THE INFLUENCE OF THE THYROID ON WOOL FOLLICLE DEVELOPMENT IN THE LAMB*

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Summary

Thyroidectomy of the newborn lamb prevents the maturation of secondary wool follicles. The administration of L-thyroxine to thyroidectomized lambs allows normal follicle development.

Body growth and wool growth are also depressed by thyroidectomy and most thyroidectomized lambs do not survive more than a few weeks without replacement therapy. The effects on wool growth do not appear to be secondary to the effects on follicle development.

The thyroxine requirements for normal wool growth and for normal secondary follicle maturation appear to be greater than the requirements for general body growth.

I. Introduction

The development of wool follicles in the lamb has been described by Carter (1943), Carter and Hardy (1947), Burns (1949, 1954), Fraser (1954), Schinckel (1953, 1955), and Short (1955). Primary follicles are fully developed and are producing fibres at birth. Development of most if not all secondary follicles commences by the time of birth but in the Merino only about 20 per cent. of secondary follicles are producing fibres at this time. In the first month after birth this proportion increases to 70–80 per cent. Further development then occurs more slowly.

The thyroid is well known to affect growth and differentiation. Simpson (1924) found that thyroidectomy of lambs retarded growth and reduced fleece weights at 13–14 months of age by 50 per cent. but no observations were reported on follicle development. The hair cycles of rats are greatly retarded by thyroidectomy (Salmon 1938; Scow and Marx 1945; Scow and Simpson 1945; Dicke 1948), and the administration of throxine to normal rats has been reported to accelerate the hair cycle (Butcher 1937). The lamb does not exhibit several cycles of wool development similar to the hair cycles in the rat, but development of individual follicles appears to be a similar process in both species.

This paper reports the results of an experiment carried out to determine the effect of the thyroid on the maturation of the secondary follicles of the lamb. The results show that the thyroid does markedly influence this process.

II. Materials and Methods

Twenty-six pregnant 6-year-old ewes of the "Egelabra" medium wool Merino strain were housed in individual indoor pens and fed *ad libitum* on a ration of 50 parts by weight of lucerne chaff, 20 parts of wheat, 10 parts of crushed oats, 10 parts of linseed meal, and 10 parts of coco-nut meal.

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The lambs were allotted at random to the following four groups:

Group A.—The six lambs in this group were untreated controls.

Group B.—Eight lambs were thyroidectomized during the first week of life. Thyroxine injections into the four surviving lambs were commenced after 11 weeks of age by which time secondary follicle development in the control group had passed its most active phase. Pure sodium-L-thyroxine was injected in a dosage of 40 mg per unit of weight (expressed as $kg^{0.73}$) per day. This was estimated to be a little more than the normal secretion rate based on the data of Schultze and Turner (1945) for young goats. The thyroxine was dissolved in 0·9 per cent. NaCl at pH 9·3 and injections given subcutaneously. Dosages were adjusted to body weight weekly.

Group C.—Six lambs were thyroidectomized during the first week of life. Thyroxine injections were given from the day of operation until 11 weeks of age. The dose rate was the same as for group B.

Group D.—Four lambs were injected daily with a dilute $Ca(OH)_2$ (pH 11·5) extract of sheep pituitary glands. The extract was freeze-dried, and, for injection, dissolved in 0·9 per cent. NaCl at pH 9·0. Solution was not complete at this pH but the fine suspended matter was not removed prior to injection. The lambs received 10 mg dry weight of extract per unit body weight in $kg^{0.73}$. The extract was assayed for thyrotrophic hormone (thyroid weight increase in chicks (Smelser 1937, 1938)) and growth hormone (body weight increase in hypophysectomized rats (Evans and Simpson 1931; Marx, Simpson, and Evans 1942)). 10 mg of the dry extract was found to contain 0·5 mg of thyrotrophic hormone activity in terms of the Armour preparation 129–31 PRR3, and 1 mg of growth hormone activity in terms of a standard of purified ox growth hormone, prepared by the method of Wilhelmi, Fishman, and Russell (1948).

The lambs were weighed and the skin sampled at birth and thereafter on Wednesday of each week except in lambs then 2 days old or younger, in which case the second sample was taken on the following Wednesday. The skin samples were taken by means of a trephine which cut a circle of skin 1 cm in diameter and were fixed in 5 per cent. formol saline. Successive samples were taken from alternate midsides, the sample on each side being taken about 1 cm behind the previous sample until the 12th sample when another row below the first was commenced in the reverse direction. After the 17th sample the interval between sampling was increased to 2 weeks, and after the 19th sample to 4 weeks so that the 22nd sample was taken at 32 weeks of age. One further sample was taken at 56 weeks of age when the experiment was completed.

