# STUDIES ON THE RATE OF WOOL GROWTH USING [$^{35}$S]CYSTINE

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*Summary*

The radioautographic method has been used to study the length growth rate of wool fibres in a sheep subjected to changing nutritional conditions.

The reproducibility of radioautographic length measurements by different observers has been examined in a detailed study of 10 fibres. The variance between observers was negligible compared with the variance between fibres.

A Corriedale wether was given nine intravenous doses of DL-[$^{35}$S]cystine at 4-day intervals. When the food intake was increased from 800 g to about 1300 g per day the absolute increase in length growth rate was similar for all fibres. When the level of nutrition was reduced to 300 g per day the length growth rate decreased in proportion to the initial rate. Although the changes in volume were approximately proportional to the initial growth rate, a differential rate of response in the largest and smallest fibres was observed. Thus the length and diameter components of volume responded independently to the nutritional changes. Clipping alone had little or no effect on the rate of wool growth when changes in skin temperature were reduced to a minimum.

Experience has shown that small doses of radioactivity can be used in studies of this type. The sensitivity of the radioautographic technique is discussed.

## I. Introduction

The advantages of the radioautographic technique for studying the rate of wool growth were indicated in a study by Downes and Lyne (1959) in which intravenous injections of $^{35}$S-labelled cystine were used to label the whole fleece. It was shown that the growth in length of individual wool fibres could be measured with good accuracy over short periods, of the order of a few days, without having to remove the fibres from the sheep until the end of the experiment.

In the present work the radioautographic technique was used to determine the effect on wool growth of changes in food intake and of clipping. At the same time, the accuracy of the method was examined more critically.

## II. Material and Methods

### (*a*) *Experimental Procedure*

A Corriedale wether (40 kg body weight) was kept in a metabolism cage in a room at 23$\pm$1°C to eliminate the influence of air temperature on wool growth. The animal was initially covered uniformly by an 8-cm thick fleece. The left side was clipped in the 2 hr before the first injection. Three small areas (A, B, and C, each about 5 by 5 cm) and two larger areas (I and II, each about 10 by 10 cm) of mid-lateral abdominal skin were clipped closely with care and defined by tattooing;

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their anteroposterior order was A, I, B, II, C. About 3 hr after the first injection a sheepskin, with its 3-cm thick fleece inwards, was used to cover the left side of the sheep. The skin was kept on for the duration of the experiment, except during the clipping, in order to minimize possible changes in the rate of wool growth due to changes in skin temperature, and thus to enable any other effects of clipping to be studied. Skin temperatures (measured with copper–constantan thermocouples held against the skin with an adhesive) on both sides of the animal were identical (38·3±0·3°C) within 3 hr.

Nine doses of DL-[$^{35}$S]cystine were injected at 4-day (±15 min) intervals into the left jugular vein and washed in with 10 ml 0·9% NaCl. The cystine (30·9 mg, 19·5 mc/m-mole at the time of the first injection) was dissolved in the minimum

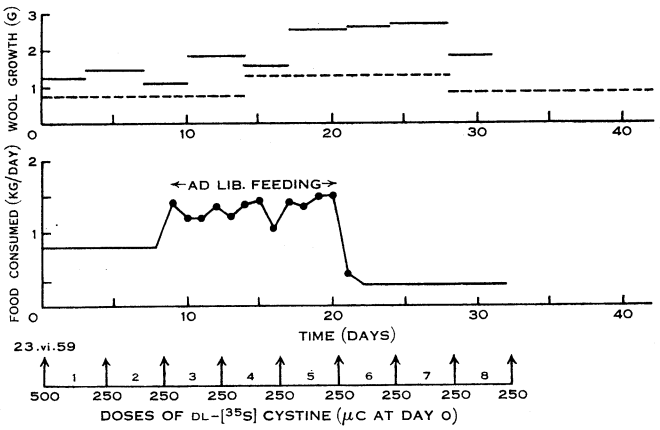


Fig. 1.—Feeding regime, timing and amount of each radioactive dose, and wool clipped. Periods between successive doses are numbered 1–8 as shown. – – – – Mass of clean dry wool clipped from areas I and II (g/14 days); ——— masses (g/day) clipped from the rest of the left side excluding the tattooed areas.

amount of HCl and diluted with 0·9% NaCl to 50 ml. Eight 5-ml portions of the stock solution were dispensed into glass tubes and kept frozen until required. The remaining 10 ml was used for the first dose. The radiochemical purity of the cystine (obtained from the Radiochemical Centre, England) was checked at intervals by eluting samples with HCl from a small "Amberlite IR120" column. In every case a single peak corresponding to cystine and accounting for more than 98% of the radioactivity was obtained. Analysis by the carrier dilution procedure confirmed the chromatographic results.

