# THE THYROID SECRETION RATE OF SHEEP AS RELATED TO SEASON, BREED, SEX, AND ITS RELATION TO SEMEN QUALITY IN THE RAM

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P. H. Nelson
Major professor

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# THE THYROID SECRETION RATE OF SHEEP AS RELATED TO SEASON, BREED, SEX, AND ITS RELATION TO SEMEN QUALITY IN THE RAM

By

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Submitted to the Graduate School of Michigan State University of Agriculture and Applied Science in Partial Fulfillment of the requirements for the degree of

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## INTRODUCTION

Research has proven that the thyroid gland plays an important role in the general metabolism of the body. Thus the thyroid is concerned with many of the body processes. The importance of the thyroid gland secretion in growth and development is shown in young animals deprived of thyroxine. These animals exhibit retarded growth and development of the sex organs. The thyroid gland has, also, been shown to be involved either directly or indirectly with the problem of "summer sterility" in rams. Rams deprived of thyroxine have shown a marked decline in semen quality and ability to reproduce. The thyroid gland may also play a role in the seasonal breeding habit of the ewe. The seasonal breeding of sheep make it difficult to maintain an orderly and uniform supply of lamb for the market. If the breeding season could be extended so that lambs could be marketed in a more uniform supply, the usefulness of these animals would be increased.

Very little information is available on the normal thyroid secretion rate of farm animals. This lack of information has made it difficult to utilize the synthetic thyroactive substances to alter the thyroid state of farm animals to increase their productivity. Many experiments have been carried out to determine the effects of added thyroactive substances on the growth, fertility, and production of farm animals. The results from these experiments have shown a great deal of variation which is due in part to different levels of administration. This indicates that knowledge of normal thyroid secretion rates is needed for further advancement in this area.

The problem of selection in farm animals is a difficult task with the limited information available to the animal breeder. If thyroid secretion rates vary between animals and are related to their productivity, measures of these rates could be a valuable tool to the animal breeder.

This study was designed to gain basic information on the normal thyroid secretion rate in sheep, to study the relationship of environmental factors to thyroid secretion rate, and to study semen quality in the ram as related to thyroid secretion rate.

The method used for determining thyroid secretion rate in sheep was developed as a preliminary experiment before the study of variations in thyroid secretion rate was carried out.

## OBJECTIVES

1. To develop a technique for determining thyroid secretion rate in intact, individual sheep which would be practical for large animal research.

2. To determine the effects of the following factors on the thyroid secretion rate of sheep:

   a. Season of the year

   b. Breed

   c. Sex

3. To determine the relationship between thyroid secretion rate and semen quality in the ram.

## REVIEW OF LITERATURE

Man has known the superficial location of the thyroid gland for many years and has been able to observe gross changes in its size. Certain substances, such as seaweed, were added to the diet to reduce the enlarged thyroids before the principle of the thyroid was established.

Shortly after the discovery of elemental iodine by Courtois, physicians were using iodine to treat goiter. In the following years, many attempts were made to identify the active principle in the thyroid substance. Iodine was finally isolated by Baumann (1896), and Bauman and Roos (1896) determined that the iodine in the thyroid was protein bound. Since these discoveries, much work has been carried out on the isolation and assay of thyroxine in the thyroid gland, (Hunter, 1910; Kendall, 1914; Kendall, 1916; Harrington, 1926; Harrington and Barger, 1927; Foster, 1929; Blau, 1935; Reineke, 1945b; Doery, 1945).

In addition to these works, the methods of McClendon (1928), Harvey (1935), Salter and Johnston (1948), and others have been used to determine the protein bound iodine in the blood serum as a measure of thyroid activity. Long et al. (1951) used the protein bound iodine content of the blood plasma as a measure for studying differences between breeds of dairy and beef cattle. They found differences between breeds and differences due to age. Lewis and Ralston (1952) used the protein bound iodine method for studying thyroid activity in dairy cows before and after calving and the thyroid activity of calves at birth, twelve hours of age, and three days of age. They also studied colostrum as compared to milk. While this method offers a practical method for measuring thyroid activity, the question has been raised as to whether protein bound iodine is actually a measure of thyroid secretion rate.

One of the most important single contributions to the knowledge of thyroid physiology was made by Magnus-Levy (1895) when he demonstrated that in Gull's disease (myxedema) metabolism was greatly reduced and treatment with desiccated thyroid material raised the metabolic rate to normal or higher. This principle of oxygen-carbon dioxide exchange has been used as a basis for modern clinical tests of metabolic activity as indicators of thyroid activity.

The introduction of synthetic goitrogens made it possible to alter the state of the thyroid gland through oral administration of these drugs. Thiouracil has been widely investigated and has been shown to interfere with the formation of thyroxine in the thyroid gland. This inhibition of thyroxine formation causes the pituitary to secrete more thyrotrophic hormone and thus an enlargement of the thyroid occurs (Kennedy, 1942; MacKenzie and MacKenzie, 1943). That the effects of thiouracil are mediated by way of the pituitary is shown by the fact that removal of the pituitary prevents this effect in thiouracil-treated animals (Astwood et al., 1943).

This inhibiting effect on thyroxine production and the resulting increase in the size of the thyroid gland has been used as a basis for a method of determining thyroid secretion rate. Thiouracil treated animals are given thyroxine in graded doses and the weight of the thyroid gland is used as an index of thyroxine secretion. Dempsey and Astwood (1943) observed that the increase in thyroid weight in thiouracil treated rats bore a quantitative relation to the thyroxine dosage. This relationship with thyroid weight in milligrams per one hundred grams of body weight can be expressed by a straight line. The point where the response curve intercepts the normal thyroid weight represents the amount of thyroxine required to maintain normal thyroid pituitary relationship. These workers determined the thyroid secretion rate of rats at several different environmental temperatures and found that thyroid secretion was depressed at high temperatures and increased at low temperatures.

In order to obtain a reliable figure for normal thyroid weight and a good curve relating thyroid weight to thyroxine dosage, it has been suggested that at least five groups of eight to ten animals be used. While this number is satisfactory in laboratory animals, it presents definite limitations in large animal research due to the expense of sacrificing animals. Another limitation of this method is the inability to determine individual thyroid secretion rates.

The introduction of "tagged" tracer substances has greatly facilitated biochemical research. These substances behave in the organism just like the natural elements according to Salter (1940). Radioactive iodine was first used as an indicator of thyroid function by Hertz et al. (1938). They found that the concentration of iodine by the thyroid gland is a biologically selective process, and that stimulation of the thyroid gland with thyrotropin increases the capacity to concentrate iodine. Hertz et al. (1940) reported that the normal thyroid contained eighty times as much iodine as other tissue. This work was done with an isotope that had a half-life of twenty-six minutes. With the availability of $Iodine^{131}$ which has a half-life of eight days, new methods for measuring thyroid activity have been suggested.

Berry (1951) injected rats with $iodine^{131}$ and measured the rate of loss of iodine from the thyroid gland by means of an external counter and determined an index of thyroid secretion rate. The validity of this method was suggested by the effect of thyrotrophic hormone, thiouracil, thiocyanate, and thyroxine on the iodine retained by the thyroid. He showed a relationship between the rate of thyroxine injection and $iodine^{131}$ retained in the thyroid. Wolff (1951) studied factors that influence the release of iodine from the thyroid using a technique similar to that of Perry.

Marked changes occur in the reproductive capacity of the ram, with the lowest fertility occurring during the periods of highest temperatures. This condition has been labeled "summer sterility." While light and temperature are recognized as highly effective factors in changing the reproductive organs from quiescence to high activity and vice versa, investigations on "summer sterility" in the ram have shown that temperature is a primary factor by MacKenzie and Berliner (1937), Berliner and Warbritton (1937), and Bogart and Mayer (1946).

Bogart and Mayer (1946) concluded that temperature affects the reproductive capacity of the ram through the thyroid gland. They also concluded that sensitivity of the reproductive organs of the ram is a reflection of the sensitivity of the thyroid to changes in environmental temperature. However, there is not complete agreement as to the role of the thyroid in male fertility. Some investigators hold the view that the thyroid has no direct effect on the testes, and that any reproductive disturbance in the male in hypothyroidism or hyperthyroidism is not due to endocrine imbalance, but to changed metabolic state (Moore, 1939); or to the complex inter-relations between the endocrine system and body metabolism as a whole (Cameron, 1945).

The thyroid secretion apparently plays an important role in maintaining the normal balance between the pituitary and reproductive glands, since the level of pituitary gonadotrophin is reduced in thyroidectomized goats (Reineke, Bergman and Turner, 1941). A further relationship was indicated by Meites and Chandrashaker (1948) who observed that in male mice the response to gonadotrophic hormone was reduced by thiouracil and augmented by feeding optimal levels of iodinated casein. Berliner and Warbritton (1937), and Bogart and Mayer (1946) observed a decrease in sperm cell concentration and an increase in the percentage of abnormal cells in thyroidectomized rams, or rams made hypothyroid by feeding thiouracil. These workers also found increased sperm cell production and lowered percentage of abnormal cells in rams given injections of pure crystalline thyroxine or fed thyroprotein during the summer months. However, there is some variation in the experimental results obtained from administration of thyroxine and thyroprotein to rams. Warwick et al. (1948) obtained some deleterious and, also, some beneficial results; Eaton et al. (1948) found no beneficial effects; and Black et al. (1950) found no improvement in sperm concentration and motility but deleterious effects on weight and libido.

