A STUDY OF THYROID ACTIVITY IN DAIRY GOATS RELATING TO AGE, LACTATION, PREGNANCY AND SEASON OF THE YEAR

by

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AN ABSTRACT

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1

The technique for measuring individual thyroid secretion rates using I-131 and l-thyroxine injections (J. Animal Sci., 14: 419, 1955) was applied to dairy goats for the purpose of determining thyroid activity in relation to age, lactation, pregnancy and season. The comparatively rapid output rate of this species ($t_{1/2}$ = 7.3 ± 0.35 days) without thiouracil treatment permits rather precise determination of the amount of l-thyroxine needed daily to prevent I-131 output. This endpoint was used to measure thyroid secretion rate. The mean thyroid secretion of pregnant goats, including 2 aged and 3 two-year-old animals, during February was 0.262 ± 0.026 mg. per 100 pounds body weight daily. A similar group of non-pregnant goats had a mean secretion rate value of 0.277 ± 0.05 mg. The mean thyroid secretion rate of the young animals in this experiment was 0.325 ± 0.019 mg. This is significantly higher than the mean value 0.187 ± 0.016 mg. found in the aged group. The mean thyroid secretion rates of a group of open non-lactating goats including a group of 5 aged and a group of 4 two-year-old animals were 0.199 ± 0.024 mg., and 0.278 ± 0.036 mg. respectively for the month of May. In July the mean values for this same group of animals were 0.099 ± 0.02 mg. and 0.177 ± 0.013 mg. respectively. In August these values were 0.197 ± 0.033 mg. and 0.213 ± 0.017 mg. respectively for the same groups, and 0.243 ± 0.03 mg. and 0.336 ± 0.018 mg. respectively for a similar group of goats in October. In comparing these data the difference between the 2 age groups was statistically significant during February, July and October. The values in July were also significantly lower

for both age groups than at any other time of the year. The mean thyroid secretion rate of a group of 4 aged and a group of 7 young lactating goats during May were respectively 0.188 $\pm$ 0.053 mg. and 0.263 $\pm$ 0.030 mg. During July the mean thyroid secretion rates of the aged and young groups were 0.05 $\pm$ 0.016 mg. and 0.07 $\pm$ 0.0074 mg. respectively. In August these values for aged and young goats were 0.121 $\pm$ 0.040 mg. and 0.177 $\pm$ 0.040 mg. respectively. No significant difference was found between the 2 age groups in either of the three experiments. However, the seasonal decline in both groups was significant. In July the mean secretion rate of the non-lactating animal was significantly higher than that of lactating goats.

Although thiouracil treatment increased the thyroidal iodine output rate, the mean thyroid secretion rate was not significantly different in thiouracilized than in normal goats.

Although no correlation with thyroid secretion rate existed, except for the month of August, there was a definitely increased thyroidal uptake of iodine during the summer months. The thyroids of non-lactating animals also took up more iodine than lactators. No correlation was found between thyroid secretion rate as determined by the substitution method and output half-time except during the month of May.

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Dedicated

to

my wife, Louise

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TABLE OF CONTENTS

LIST OF TABLES

APPENDIX TABLE PAGE

APPENDIX TABLE PAGE

LIST OF FIGURES

INTRODUCTION

Recent advances in the field of endocrinology have made it increasingly evident that an interplay of many hormones is involved in various physiological processes. The notion that one particular hormone is concerned with the adjustment of one particular process is no longer compatible with present findings. The thyroid gland presents a good example of this since it appears to play a role in conjunction with other hormones in the physiological processes of growth, lactation, reproduction and various other functions. In turn, the activity of the thyroid gland is profoundly affected by other endocrine substances as well as external and internal changes of environment.

In the past, investigators in the field of endocrinology have had their efforts hampered by the fact that necessary hormone preparations were expensive, the supply was limited and moreover they were not very uniformly standardized. However, in the case of the thyroid gland it is now possible to produce varying degrees of glandular activity. By administering certain iodinated proteins or synthetic thyroxine, varying degrees of hyperthyroidism can be produced; a hypothyroid state can be readily brought about simply by feeding with various goitrogens. The impetus of such a control over the thyroid has produced great strides in thyroid physiology, especially in the field of animal production. This is evident by the voluminous amount of material concerning the subject found in

such publications as the Journal of Dairy Science, the Journal of Animal Science and the various agricultural experiment station bulletins. However, it requires only a casual perusal of this literature to reveal many contradictory results obtained by a number of investigators. It has been pointed out by Maqsood and others that much of this antithetical information is due to a lack of knowledge of the normal thyroxine secretion rate during the time of administration of thyroidal materials. As stated by Maqsood:

> In the past, factors such as breed and strain differences, sex and age of the animal, stage of sexual development, dose, mode and duration of the hormone administered, seasonal variations in the length of daylight, and environmental temperature, all of which appear to affect the normal thyroxine secretion rate, have not been taken into consideration while studying the role of the thyroid status on maturity and fertility. This could be better understood if expressed quantitatively in terms of the amount of thyroxine secreted per day by the animal.

Until recently the techniques employed to determine thyroid secretion rates have been difficult to apply to larger animals because they necessitated slaughtering large numbers. However, a method has now been developed which employs radioactive iodine and allows a quantitative measurement of thyroid activity in the intact animal.

In the present study, the method introduced by Henneman, et al. (1955) was adapted to obtain information concerning the normal thyroid secretion rate of the dairy goat. The relative ease of handling plus the short output half-time of I-131 without the use of thiouracil make this animal ideal for such studies.

SURVEY OF LITERATURE

A. Early Investigations

Since ancient times the superficial anatomical location of the thyroid gland has made it possible to associate certain diseases with gross changes in this gland. During the time of Hippocrates it was known that certain substances such as burnt sponge and seaweed when added to the diet resulted in the relief of enlarged thyroids. As early as 1820 iodine was knowingly administered for the relief of goiter and physiological studies made it apparent that the thyroid elaborated a substance which was necessary for normal body function. Murray (1891) reported that a glycerine extract of sheep's thyroid when administered to human patients satisfactorily alleviated hypothyroidism. Magnus-Levy (1895), in his calorimetric studies, found that patients suffering from Gull's disease were markedly below normal in heat production. It was also shown by Magnus-Levy that the administration of thyroid tissue raised the level of metabolism in such patients as well as in normal persons. These findings served to stimulate much biochemical interest in the thyroid and, soon after, Baumann (1896) succeeded in demonstrating the presence of iodine in thyroid material. Kendall (1914) was the first to isolate the thyroid hormone in crystalline form and gave it the name thyroxine. Harington (1926), using improved extraction techniques, was able to isolate a sufficient quantity of thyroxine to determine the empirical

and structural formula. A year later Harington and Barger (1927) succeeded in synthesizing thyroxine.

B. Formation of Thyroid Hormone

Although it was recognized early that the thyroid contained iodine and that this element was necessary for the normal function of the gland, the accumulation of iodine by the thyroid was not directly demonstrated until Marine and Rogoff (1916) reported that within five minutes following the intravenous injection of 50 mg. of potassium iodide the iodine content of the thyroid of a dog may be increased several hundred percent. Since this time in vitro experiments involving the use of radioactive iodine and thiouracil have revealed the presence of two distinct and independent mechanisms for the handling of iodide by the thyroid gland. One mechanism is responsible for concentrating iodide and another for organic binding.

When thyroid tissue slices are incubated in a medium containing I-131 the labeled iodine is found in the tissue both as iodide ions and as iodine in organic combination (Chaikoff and Taurog, 1949). The addition of thiouracil to the medium results in iodide continuing to be accumulated, but organic binding fails to occur. In vivo experiments by Astwood (1944-45) revealed that thiouracilized rats depleted of iodine are still able to concentrate rapidly considerable quantities of iodine when injected with potassium iodide. McGinty and Sharp (1946) noted that increased amounts of dietary iodine raise the thyroid iodine content above the usual low levels in rats chronically treated with thiouracil. When the protein of the gland is then

precipitated with Somogyi's reagent this increased thyroid iodine is soluble in the supernatant fluid. In studying the effects of thiocyanate in rats Vanderlaan and Vanderlaan (1947) found that thiocyanate inhibited the preferential concentration of iodide by thyroid tissue and also caused the discharge of iodide from the thyroid.

1. Iodide Accumulation by the Thyroid Gland

Histological studies have shown the uptake of iodide by the thyroid to be directly related to the size of the thyroid epithelial cells. For this reason, it is believed that the iodide-trapping mechanism resides in these cells. Wollman (1953) observed that the power to concentrate iodide by the thyroid was dependent upon the presence of fully formed epithelial tissue and occurred in the absence of any visible colloid. It is not fully understood by what mechanism the epithelial cells are able to concentrate iodide and maintain a gradient against plasma but it is presumed to be one which requires energy. The extent and efficiency of the iodide-trapping mechanism can be quantitated by simultaneous measurement of the concentration of iodide in the thyroid and in the blood. This is expressed as a thyroid-serum or T/S ratio. In normal rats the T/S ratio has been found to be 25/1. When the animals were pretreated with propylthiouracil the ratio of thyroid iodide to serum iodide rose to a value of 250/1 (Vanderlaan and Vanderlaan, 1947).

2. Organification of Iodine

After the inorganic iodide has been trapped more than 90 percent of it is readily converted to organically bound forms. Gross

and Leblond (1947), using autoradiography, found organically bound iodide in the epithelium and colloid as early as thirty minutes after administration of radioiodine. After an interval of 24 hours it was found almost exclusively in the colloid. The organic forms of iodine have been quantitatively measured by chromatographic and colorimetric procedures. At any given time the normal thyroid has been found to contain 25 percent of total iodine as thyroxine and triiodothyronine, approximately 65 percent as diiodotyrosine and monoiodotyrosine. Some monoiodohistidine and diiodothyronine are also present (Roche and Michel, 1954).

Most of the organic iodine in the thyroid is bound to protein and believed to be in peptide linkage. Prolonged alkaline or enzymatic hydrolysis results in the liberation of iodinated amino acids. The mechanism of organification of iodine is not completely understood but at least three steps are concerned in the ultimate synthesis of thyroxine:

1. Oxidation of iodide to iodine or a more highly oxidized form.
2. Iodination of tyrosine radicals of protein to form monoiodotyrosyl and diiodotyrosyl radicals.
3. Oxidative coupling of the iodinated tyrosine radicals to form thyroxine and triiodothyronine.

Evidence has accumulated which strongly suggests that the synthesis of thyroxine is dependent upon oxidative enzyme systems. Morton and Chaikoff (1943) noted the necessity for tissue organization in the *in vitro* conversion of inorganic iodide to thyroxine and diiodotyrosine. This observation suggested to them the participation of an intracellular enzyme system. Further studies were made on the effects

of anaerobiosis and of substances that are inhibitors of cytochrome oxidase. In both cases there were marked inhibitory effects on the formation of thyroxine and diiodotyrosine. However, it is fairly well agreed that other, and probably more important, enzyme systems are also involved. This is shown by the fact that the inhibition of oxidation produced by thiourea, thiouracil and sulfa compounds is not mediated through the cytochrome - cytochrome oxidase system (Astwood, 1949). Evidence reviewed by Astwood suggested to him that the most likely enzyme to be involved directly in the synthesis of thyroid hormone is a peroxidase or a peroxidase type compound. This enzyme is capable of promoting the oxidation of iodine and, as mentioned by Westerfield and Lowe (1942), it could also carry out the oxidative coupling of two diiodotyrosine molecules to form thyroxine. Dempsey (1944) presented histological evidence of the presence of fine granules in the thyroid cell which give histochemical reactions typical of peroxidase and has shown that thiouracil inhibits these reactions. More recently Weiss (1953) reported the synthesis of organically bound iodine by cell free preparations of thyroid tissue when such homogenates were supplemented with cupric ion and tyrosine. Fawcett and Kirkwood (1953, 1954) have also shown that it is possible to extract from thyroid and salivary gland tissue a soluble enzyme which catalyzes the iodination of tyrosine in the presence of cupric ion. In view of this work it was at first believed that the requirement for copper indicated that either a copper containing enzyme was involved (Weiss, 1953) or the cupric ion participated as a non-enzymatic entity in the oxidation of iodide to iodine

(Fawcett and Kirkwood, 1953). These latter workers further suggested that the function of cupric ion in this in vitro reaction is to accept electrons directly from iodide ion and thus replace an "iodide oxidase" which causes the formation of elemental iodine in the intact thyroid gland. However, in the light of further study on the effect of iodide concentration on the rate of iodination of tyrosine Serif and Kirkwood (1956) have concluded that the iodine in this in vitro reaction is being produced by an enzyme system. They presume that this enzyme is the iodide oxidase which functions in intact thyroid and salivary gland tissue. This enzyme is believed to have the property of catalyzing an equilibrium between electron donors or acceptors and the iodide-iodine system. In this way it has the ability to catalyze either the oxidation of iodide, or the reduction of iodine, depending on the redox potential of the system. With respect to the active form of iodine in the iodination process two possibilities have been postulated by Serif and Kirkwood (1956). In the first case, in the presence of iodide oxidase two electrons are removed from iodide ion to produce I^+. This may be passed directly to an iodinating enzyme (tyrosine iodinase) and organification takes place. However, it is pointed out by these workers that this I^+ may also react with substances other than tyrosine iodinase and, therefore, it will also react with iodide to form elemental iodine. In view of this, I^+ is not thought to be the active iodinating species of iodine. The second possibility is that elemental iodine is the product of iodide oxidase and that it is released to the environment where it is taken up by tyrosine iodinase. It is further postulated that

the rate of reaction would be greatly augmented if the tyrosine iodinase were to catalyze the iodination of tyrosine by causing the elemental iodine to dissociate to I^+ as well as dissociating the phenolic hydroxyl of tyrosine.

In the past it was believed that these oxidative processes occurred within the epithelial cell and the thyroglobulin with its iodinated residues was then secreted into the colloid of the follicle. However, it has been observed by Pitt-Rivers and Trotter (1953) using radioautography that inorganic iodide accumulated in the colloid and it was found that organification of iodine took place in the periphery of the colloid near the colloid-cell interface. On the basis of these observations it is theorized that the cells continuously secrete an oxidizing enzyme into the colloid where iodination of the thyroglobulin takes place. In support of this notion are the observations of Roche. Using the best techniques the only iodinated protein that he was able to find in the thyroid was thyroglobulin, whereas Reineke and Turner (1942) have shown that it is possible to iodinate casein and other proteins. If organification of iodine took place within the cell we would, therefore, expect other proteins besides thyroglobulin to become iodinated. However, in the light of this new concept the cell, where the various proteins are available, contains only the iodide ion. The free iodine necessary for iodination of protein is found only in the colloid and in the presence of only one tyrosine-containing protein and that one is thyroglobulin.

By way of summary, the present evidence indicates that inorganic iodide is preferentially removed from circulating blood by the

thyroid epithelial cells and secreted into the colloid. In the presence of oxidative enzymes this iodide is converted to nascent iodine. Tyrosine residues contained within the protein thyroglobulin molecule are then iodinated to form monoiodotyrosine and diiodotyrosine. Two molecules of these latter residues, still in the presence of peroxidase-like enzymes, are oxidatively condensed to form thyroxyl and triiodothyronine residues of thyroglobulin. This thyroglobulin then acts as a storage form of the thyroid hormone.

3. Proteolysis of Thyroglobulin

Since thyroglobulin as such has no hormone properties it must be presumed that the next step in hormone synthesis is the liberation of the thyroid active substances. DeRobertis (1941) has shown that thyroid proteolysis leading to diffusable products of low molecular weight takes place in the thyroid. He has demonstrated the presence of a proteolytic enzyme in the colloid capable of releasing tyrosine from a protein substrate and has characterized it as a cathepsin type compound. The free iodinated products which have been found in the thyroid include monoiodotyrosine and diiodotyrosine, traces of monoiodohistidine, thyroxine, and triiodothyronine. The thyroxine and triiodothyronine contained in this pool are believed to be the source of the plasma thyroid hormone. Gross and Leblond (1951) found that after the injection of radioiodide in rats the specific activity of the free thyroxine is at first higher in the thyroid than in the plasma and later becomes approximately equal in both. It is pointed out by these workers that the passage of thyroxine from

gland to plasma is facilitated by a concentration gradient of over 100:1. More recently Taurog, Wheat and Chaikoff (1955) administered I-131 to horses and sheep. Using chromatography they analyzed a blood plasma sample obtained from the thyroid vein and found mainly thyroxine and triiodothyronine; only slight traces of iodotyrosine were detectable. This was also found earlier by Gross and Leblond (1951) using autoradiographs of paper chromatograms. These latter workers concluded that all of the iodothyronine compounds go into the circulation directly while the iodotyrosines are metabolized within the gland. The fate of the iodotyrosines then would be to undergo dehalogenation and act as hormone precursors. In this way the thyroid handles iodine with maximum efficiency. Roche has postulated that practically all of the iodine leaves the gland as hormone iodine but only after one or more cycles of organification with thyroglobulin. In support of this, Roche has shown that thyroid slices or homogenates are able to dehalogenate diiodotyrosine to monoiodotyrosine and this in turn is deiodinated to form tyrosine. This dehalogenating mechanism depends upon an enzyme system since it varies with pH and is stopped by heating. It is also shown to be inhibited by thyroxine and activated by TSH. Furthermore, incubation of thyroid slices in a medium containing iodotyrosine and thyroxine results in the deiodination of the tyrosine but not the thyroxine.

