THE THYROID SECRETION RATE OF SHEEP AS AFFECTED BY: SEASON, AGE, BREED, PREGNANCY AND LACTATION

by

Harold A. Henneman

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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Department of Animal Husbandry

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AN ABSTRACT

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This investigation was undertaken for two reasons. First, to develop a practical technique for determining thyroid secretion rates in intact, individual sheep. Second, to determine the effect of season, age, breed, pregnancy and lactation on the thyroid secretion rate of sheep.

With the background of knowledge reported by Perry (1951), Wolff (1951), and Terry (1951), preliminary investigations were conducted to develop a technique for measuring thyroid secretion in the live sheep.

External thyroid counts were taken on two control ewes to determine the time after injection of I^{131} that a definite trend of output was established. Efforts were made to maintain external thyroid counts at one hundred per cent of previous count by the subcutaneous injection of l-thyroxin This procedure did not prove practical.

An attempt was made to correlate percentage of previous count to milligrams of l-thyroxine injected. However, there was an indication that when more thyroxine was injected than what is normally secreted by the thyroid, there was a great outpouring of I^{131} from the gland. Two ewes were given overdoses of l-thyroxine and this phenomenon was confirmed.

A practical method was then devised to determine the daily thyroid secretion rate. Statistical methods were employed to extrapolate the daily secretion rate from the linear repression of l-thyroxine injections on per cent of previous external count with individual sheep.

One microcurie of I^{131} per pound of body weight was injected subcutaneously. At seven and ten days post injection, base counts were taken over the thyroid gland with the Gieger counter. A background count was taken on the outside of the foreleg. Four additional counts were taken at three-day intervals. For the first three-day period following the determination of the base counts, the sheep were handled the same as during the base period. For the second three-day period, a small quantity of l-thyroxine was injected daily; during each successive three-day period the injections of l-thyroxine were increased. It was important that the maximum amount of l-thyroxine injected daily remain below the actual secretion of the sheep.

The counts were then corrected for background and physical decay and the percentage of previous corrected count was computed. As the quantity of l-thyroxine injected daily increased the percentage of previous count increased. The predicted daily secretion rate was then extrapolated by linear regression to the point where the percentage of previous count was one hundred per cent. This level of l-thyroxine was then assumed to be the daily secretion rate.

This procedure was used to determine the secretion rate of four groups of six sheep each and the differences were analyzed by the analysis of variance.

The secretion rate in July (0.078 $\pm$ 0.020 mg.) was significantly different at the one per cent point from determinations made in January, March, May, September and December.

Pregnancy in sheep does not cause a significant difference in thyroid secretion. Lactation creates increased demands upon the thyroid gland. The secretion rates in lactating, pregnant and non-pregnant ewes during March were; 0.541 $\pm$ 0.096; 0.390 $\pm$ 0.042; and 0.346 $\pm$ 0.036 mg., respectively.

During the months of January, March, May, September and December the two-year old ewes secreted more thyroxine than four-year old ewes.

Only during the month of September did there appear to be a difference between breeds; Hampshire, 0.350 $\pm$ 0.083 mg. and Shropshire, 0.254 $\pm$ 0.036 mg.

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INTRODUCTION

Research conducted along several different lines indicates that the thyroid secretion plays an important role in the physiology of many of the life processes important to animal production.

It has been established that the thyroid hormone regulates the general metabolism of the body. Because of its action upon metabolic processes, it might be anticipated that the thyroid hormone would exert an important influence upon growth and differentiation. By depriving a young animal of thyroxine, growth is retarded and the sexual organs remain infantile.

Various experiments have been run in order to study the effect on growth and fertility of added thyroactive substances in the rations of animals. The data are very conflicting due in part, at least, to the varying levels of hyperthyroidism induced.

This study has been designed to gain more basic information on normal thyroid secretion rates in sheep and the effect of various environmental factors on the secretion rate. If thyroid secretion rates could be determined, a valuable landmark could be established for the physiological dosage range in future studies of the effects of thyroidal stimulation. Such data might also be useful in developing more efficient production methods. At the present time the feeding of iodinated protein to lactating dairy cows has been shown to increase milk and fat production.

Indirectly, previous research has indicated that the thyroid gland is involved in the problem of summer sterility in rams and may be a factor contributing to the seasonal breeding habit of the ewe. If sufficient variation in thyroid secretion rates exist between individuals within breeds, it may be possible to select on the basis of thyroid activity in an effort to increase growth rate and milk production and to overcome summer sterility in rams.

All of these factors would be of importance in increasing the productiveness and economic importance of sheep in the nation's economy. It is also believed that the methods developed in this research will be adaptable for studies with other classes of livestock.

OBJECTIVES

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

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

    a. Season of the year

    b. Age of the sheep

    c. Breed

    d. Pregnancy

    e. Lactation

REVIEW OF LITERATURE

Magnus-Levy (1895) first observed that the feeding of thyroidal materials caused an increase in the oxygen-carbon dioxide exchange. This observation has provided the basis for modern clinical metabolism determinations as one measure of thyroid status.

Mørch (1929) determined that the open-circuit type of respirometer was most suitable for the measurement of carbon-dioxide production. Teitelbaum and Horne (1941) observed that the closed-circuit type of respirometer permitted direct measurement of the volume of oxygen consumed.

Many investigators have made use of these measurements of metabolism as an indirect measure of thyroid status in laboratory animals or particularly for the assay of thyroidal materials.

With the discovery of the goitrogenic compounds, a method of determining thyroid secretion rate was developed. Goitrogens inhibit the formation of thyroxine, which permits the increased secretion of thyrotrophic hormone by the pituitary; this in turn stimulates the thyroid and causes the gland to increase in size. The enlargement of the thyroid can be prevented by the administration of thyroxine in sufficient amounts to bring the pituitary and thyroid back into normal balance.

Dempsey and Astwood (1943) observed that the decrease in thyroid weight in thiouracil-treated rats bore a quantitative relation to thyroxine dosage. The relationship between thyroid weight in milligrams per one hundred grams body weight and thyroxine dosage is a straight line. The point where the response curve intercepts the normal thyroid weight represents the amount of thyroxine required to maintain the normal thyroid-pituitary balance. These workers determined the thyroid secretion rates of rats at several different environmental temperatures. They found that the thyroid secretion rate is increased markedly at low environmental temperatures and depressed at high temperatures.

Mixner et. al. (1944) observed that the thyroid of the chick responded in a similar manner and determined that female chicks had a higher thyroid secretion rate than male chicks.

Monroe and Turner (1944) reported the secretion rate per unit of body weight is nearly constant in male rats, whereas in females the rate declines with increasing size. No significant differences from normal were found in pregnant or lactating rats.

Reineke and Turner (1945a) observed a seasonal trend in the thyroid secretion rate of young chicks. The secretion rate in the spring and summer was only about one-half the winter level.

A pronounced decline in thyroid secretion rates of mice were reported by Hurst and Turner (1945), when the temperature was increased from 80° to 87°F. Differences were observed, also, in the secretion rates of three strains of mice.

Schultze and Turner (1945) determined that growing female goats averaging 20.4 kilograms secreted 0.64 mg. of D,L-thyroxine daily while at a weight of 34.5 kilograms the secretion was 0.93 mg. of D,L-thyroxine daily.

Turner (1948) observed a pronounced decline in the thyroid secretion rate of older hens as determined by the goiter-prevention method.

For accuracy it has been suggested that five groups of animals with eight to ten animals per group be used in order to get a reliable figure for normal thyroid weight and also a good curve relating thyroid weight to thyroxine dosage. While this method is satisfactory for laboratory animals, it has definite limitations for use with large animals due to the expense of having to slaughter the animals in order to secure the thyroid weight. It is also impossible to determine individual secretion rates by this method.