The sheep was eating 800 g per day of equal quantities of lucerne chaff and wheaten chaff for 4 months before the experiment and until the day of the third injection of cystine. From then until the day of the sixth injection, i.e. for 12 days, it was allowed to eat the same ration *ad lib.* On the day of the sixth dose the animal received 450 g, and on each succeeding day until the end of the experiment 300 g of the same ration. The timing of the injections in relation to the food eaten is given in Figure 1.

The wool on areas I and II was clipped fortnightly; it was washed successively with ether, ethanol, and cold water, dried at 100°C, and weighed. Ten days after the last injection wool was clipped from areas A, B, and C, and from three corresponding areas D, E, and F on the right side. Apart from the tattooed areas, the entire left side was clipped every three or four days (Fig. 1) to see if repeated clipping of the surrounding wool produced any indirect effect on the rate of growth of the fibres in the small areas A, B, and C.

### (*b*) *Radioautographic Technique*

Wool fibres from areas A–F were cleaned in ether and dried on filter paper. They were mounted on microscope slides as follows: about 30 fibres were thoroughly wetted in a drop of freshly prepared solution of egg albumin in water (*c.* 0·1 ml,

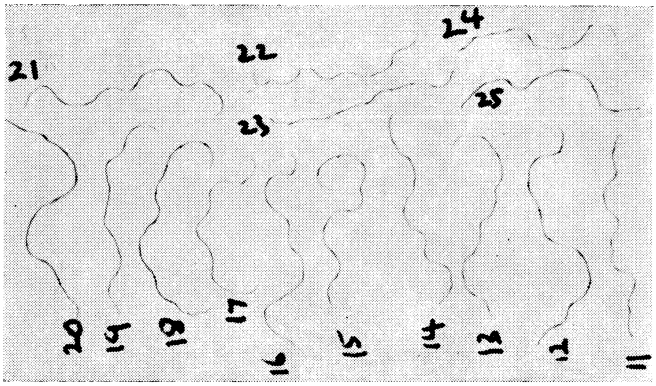

Fig. 2.—Typical preparation for length measurements of radioactive wool fibres. × 2·3.

5% w/v); the fibres were separated in the solution which was smeared over three-quarters of the slide. The slide was baked dry 25 cm below an infrared lamp for 2 hr to ensure that the fibres were held firmly. Each slide was then covered with a radioautographic stripping film ("Kodak AR50") using the technique described by Pelc (1956). After a suitable exposure time the films, still in contact with the fibres, were developed, fixed, washed, dried, and each fibre numbered to enable repeat measurements to be made. Figure 2 shows part of a typical preparation.

The lengths of 100 fibres grown on each area during each of the eight 4-day periods was measured with a flexible rule or a graduated knurled wheel after projection and magnification to ×215 (Downes and Lyne 1959). Most of the fibres measured were radioautographed for 11 days, starting 44 days after they were clipped.

During the length measurements an estimate was made of the amount of medullation along each fibre over each 4-day period. Later, the diameters of the 100 fibres from area B were measured (magnification ×500) at positions corresponding to just before and after each dose. The mean of the two measurements was taken as the diameter of the fibre at the time of each injection. The volume of fibre grown during each period was calculated by assuming that each segment was a cylinder

with a diameter equal to the mean of the diameters at the two ends. The volumes were less accurate than the lengths because the fibres were not perfect cylinders, the diameters could not be measured very accurately, and the amount of medullation increased and decreased after the food intake was respectively raised and lowered.

