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Cell Proliferation and Cortical Cell Production in Relation to Wool Growth

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Abstract

The relationship of wool growth to cell proliferation in the follicle bulb and to the subsequent migration and growth of the fibre cortical cells was investigated in 10 Peppin Merino sheep. These sheep had been maintained on a low, medium or high level of nutrient intake to ensure a wide range in wool growth. The number and mitotic activity of the germinal cells in the follicle bulb were determined after administration of colchicine. Cortical cell size was measured following isolation of the fibre cells by acid-treatment of wool.

The average fibre production of the follicle varied from $4 \cdot 1 \times 10^4$ to $13 \cdot 2 \times 10^4$ μm^3/day in these sheep. There were also substantial differences between sheep in the mitotic activity of the germinal cells in the bulb, the rate of cell proliferation being highly correlated with the average daily fibre production of the follicle ($r = +0 \cdot 88$, $n = 10$). However, the size of the germinal cell population differed from sheep to sheep and was not closely related to the level of fibre production ($r = +0 \cdot 48$, $n = 10$). The average turnover time of these cells was inversely related to fibre production and varied from $41 \cdot 6$ to $19 \cdot 4$ h ($r = -0 \cdot 82$, $n = 10$).

Multiple regression analysis of the data showed that the average daily fibre production of the follicle was largely determined by the number of germinal cells present in the bulb and their rate of proliferation ($R = +0 \cdot 95$, $n = 10$). Variations in cell turnover time and in cortical cell size were not significant in influencing the rate of fibre production. In these sheep, the average cortical cell varied in size from 658 to 1279 μm^3 and the positive correlation ($r = +0 \cdot 83$, $n = 10$) found between cell size and fibre production is considered to merely reflect an allometric relationship.

The proportion of germinal cells contributing to the fibre cortex was found to be small and variable, ranging from $9 \cdot 4$ to $17 \cdot 8\%$. Furthermore, this proportion was not related to the nutritional level of these sheep, and it is thought that the variability in the distribution of cells to the fibre may be attributed to genetic differences between sheep.

Introduction

The rate of wool growth in Merino sheep is closely related to nutrient intake (Marston 1948; Ferguson *et al.* 1949; Schinckel 1963). This response in wool fibre production reflects alterations in follicle activity; in particular, changes occur in the number of cells produced per unit time within the follicle bulb and in the subsequent migration and growth of the fibre cells (Schinckel 1962; Fraser 1965; Short *et al.* 1965).

Insufficient is known, however, of the relative significance of these various changes in determining wool fibre production. Quantitative studies have shown that the number of cells produced per hour (or mitotic activity) in the follicle bulb is of major importance in influencing fibre output (Schinckel 1961, 1962). However, sequential studies in a Merino sheep indicate that only a small and variable proportion of the bulb cells ultimately enter the fibre (Short *et al.* 1965). The remaining cells contribute

largely, if not entirely, to the inner root sheath, while cells forming the outer root sheath appear to arise from cell renewal within the outer root sheath itself (Chapman 1971; Downes, Chapman, Wilson and Till, unpublished data). Variations in the distribution of bulb cells to the fibre and inner root sheath may be important in modifying fibre production, and require further investigation. The size of the fibre cortical cell is also related to the volume of fibre produced (Schinckel 1961) but the relative contribution of changes in cortical cell size and mitotic activity to changes in fibre production appears to vary considerably (Schinckel 1961; Rougeot 1965; Short *et al.* 1965) and their roles should be further assessed.

In the proliferative region of the follicle bulb, the mitotic activity of the germinal cells is a function of the number present in the germinal population and the turnover time of these cells. Alterations in the mitotic activity of these bulb cells have generally resulted from changes in both components (Fraser 1965; Short *et al.* 1965). In one instance, however, cell number increased substantially while cell turnover time remained relatively constant (Fraser 1965). As alterations in either or both components of mitotic activity ultimately influence production, it is desirable to have more extensive information on the nature of the changes occurring within the germinal cell population of the follicle bulb.

The present investigations have examined further the relationship between fibre production and the number, mitotic activity and turnover time of cells in the proliferative region of the follicle bulb, as well as the proportion of these germinal cells entering the fibre and the subsequent size of the cortical cells in the keratinized fibre. For this purpose sheep were fed selected rations to produce widely different levels of wool growth, and the investigations were undertaken at the end of a 12 months' maintenance period.