Histological sections 7–8 μ thick were cut horizontal to the surface of the skin and stained with haemalum, picric acid, and eosin. Counts of primary (P) and secondary (S) fibres were made at a linear magnification of $\times 115$ counting six to 12 fields depending on the number of fibres present. The counts were corrected for the shrinkage which occurred during fixation and processing by measuring the area of the section.

Wool samples were clipped from areas defined by tattoo lines on the left midside region. The lines were tattooed on the day of the second skin sampling, i.e. 3–9 days

after birth; the sample areas were initially 10 sq. cm, subsequently increasing in size as the lambs grew. The wool samples were taken after consecutive periods of 4, 4, 2, 4, 5, and 4 weeks except in a few cases when the proper sampling days were missed. The samples were extracted with ether and cold water and were oven dried at 100°C prior to weighing.

Estimates of milk consumption of the lambs were obtained by test feeding at weekly intervals until 15 weeks of age. Milk consumption was recorded by weighing the lambs before and after suckling at the end of three 3-hourly periods on the day of test. In addition to milk the lambs had free access to the food of the ewes. The lambs were weaned at 18 weeks of age.

III. Results

(*a*) *Completeness of Thyroidectomy*

Variation in the effects produced by thyroidectomy indicated the presence of accessory thyroid tissue in some lambs or incomplete removal of the thyroid at the time of operation.

Table 1

WET WEIGHTS OF THYROID FRAGMENTS FOUND AT POST-MORTEM IN THYROIDECTOMIZED SHEEP*

Group	Lamb No.	Thyroid Fragment Weight (g)
B	A24	0·15
	A56	0·05
	A58	0·03
C	A32	0·01

* The weight of the combined thyroid glands of normal sheep of the same age is 4–5 g.

Autopsy at the conclusion of the experiment showed the presence of hypertrophied thyroid fragments in three lambs in group B and in one lamb in group C in the region of the thyroid isthmus. The weights of the fragments are shown in Table 1. The nature of the tissue was confirmed by histological examination. No thyroid fragments could be found in any of the other thyroidectomized lambs.

(*b*) *General Effects of Thyroidectomy*

After 2 weeks the thyroidectomized lambs in group B were noticeably less active, becoming dull, stunted, and pot-bellied. They had a typical cretinoid appearance. Muscular weakness and inability to stand was noted especially in the morning after a cool night. Four lambs in this group died and if thyroxine treatment had not been initiated after 11 weeks of age two more would probably have died. Most of the lambs in this group were constipated in the period prior to receiving thyroxine injections. The four deaths were due to the following immediate causes: peritonitis from perforation of the colon by hard faecal material; pneumonia; weakness and

inanition; and laryngeal paralysis presumably resulting from interference with the recurrent laryngeal nerve at the time of operation. Total thyroidectomy of the newborn lamb must be considered a fatal operation since, of the four lambs surviving until thyroxine injections were given, three possessed fragments of thyroid tissue.

In group C signs of thyroid deficiency began to appear about 5 weeks after the cessation of thyroxine injections. The symptoms were similar to those appearing in the younger lambs in group B.

(c) *Milk Consumption*

Milk consumption was depressed in group B relative to the other groups which were not significantly different from one another.

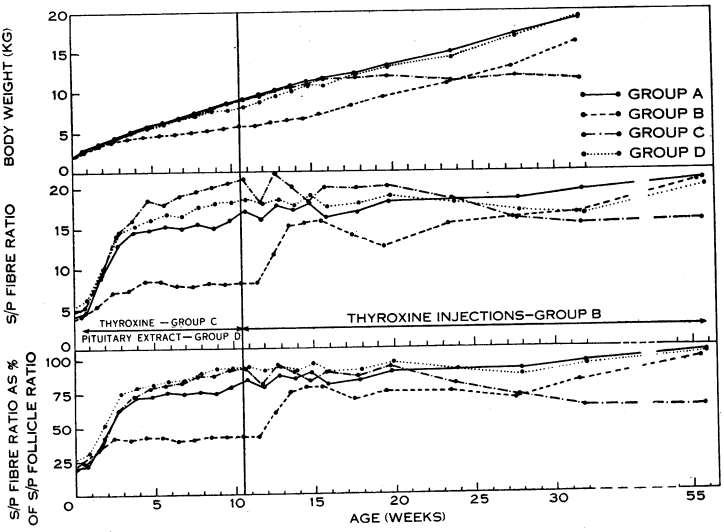

Fig. 1.—Mean body weight, S/P fibre ratio, and S/P fibre ratio as percentage of S/P follicle ratio for each group.