Since the discovery of iodine in the thyroid gland by Baumann (1896) and the fact that this iodine in the thyroid is protein-bound, (Baumann and Roos, 1896) a great deal of work has been carried out in an attempt to improve the chemical assay of thyroxine in the thyroid gland, (Hunter, 1910; Kendall, 1914; Harington, 1933; Leland and Foster, 1932; Blau, 1935; Reineke et. al., 1945b; Doery, 1945; Taurog and Chaikoff, 1946). These methods as well as those of Harvey (1935); McClendon (1928); Chaney (1940); Salter and Johnston (1948) and others have been used to determine the amount of protein-bound iodine in blood or blood serum as a measure of thyroid activity.

This determination of protein-bound iodine in blood serum as an index of thyroid activity has been used by Long, et. al. (1951) for a study in which a significant difference was found between breeds of dairy and beef cattle and also a difference due to age. Lewis and Ralston (1952) determined the plasma protein-bound iodine concentrations of cows, before and after calving; calves at birth and again at twelve hours and three days; and colostrum as compared to milk.

The above method shows promise as a practical way to measure thyroid activity. However, the question has been raised as to whether or not protein-bound iodine in the blood is actually a measure of thyroxine secretion. At the present time investigators who have used this method of protein-bound iodine determination would like a reliable method to measure thyroid secretion rate which could be used to check the results of the PBI method.

With the availability of the radioactive isotope I^{131} new methods have been suggested for determining thyroid activity. Scott et. al. (1951) have suggested a method of determination based on the Red Blood Cell I^{131}/Plasma I^{131} ratio.

Perry (1951) measured the rate of loss of radioactive iodine from the thyroid gland of the rat with an external counter and determined an index of the secretion of thyroid hormone. He checked the validity of this index by the effect of thyrotrophic hormone, goitrogens and thyroxine on the rate of loss of I^{131}. He was able to establish a relationship between dose of thyroxine and degree of inhibition of thyroid hormone secretion. However, his thyroid secretion value shows poor agreement with results obtained by the goitrogen method.

Wolff (1951) used a technique similar to Perry's in studying some factors that influence the release of iodine from the thyroid gland.

Winchester and Davis (1952) destroyed the thyroids of chicks with radioiodine and then injected varying levels of D,L-thyroxine daily to different groups. They observed that birds receiving two and four micrograms most nearly equaled the performance of comparable controls.

In this study it was hoped that sufficient support could be obtained so that at least two methods of measuring thyroid activity could be run simultaneously, one as a check against the other. However, this objective was not achieved and the writer had to settle on one technique. The technique which Perry (1951) describes was selected as a pattern from which our own technique was established by preliminary experiments.

EXPERIMENTAL PROCEDURE

A. Development of a Technique for Measuring Thyroid Secretion Rate in the Live Sheep

Terry (1951) injected subcutaneously one microcurie of radioactive iodine per pound of body weight of sheep and obtained external thyroid counts with the Geiger counter. This level of I^{131} was selected for injection subcutaneously in these experiments and was found to give reliable counts. All external thyroid counts were taken using a Nuclear Instrument and Chemical Corporation laboratory monitor with an end-window Geiger-Müller tube. The counts were taken in the area of the neck where the highest count could be obtained. The same amount of pressure was used in putting the tube against the neck, insofar as humanly possible.

The radioactive I^{131} serves only to tag or mark the thyroid gland and its secretion so that it can be measured with a Geiger tube and monitor. It was assumed that the large amounts of I^{127} present in the system perform exactly as the small amount of I^{131} which was measured.

A standard source of Co^{60} was used to check the machine at the time counts were taken on the sheep. This served to eliminate any error due to variable efficiency of the Geiger tube and monitor, as each day's readings were corrected to the standard. A shield was used on the Geiger tube when all standard, background and external thyroid counts were taken so only gamma rays were counted.

The background counts were taken on the outside of the foreleg of the sheep and these were subtracted from the external thyroid counts. This procedure corrected approximately for any gamma emissions from I^{131} in the blood stream. Lastly, the external thyroid counts were corrected to zero time for physical decay of the isotope.

All of the sheep were fed iodized salt, free-choice, in an attempt to insure uniform iodine intake.

The corrected external thyroid counts per minute are recorded in Table I, and are shown graphically in Figure 1, for two control ewes.

The curves in Figure 1 are typical of the normal uptake and output curves of I^{131} in sheep. By computation the Hampshire ewe was secreting daily, 12.27 per cent of the iodine in the thyroid gland, while the Shropshire ewe was secreting daily, 7.80 per cent. In this study we were concerned with the thyroid output and, from Figure 1, seven days after injection of I^{131} was selected as the earliest reliable time that sheep reach a rather steady output of I^{131}.

TABLE I

EXTERNAL THYROID COUNTS PER MINUTE OF TWO CONTROL EWES

Time after Injection	Hampshire Ewe	Shropshire Ewe
6 hours	797	409
20 hours	1636	1102
25 hours	2314	1488
2 days	2770	2167
3 days	5332	3538
4 days	3587	3697
5 days	2966	3418
6 days	3365	3498
7 days	2639	2967
8 days	2565	3128
10 days	2013	2368
13 days	1416	1533
16 days	892	1400
17 days	841	1186
22 days	565	925

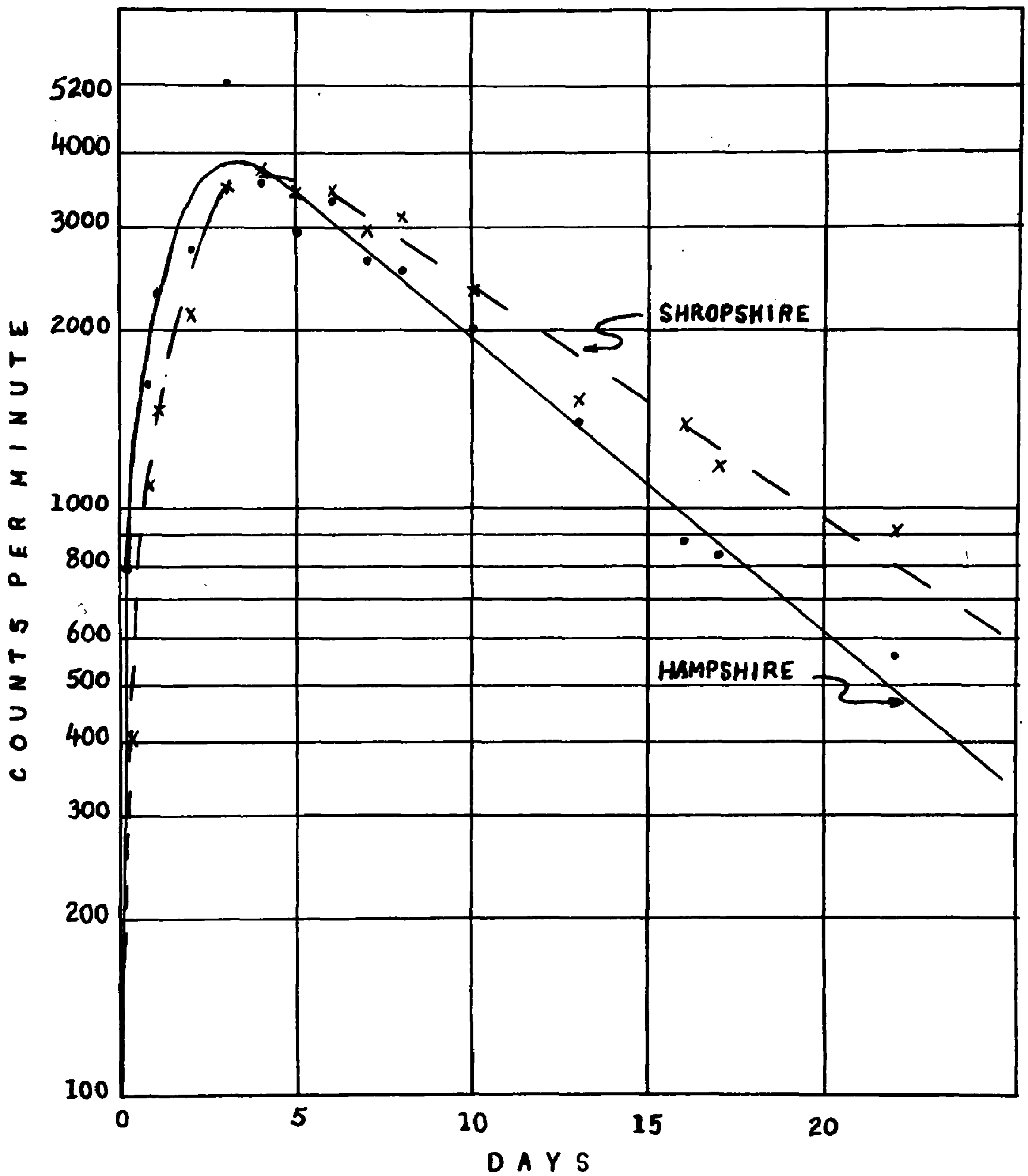

Fig. 1. External thyroid counts on two control ewes

<u>Trial I - An attempt to maintain external thyroid counts at one hundred per cent of previous count.</u> Perry (1951) maintained external counts at one hundred per cent in rats by a daily injection of 15 micrograms of l-thyroxine.

