ABSTRACT

THYROID FUNCTION IN THE LACTATING RAT, COW, AND EWE

By

Fritz L. Lorscheider

The author had previously demonstrated, using a direct-output method, that thyroid hormone secretion rate (TSR) was markedly reduced in the rat during lactation. This was postulated to be due to a relative iodine deficiency resulting from the large losses of iodine occurring in milk. Inasmuch as this TSR method was not adaptable in other than small laboratory animals, other means were sought to further substantiate observations on thyroid function as related to lactation in rats and dairy cattle, to the nonbreeding cow and heifer, and to lactation, phenothiazine treatment, and wool growth in sheep. Thyroid function was measured by a competitive protein-binding technique (Tetrasorb-125 method) which directly measures the serum thyroxine (T_4) level.

A comparison of the serum T_4 method and TSR by the direct-output method in nonlactating and lactating rats established that both methods detected a greater than 50% decrease in thyroid function by 16 days lactation. In another experiment

using heavily lactating rats suckling 12 pups each, serum T_4 at 16 days lactation was reduced to one-half that of non-pregnant, nonlactating controls. Addition of 10 times the usual amount of iodine to the diet of another group of lactating rats suckling 12 pups did not effect the serum T_4 level. In the same experiment lactating rats suckling 9, 6 and 3 pups had serum T_4 levels that were inversely correlated to lactational intensity. Serum T_4 levels remained unchanged in nonlactating rats when exogenous ovine prolactin was administered. High serum prolactin, associated with nursing, did not appear to be a direct contributing factor to the reduction in serum T_4 observed during lactation.

Holstein cows showed no change in serum T_4 during pregnancy when compared to dry-open cows. At peak lactation high producing cows had serum T_4 values that were only 50% those of dry-open and dry-pregnant cows. When milk production had declined to 28 lbs per day, at about 5 months postpartum, serum T_4 levels returned to those of dry-open or dry-pregnant cows. In cows diagnosed as nonbreeders or as having cystic ovaries, serum T_4 was markedly reduced. Nonbreeding heifers showed no reduction in serum T_4. Normal heifers had higher serum T_4 levels than those of normal adult cows.

Suffolk ewes, like Holstein cows, exhibited no change in serum T_4 during pregnancy. At 30 days lactation both Suffolk and Hampshire ewes showed a significant reduction in serum T_4 when compared with dry-pregnant or yearling ewes.

Lactating Hampshire ewes developed a considerable amount of wool slippage. Regrowth of wool was greatly enhanced and serum T_4 levels were significantly elevated to those of dry controls when 0.5 g of thyroprotein was supplied daily in the grain ration. Removal of phenothiazine from the diet of lactating Hampshire ewes resulted in an increase in serum T_4 levels to those of dry-open controls. It appeared that both phenothiazine and lactation may contribute to a reduction of serum T_4 in the ewe and could confound the problem of wool loss observed during advanced pregnancy and lactation.

It was concluded that thyroid function, as determined by serum T_4 assay, was significantly reduced in the heavily lactating rat and Holstein cow. It was postulated that the primary reason for the reduction in serum T_4 during lactation is the diversion of iodine to the mammary glands. A significant reduction in serum T_4 in cows, but not in heifers, was associated with nonbreeding and cystic ovaries. The reduction in serum T_4 in the lactating Suffolk and Hampshire ewes may have been due in part to phenothiazine normally supplied as an anthelmintic in the diet. Wool loss occurring in Hampshire ewes during late pregnancy and lactation, appeared to be associated with a reduction in serum T_4 and was believed to be due in part to lactation and in part to phenothiazine treatment. After wool slippage the regrowth of wool was greatly enhanced and the serum T_4 was elevated when thyroprotein was added to the diet.

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INTRODUCTION

Studies in many species, ranging from rat to man, have revealed that a considerable amount of iodide is secreted in milk. As a result of this implied thyroid-mammary competition for iodine it had been proposed that intense lactation might result in a functional thyroid deficiency (Flamboe and Reineke, 1959). The author has previously demonstrated that rat mammary secretion of I^{131} is of such magnitude that thyroidal I^{131} uptake is reduced by as much as eighty-six percent during lactation. These studies have also shown that the rat thyroid hormone secretion rate (TSR), as evaluated by a "direct-output" method, is markedly reduced, supposedly due to the mammary diversion of iodide with the onset of lactation (Lorscheider, 1967).

Other investigators had previously suggested that thyroid activity is increased during lactation (Turner and Cupps, 1939; Monroe and Turner, 1946; Brown-Grant, 1956; Grosvenor and Turner, 1958a), but many earlier reports have established that thyroprotein feeding increases lactational performance (Graham, 1934; Reineke and Turner, 1942; Reineke, 1943). The latter implies that lactation might be self-limiting due in part to a functional deficiency of thyroid

hormone. In women either thyroid hormone or iodine therapy has resulted in an increase in lactational performance of up to three-hundred percent (Robinson, 1947a; Robinson, 1947b; Roche et al., 1950; Miller, 1951; Romani, 1951; Miller, 1952). Wool growth, cystic ovaries, and phenothiazine treatment have also been shown to be associated with changes in thyroid function (Simpson, 1924; Reineke and Soliman, 1953; Talmage et al., 1954).

Since application of the "direct-output" method is not as yet practical in other than small laboratory animals, other means were sought in the present study to further substantiate observations on thyroid function as related to lactation in rats and dairy cattle, to the nonbreeding cow and heifer, and to lactation, phenothiazine treatment, and wool growth in sheep. To this end a competitive protein-binding technique was employed which directly measures the serum thyroxine (T_4) level. The principal analytical material used was the commercially available Tetrasorb-125 diagnostic kit.[1] This technique was applied in the present investigations instead of the more widely used protein-bound iodine (PBI) or butanol-extractable iodine (BEI) procedures which are less specific, subject to iodine contamination and influenced by the level of iodine from the diet ingested previously by the animal.

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[1]Abbott Radiopharmaceuticals, North Chicago, Illinois.

LITERATURE REVIEW

Many investigations, beginning with that of Courrier et al., (1949), have revealed that a considerable amount of iodide is secreted in milk. Subsequent reports have shown this to occur in many species including man (Honour et al., 1952; Noble and Rowlands, 1953), cows (Lengeman et al., 1955; Lengeman and Swanson, 1957), goats (Wright et al., 1955; Flamboe and Reineke, 1959; Reineke, 1961), dogs (Van Middlesworth et al., 1953; Van Middlesworth et al., 1954), rabbits (Brown-Grant, 1956; Brown-Grant, 1957; Brown-Grant and Galton, 1958), rats (Potter and Chaikoff, 1956; Potter et al., 1959; Grosvenor, 1960), and mice (Rugh, 1951).

As reviewed by Brown-Grant (1961), in man, dogs, rabbits, guinea pigs, rats and mice the milk:plasma ratio of I^{131} is 20-40 within a few hours following administration of the label and in the cow, goat and sheep the ratio is 2-3. In the goat, rabbit and several other species this milk:plasma I^{131} ratio is rapidly reduced with thiocyanate treatment (Brown-Grant, 1961; Reineke, 1961).

Iodine in the mammae of most species is present both as free iodide and as monoiodotyrosine in peptide linkage with milk protein (Potter and Chaikoff, 1956; Brown-Grant,

1961, Reineke, 1963). Apparently this organic binding of iodide in the mammae does not play a significant part in the process of iodine concentration since thiouracil does not prevent the establishment or maintenance of a high milk:plasma ratio of I^{131} even though organification is inhibited. In fact, the organic binding occurs after milk has been secreted by the alveolar cells (Brown-Grant, 1961).

Secretion of I^{131} by the functioning mammae is of such magnitude that the maternal thyroidal accumulation of administered I^{131} is decreased by 86% in the rat (Lorscheider, 1967). Other reports have indicated similar decreases of up to 50% (Brown-Grant, 1956; Iino and Greer, 1961). This mammary diversion of iodine first led Flamboe and Reineke (1959) to propose that under conditions of limited iodine supply a functional thyroid deficiency could result during lactation. The apparent increase in thyroid activity near the onset or during lactation as suggested by others (Turner and Cupps, 1939; Monroe and Turner, 1946; Brown-Grant, 1956; Grosvenor and Turner 1958a) and the lack of any specific need for increased thyroid hormone during nursing as concluded by Iino and Greer (1961) were of special interest in view of the well-established fact from many earlier reports that desiccated thyroid and thyroprotein feeding increases lactation (Graham, 1934; Reineke and Turner, 1942; Reineke, 1943). The latter investigations imply that lactation might be self-limiting due in part to a deficiency of thyroid hormone.

To elucidate the status of the thyroid during lactation, thyroid hormone secretion rate (TSR) was determined in both lactating and nonlactating rats (Lorscheider and Reineke, 1966; Lorscheider, 1967) by the thyroxine (T_4) substitution procedure (Reineke and Singh, 1955) and "direct-output" method (Reineke and Lorscheider, 1967). Reineke and Lorscheider (1967) showed that in nonlactating rats receiving adequate dietary iodine (1 μg I/g feed), TSR values determined by the two methods were not significantly different. In iodine deficient states, however, there was a large difference between results obtained by the two methods. The T_4 substitution method failed to detect differences in TSR of rats receiving two different basal diets ranging from deficient to adequate in their iodine content. By the "direct-output" method there was a high positive correlation between iodine intake and TSR, with values leveling off when iodine intake was adequate. These results were interpreted to show: (1) that the T_4 substitution method measures the demand for thyroid hormone, but represents true TSR only if iodine supplies are adequate, and (2) that the "direct-output" method is more applicable in cases where iodine supply is likely to be a limiting factor on thyroid hormone production.

Lorscheider and Reineke (1966) and Lorscheider (1967), using lactating rats on various levels of iodine intake, demonstrated that the apparent TSR by the T_4 substitution method was significantly greater than that of controls.

This finding confirmed that of Grosvenor and Turner (1958a) who employed a similar method, and their interpretation was that TSR is elevated in the rat during lactation. But by the recently developed "direct-output" method (Lorscheider and Reineke, 1966; Lorscheider, 1967) it was reported that TSR is significantly reduced during lactation even when iodine is supplied at a level considered adequate for non-lactating rats. Thus employment of the "direct-output" method revealed the actual TSR to be significantly reduced when there was either a nutritional or physiological limitation, such as lactation, on the amount of iodine available to the thyroid.

In view of these findings in the rat, and since adaptation of the "direct-output" method is not practical in species other than small laboratory animals, the recently developed Tetrasorb-125 method (Abbott Radiopharmaceuticals, North Chicago, Illinois) was employed in the present study to directly measure serum T_4. This method was favored over the more widely used protein-bound iodine (PBI) determination (Barker, 1948; Barker and Humphrey, 1950) and butanol-extractable iodine (BEI) procedure (Taurog and Chaikoff, 1948). Acland (1957) has questioned the specificity of the PBI serum precipitation step in that no discrimination is made between different iodothyronines. In some cases diiodotyrosine is also precipitated. Furthermore, several investigators (Man and Peters, 1950; Barker et al., 1951;

Benotti and Benotti, 1963) have indicated that therapy with iodine-containing compounds, which may combine with serum protein, will yield spuriously high results by the PBI determination. And the PBI may remain elevated for a period of years. Although the BEI procedure has proven somewhat more satisfactory in separating organic from inorganic iodine of plasma, this method, too, can present artifactual high values due to extraction of non-thyroxine organic iodine (Man et al., 1951). Thus PBI and BEI analyses are at best only rough estimates of thyroid function.

