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The Effect of Thyroxine on Thyroid Function

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The Effect of Thyroxine on Thyroid Function

G. W. Pipes and C. W. Turner

INTRODUCTION

The rate of secretion of the thyroid hormones plays an important role in the growth process and in the maintenance of intense milk secretion in dairy cattle. In the absence of the thyroid glands or in hypothyroidism the growth rate is slowed and milk yield may be reduced 30 to 40 percent. On the other hand, with optimal levels of thyroid homone secretion, the growth rate is accelerated and the intensity of milk secretion is increased to the point where other hormones become limiting factors in productivity (Turner, 1956).

At the present time, the selection and mating of dairy cattle are based upon the total production of milk and fat in a lactation period of 305 or 365 days. Information has not been available as to secretion rates of the various endocrine glands upon which the total productivity of the animals is dependent. As a consequence, breeders of dairy cattle have been in the dark in regard to reasons why individual cows or the progeny of sires are incapable of measuring up to the high standards of production which the breeder wishes to attain.

As a result of intense endocrine research of the past 25 years, the role of the various hormones in stimulating the growth of the udder and the intensity of milk secretion has been determined (Turner *et al.,* 1957 a & b). There are two methods by which the activity of the individual endocrine glands may be measured.

One method consists of administering exogenous hormone and observing the effect of the hormone on the intensity of milk secretion. If the milk yield increases during the period of administration, it suggests that the rate of secretion of the hormone by the cows is a limiting factor in their productivity. In other words, if the cow had inherited a higher secretion rate for the given hormone, her productivity would have been equal to that stimulated by the additional hormone (Turner *et al.,* 1957 b).

The second method of measuring the inherited rate of secretion of endocrine glands is by direct measurement of the rate a certain hormone is secreted. The present report is concerned with the problems of the quantitative determination of the thyroxine secretion rate of cattle.

In a series of earlier reports from this station (Schultze & Turner, 1945), a method was reported by which the average thyroxine secretion

rate of experimental animals and fowls was estimated using a goitrogen to block thyroxine secretion and by administering graded doses of thyroxine. With this technique, it was necessary to sacrifice the animals and determine the weight of the thyroid gland. Such a method is not applicable to the larger domestic animals due to their high value. Further, the method determined the average thyroxine secretion rate rather than the secretion rate of individual animals. As a result of recent technical progress, many new and powerful tools are available for the study of thyroid physiology—radioiodine, purified thyroid hormone, goitrogenic substances, and chromatographic techniques. New techniques and a vast store of accumulated knowledge concerning thyroid physiology now offer the possibility that direct estimations can be made of the actual rate of hormone secretion in the individual, intact animal.

The present study is an exploration of possible methods for measuring the activity of the thyroid gland in terms of actual hormone production. In addition, the present study was undertaken in hopes of gaining basic information concerning thyroid physiology as shown by the response of the thyroid-pituitary system to exogenous thyroid hormone.

REVIEW OF LITERATURE

Radioactive Iodine (I^{131}) as an Indicator of Thyroid Function

Since several comprehensive reviews of the use of I^{131} are available, Rawson and McArthur (1947), Keating and Albert (1949), Seidlin (1949), Keating et al. (1950), Riggs (1952), and Albert (1952), only a few of the basic concepts will be considered in this discussion.

I^{131} has proven to be one of the most useful of all artificially produced isotopes. Its short half-life (eight days), as well as its high energy gamma radiation, make it a powerful and versatile tool in laboratory investigations and animal studies. While numerous complexities and technical difficulties are inherent in any tracer technique, the use of radio isotopes possesses several unique advantages.

A given dose may be tagged with respect to time and dynamic analyses made of a biological system rather than a static one.

Since radioiodine free from any chemically distinguishable amounts of stable iodine is available, investigations can be conducted under strictly physiological conditions.

It can be considered that after equilibration the behavior of the injected dose reflects the movement of all of the iodine under investigation.

Tracer studies are suitable for measurement of the proportion of iodine in the body which follows each metabolic pathway and for the

study of the rate of turnover in biological systems. Unless used in combination with quantitative chemical studies, tracer methods cannot indicate the actual amounts of materials involved. Combinations of quantitative chemical techniques and tracer methods present a clear overall picture of the process under study.

Serial analysis may be made for radioactivity in tissues and body fluids. If constant geometric conditions are maintained, serial *in vivo* determinations may be made with satisfactory accuracy.

The Metabolism of Iodine as Shown by I^{131}

When radioactive iodine is injected into a normal animal, it is rapidly distributed and soon comes into equilibrium with body fluids. During a second phase of distribution, iodine decreases rapidly in the blood due to its collection by thyroid and kidney. During a third phase a leveling off of the radioactivity of the blood occurs. Chemical fractionation of the blood indicates that the leveling out phase is due to the formation and discharge of thyroid hormone into the blood. The thyroid hormone is degraded to inorganic iodine which is again distributed between thyroid and kidney. Rigorous mathematical analyses have been made of this complex situation by Oddie (1949), Riggs (1952), and Brownell (1951).

Brownell and co-workers (1953) have designed an electrical analogue computer as an aid in interpreting their experimental data.

Accumulation of Radioiodine by the Thyroid: Radioactive iodine accumulates rapidly in the thyroid followed by a slow decline. Chaikoff and Taurog (1949) observed the maximum iodine concentration in the thyroid of a rat four hours after injection. Keating and Albert (1949) found a maximum accumulation of iodine between 24 and 48 hours in the human, while Blincoe and Brody (1955) found that the thyroidal uptake of the cow was maximal between 48 and 100 hours.

Since some loss of I^{131} labeled hormone occurs before the collection of administered radioactive iodine is complete, it is impossible to measure the actual uptake by the thyroid. If serial counts are made over the thyroid gland during the declining phase and the curve is extrapolated back to zero time, an estimation may be made of the actual uptake. The actual thyroidal uptake of iodine is a fundamental parameter of thyroid function since it indicates the portion of the iodine supply which is available for hormone synthesis.

The uptake of iodine by the thyroid may or may not reflect the release of the thyroid hormone since the thyroid may store sufficient hormone to maintain a steady rate of secretion day after day despite a high or low iodine diet. If periods of sufficient length are considered, uptake and release of iodine are constant.

An estimate of uptake is the most widely used diagnostic test for thyroid function (Keating *et al.,* 1950; Kriss, 1951; Kelsey *et al.,* 1949). Measurements of thyroidal uptake are usually made 24 hours after administration. When a single measurement is made, two possibilities exist. First, collection may be far from complete in a sluggish gland; second, a considerable quantity of iodine may have been secreted by hyper-active glands.

Therefore, *in vivo* studies employing a single measurement are at best a compromise with regard to time. For serious scientific investigation, serial measurements should be made when experimental conditions permit.

Release of I^{131} from the Thyroid: The rate of release of thyroidal I^{131} has been found to be one of the most useful indices of thyroid activity since it reflects the rate of release of thyroid hormone (Wolterink and Lee, 1950; Albert, 1951; Blincoe and Brody, 1955). The apparent rate of release of I^{131} from the thyroid proceeds at a vastly different rate than uptake since metabolized hormone is degradated to inorganic iodine and as such is recycled through the thyroid gland. The turnover time (time required for one-half of the thyroidal iodine to be secreted) varies widely between species and between individuals. In the human, the biological half-life of retention of labeled iodine in the thyroid has been reported to vary from 55 days to 106 days (Hickey and Brownell, 1954). The biological half-life of thyroidal I^{131} has been reported as four to six days in normal rats and mice (Albert, 1951; Wolterink, 1952; Wolterink and Lee, 1950). Wolterink (1952) reported the following turnover rates of thyroidal iodine in domestic animals: adult ewes, 32 days; new born pigs, 9 to 15 days; dogs, 4 to 8 days; adult turkey hens, 2 days; month old chicks, 3 to 6 days. Blincoe and Brody (1955) report that the biological half-life of thyroidal iodine in the dairy cow is of the order of 12½ days.

The Excretion of Radioiodine: The major pathways of excretion of inorganic iodine are the urine and, in lactating animals, the milk. Iodine may leave the body in the form of thyroxine or a metabolite thereof (Gross and Leblond, 1947; Monroe and Turner, 1949; Myant and Pochin, 1950; Taurog, 1954) through the feces but little or none passes into the milk in this form. The major portion of organic iodine appears to be metabolized to inorganic iodine and excreted in the inorganic form through—

Urine: The major losses of iodine from the organism are through the urine. If the total amount of iodine excreted in the urine is plotted versus time, a curve results closely paralleling thyroidal accumulation and discharge. Several investigators have suggested use of the urinary excretion curve as an index of thyroid function.

Milk: In the lactating animal a large portion of the injected dose of radioiodine is excreted in the milk (Rugh, 1951; Nurnberger and Lipscomb, 1952; Campbell *et al.* 1950; Wright *et al.* 1955; Blincoe and Brody, 1955; Glascock, 1954; Lengemann *et al.* 1955).

While inorganic iodine may be lost from the animal through the sweat, through exhaled air, and through the feces, only negligible amounts escape through these avenues under normal conditions.

Feces. There is a considerable body of evidence to indicate that radioactive iodine leaves the body by the feces in the form of thyroxine. Gross and Leblond (1947) found 80 percent of the injected dose of thyroxine in the feces of the rat after 24 hours. Monroe and Turner (1949) failed to demonstrate the presence of thyroxine in the feces of normal goats and could demonstrate only 1 to 5 percent of the administered dose in the feces when exogenous hormone was administered. Myant and Pochin (1950) found 10 percent of an injected dose of radioactive thyroxine in the feces of three normal subjects within a three-day period.

Taurog (1954) reported that a conjugate of thyroxine, probably the glucuronide, was excreted more rapidly from the liver than thyroxine and was less readily absorbed from the gastrointestinal tract. The importance of this mechanism for regulating the level of endogenous circulating hormone merits further investigation.

These investigations clearly demonstrate that in both man and rat a very significant portion of an injected dose of exogenous thyroxine is lost from the body through the feces. While there is every reason to believe that endogenous thyroid hormone follows the same route as exogenous hormone, this theory has not been accepted by many workers. Iodide is absorbed so efficiently by the intestines that only traces of iodide ion escape from the body in this manner (Keating and Albert, 1949).

The Interrelationship Between Radioiodine in Tissues, Blood, Urine, and Thyroid

When a comparison is made of the radioactivity of the thyroid, the amount of radioactivity excreted in the urine, the concentration of I^{131} in the blood, and the amount of radioactivity in tissues with regard to time, it is evident that all of these curves tend to level off at about the same time, indicating that there is some factor which all have in common. As Keating and Albert (1949) have pointed out, all four of these curves have a common parameter—the rate-constant representing the proportional rate of disappearance of radioiodine from the blood.

Use of Radioiodine in Estimating Rate of Secretion of Thyroid Hormone

Stanley (1949) suggested that direct estimation of the rate of thyroid hormone formation in man could be made from simultaneous measurements of thyroidal I^{131}, urinary I^{131}, and urinary I^{127}. If the ratio between stable and radioactive iodine in the urine is known, calculations of the amount of iodine entering the thyroid can be made from *in vivo* thyroid radioactivity determinations. While this method will give an estimation of the amount of stable iodine entering the thyroid, it cannot give quantitative information concerning the synthesis and release of thyroid hormone. It is of particular interest in this regard that Hickey and Brownell (1954) have presented evidence indicating the possibility that a second iodine pool exists within the thyroid gland as iodine or a compound easily deiodinated which may be released as inorganic iodine.

Riggs (1952) has suggested the thyroid secretion rate can be calculated from excretion of stable iodine I^{127} in the urine and the uptake of I^{131} by the thyroid. It would appear that this method, as well as that of Stanley (1949), would require acceptance of the theory that iodine uptake equals hormone synthesis under the conditions of the experiment.

Wolterink and Lee (1950), who estimated the thyroid secretion rate of the rat from the biological half-life of thyroidal I^{131}, reported a good correlation with the thyroxine-thiouracil method of Dempsey and Astwood (1943). Albert (1951) estimated the thyroid secretion rate of the rat from the proportional rate of loss of thyroidal I^{131}. Since rat thyroids are known to vary widely in iodine content, it would appear difficult to measure the actual amount of thyroxine involved solely on the basis of a tracer method.

Several investigators have suggested replacement therapy techniques in estimating the thyroid hormone output (Pipes *et al.* 1950; Perry, 1951; Greer, 1951; Reineke and Singh, 1955; and Henneman *et al.* 1955). These techniques involve the administration of the thyroid hormone at varying dose rates and estimations of various thyroid states produced employing radioiodine techniques. Since these methods require the establishment of several successive states of thyroid activity, much further fundamental information must be obtained with regard to the duration of effect of thyroid hormone on the thyroid-pituitary system and the rapidity of response of this mechanism.

Effects of Thyroidally-Active Substances on Thyroid-Pituitary Relationships

Many early investigators demonstrated that the thyroid hormone

excercises a depressing effect on the thyroid stimulating function of the anterior pituitary (Cameron and Carmichael, 1920; Loeb *et al.*, 1930; Marine, 1935). Uotila (1940) found that thyroxine in large doses produced the same effect upon the thyroid as did hypophysectomy and attributed this action to suppression of pituitary function by thyroxine. Belasco and Murlin (1941) found a decreased oxygen consumption rate in the thyroid of animals fed thyroxine. Koger and Turner (1943) demonstrated that the administration of increasing amounts of iodinated casein progressively reduced the thyrotropic content of the pituitary. Purves and Griesbach (1946) demonstrated that the pituitaries of rats fed thyroid contained less than 5 percent of the normal level of thyrotropic hormone.

The use of radioactive iodine in thyroid investigations (Hertz *et al.* 1938) and the discovery of substances which inhibit the formation of thyroxine within the thyroid gland (Goitrogens) (Mackenzie and Mackenzie, 1943) have proven powerful and versatile tools in the investigation of thyroid physiology.

Thyroid function in the steady state (Dougherty, *et al.* 1951; Leblond and Cross, 1948) involves at least four simultaneous activities: 1. The trapping of inorganic iodine by the thyroid cells. 2. The formation of thyroid hormone from tyrosine and inorganic iodine. During this process, three chemical reactions appear to take place within the thyroid gland, the liberation of iodine from iodides, the iodination of tyrosine to 3, 5-diiodotyrosine, and the oxidative coupling of two molecules of the latter to form thyroxine. 3. Storage of the thyroid hormone in the colloid as thyroglobulin. 4. The release of a thyroid hormone into the blood.

The classical concept has been that all of these functions of the thyroid gland are under the direct control of the anterior pituitary as mediated through the thyrotropic hormone. Many investigators have postulated a balanced relationship between pituitary and thyroid, which in its simplest terms is a servo or feedback mechanism by which the circulating level of thyrotropic hormone controls the output of thyroxine from the thyroid gland. In turn, it has been visualized that thyroxine acts upon the pituitary to control the secretion of thyrotropic hormone. Although feedback mechanisms are common in biological systems, such a simple interplay between pituitary and thyroid is not sufficient to explain the effects of exogenous thyroxine and thyrotropic hormone or the ability of the thyroid of the hypophysectomized animal to respond to changing experimental conditions.

The regulation of thyroid activity appears to be a rather complex and poorly understood interrelationship of several mechanisms. If thyroid activity is regulated solely by the pituitary, the thyroid of the hypophy-

sectomized animal could not be influenced by any means other than the administration of thyrotropic hormone. It would also be evident that the thyroid hormone could have no action in either stimulating or depressing the activities of the thyroid gland in the absence of the pituitary. Yet the literature reveals that each of these effects can be demonstrated.

As far back as 1930, Loeb demonstrated that the simultaneous administration of thyrotropic hormone and thyroid to the rat induced less activation changes than would have been expected by thyrotropic hormone alone. Cortell and Rawson (1944) and Ingbar and Roberts (1952) reported that the effect of thyrotropic hormone was diminished when thyroxine was administered as shown by a reduced ability of the thyroid to take up I^{131}.

While Halmi *et al.* (1953) found that thyroxine did not interfere with the reaction of hypophysectomized rats to thyrotropic hormone, it appears possible that thyroxine exerts an effect upon the thyroid gland not that is directly mediated through the pituitary. One of the possible mechanisms has been suggested by the investigations of D'Angelo (1954) who found that excess thyroid hormone promotes the rapid disappearance of thyrotropic hormone from the blood. Halmi *et al.* (1953) found that thyroxine depressed thyroid activity in the rat to a greater extent than hypophysectomy. In the hypophysectomized rat, thyroxine produced a further depression of thyroid activity. These results were confirmed by Lipner *et al.* (1954), who demonstrated that the mouse thyroid was more independent of pituitary control than the thyroid of the rat.

Botkin *et al.* (1954) found that intact thyroid glands which were incubated with thyroxine responded to thyrotropic hormone by discharge of previously labeled hormone. He concluded, therefore, that thyroxine acted primarily in suppression of secretion of thyrotropic hormone or that a direct effect of thyroxine upon the thyroid was apparent only in the intact animal.

VanderLaan and Caplan (1954) and Halmi and Spirtos (1954) found that when constant amounts of thyrotropic hormone were administered to hypophysectomized rats, an inverse relationship existed between the iodine content of the diet and the ability of the thyroid to concentrate iodine. Since thyroidal uptake of iodine was high on low iodine diets and high iodine diets resulted in a low uptake, these investigators concluded that the iodine-concentrating capacity of the thyroid was not completely under the control of the pituitary.