C. Hormonal Transport and Utilization

1. Transport

The mode of transport and nature of circulating thyroid hormone after its release into the blood stream has been the object

of much investigation. It has been known for some time that the hormone is readily bound to plasma protein. Recently the use of paper zone electrophoresis to separate plasma proteins and radioactive iodine as a tracer has made it possible to study the behavior of iodine containing compounds (Albright, et al., 1956; Recant, 1956). Therapeutic amounts of I-131 were administered to euthyroid and hyperthyroid subjects, and their serum was then studied. It was found that initially most of the I-131 was unbound by protein but there was a definite localization in the albumin fraction. After several days there appeared in the normal subjects a gradually increasing localization of radioactivity in an area just ahead of the alpha-2 globulin in addition to the albumin-bound radioactivity. In the hyperthyroid subjects the sequence was essentially the same; however, the radioactivity in the inter-alpha zone reached higher levels at a more rapid rate and then declined more sharply. Albright and his coworkers suggest that this more rapid turnover of I-131 in the hyperthyroid individual is an indication that the circulating hormonal iodine is carried by an alpha globulin. Further studies involving the use of starch zone electrophoresis and paper chromotography revealed that the radioactive material present in the alpha zone consisted entirely of thyroxine. No triiodothyronine was found. In vitro experiments have shown both compounds do associate with alpha globulin but thyroxine binds more firmly and can displace triiodothyronine. This is believed to afford an explanation for the absence of triiodothyronine in the alpha globulin in in vivo experiments and

may also account for the greater speed of biologic activity of triiodothyronine.

Recant (1956) has reported an alteration in the transport mechanism of thyroxine as a result of certain conditions. She has found that 70 to 100 percent of I-131 labeled thyroxine incubated in vitro with serum from patients with nephrosis was found in the alpha-2 globulin area. In contrast, both normal and myxedematous subjects bound most of the thyroxine in the albumin fraction. The abnormal nephrotic pattern was found to be reversed within forty-eight hours after steroid therapy.

Dowling (1956) has found a marked increase of thyroxine binding to serum alpha globulin in pregnant women, beginning as early as the twenty-first day after ovulation and persisting throughout pregnancy. A similar change occurs after estrogen administration.

2. Utilization

At present one of the major problems confronting the thyroidologist concerns the metabolism of thyroxine by the peripheral cells of the body.

Gross and Pitt-Rivers (1951) have suggested that triiodothyronine is derived from a deiodination process of thyroxine by peripheral tissues. This notion carried further led to the hypothesis that thyroxine itself has no metabolic activity but acts as a precursor for triiodothyronine which is metabolically active. Direct evidence in support of this concept was afforded by Albright, et al. (1954). These workers demonstrated the deiodination of thyroxine to

triiodothyronine *in vitro* by rat kidney slices. Albright, et al. (1956) have extended these studies of thyroxine utilization to subcellular fractions of kidney tissue. When I-131 labeled thyroxine was incubated with an enzyme system derived from mitochondria the reaction product was not the expected triiodothyronine but tetraiodothyroacetic acid. When triiodothyronine was similarly incubated the reaction yielded triiodothyroacetic acid. The true significance of this reaction has not as yet been ascertained but it is believed by these workers that it may represent a degradative process. However, the point was not overlooked that this transformation may represent an intermediate compound in the formation of a specific metabolically active derivative of the thyroid hormone. Lerman and Pitt-Rivers (1956) have reported that these iodothyroacetic acid compounds act qualitatively like thyroxine and triiodothyronine but are quantitatively weaker. Tetraiodothyroacetic acid, intravenously, has 1/15th the activity of l-thyroxine in raising the B.M.R. of patients with myxedema. Triiodothyroacetic acid has 1/4th to 1/6th the activity of thyroxine.

Several theories as to mechanisms of thyroid hormone action have been postulated. Lehninger has proposed that thyroxine acts primarily on the mitochondrial membrane. He observed that thyroxine promoted water imbibition by isolated mitochondria. Lardy has postulated that oxidative phosphorylation is the primary site of action of thyroid hormone. He bases his theory on the *in vitro* evidence for the uncoupling of oxygen uptake from phosphate esterification in the presence of thyroxine.

D. Factors Influencing Thyroid Function

A variety of conditions and agents are known to influence thyroid function. These include the anterior pituitary thyrotropic hormone, environment, adrenal cortex and gonadal hormones, iodine, and antithyroid drugs.

1. Pituitary Thyrotropic Hormone

The relationship between the pituitary and thyroid has been studied in considerable detail in various laboratory animals. It is generally agreed that secretory activity of the thyroid is regulated by a balance between thyroid hormone and the anterior pituitary thyrotropin hormone (Cortell and Rawson, 1944; Keating, et al., 1944-45; Taurog, Chaikoff and Bennett, 1946; Leblond and Gross, 1948; Stanley and Astwood, 1949). It has been found by these workers that under the stimulating influence of thyrotropic hormone, inorganic iodide is more rapidly taken up, bound to protein, proteolyzed and the hormone discharged into the circulation. Associated with these processes is a hypertrophy and hyperplasia of the thyroid. With increased concentration of thyroid hormone in the blood the activity of the anterior pituitary is modified in such a way that diminished amounts of thyrotropin are secreted. With regard to the mechanism of action of thyroxine in suppressing TSH secretion D'Angelo has advanced the most recent theory. He suggests that the excess thyroxine so speeds up metabolic processes that the rate of disposal of thyrotropin from the circulation is increased and therefore lower concentrations of TSH are available to the thyroid gland. In support of

this concept is the observation that TSH and thyroxine given simultaneously result in less thyroid stimulation than when TSH is given alone. It has also been pointed out by D'Angelo that the rate of disappearance of TSH is slower in hypophysectomized, thyroidectomized, or goitrous animals. In each of these three cases the animals are existing at lower metabolic levels due to a deficiency of thyroid hormone. The sparing of TSH under these conditions may reflect an inhibition of an inactivation process due to a lowered rate of metabolism.

Although the activity of the thyroid is primarily under the control of the anterior pituitary it has recently been shown by D'Angelo that the thyroid gland is able to carry on a residual function even in the complete absence of the hypophysis. This residual function has been estimated at approximately 10 percent of the normal.

2. Temperature

It is now held as a general principle that environmental temperature modifies thyroid activity (Dempsey and Astwood, 1943; Leblond, et al., 1944; Dempsey, 1944, 1949; Schachner, Gierlach and Krebs, 1949; Blincoe and Brody, 1955; Henneman, et al., 1955). Existing evidence reveals that exposure of rats to cold leads to considerable increases in production of thyroid stimulating hormone by the pituitary, follicular cell activity resulting in a heightened production and release of thyroid hormone, and utilization of thyroid hormone by body tissues. The converse of this takes place when the

animals are exposed to high temperatures. The thyroid response to change of environmental temperature is generally assumed to be related to modifications of pituitary TSH secretion (Uotila, 1939). However, Wolf and Greep (1937) have shown that the thyroids of hypophysectomized animals do reveal minimal histologic signs of stimulation by cold. Rand, Riggs and Talbot (1952) have suggested that the mechanism involved in response to cold is an increased utilization of thyroxine by the tissues. This results in a compensatory response of the pituitary to a reduced concentration of thyroid hormone in the blood. However, Brown-Grant points out that this may be true for chronic cold experiments but it seems more probable that some neural mechanism, independent of the hormone concentration, may be involved in the rapid response to acute cold exposure. In support of this Uotila (1939), Purvis and Griesbach (1946) have found that pituitary stalk transection results in a loss of thyroid response to cold exposure.

3. Season and Light

Seasonal variations have been found to occur in the over-all activity of the thyroid in many species (Starr and Roskelly, 1940; Reineke and Turner, 1945; Henneman, et al., 1955; Lodge, Lewis and Reineke, 1956). So far no definite physiological explanation other than that due to temperature or light change has been forthcoming. Puntriano and Meites (1951) have found that continuous light induced significant reductions in thyroid weight, thyroid reaction to thiouracil and thyroid uptake of radioactive iodine in rats. Continuous

darkness was observed to have the reverse effects. These workers concluded that continuous light depresses while continuous darkness increases thyroid secretion in mice.

4. Thyroid-Gonad Relationship

There is a great deal of conflicting evidence presented in the literature concerning the relationship between the thyroid gland and the gonads. However, it seems to be agreed in general that the relationship is a reciprocal one. There is some evidence to show that castration produces a slow involution of the thyroid and a slight reduction in total metabolism (Korenchevsky, 1930). In contrast, Kippen and Loeb (1936) reported that gonadectomy in the guinea pig resulted in thyrotropin secretion and thyroid proliferation. The effects of estrogen on thyroid function are apparently related to dosage and the period over which the hormone is administered (Lerman, 1941). Both estrogens and androgens in physiologic amounts are capable of stimulating thyroid activity (Lewis and McCullagh, 1942). When the dose of estrogen is increased thyroid function is definitely depressed (Gardiner, 1949).

5. Thyroid-Adrenal Relationship

Cortical extracts of the adrenal gland have been shown to affect thyroid function (Oehme, 1936; Hoen, McGavack, Langfeld and Oehme, 1939). When cortical extracts are administered in large doses they will prevent the rise of basal metabolic rate which would ordinarily follow the administration of thyroid hormone. Tipton, et al. (1946), as a result of their enzyme studies, suggest that this

action may be due to the cortical hormones inhibiting the mechanisms which are responsible for the activation of succinoxidase and cytochrome oxidase in hyperthyroidism. When physiologic doses are administered, there is an augmentation of the characteristic action of thyroxine upon growth and calorigenesis (Vergara Soto, 1948; Hoffman, Hoffman and Talesnik, 1948). In the adrenalectomized animal there is an increase in heat production which is prevented by prior thyroidectomy (Marine and Baumann, 1922). There is also a rapid loss of thyroidal iodine following adrenal injury. Animals exposed to various situations of stress show an increase in the secretion of thyrotropin, a diminished thyroidal uptake of I-131 and a diminution of hormone in the plasma. This could be interpreted as denoting an increase in thyroid activity. The lowered amount of circulating thyroid hormone may reflect a heightened state of metabolism and result in an increase in pituitary thyrotropin release. Moreover, it has been shown that diminished storage of I-131 is associated with the more active thyroid in colder temperatures and a greater I-131 storage occurs in the summer when the thyroid is least active (Seidell and Fenger, 1913; Kendall and Simonsen, 1928). However, this picture is complicated by the findings of Meites and Wolterink (1950). They observed that rats maintained on a calorically inadequate diet had a decreased thyroidal uptake of radioactive iodine due to a decrease in size of the thyroid. The radioactivity per unit weight of the stressed animals was not significantly different from that of the well fed animals.

6. Antithyroid Compounds

The term antithyroid substance is applied to any agent which is capable of suppressing the formation of thyroid hormone by the thyroid gland of the intact animal. A vast amount of material has been published concerning these antithyroid compounds and this review does not attempt to cover all of it. Much of this literature has been summarized and the following reviews deal with their chemical classification in relation to activity (Anderson, 1951), a differentiation of the nature of their action (Astwood, 1949), and a rather complete review of the entire subject by McGavack (1951).

E. Economic Aspects of the Thyroid

1. Effect of Thyroid on Growth

It has been well established that hypothyroidism, whether induced or spontaneous, depresses growth in all animals. This effect is illustrated by the stunted growth and retarded mental and sexual development of cretins. Likewise, tadpoles immersed in water containing antithyroid drugs are delayed in their metamorphosis. Depression in growth has also been reported in thyroidectomized cattle by Brody and Frankenbach (1942) and Spielman, et al. (1945). These authors also noted that this effect was much greater in the young than in older animals. Blaxter, et al. (1949) suggested that some economic value may be derived from partial suppression of thyroid activity prior to marketing calves or older animals. Their logic for such treatment is that depression of basal metabolism and activity

would result in more of the ingested net energy being available for deposition in the tissues as fat.

There is a paucity of literature concerning the effects of mild hyperthyroidism on growth. In severe hyperthyroidism marked losses of weight occur, presumably due to an elevation in metabolism. Reineke (1946) stated that "there is some evidence that a properly regulated dosage of thyroid substance will cause an increase in growth rate at least in some species." In a later paper (1948) on the growth of swine the same author reports: "Administration of thyroprotein in proper dosages will cause some increase in the growth rate and will also increase the rate at which maturation changes occur." Millen, Nevens and Gardner (1948) administered 1.3 grams of iodinated casein per 100 pounds body weight to two dairy calves and noted a slight increase in growth above normal controls. However, when four grams per 100 pounds of body weight were given the usual symptoms of severe hyperthyroidism resulted.

2. Effect of Thyroid on Reproduction

Complete agreement has not been reached on the role of the thyroid in male fertility. There is some evidence to support the view that the thyroid has no direct effect on the testis but rather, any reproductive disturbance in the male in hypothyroidism and hyperthyroidism is due to changed metabolic status. Petersen, et al. (1941) reported that thyroidectomy in the bull, resulting in complete clinical myxedema, results in a loss of libido and interest in the estrual female. No effect on spermatogenesis was apparent since

ejaculates obtained by ampullae massaging were normal in sperm morphology, activity, longevity and fertilizing ability. When thyroidal material was administered libido was completely restored. Reineke (1946) reported favorable results when iodinated casein was fed to aged bulls. He found that ten out of fourteen animals showed increased vigor and more speedy ejaculation.

In thyroidectomized rams the picture appears to be just the reverse of that found in bulls. Bogart and Mayer (1946) noted a marked decline in sperm number and motility, and an increase in abnormal forms in thiouracilized rams. It appears that sex interest was not impaired in these experiments since semen was collected with an artificial vagina. The adverse effects on sperm production were reversed by feeding iodinated casein.

With regard to reproduction in the female, thyroidectomy in the cow results in a complete absence of normal estrual behavior (Brody and Frankenbach, 1941). However, even in myxedematous animals ovulation and a normal ovarian cycle occurs and impregnation followed by normal pregnancy can take place. Petersen and his coworkers believe that the thyroid plays only an indirect role in reproduction through maintenance of an optimum rate of metabolic processes.

3. Effect of Thyroid on Milk Production

The use of thyroidal materials in milk production is undoubtedly the most important economic aspect of thyroid physiology at the present time. The fact is now well established that the feeding of thyroidal substances will increase milk and milk-fat production.

Graham (1934) first demonstrated a definite relationship between milk secretion and the thyroid. In his thyroidectomized animals there was not a very significant difference in the fall of milk secretion when compared to that accompanying control operations. However, there was a rapid rise in milk and milk-fat production during the declining phase of lactation when a small amount of thyroid material was added to the diet of both thyroidectomized and control animals. In later experiments (1934a) he noted that there was always a marked rise in milk-fat production, but there were large variations in the increase of milk secretion. The work of Graham has been confirmed by Jack and Bechdel (1935); Herman, Graham and Turner (1938); Ralston, et al. (1940); and Blaxter, et al. (1949).

This work served to stimulate speculation on the use of iodinated proteins to increase milk production. Turner (1940) reported an increase of milk production in goats following the feeding of small amounts of iodinated protein. Later, in 1942, Reineke extended these preliminary experiments to cattle. He reported an increase in metabolism of twenty to thirty percent accompanied by an improvement in appearance and vigor and an increase in milk yield and milk-fat percentage. In 1943 Reineke reported on successful field trials of feeding synthetic thyroprotein to cattle. Since this time an iodinated casein preparation has been developed by Turner and Reineke and is now marketed under the name of protamone. It has been reported by Graham (1948) that the feeding of protamone to commercial herds in the Los Angeles milk shed resulted in increases

in milk secretion of as much as 5 pounds of milk and 0.3 pound of butterfat daily during peak production.