The development of the Tetrasorb-125 method began with the introduction of a "saturation-analysis" technique by Ekins (1960) for measuring total serum T_4. Thyroxine was extracted from serum in acid-butanol, and by electrophoretic techniques a measurement was made of the globulin-to-albumin shift of T_4 caused by the addition of exogenous tracer T_4. Murphy (1964) and Murphy and Pattee (1964) simplified Ekin's procedure which they termed "competitive protein binding". By this method T_4 was removed from serum with a single ethanol extraction. Serum T_4 was estimated by its effect on the passage of a standard labeled T_4-serum solution through a gel-filtration column.

The principle of "competitive protein binding" is derived from the fact that there is only a small amount of thyroxine-binding globulin (TBG) in plasma. Thus the TBG binding sites can be readily saturated by small amounts of exogeneous T_4.

Addition of trace amounts of T_4-I^{131} enables calculation of the protein-bound fraction of isotope. The more unlabeled T_4 which is added to the system, the greater is the decrease in TBG-bound T_4-I^{131} since both labeled and unlabeled T_4 compete for the same binding sites. If a serum extract of unknown T_4 is substituted for purified T_4 of a known amount, the unknown T_4 may be measured by the displacement of bound T_4-I^{131} from TBG. Murphy and Pattee (1964) provided a Sephadex polymer gel to serve as a binding site for the displaced T_4-I^{131}. They also used a barbital buffer and diluted standard serum to reduce binding of T_4 to prealbumin and albumin respectively. Subsequently, Murphy and Jachan (1965) further simplified their procedure by replacing the Sephadex polymer gel with a loose anion exchange resin. Murphy et al. (1966) concluded that the intact T_4 molecule is measured thus yielding determinations unaffected by iodine, and that the serum T_4 is clinically reliable when compared to normal human PBI values. Furthermore, Kaplan (1966) demonstrated that T_4-I^{125} could be bound to a TBG solution for several weeks prior to a determination. Based on the foregoing studies Abbott Laboratories in 1967 introduced the Tetrasorb-125 method which included resin impregnated sponges and a standard T_4-I^{125} TBG solution. In its present form this analysis requires an ethanol extract of serum T_4, which during a fixed incubation time and temperature, will compete with and displace a quantity of T_4-I^{125} from a prelabeled standard TBG

solution. The displaced labeled T_4 is subsequently bound to an anion resin sponge.

The potential adaptability of this serum T_4 assay procedure to sub-primate species in the present investigations, necessitated a comparison of thyroid function in lactating rats by the "direct-output" and Tetrasorb-125 methods. A preliminary report (Lorscheider, et al., 1969) indicated that lactating rats with reduced thyroid secretion rate (TSR) also showed reduced serum T_4. It was of interest to determine the relationship between serum T_4 and lactational intensity in the rat and to see if a euthyroid function could be maintained during lactation by excess iodine supplemented in the diet. Miller (1951) and Miller (1952) had shown that a high iodine intake could increase lactational performance up to 300% in women, presumably by increasing thyroid function enough for unsupplemented feeding of young (Robinson, 1947b). In view of recent suggestions that prolactin may inhibit thyroid activity (MacLeod et al., 1966; Gona, 1967; MacLeod and Abad, 1968), the effects of prolactin on thyroid function were also investigated.

Several studies have been conducted in attempts to interpret PBI determinations in dairy cattle (Long et al., 1952; Lennon and Mixner, 1959; Kossila, 1967). As these results have been somewhat inconclusive, the Tetrasorb-125 method was employed in Holstein cows to determine the status of serum T_4 during various stages of lactation. Reineke and

Soliman (1953) reported that hypothyroidism in mice resulted in their ovaries being filled with large follicles but having an absence of corpora lutea. It was of interest therefore to examine the serum T_4 status in cows diagnosed as nonbreeders or as having cystic ovaries.

Previous measurements of thyroid function in sheep have included such indices as TSR by the T_4 substitution method, thyroid histological evaluations, and thyroid uptake and output of I^{131} (Henneman et al., 1955; Hoersch et al., 1961; Griffin et al., 1962). Henneman et al. (1955) reported elevated T_4 substitution values in lactating sheep. Since the substitution method was thought to reflect apparent TSR, or thyroid hormone demand (Lorscheider, 1967; Lorscheider and Reineke, 1970), the Tetrasorb-125 method was employed in lactating Hampsire and Suffolk ewes to further assess thyroid function. In the present study serum T_4 was also investigated in lactating sheep with respect to thyroprotein and phenothiazine treatments.

It was shown many years ago (Simpson, 1924) that thyroidectomy in sheep resulted in marked wool slippage. Subsequently other investigators have confirmed that reduced thyroid function in sheep decreases wool growth and causes an alopecia-like condition (Maqsood, 1950; Ferguson et al., 1956). Attempts were made to apply T_4 therapy in the form of subcutaneous pellet implants. However, these trials met with but moderate success in reestablishing wool growth in

thyroidectomized sheep although in some animals wool length increased up to 25%. The main problems encountered were uncontrolled hormone absorption rates and the impracticality of adapting this technique to range management conditions (Ferguson et al., 1956; Ferguson, 1958; Theriez and Rougeot, 1962).

Over the past two decades a number of agricultural experiment stations and ranchers have noted an increased incidence of "spontaneous" wool slippage (apparently undocumented) in Shropshire and Southdown flocks and to a lesser extent in Hampshire flocks. In fact, this alopecia-like condition, of unknown etiology, has been partially responsible for the discontinuation of the Shropshire breed in the Michigan State University flocks. An investigation not reported herein, but currently underway at the Fred and George Buckham flock near Kalamazoo, Michigan, shows significant wool slippage in nearly all Shropshire ewes. The interesting facet of this condition is that wool slippage occurs near or at the onset of lambing (Jan.-Feb.) and disappears several weeks after weaning. As wool shearing is usually done in late fall and late spring, wool production is greatly impaired. Since this alopecia occurs in lactating Hampshire ewes to a significant degree, their thyroid function was examined by the Tetrasorb-125 method. Trials were also undertaken to supplement thyroactive casein in the diet to determine its effects on wool slippage.

Phenothiazines and their derivatives have found general application in both veterinary and human clinics as anthelmintics and as tranquilizers. One of the more common phenothiazines, thiodiphenylamine, which is used in sheep flocks, has been reported to be goitrogenic in rats (Talmage et al., 1953). This same phenothiazine was subsequently shown to be goitrogenic in sheep (Talmage et al., 1954). After further investigations it was concluded that some unknown component or metabolite of phenothiazine was responsible for at least partially depressing thyroid function (Nachimson et al., 1954; Wasserman et al., 1956). Phenothiazines may confound the problem of wool loss in lactating sheep, and in view of this possibility, a sheep trial was set up to assess the effects of phenothiazine on serum T_4.

MATERIALS AND METHODS

I. Rat Experiments

In all experiments to be reported, female Sprague-Dawley rats (Spartan Research Animals Inc., Haslett, Mich.) were used. The pre-breeding weights were 200-220 g. Animal room temperature was maintained at 76 ± 2°F and lights were on 14 hours daily.

The diet comprised two-thirds corn and one-third soybean oil meal (50% protein) plus complete vitamin and mineral salt premix supplements (Appendix A), except that KI was added at the laboratory in the amount of 1.0 μg I per g of feed. This diet was prepared at the Michigan State University Department of Animal Husbandry feed mixing plant. The diet had previously been established as providing an adequate iodine intake for nonlactating rats (Reineke and Lorscheider, 1967). Rats receiving this diet without iodine supplementation develop enlarged thyroids within two weeks. In one experiment the amount of KI added was increased to 10.0 μg I per g of feed in one group of rats.

For lactating rats whose thyroid secretion rate (TSR) was measured by the "direct-output" method, the procedure

used was that described for nonlactating rats by Reineke and Lorscheider (1967) except that after 16 days on a prescribed diet, carrier-free I^{131} was injected 2 days postpartum. But to approximate equivalent labeling of the thyroid in all groups it was necessary to administer 3 μCi of I^{131} to nonlactating rats and 8 μCi to lactating rats. TSR was evaluated from 5-15 days postpartum. Pups from certain large litters were distributed between the lactating rats to equalize the number of pups in all litters.

Protein-bound iodine (PBI) determinations were performed by an adaptation of the alkaline ashing method of Barker and Humphrey (1950). Protein was precipitated from 1.0 ml of serum by adding 1.0 ml volumes of 10% $ZnSO_4$ and 0.5 N NaOH. The latter two reagents were matched by titration with phenolphthalein as the indicator. The precipitate was washed with glass-distilled H_2O 3 times. One ml of 4 N Na_2CO_3 was added, and the mixture was dried overnight at 110° C. It was then ashed in a muffle furnace for 2½ hours at 605° C. The ash was dissolved with 2 ml 2 N HCl and 2 ml 7 N H_2SO_4 and diluted with H_2O to 11.0 ml. The timed reaction with arsenious acid and 0.38% ceric ammonium sulfate was run for exactly 15 minutes at 37° C and then stopped with brucine sulfate (Faulkner et al., 1961). Colorimetric readings were taken with a Coleman spectrophotometer (model 6/35) at 480 mμ, set for 100% transmission (T) with a distilled-water blank. Iodine content was read from a curve prepared under

identical conditions in which net percent transmittance was plotted against iodine concentration (Lennon and Mixner, 1957). Iodine values were corrected for an average 11% loss occurring during ashing. In all reagents, washings and dilutions, glass distilled water was used.

In the prolactin study nonlactating rats received subcutaneous injections of either 0.9% NaCl solution or 1.0 mg of NIH-P-S8 ovine prolactin (28.38 I.U./mg) twice daily for 14 days. Prolactin was dissolved in 0.9% NaCl solution to a concentration of 10 mg/cc.

Blood was collected by heart puncture after 14-16 days lactation in all experiments. Samples were placed in siliconized test tubes to prevent hemolysis. After 3 hours the blood was centrifuged at 2000 r.p.m. (1000 x G) for 20 minutes. In most instances enough blood was obtained from each rat to permit 3-4 ml of serum to be collected. Serum thyroxine (T_4) analyses were carried out as described later.

II. Cow Experiments

All animals used in these experiments were Holstein heifers or cows from the Michigan State University dairy herd. The lighting and environmental temperature for the animals were similar throughout the herd. Cows were confined to the barn except for about 1 hour daily. They were fed mixed alfalfa hay and corn silage, grain, and mineral-salt supplements as listed in Appendix B. The grain and mineral-salt

mixes were prepared at the Michigan State University Department of Animal Husbandry feed mixing plant.

Recording of milk weights began 1 day after calving, and milking was twice daily at 12 hour intervals. Duration of lactation periods averaged 305 days. Control and experimental groups were selected as nearly as possible on a basis of similar stages of gestation, lactation and milk yield. Some cows and heifers suspected or diagnosed as nonbreeders were also sampled for T_4 determinations.

Blood samples were withdrawn from the ventral coccygeal vein into 15 ml B-D Vacutainer tubes. All sampling was done during the period from October, 1968 to March, 1969. Serum for T_4 analysis was collected as described for rats.