Materials and Methods

Sheep and Diet

Ten medium-wool Peppin Merino sheep [nine ewes, 7 years old, and one wether (No. 3), 5 years old] were housed indoors on constant levels of feeding for a period of 12 months. All, except one (No. 5), were fed a mixture of equal parts of chopped lucerne hay and whole oats, each receiving a constant daily ration of 10, 20 or 30 g/kg body weight (based on body weight at the beginning of the feeding period); these rations were designated low, medium and high levels of feed intake respectively (Table 1). The remaining sheep (No. 5) was fed a daily ration of 15 g chopped lucerne hay per kilogram body weight, and was included in the low feed intake group.

Colchicine Treatment

Two days before the end of the 12 months' maintenance period an intravenous injection of colchicine (B.P.), at a dose rate of 0·24 mg/kg body weight, was given to each sheep at about 0945 h. This dose rate was based on earlier investigations (Schinckel 1961).

Wool Growth

Wool was clipped at 3-week intervals during the year from two adjacent patches, each about 50 cm^2, tattooed on the right midside region of each sheep. The wool samples were cleaned as described by Schinckel (1960), and the weight of clean dry wool was measured.

The mean fibre diameters of the samples clipped from the tattooed patches 19 days prior to, and 2 days after colchicine treatment were measured in an air-flow apparatus (Anderson 1954).

For each sheep the mean daily fibre production per follicle during the final 3 weeks was obtained from the weight of clean wool grown on the patches during this period and from the number of fibre-producing follicles in both patches. The percentage of fibre-producing follicles in the patches at the

time of injection was assessed from skin samples removed from an adjacent region at the time of colchicine administration and processed as described by Clarke (1960), while the total number of follicles (active and inactive) had previously been determined from the dimensions of the tattooed patches and the number of follicles per square centimetre in skin biopsies taken adjacent to the patches and processed as above.

Skin Samples and Follicle Cell Counts

Skin biopsies (1 cm diameter) were taken from near the tattooed patches on the right midside region of each sheep immediately before and at 2, 4 and 6 h after the colchicine injection. These were fixed in Zenker's fluid and processed as described by Schinckel (1961). Sections (8 μm) were cut parallel to the longitudinal axes of the follicles and stained with crystal violet and eosin. The effectiveness of colchicine in arresting mitoses was assessed from counts of mitotic nuclei in 300–500 random follicle bulb sections per sample. In each sheep the rate of accumulation of arrested mitoses was approximately linear for 6 h after the colchicine injection (Fig. 1). Furthermore, metaphase arrest was still complete at 6 h, and there was little evidence of anaphase and telophase cells, or of degenerating pycnotic metaphases. The mitotic activity and the number of germinal cells in the proliferative region of the follicle bulbs were therefore determined at 6 h after colchicine injection.

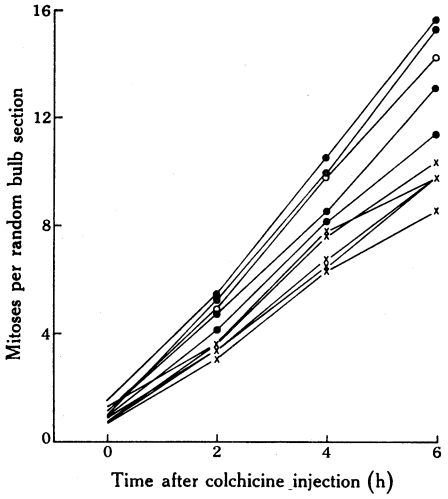

Fig. 1. Mean number of arrested mitoses per follicle bulb section (8 μm thick) in each sheep at various times after colchicine administration. The sheep maintained were on a low (×) medium (●) or high (○) level of feed intake throughout the experiment. The symbols have been omitted at 0 h due to the close proximity of the values.

The method used for estimating mitotic and germinal cell numbers and the average volume of the proliferative region of the follicle bulb was selected on the basis of previous results obtained using serial and random bulb reconstructions (Short *et al.* 1965). As the results were essentially similar, it was considered that the less tedious random bulb section method should provide a reasonable estimate of cell proliferation in the average follicle bulb of a sheep and should be particularly suitable for comparative investigations.

