A STUDY OF THYROID ACTIVITY IN DAIRY CALVES USING RADIOACTIVE IODINE AS AN INDICATOR

By

James Robert Lodge

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and
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DOCTOR OF PHILOSOPHY

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Approved Robert C. Lewis

ABSTRACT JAMES ROBERT LODGE

This study was designed to adapt the technique used by Henneman (1955) on sheep to the dairy calf and to obtain some basic information about the normal thyroid activity and secretion rate of the dairy animal.

The rate at which injected I^{131} was accumulated and excreted by the thyroid gland and the amount of exogenous thyroxine required to reduce thyroidal I^{131} output to a minimum have been used as indicators of thyroid function in dairy calves. Radioactivity of the thyroid was determined by taking external counts of the gamma radiation over the thyroid region of the neck. When 30 to 150 μc of carrier-free I^{131} were injected into 83 calves representing the five dairy breeds, the 48-hour uptake averaged 36.4 percent of the administered dose. Brown Swiss calves generally had a lower uptake than calves of the other breeds. The uptake was highest in the summer months and lowest in the spring months. Younger calves had a higher uptake than older calves.

During the declining phase of radioactivity, the I^{131} output from the thyroid was 3.1 percent daily, giving the isotope an apparent biological half-life of 31.3 days. When thiouracil was administered six to twelve days after I^{131} administration to prevent further accumulation or recirculation of I^{131}, the output rate increased to 13.8 percent daily, and the biological half-life was reduced to 6.6 days.

The thyroid hormone secretion rate was estimated by the following procedure: Daily subcutaneous injections were made of purified L-thyroxine with increments added every second day. Counts of the radioactivity over the thyroid gland were taken immediately prior to the initiation of thyroxine treatment and at the end of the 48 hours after each increase in dose. The values obtained were calculated as the percent of the previous count. The daily dosage required to raise the corrected external thyroid count to 100 percent, thus producing maximum inhibition of the gland, was assumed to represent the gland's normal secretion rate. Using this technique, the secretion rate of 42 calves averaged 0.57 mg. L-thyroxine per 100 pounds of body weight with a range of 0.36 to 0.84 mg.

Henneman, H. A., E. P. Reineke and S. A. Griffin. The thyroid secretion rate of sheep as affected by season, age, breed, pregnancy, and lactation. J. Animal Sci. 14:419. 1955.

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INTRODUCTION

The thyroid gland is interrelated physiologically with almost every organ and tissue in the body. Therefore, a deficiency or an oversupply of its hormone may be reflected in demonstrable changes in many organs and functional processes of the body.

In recent years, research in the field of thyroid physiology has been given an added stimulus by the development of methods whereby thyroid function can be regulated with relative ease. Not only is this fact of clinical importance, but also it has been found that artificially induced hypothyroidism and hyperthyroidism affect the productive processes of livestock. For example, the administration of thyroidally active substances to cows causes an increase in milk and milk-fat production. On the other hand, a hypothyroid condition brought about by the administration of certain goitrogenic agents, such as thiouracil, has been found to enhance the fattening of swine, fowl, and other domestic animals.

To administer these compounds effectively, it is first necessary tohave a clear picture of the normal thyroid activity. For example, to administer thyroprotein to an animal most effectively, it is essential to know the normal state of activity of the thyroid gland under given physiological and environmental conditions. Otherwise, one might easily give too much or too little of the hormone, since the physiological dosage range of so potent a substance is necessarily short.

Until recently, however, an accurate, comprehensive study of the normal rate of thyroid secretion has been impossible in larger animals because of the lack of a suitable technique.

This study was designed to adapt the technique used by Henneman (1955) on sheep to the dairy calf and to obtain some basic information about the normal thyroid activity and secretion rate of the dairy animal. By knowing the normal secretion rates of the young dairy animal, and knowing the correlation between this secretion rate and the animal's producing ability, it may be possible to use this technique as a tool in selecting the animals with a higher production potential at a young age. Also, by knowing what the normal secretion rate is for high producing animals, it may be possible, through stimulation of the animals' own glands or by feeding hormonal material, to increase the production of low producing animals.

REVIEW OF LITERATURE

There is a voluminous amount of material which has been published on thyroid activity, and this review does not attempt to cover all of it, but only that part which the author feels is closely related to this study.

Although early physiological studies had made it apparent that the thyroid gland produced a hormonal substance, it was not until Bauman (1896) discovered the presence of iodine in the gland that great strides were made in the isolation and identification of this hormone. Kendall (1914) first accomplished the isolation of crystalline thyroxine from thyroid tissue. However, it was not until Harington (1926) improved the technique of extraction of thyroxine that a sufficient quantity of material was obtained to determine the empirical and structural formula. A year later, thyroxine was synthesized by Harington and Barger (1927).

Economic Aspects of Thyroid Relationships

The effect of the thyroid on milk production. It is a well known fact that the use of thyroidal materials will increase milk and milk-fat production.

Graham (1934a) showed a definite relationship between milk secretion and the thyroid. He found it was hard to distinguish the drop in milk secretion following thyroidectomy from that accompanying control operations. However, the addition of small amounts of thyroid to the diet of either thyroidectomized or normal cows, during the declining phase of lactation, caused a rapid rise in milk and milk-fat production. He also reported (1934b) that thyroxine was the principle in the thyroid gland which caused the marked increase in milk-fat production. It was also observed that the rise in milk-fat production was always marked, but the rise in milk secretion showed large variations. These observations have been confirmed by a number of workers, including Jack and Bechdel (1935), Herman, Graham, and Turner (1938), and Ralston et al (1940).

Reineke (1942) was the first to report on the use of iodinated protein in cattle. When this material was fed to a three-year-old thyroidectomized Jersey heifer, it stimulated increases in metabolism twenty to thirty percent. The increased metabolism was accompanied by an improvement in the appearance and vigor and the initiation of estrous cycles. Increases were noted in the milk yield, milk-fat percentage, and yield of butterfat of lactating cows after feeding iodinated protein for a three day period.

Results of practical trials of feeding synthetic thyroprotein were reported by Reineke (1943). The thyroprotein was supplied to a number of farmers to find out the effect under applied conditions. All cows were in the declining stage of lactation when feeding started. Out of 27 cows, 23 showed increases in milk production from 6.6 percent to 60.8 percent after six days or more of feeding. The other four cows showed no change in production. The cows were fed thyroprotein continuously for periods ranging up to three months. After one to two months of elevated production, the normal decline of lactation occurred, but at a retarded rate as compared to the normal.

Since this initial work showing increased milk and milk-fat production from using thyroidally active materials, several long term studies have been conducted and a great many papers have been published on the effect of such materials on the composition of milk.

There is a lot of variation in the reports of the effect on the composition of milk. Archibald (1945) reported very little, if any, change in total solids, ash, and lactose and a decrease in casein. In contrast to this report, other workers, including Ralston et al (1940), have found an increase in the percentage of lactose and total solids. Chanda, McNaught and Owen (1952a) also found a small increase in the lactose content of milk in thyroxine treated cows corresponding to a simultaneous decrease in the chloride content.

These workers also found that the calcium, sodium, potassium, magnesium, protein, creatine, and riboflavin contents of the milk were not demonstrably affected by thyroxine treatment. Chanda et al (1952b) reported that the calcium content of the milk was unaffected, as well as the sodium, potassium, and magnesium.

Comparatively little work has been carried out on the effect of using thyroidally active materials on the vitamin content of the milk. Some of the work is conflicting and not conclusive. It has been shown by VanLandingham et al (1944) and Chanda et al (1952b) that the ascorbic acid content of the milk is decreased by one-fourth to one-third. Kemmerer et al (1946) has reported no change in the thiamine content, a decrease in riboflavin and an increase in nicotinic acid.

In a review article, Blaxter et al (1949) stated "as far as milk composition is concerned, the milk produced by cows receiving iodinated casein is perfectly safe and nutritionally adequate. The fall in vitamin C content is not of great importance as far as human consumption is concerned, for milk is not a major source of the ascorbic acid needed by the human."

Thomas and Moore (1953) reported on an experiment in which thyroprotein was fed for approximately 300 days of each lactation for the length of time that the cows remained in the herd. Of the twenty cows receiving thyroprotein in their first lactation, three cows completed their sixth lactation. Control cows were maintained under identical conditions. Cows receiving thyroprotein did not leave the herd any quicker than did control cows.

Thyroprotein caused an immediate increase in the level of milk production and in butterfat test in practically all animals. The feeding of extra nutrients to a level of 125 percent of Morrison's maximum TDN requirement was found to help sustain these increases. The feeding of thyroprotein and extra nutrients did not result in the production of a greater amount of milk over an entire lactation than in comparable cows fed extra nutrients only. The fat test and the mature equivalent fat-corrected-milk production of cows fed thyroprotein through several lactations were lower during the second and later lactations than the corresponding values for the first lactation. It was also observed that the cows fed thyroprotein decreased in their efficiency of converting energy into milk in subsequent lactations, whereas this efficiency remained constant in the control cows. Also, the mortality rate of calves born to cows fed thyroprotein under the conditions of this experiment was higher than for other calves.

The effect of the thyroid on reproduction. Little is actually known about the effect of thyroid secretion on reproduction of the dairy animal. It has been reported by Brody and Frankenbach (1942) that the thyroidectomized cow shows a complete absence of normal estrual behavior. Ovulation and a normal ovarian cycle occurs and conception, followed by a normal pregnancy, can occur.

It has been shown by Spielman et al (1945) and by Reineke and Turner (1941), respectively, that the fetal thyroid hormone can pass the placental barrier in cows and goats. There is no reason to believe the reverse can not be true, and, if this is the case, the continued feeding of thyroactive material could have an adverse effect on the fetal thyroid gland. It has been reported by Schultze and Turner (1945) that goitrogens administered to the goat passed through the placenta and resulted in fetal thyroid glands ten times the normal size.

Petersen et al (1941) found that thyroidectomy of a bull resulted in complete clinical myxedema, leading to a disappearance of libido and interest in the estrual female. Spermatogenesis was apparently not affected since ejaculates obtained by ampullae massaging were normal in sperm activity, morphology, longevity, and fertilizing ability. Libido was completely restored upon the administration of thyroidal material. Iodinated casein, fed to 14 aged bulls by Reineke (1946), produced favorable results in that ten of them showed increased vigor and a more speedy ejaculation.

The effect of the thyroid on growth and fattening. Thyroidectomy depresses the growth of all animals and this effect in cattle has been reported by Brody and Frankenbach (1942) and Spielman et al (1945). The latter authors also stated that growth depression was much more apparent in the young ruminant than in older animals. The above workers, as well as Reineke and Turner (1941), have shown that growth is completely restored by the injection of thyroxine or by feeding thyroid material or iodinated casein.

Brody and Frankenbach (1942) found a 60 percent depression in growth of a thyroidectomized Jersey calf which was not observed in 13 and 15 month old calves by Spielman and his co-workers (1945).

Blaxter et al (1949) suggested in their review that induced hypothyroidism by goitrogens to increase fat deposition prior to marketing calves or older animals might have some economic value.