III. Ewe Experiments

All sheep used in these experiments were Suffolk or Hampshire ewes from the Michigan State University sheep flocks. Animals ranged from yearlings to 5-year-olds. The ewes were confined to barn feeding and the temperature and lighting environment for the animals was similar throughout the flock. The sheep were fed alfalfa hay, grain, and a mineral-salt mix described in Appendix C. The grain and salt mixes were prepared at the Michigan State University Department of Animal Husbandry feed mixing plant. In one experiment phenothiazine was withdrawn from the salt mixture following lambing for an average of 31 days. In another experiment thyroprotein

(Protamone, Agri-Tech, Inc., Kansas City, Mo.) was added to the grain ration 4-6 days after lambing to provide 0.5 g Protamone per ewe per day (Appendix H). Ewes assigned to each control and experimental group were selected as nearly as possible on a basis of similar stages of gestation, lactation, and degree of wool slippage.

Blood samples were withdrawn from the jugular vein into 15 ml B-D Vacutainer tubes. All sampling was done in November, 1968 or the end of January, 1969 except for the phenothiazine trials where blood was taken in May, 1969. Serum was collected as described for rats and PBI determinations for some Hampshire ewes were carried out as described for rats.

IV. Serum Thyroxine (T_4) Analysis

1) Procedure

The Tetrasorb-125 method (Abbott Radiopharmaceuticals, North Chicago, Illinois) was employed, with certain modifications, for the serum T_4 analyses. The procedure was as follows: Blood serum samples were frozen and stored for periods up to several months. At the time of assay 1 ml of serum was taken from the samples which had been thawed and brought to room temperature. Two ml of 95% ethanol were added and the solution was mixed immediately by using a Vortex mixer for 30 seconds. To facilitate maximal T_4 extraction the resultant denatured protein precipitate was allowed to

stand at room temperature for 10 minutes. The mixture was then centrifuged at 2000 r.p.m. (1000 x G) for 20 minutes. Three-tenths ml of the alcoholic extract from the serum was placed in polypropylene test tubes, and while being warmed in a water bath (35-40° C) the extracts were evaporated to dryness by a steady mild air stream. After complete drying, 1.0 ml of thyroxine-binding globulin (TBG)-T_4-I^{125} solution was added to each tube and allowed to equilibrate with the dried extracted thyroxine for 10 minutes at room tepmerature. The tubes were then placed in an ice-filled Dubnoff shaker (0.5-2.0° C) for 5 minutes. At 20 second intervals one resin-impregnated sponge was placed successively in each tube and saturated by lightly depressing each sponge 5 times, using the special Abbott plunger.

The initial radioactivity count (I) of the total labeled quantity of TBG-T_4-I^{125} was obtained for each tube during the incubation period 30 minutes after adding each sponge. At exactly 1 hour of incubation the reaction was stopped in successive tubes at 20 second intervals by adding 10 ml of distilled water, depressing the sponge 5 times with the aspirator tube and immediately drawing off the solution. The sponge was washed 3 more times with about 10 ml portions of distilled water to remove residual labeled TBG. The final radioactivity count (F) was taken after 4 washes. A scintillation well-type counter (Nuclear Chicago, Model DS-5), and analyzer/scaler (Nuclear Chicago, Model 8725) was employed

for the radioactivity measurements. The percentage uptake of T_4-I^{125} on resin sponges was obtained using the formula:

$$\% \text{ Resin sponge uptake} = \frac{F \text{ (cpm)} - \text{background (cpm)}}{I \text{ (cpm)} - \text{background (cpm)}} \times 100$$

The F and I counts were measured under constant geometric conditions.

2) Standard Curves

A standard T_4 curve was run with the determinations done on any one day. The primary standard was crystalline free thyroxine purified by Dr. E. P. Reineke from monosodium thyroxine pentahydrate obtained from Baxter Laboratories, Morton Grove, Ill. The free thyroxine showed only a single component when checked by thin layer chromatography. The standard stock solution was prepared by dissolving exactly 10 mg of the purified T_4 in 95% ethanol with the aid of a minimum of 0.1 N NaOH solution and diluting with ethanol to a concentration of 5 μg per ml. Concentration of the working standard was 0.05 μg per ml. When kept refrigerated this could be used 2-3 months without deterioration. A series of standards of varying concentrations was set up for each standard curve and carried through the same procedure as the alcoholic serum extracts, as already described.

Because a 0.3 ml aliquot of the supernatant alcoholic serum extract was evaporated to dryness, the T_4 in the dried sample was one-tenth of the amount in 1 ml of serum, or

1/1,000 of the amount in 100 ml serum. Based on this ratio, the amount of working standard solution for the standard curve was computed by the following calculation, and expressed as T_4 in μg/100 ml:

$$\frac{T}{1{,}000 \times C} = V \text{ (ml)}$$

Where,

T = the T_4 equivalent of the serum extract expressed as μg per 100 ml.

C = concentration of standard solution (0.05 μg/ml)

V = volume of standard solution

When T_4, expressed as μg/100 ml, is plotted on the abscissa of linear coordinate graph paper against percent I^{125} count remaining in the resin sponge, a standard curve can be plotted. In our pilot experiments it was found that in nearly all of the species studied the serum T_4 concentration did not exceed 12 μg/100 ml. In the range 0-12 μg T_4 per 100 ml a perfectly linear relationship between T_4 concentration and resin sponge count was found to exist. Thus, instead of reading values from a plotted standard curve, the T_4 standard data were fitted by the method of least squares, and the serum T_4 values were calculated by use of the regression equation. The calculations are as follows:

$$X_u = \frac{Y - a}{b}$$

X_u = Serum T_4 (μg/100 ml) uncorrected for recovery

Y = percent resin-sponge uptake

a = intercept of the Y-axis

b = slope of the standard curve

In setting up this method we found a 77.3% efficiency for extraction of T_4 from serum with 95% ethanol.

The extraction efficiency was determined by mixing 1 ml of rat serum with 0.02 ml T_4-I^{131} (Abbott Laboratories). After 27 hours equilibration at -0.5° C, the first radioactivity measurement was obtained in the well counter. Two ml of 95% ethanol were added and the solution was treated as described previously for serum samples. The second count was obtained from 1 ml of the alcoholic extract, and the percent extraction efficiency was calculated from the difference between these counts.

To obtain true T_4 values, the results had to be corrected for extraction efficiency, as follows:

$$X_c = X_u \times \frac{100}{77.3}$$

X_c = Serum T_4 (μg/100 ml) corrected for recovery. All equations and calculations for both the serum assay procedure and standard curves were programmed on an Olivetti-Underwood desk computer (Model 101).

RESULTS AND DISCUSSION

I. Rat Experiments

A comparison of rat thyroid function as measured by the Tetrasorb-125 and direct-output methods is illustrated in Figure 1. During lactation thyroid secretion rate (TSR) falls from 2.58 to 0.99 μg T_4/100 g body wt./day ($p < .001$) and serum thyroxine (T_4) is reduced from 3.94 to 1.96 μg/100 ml ($p < .001$). Thus both methods show a significant reduction of more than 50% in the thyroid function of lactating rats. It is apparent that reduced TSR by the direct-output method will manifest itself in reduced serum T_4 by day 15 or 16 postpartum, a time estimated to be peak lactation in the rat. Individual serum T_4 values for Figure 1 are presented in Appendix D. It is interesting to note the serum T_4 value of 3.43 μg/100 ml for lactating rat no. 6. This value, which is significantly above serum T_4 levels for the other lactating rats, may be explained by the fact that on the third day postpartum 5 pups were eaten by the mother and were not replaced with foster pups. A 5/12 reduction in lactational intensity coupled with the additional source of iodine provided by the 5 pups would account for a higher serum T_4.

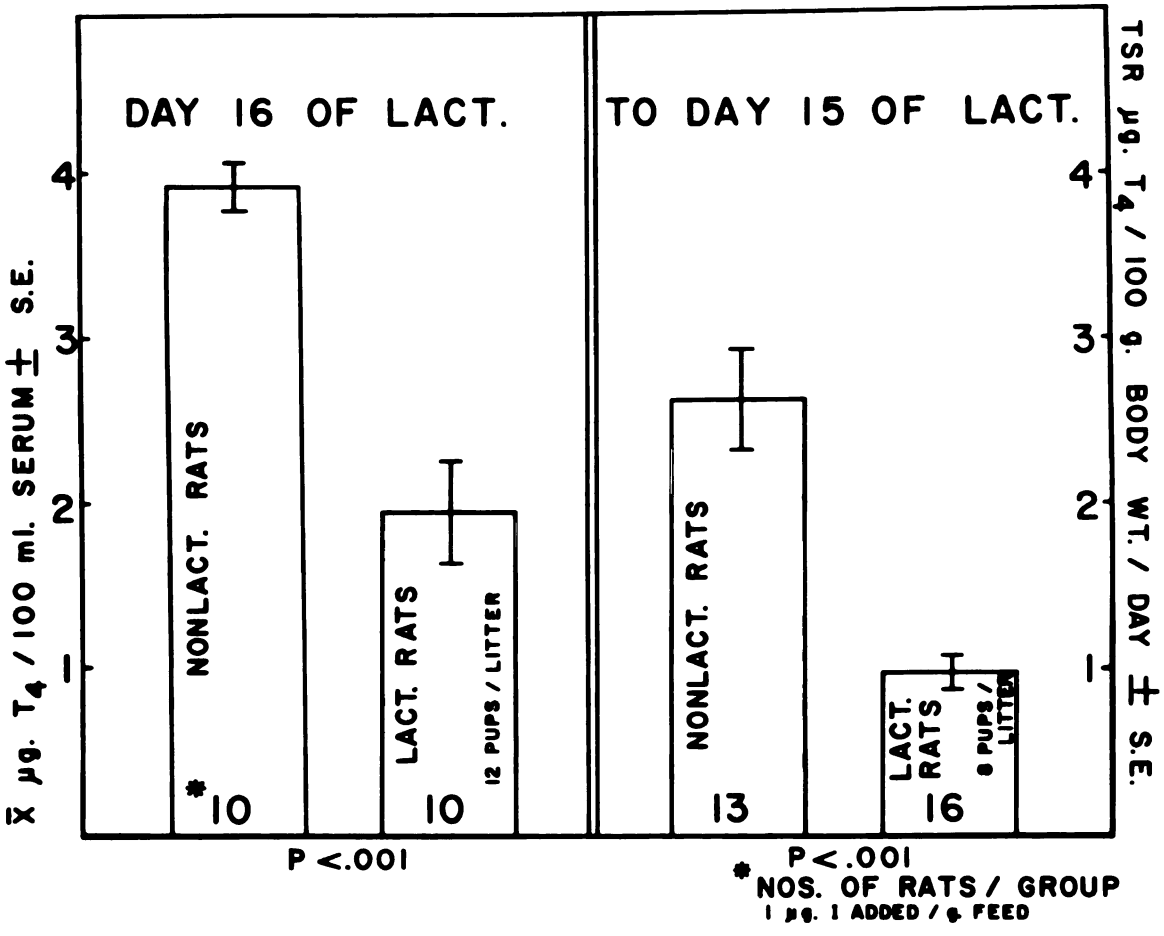


Figure 1. Comparison of serum thyroxine method and thyroid secretion rate by the direct-output method in nonlactating and lactating rats.

These data by the Tetrasorb-125 method confirm the previous interpretation by Lorscheider and Reineke (1966), Reineke and Lorscheider (1967) and Lorscheider and Reineke (1970), that the T_4 substitution method as employed by Grosvenor and Turner (1958a) cannot detect a decrease in thyroid hormone production during lactation.