In this trial with sheep a base reading was taken on the third day after injection of I^{131}. This was considered zero time and all thyroid counts were corrected for physical decay back to this time. On the seventh day after injection of I^{131} a quantity of l-thyroxine was injected subcutaneously. The l-thyroxine injected was expected to exert an effect on the level of the next day's count. At this time the l-thyroxine was used in the form supplied by Glaxo Laboratories, Greenford, Middlesex, England.

The dry thyroxine was weighed on an analytical balance and dissolved in distilled water which was made slightly alkaline with NaOH. Then enough acid (HCL) was added to make the solution slightly cloudy. This solution was then stored in the refrigerator and used within two weeks.

Four ewes were used in this trial and the data are presented in Tables II and III; also in graphic form in Figures 2 and 3.

It was assumed in this trial that when the count remained at approximately one hundred per cent of previous count, the injected l-thyroxine was sufficient to replace the normal thyroid secretion. In other words, the animal's thyroid gland was not secreting any thyroxine.

TABLE II

EFFECT OF 1-THYROXINE INJECTIONS ON EXTERNAL THYROID COUNTS OF SHROPSHIRE AND OXFORD EWES

Days Following Injection of I^{131}	Shropshire			Oxford		
	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute	% of Previous Count	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute	% of Previous Count
3 (Zero time)		5410			4580	
4		4548			4185	
5		5893			4363	
6		5369			4705	
7	0.8	5241		0.7	4369	
8	0.8	5458	104.1	0.7	4013	90.2
9	0.8		97.1	0.7		93.5
10	0.8	5148	97.1	0.7	3810	93.5
11	0.9	4665	90.6	0.8	3285	91.9
12	0.9		88.7	0.8		91.8
13	0.9	3671	88.7	0.8	3276	91.8
14	1.0	4026	109.6	0.9	3244	107.8
15		3242	80.5		3069	87.3
16		2798	86.3		2195	83.4
17		2630	93.9		1940	86.8
21		1664			1566	

TABLE III

EFFECT OF 1-THYROXINE INJECTIONS ON EXTERNAL THYROID COUNTS OF DORSET AND RAMBOUILLET EWES

Days Following Injection of I^{131}	Dorset			Rambouillet		
	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute	% of Previous Count	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute	% of Previous Count
3 (zero time)		4410			7280	
4		3974			6292	
5		4363			7156	
6		4945			6683	
7	0.6	3923		0.8	6960	
8	0.5	4959	126.4	0.9	6283	90.2
9	0.5		95.4	0.9		93.5
10	0.5	4515	95.4	0.9	5497	93.5
11	0.6	4492	99.4	1.0	5055	91.9
12	0.6		93.0	1.0		91.8
13	0.6	3884	93.0	1.0	4263	91.8
14	0.7	4298	110.6	1.1	4599	107.8
15		3258	75.8		4016	87.3
16		2680	82.2		3350	83.4
17		2408	89.8		2911	86.8
21		1744			1780	

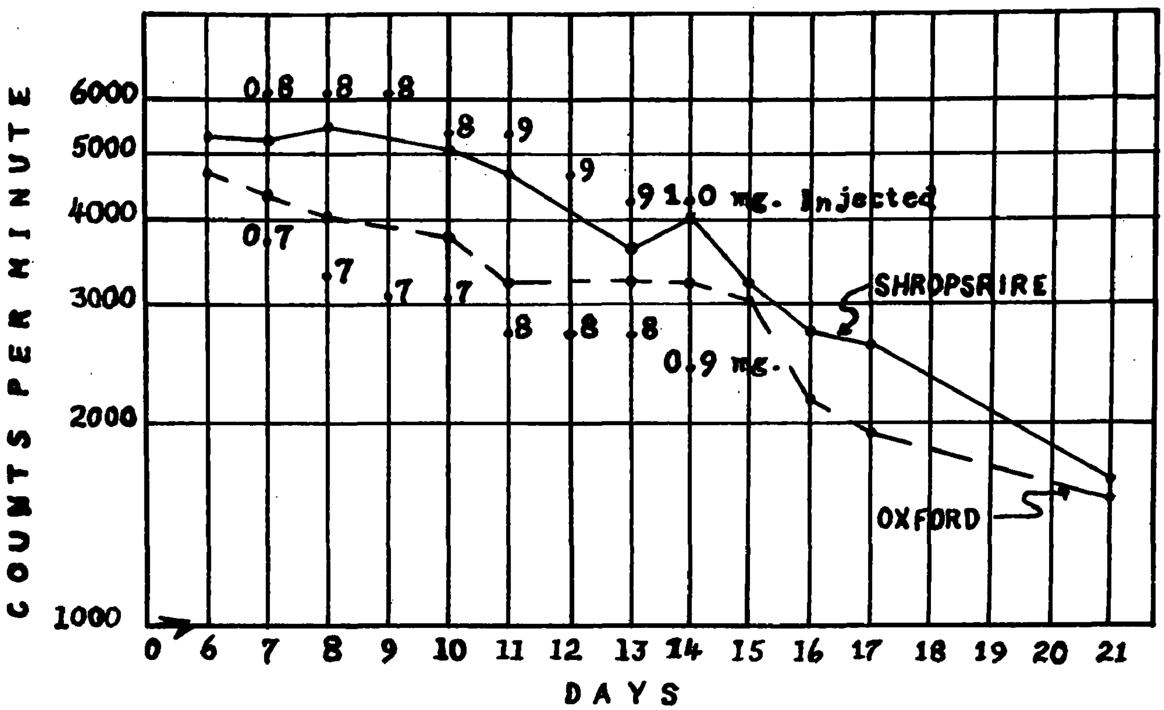

Fig. 2. Effect of l-thyroxine injections on external thyroid counts.