Very little work has been conducted on the effect of mild hyperthyroidism on growth. With the levels used in producing an increase in milk production, there has generally been a loss in body weight by the animals under treatment. Millen, Nevens, and Gardner (1948) found a slight increase in growth above normal controls when two dairy calves were given 1.3

grams of iodinated casein per 100 pounds of body weight. When four grams per 100 pounds of body weight were fed to two dairy calves, symptoms of extreme hyperthyroidism occurred.

Regulation of Thyroid Function

Marine (1935) noted that the thyroid gland is endowed with tremendous capacities for increasing or decreasing its functional activity as indicated by changes in weight, microscopic appearance, iodine content, and blood supply. Also, studies on the mitotic activity of the secreting cells of the thyroid, iodine content of the blood, histological appearance of the gland including the presence or lack of colloid in the gland, and changes of the basal metabolic rate (B.M.R.) all have indicated that the secretory activity of the thyroid is influenced by changing environment.

To interpret the meaning of such changes in the thyroid, an understanding of the normal regulatory mechanism is essential. The primary regulatory mechanism controlling the activity of the thyroid has been revealed by experimental work within the past thirty years. Such work shows that the anterior pituitary secretes a thyrotropic factor that regulates thyroid activity. Foster and Smith (1926) found that the B.M.R. was markedly lowered in rats by hypophysectomy and could be brought back to normal by pituitary implants or thyroid administration, thus showing a relationship between the anterior pituitary and the thyroid. Keating et al (1945) showed that, upon thyroid hypertrophy in the chick, there was a rapid loss of iodine stores from the gland, indicating a high degree of thyroid activity.

Further evidence of the control of the thyroid gland by the anterior pituitary has been shown through radioactive iodine studies. Randall and Albert (1951) found that hypophysectomy greatly decreased iodine uptake in the rat, and Fredrickson, Ganong, and Hume (1955) found a significant depression of uptake at all time intervals in the hypophysectomized dog. The latter workers also found a much lower blood clearance rate of I^{131} in hypophysectomized dogs compared to normal dogs. They also showed that, when thyrotropin was administered before tracer injection, the initial uptake of radioactive iodine was greater than normal and it reached a maximum earlier than in dogs not receiving thyrotropin.

Morton and his associates (1942) suggested that hypophysectomy did not severely interfere with the conversion of iodide to diiodotyrosine but the overall conversion of iodide to thyroxine was limited. Keating et al (1945) presented data showing that thyrotropic stimulation produced a prompt and early acceleration in the rate with which radioactive iodine previously stored in the thyroid was lost from it. From their work they concluded that the thyrotropic factor seemed to operate primarily as a stimulus for release of thyroid hormone into the blood stream upon a relatively sudden need for it by the body. That nervous connections are not necessary to the action, since hypertrophy of the thyroid appears to occur in autotransplants, was shown by Salmon and Sevringhaus (1936).

Reforzo-Membrives (1943) showed that thyroid hormone itself has a depressing effect on the release of thyrotropic hormone. There exists a normal balance, therefore, between the thyroid by way of its hormone and the anterior pituitary by way of its thyrotropic factor.

There are a number of factors which can shift this balance, resulting in a gland which is highly active or a gland with very little activity. Some of these factors act upon the anterior pituitary, changing its production of thyrotropic hormone, whereas others act directly on the thyroid gland. Among the agents acting through the anterior pituitary are temperature, light, and nutrition. Some of the factors acting directly on the thyroid gland are insufficient iodine intake and drugs, such as thiouracil, which prevent the formation of thyroid hormone and cause a goitrogenic effect. This goitrogenic effect is due to an increased output of thyrotropic hormone caused by an insufficient amount of circulating thyroid hormone. Many studies have been conducted to show the effect of season and temperature. Seiden and Fenger (1913) showed that the thyroid of sheep, hogs and cattle had a maximum iodine content in the late summer and a minimum iodine content in the late winter. Kendall and Simonsen (1928) confirmed and extended these observations by showing that more of a thyroxine-like fraction is contained in the thyroid of mammals in midsummer than in February.

In fowl, an opposite relationship apparently exists. Cruickshank (1929) found a higher thyroid iodine content in the winter months and a lower content in the summer months. This discrepancy between mammals and fowl can be explained on the basis that little storage of colloid takes place in the fowl thyroid during the summer such as apparently occurs in the thyroid of the mammal.

It has been established that low temperature will stimulate the thyroid to increased activity. Dempsey and Astwood (1943) found that the thyroid gland of rats produced thyroid hormone at a rate of 9.5 γ thyroxine per day when kept at a temperature of one degree Centigrade. At a temperature of 35 degrees Centigrade, the daily secretion rate was 1.7 γ thyroxine. Reineke and Turner (1945) found the highest secretion rate of young chicks in the winter and the lowest in the summer. Turner (1948) also showed a decline in thyroid secretion rate of White Leghorn hens between January and May.

Leblond et al (1944) showed that when rats were kept in a zero to two degree Centigrade temperature for 26 days, the glands fixed 2.7 times more radioiodine than controls kept at 25 degrees Centigrade. This greater fixation of radioiodine was not definite until after seven days and was absent after forty days of exposure. A diminished thyroid activity was observed as early as one day and persisted for 26 days when rats were exposed to a temperature of 32-34 degrees Centigrade.

A daily thyroxine secretion of 0.04 mg. in July was significantly lower than in any other month when determinations were made in sheep by Henneman et al (1955).

Blincoe and Brody (1955a) found that increasing the ambient temperature above the comfort zone to 35 degrees Centigrade decreased the thyroid activity 30 to 60 percent in the four breeds of cows studied. Holstein cows showed the greatest decrease and Brahman the least. Decreasing the ambient temperature below the comfort zone to -8 degrees Centigrade increased the thyroid activity 60 to 100 percent in Jersey and Brahman but not in Holstein or Brown Swiss cows. Air velocity in the range of 0.5 to 10 m.p.h. had no effect on the thyroid activity of the four breeds of cows. The addition of radiant energy (light) reduced the thyroid activity of Jersey and Holstein cows but did not affect Brahman cows. Starvation for four days markedly reduced the thyroid activity of Holstein cows. Another publication by the same authors (1955b) showed similar results in thyroid activity in Jersey and Holstein cows from temperatures above and below the comfort zone.

Puntriano and Meites (1951) found that continuous light induced significant reductions in thyroid weight, thyroid reaction to thiouracil and thyroid uptake of radioactive iodine, whereas continuous darkness had the opposite effect in mice. It was concluded that continuous light depresses while continuous darkness increases thyroid secretion in mice.

It was shown by Mulinos and Pomerantz (1940) that a poor nutritive condition depresses thyroid activity in comparison to a good nutritive condition.

The role of iodine and its effect on thyroid activity is very well demonstrated by its goitrogenicity in humans and, although not as noticeable in domestic animals, can be observed in the hairless pig and large necked calf when iodine is deficient. Many foods produce a goitrogenic effect which can be remedied by increasing the amount of iodine in the diet.

Among these are soybeans, alfalfa, and cabbage.

However, there are certain classes of compounds which, when fed, cause a goitrogenic condition that cannot be remedied by even relatively large intakes of iodine. MacKenzie, MacKenzie, and McCollum (1941) observed that rats on a diet containing sulfaguanidine had markedly enlarged thyroids compared with those of controls. Histologically the thyroids showed marked hyperplasia, the epithelium was distinctly columnar and was so increased and invaginated as to nearly extinguish the lumen. Richter and Clisby (1941) observed that administering phenylthiourea caused thyroid hypertrophy. Kennedy and Purves (1941) found that a diet containing Brassica seed caused a goitrogenic effect which was only partially prevented by iodine administration. MacKenzie and MacKenzie (1943), in extending their studies, found that many of the sulfonamides had a goitrogenic effect on the thyroid. Astwood (1943) made a detailed study of the goitrogenic action of many compounds and found that the most active compounds were substances having the thioureylene radical (NH·CS·NH). Thiourea and most of the derivatives were found to be very active although the derivatives were more toxic. Thiouracil is active and of low toxicity.

Whereas most of the above work was accomplished with rats, Mixner, Reineke, and Turner (1944), using chicks, also found an enlargement of the thyroid when thiouracil was used and suggested that the method might be used as an assay procedure for thyroxine secretion rate.

The authors investigating the effects of these goitrogenic agents (MacKenzies, 1943, and Astwood et al, 1943) report that the thyroid's histological picture indicates hyperactivity, such as a much increased acinar tissue with increased height of the epithelial cells, a greatly reduced colloid, increased size and number of follicles and hyperemia.

Although the enlargement and histological picture of the thyroid induced by the drugs indicates a hyperactive gland, studies of their indirect effects on physiological processes indicate hypofunction of the thyroid. The MacKenzies (1943), Astwood et al (1943), and Reineke, Mixner and Turner (1945) all have observed a depression of the basal metabolic rate of animals given these drugs.

The exact mechanism of action of these goitrogenic drugs has not been definitely determined, but it is generally believed to be due to interference in an enzyme system that is involved in the synthesis of the thyroid hormone.

Although excessive intake of iodine has been repeatedly shown to not prevent the goitrogenic effect of these drugs, Astwood et al (1943) and MacKenzie and MacKenzie (1943) showed that the injection of preformed thyroxine does prevent the thyroid hypertrophy when given during the treatment with the drugs.

Based on the above observations, Dempsey and Astwood (1943) conceived the idea of using thiouracil treatment simultaneously with thyroxine injections as a method of determining the thyroid's secretion rate.

Perlman, Morton and Chaikoff (1941, 1942), employing tracer amounts of radioactive iodine without carrier, conducted a series of studies on the distribution of various fractions of thyroid iodine under different experimental conditions. They have reported that, as the time interval after administering tracer iodine increases, there is a concomitant increase in the proportion of labelled iodine in the thyroxine-like fraction. These same investigators and Anderson (1942) found that, though the thyroids of hypophysectomized rats collect much less of the tracer iodine than do the thyroids of intact animals, the amount collected is real and measureable. As reported earlier, the iodine is readily converted into the diiodotyrosine-like fraction but very little of the tracer is demonstrable in the thyroxine-like fraction.

Morton and his associates (1943) also studied the formation of thyroxine and diiodotyrosine by completely thyroidectomized animals. They reported that totally thyroidectomized rats are able to convert iodide to diiodotyrosine and thyroxine. By _in vitro_ techniques (1944) they demonstrated that various sulfonamides inhibit the formation of diiodotyrosine and thyroxine without affecting the concentrating power of the thyroid slices. In another _in vitro_ study (1944) they found that the addition of a small excess of iodide to each flask inhibits the conversion of inorganic iodine to diiodotyrosine-like and thyroxine-like iodine.

Using the same _in vitro_ techniques, this group of investigators (1944) studied the effect of goitrogenic substances on the conversion of iodine by sheep thyroid slices. They found that thiourea and thiouracil strongly inhibit the formation of diiodotyrosine and thyroxine but do not prevent the collection of inorganic iodide. Potassium thiocyanate was found not only to depress the conversion of inorganic iodine but also to prevent the collection of iodine by thyroid slices.