The effect on lactation of feeding thyroidal materials has been found to be dependent upon several factors. The response in milk production with regard to dosage has been studied by Reineke, et al. (1944); Blaxter (1945) and Reece (1944). The influence of age and size of the animal has been reported on by Blaxter (1946) and Booth, et al. (1947). Blaxter has also found that in cattle breed has relatively little effect on response. In their treatise on the role of thyroidal materials in animal production Blaxter, Reineke, Crampton and Petersen (1949) state that the most important factors influencing the response of the cow to thyroprotein feeding are her stage of lactation and producing ability. This conclusion is based on the work of Herman, et al. (1938); Ralston, et al. (1940); and Blaxter (1945). Length of stimulation and nutritional status have also been studied as factors influencing lactational response (Reece, 1947; Thomas and Moore, 1948; Reineke, 1943).

With regard to the effect of feeding iodinated protein on milk composition Blaxter, et al. (1949) have concluded in their review that there is an increase in the lactose content and possibly a small decrease in the protein and non-protein nitrogen content. These authors further state that "as far as milk composition is concerned, the milk produced by cows receiving iodinated casein is perfectly safe, and nutritionally adequate."

F. Methods of Determining Thyroid Activity

1. Oxygen Consumption and B.M.R.

The observations of Magnus-Levy (1895), concerning the increase in oxygen consumption and carbon dioxide output following the administration of thyroid material, formed the basis for the most widely used method of studying thyroid activity. The method consists of determining the heat production of a resting, fasting individual in a thermo-neutral environment. This basal metabolism rate is expressed as calories per hour per square meter surface area and is usually determined indirectly by measuring the carbon dioxide and oxygen respiratory exchange. With the advent of radioactive iodine the method has fallen into more and more disuse as a diagnostic tool. It is still widely used, however, as an assay procedure for thyroxine. The technique does not lend itself very readily to the study of large animals and most especially the ruminant. It is difficult to maintain the animals in a subdued state and practically impossible to obtain a fasted ruminant animal. Gases produced in the rumen also interfere in this determination. In measuring the carbon dioxide produced there is no way of differentiating between that produced by metabolism of the body and that resulting from bacterial fermentation.

2. Body Weight and Replacement Therapy

The loss of body weight in thyroidectomized animals and the restoration of growth following the administration of thyroid active materials has been used as a method of assay. However, this type of

study has not found wide acceptance because it lacks the necessary sensitivity for accurate quantitative measurements.

3. Goitrogenic Method

Following the discovery of certain compounds such as thiourea and thiouracil which prevent the formation of thyroid hormone, Dempsey and Astwood (1943) described a new method of assay based on the action of these compounds. Mixner, Reineke and Turner (1944) reported a similar type of assay using the one-day old chick as the test animal. In the absence of thyroid hormone there is a consequent increased production of pituitary thyrotropic hormone. As a result there is a marked stimulation of the thyroid and this is reflected in histological changes and increased size of the gland. These changes vary directly with the rate of secretion of the thyrotropic hormone and indirectly with the amount of circulating thyroid hormone. The end point used in this determination is that amount of exogenous thyroxine needed daily to maintain a normal hormonal balance between the thyroid and pituitary as determined by the absence of weight change in the thiouracilized animal. Reineke, Mixner and Turner (1945) found good agreement between the data obtained by this method and that obtained from basal metabolism measurements. The technique has gained widespread acceptance and most of our present day knowledge of thyroid activity and factors affecting the activity have resulted from its use. However, the application of this method to the large domestic animal has been limited by the necessity for slaughtering large numbers.

4. PBI Method

Because of the limitations of the goitrogenic technique in larger animals many investigators in this field have used protein-bound iodine measurements as an indication of thyroid activity. It has been confirmed by Morton, Perlman, and Chaikoff (1941); Morton, et al. (1942) that disturbances in thyroid activity are reflected in the thyroxine-like fraction of plasma iodine. Long and his co-workers (1951) measured the protein bound iodine content of serum in dairy cattle and found significant differences between breeds. However, in connection with the determination of protein-bound iodine it has been pointed out by Salter, et al. (1949) that inorganic iodine, when present in excess, is capable of forming a loose combination with protein, and may be precipitated with the hormone active fractions. Values for protein-bound iodine may therefore become meaningless if iodine is being taken in by the subject. In a review, Rapport and Curtiss (1950) have stated that the chief disadvantage of the PBI method of determining thyroid activity is that iodine in any form has a demonstrable effect on protein-bound iodine levels in the serum. In this regard, Reece and Man (1952) have shown the presence of a nonthyroidal fraction in the plasma protein-bound iodine of cattle. Lewis (1952) observed no effect on PBI of cattle when the ration was supplemented with iodized salt. However, he further stated that the iodine in the supplemented ration may not have been in excess of the animal's needs and if an excess were present the results might have been different. Although there is much evidence for and against the PBI method of ascertaining

thyroid activity many clinicians still regard it as a valuable laboratory aid in diagnosis of thyroid function.

5. Thyroid Studies with Radioactive Iodine

Soon after the discovery of artificial radioactivity by Joliot and Curie (1934), Fermi reported the successful preparation of radioactive iodine. Following the development of the cyclotron by Lawrence and Cooksey (1936) this isotope became available to investigators. Since this time much knowledge of the thyroid has been gained through the use of radioiodine.

Kelsey, Haines and Keating (1949) have identified fourteen radioactive isotopes of iodine. Four of these have been employed in biological investigations. I-128 with a half-life of 25 minutes was the first to be prepared. This was accomplished by the action of slow neutrons on iodine. I-126 with a half-life of 13 days is prepared by the action of fast neutrons on iodine. The bombardment of metallic tellurium with slow neutrons in the chain reacting pile gives rise to I-131 with a half-life of 8 days; I-130 with a half-life of 12.6 hours also results from the bombardment of tellurium. I-131 is used almost exclusively in biological studies at the present time because of its convenient half-life and it can be obtained in carrier-free form.

Hertz and his coworkers (1938, 1940, 1941) were the first to report the use of radioiodine in following the distribution of this element within the body. The action of factors which affect the thyroid, such as thyrotropin and iodine were further elucidated by these workers.

Morton, Chaikoff, et al. (1943, 1944), using *in vitro* techniques involving the incubation of thyroid slices in Ringer's solution containing tracer amounts of radioactive iodine, were able to determine the fractions of iodine-containing compounds in the thyroid gland.

Hamilton and Soley (1938, 1939, 1940) first reported the use of radioactive isotopes in humans. By making measurements directly over the thyroid they were able to follow the uptake of iodine and compared the values obtained from normal persons with those from patients with various thyroid diseases. Werner, Quimby and Schmidt (1949), working with humans, reported the uptake of radioiodine by the thyroid after an interval of 24 hours post-injection. It was deemed by these workers as the most favorable time for assaying thyroid function in humans because at this time uptake has leveled off in both the euthyroid and hyperthyroid gland. Measurements at the 24 hour period also provide a normal uptake range whereby a large majority of patients with hyperthyroidism and hypothyroidism can be distinguished from those with normal function. At the present time the 24 hour thyroidal uptake of radioiodine is probably the most widely used technique in diagnosing thyroid disorders. It has the advantage of being simple to perform and requires minimal cooperation by the patient and minimal dose of radioiodine. However, in the past few years some criticism of this method has been expressed. In view of various factors which may have an effect on the blood iodide pool, from which the thyroid draws for its hormonal iodine, it is felt that uptake determinations do not always reflect

true thyroid activity. Such variables as differences in excretion rates, enterohepatic circulation, and exogenous iodine from known and unknown sources would be expected to affect the blood iodide pool. McConahey, et al. (1956) have reported on a clinical appraisal of seven radioiodine tests in humans. These included: (1) 6 hour uptake, (2) 24 hour uptake, (3) 24 hour excretion, (4) accumulation rate, (5) extrarenal disposal rate, (6) conversion ratio, and (7) thyroidal iodide clearance. These workers stated that all seven were relatively accurate for diagnosis of exophthalmic goiter in the 92 patients studied. It was felt that the 6 hour uptake and thyroidal iodide clearance were the most precise of the tests studied because they distinguished sharply between the patients with exophthalmic goiter and those without thyroid disease. However, it was pointed out by these investigators that radioiodine tests are of limited usefulness in diagnosis of myxedema. They concluded that the clinical interpretation of uptake studies is always hindered by the knowledge that exogenous iodine may frequently be the cause of abnormal results.

More recently, Jaimet and Thode (1956) reported that although the saliva-plasma iodine ratio and thyroid activity gave a good correlation, a much better indication of thyroid function could be obtained when the saliva-PBI ratio at 24 hours was used. This was found to be almost 100% accurate when other radioiodine tests gave inconclusive results. Observed S-PBI ratios ranged from 0.14 to 44 for hyperthyroids, from 150 to 250 for normal subjects and from 400 to over 1900 for hypothyroid individuals.

Wolff (1951) used the measurement of output half-time of radioiodine from the rat thyroid as a method of studying the various factors which influence the release of iodine. He found a value of 3.3 days in control animals. Propylthiouracil treated animals had an accelerated release with a half-life of 1.6 days. Hypophysectomized and thyroxine treated rats had similar values of 24 and 26 days. respectively. It was also shown by Wolff that thyrotropin action had a latent period of two to three hours before any significant effect could be shown on I-131 release. Albert and Tenny (1951) made similar observations using the method of proportional rate of disappearance of thyroidal I-131 as percent per day.

Perry (1951) also reported a method of determining an index of thyrcidal hormone release based on the decline of radioactivity in the gland. Besides showing that secretion is markedly inhibited by exogenous thyroxine Perry further demonstrated a relationship between the amount of thyroxine given and the degree of inhibition of hormone release. Such a relationship was also shown by Reineke and Singh (1955). This was accomplished by measuring the radioactivity of the thyroids of several groups of animals after each group had been injected with a different dose of l-thyroxine ranging from 0 to 10 micrograms. This radioactive count was expressed as a percent of the activity determined previous to thyroxine injection. By plotting the percent of initial activity against micrograms of l-thyroxine injected a standard curve was obtained by Perry whereby unknown thyroid active materials could be assayed. From the quantities of thyroxine required to block the release of radioiodine Perry found

that the daily thyroid hormone demand of a 200 gram rat was approximately 10 micrograms of l-thyroxine.

Based upon Perry's work, Henneman, et al. (1952) developed a method whereby thyroid secretion rate in individual sheep could be quantitatively measured. This procedure consists of injecting the animals with radioactive iodine and determining the thyroidal uptake and output of iodine. This is done by making radioactive measurements directly over the thyroid gland. After peak uptake of iodine has occurred and output is established, daily injections of l-thyroxine are begun. Every third day the injection dose is increased by an appropriate increment. With increasing amounts of injected thyroxine there is a correspondingly increased amount of inhibition of the thyroid-pituitary system resulting in smaller amounts of iodine leaving the gland. The degree of inhibition of the thyroid-pituitary system is measured by determining the percent of preceding radioactive count. The end-point then is that amount of exogenous l-thyroxine needed daily to prevent I-131 output; or, that point where the radioactive count becomes 100% of its preceding count. In experiments on rats Reineke and Singh (1955) have further extended and confirmed this method. Since this time it has been used to study various environmental and physiological factors in dairy calves (Lewis, Reineke and Lodge, 1955; Lewis, Lodge and Reineke, 1956; Pipes, et al., 1956; Lodge, 1957), genetic differences in thyroid activity of several strains of inbred F_1 hybrid mice (Amin, et al., 1956, 1957), and the influence of senescence on thyroid function (Wilansky, et al., 1957).

MATERIALS AND METHODS

A. Experimental Animals

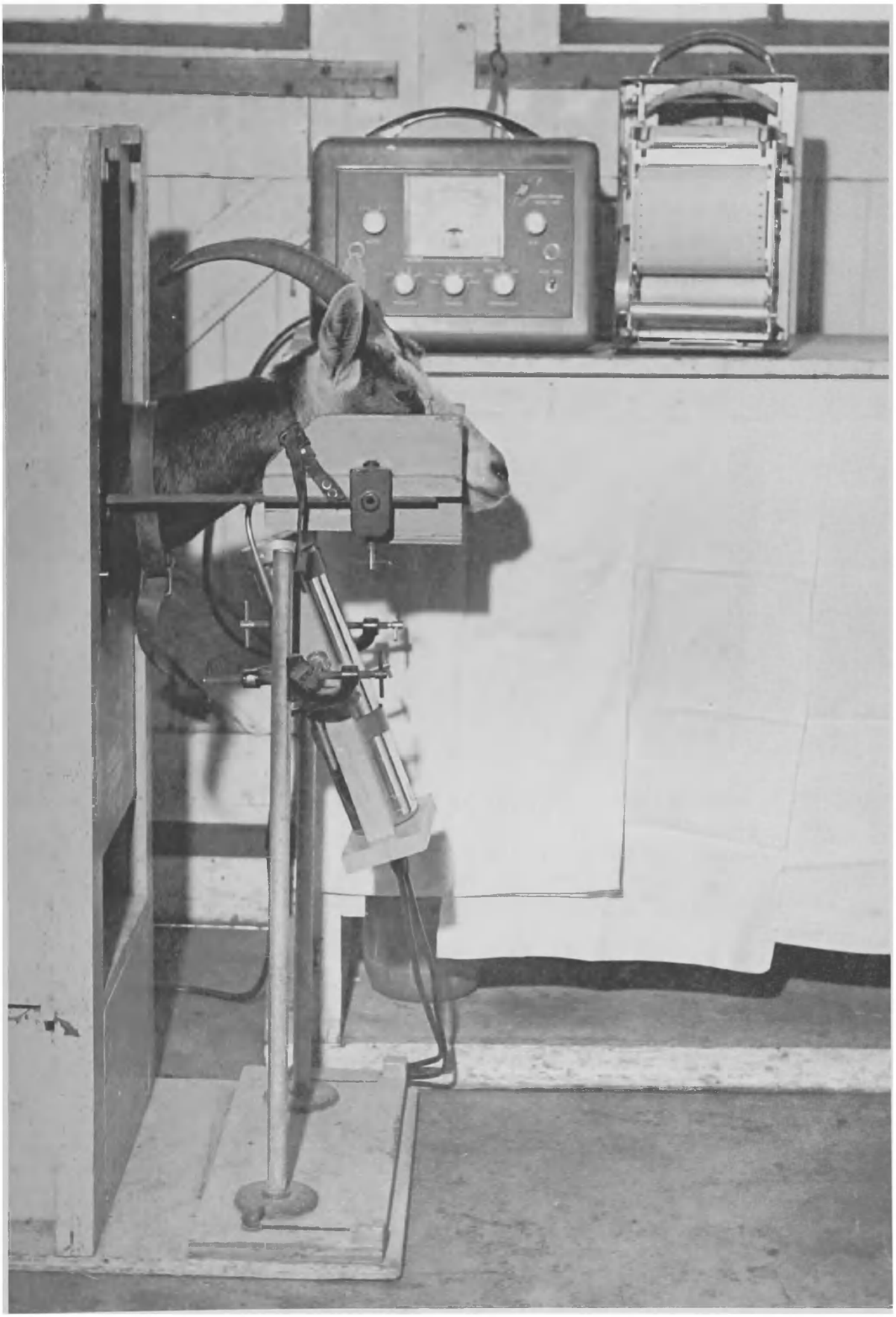
The experimental animals used in this work were obtained from a herd of goats maintained by the physiology department of Michigan State University. These animals were female, of mixed breeding of the dairy type. They were divided into groups according to age and to whether they were pregnant or non-pregnant, lactating or non-lactating. Environmental temperature was not controlled except for supplementary heat in the colder weather when the animals were kept inside the barn. The feeding regimen consisted of pasturage in the summer and hay in the winter. This roughage was supplemented throughout the year with a grain concentrate manufactured by Darling and Company. The manufacturers guaranteed analysis of iodine content of this ration was reported to be 0.008 percent.

