Effects of Induced Mild Hyperthyroidism on Serum Protein-Bound Iodine, Thyroxine Distribution Volume, and Biological Half-Life of Thyroxine-$^{131}$I in Dairy Cattle ¹ ²

T. R. BAUMAN, R. R. ANDERSON, and C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

Abstract

Lactating and dry dairy cattle of several breeds were injected with exogenous L-thyroxine at levels 25 and 50% in excess of the normal thyroid hormone secretion rate. The biological half-life of L-thyroxine $^{131}$I became shorter as the degree of induced hyperthyroidism increased. Serum protein-bound iodine levels increased markedly above control values as the level of injected L-thyroxine increased. Thyroxine distribution volume showed an apparent increase over control values at 125 and 150% of normal thyroid secretion rate, but values at the 150% level declined below those at the 125% level. Thyroid secretion rates of control animals as determined by replacement technique and isotope dilution technique were in fair agreement with each other. The calculated utilization rates of L-thyroxine at the 125 and 150% of normal thyroid secretion rate level were two to three times higher than the known amounts of L-thyroxine injected daily. Excess thyroxine, up to 50% above normal secretion rate, seemed to have been eliminated from the body by rapid removal from the blood after the thyroxine-binding proteins were saturated.

In a previous study on the biological half-life $(t_{1/2})$ of L-thyroxine $^{131}$I in dairy cattle, a marked decrease in the half-life was observed when exogenous L-thyroxine (L-T$_{4}$) was injected at levels above the normal thyroid secretion rate (13). Other workers have reported decreased half-life of L-T$_{4}$ in human hyperthyroid patients (16).

Among the important factors that serve to regulate half-life rates of L-T$_{4}$ from the blood are the thyroxine-binding proteins. Oppenheimer et al. (10) and Webster et al. (18) suggested that the distribution of thyroxine between extra- and intracellular compartments was accomplished by the carrier proteins, with a direct transfer of L-T$_{4}$ from one binding site to another. Biological half-life rates depend mainly on competition between those forces tending to retain hormone (ability of serum protein to bind L-thyroxine) and all other forces tending to remove it from the circulation. The half-life is consistently related, inversely, to the concentration of unsaturated L-T$_{4}$-binding sites associated with thyroxine-binding globulin (TBG). The amount of free thyroxine in the blood at any time is only 1% or less of the total thyroxine (14).

Berson and Yalow (1) and Ingbar and Freinkel (6) reported a negative correlation between half-life of L-thyroxine and serum plasma-bound iodine levels. The relationship between decreased half-life of L-T$_{4}$ and increased bound iodine as seen in hyperthyroidism would lead one to assume that L-T$_{4}$ was being utilized at a rapid rate.

Our experiment was undertaken to determine in dairy cattle, injected subcutaneously each day with L-thyroxine at levels 25 and 50% above their normal thyroxine secretion rate, the kinetics of thyroxine utilization as measured by the thyroxine body-pool turnover method.

Materials and Methods

Normal thyroid secretion rates (TSR) were determined in lactating and dry dairy cattle of several breeds. Exogenous thyroxine was injected daily in quantities 25 and 50% above normal secretion rate levels for varying periods of time (from 4 to 14 weeks) prior to determining the thyroxine distribution volume (TDV), serum PBI levels, half-life of L-T$_{4}$, and calculated utilization rates of L-T$_{4}$. In all cattle the 125% level of exogenous thyroxine was administered prior to the 150% level.

Thyroid secretion rates were determined by a thyroxine replacement technique in which exogenous thyroxine was injected subcutaneously daily to inhibit the release of previously labelled thyroxine from the thyroid gland via inhibition of TSH secretion (11).

Received for publication August 21, 1968.
1 Contribution from the Missouri Agricultural Experiment Station, Journal Series no. 2808. Approved by the Director.
(1-methyl-2-mercaptoimidazole) was given orally in gelatin capsules each day at the rate of 4.0 g/455 kg body weight, to prevent recycling of $^{131}$I from metabolized thyroxine.

The biological half-life of L-T$_4$ was determined by injecting 200 $\mu$g L-thyroxine-$^{131}$I (approx 0.0052 mg L-T$_4$) intravenously and collecting blood samples at 12- or 24-hr intervals post-injection up to five days. Methimazole was administered orally at the rate of 4.0 g/455 kg body weight per day during control determination to block recycling of $^{131}$I. Total $^{131}$I counts were made on 3 ml of plasma which had been precipitated and washed twice with cold 10% trichloroacetic acid. Regression (k) values and half-life were calculated by the method of least squares.