## EXPERIMENTAL PROCEDURE

### A. Development of a Technique for Measuring Thyroid Secretion Rate in Intact Sheep

The development of the technique was worked out in co-operation with H. A. Henneman. It was presented in a thesis entitled "The Thyroid Secretion Rate of Sheep as Affected by: Season, Age, Breed, Pregnancy and Lactation," submitted by H. A. Henneman (1953). It was published in the Journal of Animal Science, Vol. 14, No. 2, May 1955 with the writer of this thesis as junior author.

1. Determination of normal uptake and output curves of iodine$^{131}$ in sheep thyroid glands. One Hampshire and one Shropshire ewe were injected with one microcurie of $I^{131}$ per pound of body weight. This level of $I^{131}$ was based on the work of Terry (1951) and was found to give reliable counts. The $I^{131}$ serves as a means of locating the iodine in the body and is believed to act the same as the $I^{127}$ which is present in large quantities. Iodized salt was fed free choice to the animals to insure a uniform uptake of iodine.

External counts were taken with a Nuclear Instrument and Chemical Corporation Laboratory monitor with an end window Geiger-Muller tube. A metal shield was used on the Geiger-

Muller tube so that only gamma rays were counted. The thyroid counts were taken in the area of the neck where the highest counts could be obtained. The tube was held firmly against the sheep and the pressure was kept as equal as possible for each count. Background counts were taken on the outside of the foreleg to compensate for gamma emission by $I^{131}$ in the blood stream. The wool was sheared from the neck and the foreleg to facilitate the handling of the tube against the sheep.

To compensate for variations in the efficiency of the monitor and the tube, a standard source of $Co^{60}$ was used to check the machine each day before taking counts on the sheep. All counts were corrected to this standard. The background counts were subtracted from the thyroid counts and this figure was then corrected to zero time for physical decay of the $I^{131}$.

The corrected external counts per minute for the two ewes are presented in Table I and shown graphically in Figure 1. The curves in Figure 1 are typical of uptake and output curves of $I^{131}$ in the sheep thyroid gland. From these curves, seven days after injection of $I^{131}$ was selected as the earliest time that the sheep thyroid gland reaches a somewhat steady output of $I^{131}$.

2. An attempt to maintain external thyroid counts at one hundred percent of previous count. One Shropshire ewe, one Oxford ewe, one Dorset ewe, and one Rambouillet ewe were injected with $I^{131}$ and a base reading was taken on the third

TABLE I

EXTERNAL THYROID COUNTS PER MINUTE OF TWO CONTROL EWES

| Time after Injection | Hampshire Ewe | Shropshire Ewe |
|---|---|---|
| 6 hours | 797 | 409 |
| 20 hours | 1644 | 1107 |
| 25 hours | 2331 | 1499 |
| 48 hours | 2841 | 2223 |
| 72 hours | 5626 | 3733 |
| 4 days | 3922 | 4042 |
| 5 days | 3383 | 3899 |
| 6 days | 4025 | 4184 |
| 7 days | 3325 | 3738 |
| 8 days | 3420 | 4170 |
| 10 days | 3028 | 3562 |
| 13 days | 2602 | 2817 |
| 16 days | 2040 | 3200 |
| 17 days | 2067 | 2915 |
| 22 days | 1509 | 2347 |

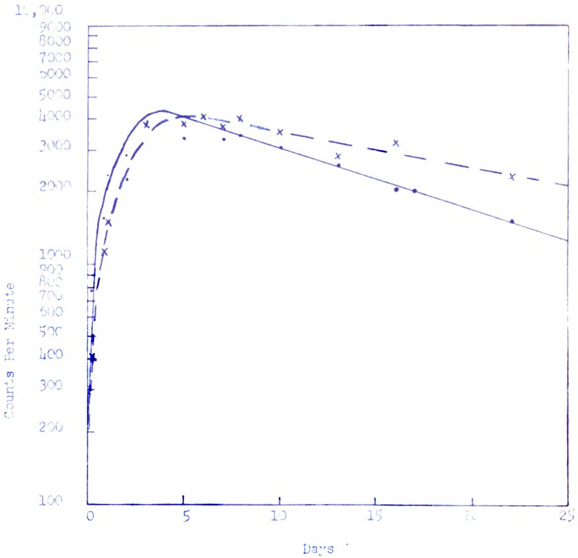



Figure 1 Typical External Thyroid Counts of Two Control Ewes

day. This was used as zero time and all corrections for physical decay were made at this time.

Dry thyroxine from Glaxo Laboratories, Greenford, Middlesex, England, was weighed on an analytical balance, dissolved in distilled water made slightly alkaline with NaOH, and acid (HCL) added to make the solution slightly cloudy. This solution was stored in a refrigerator and used within two weeks.

After injection of $I^{131}$, a subcutaneous injection of l-thyroxine was given to observe the effect on the next day's counts. Perry (1951), with rats, was able to maintain counts at one hundred percent by injection of 15 micrograms of l-thyroxine daily.

The hypothesis was, that as the amount of l-thyroxine injected daily approached the normal output of the sheep's thyroid gland, 100 percent of previous count could be maintained. From these data it is apparent that the injections of l-thyroxine materially affected the slope of the output curve. However, it was also apparent that a great many injections and readings would be needed to obtain a reasonable estimate of thyroid secretion rate. This did not appear to be practical .

The data from this trial are presented in Tables II and III and graphically in Figure 2.

TABLE II

EFFECT OF L-THYROXINE INJECTIONS ON EXTERNAL THYROID COUNTS OF SHROPSHIRE AND OXFORD EWES

| Days Following Injection of $I^{131}$ | Shropshire | | | Oxford | | |
|---|---|---|---|---|---|---|
| | Thyroid Counts Per Minute | Percent of Previous Count | Mg. of L-Thyroxine Injected | Thyroid Counts Per Minute | Percent of Previous Count | Mg. of L-Thyroxine Injected |
| 3 (Zero Time) | 5410 | | | 4580 | | |
| 4 | 4579 | 84.6 | 0.8 | 4214 | 92.0 | 0.6 |
| 5 | 6046 | 132.0 | | 4476 | 106.2 | |
| 6 | 5665 | 93.7 | 0.7 | 4965 | 110.9 | 0.6 |
| 7 | 5731 | 101.2 | 0.8 | 4777 | 96.2 | 0.7 |
| 8 | 6226 | 108.6 | 0.8 | 4577 | 95.8 | 0.7 |
| 9 | | | 0.8 | | | 0.7 |
| 10 | 6486 | 104.2 | 0.8 | 4800 | 104.9 | 0.7 |
| 11 | 6220 | 95.9 | 0.9 | 4380 | 91.3 | 0.8 |
| 12 | | | 0.9 | | | 0.8 |
| 13 | 5521 | 88.8 | 0.9 | 4928 | 112.5 | 0.8 |
| 14 | 6461 | 117.0 | 1.0 | 5205 | 105.6 | 0.9 |
| 15 | 5561 | 86.1 | | 5265 | 101.2 | |
| 16 | 5142 | 92.5 | | 4033 | 76.6 | |
| 17 | 5186 | 100.9 | | 3826 | 94.9 | |
| 21 | 4412 | 85.1 | | 3151 | 82.3 | |

TABLE III

EFFECT OF L-THYROXINE INJECTIONS ON EXTERNAL THYROID COUNTS

OF DORSET AND RAMBOUILLET EWES

| Days Following Injection of $I^{131}$ | Dorset | | | Rambouillet | | |
|---|---|---|---|---|---|---|
| | Thyroid Counts Per Minute | Percent of Previous Count | Mg. of L-Thyroxine Injected | Thyroid Counts Per Minute | Percent of Previous Count | Mg. of L-Thyroxine Injected |
| 3 (Zero Time) | 4410 | | | 7280 | | |
| 4 | 4001 | 90.7 | 0.5 | 6335 | 87.0 | 1.0 |
| 5 | 4476 | 111.9 | | 7341 | 115.9 | |
| 6 | 5218 | 116.6 | 0.5 | 7052 | 96.1 | 0.8 |
| 7 | 4290 | 82.2 | 0.6 | 7611 | 107.9 | 0.8 |
| 8 | 5656 | 131.8 | 0.5 | 7166 | 94.2 | 0.9 |
| 9 | | | 0.5 | | | 0.9 |
| 10 | 5689 | 100.6 | 0.5 | 6926 | 96.7 | 0.9 |
| 11 | 5990 | 105.3 | 0.6 | 6740 | 97.3 | 1.0 |
| 12 | | | 0.6 | | | 1.0 |
| 13 | 5842 | 97.5 | 0.6 | 6412 | 95.1 | 1.0 |
| 14 | 6896 | 118.0 | 0.6 | 7380 | 115.1 | 1.1 |
| 15 | 5589 | 81.0 | | 6888 | 93.3 | |
| 16 | 4926 | 88.1 | | 6158 | 89.4 | |
| 17 | 4749 | 96.4 | | 5740 | 93.2 | |
| 21 | 4026 | 84.8 | | 4721 | 82.2 | |