Figure 2 illustrates levels of serum T_4 in the rat at several levels of lactational intensity and elevated iodine intake. Individual serum values are listed in Appendix E. It can be seen in groups A, B, C, and D, that as the number of pups per litter is increased from 0 to 3, 6, and 12, respectively, serum T_4 is significantly reduced. A high inverse correlation exists between serum T_4 and lactational intensity ($r = -0.83$, $p < .001$). Even with only 3 pups per litter the serum T_4 of a nursing rat is significantly reduced ($p < .05$) below that of nonlactating controls.

The normal level of dietary iodine necessary for euthyroid function in a nonlactating rat is 1.0 μg per g feed (Reineke and Lorscheider, 1967). When 10 times this amount of iodine is added to the diet of lactating rats (Group E, Figure 2) there is no significant increase in serum T_4 when compared to that of similar rats receiving 1.0 μg of I per g feed (Group D). Previous studies in women have indicated that iodine therapy could be used in the treatment of inadequate lactation (Robinson, 1947b; Miller, 1951; Miller, 1952). Presumably the supplemented iodine was sufficient to

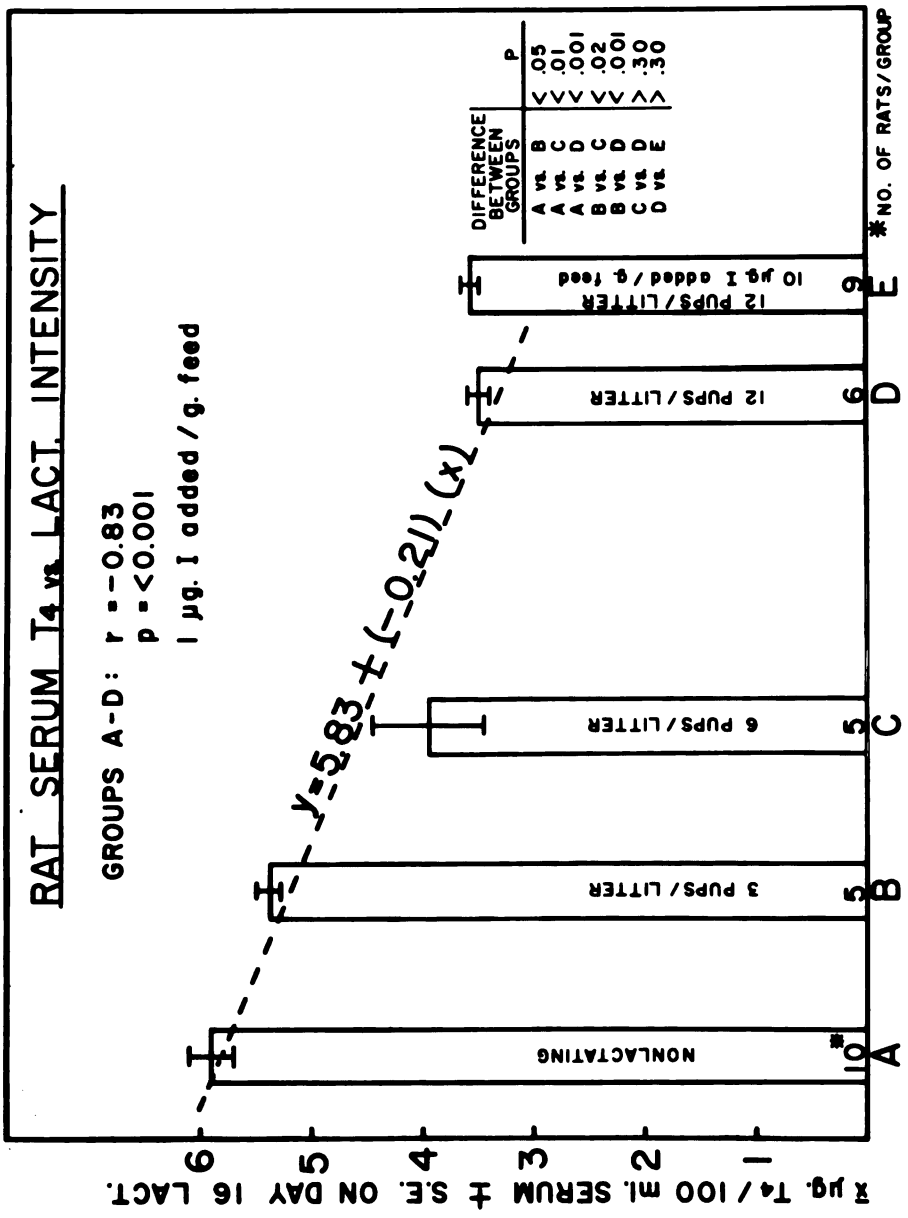


Figure 2. Rat serum thyroxine vs. lactational intensity and elevated iodine intake.

saturate the mammary iodide space thereby permitting enough iodine to be diverted to the thyroid for increased hormone production. However, in these circumstances some difficulty has been encountered in quantitating the dose of iodide (Friend, 1960). In the case of the lactating rat, either it does not respond to iodine therapy by increasing T_4 production, or the rat requires an iodide supplement far in excess of the elevated level provided in the present investigation. When sufficient serum was available, PBI determinations were run on the same rats for which data are presented in Figure 2. As shown in Appendix E the lactating rats receiving 10 times the normal iodine intake (Group E) had an average PBI value of 2.63 μg I per 100 ml of serum, similar to that of nonlactating rats in Group A which had a PBI of 2.70 μg I per 100 ml of serum. The fact that this similarity in PBI values between these two groups is not reflected in a similarity in serum T_4 values further illustrates the possible error previously discussed (Man and Peters, 1950; Barker et al., 1951; Acland, 1957) in using PBI as an index of thyroid function.

It is shown in Table 1 that exogenous ovine prolactin had no significant effect on serum T_4 in the nonlactating rat ($p > .90$). MacLeod et al. (1966) transplanted tumor-bearing pituitaries secreting large amounts of prolactin and growth hormone into rats, and reported a depression in thyroid I^{131} uptake and a decrease in host pituitary thyroid

TABLE 1

EFFECT OF PROLACTIN INJECTIONS ON SERUM THYROXINE IN THE ADULT FEMALE RAT

All rats received 1.0 μg I^- added/g feed.

Saline injected rats		Prolactin injected rats (1 mg prolactin* injected subcutaneously twice daily for 14 days)	
Rat no.	μg T_4/ 100 ml serum	Rat no.	μg T_4/ 100 ml serum
1	6.17	12	6.28
2	4.95	13	5.53
3	5.80	14	5.53
4	5.85	15	6.39
5	6.02	16	5.85
6	7.03	17	5.54
7	6.17	18	5.73
8	6.17	19	6.71
9	5.32	20	6.28
10	6.81	21	6.17
	$\bar{X}$ = 6.03		$\bar{X}$ = 6.00
	S.E. = 0.19		S.E. = 0.13
	N = 10		N = 10

*NIH-P-S8 Ovine Prolactin 28.38 I.U./mg (10 mg. prolactin/ 1.0 ml saline)

stimulating hormone (TSH) content. They concluded that suppression of thyroid function was mediated by an inhibitory effect of the tumor hormones on the pituitary of the rat. In a subsequent study MacLeod and Abad (1968) reported free T_4 tended to be slightly lower in similar tumor bearing rats. However, this decrease in free T_4 was not statistically significant. Gona (1967) has suggested that prolactin has a goitrogenic effect in the frog. Prolactin reduced thyroidal I^{131} uptake and this reduction was not changed by injections of TSH. The impairment of metamorphosis caused by prolactin could be counteracted by T_4 administration. Gona concluded that prolactin had no direct effect on the frog pituitary TSH content, but rather has a direct effect on the frog thyroid. However, Grosvenor and Turner (1958b) showed by bioassay techniques that the suckling stimulus caused a fall in pituitary prolactin stores in the lactating rat. More recently, Johke (1969) has shown by radioimmunoassay an increased plasma prolactin level following milking in the cow and goat. Amenomori et al. (1970) have also shown by radioimmunoassay that serum prolactin increases are directly correlated to increases in suckling stimuli in rats with litter sizes ranging from 0 to 12 pups.

In view of the earlier reports by others it could be suggested that the progressive decrease in rat serum T_4 as lactational intensity increases (Figure 2) is due to the concomitant increase in serum prolactin which in turn exerts

a progressively greater suppression on thyroid function. However, the use of thyroid I^{131} uptake as an index of thyroid activity is questionable since hyper- or hypothyroid function can be accompanied by either high or low I^{131} uptake. The present study (Table 1) would indicate that exogenous ovine prolactin, at the level administered, has no influence on thyroid function when evaluated by serum T_4 measurements.

Chen and Meites (1969) have shown that T_4 has no effect on hypothalamic prolactin inhibiting factor (PIF) content, but does stimulate an increase in pituitary prolactin. Thiouracil reduced pituitary prolactin levels. They concluded that T_4 acts directly on the anterior pituitary to enhance prolactin production and that a lack of T_4 when thiouracil is administered will depress prolactin secretion. This reduction in prolactin secretion in the absence of adequate T_4 would partially explain why lactation can be enhanced by the administration of thyroactive substances as reviewed by Blaxter et al., (1949). If prolactin were an antithyroid agent as suggested by others (MacLeod et al., 1966; Gona, 1967; MacLeod and Abad, 1968), then it would be inhibiting its own production via the thyroid. The evidence to date would indicate that the primary reason for a reduction in serum T_4 during lactation is the mammary diversion of iodine coupled with a possible decrease in total serum thyroid-binding protein (TBP). The latter could be due to

the increased demand for protein synthesis during lactation. There is also the possibility that high endogenous prolactin secretion could indirectly exert an antithyroid effect by enhancing lactation, thereby increasing the mammary drain on iodine supplies which would ultimately further decrease serum T_4.

Figure 3 illustrates a proposed schema for the thyroid-mammary-kidney distribution of iodine. The size of the arrows are indicative of the relative magnitude of the components of this iodine cycle. This chart shows that subnormal iodine intake in the nonlactating rat will result in less thyroid hormone production and eventually in a hypothyroid condition characterized by elevated TSH output and thyroid gland hyperplasia. The two primary competitors for dietary iodine in the nonlactating rat are the kidney and the thyroid. In the lactating rat a third competitor for dietary iodine is introduced, namely the mammary gland. As a result a functional hypothyroidism is produced, similar to that observed in a nonlactating rat on reduced iodine intake, even though the lactating rat receives an iodine allowance that would be adequate in the nonlactating state. As indicated, some species such as the rat, recycle iodine via the urine of the suckling infants. This phenomenon has been well documented by several investigators (Capek and Jelinek, 1956; Samel et al., 1963; Samel and Caputa, 1965; Beltz and Reineke, 1968). It does not appear that the net

