THE INFLUENCE OF PREPARTUM PLANE OF NUTRITION ON THYROID ACTIVITY AND MILK PRODUCTION OF DAIRY CATTLE

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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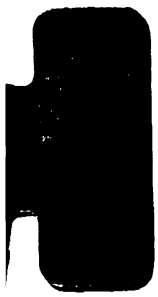
1964



ABSTRACT

THE INFLUENCE OF PREPARTUM PLANE OF NUTRITION ON THYROID ACTIVITY AND MILK PRODUCTION OF DAIRY CATTLE

by LaVern A. Rice

Thyroxine secretion rates were measured in nine animals (six heifers and three cows) to determine what relationship there was between thyroid activity and both prepartum grain intake and age of animal as reflected in milk production. These nine animals were a random sample from an experiment of 93 Holsteins which was designed to determine the effect of prepartum grain feeding on mammary edema and milk production. Milk production for 45 days postpartum, roughage intake, and grain intake for the remaining 84 animals (30 cows and 54 heifers) was compared with that of the nine animals.

In both the trial and the experiment, group I and II were fed alfalfa mixed hay ad libitum before and after parturition and corn silage (30 lb/day) from 5 days to 45 days postpartum. Group I received grain ad libitum from 21 days prepartum to 45 days postpartum while group II received grain ad libitum only after 5 days postpartum. The grain ration was a standard 14.5% crude protein mix calculated to contain 1.08 g iodine per ton.

Each of the nine animals were injected with sodium L-thyroxine-pentahydrate* on the third day following calving. Seven animals received 20 mg injections while one received 15 mg and another received 25 mg. A pre-injection blood sample was taken with postinjection samples taken on an average of 30, 44, 54, and 72 hours. Serum protein-bound iodine (PBI) was measured to determine the turnover rate of chemical thyroxine from which thyroxine secretion rate (TSR) was computed.

Pre-injection PBI ranged from 4.03 to 37.60 ug%, with an average of 16.27 for the nine animals at 3 days postpartum. These values did not appear to be related to iodine intake. There was a range from 24.21 to 104.33 L, with an average of 51.75 for thyroxine volume of distribution. The extrathyroidal thyroxine (ETT) pool ranged from 5.91 to 16.79 mg, with a mean of 9.98. Individual values were about three times higher than has been reported. The normal PBI may have contributed to the higher ETT pool. Time-half for disappearance of injected thyroxine averaged 99.3 hours, which was considerably above reported values. Thyroxine fractional turnover rate (TFTR), the proportion of the extrathyroidal thyroxine pool leaving per day, ranged from 0.053 to 1.765, with a mean of 0.406. Thyroxine secretion rate mg/day ranged from 0.46 to 16.96, with a mean of 3.77. There was no significant difference found for cows versus heifers or for prepartum grain versus no prepartum grain for thyroxine volume of distribution, extrathyroidal thyroxine, time-half, TFTR, and TSR. However, there appeared to be a difference for TFTR and TSR which can be attributed to the one cow in the no prepartum grain group. This cow had the highest TSR and TFTR of all the animals.

For those animals only on the edema experiment milk production was significantly greater ($P<0.05$) for cows than for heifers. Grain consumed in the edema experiment was greater ($P<0.05$) for cows than for heifers and for the animals on prepartum grain than for those on no prepartum grain. For the same comparisons in the thyroxine trial the difference was significant ($P<0.05$) only for cows versus heifers. No significant difference was found for milk production for either age of animal or grain treatment.

In both the experiment and the trial the three week prepartum grain treatment seemed to increase milk production by 2 lb/day above no prepartum grain feeding; however, the difference was not significant. Also, the cows outproduced the heifers in the experiment and the trial. The milk production for age of animal and grain treatment indicate a similar response for both the trial and experiment.

Concerning a change in thyroid activity and the probable relationship to grain treatment and age of animal as reflected in milk production, no conclusion may be made from the data compared on the basis of cows versus heifers and prepartum grain versus no prepartum grain.

Body temperatures of the cows and heifers were consistantly above the normal average for cows of 101.5° F. Thyroxine treatment seemed to have an effect on the temperatures. Seasonal variation was not detectable from the results of this trial.

*Supplied by Smith Kline & French Laboratories, Philadelphia, Penn.

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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 Dairy

1964

ACKNOWLEDGMENTS

The author wishes to express appreciation to Dr. R. S. Emery, Associate Professor of the Department of Dairy, for his assistance and guidance during the course of this study and the preparation of this thesis.

The author expresses appreciation to Dr. C. A. Lassiter, Head of the Department of Dairy, for making available the Graduate Research Assistantship.

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INTRODUCTION

Numerous studies have been conducted with the objective of increasing milk production of dairy cows. Considerable attention has been given to prepartum feeding of various quantities of grain. Also, postpartum regimens have been considered in connection with the prepartum period. Both positive and negative milk production responses have been obtained when feeding grain prepartum.

Another potential area of consideration has been the effect of the endocrine system on milk production and secretion. Endocrine secretions influence the metabolic systems synthesizing milk; thus, one hormone or a combination of hormones could affect the productive ability of the animal. Several hormones have been studied during the various phases of lactation. Production has been increased with the use of pituitary growth hormone and crude pituitary extracts. Oxytocin and prolactin have been questionable as stimulants of production. Depressing effects have been demonstrated with the use of estrogens and adrenocorticotrophic hormones. Milk yield has been depressed by thyroidectomy with recovery following thyroid treatment. Thyroxine and iodinated casein preparations have stimulated higher production. The importance of thyroid activity has been emphasized.

Two groups of animals were studied to determine whether there would be a change of thyroid activity associated with prepartum grain feeding and age of animal as reflected in milk production. One group of animals received grain prepartum while the other group received no grain prepartum. A recent method was employed for estimating thyroid secretion rate.

LITERATURE REVIEW

Thyroid Gland

Although all vertebrates have thyroids, there is considerable variation in the degree of proximity of the follicles to one another. Although thyroxine and its precursors are found in many invertebrates, there is seldom present any resemblance of developed thyroid follicles (98). Being an embryonic derivative of the alimentary tract, the thyroid developes from an evagination of the floor of the embryonic pharynx into a bilobed gland, situated on the anterior tracheal surface (98). The gland is composed of an aggregation of cystlike follicles which are lined by secretory epithelium of cuboidal or low columnar cells. Vascularized connective tissue occupies the interfollicular spaces. The thyroidal epithelium secretes, within the follicle, an amber globulin, a colloid which is the storage product (98). The important protein in the colloid is thyroglobulin, a glycoprotein, which is considered the storage form of the thyroid hormone (98). It appears that thyroglobulin is continually produced by the thyroid cells and is passed into the follicular lumen and stored (98). When the protein is hydrolyzed with catheptases under physiologic conditions iodinated amino acids are yielded (98). The important iodinated amino acids are monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (TRIT), and tetraiodothyronine (thyroxine) (72,98).

Iodine Metabolism

The several forms of dietary iodine are mostly reduced to iodide

before absorption from the gastrointestinal track. In the blood plasma inorganic iodide is the form present and its concentration is low (98). More inorganic iodide is concentrated by the thyroid follicular epithelium than by other body tissues (98). Inorganic iodide is concentrated by the thyroid, partially converted to organic iodine (72), and partially returned to the circulation (72). Most of the thyroidal iodine is present as organic iodides, with inorganic iodides comprising about 1% of the thyroid iodine content under physiologic conditions (98). In the follicular epithelium, enzyme systems convert the inorganic iodides to elemental iodine (I_2) or iodate (IO_3^-) by oxidation (98). The suggestion has been made that an "iodase", which may convert iodide to iodine, mediates organic binding (72). A thyroidal peroxidase has been postulated for this conversion and peroxidase activity has been indicated in both thyroid cells and follicular colloid of rat tissues (72).

Thyroxine and related compounds are probably formed in the cells of the secretory epithelium or from precursors in the follicular lumen (98). Evidence is presented that thyroglobulin, which is mainly uniodinated, becomes iodinated in the colloid (66). The iodination of the amino acid tyrosine probably proceeds while bound to thyroglobulin, forming MIT, DIT (72), and finally TRIT and thyroxine, which are the organically bound forms of thyroidal iodine (72). Two of the bound DIT may couple to form thyroxine and the hormone TRIT may be formed by coupling MIT and DIT or by loss of iodine from thyroxine (72,98).

Thyroid Hormone Release

Under normal conditions thyroxine and TRIT, but not MIT or DIT are secreted into the circulatory system (72). Hormones must be liberated and secreted into the circulation before they can perform their physiological functions. There is an indication of protease secretion by thyroidal epithelial cells which splits thyroglobulin into smaller molecules plus iodinated derivatives of tyrosine (98). Thyroxine and TRIT passage probably is due to a concentration gradient existing between the colloid and fluids of the tissues. The principle circulating hormone is thyroxine with small amounts of TRIT being present (98). Previously the thyroidal hormone was thought to be a peptide or a polypeptide containing thyroxine and DIT (72). Monoiodotyrosine and DIT do not leave the follicle, but are deiodinated within the thyroid cells and recycled to synthesize more thyroglobulin. Deiodination is mediated by an enzyme, deiodinase, which is specific for DIT and MIT as substrate (98). No detectable action of this enzyme has been obtained on either thyroglobulin-bound DIT and MIT or on bound thyroxine or TRIT (72). The indication is that if thyroxine is free from blood proteins the deiodinase of pig thyroid will deiodinate it, but no TRIT has been detected (72).

Thyroidal hormones are bound to the circulating serum proteins, albumin and alpha globulin (72). Taurog and Chaikoff (96) reported that most of the iodine in the blood was in the albumin with alpha globulin containing the greatest concentration. Later indication was that most of the binding was between the alpha 1 and alpha 2 globulin fraction, this is referred to as thyroxine binding protein (TBP) (72).

This protein transports the thyroidal hormone from the thyroid gland through the circulation to the peripheral tissues and cells. The binding to TBP is not a peptide linkage as is the binding to thyroglobulin (72). More TBP is present in plasma than is required to bind the normal concentration of thyroxine, also there is increased TBP during pregnancy (72,98).

The mechanism of action and the form in which the hormones act on the peripheral tissues is not well understood (98). The indication is that hormones circulating through the tissues, being freed from their protein carriers, pass through the capillary walls and act upon the tissue cells (98). Freinkel, Ingbar, and Dowling (30) indicated that whether thyroxine and TRIT enter into the cells free or bound to TBP is not known. There is little known concerning the binding of the thyroid hormones by proteins of the tissues; however, the suggestion has been made that a small amount of free hormone enters the cell because of binding sites on the cell (72). Tata (95) detected the binding of physiologic concentrations of levorotatory thyroxine and 3,5,3' TRIT by a protein fraction in the rat skeletal muscle. This protein appeared to differ from the TBP of serum (72). While thyroxine is the main thyroid hormone, TRIT is a more active form and thyroxine may be converted to TRIT before affecting the peripheral tissues (98). There may be several hormones working together at the tissue level or perhaps the active form is still to be discovered. The metabolism of thyroid hormone at the peripheral sites is generally accepted as being partially controlled by the interactions with the proteins of the extracellular fluids. These proteins serve to regulate the delivery

rate of the hormones to the peripheral tissues (43).

Under normal conditions thyroid stimulating hormone (TSH) from the pituitary is the main controller of thyroid function. Thyroid stimulating hormone stimulates the output of thyroxine from the thyroid, when the thyroxine level is low in the circulation, and high levels depress TSH output (98).

Indirect Measurements of Thyroid Activity

Since thyroid activity controls the various functions of the body a hyperactive or hypoactive thyroid would alter these processes. The activity of the thyroid may be greater in one animal than another, but neither would be considered hyperactive. Certain criteria of thyroid activity are desirable to estimate the degree of activity. From these indexes the productive ability of an animal may be indicated. These criteria do not measure directly the activity, but only indicate the deviation from the "normal" or relative function of the gland.

Total iodine of blood serum. Schultze and Turner (84) made the following observation from their review of literature. The changes of total blood iodine seems to be of doubtful value as an index of thyroid activity, since the iodine intake influences these values and iodine intake above the necessary requirement does not influence metabolic rate. Blood iodine concentration and basal metabolism are correlated but may be obscured by inaccurate analytical techniques, excessive exogenous iodine intake, and a changing physiological state (82). This seems to indicate that there are conditions in which the concentration of blood iodine varies with changes of thyroid activity (84).