B. Apparatus

Figure 1 shows the apparatus that was used to hold the animal in place for determining radioactive count and also the radiation counting equipment. This apparatus was quite flexible and allowed many changes in adjustment to be made to accommodate various sized animals. The radiation counting equipment consisted of a scintillation counter, Nuclear Chicago count rate meter, Model 1620, and an Esterline-Angus recorder. The standardization of this equipment was checked each time it was used by means of a Co^{60} radiation

Figure 1. Apparatus and radiation counting equipment used in determining thyroidal radioactivity in the goat.

Figure 1



source. A spacer mounted on the tube holder facilitated the duplication of geometry in successive determinations. It was found that maximum count was obtained when the ring at the top of the spacer was placed just behind the thyroid cartilage. This was therefore used as a convenient landmark in positioning the tube for each determination.

C. Thyroxine

The l-thyroxine used was obtained from the Glaxo Laboratories, Greenford, Middlesex, England. This material was further purified by repeated recrystallizations by Dr. E. P. Reineke. Twenty mg. of crystallized thyroxine were weighed on an analytical balance and then dissolved in a small amount of 0.1N NaOH. Enough 0.1N HCl was then added to make the solution slightly cloudy which signifies the point where the monosodium salt of thyroxine is formed. This suspension was then diluted with an appropriate amount of distilled water to obtain the desired concentration. This stock solution was stored in the refrigerator for short periods of time and the solutions for injection were made up from it.

D. Radioactive Iodine

The radioiodine used in these experiments was obtained from the AEC laboratories at Oak Ridge, Tennessee. Fifty microcuries of this carrier-free I-131 was injected subcutaneously in the shoulder region of the experimental animals. To facilitate the determination of the percent of injected dose taken up by the thyroid a standard

containing 1/4 of the injected dose was made up in small glass planchettes. The radioactive iodine contained in this material was stabilized by the addition of 0.2 cc of a casein suspension containing an excess of KI and $NaHSO_3$. The radioactivity contained in this standard was measured each time a determination was made on the animals and at the same geometry. Appropriate corrections for background, physical decay and dilution were made on each of these counts.

E. Thiouracil

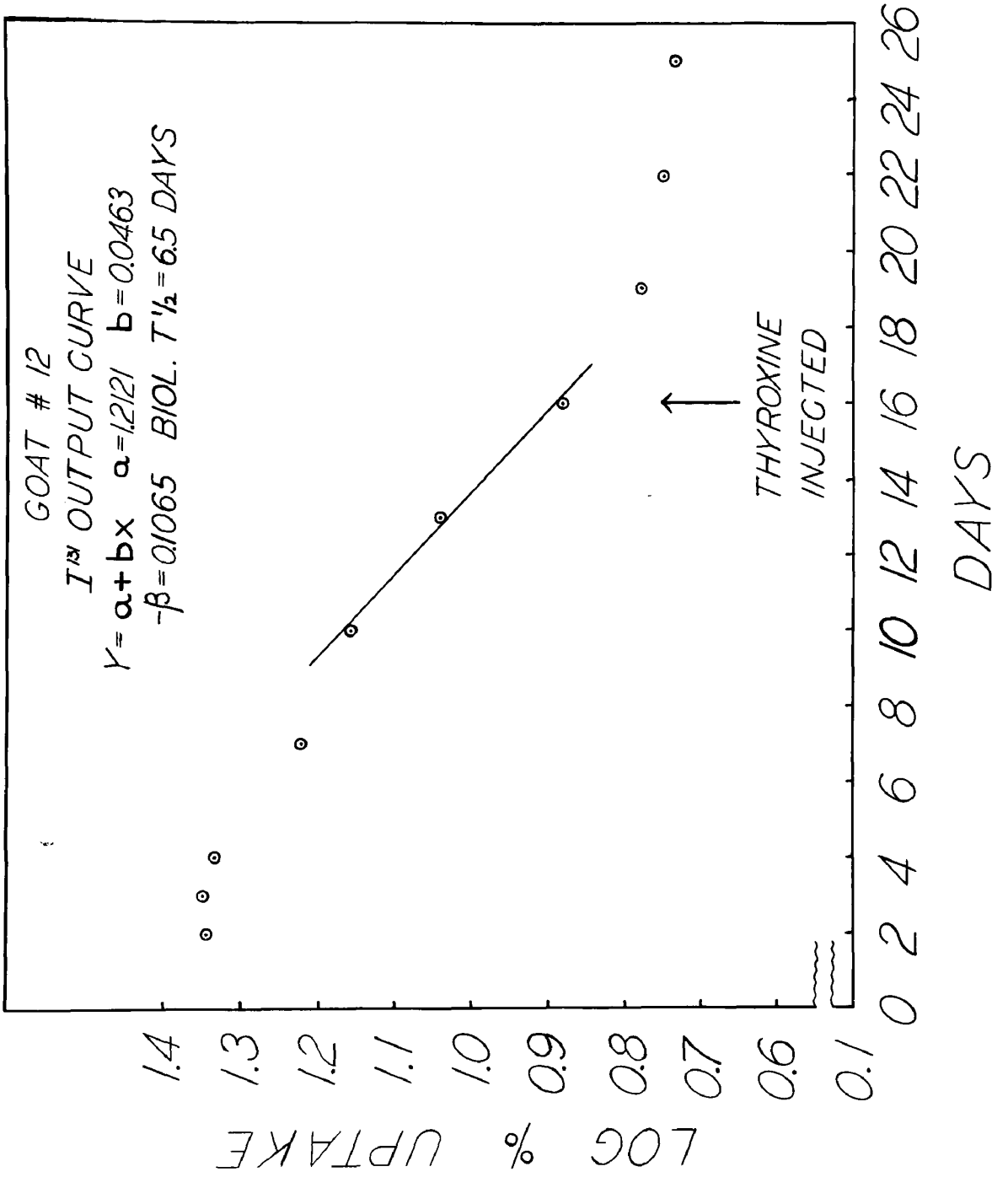
In Experiment 5 a group of five animals was given thiouracil to compare their thyroid secretion rates with those of normal animals. Two days after peak uptake occurred thiouracil treatment was begun. The dose, given by means of capsules, was 0.1 gm. per kilogram body weight twice daily at twelve hour intervals.

F. Method

The application of the method of Henneman, Reineke and Griffin (1955) to goats and the validity of the acquired data is represented by the following two figures. Figure 2 shows an uptake and output curve of I-131 and the influence of thyroxine injections in graded doses on the output portion of the curve. The data are plotted with the log of percent of the injected dose taken up by the gland on the ordinate, and time in days after the injection of 50 uc of I-131 on the abscissa. These data were obtained by making daily counts directly over the thyroid region until maximum uptake

Figure 2. A graphic illustration of thyroidal I-131 uptake and output, and the influence of thyroxine injections in graded doses on the output portion of the curve.

Figure 2


GOAT # 12
I¹³¹ OUTPUT CURVE
Y = a + bx a = 1.2121 b = 0.0463
-β = 0.1065 BIOL. T½ = 6.5 DAYS
THYROXINE INJECTED
LOG % UPTAKE
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.1
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS

had been established. After that, counts were made every third day. These radioactive measurements were corrected for decay and both general and body background. Body background counts were made over the external thigh region. Output half-times were determined from the data obtained from the middle portion of the curve just prior to the start of thyroxine injections. A straight line was fitted to these points by the method of least squares. The latter part of the curve reflects the influence of graded doses of l-thyroxine. When the data contained in this part of the curve are plotted as percent of preceding count on the ordinate against micrograms of l-thyroxine injected daily we obtain a curve as shown in Figure 3. As the dose of l-thyroxine is increased the degree of inhibition of output is also increased so that the percent of previous count becomes greater. By using the prediction equation:

$$Y = a + (bx)$$

or as in this case $$x = \frac{Y - a}{b}$$

where $Y = 100$

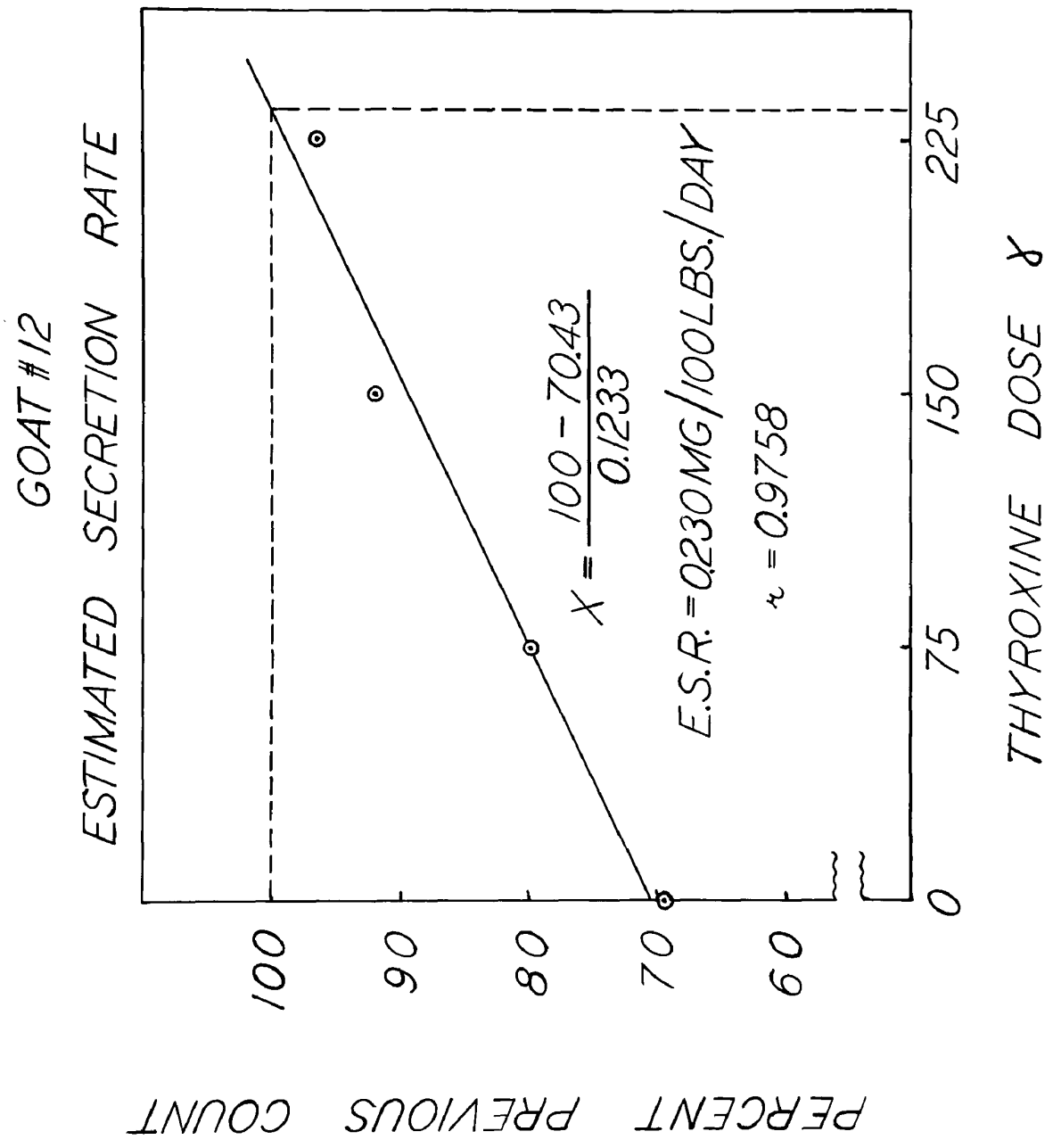
$a = 70.43$

$b = 0.1233$

it is possible to extrapolate to 100 percent previous count. This point represents complete inhibition of output, or that dose of exogenous l-thyroxine which satisfies the animal's requirement for thyroid hormone, and it is presumed that TSH secretion from the pituitary is not stimulated.

Figure 3. Graphic illustration of the dose response relationship between thyroidal I-131 output and thyroxine injections, and the extrapolation of the data in order to obtain the estimated thyroid secretion rate.

Figure 3


GOAT #12
ESTIMATED SECRETION RATE
PERCENT PREVIOUS COUNT
100
90
80
70
60
0
75
150
225
THYROXINE DOSE γ
$X = \frac{100 - 70.43}{0.1233}$
E.S.R. = 0.230 MG/100 LBS./DAY
r = 0.9758

EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1

This experiment was conducted during the month of February to determine whether or not there was a difference in thyroid secretion between young and aged, pregnant and non-pregnant animals. Twenty goats were selected for this study and divided into four groups. Group 1 consisted of 5 aged, pregnant animals having an age range of 4½ to 5½ years. Group 2 consisted of 5 aged, non-pregnant animals with an age range of 3½ to 5½ years. Five 2-year-old pregnant animals were designated as Group 3 and five 2-year-old non-pregnant animals as Group 4.

After the output of I-131 had been established an initial dose of 75 micrograms of l-thyroxine was administered. This dose of exogenous thyroxine was increased every third day by a 75 microgram increment.

Although 20 goats were used in this experiment, the estimated secretion rates were obtained for only 10 animals. Of these, two were aged pregnant and 2 aged non-pregnant; three were young pregnant and three young non-pregnant. Faced with this reduced number it was felt that more indicative results could be obtained if the data from the pregnant animals, both young and old, were placed in one group and compared with the data obtained from all the non-pregnant animals.

The results of this experiment are shown in Tables I and II. The mean estimated secretion rate, expressed as mg./100 lbs./day, for the pregnant animals was found to be 0.262 ± 0.026. For the non-pregnant animals a value of 0.277 ± 0.05 was obtained. When these mean values were subjected to the "Student t" test no significant difference was found to exist.

Once it had been established that under the conditions of this experiment pregnancy had no effect on the estimated secretion rate the data obtained for all the young animals were combined in one group and compared with the data from all the aged animals. These results are shown in Tables III and IV. The mean secretion rate, expressed as mg./100 lbs./day, for the aged animals was found to be 0.187 ± 0.016. For the young animals a value of 0.325 ± 0.019 was obtained. This time the application of the t test revealed a highly significant difference, as shown in Table VI.

Experiment 2

This experiment was conducted during the month of May. The object of this study was to determine the relation between lactation and the thyroid secretion rate.

Twenty goats were again selected for this experiment and divided into 4 groups according to age and whether lactating or non-lactating. All of the lactating animals were approximately 2 months post-partum at the time the thyroid secretion rate was determined, most of them having kidded around the middle of March. These animals were at peak lactation at this time. Group 1 consisted of 4 aged,

TABLE 1. EXPERIMENT 1

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL PREGNANT GOATS

FEBRUARY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
1	Saanen	5½	140	0.175	7.00	4.44	0.9563	4	8.47
8	Nubian	4½	110	0.245	6.77	28.35	0.9489	4	35.27
5 cu	Saanen	2	95	0.304	7.93	48.71	0.918	2	48.71
10	Saan.-Nub.	2	120	0.267	7.93	14.22	0.9964	4	23.20
23	Saanen	2	92	0.321	5.69	6.52	0.9767	4	11.85
Mean				0.262 ± 0.026*	7.06	20.47		3.6	25.5

*Standard Error

TABLE II. EXPERIMENT 1

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL NON-PREGNANT GOATS

FEBRUARY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
2	Saanen	5½	130	0.128	8.37	26.96	0.9381	4	31.06
4	Saanen	3½	120	0.198	6.86	4.25	0.9523	5	7.45
12 cu-	Saanen	2	95	0.407	8.93	29.09	0.9978	4	35.55
17	Saanen	2	97	0.320	5.58	8.81	0.846	4	12.18
22	Saanen	2	100	0.333	7.93	15.74	0.7916	4	21.82
Mean				0 277 ± 0.05*	7.53	16.97		4.2	21.6

*Standard Error

TABLE III. EXPERIMENT 1

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED GOATS

FEBRUARY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
1	Saanen	5½	140	0.175	7.00	4.44	0.9563	4	8.47
2	Saanen	5½	130	0.128	8.37	26.96	0.9381	4	31.06
4	Saanen	3½	120	0.198	6.86	4.25	0.9523	5	7.45
8	Nubian	4½	110	0.245	6.77	28.35	0.9489	4	35.27
Mean				0.187 ± 0.016*	7.25	16.00		4.25	20.56

*Standard Error

TABLE IV. EXPERIMENT 1

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG GOATS

FEBRUARY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
5 cu	Saanen	2	95	0.304	7.93	48.71	0.9180	2	48.71
10	Saanen.-Nub.	2	120	0.267	7.93	14.22	0.9964	4	23.20
12 cu	Saanen	2	95	0.407	8.93	29.09	0.9978	4	35.55
17	Saanen	2	97	0.320	5.58	8.81	0.8460	4	12.18
22	Saanen	2	100	0.333	7.93	15.74	0.7916	4	21.82
23	Saanen	2	92	0.321	5.69	6.52	0.9767	4	11.85
Mean				0.325 ± 0.019*	7.33	20.50		3.67	25.6

*Standard Error

lactating animals ranging from 4 to 6 years of age. Group 2 contained 7 young, lactating animals and were all 2 years of age. Five aged, non-lactating, 5- to 6-year-old animals formed Group 3 and Group 4 was composed of four 2-year-old, non-lactating animals.