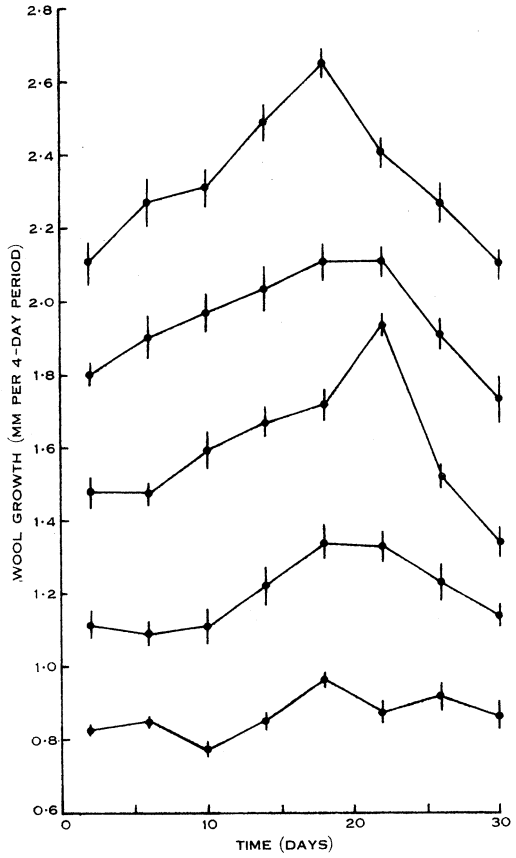


Fig. 3.—Accuracy of radioautographic length measurements of five individual fibres. Each point represents the mean of 10 measurements of length of wool grown during each 4-day period. Standard deviations of single measurements are shown as vertical lines.

## III. Results

### (*a*) *Accuracy of Method*

The lengths of 10 fibres taken randomly from the 100 fibres from position C were measured by five observers on two occasions. Figure 3 shows typical results from five fibres. Most standard deviations of single measurements were less than $\pm 4\%$ of the mean. It is thought that the absolute errors of measurement in the bigger fibres were larger because of the greater difficulty in defining the start of the radioactive band. The mean growth rates for each group of 10 fibres during

each of the eight periods are shown in Table 1. The largest standard deviation from the mean of each set of results was $\pm 1 \cdot 5\%$. The maximum observer difference, as judged by the mean lengths of the 10 fibres over the whole eight periods, was about 1%.

Nine of the fibres used for the accuracy test were removed from the slides and film, soaked in distilled water for about 15 min, and radioautographed again. The second exposure began 146 days after the commencement of the first, that is 232 days

TABLE 1

REPRODUCIBILITY OF RADIOAUTOGRAPHIC LENGTH MEASUREMENTS BY FIVE OBSERVERS

| Period | Mean Length (mm) of Wool Grown by 10 Fibres during the Eight 4-day Periods of the Experiment* | | | | | | | | | | Mean ± S.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Observer | | | | | | | | | | |
| | A | | B | | C | | D | | E | | |
| 1 | 1·30 | 1·29 | 1·30 | 1·32 | 1·28 | 1·27 | 1·30 | 1·30 | 1·30 | 1·31 | 1·30 ± 0·015 |
| 2 | 1·33 | 1·32 | 1·36 | 1·35 | 1·34 | 1·38 | 1·36 | 1·36 | 1·32 | 1·33 | 1·34 ± 0·020 |
| 3 | 1·40 | 1·39 | 1·40 | 1·41 | 1·37 | 1·38 | 1·40 | 1·39 | 1·39 | 1·40 | 1·39 ± 0·012 |
| 4 | 1·49 | 1·46 | 1·47 | 1·48 | 1·44 | 1·48 | 1·48 | 1·49 | 1·49 | 1·43 | 1·47 ± 0·022 |
| 5 | 1·58 | 1·57 | 1·56 | 1·57 | 1·59 | 1·57 | 1·58 | 1·59 | 1·54 | 1·59 | 1·57 ± 0·016 |
| 6 | 1·51 | 1·52 | 1·55 | 1·54 | 1·51 | 1·53 | 1·56 | 1·52 | 1·54 | 1·55 | 1·53 ± 0·018 |
| 7 | 1·43 | 1·43 | 1·45 | 1·46 | 1·46 | 1·46 | 1·46 | 1·48 | 1·42 | 1·45 | 1·45 ± 0·018 |
| 8 | 1·33 | 1·35 | 1·34 | 1·32 | 1·32 | 1·29 | 1·33 | 1·33 | 1·33 | 1·31 | 1·32 ± 0·018 |
| Total length | 11·37 | 11·33 | 11·43 | 11·45 | 11·31 | 11·36 | 11·47 | 11·46 | 11·33 | 11·37 | |