Protein-bound iodine. Protein-bound iodine (PBI) concentration in blood plasma has been used as an index of net thyroid function (84) and is used as a diagnostic test for hyperthyroidism and hypothyroidism (37). The PBI level increases in proportion to the degree of hyperthyroidism and decreases in the hypothyroid state (37). Sorensen (89) concluded that PBI values in general are good indicators of thyroid function, but are still open to discussion (90). They are a reliable index if the thyroid function is steady, but if glandular secretion and peripheral tissue consumption vary at the same time, the change in PBI may be less marked (90). Thus, PBI concentration may increase when the thyroid secretion rate is low (89). Blood PBI values do not measure thyroid output directly, they only represent a balance between thyroid output, distribution to the tissues, metabolism, and excretion (39).

Factors influencing PBI levels. Error may be introduced due to the presence of other iodine compounds in the plasma (37). Cows receiving thyroprotein (52,59), L-thyroxine (94), and iodide as potassium iodide (58) have shown marked increases in PBI. King and Lee (48) obtained increased PBI in heifers fed a trace mineral salt containing cuprous iodide and 3, 5-diiodosalicylic acid as opposed to those fed plain salt.

Grass silage. Asplund et al. (2) estimated PBI in calves and dairy cows and found a seasonal rise in two successive years which was associated with the use of silage made in June of both years. The maximum PBI was reached in March followed by a rapid decline (2). Grass silage, suspected of causing higher than normal PBI values, increased PBI 230% over that of rats fed a control ration (4).

Age. Long et al. (56) and Lewis and Ralston (52) have shown that younger animals possess higher PBI levels which decline with age similarly as basal metabolic rate declines in humans (57). Protein-bound iodine in cattle has been demonstrated to decrease with an increase in age with a significant correlation of -0.64 (90).

Pregnancy. In pregnant heifers PBI concentration appeared to be significantly higher than in the nonpregnant twins (90). The PBI values obtained at the time of calving were significantly lower than before or after calving (53). Robertson et al. (80) also found that there was a marked drop in PBI at parturition and during the two week period following with a gradual return to normal values.

Environmental temperatures. Reduced environmental temperatures had little effect on PBI levels (31). Sorensen (90) concluded from a review of literature that results for PBI levels diverge at different environmental temperatures. For the rat at 7 to 10° C there was an increase in PBI over the controls at 25° C (90). In contrast rats have shown a slight decrease in PBI under cold temperatures of 1 to 7° C as compared to controls at room temperature (90). Cattle under cold conditions showed only a slight PBI increase (90). The serum PBI of pigs was found to increase in winter and to decrease in summer (92). High PBI levels of Ayrshire cattle, aged 3 months to 18 months, occurred mostly in the Spring with few in late Fall (3). For two breeds of cattle, the PBI was lower in November than in June and August (52).

Stresses. Certain stresses influence PBI in cattle. Cows with ketosis had a mean PBI value which was significantly lower than the mean for normal cows (80).

Breed differences. The ranking for 116 individual dairy and beef cattle was 4.11, 3.51, 3.37, 3.19, 2.73, and 2.19 ug % for Jersey, Guernsey, Brown Swiss, Ayrshire, Holstein, and beef breeds, respectively (57). Bullocks 18-25 months old had PBI significantly lower than heifers of similar age (90).

Basal metabolic rate. It has been indicated that thyroidectomy would reduce the basal metabolic rate (BMR) by nearly half, while the feeding of sufficient thyroid extract would nearly double the BMR (7). Metabolic rate was then used as an index of thyroid function. In severe hyperthyroidism of humans BMR usually increases by approximately 40-60% (37). The mean BMR of rats, fed a grass silage suspected of causing higher than normal PBI values, increased 15% after seven days of treatment over rats fed a control ration (4). A significant corrlation between BMR and PBI was found (4). Burns, Colby, Gougler, and Kunkel (12) found a positive correlation between BMR and PBI in cattle. The stimulus to increased thyroid activity by the anterior pituitary factor (thyrotrophic factor) is indicated by increased BMR (84). With the absence of the thyrotrophic factor, gland weight increases. Depending on the factors causing the increased weight there is either decreased hormonal blood iodine or increased hormonal blood iodine resulting in decreased BMR and increased BMR, respectively (84). Basal conditions for estimating BMR are difficult to obtain in large animals.

Thyroid gland weight. Increased weight of the thyroid gland has been used as an index of thyroid activity and indicates stimulus by the thyrotrophic factor (84). Marine and Lenhart (61) and Seidell and Fenger (86) found an inverse relationship between gland weight and per cent

iodine content of sheep, cow, and pig glands. Thyroid activity is inversely proportional to the iodine content of the gland (61). There has been reported a marked seasonal variation in iodine content of the thyroid glands from cattle, pigs, and sheep (86). The iodine content was more than two times as high in summer than in the winter. The seasonal variation was later verified by Kendall and Simonsen (47) in pigs and was also indicated for thyroxine content. The weight index is complicated by weight changes that may occur due to alterations in the vascularity and stroma along with changes in the amount of colloid and size and number of acinar cells. Increased weight changes can be due to increased colloid or increased epithelial constituents (84). A disadvantage of this method is the necessity of sacrificing large animals.

Histology of the gland. Histological changes of the gland are an indication of thyroid activity. The thyrotrophic factor results in hyperplasia, loss of follicular colloid, increased secretory cell height, increased secretory tissue, and increased mitotic activity of the cells (84). The weight of the gland varied directly with the degree of hyperplasia (61). Epithelial cell height is a fairly reliable index of thyroid activity (84). Greater secretory activity may be indicated by increased mitotic activity of the epithelial cells. This criterion alone cannot determine which of the secretory phases, colloid accumulation or increased thyroid hormone output, is being augmented (84).

Milk secretion. A simple criterion for thyroid status would be the exogenous administration of thyroid hormone to observe the affect on the intensity of milk secretion. With increased milk yield the inference would be that the hormone secretion rate was the limiting

factor (99). However, other hormonal and environmental factors could also limit milk production.

Milk alkaline phosphatase level. Chanda (15) reported that milk phosphatase titre is sensitive and specific enough to assay the potency of thyroid active and anti-thyroid drugs. A marked decrease in milk alkaline phosphatase was observed when lactating cows were treated with 10 mg DL-thyroxine and the milk phosphatase level increased with thiouracil treatment (15). The response of phosphatase level to the treatment varied with the initial phosphatase titres and the stage of lactation. Within the species the higher milk yields associated with lower milk phosphatase titres were attributed to the natural difference in gland activity (15). The response of milk phosphatase was proportional to the level of L-thyroxine administered (15).

Conversion ratio of I^{131}. There are methods which use radioactive I^{131} to determine thyroid activity. A chemical diagnosis of thyroid pathology is the conversion ratio (ratio of thyroxine-like I^{131} to total I^{131} in the blood plasma) (6). In thyroidectomized rats the rate of incorporation of radioactive iodine into the protein-bound fraction of plasma is depressed and is increased with the injection of thyrotrophic hormone (14). The ratio of radioactivity in counts per second in the protein fraction to the total plasma radioactivity in counts per second multiplied by 100 gives the conversion ratio in per cent (17). In humans those ratios above 50% included the hyperthyroid individuals while those below 10% included the hypothyroid ones (17). This index of thyroid activity, because it is a ratio, is independent of errors in the preparation of the I^{131} standard; however, since the index is

calculated from two non-identical measurements its error becomes relatively large (6). The conversion ratio in the rabbit and cow by the gland at 95° F is below that at 50-60° F air temperature (6).

Direct Measurements of Thyroid Activity

Maximum uptake of I^{131}. Clinically, maximum uptake of I^{131} by the thyroid is the most frequently used index of thyroid activity (6). The maximum uptake depends on the rate of uptake and release of I^{131} by the gland and the excretion of I^{131} in the urine and milk (6). The maximum uptake and rate of uptake of I^{131} by the gland is indicative of the uptake (iodide fixation) phase of the thyroid (6). However, they may not indicate the release of thyroid hormone, because the iodine may be stored and the hormone released as required. There could be a change in the synthesis and release of the hormone from the gland without a corresponding change of iodine uptake (6). This measurement may be made directly as per cent of injected dose on time and is a good index, if the rate of excretion of I^{131} is relatively constant. The release of I^{131} from the thyroid is sufficiently slow to have little affect on the maximum uptake (6).

Thyroid hormone release rate. Blincoe and Brody (6) considered the hormone release rate constant, k_4, as the most useful parameter of thyroid activity. Pipes, Premachandra, and Turner (70) used the release technique as a criteria of thyroid function. They corrected the observed rate constant (k'_4) for recycling of I^{131} to the gland, by the method of Brownell (10) to obtain the true rate constant (k_4) which would be higher (70). The recycling I^{131} must be assumed to be re-

utilized by the gland as was the initial radioactive iodine to use the equation of Brownell (70). The same method was used with the addition of thiourcil to block recycling. A new steady state was obtained with an increased observed rate constant for thyroidal I^{131} release. Increased true and observed rate constants indicated increased functional activity but measured only the relative ratio of thyroid function rather than the actual amount of hormone synthesized (70). Small differences in thyroidal I^{131} release rates did not measure thyroid function with accuracy because the release rates were not followed by larger differences in thyroxine secretion rates (75).

Factors influencing uptake and release measurements. There has been found pronounced species and ration differences in the amount of I^{131} absorbed by the thyroid gland. The release rate of I^{131} was faster in cattle on high protein intake (40). The release rate of I^{131} was greater for mixed hay and predominantly legume silage than for grass silage (100). Lundgren and Johnson (60) indicated from a review of literature that earlier studies showed thyroid activity and feed intake were influenced by climatic and environmental factors. In three breeds of heifers the I^{131} release declined with an increase of environmental temperature above 80° F (44). There are results which suggest that thyroxine I^{131} disappearance rate from the blood is depressed directly by high environmental and body temperature instead of depressed feed intake (60).

Thyroid activity depends on ambeint temperature. The I^{131} uptake by the gland at 95° F in the rabbit and cow is below that at an air temperature of 50-60° F (6). Thyroidal uptake of radioactive iodine

in rats increases at 0° C and decreases at 34° C as compared to room temperature (50). However, in three breeds of calves the highest uptake was in the summer and the lowest in the spring (55). Younger calves had a higher average uptake than older ones.

Grosvenor (35) showed a depressed thyroid uptake of I^{131} during lactation in rats. In lactating sheep the mammary gland was three times more efficient than the thyroid in removing iodide from the blood (25). Since the mammary gland competes with the thyroid for dietary iodide, thyroxine synthesis may be reduced when iodide intakes are suboptimal which could lower thyroid secretion rate or activity (36).

Grosvenor (36), in reviewing the literature, found that mammary glands of mice, rats, rabbits, dogs, sheep, goats, humans, and cows accumulated variable percentages of injected I^{131} per unit time. The loss of I^{131} into milk of mice, rats, and goats was great enough so that the thyroid uptake was reduced (36). Rats fed a low iodine diet accumulated five times as much I^{131} in the mammary gland as the thyroid (36). Goats (26,79,102) and cows (51,76) have secreted varying amounts of the injected I^{131} into the milk with cows secreting the least. Thus, cattle may not have as great a competition for blood iodide as sheep.

Thyroidal-I^{131} release rate and specific activity of circulating hormone. To make repeated measurements of cows and goats during growth, pregnancy, and lactation methods that do not require sacrificing of the animals are needed (69). Sorensen (89) developed a method using the release rate constant and specific activity of the circulating thyroid hormone to estimate thyroxine secretion rate (TSR). This method permitted a study of the aspects of iodine metabolism and thyroid function

without administering goitrogens or thyroidally active substances to experimental animals (89). The estimated secretion rate of radioactive thyroid hormone was divided by the specific radioactivity of the circulating thyroid hormone and multiplied by the factor .03673 to obtain TSR mg L-thyroxine/day (92). The assumption was made that the specific activities of the thyroid hormone secreted at a given instant and that circulating were equal (92). The indication was that I^{131} uptake, normal release rate, and thiouracil blocking of iodine release rates were not satisfactory measures of actual TSR, that is, as indexes of thyroid activity (75). This method of Sorensen's (89) and the following ones require much time and are restricted by the use of radioisotopes.