The initial dose of l-thyroxine administered was 75 micrograms, this being increased by a 75 microgram increment every third day.

The animals were no longer kept inside the barn at this time but were left out to pasture. The mean temperature for the duration of this particular experiment as recorded by the U. S. Weather Bureau in East Lansing was 57° F. with a standard deviation of $\pm$ 8.4°.

The data obtained for each individual animal in this experiment are shown in Tables A through D in the Appendix. The mean secretion rate of 0.188 $\pm$ 0.053 for the aged lactating goats was not significantly different (Table VII) from the mean secretion rate of 0.263 $\pm$ 0.050 obtained for the young lactating animals. Similarly, no significant difference was found between the mean secretion rates of lactating and non-lactating goats when animals of similar age groupings were compared. However, the non-lactating animals do show a higher average rate of secretion. The values obtained for the aged and young, non-lactating animals respectively were 0.199 $\pm$ 0.024 and 0.278 $\pm$ 0.036. The difference between these two means is significant at the 10 percent level.

Experiment 3

This study was conducted during the month of July to obtain more information concerning thyroid secretion rates in lactating animals as compared to non-lactating animals as well as obtaining data with regard to summer temperatures. The same goats that had been used in Experiment 2 were used in this study and were again placed in the same groups. The animals were at this time in the declining phase of lactation.

The mean temperature during the time of this experiment was 71° F. $\pm$ 4.2°. Because of the decrease in thyroid function that was expected at this time of the year the initial dose of l-thyroxine was reduced from 75 micrograms to 25 micrograms. This injected dose was increased every third day by an increment of 25 micrograms. It was soon found, however, that in most of the non-lactating animals this amount of thyroxine was too small to have any effect on the inhibition of thyroidal I-131 output. For this reason the administration of thyroxine was stopped for a period of three days in the animals showing no response. A new series of injection doses was then begun starting with 50 micrograms and increasing by 50 microgram increments.

The results of this study for each goat will be found in Tables E through H in the Appendix. With regard to the lactating animals, a relationship similar to that found in Experiment 2 appears to exist between the thyroid secretion rates obtained at this time for the young and aged lactators. As shown in Table VII, no

significant difference was found when the mean secretion rate of 0.05 ± 0.016 for the aged lactating goats was compared with the value of 0.07 ± 0.0074 obtained for the young lactators. However, the seasonal decline for both groups was significant.

In contrast to the previous experiment a significant difference was found at this time between the mean secretion rates of lactating and non-lactating goats when animals of similar age groupings were compared (Table VII). The values obtained for the aged and young non-lactating animals respectively were 0.099 ± 0.02 and 0.177 ± 0.013. As shown in Table VI, these values are significantly different. The seasonal decline for both of these groups, as shown by a comparison with the values obtained in Experiment 2, was also significant.

Experiment 4

In view of the unexpected results that were obtained in the two previous experiments with lactating animals it was decided that another study should be made. The same animals were again divided into the same groups and the experimental procedure again repeated during the month of August. Lactation in some of the goats had by this time almost stopped. The mean temperature for the duration of this experiment was 70.1° F. ± 5.1°.

Since it had been found in the previous study that the amount of thyroxine administered to some of the animals was not adequate, the dose was therefore increased to 30 ug for the lactating animals and to 60 ug for the non-lactators. This amount was

increased every third day by an increment of 30 and 60 ug, respectively. In spite of the increased amount of injected thyroxine, four of the young lactators showed no response to the hormone as judged by thyroidal I-131 output. The treatment was again stopped in these animals for a period of three days and a new series was started at the level of 40 ug and increased every third day by an equal amount. In this way a good response was achieved and successful results were obtained.

There were also included in this study 5 immature goats which had been born the previous spring. Earlier attempts at measuring the secretion rate of these kids had not met with much success. Because of their small stature in proportion to the apparatus that was used for holding the goats in place it proved difficult to get a true measure of radiation over the thyroid region during successive determinations. However, by adding a few new adjustments and along with their increase in size it was possible at this time to determine thyroid secretion rates in these animals. The thyroxine dose given was 30 ug and was increased by this amount every third day.

The results of this experiment are shown in Tables J through N in the Appendix. Again, similar to the previous two experiments, no significant difference was found between the thyroid secretion rates of the young and aged lactating animals. These mean values were respectively 0.177 ± 0.04 and 0.121 ± 0.04. In contrast to the previous experiment no significant difference was found between the mean secretion rates of lactating and non-lactating goats when

animals of similar age groupings were compared. However, the lactating animals again showed a higher average rate of secretion. The values obtained for the aged and young, non-lactating animals respectively were 0.197 $\pm$ 0.33 and 0.213 $\pm$ 0.0168. As may be noted in Table VI the aged animals show a significant seasonal increase. No significant difference appears to exist between the average secretion rates of these young and aged animals at this time. The value of 0.262 $\pm$ 0.051 obtained for the immature goats was not significantly different from the average secretion rate obtained for the young, non-lactating animals.

Experiment 5

The object of this investigation which was carried out during the month of October was threefold:

1. To obtain data on animals of various ages during fall weather.
2. To determine if any effect on thyroid activity existed during the period of estrus.
3. To determine whether or not thiouracil treatment had an effect on thyroid secretion rate values.

To accomplish this, 24 goats were divided into 4 groups. Eight animals ranging in age from 3 to 6 years were designated as Group 1. Group 2 consisted of six 2½-year-old goats and Group 3 contained five 2½-year-old thiouracil-treated animals. Group 4 was made up of 5 immature, 8-month-old animals. All mature animals were showing the usual signs of estrual behavior at this time.

The recorded temperature during this experimental period was 50.15° F. ± 6.6°. The initial dose of thyroxine administered to the mature animals was 75 ug. This initial amount was increased by a 75 ug increment every third day. The immature animals were given a 40 ug dose and this was increased every third day by 40 ug.

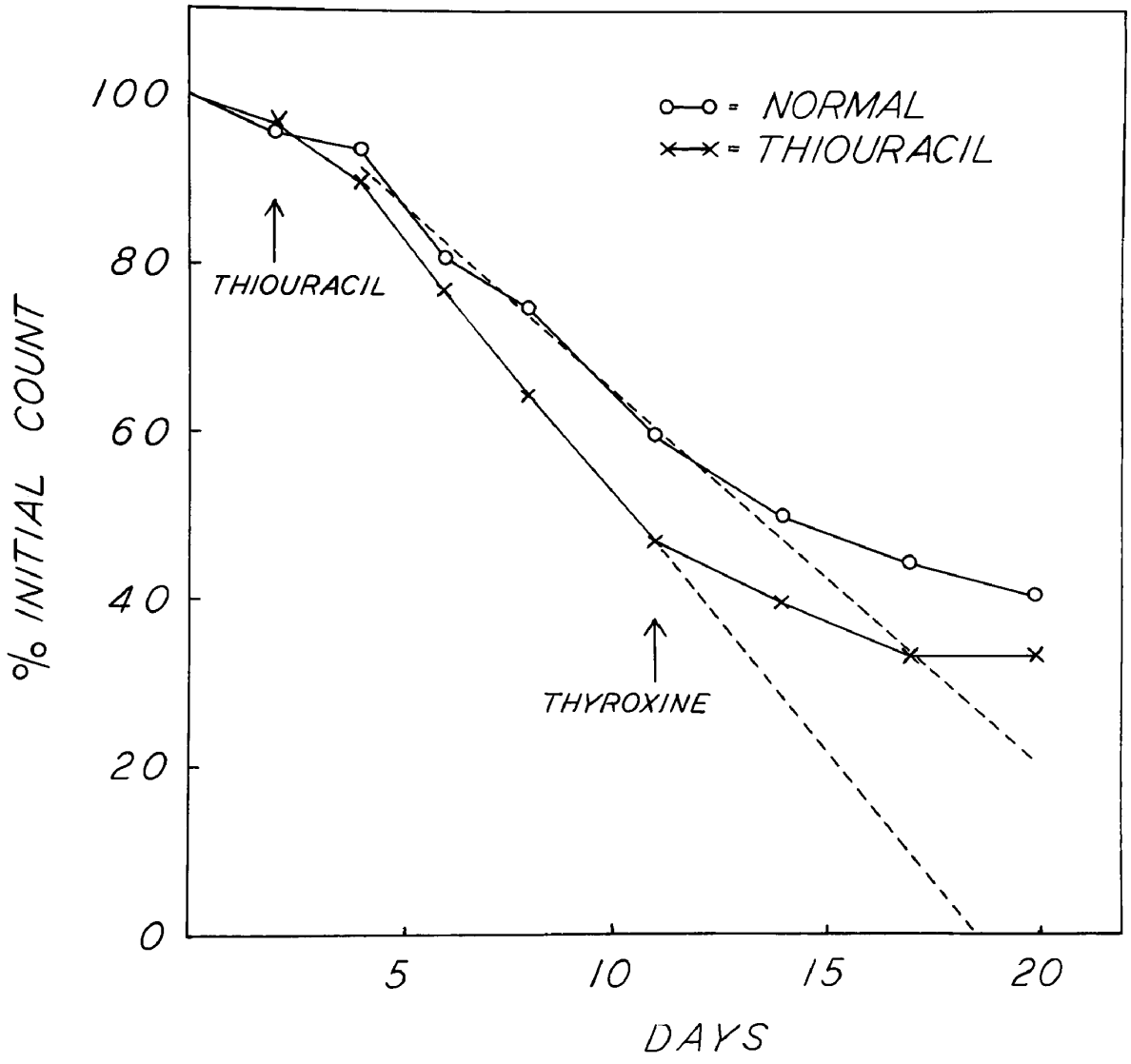
Although 24 goats were used in this study, secretion rates could be determined on only 22 animals. One aged animal died during the experiment and was found to be heavily parasitized with stomach worms. Another animal in the thiouracil-treated group also died during the experiment. However, no autopsy report was received on this animal and cause of death was not established.

Figure 4 shows output curves for the two groups of 2-year-old animals, one group normal and the other thiouracil-treated. The graph is defined by percent of initial count on the ordinate and time in days on the abscissa. Peak uptake was taken as initial count. The top curve represents an average percent initial count of 6 animals. The bottom line is an average of 4 animals. Two days after peak uptake had occurred thiouracil treatment was begun. The dose given was 0.1 gram per kilogram body weight twice daily at 12 hour intervals. The output half-time for the normal is 11.07 days and for the thiouracil group 7.2 days. Eleven days after peak uptake thyroxine injections were begun and the thyroid secretion rate was determined.

The results of this experiment as shown in Tables O through R in the Appendix reveal that a significant difference exists under the conditions of this experiment between the mean secretion rate

Figure 4. Graphic illustration of thyroidal I-131 uptake and output for both normal and thiouracilized animals, and the effect of thyroxine injections in graded doses on the output portion of these curves.

Figure 4


100
80
60
40
20
0
% INITIAL COUNT
5
10
15
20
DAYS
o—o = NORMAL
×—× = THIOURACIL
THIOURACIL
THYROXINE

of the aged animals (0 243 ± 0.03) when compared to the young and immature goats. No significant difference was found between the values determined for the young (0.336 ± 0.018) and immature (0.403 ± 0.037) animals. When the mean secretion rate of 0.379 + 0.04 mg./100 lbs./day obtained for the young, thiouracilized goats was compared to that calculated for the young, normal animals no significant difference was found. This particular part of the experiment will be discussed in greater detail in a later section.

Although all mature animals were showing the usual signs of estrual behavior during this experimental period there appeared to be no significant difference between thyroid activity at this time when compared to thyroid secretion rates obtained during February.

In order to summarize the data obtained from all five experiments the mean values for secretion rates, output half-times, peak and 48 hour uptake for the various groups of animals and the respective time of year are recorded in Table V. Figures 5 and 6 also give the same data in graphic form. The mean t½ values for all normal open goats for the entire year ranged from a low of 6.70 days recorded for the young goats during the month of May to a high of 12.55 days for the aged animals during the month of July. Upon closer examination of the data it may be seen that during February and May both aged and young groups of goats showed a much faster output rate as compared to July, August and October. During February and May these values ranged from 6.70 to 7.33 days; for July, August and October the range for these output half-time values was 10.35 days recorded for the aged animals in August to 12.55 days for the aged animals in July.

Figure 5. A graphic summary comparing the mean thyroid secretion rate data obtained from all five experiments for the various groups of non-lactating animals at various times of the year.

