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To the Graduate Council:

I am submitting herewith a thesis written by John J. Sullivan entitled "The Effect of Feeding L-Thyroxine to Dairy Cows Upon Plasma Protein-Bound Iodine and Fecal Thyroxine." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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THE EFFECT OF FEEDING L-THYROXINE TO DAIRY COWS UPON PLASMA PROTEIN-BOUND IODINE AND FECAL THYROXINE

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
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Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

John J. Sullivan

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INTRODUCTION

When thyroactive compounds are fed to lactating cows, milk production increases. Observations have shown, however, that the production stimulus is temporary, and subsequently a decrease in persistency of milk production results. It has been shown that plasma protein-bound iodine and excretion of thyroidally active compounds are good indicators of the metabolism of thyroxine. This study was made to determine the effect of L-thyroxine dose-level and length of time it is fed to lactating cows upon the amounts of thyroxine equivalents in the blood and feces.

REVIEW OF LITERATURE

Anatomy of the Thyroid Gland

There is a thyroid gland in all vertebrates, with the normal size varying between species roughly proportional to body weight. The vascular supply is very rich, and innervation abundant. Microscopically, the gland is composed of spheroidal structures, called follicles, acini, or alveoli, surrounded by connective tissue. The follicles are lined by a secretory epithelium composed of a single layer of cells and a lumen which contains a secretion called colloid (13).

Synthesis of Thyroxine and Release

Tetraiodothyronine (hereafter called thyroxine) was first isolated from the thyroid tissue in 1914 (28). Harrington and Salter (22) in 1930 improved the technique of extraction of thyroxine and obtained sufficient yield to determine the empirical formula $C_{15}H_{11}O_4NI_4$, the structure, and the fact that it is levorotatory (L^-).

Circulating iodide originating from the diet and iodide from the breakdown of iodinated products of the thyroid are trapped and concentrated by the thyroid follicle (17, 24, 42). The mechanism of iodide-trapping has been found to be independent of its organic binding. Thiouracil-

treated rats, in which thyroxine synthesis is blocked, were injected with inorganic iodide, and a linear relationship was found in the curves depicting inorganic iodide concentration in the plasma and the goitrous thyroid, with the latter at intervals having 200 to 300 times greater concentration than the plasma. The linear relationship demonstrated that thyroid iodide concentration depends upon plasma iodide concentration (51).

Along with thyroxine, diiodotyrosine and monoiodotyrosine, all of which are amino acids, have been identified in the thyroid gland (19). It is believed that thyroxine synthesis occurs with the oxidation of iodide to iodine, the iodination of tyrosine to monoiodotyrosine followed by diiodotyrosine, and the oxidative uniting of two molecules of diiodotyrosine to form thyroxine with the loss of one molecule of alpha-aminopropionic acid. The resulting amino acids combine with other amino acids to form thyroglobulin, and this is stored in the lumen of the follicle as colloid (9, 35). Tissue organization was found to be of most importance in the <u>in vitro</u> conversion of inorganic iodide to diiodotyrosine and thyroxine. Little formation of either compound was observed when tissue organization was disrupted by homogenization, a finding that suggests the participation of an intracellular enzyme system in these syntheses (40).

Thyroglobulin per se does not appear in the blood stream; hence some mechanism of release of the thyroid hormone

must be present. De Robertis (11) reported that the colloid of the thyroid follicles contained a proteolytic enzyme. He suggested the hypothesis that thyroglobulin is hydrolyzed in the lumen of the follicles and that the smaller molecules resulting become available for transport through the follicular walls into circulation.

Steady State of Thyroid Hormone

Dougherty et al. (12) showed that the uptake of iodine and release of hormonal iodine are in a steady state. When rats were injected with radioactive iodine, it was found that on a low or normal iodine diet, the uptake and release of iodine-containing hormone occurred simultaneously, continuously, and at an approximately constant rate.

With normal humans, Inbar and Freinkel (25) found that the rate of disappearance of thyroxine from the blood stream due to peripheral degradation and the rate of release of thyroxine from the thyroid gland were almost the same. A correlation of 0.84 ± 0.19 was found between the two rates.

Regulation of the Thyroid Gland

Nervous regulation. Williams (63) transplanted thyroid tissue into the ears of rabbits. When stained sections of a graft 49 days old with no nerves were compared with sections of normal thyroids, no significant histological difference was noted. Uotila (61) confirmed this observation by

removing the nerves to the thyroid gland in rats. He found that nerves were not necessary for a continued histologically normal thyroid function.

Hormone regulation. Following hypophysectomy of rats a fall in metabolic rate was observed by Smith (48). When the rats were treated with transplants of anterior pituitary or dessicated thyroid, the metabolic rate was increased. On the basis of these and other similar studies, there is general agreement that the anterior pituitary secretes a hormone which specifically stimulates the thyroid gland; and this has been called thyroid-stimulating hormone, thyrotrophic hormone, or thyrotrophin.

Leblond and Sue (34) observed that hypophysectomized rats concentrated much less of the administered tracer iodine in their thyroids than did intact controls. Also noted was that the thyroids of guinea pigs previously treated with thyrotrophin trapped more iodine in their thyroids than untreated controls.

Taurog et al. (56) injected normal and hypophysectomized rats with I^{131} and then measured radioactive thyroxine in the gland. It was found that hypophysectomy reduced thyroid thyroxine, but it was increased within seven hours after a large injection of thyrotrophin. Three injections of thyrotrophin restored the I^{131} thyroxine content of newly-formed thyroglobulin almost to normal.

Keating et al. (27) injected I^{131} into cockerels and measured the normal rate, and the rate with injections of thyrotrophin, at which I^{131} was released from the gland. This was done to obtain a measure of the thyroid hormone secreted by the gland. It was found that thyrotrophin produced a prompt acceleration in the rate of loss of iodine previously stored in the thyroid.

Factors Affecting Thyroid Activity

Thyroxine. Pipes and Ruppert (43) administered I^{131} to dairy cattle and measured its release from the gland, presumably thyroid hormone secretion. It was found that a single intravenous dose of 0.5 mg. of L-thyroxine per 100 lb. of body weight inhibited thyroid activity for 3 to 4 days in mature animals. Astwood (5) found that atrophy of the gland resulted if thyroxine treatment was continued long enough.

Thiouracil. This drug and others have been shown to prevent thyroxine synthesis by the thyroid gland. The anatomical changes that occur in the thyroid gland following the administration of thiouracil are a rapid development of hypertrophy and hyperplasia. These changes were found to be identical with those following the administration of thyrotrophin. This was taken to mean that the goitrous thyroid following the administration of thiouracil resulted from the

action of thyrotrophin (5, 29).

Iodine. A dietary deficiency in iodine and a consequent deficiency in thyroid hormone synthesis lead to compensatory changes in the thyroid similar to those resulting from thiouracil. In contrast, if there is a dietary excess of iodine, thyroid hormone synthesis is reduced and the thyroid gland atrophies (5, 64).

Environmental temperature. Uotila (61) observed with normal rats that exposure to cold (5° to 6°C.) resulted in an increased output of thyroid hormone by the thyroid. The basal metabolism was increased so that a fatal drop in body temperature was prevented. This did not occur with hypophysectomized rats; thyroid atrophy occurred which could not be compensated by cold stimulation. Therefore, it was concluded that there is a nervous stimulation of the anterior pituitary that causes it to release thyrotophin and that the hormone stimulates the thyroid.

Dempsey and Astwood (10) administered ad libitum thiouracil as 0.1 per cent solution in the drinking water of rats. Graded doses of D, L-thyroxine were injected daily; and as dose increased, the thyroid became smaller. The dose that maintained a normal thyroid weight was interpreted as the normal daily D, L-thyroxine secretion rate. This technique for determining the thyroid hormone secretion rate is called the goiter-prevention method. The D, L-thyroxine

secretion rate was determined at various environmental temperatures. It was found that at 33.8° ± 1°F., 9.5 mcg. of D, L-thyroxine daily were necessary to maintain normal thyroid weight. At 95° ± 1°F., only 1.7 mcg. of D, L-thyroxine daily were necessary to maintain normal thyroid weight.

Using what is called the extrapolation technique, many workers have estimated the thyroid activity under seasonal environments. This method, different from the preceding one, does not require destroying the animal. It involves the intravenous injection of I^{131}, followed by the oral administration of thiouracil to prevent reutilization of I^{131} by the thyroid gland. Direct counts of radioactivity in the thyroid region are made while daily injections of L-thyroxine in increasing levels are given. The thyroid hormone secretion rate is then measured as the amount of L-thyroxine that must be given daily to suppress the output of previously collected I^{131} from the gland. The thyroid secretion rates, expressed as mg. L-thyroxine per 100 lb. of body weight, found with dairy cattle are 0.2 to 0.7 in the winter and 0.1 to 0.3 in the summer (44); 0.077 to 0.195 in the fall and 0.070 to 0.205 in the spring (21).

Freinkel and Lewis (15) studied the effect of lowered environmental temperature upon peripheral tissue degradation of injected I^{131}-labeled L-thyroxine in sheep. The temperature

difference was produced by using sheep that were shorn and others unshorn. Disappearance of labeled L-thyroxine from the blood stream occurred at two rates. The first disappearance rate was very quick, and this was attributed to the distribution of the thyroxine into tissues and fluids. The second disappearance rate was much slower and was attributed to the peripheral metabolic destruction and excretion of thyroxine. It was found that the biological half-life in the shorn sheep averaged 25 hours while that in the unshorn sheep averaged 38 hours. The enhanced rate of thyroxine degradation in shorn sheep was accompanied by significant histological evidence of thyroidal hypertrophy. They also found that a reduced environmental temperature lowered the circulating protein-bound iodine (PBI) in athyroidal animals maintained on constant amounts of exogenous hormone while it did not change significantly in normal animals subjected to the same environment.

Age and breed. Using the extrapolation technique upon dairy cattle, Johnson and Cheng (26) found a gradual increase in the secretion rate from one month to one year of age. The greatest increase occurred up to three months of age. With constant environmental temperatures of 50 or 80°F., Jersey calves maintained higher thyroid activity than Holstein and Brown Swiss calves.

The Nature of the Circulating Hormone

Taurog and Chaikoff (52) demonstrated that the circulating hormone can be precipitated with the plasma proteins. When crystalline thyroxine was added to plasma, it behaved like the naturally-occurring PBI under various chemical treatments, such as being soluble in butanol. When this method was used, 80 per cent of the added thyroxine was recovered; therefore, it was suggested that 80 per cent of the circulating hormone acts like thyroxine.

By the use of paper chromotography and the injection of I^{131} Tong et al. (59) found two iodine-containing components in the blood of normal rats. These were identified as thyroxine and inorganic iodide by comparing their position on the chromatograms with those made with known thyroxine and inorganic iodide. Monoiodothyrosine and diiodotyrosine were not found in the plasma of normal rats. Taurog et al., (53) by the same method as above, determined that 10 to 15 per cent of the radioiodine in rat plasma was in the form of inorganic iodide while 85 to 90 per cent was thyroxine.

