidal cells become columnar.

With consideration of these premises, measurement of mean cell heights of follicular cross sections employs the following geometric equations:

$$C = (2\pi)r \quad \text{or} \quad r = \frac{C}{2\pi} \tag{5}$$

where: C = circumference
r = radius

$$A = (\pi)r^2 \quad \text{or} \quad r^2 = \frac{A}{\pi} \tag{6}$$

where: A = area of circle
r = radius

r_o = radius of outer circle

r_i = radius of inner circle

thus: $r_o - r_i$ = mean cell heights (7)

Appendix D presents the steps followed in the present study for applications of equations 6 and 7 to estimate thyroid follicle cell height.

RESULTS AND DISCUSSION

The T_3:T_4 activity ratio of 4.60, derived in Appendix C and applied in the direct output method (Table 2), compares favorably with values reported by others (Barker, 1955). By expressing TSR values in terms of T_4 activity, comparisons may be made of results obtained by the direct output method with those obtained by the T_4 substitution method.

Such a comparison is made of non-lactating rats (Table 3) at three levels of iodine intake. The T_4 substitution method evidenced no significant difference in TSR between groups on different iodine diets. The slightly elevated TSR value of 2.53 μg T_4/100gm. body wt./day on the 1.30 μg iodine diet may be attributed to experimental error. Employment of the direct output method significantly reduced TSR values on diets with 0.00 and 0.65 μg iodine added when compared to the 1.30 μg iodine diet, and the TSR of subjects on the 0.65 μg diet tended to be higher than that of subjects on the low iodine diet. That this latter difference is not significant may reflect the possibility that sublimation may have reduced the iodine level of the 0.65 μg iodine diet. But the TSR (2.53 and 2.29 μg T_4/100 gm.) as calculated by either method is essentially the same ($P > .50$) on the 1.30 μg iodine diet. This seems to indicate similarity in the two methods of

Table 3. Influence of iodine supplementation on the thyroid secretion rate of non-lactating (control) rats as measured by the thyroxine substitution and direct output methods. The number of rats in each trial is shown in parentheses.

Group	Diet (μg I added/ gm. feed)	TSR T_4 Substitution Method (μg T_4/100 gm.)	TSR Direct Output Method (μg T_4/100 gm.)
I	none	2.05 (7)	0.65 (5)
		$P^* > .50$	$P < .15$
II	0.65	2.05 (9)	0.80 (10)
		$P > .25$	$P < .05$
III	1.30	2.53 (7)	2.29 (7)

*P - Mann-Whitney U test (Siegel, 1956).

evaluating TSR when iodine supply is sufficient. The 1.30 μg iodine diet is somewhat in excess of previously reported values (Parker et al., 1951) but compares favorably with Wayne and Purina normal diets of 1.00-1.50 μg iodine per gram of feed.

These data presented in Table 3 agree favorably with data previously reported by Reineke (1964): first, that no difference is exhibited in TSR by the T_4 substitution method employed in rats on various levels of dietary iodine, and second, that TSR by the direct output method is significantly decreased when iodine supply is limited and that a correlation can be shown between TSR and iodine availability. From the foregoing results it may be concluded that the T_4 substitution method measures the apparent thyroid hormone demand, but can represent the actual TSR only if iodine supply is adequate. Thus, under conditions of limited iodine, such as lactation, the direct output method would appear more applicable.

Because of the lengthy laboratory procedure and large numbers of animals needed for adequate statistical evaluation, the goitrogen technique of Dempsey and Astwood (1943) for measuring TSR has proved relatively impractical. By this indirect method TSR was reported to be 5.20 μg T_4/100 gm. at 25°C. The thyroxine degradation procedure involves protein bound iodine (PBI) determinations which iodine intake may influence markedly (Sterling et al., 1954; Ingbar and

Freinkel, 1955) and by this indirect method TSR for the female rat has been estimated at 1.22 μg T_4/100 gm. but, this method does not correct for the T_3 component. Previous TSR values by the T_4 substitution method ranged from 1.70 to 2.21 μg T_4/100 gm. in the adult female rat (Reineke, 1964; Reineke and Singh, 1955), and this agrees favorably with data of the present study. The slight elevation of the present TSR may represent the goitrogenic effects of trace amounts of thiocyanate (SCN) in the corn-soybean protein. It is recognized that fluctuations of TSR in the rat may indicate not only differences in methodology, but also variances due to age, sex, strain and environmental temperature.