Determination of thyroxine distribution volume was calculated by the method of Gregerman and Crowder (3). This method, also used to calculate thyroxine secretion rate, is based upon the assumption that the utilization rate and secretion rate of thyroxine are in equilibrium; hence, measurement of utilization rate is a measure of secretion rate. This was verified in human euthyroid subjects by Ingbar and Freinkel (6). Thyroid distribution volume was obtained by a visual semilog plot of radioactivity (per cent administered dose/milliliter of plasma) versus time. By extrapolation of the curve back to zero time, the theoretical concentration in per cent dose per milliliter was obtained. One hundred divided by this value gave distribution volume expressed in milliliters.

Serum protein-bound iodine values were determined on individual serum samples taken near the end of each period of L-thyroxine treatment. Protein-bound iodine was determined on a technicon Auto-Analyzer by the Pathological Diagnostic Laboratories in Columbia. This method employs the catalytic effect of iodine on the reduction of ceric ion by arsenious acid after the organic iodine has been freed by acid digestion and distillation. All determinations were made with clinical controls. Protein-bound iodine values were multiplied by 1.54 to give the equivalent amount of plasma thyroxine (PT) expressed as micrograms thyroxine per milliliter of plasma. Thyroxine utilization or secretion rate was then calculated as the product of thyroxine distribution volume × plasma thyroxine × k.

Two groups of cows were utilized during the experiment; both were kept under dry-lot conditions with free access to hay and water at all times. The lactating cattle were given all they could eat of a high-energy dairy ration at each milking. Group I was composed of 19 lactating cows of several breeds weighing from 386 to 709 kg on which normal thyroid secretion rates (by replacement technique), half-life of L-T$_4$, at 25 and 50% above the secretion rate, and distribution volumes were determined. The cattle in Group I were designated for a production experiment. As a result, the control half-life of L-thyroxine prior to injecting it at levels 25 and 50% above normal secretion rate was not obtained. However, Pipes et al. (12) and Premachandra and Turner (13) reported values of 2.47 and 2.32 days, respectively, for normal half-life of L-thyroxine-$^{131}$I in dairy cattle. A mean value of their data (2.39 days) was used as the control value for the cows involved in Group I. Group II was composed of six dry cows of several breeds weighing from 500 to 898 kg on which thyroid secretion rates (by both replacement and dilution techniques), half-life of L-T$_4$, distribution volume, and protein-bound iodine were determined.

Group means were compared using Student's "t" test.

**Results**

Data accumulated from cows in Group I, showing the decrease in half-life with increasing degrees of induced hyperthyroidism and

| Table 1. Biological half-life of L-thyroxine $^{131}$I and thyroxine distribution volume in lactating cattle. |
|-------------------------------------------------|-------------------------------------------------|
| 25%                                             | 50%                                             |
| **t$_{1/2}$**                                   | **t$_{1/2}$**                                   |
| Hours:                                          | Days:                                           |
| Range                                          | Range                                          |
| 21.4-46.3                                      | 0.80-1.93                                      |
| Mean ± SE                                      | Mean ± SE                                      |
| 33.8 ± 4.0                                     | 1.41 ± .067                                    |
| No. of animals                                 | No. of animals                                 |
| 19                                              | 17                                             |
| TDV:                                           | TDV:                                           |
| In liters                                      | In liters                                      |
| Range                                          | Range                                          |
| 131.5-285.7                                    | 90.9-163.9                                     |
| Mean ± SE                                      | Mean ± SE                                      |
| 189.7 ± 11.2                                   | 127.2 ± 6.3                                    |
| As % body weight                               | As % body weight                               |
| 25.5-41.4                                      | 17.7-31.9                                      |
| Mean ± SE                                      | Mean ± SE                                      |
| 39.1 ± 1.18                                    | 24.0 ± 1.08                                    |
| No. of animals                                 | No. of animals                                 |
| 19                                              | 17                                             |

$P<.01$ $^a$ vs. $^b$; $^c$ vs. $^d$; $^e$ vs. $^f$.

J. DAIRY SCIENCE VOL. 52, NO. 2
changes in thyroxine distribution volume under each treatment are presented in Table 1. Thyroxine distribution volume increased at 25% above normal thyroid secretion rate and declined from the 125% level at the 50% above secretion level. However, the distribution volume in the latter situation was still higher than the normal control volume reported by Mixner and Lennon (7) and Yousef and Johnson (19). Tests for significance are given in Table 1.

Data given in Table 2, concerning Group II, indicate much the same trend as Group I. Increased levels of serum protein-bound iodine were noted with each increase in the amount of exogenously administered thyroxine. The mean half-life decreased as the degree of induced hyperthyroidism increased. Total distribution volume at the 25% above normal secretion level exhibited an apparent increase; at the 50% level an apparent decrease from the 25% treatment volume was noted. It should be emphasized, again, that the calculated thyroid distribution volume for the 50% above normal thyroid secretion rate group was greater than the normal control.