Figure 5

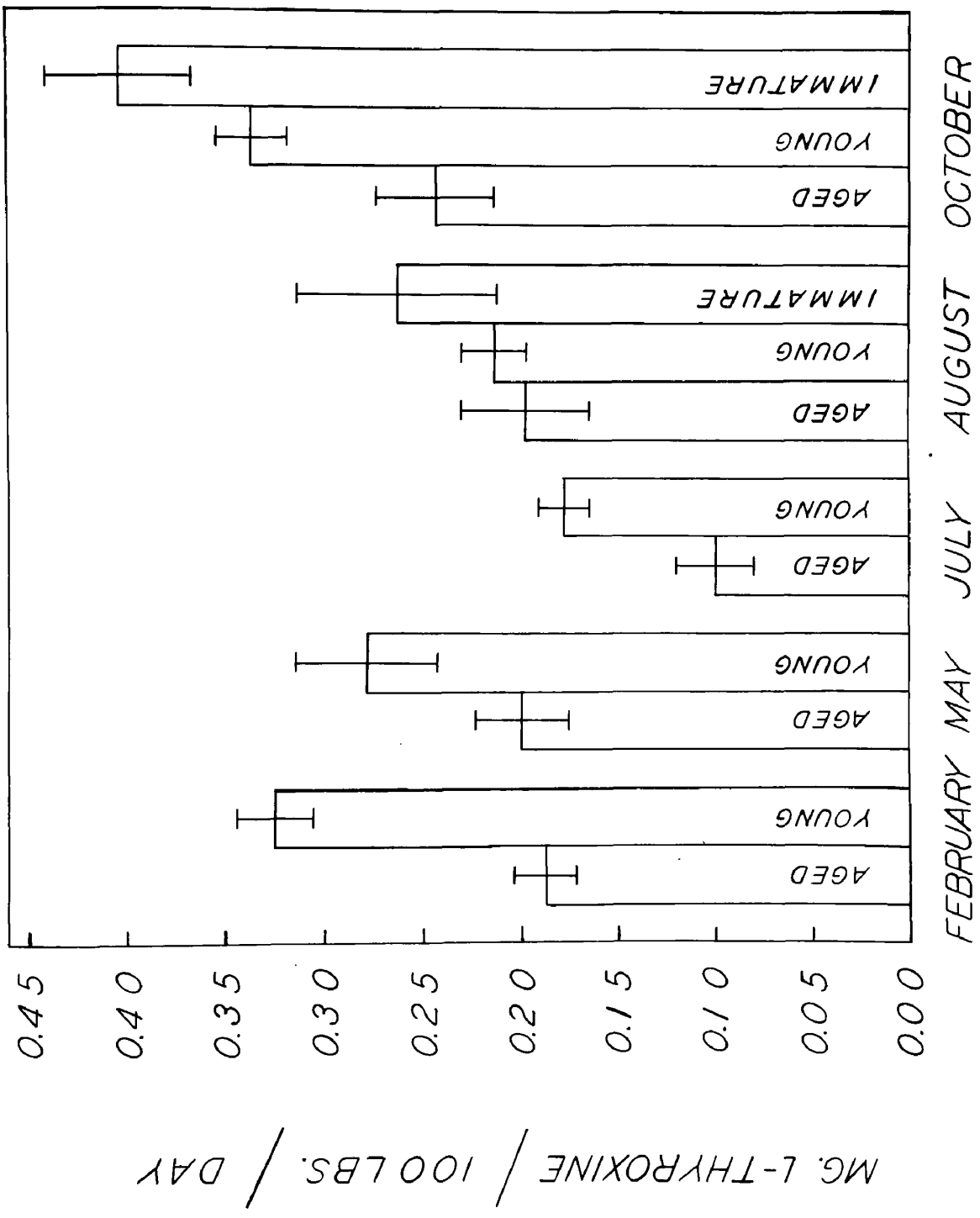
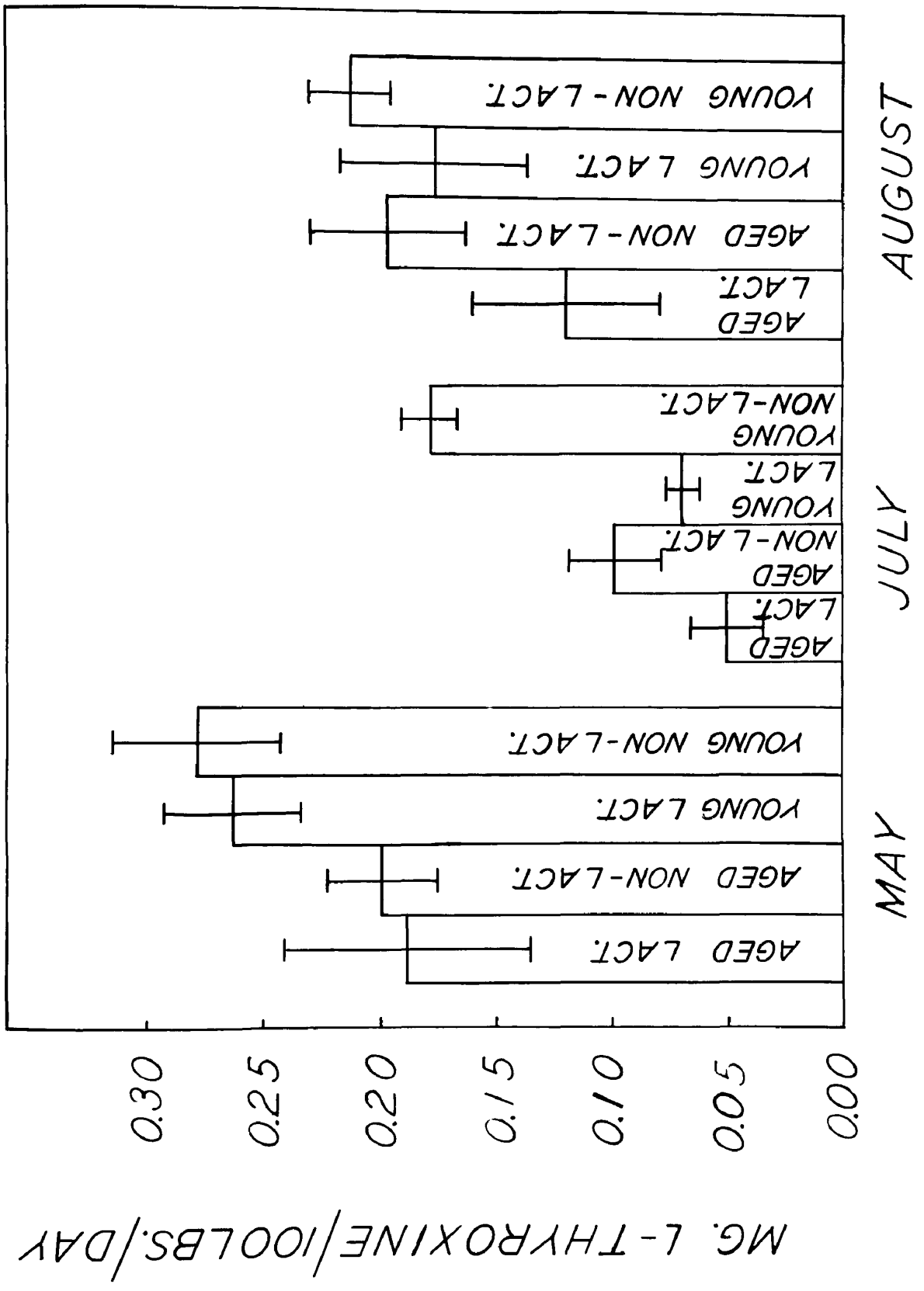

MG. L-THYROXINE / 100 LBS. / DAY
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
FEBRUARY
MAY
JULY
AUGUST
OCTOBER
YOUNG
AGED
IMMATURE

Figure 6. A graphic summary comparing the mean thyroid secretion rate data obtained at various times of the year for different age groups of lactating and non-lactating animals.

Figure 6


MG. L-THYROXINE/100LBS./DAY
0.00
0.05
0.10
0.15
0.20
0.25
0.30
MAY
AGED LACT.
AGED NON-LACT.
YOUNG LACT.
YOUNG NON-LACT.
JULY
AGED LACT.
AGED NON-LACT.
YOUNG LACT.
YOUNG NON-LACT.
AUGUST
AGED LACT.
AGED NON-LACT.
YOUNG LACT.
YOUNG NON-LACT.

With regard to both 48 hour and peak thyroidal iodine uptake studies, the greatest average amount taken up by the open goats occurred in May. The mean 48 hour uptake recorded for aged and young goats during this time was 31.9 and 32.40 percent of the injected dose, respectively. The peak uptake values during May were 44.06 for the aged and 35.68 percent of the injected dose recorded for the young animals. The lowest average iodine uptake values were observed during the colder weather. In October the average amount taken up in 48 hours for the aged goats was 11.40 percent of the injected dose, whereas in the young animals this value was 9.55 percent. Average peak iodine uptake for October was 15.14 and 14.80 percent of the injected dose for the aged and young goats, respectively. These October values were the lowest recorded for the entire year. In February the mean percent of injected dose taken up in 48 hours by the thyroids of aged and young goats respectively was 16.00 and 20.50 Peak uptake values during this same experimental period were 20.56 and 25.60 for the aged and young goats respectively. In contrast to the open animals the lactating goats took up smaller amounts of iodine. Forty-eight hour uptake studies reveal a range in values from a low of 6.90 percent of injected dose occurring in the young lactators during August to a high of 15.20 found in the young animals during May. With regard to the influence of season on iodine uptake in lactating animals a trend somewhat different is noted when these animals are compared to the open goats. In both cases the highest mean values were recorded in May. The average peak amounts taken up by the lactators

during May was 15.47 and 16.70 percent of the injected dose for the aged and lactating goats, respectively. However, the lowest peak uptake values were observed during July. For the aged animals this mean value was found to be 9.58; for the young animals 10.41 percent of the injected dose was taken up by the thyroid. In August again there was an increased amount taken up as indicated by the values of 14.05 and 10.53 for the aged and young animals, respectively. The reason for the lower values occurring during the summer is believed to be due to a loss of I-131 by way of the mammary gland. This aspect will be discussed in more detail in a later section.

Output rates for the lactating animals were similar to those observed in the open goats. The greatest output rate occurred in May. The mean t½ values recorded during this month for the aged and young goats respectively was 7.59 and 8.96 days. During July and August higher values were observed, as indicated by the range of 13.40 days output half-time recorded for the young lactators during August to a low of 10.05 days for the young lactating goats during July.

The significance of these findings pertaining to thyroidal I-131 uptake and output for both young lactators and non-lactating animals, as well as aged lactating and open goats, will be discussed further in a later section.

For the purpose of comparing the mean thyroid secretion rate data, Tables VI and VII have the test statistic, or student t

value, and degrees of freedom recorded at the intersection of the appropriate comparison columns.

In order to study the relationship between thyroid secretion rate and uptake as well as secretion rate and output of radioactive iodine, correlation coefficients for these respective values were calculated. These data may be found in Table VIII. As may be noted in these tabulations, when the data concerning 48 hour uptake and secretion rate for the entire year are compared, a correlation coefficient value of 0.067 is obtained. This reveals no significant correlation. Similarly, with regard to a comparison of output and secretion rate during the entire year, the correlation coefficient value of 0.1685 revealed no significant correlation. When these data are broken down into sub-groups according to months again no correlation was observed, except in the experiment during August when an r value of 0.53 (P less than 0.01) was found for 48 hour uptake versus secretion rate. Similarly, in comparing output half-time with secretion rate no significant correlation was observed, except during the month of May. At this time an r value of 0.56 (P less than 0.01) was found. However, it has been pointed out by Montoye (1955) that the possibility of one significant value out of several which are not significant may be due purely to chance. In this regard, Wilkinson (1951) has published binomial expansion tables whereby it may be ascertained just what the probability is of obtaining, in a given number of statistical computations, a significant correlation which is due entirely to chance. In the case of the present data, one significant correlation

(P less than 0.01) out of six was observed. According to Wilkinson's findings the probability of this happening simply by chance is 5.8 percent.

TABLE V

A SUMMARY OF THE MEAN VALUES OBTAINED FOR OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE, PEAK PERCENT UPTAKE AND THYROID SECRETION RATES FOR ALL GROUPS AT VARIOUS TIMES OF THE YEAR

		Aged	Young	Young Thiouracil	Immature
Feb.	T½	7.25	7.33	-	-
	48 Hr. Uptake	16.00	20.50	-	-
	Peak Uptake	20.56	25.60	-	-
	Sec. Rate	0.187 ± 0.016	0.325 ± 0.019	-	-
May	T½	7 27	6.70	-	-
	48 Hr. Uptake	31.90	32.40	-	-
	Peak Uptake	44.06	35.68	-	-
	Sec. Rate	0.199 ± 0.024	0.278 ± 0.036	-	-
July	T½	12.55	10.69	-	-
	48 Hr. Uptake	34.60	28.73	-	-
	Peak Uptake	36.20	31.00	-	-
	Sec. Rate	0.099 ± 0.024	0.177 ± 0.013	-	-
Aug.	T½	10.35	11.40	-	8.58
	48 Hr. Uptake	18.90	13.60	-	20.30
	Peak Uptake	27.58	19.88	-	24.16
	Sec. Rate	0.197 ± 0.033	0.213 ± 0.017	-	0.262 ± 0.051
Sept.	T½	10.70	11.07	7.20	10.32
	48 Hr. Uptake	11.40	9.55	6.70	16.20
	Peak Uptake	15.14	14.80	10.38	18.13
	Sec. Rate	0.243 ± 0.03	0.336 ± 0.018	0.379 ± 0.042	0.403 ± 0.037

		Aged Lactating	Young Lactating	Pregnant	Non-Pregnant
Feb.	T½	-	-	7.06	7.53
	48 Hr. Uptake	-	-	20.47	16.97
	Peak Uptake	-	-	25.50	21.60
	Sec. Rate	-	-	0.262 ± 0.026	0.277 ± 0.05
May	T½	7.59	8.96	-	-
	48 Hr. Uptake	14.00	15.20	-	-
	Peak Uptake	15.47	16.70	-	-
	Sec. Rate	0.188 ± 0.053	0.263 ± 0.030	-	-
July	T½	10.43	10.05	-	-
	48 Hr. Uptake	7.70	8.16	-	-
	Peak Uptake	9.58	10.41	-	-
	Sec. Rate	0.050 ± 0.016	0.070 ± 0.0074	-	-
Aug.	T½	10.33	13.40	-	-
	48 Hr. Uptake	9.50	6.90	-	-
	Peak Uptake	14.05	10.53	-	-
	Sec. Rate	0.121 ± 0.040	0.177 ± 0.040	-	-
Oct.	T½	-	-	-	-
	48 Hr. Uptake	-	-	-	-
	Peak Uptake	-	-	-	-
	Sec. Rate	-	-	-	-

TABLE VI

THE t VALUES AND DEGREES OF FREEDOM OBTAINED WHEN MEAN SECRETION RATES OF VARIOUS GROUPS OF ANIMALS ARE COMPARED WITH REGARD TO SEASON AND AGE

		FEBRUARY				MAY			
		Young		Aged		Young		Aged	
		t	D.F.	t	D.F.	t	D.F.	t	D.F.
Feb.	Young	-	-	**** 13.50	9	1.15	10	-	-
	Aged	**** 13.50	9	-	-	-	-	0.420	9
May	Young	1.15	10	-	-	-	-	* 1.8	9
	Aged	-	-	0.42	9	* 1.8	9	-	-
July	Young	**** 6.43	10	-	-	** 2.64	8	-	-
	Aged	-	-	**** 3.44	9	-	-	**** 3.20	10
Aug.	Young	**** 4.42	10	-	-	1.63	8	-	-
	Aged	-	-	-	-	-	-	0.049	10
Oct.	Young	0.42	12	-	-	1.44	10	-	-
	Aged	-	-	1.65	12	-	-	1.14	13

**** Probability 0.01
*** " 0.02
** " 0.05
* " 0.10

TABLE VI -- Continued

JULY				AUGUST				OCTOBER			
Young		Aged		Young		Aged		Young		Aged	
t	D.F.	t	D.F.	t	D.F.	t	D.F.	t	D.F.	t	D.F.
**** 6.43	10	-	-	**** 4.42	10	-	-	0.42	12	-	-
-	-	**** 3.44	9	-	-	-	-	-	-	1.65	12
** 2.64	8	-	-	1.63	8	-	-	1.44	10	-	-
-	-	**** 3.20	10	-	-	0.049	10	-	-	1.14	13
-	-	**** 3.78	9	1.69	8	-	-	**** 7.16	10	-	-
**** 3.78	9	-	-	-	-	** 2.54	10	-	-	**** 4.00	13
1.69	8	-	-	-	-	0.433	9	**** 4.97	10	-	-
-	-	** 2.54	10	0.433	9	-	-	-	-	1.03	13
**** 7.16	10	-	-	**** 4.97	10	-	-	-	-	*** 2.66	14
-	-	**** 4.00	13	-	-	1.03	13	*** 2.66	14	-	-

TABLE VII

THE t VALUES AND DEGREES OF FREEDOM OBTAINED WHEN MEAN SECRETION RATES OF VARIOUS GROUPS OF ANIMALS ARE COMPARED WITH REGARD TO AGE, SEASON AND LACTATION

LACTATING

			May				July				August			
			Young		Aged		Young		Aged		Young		Aged	
			t	D.F.	t	D.F.	t	D.F.	t	D.F.	t	D.F.	t	D.F.
Lactating	May	Young	-	-	1.214	10	6.246^{****}	12	-	-	1.72	13	-	-
		Aged	1.214	10	-	-	-	-	2.49^{**}	6	-	-	1.009	7
	July	Young	6.246^{****}	12	-	-	-	-	1.135	8	2.63^{**}	12	-	-
		Aged	-	-	2.49^{**}	6	1.135	8	-	-	-	-	1.648	6
	Aug.	Young	1.72	13	-	-	2.63^{**}	12	-	-	-	-	0.990	10
		Aged	-	-	1.009	7	-	-	1.648	6	0.990	10	-	-
Non-Lactating	May	Young	0.320	10	-	-								
		Aged	-	-	0.189	8								
	July	Young					7.153^{****}	9	-	-				
		Aged					-	-	1.913^{*}	7				
	Aug.	Young									0.830	10	-	-
		Aged									-	-	1.466	8

**** Probability 0.01
*** " 0.02
** " 0.05
* " 0.10

TABLE VIII

THE VALUES OBTAINED FOR COEFFICIENTS OF CORRELATION (r) WHEN 48 HOUR PERCENT UPTAKE AND OUTPUT HALF-TIME IS COMPARED WITH THYROID SECRETION RATE

	February		May		July		August		October		Entire Year	
	r	D.F.*	r	D.F.	r	D.F.	r	D.F.	r	D.F.	r	D.F.
Uptake vs. Secretion Rate	0.1939	9	0.0143	18	0.3670	17	0.5373**	24	0.3262	17	0.067	89
Output vs. Secretion Rate	0.0487	9	0.5624**	19	0.2090	17	0.2865	24	0.0285	17	0.1685	90

* Degrees of freedom

** Probability 0.01

GENERAL DISCUSSION

As pointed out previously the method employed in this study has the advantage of allowing the quantitative measurement of thyroid secretion rate in individual intact animals. Since it is not necessary to slaughter or in any way change the animal physiologically it is therefore possible to follow thyroid activity in the same animals over a long period of time. A further point in favor of this method is its relative ease of technique along with a minimum of animal manipulation. However, there are certain precautions which should be kept in mind when obtaining data by this method. In the present study a dose of l-thyroxine equal to approximately 1/3 to 1/4 of the animal's daily secretion rate had to be administered initially and increased by that increment every third day. For this reason, the usual time required for actual measurement of secretion rate was either 9 or 12 days. It was felt that during this experimental time a great deal of change in thyroid activity could possibly occur. Since this method is one which involves substitution its degree of accuracy is dependent upon a minimum of variation in thyroid secretion during the experimental period. As a hypothetical example, if the first two points on the response curve were obtained during a period of low thyroid activity, these points representing percent of previous count would be high. Likewise, if the last two points were taken during a time of high activity, the percent of previous count would be relatively lower than would be

expected had the secretion rate remained constant. As a result of this the degree of slope for the curve would be lessened to such an extent that an abnormally high secretion rate would be calculated which would not at all reflect the true thyroid activity. However, in view of the good agreement between the data obtained in the present study, as well as the data of previous investigators using this method, when compared with that obtained by other techniques it appears that the hypothetical example cited above is a relatively rare circumstance. The more probable situation which exists is that the variations in activity are of short duration and are such that periods of high activity are compensated by periods of low activity so that a radioactive measurement taken every third day represents an average between these various changes.