Though most attempts to evaluate TSR have used rats receiving normal levels of iodine, the present studies which varied the dietary levels of iodine reveal no differences in the TSR as a function of diet when using the T_4 substitution method. But this method bypasses the thyroid and assesses only indirect parameters of thyroid function. Thus, the present study employs the direct output method which yields significantly lower TSR on reduced iodine intake, and this apparently substantiates the theory first proposed by Flamboe and Reineke (1959) that if inadequate amounts of iodine are taken up for normal hormone synthesis, thyroid secretion rate may decrease.

Table 4 presents the TSR of lactating and control rats by T_4 substitution and direct output at three levels of

iodine intake. Figure 2 is a graphical illustration of the TSR data contained in Table 4. It should be noted that the non-lactating control data of Table 4 is that previously discussed in Table 3. It, therefore, becomes appropriate to discuss these data in context since lactating and control data for both TSR evaluation methods were run simultaneously for a given dietary level of iodine.

Table 4 indicates and Figure 2 illustrates that, with the T_4 substitution method at 0.00 and 0.65 μg iodine added/gm. diet, TSR of lactating rats is significantly elevated above control TSR values. This finding is in accord with previous results obtained by this method (Grosvenor and Turner, 1958) that showed elevated TSR during lactation. This method appears to reflect elevated TSH output caused by a hyperactive pituitary as a result of pre-existing iodine deficiency instead of an increase in TSR. Based on a normal Wayne diet of 1.00 μg I/gm. feed, the slightly increased 1.30 μg showed no difference between lactating and control TSR values. Furthermore, T_4 may actually enhance lactation so that more hormone is required to maintain the increased lactation state. This would necessitate additional T_4 for TSH suppression and thereby result in a positive feedback cycle.

Using the direct output method (Table 4) it is evident that with inadequate iodine intake (Group I) thyroidal iodine is markedly reduced. This reflects the mammary drain on limited iodine which would otherwise be available for thyroid