It would appear that if thyrotropic hormone were the sole regulator of thyroid function, and if thyrotropic hormone acted at the same threshold on both the accumulation and discharge of iodine of the thyroid

gland, it would be impossible for an animal to achieve iodine balance. On a low iodine diet the increased amounts of thyrotropic hormone necessary for higher iodine uptake would also result in more rapid discharge of hormone from the thyroid gland.

Halmi and Spirtos (1955) reported that when propyl thiouracil was administered to hypophysectomized rats receiving constant amounts of thyrotropic hormone, the response of the thyroid gland to dietary iodine concentration was abolished. These investigators suggested that thyroxine or thyroid hormone within the thyroid gland acted as an inhibitor to the uptake of iodine.

VanderLaan and Caplan (1954) demonstrated that intact rats chronically treated with propyl thiouracil, responded to concurrent administration of thyroxine and propyl thiouracil by decreased ability of the thyroid to collect iodine. Presumably the thyroid gland had been purged of organic iodine due to prolonged treatment with thiouracil and was unable to form thyroid hormone while thiouracil and thyroxine were being administered. Under these conditions, iodine uptake could not be blocked by stores of iodine within the thyroid gland.

Rawson and Money (1949) have suggested that the increased collection of iodine observed when thyrotropic hormone is administered is due to the release of thyroid hormone. Increased collection of iodine and synthesis of thyroid hormone follow due to the purging action of thyrotropic hormone. In view of the work of VanderLaan and Caplan (1954), as previously discussed, it is evident that thyrotropic hormone acts upon both the iodine collecting and the iodine releasing functions of the thyroid.

While there is little doubt that the primary effect of exogenous thyroxine on the thyroid gland is mediated by the anterior pituitary, there is a considerable body of evidence to indicate that all of the functions of the thyroid gland may not be inhibited at the same rate and to the same degree when exogenous thyroid hormone is administered at varying dose rates. In view of this possibility, the effect of thyroid hormone upon the uptake of iodine by the thyroid and the release of thyroid hormone will be discussed in separate sections.

The Effect of Thyroid Hormone on the Uptake of Iodine by the Thyroid Gland:

The qualitative effect of thyroid hormone on the uptake of iodine has been studied in several species. A reduced uptake of iodine has been reported by Cortell and Rawson (1944) in the rat; Joliet *et al.* (1945) in the guinea pig; Werner *et al.* (1948) in the human subject; Cornwall

(1950) in the chick; and Querido *et al.* (1953) in the mouse.

Rate of Response:

Few detailed studies have been made for the time required for exogenous thyroid hormone to exert an effect upon thyroid pituitary balance. Most investigators have continued administration for one week or longer before repeating iodine uptake studies. Greer (1951), who studied the effects of oral thyroid administration in the normal human subject, found depression was more complete after the eighth day of treatment as compared with the fourth.

Johnston *et al.* (1951) investigated the effect of prolonged administration of desiccated thyroid. In patients receiving 0.5 grains (32.5 mg.) of thyroid daily there was a rapid decline in the ability of the gland to take up radioiodine, which reached its lowest level after one to two weeks. At the same dose level, the radioiodine uptake rose rapidly to levels as high or higher than the original. When the dose was doubled, iodine uptake rapidly declined again. In some cases a second rise was observed, while in others iodine uptake diminished slowly over a four-week period until hypothyroid levels were reached.

Overbeek *et al.* (1953) found that iodinated casein produced a more profound effect upon iodine uptake in the rat when it was administered for 12 days as compared with 8. Querido *et al.* (1953) found that the uptake of iodine was greater in the mouse after five days of treatment with iodinated casein as compared with nine days.

Duration of Effect.

Werner *et al.* (1948) reported that in one case iodine collection failed to return to normal ten weeks after cessation of desiccated thyroid. Keating *et al.* (1950) found that in eight out of nine patients who had received desiccated thyroid within the previous 30 days, iodine accumulation of the thyroid was nil. In one case, however, iodine uptake returned to normal seven days after discontinuation of desiccated thyroid.

Greer (1951), in a study of a larger number of normal human subjects, found thyroidal uptake was normal one week after cessation of therapy. A few subjects showed marked depression for 6 to 11 weeks. Recovery of the ability of the thyroid to accumulate iodine was as rapid in subjects who had received thyroid for several years as in those who had received treatment for only one week. Johnston *et al.* (1951) reported that a three-week interval was required for recovery from desiccated thyroid therapy as indicated by radioactive iodine uptake.

The results of these investigations are difficult to reconcile with the

work of Hughes (1945) and Monroe and Turner (1949), who found that the effect of thyroxine upon the thyroid pituitary system was of short duration, as shown by the ability of the thyroid gland to hypertrophy rapidly under the influence of goitrogen when thyroxine was withdrawn. It can only be postulated that the thyroid gland may temporarily lose its ability to respond to the thyrotropic hormone after prolonged administration of large amounts of thyroxine or that there may be a similar delay in the resumption of pituitary function.

The duration of effect of thyroid hormone may vary considerably with species. Overbeek *et al.* (1953) reported that thyroidal uptake of iodine was resumed 48 hours after iodinated casein was withdrawn from the rat. In addition, the rate of metabolism of thyroid hormone may vary widely between species.

Dose Response.

Cortell and Rawson (1944) found that 20 micrograms of D,L-thyroxine depressed iodine uptake to the level of hypophysectomy in the rat. Joliet *et al.* (1945) found that large doses of thyroxine (0.25 milligrams per day) prevented the uptake of iodine by the thyroid gland of the rat and the guinea pig. Werner *et al.* (1948) reported that 120 milligrams daily of desiccated thyroid depressed the uptake of I^{131} in man to 4 percent of administered dose of I^{131}.

Greer (1951) found that 3 grains (195 milligrams) reduced iodine uptake to about 10 percent of the injected dose of I^{131}. The amount of desiccated thyroid required to produce marked thyroid depression varied from 1 to 3 grains (64.8 to 194.4 mg.) in the normal human subject. In one case, a daily dose of 9 grains (583.2 mg.) of desiccated thyroid was required to reduce the 24 hour uptake of I^{131} to 10 percent. No further depression was noticed when 12 grains (777.6 mg.) were given daily. A rather wide variation existed between normal subjects, both in uptake of iodine and response to desiccated thyroid. Some individuals had uptakes as high as 7 or 8 percent while receiving desiccated thyroid and increased dose rates did not produce further depression. In other cases, the lowest dose given reduced iodine uptake to 2 percent.

Considerable variation in response to desiccated thyroid has been noted by Stanley and Astwood (1949), who found that 1.5 grains (97.2 mg.) desiccated thyroid produced nearly complete inhibition in some individuals while 3 grains (194.4 mg.) produced only a moderate inhibition in others. These investigators found that 4 to 8 grains (259.2 to 518.4 mg.) of thyroid daily produced a complete or very nearly complete suppression of iodine uptake in normal subjects.

Perlmutter *et al.* (1952) reported that 120 milligrams of desiccated thyroid depressed thyroidal uptake in man to hyperthyroid levels. Papper *et al.* (1952) found that 0.2 to 0.5 milligrams of L- thyroxine, sodium, suppressed the uptake of I^{131} in normal patients but only partially suppressed the uptake in cases of non-toxic goiter. Starr and Liebhold (1953) reported that 0.075 milligrams of L-thyroxine reduced I^{131} uptake to about 50 percent of normal in man. Greer and Smith (1954) found that the administration of 180 milligrams of thyroid for one week depressed the uptake of I^{131} to below 20 percent in most cases, but that 540 milligrams did not suppress uptake in thyrotoxic patients. These investigators suggested that this procedure be used in diagnosis of hyperthyroidism.

Starr *et al.* (1955) investigated the effects of triiodothyronine on the uptake of iodine. Thyroid accumulation was reduced by about 75 percent by 0.0088 milligrams per day. Continued administration at the 0.14 milligram level did not produce further depression. All investigators are in agreement that thyroidally-active substances suppress the uptake of I^{131} of the thyroid gland. Results vary rather widely, which is probably due to the different methods used in different laboratories to measure iodine uptake. A large degree of variation is evident between individuals.

Effects of Thyroidal Substances Upon the Release of I^{131} from the Thyroid Gland

The Response of Thyroid-Pituitary System—Perry (1951), Wolff (1951), and Reineke and Singh (1955) found that thyroxine inhibited the release of thyroidal iodine in the rat within 20 hours of injection. The data of Anderson (1954) indicates that thyroxine or triiodothyronine exerts its maximum effect in the rat within 24 hours as shown by retention of I^{131} in the thyroid.

It is difficult to reconcile these findings with the work of Randall and Albert (1951) and VanderLaan and Greer (1950), who found a maximum depression in the uptake of I^{131} by rats five to six days after hypophysectomy, unless it is assumed that the administration of thyroxine produces a more rapid effect than hypophysectomy. It is also possible that iodine collection and discharge by the thyroid are not inhibited at the same rate by the absence of thyrotropic hormone.

Randall *et al.* (1951) have shown that hypophysectomy reduces the rate of loss of iodine from the thyroid of the rat to about one-eighth of normal, which corresponds rather closely with the uptake of iodine by the hypophysectomized rat.

Reineke and Singh (1955), as well as Hennemann *et al.* (1955), observed that when thyroxine was administered in excess of the thyroid

secretion rate, the radioactivity of the thyroid increased, indicating that iodine collection was occurring at a more rapid rate than discharge. It is interesting to note in this regard that Greer and Smith (1954) found, in some cases, that part of the iodine which accumulated in the thyroids of patients receiving large doses of desiccated thyroid could be discharged by administration of thiocyanate, indicating that the iodine was present in inorganic form.

Hennemann *et al.* (1955), in estimating the thyroid secretion rate of sheep, gave three consecutive doses at 24-hour intervals. Presumably this period of time was to allow the thyroid-pituitary system to reach equilibrium.

Dosage and Duration of Effect—Perry (1951) found that in the rat the effect of five daily doses of 15 micrograms of L-thyroxine persisted for four days after discontinuing treatment. Anderson (1954) found that the magnitude of dose affected the duration of effect in the rat. Ten micrograms of D, L-thyroxine inhibited the release of I^{131} for at least 48 hours. The 2.5 and 5.0 microgram levels of L-triiodothyronine produced an inhibiting effect for about 24 hours. These data indicate that despite the much greater biological activity of triiodothyronine, its effect upon the thyroid-pituitary system is much shorter than that of thyroxine. Reineke and Singh (1955), in estimating the thyroid secretion rate of the rat, progressively increased dosage every two days. Evidently at lower dosage rates, the pituitary-thyroid system of the rat comes to equilibrium more rapidly.

A review of the literature indicates that the effect of exogenous thyroid hormone is more rapid and more pronounced upon release of thyroidal hormone than upon uptake of iodine by the gland.

EXPERIMENTAL

While the literature reveals that techniques have been developed to measure wide variations in thyroid activity, such as those found in cases of hyperthyroidism and hypothyroidism, no generally accepted methods are available for estimation of the thyroid secretion rate in terms of actual hormone production. As previously discussed, radioactive tracer techniques, unless combined with chemical measurements, cannot indicate the quantitative amount of thyroid hormone involved.

In measuring thyroid hormone secretion rate in terms of actual production in the intact animal, replacement techniques appeared particularly promising. Since the thyroid-pituitary system acts as a servo-mechanism to maintain a constant supply of thyroid hormone in the blood, exogenous thyroid hormone should replace an equal part of the normal secre-

tion without increasing the total amount present in the blood after equilibration. When thyroxine is administered at rates exceeding the normal secretion, thyroid activity should be depressed at least to the level of the hypophysectomized animal.

It would appear feasible to measure thyroid activity by administration of increasing amounts of thyroid hormone and parallel measurement of thyroid activity as shown by radioiodine techniques. The minimum dose required to produce maximum depression of thyroid activity could be used as an index of the normal thyroid hormone secretion rate. A second approach would be the administration of thyroid hormone in excess of the normal secretion rate and a progressive reduction of the dose until thyroid activity is resumed as indicated by radioiodine. In this study, both avenues of approach were investigated.

In the application of either of these theories, several basic questions arise. What is the normal iodine metabolism of the species under investigation? As reported by Wolterink (1952) iodine metabolism progresses at vastly different rates in various species. Other questions that arise in application of these techniques are also of importance. How rapidly does the thyroid-pituitary system respond to exogenous hormone? What is the duration of effect of administered hormone on thyroid-pituitary equilibrium?

While realizing that a practical method of estimation of the thyroid hormone secretion rate must be based on *in vivo* measurements, original investigations were made on blood samples. Two factors were involved in our choice of blood samples as a criterion of thyroid function. First, the protein-bound-iodine (PBI^{131}) or thyroxine-like fraction of blood represents a summation of all thyroid function, i.e. synthesis and release of thyroid hormone. Second, the specialized equipment necessary for highly reproducible *in vivo* measurement of thyroidal I^{131} was not available at that time.

METHODS

Since operating procedures varied with the species studied, details of each experiment will be included in the appropriate section. Only general operating techniques will be discussed here.

Radioiodine Solutions.

Carrier-free sodium radioiodine (NaI^{131}) was diluted to the required concentration with slightly alkaline distilled water. At the same time, standards (a portion of injected dose) were prepared for future comparisons. All standards were prepared in series of three or five.

Total Radioiodine in Blood.

Blood plasma separated from heparinized blood was transferred to 2.5-centimeter diameter, cupped, stainless steel planchets. Samples were dried by infrared heat lamps positioned at a sufficient distance to prevent formation of uneven surfaces. In this procedure as well as in all following procedures, known amounts (a portion of injected dose) were added to non-radioactive blood or fractions thereof, and dried under identical circumstances.

Protein-Bound-Radioiodine.

The protein-bound-iodine fraction of blood was determined according to the procedure of Chaikoff *et al.* (1947). Plasma proteins were precipitated with dilute trichloroacetic acid, separated by centrifugation, and washed twice with the precipitating agent. The precipitating proteins were dissolved in 2 N NaOH and dried as previously described.

Inorganic Iodine.

The radioactivity of the inorganic iodine fraction of blood was determined from an aliquot of the first two portions of trichloroacetic acid used in the preceding procedures. After neutralization with NaOH, the samples were dried as previously described. Appropriate corrections were employed for the small amount of inorganic iodine lost in the third trichloroacetic acid wash.

Butanol-Soluble Radioiodine.

The thyroxine-like fraction was determined by the method of Taurog and Chaikoff (1948) as modified by Blincoe and Brody (1955). Plasma samples were extracted once with a double volume of n-butanol and twice with equal portions of n-butanol. The extracts were combined and extracted twice with Blau's reagent (4 N NaOH and 5 percent Na_2CO_3). The n-butanol fraction was concentrated under reduced pressure at 50 to 70°C. The residue, consisting of 1 to 3 milliliters, was transferred to the previously described planchets and evaporated almost to dryness under infrared heat lamps. The distillation flasks were successively washed three times with 2-milliliter portions of n-butanol. These portions were added to planchets as soon as the required volume was provided by evaporation. In cases where it appeared that poor geometry or contamination might conceivably lead to errors, the planchets were counted and allowed to remain in the laboratory until radioactive decay had reduced the count rate to almost background levels. At this time, known amounts of radioactivity were added to each planchet in the series with sufficient water to

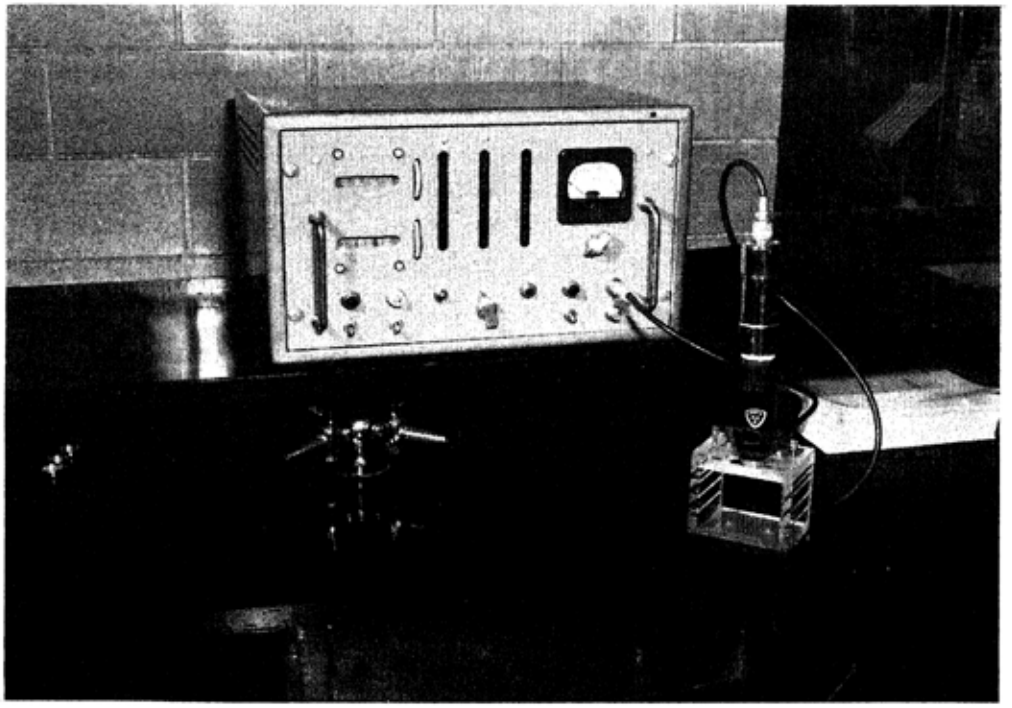

Figure 1—Scaler and GM tube used in measurement of PBI^{131} and BSI^{131}.