(d) *Growth*

The mean body weight of each group is shown in Figure 1. In group B growth virtually ceased 2 weeks after thyroidectomy except in two lambs (A24 and A58) which grew at nearly normal rate. It may be seen from Table 1 that these two lambs had thyroid fragments at post-mortem. Growth recommenced in the other two surviving lambs of this group 4 weeks after thyroxine injections were started at 11 weeks of age and the growth curve of this group became parallel to that of the control group.

The growth of lambs in group C was similar to that of the controls during the period they received thyroxine injections. Five weeks after the cessation of injections further body growth ceased and body weight remained level until the conclusion of the experiment. The milk supply of one ewe in group C was poor and was supplemented by bottle feeding. However, the growth rate of the lamb A32 was well below normal and the mean body weight curve for this group in Figure 1 excludes the data for this lamb.

The lambs in group D receiving pituitary extract grew at a similar rate to that of the controls during the period of injections. It may be noted that the growth of groups C and D was not increased above normal by the administration of thyroxine and pituitary extract respectively during the first 11 weeks of age.

(e) *Secondary Wool Follicle Maturation*

The ratio of secondary to primary (S/P) fibres appears to be the most suitable measure of the maturation of secondary follicles in the lamb (Carter 1943; Schinckel 1955). The validity of the measure requires that primary follicle development be substantially complete by the time of birth.

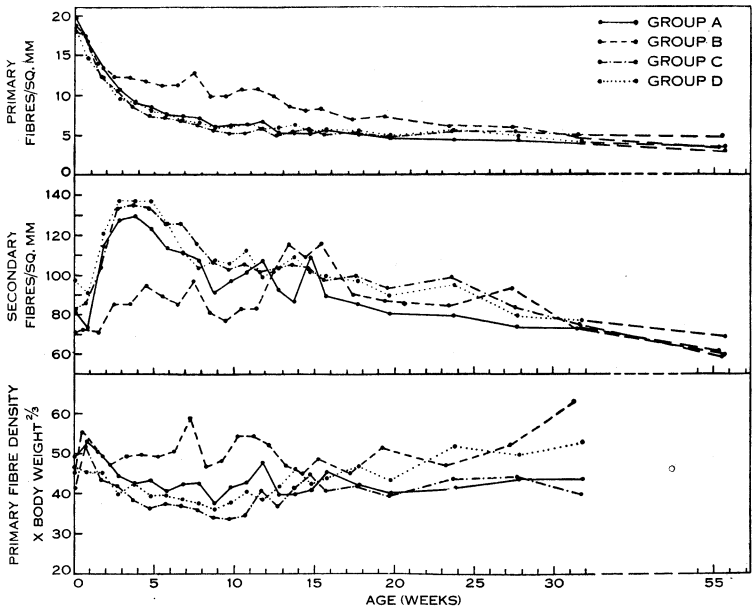

Fig. 2.—Mean primary fibre population density, secondary fibre population density, and primary fibre population density × body weight in kg$^{\frac{2}{3}}$ for each group.

No histological evidence of further primary follicle development after birth was found in the present experiment and estimates of the total primary fibre population given by the product of body weight to the $\frac{2}{3}$ power and primary fibre population density for each group show no consistent increase with age (Fig. 2). The greater values of the product for group B during the first 11 weeks of age are probably due to the increased amount of skin taken in the biopsy sample caused by the huddled attitude of these lambs. A similar increase in primary density is also likely to be the explanation of the increased values of the product for all groups for the early samples.

Figure 1 shows the S/P fibre ratio and this ratio expressed as a percentage of the S/P follicle ratio at birth. The individual data for these percentages are shown in Figure 3. The expression of the data in this form removes variability resulting from

differences in the potential mature S/P fibre ratio but, on the other hand, introduces variability due to the sampling error of the measurement of S/P follicle ratio at birth.