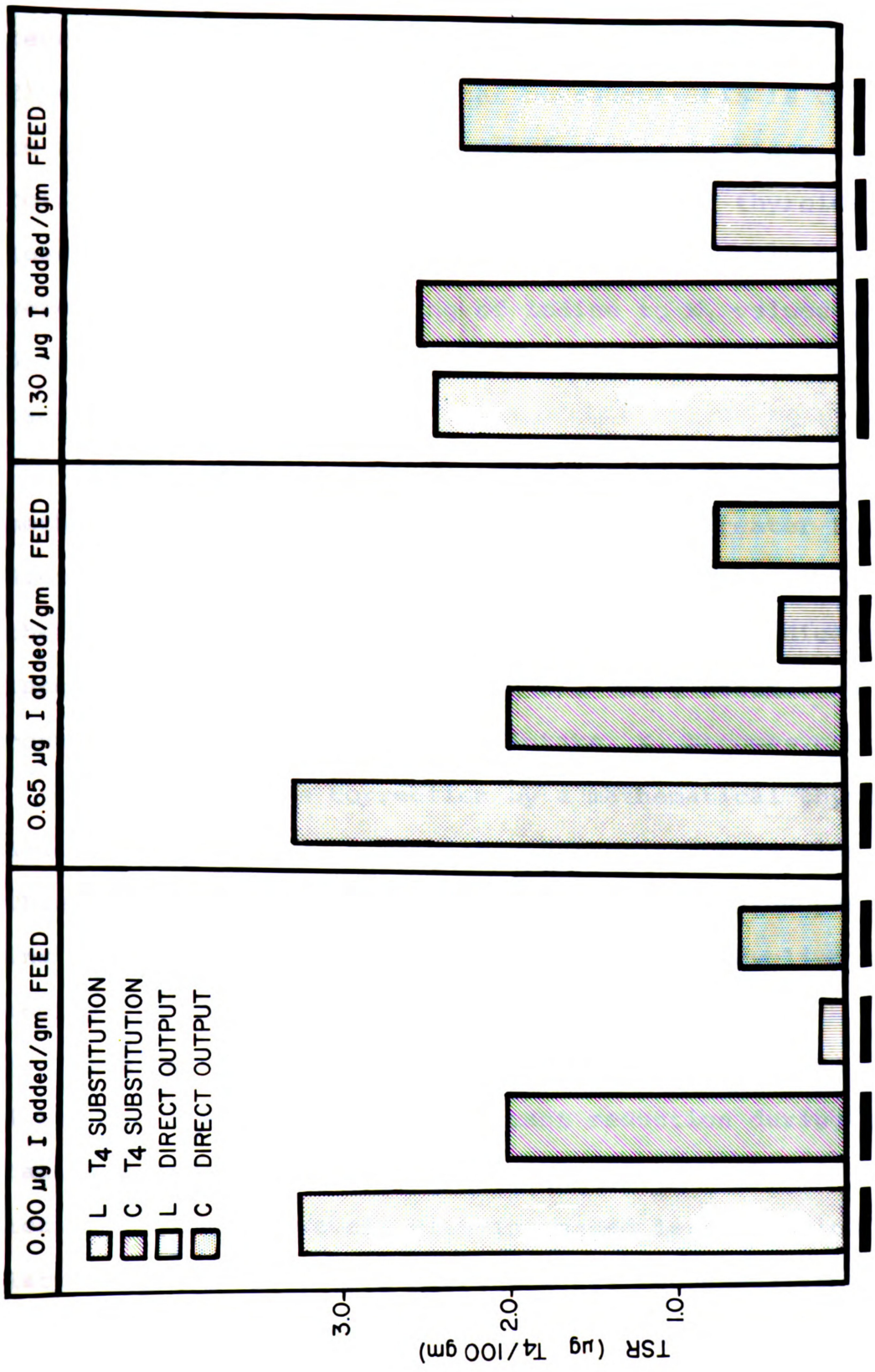
Table 4. Thyroid secretion rates of lactating (L) and control (C) rats by T_4 substitution and direct output methods at three levels of iodine intake. The number of rats in each trial is shown in parentheses. TSR is expressed as μg T_4/100 gm. body wt./day.

Group	Diet (μg I added/ gm. feed)	T_4 Substitution TSR (μg T_4/100 gm.)			Direct Output μg Total Thyroidal Iodine		Direct Output TSR (μg T_4/100 gm.)		
		L	C	L/C	L	C	L	C	L/C
I	none	3.31 (8)	2.05 (7)	1.61	1.11 (9)	3.89 (5)	0.17 (9)	0.65 (5)	0.26
	P*	< .012			< .002		< .006		
II	0.65	3.43 (10)	2.05 (9)	1.67	3.53 (9)	3.42 (10)	0.38 (9)	0.80 (10)	0.48
	P	< .006			> .50		< .05		
III	1.30	2.48 (7)	2.53 (7)	.98	13.62 (10)	21.32 (7)	0.78 (10)	2.29 (7)	0.34
	P	> .50			> .50		< .05		

*P = Mann-Whitney U test (Siegel, 1956), a nonparametric analysis if the assumption of variance homogeneity is not fulfilled; otherwise an unpaired t-test is employed.

[illegible]
Values for TSR are expressed as µg T_4/100 gm. body wt./day.