Thyroxine substitution and gland weight. The method used extensively in domestic animals is the thyroxine substitution technique. Dempsey and Astwood (19) indicated actual hormone secretion would be determined after blocking thyroid hormone synthesis by thiouracil and then measuring the daily dose of thyroxine required to prevent the enlargement of the thyroid gland. Killing of animals or thyroidectomy is required for gland investigation and is not suitable for large domestic animals. Error may be introduced if there is preformed thyroid hormone in the gland, but with a suitably long treatment period this error is not significant. Goitrogenic drugs may partially inhibit the action of preformed thyroxine but this does not seem to be indicated (19). This substitution method is based on two assumptions, one is that thiouracil completely inhibits the formation of thyroxine, and the other is that the restoration or maintenance of the normal gland weight by the use of thyroxine in thiouracil treated animals is a valid measure of normal

thyroid secretion rate (19). Schultze and Turner (84) determined TSR in lactating goats and cows by using the rate of milk production as an index of the amount of thyroid hormone in the blood. Thyroxine secretion rate was considered equal to the amount of DL-thyroxine (10 mg) required to maintain normal milk flow when administered in conjunction with thiourea or thiouracil. This eliminates the requirement of a larger dose to stimulate depressed milk secretion back to normal than is needed to maintain normal milk flow. This procedure may necessitate consideration of the normal decline in milk production (84).

Thyroxine substitution and blocking of injected I^{131}. A substitution method based on the daily minimum amount of thyroxine administered which completely blocks the release of previously injected I^{131} taken up by the thyroid gland (74) has been used by Henneman, Griffin, and Reineke (38); Lewis, Reineke, and Lodge (54); and Flamboe and Reineke (26) in sheep, dairy cattle, and goats, respectively. The TSR was based on the amount of thyroxine needed to make the gland radioactive count 100 per cent of the previous count (26). Lewis *et al.* (54) used thiouracil to block the recycling of I^{131} while making the usual gland counts. Flamboe and Reineke (26) indicated that the thyroidal I^{131} output was rapid enough so the blocking of I^{131} recycling with thiouracil treatment of goats was not significantly different. In correlating thyroidal I^{131} uptake, per cent of injected dose, and output values ($t_{\frac{1}{2}}$) with thyroxine secretion rate values no significance was found. The former were not considered reliable indexes of thyroid function in goats under normal environmental temperatures (26).

Pipes and Ruppert (71) using the substitution technique administered

L-thyroxine dosage above thyroxine secretion rate with a progressive decline in dose after the maximum concentration of I^{131} in the gland was reached to obtain thyroxine secretion values. These counts were made on the blood plasma. If the purpose is to determine metabolic rate and thyroid activity as affected by environmental temperature the method of using thyroxine to block gland activity when I^{131} is given, is of little use, because thyroxine will alter the metabolism of the animal (69).

Factors influencing thyroxine secretion rate. There are many factors which affect the thyroxine secretion rate (TSR) and other indexes which need to be taken into account to properly evaluate the thyroid status of a given animal and to relate the thyroid activity to other aspects of the animal such as milk production.

Season. In two year old open ewes the mean daily TSR for July was significantly different than any other month studied (39). Premachandra, Pipes, and Turner (75) working with the substitution technique in cattle found the TSR reduced three fold in summer versus winter. The rate of change for TSR has been found to be not directly proportional to the rate of seasonal change under decreasing ambient temperature (75). Thyroid secretion rate in rats was higher at an environmental temperature of 0^{o} C than at 35^{o} C (19).

Pregnancy. Schultze and Turner (84) using the gland weight substitution technique found no particular difference between pregnant and nonpregnant animals in thyroid activity. When age, breed, and month of year was corrected there was no significant differences in the TSR of pregnant sheep (39). In lactating two year old ewes there was higher thyroid output than for bred or open two year olds (39). Similar results

were reported by Flamboe and Reineke (26) for goats; the pregnant ones were lower than the nonpregnant but TSR for the two groups were not significantly different. There was a lower TSR in both aged and young lactating goats than for non-lactating controls of similar age (26).

Age. There was a decline in TSR with increase in age in both sheep (39) and goats (26). The TSR increased with increased body weight in the growing goat. The increase in TSR with increased body weight was more rapid in the younger ones, but apparently as maturity was attained the TSR leveled off (84).

Sex. Slight differences in TSR between mature males and females was indicated but the difference was reversed with the strain of mice studied. If male mice were castrated, after 16 weeks elapsed between castration and TSR measurements, TSR was found to decrease (41).

Turnover of I^{131} and thyroxine secretion rate. Thyroxine degradation (or secretion rate) has been determined in a few days time with the use of I^{131} labeled thyroxine turnover techniques (31,93). Sterling and Chodos (93) and Freinkel and Lewis (31) based the technique on the decline of plasma radioactivity. The turnover rate indicates the fraction of the body extrathyroidal organic iodine (EOI) pool synthesized and degraded daily. The product of EOI times turnover rate gave the rate of formation and degradation, which were identical if a steady state existed and the EOI pool was constant. The calculations depend on the assumption of a steady state (93). Thus thyroxine eliminated from the pool must equal secretion into the pool (42).

Post and Mixner (74) used the above technique to determine thyroxine secretion rate (TSR) in dairy cattle. They used three sources to

represent the turnover of I^{131} labeled L-thyroxine. The disapearance of radioactivity was determined from two sources; whole plasma and precipitated and washed plasma protein. The decline of specific activity was determined from plasma PBI which was the third source. From each turnover curve, separate estimates of TSR were calculated for measuring thyroid activity (74). The third method was based on the rate of dilution of labeled thyroxine by newly synthesized unlabeled hormone (74) with TSR the product of thyroxine pool size (ETT) and thyroxine fractional turnover rate. Post and Mixner (74) indicated that since plasma protein PBI was more representative of plasma thyroxine I^{131} than whole plasma I^{131}, that the former may be a more accurate estimate. Both were considered measures of thyroxine degradation per unit time (74).

Mixner and Lennon (63) proposed a modification which eliminates the need of radioiosotopes, in which the TSR is estimated from the turnover of normal thyroxine. This was based on the decline of total PBI and PBI response (total PBI minus normal PBI or asymptoic PBI levels) (63).

Factors altering methods. Factors which would alter the above methods would be similar to those already discussed concerning rates of release and uptake as well as those influencing PBI since PBI is used in the thyroxine turnover techniques.

Loss of iodine. The loss of iodine in PBI assays can contribute to error in estimates of some of the parameters used in calculating TSR (74). Low normal PBI underestimate extrathyroidal thyroxine pool and thyroxine secretion rate when using the plasma I^{131} and plasma protein I^{131} methods. The volume of distribution is overestimated in the isotope dilution method, but is of small importance since the volume of

distribution is not used in calculating thyroxine secretion rate (74).

Recycling of released I^{131}. As with the estimation of the release and uptake of I^{131} a source of error for the radiothyroxine turnover measurements would be in the recycling of metabolically released I^{131} from the labeled thyroxine (74). Recycling if sufficient would reduce the rate of disappearance from the plasma of radioactivity and at the same time thyroxine fractional turnover rate would be underestimated (74).

Dosage of thyroxine. The chemical turnover method involves non-physiological amounts of thyroxine, but the indication is that a single large dose of normal thyroxine did not alter significantly the turnover rate of radioactive thyroxine, nor did it hinder the accurate measurement of thyroxine turnover and thyroxine secretion rate by the chemical total PBI method (74).

Comparison of methods. Post and Mixner (73) obtained a correlation of 0.87 between the TSR/100 lb. body weight (BW) as determined by the stable and radioactive thyroxine methods. Later they reported work from the stable thyroxine, radioactive thyroxine, and thyroxine inhibition of thyroidal I^{131} release methods (74). The mean daily thyroxine secretion rate in mg/100 lb. BW were not significantly different. This precision of thyroxine secretion rate measurement is considered fairly good (74). Johnston et al. (45) obtained a correlation of -0.11 for the rate constant (hour slope of destruction curve) and PBI. There was a correlation of -0.03 for thyroxine secretion rate and PBI. A correlation of 0.42 for metabolic rate and rate of thyroxine removal from the blood was obtained. Thus, PBI was a questionable index of thyroid function.

Metabolic rate was most closely related to thyroxine secretion rate with a correlation of 0.90 (45). Post and Mixner (74); however, found a significant relationship between thyroid secretion rate and normal PBI when correlating the measures within the radiothyroxine (isotope dilution) and chemical thyroxine (total PBI) turnover methods (74). They adapted the radiothyroxine turnover technique to the turnover of chemical or stable thyroxine and compared the two methods. They also considered the thyroxine substitution technique (74). The radiothyroxine (isotope dilution) method is assumed to be the most accurate in theory since it measures thyroxine secretion rate rather than thyroxine degradation and is not affected by iodine losses in PBI assays. The chemical thyroxine (total PBI) method was found to compare favorably with the isotope dilution method (74).

Possible Pathways of Hormone Metabolism

Six reactions which may occur at various points on the thyroxine molecule transforming it in the body tissues are "deiodination, oxidation of the phenol, conjugation of the phenol, rupture of the diphenyl ether linkage, oxidative deamination of the alanine side chain, and decarboxylation" (72). The main organs of catabolism of thyroxine are the liver and kidneys (98).

Deiodination. Deiodination is considered the most important reaction in the metabolism of thyroid hormones (72). The salivery glands, abdominal organs, and extravascular tissue all seem to have some deiodinating ability for thyroid hormones (29), with the liver and kidney being of great importance (72). The kidney may have a part in converting

thyroxine to the more active form of the hormone since thyroxine was deiodinated to triiodothyronine (TRIT) by kidney slices but not homogenates (97). Rat kidney mitochondria converted thyroxine and TRIT to the acetic acid analogues, tetraiodothyroacetic acid and triiodothyroacetic acid (97). Thyroxine and TRIT conjugate to glucuronides and pass into the intestines with the bile (98). Deiodination was more rapid in hyperthyroid than hypothyroid humans and animals (72).

Oxidation. Oxidation of the phenol of thyronine and some halogenated derivatives of tyrosine can be mediated by the ascorbic acid-Fe++ -O_2 system and by tyrosinase of mushrooms. However, thyroxine is not oxidized by either system (72).

Conjugation. Conjugation of the phenolic group of thyroxine leads to the formation of glucuronide and sulphate esters, the former being the more important (72). This occurrs mostly in the liver with the conjugate being secreted in the bile. The kidney also forms thyroid hormone conjugates. The sulphate ester is formed with triiodothyronine which may be its form of transport to target cells (72). A small amount of conjugated thyroxine is excreted by the kidneys (98).

Biological rupture of the diphenyl ether linkage. The rupture of this linkage in thyroxine would be the reverse of its postulated synthesis by condensation of two diiodotyrosine molecules (72). Reports have indicated detection of small quantities of diiodotyrosine in urine, but they are open for criticism (72). Evidence exists for enzymic splitting of the linkage by mushroom tyrosinase (72).

Oxidative deamination. The occurrence of oxidative deamination on the alanine side chain of iodothyronine is not known in normal animals,

but has been shown in different unphysiological situations. When large doses of labeled thyroxine and triiodothyronine were given to thyroidectomized rats alpha keto acid analogues were found in the bile. However, there is an indication that the acetic acid analogues may be metabolites of endogenous hormones (72). Acetic acid analogues have been detected in rat bile, kidney, muscle, and homogenates of brain tissue and in the kidney and liver of mice (72). A soluble enzyme has been prepared from rat kidney which will catalize oxidative deamination of thyronine and triiodothyronine (97). A pathway through the keto acid and then to the acetic acid by decarboxylation has been postulated (72). The deiodination and phenolic conjugation are quantitatively more important than oxidative deamination (72).

Decarboxylation. This pathway has lead to thyroxamine *in vitro* from thyroxine, but is of questionable physiological significance (72).

Some of the thyroxine, triiodothyronine, and the deaminated metabolites are excreted with the bile in unconjugated form and small amounts of free thyroxine are excreted through the kidneys. Certain metabolites of the hormones may be resorbed from the intestine and circulated through the liver. The iodides produced may be reused by the thyroid or excreted by the kidneys (98). There is very little of the organic forms lost in this manner (98). Urinary iodide is the greatest degradation product of organic iodine compounds and nearly 97% of this recycling and filtered iodide is reabsorbed by the kidney (72). Most of the freed iodine is conserved by the metabolism of the hormones of the thyroid (98).

Thyroxine is bound to serum proteins as well as to proteins of fluids and tissue (72). Thyroxine is bound to pre-albumin which has a

high affinity for thyroxine while the beta and gamma globulins have very little thyroxine binding capacity. However, the physiological significance of this binding to pre-albumin is not known (72). In extravascular fluids such as thoracic duct lymph, femoral lymph, ascites fluid, and joint fluid, the thyroxine-binding protein and albumen are the main proteins binding thyroxine (72).