After thyroidectomy there was little further secondary follicle maturation in the lambs of group B except in one lamb (A24) which showed normal body growth and which had the largest thyroid fragment remaining at the conclusion of the experiment. However, follicle maturation showed a sudden increase in this lamb after the start of thyroxine injections at 11 weeks of age. The other lamb in group B with normal body growth (A58) showed a more marked depression of secondary follicle development. This lamb also responded immediately to thyroxine injections. The other two lambs in group B which survived the experiment showed a much slower follicle maturation response to thyroxine but eventually maturation was complete at 12 months of age.

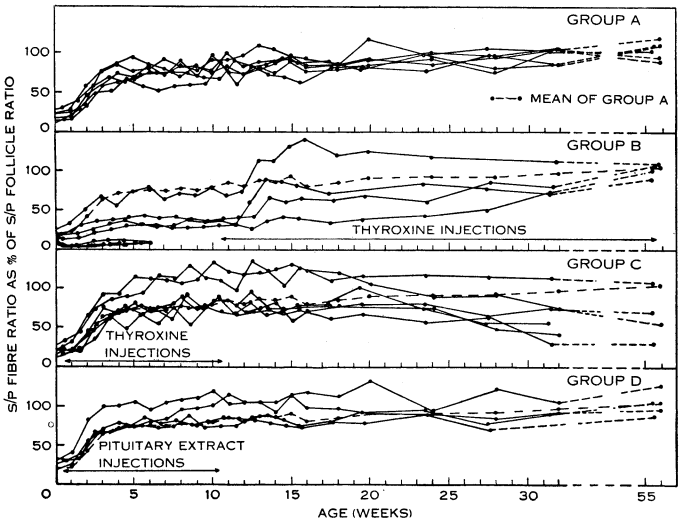

Fig. 3.—Individual values of S/P fibre ratio at various ages as a percentage of S/P follicle ratio for each group.

The depression of secondary follicle maturation may be seen in Figure 3 to have been much more marked in the four lambs which died than in the surviving lambs of group B in which thyroid deficiency may have been less severe. However, except in one instance, the body growth of the four lambs which died was similar to that of the survivors. Apparently a level of thyroxine secretion sufficient for general body growth is not sufficient for the normal rate of secondary follicle maturation. The results also indicate that the effect of thyroidectomy on secondary follicle maturation is not a secondary effect of diminished growth and food intake. This conclusion may be examined by plotting the S/P fibre ratio against body weight to the $\frac{2}{3}$ power from birth until thyroxine injections were commenced in group B (Fig. 4). The individual data are shown for the control group separately with each of the treated groups. It may be seen that at comparable body weights follicle maturation was less in the thyroidectomized lambs of group B.

The secondary fibre population density of the lambs in group B (see Fig. 2) was far below that of the other groups during the active stage of secondary follicle development. This supports the conclusion from Figure 4 that a crowding effect due to lack of skin growth was not the cause of the depression of secondary follicle maturation. Figure 2 also shows that the increase in follicle development in the first few weeks of life in normal lambs is not due to increased "living" space for follicles.