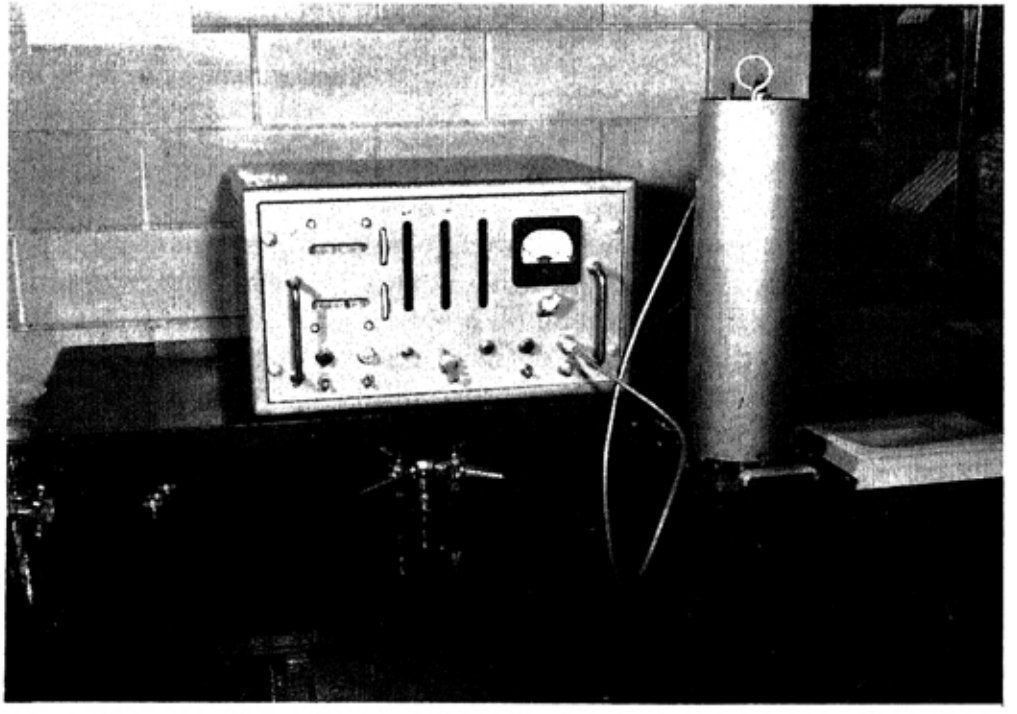

Figure 2—Scintillation counter and decade scaler.

dissolve the residue. The liquid was evaporated to dryness, the radioactivity of a planchet was again determined, and corrections were applied if necessary to the preceding count. By use of such internal standards each planchet provided its own self-absorption correction.

Determination of Radioactivity.

Samples and standards were counted together with one of two counting systems. The first consisted of a thin mica end window GM tube connected to a scaler. The second was composed of a scintillation counter employing a cylindrical anthracene crystal, 2.5 centimeters in diameter, coupled to a photomultiplier tube and decade scaler (Figs. 1 & 2). The standard error of counting ranged from 3 to 4 percent. All samples were prepared in series of three when the volume of plasma permitted. Standards were employed in series of three or five. The conventional corrections were made for radioactive decay, self-absorption, and background.

Thyroxine Solutions.

In the earlier phases of this investigation, crystalline D, L-thyroxine (free acid), as provided by the British Drug House, was employed. When crystalline L-thyroxine became available, the free acid, as provided by Glaxo Laboratories Ltd. was employed. The purity of this product is reported to range between 95 and 98 percent.

Since the activity per unit of weight of L-thyroxine is twice that of D, L-thyroxine (Reineke and Turner, 1945), the thyroxine used is identified in each experiment. All thyroxine solutions were dissolved in a minimum amount of 0.1 N NaOH and diluted nearly to volume with distilled water. Sufficient 0.1 N HCl was added until precipitation became slightly evident. For final injection, the pH was adjusted again with 0.1 N NaOH until the solution became clear. All solutions were kept under refrigeration. Injections were made daily according to body weight. Administration in all species was by the subcutaneous route.

RESULTS

Effect of Thyroxine Upon Thyroid Function in the Rat

Rats were used in the initial studies because of their convenience as a laboratory animal, their ready availability in large numbers, and the extensive data available on the thyroid secretion rate of the Missouri strain.

Methods and Materials.

The experimental animals used were Wister albino rats of the Missouri strain. Since the thyroid secretion rate of rats has been shown to

vary with sex and body weight (Monroe and Turner, 1946), only rats of one sex were used in each experiment. Rats were divided into groups according to body weight. Each experimental lot was then balanced by rats from each of the weight groups.

The weights of individual rats ranged from 90 to 200 grams but the total body weight for each group remained relatively constant. Blood samples were taken by heart puncture while the animals were under ether or nembutal anesthesia. One-milliliter blood samples were taken from each animal and added to a pool common to the group. Temperatures in the laboratory ranged from 70° to 80° F except as noted.

Radioactive iodine I^{131} was administered interperitoneally. Injections were made on the basis of body weight at approximately 10 microcuries per 100 grams.

Thyroids were removed, dissected free of other tissues, and digested in 2 N NaOH prior to counting.

Effect of Graded Doses of Thyroxine on Thyroidal Uptake of I^{131} and Formation of PBI^{131}.

Five groups of female rats were injected daily for seven days at the following levels of D,L-thyroxine; 0, 2.5, 5.0, 7.5 and 10.0 micrograms per 100 grams body weight. On the seventh day I^{131} was administered. Twenty-four hours later, blood samples were drawn from all groups.

Thyroxine at the rate of 2.5 micrograms per day reduced the uptake of the rat thyroid to about 30 percent of the thyroidal uptake to the control animals (Fig. 3). Further increases in dose rate did not significantly reduce uptake of I^{131}.

Maximum depression of protein-bound-iodine in the blood (Fig. 3) resulted from the 5-microgram dosage. Further depression in thyroid activity was not evident at the 7.5 and 10.0 microgram levels.

The activity of the PBI^{131} fraction indicated that the thyroid secretion rate of the rat, under our laboratory conditions, ranged roughly between 2.5 and 5.0 micrograms per 100 grams body weight. Unfortunately, the majority of the dose rates were above the secretion rate and closer estimation was impossible.

Response of Rat Thyroid-Pituitary System to Thyroxine.

Since preliminary experiments indicated the feasibility of further study, observations were made on the rapidity of the response of the thyroid-pituitary system to thyroxine.

Groups of rats receiving daily injections of 4 micrograms of thyroxine indicated a maximum depression of PBI^{131} after thyroxine had been

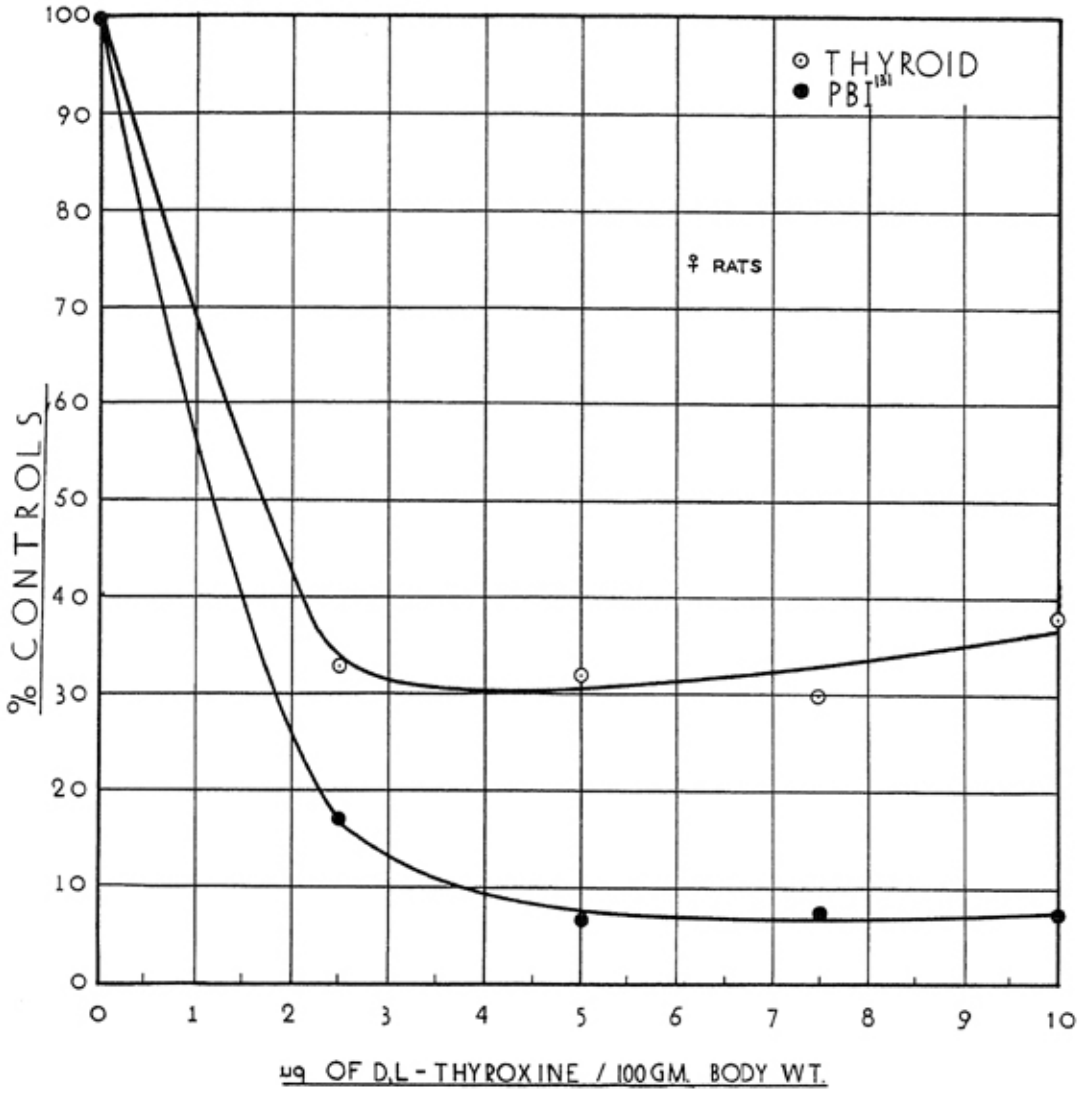

Figure 3—The effect of graded doses of thyroxine on thyroid I^{131} and PBI^{131}.

administered for one or two days as compared with four, six and eight days of treatment. It was also of interest to note that the effect of eight daily doses of thyroxine did not produce a greater depression of thyroid activity than did two doses (Fig. 4).

In another series of experiments designed to study the response of the thyroid-pituitary system to administered thyroid hormone, one group of female rats was injected with 8 micrograms of thyroxine at both 6 and 24 hours prior to the administration of I^{131}. A second group received one injection at the 8 microgram level 24 hours before I^{131}. The third group received 8 micrograms of thyroxine 12 hours prior to I^{131}.

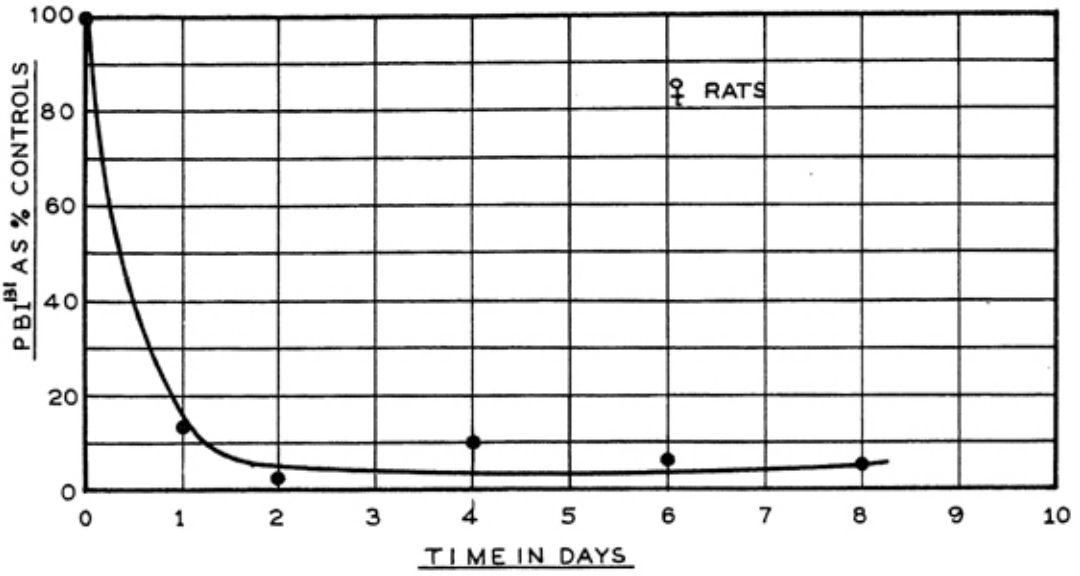

Figure 4—The rate of response of the rat thyroid-pituitary system to thyroxine (Micrograms of D, L-thyroxine daily).

The experiment was duplicated at the 2-microgram level of thyroxine administration. Both the 2 and 8 microgram dosage ranges produced a maximum effect after 24 hours as compared with 60 hours. Therefore, it seemed evident that a single injection at either of these two dosage levels resulted in a rapid and stabilized depression of thyroid activity (Fig. 5).

Thyroxine injected 12 hours prior to iodine administration did not appear to produce as profound an effect at the 8 microgram level as did

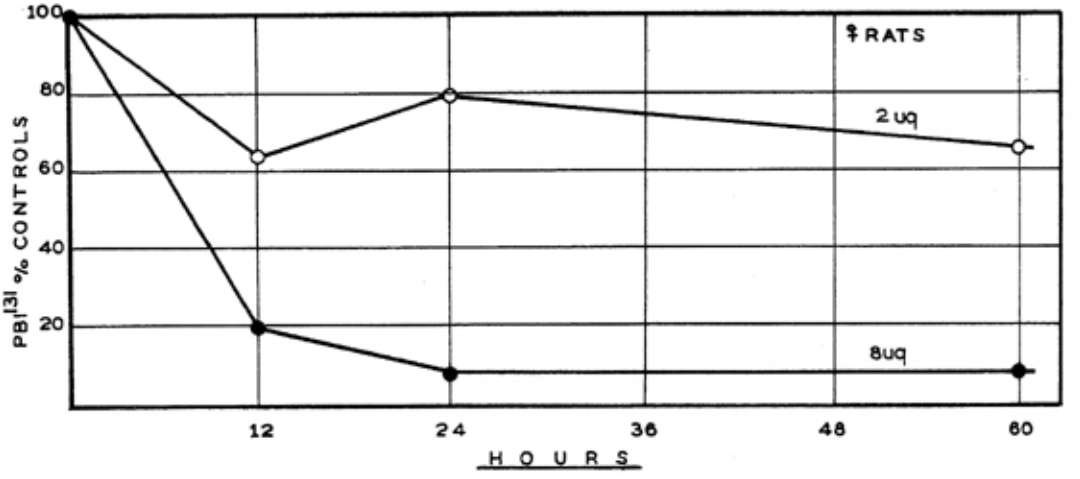

Figure 5—The rate of response of the rat thyroid to 2 and to 8 micrograms of D, L-thyroxine.

the same dosage injected at the 24 hour interval. However, since the 2-microgram level of thyroxine administration produced an effect as great as that produced by administration at 24 and 60-hour intervals, the problem merits further study. The results of this investigation are in accord with experiments of Reineke and Singh (1955) and Perry (1951), who found that the effect of thyroid hormone on the release of iodine by the thyroid gland was evident 24 hours after administration of thyroxine as indicated by reduced rate of loss of thyroidal iodine in the rat.

Estimating the Thyroid Secretion Rate of the Rat:

Estimates of the thyroid secretion rate of female rats were obtained by administering D,L-thyroxine at dose rates below the level of normal secretion (Fig. 6). A regression line obtained by the method of least squares indicated that the normal thyroid secretion rate of young female rats was 5.63 micrograms per 100 grams body weight.

The observations of Reineke and Singh (1955) are in agreement with the present study. These investigators found that the thyroid secretion rate of young female rats ranged between 2.21 to 2.56 micrograms of L-thyroxine. Since L- thyroxine possesses twice the effectiveness of the D,L-isomer (Reineke and Turner, 1945) these findings indicate a secretion rate of 4.42 to 5.2 as compared with 5.63 micrograms found in our study.

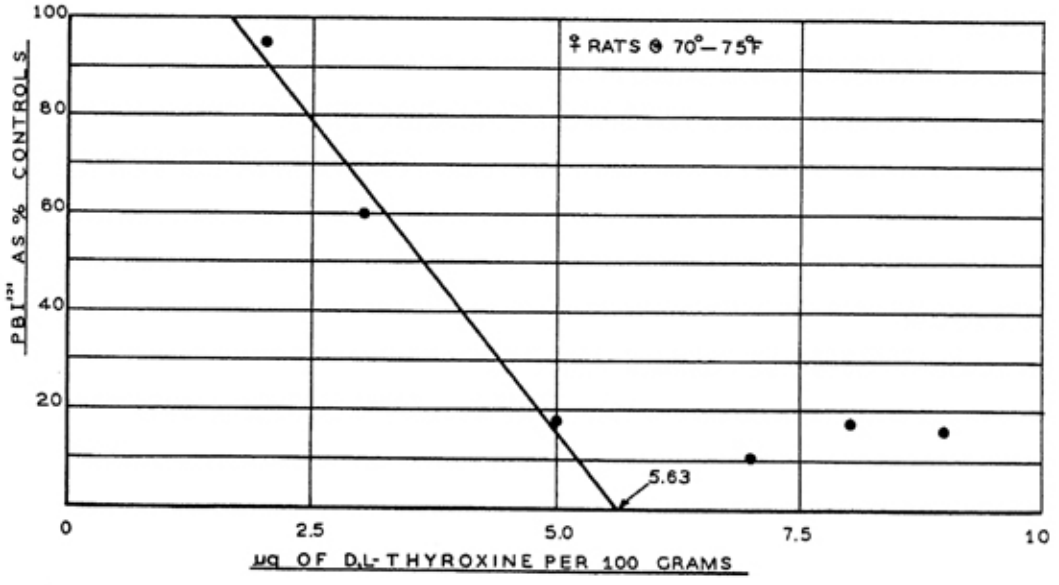

Figure 6—The thyroid secretion rate of the rat at 70° F.