This group of workers (1944) also studied the influence of thiouracil on the formation of thyroxine and diiodotyrosine by the intact thyroid gland of the rat. The feeding of thiouracil for seven days depressed the uptake of radioactive iodine. The thyroid of rats receiving thiouracil contained only about half the amount of radio-thyroxine and about one-third the amount of radio-diiodotyrosine found in the normal glands.

Methods of Thyroid Study

Magnus-Levy (1895) administered thyroid material to a normal individual and found a marked increase in oxygen consumption and carbon dioxide output. From this observation arose the most widely used method of studying thyroid activity. It is still used in diagnosing thyroid disorders in humans, although in the past few years the use of radioactive iodine as a diagnostic tool has gained much prominence. This method has also been widely used in laboratory animals as an assay procedure for thyroxine. However, in the large ruminant, it loses its usefulness. For an accurate determination of the basal metabolic rate, the individual or animal must be in a fasted, rested, subdued state. It is very difficult to keep large animals in a subdued state and almost impossible to obtain a fasted ruminant animal. Further complications arise from the gases in the rumen. In measuring the carbon dioxide produced, not only is that produced by metabolism of the body measured but also that produced by bacterial fermentation in in the rumen. The other gases produced in the rumen also interfere in this determination.

The loss in body weight of animals and restoration of growth in thyroidectomized animals have been used in studying thyroidally active materials. However, these methods are only qualitative and not sensitive enough for quantitative measurements.

The discovery of goitrogenic compounds, referred to earlier, made possible a new type of thyroid study based on maintenance of the hormone balance between the thyroid and pituitary glands. The method involves the use of a goitrogenic agent such as thiourea or thiouracil that has a thyroidectomy effect and results in thyroid enlargement. By the subcutaneous injection of graded dosages of thyroxine, the weight of the thyroid is reduced in thiouracil- or thiourea-treated animals and the weight reduction in general is proportional to the thyroxine dosage. Thus, the establishment of a normal thyroxine-thyrotropin equilibrium by injection of thyroxine in thiouracil-treated animals results in a thyroid weight equal to that in control animals. The quantity of thyroxine required to bring about this result is considered to be an estimate of the normal thyroid secretion rate by the glands of a particular group of animals.

Dempsey and Astwood (1943) are given credit for proposing this method and using it to determine the secretion rate of rats. Reineke, Mixner and Turner (1945) found that values obtained by this goitrogenic method compared very well with those from basal metabolic rate measurements. Many workers have used this technique in the study of thyroid activity and the factors affecting the activity. Undoubtedly, most of the knowledge gained about the thyroid secretion rate of different animals today has been obtained by this method. However, this method does not readily lend itself to the study of thyroid activity in the large domestic animal, such as the dairy cow, due to the fact that the animals need to be sacrificed to obtain the results.

A method which has been used in larger animals with some degree of success has been the protein-bound-iodine method, better known as the PBI method. Curtis et al (1933) reviewed the early results of blood iodine determinations in humans which established the relation between the level of blood iodine and thyroid status. He concluded that there is good agreement between the blood iodine level and thyroid function. Upon further study, it was found by Turner et al (1940) and Salter and his workers (1941) that the total blood iodine failed to reflect the actual thyroid status under all circumstances. Clark and Boyd (1940) were unable to show any seasonal variation in thyroid activity of pigeons and chickens through the blood iodine, even though it is known that such a variation does exist.

Because of these findings, workers turned to using the protein-bound-iodine fraction of the plasma, serum, or blood. Long and co-workers (1951) found significant differences in protein-bound-iodine content of serum between breeds of dairy cattle. Reece and Man (1952) have presented evidence of a nonthyroidal iodine fraction in the plasma protein-bound-iodine of cattle.

In a review, Rapport and Curtis (1950) state that the level of both the PBI and inorganic iodine is demonstrably affected by iodine in any form and that this is its chief disadvantage.

Lewis (1952), in an extensive study in dairy cattle, observed no effect when cows which had not previously received iodized salt were supplemented with it. However, he also stated that the amount of iodine in the supplemented diet may have been no more than adequate and, if the supplemental iodine had been in excess of the animals' needs, the results might have been different.

Since the discovery of artificial radioactivity by Joliot and Curie (1934), a new era has developed in the study of metabolic processes.

As Marine (1915) demonstrated, the thyroid gland is unique in that it has the ability to collect iodine selectively in relatively large quantities. It is, therefore, not surprising that studies with the radioactive isotopes of iodine have proved readily applicable in the study of thyroid physiology.

Five isotopes of radioactive iodine have been described in detail by Livingood and Seaborg (1938). The first available radioactive iodine was prepared by the action of slow neutrons on iodine. Its atomic weight is 128 and its half life is 25 minutes, which limits its usefulness in biological studies. Bombardment of iodine with fast neutrons gives rise to a radioactive element which has a half life of 13 days and atomic weight of 126. Another radioactive isotope of iodine, having a half life of four days and an atomic weight of 124, arises from the bombardment of antimony with helium ions. The bombardment of metallic tellurium gives rise to two radioactive isotopes of iodine. One has a half life of 12.5 hours and an atomic weight of 130. The other has a half life of 8 days and an atomic weight of 131. This last is the one in most common use today and its eight day half life makes it very convenient for use in biological experimentation.

Hertz and his associates (1938, 1940, 1941) were the first to follow the distribution of radioiodine within the body after its administration for the purpose of obtaining information concerning thyroid function. These workers demonstrated that the normal thyroid collects far greater amounts of administered labelled iodine than do other tissues. The normal thyroid of rabbits was found to collect up to eighty times the quantity to be expected from uniform diffusion into the general body tissues. They also found that a gland, made hyperplastic with the thyrotropic hormone, collected up to several hundred times the quantity expected from uniform diffusion. They also demonstrated that the thyroid of animals previously treated with iodine collects smaller amounts of the administered tracer iodine than does the thyroid of normal animals. They concluded from comparing thyroid iodine collection, acinar cell height, relative thyroid size, and basal metabolic rate that the result of thyrotropic hormone injections may be regarded as an initial stimulation, followed by an involution, if iodine is administered, and functional depression after prolonged thyrotropic stimulation.

Chaikoff and his associates (1943, 1944) utilized radioactive iodine for the study of iodine metabolism with *in vitro* techniques. They incubated thyroid slices in Ringer's solution containing tracer amounts of radioactive iodine and then determined the fractions of inorganic, diiodotyrosine-like and thyroxine-like radioactive iodine in the thyroid tissue. They found that slices of thyroid from dogs and sheep convert less iodine into organic fractions than do those from rats.

Hamilton and Soley (1938, 1939, 1940) were the first to report the use of radioactive isotopes in tracing the course of iodine given to human beings. They compared the uptake of labelled iodine by the thyroid of normal controls with that of patients with various thyroid diseases. These measurements were made by placing a Geiger counter tube over the isthmus of the thyroid gland and measuring the gamma rays emitted from the radioactive iodine that had been collected in the thyroid.

Werner, Quimby and Schmidt (1949) used the 24 hour uptake of radioactive iodine as the most stable time at which to assay thyroid function in humans. The uptake by the euthyroid gland has leveled off, as has that by the gland in hyperthyroidism, at that time. Moreover, measurements at the 24 hour period provide a range of normal uptake which separates the great majority of patients with hyperthyroidism and hypothyroidism from those with normal function. These same workers (1950) determined from the 24 hour uptake that there was no statistically significant effect of season on average uptake by euthyroid individuals. They also found a slight but progressive decrease in uptake with each decade in age and that women had a slightly higher average uptake than men.

Werner et al (1950) compared the tracer technique with the basal metabolic rate determination and with the serum precipitable iodine determination. They found errors in all three methods in diagnostic studies. However, the tracer technique gave a smaller percentage of error than did the basal metabolic rate determination. They found by combining two or more of these tests a high degree of accuracy in diagnosis was obtained.

Morton, Perlman and Chaikoff (1941) found that, two hours after labelled iodine was injected, four to nine percent of it was found deposited in the whole of the thyroid gland removed from each of the normal or control guinea pigs, whereas 12 to 23 percent was taken up by the glands that had been made hyperactive by thyrotropic hormone treatment. At the 26 hour interval, the whole normal glands contained from 14 to 27 percent of the administered labelled iodine and the hyperactive glands, from 32 to 41 percent. In both cases, however, the major portion of the labelled iodine held by the glands was not free but approximately 90 percent of it was organically bound.

There are a number of ways by which factors can be shown to influence the release of iodine from the thyroid gland. Wolff (1951), using the biological half-life as a measure, found it to be 3.3 days in control rats. Rats receiving 0.03 percent propylthiouracil in their diet had an accelerated release with a half-life of 1.6 days. Hypophysectomy resulted in a half-life of 24 days which was returned to normal in 24 hours by thyrotropin treatment. Treatment with 15 μg of thyroxine daily had an effect similar to hypophysectomy. In the presence of propylthiouracil, thyroxine prolonged the biological half-life of I^{131} in the thyroid to 26 days. Controls receiving an equivalent amount of iodide had a 1.4 day half-life. In the absence of propylthiouracil, the thyroxine and control values were 24 and 3.7 days respectively. Thyrotropin treatment markedly accelerated the release of I^{131} from the thyroid. In addition, it was demonstrated that the action of thyrotropin showed a latent period of two to three hours before any significant effect could be shown on I^{131} release. He also found that large doses of iodide failed to influence the release of previously injected I^{131} from the gland.

Albert and Tenney (1951), using the method of a proportional rate of disappearance of thyroidal I^{131} as percent per day, found that thiouracil accelerated thyroidal secretion six fold, thyroxine inhibited the release, and sodium iodide induced either no change or a depression of secretory rate.

Perry (1951) proposed a method for measuring thyroid hormone secretion in the rat and suggested its use as an assay procedure on thyroid extracts. By measuring the rate of loss of radioactive iodine from the thyroid gland of the rat, an index of the secretion of thyroid hormone may be obtained. The validity of this index has been suggested by the effect of such influences as thyrotropic hormone, thiouracil, thiocyanate, and thyroxine. It has been found that secretion is markedly inhibited by the administration of quantities of thyroxine of the order of 10 μg. With lower doses of thyroxine, a relationship between dose and degree of inhibition of thyroid hormone may be demonstrated.

Based on these facts, a rapid and simple procedure for the bioassay of thyroid preparations was proposed.

From Perry's work, Henneman et al (1952) reported the development of a method for quantitatively measuring the thyroid secretion rate of individual intact sheep. The procedure introduced by these authors involves direct counts of radioactivity in the thyroid region to determine the amount of thyroxine that must be given daily to suppress the output of previously collected I^{131} from the gland. The method was confirmed and extended by experiments in rats (Reineke and Singh, 1954). It has been used by Henneman et al (1955) and Singh et al (1954) to study some of the environmental and physiological factors related to thyroid function in sheep. These findings were the basis for the method used in this study.

A method reported by Pipes and Ruppert (1955) and Pipes and Turner (1956) differed from the above in that radioactivity counts were taken on samples of blood plasma instead of directly over the thyroid. It has the disadvantage of being considerably more tedious to apply. Administration of sufficient quantities of I^{131} to insure satisfactory counting levels in blood plasma also introduces serious danger of thyroid injury in the animals through excessive radiation. It might be added that these workers have since changed to the method employed in this study as reported by Pipes, Premachandra and Turner (1956).

