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Measuring short periods of wool fibre growth using radiolabelling

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ABSTRACT

Brief periods cannot be measured with traditional autoradiographic methods on wool fibres because the labelled points overlap. A dose of [^{35}S]cysteine was injected into 24 sites across the side of five Lincoln ewes. After 48 hours a second injection was administered to one site on each ewe, followed by an injection into another site every two hours until all 24 sites had been labeled twice. Three days later all 24 sites were injected again. Following autoradiography the distance between radiolabelled points was traced using a digitizer (50 fibres per site). A linear increase in length between points 1 and 2 was observed between sites ($r^2 = 0.920$) and the regression equation showed the fibre increased in length by 29.2 µm per site ($P < 0.01$). However, a linear decrease in length was observed down ($P < 0.001$) but not along ($P = 0.813$) the midside over the period delineated by injections 1 and 3. Removing the effect of site gave growth rates that reflected the overall mean of 28.9 µm/hour for all sites. This method could be used to determine changes in growth rate over a few hours or the response to a growth factor, hormone or nutrient over a time course of several hours.

Keywords: fibre length; fibre growth rate; sheep; autoradiography.

INTRODUCTION

The rate of wool growth in terms of length of the fibre can be measured using radiolabelled amino acids (Woods and Orwin, 1988; Nelson and Woods, 1992). An isotopic amino acid is injected into the skin and taken up into the wool fibre. A second injection is made some time later, and the length grown between two injections can be measured following autoradiography. However, the isotope moves slowly from the skin into the fibre. Also, the radioisotope emits in a random direction, and when the autoradiography film is laid on one side of the fibre, some of these emissions are picked up in a comparatively large spot on the autoradiograph. The use of gel photographic emulsion can overcome some of this as the emulsion surrounds the fibre (Friend and Robards, 1995). These factors preclude the measurement of time periods less than several days, because the two injection points become indistinguishable on the autoradiographs. A modified technique to overcome this time limitation is described here.

MATERIALS AND METHODS

Mature Lincoln ewes (n = 5) were housed in individual pens in a controlled environment room in which the lights were automatically switched on at 0600 hours and off again at 1800 hours. The temperature was maintained at 23 ± 2 °C. Each day the ewes were fed 800g of a pelleted ration and 400g of lucerne chaff and the room cleaned at 0900 hours. The sheep were accustomed to the lighting and feeding protocol for a pre-treatment period of eight weeks.

At 1600 hours on day 1 of the experimental period [^{35}S]cysteine (0.5 µCi in 0.1 ml of saline) (Amersham Laboratories, Amersham, Buckinghamshire, UK) was injected intradermally into 24 sites across the right midside of each ewe. Prior to injection, the sites were delineated by small circles marked using a felt-tipped pen. These sites were placed at points 10 cm apart on a grid pattern. The grid pattern was 50 cm long in the anterior to posterior direction and 30 cm deep dorsoventrally.

After 48 hours, a second injection was administered to one of the sites on each ewe. This was followed by an injection into another site two hours later, and this procedure was repeated every two hours until all 24 sites had received a second injection.

At 1600 hours on day 7, all 24 sites were injected again. The ewes remained in the same feeding and lighting conditions for a further week to allow the radiolabelled points on the fibres to grow out of the skin to a level where they could be harvested using small animal clippers.

The wool samples were washed, individual fibres (n = 50) were separated and stuck to microscope slides. The slides were then exposed to "Agfa-Structurix" autoradiography films (D7pFW, Agfa-Gevaert, Belgium) for six weeks and subsequently developed.

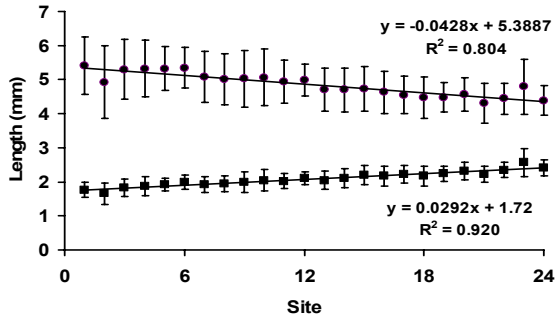
The radiolabelled points were characterised by an initial round spot, followed by a "comet tail" that could be traced to the next labelled point (Figure 1). The fibres could thus be measured directly from the autoradiography films using a digitising tablet (Summagraphics, Scottsdale, Arizona, USA).