Serial longitudinal sections of the 6 h biopsies were cut at both 4 and 8 μm thickness, stained with haematoxylin, eosin and picric acid and then examined with a projection microscope. For each sheep the number of mitotic and interphase nuclei in the proliferative region, as defined by Short *et al.* (1965), was counted in 220–450 random bulb sections at both thicknesses (magnification for 4 μm, × 500; for 8 μm, × 1250). The histological sections examined, each including *c.* 30 bulb sections, were chosen so that a bulb was represented by only one count. The areas of the counted regions were delineated on tracing paper which was then cut out, weighed, and the area estimated as previously described (Short *et al.* 1965).

For each sheep, the mean area of the proliferative region in the random bulb sections was taken to represent the mean area of the zone in serial sections through an average follicle bulb. The diameter of the average follicle bulb was taken as the mean of the maximum bulb widths of 150 follicles. The latter was measured by partial reconstructions of 50 and 100 follicles in 4 μm and 8 μm histological

sections respectively. Assuming that the bulb is symmetrical in cross section, the volume of the proliferative region in the average follicle bulb can then be obtained from the mean area of the zone × mean maximum bulb width.

The true number of mitotic and interphase nuclei present in a 4 μm thick section of the proliferative region and of known mean area was derived from the difference between the apparent numbers of these nuclei present in the average 8 and 4 μm random section (Abercrombie 1946; Short *et al.* 1965). The number of these nuclei present in the proliferative region of the average follicle bulb was then obtained using mean maximum bulb width/section thickness (4 μm). For each sheep, the turnover time of the germinal bulb cells was derived from the total number of nuclei and the number of mitoses per hour present in the proliferative region of the average follicle bulb.

Cortical Cells

At the time of colchicine injection, small staples of wool were clipped from the left midside region of each sheep. A small snippet (*c.* 3 mg, and several millimetres in length) was cut from the base, cleaned, and digested in 10 ml of 6 M HCl at 60°C for 45 min (Short *et al.* 1965). For each sheep the length and width of 250 cortical cells were measured at ×900 and the average cortical cell volume calculated as described by Short *et al.* (1965).

The number of cortical cells contributing hourly to the fibre cortex was determined from cortex volume per hour and cortical cell size. For this purpose it was assumed that the mean thickness of the fibre cuticle was 0·5 μm; this thickness was based on electron microscopic examination of representative fibres from these sheep.

Statistical Analysis

The relationship of the daily fibre production of the follicle to various follicle parameters, namely number of mitoses per hour, number of germinal cells, cell turnover time, and fibre cortical cell volume, was determined by multiple linear regression analyses employing both forward and backward stepwise elimination to confirm the relative contribution of each variable to fibre production. At each step of adding or deleting a variable, the *t*-test was used for testing the significance of the change in the residual sum of squares. Multiple correlation coefficients were adjusted for the number of variables in the relationship.

Correlation coefficients, regression equations and analyses of variance were calculated after Snedecor (1956).

Results

Wool Growth

The daily clean dry wool production of the 10 sheep during the last 3 weeks of the maintenance period ranged from 24 to 148 mg/day for the combined tattooed patches and generally reflected the feed intake level of the animal (Table 1). The total number of fibre-producing follicles in both patches also varied considerably between sheep (Table 1) but was not significantly correlated with the wool production ($r = +0{\cdot}47$, $n = 10$). In all except two sheep the proportion of inactive, or resting, follicles was negligible (less than 1%); the remaining two sheep (Nos 1 and 4) on a low level of feed intake had 22 and 25% of follicles inactive. Both the daily fibre production of the active follicle and the mean diameter of the fibre were generally found to be greater in sheep of higher wool production (Table 1).

Colchicine Treatment

The skin biopsies taken after colchicine injection showed that the dose rate used has effectively arrested mitoses (Fig. 1). Previous investigations had indicated that the dose margin between complete mitotic arrest and death was narrow (Schinckel 1961). In the present experiment the colchicine treatment resulted in the death of five

sheep within 2–6 days of injection (Nos 2, 3, 4, 7 and 8). The remaining sheep shed their fleece 14 days after receiving colchicine, and two of these subsequently died 1 week later (Nos 6 and 10).