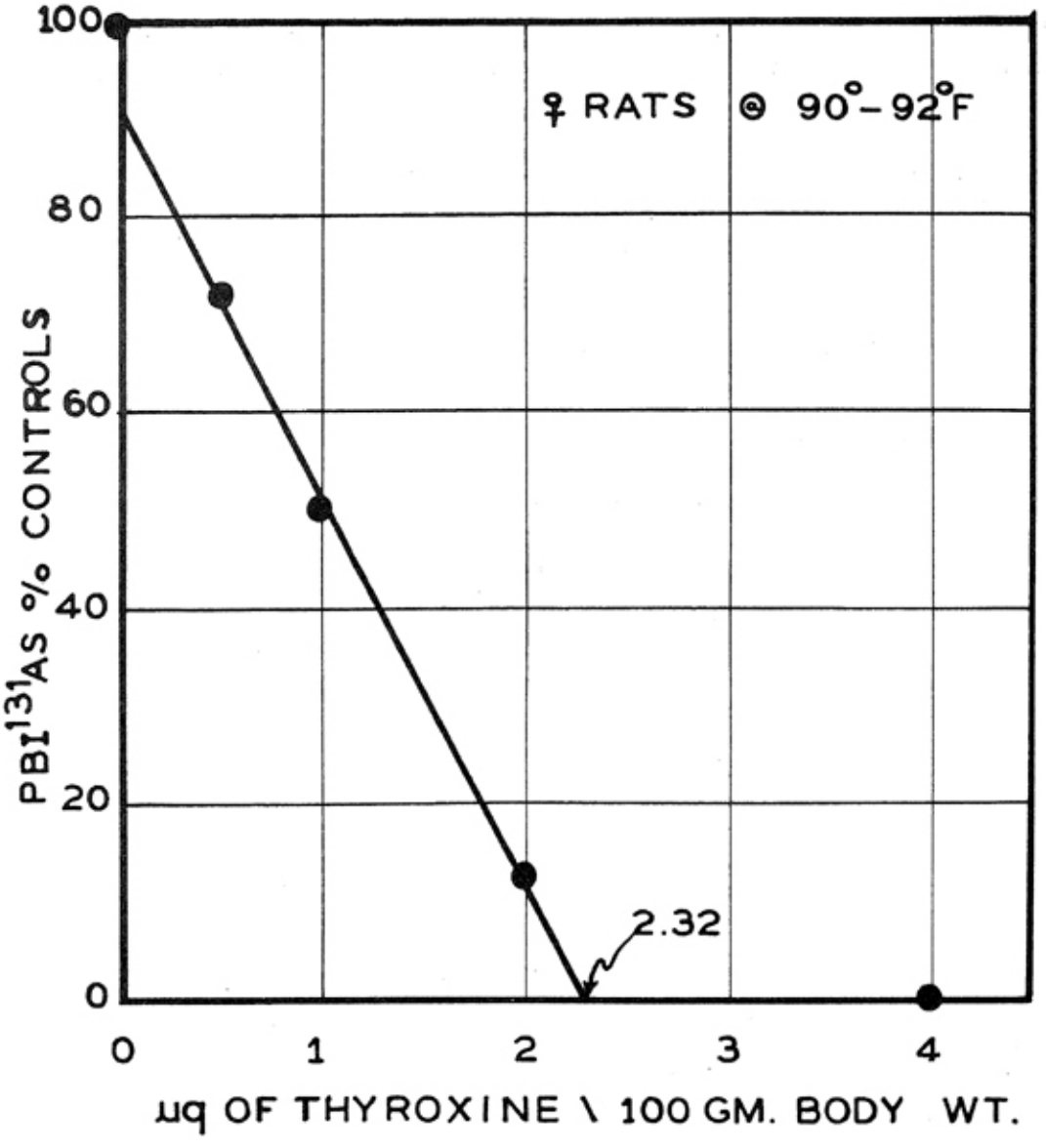

Figure 7—The thyroid secretion rate of the rat at 90° F. The secretion rate is reduced as environmental temperature increases.

The Effect of Temperature Upon the Thyroid Secretion Rate of the Rat:

Since Dempsey and Astwood (1943) had investigated the effect of temperature upon the thyroid secretion rate of the rat as indicated by the thiouracil-thyroxine technique, it was decided to evaluate the proposed technique by measuring the thyroid secretion rate of rats held at higher temperatures. Young female rats held at temperatures of 90°

to 92° F during the experiment secreted thyroxine at the rate of 2.32 micrograms per 100 grams body weight (Fig. 7).

The results of this investigation are in accord with those of Dempsey and Astwood (1943), who found that the rat at a temperature of 77° F secreted a quantity of thyroid hormone equivalent to 5.2 micrograms of L-thyroxine daily. At 95° F these investigators found an indicated thyroid secretion rate of 1.7 micrograms of L-thyroxine. Since the thyroxine used by these investigators was prepared from dessicated thyroid, some question may exist as to whether the material used was L-thyroxine or the D,L-isomer. It is evident, however, that the proposed technique shows the expected reduction in thyroid secretion rate at high temperature.

The thyroid secretion rate of 5.63 micrograms of D,L-thyroxine, as indicated in the present experiment, is relatively close to the value found by Monroe and Turner (1946). Their work shows a rate of 5.19 milligrams per 100 grams body weight on albino rats of the Missouri strain under similar temperature conditions.

Statistical treatment of the data from these experiments yielded highly significant correlation coefficients. Greater statistical significance could be obtained by increasing the number of observations at levels below the secretion rate. Since thyroid secretion rate varies with sex, body weight and temperature, every effort should be made to control these factors as closely as possible. In the present study, body weight and temperature could not be as closely controlled as desired for detailed studies.

Since previous experiments had demonstrated that progressively increasing doses of thyroxine decreased the formation of protein-bound-iodine in the blood, these findings were further investigated by studying the ratio of inorganic iodine to organic iodine (PBI) in the blood of rats treated with thyroxine.

Blood samples were taken from five groups of untreated male rats at intervals ranging from 3 to 144 hours after I^{131} was injected. Inorganic iodine was rapidly converted to organically-bound-iodine with the ratio becoming practically constant 20 hours after I^{131} injection (Fig. 8). These findings are in accord with those of Chaikoff and Taurog (1949) and indicate little difference between rats used in the two laboratories.

Increasing doses of thyroxine progressively reduced the ability of the rat thyroid to transform inorganic iodine to "thyroxine-like" iodine (Fig. 9). The maximum effect of thyroxine upon the conversion ratio was found to be between 2 and 3 micrograms. These findings confirm our previous studies on the effect of thyroxine on thyroid function in the rat. Detailed studies of the thyroid secretion rate of the rat were not made using the conversion ratio as an index of thyroid activity.

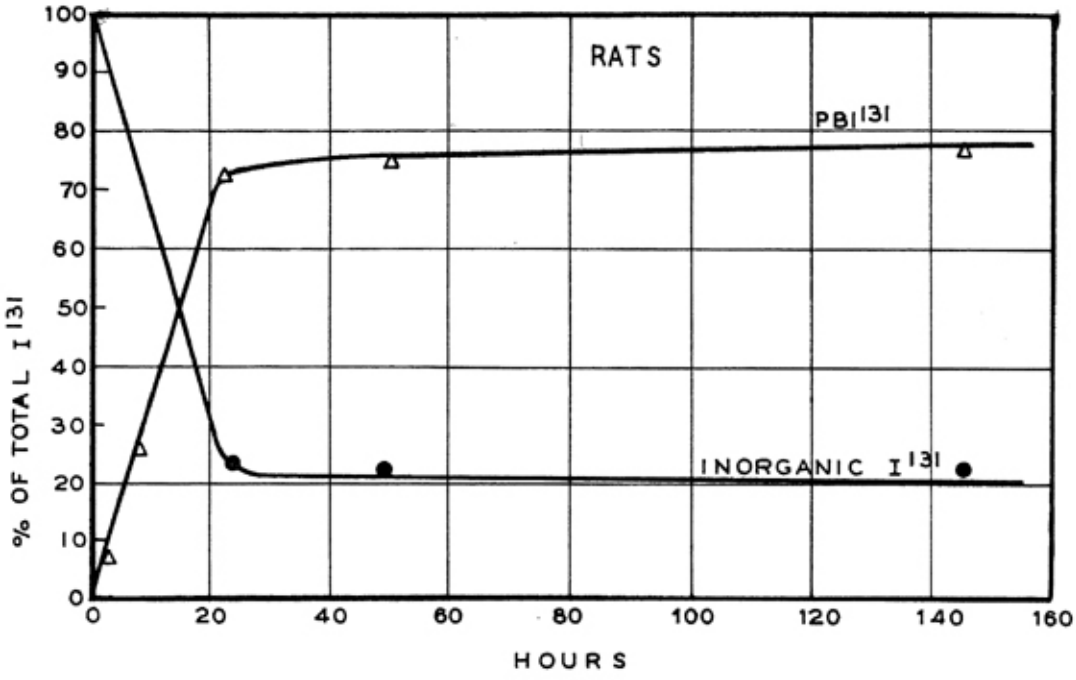

Figure 8—I^{131} metabolism in the rat. Note that the inorganic iodine injected is rapidly converted to protein-bound-iodine.

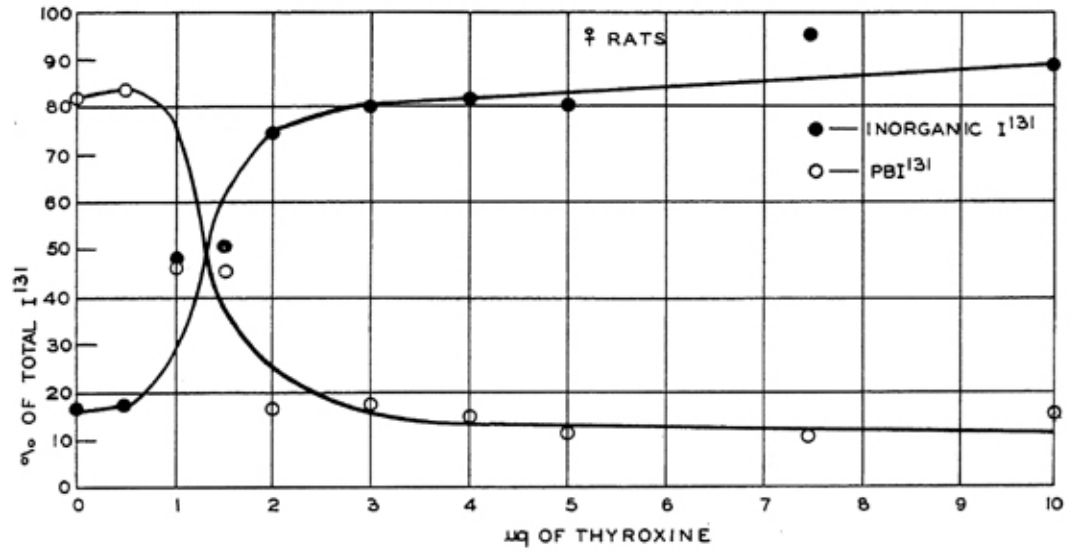

Figure 9—The effect of increasing doses of D,L-thyroxine on the organic binding of I^{131}. Thyroxine progressively reduced the ability of the rat thyroid to transform I^{131} to PBI^{131}

Effect of Thyroidally-Active Substances on the Thyroid Function of the Goat

Previous investigations had demonstrated the feasibility of estimating the thyroid secretion rate of groups of animals by pretreatment with thyroxine and subsequent administration of I^{131}. An attempt was made in this investigation to measure the secretion rate of individual animals.

The goat was used as an experimental animal in these studies, our primary interest being the estimation of thyroid secretion rate in relation to the productive ability of dairy animals. Another factor in the choice of the goat was existence of data gathered by Schultze and Turner (1945) on the secretion rate of the goat by the thiouracil-thyroxine technique.

Preliminary experiments indicated that the technique employed with rats and fowls would not be practical due to the longer biological half-life of I^{131} in the goat. For that reason a modification of the method suggested by Biellier (1955) was employed.

This method was based upon labeling the thyroid's store of iodine by an intravenous injection of I^{131} and subsequent administration of thyroxine at rates exceeding the estimated secretion rate. Under these conditions, it was expected that the administered thyroxine would inhibit the secretion of thyrotropic hormone. In the absence of thyrotropic hormone, thyroid function would be blocked and the I^{131} tagged thyroid hormone would disappear from the blood until the effect of the exogenous thyroxine was terminated or until the dosage was reduced below the normal secretion rate. At this time, the thyrotropic function of the pituitary and the activity of the thyroid would become evident as indicated by an increase of I^{131} labeled thyroid hormone in the blood.

Methods and Materials:

The goats used were of mixed breeding of dairy type. Some were purchased locally and others were raised in the experimental herd.

Studies were made throughout the fall, winter, and spring seasons at environmental temperature.

About 70 microcuries of I^{131} per 100 pounds body weight were injected into the jugular vein. After five to seven days, blood samples were taken from the jugular vein and the radioactivity of the butanol-soluble I^{131} (thyroxine-like fraction) was determined. Immediately after taking the first blood sample, L-thyroxine was injected on a basis of body weight. The radioactivity of the blood samples taken after injection was expressed as percentage of the activity found in the original blood sample.

Effect of a Single Dose of L-Thyroxine and L-Triiodothyronine on Thyroid Function of the Goat:

It seemed of interest to compare the effectiveness and duration of action of thyroxine and triiodothyronine in the goat, since Anderson (1954) had reported that triiodothyronine was more potent and had a shorter duration of action than thyroxine in the rat.

When L-thyroxine was compared with L-triiodothyronine (Glaxo Laboratories Ltd.) in two mature female goats, at a level of 1.2 milligram per 100 pounds body weight, both were found to have a prompt and profound effect upon thyroid function (Fig. 10). The maximum apparent effect of both hormones were observed on the third day. While samples were not obtained on the fourth day, thyroid function was resumed on the fifth day as indicated by the increasing radioactivity of the blood. On the seventh day after injection of the hormones, the radioactivity of the blood was at a higher level than on the fifth day, confirming the observations made on the fifth day.

While only two animals were used in this experiment, serial blood samples indicated prolonged depression of thyroid activity. The progres-

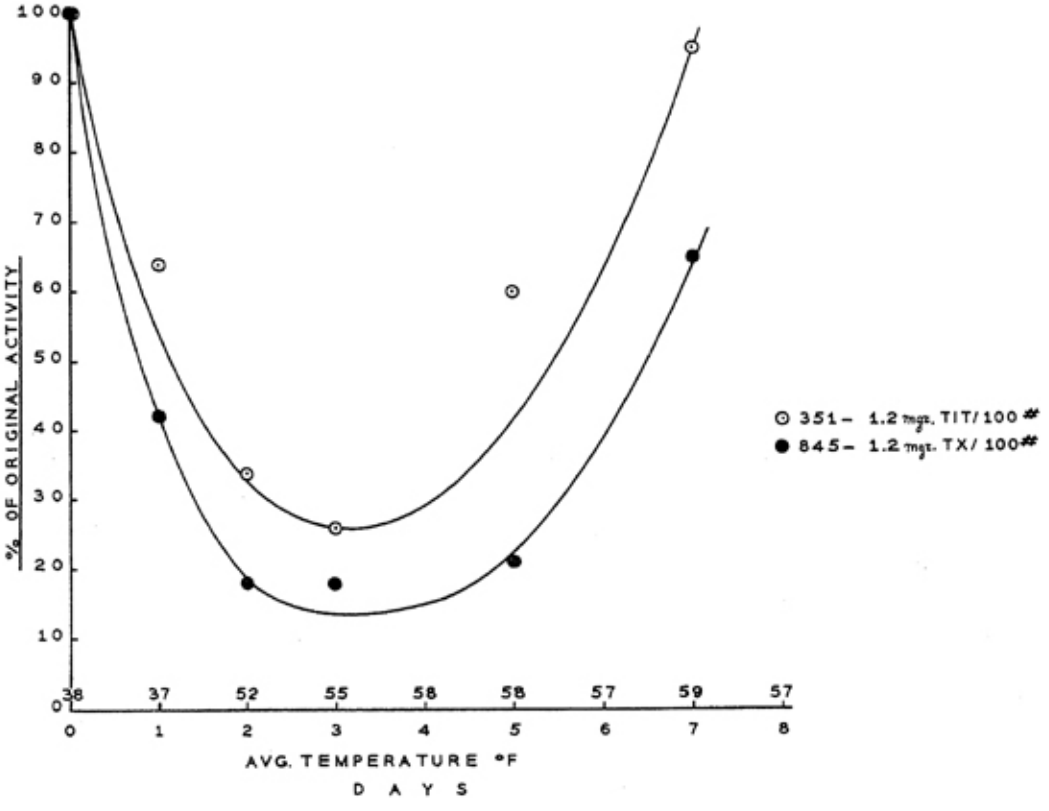

Figure 10—The effect of a single dose of L-thyroxine and L-triiodothyronine on the thyroxine-like I^{131} fraction of blood in the goat.

sive depression of the radioactivity of the blood until the third day after hormone injection indicated a slower response of the pituitary-thyroid system to exogenous hormone or a slower rate of removal of I^{131} tagged hormone from the blood than is found in the rat. Average daily temperatures ranged between 38° F and 59° F during the experiment.