Figure 2. Thyroid secretion rates of lactating (L) and control (C) rats by T_4 substitution and direct output methods at three levels of iodine intake. Values for TSR are expressed as μg T_4/100 gm. body wt./day.



incorporation. Even in cases where lactating and control levels of iodine are similar (Group II) K'_4 values (Equation 2) tended to be lower in lactating rats. This is contrary to an earlier report where a simultaneous decrease in thyroidal uptake was observed with an increased thyroidal release rate of isotope (Brown-Grant, 1956). When K'_4 values were corrected for recycling of iodine to K_4 values (Equation 3), release rates were still not necessarily greater during lactation.

In applying the Brownell equation to the direct output method it may be generally stated that the greater the dietary deficiency in iodine for non-lactating (control) rats, the greater the attempt to conserve iodine as indicated by larger U uptake values (Equation 3). As U becomes larger in control rats on reduced iodine intake, K_4 becomes correspondingly larger. This correction by a mathematical thyroid analog in lactating animals is apparently not as significant. This may possibly be the result of mammary iodine partitioning acting as an effective trap to prevent recycling of iodine.

The TSR as measured by the direct output method (Table 4 and Figure 2) shows a significant reduction during lactation when compared to controls on all three levels of iodine intake. However, with increased levels of iodine, the lactating TSR values are significantly increased to approach control values. In addition, histological evaluation, as

described in Appendix D, indicated that endogenous TSH stimulation had effected a significant hyperplasia of thyroid follicular cells (Table 5) of lactating rats on 0.00 μg of added iodine, with a similar tendency for lactating rats on 0.65 μg of added iodine. This hyperplasia was presumed to indicate a functional hypothyroidism. Long term PBI and histology studies of Grosvenor (1962) tend to agree with these observations.

It should be noted that the direct output lactating TSR of 0.78 μg T_4/100 gm. may actually be larger than that calculated. Recent studies indicate that I^{131} output rates may be masked by recycling of I^{131} back to the mother by way of the litter urine. Capek and Jelinek (1956) demonstrated that micturition in neonate rats is dependent on perineal stimulation by the mother for up to 16 days post partum. In current research this phenomenon has been observed to occur in rabbits as well. Present studies and those reported by others (Samel et al., 1963; Samel and Caputa, 1965) have demonstrated a significant amount of I^{131} from tagged rat and rabbit neonates to be present in the untagged mother 24 hours post I^{131} injection. Since milk contains a high iodine titer, it was proposed from this observation that the recycling of iodine, via the litter urine injested by the mother, acted as a conserving mechanism for iodine.

The direct output method is based on three assumptions as applied to this study. First, that all of the iodine

Table 5. Mean cell heights of thyroid follicles in lactating (L) and control (C) rats at three levels of iodine intake.

	0.00 μg I added/gm. feed		0.65 μg I added/gm. feed		1.30 μg I added/gm. feed	
	L	C	L	C	L	C
Number of rats	9	5	9	10	10	7
$\bar{x}$ cell ht. (μ)	17.33	12.80	13.22	11.65	11.43	11.72
P*	< .002		= .075		> .50	

* Mann-Whitney U-test.

normally released from the gland is in hormonal form; second, that T_4 and T_3 are released in the same proportions in which they are found in the thyroid; and third, that under conditions of iodine deficiency and/or during lactation the ratio of T_3 to T_4 released is not significantly altered.