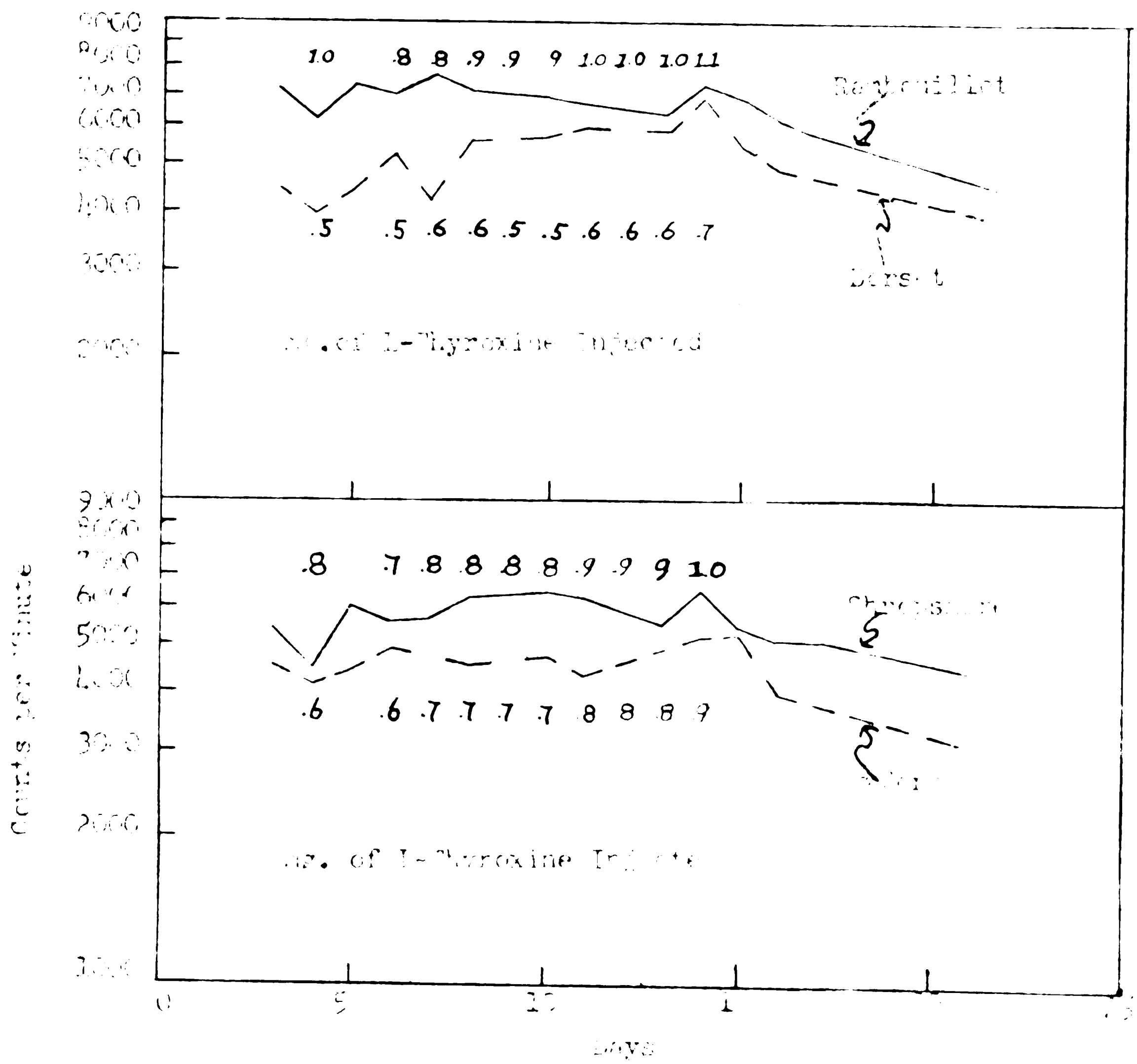



Figure 2 The Effect of L-Thyroxine Injections on Patent Thyroid Counts

3. <u>Predicting secretion rate by linear regression with three groups of sheep</u>. Three groups of four ewes were used in this trial. Each ewe was injected with one microcurie of $I^{131}$ per pound of body weight and a base count taken at seven days post injection. The three groups of ewes were designated as A, B, and C groups. L-thyroxine was injected subcutaneously for three days to permit the thyroid gland to adjust its activity: Group A received .4 mg. l-thyroxine daily, Group B received .8 mg., and Group C received 1.2 mg. daily for three days. At the end of this period, counts were taken and percent of previous count determined for each ewe.

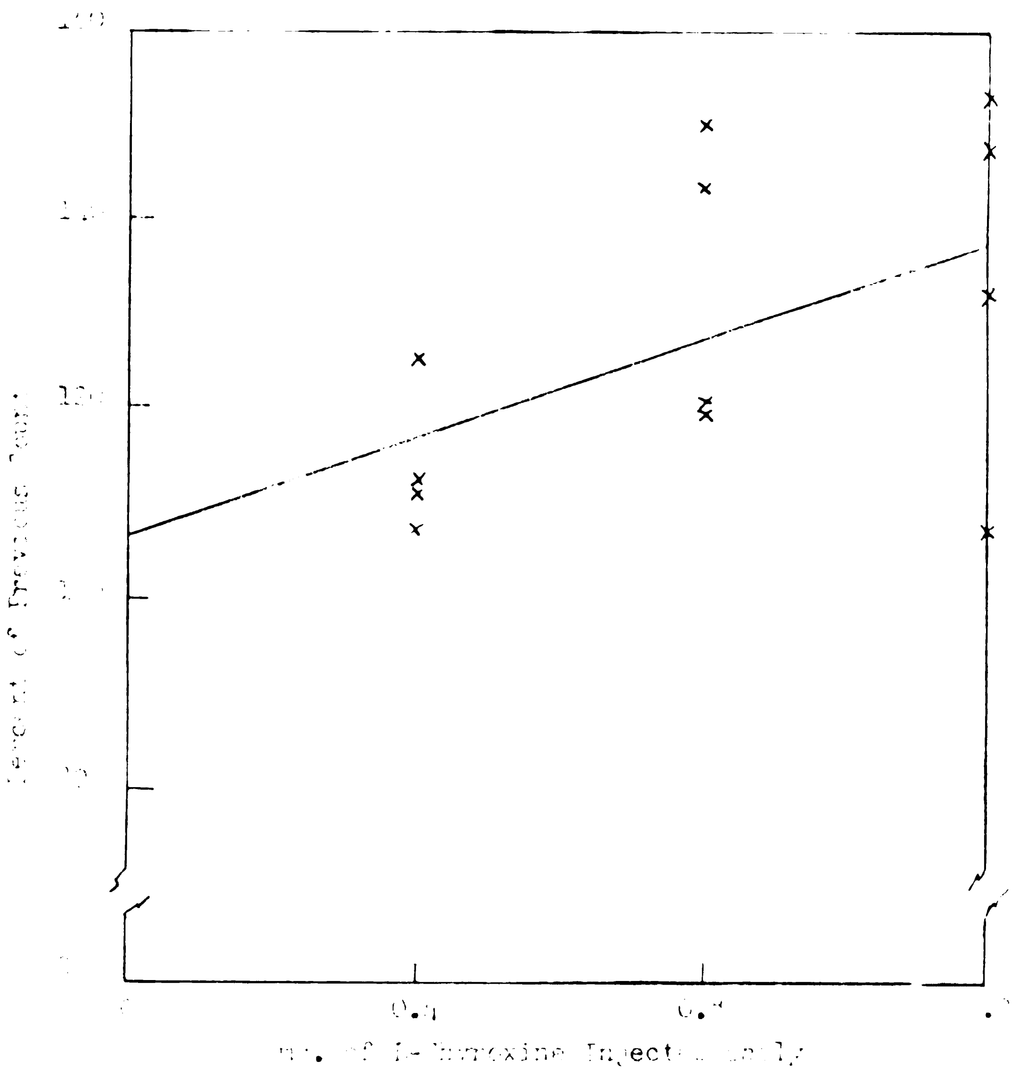
These data are presented in Table IV and Figure 3. It was unfortunate that all of the selected levels of l-thyroxine injections yielded percentages of previous count in excess of 100 percent. Therefore, any predicting equation for estimating daily thyroid secretion rate would be meaningless as demonstrated by the regression line drawn from the data. However, the data did demonstrate that percentage of previous count could be obtained that were greater than 100 percent. In other words, the thyroid was accumulating and storing additional $I^{131}$ from the blood stream from seven to ten days after injection of $I^{131}$.

Due to the fact that the design of this trial would give only an average secretion rate for a group of sheep an attempt was made in further trials to work with individual sheep.