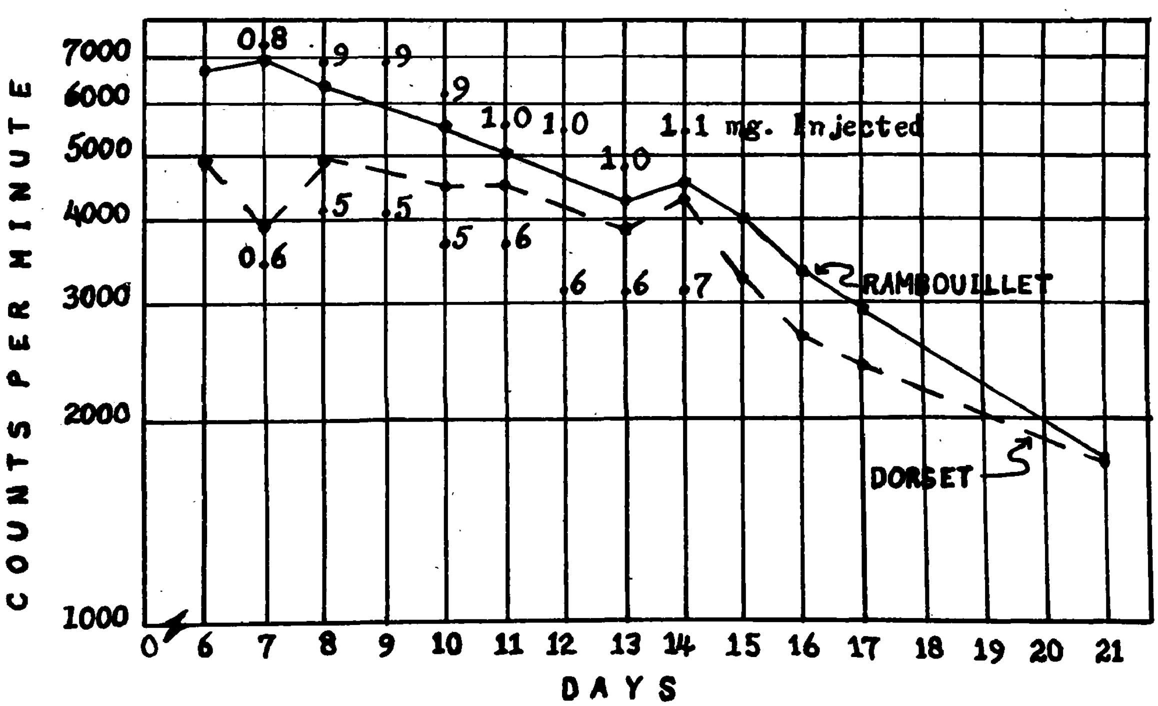

Fig. 3. Effect of l-thyroxine injections on external thyroid counts.

However, as the trial progressed it appeared less and less likely that a very good estimate of daily thyroid secretion rate could be made. It was more or less a trial and error method and it was obvious that a great many readings and injections would be necessary before a reasonable estimate of secretion rate could be made.

At the start of the trial it was believed that if more than the daily output of thyroxine was injected the counts in the thyroid would remain at one hundred per cent. However, with the Rambouillet and Shropshire ewes especially, the per cent of previous count was decreasing as the thyroxine injections were increased. Also, when all four ewes received their largest injections of thyroxine the greatest drop in counts was observed.

Probably, in the case of the Oxford ewe, the daily secretion rate was approached when 0.8 mg. of l-thyroxine was injected daily. However, the method did not appear to be very practical and therefore a second trial was started to test a different hypothesis.

Trial II - Predicting secretion rate by linear regression using three groups of ewes. In the second trial twelve ewes were injected with one microcurie of I^{131} per pound of body weight and seven days later a base count was taken on the thyroid gland. The four ewes in Group A were then injected daily for three days with 0.4 mg. of l-thyroxine. The four ewes in Group B received a similar schedule of l-thyroxine injections of 0.8 mg. daily and Group C received daily l-thyroxine injections of 1.2 mg. daily. Injections of l-thyroxine were made for three days in order that the thyroid gland might adjust its secretion to the daily injections. Three days after the base counts were taken of I^{131} in the thyroid, a second external count was taken and corrected for physical decay. The percentage of previous count was then determined for each ewe and the predicting equation was determined. In this trial the predicting equation was $Y = -.504 + 1.264\,x$. When x is equal to 1 (or 100%), Y = 0.76 mg. with a standard error of the predicting line of 0.28 mg. (Figure 4). This value of 0.76 mg. was then assumed to be the average daily thyroid secretion rate of these twelve ewes.

However, from the data, which appear in Table IV and Figure 4, it appeared that, at the highest level of l-thyroxine injection (1.2 mg. daily), the animals' thyroid glands were actually secreting as much or more I^{131} than when only 0.8 mg. of l-thyroxine was injected. From the plotted data in Figure 4 it appears that when the injections of exogenous thyroxine surpass the normal daily secretion the data no longer fit the straight line. This same observation was made in Trial I and therefore a third trial was started to check this assumption.

<u>Trial III - The effect of large daily injections of l-thyroxine.</u> Continued external thyroid counts were taken on two sheep that were included in the previous trial. These data along with the l-thyroxine injections are included in Table V and Figure 5. Shropshire 215 received l-thyroxine injections of 0.8 mg. daily for three days and the thyroid counts remained at approximately one hundred per cent of previous count. Then the l-thyroxine injections were increased to 1.5 mg. daily and the external counts fell rapidly, even more rapidly than when no thyroxine was injected.

TABLE IV

MILLIGRAMS OF 1-THYROXINE INJECTED DAILY AND PERCENTAGE OF PREVIOUS COUNTS FOR THE TWELVE EWES

Group A	Mg. of 1-Thyroxine Injected Daily	Per Cent of Previous Count
Shropshire 209	0.4	90.7
Hampshire	0.4	91.9
Shropshire 929	0.4	102.2
Shropshire 916	0.4	86.5
	Average	92.0
Group B		
Dorset	0.8	115.7
Shropshire 215	0.8	121.5
Shropshire 911	0.8	96.7
Shropshire 923	0.8	97.3
	Average	112.3
Group C		
Rambouillet	1.2	119.2
Oxford	1.2	87.1
Shropshire 940	1.2	106.8
Shropshire 952	1.2	122.8
	Average	106.4