* Each observer measured the same 10 fibres, selected at random from position C, on two different occasions.

after the first dose, and was increased to 41 days because of the decay of the $^{35}S$ in the intervening period. The length measurements were then repeated by two of the observers and were found to be the same as the original ones within experimental error.

### (*b*) *Wool Growth and Nutrition*

The mass of wool grown on all areas was related to the amount of food eaten (Fig. 1). This is best seen from the masses obtained from areas I and II. The masses obtained from the rest of the left side of the sheep were much less reliable because of the difficulties in clipping wool reproducibly and quantitatively at intervals of only 3 or 4 days. In view of the inaccuracy of the results from these clippings, it is considered that the apparent lag which they indicate in the response to the reduced plane of nutrition is not significant.

The results of the radioautographic length measurements and especially the rapid response of wool growth to changes in the plane of nutrition are shown in Figure 4. The average length of wool grown by 600 fibres, 100 from each of the six

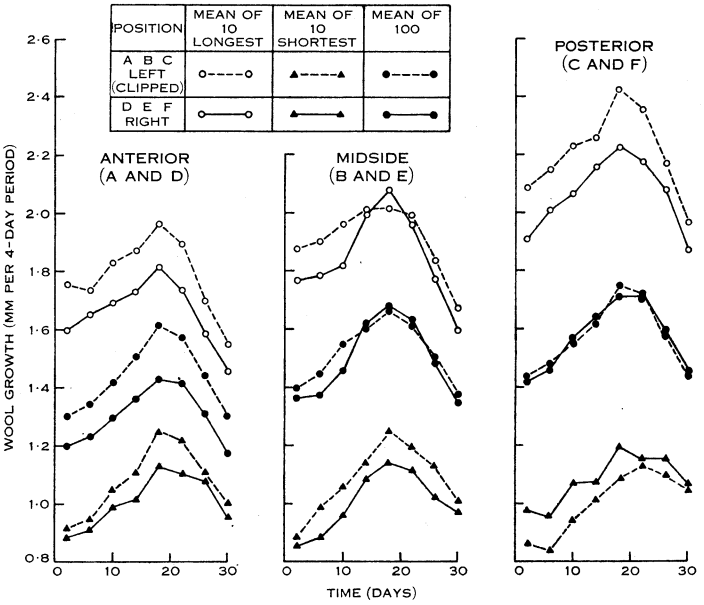


Fig. 4.—Results of the radioautographic length measurements on 100 fibres from each of the six positions (A–F) during the eight 4-day periods.

areas (A–F), was 1·35 (S.D. ±0·31) mm during the first 4 days. Maximum growth of 1·64 (±0·34) mm occurred during the last 4 days of the *ad lib.* feeding, representing

TABLE 2

MEAN LENGTHS AND STANDARD DEVIATIONS OF 100 FIBRES FROM POSITIONS A–F DURING PERIODS 1 AND 8

| Position | | Mean Length (mm) ±S.D. | |
|---|---|---|---|
| | | Period 1 | Period 8 |
| Anterior | A | 1·30±0·26 | 1·30±0·18 |
| | D | 1·19±0·24 | 1·17±0·22 |
| Midside | B | 1·40±0·33 | 1·37±0·21 |
| | E | 1·36±0·29 | 1·34±0·22 |
| Posterior | C | 1·44±0·39 | 1·44±0·30 |
| | F | 1·42±0·30 | 1·45±0·25 |

an increase of approximately 20%. The average growth rate then fell to 1·34 (±0·25) mm during the last experimental period.