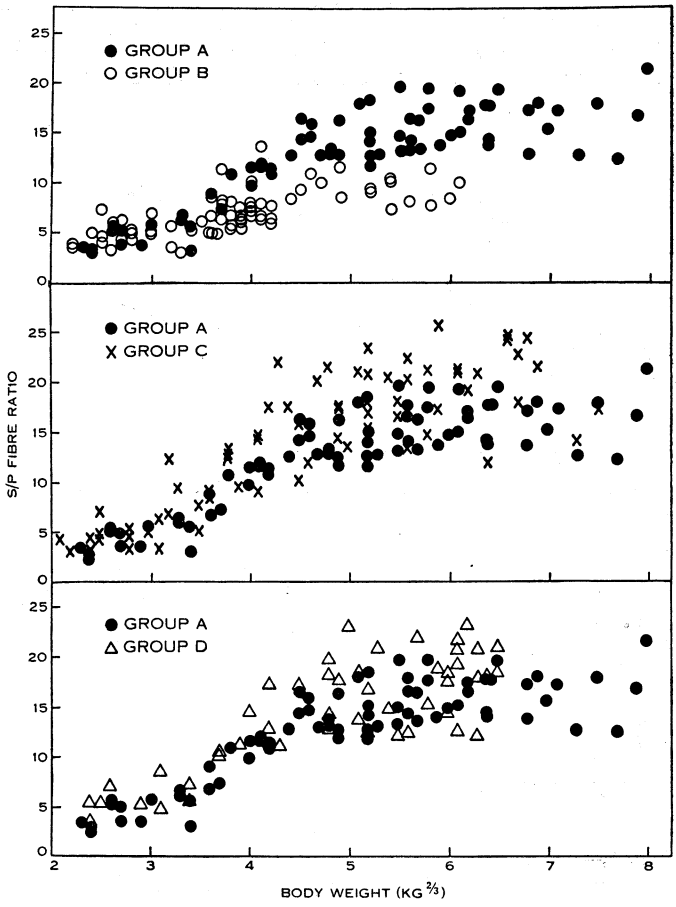

Fig. 4.—Relation of S/P fibre ratio to body weight in kg$^{\frac{2}{3}}$. Group A is compared separately with groups B, C, and D.

Rather the development proceeds in spite of increasing fibre population density. The primary fibre density was of course greater in the lambs of group B due to their diminished growth.

The thyroidectomized lambs of group C which received thyroxine during the period of active follicle development showed an apparently more rapid secondary follicle maturation than the control lambs. However, the mean S/P fibre ratio, expressed as a percentage of the S/P follicle ratio (Fig. 1), during the period of injections for group C was not significantly greater than for the controls. After injections

ceased there was a gradual decline in the S/P fibre ratio owing to the atrophy of some secondary follicles. Some primary follicles also shed their fibres but to a lesser degree than did the secondaries.

One lamb in group C which grew at a subnormal rate due to the failure of its dam's milk supply showed a depressed secondary follicle maturation but maturation was greater than in the lambs of group B despite a similar body growth.

The lambs of group D which received injections of sheep pituitary extract during the period of active secondary follicle maturation also showed an apparently more rapid development than the control lambs but again the difference was not statistically significant.

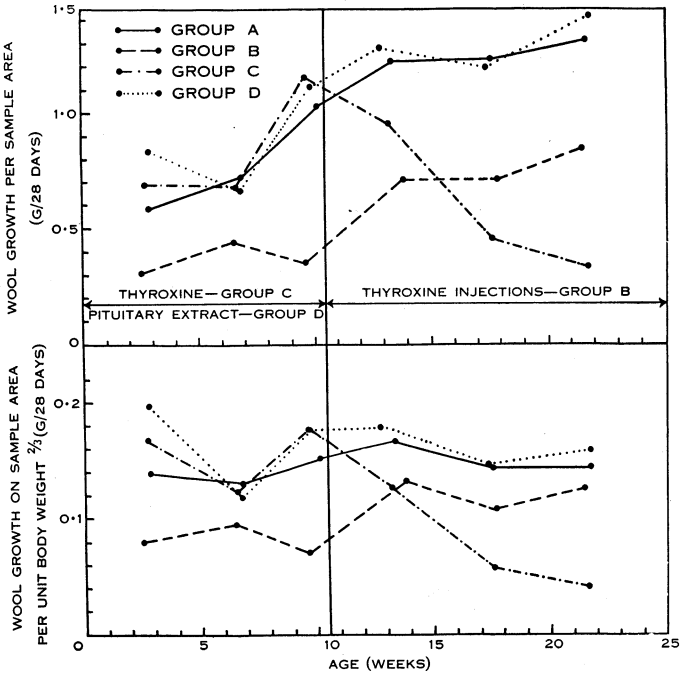

Fig. 5.—Mean wool growth per sample area and wool growth on sample area per unit body weight in kg$^{\frac{2}{3}}$ for each group.

(*f*) *Wool Growth Rate*

When the weight of wool grown per unit time on the tattooed sample areas is used as a measure of the total wool growth rate of the lamb, it is subject to the error introduced by variation in size of the lambs when they were tattooed. This error can be corrected by multiplying the individual wool weights per sample area throughout the experiment by a factor for each lamb. This factor is given by $W^{\frac{2}{3}}$(individual)/$W^{\frac{2}{3}}$(mean of all lambs), where W equals body weight at the time of tattooing.