Gordon et al. (16) added thyroxine to plasma and it became protein bound. The resulting thyroxine complex behaved in electrophoresis like the thyroid hormone.

Larson and Albright (30) demonstrated that the circulating hormone associated with a specific alpha globulin. It

was found that the amino, carboxyl, and phenolic groups were essential for the binding of thyroxine, with the amino group being the most significant although this also was a weak bond. Triiodothyronine was found to have less affinity for this protein.

Metabolic Fate of Thyroxine

Gross and Leblond (17) injected intravenously a large dose (0.8 mg.) of I^{131}-labeled D, L-thyroxine into rats and studied its distribution in the organs and tissues. Two hours following the injection, not more than an average of 2 per cent of the radioactivity was detectable in the entire volume of circulating plasma, but an average of more than 50 per cent of it was detected in the gastrointestinal tract (stomach and intestines), liver, and pancreas. The I^{131} was still mainly in the form of thyroxine except in the stomach, where it was demonstrated as iodide. In addition, significant radioactivity concentration was shown in the kidneys, lungs, adrenals, ovaries, and lymphatic tissue. Twenty-four hours following the injection, an average of 80 per cent of the dose was found in the feces; and approximately one-half of the fecal I^{131} was butanol soluble, presumably thyroxine. An average of 11 per cent of the dose was determined as inorganic iodine in the urine.

These investigators also demonstrated that the route of entry of thyroxine into the gastrointestinal tract was

mostly by way of the liver and bile duct. Segments of the gastrointestinal tract and the bile duct were ligated. This resulted in an increase in the radioactivity of the liver and blood, while a corresponding decrease in radioactivity was found in the intestine.

In another experiment Gross and Leblond (18) injected intravenously 0.01 to 20 mcg. of I^{131}-labeled L-thyroxine into rats. Distribution was found to be similar to that reported in the preceding experiment, although in lower proportions. As the dose increased, both the percentage of the dose excreted in the feces and the proportion present as thyroxine-like material increased. Tracer doses were excreted in both urine and feces, mostly in a non-thyroxine form, and 75 per cent was destroyed in 24 hours.

Monroe and Turner (39) using the goiter-prevention method, analyzed urine and feces of thyroxine-injected goats for thyroidal activity. The goats were injected subcutaneously with 10 mg. of D, L-thyroxine daily for a period of 10 days. Chicks were used for the assay, and the addition of thiouracil to the normal ration caused marked hypertrophy of their thyroid glands. The inclusion of normal goat urine or feces in the feed of the chicks did not counteract the hypertrophy. It was concluded, therefore, that thyroxine per se is not normally excreted in significant amounts. Feces collected from the treated goats during the first five days

of the injection period made only a slight reduction in thyroid size. The feces collected during the second five days of the injection period caused a marked reduction in thyroid size. It was suggested that the difference observed may have been the result of some initial retention of the hormone or of a greater amount of hormone destruction in the first five days. Urine from treated goats did not counteract the hypertrophy. It was suggested that thyroid hormone destruction predominated over excretion, since only 5 per cent of the injected dose of D, L-thyroxine was accounted for in the feces.

Monroe and Turner (39) also injected single, increasing doses of D, L-thyroxine into thiouracil-fed chicks. The time between the injection and the appearance of known effects of thiouracil was prolonged with increasing doses of D, L-thyroxine up to a certain dose, beyond which the time interval remained the same. It was suggested that dosages above the normal level stimulate mechanisms, probably in the liver, to destroy increasing amounts of the hormone.

Taurog et al. (54) injected I^{131}-labeled L-thyroxine into rats. Upon the intravenous injection of 100 mcg., more than 70 per cent of the injected dose appeared in the bile in 26 hours. Following intravenous injection of 15 mcg., 50 per cent was recovered in 24 hours. In view of this it was suggested that large doses of thyroxine are more rapidly metabolized. During the first hour after an intravenous

injection, practically all the I^{131} that appeared in the bile was in the form of thyroxine, as determined by paper chromatography. However, during the second hour after an intravenous injection and during the first hour after a subcutaneous injection, the labeled thyroxine appeared mostly in the form of an unidentified component which these workers designated as compound U.

Albert and Keating (3) injected intravenously physiological quantities (0.25 and 0.6 mcg.) of I^{131}-labeled L-thyroxine into rats. One minute after injection 38 per cent of the radioactivity was in the entire blood volume, 30 per cent in the liver, 9.5 per cent in the gastrointestinal tract, and the remainder in the rest of the carcass. Following the initial distribution, radioactivity increased in the gastrointestinal tract, due to diffusion chiefly by way of the bile, but also by direct secretion as shown by the initial distribution. For the first few hours after injection, the ratio of liver to blood concentrations remained about the same, although they both went down. Following equilibrium with the gastrointestinal tract, the liver was observed to concentrate thyroxine, for there was an accumulation of radioactivity which was greater than that in the blood. After 16 hours, the liver contained more than one-half of the total radioactivity in the body. Reabsorption of thyroxine or I^{131} from the intestines and the rapidity at which this

occurs were emphasized by the fact that more than 100 per cent of the radioactivity was secreted into the intestines per hour while only 3 per cent per hour left the intestines in the feces.

Albert et al. (2) reported on the absorption of thyroxine from isolated portions of the gastrointestinal tract of the rat. Using I^{131}-labeled L-thyroxine, these workers reported 20-25 per cent absorption from the small intestine.

Taurog et al. (55) injected subcutaneously 80 mcg. of I^{131}-labeled L-thyroxine into rats. The radioactive iodine in the 0 to 21 hours' sample of urine was 95 per cent inorganic iodine and 5 per cent compound U, as shown by paper chromatography. The 0 to 44 hours' sample of bile accounted for 50 to 60 per cent of the injected I^{131}, while after 44 hours the feces contained 42 to 48 per cent of the injected I^{131}. Therefore, it appears that there was a small amount of reabsorption, since the amount of injected I^{131} appearing in the feces was smaller than the amount that appeared in the bile. Chromatograms of the feces showed that they contained considerable amounts of inorganic radioactive iodine. Thyroxine was also shown to be present, but compound U was completely absent. Since it is known that the most common detoxifying agent of phenolic compounds in the animal body is glucuronic acid, compound U was treated with the enzyme β-glucuronidase. The results showed that it was very active in splitting the

excretion product of the bile, with the formation of thyroxine, suggesting that compound U is a glucuronide of thyroxine. Intestinal contents removed from the lower small intestine and from the large intestine had the same effect, while enzymes of the upper intestinal tract did not. It was hypothesized that Escherichia coli, which are present in the lower intestinal tract, could act as a plentiful source of β-glucuronidase. Physiological doses of I^{131}-labeled L-thyroxine showed bile I^{131} in the form of compound U, and it was suggested that this might be a normal physiological process.

These workers also incubated tissue slices of liver, kidney, spleen, heart, diaphragm, and brain, to which were added 2.5 mcg. of I^{131}-labeled L-thyroxine. After 2 hours, formation of large amounts of compound U had occurred in the liver slices. Activity was also noted in the kidney slices, but all other tissues gave negative results.

Briggs et al. (7) obtained bile from rats injected with 100 to 125 mcg. of I^{131}-labeled L-thyroxine which contained both compound U (approximately two-thirds) and thyroxine (approximately one-third) as revealed by paper chromatography. This was slowly infused by way of the bile duct into the duodenuna of recipient rats, and approximately 30 per cent of the radioactivity was absorbed. The bile secreted by the recipient rats contained approximately one-third of the

absorbed radioactivity, which meant that of the iodine secreted into the bile following the injection of thyroxine, only about 10 per cent underwent enterohepatic circulation.

In another experiment, Briggs et al. (7) added purified samples of I^{131}-thyroxine or I^{131}-compound U from bile to nonradioactive bile. The bile containing added I^{131}-thyroxine was infused into the duodenuna of recipient rats by way of the bile duct. The absorption of I^{131}-thyroxine varied from 41 to 69 per cent, and the bile collected contained 17 to 31 per cent of the administered I^{131}. In like manner with the bile containing I^{131}-labeled compound U, I^{131} was absorbed to the extent of 24 to 40 per cent and the bile collected contained 9.6 to 20.3 per cent of the administered I^{131}.

O'Neal (41) injected intravenously D, L-thyroxine in thyroidectomized dogs. The disappearance rates of plasma PBI into its volume of distribution were not significantly different upon the injection of 500 or 5000 mcg. of thyroxine. Disappearance of PBI due to metabolic destruction and excretion upon the injection of 500 and 5000 mcg. of thyroxine, however, resulted in an average half-life of 24.6 and 9.4 hours respectively. At 50 hours the dogs had disposed of 94.1 per cent of the larger dose and 75.4 per cent of the smaller dose. It was suggested that the dog is able to rid itself of excess hormone because of the increased rate of disposal of the large dose of thyroxine from the blood stream.

Aside from thyroxine and iodide Gross and Pitt-Rivers (20) found that two other unidentified compounds existed in the body and identified one as L-triiodothyronine. Experimental evidence since has shown that deiodination of thyroxine to triiodothyronine took place in rat kidney slices *in vitro* (4). Triiodothyronine has been found to be more active metabolically than thyroxine, and it was hypothesized that this may be the active form of the thyroid hormone at the cellular level (23).

Van Arsdel *et al.* (62) injected intravenously 5 to 8 microcuries of I^{131}-labeled L-thyroxine and I^{131}-labeled L-triiodothyronine into rats. At intervals studied, the concentration of triiodothyronine in plasma was much lower than that of thyroxine; and somewhat lower also in the liver, except within 5 minutes after administration when it was significantly higher. Triiodothyronine was present in higher concentrations in kidney tissue and skeletal muscle during the first 12 hours after administration when its concentration dropped to below that of thyroxine. It was also noted that thyroxine concentration in the liver increased to levels above the plasma 3 to 6 days following the administration of labeled thyroxine.

Flock *et al.* (14) studied the metabolism of L-thyroxine and L-triiodothyronine in the dehepatized dog. Deiodination was markedly diminished. The total amount of I^{131} excreted

in the urine by the dehepatized dog was found to be about the same as in the normal dog, though very little was excreted as inorganic iodide. Larger amounts of intermediary compounds were found in the tissues and urine of the dehepatized dog. However, the presence of radioactive inorganic iodide in tissues suggested that extrahepatic tissues have a potential deiodination capacity, for they were able to deiodinate about the same percentage of injected compound, whether 25 mcg. or 25 mg. doses were given. Thyroxine, inorganic iodide, and an unidentified compound were excreted in the urine by the dehepatized dog after the administration of thyroxine, but the most abundant compound was the glucuronide of thyroxine. In contrast, the urine of dehepatized dogs injected with triiodothyronine contained a large amount of inorganic iodide, with small amounts of both free triiodothyronine and glucuronide of triiodothyronine, although the most abundant compound in the urine was the same unidentified compound mentioned previously. The urine from normal dogs injected with both compounds contained large amounts of inorganic iodide. Within the first few hours after thyroxine administration, however, sometimes free thyroxine was excreted.