Tissues other than the thyroid may form thyroxine in mammals (98). Under the proper conditions of incubation, iodine will be taken up by tyrosine in the protein forming diiodotyrosine (DIT) and monoiodotyrosine (MIT). Two DIT or one each of DIT and MIT may couple to form thyroxine or triiodothyronine, respectively (72). Tyrosine is present in tissue and with the proper enzyme oxidizing system thyroxine may be formed in the presence of iodine (98). Thus, the synthesis of thyroxine may be regarded as a biologic reaction; however, the thyroid is a specialized organ of concentration and storage of the hormone (98). There is indication for the presence of a specific thyroxine-binding protein fraction of skeletal muscle in the rat and rabbit, but the binding capacity of the cellular thyroxine binding protein is weaker than serum thyroxine-binding protein rendering cellular thyroxine binding protein more difficult to identify in vascular tissue of the liver and kidney (72).

Physiological Activity of Hormones

The thyroid hormone increases cellular oxidations, energy production or calorigenesis, and oxygen consumption. Calorigenic action is expressed as basal metabolic rate (BMR) which is a measure of heat pro-

duction. After thyroidectomy BMR falls rapidly and may be elevated by thyroid hormone to above normal levels. The acceleration of respiration and oxygen consumption of excised tissues of hyperthyroid animals indicates increased metabolism (72,98). The thyroid hormone is one among several hormones regulating calorigenesis.

In the regulation of body temperature there is an interaction of neural and endocrine factors and the thyroid has an important position. When the environmental temperatures get too low the thyroid hormone stimulates heat production. Metabolic rate has been increased in animals which have been chilled and treated with thyroxine (72).

Protein metabolism. Thyroid hormone may produce either a catabolic or an anabolic effect on protein metabolism depending on the dose level and metabolic state of the animal (72,98). The restoration of nitrogen retention, normal growth rate, liver size, and plasma globulin level, which are deficiencies of hypothyroidism, were brought about by sufficient doses of thyroid hormone (98). Thyroxine has stimulated the growth of most tissues in the absence of the pituitary hormones (98). It has been noted that in hypothyroidism urea excretion was below normal and with replacement therapy the converse was seen (72). Metabolism of creatine and creatinine were altered in both hypothyroidism and hyperthyroidism. Generally, the secretion or administration of excess thyroid hormone has caused creatinuria and increased serum creatine level with a decreased creatinine concentration in urine and blood. The thyroid hormone brought a response in metabolism proportional to dose level (72).

Fat metabolism. Increased serum cholesterol and phospholipid levels

in hypothyroidism may be restored to normal with the administration of thyroxine (98). Cholesterol content of the liver was markedly increased due to thyroidectomy or thiouracil treatment, but there was only a small increase in total fat (72). The content of cholesterol was reduced in the liver during hyperthyroidism. Thyroid hormone increases oxidative processes of the body which may deplete the carbohydrate stores and mobilize fat from the tissue deposits to the liver with an increase in ketone body formation (98). The levels of the various classes of lipids have been shown to vary inversely with thyroid function with cholesterol showing the greatest regularity of response (72). There is an indication for the direct action of the hormone on fat metabolism. The direct action apparently is on lipid synthesis and degradation rather than on fat absorption and digestion (72). The conversion of carbohydrates to fats is concluded to be accelerated by thyroxine treatment (72). Pitt-Rivers and Tata (72) concluded that thyroid hormone accelerated the rate of synthesis, breakdown, and excretion of cholesterol and phospholipids. During thyroid deficiency the increased serum lipid levels are probably due to retarded degradation rather than synthesis (72).

Carbohydrate metabolism. Thyroid hormone accelerated monosaccharide absorption from the alimentary tract (98). Liver stores of glycogen were decreased due to liver glycogenolysis, while the blood sugar levels tended to increase. Hypothyroidism increased liver glycogen without changing the fat content (98). Pitt-Rivers and Tata (72) doubted the physiological role of thyroid hormone in liver glycogen mobilization because of the large doses of hormone which have been

given in studies with cats and dogs.

Vitamin metabolism. Thiamine, riboflavin, nicotinamide, and B_{12} requirements and turnover were increased with hyperthyroidism and decreased with hypothyroidism. This affect may have been secondary to stimulation of general metabolism by thyroid treatment since many vitamins are believed to be co-factors for enzyme systems which are influenced by alteration of metabolic rate (72).

Electrolyte metabolism. Extracellular retention of sodium, chloride, and water and reduced blood volume have been associated with hypothyroidism (98). Diuresis, urinary loss of sodium, and increased plasma volume occurred with administration of thyroid hormone. In the normal individual thyroxine had a diuretic action and potassium was the main electrolyte excreted probably due to mobilization of intracellular fluid. Under hyperthyroid conditions calcium mobilization from the skeleton was increased along with calcium loss through the urine and feces (98).

Activity of the Hormones and Their Analogues

The hormones 3, 5, 3' TRIT and thyroxine are known to posses biological activity; however, TRIT has been found to be seven times more active than thyroxine, and the effect of TRIT is produced in less time (98). Large numbers of hormonal analogues have been prepared which exhibit biologic potencies quite different from the hormones of the gland. Deaminated analogues, tetraiodothyroacetic (TETRAC) acid and triiodothyroacetic (TRIAC) acid have been demonstrated in peripheral tissues, but not in blood (98). Biologically, TETRAC and TRIAC are less active

than their precursors, but do uncouple oxidative phosphorylation in mitochondrial preparations. Triiodothyroacetic acid is weaker than thyroxine and TRIT in preventing goiter and elevating oxygen consumption of the rat, while tissue metabolism is stimulated by TETRAC (98). Triiodothyropropionic acid is 300 times more potent than thyroxine in stimulating metamorphosis in Rana pipien tadpoles (98).

Effects of Hormones and Other Factors on Milk Secretion

Hormones are generally known to have an effect on the intensity of milk secretion and production. However, the results have been variable. When there is an increase due to treatment, the hormone or a combination of the hormone with other factors is considered the limiting factor for milk production.

Thyroid hormone and iodinated casein. Variation in thyroid activity among animals is to be expected and may be due to the genetic make up of the animal or the results of environment or condition. Since thyroxine is the main biologically active thyroid hormone, metabolic processes would be inhibited and milk production could be reduced due to the absence of thyroxine. Sorensen (91) showed by butterfat measurement that thyroid activity influenced the rate of milk production. Flamboe and Reineke (26) showed no increased thyroid activity in lactating as apposed to nonlactating goats. Henneman, Reineke, and Griffin (39) showed an increase in thyroid secretion rate during lactation in sheep. Falconer (25) found no change during lactation for thyroid activity in sheep.

Thyroprotein and thyroid hormone treatment has been reported to stimulate an increase in milk production (68). Snythetic L-thyroxine has given

similar results (77). Relative to thyroprotein feeding, in short term trials, milk production has varied from a decrease of 8% to an increase of 61% (67). Similar results are indicated for long term trials, when fed over the major portion of lactation or successive lactations. Results indicate that under optimal conditions an increase of 10-25% may be expected in milk production over a period of four to ten weeks. The net effect of thyroprotein feeding may be small or nil when prorated over the total lactational period due to decreased persistancy in late lactation (67,68). A summary of the stimulating action of thyroid active materials on milk production (67) agrees with more recent studies (68).

Growth hormone. The galactopoetic effects of growth hormone (GH) have been demonstrated (101). With varying conditions positive results ranging from 8%-50% increase in milk production have been obtained with GH injections (16,18,22,46). The effects of GH continue after discontinuance of the injections (68). In the various experimental trials GH has been administered early in lactations and in others GH has been withheld until the last half or third of the lactation. The data were not sufficient as reported by the National Research Council-Subcommittee on Hormones (68) to allow complete evaluation, but the effects seemed to be equal in early or late lactation.

Estrogens. Research has well established that estrogen administration during established lactation will often inhibit milk secretion, which may be incomplete or only temporary (27). Results indicate that in lactating cows diethylstilbesterol, estrogens in general, and

diethylstilbesterol dipropionate produce a decline in milk yield (28,78). Mixner, Meities, and Turner (65) working with goats in the advanced stages of lactation found the inhibitory effect of diethylstilbesterol to be proportional to the dosage, but inhibition was only temporary and the milk yield often returned to the previous or a higher level following treatment. Browning et al. (11) obtained a slight inconclusive increase in production using diethylstilbesterol on five pairs of identical twin cows. In heifers pre-treated with estrogen and progesterone combinations to induce mammary growth and further estrogen injections to initiate lactation there was an induced lactation comparable to related animals. The related animals were not described (81). When the peak milk production passed the animals received 10 mg of diethylstilbesterol daily for four weeks and this treatment reversed the decline in production. There was a further increase in milk production with four to eight weeks of thyroxine injections (81). A summary of data up to 1952 concerning the use of estrogen to stimulate lactation in cattle and goats indicated that their use could not be recommended because results were too variable and uncertain (68).

Oxytocin. Donker, Koshi, and Petersen (21) working with identical twins treated one of the pair with oxytocin. Comparing the second and third lactations over a 10 week period the experimental one gave approximately two times more milk than the control. However, a trial in which 62 cows were used showed that removal of complimentary milk with weekly use of oxytocin had no discernable effect on milk yield (49).

Lactogenic and Adrenocorticotrophic Hormone. Cotes et al. (18) indicate that purified lactogen (prolactin) was found to have some

insignificant galactopoetic effect. Donker and Petersen (23) indicated that prolactin depressed production in two animals by 50 and 35%. Wrenn and Sykes (101) obtained only slight stimulatory effects of prolactin if any on induced lactation. The indication is for milk production to be depressed with adrenocorticotrophic (ACTH) hormone treatment (68). This depression in milk yield has been shown to be proportional to the level and length of injection (87).

Crude Pituitary Extracts. Asimov and Krouze (1) showed that a crude preparation of the anterior pituitary produced a temporary increase in milk production. With crude pituitary preparation a 9% increase in production has been indicated (68).

When cows were treated with stilbesterol alone they responded to GH, prolactin, and thyroid stimulating hormone with higher yields than those pre-treated with a combination of stilbesterol and progesterone (101). Also, a combination of GH and lactogen gave an increase of 12% in milk yield (68).

Feed factors. There have been variable results from the feeding of concentrates at various prepartum levels on subsequent milk production of dairy cattle. Blaxter (5) and Broster, Ridler, and Foot (8) have obtained positive results while Schmidt and Schultz (83) and Greenhalgh and Gardner (33) have shown negative results. The condition of the animal during the dry period could be a factor in the results obtained.

Mode of Action of Thyroid Hormones

One proposed concept is that thyroxine and related compounds un-

couple oxidative phosphorylation to produce effects on tissues such as reduced rate of adenosine triphosphate synthesis, which would increase the respiratory rate and cause a compensatory increase in oxidative reactions (98). Oxidative phosphorylation may be influenced by thyroid substances which may induce a change in permeability of mitochondrial membranes (98). Due to thyroxine action on metabolic systems, another concept suggests that increased metabolic rate is secondary to the stimulation of energy-requiring reactions (98). Salts of Mn, Ca, Cu, Fe, Co, and Zn chelate with thyroxine and inhibit oxidation. In the liver and other tissues cytochrome oxidase, cytochrome C, and succin-oxidase systems are increased when thyroid hormone is given to the animal (98). Thyroidectomy will generally diminish these enzyme systems, but whether this action is indirect or direct is not known. The mode of thyroid hormone action remains to be clarified in spite of these extensive researches (98).

Goitrogens

Goitrogens interfere with synthesis of thyroid hormones, causing the blood level of thyroxine to fall. This, inturn, enhances the output of thyroid stimulating hormone (TSH) from the pituitary. The thyro-trophic hormone causes hypertrophy of the thyroid tissues (98).

Two major categories of compounds with goitrogenic activity are the thiocarbamide derivatives, such as thiourea, thiouracil, and propyl-thiouracil, and compounds with an amino benzene ring in the structure, such as the sulfa drugs, paraaminobenzoic acid, and paraaminosalicylic acid (98).

Foods such as rape, cabbage, kale, and turnips are active goitrogens and contain 5-vinyl-2-thiooxazolidine (98). The sulfonylureas used in treating diabetus mellitius have similar effects.

One group of antithyroid drugs or compounds are those which block the iodine uptake by the gland, such as thiocyanate and perchlorate (98). These drugs do not block iodination, oxidation, and coupling within the gland cells (98). Another group inhibits the organic binding of iodide once it enters the gland. The sulfonamides and the derivatives of thiourea belong to this group (72,98).