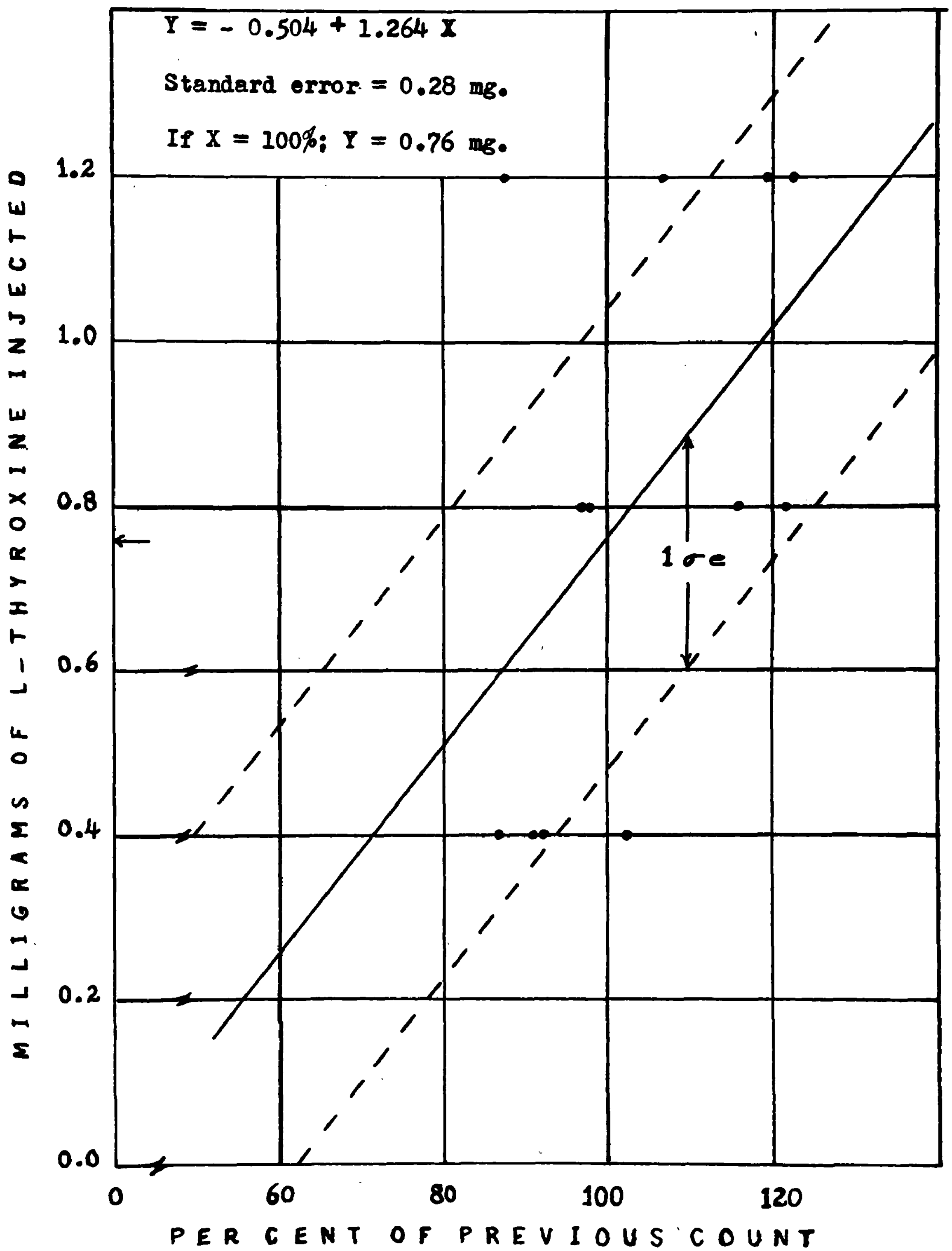

Fig. 4. Predicting equation using data obtained on twelve ewes

TABLE V

EXTERNAL THYROID COUNTS OF TWO EWES RECEIVING LARGE DOSES OF INJECTED 1-THYROXINE

Date	Shropshire 215		Shropshire 209	
	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute	Mg. of 1-Thyroxine Injected	Thyroid Counts Per Minute
Nov. 3		1340		2798
Nov. 4		1140		2630
Nov. 8				1664
Nov. 9	0.8	903	0.4	1400
Nov. 10	0.8		0.4	
Nov. 11	0.8		0.4	
Nov. 12	1.5	918	1.5	1057
Nov. 13	1.5		1.5	
Nov. 14	1.5	759	1.5	827
Nov. 15	1.5	577	1.5	722
Nov. 16		428		542
Nov. 17		375		469

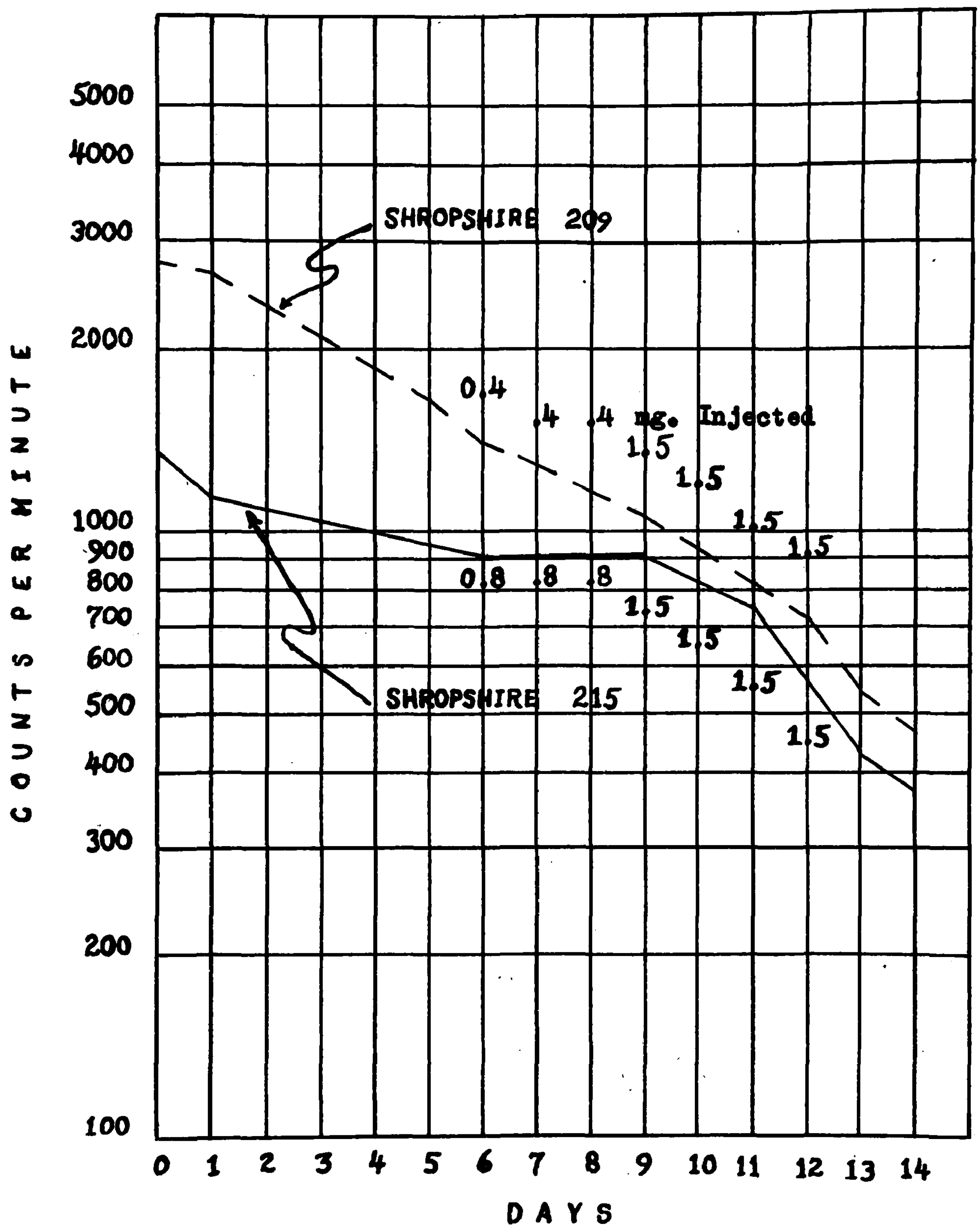

Fig. 5. Effect of large daily injections of l-thyroxine on external thyroid counts.

Shropshire 209 received only 0.4 mg. of l-thyroxine daily by injection and as expected the counts were not held at one hundred per cent. However, when this ewe received 1.5 mg. of thyroxine daily the percentage decline in counts was even greater.

These results confirmed earlier suspicions that when more thyroxine is injected than what is normally secreted by the thyroid gland, there is a great outpouring of I^{131} from the thyroid. Cornwall (1950) observed that the secretion rate of chicks receiving one microgram of exogenous thyroxine was inhibited more than in groups of chicks injected with two and three microgram doses. Therefore, it was concluded that in order to predict the daily thyroid secretion rate by correlating daily injections of l-thyroxine to percentages of previous count, it would be necessary to inject thyroxine in quantities which were below the daily thyroid secretion rate. Thyroxine secretion could then be estimated by extrapolating the prediction line so obtained to the point where no further discharge of I^{131} was indicated.

<u>Trial IV - Extrapolating the thyroid secretion rate from varying levels of thyroxine injection and a series of external counts on individual lambs.</u> Previous to the start of this trial the stock supply of l-thyroxine was further purified by Dr. E. P. Reineke. The activity of the l-thyroxine increased, necessitating a lower level of thyroxine injections if reliable estimates of secretion rate were to be obtained.

Four lambs were used in this trial. One microcurie of I^{131} per pound of body weight was injected subcutaneously. At seven (zero time) and ten days, post injection of I^{131}, base counts were taken over the thyroid gland with the Geiger counter. A background count was taken on the outside of the foreleg. Four more counts were taken at three-day intervals. For the first three-day period following the determination of the base counts the sheep were handled the same as during the base period; that is, no l-thyroxine was injected. For the second three-day period 0.1 mg. of l-thyroxine was injected daily and a reading of external counts was made. During the next three-day period 0.25 mg. of thyroxine was injected daily into each lamb and then for the last three-day period, 0.5 mg. of thyroxine was injected.

These counts were then corrected for background and physical decay, to zero time, and the percentage of previous count was computed. As the quantity of l-thyroxine injected daily was increased, the percentage of the previous count increased, which indicated that the thyroid itself was secreting less thyroxine.

The data are presented in Table VI.