Reports have appeared recently that tetraiodothyropyruvic acid and triiodothyropyruvic acid have been found in bile and urine of rats. Roche et al. (45) stated that this

probably occurred by deiodination and oxidative deamination of the alanine residue of the thyroid hormone through an enzyme system. Continued experimentation was carried on to determine if there was further degradation. Thyroidectomized rats were injected with physiological doses of I^{131}-labeled L-triiodothyronine. Analysis of kidney tissue extracts revealed that the injected hormone and three of its derivatives were present: iodide, diiodothyronine, and triiodothyroacetic acid. It was suggested that diiodothyronine was produced by deiodination of triiodothyronine and that triiodothyroacetic acid was produced by oxidative decarboxylation of triiodothyropyruvic acid. The presence of diiodothyronine has special significance because it can be destroyed by oxidases effective on thyronine and on moniodothyronine, but not on thyroxine and triiodothyronine. Therefore, a hypothesis for the metabolism of thyroid hormones was suggested; it may take two pathways, deiodination followed by rupture of the phenoxy bond, with or without oxidative degradation of the alanine residue.

Tomita et al. (58) demonstrated the enzymatic conversion of thyroxine to tetraiodothyroacetic acid and triiodothyroacetic acid by the mitochondrial fraction of the kidney of the rat.

Barker (6) compared the metabolic activity resulting from pharmacologic doses of L-triiodothyronine, L-thyroxine,

and L-triiodothyroacetic acid administered to rats 4 days premortem. Triiodothyroacetic acid was about a quarter more active than thyroxine, but far short of triiodothyronine, which was more than four times as active as thyroxine.

Larson and Albright (31) injected 0.01 mcg. each of I^{131}-labeled L-thyroxine, I^{131}-labeled L-triiodothyronine, and I^{131}-labeled L-triiodothyroacetic acid into rats and studied their distribution. The experiment revealed that triiodothyronine and triiodothyroacetic acid left the plasma much more rapidly than thyroxine. Triiodothyronine reached high levels in the kidney, liver, heart, and skeletal muscle, while triiodothyroacetic acid accumulated only in the liver. The initial levels were higher than thyroxine, but rapidly fell to a lower concentration. More triiodothyroacetic acid was excreted unchanged in the feces than in the urine. Approximately 19 per cent was deiodinated and the iodide was excreted in the urine. Almost the same amount of triiodothyronine was excreted unchanged in the urine and feces, but 55 per cent was deiodinated and appeared as iodide in the urine. Since triiodothyroacetic acid was not observed in the tissues studied, it was suggested that this may be the reason for the weak metabolic effect exhibited by administration of exogenous triiodothyroacetic acid.

Absorption of Orally Administered Thyroidally Active Compounds by Ruminants

Turner and Reineke (60) studied the effect of the route of administration of thyroidally active compounds upon their utilization. The physiological effect was measured by the decline in body weight of sheep during a two-week period. Thyroprotein was administered subcutaneously and orally. It was found that 5 per cent of the thyroxine in the thyroprotein absorbed from a subcutaneous injection was absorbed from the digestive tract following oral administration. Repeating the same comparison with D, L-thyroxine, it was observed that the oral administration was 12.5 per cent as effective as the subcutaneous route. Preparations of thyroprotein were administered directly into the abomasum of sheep and this method was compared with administration by mouth to determine the destruction of thyroprotein by the rumen. Body weight reduction was the same in both cases, whereupon it was concluded that the low oral biological value of thyroprotein was not due to destruction in the rumen.

Monroe and Turner (39) fed 1.5 gm. of thyroprotein, containing 25.5 mg. D, L-thyroxine to goats, daily for three weeks. During this period the feces were collected and assayed for thyroidal activity, using the goiter-prevention method with chicks. The difference in activity between the thyroprotein in the feed and that in the feces was taken to

indicate the extent of absorption or metabolism of the thyroprotein. This was found to be between 78 and 88 per cent in terms of an equivalent amount of D, L-thyroxine.

Mixner and Lennon (37) fed a single dose of 25 gm. of thyroprotein, containing 265 mg. of thyroxine, to lactating Holstein cows. At a different time the cows were injected intravenously with L-thyroxine. The PBI increase per milligram of thyroxine administered was determined for the thyroxine from both sources. The injected thyroxine was rated as 100 per cent efficient, and the relative mean efficiency of absorption of the thyroxine in thyroprotein was calculated to be 12.3 $\pm$ 1.33 per cent.

Mixner and Lennon (38) in the same manner studied the absorption of L-thyroxine and thyroxine in thyroglobulin. Single doses of 244.8 or 367.2 mg. of L-thyroxine and 1000 or 750 mg. of thyroglobulin were administered to one Guernsey and four Holstein lactating cows in veterinary capsules. The mean oral efficiency of absorption of thyroxine was 11.62 per cent and of thyroglobulin was 15.68 per cent.

Observations Upon the Stimulus to Lactation of Cows Fed Thyroidally Active Compounds

Seath et al. (47) fed thyroprotein to dairy cows from approximately the fifth through the last month of lactation. Dosages fed were 12 to 20 gm. daily, depending on initial body weight, response to milk production, and changes in body

weight. An increase in milk production was obtained for the first five and six weeks of treatment; but following this, control cows averaged higher in production. An average body weight loss of 32 lb. occurred during the first 8 weeks, and then the average weight held constant among the treated cows. During the same period the control cows gained 36 lb.

Reece (46) fed dairy cows, past their lactational peaks, 15 gm. of thyroprotein daily for 12 or more weeks. An increase in milk production was observed, with the highest weekly average production occurring during the second week of thyroprotein feeding. A gradual decrease followed, until the tenth week when daily milk production was nearly identical to that of the week prior to thyroprotein feeding.

Leech (32) fed 15, 20, and 25 gm. of thyroprotein per day to dairy cows. Treatment was generally started 12 weeks after calving and continued until the cow went dry, but in any case for not longer than 23 weeks. Milk yields of the treated groups exceeded the control group and were greatest during the third to fifth week of treatment. Response to the 15 and 20 gm. doses then gradually decreased, and vanished after 11 weeks of treatment. In contrast, the groups of cows receiving 25 gm. appeared to maintain a gain in milk yield so long as the treatment continued. There

was, however, a slight decrease in production following the initial response, and thereafter the decline was parallel to that of the control cows. Because milk yield response disappeared with the lower dosage levels of thyroprotein, it was explained that these doses cancelled thyroid activity and, therefore, were not effecting a permanent increase in metabolic rate. If milk yield is a function of metabolic rate per se, then a permanent increase in milk production would not be expected in the presence of a normal metabolic rate. It was postulated that with 25 gm., a deactivation of the thyroid gland as well as a rise in blood thyroxine would result in a permanent rise in milk yield. Body weight loss was found to be approximately proportional to dose given. This effect was found to pass off by the end of 23 weeks of treatment, when the body weight of treated animals equalled that of control animals.

Swanson (49) fed 15 gm. of thyroprotein to dairy cows per day, starting when a definite decline in milk production occurred and continuing until the end of lactation. Concentrates were fed to treated animals to maintain uniform physical condition of all cows. A peak milk response was noted in the third and fourth week, which declined to control levels by the tenth week of treatment. Although the concentrate ration was increased, a body weight loss

occurred in the treated animals. Weight gains were resumed when the production stimulus was no longer in effect. Histologically, the thyroid glands of treated cows were almost inactive.

Thomas and Moore (57) fed thyroprotein to dairy cows, from approximately the second through the last month of lactation. Extra feed was given to both treated and untreated animals. An increase in milk production and a decrease in body weight was observed in the animals treated. Treated cows lost an average of 24 lb., while control cows gained an average of 94 lb. The treated animals began gaining body weight, however, when the production stimulus declined.

Aitken et al. (1) started feeding 20 gm. of thyroprotein or 80 mg. of L-thyroxine at the beginning of the seventeenth week of lactation to Red Polls and Ayrshires. The thyroxine was fed for 15 weeks and was gradually withdrawn during the last 3 weeks. Feed was provided in excess of production requirements. Milk production increased to a maximum between the fourth and fifth week of treatment. Thereafter, an accelerated reduction in yield followed. With the Red Polls, however, the decline was not as great. The Ayrshires lost weight during the treatment period; the Polls gained, but considerably less than did the controls. It was suggested that the lesser decrease in milk production during treatment was due to the Red Polls'

being fleshier, so that there was an extra reserve available for conversion to milk. The fact that the hormonal effect had diminished half way through the treatment prompted the suggestion that a natural reaction of the organism occurs to offset the excessive drain from body reserves caused by the enhanced yield. It was suggested that such a compensating effect might operate at all except excessive levels of administration.

The effect of feeding various levels of L-thyroxine upon milk production was reported in a thesis by Miller (36). The dosages fed were 60, 80, and 100 mg. per day. The cows were fed one of the dose-levels for 20 days, followed by no treatment for 10 days; and the remaining two dose-levels were fed and stopped in the same time sequences. In another experiment, 100, 120, and 140 mg. were compared in the same manner. The results showed that the 20-day response of increased milk production was correlated to dosage up to the 100 mg. level, beyond which the response was no greater than at the 100 mg. level.

Summary of Literature Review

Thyroxine, which is an amino acid, is synthesized under the influence of intracellular enzymes by the iodination of tyrosine and the coupling of two diiodotyrosine molecules. After thyroxine is synthesized it either goes directly into circulation or is stored in the lumen

of the thyroid follicle in a protein complex, the thyroglobulin.

Thyrotrophin, a hormone produced by the anterior pituitary, regulates the ability of the thyroid to concentrate iodide from circulation, to synthesize, and to discharge thyroid hormone. There is a system of balances also regulating thyroid activity. High amounts of circulating iodine and thyroxine suppress thyroid activity and eventually cause thyroid atrophy or reduced activity. With low amounts of circulating iodine and subsequently low production of thyroxine, thyroid hypertrophy and hyperplasia will result in an effort to overcome the deficiency. This same thyroid condition occurs upon the administration of thiouracil, which blocks thyroxine synthesis. In both cases it has been shown to be due to an increased production of thyrotrophin. Moreover, thyroid gland activity varies inversely with environmental temperature. The effect of environmental temperature results from a nervous stimulation of the anterior pituitary in the production of thyrotrophin.

Most of the circulating protein-bound iodine is in the form of L-thyroxine which is weakly associated with the alpha globulin.