EXPERIMENTAL PROCEDURE

The experimental animals were from the Michigan State University dairy herd and included the Jersey, Guernsey, Holstein, Brown Swiss, and Ayrshire breeds. The average age was 5.2 months with a standard deviation of 2.2 months. The calves received the regular calf rations used at the University. In the early experiments the ration did not include supplemental iodine. However, for the later experiments, the ration was supplemented with iodine at the level recommended to dairy farmers.

A tracer dose of carrier-free I^{131}, up to 150 μc, was injected either subcutaneously or intravenously into each heifer. The uptake of I^{131} by the thyroid gland was followed by taking counts of the radioactivity from the thyroid area of the neck at hourly, daily, or alternate day intervals. As far as possible, the same amount of pressure was applied on the neck of the calf each time the counts were taken. The count used in the calculations was the maximum repeatable count obtained from this region of the neck. All counts were corrected for background and the physical decay of the isotope. A Co^{60} source was used to standardize the instruments.

During the earlier studies, a shielded end-window TGC-8 Geiger-Mueller Tube (Tracerlab) was used with a model 1615B count rate meter (Nuclear). A scintillation counter and model 1620 count rate meter (Nuclear) were used for the later work.

Once the slope of the output curve was established, L-thyroxine, purified by repeated recrystallizations, was administered daily by subcutaneous injection. The thyroxine was recrystallized and furnished by Dr. E. P. Reineke from the Physiology Department of Michigan State University. The crystalline thyroxine was weighed and then dissolved in a dilute NaOH solution. Enough HCl was added to make the solution slightly cloudy before it was diluted with distilled water to obtain the desired concentration. Only enough of the L-thyroxine for a few days use was put into solution, and this was kept under refrigeration. The dosages were started at a low level and increments were added each second day. At the end of each period of a given thyroxine level, an external thyroid count was taken. In this way, the effect of each increment upon the rate of I^{131} output by the thyroid was determined.

The thyroxine secretion rate was estimated by plotting each corrected thyroid count, calculated as the percentage of the last previous count, against the thyroxine dosage. The amount of thyroxine required to raise the percent of previous count to 100, or to produce a leveling off of I^{131} output to a minimum rate, was considered to represent the normal secretion rate of the thyroid gland.

RESULTS AND DISCUSSION

Preliminary trials were conducted in order that the author could gain experience in the counting technique and in handling isotopes. From these early trials, it was found that the thyroid gland of dairy calves collected a substantial amount of the injected radioactive iodine in a relatively short period of time, but that the output was very slow. The biological half-life was in the neighborhood of twenty days. Since attempts to measure the thyroxine secretion rate of the calves in the preliminary experiments were unsuccessful due to the slow rate at which the I^{131} was eliminated from the thyroid of the experimental animals, some adjustment had to be made in the technique previously employed. In addition, it was found that the counts were so erratic that it was impossible to establish a valid output rate. Figure 1 illustrates this situation. A reasonable explanation for the apparent lack or irregularity of I^{131} output seemed to be that the iodine was salvaged from the metabolized hormone and recycled through the gland.

In earlier work by Lewis and Reineke (unpublished), it had been found that oral dosages of 50 mg. of sodium iodide given daily or on alternate days did not prevent the apparent recirculation even when the treatment was continued for one to two weeks.

After this preliminary work, a number of trials were directed toward determining the rate and degree of uptake by the thyroid gland and the thyroid secretion rate of a number of calves. These trials will be reported and discussed individually.

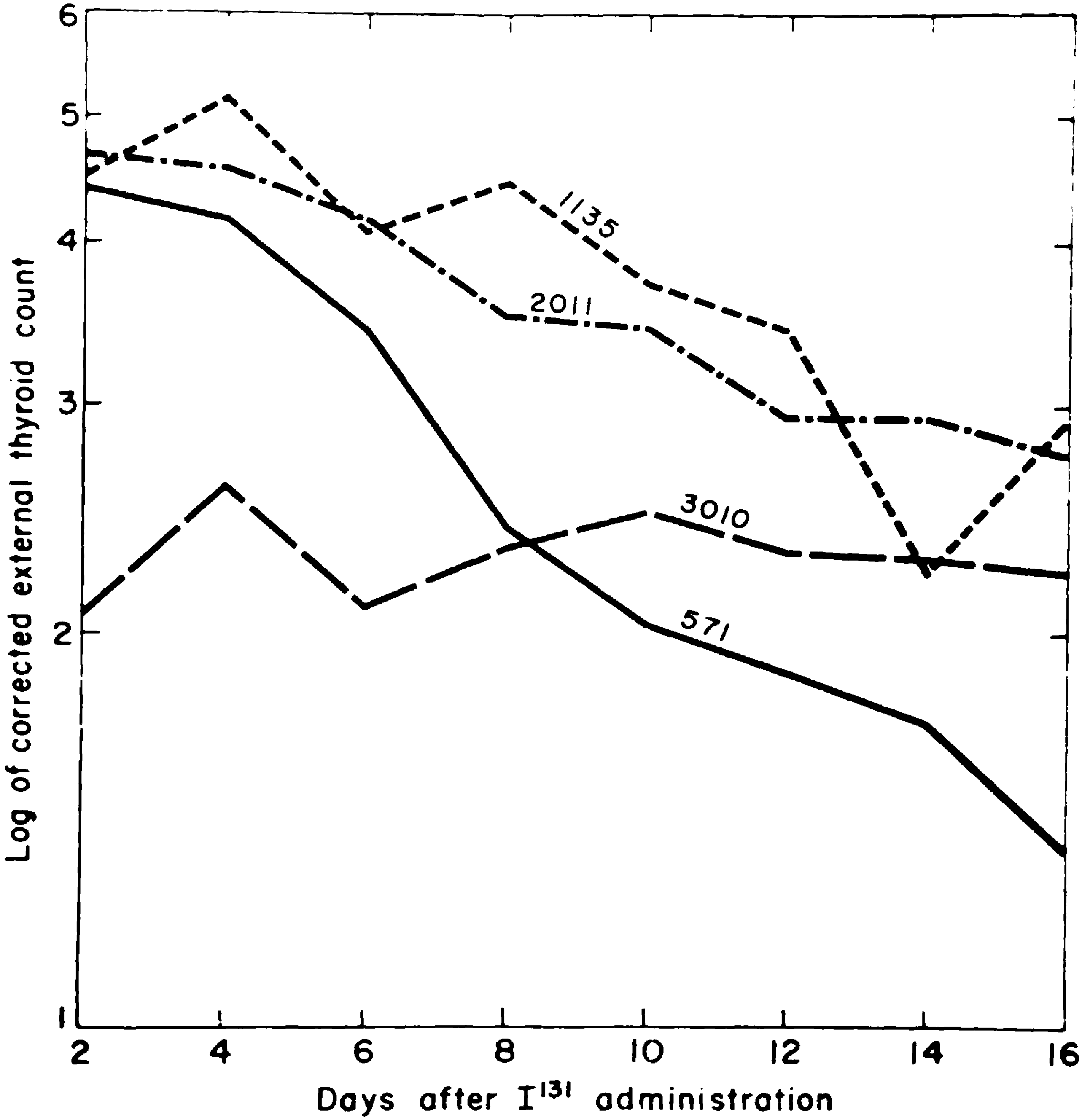

Figure 1. The I^{131} output rate of four heifers. One animal appeared to have no output whereas the others showed a variable rate between counts.

Trial 1

Eight calves (two Holsteins, four Jerseys, and two Ayrshires) were given subcutaneous injections of 150 μc of I^{131}. Forty-eight hours after the injection, counts were taken over the thyroid gland area of the neck, and the percent of the injected dose taken up by the thyroid was calculated. To permit comparison of the relative percent of the I^{131} that was collected by the thyroid, aliquots of the injection solution were counted in a "phantom neck," similar to that described by Blincoe (1953). This method was used in determining the percent of iodine uptake by the calves in this trial and all subsequent trials. The percentage of the 48-hour uptake of these calves can be found in Table I.

Since thiouracil is known to prevent incorporation of iodine into thyroxine, it was thought that the administration of this drug would prevent the recycling. It had been demonstrated by Goldsmith et al (1951) that compounds related to thiouracil increased the excretion rate of a tracer dose of radioactive iodine from the human thyroid gland. This same observation was made by Wolf (1951) in rats. Reineke and Singh (1955) reported similar thyroid secretion rates in normal and thiouracil-treated rats.

A level of 0.2 g. thiouracil per kilogram of body weight daily was used in this trial because, in the preliminary trials, an oral dose of 0.1 g. of thiouracil was not effective in preventing the recirculation. Ely et al (1948) reported that 0.2 g., administered in divided doses at 12-hour intervals,

TABLE I

THE EFFECT OF THIOURACIL ON THE OUTPUT RATE AND BIOLOGICAL HALF-LIFE OF I^{131}

Calf No.	Calf Breed	Age	Wt.	48 Hr. Uptake	Daily Output Rate[1] 1-12	Daily Output Rate[1] 12-18	Biol Half-Life[2] 1-12	Biol Half-Life[2] 12-18
		(mos.)	(lbs.)	(%)	(%)		(days)	
261	A	6.5	380	49.0	4.3	3.1	16.1	22.4
590	H	5.5	340	51.0	1.3	2.3	53.3	30.1
1143	J	10.0	465	37.9	4.7	12.1	14.7	5.7
1145	J	8.5	400	33.9	4.3	7.7	16.1	9.0
262[T]	A	6.0	355	35.9	1.1	12.3	63.0	5.6
591[T]	H	5.5	330	53.0	0.8	21.3	86.6	3.3
1144[T]	J	9.0	408	39.8	4.0	28.6	17.3	2.4
1146[T]	J	7.0	300	48.4	6.7	36.7	10.3	1.9

[1] Daily output rate calculated from the formula, $-\beta = \frac{2.3}{t} \log \frac{AT}{AO} \times 100$, where $-\beta$ = daily output rate in percent, t= experimental time in days, AT= count at end of experimental time, AO= count at start.

[2] Biological half-life calculated from the formula, $T\ 1/2 = \frac{.693}{-\beta}$

[T] Thiouracil treatment 12 to 18 days

effectively maintained a relatively high concentration of thiouracil in the blood of calves.

Four of the calves received thiouracil by capsule starting on the twelfth day after the injection of the isotope. Half of the daily dosage was given in the morning and the remainder was given approximately twelve hours later. The other four calves were used as controls. The effect of this level of thiouracil on the output of I^{131} is demonstrated in Figure 2.