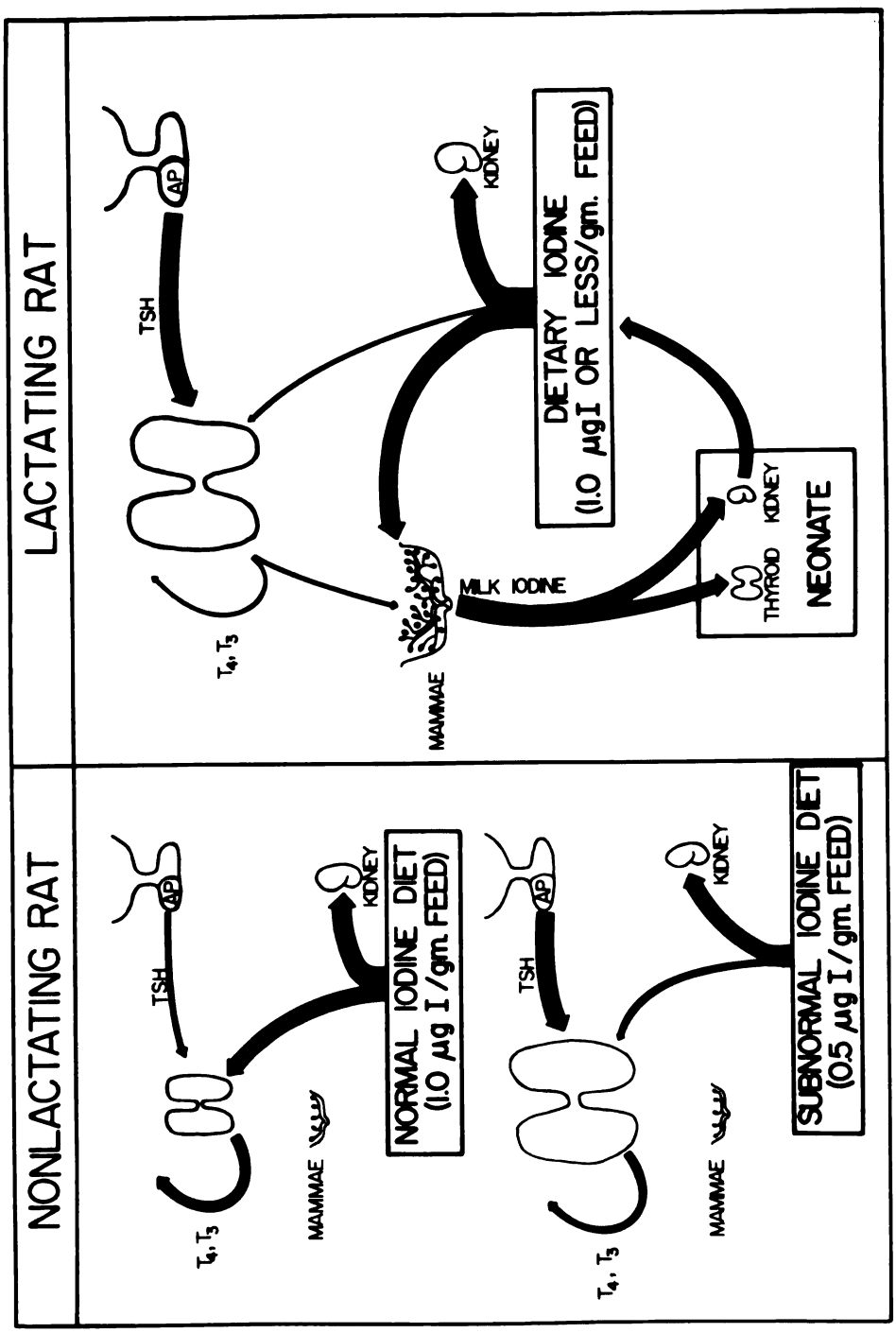


Figure 3. Thyroid, mammary and kidney distribution of iodine in the non-lactating and lactating rat.

opposing factors (total thyroid I and thyroid I^{131} release rate) in this maternal recycling of neonatal iodine can appreciably influence the results obtained by direct-output TSR in the lactating rat (Lorscheider and Reineke, 1970). This conclusion is supported by the present study which shows the serum T_4 to be significantly reduced during lactation.

II. Cow Experiments

Figure 4 illustrates serum T_4 values in 4 groups of Holstein cows during various stages of gestation and lactation. Individual values are listed in Appendix F. It can be seen that serum T_4 in nonlactating cows remains unchanged during pregnancy (5.66 μg T_4/100 ml) when compared to nonlactating nonpregnant cows (6.20 μg T_4/100 ml). This is unlike the condition found in women (Arango et al., 1968) where serum T_4 is reported to be elevated during pregnancy. Dairy cattle normally increase to a peak in milk yield 60 days or more after calving, then gradually decline until dried off at about the 7th to 8th month of gestation. As shown in Figure 4 serum T_4 is markedly reduced in high producing nonpregnant cows (3.39 μg T_4/100 ml) followed by a recovery in serum T_4 to normal levels during advanced lactation and pregnancy (5.63 μg T_4/100 ml). The magnitude of serum T_4 reduction in the high producing cows is similar to the 50% reduction observed in lactating rats (Figure 1).

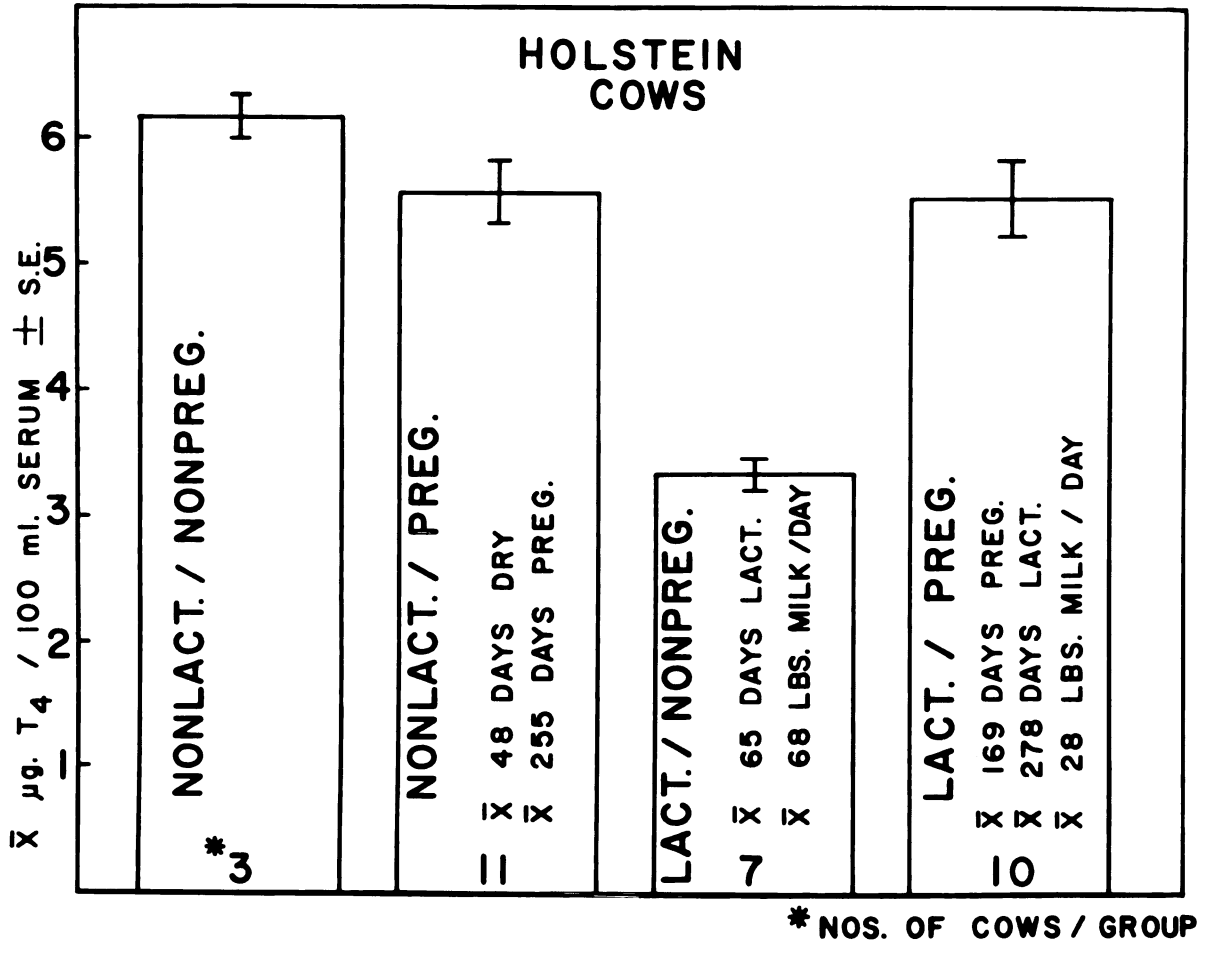


Figure 4. Serum thyroxine in Holstein cows during various stages of gestation and lactation.

These results support the trends shown in previous studies on Ayrshire cows (Kossila, 1967) in which protein-bound iodine (PBI) values tended to be lower at peak lactation with gradual increases during the first 5 months postpartum. Furthermore, in a comparison of five breeds of dairy cows, PBI values tended to be lowest in Holstein cows (Long et al., 1952). Inasmuch as Holsteins are the highest milk producers, the depletion of serum T_4 during lactation may be the most pronounced in this breed.

A paired comparison of serum T_4 levels in Holstein cows during pregnancy and different stages of lactation is shown in Table 2. At 30 days lactation serum T_4 was significantly reduced from 5.66 to 4.63 μg T_4/100 ml ($p < .001$). At 93 days lactation serum T_4 was reduced still farther to 3.90 μg T_4/100 ml. This paired comparison was terminated in mid-March in order to avoid the seasonal changes in light and temperature known to influence the thyroid function of farm animals (Henneman et al., 1955; Hoersch et al., 1961; Griffin et al., 1962).

One might expect that the high inverse relationship between lactational intensity and serum T_4 as exists in the rat, might also be present in cows. However, a close inspection of the individual milk records in both of the present studies (Appendix F and Table 2) does not reveal such an inverse relationship within a given cow group. The higher milk producers did not necessarily have the lowest serum T_4

TABLE 2

PAIRED COMPARISON OF SERUM THYROXINE IN HOLSTEIN COWS DURING PREGNANCY AND DIFFERENT STAGES OF LACTATION

	GROUP A 48 days dry 255 days preg.	GROUP B 30 days lactation		GROUP C 93 days lactation est. 20 days preg.	
Blood Sampling Date	11-21-68	1-14-69		3-18-69	
Cow No.*	µg T_4/100 ml serum	µg T_4/100 ml serum	Milk Rec. (lbs.) 11-13-69	µg T_4/100 ml serum	Milk Rec. (lbs.) 3-17-69
800	4.28	3.43	90.7	3.14	56.5
970	5.30	4.06	83.7	3.30	47.0
983	5.40	4.00	88.7	4.13	81.0
904	5.71	5.11	95.3	5.22	78.0
961	5.36	5.10	68.3	4.74	35.0
957	7.39	5.50	86.7	4.43	66.5
766	5.15	5.48	97.0	5.50	74.5
989	5.59	4.92	88.7	2.48	72.0
768	5.97	4.47	76.0	killed	(leaked milk)
801	6.81	4.19	84.3	3.31	70.5
795	5.35	4.63	82.3	2.75	92.5
	$\bar{X}$ = 5.66	$\bar{X}$ = 4.63	$\bar{X}$ = 85.6	$\bar{X}$ = 3.90	$\bar{X}$ = 67.3
	S.E. = 0.25	S.E. = 0.20		S.E. = 0.33	

Group Serum T_4, paired t-tests

$A > B$, $p < .001$

$A > C$, $p < .001$

*For individual case histories refer to Appendix F, nonlactating/nonpregnant cows.

values. This is attributed to the fact that lactational performance is a function of both intensity and duration. The overall trend between groups though, was for markedly lower serum T_4 levels during the first 3 to 4 months following calving, a time when milk production is highest. Tucker and Reece (1961) showed that the increase in milk output due to thyroprotein feeding was much greater in cows at peak lactation. They concluded that thyroid hormone secretion may be a limiting factor on milk secretion at the peak of lactation. Sorensen (1958) reported that a high TSR of cows was highly correlated with high milk yield. The present investigations would tend to support the conclusions of Tucker and Reece in that serum T_4 is markedly reduced during high lactation.