TABLE IV

MILLIGRAMS OF L-THYROXINE INJECTED DAILY AND PERCENTAGE OF PREVIOUS COUNTS FOR TWELVE EWES

| Group A | Mg. L-Thyroxine Injected Daily | Percent of Previous Count |
|---|---|---|
| Shropshire 209 | 0.4 | 111.4 |
| Hampshire | 0.4 | 112.7 |
| Shropshire 929 | 0.4 | 125.6 |
| Shropshire 916 | 0.4 | 107.6 |
| | Average | 114.3 |
| Group B | | |
| Dorset | 0.8 | 143.3 |
| Shropshire 215 | 0.8 | 150.0 |
| Shropshire 911 | 0.8 | 119.7 |
| Shropshire 923 | 0.8 | 120.9 |
| | Average | 133.5 |
| Group C | | |
| Rambouillet | 1.2 | 147.6 |
| Oxford | 1.2 | 107.6 |
| Shropshire 940 | 1.2 | 132.4 |
| Shropshire 952 | 1.2 | 152.8 |
| | Average | 135.1 |



[illegible] 5 The [illegible] of Thyroxine [illegible]
[illegible] of [illegible]

4. <u>The effect of large daily injections of l-thyroxine</u>. Cornwall (1950), working with chicks, observed that one microgram of exogenous thyroxine daily inhibited the thyroid secretion rate more than three micrograms.

In this trial, two sheep from Trial 3 were used and more counts were taken. Shropshire ewe 215 received a daily injection of .8 mg. l-thyroxine for three days and the thyroid counts increased to 150 percent of previous count. For the next three days, Shropshire ewe 215 received an injection of 1.5 mg. l-thyroxine daily and external counts declined. Shropshire ewe 209 received an injection of .4 mg. l-thyroxine daily for three days and counts increased to 111 percent. For the next three days, Shropshire ewe 209 received an injection of 1.5 mg. l-thyroxine daily and the percentage of previous count declined.

The results presented in Table V and Figure 4 indicate that when the amount of thyroxine injected exceeds the daily secretion rate the percentages of previous count are unreliable. From this, it was concluded that for prediction of daily thyroid secretion rate by correlating daily injections of l-thyroxine to percentage of previous count, the level of l-thyroxine injection must be lower than the normal secretion rate of the animal. If this were done, then the secretion rate could be estimated by extrapolation of the prediction line thus obtained.

TABLE V

EXTERNAL THYROID COUNTS OF TWO EWES RECEIVING LARGE DOSES OF INJECTED L-THYROXINE

| Date | Shropshire 215 | | Shropshire 209 | |
|---|---|---|---|---|
| | Mg. of L-Thyroxine Injected | Thyroid Counts Per Minute | Mg. of L-Thyroxine Injected | Thyroid Counts Per Minute |
| Nov. 3 | | 800 | | 1670 |
| Nov. 4 | | 670 | | 1684 |
| Nov. 8 | | | | 1433 |
| Nov. 9 | 0.8 | 588 | 0.4 | 1075 |
| Nov. 10 | 0.8 | | 0.4 | |
| Nov. 11 | 0.8 | | 0.4 | |
| Nov. 12 | 1.5 | 882 | 1.5 | 1198 |
| Nov. 13 | 1.5 | | 1.5 | |
| Nov. 14 | 1.5 | 855 | 1.5 | 1139 |
| Nov. 15 | 1.5 | 790 | 1.5 | 1087 |
| Nov. 16 | | 724 | | 877 |
| Nov. 17 | | 688 | | 822 |

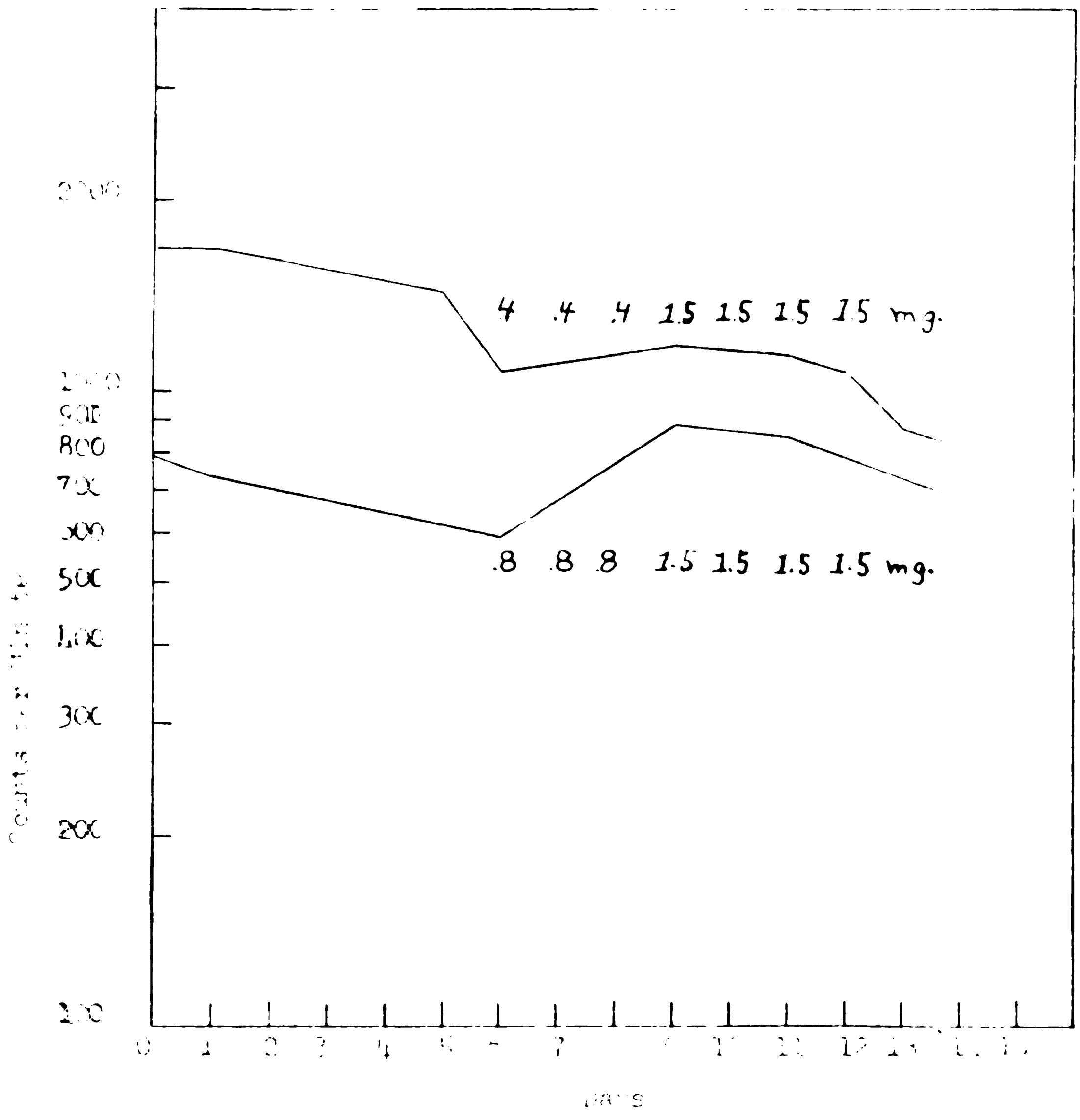



Figure 4 Effect of Large Daily Injections of [illegible] on External Thyroid Counts

5. <u>Use of varying doses of thyroxine injection and a series of external counts on individual lambs to extrapolate thyroid secretion rate</u>. Four lambs were injected subcutaneously with one microcurie of $I^{131}$ per pound of body weight. At seven days (zero time) and ten days post injection $I^{131}$, base counts were taken over the thyroid gland. Background counts were taken on the outside of the foreleg.

The experiment was then divided into four three-day periods:

Three-day period I; the lambs were treated the same as during the base period. Thyroid and background counts were taken at the end of the period.

Three-day period II; the lambs were injected subcutaneously with 0.1 mg. 1-thyroxine daily. Thyroid and background counts were taken at the end of the period.

Three-day period III; the lambs were injected subcutaneously with 0.25 mg. 1-thyroxine daily. Thyroid and background counts were taken at the end of the period.

Three-day period IV; the lambs were injected subcutaneously with 0.5 mg. of 1-thyroxine daily. Thyroid and background counts were taken at the end of the period.

Prior to the start of this trial, the stock supply of 1-thyroxine was further purified by Dr. E. P. Reineke. The activity of the thyroxine was increased and lower levels of injection were used.

The counts were corrected to the $Co^{60}$ standard, corrected for background, and, physical decay back to zero time, and the percentage of previous count computed. As the quantity of l-thyroxine injected daily was increased, the percentage of previous count increased. This would indicate that the thyroid was secreting less thyroxine as the quantity of l-thyroxine injected daily was increased.

The data were analyzed statistically and the correlation between percentage of previous count and mg. of l-thyroxine were obtained for each lamb and on an intra-lamb basis also. The regression coefficients were calculated and the predicting equation (y=a+bx) was used to estimate the daily thyroxine secretion rate of each lamb. It was assumed that where the predicting line crossed 100 percent of previous count would represent the daily secretion rate. The estimated secretion rates, and the correlation and regression coefficients are presented in Table VI and the predicting lines are shown in Figure 5.