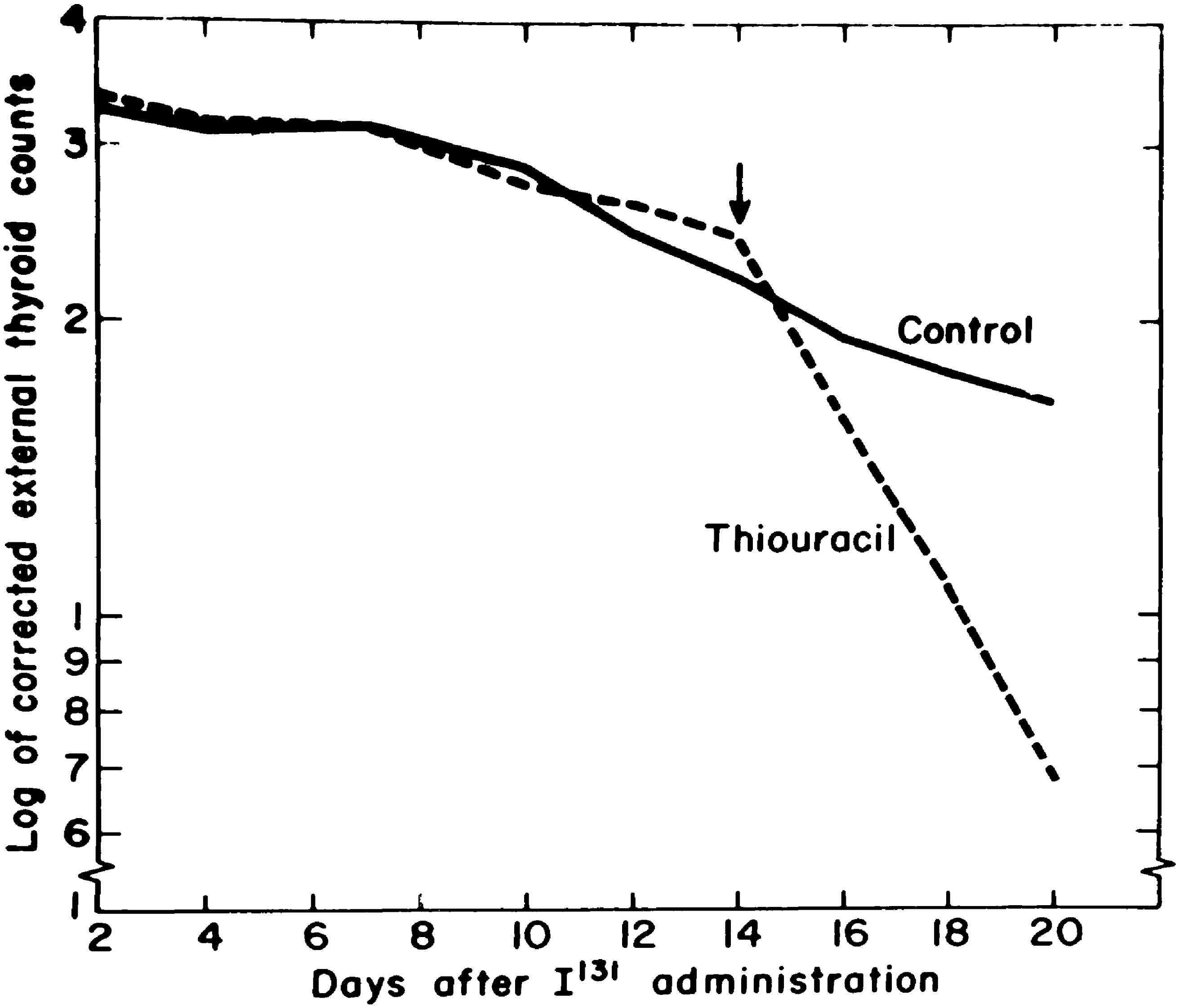

Figure 2. The effect of administering 0.2 g. of thiouracil per kg. of body weight, given in two equal doses daily, on the I^{131} output rate. The control and experimental groups consisted of four calves each.

The results of thiouracil treatment on daily output rate and biological half-life are shown in Table I. It can be seen readily that the level of thiouracil used in this trial effectively increased the net output of I^{131}.

When it had become obvious that the detectable output was greatly increased by thiouracil treatment, thyroxine was injected subcutaneously at the rate of 1 mg. per 100 pounds of body weight to all calves daily for a period of three to four days. Even though the injection period was short and the counts on the calves were very low, it was demonstrated that thyroxine did decrease the rate at which the I^{131} was eliminated.

Trial 2

In the month of March, eight calves (two Holsteins, two Jerseys, and four Brown Swiss) were injected subcutaneously with 150 μc of I^{131}. The 48-hour uptake was determined (Table II), and, on the fourth day after injection of the isotope, all calves were started on thiouracil at the level found to be effective in the previous trial. The same effect from the thiouracil treatment was not observed in all the calves that had been seen in the previous trial. This lack of effect from the thiouracil will be discussed further in a later trial.

In this particular trial, the supply of thiouracil which was being used was exhausted on the sixth day and a supply of methylthiouracil which was several years old was substituted. Several of the calves which did not show a good response to the thiouracil were given increased amounts to a level of 0.3 or 0.4 g. per kilogram of body weight. When a new supply of

TABLE II

IRREGULAR EFFECT OF THIOURACIL IN MARCH, 1955

Calf No.	Calf Breed	Age	Wt.	48-hour Uptake	Daily Output Rate	Biol. Half-life
		(mos.)	(lbs.)	(%)	(%)	(days)
594	H	3.0	205	37.5	5.0	13.9
595	H	2.0	168	52.7	13.3	5.2
1149	J	3.0	140	50.6	5.7	12.2
1150	J	2.0	91	___*	20.3	3.4
3025	BS	3.5	235	42.5	1.5	46.2
3026	BS	3.0	200	30.1	8.9	7.8
3029	BS	2.0	265	41.1	3.5	19.8
3030	BS	1.0	130	18.1	5.3	13.1

* Count was above the count rate meter range.

thiouracil was received, the level was reduced to 0.2 g. per kilogram. The higher levels of methylthiouracil appeared to have no effect on the output rate but did, however, have an apparent adverse effect on some of the calves. Calf number 3029 had to be removed from the experiment because of a bad cold and generally run-down condition.

Thyroxine was administered in graded dosages to those calves which did show acceptable output rates. The dosage rates used were 0.1, 0.3, 0.6, 0.9, and 1.2 mg. thyroxine per 100 pounds of body weight. Because of the relative lack of effectiveness of thiouracil in this trial, no actual secretion rates were estimated. However, it appeared that the maximum inhibition of the thyroid gland occurred between 0.3 to 0.9 mg. per 100 pounds.

Trial 3

In May, one Holstein, two Jerseys, one Guernsey, and four Brown Swiss calves were injected subcutaneously with 135 μc of I^{131}. The 48-hour uptake of the I^{131} was again determined and can be found in Table III. The uptakes are considerably lower than those reported in the previous trials. This lower uptake will be discussed in more detail later, but unseasonably warm weather was noted during the 48 hours of the uptake phase, which might have had an influence upon the amount of iodine collected by the glands.

On the sixth day after injection, thiouracil, at the level of 0.2 g. per kilogram of body weight, was started on all eight calves. After two counts were obtained on the thiouracil treatment, it was observed that the treatment was effective in increasing the output of I^{131} from the glands of the calves. The count from most of the calves was between 70 and 80 percent of the previous count, which the author feels is a sufficient output to permit obtaining secretion rate values.

Thyroxine injections were started after the second count, ten days after injection of the isotope. Thyroxine was given to all calves at a level of 0.3 mg. per 100 pounds of body weight. This level of thyroxine had shown very little, if any, response on the calves used previously and was the basis for starting the calves on this level. The plan had been to increase the level of thyroxine by increments of 0.1 mg. every second day, but, through an error, the second level was 0.6 mg. instead of 0.4 mg. The increments thereafter were 0.1 mg. as

TABLE III

ESTIMATED THYROXINE SECRETION RATE
OF CALVES IN MAY, 1955

Calf No.	Calf Breed	Age	Wt.	48-hour Uptake	Daily Output Rate	Biol.* Half-life	Est. Thy. Secr. Rate
		(mos.)	(lbs.)	(%)	(%)	(days)	(mg./100#)
592	H	5.5	395	25.5	7.5	9.2	0.75
1147	J	7.5	315	18.9	13.5	5.1	0.78
1148	J	6.5	320	17.5	18.4	3.8	—
2022	G	7.0	355	29.4	4.7	14.7	0.77
3023	BS	6.5	395	20.5	8.6	8.1	0.84
3024	BS	5.5	410	26.3	10.5	6.6	0.51
3027	BS	4.0	415	15.2	13.3	5.2	0.73
3028	BS	4.0	290	11.9	4.3	16.1	0.64

* The biological half-life was calculated for the period of time when the calves were under thiouracil treatment.

planned until the end of the experiment. Thyroxine was injected once each day at approximately the same time. Thus, the animals received two injections at each level of thyroxine, and thyroid counts were taken at the end of each period. The results of this trial can be found in Table III. As is shown, secretion rates were obtained on seven of the eight calves. Calf number 1148 had to be dropped from the experiment because the count became too low due to a relatively low uptake and a high output rate.

Figure 3 shows the counts obtained from six of the calves during this trial. These values give typical curves produced