It should also be mentioned that a certain amount of difficulty may be encountered in obtaining a good correlation for these dose response curves as a result of variable activity during the experimental period. As an example of this, if thyroid activity should be higher than normal as a result of an untoward environmental change, or some such variable factor, then the percent of previous count based on I-131 output from the gland would be low. The next value may be based on a return to normal I-131 output but, because the percent of preceding count is contingent upon the previous low value obtained, This subsequent value will be represented by a point which is abnormally high on the curve. The problem is further compounded by its inherent perpetuation resulting in alternately low and high points throughout the rest of the curve. A

straight line fitted to such a curve would lie along the average between the high and low extremes and give a good estimate of secretion rate but the variation about the regression line would be large. This same type of curve may also result from improper technique on the part of the investigator when measuring the amount of radioactivity over the thyroid. Because of this, positioning of the scintillation counter and the duplication of geometry during successive determinations is of paramount importance. In the present study very good correlations were obtained.

A further point in regard to the technique concerns the theory upon which it is based. The decline in thyroidal radioactivity depends upon the loss of I-131 from the gland as hormonal iodine (Lerman, 1940; Taurog, Wheat and Chaikoff, 1945; Perlman, et al., 1941; Perry, 1951). The rate of hormone secretion, and therefore loss of I-131, depends upon the amount of pituitary TSH in the blood stream (Taurog, Chaikoff, and Bennett, 1946; Astwood, 1949). TSH release from the pituitary, in turn, depends upon the plasma concentration of thyroid hormone (Uotilla, 1940; McGavick, 1951). Therefore, since the administration of exogenous hormone results in a greater concentration of thyroid hormone in the plasma, an increase in inhibition of TSH release would result from such a procedure. The degree of inhibition would be proportional to the amount of hormone in the blood from the exogenous source plus the amount from the glandular source minus the amount metabolized by body tissue. These relationships may be theoretically pictured mathematically as:

$$A = B + C - D \qquad (1)$$

where A = plasma concentration of thyroxine

B = amount of exogenous hormone administered

C = amount of hormone released by the gland

D = amount of hormone metabolized and lost by excretion

and, since C is proportional to the amount of pituitary TSH in the blood, it may be stated that

$$C = Ke \qquad (2)$$

where e = amount of TSH in the blood stream

K = constant representing the proportional relationship between amount of hormone released by the gland and blood TSH levels;

and further, e is inversely proportional to the amount of thyroid hormone in the blood. This may be represented by the equation

$$A = \frac{1}{e} k \qquad (3)$$

where k = constant representing the proportional relationship between blood thyroxine and blood TSH levels.

With these equations in mind, it follows that as A is increased by an amount of B of exogenous hormone, the value "e" for the blood TSH level is proportionately decreased (3); and as "e" is decreased, the amount of blood thyroxine C is also proportionately decreased (2). Therefore, the smaller the value for C, the smaller need be the amount B required to decrease it. It is on this basis that the unknown amount C can be measured simply by substituting for it the known amount B. The point of complete substitution can be determined

on the basis of no loss of I-131 from the gland, as indicated by the measure of radioactivity in the thyroid gland. In actual practice this point is arrived at by extrapolating the data obtained from administering several graded doses of thyroxine. For each of these graded doses equal to B in our equation an amount C is still being secreted by the gland. In effect, what is being measured by this method is the metabolic demands of the body on the thyroid. In view of the foregoing equations an alternate method of calculating the secretion rate might be arrived at. This would involve a slight modification in the procedure so that only one injected dose level would be required. The percent decrease in the value of C as a result of this injected dose would equal the percent of the secretion rate injected. The percent decrease in C would equal the difference in percent output as determined before and after thyroxine treatment. In other words, the secretion rate is calculated by multiplying the injected dose level by the quotient arrived at as a result of dividing the percent output before thyroxine treatment by the difference in percent output before and after treatment. This treatment is of course theoretical and would require substantiation by experimental trial.

As may be noted in the tabulation of results, the peak amount of radioactive iodine taken up by the thyroid, as well as 48 hour uptake, reveals a great deal of variation within and between experiments. Although no conclusions as to the causes of these differences can be drawn from this study the major factors involved appear to be season and whether the animals were lactating or not.

The data obtained in these experiments indicate the highest peak uptake occurred during the late Spring and Summer months and involved the non-lactating animals. Of these, the aged non-lactators had the greatest uptake in all three of the Summer experiments.

With regard to the comparison of iodine uptake in lactating and non-lactating animals, the results can be explained on the basis of the permeability of the mammary epithelium to iodide. Because of this iodine permeability, the size of the blood iodide pool is reduced considerably in these animals by way of secretion into the milk. Therefore, the thyroid of the lactating animal does not have access to as much iodine as the non-lactator and consequently not as much is taken up.

The findings regarding seasonal variations in thyroidal iodine concentrations are somewhat similar to those reported in cattle by Seidell and Fenger (1913) and Lodge (1957). These investigators noted that the highest iodine content of cattle thyroids was found in the later Summer and the minimum iodine content in the late Winter and Spring. In this last respect the data obtained in goats show a marked difference. Lodge found that the minimum iodine uptake occurred in Spring (April - June), whereas in the present study the greatest amount of iodine was found to be taken up by the goat thyroid during the month of May. Although it may be stated that in general both goat and cattle thyroids take up more iodine in Summer than in Winter, under the conditions of these experiments, except during the month of August, there is no correlation between

iodine uptake and secretion rate in the goat, as may be noted in Table VIII.

In view of the widespread use of thyroidal I-131 uptake as an indication of thyroid activity, it is believed that these data are quite pertinent. The use of such uptake studies as a diagnostic tool is based on the assumption that the more active gland retains the most iodine. The data herein reported, as well as those obtained by the previously mentioned investigators, seems to indicate just the opposite to be true. Lodge (1957) points out that such data suggests "that during the Summer, when the actual secretion is low, a large amount of iodine is collected by the gland and stored for future use." Likewise, during the Winter months when secretion rate is high the lower concentration of radioactive iodine measured in the gland represents the amount taken up minus the amount of hormonal iodine secreted. In other words, thyroidal iodine equals iodine uptake minus iodine output. Therefore, if thyroidal iodine were to remain constant, output would equal uptake and a decrease in thyroidal iodine would indicate an output greater than uptake. Likewise, an increase in storage of thyroidal iodine would be the result of an uptake greater than output. This is in contrast to the so-called "steady state", as reported by Dougherty, et al. (1951). Evidence obtained by these workers tends to show that an increase in secretion rate is compensated for by an increase in iodine uptake and, conversely, a decrease in secretion rate is accompanied by a decrease in iodine being taken up by the thyroid. As mentioned before, the results obtained under the conditions of the present experiments

seem to favor the theory that the amount of thyroidal iodine is equal to the algebraic sum of uptake and output. In either case the measurable perameter of thyroidal iodine at any given time is dependent upon two variables, namely uptake and output. Uptake in turn may be influenced by any circumstance which will alter the blood iodide pool and this may account for the large variations found in individual animals. An example of this is the lactating goat which has already been discussed. Other cases may be individual differences in enterohepatic circulation and renal excretion. Exogenous iodine from known and unknown sources would also be expected to affect the blood iodide pool. Considering these factors, the large amount of variation in uptake within and between experiments and its lack of correlation with the measured secretion rate is not too surprising.

In reviewing the data concerning the thyroidal output half-time of iodine it will be noted that the greatest rate of release occurred in the Winter and Spring. The values obtained for Summer and Fall for all groups of animals, except the immature, fall within a range of 10.05 to 13.4. This difference between Winter and Summer is to be expected since the thyroid is undergoing greater activity during the Winter and therefore releasing a greater amount of hormonal iodine. However, if this explanation is accepted there are certain incongruities to be noted in the Summer and Fall data. Although the thyroid secretion rate is significantly greater in the Fall than in the Summer the output half-time values recorded for both of these periods are approximately the same. Likewise, the

secretion rate values for Fall and Winter are not significantly different, although a great deal of difference is found between the output half-times for these two periods. This situation not only exists between various times of the year but also within experiments as well. This may be noted in Table VIII. The reason for this lack of correlation, except for the one instance during the month of May, between thyroid secretion rate and output cannot be fully explained on the basis of the present findings but it is strongly felt that more indicative results could be obtained if more controlled environmental conditions as well as animals of more uniform breed were to be used. A further point to be discussed in regard to this question, however, concerns the recycling of iodine and the variability of iodine intake. Directly bearing on this subject is the experiment involving the thiouracilized goats. A comparison between the output half-times of the thiouracilized and non-thiouracil treated animals reveals a difference of approximately the same magnitude as found between Winter and Summer $t_{1/2}$ values. Nevertheless, no significant difference was found between the mean secretion rates recorded for these two groups. As may be noted in Tables P and Q in the Appendix, the mean secretion rate for the normal, young goats is 0.336 ± 0.018. For the thiouracil treated animals the value is 0.379 ± 0.042. A secretion rate of 0.502, which is abnormally high, was calculated for goat number 16 of the thiouracilized group. It should be mentioned here that during the last part of the experiment this particular animal appeared to be quite ill and died one week after the termination of the experiment.

When the data obtained from this goat is eliminated the mean secretion rate for the thiouracilized group is 0.338 $\pm$ 0.015.

The difference in t½ values between normal and thiouracil treated animals, even though their average secretion rates were not different, could be attributed to two causes: 1) In the case of the non-thiouracilized animals, a certain amount of recycling of I-131 as well as I-127 would be expected to take place. This would be reflected in a slower decline of radioactive count in the thyroid and result in a longer output half-time. 2) The second case, which is probably the more important, is the difference in composition of the thyroid iodine pools over a period of time. In the thiouracilized animal the organification of iodine is blocked and therefore this iodine pool would be expected to diminish over a period of time. However, the proportion of non-radioactive iodine to radioactive iodine in the pool would remain constant and the thyroid hormone released from the gland would therefore be labeled with the same amount of I-131 at one instant as at a later time. But in the case of the normal animal, iodide uptake and organification is unimpeded. Therefore, the size of the iodine pool should remain relatively constant over a short period of time, but its composition with regard to I-127 and I-131 would be changing. The change would be such that I-131 in the pool is continually being diluted by I-127 and the proportion of non-radioactive iodine to radioactive iodine would be growing larger. I-131 labeling of the hormone then would become increasingly smaller. The end result of such a situation is that not as much radioactive iodine leaves the gland over a given period

of time because of the diluting effect on the pool by I-127. This also would be reflected in a longer output half-time. In either case, although there is a difference in t½ values, the same amount of hormone would be expected to leave the gland if all other things are considered equal. Since the method employed in this study is one of substitution and acts through inhibition of thyroid stimulating hormone from the pituitary, it would be expected that similar secretion rate values would be calculated.

Although the circumstances involved in obtaining the data from normal animals in the other experiments are admittedly not comparable to those in the study just cited, the possible importance of individual variations in recycling and iodine intake and their effects on output half-time may be readily appreciated.

Seasonal differences in thyroid secretion rates have been observed previously in both goats (Schultze and Turner, 1945) and sheep (Henneman, et al., 1955). The seasonal changes were noted in goats by means of the goitrogen technique, whereas the values obtained for sheep were determined by the same method as used in the present study. The mean secretion rates reported by Henneman, et al. for open two-year-old ewes ranged from a high of 0.20 milligrams daily during the month of March to a low of 0.04 milligrams for the month of July. The latter value was found to be significantly lower than at any other month of the year. The next lowest secretion was observed in September (0.14 mg.). This was found to be significantly different from the value recorded for March. No quantitative figures concerning seasonal variation were included in the publication of

Schultze and Turner but it was pointed out that the dosage levels of thiourea and thyroxine which caused the thyroid weight to return to normal in other similar animals in warm weather resulted in much greater thyroid weight when employed in the Winter. On this basis it was concluded by them that a much higher secretion rate existed in these animals in cold weather. Seasonal studies in chicks have also been reported. Reineke and Turner (1945) observed the summer secretion rate in young chicks to be approximately one-half the Winter level.

The results of the present study, it will be noted (Table V and Figure 5), tend to confirm the previous observations. In comparison to sheep, goats of a comparable age and approximate weight appear to have a much higher rate of thyroid activity. The mean secretion rate for the two-year-old goat was found to range from a high of 0.336 $\pm$ 0.018 mg. l-thyroxine per 100 pounds per day during the month of October to a value of 0.177 $\pm$ 0.013 for July. Similar to sheep, the secretion rate in goats for July was significantly lower than any other time of the year studied except August. Whether the approximately 50 percent reduction in thyroid secretion in summer is due primarily to the depressing effect of high temperature, increased light exposure or a combination of both cannot be determined on the basis of present findings since these factors were not controlled.

For the purpose of comparing the mean secretion rates of all non-lactating animals according to age groups and for various times

of the year, Table VI lists the t values and degrees of freedom at the intersection of the appropriate comparison columns.

In considering the data concerning the relationship of age and thyroid activity it was found that in February, July and October there was a highly significant difference between the mean secretion rates of the young and aged goats. Although the older goats were secreting thyroxine at a lower level in all experiments, the reason that no significant difference was found in May or August cannot be fully explained at this time. It is possible that this may be attributed to changing environment during these times resulting in a greater variability of response in individual animals. This is reflected to some extent in the larger standard errors found at these times. Similar findings of variations in age groups have been reported for other species. Turner (1948) observed a pronounced decrease in secretion rate of older hens. Using the protein-bound iodine technique, Long, et al. (1951) have observed differences due to age in cattle. Henneman, et al. (1955) have reported a significant difference due to age when 2-year-old ewes were compared to 4-year-old ewes during various times of the year with the exception of July and September. However, in all cases the older ewes were secreting thyroxine at a lower level than the younger animals.

In the study on the effect of pregnancy on thyroid secretion the results obtained are in agreement with those obtained by Henneman, et al. (1955). These investigators found no significant difference in daily secretion rate between pregnant and non-pregnant ewes. It has also been reported by Monroe and Turner (1948) that

no significant difference from normal exists in pregnant rats. In contrast, Rugh (1951) observed that the lactating mouse thyroid was better protected from I-131 radiation damage presumably because of greater output from the gland.

Previous studies involving lactating animals have shown that thyroid activity is significantly higher in these animals when compared to non-lactators. Henneman, et al. (1955) reported a secretion rate of 0.20 mg. of l-thyroxine daily for open 2-year-old ewes and a value of 0.32 mg. for lactating 2-year-old animals during the month of March. A similar significant difference was noted in May. In contrast to these observations the results of the present experiments, as recorded in Tables A through M (Appendix), show that the non-lactating goats had a significantly higher secretion rate in July than did the lactating animals of corresponding ages. In May and August there was no significant difference between comparable groups but the non-lactators again revealed a higher secretion rate. Although these results elude explanation at the present time, experiments utilizing adrenocortical hormones and their effect on thyroid activity as measured by this substitution method might prove to be a productive area of investigation. Very few findings appear in the literature regarding the problem of the effect of lactation on thyroid secretion rate. To this author's knowledge there are no data concerning secretion rate in lactating domestic animals which were obtained during the summer months with which to compare the present findings. Confronted with this lack of information, it may be theorized that during the higher temperatures of summer the

process of lactation may place an added stress on the animals which would shift the endocrine balance, possibly via the adrenals in such a way that thyroid secretion rate is reduced. It may be under these circumstances that the feeding of thyroid materials offsets this condition and in this way they exert their beneficial effects.