Estimating Thyroid Secretion Rate of the Goat:

Since the previous experiment indicated that the dosage rate was outside of physiological limits, appropriate reductions were made in dose range. To allow time for the pituitary-thyroid system to equilibrate with injected hormone, the initial dose was repeated for four consecutive days. On the fifth day dosage was reduced and continued at this level for three consecutive days before a lower dose was employed. At all lower dose levels, injections were made for three consecutive days or until increases in radioactivity of blood were noted with two consecutive samples.

Two mature female goats, No. 363 and No. 446, were injected with L-thyroxine on a decreasing dosage schedule after the administration of I^{131}. Thyroid activity was depressed, compared with a control animal at the 0.1 and 0.075 milligram per 100 pounds body weight level (Fig. 11).

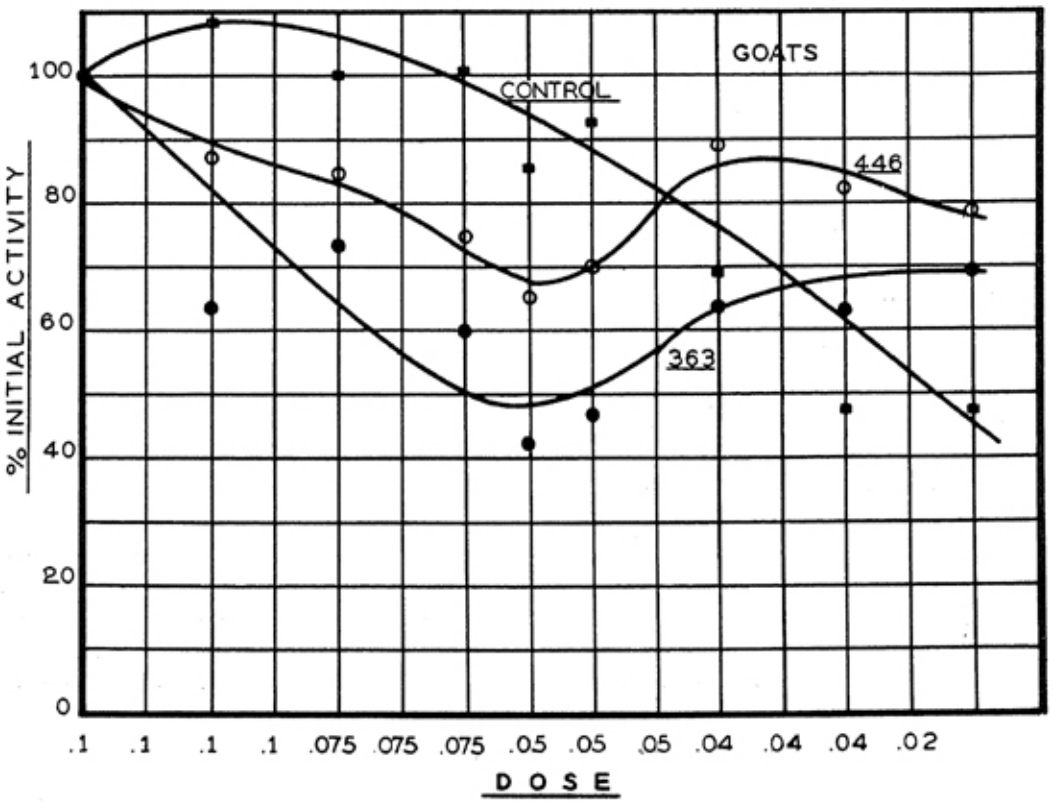

Figure 11—Estimation of the thyroid secretion rate of the mature female goat in terms of L-thyroxine.

On the day following the first injection of 0.05 milligrams, the radioactivity of the "thyroxine-like" iodine fraction of blood increased. On the day following the third injection of 0.05, a further increase was noted.

From this experiment, the thyroid secretion rate of the goat was estimated as ranging between 0.075 and 0.05 milligram per 100 pounds body weight daily.

Since the depression of thyroid activity as shown by the radioactivity of the blood was less than that observed when larger single doses (1.2 milligrams) were administered, the possibility existed that thyroid function was not completely inhibited. To investigate this possibility, two mature goats, female No. 351 and male No. 633, were injected at higher dosage levels, ranging during the experiment from 0.2 to 0.02 milligram per 100 pounds body weight (Fig. 12).

Two days after the dosage level had been changed from 0.10 to 0.04 milligram per 100 pounds body weight, an increase was observed in the radioactivity of the blood of No. 351. A further increase was observed when the dosage was lowered to 0.2 milligram per 100 pounds body weight. The male goat, No. 633, exhibited a marked depression of thyroid activity from

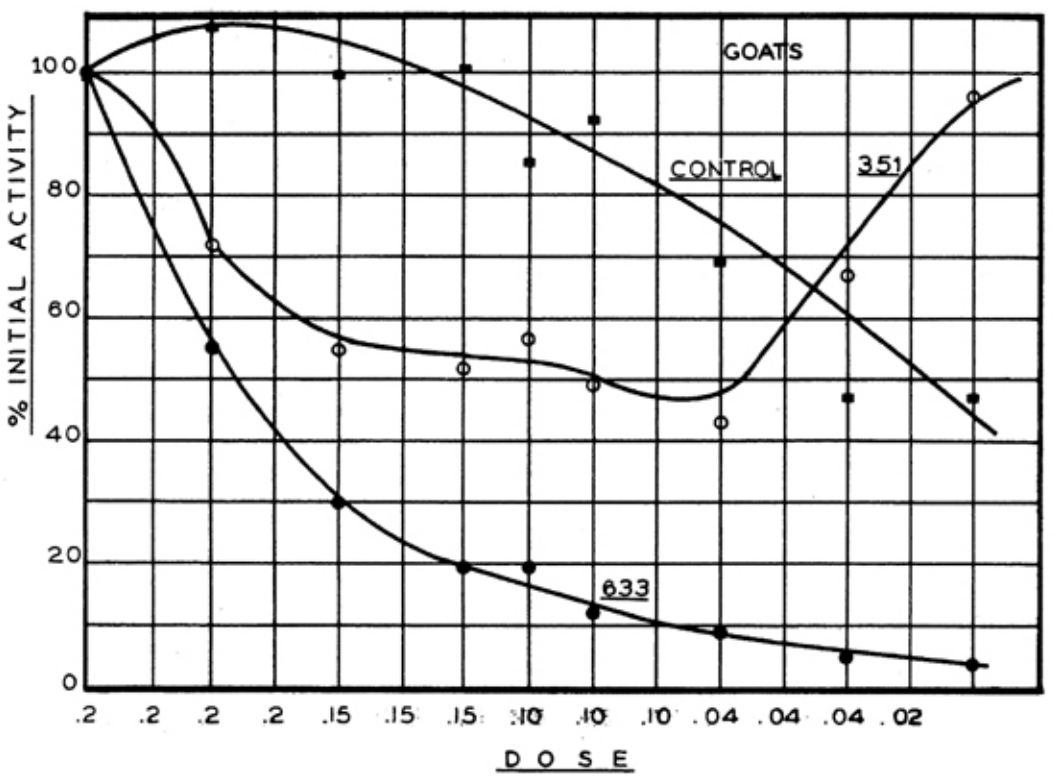

Figure 12—Response of the pituitary-thyroid system of the goat to varying levels of L-thyroxine as shown by the thyroxine-like I^{131} fraction of blood.

the initial dosage, and thyroid activity was not resumed at any of the lower thyroxine dosage levels.

Since No. 351 responded rapidly when the dosage was changed from 0.10 to 0.04 milligrams, it appeared that carry-over of thyroxine from previous dosages was not an important factor in this investigation. It was assumed that No. 633 had a secretion rate less than the lowest dose rate employed. It also appeared that as long as the initial dose was greater than the secretion, comparable estimates of thyroid activity would be obtained.

Two female goats, No. 351 and No. 363, were injected with thyroxine in decreasing dose ranges between 0.1 and 0.02 milligram per 100 pounds body weight (Fig. 13). The thyroids of both animals resumed function after receiving the third dose of 0.05 and at 0.04 milligram per 100 pounds body weight. This corresponds with a previously estimated secretion rate of 0.05 to 0.04 for No. 363 (Fig. 11) and a previously estimated secretion rate of 0.1 to 0.04 for No. 351 (Fig. 12). Average daily temperature ranged between 52° F and 21° F.

These estimated thyroid hormone secretion rates are much lower than the average values reported by Schultze and Turner (1945), who found that mature female goats secreted the equivalent of 0.60 milligram of L-thyroxine daily as shown by the thiouracil-thyroxine method.

Both investigations can be criticized on the basis of the small number of animals employed. In the present studies, the goats were observed

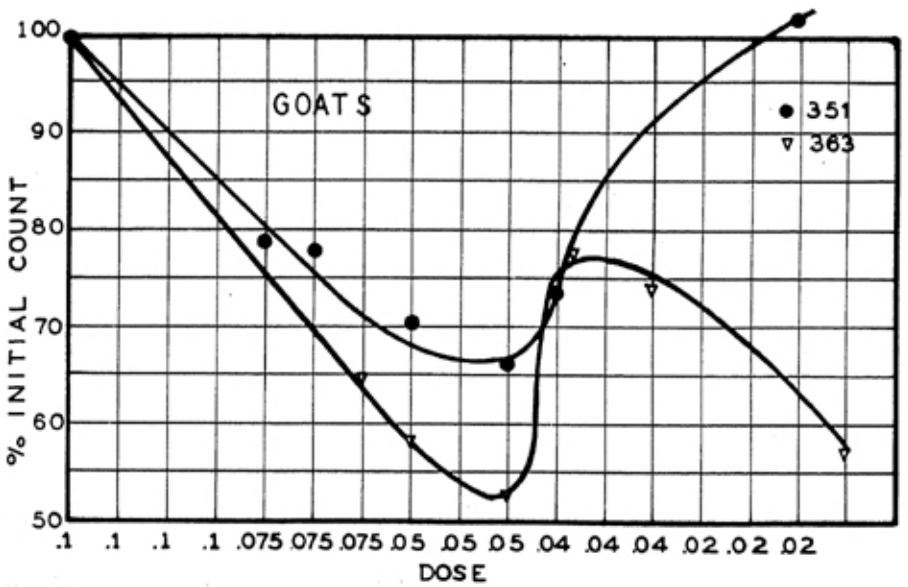

Figure 13—Estimation of the thyroid secretion rate of the goat in terms of L-thyroxine.

to lose weight during the experiments, presumable due to blood sampling. Since reduced food intake (Blincoe and Brody, 1955) has been shown to reduce thyroid activity, the possibility exists that repeated blood sampling and handling of the animals could have reduced thyroid activity although only about 400 milliliters of blood were drawn during the entire experiments.

Effect of Thyroxine on Thyroid Function of Dwarf Beef Animals and Dairy Heifers

Both dwarf beef animals and dairy animals were available so attempts were made to compare their relative thyroid activities by the methods previously employed with goats.

Methods and Materials

The dwarf beef animals employed in these experiments were of the Angus and Hereford breeds. While these animals had the typical appearance of thyroidectomized cattle, (Brody, 1945) short legs, "pot belly" and short dished face, previous investigations, (Crenshaw, 1955) showed normal qualitative metabolism of I^{131} and normal response to thyroxine and thyrotropic hormone. These animals ranged in weight from 200 to 450 pounds and were from one to three years old. Only females were employed in this study.

The dairy animals, with the exception of No. 158, were sterile Jersey heifers ranging in age from three to four years. During the study these animals were also being used in an investigation of experimental induction of lactation. During the course of the thyroid studies these animals received daily subcutaneous injections of 100 milligrams of progesterone and 100 micrograms of estradiol benzoate in oil. These facts are noted since Wolterink *et al.* (1950) reported that estrogen increased thyroid activity in the rat, while Reineke and Saliman (1953) found that simultaneous injection of progesterone and estrogen inhibited thyroid function in this species.

No. 158 was a year old Jersey heifer which did not receive estrogen-progesterone treatment during the experiment.

Effect of a Single Dose of Thyroxine on the Pituitary-Thyroid System of Cattle—Since a single large dose of thyroxine was found to have a prolonged effect on the pituitary-thyroid system of the goat, similar studies were made in cattle.

As in the goat, 70 microcuries of I^{131} per 100 pounds body weight were injected into the jugular vein. Five to seven days were allowed for the maximum uptake and conversion of inorganic I^{131} to thyroxine be-

fore exogenous thyroxine was administered.

Dwarf Beef Cattle—Two dwarf beef animals, No. 127 and No. 641, were given a single injection of L-thyroxine at 0.25 and 0.5 milligrams per 100 pounds body weight, respectively. Dwarf No. 176 served as a control in this experiment. At both dose rates, a progressive reduction in the I^{131} tagged hormone of the blood was observed during the first three days after administration of thyroxine (Fig. 14). While blood samples were not obtained on the fifth and sixth days, on the seventh and ninth days large increases were noted in the radioactivity of the blood, indicating that thyroid function was resumed.

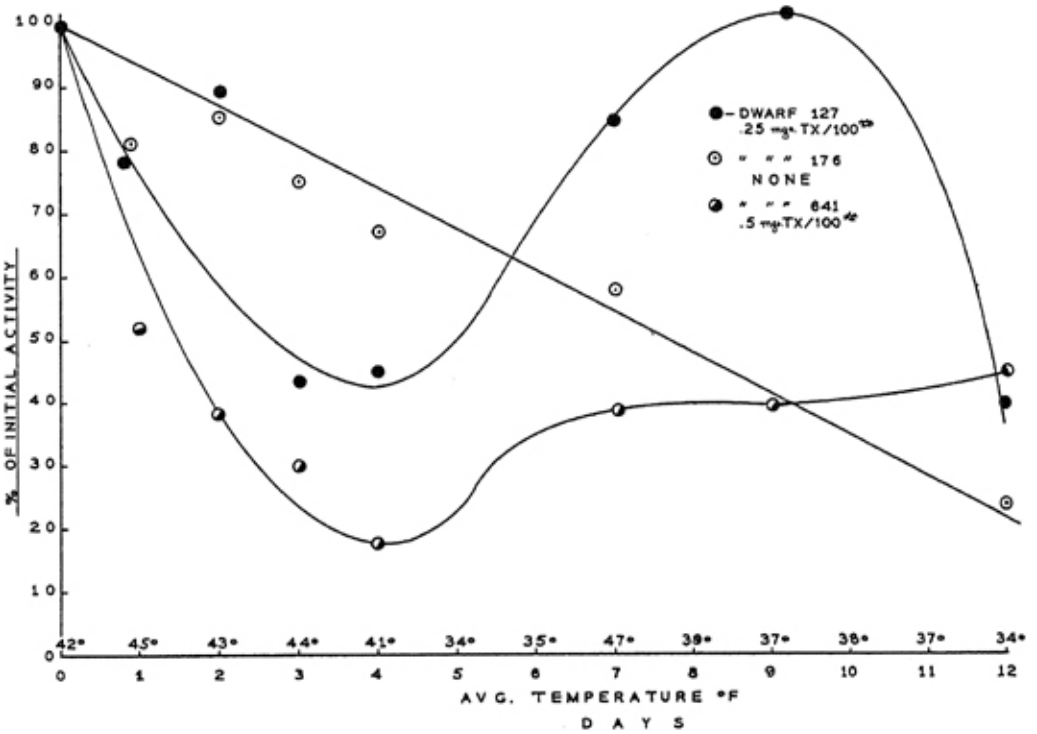

Figure 14—The effect of thyroxine upon thyroid function of dwarf beef animals as shown by the thyroxine-like I^{131} of blood.