If the first assumption is not met it would result in an overestimation of hormone output. Pitt-Rivers and Rall (1961) have suggested evidence in thyroid-blood equilibrium studies to support the second assumption. Rosenberg et al. (1966) have shown that despite early heterogeneous labelling and turnover of iodine, after a few days homogeneous labelling is attained. In regard to the third assumption, the in vivo studies of Matsuda and Greer (1965) which measured thyroid venous effluent in the rat, have suggested that under elevated TSH stimulation there is a substantial proportional increase in the secretion of iodotyrosines, chiefly DIT, and in the amount of iodothyronines, mainly T_3. Further, Shimoda and Greer (1966) have suggested that rats under iodine deficient conditions, propylthiouracil, or TSH all evidenced increased in vitro labelling of iodothyronines, mainly T_3. However, Heninger and Albright (1966) measured total thyroidal quantities of T_4 and T_3 in iodine deficient rats to be 0.17 μg and 0.10 μg respectively per gland. Using these values the T_4 plus T_3 equivalent of iodine in terms of T_4 is calculated to be 2.23 from Table 2 and a final correction

factor of 3.42 is obtained (Reineke and Lorscheider, in publication). Even if this factor is applied to the present data the increase in TSR is still markedly reduced in lactating subjects. Thus, the apparently more efficient shift to T_3 production under iodine deficiency fails to compensate adequately in elevating TSR to normal.

In view of these findings the T_4 substitution method appears to reflect the physiological demand for thyroxine and/or may represent a latent TSH suppression by exogenous T_4 as a result of iodine deficiency. However, this estimate represents the true hormone output only if the thyroid is receiving sufficient iodine to meet the hormone demand. The direct output method appears to be a more valid measurement of actual thyroxine released under conditions such as lactation which limit iodine available for thyroid hormone synthesis. With an adequate supplement of iodine, estimates of thyroid secretion rates are similar, employing either method of evaluation, under non-lactating conditions.

During lactation there is sufficient loss of iodine in the milk that secretion of thyroxine is reduced below normal, resulting in a functional hypothyroidism, as evidenced by histology, unless adequate iodine is supplied in the diet. By increasing the intake of iodine, the deficiency in hormone production is significantly alleviated. However, the exact extent of alleviation could not be assessed at this time since recycling of I^{131} by way of the litter urine

back to the mother masks, to some degree, the true output rate of hormone. Once this is clarified, it might still prove necessary to determine whether this disparity between lactating and control animals can be overcome by additional iodine supplementation.

The proposed thyroid-mammary-kidney distribution of iodine is illustrated in Figure 3. The size of the arrow is indicative of the approximate proportional distribution for several species. The chart shows that lactating females do not receive an adequate supply of thyroidal iodine even when their dietary intake is doubled to normal. Thyroid hormone enhances milk production. However, increased mammary secretion results in increased competition with the thyroid for available iodine, thus tending to make lactation a self-limiting process. The chart also shows that the thyroid does not take up more iodine than it requires, in non-lactating females, so that the amount going to the thyroid remains constant. As indicated, some species recycle iodine via the litter urine. This phenomenon tends to complicate the evaluation of certain thyroid-mammary parameters.

In support of the data presented herein, it is interesting to note that clinical studies have indicated low serum butanol extractable iodine values in women during the latter part of the third trimester (Man et al., 1964), near the onset of lactation. In cattle, although some data has been inconclusive (Lennon and Mixner, 1959), a positive correlation