Concerning the metabolic fate of thyroxine, it is deiodinated in the tissues, primarily in the liver, with

deiodination possibly followed by the rupture of the phenoxy bond of the thyroxine molecule. Degradation of the alanine residue of thyroxine and its intermediate, triiodothyronine, to their pyruvic and acetic analogues has been observed, and it is a matter of conjecture whether these are the active forms of the hormone at the cellular level. Thyroxine and its intermediates that escape destruction are conjugated (as glucuronides) by the liver, excreted in the bile with inorganic iodide, and then either absorbed or excreted in the feces. The conjugates, however, are hydrolyzed to the free state before excretion in the feces, whereas they are not when excreted in the urine. With endogenous and physiological doses of thyroxine, in contrast to large doses, the most abundant compound excreted is inorganic iodide with very little of thyroxine and its intermediates. The evidence indicates that the animal body is able to rid itself of excess thyroxine. An increased rate of disposal from the blood stream and in the feces and urine has been observed with large doses.

Oral administration of thyroidally active compounds is not as effective as administration by injection. The figures on absorption by ruminants are complicated by the facts that some hormone destruction occurs in the gastro-intestinal tract, that absorbed hormone may be excreted back through the intestinal wall, and that the hormone may undergo

enterohepatic circulation and then be excreted directly or reabsorbed.

Upon the feeding of thyroidally active compounds to dairy cows, an increase in milk production occurs, followed by a decline. The decrease in milk production is more rapid than in normal lactation, suggesting a reduction in persistency. The evidence indicates that this is due to a compensating physiological reaction in the cow; but whether this reaction is due to an excessive loss in body weight or to the effects of the thyroactive substances or secondary hormone effects is not known.

EXPERIMENTAL PROCEDURE

Studies of the plasma protein-bound iodine and excretion of thyroidally active material were made with L-thyroxine-fed dairy cows in the University of Tennessee herd. The purpose of the study was to determine the effect of dose-level and length of time thyroxine is fed to cows upon the amounts of thyroxine equivalents in blood and feces.

Cows and Thyroxine Dosage Plan

Four lactating cows, one set of identical Guernsey twins (nos. 9 and 10), one Jersey (no. 23) and one Jersey-Holstein cross (no. 51), were administered oral doses of sodium L-thyroxine (hereafter referred to as thyroxine) during the summer months. The twins (9 and 10) were fed 110 mg. per day for 15 weeks. The two other cows (23 and 51) were fed varying daily dosages of thyroxine with the level changed weekly: they were started at 60 mg. per day for the first week, with 80, 100, 120, 140, 160, 100, 80, and 80 mg. following in consecutive weeks. The twins were 6 years old and in their fourth lactation during this experiment. Treatment was started the 140th and 175th day of lactation with cows 9 and 10 respectively. Cow 23 was 7 years old and in the fourth lactation. This cow had had mastitis several times previous to thyroxine treatment, which started the 132nd day of lactation. Cow 51 was 2 years old and in the first

lactation. Treatment started the 133rd day of lactation. The physical condition of all the cows was good at the beginning of treatment. The thyroxine was first mixed in corn starch, which was then mixed with soybean meal both for the purpose of diluting the thyroxine. One half of the daily dose for each cow was weighed out and put in separate small envelopes, and one of these half-doses was fed along with the regular grain ration at the morning, and one at the evening milking (12 hr. intervals).

Collections of Feces and Blood

During collections of feces the cows were kept in tie stalls and were fed a good quality hay. Feces from cows 9 and 10 were collected for a 24-hour period once a month. From 23 and 51, weekly collections were made during the last 24 hours they were on each particular dosage level. Feces were also collected from the cows before and after feeding of thyroxine. After each collection period, the feces from each cow were weighed separately wet, and about half of the total was weighed into shallow pans. They were then dried in a forced-air oven at 45°C., after which an air-dry weight was taken. From the weight of the wet and air-dry feces, an air-dry percentage was calculated. This was multiplied by the total wet feces excreted, and the total air-dry weight of the feces was determined. The dried

feces were ground in a Wiley Mill, labeled, and stored in paper bags until assayed.

During the afternoon of each feces collection period, about 10 ml. of blood were collected from the jugular vein of each cow into a test tube containing 0.05 cc. of heparin solution. Plasma from this blood was used for PBI determination. The method of determining PBI was that of Brown (8) as revised by Lennon and Mixner (33) and modified for use with an Evelyn photoelectric colorimeter.

Materials and Methods

The goiter-prevention method as described by Dempsey and Astwood (10) and by Monroe and Turner (39) was used for the biological assay of the feces for thyroid hormone activity. One day-old white Leghorn cockerels purchased from a local hatchery were used in these assays. Usually 200 chicks were used at one time. The chicks were housed in brooder cages and were divided into groups of 10 to 20 each. In each lot of chicks used for assays one group served as control and the other groups received thiouracil plus thyroxine or feces. Graded levels of thyroxine plus thiouracil were mixed with the basal ration in a tumbler mixer. Each collection of feces was treated in a similar manner. The assay trials were conducted for three weeks. At the end of each trial the chicks were killed by ether asphyxiation and the body weights were recorded in gm.

The thyroids were dissected out and collected on moistened filter paper in a covered petri dish. The combined weight of the thyroid glands from each chick was determined on a precision balance to the nearest tenth of a mg. and coverted to mg. per 100 gm. of body weight. The mean thyroid weight per 100 gm. body weight was calculated for each group of chicks.

Preliminary assays. The purpose of the preliminary assays was to establish a range of thyroxine dosage that would permit the determination of the level that maintains normal size thyroids, as well as for production of above normal sized thyroids to eliminate as much error as possible in the thyroidectomy.

Rations of simpler formula, lower quality or lower in iodine were formulated and compared with a commercial turkey poult starter. The latter was a very complex formula, with added antibiotics, B-complex vitamins, and trace minerals including iodine. Thiouracil was fed at a level of 0.10 per cent of the ration, and the hover heat (90° to 95°F.) of the brooders was left on for the duration of the trial. This trial was conducted in an attic room at a time when the days were quite warm and the nights were cool. There was very little difference in the average thyroid gland weights from the chicks on each ration, indicating very slight effect of the feed upon the reliability of the assays.

A thyroxine standard trial was conducted in the fall. Thiouracil was fed at a level of 0.10 per cent along with graded doses of thyroxine at 0.000005, 0.000010, 0.000015, and 0.000020 per cent of the basal ration to different groups of chicks. The basal ration in this and subsequent tests was a commercial turkey poult starter. Room temperature was kept at 75°F. overnight by thermostatically controlled heaters. Daytime room temperature was considerably warmer. The hover heat of the brooders was on for the duration of the trial. The chicks receiving thyroxine did not have thyroids of desirable size, and the spread between groups was small.

Another trial was conducted comparing three levels of thiouracil, 0.10, 0.15, and 0.20 per cent of the ration. The same graded doses of thyroxine were used as in the preceding trial. Also in this trial, duplicate groups were fed the test rations only during the last week of the three-week trial. During the initial 2 weeks the latter groups of chicks were fed the control ration. The hover temperature of the brooders was turned off after 2 weeks and room temperature was thermostatically controlled at 70°F. From the chicks fed the experimental ration for 3 weeks, approximately the same average thyroid weight resulted from 0.15 as from 0.20 per cent thiouracil, and both dosages gave larger, more desirable sized thyroids than those from

the chicks fed at the 0.10 per cent level. Mortality from the 0.20 per cent thiouracil group was high, however, and depression of body weight was observed. Also it was noted that the thyroxine dosage could not be narrowed to the extent that it was in this trial, since the variability of thyroid weights within dosage groups yielded averages that confused the interpretation of the data. The average thyroid weights from the groups fed the experimental rations 1 week showed that this was not long enough to produce thyroids of desirable size, and it did not obtain a desirable spread between the average thyroid weights of thyroxine-fed groups.

A controlled experiment was conducted to demonstrate the effect of high room temperature in reducing the production of thyrotrophin by the anterior pituitary, which subsequently reduces thyroid hypertrophy in the thiouracil-treated chicks. Two groups of chicks were administered thiouracil and thyroxine as 0.15 and 0.000010 per cent of the ration respectively. One group was kept in an air conditioned room in which the temperature was controlled to stay within the range of 73° to 75° F. The other group was kept in a non air conditioned room exposed to summer temperatures which were recorded. The average hourly temperatures are shown in Figure 1. The average thyroid gland weight for the chicks kept in the air conditioned room was 53.0 mg. per 100 gm. of body weight. In contrast, the average