Table 1. Level of nutrition and fibre production at the time of colchicine administration in 10 sheep maintained for 12 months on constant rations

Feed intake level	Sheep No.	Clean wool per 100 cm^2 patches (mg/day)	10^{-3} × No. of fibres in 100 cm^2 patches	10^{-4} × Fibre volume[A] per follicle (μm^3/day)	Fibre diameter (μm)
Low	1	24	438	4·12	18·5
	2	38	705	4·08	19·0
	3	48	734	4·98	17·7
	4	65	880	5·65	18·0
	5	71	780	6·97	21·2
Medium	6	63	568	8·48	22·9
	7	78	561	10·60	23·6
	8	88	877	7·66	21·2
	9	94	573	12·60	25·5
High	10	148	860	13·20	27·7

[A] Fibre volume = clean wool weight per active follicle/density of wool. Density of clean dry wool was taken as 1·3052 g/cm^3 (Van Wyk and Nel 1940).

Cell Population and Proliferation in the Follicle Bulb

For each sheep the mean maximum follicle bulb width, and the area and true number of mitotic and interphase nuclei present in the proliferative region of an average 4-μm thick bulb section are given in Table 2. The size of the follicle bulb was related to the fibre production of the follicle, the correlation between daily fibre volume and bulb width being +0·80 (n = 10). The cell population in the proliferative region of the average follicle bulb of each sheep (Table 3) consisted of a variable number of mitotic and interphase nuclei, the total number of cells present ranging from 395 to 891. Although the number of germinal cells was not closely correlated with fibre production (r = +0·48, n = 10, Table 5), analysis of variance showed that the feed intake level of these sheep had a significant influence on total cell number in the follicle bulb. The proliferative region of the bulb varied in size from approximately 2×10^5 to 4×10^5 μm^3 and was closely related to the total number of germinal bulb cells (r = +0·81, n = 10). The mean volume of the germinal cells ranged from an estimated 353 to 555 μm^3 (Table 3). The rate of cell proliferation in the bulb varied from a minimum of 13·6 to a maximum of 39·2 mitoses per hour and was highly correlated with the volume of fibre produced per day (r = +0·88, n = 10, Table 5).

The turnover time of cells in the average follicle bulb decreased from 41·6 h for a sheep on a low level of feed intake to 19·4 h for a sheep on a high feed intake (Table 3). Analysis showed that cell turnover time was inversely related to the daily fibre production of the follicle (r = −0·82, n = 10, Table 5).

Cortical Cells

Mean cell width varied from 5·2 to 7·1 μm and mean cell length from 88·1 to 106·0 μm (Table 4). These two cell dimensions appeared to vary independently,

regardless of whether the cells were obtained from the same or different sheep. Mean cortical cell width, however, was significantly correlated with the mean fibre diameter of the sheep ($r = +0 \cdot 90$, $n = 10$). The average cortical cell in these sheep varied in size from 658 to 1279 μm^3 (Table 4), the size of the cell being significantly correlated with fibre volume growth rate ($r = +0 \cdot 83$, $n = 10$, Table 5).

Table 2. Width of the follicle bulb, and the area and true number of nuclei in the proliferative region of an average bulb section 6 h after injection of colchicine

Sheep No.	Mean maximum bulb width (μm)	Proliferative area in average bulb section (μm^2)	No. of nuclei per 4-μm thick bulb section: Mitotic	Interphase
1	74·8	3430	5·7	30·7
2	68·4	3020	4·8	28·4
3	69·5	3170	5·4	20·9
4	67·8	3210	7·4	28·9
5	69·6	3150	5·6	17·0
6	77·0	3130	6·8	28·3
7	83·2	3600	9·7	31·0
8	86·8	4380	7·9	30·9
9	90·5	4470	10·4	29·0
10	83·2	3620	8·7	19·5

In these sheep, the relationship between fibre cortex volume per hour and cortical cell size showed that the number of cortical cells entering the cortex varied from 1·7 to 4·6 cells per hour (Table 4). Comparison of this rate with the number of mitoses