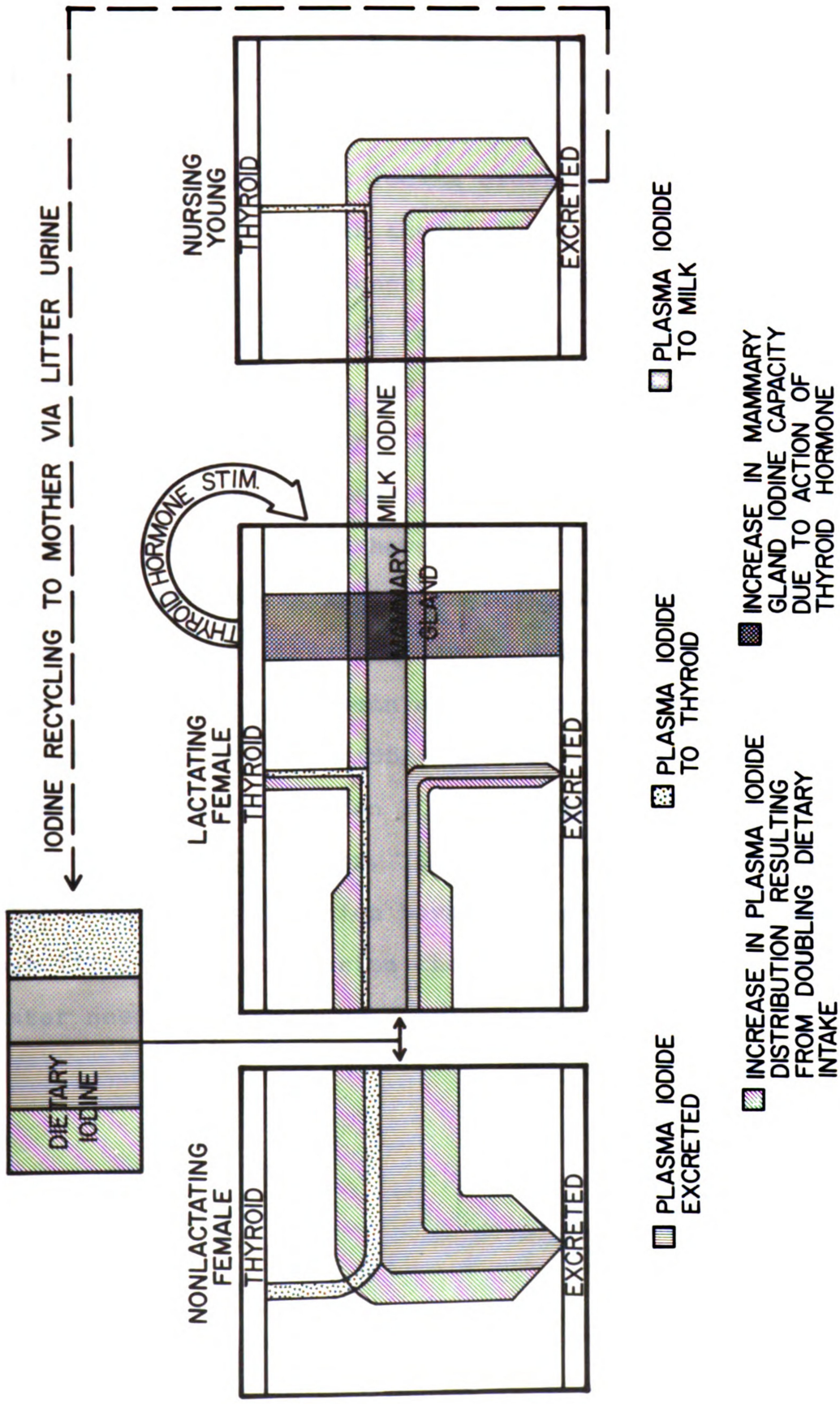


Figure 3. Thyroid-mammary competition for iodine.

has been shown to exist between thyroid function, as measured by PBI, and lactational performance (Sorenson, 1958).

Prior to the advent of iodized salt, it was noted that young women living in low iodine areas began to show enlargement of their thyroid with the birth of their first child. This problem is still of considerable clinical significance (Aboul-Khair et al., 1964). Goiter was usually attributed to the stresses of pregnancy and at present increased requirements occurring in pregnancy warrant iodine supplementation (Friend, 1960). In addition to the dairy herd applications of thyroid hormone, which have been reviewed (Blaxter et al., 1949), similar success in increasing milk yield has resulted in women (Robinson, 1947a; Romani, 1951; Roche et al., 1950). Iodine therapy has also been used in the treatment of inadequate lactation (Miller, 1951; Miller, 1952) and has increased average milk yield in women 300%, enough for unsupplemented feeding of young (Robinson, 1947b). Other implications of thyroid-mammary dysfunction have been adequately reviewed by Prout (1966). In conclusion these findings demonstrate a greater need for iodine by nursing mothers.