Figure 4 also shows that the changes in nutrition produced different growth patterns in fibres of different length, namely the rate of growth of the 10 longest fibres from all areas during the last period was below that of the first period, whereas the 10 shortest ones were still growing at rates above the initial rate. This was confirmed by calculating the standard deviations of the lengths for each group of 100 fibres during periods 1 and 8. In every case the standard deviation was larger

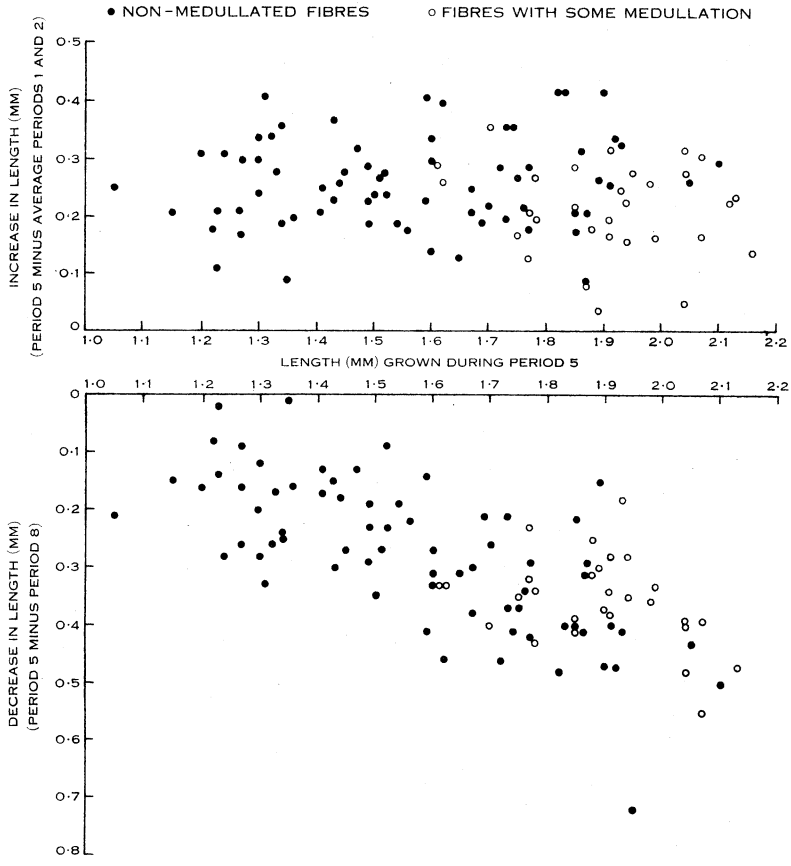


Fig. 5.—Changes in length of the 100 fibres from position B. Each point in the upper figure shows the difference between the length grown during period 5 and the average length for periods 1 and 2. The lower figure shows the corresponding differences for periods 5 and 8.

for period 1 than for period 8 (Table 2). Figure 5 illustrates these differences in another way; the average increase in *length* of wool grown as a result of the increased plane of nutrition was about the same for all fibres, irrespective of the presence or absence of medullation; that is the percentage increase in length was greater for the smaller fibres. The response to the sudden drop in nutrition was different: the bigger the fibre the larger was the decrease in length and this change was approximately proportional to the fibre length. Similar results were obtained for the other areas.

Figure 6 shows a comparison of the length and volume growth rates for the 100 fibres from area B. Subject to the errors described in the methods, the distribution of values for both length and volume were markedly less during the last period compared with the first.

The true volumes of keratin produced by the medullated fibres must be less than the calculated values, because the medulla contains air spaces (Wildman 1954). Only fibres whose initial growth rates were higher than $8 \times 10^{-4}$ mm$^3$ per 4-day period were medullated, but no corrections for the medullation changes were made.