Several problems arise when using injections of varying levels of thyroxine and obtaining percentage of previous count. Tracer doses of $I^{131}$ that were believed would not impair thyroid activity were used. Thus only four or five readings at three day intervals could be taken with the G.M. tube before the external thyroid counts had dropped to a level two or three times the background count.

TABLE VI

CORRELATION AND REGRESSION COEFFICIENTS AND ESTIMATED DAILY L-THYROXINE SECRETION

| Item | Degrees of Freedom | r | Level of Significance | b | Estimated Daily Secretion |
|---|---|---|---|---|---|
| | | | | | mg. |
| Crossbred 52 | 3 | .992 | .01 | .421 | .37 |
| Crossbred 69 | 3 | .981 | .05 | .619 | .39 |
| Shropshire | 2 | .983 | .15 | .454 | .23 |
| Hampshire | 3 | .837 | .10 | .424 | .57 |
| Intra-lamb | 11 | .928 | .01 | .485 | .39 |

A statistical predicting equation based on only four items was not desirable from a statistical standpoint. It was observed that when a reading of about 100 percent of previous count was obtained the injection of a higher level of l-thyroxine resulted in an unreliable percentage of previous count. In some cases the percentage of previous count was higher, in some cases very little change, and in some cases it was lowered. Therefore, in this study after a reading of about 100 percent of previous count was obtained any further readings using a higher level of l-thyroxine injection were not used.

In order to study differences in thyroxine secretion level between groups of sheep it was necessary to obtain

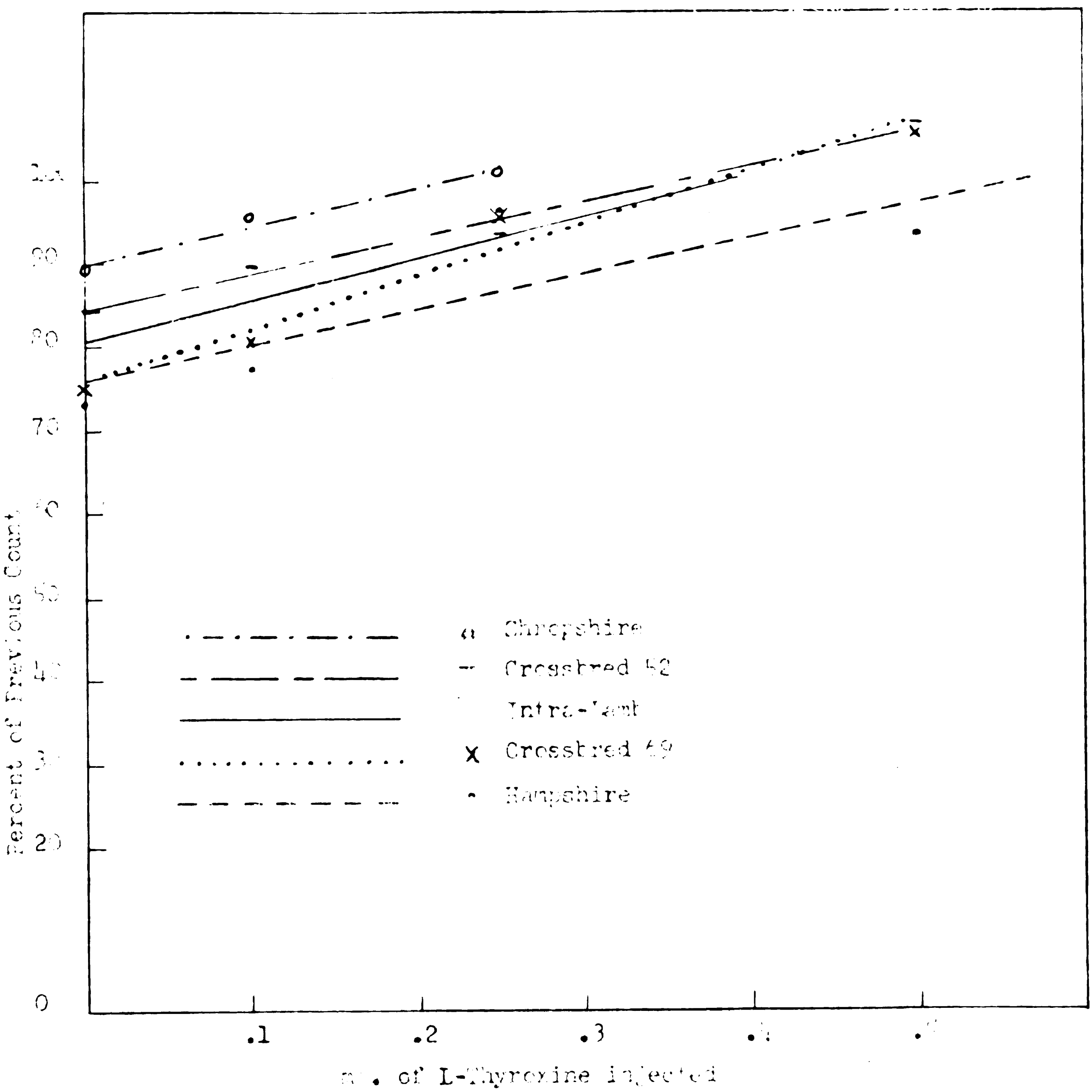



Figure 5 Extrapolation of Daily Secretion Rate From Prediction Equation

estimates of individual secretion for use in statistical analysis. However, it was realized that intra-lamb correlation and regression coefficients are more accurate for estimating the secretion rate of an entire group of sheep.

B. Study of Thyroid Secretion Rate Using the Extrapolation Technique

Twelve rams and six ewes were used in this experiment to study the thyroid secretion rate of sheep as affected by season, breed, sex, and its relation to semen quality in the ram. The sheep were divided into the following groups:

6 Yearling Hampshire Rams

6 Yearling Shropshire Rams

6 Open Yearling Shropshire Ewes

The Shropshire ewes were selected from the college flock and were all sired by the same ram. The rams were purchased from several different breeders and their previous history was not known. This may have introduced an additional source of variation. Due to the death of some sheep during the year the numbers in the groups were not constant.

Open Shropshire ewes were used for comparing the sexes to eliminate the effects of pregnancy and lactation. However, half the ewes were accidentally bred which left only three ewes for determination in January and March. When these ewes lambed, the lambs were removed and the ewes considered as dry.

The rams were handled as one flock under similar feeding conditions. They were kept in the barn during the winter months and allowed to run in a small pasture adjoining the barn during the summer. Iodized salt was fed free choice. The ewes were handled similarly to the rams.

When external thyroid counts were made, the counting was started at the same time each day, and the sheep were taken in the same order so that all readings were seventy-two hours apart. The amount of l-thyroxine injections had to be estimated due to lack of information on thyroid secretion rate. This made it necessary to take an additional three day period in some cases. During September the injected doses of l-thyroxine were too low to give a spread in percentage of previous count. Therefore an additional three day trial was run using a larger dose of l-thyroxine.

Semen collections were made with the use of the artificial vagina and a ewe held by a helper. Each semen specimen consisted of one ejaculate. Semen was collected from the rams every seven to ten days. However, the semen figures are based on the semen collection made just prior to the start of each trial. This was done to discount the effects of thyroxine injections during the thyroid secretion determination.

Motility ratings were made immediately at the barn by placing a drop of undiluted semen on a hanging drop slide and observing under both high and low powers of the microscope.

The ratings were based on the percent of cells showing progressive movement.

The concentration of sperm cells was determined by the use of a Coleman Universal Spectrophotometer. The semen was diluted 1:1000 with normal saline and read against a blank of normal saline. A curve for converting the Spectrophotometer readings to sperm numbers was plotted by counting the samples in a haemacytometer and taking corresponding readings on the Spectrophotometer.

The percent of abnormal cells was determined by counting three fields on a smeared and fixed slide.

The semen concentration figures for July were discarded as the Spectrophotometer was found to be inaccurate at this time.

## RESULTS AND DISCUSSION

### A. Seasonal Differences in Thyroid Secretion Rate

The Shropshire rams and Hampshire rams were treated as different groups in studying the effects of season on thyroid secretion rate. The daily secretion rates during the different months for the Shropshire rams are given in Table IX and for the Hampshire rams in Table X. Analysis of variance was used to study the differences in thyroid secretion during different months of the year. The results for the Shropshire rams are given in Table VII and the results for the Hampshire rams in Table VIII. The "F" value of 6.50** for the Shropshire rams and the "F" value of 6.18** for the Hampshire rams indicated a significant difference between at least two of the means at the 1 percent level in both breeds.

TABLE VII

SHROPSHIRE RAMS -- SEASONAL VARIATION

ANALYSIS OF VARIANCE

| Source | Degree of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 22 | .0299 | |
| Months | 5 | .0197 | .0039 |
| Error | 17 | .0102 | .0006 |

TABLE VIII

HAMPSHIRE RAMS -- SEASONAL VARIATION

ANALYSIS OF VARIANCE

| Source | Degree of Freedom | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 27 | .0598 | |
| Months | 5 | .0340 | .0068 |
| Error | 22 | .0258 | .0011 |

The "Student T" table was then used to test for differences between the individual means.