SUMMARY AND CONCLUSIONS

It has been proposed that with limited iodine (I) intake, lactation may become self-limiting due to reduced thyroid function resulting from a mammary-kidney-thyroid competition for I. Studies were conducted using lactating (L) and non-lactating (C) rats on specific dietary I levels by employing isotopic direct output and thyroxine (T_4) substitution methods to measure thyroid secretion rate (TSR).

With the direct output method thyroids were excised for histology and total I analysis. As an estimate of TSR, thyroid output rate times total I content, was expressed as T_4 equivalents/100 gm. body wt./day using the factor 1.53 for conversion of I to T_4 and the proportionality factor 1.52 to adjust for T_3 activity. On low I diet, mean total thyroidal I was 1.1 μg in L and 3.9 μg in C. TSR was 0.65 μg T_4 for C and 0.17 μg T_4 for L rats. Follicular hyperplasia was evident in L, indicating a functional hypothyroidism. Using the T_4 substitution method the apparent TSR was 3.3 μg T_4 and 2.0 μg T_4 in L and C, respectively. On higher I intake (0.65 μg I^- added/gm. feed) TSR by the direct output method was 0.80 μg T_4 in C and 0.38 μg T_4 in L rats. Follicular hyperplasia was again evident in L rats. TSR by the T_4 substitution method was similar on both diets. With a normal supplement of iodine (1.3 μg I^- added/gm. feed),

estimates of TSR were similar, employing either method of evaluation--under non-lactating conditions.

It was concluded that the direct output method was more valid for evaluating TSR during lactation than was the T_4 substitution method. The latter method can represent the true hormone output only if the thyroid is receiving sufficient iodine to meet the hormone demand. Under conditions employed it was evident that during lactation TSH was elevated, but I was insufficient to support the demand for thyroid hormone.

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APPENDICES

APPENDIX A

RAT FEED MIXTURE

Ingredient	Lbs. per 100# mix
Shelled yellow corn ground through 1/8 screen	68.8
Soybean oil meal (50% prot.)	28.0
Dicalcium phosphate	1.8
Limestone	0.6
Dawes & Forbes Vit. B Supplement	0.1
Dawes & Forbes Vit. B_{12} Supplement (6 mg B_{12}/lb.)	0.2
Std. Brand 9F yeast 9000 i.u. (Vit. D_2/gm.)	5.0 gm.
Pfizers Vit. A Supplement (10,000 i.u. Vit. A/gm.)	15.0 gm.
Special Mineral Salt Premix*	0.5

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* Special Mineral Salt Premix

Element	Per cent Element	Compound	Per cent Compound
Mn	0.524	$MnSO_4 \cdot H_2O$ (Baker)	1.612
Cu	0.054	$CuSO_4$ (anhyd) (Baker)	0.136
Fe	0.270	$FeSO_4 \cdot 2H_2O$ (Baker)	0.909
Zn	0.800	$ZnSO_4 \cdot H_2O$ (Mallinckrodt)	2.196
NaCl (plain)		(Morton's)	95.134

APPENDIX B

QUANTITATIVE IODINE DETERMINATION IN THE THYROID

Dry ashing with Na_2CO_3 is used in lieu of Barker's (1948) distillation procedure for total thyroidal iodine determinations (Reineke, unpublished).

I. Drying and incineration.

Place 1 ml. of 4 N Na_2CO_3 in Pyrex test tube.

Add weighed lobe of thyroid to tube and place in drying oven for 15 hours at 90-95°C.

Incinerate dried residue in muffled furnace for 2-2.5 hrs. at 600°C.

II. Dissolving iodine from ash.

After incineration add:

2.0 ml. 2 N HCl

2.0 ml. 7 N H_2SO_4

21.0 ml. glass D·H_2O

Mix well with glass stirrer after making up to 25.0 ml. volume and centrifuge at 2000 R.P.M. for 20 min.