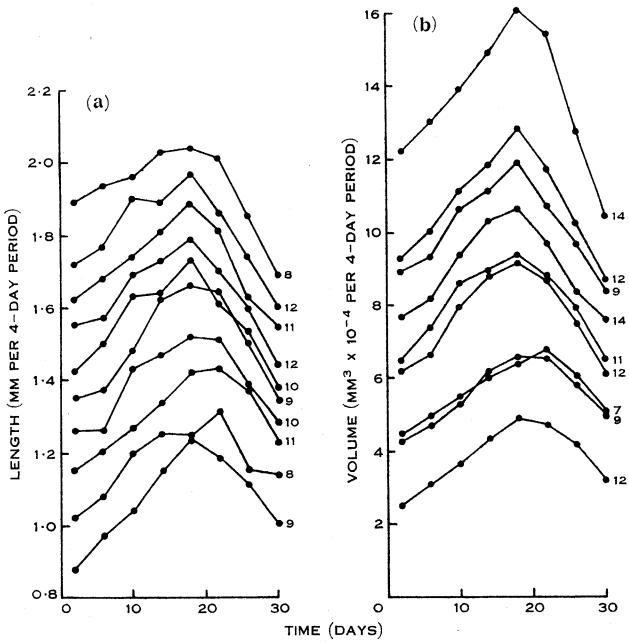


Fig. 6.—Comparing the changes in length and volume for the 100 fibres from position B. (*a*) Fibres were classified into 10 length groups according to the average length grown during the first two periods ($<1 \cdot 0$, $1 \cdot 0$–$1 \cdot 1$, $1 \cdot 1$–$1 \cdot 2$, etc. and $>1 \cdot 8$ mm per 4-days), and the mean lengths for each group during each period calculated. (*b*) Fibres were classified into nine diameter groups according to the average diameter during the first two periods ($<20$, 21 and 22, 23, 24, 25, 26, 27, 28 and 29, and 30–32 $\mu$), and the mean volumes calculated. A similar set of volume curves was obtained when the fibres were classified by lengths as in (*a*). The number of fibres in each group is shown at the right of each diagram.

Nevertheless, the results showed that the increase in the volume growth rate after the increased food intake was not directly proportional to the initial rate. The average increase was 61% for the smallest 10 fibres and 43% (an upper limit because of the changes in medullation) for the largest 10 fibres from each of the six areas.

### (*c*) *Effect of Clipping*

During the period between the first and second injections, the only period when the effect of clipping could be measured directly, the mean lengths of 300 fibres

from each side of the sheep differed by 4% but this difference was not statistically significant.

Clipping the left side of the sheep every 3 or 4 days did not influence the rate of growth of the fibres clipped from the small areas A, B, and C 10 days after the last injection, when compared with fibres from corresponding positions on the other side of the animal. It is concluded that the direct or indirect effect of clipping, if any, is very small.

## IV. Discussion

The results reported here and in our preceding paper (Downes and Lyne 1959) show that the radioautographic technique can be used to measure changes in wool growth rates over short periods with good accuracy. The main source of error is the difficulty of locating the points on each fibre corresponding to the injection times. Experience has shown that this can be done to within $\pm 30\ \mu$, which corresponds to about $\pm 2$ hr for fibres growing 0·36 mm per day. Since this is approximately a constant error, the longer the interval between doses the greater is the accuracy of the length measurement. The length of a single fibre grown over a 4-day period can be measured with a standard error of less than $\pm 2\%$ by taking the mean of several measurements as indicated above. For an 8-day period the error would be $\pm 1\%$. However, in studies of a fibre population, a larger number of fibres may be measured with a lower accuracy since the variance between observers is negligible compared with the variance between fibres.

In using the radioautographic technique, the number of fibres that need to be measured will depend on the purpose of the experiment. If an accurate value for the mean length growth rate of the whole fibre population is required then, as with conventional techniques, several hundred fibres must be measured. However, one of the main advantages of the technique is that is enables relative measurements to be made over successive short periods, of the order of 3 or 4 days. In this case a few fibres are sufficient. The results confirm this since the percentage changes in length were practically the same for 10 fibres taken at random as for the whole hundred in each group, even though the individual fibres did not respond in exactly the same way.

The experience gained has shown that much smaller doses of radioactive cystine could have been used. For example, fibres exposed to the radioautographic film about three half-lives after the first injection still produced sufficiently clear "spots" with exposures of about 40 days. Thus the standard dose used, 250 $\mu$c of DL-cystine, could have been reduced to about 30 $\mu$c provided the fibres had been radioautographed without delay. In addition, since the sheep evidently does not use D-cystine (Downes, unpublished data), the dose could presumably have been halved again if the pure L-isomer had been injected.