In the Hampshire rams the daily secretion rates in May and July were significantly different at the one percent level from all the other months in which readings were taken. The secretion rates being .06 mg. of thyroxine daily in May and .03 mg. in July as compared to .11 mg. in September and December. There was not a significant difference between the secretion rates in May and July.

In the Shropshire rams the daily secretion rate in July was significantly different at the one percent level from all other months in which determinations were made. The secretion rate in July being .02 mg. thyroxine daily as compared to .10 in September and December. While the difference between the secretion rates in March and May was not significant an apparent decline had set in at this time. Also there were only two entries in the May determination which made larger differences necessary for significance.

The increase in secretion rate from January may be due in part to the fact that all the college sheep were shorn in February.

These results are in agreement with published research. Mills (1918), reported that high environmental temperature increased thyroid activity in the rabbit. Dempsey and Astwood (1943), reported similar results with rats. Berliner and Warbritton (1937), reported that rams placed in a hot room showed decreased thyroid activity. Reineke and Turner (1945), observed a seasonal variation in thyroid secretion rate of young chicks, with the secretion rate in the winter being about double the secretion rate in spring and summer. Brody and Proctor (1932), reported a seasonal rhythm in energy metabolism.

Apparently high summer temperature tends to reduce the thyroid activity which is expressed in lowered metabolic rate and fertility.

A seasonal variation in daily thyroid secretion in sheep is shown by the data, with the thyroid secretion rate being inversely related to environmental temperature.

TABLE IX

MILLIGRAMS OF L-THYROXINE SECRETED DAILY

Shropshire Rams

| Ram No. | January Secretion Mg. | P* | March Secretion Mg. | P* | May Secretion Mg. | P* | July Secretion Mg. | P* | September Secretion Mg. | P* | December Secretion Mg. | P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | .08 | .30 | .08 | .20 | .04 | .30 | - | - | - | - | - | - |
| 187 | .05 | .10 | - | - | - | - | .03 | .20 | .09 | .25 | .06 | .10 |
| 192 | .07 | .20 | - | - | .06 | .05 | .02 | - | .11 | .25 | .11 | .05 |
| 241 | - | - | .14 | .20 | - | - | - | - | .10 | .02 | - | - |
| 967 | .09 | .30 | .06 | .30 | - | - | .02 | .10 | .07 | .01 | .12 | .05 |
| 973 | - | - | .07 | .30 | - | - | .02 | .20 | .12 | .01 | .12 | .01 |
| Total | .29 | | .35 | | .10 | | .09 | | .49 | | .41 | |
| Mean | .07 | | .09 | | .05 | | .02** | | .10 | | .10 | |

P* - Probability that the regression coefficient is significantly different from 0.
** - July secretion significantly different from all other months at one percent level of significance.

TABLE X

MILLIGRAMS OF L-THYROXINE SECRETED DAILY

Hampshire Rams

| Ram No. | January | | March | | May | | July | | September | | December | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* |
| 0 | - | - | - | - | .05 | .30 | .03 | .05 | .16 | .10 | .12 | .05 |
| 39 | - | - | .10 | - | - | - | .03 | .10 | .11 | .25 | .07 | .30 |
| 42 | .11 | .15 | .11 | .20 | .02 | .30 | .03 | .05 | .12 | .20 | .12 | .10 |
| 45 | .14 | .20 | .13 | .01 | .17 | .10 | .04 | .30 | .09 | .05 | .13 | .01 |
| 47 | .08 | .05 | .12 | .30 | .03 | - | - | - | - | - | - | - |
| 270 | - | - | .17 | .01 | .03 | .30 | .04 | .30 | .10 | .25 | .12 | .10 |
| Total | .33 | | .63 | | .30 | | .17 | | .58 | | .56 | |
| Mean | .11 | | .13 | | .06** | | .03** | | .11 | | .11 | |

P* - Probability that the regression coefficient is significantly different from 0.
** - July and May secretion significantly lower than all other months at one precent level of significance.

B. The Effect of Breed on Thyroid Secretion Rate

Yearling Shropshire rams and Yearling Hampshire rams were used for studying breed differences. The data used for this study are presented in Table IX for the Shropshire rams and Table X for the Hampshire rams.

The analysis of variance showed a significant difference between the secretion rate of Shropshire rams and Hampshire rams at the one percent level. The "F" value obtained was 4.43**. The Hampshire rams had a higher secretion rate than the Shropshire rams at every period that determinations were made. However the Hampshire rams and the Shropshire rams showed the same general pattern of seasonal variation in thyroid secretion rate. These relationships are shown in Figure 6.

It is interesting that the secretion rate for Shropshire rams in July (.02 mg.) was about 22 percent of the secretion rate in September (.09 mg.) while the secretion rate for the Hampshire rams in July (.03 mg.) was about 29 percent of the secretion rate in September (.11 mg.). This indicates that the Shropshire rams not only secreted thyroxine at a lower rate than Hampshire rams but also show a greater percentage decline in thyroid secretion rate in the summer months.

Berliner and Warbritton (1937) reported that Shropshire rams showed less thyroid activity than Hampshire rams when subjected to high temperatures. Long et al. (1951) observed differences in secretion rate between breeds of dairy and

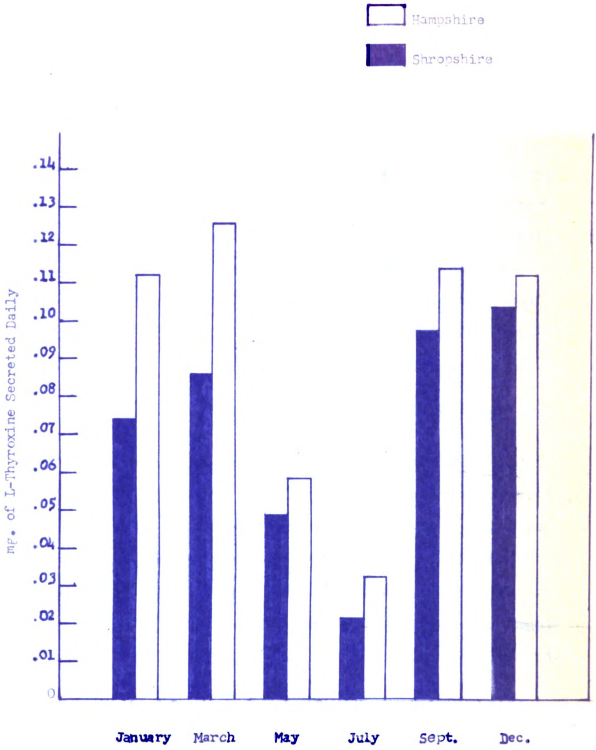



Figure 6

Comparison of Secretion Rate in Hampshire and Shropshire Rams.

beef cattle. Henneman (1953) found no significant difference between the thyroid secretion rates of Hampshire and Shropshire ewes. However, he did find that the secretion rate of the Hampshire ewes was higher at every period of the year in which determinations were made.

The Hampshire is generally believed by practical sheep breeders to have an earlier breeding season than the Shropshire. The higher secretion rate of the Hampshire may play a part in earlier breeding. This may offer the animal breeder a new tool for selection of sheep to lengthen the breeding season of sheep. More research in this field is needed as a means of increasing the usefulness of sheep.

### C. The Effect of Sex on Thyroid Secretion Rate

The open, yearling Shropshire ewes and the yearling Shropshire rams were used to study the differences between sexes. The data used for this comparison are presented in Table IX for the Shropshire rams and Table XI for the Shropshire ewes. The analysis of variance showed a difference between Shropshire rams and Shropshire ewes which was significant at the one percent level. The "F" value obtained was 10.1**.

Both the ewes and the rams showed a similar seasonal variation in thyroid secretion rate, however, the ewes had a higher secretion rate at every period that determinations were made. Certain differences did exist between the seasonal variation patterns of the ewes and rams. The secretion rate of the ewes dropped only 15 percent from March (.20 mg.) to May (.17 mg.) while the secretion rate of the rams dropped 45 percent from March (.09 mg.) to May (.05 mg.). From May to July the ewes dropped 76 percent from .17 mg. in May to .04 mg. in July while the rams dropped 51 percent from .05 mg. in May to .02 mg. in July. It would appear from this that the rams start the seasonal decline in thyroid secretion rate earlier than the ewes and decline at a more gradual rate. These relationships are shown in Figure 7.

It is important that the ewes used in this study were not bred thus removing the effects of pregnancy and lactation on thyroid secretion rate. Henneman (1953) reported that the thyroid secretion rate increased during lactation. Therefore under normal farm conditions it is probable that the thyroid secretion rate of ewes will show greater variation than the thyroid secretion rate of rams.

Mixner et al. (1944), and Schultze and Turner (1945) found that female chicks consistently showed a higher secretion rate than males. Schultze and Turner (1945) also showed that growing female goats showed a higher secretion rate than growing male goats.