III. Colorimetry.

Pipette in duplicate 1 ml. of the diluted digest into colorimeter tubes. Add 4.0 ml. of glass distilled H_2O to bring up to 5 ml. volume.

Add:

0.5 ml. arsenious acid reagent (Hycel) from a blowout pipette. Place colorimeter tube in 27°C water bath for 15 min.

Then add:

0.5 ml. of 40% ceric ammonium sulfate reagent (Hycel) at 1 min. intervals to each tube. Let each tube stand in water bath exactly 15 min.

After 15 min. add 0.5 ml. of 1.0% brucine solution* and read at 480 mμ on a Coleman Universal Spectrophotometer.

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*Make fresh brucine solution daily--add 5 drops of 7 N H_2SO_4 to facilitate dissolving brucine for each 10 ml. of reagent.

APPENDIX C

COMPARATIVE ACTIVITY OF L-THYROXINE AND L-TRIIODOTHYRONINE

(Reineke and Lorscheider, in publication)

Two groups, each containing 6 female CFN rats, were maintained on a Remington low-iodine diet: one group receiving parenteral injections of L-thyroxine and the other group parenteral injections of L-triiodothyronine. Thyroxine was injected successively for 2-day intervals at levels of 0.0, 0.5, 1.0, 1.5 and 2.0 μg/100 gm. body wt. and dosages for triiodothyronine were 0.0, 0.108, 0.216, 0.324, and 0.432 μg/100 gm. body wt. The thyroid blocking dose is that level of hormone that reduces further output of I^{131} to 97.5% of the previous count. Thyroidal I^{131} activity is determined by successive external thyroid counts at 2-day intervals. The ratio of T_3:T_4 activity is shown to be 4.09 per unit of wt., 3.42 per mole or 4.60 per unit of iodine.

Group	Number of Rats	Compound tested	Thyroid blocking dose, μg/100 gm. at 97.5%	r
I	6	L-thyroxine, parenteral	1.700	.707
II	6	L-triiodothyronine, parenteral	0.416	.755

Comparative activities:

Weight basis---------- $T_3:T_4 = \frac{1.700}{0.416} = 4.09$

Molar basis---------- $\frac{1.70}{776.93^*} = 0.00219$ moles T_4

$\frac{0.416}{651.02^*} = 0.00064$ moles T_3

$T_3:T_4 = \frac{0.00219}{0.00064} = 3.42$

Iodine basis---------- Per cent $T_4{}^{I^-} = \frac{507.64^*}{776.93} = 65.34\%$

Per cent $T_3{}^{I^-} = \frac{380.73}{651.02} = 58.48\%$

1.700 μg T_4 (0.6534) = 1.111 μg $T_4{}^{I^-}$

0.416 μg T_3 (0.5848) = 0.243 μg $T_3{}^{I^-}$

$T_3:T_4 = \frac{1.111}{0.243} = 4.60$

* M.W. T_4 776.93

M.W. T_3 651.02

M.W. I 126.91

APPENDIX D

PROCEDURE AND CALCULATIONS FOR HISTOLOGICAL EVALUATION

1. Thyroid lobes were fixed in Dietrich's solution and stained with hematoxylin-eosin.

2. Photomicrographs were taken with 35 mm. Ektachrome film at 960 X.

3. Images were projected beneath a translucent drawing board and the outer and inner areas of the concentric circles, delineated by apical and basal boundaries of the thyroid follicular cells, were measured in cm. with an area planimeter.

4. $\frac{A}{\pi} = r^2$

5. $r = \sqrt{\frac{A}{\pi}}$

6. $r_o - r_i$ = mean cell height (cm.)

7. This projection system used a correction factor of 1 cm. = 9.8 μ or 0.102 cm. = 1.0 μ

8. Mean cell height (microns) = $\frac{\text{cm. mean cell ht.}}{0.102 \text{ cm./}\mu}$