Dairy Cows—Concurrently, a similar experiment was conducted with two mature dairy cows (Fig. 15). Thyroxine was administered at the rate of 0.5 milligram per 100 pounds body weight to each of the animals. As had been observed in goats (Fig. 10) and dwarf beef animals (Fig. 14), a progressive decrease in the radioactivity of the blood occurred during the first three to four days, followed by a rise on the seventh day. Blood samples taken on the ninth and twelfth days after thyroxine injections were higher than on the fourth day but indicated a progressive decrease as the

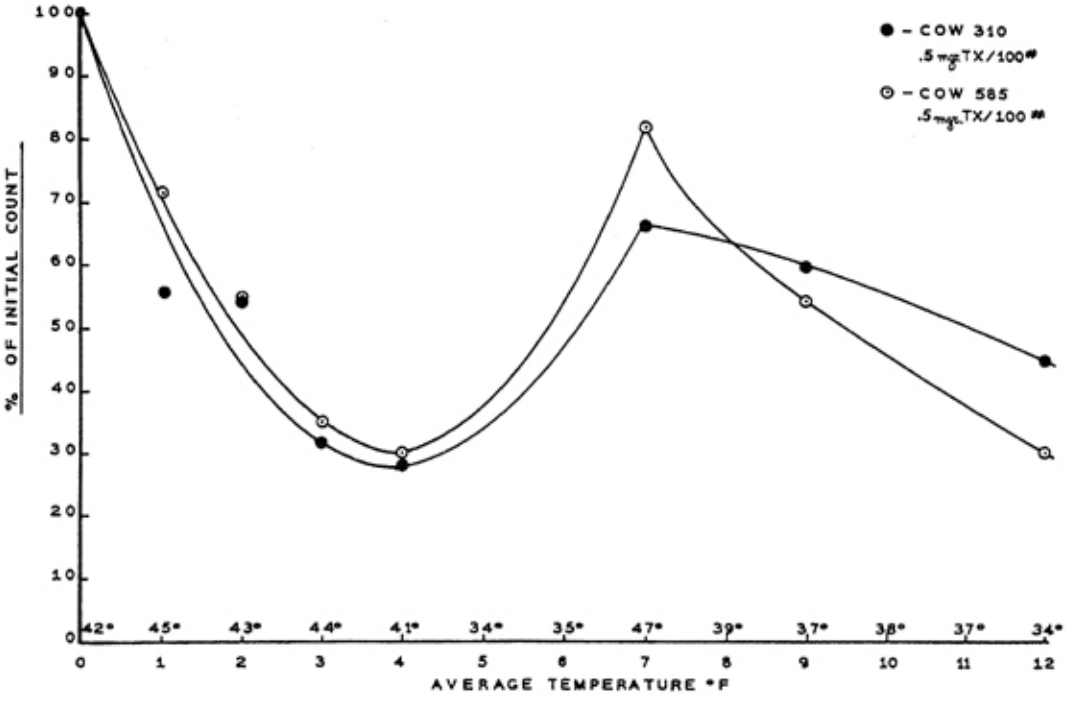

Figure 15—The effect of a single dose of L-thyroxine upon the thyroid function of the mature dairy cow as shown by the thyroxine-like I^{131} of the blood.

secretion of I^{131} tagged thyroid hormone followed its normal course. The average daily temperature ranged between 34° F and 47° F.

In each of these short term experiments, thyroid function was never completely inhibited, regardless of the size of the administered dose. These findings are in accord with those of Wolff (1951) and Albert (1951), who found a varying degree of autonomous thyroid activity in the hypophysectomized rat. While similar observations have been made in the human (Greer, 1951), further studies should be made in cattle after prolonged administration of thyroidally-active materials.

Estimates of the Thyroid Secretion Rate of Cattle—While previous experiments indicated that the thyroid secretion rate of cattle was far lower than previous estimates as shown by the duration of effect of a single dose, investigations were made in dairy cattle using a large dose of thyroxine to inhibit thyroid function as completely as possible. This was followed by progressively decreasing doses until thyroid activity was resumed.

Dairy Cattle—Three non-lactating Jersey cows were used in an attempt to determine the thyroid secretion rate. A dose of 0.5 milligram of L-thyroxine per 100 pounds body weight was administered to two ani-

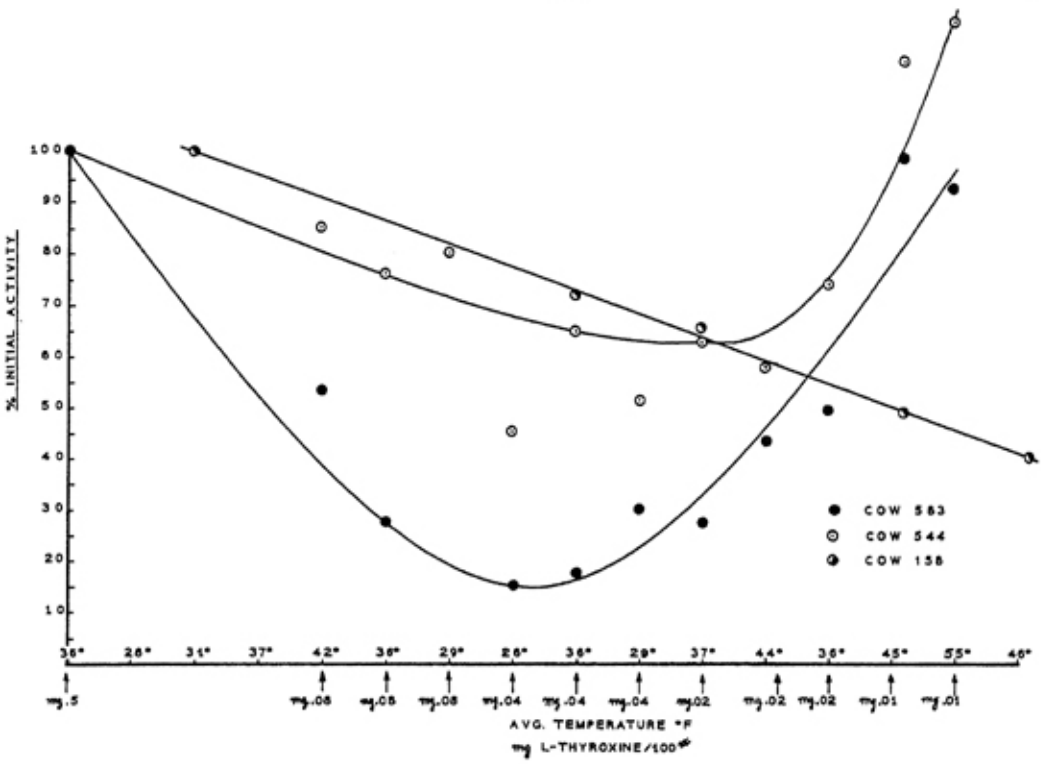

Figure 16—Estimation of the daily thyroid secretion rate of the mature dairy cow. (Cow No. 158 served as a control animal and did not receive thyroxine.)

mals (No. 583 and No. 544) five days after the injection of 70 microcuries of I^{131} per 100 pounds body weight. One animal (No. 158) was used as a control. Four days after the injection of the 0.5 milligram dose, 0.08 milligram per 100 pounds body weight was injected for three consecutive days. At three-day intervals the dose was progressively reduced to 0.04, 0.02 and 0.01 milligram per 100 pounds body weight. Apparently, thyroid activity of the two treated animals was progressively reduced until the 0.04 milligram level was reached (Fig. 16). During the 0.04 and 0.02 and 0.01 dosage range, thyroid activity was rapidly resumed in cow No. 583 and cow No. 544. It can be estimated from these results that the thyroid secretion rate of these animals ranged between 0.04 and 0.02 milligram per 100 pounds body weight per day.

A survey of the literature fails to reveal data on the thyroid secretion rate of cattle other than that of Schultze and Turner (1945). They found that the administration of 10 milligrams of D,L-thyroxine returned milk secretion to approximately normal in the thiourea fed cow. From these data, a daily thyroid secretion rate equivalent to 0.5 milligram of L-thyroxine per 100 pounds body weight can be calculated. Since the effect of

lower thyroxine dose rates was not reported by these investigators, it is possible that much lower dose ranges could have returned these animals to normal lactation.

In Vivo Measurement of Thyroidal I^{131} in the Bovine

In this phase of the investigations, equipment became available for *in vivo* determination of thyroidal I^{131} and a method for obtaining highly reproducible measurements was developed.

Equipment Development.

Several investigators have developed methods for determination of thyroidal I^{131} in laboratory animals (Fish *et al.,* 1952; Blincoe, 1953; Brown-Grant, 1955; Fredrickson *et al.,* 1955). Extensive investigations have been made in developing methods for measurement of I^{131} in the thyroid gland of the human. Fundamentally, the latter methods consist of locating one or more receptors (GM tubes or scintillation counters) at approximately 30 centimeters from the thyroid gland. Perlmutter *et al.* (1951) demonstrated that at a distance of 30 centimeters between the radioactive source and the receptor, a variation in location of 1 centimeter produced a mean of 3 percent difference, compared with a mean of 42 percent difference at a distance of 1 centimeter. The inverse-square law, i.e., the intensity of radiation is inversely proportional to the square of the distance between the source of radiation and the point of measurement, indicates that precision measurements of thyroidal I^{131} should be made at as great a distance as the sensitivity of the detector will permit.

Equipment for Restraining the Animal—Since the distance between the thyroid gland and the receptor had to remain constant during the period of measurement, the head and neck of the animals were immobilized with a Ranger Speed Table.* (Fig. 17) This head holder was attached to a Ranger Cattle Chute or attached to the metal upright bars of a cattle stall (Fig. 18). The head holder was attached to the chute at a point 31 inches above the floor. Portions of the head holder directly below the throat were removed to allow access to the thyroid region.

After a short training period, dairy cows and calves can be handled in this apparatus without excitement. Dairy animals that are trained to lead can be handled with the head holder attached to a cattle stall. When handling beef animals or untrained dairy animals, restraint in a cattle chute is required during the training period.

Radiation Detecting Equipment—A scintillation counter was employed. This instrument is 30 to 50 times more efficient than the GM tube. The one used contained a 1″ x 1″ NaI (thallium activated) crystal, and was

*Delphi Products Co., Delphi, Ind.

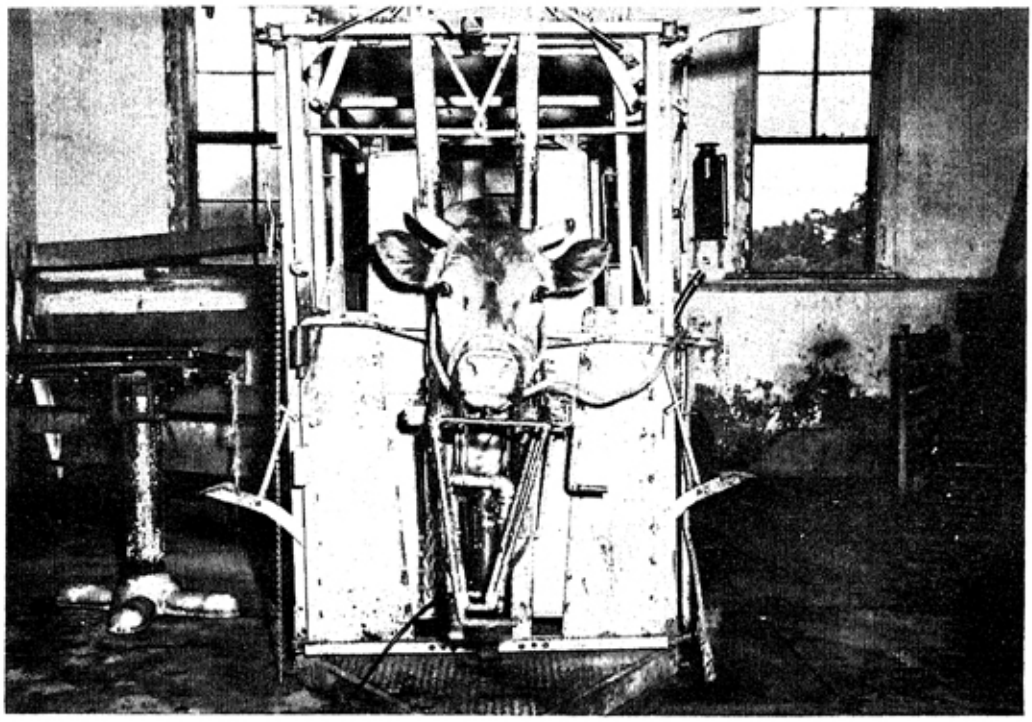

Figure 17—*In vivo* determination of thyroidal I^{131} in the bovine.

Figure 18—Scintillation counter and head holder attached to a cattle stall.

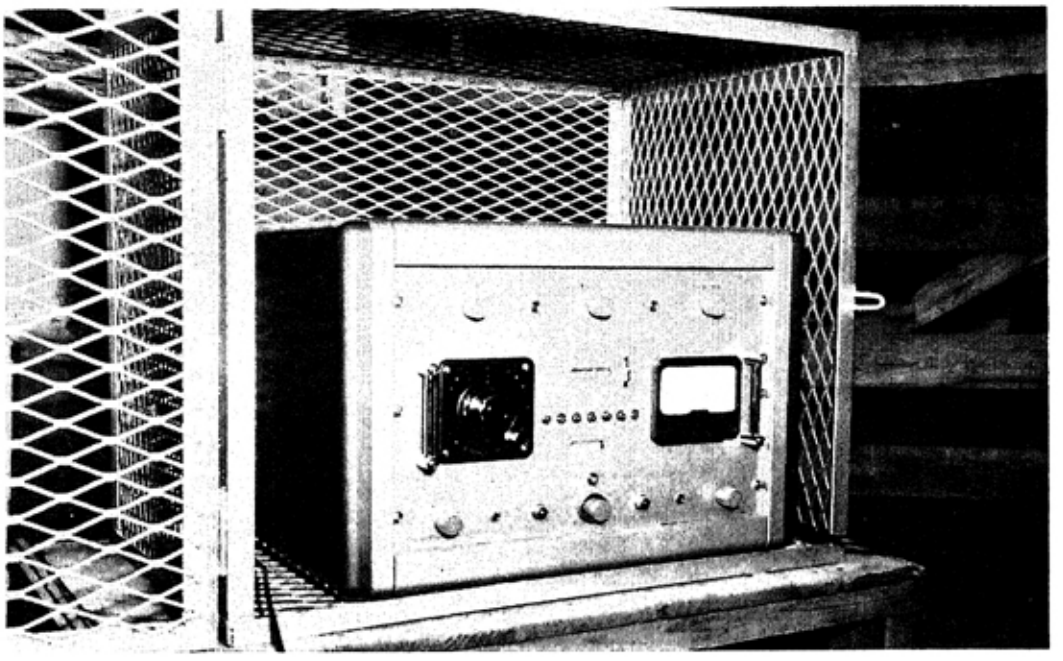

Figure 19—Combination analytical rate meter and scaler unit employed for measurement of thyroidal I^{131}.

connected by a 20-foot coaxial cable to a combination scaler and analytical rate meter (Fig. 19).

The head holder was modified to permit attachment of the scintillation counter holder on a flexible arm. The flexible arm allowed adjustment of the counter in vertical and horizontal positions under the thyroid regions of the throat of the animal.

To develop a sturdy spacer, two steel rods, 6 millimeters in diameter and 30 centimeters in length, were attached to the scintillation counter holder. The upper end of the steel rods terminated in a steel ring 10½ centimeters in diameter, which was reinforced by a bisecting steel rod (Fig. 20). A spring balance was inserted between the counter holder and the flexible arm to allow the adjustment of uniform pressure between the throat and spacer (Fig. 21). This device minimized the effect of variable dewlap size and provided for reproducible geometry.

In operation, the front of the steel spacer ring was placed at the posterior aspect of the cricoid cartilage. The spacer ring in this position covered the first two or three tracheal rings. These anatomical landmarks in the region of the thyroid gland were easily located by palpation of the neck. Maximum counting rates were consistently observed in this region.

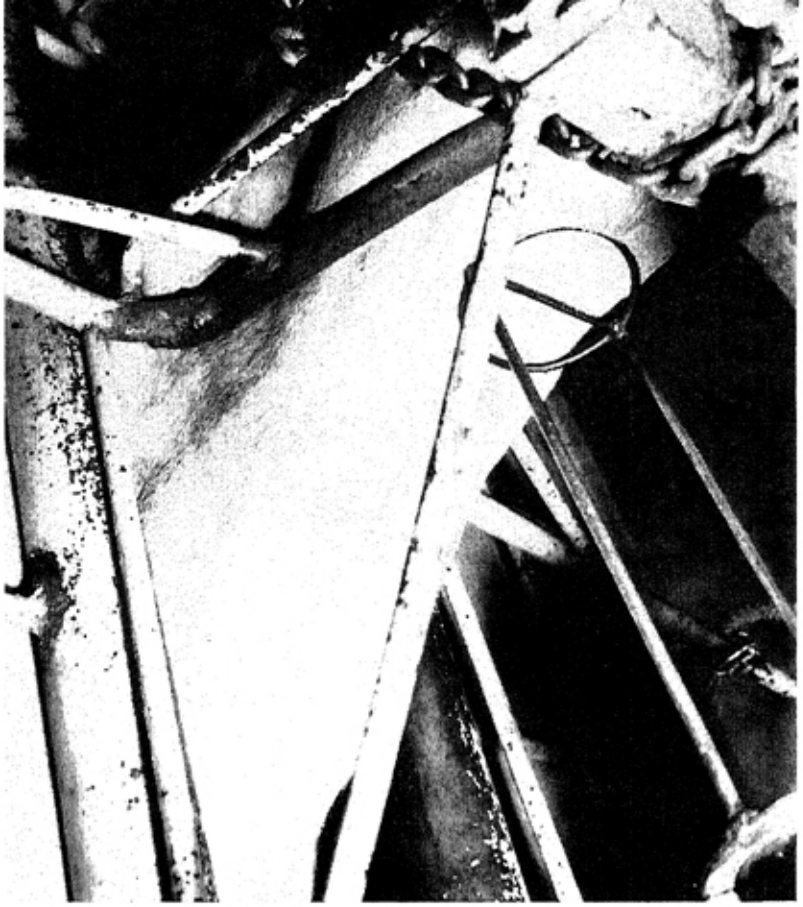

Figure 20—Spacer and steel ring located over the thyroid gland.