Thyroid secretion rates obtained by the replacement technique as compared to the dilution technique were in fair agreement in the controls. However, the calculated utilization rates of L-T, greatly exceeded the daily injected dose of thyroxine at both the 25 and 50% above secretion levels.

**Discussion**

Recently Blincoe and Weeth (2) have reported that thyroxine-<sup>131</sup>I added to bovine serum migrated during electrophoresis, primarily in the a-globulin fraction. Many other workers, in studying human thyroxine distribution and transportation, have determined that thyroxine is distributed among thyroxine-binding globulin, serum prealbumin, and albumin, with varying degrees of affinity and capacity assigned to each fraction (5, 9, 10, 17, 18).

Since these proteins are responsible for distribution of thyroxine in the vascular and extravascular spaces, they must play an important role in determining the calculated thyroxine distribution volume. Yousef and Johnson (19) have reported the thyroxine distribution volume in normal cattle, under different temperature conditions, to vary from 14.3 to 20.9% of body weight. Mixner and Lennon (7) studied thyroxine secretion in lactating cattle and bull calves and found this volume to vary from 9.6 to 23.9% of body weight.

In this study, an increase in thyroxine dis-
distribution volume from 18.5% body weight in controls to 37.3% body weight in cattle injected with thyroxine 25% in excess of normal thyroid secretion was noted (Table 2). When the level of injected thyroxine was increased to 50% in excess of normal, the determined thyroxine distribution volume was 24.5% body weight, a decline of 12.8% from the 125% treatment, but still 6% above control values (Table 2). A similar decline was observed in lactating cattle (Table 1).

Blincoe and Weeth (2) determined that only about 71% of thyroxine was bound to plasma protein in cattle, but they did not suggest that the thyroxine-binding protein was saturated. Likewise, Slebozinski (15) reported that the thyroxine-binding globulin in newborn pigs was only 67% saturated with thyroxine, although the quantity of L-T₄ needed to completely saturate the system was a small fraction of the daily production of hormone. It appears from the data presented that L-thyroxine given at a level of 25% in excess of normal secretion can be bound by plasma proteins, as evidenced by an apparent increase in thyroxine volume. However, at a level 50% in excess of normal all the binding sites have been saturated and an apparent decrease in thyroxine volume is observed. It is for this reason that the increase and decrease in thyroxine volume is not believed to be physiological in nature, but rather a reflection of the degree of saturation of the thyroxine binding sites. In effect then, the daily injection of L-thyroxine at 25%, and even more so at 50% in excess of normal secretion rate, can be compared to running water into a sink full of water. The excess in the blood must run out rapidly, because all of the binding sites are saturated and the most likely excretory pathway for the excess thyroxine is to the bile via the liver.

It follows then that if the thyroxine is eliminated rapidly, primarily from the blood, because the vascular and extravascular binding sites are saturated, it would not have as great a physiological effect as might be expected as long as the liver is not overwhelmed with excess L-T₄. In a previous report on the same lactating cattle (4), a 1.1 C rise in body temperature, some increases in milk yields, and body weight gains above control values were noted. In the case of the dry cows no weight loss was observed; body temperatures were not taken.

When one examines the data presented on the cattle receiving L-thyroxine 25% in excess of normal thyroid secretion rate, 0.21 mg L-T₄/45.5 kg body weight was injected daily, but the calculated utilization rate was 0.68 mg/45.5 kg body weight or three times as much as injected. At the 50% excess L-T₄ level, a mean of 0.32 mg L-thyroxine/45.5 kg body weight was injected daily, but the calculated utilization rate observed was 0.78 mg/45.5 kg body weight, slightly more than two times more than injected. Since the methods for determining secretion-utilization rates have been confirmed only under euthyroid conditions (6), the accuracy of the values determined is questionable when hyperthyroidism is a factor.

Alterations in thyroxine distribution volume do not appear to be physiological, which is in agreement with Oddie’s review (8) on thyroxine turnover studies. It appears that excess thyroxine (in amounts up to 50% above normal secretion rate) is eliminated from the body of the cow after complete saturation of the protein carrier systems, and the excess probably is excreted rapidly into the bile. Since the excess thyroxine is cleared from the blood very rapidly, and apparently not uniformly mixed in the body pool, it might be expected that much of it is eliminated unmetabolized.

Acknowledgment

The authors thank Dr. W. R. Bynum of the Pathological Diagnostic Labs, Columbia, Missouri, for PBI analysis.

References


J. DAIRY SCIENCE VOL. 52, NO. 2
KINETICS OF THYROXINE UTILIZATION

as calculated from plasma levels, turnover rates and volumes of distribution of thyroxine. Proc. XVth Int. Dairy Congr., 1: 20.