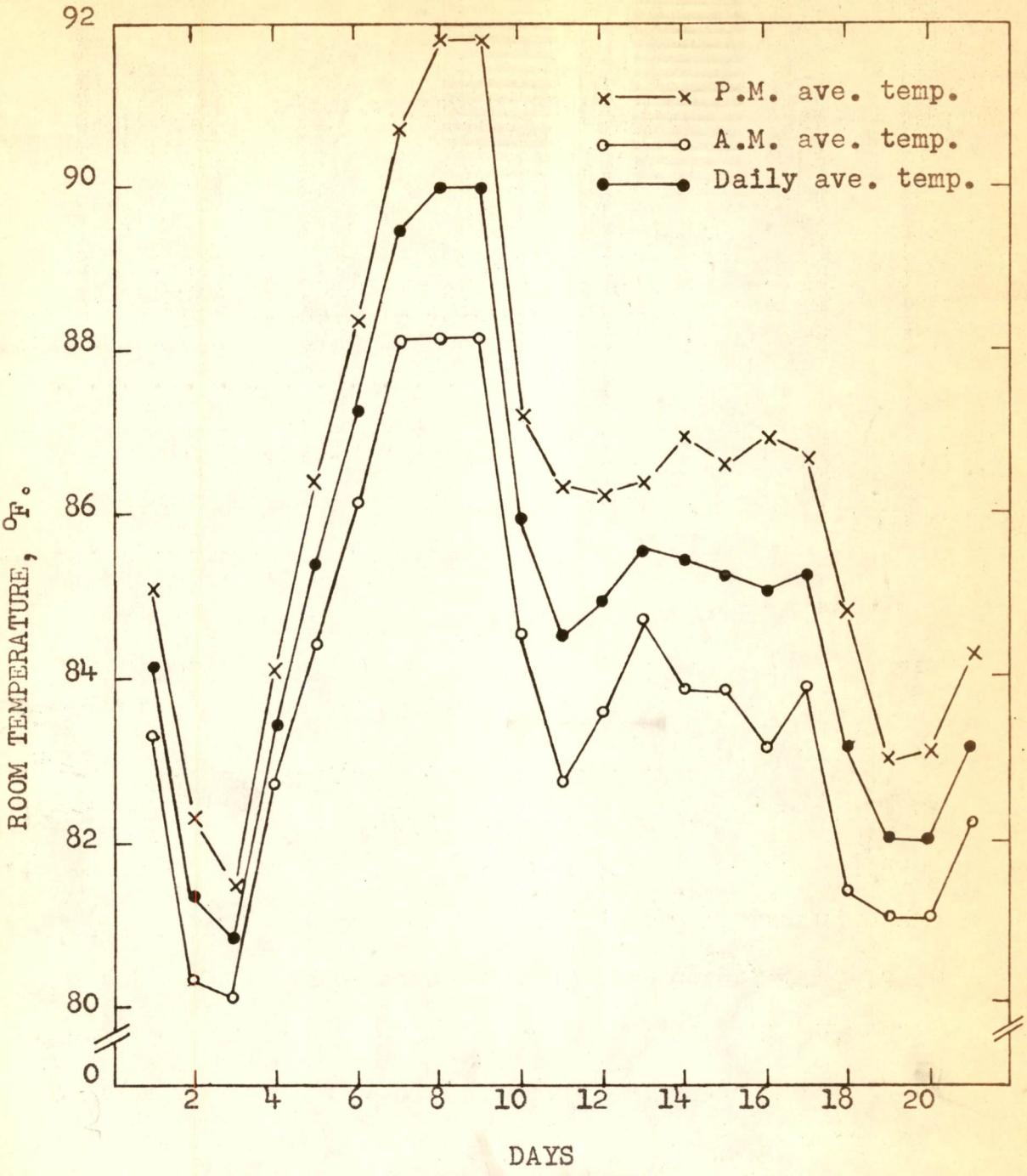


Figure 1. Average A.M., P.M., and daily temperatures of room in which the effect of summer temperatures upon thyroid size of thiouracil-fed chicks was observed.

thyroid weight of the chicks exposed to higher temperatures was 32.0 mg. per 100 gm. of body weight. This shows the importance of careful temperature control for bioassays of thyroxine.

Experimental assays. A commercial turkey poult starter was used for the basal ration because preliminary tests showed that it was a very nutritious, palatable feed. Thiouracil was fed at a level of 0.15 per cent of the feed. This amount produced maximum size thyroids without ill effects upon the chicks under the conditions of the preliminary tests. Thyroxine was fed at 0.000010, and 0.000020 per cent of the feed, for the amount of thyroxine necessary to maintain normal thyroid weight had been found to be within this range. These thyroxine standards were included in each lot of chicks assayed because of the variability of the amount of thyroxine that would maintain normal thyroid weight with different environmental temperatures of different lots of chicks. Levels of feces included in the feed were regulated according to the level of thyroxine the cow was receiving at the time of collection. Three levels were used to try to get one level producing thyroid weights within the range of the thyroxine standards. The experimental rations were fed for the complete duration of each assay period, 3 weeks. Fifteen to 20 chicks were fed each assay mixture. The hover temperature of the brooders

usually was started at 90° to 95°F. and the heat was gradually decreased until it was entirely off in the third week.

Example of Determining the Amount of Equivalent L-Thyroxine in the Feces

The average thyroid weights per 100 gm. body weight of the chicks fed thyroxine and feces were plotted against the level at which they were fed (Figure 2). The points were connected; and the point at which the line crossed the average thyroid weight of the control chicks was considered the amount of thyroxine necessary to maintain normal thyroid weight. In the trial used here for illustration this point was established at 0.0000216 per cent of thyroxine in the feed, and at 5.58 per cent of feces in the feed. From the average amount of ration consumed daily in these groups, it was determined that 4.676 mcg. of thyroxine and 1.206 gm. of feces were consumed per chick per day. Assuming that 1.206 gm. of feces contains 4.676 mcg. of thyroxine, the percentage of thyroxine in the feces was calculated and multiplied by the total amount of air-dry feces excreted (3908.30 gm.). This cow, therefore, excreted the equivalent of 15.15 mg. of L-thyroxine daily.

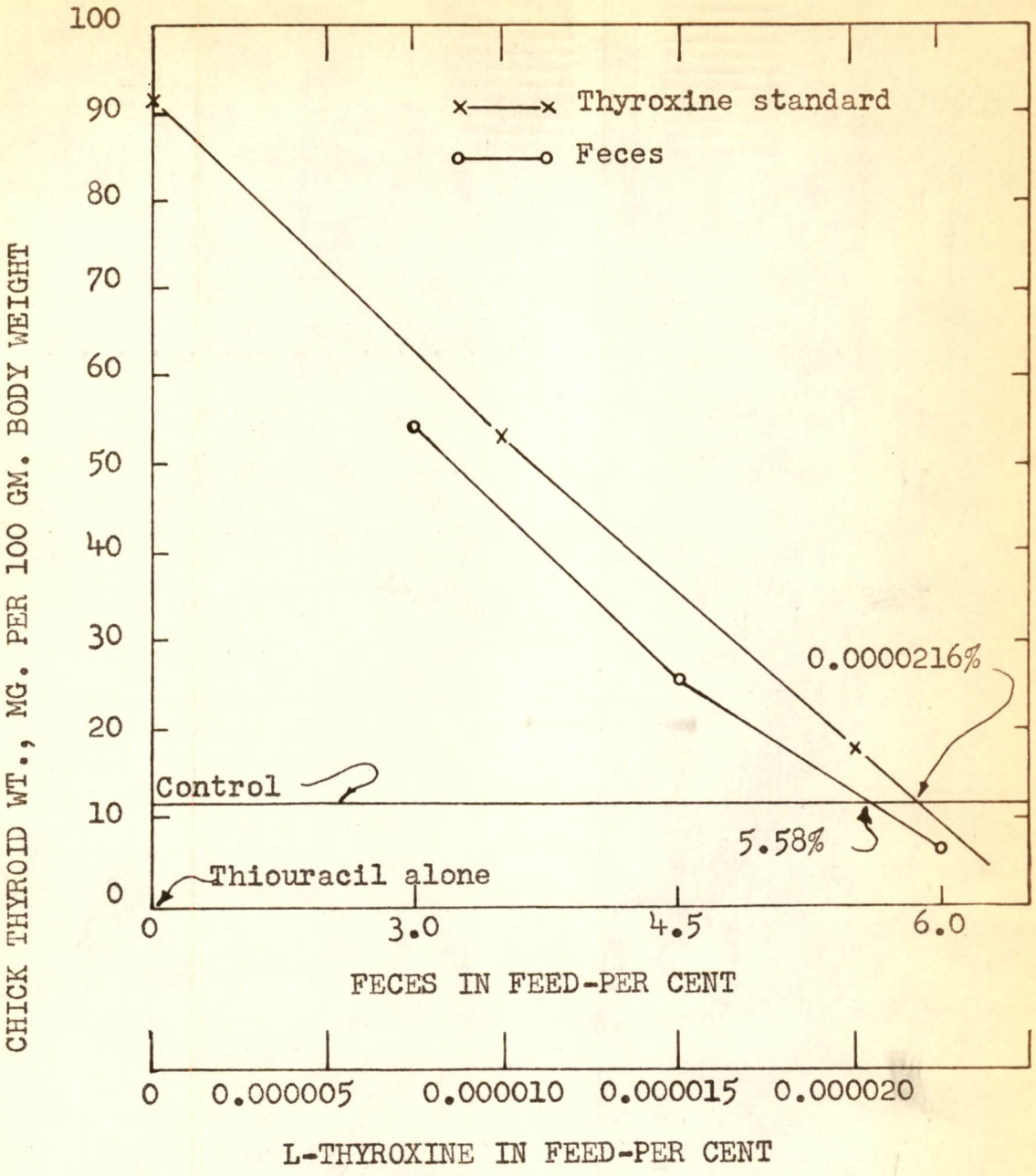


Figure 2. Thyroidal activity in feces of a cow fed L-thyroxine and the L-thyroxine standard.

RESULTS

Changing L-Thyroxine Dose-Level Weekly

In both cows, 23 and 51, the plasma PBI concentration went up when thyroxine feeding started and reached a maximum in the second week of treatment, when 80 mg. were fed daily (Figures 3 and 4). For cow 23, about the same maximum PBI concentration continued through the third week, when 100 mg. were fed daily. In cow 51, the PBI level was maintained near the maximum for the third and fourth weeks, when 100 and 120 mg. daily were fed, respectively. Cow 51 tended to have almost two times or more the PBI concentration of cow 23 throughout the experiment, even when they were not fed thyroxine. There was a general trend in both cows toward a lower PBI concentration after the maximum had been reached, even when still greater dosages were being fed each week. Within one week after thyroxine was withdrawn, the PBI concentration fell to approximately the same level as before treatment.

The actual excretion of thyroxine, although erratic, generally increased with increasing daily dose-levels. The percentage of dose excreted when increasing dose-levels of thyroxine were fed generally declined. Cow 23 excreted considerably more thyroxine than cow 51 when 60 mg. were fed daily; but under continued treatment with increasing doses, both cows tended to excrete the same quantity.