Iodine deficiencies in animals cause goiter, because thyroxine is not synthesized. The low level of thyroxine causes the release of TSH which stimulates the thyroid gland to hyperplasia of the cells and increases the gland weight (98).

EXPERIMENTAL PROCEDURE

Design. This trial was part of a larger experiment designed by Dairy Department personnel; Emery, Snyder, Armstrong, and Hafs to determine the effect of prepartum grain feeding on mammary edema and milk production. The experiment involved Holstein heifers, due to calve after March 1, 1961, and cows which were selected at random. There were two groups, group I with 15 cows and 28 heifers and group II with 15 cows and 26 heifers, with a total of 84 animals. Both groups were fed hay ad libitum before and after parturition and corn silage (30 lb/day) from 5 days to 45 days postpartum. Group I received grain ad libitum from 21 days prepartum to 45 days postpartum while group II received grain ad libitum only after 5 days postpartum. Hereafter, group I and II will be known as prepartum grain and no prepartum grain fed, respectively.

Ration. Corn silage and alfalfa mixed hay were fed with a 14.5% crude protein grain mixture consisting of ground shelled corn, ground oats, soybean meal (50% CP), trace mineral salt, dicalcium phosphate, and molasses. According to the manufacturer, the trace mineral salt contained 0.013% iodine as stearated potassium iodide and contributed a calculated 1.08 g iodine per ton of grain mix. A salt mix also was available with a 1:1 ratio of trace mineral salt to dicalcium phosphate.

Animals. Nine animals were picked at random from the mammary edema experiment for this thyroxine trial. In group I there were four heifers and two cows and in group II there were two heifers and one cow. The cattle ranged in age from approximately 21 months to 42 months. Six

animals were treated in February and March and three in June and July. On the third day following calving each animal was injected via the jugular vein with sodium L-thyroxinepentahydrate* (1.145 gm L-thyroxine-sodiumpentahydrate equivalent to 1.000 gm L-thyroxine free acid). The powder was reconstituted with 200 ml of 10% ethyl alcohol plus 0.05 g $NaCO_3$. Later an equal volume of propylene glycol was added and this rendered the powder fully soluble. Animal 235 received 25 mg of sodium L-thyroxinepentahydrate and animal 220 received 15 mg while all others received 20 mg.

Rectal temperatures. Rectal temperatures were measured before injection and at about 8:30 to 11:00 am and 4:00 to 5:30 pm daily, for a maximum of 225 to 459 hours following thyroxine treatment. Temperatures were taken for only 170 to 180 hours for animals 235, 220, 214, and 663. From 10 to 15 temperatures were taken per animal over a period of 6 to 12 days.

Method for determining thyroxine secretion rate. The turnover rate of chemical thyroxine (total PBI) (74) was used to determine thyroxine secretion and its components. The method has given good results when compared to an isotope dilution method (74) and is desirable where radioisotopes are restricted.

Blood samples. An initial blood sample was taken pre-injection from each animal except heifer 235 for which a sample nine days postpartum was substituted for the pre-injection sample. Postinjection samples were taken on an average of 30, 44, 54 and 72 hours. The samples were collected without an anticoagulant and stored at 3° C for one to four days.

*Supplied by Smith Kline & French Laboratories, Philadelphia, Penn.

Serum was separated from the whole blood by centrifugation and was stored in a deep freeze. The analyses were made after one to nine months of storage.

Analytical procedures. The dry ashing method of Brown, Reingold, and Samson (9) was used, with slight modification, to analyze for plasma PBI. Except for the anhydrous sodium carbonate, which was used to make a chloride solution to be described, all reagents were made without alteration from the above method. Monohydrate sodium carbonate rather than the anhydrous form was used for part of the work; however, the monohydrate was dried at 600° C for 2.5 hours as was the procedure for the anhydrous form. All reagents were made with double distilled deionized water which was also used for the final rinse of the glassware. Brucine sulfate (34) was used to stop the color reaction in all but four analyses. The PBI values were not apparently influenced by brucine. Alkaline incineration for protein-bound iodine was conducted as follows:

I. Pipette 7 ml of double distilled deionized water into each tube.

II. Pipette the following into each tube.

A. Add 1 ml of serum with mixing.

B. Add 1 ml of 10% $ZnSO_4$; mix with glass rod.

C. Add 1 ml of 0.5 N NaOH; mix thoroughly with a glass rod for even distribution of alkali and precipitation of zinc proteinate. Rotate rod to remove material.

III. Allow the precipitate to stand for five minutes.

A. Centrifuge the mixture for ten minutes at 2,000 rpm to sediment protein.

B. Decant and discard the supernatant liquid.

C. Wash the protein three times by centrifuging with 10 ml portions of the distilled water.

IV. Suspend the washed protein in 1 ml of 4 N Na_2CO_3 with glass rod and thoroughly stir.

A. Rinse the rod with 0.5-1.0 ml of the distilled water before removing.

B. Heat the carbonate mixture overnight in a hot air oven at 90-95° C until dried.

C. Place the tubes of dried powder in a muffle furnace.

1. Furnace set at 800° C for half an hour the day before incineration and allowed to cool.

2. Incineration conducted for 2.5 hours at 600° C and samples left in over night to allow for cooling.

V. To residue cooled to room temperature add cautiously exactly 2.5 ml 2 N HCl. Occasionally swirl during a 10 minute period to dissolve iodide from the ash. Avoid excess bubbling. Mix with glass rod to dissolve some of the white residue and to help dissipate H_2S gas.

VI. Add exactly 10 ml of distilled water.

A. Mix thoroughly with the tube contents.

B. Centrifuge contents for 10 minutes at 2,000 rpm to pack insoluble residue (carbon and some silica).

C. Pour the supernatant liquid into clean dry test tubes.

D. Pipette 2, 3, and 5 ml portions of supernatant into dry tubes.

E. To the 2 and 3 ml portions add enough of the chloride solution to make a final volume of 5 ml which maintains the proper chemical composition and concentration of the color reactants.

VII. Treat all 5 ml volumes as follows:

A. Add 0.5 ml of arsenious acid.

B. Hold the reaction mixture at a constant water bath temperature of 37° C.

C. Exactly 0.5 ml of ceric sulfate solution is added at 2 minute intervals.

D. Colorimeter readings are made at 15 minute intervals thereafter for each sample or at this time 0.2 ml of brucine is added on the same time schedule, with the readings made later.

After approximately 20 hours at 95° C the oven temperature was increased to 110 to 120° C to hasten the drying of a few analyses. In a few cases air was dried over sulfuric acid and drawn through the oven but this procedure was not helpful. Protein-bound iodine was not apparently influenced by the drying procedure.

A standard curve was made with each analysis from a series of iodine dilutions ranging from 0.00 to 0.10 ug per 5 ml. The dilutions were prepared from a stock potassium iodide (KI) solution plus a chloride solution

of anhydrous Na_2CO_3, 2 N HCl, and distilled water. The iodine dilutions were refrigerated until further use as was the stock solution.

The ceric ammonium sulfate solution was adjusted to read an OD of 1.0 at a wave length of 420 mu with a blue light in a Beckman Model B Spectrophotometer. The optical density of the ceric solution was found to vary only slightly or none at all.

Iodine dilutions, serum samples, reagent blanks, and serum PBI standards* were read against the 0.00 iodine dilution for the standard curve at step 7d of the dry ashing outline. The iodine dilutions were brought to room temperature before the arsenious acid step. All items to be read received the acid followed by ceric ammonium sulfate. Reagent blanks and standard sources were treated exactly as the serum samples except only 5 ml portions were used. The reagent blanks were included with each analysis while the standards were included for only 13 analyses.

In most analyses the 0.00 iodine dilution which had the highest initial OD (A) was adjusted to an OD of 1.0 which resulted in a new lower OD for the duplicate (B). The rest of the tubes were read against the average of the OD of A and B. In a few analyses the average OD was taken of both A and B without the adjustment whether they were above or below an OD of 1.0 initially. Four times one iodine blank only was used and adjusted to an OD of 1.0 before the rest of the tubes were read. The above adjustments did not appear to influence the PBI values which were obtained. The iodine blank was checked occasionally during the readings and found not to change in value.

*Versatol Supplied by General Diagnostics Division Warner-Chilcott Division, Morris Plains, New Jersey.

Computation of PBI. Optical density values of the iodine dilutions were plotted on the Y axis of coordinate paper with their respective iodine concentrations on the X axis to form the standard curve. From the curve the iodine concentration in ug/5 ml of the serum samples, PBI standards, and reagent blanks were determined and multiplied by 625, 417, and 250 for the 2, 3, and 5 ml serum samples, which converted the PBI concentrations to ug/100 ml of serum. The results for the 2 and 3 ml portions were of similar magnitude as the 5 ml portion of redissolved PBI ash.

Combining of PBI values. In five of the 16 sets of analyses the 2, 3, and 5 ml portions were run for each sampling time while in the other 11 the 5 ml portion was run only for the pre-injection and last sample. The 2 and 3 ml portions of serum were averaged together for a given analysis. Some alterations were made because, generally, the OD readings were used only from the linear portion of the standard curve. Two exceptions to this were animals 234 and 663 which may bias the results somewhat. Only a few 5 ml portions were used and averaged with the other portions because the OD readings were not on the linear portion of the curve. In both sets of analyses for animal 234, PBI from only the 3 ml portions were used because the readings for the 2 ml portions were erratic within and between analyses in comparison to the 3 ml portions. In four animals 214, 707, 235, and 671 there was one set of analysis in which one PBI value for a given time was not available.

The PBI from the two sets of analyses performed for animals 220, 235, 214, 707, 671, and 234 were averaged except for 663 for which only

one set was available. Animals 210 and 229 had three and four sets of analyses of which only one and two sets were usable, respectively. The OD of one set of analysis for 210 were too low on the standard curve. The other set gave erratic readings for the 3 ml portion and there were only 2 values for the 2 ml portion. For 229 one set gave no results while the other set was not comparable with sets three and four which were used. There were 16 usable PBI analysis out of a total of 20. The reagent blank values were subtracted from the proper serum PBI values before preceding with the calculations.

Calculations. Results were calculated according to the method of Freinkel and Lewis (31) as used by Post and Mixner (74). The average PBI values converted to the natural log for each cow and the corresponding sampling time were used to estimate the rate constant or thyroxine turnover rate per hour (b) according to the expression:

$$b = \frac{\Sigma X \ln Y - \frac{\Sigma X \ \Sigma \ln Y}{N}}{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}$$

where the denominator = total sum of squares

numerator = total sum of products

To obtain PBI at zero time (Yo) with theoretically complete mixing of chemical thyroxine, the following expression was used by rearrangement of the least squares equation:

$$\ln Yo = \ln \bar{Y} + b\ (X - \bar{X})$$

where $\ln \bar{Y}$ = the average of the PBI values in natural logs

b = rate constant in hours

X = time of sampling in hours

$\overline{X}$ = average time of sampling in hours

lnYo = PBI at zero time in natural logs

The anti log of lnYo is PBI in ug %.

Time-half was estimated from:

$$t_{\frac{1}{2}} = \frac{.69315}{b}$$

where .69315 = ln of 2

b = rate constant in hours

Thyroxine fractional turnover rate per day (TFTR) was equal to:

$$\text{TFTR/day} = b \times 24 \text{ hours}$$

where b = rate constant in hours

Thyroxine volume of distribution (TVD) in liters was obtained by:

$$\text{TVD(L)} = \frac{\text{Dose of thyroxine mg} \times 100}{\text{Yo}/0.653}$$

where Yo = extrapolated PBI at zero time

0.653 = constant for converting PBI to thyroxine

Daily thyroid secretion rate (TSR) mg was the product of TVD (L), TFTR/day, and normal pre-injection PBI ug % divided by 65.3:

$$\text{TSR mg} = \frac{\text{TFTR} \times \text{TVD} \times \text{PBI}}{65.3}$$

Extrathyroidal thyroxine (ETT) mg was determined as:

$$\text{ETT mg} = \frac{\text{TSR mg}}{\text{TFTR/day}}$$

Due to the zero pre-injection PBI value of animal 671 and the single blood sample available for the other eight animals the post-injection PBI of the eight animals were used to make an estimate of pre-injection PBI. This was done according to the following expression:

$$\ln Yp = \ln Yo - bXp$$

where Yp = the normal pre-injection PBI ug %

Yo = PBI at zero time

b = the rate constant in hours

The equation was solved for, Xp, which was the time in hours at which the PBI had declined to the pre-injection value. The Xp were computed for each Yp for the eight animals. Each Xp was divided by time-half and the eight quotients were averaged. The average was used to compute the estimated pre-injection PBI, $\hat{Y}p$, for each of the eight animals by the expression: $\ln \hat{Y}p = \ln Yo - b\hat{X}p$. Using the average, a computed estimate for animal 671 was obtained for the pre-injection PBI which was substituted for the zero value of this animal. Both the pre-injection and estimated pre-injection PBI values were used to calculate TSR and ETT. The level of probability for a significant difference was used in the analysis of variance according to the methods of Dixon and Massey (20). The sources of variance were prepartum grain versus no prepartum grain and cows versus heifers. The interaction sum of squares and degrees of freedom were combined with the error term because there was only one animal in the cow-no prepartum grain group.