Rougeot (1959) has shown that it is possible to label the wool fibres over a small area of skin by a subcutaneous dose of much smaller amounts of [$^{35}$S]cystine. In his experiment doses of 1–10 $\mu$c were injected at 21-day intervals, without producing any visible lesions. However, to our knowledge, it has not been shown whether subcutaneous injections can be made into the same region of the skin at intervals of only a few days without altering the rate of wool growth in that area. Since some

local damage must be produced and since cystine is known to concentrate in healing tissue (Williamson 1959) it is possible that the rate of wool growth could be affected. Intravenous doses, on the other hand, although requiring more radioactivity, label the whole fleece and can be given at convenient sites well away from the regions to be studied.

The biggest fibres grew about three times as fast as the smallest. To explain this, a mechanism for wool growth could be postulated in which the growth stops or becomes very slow for periods of time which might differ for the various follicles. If the extraction of cystine from the blood by the follicles also stops or becomes negligible during such periods, and if these periods are distributed randomly in time one would expect to find fibres with one or more of the radioactive areas missing. However, since every fibre examined showed the nine radioactive spots, and since the time interval between an injection of labelled cystine and the first arrival of the radioactivity at the site of incorporation is no more than a few minutes (Downes and Lyne 1959) the fibres were evidently all growing at the time of each injection. Moreover, in spite of the individual variations, the fibres all responded qualitatively in the same way to the nutritional changes, so that any irregularities in the growth rate must have been for periods much smaller than 4 days. Since each dose was given at the same time of day ($\pm$15 min) the evidence does not exclude the possibility of diurnal or more frequent variations in the rate of fibre growth.

Figure 4 shows that the response to both an increase and decrease in nutrition was quite rapid—certainly much less than 4 days. Similar changes were observed in the wool taken from all six sites examined. The drop in food intake after the sixth dose was much larger than the increase after the third dose but the average length of wool grown during the last period had only fallen to a value about the same as those for the first two periods. This suggests that the wool growth rate responded more slowly to the decrease than to the increase in the food eaten.

The results in Figures 4, 5, and 6 appear to provide the first direct demonstration of a differential response of length growth rate to changes in nutrition. The absolute increases in length growth rate as a result of the increased nutrition were about the same for all fibres; that is, the shorter the fibre the larger the *percentage* increase in length grown per day. When the nutritional level was reduced the decrease in length growth rate was approximately proportional to the original rate. Although the measurements of volume were much less accurate than those of length, the results showed that the changes in volume were not simply proportional to the initial fibre size; the bigger the fibre the smaller the percentage increase. Moreover, at the end of the experiment there was a smaller distribution of fibre volumes than at the beginning (Fig. 6). Thus there was a differential response of volume as well as of length. These differential responses might have been due to different *rates* of change for the various fibres. If the animal had been allowed to come to a new equilibrium the original relationship between the fibres might have been restored. An experiment to examine this is in progress. The present results suggest that, although nutritional changes may produce minor variations in the *relative* growth rates of different follicles, these growth rates are largely determined by properties inherent in the follicles themselves.

On the basis of indirect evidence, other authors have reported differential responses in diameter. For example, Marston (1955) reported a relatively greater response in the diameters of the "stronger" fibres to a higher plane of nutrition. He also stated that "the length of the fibres is similarly influenced by the rate of wool growth though the extent of the change may vary independently of the mean fibre diameter". Our results support this statement. Short, Fraser, and Carter (1958), as a result of a statistical analysis of the diameters of primary and secondary fibres as measured in skin sections taken from a number of sheep, concluded that primary and secondary fibres did show a differential response. However, their results refer only to changes in diameter.

The results show that the effect of clipping alone, if any, is very small. Since the clipped side was kept covered with a thick fleece any effects of clipping due to changes in skin temperature were probably avoided. The fibres from positions A, B, and C were clipped twice—just before the first injection and 10 days after the last —and could therefore have been affected directly by the clipping only once. The repeated clipping of the surrounding wool on the rest of the left side could have produced indirect effects. However, the difference of 4% between the mean lengths grown in the first period by the 300 fibres taken from each side of the sheep was not statistically significant. The mean difference for the eight periods was also about 4%.

## V. Acknowledgments

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