SUMMARY AND CONCLUSIONS

The substitution technique of Henneman, Reineke and Griffin (1955) for measuring thyroid secretion rates in individual intact animals was adapted to the study of thyroid activity in dairy goats. The application of this method to these animals is described and discussed.

The percent of the injected dose of radioactive iodine taken up by the thyroid was also investigated in goats of various ages, during pregnancy and lactation and at various times of the year. It was found that although no correlation with thyroid secretion rate existed, with the exception of the experiment during August, there was a definitely increased uptake of iodine during the summer months. It was also noted that the thyroids of lactating animals take up less iodine than non-lactators. The implication of these findings is discussed.

Output half-times were determined for the same animals. Again, no correlation with secretion rates was noted except for the month of May. These data are discussed in relation to thyroid iodide pools. In connection with this, the experiment concerning thiouracilized and non-thiouracil treated animals is examined. It was observed in these two groups of animals that although the rate of release of I-131 was different, secretion rates determined by the substitution technique were the same.

The thyroid secretion rate values obtained for goats of various ages, during pregnancy and lactation and at various times of the year are herein recorded and discussed. The observations made in the present study that season and age affect thyroid activity are in line with the findings of previous investigators. That pregnant animals do not differ from non-pregnant animals with regard to thyroid secretion is also in agreement with previous findings. In sharp contrast to reports in the literature it was found in these experiments that non-lactating goats secreted thyroxine at a higher level during summer months than lactating animals. Differences during the remainder of the year were non-significant. Based on the present findings no explanation for this occurrence is given. However, it is theorized that the adrenal cortex may be implicated.

Conclusions which may be drawn from this work include:

1. Higher temperatures effect a lower level of hormone secretion on a body weight basis in the goat thyroid and, conversely, lower temperatures result in a higher level of secretion.

2. The relationship between age and thyroid activity is such that lower levels of hormone secretion on a body weight basis occur in the older goat as compared to the younger animal.

3. Although thiouracil treatment increases the thyroidal I-131 output rate the thyroid rate as measured by the substitution technique is not significantly different in thiouracilized than in normal goats.

4. Pregnancy in the goat does not effect a change in thyroid activity as judged by hormone secretion on a body weight basis.

5. Radioactive iodine uptake studies do not constitute a reliable indication of thyroid activity in the goat.

6. Release rates of radioactive iodine from the thyroid are not always dependable in determining thyroid activity in the goat.

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APPENDIX

FORMULAE USED IN CALCULATIONS

With regard to statistical treatment and computations, it has already been pointed out that straight lines were fitted to all curves by the method of least squares. In the case of calculating output half-times, the formula used for fitting the straight line was

$$Y = a + (bx)$$

where Y = log of percent of injected dose taken up by the thyroid
x = days after output of I-131 has been established
a = intercept
b = slope

The determination of b was obtained by the formula:

$$b = \frac{Sxy - \frac{(Sx)(Sy)}{n}}{Sx^2 - \frac{(Sx)^2}{n}}$$

where N = the number of observations
S = summation of

The formula for determining a :

$$a = \bar{Y} - (b\bar{x})$$

where $\bar{Y}$ = mean value of Y
$\bar{x}$ = mean value of x

The calculation of output half-time ($t\frac{1}{2}$) was obtained by the formula:

$$t\frac{1}{2} = \frac{0.693}{-B}$$

To determine -B : $$-B = \frac{2.3}{T} \log \frac{A_t}{A_o}$$

where A_o = initial activity

A_t = activity at an arbitrary time interval after A_o
T = the arbitrary time interval between A_o and A_t

The values for A_o and A_t were taken from points situated on the fitted line.

In calculating the estimated secretion rate, the formula again used to fit a straight line was:

$$Y = a + (bx)$$

where Y = percent of previous count
x = daily l-thyroxine dose in micrograms

As was mentioned previously, a different form of this equation was used to predict the dose at which 100 percent of previous count occurred or the amount of l-thyroxine which would have been required to completely inhibit I-131 output from the thyroid.

A coefficient of correlation was determined on each of the estimated secretion rate curves using the formula:

$$r^2 = \frac{aSy + bS(xy) - N(\bar{Y}^2)}{SY^2 - N(\bar{Y}^2)}$$

where r = correlation coefficient
Y = percent of previous count
x = daily thyroxine dose in micrograms
a = intercept
b = slope
N = number of observations
$\bar{Y}$ = mean percent of previous count
S = summation of

The "r" value for each individual determination will be found in the tabulated results in the following section.

The mean estimated secretion rate for each group of animals was determined and the standard error of the mean was calculated using the formula:

$$S.E. = \frac{\sqrt{\frac{Sx^2 - \frac{(Sx)^2}{N}}{N - 1}}}{\sqrt{N}}$$

where x = estimated secretion rate
N = number of observations

To determine the existence of significant differences between the means of any two groups, the following calculation was made:

$$T = \frac{m_1 - m_2}{\sqrt{(S.E._1)^2 + (S.E._2)^2}}$$

where T = test statistic
m_1 = mean of group 1
m_2 = mean of group 2
$S.E._1$ = standard error of group 1
$S.E._2$ = standard error of group 2

TABLE A. EXPERIMENT 2

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENT (r) FOR AGED LACTATING GOATS

MAY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
1	Saanen	6	138	0.185	8.66	8.7	0.9128	7	12.0
4	Saanen	4	104	0.132	6.27	5.4	0.9146	7	7.1
8	Nubian	5	95	0.206	8.94	19.8	0.9200	3	20.5
12	Nubian	4	104	0.230	6.50	22.1	0.9758	3	22.3
Mean				0.188 ± 0.053*	7.59	14.0		5	15.47

* Standard Error

TABLE B. EXPERIMENT 2

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG LACTATING GOATS

MAY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
5 cu	Saanen	2	96	0.271	6.43	15.0	0.9761	2	15.0
11	Tog.*	2	97	0.229	10.25	9.2	0.9755	2	9.2
15	Saanen	2	98	0.166	7.16	19.2	0.9397	7	22.3
16	R.A.**	2	95	0.210	5.85	14.2	0.9841	3	15.6
18	Saanen	2	83	0.360	14.56	11.4	0.9978	3	15.5
19	Nubian	2	120	0.223	7.80	19.6	0.9853	2	19.6
23	Saanen	2	96	0.380	10.65	18.2	0.7572	3	19.7
Mean				0.263 ± 0.030***	8.96	15.2		3.14	16.7

* Toggenberg
** Rock Alpine
*** Standard Error

TABLE C. EXPERIMENT 2

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED NON-LACTATING GOATS

MAY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
2	Saanen	6	136	0.206	6.56	-	0.8817	4	57.7
5	Saanen	5	129	0.109	6.30	21.4		7	32.1
6	Tog.-Nub.	6	120	0.243	7.77	39.8	0.8724	7	50.5
7	Nubian	5	133	0.205	6.96	46.1	0.8646	3	50.3
9	Tog.-R.A.	6	93	0.232	8.76	20.3	0.9536	7	29.7
Mean				0.199 ± 0.024*	7.27	31.9		5.6	44.06

* Standard Error

TABLE D. EXPERIMENT 2

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG NON-LACTATING GOATS

MAY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
7 cu	R.A.	2	91	0.190	5.90	48.3	0.9542	3	49.5
17	Saanen	2	100	0.361	8.10	17.7	1.0000	4	21.5
20	Tog.	2	80	0.298	6.46	32.7	0.9829	3	36.7
22	Saanen	2	100	0.264	6.34	30.8	0.8408	4	35.0
Mean				0.278 ± 0.036*	6.7	32.4		3.5	35.68

* Standard Error

TABLE E. EXPERIMENT 3

THF VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED, LACTATING GOATS

JULY

Goat #	Breed	Age	Wt Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
1	Saanen	6	117	0.0368	10.95	5.7	0.9572	4	8.8
4	Saanen	4	109	0.0312	10.18	3.6	0.9792	3	4.3
8	Nubian	5	95	-	-	-	-	4	10.4
12	Nubian	4	96	0.0827	10.17	13.7	0.9719	4	14.8
Mean				0.05 ± 0.016*	10.43	7.7		3.75	9.58

* Standard Error

TABLE F. EXPERIMENT 3

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIME, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG, LACTATING GOATS

JULY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake	
								Day	%
5 cu	Saanen	2	85	0.068	15.07	6.7	0.8946	4	10.0
11	Tog.	2	95	0.042	9.3	9.9	0.9950	4	13.0
15	Saanen	2	87	-	-	-	-	3	11.2
16	R.A.	2	85	0.091	9.3	10.4	0.9761	3	12.9
18	Saanen	2	86	0.072	10.5	7.6	0.9901	4	10.3
19	Nubian	2	109	0.088	10.9	8.6	0.7378	4	8.9
23	Saanen	2	80	0.060	8.1	5 8	0.9688	3	6.6
Mean				0.07 ± 0.0074*	10.05	8.16		3.57	10.41

* Standard Error

TABLE G. EXPERIMENT 3

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED, NON-LACTATING GOATS

JULY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
2	Saanen	6	128	0.085	8.48	55.9	0.9586	2	55.9
5	Saanen	5	113	0.050	12.70	14.5	0.8452	4	16.5
6	Tog.-Nub.	6	118	0.073	15.13	31.7	0.9883	4	36.4
7	Nubian	5	125	0.128	10.53	44.7	0.9187	2	44.7
9	Tog.-R.A.	6	97	0.161	15.89	26.1	0.9635	3	27.5
Mean				0.099 ± 0.02*	12.55	34.6		3	36.2

* Standard Error

TABLE H. EXPERIMENT 3

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE, AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG, NON-LACTATING GOATS

JULY

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
7 cu	R.A.	2	88	0.104	9.26	27.7	0.9288	3	31.8
17	Saanen	2	94	0.288	12.24	20.7	0.9803	4	25.7
20	Tog.	2	80	0.178	11.03	30.8	0.9872	2	30.8
22	Saanen	2	102	0.141	10.22	35.7	0.9695	2	35.7
Mean				0.177 ± 0.013*	10.69	28.73		2.75	31.0

* Standard Error

TABLE J. EXPERIMENT 4

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED, LACTATING GOATS

AUGUST

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake	
								Day	%
1	Saanen	6	117	0.072	14.20	5.6	0.9662	4	9.1
4	Saanen	4	109	0.061	9.15	1.6	0.9912	4	3.7
8	Nubian	5	95	0.114	10.68	12.4	0.9150	4	18.4
12	Nubian	4	96	0.237	7.29	18.3	0.9872	4	25.0
Mean				0.121 ± 0.040*	10.33	9.5		4	14.05

* Standard Error

TABLE K. EXPERIMENT 4

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG, LACTATING GOATS

AUGUST

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
5 cu	Saanen	2	85	0.220	14.62	7.3	0.8701	4	11.4
11	Tog.	2	95	0.126	14.56	7.0	0.9864	4	11.5
15	Saanen	2	87	0.200	10.68	5.7	0.9912	4	9.3
16	R.A.	2	85	0.137	11.00	10.1	0.9977	3	15.3
18	Saanen	2	86	0.136	15.30	4.8	0.9887	3	6.6
19	Nubian	2	110	0.122	15.07	6.0	0.9924	4	9.2
23	Saanen	2	80	0.298	12.29	7.4	0.9760	3	10.4
Mean				0.177 ± 0.04*	13.4	6.9		3.57	10.53

* Standard Error

TABLE L. EXPERIMENT 4

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED, NON-LACTATING GOATS

AUGUST

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
2	Saanen	6	128	0.181	7.4	28.3	0.9526	4	42.5
5	Saanen	5	113	0.111	12.16	15.5	0.9944	3	22.1
6	Tog.-Nub.	6	119	0.271	18.24	22.0	0.9956	4	31.8
7	Nubian	5	125	0.139	10.17	15.6	0.9522	4	22.3
9	Tog.-R.A.	6	97	0.285	10.42	13.1	0.9628	3	19.2
Mean				0.197 ± 0.033*	10.35	18.9		3.6	27.58

* Standard Error

TABLE M. EXPERIMENT 4

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG, NON-LACTATING GOATS

AUGUST

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
7 cu	R.A.	2	88	0.244	11.20	23.3	0.9456	4	33.6
17	Saanen	2	94	0.172	12.01	8.5	0.9341	4	11.5
20	Tog.	2	80	0.236	10.15	10.6	0.9687	4	16.5
22	Saanen	2	102	0.199	12.16	12.1	0.9890	4	17.9
Mean				0.213 ± 0.0168*	11.4	13.6		4	19.88

* Standard Error

TABLE N. EXPERIMENT 4

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL IMMATURE GOATS

AUGUST

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
25		5 mo.	44	0.172	5.04	40.6	0.8936	4	41.8
26		5 mo.	36	0.441	5.88	22.3	0.6050	3	23.2
27		5 mo.	39	0.216	12.50	14.3	0.7874	3	20.6
28		5 mo.	28	0.309	8.21	20.5	0.8963	3	28.0
29		5 mo.	30	0.173	11.29	4.0	0.9991	3	7.2
Mean				0.262 ± 0.051*	8.58	20.3		3.2	24.16

* Standard Error

TABLE O. EXPERIMENT 5

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL AGED GOATS

OCTOBER

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
2	Saanen	6	130	0.203	8.37	11.0	0.9675	4	15.5
4	Saanen	3	114	0.167	11.25	2.4	0.9864	5	5.3
5	Saanen	5	123	0.233	8.76	11.4	0.9356	4	13.4
6	Tog.-Nub.	6	125	0.155	16.46	14.0	0.9520	4	20.9
7	Nubian	5	130	0.222	10.61	10.4	0.9966	4	12.3
8	Nubian	5	97	0.375	10.56	11.7	0.9955	4	16.5
9	Tog.-R.A.	6	104	0.192	11.07	15.8	0.9719	4	19.1
12	Nubian	3	87	0.394	8.86	14.1	0.9895	5	18.1
Mean				0.243 ± 0.03*	10.7	11.4		4.25	15.14

* Standard Error

TABLE P. EXPERIMENT 5

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG GOATS

OCTOBER

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
5 cu	Saanen	2	87	0.362	12.01	9.1	0.9980	5	14.9
7 cu	R.A.	2	91	0.349	8.12	11.2	0.9867	7	18.2
11	Tog.	2	96	0.358	12.60	10.7	0.9217	5	17.0
19	Nubian	2	101	0.345	13.50	1.5	0.9009	9	7.3
22	Saanen	2	109.5	0.245	11.55	13.5	0.9841	4	15.7
23	Saanen	2	86.5	0.354	8.66	11.3	0.9921	7	15.7
Mean				0.336 ± 0.018*	11.07	9.55		6.17	14.8

* Standard Error

TABLE Q. EXPERIMENT 5

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL YOUNG, THIOURACILIZED GOATS

OCTOBER

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
15	Saanen	2	91	0.308	6.20	7.1	0.9834	7	11.0
16	R.A.	2	79	0.502	7.97	6.0	0 9961	4	9.3
18	Saanen	2	66.5	0.355	7.23	3.9	0.9032	4	7.2
20	Tog.	2	81.5	0.351	6.80	9.8	0.9899	5	14.0
Mean				0 379 ± 0.0423*	7.2	6.7		5	10.38

* Standard Error

TABLE R. EXPERIMENT 5

THE VALUES OBTAINED FOR THYROID SECRETION RATES, OUTPUT HALF-TIMES, 48 HOUR PERCENT UPTAKE AND CORRELATION COEFFICIENTS (r) FOR INDIVIDUAL IMMATURE GOATS

OCTOBER

Goat #	Breed	Age	Wt. Lbs.	Thyroid Secretion Rate mg./100 lbs./day	T½ d.	48 Hr. % Uptake	r	Peak Uptake Day	Peak Uptake %
25		8 mo.	43.5	0.402	9.67	24.5	0.9096	3	23.8
26		8 mo.	40	0.478	7.29	17.3	0.9768	2	17.3
27		8 mo.	45	0.429	13.56	15.3	0.9865	4	20.4
29		8 mo.	28.5	0.302	10.79	7.5	0.9999	5	11.0
Mean				0.403 ± 0.037*	10.32	16.2		3.5	18.13

* Standard Error