Feed intake and milk production. The feed intake and milk production data for the 84 animals on the edema experiment were expressed as an average per day as were the data for the thyroxine trial. Because three animals in the edema experiment did not receive corn silage the hay and silage intake was expressed on a dry matter basis for all animals. The silage intake was multiplied by 0.333 and the product was

added to the hay intake to obtain total dry matter intake. There were five animals which received high moisture corn in place of grain during the last part of the edema experiment. Thus, the data for these animals were only for the period up to the feeding of the high moisture corn. For several days one animal received grass silage which was divided by four and the resulting dry matter was included with the hay. Three heifers did not have recorded hay weights for the prepartum period of the experiment. Therefore the average prepartum days of the remaining animals of about 17 and 13 days for group I and group II, respectively, were multiplied by the average daily intake for the heifers within the proper treatment group to obtain estimated hay weights for the missing data. In one case the average intake per day for the animal was used to obtain missing hay weights for the first three days postpartum.

The grain intake, roughage intake, and milk production per day were analyzed by analysis of variance. In the thyroxine trial the interaction was treated in the same manner as for the thyroxine data. The degrees of freedom for the three estimated hay weights were not adjusted. Milk weights per day were based on the number of days within the 45 day postpartum period for which experimental data was available. The average of 17 days prepartum grain feeding for group II was added on to the number of postpartum days of group I to properly adjust the grain intake per day against the milk production per day for the two groups. This also was done for the thyroxine trial.

RESULTS AND DISCUSSION

Pre-injection protein-bound iodine. The initial PBI presented in table 1 may be somewhat high; however, the values in literature have been variable. Therefore, the values of this trial may be acceptable. The normal or initial PBI were from 4.03 to 37.60 ug% with an average of 16.27. From the procedure described previously a computed PBI of 12.71 ug% was obtained for cow 671. This value was substituted for the zero initial PBI of the cow. A correlation of 0.73 was determined for the initial and estimated PBI with the computed PBI excluded.

Long et al. (57) have reported a range of 1.8 to 4.0 ug% for Holsteins although the animals had access to iodized salt. Lewis and Ralston (52) have obtained a range of 3.0 to 15.3 and 2.1 to 16.5 for heifers and cows, respectively, of all dairy breeds. Supplementary iodine was not added to the ration. Asplund et al. (2) have observed in Aryshires mean PBI of 21.3 to 26.7 during portions of three successive years.

Potassium iodide (KI) as a source of iodine has not affected PBI when fed at or somewhat above 22 mg/day (58). Following parturition in the present trial the highest average grain intake per day per animal of 31.7 lb supplied about 22.3 mg KI/day. Possibly, the iodine from other sources was not great enough to increase the iodine intake appreciably above the amount (17.1 mg) supplied by this quantity of KI. There seemed to be no relationship between PBI concentration and calculated iodine intake.

Thyroxine volume of distribution. Presented in table 2 are the

Table 1. Pre-injection PBI values

Animal no.	Normal pre-injection PBI[a]	Estimated pre-injection PBI[a]
	ug %	
235H[e]	27.29[d]	32.35
220H	17.02	10.88
214H	16.59	12.74
707H	12.89	20.99
663C	37.60	21.45
210C	6.22	6.97
234H	12.08	13.45
229H	4.03	6.00
671C	12.71[c]	12.71[b]
Mean	16.27	15.28

[a]Correlation 0.73 between normal and estimated PBI, with the value for cow 671 excluded from the calculations.

[b]The computed PBI as described in the experimental procedure.

[c]The computed PBI substituted for the zero PBI of cow 671.

[d]Nine day postinjection PBI sample.

[e]H and C equal Heifer and Cow.

thyroxine volumes of distribution (TVD). The values ranged from 24.21 to 104.33 L with a mean of 51.75. Compared to the other individual values the two highest TVD were from heifer 229 and cow 210 of 104.33 and 89.77 L, respectively. In contrast to values which have been reported most of the individual TVD were somewhat lower. Even though there were individual variations the values may be considered reasonable.

Mixner, Kramer, and Szabo (62) have reported mean TVD of 59.91 and 67.82 L. Post and Mixner (74) have found a mean TVD of 52.4 L and Mixner and Lennon (63) have obtained a mean of 67.81 L.

The TVD may be overestimated or underestimated owing to the extrapolated zero time PBI. Also, the PBI response above a given normal PBI may vary among individual animals receiving an equal dose of thyroxine. The response would indicate a similar variance in zero time PBI. Total zero time PBI (table 2) were from 12.52 to 67.43 ug%. From the data of Mixner and Lennon (64) PBI responses were derived which ranged from 17.54 to 44.33 ug% above a given normal PBI. Thus, varied PBI response may partially account for the differences found in individual zero time PBI of the present trial. Zero time PBI and TVD tended to have an inverse relationship indicating the affect of PBI on TVD.

Thyroxine volume of distribution is the space in which thyroxine is distributed in the body (42). For a given TVD a higher turnover rate would imply a greater volume (L) of TVD whose hormone is replaced daily and vice versa due to degradation and utilization of thyroid hormone.

As presented in table 3 no significant difference of TVD means was attained for prepartum grain versus no prepartum grain or for cows

Table 2. Thyroxine volume distribution, extrathyroidal thyroxine, and zero time PBI

Animal no.	Thyroxine volume distribution	Extrathyroidal[a] thyroxine	Zero time PBI
	L	mg	ug %
		Prepartum grain	
235H[c]	24.21	10.12	67.43
220H	43.19	11.26	22.68
214H	49.18	12.49	26.56
707H	29.95	5.91	43.60
663C	29.16	16.79	44.79
210C	89.77	8.55	14.55
		No prepartum grain	
234H	46.58	8.62	28.04
229H	104.33	6.44	12.52
671C	49.38	9.61[b]	26.45
Mean	51.75	9.98	

[a]The values are based on normal pre-injection PBI.

[b]The value is based on the computed PBI which is substituted for the zero normal PBI of cow 671.

[c]H and C equal Heifer and Cow.

Table 3. Mean thyroxine volume distribution and extrathyroidal thyroxine

Treatment	No.	Thyroxine volume distribution	Extrathyroidal[a] thyroxine	Extrathyroidal[b] thyroxine
		L	mg	mg
Heifers	6	49.57	9.14	9.60
Cows	3	56.10	11.65	9.59
Prepartum no grain	3	66.76	8.22	9.60
Prepartum grain	6	44.24	10.85	9.59
Heifers no prepartum grain	2	75.45	7.53	9.60
Heifers prepartum grain	4	36.63	9.94	9.60
Cows no prepartum grain	1	49.38	9.61	9.61
Cows prepartum grain	2	59.46	12.67	9.58
All treatments	9	51.75	9.98	9.57

[a]Values are based on the normal pre-injection PBI.

[b]Values are based on the estimated pre-injection PBI.

versus heifers.

Extrathyroidal thyroxine. Extrathyroidal thyroxine (ETT) values are presented in table 2. The range was from 5.91 to 16.79 mg, with a mean of 9.98. Of the values obtained the highest and the lowest ETT were 16.79 mg for cow 663 and 5.91 mg for heifer 707, respectively. The individual extrathyroidal thyroxine values were approximately three times greater than those in the literature.

Mixner et al. (62) have demonstrated mean ETT of 2.83 and 2.80 mg. Post and Mixner (74) have observed a mean ETT of 4.10 mg.

Contributing to the higher ETT values were the higher initial PBI obtained. The variation from the mean ETT was small; about 4 to 7 mg. The small variation was attributed to the various combinations of the two variables, initial PBI and TVD.

The extrathyroidal thyroxine pool is contained within the TVD (42). The thyroid hormone is degraded from this pool at various rates to be utilized by tissues and metabolic systems of the body. The ETT pool of the present trial may be indicative of a greater than "normal" thyroxine requirement. However, this was not supported by the thyroxine fractional turnover rate and thyroxine secretion rate to be discussed.

The mean ETT values in table 3 are listed by age of animal and grain treatment. No significance was found for ETT based on normal or initial PBI.

Rate constants. The rate constant is the proportion of the ETT leaving the pool each hour. Thyroxine turnover rate constants/hour (b) are listed in table 4. The mean was 0.0169 and the highest and lowest values were 0.0022 from cow 671 and 0.0735 from heifer 234, respectively.

Table 5 shows the rate constant means for age of animal and grain treatment. There were no significant differences for prepartum grain versus no prepartum grain or for cows versus heifers.

Thyroxine fractional turnover rates. Thyroxine fractional turnover rates/day (TFTR) are presented in table 4. The range for TFTR was from 0.053 to 1.765 with a mean of 0.406. Thyroxine fractional turnover rates for cow 671 and heifer 234 were the highest and lowest, respectively. Generally, the mean and individual values were lower than the values which have been reported.

Mixner et al. (62) have reported mean TFTR of 0.593 and 0.655. Mixner and Lennon (63) obtained a mean TFTR of 0.676. Post and Mixner (74) have indicated a mean rate of 0.369.

In comparison to the other individual TFTR obtained, the highest value of 1.765 may be interpreted as meaning greater hormonal utilization for metabolic processes such as milk synthesis. The highest TFTR may be indicative of an active thyroid. Lower individual TFTR indicate possibly decreased hormone utilization.

The mean TFTR are presented in table 5 by age of animal and grain treatment. Thyroxine turnover appeared to be faster in cows than in heifers and faster in the absence of grain than in its presence ($P<0.25$). However, each of these differences can be attributed to the one cow (671), with the highest TFTR, in the no prepartum grain group. Further support was given to the possible influence of this cow when considering the mean TFTR of 0.296 for heifers on prepartum grain which was not significantly different than the mean of 0.174 for heifers on no prepartum

Table 4. Thyroxine turnover rate, thyroxine fractional turnover rate, and time-half

Animal no.	Thyroxine turnover rate/hr	Thyroxine fractional turnover rate/day	Time-half hr
		Prepartum Grain	
235H[a]	0.0179	0.429	38.8
220H	0.0058	0.139	119.9
214H	0.0075	0.179	92.9
707H	0.0182	0.438	38.0
663C	0.0044	0.106	156.5
210C	0.0103	0.248	67.2
		No Prepartum Grain	
234H	0.0022	0.053	314.6
229H	0.0122	0.294	56.7
671C	0.0735	1.765	9.4
Mean	0.0169	0.406	99.3

[a]H and C equal Heifer and Cow.

Table 5. Mean thyroxine turnover rate, thyroxine fractional turnover rate, and time-half

Treatment	No.	Thyroxine turnover rate/hr	Thyroxine fractional turnover rate/day	Time-half hr
Heifers	6	0.0106[a]	0.255[c]	110.1
Cows	3	0.0294[a]	0.706[c]	77.7
Prepartum no grain	3	0.0293[b]	0.704[d]	126.9
Prepartum grain	6	0.0107[b]	0.257[d]	85.5
Heifers no prepartum grain	2	0.0072	0.174	185.6
Heifers prepartum grain	4	0.0123	0.296	72.4
Cows no prepartum grain	1	0.0735	1.765	9.4
Cows prepartum grain	2	0.0074	0.177	111.8
All treatments	9	0.0169	0.406	99.3

[a,b,c,d]Values with common letter may differ ($P<0.25$).

grain. However, the mean TFTR of 0.706 for cows was not significantly greater than the value of 0.255 for the heifers. Furthermore, the difference was not significant between TFTR of 0.704 for no prepartum grain and 0.257 for prepartum grain treatments. No positive relationship was indicated between thyroid activity and either prepartum grain feeding or age of animal.

Time-half. The time-half values are shown in table 4. An average of 99.3 hours and a range of 9.4 to 314.6 hours was obtained for the disappearance of injected thyroxine. The highest and lowest values were for heifer 234 and cow 671, respectively. In this trial a reverse order existed between the rate constant and time-half values. This result was to be expected because of the relationship; time-half = .69315 ÷ b. These time-half values were found to be somewhat higher than those which have been reported.