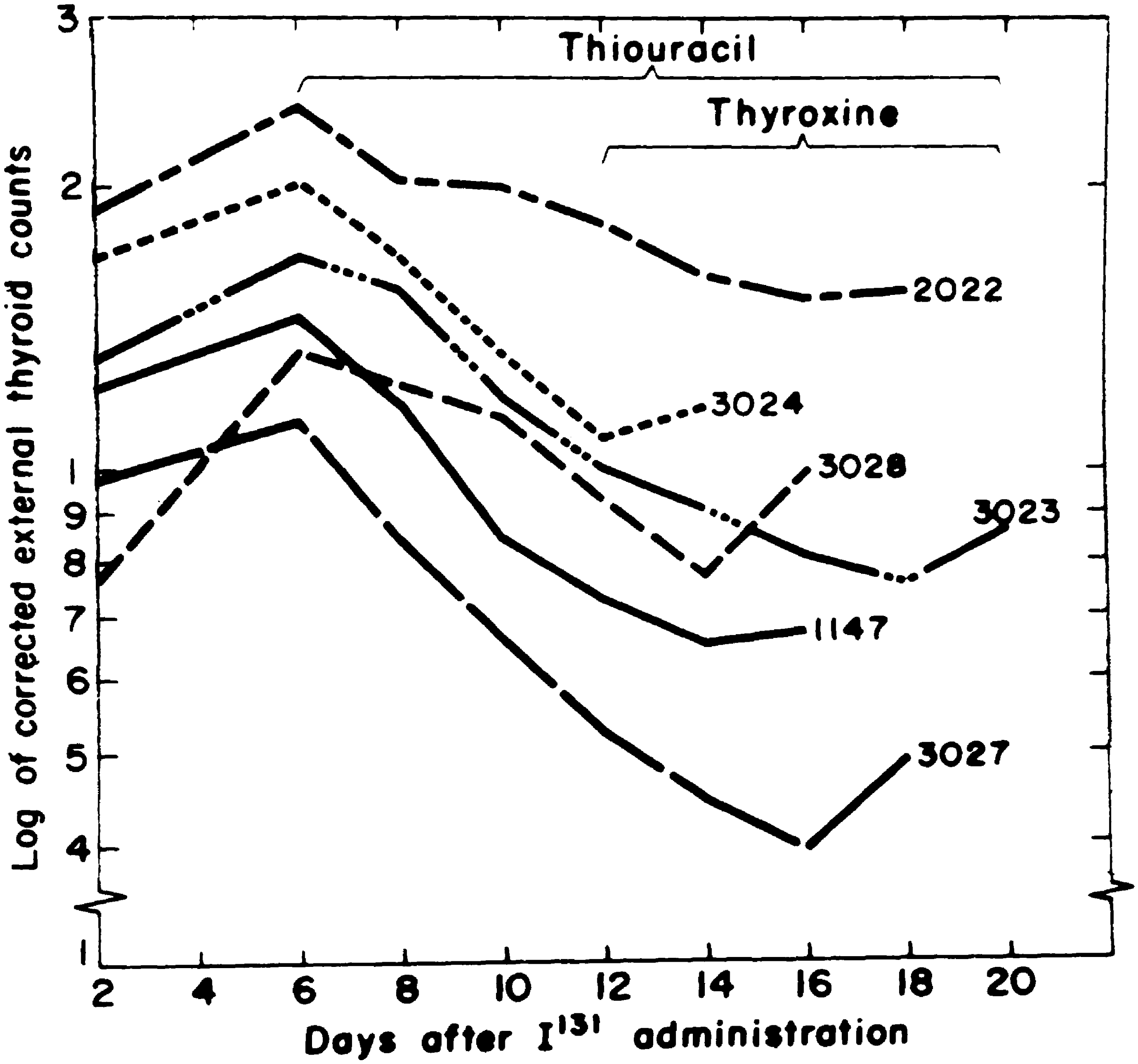

Figure 3. Log of the external thyroid counts of six calves treated with thiouracil followed by thyroxine. Thiouracil was administered orally starting on the sixth day after I^{131} injection. Thyroxine, in graded doses, was injected subcutaneously daily starting on the twelfth day with increments added each second day.

by calves under the treatment employed. As the thyroxine dosage was progressively increased, its inhibitory effect upon the thyroid gland was marked by a decreased rate of I^{131} output. The thyroxine secretion rate was estimated by plotting each thyroid count, expressed as the percent of previous count, against thyroxine dosage (Figure 4). The amount of thyroxine required to raise this figure to 100 percent has been taken as the animal's thyroxine secretion rate. It also was found that when the thyroid gland was completely inhibited, that is, when the percent of previous count reached 100, the effect of any additional increases in thyroxine was unpredictable. This was also noted by Henneman et al (1955) in sheep.

Trial 4

Two Holsteins, three Jerseysand three Brown Swiss heifers were injected subcutaneously with 30 uc of I^{131} in August. The 48-hour counts were obtained and the uptake calculated as previously. A scintillation counter was used for obtaining the remainder of the counts and in all subsequent trials. The same procedure was followed as in the previous trial in that thiouracil, at 0.2 g. per kilogram of body weight, was started on all calves six days after the injection of the isotope. Due to an apparent delayed effect of thiouracil, only the three Jersey calves were started on 0.3 mg. of thyroxine per 100 pounds of body weight per day on the tenth day. All calves were given injections of 0.4 mg. thyroxine on the twelfth day and were continued on increments as has been explained previously.

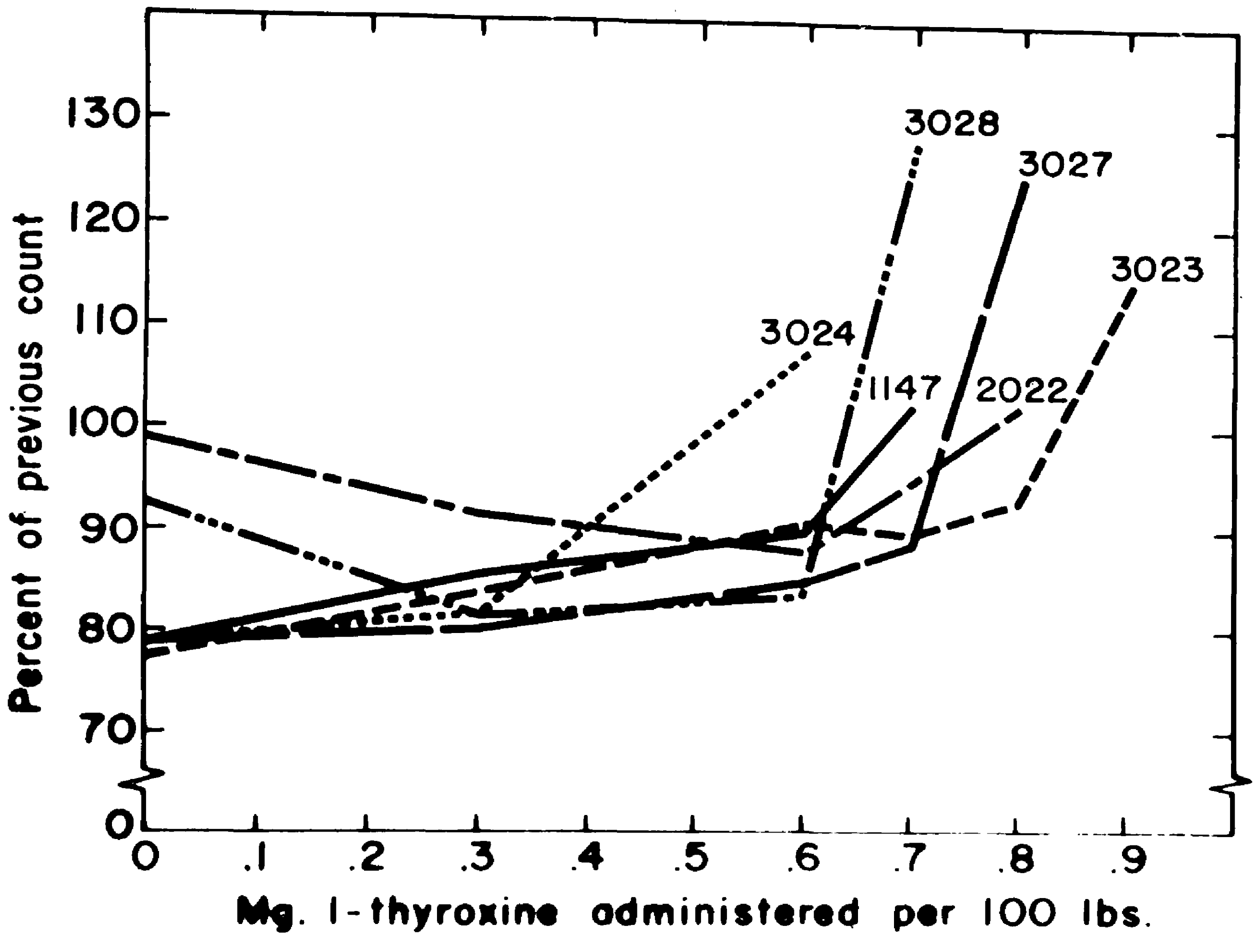

Figure 4. Corrected external thyroid counts calculated as the percent of the last previous count. The daily amount of thyroxine required to raise the percent of previous count to 100 was assumed to represent the daily secretion rate of the thyroid gland.

TABLE IV

ESTIMATED THYROXINE SECRETION RATE OF CALVES IN AUGUST, 1955

Calf No.	Calf Breed	Age	Wt.	48-hour Uptake	Daily Output Rate	Biol. Half-life*	Est. Thyroid Sec. Rate
		(mos.)	(lbs.)	(%)	(%)	(days)	(mg./100#)
596	H	5.0	335	59.3	15.5	4.5	0.57
598	H	4.5	295	70.3	9.2	7.5	0.53
1151	J	5.0	245	80.8	24.0	2.9	0.47
1152	J	4.5	220	70.8	30.4	2.3	0.52
1153	J	4.5	260	59.3	26.3	2.6	0.57
3031	BS	5.5	405	67.2	27.3	2.5	0.60
3032	BS	5.0	350	65.6	19.1	3.6	0.54
3033	BS	3.5	250	44.5	11.6	6.0	0.56

* The biological half-life was calculated for the period of time when the calves were under thiouracil treatment.

The results of this trial are found in Table IV. The average thyroxine secretion rate obtained in this trial was somewhat lower than that found in the previous trial. This is probably due to a temperature effect, since it is assumed that the thyroid activity is depressed during hot weather.

Trial 5

In this trial, 12 calves (four Guernseys, four Jerseys and four Brown Swiss) were injected subcutaneously with 50 μc of I^{131}. After the 48-hour uptake was determined, the calves were divided into four groups with one calf of each of the three breeds in each group. Since it has only been assumed that the effective length of time of a single dose of thyroxine is 24 hours or less, this trial was designed to determine the length of time a single dose of thyroxine is effective in normal and thiouracil-treated calves. It was also designed to evaluate the possibility of a cumulative effect of thyroxine administration as well as to obtain measurements of thyroxine secretion rates of normal and thiouracil-treated calves.

The first group of three calves was to receive only single injections of thyroxine with counts obtained daily to determine the length of time a single dose is effective. The output rate of I^{131} was so slow and variable from day to day that no thyroxine was administered until the 22nd day after the injection of the isotope. At that time, a single dose of 0.1 mg. thyroxine per 100 pounds of body weight was administered. Because of the daily variations found before the injection, the effect of this dosage, if any, was not clear.

The second group of three calves was started on thiouracil on the sixth day after injection of the isotope and on the twelfth day a single injection of 0.6 mg. thyroxine per 100 pounds of body weight was given. The output of I^{131} was slowed in two of the three animals with the effect lasting not more than two days. However, a new reduction in output was observed on the fourth day. A similar effect was also noted after 0.3 mg. of thyroxine per 100 pounds of body weight was injected on the 20th day. No explanation can be given for the delayed effect.

The calves in group three were treated the same as those in group one except that a dosage of 0.3 mg. thyroxine per 100 pounds of body weight was given on the 22nd day with the idea of obtaining the thyroid secretion rates of the calves. The results of this dosage were no more distinguishable than in the first group.

The thyroxine secretion rates of the calves in the fourth group were obtained in the manner established in the previous trial.

The results of this trial can be found in Table V, and further effects of thiouracil on the output rate of I^{131} can be noted. It also confirms the need for thiouracil treatment in obtaining thyroxine secretion rates of dairy calves. The length of time that a single dose of thyroxine is effective could not be established, but, from the indication found in the group receiving thiouracil, further work should be conducted along this line.