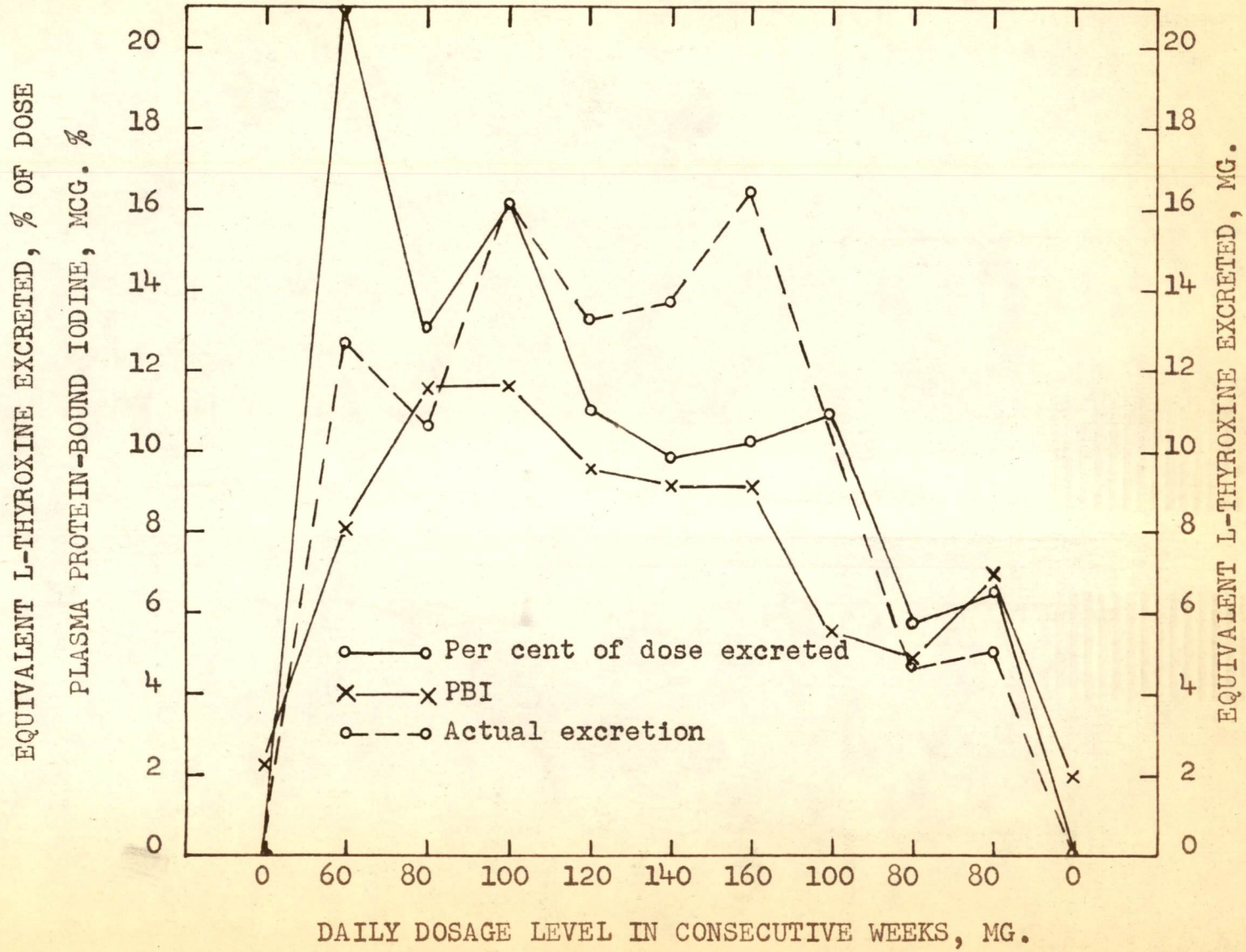


Figure 3. Effect of feeding L-thyroxine upon plasma and fecal thyroxine of cow 23.

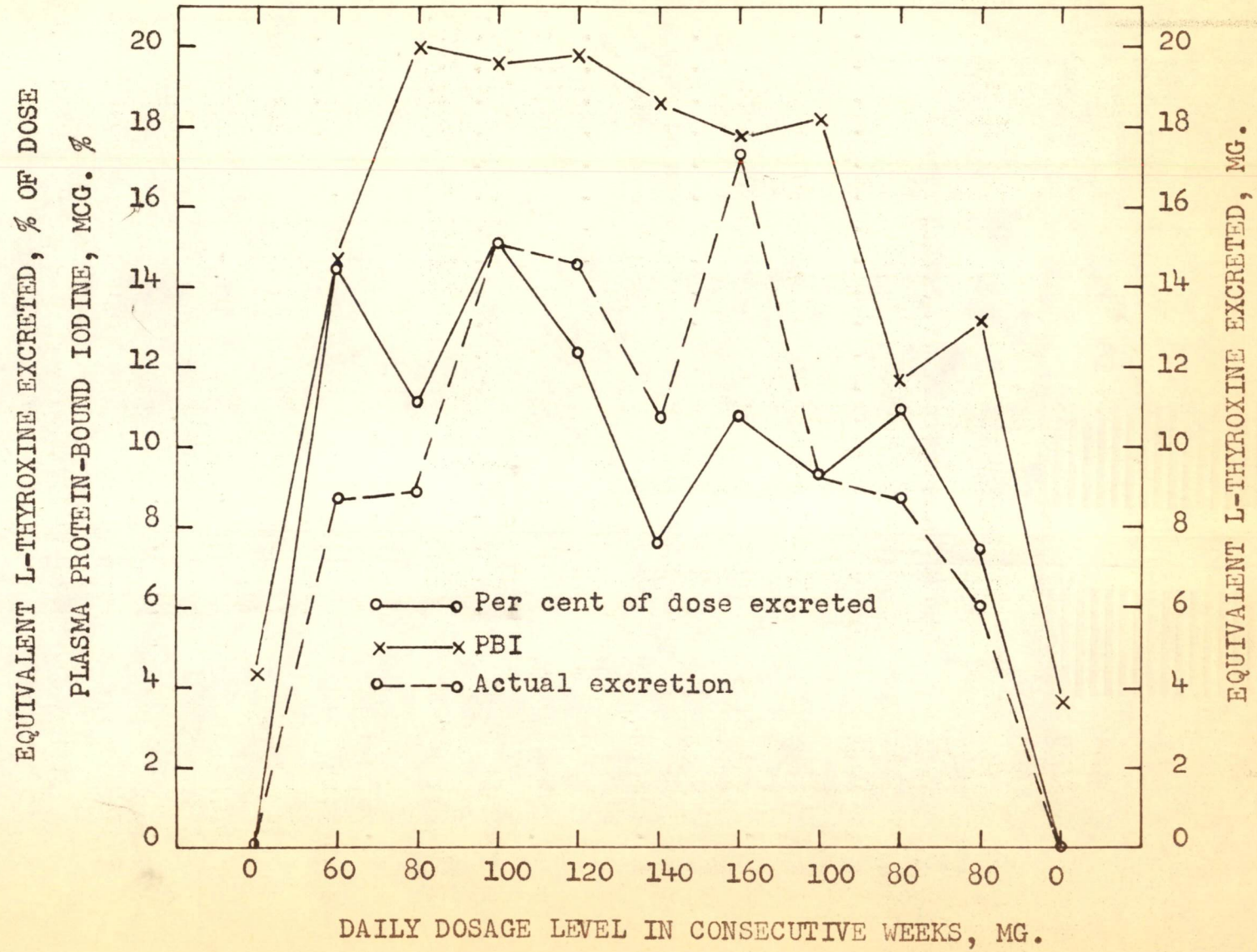


Figure 4. Effect of feeding L-thyroxine upon plasma and fecal thyroxine of cow 51.

Upon lowering the dose-level of thyroxine, actual excretion by both cows decreased. The decrease, however, followed separate patterns for the two cows. The percentage of dose excreted by cow 23 remained about the same for the first week at the lowered dose-level of 100 mg. daily, and then it dropped in the following week when 80 mg. were fed daily. With cow 51 the percentage of dose excreted remained about the same for the first two weeks, when successively lowering weekly dose-levels of first 100 and then 80 mg. were fed daily, and dropped in the following week when 80 mg. daily were still being fed. The amount of thyroxine excreted by cow 23, when 80 mg. of thyroxine were fed daily, was much lower in the latter part of the experimental period than in the initial part of the period; but with cow 51 it was about the same or only slightly lower.

The drop in excretion noted in cow 51 and not in cow 23 when 140 mg. of thyroxine were fed daily may have been due to a more extreme breakdown and destruction of thyroidally active compounds in the digestive tract. During this collection period, cow 51 had very watery feces, mild diarrhea.

Feces samples procured at control periods before and after thyroxine feeding did not contain thyroidally active compounds in significant quantities, as thyroid hypertrophy of the thiouracil-fed chicks was not reduced by such feces when fed as high as 10 per cent of the ration.

Continuous Feeding of 110 mg. of L-Thyroxine for 15 Weeks

The plasma PBI concentration of the identical twins followed parallel patterns, even though cow 9 was started on treatment 9 weeks before cow 10 (Figure 5). The trend in PBI concentration was in a downward direction as treatment continued, except for the 9 and 10-weeks' samples of blood. A reasonable explanation was not discovered for the increased PBI in both twins at that time. The percentage of thyroxine excreted in the feces collected in the third week of treatment was approximately the same as for cows 23 and 51 during the time when 120 to 160 mg. of thyroxine per day were fed, but cow 9 excreted more than cow 10. Feces samples collected from cow 9 showed continuously decreasing amounts of thyroxine as treatment progressed. Cow 10 had a sharp drop in excretion in the seventh week and returned in the twelfth week to approximately the same excretion as in the third week.

The drop in thyroxine excretion at the seventh week of treatment in cow 10 may have been for the same reason as suggested for cow 51, since 10 also had diarrhea during the collection period. Since 10 and 51 were the only cows that had diarrhea and since both had a sharp drop in the excretion of thyroxine at this collection, it is highly suggestive that diarrhea and a more extreme destruction of thyroxine in the digestive tract may be correlated. Feces samples collected one week after treatment was discontinued did not contain

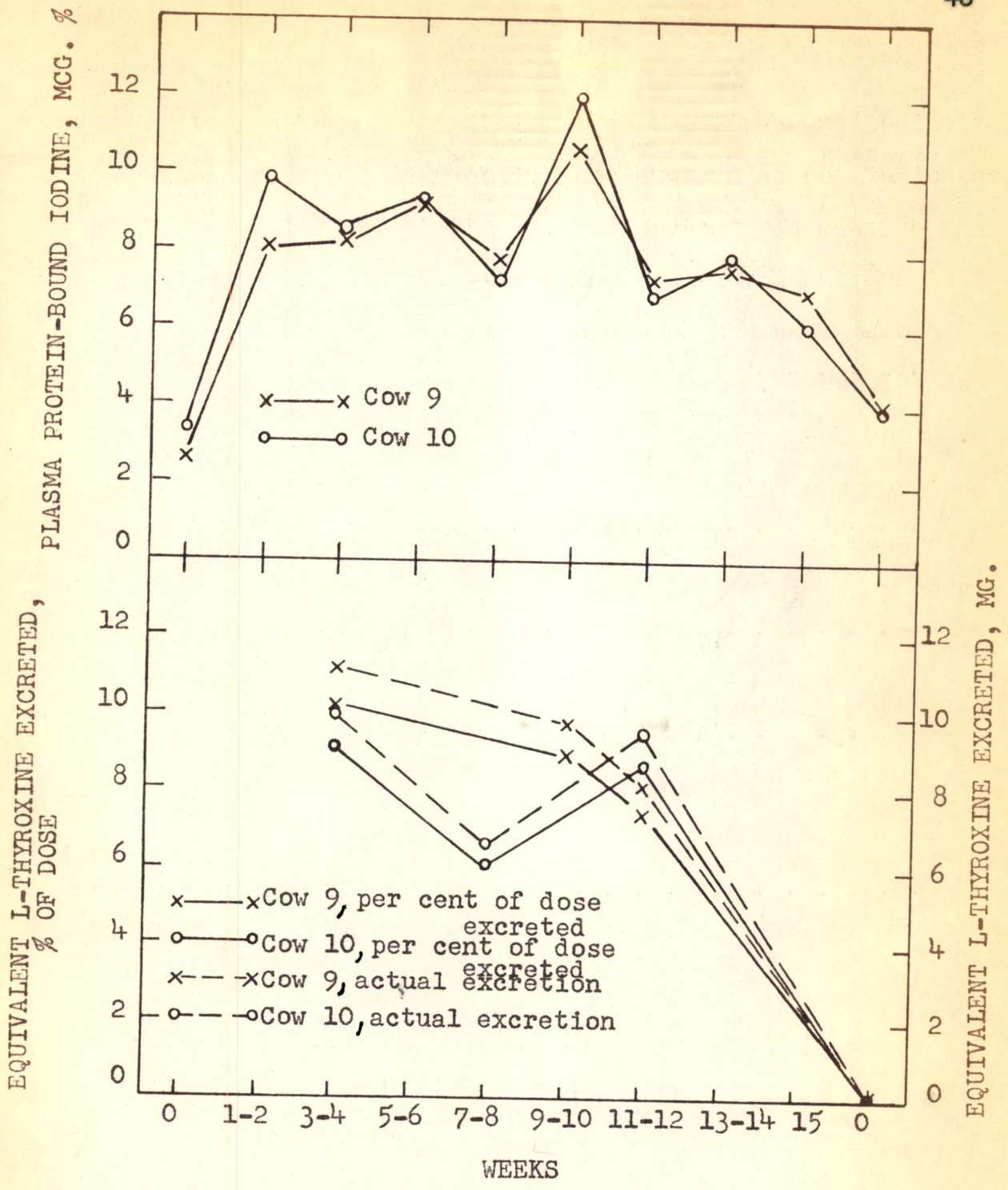


Figure 5. Effect of feeding 110 mg. of L-thyroxine upon plasma and fecal thyroxine of identical twin cows (9 and 10).

thyroidally active compounds in significant quantities.