Post and Mixner (74) obtained a mean time-half of 43.3 hours; however, the mean time-half of the present trial was approximately 2.29 times greater. The greater time-half was attributed to the rate constants per hour which were lower since the TFTR per day in the present trial were lower than reported values.

A decreased utilization of thyroid hormone was suggested by the time-half values obtained. Time-half gives only an indication of thyroid activity in comparison to another animal or physiological state rather than expressing activity in quantitative terms. As shown in table 5, no significant differences were found for time-half means for prepartum grain versus no prepartum grain and heifers versus cows.

Thyroxine secretion rates. The results for individual thyroxine secretion rates (TSR) mg/day are shown in table 6. A range from 0.46 to 16.96, with a mean of 3.77 mg/day was observed. Compared to values reported in the literature the values of this trial may be considered acceptable except for the highest and lowest TSR.

Mixner and Lennon (63) have reported a mean TSR/day of 2.52 mg. Mixner et al. (62) have obtained lower mean TSR of 1.62 and 1.76 mg. A similar mean TSR of 1.59 was reported by Post and Mixner (74). Sorensen (89) presented TSR with a range from 1.0 to 11.3 mg with a mean of 4.7.*

Thyroxine secretion rate was influenced by the extrathyroidal thyroxine (ETT) and TFTR. In this trial the combinations of the higher ETT and lower TFTR resulted in similar individual thyroxine secretion rates.

The highest TSR of 16.96 was from cow 671 and the lowest TSR of 0.46 was from heifer 234. The highest and lowest TFTR were in the same order for the two animals. Extrathyroidal thyroxine pools were similar for both animals. Thus, the TSR obtained for the cow and heifer were influenced by the TFTR. In comparison to the other TSR of the trial the highest TSR may be indicative of a more active thyroid. The greater activity may be considered to be a response to the requirement caused by higher utilization of thyroid hormone. From the individual values thyroid activity seemed to be about "normal" when compared with values which have been reported.

The TSR based on the normal pre-injection PBI are listed in table 7. The mean TSR were 2.44 for prepartum grain, 6.44 for no prepartum grain,

*The mean was calculated from only seven of eight values given.

Table 6. Total daily thyroxine secretion rate

Animal no.	Thyroxine secretion[a] rate	Thyroxine secretion[b] rate
	mg	
	Prepartum Grain	
235H[d]	4.34	5.14
220H	1.56	1.00
214H	2.24	1.72
707H	2.59	4.22
663C	1.78	1.02
210C	2.12	2.38
	No Prepartum Grain	
234H	0.46	0.51
229H	1.89	2.82
671C	16.96[c]	16.96[c]
Mean	3.77	3.97

[a]Values based on normal pre-injection PBI.

[b]Values based on estimated pre-injection PBI.

[c]Computed PBI used to calculate the value.

[d]H and C equal Heifer and Cow.

Table 7. Mean total daily thyroxine secretion rate

Treatment	No.	Thyroxine secretion[a] rate	Thyroxine secretion[b] rate
		mg	
Heifers	6	2.18[c]	2.57
Cows	3	6.96[c]	6.78
Prepartum no grain	3	6.44	6.76
Prepartum grain	6	2.44	2.58
Heifers no prepartum grain	2	1.18	1.66
Heifers prepartum grain	4	2.68	3.02
Cows no prepartum grain	1	16.96	16.96
Cows prepartum grain	2	1.95	1.70
All treatments	9	3.77	3.97

[a]Values are based on the normal pre-injection PBI.

[b]Values are based on the estimated pre-injection PBI.

[c]Values may differ ($P<0.25$).

2.18 for heifers and 6.96 for cows. No significant differences were found between the mean thyroxine secretion rates for prepartum grain versus no prepartum grain and cows versus heifers. The thyroxine secretion rate appeared to be faster in cows than in heifers ($P<0.25$) and faster in the absence of grain than in its presence. As for TFTR, each of these differences can be attributed to the one cow (671) in the no prepartum grain group. An apparent faster rate was indicated for the heifers on prepartum grain than for those on no prepartum grain ($P<0.25$). This difference can be attributed to one high rate of 4.34 mg in the presence of grain and to one low rate of 0.46 mg in the absence of grain. Nevertheless, the mean values for heifers-prepartum grain versus heifers-no prepartum grain reflected the effect of cow 671. There was no significant difference and no apparent differences ($P>0.25$) for TSR mg per day per 100 lb body weight based on the normal PBI. No positive relationship was shown between thyroid activity and either prepartum grain feeding or age of animal.

Milk production and feed intake. The thyroxine trial means for the animal and grain groups for daily milk production, grain intake, and roughage intake are presented in table 8. Average milk production was 59.2 lb for cows versus 49.1 lb for heifers and 53.7 lb for prepartum grain versus 50.0 lb for no prepartum grain. The differences were not significant, but the heifers versus cows differed slightly ($P<0.25$). The means for roughage intake per day were not significantly different for prepartum grain versus no prepartum grain or for cows versus heifers. Cows consumed 24.5 lb of grain and heifers consumed 18.5 lb of grain.

Table 8. Mean roughage intake, grain intake, and milk yield for thyroxine trial

Treatment	No.	Roughage[a] intake	Grain[b] intake	Milk[c] yield
		lb/day		
Heifers	6	12.1	18.5[d]	49.1[f]
Cows	3	13.8	24.5[d]	59.2[f]
Prepartum no grain	3	13.7	17.3[e]	50.0
Prepartum grain	6	12.2	22.1[e]	53.7
Heifers no prepartum grain	2	12.1	16.5	49.7
Heifers prepartum grain	4	12.1	19.5	48.8
Cows no prepartum grain	1	16.9	18.9	50.8
Cows prepartum grain	2	12.3	27.3	63.5
All treatments	9	12.7	20.5	52.5

[a]On a per day basis silage as roughage is figured for the total postpartum period. Hay is figured for total period of the trial.

[b]Grain is figured for total period of the trial.

[c]Yield for which data was available during 45 days postpartum.

[d]Significant difference ($P<0.05$).

[e]Values may differ ($P<0.10$).

[f]Values may differ ($P<0.25$).

This difference was significant ($P<0.05$). Average grain intake of 22.1 lb and 17.3 lb for prepartum grain and no prepartum grain, respectively, may differ ($P<0.10$).

Similar data are presented in table 9 for the edema experiment. Milk production was significantly greater ($P<0.05$) for cows (65.3 lb) than for heifers (44.7 lb), whereas no significant difference was found between the production for prepartum grain (53.0 lb) and no prepartum grain (51.1 lb). The grain intake for cows (22.3 lb) was significantly greater ($P<0.05$) than the intake for heifers (19.0 lb). Average consumption of grain was 23.9 lb and 19.0 lb for prepartum grain and no prepartum grain, respectively. The difference was significant ($P<0.05$). The difference between grain treatments was greater than the difference between cows and heifers. Roughage intake was slightly greater for the cows than for the heifers. The animals in the no prepartum grain group consumed significantly greater ($P<0.05$) quantities of roughage than those in the prepartum grain group. The greater intake of roughage for the no prepartum grain group was to be expected since the grain intake was less than for the prepartum grain group and vice versa.

The trial and the experiment were qualitatively similar since the cows outproduced the heifers. Also, the three week prepartum grain treatment for both the experiment and the trial probably increased production about 2 lb/day above the no prepartum grain treatment, although it was not a significant increase.

Schmidt and Schultz (83), Greenhalgh and Gardner (33), and Castle and Watson (13) reported no significant difference in milk production

Table 9. Mean roughage intake, grain intake, and milk yield for edema experiment

Treatment	No.	Roughage[a] intake	Grain[b] intake	Milk[c] yield
		lb/day		
Heifers	54	14.5	19.0[d]	44.7[f]
Cows	30	16.3	22.3[d]	65.3[f]
Prepartum no grain	41	16.8[g]	16.2[e]	51.1
Prepartum grain	43	13.5[g]	23.9[e]	53.0
Heifers no prepartum grain	26	16.2	15.3	43.0
Heifers prepartum grain	28	13.0	22.4	46.4
Cows no prepartum grain	15	17.9	18.0	65.2
Cows prepartum grain	15	14.6	26.6	65.3
All treatments	84	15.1	20.2	52.1

[a,b,c]See footnote (a,b,c), respectively, table 8.

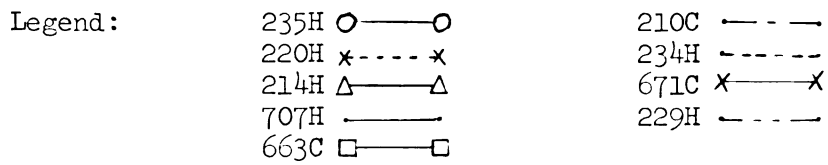
[d,e,f,g]Values with common letter significantly different ($P<0.05$).

when comparing various levels of prepartum grain feeding for periods of five to eight weeks in length. However, Blaxter (5) reported positive results in production between two groups when one group received grain for six weeks prepartum. Factors possibly influencing milk production are presented in table 1 and 2 of the appendix.

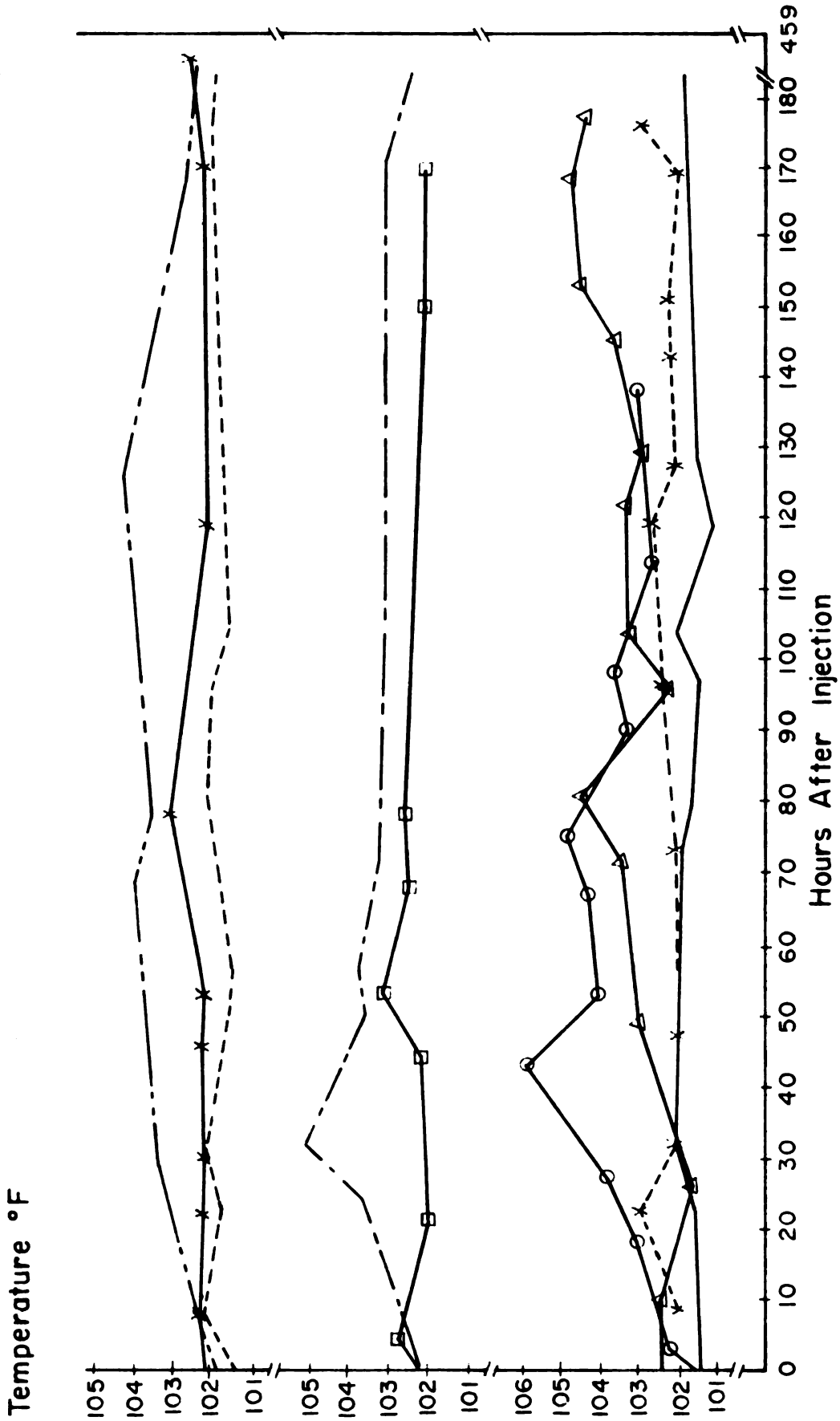
After comparing TFTR and TSR, with milk production and grain intake for cows versus heifers and prepartum grain versus no prepartum grain, no positive evidence was found for a change of thyroid activity associated with prepartum grain treatment and age of animal as reflected in milk production. A more sensitive technique may be required to detect significant differences in such a trial as was conducted. Also, an abnormal glandular state may be manifested following stress of parturition. These factors may have complicated the results of the trial.