The sex of an animal has an affect on the thyroid secretion rate, thus sex must be taken into consideration when determining the level of administration of thyro-active substances. Levels of administration that are effective in one sex may not give similar results in the other sex.

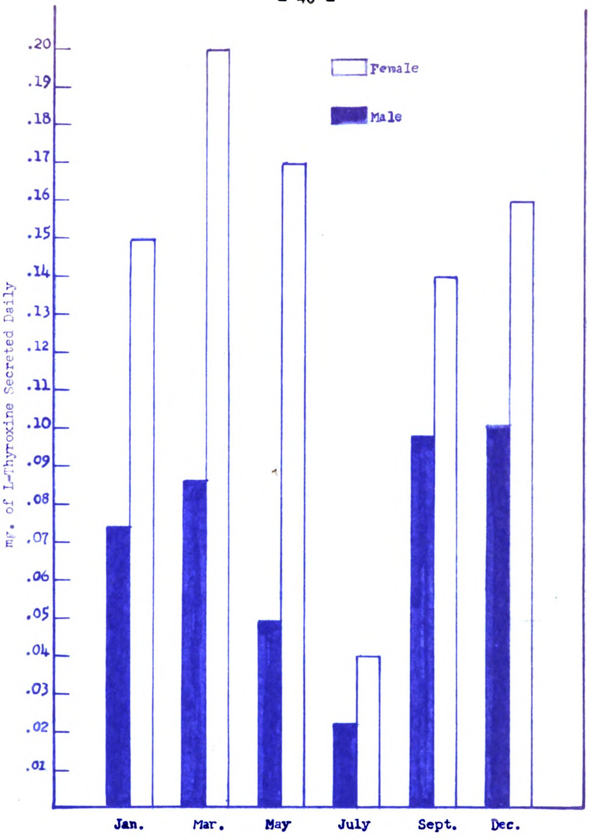



Figure 7

Comparision of Thyroid Secretion Rate of Shropshire Ewes and Shropshire Rams.

## D. The Relationship of Thyroid Secretion Rate to Semen Quality

Shropshire rams and Hampshire rams were used to study the relationship of thyroid secretion rate to semen quality. It was not possible to obtain equal numbers of samples for each period due to the death of some animals and the refusal of some rams to serve the artificial vagina. The semen quality evaluations were based on samples collected just prior to the beginning of the thyroid determination to eliminate as far as possible the effects of the thyroxine injections. Concentration of sperm, motility of sperm, and percent of abnormal sperm were used to evaluate the semen. The data from which relationships were studied are presented in Tables VIII, XII, XIII, XIV for the Shropshire rams and in Tables X, XII, XIII, XIV for the Hampshire rams.

Both the Shropshire and Hampshire rams showed a seasonal decline in semen quality which closely followed the changes shown in thyroid secretion rate. There were some individual and some breed fluctuations but the general trend was similar in all cases. As the thyroid secretion rate declined the semen quality declined in all factors studied and when the thyroid secretion rate rose the semen quality rose by the same standards. However there was an apparent time lag between the changes in thyroid secretion rate and changes in semen quality. Due to this time lag between changes in thyroid secretion rate and semen quality it was not possible to

TABLE XI

MILLIGRAMS OF L-THYROXINE SECRETED DAILY

Shropshire Ewes

| | January | | March | | May | | July | | September | | December | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ewe No. | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* | Secretion Mg. | P* |
| 923 | .18 | .25 | .15 | .01 | - | - | .03 | .05 | .14 | .05 | .15 | .05 |
| 916 | - | - | .25 | .05 | .19 | .05 | - | - | .13 | .05 | - | - |
| 940 | .12 | .05 | .20 | .05 | .16 | .25 | .07 | .06 | .19 | .05 | .16 | .10 |
| 929 | - | - | - | - | .09 | .25 | .03 | .01 | .12 | .05 | .17 | .05 |
| 952 | - | - | - | - | .23 | .10 | .04 | .30 | .12 | .05 | .15 | .05 |
| Total | .30 | | .60 | | .67 | | .17 | | .70 | | .63 | |
| Mean | .15 | | .20 | | .17 | | .04** | | .14 | | .16 | |

P* - Probability that the regression coefficient is significantly different from 0.
** - July secretion significantly lower than all other months at one percent level of significance.

TABLE XII

CONCENTRATION OF SPERM IN THOUSANDS PER MILLIMETER

| | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|
| Shropshire | | | | | | | | | | |
| 0 | 4500 | 4350 | 3300 | - | - | - | 2400 | 2400 | 2850 | 3300 |
| 241 | 6150 | 5400 | 5400 | 3150 | - | 700 | 2100 | 2200 | 4000 | 3800 |
| 967 | 3500 | 4650 | 6600 | 3500 | - | 100 | 5500 | 1050 | 3300 | 2500 |
| 192 | 4000 | 4050 | 5400 | 3000 | - | - | 1100 | 1500 | 2700 | 2450 |
| 973 | 2900 | 3300 | 3000 | 3200 | - | 500 | 1450 | 2450 | 3000 | 3450 |
| Mean | 4210 | 4350 | 4140 | 3212 | - | 433 | 2510 | 1920 | 3170 | 3100 |
| Hampshire | | | | | | | | | | |
| 42 | 4150 | 2900 | 2700 | 1950 | - | 100 | 100 | 300 | 3000 | 3600 |
| 45 | - | - | - | - | - | - | 500 | 1100 | 1500 | 1800 |
| 47 | 4000 | 4800 | 3300 | 2900 | - | - | - | - | - | - |
| 270 | 4150 | 4300 | 3600 | 3500 | - | 600 | 100 | 100 | 1100 | 1800 |
| Mean | 4100 | 4000 | 3200 | 2783 | - | 350 | 250 | 500 | 1866 | 2400 |

TABLE XIII

PERCENT OF SPERM SHOWING PROGRESSIVE MOTILITY

| | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Shropshire** | | | | | | | | | | |
| 0 | 70 | 30 | 60 | - | - | - | 70 | 90 | 80 | 90 |
| 241 | 10 | 50 | 80 | 70 | 60 | 70 | 80 | 90 | 80 | 90 |
| 967 | 80 | 60 | 70 | 10 | 10 | - | 10 | 40 | 80 | 70 |
| 192 | 70 | 60 | 60 | 10 | 10 | 10 | 40 | 50 | 70 | 70 |
| 973 | 80 | 80 | 70 | 70 | 40 | 10 | 70 | 20 | 90 | 90 |
| Mean | 62 | 56 | 68 | 40 | 30 | 22 | 54 | 58 | 80 | 82 |
| **Hampshire** | | | | | | | | | | |
| 42 | 60 | 20 | 40 | 70 | 20 | 10 | 20 | 70 | 80 | 90 |
| 45 | - | - | - | - | - | - | 80 | 70 | 80 | 90 |
| 47 | 60 | 70 | 80 | 70 | - | - | - | - | - | - |
| 270 | 80 | 10 | 60 | 10 | - | - | 10 | 40 | 80 | 80 |
| Mean | 73 | 33 | 60 | 50 | 10 | 10 | 36 | 60 | 80 | 86 |

TABLE XIV

PERCENT OF ABNORMAL SPERM

| | March | May | July | September | December |
|---|---|---|---|---|---|
| **Shropshire** | | | | | |
| 0 | 39 | 28 | - | 48 | 28 |
| 241 | 20 | 16 | 43 | 48 | 44 |
| 967 | 20 | 23 | 68 | 40 | 25 |
| 187 | - | - | - | - | - |
| 192 | 20 | 29 | 90 | 46 | 31 |
| 973 | 41 | 24 | 49 | 100 | 27 |
| **Average** | 28 | 24 | 62 | 56 | 20 |
| **Hampshire** | | | | | |
| 42 | 38 | 25 | 52 | 66 | 27 |
| 45 | - | - | 44 | 37 | 29 |
| 47 | 14 | 23 | 45 | 80 | 15 |
| 270 | 23 | 43 | - | - | - |
| **Average** | 25 | 29 | 48 | 61 | 34 |

obtain a statistical correlation. The relationship between thyroid secretion rate and semen quality factors studied are presented in Figure 8 for the Shropshire rams and Figure 9 for the Hampshire rams.

There has not been complete agreement among research workers as to the role of the thyroid in male fertility. Bogart and Mayer (1946) concluded that temperature affects the reproductive mechanism of the ram through the thyroid gland and that the sensitivity of the reproductive organs of the ram is a reflection of the sensitivity of the thyroid to changes in environmental temperature. Moore (1939) believed that the thyroid gland had no direct effect on the testes and that reproductive disturbances were due to changed metabolic state. Cameron (1945) believed that the effects were due to complex inter-relations between the endocrine system and body metabolism as a whole.

Apparently the thyroid secretion is concerned with the normal balance between the pituitary and reproductive glands, since the level of pituitary gonadotrophin is reduced in thyroidectomized goats according to Reineke, Bergman and Turner (1941). The time lag observed between changes in thyroid secretion rate and changes in semen quality indicate that there is a relationship between the body metabolism and semen quality. Magsood and Reineke (1950) observed that the optimal temperature and the optimal level of hormone therapy were the same for body growth and testes stimulation.