After one collection, feces from a thyroxine-fed twin were accidently dried at a temperature of 110°C., whereas feces from the same cow collected a week later were dried at 45°C. Upon the assay of both feces samples, it was found that while increasing levels of the overheated feces did cause some reduction in thyroid hypertrophy in thiouracil-fed chicks (indicating thyroxine activity), the reduction was very slight in comparison to that obtained with the feces dried at 45°C. It appears, therefore, that high drying temperatures could cause considerable destruction of thyroidally active compounds in the feces.

DISCUSSION

The results reported herein substantiate observations in previous investigations (17, 18, 39) that upon administration of exogenous thyroxine to animals, larger than normal amounts of thyroidally active compounds appear in the feces. It has been shown with injections that the main route of elimination is by way of the liver and bile (3, 17, 54). Since the results reported here are from cows fed thyroxine, it can be inferred from the evidence of others (7, 55) that the thyroxine was absorbed and then after enterohepatic circulation, was eliminated back into the intestinal tract.

The decrease in plasma PBI concentration observed here with increasing dose-levels of thyroxine is in agreement with the observations by O'Neal (41). He found that larger doses of thyroxine disappear more rapidly from the blood stream than smaller doses. It was found from experiments here with cows that the percentage of the dose excreted decreased with increasing dose-levels of thyroxine. This supports Monroe's (39) hypothesis that thyroxine destruction rather than excretion was the predominating factor in the elimination of excess thyroxine by the animal body and that excess thyroxine produced a stimulatory effect, probably in the liver, for the destruction of thyroxine. Experimental evidence has shown that destruction occurs mainly in the liver as Flock et al. (14) studied and found that deiodination

was diminished greatly in dehepatized dogs.

An increasing rate of destruction is indicated by the declining plasma PBI concentration when the same dose-level of thyroxine was fed for 15 weeks. The progressively decreasing amounts of thyroxine in the feces from cow 9 as treatment proceeded support this assumption, but the feces of cow 10 had approximately the same amount of thyroxine in the twelfth week as in the third week of treatment. This difference noted between cows 9 and 10, as well as the greater excretion of thyroxine by cow 9 in the feces collected at the third week of treatment, suggests the following hypothesis: the greater ability of cow 9 to excrete and destroy exogenous thyroxine may be due to these mechanisms holding over from year to year and becoming cumulatively more effective; for cow 9 had been fed thyroxine in previous lactations, whereas cow 10 had not.

The decline in plasma PBI concentration may be a cause of the decrease in persistency of stimulated milk production induced by feeding thyroactive compounds shown in many experiments (1, 32, 46, 47, 49). Cow 51 had a much higher plasma PBI concentration than cow 23 throughout the experiment on changing levels of thyroxine. This may be due to two reasons. One is that upon the first week's feeding of thyroxine, cow 23 excreted more thyroidally active compounds than did cow 51. Assuming that the absorption was the same

for both cows, it can be inferred that cow 23's liver was more capable of excreting thyroxine into the bile than was cow 51's liver. The second reason is that cow 23 was capable of destroying greater amounts of thyroxine. This can be inferred from the fact that cow 23 excreted lower amounts of thyroidally active compounds when 80 mg. of thyroxine were fed daily in the latter part of the experimental period than in the initial part of the period, whereas with 51 excretion was about the same or only slightly lower. The greater ability of cow 23 to excrete and destroy exogenous thyroxine may be due to the same reason previously mentioned for cow 9, because cow 23 also had been fed thyroxine in lactations prior to this experiment, whereas cow 51 had not. Allowance must be made, however, for the possibility that the extent to which cows 23 and 51 differed may be due to inherent cow differences.

Miller (36) found that daily thyroxine levels higher than 100 mg. did not give a greater stimulus to milk production. The report herein shows that maximum plasma PBI concentrations were reached with 80 mg. and continued with 100 mg. This would substantiate Miller's lactation results, since a higher blood thyroxine concentration did not occur to cause a higher metabolic rate for a greater stimulus in milk production. Swanson and McFee (50), however, found an increase in plasma PBI concentration from 80 to 110 to 140 mg. of

thyroxine when different groups of cows were fed each dose-level. The difference is probably due to an increased thyroxine excretion and destruction ability of the cows. Which in turn may have been induced by greater body stress; for the experiments reported herein were conducted in the summer, and those reported by Swanson and McFee (50) were in the winter.

Excretion figures presented in the results should not be considered exact, but only relative, for the following reasons. Destruction of thyroxine occurs in the digestive tract and bowels (17). Thyroxine undergoes enterohepatic circulation and is reabsorbed (7, 55). Thyroxine intermediates and analogues are excreted (14, 31, 54, 55) and have different metabolic activities (6). There may be some thyroxine retention by the liver (3, 36, 61). The chick assay method is not very precise.

Other workers have found that excretion of thyroidally active substances occurs in the urine after the administration of thyroxine (14, 31). Monroe (39), however, found with the goiter-prevention assay method that the urine of thyroprotein-fed goats did not cause any reduction in thyroid hypertrophy in thiouracil-fed chicks. Therefore, the assays reported here did not include the urine from the cows.

SUMMARY

Studies were conducted to determine the effect of varying dose-level and length of time L-thyroxine is fed upon plasma protein-bound iodine and fecal thyroxine in lactating cows. Two cows were fed varying daily dosages of L-thyroxine, with the level changed weekly. They were started at 60 mg. per day for the first week, with 80, 100, 120, 140, 160, 100, 80 and 80 mg. following in consecutive weeks. Plasma protein-bound iodine concentration although twice as high for one cow as for the other, reached a maximum above initial levels when 80 mg. were fed. The actual excretion of equivalent L-thyroxine in the feces generally increased as dose-level increased. However, the percentage of dose excreted decreased until 120 mg. of thyroxine were fed daily and then tended to remain constant through the 160 mg. daily dose.

The plasma PBI concentration of identical twin cows fed 110 mg. of L-thyroxine daily for 15 weeks followed parallel patterns, with a trend toward a decline as treatment continued. Feces samples collected from one twin during treatment showed that the actual excretion and the percentage of dose excreted decreased as treatment progressed, while its mate showed approximately the same degree of thyroxine excretion throughout the treatment period.

The results indicate that the cow's body rids itself of excess thyroxine. It appears that the most important mechanism is destruction and that this is stimulated to be more effective by increasing doses of thyroxine. Because of this the plasma PBI level resulting from exogenous thyroxine will not always be proportional to dose.

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REFERENCES

REFERENCES

(1) AITKEN, J. W., BOYNE, A. W., and CRITCHON, J. A. The Effects of Feeding Iodinated Casein and L-Thyroxine Upon the Health, Reproduction, and Yield of a Dual Purpose Breed of Cattle and a Purely Dairy Breed. J. Dairy Research, 20: 291. 1953.

(2) ALBERT, A., TENNEY, A. and LORENZ, N. The Absorption of Thyroxine from the Gastrointestinal Tract of the Rat. Endocrinology, 50: 374. 1952.

(3) ALBERT, A. and KEATING, F. R. J. The Role of the Gastrointestinal Tract, Including the Liver in the Metabolism of Radiothyroxine. Endocrinology, 51: 427. 1952.

(4) ALBRIGHT, E. C., LARSON, F. C., and TUST, R. H. In Vitro Conversion of Thyroxine to Triiodothyronine by Kidney Slices. Proc. Soc. Exptl. Biol. and Med., 86: 137. 1954.

(5) ASTWOOD, E. B. Mechanism of Action of Various Antithyroid Compounds. Ann. N.Y. Acad. Sci., 50: 419. 1949.

(6) BARKER, S. B. Metabolic Actions of Thyroxine Derivatives and Analogs. Endocrinology, 59: 548. 1956.

(7) BRIGGS, F. N., TAUROG, A., and CHAIKOFF, J. L. The Enterohepatic Circulation of Thyroxine in the Rat. Endocrinology, 52: 559. 1953.

(8) BROWN, H., REINGOLD, A. M., and SAMSON, M. The Determination of Protein-Bound Iodine by Dry Ashings. J. Clin. Endocrinol. and Metabolism, 13: 444. 1953.

(9) CHAIKOFF, I. L. and TAUROG, A. Studies on the Formation of Organically-Bound Iodine Compounds in the Thyroid Gland and their Appearance in Plasma as Shown by the Use of Radioactive Iodine. Ann. N.Y. Acad. Sci., 50: 377. 1949.

(10) DEMPSEY, E. W. and ASTWOOD, E. B. Determination of the Rate of Thyroid Hormone Secretion at Various Environmental Temperature. Endocrinology, 32: 509. 1943.

(11) DeROBERTIS, E. Proteolytic Enzyme Activity of Colloid Extracted from Single Follicles of Rat Thyroid. Anat. Record, 80: 219. 1941.

(12) DOUGHERTY, J., GROSS, J. and LEBLOND, C. P. Steady State of the Thyroid Hormone. Endocrinology, 48: 70. 1951.

(13) DUNCAN, G. G. Diseases of Metabolism. 3rd ed. W. B. Saunders Co., Philadelphia and London. 1953.

(14) FLOCK, E. V., BOLLMAN, J. L., GRINDLEY, J. H., and ORVIS, A. L. Metabolism of L-thyroxine and L-Triiodothyronine in the Absence of the Liver. Am. J. Phys., 87: 407. 1956.

(15) FREINKEL, N. and LEWIS, D. The Effect of Lowered Environmental Temperature on the Peripheral Metabolism of Labeled Thyroxine in the Sheep. J. Physiol., 135: 288. 1957.