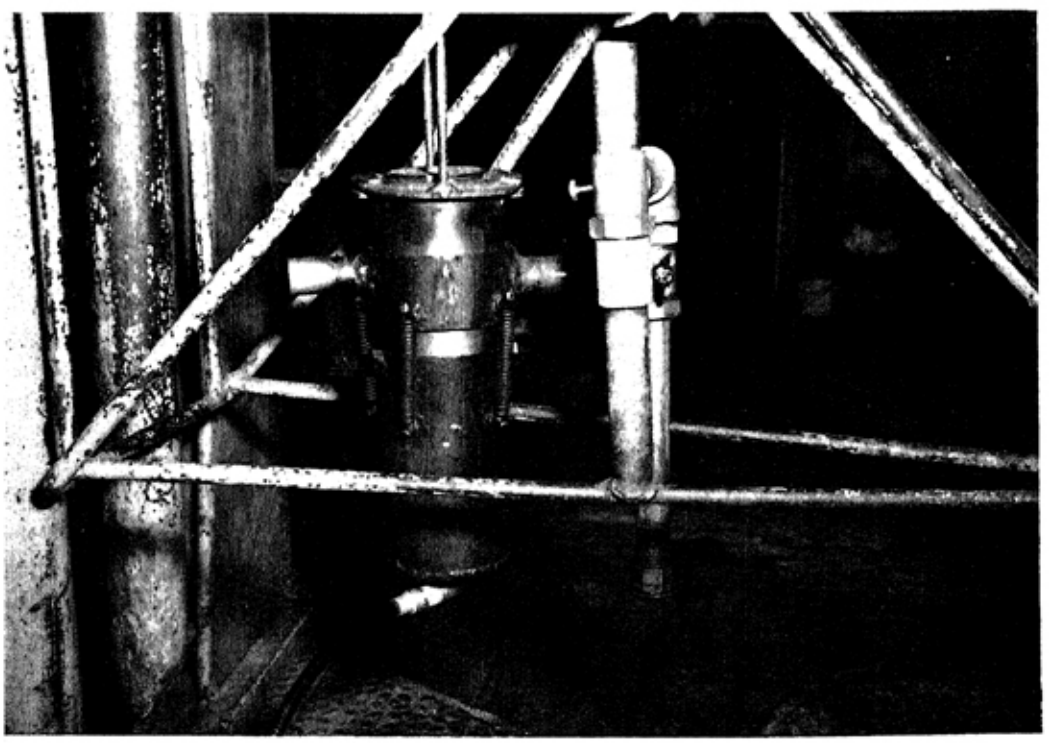

Figure 21—Scintillation counter and flexible arm in position for operation.

Experimental Methods—The animals available for the preliminary study were dairy and dwarf beef cattle fed and housed in a unit separate from the University herd. All animals were allowed access to iodized salt during the experimental period.

From 100 to 300 microcuries of carrier-free radioiodine (NaI^{131}) were injected intravenously into each animal at the beginning of the experimental period, the amount injected varying with the duration of the experiment. The results have been expressed in terms of percentage of the injected dose. Corrections were made for the radioactive decay of I^{131}. Corrections were not made for absorption of gamma radiation by the neck or the spacer ring, since these corrections were assumed to be constant for each individual animal throughout the experiment. The discriminator circuit was adjusted so that low energy gamma radiation due to backscatter was eliminated from the measurements.

Uptake and Release of I^{131} by the Bovine Thyroid

The three Jersey cows, ranging in weight from 900 to 1200 pounds, were injected with carrier-free NaI^{131}, and measurements of thyroidal I^{131} were made daily with the described equipment for 14 days. Maximum observed uptake of I^{131} by the bovine thyroid varied from 48 to 72 hours after injection. After this period of time the release of I^{131} as thyroid hormone exceeded the rate of uptake. Thyroid radioactivity began an exponential decline, with the rate of decline being dependent primarily upon the rate of release of thyroid hormone and secondarily upon the uptake rate of I^{131} from metabolized thyroid hormone.

When I^{131}-tagged thyroxine is released into the blood, it is metabolized in various cells of the body and I^{131} is removed from the carbon skeleton of the thyroxine molecule. As a result of this process, I^{131} again becomes available to the thyroid and the kidney. It is assumed that such I^{131} is collected by the thyroid in exactly the same manner as the initial injection of I^{131}. This process is called "recycling" or ' reutilization" of iodine. The true rate of release of thyroid hormone is masked to the extent and degree that recycling I^{131} occurs.

When lines are fitted by the method of least squares to the data obtained during the release phase, it can be seen that the daily measurements follow very closely the calculated slope and that average daily temperature variations (19 to 40° F) do not affect daily measurements (Fig. 22).

Standard errors apparently were quite low when compared with the data of Blincoe and Brody (1955).

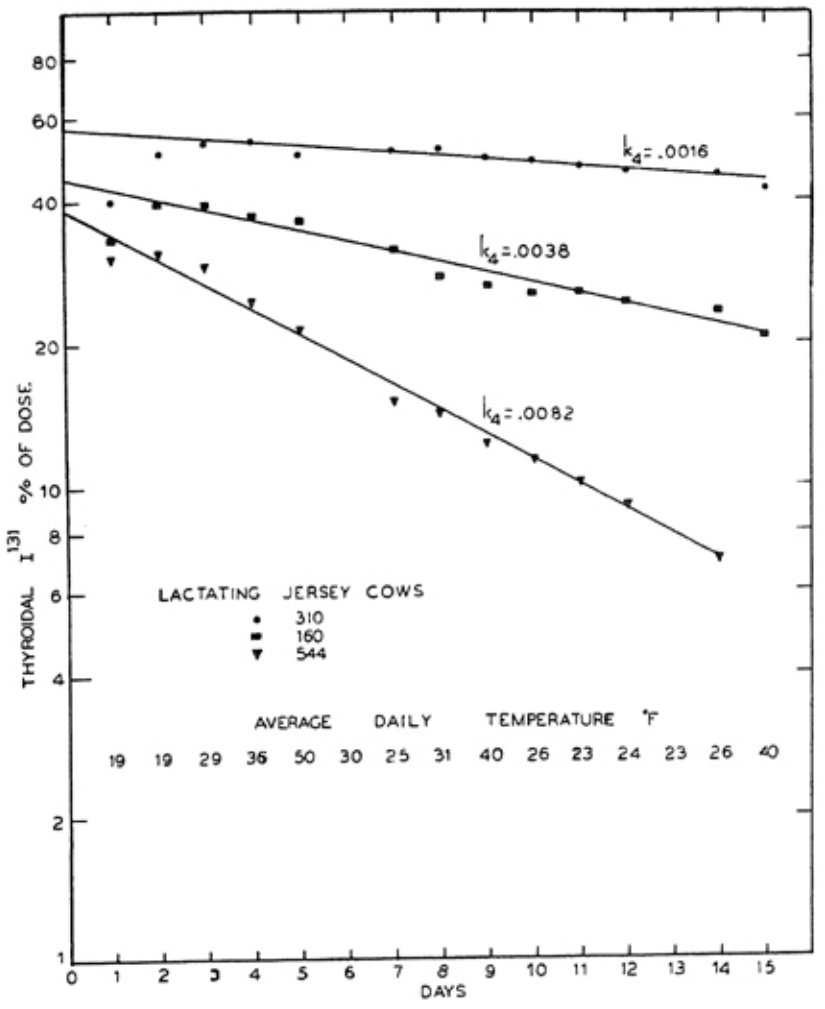

Figure 22—Uptake and release of I^{131} from the bovine thyroid. The k_4 values are rate constants for release of I^{131} corrected for reutilization.

Rate of Response and Duration of Action of Thyroxine in Dwarf Beef Animals

Since previous investigations indicated that the lowest levels of thyroxine-like I^{131} in the blood were found three to four days after injection of thyroxine the rate of response of the thyroid to injected thyroxine was investigated by *in vivo* measurement as well as by blood studies.

Two dwarf beef animals were injected with radioiodine and measurements of thyroidal I^{131} were made for a five day period. At this time a single injection of 0.5 milligram of L-thyroxine was made and a blood sample taken. Due to the low ambient temperature during the experiment the release of I^{131} from the thyroid was rapid and the effects of the thyroxine injection were readily apparent. Within one day the effect of the single dose was apparent in both animals and the loss of thyroidal I^{131} was inhibited for three or more days (Fig. 23).

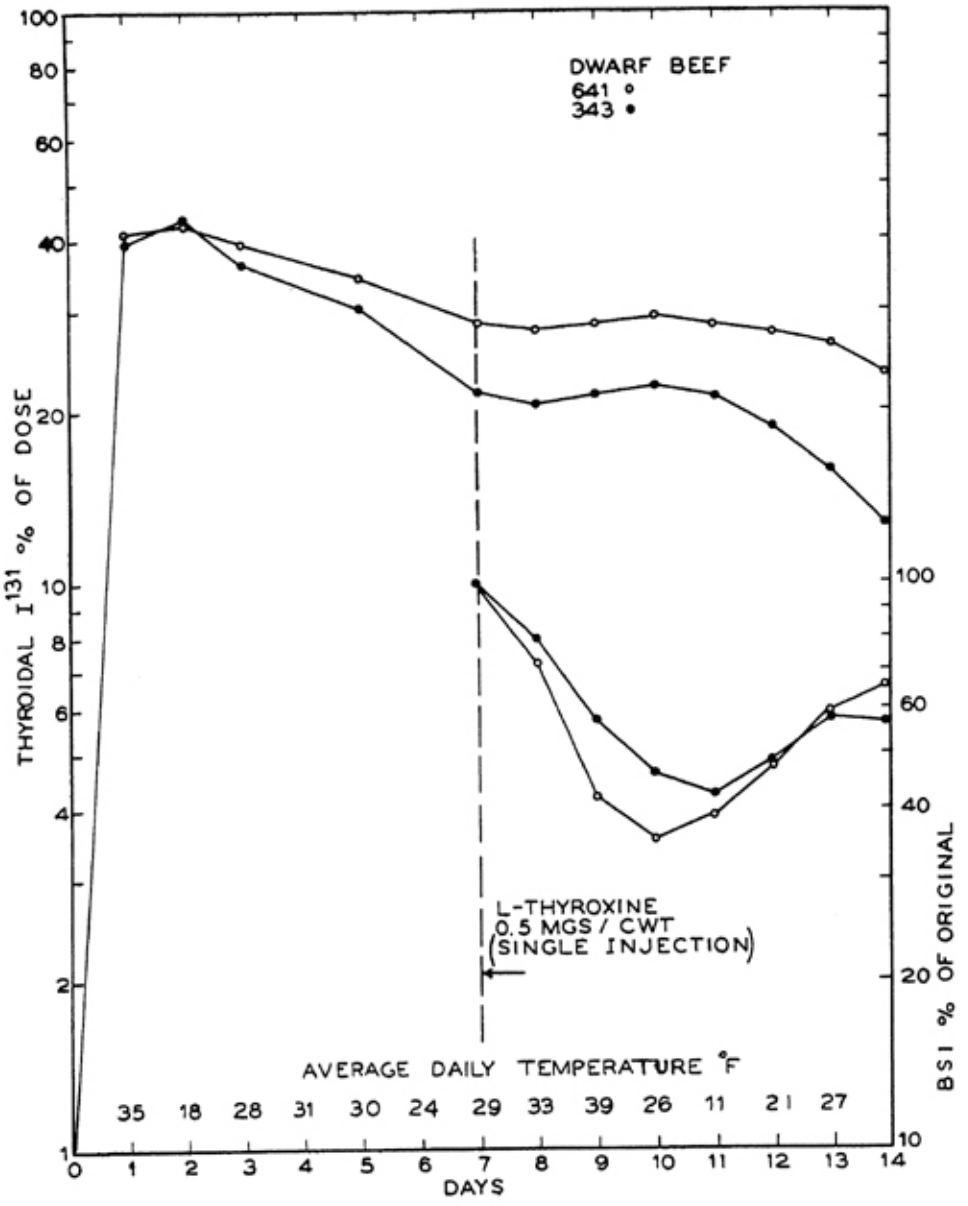

Figure 23—The effect of a single injection of thyroxine on the release of thyroidal I^{131} and blood levels of thyroxine-like I^{131} (Butanol soluble I^{131}).

The lowest levels of "thyroxine-like I^{131}" in blood were found three to four days after injection of thyroxine (Fig. 23). This experiment clearly demonstrates that the effect of thyroxine on the thyroid-pituitary system of cattle is rapid and that the slow rate of disappearance of I^{131} from the blood is due to the rate of tissue utilization of preformed I^{131} tagged hormone.

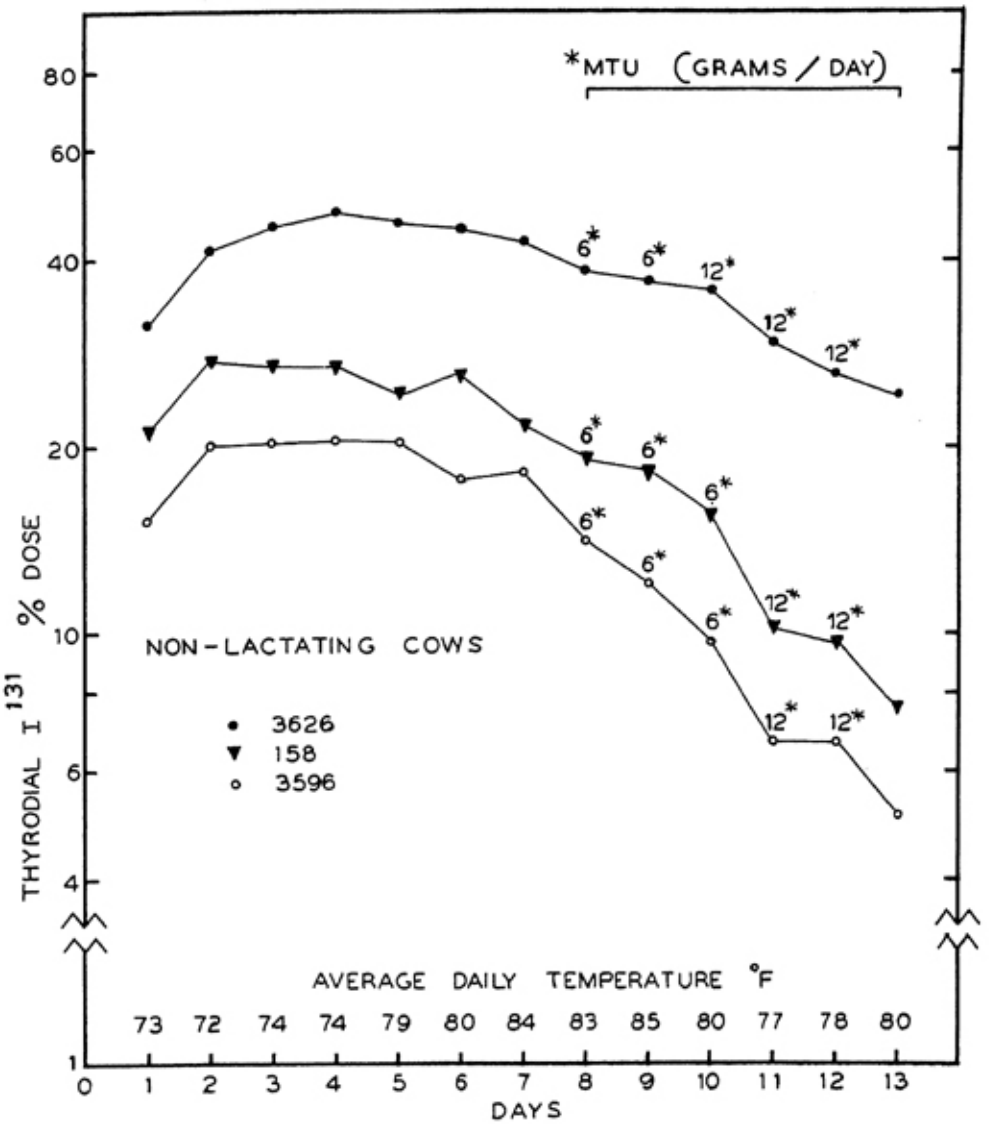

Figure 24—The effect of varying doses of 6-methyl thiouracil (MTU) on thyroid function in cattle.

Effects of Goitrogens on Thyroid Function in Cattle

Since the release of thyroidal I^{131} proceeds at a slow rate in cattle, especially at higher temperatures, goitrogens were administered to block the reutilization of I^{131} from the thyroid hormone. In order to establish the dose rate, three non-lactating cows were injected with radioiodine and measurements of thyroidal I^{131} were made for seven days. On the seventh day 6-methyl thiouracil was administered by capsule at the rate of 6 grams per day.

In all animals except No. 3626 an apparent increase in the rate of release of thyroidal I^{131} was observed (Fig. 24). After two to three days,

the dose was increased to 12 grams per day and all animals responded at this dosage level. Since the largest animal weighed about 1200 pounds, it was concluded that one gram per 100 pounds body weight was sufficient to prevent the reutilization of I^{131} by the thyroid. Since the counting device was in an early stage of development, large errors were evident in day to day measurements but the effect of thiouracil was readily apparent. Subsequent experiments indicated that 1 gram of 6-methyl thiouracil or thiouracil per day per 100 pounds body weight produced the maximum effect in mature dairy animals and dwarf beef animals.

Estimation of the Thyroid Secretion Rate of Dairy Cattle

Four non-lactating dairy cows were injected with I^{131}. After a control period of four days during which daily measurements of thyroidal I^{131} were made, thiouracil was administered at the rate of 1 gram per 100 pounds body weight. At the same time L-thyroxine was administered by the subcutaneous route at 0.02, 0.04, 0.08 and 0.16 milligram per 100 pounds body weight on successive days. In all animals and at all levels of thyroxine injection, thyroid function was not inhibited, as indicated by a rapid rate of release of I^{131} in the thiouracil treated animals (Fig. 25).