Trial 6

Since Michigan lies in a goitrogenic area and the rations did not include any supplemental iodine, it was thought that the addition of iodine in the ration might influence the uptake and output of I^{131}. On the first of December, iodized salt containing 0.02 percent potassium iodide was mixed in the concentrate ration at the recommended level of one percent. Prior to this trial, two calves (one Guernsey and one Ayrshire) were injected subcutaneously with 50 μc of I^{131}. The uptakes were calculated as usual and were found to be no lower than the uptakes of calves the previous year which is reported in

TABLE V

EFFECT OF SINGLE INJECTIONS OF THYROXINE AND ESTIMATED THYROXINE SECRETION RATES IN NORMAL AND THIOURACIL TREATED CALVES

Calf No.	Calf Breed	Age	Wt.	48-hour Uptake	Daily Output Rate	Biol. Half-life	Est. Thyroid Sec. Rate
		(mos.)	(lbs.)	(%)	(%)	(days)	(mg./100#)
Group 1 - No Thiouracil							
1151	J	6.5	310	36.4	3.4	20.4	
2023	G	8.5	425	35.3	2.9	23.9	
3033	BS	5.0	390	21.8	4.6	15.1	
Group 2 - Thiouracil							
1150	J	8.5	425	28.0	10.5	6.6	
2024	G	7.5	455	31.2	12.2	5.7	
1153	J	6.0	365	28.6	11.3	6.1	
Group 3 - No Thiouracil							
1152	J	6.0	300	33.3	2.3	30.1	
2026	G	4.0	270	46.8	4.5	15.4	
3030	BS	8.0	500	24.9	3.6	19.3	
Group 4 - Thiouracil							
3032	BS	6.5	465	26.0	16.8	4.1	0.41
2027	G	3.5	260	74.5	16.9	4.1	0.53
3031	BS	7.0	520	27.5	19.6	3.5	0.36

Table I. The uptake of one calf could not be calculated at 48 hours because the count was beyond the range of the count rate meter or, in other words, in excess of 60 percent. The other calf had an uptake of 49.2 percent of the injected dose. Counts were obtained every second day for 30 days. The calculated percent of daily output rate was 1.9 and 0.8 percent, which gives a calculated biological half-life of 36.5 and 86.6 days respectively. It appeared that, if the addition of iodized salt to the ration had any effect, it depressed the output rate of I^{131}. However, there was a period of approximately three weeks in late February and early March when the iodized salt was not included in the ration so the values obtained on the two calves might not give a true picture.

At the time this trial was started, iodized salt was placed in the mangers of the calves. Two days later, it was included in the ration. Eight calves (four Jerseys, two Holsteins and two Brown Swiss) were injected subcutaneously with 50 μc of I^{131} in early March. It can definitely be seen that the iodized salt had no detrimental effect on the uptake when the values obtained in this trial (Table VI) are compared to those of Trial 2 (Table II), which were obtained the previous year when no iodized salt was included in the ration. It was particularly desirable to learn if thiouracil administration would be necessary to obtain a sufficient output rate of the calves. It was obvious after eight days that the output was no greater than had previously been found so thiouracil treatment was started at the same level as in the previous trials. The same procedure was also followed, with the exception that thyroxine was started at a level of 0.4 mg. per 100 pounds of body weight rather than at 0.3 mg. because the counts were getting low by the time the injections of thyroxine were made. It was concluded from this trial that, even though iodized salt was added to the ration, thiouracil treatment was still necessary to remove variation from and to steepen the output curve.

TABLE VI

EFFECT OF THE ADDITION OF IODIZED SALT TO THE RATION ON THE 48-HOUR UPTAKE AND ON THE NET OUTPUT RATE OF I^{131}

Calf No.	Calf Br.	Age	Wt.	48-hr. Uptake	Daily Output Rate Bef.*	Daily Output Rate Aft.*	Biol. Half-life Bef.*	Biol. Half-life Aft.*	Est. Thyroid Sec. Rate
		(mos.)	(lbs.)	(%)	(%)		(days)		(mg./100#)
599	H	7.0	430	31.5	1.5	8.7	46.2	8.0	0.64
600	H	5.0	315	31.1	1.9	8.0	36.5	8.7	0.51
1151	J	12.0	500	34.7	4.4	8.7	15.8	8.0	0.47
1152	J	11.0	490	26.6	1.8	8.7	38.5	8.0	0.51
1154	J	8.0	410	33.8	4.3	13.0	16.1	5.3	0.60
1155	J	6.0	275	40.0	2.3	9.6	30.1	7.2	0.60
3034	BS	6.0	385	24.9	1.7	13.1	40.8	5.3	0.62
3037	BS	5.0	310	27.5	1.1	12.2	63.0	5.7	0.49

* Bef. = Before thiouracil; Aft. = After thiouracil

Trial 7

It was thought that intravenous injections of the isotope might possibly have a different effect on the uptake and output rate than was observed from subcutaneous injections. The calves (six Holsteins and two Brown Swiss) were injected intravenously with 50 μc of I^{131} in April. The injections were made in the jugular vein and were successful in six of the eight calves. In the other two calves, a portion of the I^{131} appeared to have been deposited in the tissue outside the vein.

To find if the kind of injection would have any effect on the output rate, no further treatment was given the calves until 18 days after the injection of the isotope. It was very obvious by this time that the intravenous injection was not going to increase the output rate, so thiouracil treatment was started. As was found the previous year in the month of March, thiouracil had an irregular effect on the output rate. There is no readily available explanation for this lack of effect. Highley et al (1954) found that the iodine level in the diet prior to the feeding of thiouracil had a pronounced effect on the response to thiouracil, in that it exerted its maximum effect on the thyroid gland of rats when the iodine level of the diet was near the minimum level required to prevent thyroid enlargement in the normal rat. This, however, could not be the cause in this case since it happened when calves were not on supplemental iodine as well as when they were. This lack of response has also been indicated by Turner (1948) when he was evaluating the use of the thiouracil-thyroxine method for estimating the thyroid secretion rate of White Leghorn hens. He found that the method was satisfactory for a period in the fall and winter when thyroid enlargement under the influence of thiouracil is at a maximum. During the spring and summer, however, the range of thyroid weight response was too small to permit one to distinguish any except gross differences in the thyroidal potency of test substances. This lack of response to thiouracil during this time needs further investigation.

Secretion rates could not be obtained for the calves in this trial because of the lack of response to thiouracil. The results can be seen in Table VII.

TABLE VII

EFFECT OF INTRAVENOUS INJECTIONS OF THE ISOTOPE, I^{131}, ON THE 48-HOUR UPTAKE AND ON THE NET OUTPUT RATE

Calf No.	Calf Breed	Age	Wt.	48-hr. Uptake	Daily Output Rate Bef.*	Daily Output Rate Aft.*	Biol. Half-life Bef.*	Biol. Half-life Aft.*
		(mos.)	(lbs.)	(%)	(%)		(days)	
601	H	6.0	380	24.7	0.7	3.9	99.0	17.8
602	H	6.0	375	19.5	2.3	_	30.1	_
603	H	6.0	330	31.2	0.6	7.4	115.5	9.4
605	H	4.0	290	53.5	1.4	5.8	49.5	11.9
606	H	4.0	245	43.2	1.1	_	63.0	_
607	H	3.0	165	39.0	_	7.7	_	9.0
3039	BS	5.0	300	21.2	3.3	_	21.0	_
3040	BS	4.0	275	29.9	1.5	2.8	46.2	24.8

* Bef. = Before thiouracil; Aft. = After thiouracil

It was thought that the I^{131} in the gland was possibly in the inorganic form, since thiouracil did not increase its output. Thiocyanate has been shown to cause the release of inorganic iodine from the thyroid gland. At the termination of this trial, potassium thiocyanate, at a level of 10 mg. per pound of body weight, was injected intravenously in two of the calves, of which one had received thiouracil and one had not. There was no change in the radioactivity from the thyroid region during a five hour period after injection of the thiocyanate. This would indicate that there was no inorganic iodine present. However, the effective dosage level of thiocyanate for dairy calves is unknown, so the level used may have been too low.

Trial 8

In May, eight calves (three Jerseys, three Brown Swiss and two Holsteins) were injected intravenously with 35 μc of I^{131}. The main purpose of this trial was to obtain the thyroxine secretion rate of the heifers. The usual procedure was followed including the determination of the 48-hour uptake, the administration of thiouracil started the sixth day after the injection, and graded doses of thyroxine given on the tenth day after injection. Nothing unusual was observed in this trial, and the results may be found in Table VIII.

TABLE VIII

ESTIMATED THYROXINE SECRETION RATE OF DAIRY CALVES IN MAY, 1956

Calf No.	Calf Breed	Age	Wt.	48-hr. Uptake	Daily Output Rate*	Biol. Half-life *	Est. Thyroid Sec. Rate
		(mos.)	(lbs.)	(%)	(%)	(days)	(mg./100#)
608	H	3.0	200	40.4	4.4	15.8	0.62
609	H	2.0	180	36.9	7.9	8.8	0.53
1157	J	4.0	215	24.8	5.0	13.9	0.56
1158	J	4.0	170	32.4	8.3	8.3	0.54
1159	J	3.0	120	19.8	14.7	4.7	0.55
3041	BS	4.0	240	14.9	5.3	13.1	0.37
3042	BS	4.0	270	14.6	8.5	8.2	0.49
3043	BS	4.0	250	30.3	4.6	15.1	0.38

* Calculated for the period of time when the calves were under thiouracil treatment.

Trial 9

This trial was conducted in the same manner as the previous one and was designed primarily to obtain secretion rates of the eight calves injected. In this trial in August, two Holstein, two Brown Swiss, two Jersey and two Guernsey heifers were injected intravenously with 50 uc of I^{131}. Thiouracil and thyroxine treatments were started as usual with the exception of one Jersey calf, which appeared to have a fairly steep and consistent output rate. The thyroxine secretion rate of this calf was determined without the use of thiouracil. The result, which can be found in Table IX, was very similar to that found from the other calves.

TABLE IX

ESTIMATED THYROXINE SECRETION RATE OF DAIRY CALVES IN AUGUST, 1956

Calf No.	Calf Breed	Age	Wt.	48-hr. Uptake	Daily Output Rate[a]	Biol. Half-life[a]	Est. Thyroid Sec. Rate
		(mos.)	(lbs.)	(%)	(%)	(days)	(mg./100#)
610	H	5.0	250	29.0	8.8	7.9	0.62
612	H	5.0	265	30.2	10.4	6.7	0.65
1160	J	5.0	210	—[b]	32.1	2.2	0.57
1161	J	5.0	215	—[b]	12.4[c]	5.6[c]	0.57[c]
2033	G	5.0	175	—[b]	9.3	7.5	0.59
2034	G	4.0	180	—[b]	19.0	3.6	0.65
3044	BS	5.0	235	33.1	13.9	5.0	0.57
3045	BS	5.0	245	33.6	12.1	5.7	0.58

[a] Calculated for the period of time when the calves were under thiouracil treatment.
[b] Count was above the count rate meter range.
[c] Calf number 1161 received no thiouracil.