(16) GORDON, A. H., GROSS, J., O'CONNOR, D., and PITT-RIVERS, R. Nature of Circulating Thyroid Hormone-Plasma Protein Complex. Nature, 169: 19. 1952.

(17) GROSS, J. and LEBLOND, C. P. Distribution of a Large Dose of Thyroxine Labeled with Radioiodine in the Organs and Tissues of the Rat. J. Biol. Chem., 171: 309. 1947.

(18) GROSS, J. and LEBLOND, C. P. Metabolism of the Thyroid Hormone in the Rat as Shown by Physiological Dose of Labeled Thyroxine. J. Biol. Chem., 184: 489. 1950.

(19) GROSS, J. and LEBLOND, C. P. The Presence of Free Iodinated Compounds in the Thyroid and Their Passage into Circulation. Endocrinology, 48: 714. 1951.

(20) GROSS, J. and PITT-RIVERS, R. 3: 5: 3' Triiodothyronine Biochem. J., 53: 652. 1953.

(21) HAMBLIN, F. B., JOHNSON, J. E., and SCHRADER, G. Protein-Bound Iodine and I^{131} Extrapolation Technique as Methods of Determining Thyroid Activity in Dairy Cows. J. Dairy Sci., 41: 728. 1958.

(22) HARRINGTON, C. R. and SALTER, W. T. Chemistry of Thyroxine. The Isolation of L-Thyroxine from the Thyroid Gland. Biochem. J., 24: 456. 1930.

(23) HEIMBERG, M., PARK, J. H., ISSACS, A. and PITT-RIVERS, R. The Effects of the Acetic Acid Analogs of Thyroxine and Triiodothyronine on Glycolysis in Ascites Tumor Cell In Vitro. Endocrinology, 57: 756. 1956.

(24) HERTZ, G. and ROBERTS, A. Radioactive Iodine as an Indicator in Thyroid Physiology; Iodine Collection as Criterion of Thyroid Function in Rabbits Injected with Thyrotrophic Hormone. Endocrinology, 29: 82. 1941.

(25) INBAR, G. H. and FREINKEL, N. Simultaneous Estimation in Rats of Thyroxine and Thyroid Hormone Synthesis. J. Clin. Invest., 34: 808. 1955.

(26) JOHNSON, A. D. and CHENG, C. S. Effect of Constant Environmental Temperature (50° and 80°F.) on Thyroid I^{131} Activity of Holstein, Brown Swiss, and Jersey Calves During Growth. J. Animal Sci., 16: 1062. 1957.

(27) KEATING, F. R. Jr., RAWSON, R. W., PEACOCK, W., and EVANS, R. D. The Collection and Loss of Radioactive Iodine Compared with the Anatomic Changes Induced in the Thyroid of the Chick by the Injection of Thyrotrophic Hormone. Endocrinology, 36: 137. 1945.

(28) KENDALL, E. C. A Method of Decomposition of the Proteins of the Thyroid, with a Description of Certain Constituents. J. Biol. Chem., 20: 501. 1915.

(29) LARSON, R. A., KEATING, F. R. Jr., PEACOCK, W., and RAWSON, R. W. A Comparison of the Effect of Thiouracil and Injected Thyrotrophic Hormone on the Collection of Radioactive Iodine and the Anatomic Changes Induced in the Thyroid of the Chick. Endocrinology, 36: 149. 1945.

(30) LARSON, F. C. and ALBRIGHT, E. C. The Specificity of Thyroxine Binding by Serum Alpha Globulin. Endocrinology, 56: 739. 1955.

(31) LARSON, F. C. and ALBRIGHT, E. C. Distribution of 3: 5: 3'-Triiodothyroacetic Acid in the Rat. Endocrinology, 63: 183. 1958.

(32) LEECH, F. B. The Galactopoietic Effect of Iodinated Casein: Dose Response Relationships During Prolonged Treatment. J. Endocrinol., 7: 42. 1950.

(33) LENNON, H. D. J. and MIXNER, J. P. Some Factors Affecting the Determination of Plasma Protein-Bound Iodine Using the Alkaline Fusion-Ceric Sulfate Method. J. Dairy Sci., 40: 35. 1957.

(34) LEBLOND, C. P. and SUE, P. Iodine Fixation in the Thyroid as Influenced by Hypophysis and Other Factors. Am. J. Physiol., 134: 549. 1941.

(35) LEBLOND, C. P. and GROSS, J. Thyroglobulin Formation in the Thyroid Follicle Visualized by the "Coated Autograph" Technique. Endocrinology, 43: 306. 1948.

(36) MILLER, W. H. Feeding Thyroxine and Thyroactive Protein to Milking Cows. M.S. thesis. Univ. of Tennessee. 1957.

(37) MIXNER, J. P. and LENNON, H. D. Jr. Efficiency of Absorption of Thyroxine in Thyroprotein from the Gastrointestinal Tract of Lactating Dairy Cattle. J. Dairy Sci., 41: 728. 1958.

(38) MIXNER, J. P. and LENNON, H. D. Jr. Efficiency of Absorption of Crystalline L-Thyroxine and of Thyroxine in Thyroglobulin from Gastrointestinal Tract of Lactating Cows. J. Dairy Sci., 42: 927. 1959.

(39) MONROE, R. A. and TURNER, C. W. The Metabolism of Thyroxine. Missouri Agr. Exptl. Sta. Research Bull., 446. 1949.

(40) MORTON, M. E. and CHAIKOFF, I. L. The Formation In Vitro of Thyroxine and Diiodothyrosine by Thyroid Tissue with Radioactive Iodine as Indicator. J. Biol. Chem., 147: 1. 1943.

(41) O'NEAL, L. W. Plasma Protein-Bound Iodine After Intravenous Injection of Thyroxine in Thyroidectomized Dogs. Endocrinology, 53: 358. 1953.

(42) PEARLMAN, I., CHAIKOFF, I. L., and MORTON, M. E. Radioactive Iodine as an Indicator of the Metabolism of Thyroxine. I. Turnover of Iodine in the Tissues of the Normal Animal with Particular Reference to the Thyroid. J. Biol. Chem., 139: 433. 1941.

(43) PIPES, G. W. and RUPPERT, A. Estimation of the L-Thyroxine Secretion Rate of Dairy Animals. J. Dairy Sci., 38: 610. 1955.

(44) PREMACHANDRA, B. N., PIPES, G. W., and TURNER, C. W. A Study of Thyroxine Secretion in Cattle. J. Animal Sci., 16: 1063. 1957.

(45) ROSCHE, J., MICHEL, R., JOUAN, P., and WOLFE, W. The Recovery of 3: 5: 3'-Triiodothyroacetic Acid and 3: 3'-Diiodothyronine. Endocrinology, 59: 425. 1956.

(46) REECE, R. P. Thyroprotein in the Ration of Dairy Cattle. I. Its Influence on Milk Production, Fat Tests, Heart Rate, and Body Weight. J. Dairy Sci., 33: 127. 1950.

(47) SEATH, D. N., BRANTON, C., and GROTH, H. H. Effect of Feeding Iodinated Casein on Production and Health of Milking Cows. J. Dairy Sci., 26: 509. 1945.

(48) SMITH, A. E. Hypophysectomy and a Replacement Therapy in the Rat. Am. J. Anat., 45: 205. 1930.

(49) SWANSON, E. W. The Effect of Feeding Thyroprotein to Dairy Cows During the Decline of Lactation in Successive Lactations. J. Dairy Sci., 34: 1014. 1951.

(50) SWANSON, E. W. and McFEE, A. F. Effect of Feeding L-Thyroxine upon Plasma Protein-Bound Iodine in Lactating Cows. J. Dairy Sci., 42: 928. 1959.

(51) TAUROG, A., CHAIKOFF, I. L., and FELLER, D. D. The Mechanism of Iodine Concentration by the Thyroid Gland: Its Non-Organic Iodine-Binding Capacity in the Normal and Propylthiouracil-Treated Rat. J. Biol. Chem., 171: 189. 1947.

(52) TAUROG, A. and CHAIKOFF, I. L. The Nature of the Circulating Thyroid Hormone. J. Biol. Chem., 176: 639. 1948.

(53) TAUROG, A., CHAIKOFF, I. L., and TONG, W. The Nature of Plasma Iodine as Revealed by Filter Paper Partition Chromatography. J. Biol., Chem., 184: 99. 1950.

(54) TAUROG, A., BRIGGS, F. N., and CHAIKOFF, I. L. I^{131}-Labeled L-Thyroxine. I. An Unidentified Excretion Product in Bile. J. Biol. Chem., 191: 29. 1951.

(55) TAUROG, A., BRIGGS, F. N., CHAIKOFF, I. L. I^{131}-Labeled L-Thyroxine. II. Nature of the Excretion Product in Bile. J. Biol. Chem., 194: 655. 1952.

(56) TAUROG, A., TONG, W., and CHAIKOFF, I. L. Thyroid I^{131} Metabolism in the Absence of the Pituitary: The Hypopthsectomized Rat Treated with Thyrotrophic Hormone. Endocrinology, 62: 664. 1958.

(57) THOMAS, J. W. and MOORE, L. A. Thyroprotein Feeding to Dairy Cows During Successive Lactations. J. Dairy Sci., 36: 657. 1953.

(58) TOMITA, K., LARDY, H. A., LARSON, F. C., and ALBRIGHT, E. C. Enzymatic Conversion of Thyroxine and Triiodothyronine to Corresponding Acetic Acid Analogues. Endocrinology, 59: 252. 1956.

(59) TONG, W., TAUROG, A., and CHAIKOFF, I. L. Nature of Plasma Iodine Following Destruction of the Rat Thyroid with I^{131}. J. Biol. Chem., 195: 407. 1952.

(60) TURNER, C. W. and REINEKE, E. P. The Relation of the Route of Administration of Thyroxine, Thyroprotein, and Intermediate Products Upon their Utilization by Ruminants. Missouri Agr. Exptl. Research Bull., 397. 1946.

(61) UOTILA, U. U. The Role of the Cervical Sympathetics in the Regulation of Thyroid and Thyrotrophic Function. Endocrinology, 25: 63. 1939.

(62) VAN ARSDEL, P., HOGNESS, J. R., WILLIAMS, R. H., and ELGEE. N. Comparative Distribution and Fate of I^{131}-Labeled Thyroxine and Triiodothyronine. Endocrinology, 55: 332. 1954.

(63) WILLIAMS, R. G. Microscopic Studies of Living Thyroid Follicles Implanted in Transparent Chambers Installed in the Rabbit's Ear. Am. J. Anat., 62: 1. 1937.

(64) WOLFF, J. and CHAIKOFF, I. L. Plasma Inorganic Iodide as a Homeostatic Regulator of Thyroid Function. J. Biol. Chem., 174: 555. 1948.