Table 3. Number of nuclei present in the proliferative region of the average follicle bulb, and the size, mitotic activity and turnover time of the cells 6 h after injection of colchicine

Sheep No.	No. of nuclei per average bulb: Mitotic	Interphase	Total	$10^{-5} \times$ Bulb[A] volume (μm^3)	Cell volume (μm^3)	No. of mitoses per h	Turnover time (h)
1	106	574	680	2·6	377	17·7	38·3
2	82	485	567	2·1	364	13·6	41·6
3	94	363	457	2·2	481	15·7	29·1
4	126	489	615	2·2	353	21·0	29·3
5	98	297	395	2·2	555	16·4	24·1
6	131	544	675	2·4	357	21·8	31·0
7	203	645	848	3·0	353	33·8	25·1
8	171	671	842	3·8	452	28·4	29·6
9	235	656	891	4·1	454	39·2	22·7
10	182	406	588	3·0	511	30·3	19·4

[A] i.e., proliferative region.

per hour in the follicle bulb indicated that the proportion of proliferated cells entering the fibre cortex was small and variable, ranging from 9·4 to 17·8%. Furthermore, analysis of variance showed that the nutritional level of these sheep had no significant effect on the relative proportion of cells contributing to the fibre.

The Determinants of Fibre Production

In the regression analyses of the data, the multiple correlation coefficient between fibre production and the number of mitoses per hour, total number of germinal cells, their turnover time and cortical cell volume was 0·955, and between fibre production

Table 4. Size and rate of production of cortical cells

Sheep No.	Mean cortical cell dimensions:[A] Width (μm)	Length (μm)	Cortical cell volume (μm^3)	Fibre cortex volume (μm^3/h)	No. of cortical cells per h	Proportion of bulb cell entering fibre[B] (%)
1	5·4±0·1	92·1±0·6	709	1537	2·2	12·2
2	5·8±0·1	98·2±0·8	876	1525	1·7	12·8
3	5·2±0·1	94·0±0·7	658	1846	2·8	17·8
4	5·3±0·1	93·2±0·8	675	2100	3·1	14·8
5	6·0±0·1	94·7±0·7	904	2637	2·9	17·8
6	6·0±0·1	99·8±0·8	928	3233	3·5	16·0
7	6·9±0·1	103·2±0·7	1279	4071	3·2	9·4
8	5·4±0·1	106·0±0·8	803	2896	3·6	12·7
9	6·4±0·1	96·3±0·7	1049	4846	4·6	11·8
10	7·1±0·1	88·1±0·7	1176	5096	4·3	14·3

[A] Mean ± standard error.
[B] Derived from (No. of cortical cells per h)/(No. of mitoses per h).

and mitoses per hour and germinal cell number was 0·947 (Table 5). Although the inclusion of cortical cell volume, or both cortical cell volume and cell turnover time

Table 5. Correlation coefficients for relationships between daily fibre production and mitoses per hour, germinal cell number, cell turnover time and cortical cell volume in the average wool follicles of 10 sheep

(*a*) Matrix of simple correlation coefficients

	Fibre	Mit.	No.	T. time	Vol.
Daily fibre volume (fibre)	1·000				
Mitoses per h (mit.)	0·882	1·000			
Cell number (No.)	0·477	0·798	1·000		
Turnover time (t. time)	−0·823	−0·658	−0·105	1·000	
Cortical cell volume (vol.)	0·828	0·693	0·397	−0·561	1·000

(*b*) Adjusted multiple correlation coefficients

	Fibre		Fibre
Mit. + No.	0·947	Mit. + No. + t. time	0·941
Mit. + t. time	0·921	Mit. + No. + vol.	0·963
Mit. + vol.	0·912	Mit. + t. time + vol.	0·954
No. + t. time	0·884	No. + t. time + vol.	0·942
No. + vol.	0·792	Mit. + No. + t. time + vol.	0·955
Vol. + t. time	0·914		

improved the correlation (R = 0·963, 0·955 respectively, n = 10, Table 5), these two variables made no significant contribution to daily fibre production. As most of the variance in fibre production is accounted for by mitotic activity and cell number,

the relationship between these components is expressed in the following regression equation:

$$Y = 3\cdot36(\pm1\cdot48) + 0\cdot534(\pm0\cdot069)X_1 - 0\cdot013(\pm0\cdot004)X_2,$$

where Y is fibre production, X_1 is the number of mitoses per hour, X_2 is the germinal cell number, and the values in parentheses are standard errors.