In Table 3 serum T_4 values are shown for Holstein cows diagnosed as nonbreeders or as having cystic ovaries. It can be seen that the highest value (3.07 μg T_4/100 ml) is only half that of values for comparable nonlactating nonpregnant control animals (Figure 4 and Appendix F). Reineke and Soliman (1953) reported that hypothyroidism in mice is associated with cystic ovaries. The present study suggests that a similar relationship may be true in cows. Further investigations in cows are currently underway to establish the feasibility of employing the serum T_4 assay as a diagnostic tool for the detection of cystic ovaries and other causes of reproductive failure.

TABLE 3

SERUM THYROXINE VALUES IN HOLSTEIN COWS DIAGNOSED AS NONBREEDERS OR AS HAVING CYSTIC OVARIES*

NONPREGNANT/NONLACTATING
(blood sampling 10-1-68)

Cow No.	Birth Date	Pathology	μg T_4/100 ml serum
960	7-6-65	cystic ovaries	3.07
909	11-12-62	cystic ovaries	2.94
886	6-21-66	nonbreeder	1.48
866	9-8-66	nonbreeder	1.76
709	3-7-61	nonbreeder	0.64

*Cystic ovaries--post mortem diagnosis.

TABLE 4

SERUM THYROXINE VALUES IN NORMAL AND NONBREEDING HOLSTEIN HEIFERS

All heifers bled on 2-21-69

NORMAL HEIFERS

Heifer No.	Birth Date	μg T_4/100 ml serum
997	11-16-67	9.58
1000	11-30-67	12.37
1005	12-12-67	10.77
1006	12-13-67	9.98
1010	1-3-68	5.98
1011	1-4-68	9.58
1012	1-5-68	7.18
1016	1-29-68	5.19
		$\bar{X}$ = 8.83
		S.E. = 0.87

NONBREEDING HEIFERS*

Heifer No.	Birth Date	Nos. of Previous Inseminations	μg T_4/100 ml serum
218	3-22-67	12	11.18
235	4-9-67	8	8.38
270	7-5-67	10	9.58
286	6-21-67	11	7.18
			$\bar{X}$ = 9.08
			S.E. = 0.85

*Etiology unknown

The relationship between serum T_4 and the incidence of nonbreeding in Holstein heifers, not diagnosed as having cystic ovaries, is somewhat different (Table 4). Average serum T_4 values for controls and nonbreeders are 8.83 and 9.08 μg T_4/100 ml respectively. No significant difference was found between these two groups ($p > .90$). The average control serum T_4 value is significantly higher ($p < .05$) in heifers (8.83 μg T_4/100 ml) than that reported for mature nonlactating cows (6.20 μg T_4/100 ml) as shown in Figure 4 and Appendix F. This would agree with reports by others (Long et al., 1952) that in general younger cows have a higher PBI. However, heifers have never been through a lactation and are therefore not directly comparable to cows.

III. Ewe Experiments

Serum T_4 values in Suffolk and Hampshire ewes during gestation, lactation and thyroprotein feeding are shown in Figure 5. Individual values for the Suffolk ewes are listed in Appendix G. Nonlactating nonpregnant Suffolk yearlings have a serum T_4 value of 13.52 μg T_4/100 ml as opposed to 11.90 μg T_4/100 ml for nonlactating pregnant Suffolk ewes ($p > .10$). Thus, as in the cow, pregnancy does not elevate serum T_4 of ewes. In pregnant women, serum T_4 does rise (Arango et al., 1968). At 30 days lactation, estimated to be peak lactation in ewes, serum of T_4 of Suffolks is significantly reduced (9.32 μg T_4/100 ml) below that of pregnant

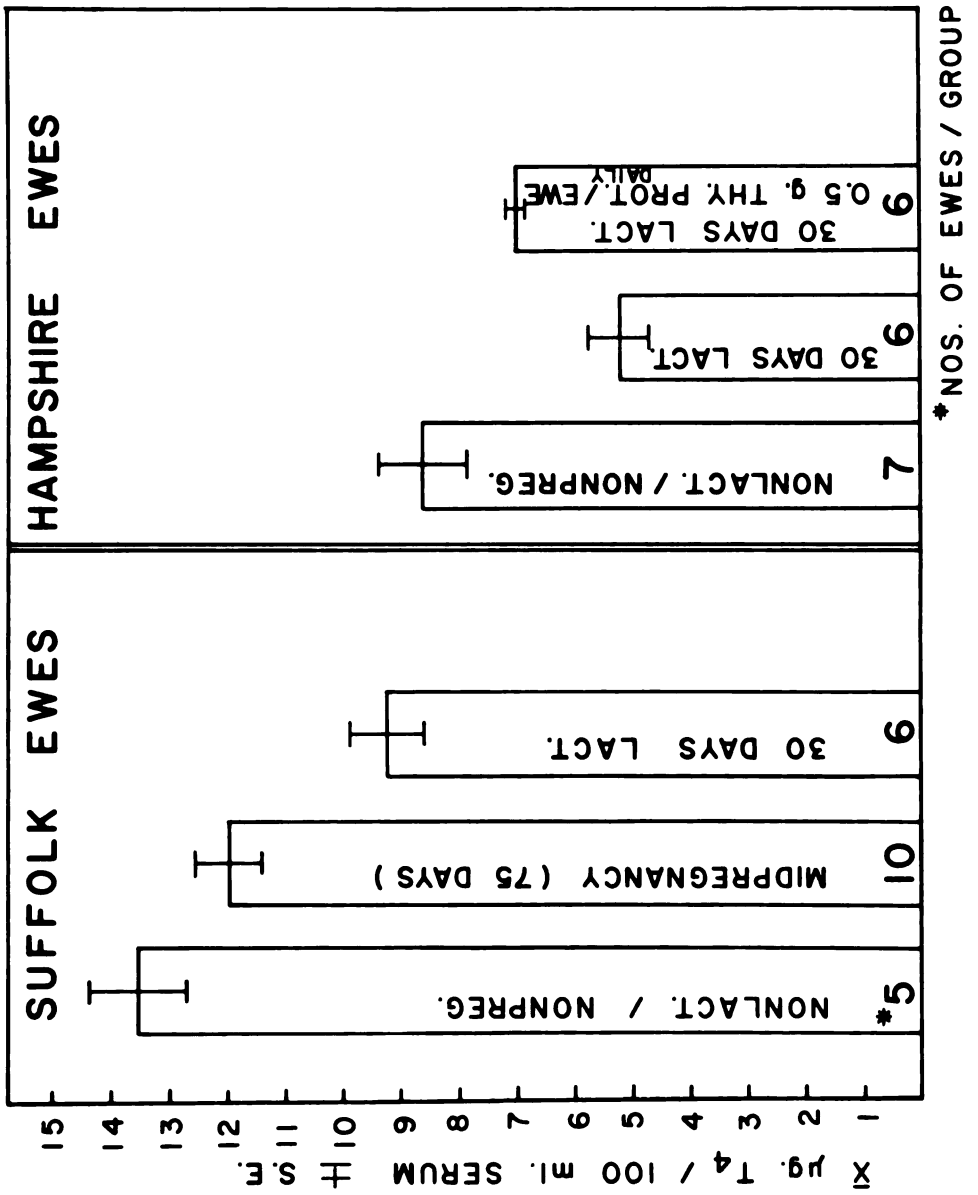


Figure 5. Serum thyroxine in Suffolk and Hampshire ewes during gestation, lactation, and thyroprotein feeding.

ewes ($p < .02$). In Hampshire ewes (Figure 5 and Appendix H), serum T_4 is likewise significantly reduced at 30 days lactation from 8.59 μg T_4/100 ml in yearling controls to 5.27 μg T_4/100 ml ($p < .01$). Thus both breeds of ewes exhibit a reduction in serum T_4 during lactation similar to that observed in the rat and cow. If the serum T_4 of the Suffolk and Hampshire yearling controls is compared, 13.52 and 8.59 μg T_4/100 ml respectively, there is a significant breed difference in thyroid hormone levels ($p < .01$).

Henneman et al. (1955) reported that TSR is not altered in sheep during pregnancy. The serum T_4 values for yearling and pregnant Suffolk ewes in the present study tend to confirm this finding. When the T_4 substitution method was employed in previous investigations (Henneman et al., 1955; Griffin et al., 1962), apparent TSR was found to be elevated in sheep during lactation. The present data, showing reduced serum T_4 in lactating sheep, are consistent with the previous rat studies (Lorscheider and Reineke, 1966; Reineke and Lorscheider, 1967; Lorscheider and Reineke, 1970). These experiments show that the T_4 substitution method reflects the thyroid hormone demand, which is greater during lactation, but cannot detect the actual decrease in thyroid function that occurs in lactating animals. The direct-output TSR and serum T_4 methods for measuring thyroid function are more applicable in any case where iodine availability is likely to be a limiting factor.

Figure 5 shows that lactating Hampshire ewes fed thyroactive protein had significantly higher serum T_4 values (7.01 μg T_4/100 ml) than did a comparable lactating group not fed thyroprotein (5.27 μg T_4/100 ml) ($p < .02$). Individual Hampshire serum T_4 values for these two groups are listed in Appendix H. It is interesting to note that lactating ewe number 421, which was not fed thyroprotein, has the lowest serum T_4 in its group (3.54 μg T_4/100 ml). This particular animal also had the highest degree of wool slippage at lambing in its group. Lactating ewe number 505 on the thyroprotein treatment also had the highest degree of wool slippage at lambing in its group. Plate 1 shows that ewe number 421 has a negligible regrowth of wool 2 1/2 months after lambing. Plate 2 shows a 2.0 cm. regrowth of wool on ewe number 505 2 1/2 months after lambing and thyroprotein treatment. Based on these data, it would appear that severe wool slippage is associated with a marked reduction in serum T_4 during lactation. Thyroprotein supplemented in the diet will significantly raise serum T_4 and facilitate the regrowth of wool. More extensive trials are currently underway in Shropshire and Hampshire ewes to further assess the effectiveness of thyroprotein feeding in alleviating the alopecia-like condition which develops during late pregnancy and lactation. One theory proposed is that thyroid hormones exert an effect on mitochondrial membranes to increase respiratory exchange and energy transfer (Tapley and Hatfield, 1962) and

Plate 1. Control Hampshire ewe number 421 about 2 1/2 months after lambing. Note the negligible regrowth of wool.