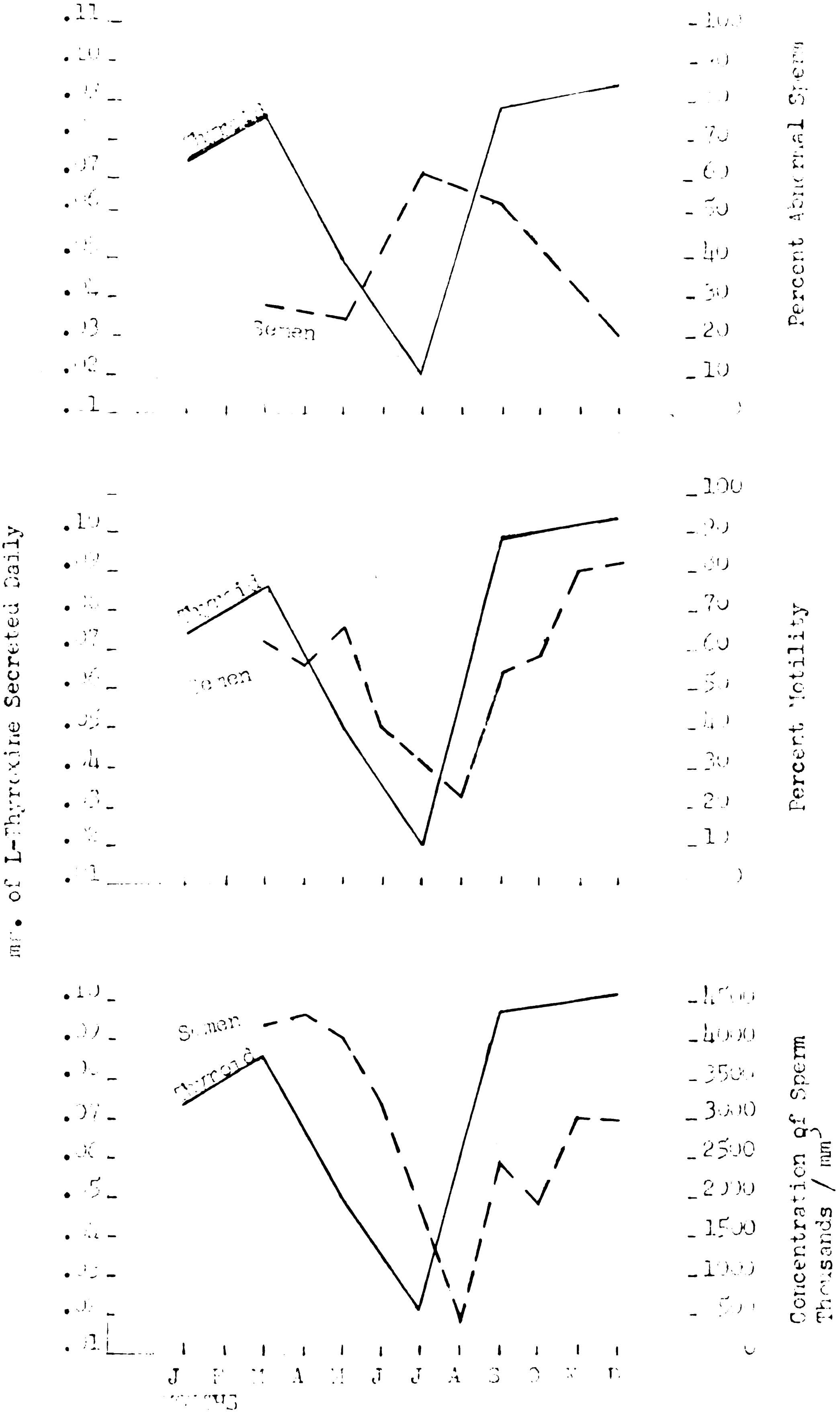



FIGURE 8
The Comparison of Thyroid Secretion Rate to Semen Characteristics in Shropshire Rams.

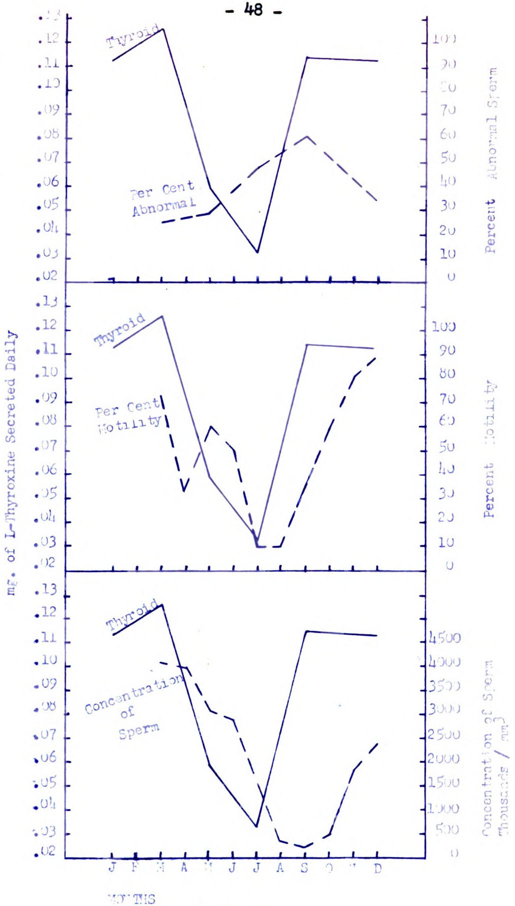



FIGURE 9

The Comparison of Thyroid Secretion Rate to Semen Characteristics in Hampshire Rams.

Many experiments have been conducted on "summer sterility" in rams, McKenzie and Berliner (1937); Berliner and Warbritton (1937); Green (1940) and Bogart and Mayer (1946). The results of these workers show a progressive decline in semen quality during the summer months with recovery during the breeding season in the fall. McKenzie and Berliner (1937) observed that Shropshire rams showed a greater decline in semen quality that extended over a longer period of time than Hampshire rams. In this experiment superiority of Hampshire rams was not observed.

Research has also been conducted on the use of thyro-active substances to improve the semen quality of rams during the summer months, Warwick et al. (1948); Eaton et al. (1948); and Black (1950). There is a great deal of variation in the results of these workers. Some found beneficial effects, some deleterious effects and some no effect. It appears that this variation is due in part to the different levels of administration. Wilworth et al. (1954) working with Rhode Island Red Cocks found that there was an optimal level of thyroprotein administration for increasing semen quality. When higher levels were given the quality of the semen fell below that of the controls.

The establishment of normal thyroid secretion rates for rams provides a guide for further work in the administration of thyro-active substances to improve semen quality. Further research in this area should be conducted.

Many factors affect the thyroid secretion rate of sheep. While breed has been shown to have an effect on the thyroid secretion rate it appears that season and sex have a greater effect.

These results indicate that the extrapolation technique should be refined so that differences in individuals can be studied. Individual differences will give the animal breeder more information for selection to improve not only the efficiency of sheep but other types of farm livestock as well. With increasing competition from other food sources it is important that every possible means of increasing the efficiency of livestock production be used.

## SUMMARY AND CONCLUSIONS

In preliminary experiments a technique was developed for measurement of daily thyroid secretion rate in intact individual sheep. A study was then carried out to determine the effects of season, breed, sex, and the relation of semen quality to thyroid secretion rate. Six Hampshire rams, six Shropshire rams, and six open Shropshire ewes were used for the study. Thyroid secretion rate determinations were made approximately every other month for one year. Semen was collected from the rams every seven to ten days during the experiment.

From these data the following conclusions were reached:

1. A technique for measuring daily thyroid secretion rate in intact individual sheep is described. This method is practical for research in large animals. While this method is suitable for studying differences between groups it is not refined enough for comparison of individuals.

2. The thyroid secretion rate of sheep is greatly affected by the season of the year. The secretion rate in July was significantly different from the secretion rate in all other months that determinations were made. A seasonal variation in thyroid

secretion rate of sheep exists with the lowest level of secretion in the summer months and the highest levels in the fall and winter.

3. There is a difference in the thyroid secretion rate of Hampshire rams and Shropshire rams. This difference is significant at the one percent level. Hampshire and Shropshire rams both show the same seasonal variation in thyroid secretion rate with the secretion rate for the Hampshire rams higher than the secretion rate of Shropshire rams during every month in which determinations were made.

4. There is a difference in the thyroid secretion rate of Shropshire rams and Shropshire ewes. This difference is significant at the one percent level. The Shropshire rams and the Shropshire ewes exhibit the same seasonal variation in thyroid rate with the ewes showing a higher secretion rate in every month that determinations were made. Both the rams and ewes showed the lowest secretion rate in July.

5. There is a relationship between semen quality in the ram and thyroid secretion rate. The seasonal variation in thyroid secretion rate was closely paralleled by the seasonal change in semen quality. An apparent time lag exists between changes in thyroid secretion rate and changes in semen quality.

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