FIGURE 1. An autoradiograph of Lincoln wool fibres, showing three labelled points, with a trace or "comet tail" joining them. Scale bar indicates 5 mm.

RESULTS

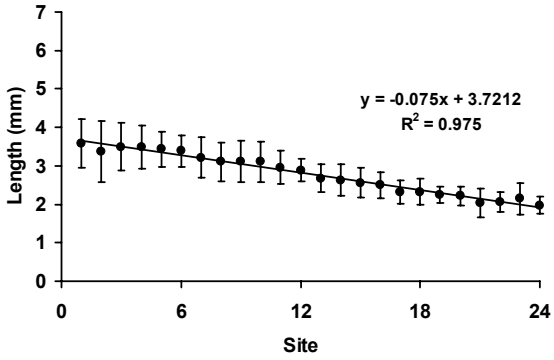
The variation between animals was considerable ($P < 0.001$), with overall mean growth rates of 4069 ± 546, 4453 ± 561, 4877 ± 1009, 5275 ± 687 and 5422 ± 939 µm for the five ewes in ascending order of fibre length grown during the seven day period. A linear increase in distance between the first and second radiolabelled points was observed between sites ($r^2 = 0.920$). The data are summarized in Figure 2 for all five animals. The mean difference between sites was 29.2 µm or 14.6 µm/hour.

FIGURE 2: The average distance between labelled points on wool fibres from 24 injection sites on each of five Lincoln ewes. The first and third labelled points were separated by 7 days and the distance between them is indicated by circles. The distance between the first and second labelled points is indicated by squares, where the time interval between the labelled points increased by two hours beginning on day 2 at site 1. (Error bars indicate standard deviation).



However, during the seven day period a decrease in total length of fibre between the first and third radiolabelled points was evident between sampling sites 1 to 24 ($r^2 = 0.804$). The reason for this was a gradual decline in total length of fibre grown between the sites in the dorso-ventral direction ($P < 0.001$) but not in the anterior to posterior direction ($P = 0.813$) across the side of the sheep. The average length of fibre grown during the seven day period between labelled points 1 and 3 was 4883 µm, while at the most dorsal and anterior site fibers averaged 5441 µm and only 4325 µm at the most posterior and ventral. This was equivalent to a reduction of 42.8 µm per site or 21.4 µm/hour over seven days or 3.0 µm/hour/day.

FIGURE 3: The average distance between the second and third labelled points on wool fibres from 24 injection sites on each of five Lincoln ewes. The second and third points were initially separated by 5 days, declining by two hours intervals to 3 days. (Error bars indicate standard deviation).



The length of fibre grown between injections 2 and 3 at each site is displayed in Figure 3. Essentially this shows the additive effects of the decrease in length between the sites due to the decrease in fibre growth rate, and the reduced time interval between injections 2 and 3 for the 24 sites. The slope of the regression calculated from these lengths was -75 µm per site or -37.5 µm/hour. This is a considerably greater difference between sites than estimated from the sites for injections 1 and 2. Since the average time period between the first and second injections was three days, the rate of growth was underestimated by 9 µm/hour, while it was overestimated by -12 µm/hour for the four days between the second and third injections. This yields estimates of 23.6 µm/hour and - 25.5 µm/hour. Disregarding whether the slope of the regression was negative or positive, the average slope of these two regression lines was 24.5 µm per hour. This is similar but not identical to the rate of 29.1 µm/hour that can be calculated from 4883 µm grown from the average site in a period of seven days.

The effect of site and sheep were removed by transforming the data. The relationship between these two lengths is linear and would pass through the origin so that the criteria for transformation to percentages set out by Scobie and Saville (2000) are satisfied. The length of fibre grown between injections 1 and 2 was calculated as a percentage of the length grown between injections 1 and 3.