Discussion

Previous investigations of cell proliferation in the follicle have involved the use of colchicine, colcemid or tritiated thymidine (Bullough and Laurence 1958; Cattaneo *et al.* 1961; Schinckel 1961; Fraser 1963; Fraser 1965; Short *et al.* 1965; Downes *et al.* 1966). Although it is possible that these agents may retard or stimulate cell proliferation (Bertalanffy 1964; Schultze 1969; Fitzgerald and Brehaut 1970), colchicine and thymidine have provided similar estimates of turnover time in both the same and comparable tissues (Bertalanffy 1964; Tannock 1965). For accurate estimation of cell proliferation with colchicine, however, it is important to establish a dose–response curve and a linear collection of metaphases after injection (Tannock 1965). The data in the current investigation together with the previously established dose–response curves (Schinckel 1961) satisfy both criteria, and metaphase arrest was still complete at 6 h after colchicine injection.

The present results show that the growth of the wool fibre in different sheep is determined largely by two factors, namely the mitotic activity and the total cell population in the proliferative region of the bulb. Mitotic activity is known to be a major factor influencing wool growth in different sheep phenotypes (Schinckel 1961) and in the same sheep under widely differing nutritional conditions (Schinckel 1962; Short *et al.* 1965). The effect of hormones on mitotic activity in the follicle has not been elucidated, although thyroxine is thought to have little or no effect (Rougeot 1965). Despite the fact that cell proliferation is an important rate-limiting process in fibre production, insufficient is known of the conditions which will modify the mitotic activity of the wool follicle.

Changes in either or both cell turnover time and germinal cell number can alter the mitotic activity of cells in the follicle bulb (Fraser 1965; Short *et al.* 1965). It has been shown that bulb cell turnover time is influenced by variations in the nutritional intake of the sheep (Short *et al.* 1965). In the current investigations based on a number of sheep on different planes of nutrition, turnover times ranged from 19·4 to 41·6 h, and the inverse association with fibre production is further indication that turnover time is affected by nutrition. Temperature, hormones and deprivation of food are some of the various factors known to alter the duration of cell turnover time (Schultze 1969), and it is conceivable that some or all of these factors may influence cell proliferation in the wool follicle. However, it has been observed that improved seasonal, and hence nutritional, conditions will not always produce a change in cell turnover time (Fraser 1965). This situation may well be explained by the concept of a minimum value for bulb cell turnover time as postulated by Black and Reis (1979).

Although the number of cells in the germinal population is not closely correlated with fibre production, it is nevertheless the other main factor determining wool growth. Alterations in cell number and mitotic activity together account for *c.* 90% of the variance in fibre production between sheep ($R = +0\cdot947$). In these Merino sheep, the population of the average follicle bulb varied from 395 to 891 cells. This

variation in cell number probably reflects individual variation between sheep receiving the same nutrient intake, as well as an effect which is attributable to nutrition. Other data from individual sheep indicate that the number of cells in the bulb population appears to be influenced by the animal's nutritional status; increases of 12–49% occurred in three sheep when nutrition was improved (Fraser 1965; Short *et al.* 1965). Such population changes presumably arise by the retention of a greater number of newly formed cells in the germinal region such as occurs in epidermis (Van Scott 1965). This alone would cause an increase in the mitotic activity of the bulb. An increase in the number of cells capable of division implies that the balance between cell proliferation and cell differentiation has been modified. Improved nutrition may well result in improved blood supply or nutrients available to the follicle and thus enhance the capacity of the cell to divide.

Isolation of cortical cells from wool by enzymic or chemical procedures generally results in some loss of material and chemical modification of the cells (Ward *et al.* 1955; Leveau 1958; Simmonds and Bartulovich 1958; Lundgren 1965). With HCl treatment and suitable reaction conditions, however, these disadvantages can be minimized (Leveau 1956*a*, 1956*b*), and cortical cell dimensions measured under optimal conditions (Short *et al.* 1965) should enable valid comparisons to be made of variations in cortical cell size.