TABLE VI

MILLIGRAMS OF l-THYROXINE INJECTED DAILY AND PERCENTAGES OF PREVIOUS COUNT OF FOUR LAMBS

Lamb	% Previous Count No Thyroxine Injection	% Previous Count 0.1 mg. Thyroxine	% Previous Count 0.25 mg. Thyroxine	% Previous Count 0.5 mg. Thyroxine
Shropshire	72.2	90.9	93.8	81.2
Cross Bred 52	71.2	84.5	66.3	93.8
Cross Bred 69	80.6	66.3	80.6	92.6
Hampshire	59.4	63.7	80.7	82.1

By combining the data of all four lambs there were sixteen observations which could be analyzed statistically and an average secretion rate for the entire group determined. This procedure was followed and is expressed graphically in Figure 6. From the predicting equation, $Y = -.585 + 1.03\,x$, the average daily secretion rate for the four lambs was found to be 0.445 mg. when x was equal to one hundred per cent. The standard error of the predicting line was 0.15 mg. The standard deviation of the "b" value was $\pm$ 0.37 mg. and "b" was significantly different from zero at the two per cent level. The correlation coefficient between milligrams of l-thyroxine injected and per cent of previous count was 0.552, which was significant at the five per cent level.

Without question, this method of analysis would be the most accurate for predicting an average daily secretion rate for a group of sheep, as there are more observations to support the predicted value. However, in the study to be presented it was mandatory to obtain predicted values for individual sheep so that an estimate of variation within groups could be obtained. This could be used as a basis for testing significance between group means by the method of analysis of variance. It was fully realized that the predicted values for individual sheep, which were based on only four observations, were not very reliable.

By computing the predicted values for each of the four lambs the secretion rates were as follows: Shropshire: 0.263 mg.; Crossbred 52: 0.428 mg.; Crossbred 69: 0.503 mg.; and Hampshire: 0.695 mg. None of

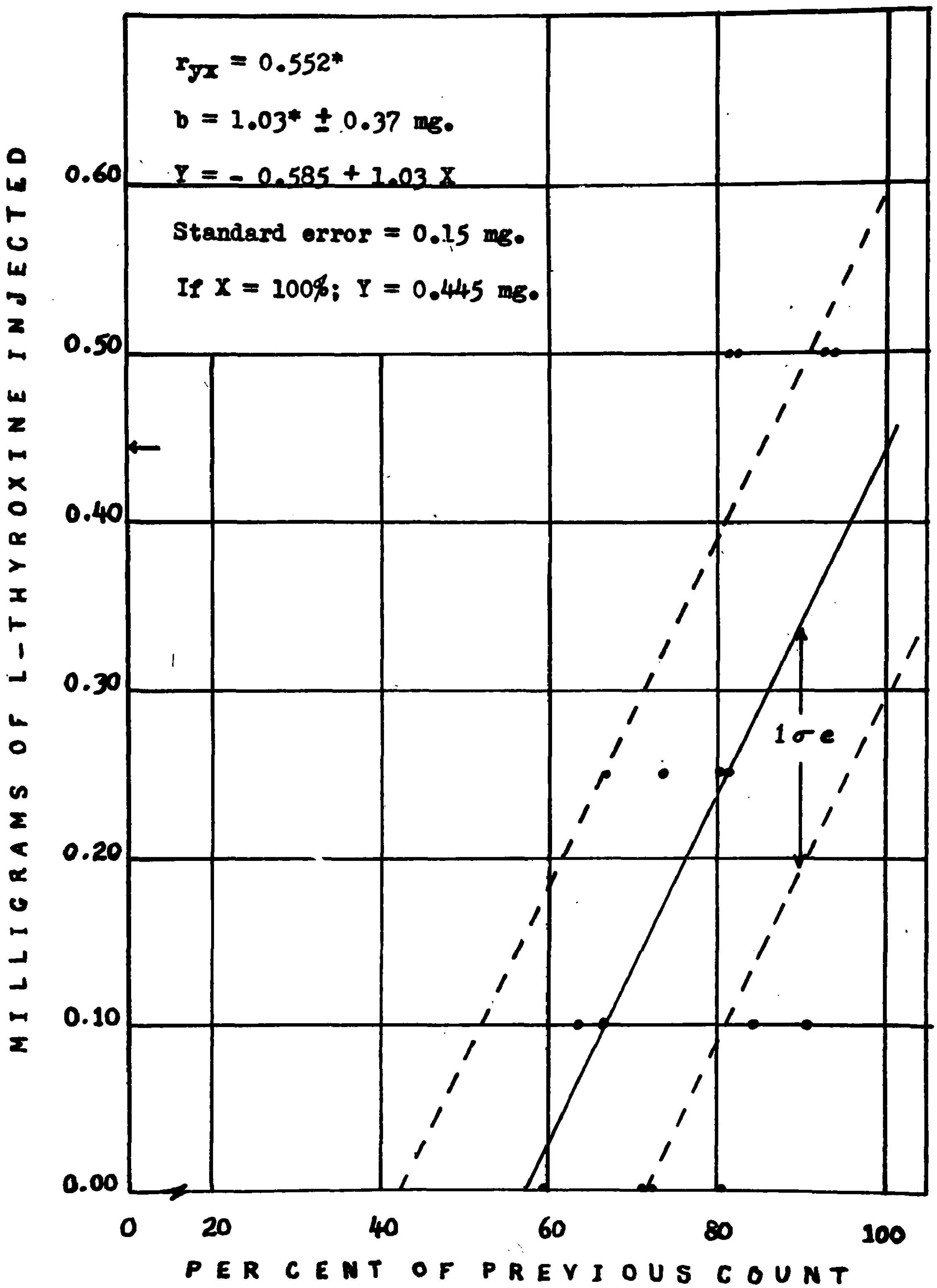

Fig. 6. Predicting daily thyroid secretion rate from per cent of previous count on a group of four lambs.

the "b" values were significantly different from zero at the five per cent level, nor were the correlations, between injected thyroxine and per cent of previous count, significant at the five per cent level. However, the average daily secretion rate for the group as determined from the individual predicted values, was 0.472 mg. as compared to 0.445 mg. which was obtained by the previously mentioned statistical method.

This method of procedure for obtaining and analyzing the data was accepted as the one to use in studying the differences in thyroid secretion rate in sheep. From the data to be presented in the next section it will be apparent that this extrapolation technique gave good repeatable results. The results obtained are in line with variations in secretion rate which have been reported in other species where other techniques for estimating secretion rate have been used.

The extrapolation technique is well suited to the study of thyroidal secretion in large animals as the productive capacity of the animal is not impaired.

B. Application of the Extrapolation Technique to the Study of Differences in Thyroid Secretion Rate

Twenty-four sheep were selected at the beginning of the year to be used in studying the differences in thyroid secretion rate due to season, age, breed, pregnancy and lactation. They were divided into four groups of ewes with six animals in each group as follows:

6 Open Shropshire ewes dropped in 1950, average weight 114 pounds.

6 Bred Shropshire ewes dropped in 1950, average weight 117 pounds.

6 Bred Shropshire ewes dropped in 1948, average weight 139 pounds.

6 Bred Hampshire ewes dropped in 1950, average weight 141 pounds.

All of the Shropshire ewes dropped in 1950 were sired by the same ram. All of the Hampshire ewes were sired by the same ram. It was impossible to select six 1948 ewes from the same sire.

Due to deaths of lambs or ewes during the year the number of sheep in each group was not maintained. Half of the open ewes were accidentally bred and, therefore, only three ewes remained in the open group for the determinations in January and March. When these ewes lambed, the lambs were immediately removed and the ewes were considered as dry while the other ewes were milking.

The entire group of ewes were handled in one flock under similar feeding conditions and iodized salt was fed free-choice.

When the external thyroid counts were made the sheep were selected in the same order each time and the readings were taken at the same hours of the day. Thus, all readings were 72 hours apart.