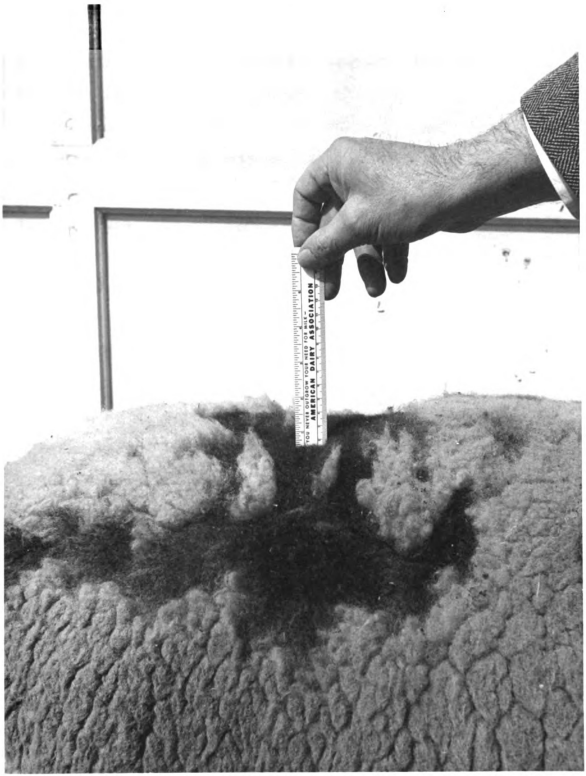


Plate 2. Hampshire ewe number 505 about 2 1/2 months after lambing and thyroprotein treatment. Note that wool has regrown to a length of 2.0 cm.

that secondarily, T_4 stimulates increased amino acid incorporation into newly synthesized protein (Sokoloff and Kaufman, 1961) for wool growth.

Suffolk ewes have a higher serum T_4 level than do Hampshire ewes, as previously discussed. This may explain why Suffolks rarely show wool slippage during lactation. The fact that the incidence of wool slippage is greater in Shropshire than Hampshire ewes could mean that the serum T_4 is lower in the Shropshire breed than in the Hampshire breed. The study by Henneman et al. (1955) would tend to support this hypothesis. In their study the mean TSR of nonlactating Shropshire and Hampshire 2-year-olds was 0.17 and 0.28 mg T_4 per day, respectively, during January. Griffin et al. (1962) also reported a significantly reduced TSR in Shropshire rams as compared to Hampshire rams, regardless of the season of year.

The problem of postpartum alopecia is not exclusively confined to Hampshire and Shropshire ewes. Morris (1969) reported a similar condition in lactating bitches, especially in Great Danes, Beagles, and many long-haired show dogs. The cause was hypothesized to be a low-energy maintenance ration. The etiology of postpartum Alopecia areata in man remains unknown and as yet no causal therapy has been found. Gip et al.(1969) reported in their study that women with Alopecia areata exhibited a manifest improvement during pregnancy followed by a marked deterioration after

parturition. It is interesting that hair loss should be alleviated during pregnancy (a time of elevated serum T_4 in women) followed by a severe loss after parturition (at the onset of lactation) when serum T_4 is probably reduced as it is in the rat, cow, and ewe. No records on breast-feeding were reported in Gip's investigation. Thyroxine therapy has been utilized in the past as an effective treatment for increasing milk yield in women suspected of having reduced thyroid function (Robinson, 1947a; Roche et al., 1950; Romani, 1951). If the etiology of postpartum alopecia is related to the status of the thyroid, T_4 therapy might also prove beneficial in this instance.

Table 5 shows serum T_4 values in lactating Hampshire ewes fed phenothiazine, which is routinely used as an anthelmintic. Again, as in Figure 5, it can be seen that serum T_4 is significantly reduced during lactation from 8.44 to 6.44 μg T_4/100 ml ($p < .02$). When phenothiazine was withheld from the diet the average serum T_4 level during lactation was significantly elevated to 8.41 μg T_4/100 ml ($p < .02$). While no statistical correlation was found between the number of days off phenothiazine and serum T_4, it is interesting to note that ewe number 229 had the highest serum T_4 value in its group (10.25 μg T_4/100 ml) and was off phenothiazine treatment the longest (37 days). Ewe number 7221 had the lowest serum T_4 value in this group (6.71 μg T_4/100 ml) and was off phenothiazine the shortest period of

TABLE 5.

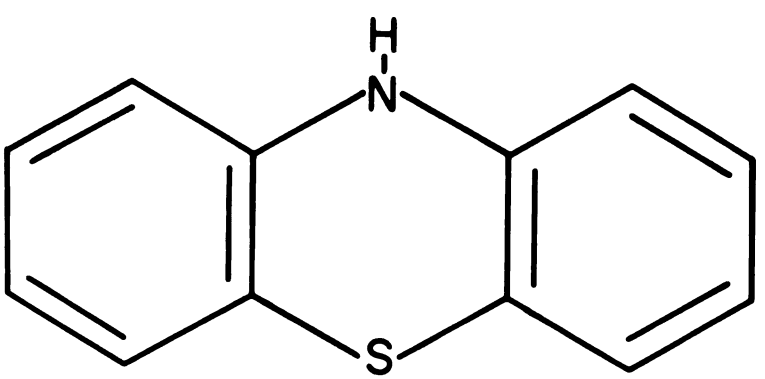
HAMPSHIRE EWES

SERUM T_4 /LACTATION vs. PHENOTHIAZINE TREATMENT

	EWE NO.	DAYS LACTATION	NO. OF LAMBS	DAYS OFF *PHENOTHIAZINE	µg. T_4/100ml. SERUM	MEAN ± S.E.
NONLACTATING	809	———	—	0	10.07	8.44 ± 0.44
	814	———	—	0	8.30	
	818	———	—	0	7.44	
	833	———	—	0	8.05	
	836	———	—	0	8.36	
LACTATING	704	32	1	0	5.62	6.44 ± 0.31
	503	31	2	0	7.14	
	619	27	2	0	6.83	
	605	24	2	0	5.76	
	535	23	1	0	6.83	
LACTATING	229	37	2	37	10.25	8.41 ± 0.60
	7252	33	1	33	7.88	
	534	30	2	30	8.05	
	607	24	1	24	9.15	
	7221	20	1	20	6.71	

*1 PART PHENOTHIAZINE : 9PARTS SALT - MINERAL MIX

PHENOTHIAZINE : $C_{12}H_9NS$



time (20 days). Based on these data it would appear that phenothiazines influence thyroid function and may confound the problem of wool loss in lactating sheep.

The effect of phenothiazines in blocking thyroid I^{131} uptake has been observed in a number of different farm animals (Talmage et al., 1954). In studies using the rat and chick as experimental models (Nachimson et al., 1954; Wasserman et al., 1956), the thyroid uptake of I^{131} was likewise blocked when these animals were fed phenothiazine. At first it was suggested that iodine, introduced as a catalyst in the production of phenothiazine, depressed thyroid I^{131} uptake by isotope dilution or thyroid saturation of iodide. However, when highly purified iodine-free phenothiazine was fed, thyroid I^{131} uptake was still significantly depressed. In fact, it was shown that the iodine adsorbed to phenothiazine is not readily dissociated and consequently is not available in the circulation as a free iodide ion. It was concluded in both studies that some compound or metabolite of phenothiazine, as yet unknown, was at least partially responsible for suppressing the thyroid. The present study shows that removal of phenothiazine from the diet of lactating ewes will result in higher serum T_4 levels. It may be that removal of phenothiazine from the diet of nonlactating Hampshire or Shropshire ewes will raise their serum T_4 also. Trials currently underway are designed to show whether a combination of thyroprotein

feeding and/or withdrawal of phenothiazine will alleviate the problem of wool loss in lactating sheep.

SUMMARY AND CONCLUSIONS

A serum thyroxine (T_4) analysis was employed to assess thyroid function as related to lactation in rats and dairy cattle, to the nonbreeding cow and heifer, and to lactation, phenothiazine treatment and wool growth in sheep. The results of these studies are as follows:

1. A comparison of serum T_4 and thyroid secretion rate (TSR) by the direct-output method in nonlactating and lactating rats established that both methods detected a greater than 50% decrease in thyroid function during lactation. The serum T_4 method was found to be valid and applicable in cases where the direct-output method was not practical.

2. Rat serum T_4 levels were inversely correlated with lactational intensity, and the markedly reduced serum T_4 observed in high-lactating rats could not be counteracted with 10 times the normal dietary iodine.

3. Exogenous ovine prolactin, when administered to nonlactating rats, had no effect on serum T_4. It was concluded that high serum prolactin, associated with nursing, was not a directly contributing factor to the reduction of serum T_4 observed during lactation.

4. A schema was proposed for the thyroid-mammary-kidney distribution of iodine. The evidence presented suggested that the primary reason for a reduction in serum T_4 during lactation was the diversion of iodine to the mammary glands.

5. Holstein cows showed no change in serum T_4 during pregnancy. During the period of intense lactation serum T_4 was reduced by 50%. When the intensity of lactation declined, at about 5 months postpartum, serum T_4 returned to a euthyroid level.

6. In cows diagnosed as nonbreeders or as having cystic ovaries, serum T_4 was markedly reduced. This finding agrees with previous conclusions that cystic ovaries may be associated with a hypothyroid condition.

7. Serum T_4 values for heifers were higher than those of adult cows and the difference was attributed primarily to age. Nonbreeding heifers showed no reduction in serum T_4. However, these animals had never lactated and therefore were not directly comparable to cows.

8. Suffolk ewes, like the cow, exhibited no change in serum T_4 during pregnancy. During lactation both Suffolk and Hampshire ewes showed a significant reduction in serum T_4.

9. Lactating Hampshire ewes developed a considerable amount of wool slippage. The regrowth of wool was greatly enhanced by thyroprotein treatment.

10. Removal of phenothiazine from the diet of lactating Hampshire ewes significantly raised their serum T_4. Thus both phenothiazine and lactation may contribute to a reduction of serum T_4 in the ewe and could confound the problem of wool loss observed during advanced pregnancy and lactation.

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APPENDICES

APPENDIX A

RAT FEED MIXTURE

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

*Special Mineral Salt Premix

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

APPENDIX B

M. S. U. STANDARD DAIRY RATION

Ingredient	Lbs. of feed per batch
Shelled corn	1200
Oats	500
Soybean oil meal (50-55% protein)	300
Liquid molasses	150
Dicalcium phosphate	20
Trace Mineralized Salt* (Morton's)	20

*TMS Premix

Element	Compound	Minimum per cent of compound in TMS
Zn	Zinc oxide	1.00
Mn	Manganous oxide	0.80
Fe	Iron oxide	0.50
S	Ferrous sulfate	0.24
Cu	Copper oxide	0.10
I	Calcium iodate	0.01
Co	Cobalt carbonate	0.01
Na	Sodium chloride	94.00

Cattle had free access to mixed alfalfa hay and corn silage.

APPENDIX C

M. S. U. SHEEP RATION

I. Alfalfa brome hay fed but not weighed. Ewes consumed approximately four pounds per day.

II. Grain

a.) Pre-lambing, 1 lb. per head per day of the following ration to all lots.

Ingredient	Per cent
Whole oats	55.00
Corn	13.75
Wheat	20.00
Wheat bran	10.00
Wet molasses	1.00
Aurofac 10	0.25
	100.00

b.) Post-lambing, 2 lbs. per head per day of the following ration to all lots.

Ingredient	Per cent
Whole oats	53.00
Corn	25.00
Wheat bran	15.00
Soybean oil meal	5.00
Wet molasses	2.00
	100.00

III. Phenothiazine and trace mineral salt (TMS) supplementation. This mixture was weighed into self-feeding salt boxes once each week. Average consumption of TMS is usually 1 lb. per ewe per month.