In this group of Merino sheep, differences were observed in the average length and width of the cortical cells, but there was no apparent relationship between these two cell dimensions either within or between sheep. The positive correlation found between cell width and fibre diameter is further evidence that larger fibres are generally composed of larger cortical cells (Schinckel 1961; Appleyard 1967). In the current investigations the average size of the cortical cell varied considerably between sheep and was correlated with fibre production. Initially it was thought that changes in cortical cell volume may have partly contributed to differences in the rate of fibre output (Schinckel 1961). Since then an increasing amount of evidence has indicated that changes in cortical cell number rather than changes in cortical cell size account for most of the differences in fibre growth (Short *et al.* 1965; Appleyard 1967). It is now apparent from the present study that changes in cortical cell size do occur, but are not significant in determining variations in fibre production between sheep. The relationship observed between cortical cell size and the volume of fibre produced is presumably merely allometric, and not one of cause and effect.

Although the average follicle bulb is producing some 14–39 new cells per hour, only a small proportion of these cells actually contribute to the fibre. The current estimates show that 9–18% of the cells were destined for the fibre cortex, and this distribution appears to vary randomly from sheep to sheep regardless of nutrient intake and wool growth levels. A similar distribution was observed by Short *et al.* (1965); however, in that particular Merino sheep improved nutrition resulted in increased fibre production and a 3% increase in the proportion of cells entering the fibre cortex. Alterations in the distribution of cells would not be apparent in the present investigations, but it is doubtful if such slight shifts in distribution would generally be significant in influencing fibre production. However, in some situations such as the seasonal thinning of primary wool follicles in Herdwick sheep and in the hair constrictions of various animals, quite pronounced shifts do occur in the movement of cells to the fibre and inner root sheath (Priestley and Rudall 1965; Straile 1965). In these instances, changes in the cell distribution may play a more significant

role in influencing fibre production. The possibility that such changes arise from the presence in the germinal cell population of several cell types having differing cell turnover times is not entertained here. It is obvious that a greater understanding is needed of the factors which regulate the distribution of cells from the follicle bulb, and the extent to which this shifting of cells affects the rate of fibre production.

The formation of a wool fibre from a small proportion of germinal bulb cells is a relatively inefficient process in terms of cell production. In the follicle, the functional role of the inner root sheath may be important in placing restrictions on the proportion of cells entering the fibre. In addition to this, the variability observed in Merino sheep suggests that the proportion is determined genetically and may be subject to slight fluctuations imposed by nutrition. Comparative studies in three breeds of sheep indicate that the proportion of the follicle cross-section occupied by fibre varies between breeds (Vsevoldov and Prusova 1966); this may reflect genetic differences between breeds in the proportion of cells forming the fibre. By careful selection of sheep, both within and between breeds, it may be possible to improve the relative efficiency of fibre production by the follicle.

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References

Abercrombie, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–47.

Anderson, S. L. (1954). The air-flow method of measuring wool fibre fineness. *J. Text. Inst.* **45**, P312–16.

Appleyard, H. M. (1967). Observations on cortical cell size and arrangement in some animal fibres. *J. R. Microsc. Soc.* **87**, 1–6.

Bertalanffy, F. D. (1964). Tritiated thymidine *versus* colchicine technique in the study of cell population cytodynamics. *Lab. Invest.* **13**, 871–86.

Black, J. L., and Reis, P. J. (1979). Speculation on the control of nutrient partition between wool growth and other body functions. In 'Physiological and Environmental Limitations to Wool Growth'. (Eds J. L. Black and P. J. Reis.) (University of New England Publishing Unit: Armidale.)

Bullough, W. S., and Laurence, E. B. (1958). The mitotic activity of the follicle. In 'The Biology of Hair Growth'. (Eds W. Montagna and R. A. Ellis.) pp. 171–87. (Academic Press: New York.)

Cattaneo, S. M., Quastler, H., and Sherman, F. G. (1961). Proliferative cycle in the growing hair follicle of the mouse. *Nature (London)* **190**, 923–4.

Chapman, R. E. (1971). Cell migration in wool follicles of sheep. *J. Cell Sci.* **9**, 791–803.

Clarke, W. H. (1960). A histological technique for the study of the skin follicle population in sheep. In 'Biology of the Fleece'. Tech. Pap. Anim. Res. Lab. CSIRO Aust. No. 3, pp. 92–7.