General Discussion

As can be noted, there is considerable variation in the 48-hour uptake within and between trials. Although the causes of these differences have not been completely resolved in this study, the major factors involved appear to be season, breed and age. The average 48-hour uptake of 83 calves has been 36.4 percent of the injected dose. The highest uptake has been in the summer months and the lowest in the spring months as seen in Table X. When compared to conclusions drawn by Seiden and Fenger (1913) that the highest iodine content of cattle thyroids was found in the late summer and the minimum iodine content in the late winter, this difference in uptake throughout the year is not too surprising. Table XI shows the average 48-hour uptake of the 83 calves according to breed. The greatest difference appears to be between the Brown Swiss and the other breeds. The uptake of the Brown Swiss calves averaged about 10 percent less than either the Jerseys or the Holsteins. So few Ayrshires and Guernseys were used in this study that comparisons cannot be made, but, from the few that were used in this study, it would seem that their uptakes are not different from those of the Jerseys or Holsteins. There is not a large difference in the 48-hour uptake of the calves of different ages in this study. However, there is an indication that the younger calves have a higher uptake than the older ones. The distribution of the calves in each season, breed and age classification makes it difficult to carry out a statistical analysis, but it appears that there may be a breed-season

TABLE X

EFFECT OF SEASON ON THE 48-HOUR UPTAKE OF I^{131} BY THE THYROID GLAND OF DAIRY CALVES

Season	Number of Animals	48-hour Uptake	Standard Deviation
		(%)	
Winter (Jan-Mar)	24	38.4	9.7
Spring (Apr-June)	24	26.7	10.4
Summer (July-Sept)	12	53.6	18.5
Fall (Oct-Dec)	23	35.5	15.5

TABLE XI

EFFECT OF BREED ON THE 48-HOUR UPTAKE OF I^{131} BY THE THYROID GLAND OF DAIRY CALVES

Breed	Number of Animals	48-hour Uptake	Standard Deviation
		(%)	
Ayrshire	3	44.7	—
Brown Swiss	26	29.3	13.8
Guernsey	8	38.9	15.7
Holstein	22	37.9	13.6
Jersey	24	40.9	17.4
Overall	83	36.4	

interaction which means that, when talking about a breed or season difference, one has to specify the particular breed and season which is being compared. The average 48-hour uptake of I^{131} by the Jersey calves was higher than that of the Brown Swiss in the winter, summer and fall, but was about the same in the spring. The Holsteins had a higher percentage of uptake than the Brown Swiss in the winter and spring but dropped to the same level in the summer and fall. Blincoe and Brody (1955) have shown that changing the ambient temperature has a greater effect on some breeds than on others, and this could be the cause of this apparent interaction observed in this study.

The addition of supplemental iodine, at the level used in this study, has no apparent effect on the uptake of I^{131} by the thyroid gland of calves. However, the amount administered was probably not in excess of the animals' needs and further study should be conducted to determine the effect of an excess amount of iodine on the uptake.

Neither did intravenous injections have an effect on the uptake of I^{131} by the thyroid gland. Hourly counts were obtained from the calves receiving the intravenous injections for a period of eight hours to show the rate of uptake of the isotope. The percent of 24 hour count for the first eight hours is shown in Table XII for 20 calves. When the percent of the 24- or 48-hour count is plotted, the accumulation of I^{131} over a period of 48 hours is curvilinear but may approach linearity over a small segment of time, particularly in the first few hours after injection. When plotted in this manner,

TABLE XII

THE PERCENT OF 24-HOUR COUNT AS A MEASURE OF RATE OF UPTAKE FOR THE FIRST FEW HOURS AFTER INJECTION OF I^{131}

Hour	Overall Average	Eight Holstein (Ave.)	Seven Br. Swiss (Ave.)	Four Jersey (Ave.)	One Guernsey
1	8.6	11.0	5.6	8.3	12.1
2	14.3	16.7	10.5	15.1	18.4
3	19.7	22.0	15.7	19.8	28.2
5	29.9	33.1	25.1	29.4	39.4
6	33.7	36.8	29.1	35.0	43.0
7	37.5	41.9	30.0	40.0	44.3
8	43.4	46.5	38.4	44.3	50.3
24	100.0	100.0	100.0	100.0	100.0

there seems to be no difference in the rate of uptake by the calves of different breeds as was found in the percent of I^{131} uptake at 48-hours. The thyroid gland of the Brown Swiss calves collected about the same percentage of the 48-hour uptake at each period of time as did the calves of other breeds.

Since the thyroid gland of some animals has the ability to store iodine, the percent of I^{131} uptake is not a good measure of thyroid output in these animals. It has been well demonstrated that in several other species the winter and spring months are the months of high thyroid secretion, whereas the summer months are a period of low secretion. As mentioned before, the iodine content of the thyroid gland of mammals is high in the summer and low in the spring, so it is not a good measure of output. The percent of I^{131} uptake found in this study would also suggest that during the summer, when the actual secretion is low, a large amount of iodine is collected by the gland and stored for future need by the body.

The output rate of I^{131} would be a much better measure of thyroid output, since it actually measures the rate at which iodine leaves the gland. As has been pointed out earlier, the apparent output rate of dairy calves is very slow, which is probably due to recirculation of metabolized hormonal iodine, so could not be used as an indication of activity. Because the use of thiouracil was necessary to obtain the secretion rates of the calves, there may be some influence on the output from the gland. Thiouracil definitely increases the output as was demonstrated here and in studies by other workers. The results on 22 untreated and 50 treated calves are shown in Table XIII. Whether this is the true output rate or not is not known. However, as long as there is enough stored hormone in the thyroid gland to meet the needs of the animal, there should be no increase above the normal output of the hormone. If there were not enough hormone stored in the gland, there would then be an increased production of thyrotropic hormone from the anterior pituitary which would cause an increase in thyroid activity and eventually an increase in the size of the thyroid gland. There were no histological studies made on any of the thyroid glands from the calves used in these trials. In order to know the effect, if any, on the thyroid gland by the thiouracil as used in this technique, histological studies need to be made. It would appear, though, that if there is an increased output of thyrotropic hormone, there should also be

TABLE XIII

EFFECT OF THIOURACIL OF I^{131} OUTPUT OF THE THYROID GLAND

Group	No. of Animals	Daily Output Rate	Standard Deviation	Biol. Half-life	Standard Deviation
		(%)		(days)	
Untreated	22	3.1	1.6	31.3	20.3
Treated	50	13.8	7.7	6.6	3.6

a further break in the output curve demonstrated by an increased output of I^{131} as the thyrotropic hormone is increased. This effect was not observed in this study. Reineke and Singh (1955) reported that rats, given thiouracil to prevent further thyroidal combination of I^{131} during treatment, yielded thyroxine secretion rate values ten percent higher than those not receiving thiouracil. No such comparison could be made in this study since it was impossible to obtain secretion rates on the calves without thiouracil as demonstrated in Trial 5.

To the author's knowledge, there has been only one previous report of the thyroid secretion rate of dairy animals. This report, made by Monroe and Turner (1948), stated that a calf weighing 72.6 kg. secreted 1,500 µg D,L-thyroxine per day or 2.06 µg per 100 G. of body weight. This amount is equivalent to a 160 pound calf secreting 0.47 mg. per 100 pounds of body weight of L-thyroxine. The value was obtained by the method of Dempsey and Astwood (1943) in which the thyroid weight of two thiouracil treated calves (one calf received 1,000 µg and the other, 1,500 µg of D,L-thyroxine daily) were compared with the average thyroid weight of three normal calves.

They concluded from this comparison that the daily thyroid secretion rate was that given above. Since the number of calves involved in their study was small and the time of year when the value was obtained was not stated, it is hard to make a comparison. However, their value does fall in the range of the values obtained in this study. The average secretion rate of 42 calves in this study was 0.57 mg. per 100 pounds of body weight with a standard deviation of 0.105 mg. The secretion rate of each individual calf can be found in the tables of each trial. The average secretion rate by breed can be found in Table XIV. There is very little difference in the averages of the breeds. The Brown Swiss calves have the lowest average secretion rate, as might be expected because of the temperment of this breed. There is much more variation between individuals within a breed than between breeds.

A large seasonal difference in secretion rate found in other animals was not demonstrated in this study due to the fact that the calves were kept in the barn at all times from birth until after each trial and were not subjected to extreme environmental changes. There was a slight difference in the secretion rate values in studies made in May, 1955, and those in August of the same year. In comparing the May values (Table III) with those in August (Table IV), the latter were generally lower. These findings support the usual assumption that thyroid activity is depressed during hot weather. The next year, however, trials conducted during the same months did not show this difference, as can be seen in comparing the values of Table VIII with those of Table IX. This difference between

TABLE XIV

ESTIMATED THYROXINE SECRETION RATE
OF CALVES BY BREED

Breed	Number of Animals	Est. Thyroid Sec. Rate (mg./100 lbs.)	Standard Deviation
Brown Swiss	16	0.54	0.4
Guernsey	4	0.63	0.1
Holstein	9	0.60	0.08
Jersey	13	0.56	0.076
Overall	42	0.57	0.105

the two years can possibly be explained by the fact that the summer of 1955 was unusually hot and the summer of 1956 was unusually cool. It would have been interesting to have had the average barn temperature during the period of time when each trial was conducted to see how much variation there was throughout the year. It is the author's feeling that the environmental temperature, except during the summer of 1955, was seldom outside the range of the comfort zone of cattle. If this were true, then the secretion rate values obtained would not be expected to show any great seasonal variations but only those differences due to individual calves.

Because very few of the heifers used in this study are now in production, it is too early to determine if there is any correlation between the uptake of iodine or the thyroid secretion rate as a calf and the heifer's actual production. It is assumed that such a correlation does exist and a study to determine the degree to which it exists would be interesting. Also, a study to obtain secretion rates on the same animals while they are in production to determine the difference due to age and lactation might prove worthwhile.

SUMMARY

A thyroxine substitution technique for measuring the daily thyroid secretion rate of intact dairy calves is described. This method consists of injecting each calf with a tracer dose of I^{131}, either subcutaneously or intravenously. Forty-eight hours later, a count is obtained from the thyroid region of the neck and the uptake of the isotope is determined. On the sixth day after the injection of the isotope, oral treatment of 0.2 g. of thiouracil per kg. of body weight is started. Half of the daily dosage of thiouracil, administered by capsule, is given in the morning and the remainder given approximately twelve hours later. After four days of the thiouracil treatment, injections of thyroxine are started.

In this study, daily injections of L-thyroxine were given in graded doses of 0.1 mg. The level was increased every second day, and a thyroid count was obtained on each dosage level just prior to the injection of the increased dose. The daily thyroid secretion rate was estimated by plotting the corrected thyroid count, computed as percent of the previous count, against the level of L-thyroxine. The point at which the line crossed 100 percent was taken as the thyroid secretion rate of that animal.

The 48-hour uptake of injected I^{131} averaged 36.4 percent of the administered dose for 83 calves. Although the uptake varied between individuals and trials, Brown Swiss heifers had a comparatively lower uptake than the other breeds. The addition

of iodized salt to the ration had no apparent effect on the percent of I^{131} collected by the thyroid gland. Injecting the isotope intravenously made no difference in the 48-hour uptake. The calves showed the highest average uptake in the summer and the lowest in the spring. Younger calves had a higher average uptake than did the older calves.

The experimental animals demonstrated a very slow output rate which was assumed to be due to a recycling of metabolized hormone iodine. The output rate could not be increased by subcutaneous administration of potassium iodide during the experimental period or by including iodized salt at the recommended level for dairy cattle, but was increased when thiouracil, at the rate of 0.2 g. per kg. of body weight, was given orally each day.

The average daily thyroxine secretion rate of 42 calves was 0.57 mg. per 100 pounds of body weight with a range of 0.36 to 0.84 mg.

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