In subsequent study the amount of thyroxine injected was increased to an initial dose of 0.2 milligram per 100 pounds body weight for two successive days. Measurements of thyroidal I^{131} were made on the third day. This regime was continued with dosages of 0.4 milligram and 0.6 milligram of L-thyroxine daily. In all animals inhibition of thyroid function was observed at or before the end of the 0.6 milligram period, indicating that sufficient thyroxine was present in the animals to prevent the secretion of thyroidal I^{131} (Fig. 26). However, in all animals, thyroid function was inhibited for seven days after the injection of thyroxine was discontinued. After this period thyroid function was resumed, as indicated by a rapid decrease in thyroidal I^{131}.

Two interpretations can be made of the data obtained. First, the rate of metabolism of thyroxine in the dairy cow was so slow that considerable amounts of thyroxine remained in the body for long periods after cessation of injection. However, in the rabbit, Brown-Grant (1955), and in the fowl, Biellier (1955), demonstrated that the metabolism of thyroxine is rapid and that thyroid function rapidly returns to normal levels. The second interpretation of the observed data could be that the recovery of the pituitary-thyroid system after replacement therapy with thyroxine requires several days before normal function is established.

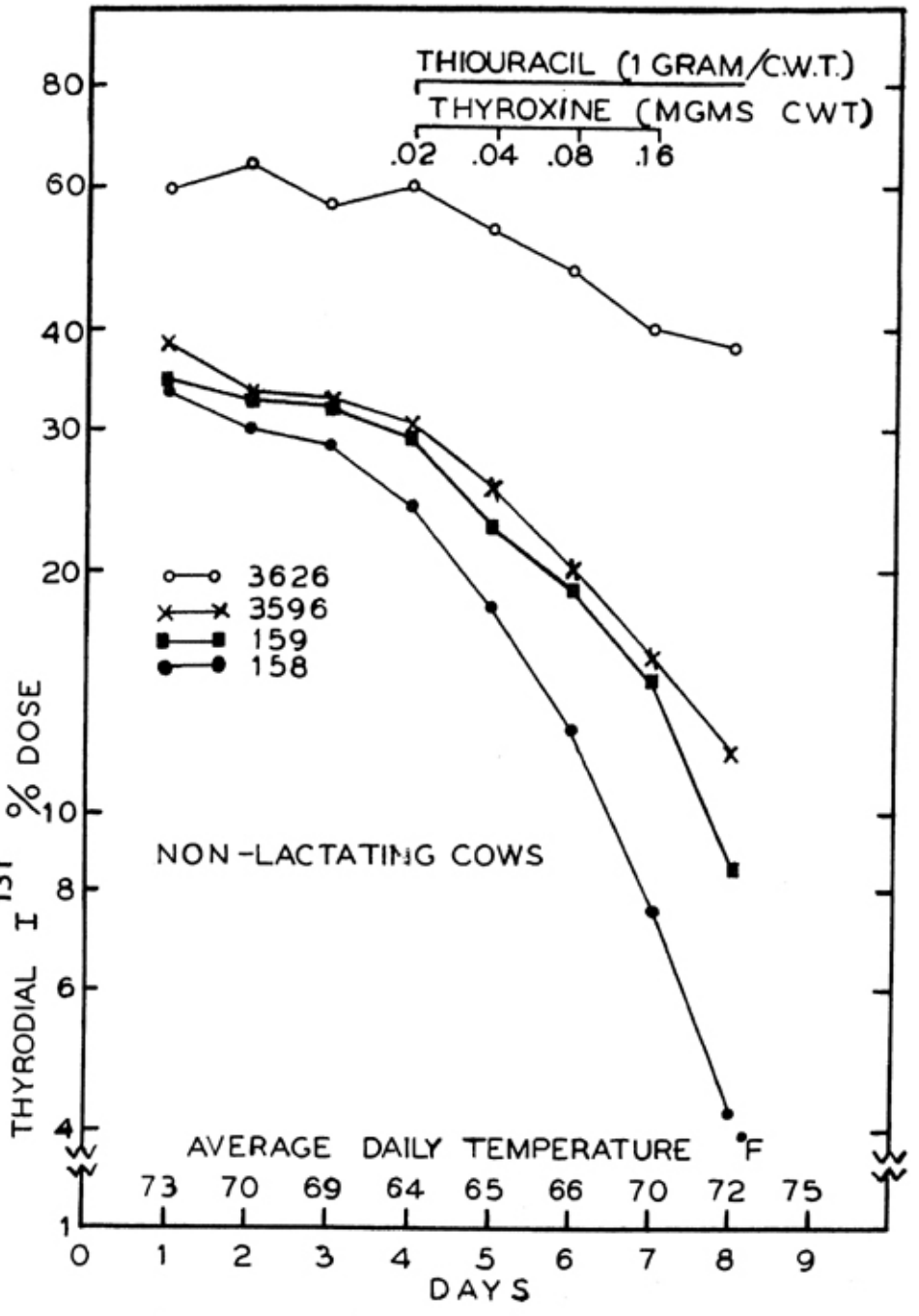

Figure 25—The effect of low dosages of thyroxine and optimum dosages of thiouracil on thyroid function.

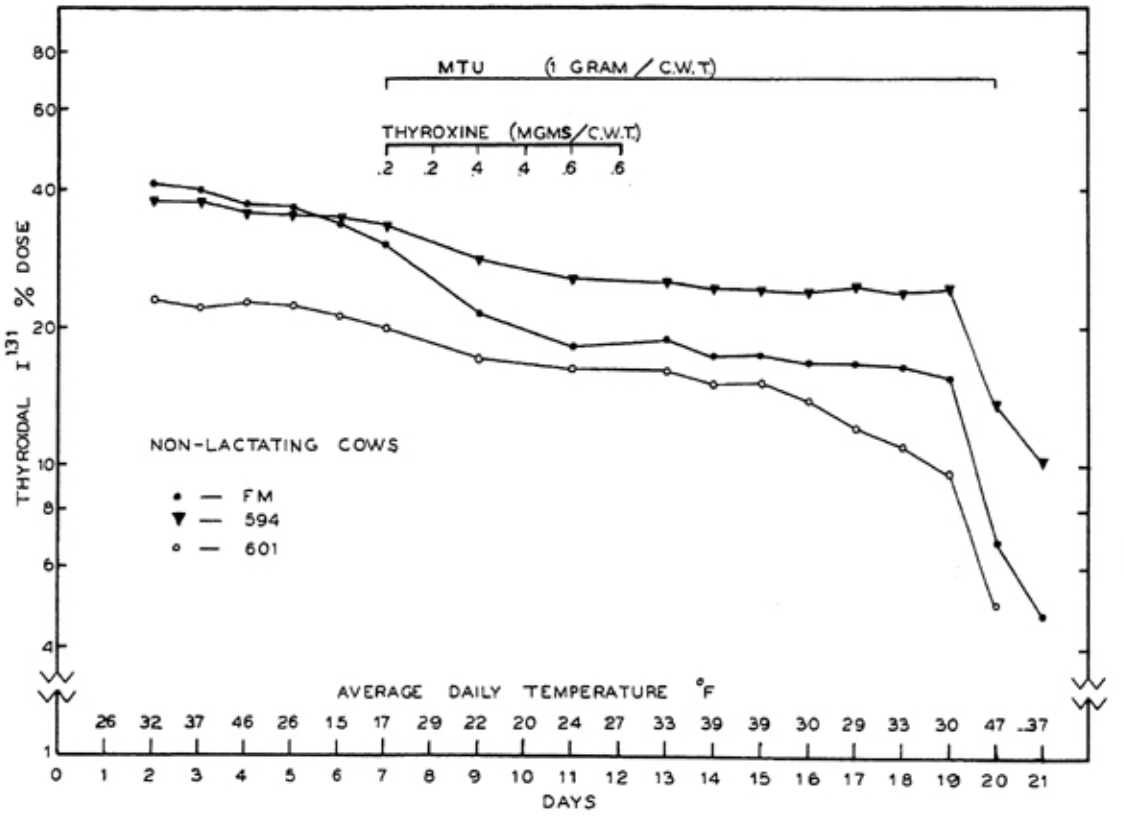

Figure 26—The inhibition of thyroid function by thyroxine in thiouracilized cattle.

DISCUSSION

In the absence of established methods for estimation of the thyroid hormone secretion rate of intact animals, several approaches to this problem were investigated. In the rat, the response of the pituitary-thyroid system to thyroxine is rapid as shown by a prompt depression of the organically bound iodine of blood. Similar results have been observed in the chicken and turkey (Biellier, 1955).

At thyroxine dosage ranges below the normal secretion rate, a quantitative inverse relationship was found between exogenous thyroxine and the "thyroxine-like" iodine of the blood. This relationship lends itself readily to estimation of the thyroid secretion rate of animals and can be made the basis of assay for thyroidally-active materials.

When comparisons are made between groups of animals with a single dose level for each group, the duration of effect of exogenous hor-

mone is not of particular importance. On the other hand, if the thyroid hormone secretion rate of individual animals is to be estimated by administration of varying dosages of thyroxine and subsequent measurement of I^{131} metabolism, the duration of effect of single dosages upon the pituitary-thyroid system becomes of critical importance.

In the present studies on large animals, estimates have been made of the duration of the effect of large doses of thyroxine by investigation of changes in the thyroxine-like I^{131} fraction of blood, since smaller physiological doses do not appear to allow sufficient time for tissue utilization of the I^{131} tagged hormone in the blood. It should be pointed out in this regard that while results obtained in the present investigation indicate that thyroid function is at its lowest level two to three days after the administration of thyroid hormone, as shown by "thyroxine-like" I^{131} levels in blood, administered thyroid hormone actually exerts its effect rapidly upon the pituitary-thyroid system, as shown by thyroidal I^{131} measurements.

Since thyroid function is rapidly inhibited by exogenous thyroxine, the prolonged decline of I^{131} tagged hormone in the blood may be due to a slow rate of tissue metabolism of preformed hormone in the blood.

Calculations made from the data of Blincoe (1955) indicate a half-life of thyroid hormone in the blood of dairy cattle ranging from 19 to 106 hours, and further suggest that a slow rate of hormone metabolism is responsible for the prolonged decline of blood thyroxine-like I^{131} after thyroxine is administered. The investigations of Greer (1951), however, indicate a slow response of the pituitary-thyroid system to exogenous thyroid hormone in the human as shown by a reduced thyroidal uptake of I^{131} after eight days of medication as compared with four.

When thiouracil and thyroxine were administered simultaneously to dairy animals which had received I^{131}, the rate of release of thyroidal I^{131} was reduced as the dose of thyroxine was increased until complete inhibition of endogenous thyroid hormone was observed. Relatively large doses of thyroxine were required to inhibit thyroid function (0.2 to 0.4 mg. per 100 pounds body weight) but the effect of exogenous thyroxine on thyroid function continued for several days after cessation of injections. These data as well as the data obtained from blood studies indicate that thyroxine metabolism progresses at a slow rate in large animals.

The data suggest that estimates of the daily secretion of thyroxine in cattle are too low by the blood methods and to high by the *in vivo* measurement technique. In both cases, the carry-over of previous doses of thyroxine appears to mask the true picture to some extent.

The problem involved may be indicated by an example of endo-

genous thyroid hormone secretion. If a given cow secretes one unit of hormone during a 24-hour period and if the hormone were completely metabolized during a 24-hour period, the level of circulating hormone would equal the secretion rate. However, if the thyroid hormone secreted each day was not metabolized as rapidly as it was secreted, then some of the hormone secreted one day would still be biologically active the next day. If the daily hormone secretion was not completely metabolized for three or more days, then the amount of circulating hormone would exceed the daily secretion rate. At the present time it is not known how long a single day's secretion of thyroxine by a cow is biologically effective. It is suggested that the answer to this problem may be determined by the injection of a single dose of I^{131}-tagged thyroxine into a cow (the estimated daily secretion rate) and then determining the time required for its metabolism i.e., its excretion. Thiouracil would be administered during the test period to prevent the reutilization of the metabolized I^{131}. With data on the half-life of radioactive thyroxine available, it will be possible to compute the time required for a series of daily doses of thyroxine to reach a state of equilibrium in the body and at the cessation of injections, the time required for thyroxine to be metabolized, i.e. to become no longer physiologically active.

As indicated above it is our belief that the time required for the pituitary-thyroid system to return to normal function when thyroxine injections are discontinued is due to the slow metabolism of the residual thyroxine in the body. It is possible that the pituitary-thyroid system may fail to return to normal equilibrium even when the exogenous thyroxine has been metabolized. While this alternative is tentatively rejected, it will be possible to determine if it is true when data becomes available on the average rate of metabolism of thyroxine in the bovine.

In initiating these studies to develop methods by which the thyroxine secretion rate could be estimated in the intact animal, the equipment available required that study of radioactivity of the blood be made. These studies have contributed much to our understanding of thyroid physiology. The equipment described in this bulletin now makes possible highly repeatable *in vivo* measurements of thyroid gland radioactivity. In contrast to the disturbances inherent in obtaining frequent blood samples, the measurement of radioactivity of the thyroid gland reduces the disturbance to the trained animal, avoids tedious and cumbersome laboratory work with the blood, and permits day to day observations of the changes occurring in the thyroid gland.

While the estimates of the thyroxine secretion rate obtained by the method described may be slightly above the actual thyroxine secretion

rate due to the slow rate of metabolism of thyroxine in the bovine and its carry-over effect, study of this problem may make possible a correction for this effect. In the meantime the method described will provide a valuable index of the variation in thyroid activity in cattle under standardized conditions.

Thiouracil or 6-methyl thiouracil at the rate of 1 gram per 100 pounds body weight has been shown to inhibit the reutilization of I^{131} by the thyroid of the mature bovine. Larger doses may be required for young animals. Smaller dosages may be used if the dose is divided and administered at intervals during the day since thiouracil is rapidly metabolized.

Two additional criteria of thyroid function in the bovine are suggested by these investigations, first, the rate constant for release of thyroidal I^{131} corrected for reutilization and, second, the rate constant for release of thyroidal I^{131} when reutilization is blocked by the administration of goitrogen. Since the two values differ considerably, it should be pointed out that the former value is based upon a mathematical correction for reutilization of iodine whereas the latter value is based upon direct observations of the release of thyroidal I^{131} while reutilization is inhibited by goitrogens.

The latter method provides a sensitive criteria of thyroid hormone release. For comparing the relative thyroid activity of groups of animals or of individual animals under varying physiological states where estimated thyroxine secretion rates are unimportant this latter criteria of thyroid activity is recommended.

SUMMARY

1. Methods have been proposed for the determination of the thyroid hormone secretion rate of intact animals by the administration of graded levels of thyroxine and concurrent studies of the extent of inhibition of thyroid function as shown by I^{131} blood changes and by *in vivo* measurements of the thyroid gland.

2. The pituitary-thyroid system of the rat was found to show a rapid quantitative response to exogenous thyroxine as indicated by reduced levels of blood I^{131} tagged hormone.

3. Using the blood method, the average daily thyroid hormone secretion rate of the female rat of our stock was estimated as 5.63 micrograms of D,L-thyroxine per 100 grams body weight at an environmental temperature of 70° F., compared with the estimate of 2.32 micrograms at a temperature of about 90° F. These data are quite similar to estimates of the thyroxine secretion rate of our rats by the goitrogen technique.

4. In several species, after thyroxine was administered, a progressive depression of the I^{131} labeled thyroid hormone in blood was observed for three or more days, followed by a progressive increase.

5. The duration of physiological action of exogenous thyroid hormone has been measured in the goat, in dwarf beef cattle, and in dairy cattle by the administration of I^{131}, followed, after 2 or 3 days, by the injection of thyroxine and serial assays of the radioactivity of the thyroid hormone in the blood.

6. In the goat a single injection of 1.2 milligrams per 100 pounds body weight of L-thyroxine of L- triiodothyronine was found to inhibit thyroid function for at least three days.

7. In the mature dairy cow a single injection of 0.5 milligram of L-thyroxine per 100 pounds body weight inhibited thyroid function for at least three days.

8. In dwarf beef cattle single injections of L-thyroxine at levels of 0.25 or 0.50 milligram per 100 pounds body weight inhibited thyroid function three or more days. These observations are believed to indicate that dwarf beef cattle have pituitaries and thyroid glands comparable to normal cattle in response to exogenous thyroxine.

9. These findings are interpreted as indicating a slow rate of metabolism of the thyroid hormone in the body rather than a slow rate of response of the pituitary-thyroid system to exogenous hormone.

10. Preliminary investigations employing a modification of the radio-iodine-thyroxine technique proposed by Biellier (1955) indicated a daily L-thyroxine secretion rate ranging from 0.04 to 0.075 milligram per 100 pounds body weight in mature female dairy goats.

11. Employing similar techniques, estimates of the daily L-thyroxine secretion rate per 100 pounds body weight ranged between 0.02 and 0.04 milligrams in mature non-lactating Jersey cows.

12. Equipment permitting precision *in vivo* measurements of thyroidal I^{131} in cattle has been described.

13. Thiouracil or 6-methyl thiouracil at the rate of one gram per 100 pounds body weight has been shown to inhibit the reutilization of I^{131} from metabolized thyroxine in mature dairy animals. This amount may be considered the upper level of dosage required to inhibit thyroid function.

14. Estimates of the daily L-thyroxine secretion rate by *in vivo* measurement of thyroidal I^{131} during concurrent administration of thiouracil and increasing dosages of L-thyroxine ranged between 0.2 and 0.6 milligram per 100 pounds body weight in dairy cattle, but carry-over of unmetabolized thyroxine was observed for as long as seven days after injection ceased. This method is considered satisfactory as a practical index of thyroidal function if standard conditions are maintained.

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