Due to the fact that no previous estimates were available regarding secretion rates, the problem of how much l-thyroxine to inject, could only be estimated. However, the calculations were made immediately so that if it was obvious that too much thyroxine was being injected, additional three-day trials were run with a lower level of injection. During September too small amounts of thyroxine were injected to give a good spread in percentages of previous count and, therefore, an additional three-day trial was run using a higher level of injection. During July it appeared that the thyroid counts remained on a plateau for a longer period and the steady output curve was delayed. Thus, another reading was required.

These problems made it necessary to use good unbiased judgment in regard to reliable readings for use in the statistical calculations and analysis of variance.

RESULTS AND INTERPRETATION

A. Seasonal Difference in Thyroid Secretion Rate

The group of open Shropshire ewes were used to study the effect of season of the year upon daily thyroid secretion rate to alleviate any differences which may be due to pregnancy and lactation.

To study the differences in thyroid secretion rate during different months of the year the analysis of variance was used as illustrated in Table VII.

TABLE VII

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Squares	Experimental Error
Total	22	0.2565		
Months	5	0.1611	0.0322	
Error	17	0.0954	0.0056	0.075

The "F" value ($\frac{0.0322}{0.0056} = 5.7^{**}$) indicates a significant difference between at least two of the means at the one per cent level. The standard deviation of the means and standard deviation of the difference of the means was computed. The "Student t" table was then used to test for the significance of differences between the means.

There was a highly significant difference between the daily thyroid secretion rate in July as compared to the other months of the year in which readings were taken, (Table VIII). All of the college sheep were shorn in February which may account for the slight increase in secretion rate during March (0.346 mg.) as compared to January (0.287 mg.). Even though the secretion during May was not significantly different from the March reading there seems to be a trend downward in secretion rate. Also, in September, (0.286 mg.) when Shropshire ewes are generally entering their normal breeding season, their thyroid secretion rate was significantly greater than in July, (0.078 mg.) when very few ewes of the Shropshire breed have been observed to come into estrus.

These results were in line with previously reported research. Reineke and Turner (1945a) observed a seasonal trend in thyroid secretion rate of young chicks, the rate in spring and summer being only about one-half the winter level.

Brody and Procter (1932) reported a seasonal energy metabolism rhythm in sheep which they believed to be associated with the seasonal sex rhythm. Brody (1938) has also observed this seasonal metabolic rhythm in young goats prior to the first lactation.

Meites and Chandrashaker (1949) and Johnson and Meites (1950) concluded that thyroprotein treatment increased gonadotrophic response in the female mouse. Evans and Simpson (1930) observed a reduced secretion of the pituitary gonadotropins from thyroidectomized rats. Maqsood and Reineke (1950) reported that modifications in the thyroid status and environmental temperature can have a pronounced effect on sexual function as well as body growth.

TABLE VIII

MILLIGRAMS OF 1-THYROXINE SECRETED DAILY BY OPEN 1950 SHROPSHIRE EWES

1950 Shropshire Ewes	Months in Which Readings Were Taken					
	January	March	May	July	September	December
940	.220 mg.	.300 mg.	.276 mg.	.108 mg.	.383 mg.	.156 mg.
923	.260	.389	.309	.065	.277	
916	.380	.348	.236		.285	
952			.371	.081	.220	.359
929			.115	.056	.264	.340
Mean	.287	.346	.261	.078 **	.286	.285
Standard Deviation	.068	.036	.086	.020	.060	.112

** Significant at the 1% level.

Berliner and Warbritton (1937), and Bogart and Mayer (1946) concluded that high summer temperatures were a major factor in the production of summer sterility in rams and, also, the thyroid was of major importance in the reproductive physiology of the ram.

Apparently, high summer temperatures caused a reduced thyroidal secretion which was responsible for lowered sexual activity. While a great many experiments have been reported on the effect of added thyroxine on semen quality: Berliner and Warbritton (1937); Anderson (1945); Bogart and Mayer (1946); Warwick et. al. (1948); and Eaton et. al. (1948), the results are very conflicting. This may be due to the varying levels of thyroxine administration employed by the different investigators. The experiment reported herein should provide a valuable landmark in determination of the physiological dosage range for different seasons of the year in studying the effects of thyroidal stimulation.

B. The Effect of Pregnancy and Lactation on Thyroid Secretion Rate

Comparisons between ewes that were not pregnant and ewes that were pregnant, during the months of January, March and December, indicate that there was no significant difference in the daily thyroid secretion rate. This would indicate that pregnancy does not make great demands on the thyroid gland for increased secretion.

The data for making these comparisons between open, pregnant and lactating ewes are given in Tables VIII and IX.

During March, there was a significant difference at the five per cent level between non-pregnant ewes (0.346 mg.) and lactating ewes

TABLE IX

MILLIGRAMS OF 1-THYROXINE SECRETED DAILY BY PREGNANT AND LACTATING 1950 SHROPSHIRE EWES

1950 Shropshire Ewes	January	March		May	July	September	December
	Bred	Bred	Lactation	Lactation	Dry	Open	Bred
952	.295	.424					
929	.244	.324					
919	.279	.392		.339	.068	.242	.198
911	.204	.400		.312	.083		
902		.320		.312		.242	.366
924	.404		.559	.372	.062	.278	.247
903	.358		.644	.316	.071	.207	
905	.326		.384		.062	.300	.329
927	.315		.576				
Mean	.303	.390	.541	.330	.069	.254	.285
Standard Deviation	.059	.042	.096	.021	.008	.036	.076

(0.541 mg.) of the same age and breed. In May, the difference between open ewes and lactating ewes was not as great, but there was a difference at the ten per cent level of significance. Also, during March, and at the ten per cent level of significance there is a difference between pregnant ewes (0.390 mg.) and lactating ewes (0.541 mg.) of the same breed and age.

Very few data appear in the literature in regard to this problem. Monroe and Turner (1944) reported no significant differences from normal in pregnant and lactating rats. Rugh (1951) observed that the mouse thyroid was protected from radiation damage by the process of lactation indicating there was a greater output of radioactive iodine from the thyroid gland.

The literature regarding the response of lactation to the feeding of thyroidal materials has been recently reviewed by Blaxter et. al. (1949). It was shown by Graham (1934a, 1934b) and others that thyroid feeding or thyroxine injection increased both the milk and fat yield of dairy cows. The effects of iodinated casein feeding were first reported by Reineke (1942). The increase in milk yield obtained by feeding thyroidal materials has amounted to as much as fifty per cent, (Reineke, 1943; Archibald, 1945). Where milk yield increased five to twenty per cent, fat yield was increased twenty-five to fifty per cent, (VanLandingham et. al., 1944).

C. The Effect of Age on Thyroid Secretion Rate

In studying differences in thyroid secretion rate due to the effect of age, the group of Shropshire ewes that was dropped in 1950 was compared to the group of Shropshire ewes that was dropped in 1948. All of these ewes were bred, lambed and nursed their lambs.

By use of the analysis of variance, significant differences were obtained between these two different age groups except in the month of July when all of the sheep were secreting thyroxine at a very low level. In January, there was a difference between the means which was significant at the five per cent level between bred 1950 ewes (0.303 mg.) and bred 1948 ewes (0.167 mg.). In March, the difference between the bred ewes was significant only at the ten per cent level; however, the difference in secretion rate for the two groups of lactating ewes was significant at the one per cent level.

The data used for the age comparisons are presented in Tables IX and X.

During the month of May, while the ewes were nursing lambs, the younger ewes, dropped in 1950, secreted more thyroxine (0.330 mg.) than the ewes dropped in 1948 (0.114 mg.). The difference was significant at the one per cent level.

By considering all ten of the 1950 Shropshire ewes as one group (0.270 $\pm$ 0.045 mg.) and comparing them to the mean secretion rate of the 1948 Shropshire ewes, there was a significant difference between the means at the five per cent level of significance during September. Also, in December after the ewes were bred, there was a significant difference due to age, at the five per cent level.