The transformed data yielded the regression equation y = 0.978x + 31.3 (r^2 = 0.995), where y is the percentage of 7 days growth and x is site. This says that for each two hours the change in fibre length (47.8 µm) was 0.978 % of the total length grown over the seven days, or 23.9 µm/hour.

DISCUSSION

This technique will be a powerful tool to study short term changes in wool growth rate. Lincoln sheep were chosen to evaluate this method as they are a breed with a very fast rate of wool growth. These well fed ewes grew wool at almost 700 µm/day. Even at this rate, one can clearly see from Figure 1 that the 66 hour interval between the first and second injection and the 102 hour interval between the second and third for these particular fibres, that points separated by two hours would be indistinguishable. This same pattern was also evident in the trial of Downes *et al.* (1964) with periods of 11 and 7 days separating the three injections in their example. From their work in Corriedale sheep, periods of less than a day would be difficult to measure unless the dose rate and therefore the extent of labelling were reduced. By comparing between separately labelled sites on the midside of each animal, we can measure very short periods of wool growth. By comparing the slope of the regression about an inflexion point we may also be able to determine the effect of a nutrient, growth factor or hormone that changes fibre growth rate. For example catecholamines are known to produce a rapid change in cell division rate in the wool follicle (Scobie *et al.* 1994), but may have either no effect on fibre formation or a transient effect. A transient effect over a short period could be detected with the method outlined here, but not with the method of Downes *et al.* (1964).

FIGURE 4: A pooled frequency distribution of fibre lengths grown by Lincoln ewes during a seven day period with the effect of site and sheep removed, the curve has been smoothed.

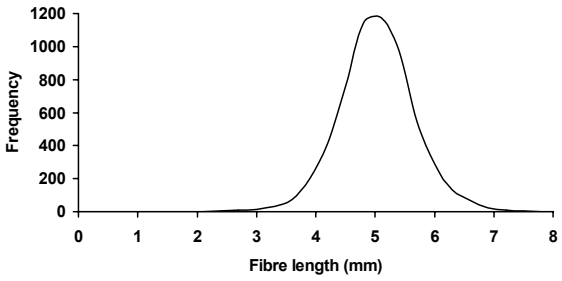


Figure 4 provides a pooled fibre length distribution for fibres from these sheep. The distribution has been transformed to the overall mean by subtracting the mean for the individual site and adding the overall mean for the five sheep. The frequency distribution curve has also been smoothed. For all intents and purposes of analysis, the frequency distribution in Figure 4 resembles a normal distribution. Random samples from this population would be adequate, were it not for the systematic changes in length down the midside. It is therefore essential that this be accounted for by a control period for each sample site and transformation of the treatment period measurements to a percentage of the growth rate during the control period. Note that as measured here the "control" period injections one and three, overlay the "treatment" period injections one and two. For treatments likely to affect the rate, the control period must come first, but in terms of measurement and analysis this makes no difference.

A pooled estimate of the standard deviation of length over the first two days is 220 µm. From power analysis, using only two sample sites to detect a difference of 30 µm with this amount of variation at the 5% level of significance and with 90% power, would require 1132 fibres per sample. Each injection site labels around one square centimeter of skin (Friend and Robards, 1995), which in this breed would label 1400 fibres, with around 14 follicles/mm² (Scobie and Young, 2000). Since the total rate of fibre growth is 30 µm per hour, then if growth was completely stopped for one hour by some treatment, this would scarcely be detectable using only two sites, even if all of the fibres one can harvest from each site were measured. If we consider using breeds like the Merino which grow fibre at rates less than half that of the Lincoln sheep studied here, the accuracy of length measurement will be relatively lower and these power calculations would be changed. On the other hand with greater follicle density in the Merino, there will be more fibres over the injection site to be measured.

Using the method described here to detect a change in the slope of the curve could be achieved with fewer fibres across fewer sites. The large number of sites and fibres used here helped to characterise the fibre populations and ensure that there were no inherent short term fluctuations such as a circadian rhythm of fibre growth. At least five sites pre-treatment and five sites post-treatment are recommended to give a good indication of slope of the line, or to indicate when curvature might become evident due to changing rates of growth.

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