Ingredient	Per cent
Trace Mineral salt* (Morton's)	60.00
Dicalcium phosphate	30.00
Phenothiazine N.F. (green)	10.00
	100.00

In cases where phenothiazine was removed:

Ingredient	Per cent
Trace mineral salt* (Morton's)	67.00
Dicalcium phosphate	33.00
	100.00

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*TMS Premix

Element	Compound	Minimum per cent of compound in TMS
Zn	Zinc oxide	1.00
Mn	Manganous oxide	0.80
Fe	Iron oxide	0.50
S	Ferrous sulfate	0.24
Cu	Copper oxide	0.10
I	Calcium iodate	0.01
Co	Cobalt carbonate	0.01
Na	Sodium chloride	94.00

APPENDIX D

SERUM THYROXINE MEASUREMENTS IN NONLACTATING AND LACTATING RATS (INDIVIDUAL VALUES)

All rats received 1.0 μg I^- added per g. feed.
Lactating rats each nursed 12 pups.
Lactators were bled at 16 days postpartum.

Nonlactating rats		Lactating rats	
Rat no.	μg T_4/ 100 ml. serum	Rat no.	μg T_4/ 100 ml. serum
13	3.35	2	2.25
15	3.95	3	2.47
16	3.64	4	2.61
17	3.68	6	(ate 5 pups on 3rd day postpartum) 3.43
18	4.16	7	2.72
19	4.11	8	1.93
20	3.17	9	0.78
21	4.16	10	0.95
22	4.62	11	0.80
23	4.54	12	1.63
$\bar{X}$ = 3.94		$\bar{X}$ = 1.96	
S.E. = 0.15		S.E. = 0.26	
N = 10		N = 10	

t = 6.12
p < .001

APPENDIX E

RAT SERUM T_4 AND PBI VS. LACTATIONAL INTENSITY (INDIVIDUAL VALUES)

Groups A-D received 1.0 μg I^- added/g feed.
Group E received 10.0 μg I^- added/g feed.
Lactators bled at 16 days postpartum.

Group A
(nonlactating)

Rat. no.	μgT_4/100 ml serum	PBI (μg/100 ml serum)
1	7.13	1.86
2	5.29	2.12
3	5.52	1.63
4	5.52	2.08
5	5.98	3.10
6	5.78	3.83
7	6.05	4.93
8	5.51	2.51
9	5.22	2.31
10	7.15	———
	$\bar{X}$ = 5.92	$\bar{X}$ = 2.70
	S.E. = 0.22	S.E. = 0.36

Group B
(3 pups per litter)

Rat. No.	μgT_4/100 ml serum	PBI (μg/100 ml serum)
11	5.52	1.85
12	5.06	
13	5.52	1.66
14	5.29	1.36
15	5.52	———
	$\bar{X}$ = 5.38	$\bar{X}$ = 1.62
	S.E. = 0.09	S.E. = 0.14

Group C
(6 pups per litter)

Rat no.	μg T_4/100 ml serum	PBI (μg/100 ml serum)
16	5.52	1.97
17	4.14	1.57
18	4.14	2.12
19	2.99	1.50
20	2.93	
	$\bar{X}$ = 3.96	$\bar{X}$ = 1.79
	S.E. = 0.48	S.E. = 0.14

Group D
(12 pups per litter)

Rat no.	μg T_4/100 ml serum	PBI (μg/100 ml serum)
21	3.44	0.99
22	3.91	1.14
23	3.44	1.24
24	3.21	1.26
25	3.21	1.80
26	3.68	1.54
	$\bar{X}$ = 3.48	$\bar{X}$ = 1.32
	S.E. = 0.11	S.E. = 0.10

Group E
(12 pups per litter; 10 x normal I^- allowance)

Rat no.	μg T_4/100 ml serum	PBI (μg/100 ml serum)
28	3.44	2.35
29	3.68	3.00
30	3.68	2.30
31	3.91	
32	3.21	
33	3.68	2.43
34	3.21	
35	3.68	2.99
36	3.68	2.72
	$\bar{X}$ = 3.57	$\bar{X}$ = 2.63
	S.E. = 0.08	S.E. = 0.10

APPENDIX F

SERUM THYROXINE MEASUREMENTS, REPRODUCTION, AND MILK RECORDS OF HOLSTEIN COWS (INDIVIDUAL VALUES)

Nonlacting-Nonpregnant (blood sampling 10-1-68)

Cow No.	Birth Date	Body Wt. (lbs.)	No. of Previous Calves	µg T_4/100 ml serum
263	6-18-62	1415	2	6.32
815	3-4-64	1200	2	6.42
882	5-25-66	1260	1	5.86
				$\bar{X}$ = 6.20
				S.E. = 0.17

Nonlactating-Pregnant (blood sampling 11-21-68)

Cow No.	Birth Date	Body Wt. (lbs.)	No. of Previous Calves	Date Dried Off	Subsequent Calving Date	µg T_4/100 ml serum
800	12-9-64	1242	2	10-15-68	11-27-68	4.28
970	4-22-62	1576	4	9-18-68	12-8-68	5.30
983	1-1-62	1480	4	9-1-68	12-9-68	5.40
904	10-15-64	1576	3	9-18-68	12-9-68	5.71
961	11-22-65	1368	2	10-15-68	12-13-68	5.36
957	7-12-65	1357	2	10-15-68	12-5-68	7.39
766	10-14-63	1534	3	10-15-68	12-15-68	5.15
989	4-29-65	1690	1	11-1-68	12-25-68	5.59
768	12-8-63	1796	3	10-13-68	12-26-68	5.97
801	12-23-64	1732	1	10-1-68	12-30-68	6.81
795	10-23-64	1785	2	9-18-68	12-28-68	5.35
						$\bar{X}$ = 5.66
						S.E. = 0.25

Based on drying and subsequent calving dates this group averaged 48 days dry and 255 days pregnant at the time of blood sampling. Gestation period 280 days.

Lactating-Nonpregnant
(Blood sampling 10-1-68)

Cow No.	Birth Date	Body Wt. (lbs.)	Nos. of Previous Calves	Last Calving Date	Milk Rec. (lbs.) 10-1-68	µg T_4/100 ml serum
319	2-15-64	1360	3	9-3-68	37.5	3.08
796	11-4-64	1400	3	9-6-68	71.5	3.52
780	2-26-64	1607	2	7-1-68	60.0	3.62
1027	6-12-65	1114	2	8-13-68	73.0	3.35
799	11-18-64	1498	2	6-20-68	62.0	3.17
716	7-4-61	1194	4	6-28-68	92.0	3.81
953	11-7-64	1484	2	7-17-68	77.0	3.16

$\bar{X}$ = 3.39
S.E. = 0.10

Based on the last calving dates this group averaged 65 days lactation with a milk output of 68 lbs. per day at the time of blood sampling

Lactating-Pregnant
(blood sampling 11-26-68)

Cow No.	Birth Date	Body Wt. (lbs.)	No. of Previous Calves	Previous Calving Date	Subsequent Calving Date	Milk Rec. (lbs.) (11-25-68)	µg T_4/ 100 ml serum
902	7-6-64	1885	2	3-8-68	6-19-69	25.5	5.70
991	8-22-64	1330	2	2-12-68	6-12-69	44.0	7.93
838	3-6-66	1220	1	5-25-68	5-17-69	29.5	4.39
811	10-18-64	1840	2	11-16-67	5-14-69	15.0	5.52
702	11-30-60	1550	4	1-2-68	5-24-69	44.0	6.46
869	10-28-64	1815	2	2-28-68	5-13-69	15.5	6.14
873	3-13-64	1578	2	4-5-68	5-2-69	18.5	5.23
771	1-6-64	1480	3	2-19-68	4-20-69	17.5	4.19
891	6-5-65	1438	1	1-25-68	4-26-69	15.5	5.50
837	3-4-66	1122	1	2-22-68	4-7-69	55.0	5.23

$\bar{X}$ = 5.63
S.E. = 0.34

Based on an average gestation period of 280 days and the previous and subsequent calving dates this group averaged 169 days pregnant and 278 days lactation with a milk output of 28 lbs per day at the time of blood sampling.

APPENDIX G

SERUM THYROXINE MEASUREMENTS IN YEARLING, PREGNANT, AND LACTATING SUFFOLK EWES (INDIVIDUAL VALUES)

Yearling Control Ewes (blood sampling 1-29-69)

Ewe No.*	μg T_4/100 ml serum
8-38	16.34
8-42	13.15
8-04	13.97
8-21	11.16
8-26	12.99
	$\bar{X}$ = 13.52
	S.E. = 0.84

Estimated Mid-pregnant Ewes (blood sampling 11-19-68)

Ewe No.	μg T_4/100 ml serum
4-35	10.53
6-11	13.68
7-29	10.91
4-13	8.19
4-33	12.47
7-23	13.94
4-29	12.79
3-06	11.63
6-30	10.92
7-34	13.95
	$\bar{X}$ = 11.90
	S.E. = 0.58

Lactating Ewes
(blood sampling 1-29-69)

Ewe No.	Lambing Date	No. of Lambs	μg T_4/100 ml serum
7-23	12-31-68	1	10.83
3-06	12-28-68	2	10.83
4-35	1-3-69	1	7.71
7-29	1-2-69	1	8.75
4-13	1-21-69	1	10.49
6-30	12-31-68	2	7.36
		$\bar{X}$ =	9.32
		S.E. =	0.67

*First digit of ewe no. indicates year of birth, during Jan. or Feb.

APPENDIX H

SERUM THYROXINE AND PBI MEASUREMENTS IN YEARLING, LACTATING, AND LACTATING THYROPROTEIN-FED HAMPSHIRE EWES (INDIVIDUAL VALUES)

All ewes bled on 1-29-69

Yearling Control Ewes

Ewe No.*	μg T_4/100 ml serum	PBI μg/100 ml serum
8-25	8.05	3.82
8-09	7.01	5.06
8-36	12.57	4.94
8-44	10.14	4.01
8-18	7.36	4.67
8-33	6.66	9.69
8-14	8.40	4.17
	$\bar{X}$ = 8.59	$\bar{X}$ = 5.19
	S.E. = 0.80	S.E. = 0.77

Lactating Ewes

Ewe No.	Lambing Date	No. of Lambs	μg T_4/100 ml serum	PBI μg/100 ml serum
7-22	1-22-69	1	7.36	6.47
5-06	1-13-69	2 foster	4.23	6.31
5-14	12-20-68	2	5.62	4.81
4-21[a]	1-11-69	1	3.54	5.61
5-22	1-9-69	1	5.62	5.10
5-26	1-25-69	3	5.27	4.87
			$\bar{X}$ = 5.27	$\bar{X}$ = 5.53
			S.E. = 0.53	S.E. = 0.30

Lactating Thyroprotein-Fed Ewes
(0.5 g Protamone[1]/ewe/day mixed with
grain beginning 4-6 days postpartum)

Ewe No.	Lambing Date	No. of Lambs	μg T_4/100 ml serum	PBI μg/100 ml serum
5-01	1-1-69	2	7.36	4.56
3-07	1-31-68	2	6.32	6.28
7-10	1-8-69	2	7.01	8.48
5-05[b]	1-9-69	2	6.66	7.59
5361	1-18-69	1	7.36	6.81
7-12	1-27-69	2	7.36	8.02
			$\bar{X}$ = 7.01	$\bar{X}$ = 6.96
			S.E. = 0.17	S.E. = 0.58

*First digit of ewe no. indicates year of birth, during Jan. or Feb.

[1]Thyroactive casein--10X activity of U. S. P. desiccated thyroid--contains 5% nonhormonal iodine.

[a]Ewe 4-21, severe wool loss 1-29-69, no wool regrowth 3-23-69, see Plate 1.

[b]Ewe 5-05, severe wool loss 1-29-69, 60% wool regrowth 3-23-69, see Plate 2.