Turner (1948) observed a pronounced decline in the thyroid secretion rate of older hens. The medical profession has also observed a

TABLE X

MILLIGRAMS OF 1-THYROXINE SECRETED DAILY BY PREGNANT AND LACTATING 1948 SHROPSHIRE EWES

1948 Shropshire Ewes	January	March		May	July	September	December
	Bred	Bred	Lactation	Lactation	Dry	Open	Bred
517	.191	.202		.139	.064	.110	
552		.188		.130	.073	.145	.158
546	.099		.341	.096	.041	.189	.140
558	.274		.191	.088	.038	.408	.220
502			.106	.117	.057	.111	.194
541	.103		.277				
Mean	.167	.195	.229	.114	.055	.193	.178
Standard Deviation	.072	.007	.089	.019	.013	.124	.036

lowering of the metabolism in humans with advancing age. By use of the protein-bound iodine technique, Long et. al. (1951) has observed differences due to age in cattle.

Here again it would be very important to take into consideration the age of the animal if experiments are designed involving added thyroxine or the feeding of iodinated casein.

D. The Effect of Breed on Thyroid Secretion Rate

Two breeds of sheep were used in this study in an attempt to determine whether or not there was any difference in daily thyroid secretion rate due to breed. The Hampshire and Shropshire ewes were all dropped in 1950, and were bred, lambed and nursed their lambs.

Data on the Shropshire ewes are presented in Table IX, and data on the Hampshires in Table XI.

While the thyroid secretion rate of the Hampshire ewes was slightly higher than that of the Shropshires at the time of every determination, a significant difference at the ten per cent point was reached only during the month of September, (Hampshires 0.350 mg., Shropshires 0.254 mg.). A higher level of significance was not obtained even when all ten Shropshires were considered as one group.

Apparently, differences between these two breeds in thyroid secretion rate are not as large as the differences due to season, age and lactation. In order to detect the smaller differences, either more sheep should be included in each group, or a more refined technique for estimating secretion rate will need to be designed.

TABLE XI

MILLIGRAMS OF 1-THYROXINE SECRETED DAILY BY PREGNANT AND LACTATING 1950 HAMPSHIRE EWES

1950 Hampshire Ewes	January	March		May	July	September	December
	Bred	Bred	Lactation	Lactation	Dry	Open	Bred
834	.172		.515	.339	.054	.352	.324
809	.441	.426		.388	.069	.434	.413
823	.347	.306		.422	.056	.283	.318
831	.469	.220		.222	.134	.252	.293
832	.503	.352		.304		.431	.317
816	.402	.710					
Mean	.389	.403	.515	.335	.078	.350	.333
Standard Deviation	.109	.161		.069	.033	.083	.045

It is quite significant that a difference was found, even at the ten per cent level of significance, during the month of September. In practical sheep raising, the Hampshire is generally considered to be an earlier lambing breed than the Shropshire. Perhaps, there is a relationship between the early breeding characteristic and thyroid secretion.

Berliner and Warbritton (1937) observed that Shropshire rams were more subject to summer sterility than Hampshire rams, and the thyroids of Hampshires were more active. They also alleviated summer sterility by the administration of thyroxine. Green (1940) reported decreasing semen quality in Shropshire rams from January through July and an increase in semen quality from July to October, which was maintained through December. These observed breed differences in rams may also be associated with the early breeding characteristic of ewes.

Mixner and Upp (1947) reported that "double cross" hybrid chicks had a considerably higher thyroid secretion rate than either "single cross" chicks or ordinary breeds. Hurst and Turner (1948) observed differences in secretion rates in three strains of mice. These studies were made by the goitre-prevention technique.

Long et. al. (1951) used the technique of protein-bound iodine determination of blood serum and observed differences between breeds of dairy and beef cattle.

While the breed of sheep may have to be taken into consideration in determining the physiological dosage range of thyroidal stimulation, it appears much more important that season, age and lactation receive more emphasis.

The establishment of these landmarks of normal thyroid secretion rates in sheep will aid in studying the effect of feeding thyroidal materials. These studies may lead to practical recommendations for increasing milk production of ewes and consequently, lamb growth. Under such conditions, more of the producers could take advantage of marketing lambs early when prices tend to be more favorable.

There is also a need for more study on the role of thyroxine in the seasonal breeding habit of the ewe. Practical experiments are also needed on the use of induced hyperthyroidism for lengthening the breeding season of sheep. By increasing the length of the breeding season, the lamb crop could be spread out over a longer period of time, thus reducing the peaks and depressions which now occur in the number of lambs marketed at different times of the year. A more uniform supply of lamb would increase merchandising efficiency.

Further research should be conducted in an effort to refine the extrapolation technique so that it might be applied to the study of individual variations in secretion rate among animals. It also would be well to check the results of this new method against the goitre-prevention technique. This could easily be accomplished with a group of feeder lambs without too much expense.

An interesting avenue of research would be that of correlating the daily thyroid secretion rate to milk production in the ewe. Milk production might be indirectly measured by growth rate of the young lamb. A correlation of thyroid secretion to daily gain in feeder lambs would also be of importance.

Finally, if there is sufficient variation in thyroid secretion rate among individual sheep, the geneticist may be interested in selecting sheep on the basis of thyroid activity. The development of more productive strains of sheep are a necessity in a competitive economy. If such characteristics as early breeding, high milk production and rapid growth rate are positively correlated with an optimum thyroid secretion, more rapid advancement toward improved strains of sheep could be made by selecting for thyroid secretion rate only rather than selecting for the three mentioned characteristics independently.

Many of the above suggestions need not be limited to sheep but should embrace the field of large animal research.

These suggested avenues of further research may not all be practical but they should add to our knowledge of the physiologic processes upon which livestock production is based.

SUMMARY AND CONCLUSIONS

After a technique was developed for measuring daily thyroid secretion rate in intact individual sheep, a study was made to determine the effect of season of the year, age of the sheep, breed, pregnancy and lactation upon thyroidal secretion.

Twenty-four ewes were divided into four groups of six ewes each as follows:

Open 1950 Shropshire ewes
Bred 1950 Shropshire ewes
Bred 1948 Shropshire ewes
Bred 1950 Hampshire ewes

Approximately every other month for one year a daily thyroid secretion rate determination was made. These data were then analyzed statistically to arrive at the following conclusions.

1. A technique is described for measuring the daily thyroid secretion rate in intact, individual sheep which is practical for large animal research. This method has an application in studying differences between groups of sheep, but is not refined enough to give reliable individual secretion rates.

2. The season of the year has a great effect upon thyroid secretion in sheep. In July, the daily secretion was 0.078 mg.

± 0.020 mg. which was only thirty per cent of the next lowest figure obtained in May, (0.261 ± 0.086 mg.). This difference was significant at the one per cent point.

3. Pregnancy in sheep does not cause a significant difference in thyroid secretion.

4. Lactation creates increased demands upon the thyroid gland. At the onset of lactation, during March, there was a significant difference at the five per cent level between ewes that were nursing lambs, (0.541 ± 0.096 mg.) and ewes that were not bred, (0.346 ± 0.036 mg.). There was, also, an increase over the secretion rate of pregnant ewes (0.390 ± 0.042 mg.) which was significant at the ten per cent level. After the ewes had been milking from six to eight weeks, the increase in thyroidal secretion rate due to lactation was not as great. The lactating ewes secreted 0.330 ± 0.021 mg., while the open, dry ewes secreted 0.261 ± 0.086 mg. This difference was significant at the ten per cent level of significance.

5. During the months of January, March, May, September and December, there were significant differences in thyroidal secretion rate due to an age difference of two years. The two-year-old ewes had a higher secretion rate than

four-year-old ewes. In July, both ages of sheep were secreting thyroxine at very low levels and there was no significant difference due to age of the sheep.

6. While Hampshire ewes had a thyroid secretion rate slightly higher than Shropshire ewes of the same age, there was no significant difference due to breed except during the month of September. At that time, the thyroidal secretion of the Hampshires (0.350 $\pm$ 0.083 mg.) was significantly greater at the ten per cent level than Shropshires, (0.254 $\pm$ 0.036 mg.).

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