Body temperatures. Animals in the present trial showed body temperatures (Fig. 1) above the normal range from 100.4 to 102.8° F (24). During a 43.5 hour interval immediately following the injection of thyroxine, the smallest heifer in the trial (235) showed an uniform rise in body temperature from 101.5 to 105.8° F. The heifer weighed 964 lb and received the largest L-thyroxine injection of 25 mg. The injection and body weight together may have contributed to the 43.5 hour postinjection temperatures observed. A further complicating factor associated with the peak temperature was an udder inflamation manifested during the eight-hour period following the fever. About 40 to 50 hours following the udder inflamation there was a decline from 105.8 to 103.2° F. The combination of body weight, thyroxine dose, and udder inflamation seemed

Figure 1. Body temperature by hours after thyroxine injection.



The H and C represent heifers and cows, respectively. For animals 707, 210, 234, 671, and 229 the temperatures taken after approximately 180 hours are plotted in the 180-459 hour portion of the temperature graph. The five temperatures represent three, six, four, six, and six temperatures, respectively, for the five animals.


Temperature °F
105
104
103
102
101
105
104
103
102
101
106
105
104
103
102
101
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
459
Hours After Injection

to have prolonged the higher temperature of the heifer. Cow 210 showed a steady increase in body temperature from 102.2 to 105.1° F. The period of temperature rise began soon after the injection of 20 mg thyroxine and reached the peak temperature in 32 hours. Udder inflamation may have been involved, although it was not apparent until about 17 hours following the highest temperature. The temperatures remained somewhat above 103.0° F throughout the period. Consistantly high temperatures above 102.8° F ranged from 103.4 to 104.2° F for heifer 229. The temperature continued from about 30 to 125 hours following the thyroxine injection. An explanation for the temperatures above 103.0° F for 210 and 229 may be the high environmental temperatures during the first of August. There was considerable fluctuation of temperatures from approximately 72 to 175 hours following injection for cow 214. No explanation was found for the range in temperature from 101.6 to 104.0° F.

Cows 671, 210 and heifer 229 were injected in July and August (Summer) while the remaining animals were injected in February and March (Winter). The observed temperatures above the normal range of 100.4 to 102.8° F occurred during July, August, February, and March but were not apparently influenced by the seasonal temperature. However, there were not sufficient numbers of animals within a season to accurately evaluate a seasonal effect. Average temperatures for all animals up to approximately 180 hours following injection ranged from 101.7° F for heifer 707 to 103.5° F for heifer 235. The trend for the temperatures to be above the normal average of 101.5° F may be due to thyroxine treatment.

Thyroxine and thyroprotein have influenced body temperature. Over

an eleven week period cows treated with thyroprotein averaged 0.68° F and ranged from 0.2 to 1.2° F higher than the controls (85). Extended hot periods of 96° F affected body temperature when iodinated casein has been fed (32). The controls had an average temperature of 102.3° F in contrast to 105.0° F for thyroprotein treated cows. Cows treated in summer with L-thyroxine showed a gradual rise from the preliminary period temperature (77). However, the elevated temperatures in the experimental cows have been very small and the fluctuations have been comparable in both treated and nontreated cows (77).

Protein-bound iodine standard. Two lots of Versatol (serum standard) were analyzed. The PBI analysis of the serum standards are in table 5 of the appendix. The coefficient of variation was 28.4% and the SE was 0.623 ug% (88).

SUMMARY

Thyroxine secretion rates were measured in nine animals (six heifers and three cows) to determine what relationship there was between thyroid activity and both prepartum grain intake and age of animal as reflected in milk production. These nine animals were a random sample from an experimental population of 93 Holsteins which was designed to determine the effect of prepartum grain feeding on mammary edema and milk production. Milk production for 45 days postpartum, roughage intake, and grain intake for the remaining 84 animals (30 cows and 54 heifers) was compared with that of the nine animals.

In both the trial and the experiment, group I and II were fed alfalfa mixed hay ad libitum before and after parturition and corn silage (30 lb/day) from 5 days to 45 days postpartum. Group I received grain ad libitum from 21 days prepartum to 45 days postpartum while group II received grain ad libitum only after 5 days postpartum. The grain ration was a standard 14.5% crude protein mix calculated to contain 1.08 g iodine per ton.

Each of the nine animals were injected with sodium L-thyroxine-pentahydrate on the third day following calving. Seven animals received 20 mg injections while one received 15 and another received 25 mg. Rectal temperatures were taken both pre-injection and postinjection. A pre-injection blood sample was taken with postinjection samples taken

on an average of 30, 44, 54, and 72 hours. Serum protein-bound iodine (PBI) was measured to determine the turnover rate of chemical thyroxine from which thyroxine secretion rate (TSR) was computed.

Pre-injection PBI ranged from 4.03 to 37.60 ug%, with an average of 16.27 for the nine animals at 3 days postpartum. These values did not appear to be related to iodine intake. There was a range from 24.21 to 104.33 L, with an average of 51.75 for thyroxine volume of distribution. The extrathyroidal thyroxine (ETT) pool ranged from 5.91 to 16.79 mg, with a mean of 9.98. Individual values were about three times higher than has been reported. The normal PBI may have contributed to the higher ETT pool. Time-half for disappearance of injected thyroxine averaged 99.3 hours, which was considerably above reported values. Thyroxine fractional turnover rate (TFTR), the proportion of the extrathyroidal thyroxine pool leaving per day, ranged from 0.053 to 1.765, with a mean of 0.406. Thyroxine secretion rate mg/day ranged from 0.46 to 16.96, with a mean of 3.77. There was no significant difference found for cows versus heifers or for prepartum grain versus no prepartum grain for thyroxine volume of distribution, extrathyroidal thyroxine, time-half, TFTR, and TSR. However, there appeared to be a difference for TFTR and TSR which can be attributed to the one cow in the no prepartum grain group. This cow had the highest TSR and TFTR of all the animals.

For those animals only on the edema experiment milk production was significantly greater ($P < 0.05$) for cows than for heifers. Grain consumed in the edema experiment was greater ($P < 0.05$) for cows than for heifers

and for the animals on prepartum grain than for those on no prepartum grain in the edema experiment. For the same comparisons in the thyroxine trial the difference was significant ($P<0.05$) only for cows versus heifers. No significant difference was found for milk production for either age of animal or grain treatment. Both the milk production and grain consumption may be due to the small number of animals in the trial. In both the experiment and the trial the three week prepartum grain treatment seemed to increase milk production by 2 lb/day above no prepartum grain feeding; however, the difference was not significant. Also, the cows outproduced the heifers in the experiment and the trial. The milk production for age of animal and grain treatment indicate a similar response for both the trial and experiment.

Concerning a change in thyroid activity and the probable relationship to grain treatment and age of animal as reflected in milk production, no conclusion may be made from the data compared on the basis of cows versus heifers and prepartum grain versus no prepartum grain.

The blood turnover of chemical thyroxine may not be a sensitive enough technique for such a trial as was conducted. Also, the glandular system is probably in an abnormal state following the stress of parturition. These factors may have complicated the results somewhat.

Body temperatures of the cows and heifers were consistantly above the normal average for cows of 101.5° F. Thyroxine treatment seemed to have an effect on the temperatures. Temperatures were the highest for cow 210 and heifer 235 of 105.1 and 105.8° F, respectively. Udder inflamation was implicated with the temperature rise in both animals. However, light body weight and a large thyroxine dose (25 mg) were added

complicating factors for cow 235. The trend for heifer 229 and cow 210 to remain above 103° F may have been due to the high environmental temperatures during August. Seasonal variation was not detectable from the results of this trial.

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APPENDAGES

Table 1. Factors possibly influencing milk production for edema experiment

	Number	Mastitis	Milk fever	Uterine inflamation	Retained placenta	Heat	POP[a]	Off feed
Heifers	54	16	0	16	1	4	4	2
Cows	30	15	2	5	2	0	0	2
Prepartum no grain	41	14	0	12	1	1	1	1
Prepartum grain	43	17	2	9	2	3	3	3
Heifers no grain	26[b]	6	0	9	0	1	1	1
Heifers grain	28[c]	10	0	7	1	3	3	1
Cows no grain	15	8	0	3	1	0	0	0
Cows grain	15[d,e]	7	2	2	1	0	0	2
All treatments	84	31	2	21	3	4	4	4

[a]Purified oxytocin principle.

[b]One vaginal laceration and one torn vagina with a pulled calf.

[c]One vaginal laceration with a dead calf.

[d]One malfunctioning milk meter and one inflamation of kidney.

[e]One dead calf.

Table 2. Factors possibly influencing milk production for thyroxine trial

	Number	Mastitis	Milk fever	Uterine inflamation	Retained placenta	Heat	POP[a]	Off feed
Heifers	6	1	0	2	0	0	1	0
Cows	3	1	0	0	0	0	0	1
Prepartum no grain	3	0	0	1	0	0	0	0
Prepartum grain	6	2	0	1	0	0	1	1
Heifers no grain	2	0	0	1	0	0	0	0
Heifers grain	4	1	0	1	0	0	1	0
Cows no grain	1	0	0	0	0	0	0	0
Cows grain	2	1	0	0	0	0	0	1
All treatments	9	2	0	2	0	0	1	1

[a]Purified oxytocin principle

Table 3. Total daily thyroxine secretion rate per 100 lb body weight

Animal no.	Thyroxine secretion[a] rate	Thyroxine secretion[b] rate
	mg/100 lb BW	
	Prepartum Grain	
235H[d]	0.4502	0.5337
220H	0.1315	0.0840
214H	0.2007	0.1414
707H	0.2397	0.3905
663C	0.1156	0.0659
210C	0.1626	0.1822
	No Prepartum Grain	
234H	0.0455	0.0506
229H	0.1658	0.2468
671C	1.0669[c]	1.0669[c]
Mean	0.2865	0.3069

[a]Values based on normal pre-injection PBI.

[b]Values based on estimated pre-injection PBI.

[c]Computed PBI used to calculate this value.

[d]H and C equal Heifer and Cow.

Table 4. Mean total daily thyroxine secretion rate per 100 lb body weight

Treatment	No.	Thyroxine secretion[a] rate	Thyroxine secretion[b] rate
		mg/100 lb BW	
Heifers	6	0.2056	0.2412
Cows	3	0.4484	0.4383
Prepartum no grain	3	0.4261	0.4548
Prepartum grain	6	0.2167	0.2329
Heifers no prepartum grain	2	0.1057	0.1487
Heifers prepartum grain	4	0.2555	0.2874
Cows no prepartum grain	1	1.0669	1.0669
Cows prepartum grain	2	0.1391	0.1240
All treatments	9	0.2865	0.3069

[a]Values are based on normal pre-injection PBI.

[b]Values are based on estimated pre-injection PBI.

Table 5. Protein-bound iodine of serum standards[a]

Lot 1[b]		Lot 2[b]	
	ug %		
	2.00	6.95	
	6.40	8.83	
	4.86	8.64	
	3.01		
Total	16.27	24.42	40.69
Mean	4.07	8.14	5.81

	Sum Squares	df	Mean Squares
Lot 1 versus Lot 2	28.43	1	28.43
Within	13.60	5	2.72
Total	42.03	6	

$F_{.95}(1,5) = 6.61$ $F = 10.45$

SD = $\sqrt{2.72} = 1.65$ SE = $1.65/\sqrt{7}$

Coef. vari. = $1.65 \div 5.81 = 28.4\%$ $1.65 \div 2.646 = 0.623$ ug%

[a]Versatol supplied by General Diagnostics Division Warner-Chilcott Division, Morris Plains, New Jersey.

[b]Two lots, 1 and 2, with indicated PBI of 7.2 and 6.3 ug%, respectively, were analyzed. The PBI obtained are shown.