The wool growth rates per sample area for each group adjusted in this way are shown in the upper half of Figure 5. The regression coefficient for the regression of log wool growth per sample area on log body weight for the period means of groups

A and D (which did not differ significantly from one another) was found to be $0{\cdot}676 \pm 0{\cdot}200$. This indicates that the sample area wool growth per unit body weight to the $\frac{2}{3}$ power was independent of body weight in these two groups from birth until 6 months of age when wool growth measurements were terminated. Figure 5 also shows the wool growth rates on the sample area per unit body weight in $kg^{\frac{2}{3}}$. The relation of wool growth per sample area to body weight in $kg^{\frac{2}{3}}$ for groups A and D is shown in Figure 6.

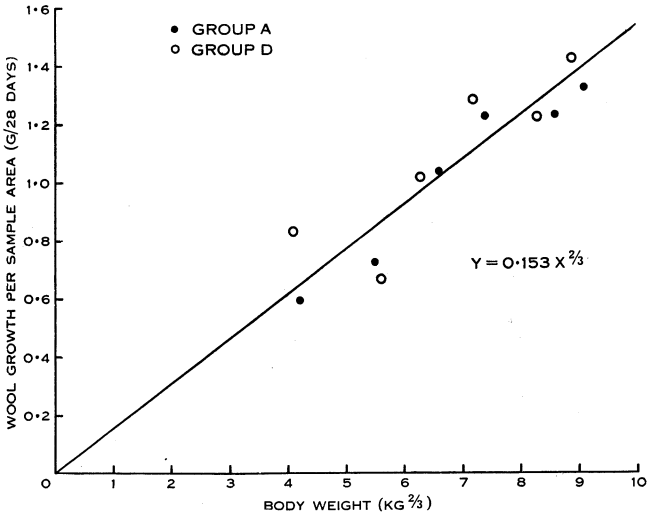

Fig. 6.—Relation of wool growth per sample area to body weight in $kg^{\frac{2}{3}}$ for groups A and D.

In group B wool growth per sample area was considerably depressed but to a lesser extent in the two lambs in this group which showed normal body growth. Thyroxine caused an increase in wool growth but it remained substantially less than in the control lambs up till 6 months of age. Wool growth per unit body weight in $kg^{\frac{2}{3}}$ was also depressed during the pre-injection period and did not reach normal after thyroxine injections were given. Thus the depression of wool growth per sample area cannot be attributed to the smaller size of these lambs. The extent to which wool growth per unit body weight in $kg^{\frac{2}{3}}$ of different lambs approached the control level when thyroxine was given was not related to the corresponding S/P fibre ratio or to fibre population density. Furthermore, in any wool growth period there was no relation between wool growth per unit body weight in $kg^{\frac{2}{3}}$ and S/P fibre ratio or fibre population density within any of the groups.

In group C wool growth per sample area and per unit body weight in $kg^{\frac{2}{3}}$ was similar to that of the control lambs while they received thyroxine. When thyroxine injections ceased, wool growth, expressed in both ways, declined sharply.

IV. Discussion

The present experiment shows that the thyroid is necessary for the completion of secondary follicle development in the lamb as indeed it is for growth and develop-

ment in general. However, it appears that the requirement of the follicles for thyroxine is greater than that of the body for growth in general. Natural conditions might exist under which thyroxine secretion might be inadequate for normal secondary follicle maturation.

In the present experiment depression of secondary follicle maturation for 11 weeks by thyroid deficiency did not lead to permanent depression of the S/P fibre ratio. However, it did cause an effect which took months of thyroxine injections to repair and it is possible that a more prolonged period of thyroid deficiency could lead to a permanent loss of secondary follicles.

Current experiments at this Laboratory (Schinckel and Short, unpublished data) show that restricted post-natal nutrition delays the maturation of secondary follicles, but it is too early to state whether there is a permanent depression of secondary follicle development.

Poor nutrition leads to depression of secretion of thyrotrophic hormone and hence of thyroxine in the guinea pig (Stephens 1940) and this effect, if present in the sheep, may account at least in part for the delayed follicle development of lambs fed on a restricted diet.

Schinckel (1953) has shown that the lambs from twin births have a retarded maturation of secondary follicles compared with single lambs. Not only is maturation retarded but also the S/P fibre ratio of twins appears to remain permanently below that of singles. However, it is not clear to what extent the difference between singles and twins results from post-natal nutrition.

Depression of wool growth rate accompanied the depression of follicle maturation caused by thyroid deficiency but the effect on wool growth did not appear to be a consequence of the lowered follicle population but rather to be independent of it. This suggests that the two effects may be referable to different metabolic defects which occur to different relative extents in different lambs.

V. Acknowledgments

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