Downes, A. M., Chapman, R. E., Till, A. R., and Wilson, P. A. (1966). Proliferative cycle and fate of cell nuclei in wool follicles. *Nature (London)* **212**, 477–9.

Ferguson, K. A., Carter, H. B., and Hardy, M. H. (1949). Studies of comparative fleece growth in sheep. I. The quantitative nature of inherent differences in wool-growth rate. *Aust. J. Sci. Res.* **B2**, 42–81.

Fitzgerald, P. H., and Brehaut, L. A. (1970). Depression of DNA synthesis and mitotic index by colchicine in cultured human lymphocytes. *Exp. Cell Res.* **59**, 27–31.

Fraser, I. E. B. (1963). The use of colchicine and colcemid for metaphase stasis in the matrix cells of wool follicles. *Aust. J. Biol. Sci.* **16**, 211–17.

Fraser, I. E. B. (1965). Cellular proliferation in the wool follicle bulb. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 427–45. (Angus and Robertson Ltd: Sydney.)

Leveau, M. (1956*a*). Colorimetric differentiation of cortical cells isolated from the ortho- and para-cortex of wool. (In French.) *Bull. Inst. Text. Fr.* **60**, 61–4.

Leveau, M. (1956*b*). Wool: separation of ortho- and para-cortical cells. (In French.) *Bull. Inst. Text. Fr.* **63**, 91–6.

Leveau, M. (1958). Distribution of cystine between the ortho- and the para-cortex of wool. (In French.) *Bull. Inst. Text. Fr.* **74**, 75–81.

Lundgren, H. P. (1956). Separation of cortical cells of two types from disintegrated wool by means of density gradient columns. Proc. Int. Wool Text. Res. Conf., Aust. Vol. F, pp. 200–5. (CSIRO Aust.: Melbourne.)

Marston, H. R. (1948). Nutritional factors involved in wool production by Merino sheep. I. The influence of fodder intake on the rate of wool growth. *Aust. J. Sci. Res.* **B1**, 362–75.

Priestley, G. C., and Rudall, K. M. (1965). Modifications in the Huxley layer associated with changes in fibre diameter and output. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 165–70. (Angus and Robertson Ltd: Sydney.)

Rougeot, J. (1965). The effect of thyroid hormones on the morphology of the wool cuticle. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 625–40. (Angus and Robertson Ltd: Sydney.)

Schinckel, P. G. (1960). Variation in feed intake as a cause of variation in wool production of grazing sheep. *Aust. J. Agric. Res.* **11**, 585–94.

Schinckel, P. G. (1961). Mitotic activity in wool follicle bulbs. *Aust. J. Biol. Sci.* **14**, 659–76.

Schinckel, P. G. (1962). Variation in wool growth and of mitotic activity in follicle bulbs induced by nutritional changes. *Anim. Prod.* **4**, 122–7.

Schinckel, P. G. (1963). The potential for increasing efficiency of feed utilization through newer knowledge of animal nutrition: C. Sheep and goat. Proc. Wld. Conf. Anim. Prod. 1st, Rome, Vol. 1, pp. 199–248. (European Association for Animal Production : Rome.)

Schultze, B. (1969). 'Physical Techniques in Biological Research'. (Ed. A. W. Pollister.) Vol. IIIB. (Academic Press: New York.)

Short, B. F., Wilson, P. A., and Schinckel, P. G. (1965). Proliferation of follicle matrix cells in relation to wool growth. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 409–26. (Angus and Robertson Ltd: Sydney.)

Simmonds, D. H., and Bartulovich, J. J. (1958). The amino acid composition of fractionated cortical cells from wool. *Text. Res. J.* **28**, 378–81.

Snedecor, G. W. (1956). 'Statistical Methods'. 5th edn. (Iowa State University Press: Iowa.)

Straile, W. E. (1965). Root sheath–dermal papilla relationships and the control of hair growth. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 35–57. (Angus and Robertson Ltd: Sydney.)

Tannock, I. F. (1965). A comparison of the relative efficiencies of various metaphase arrest agents. *Exp. Cell Res.* **47**, 345–56.

Van Scott, E. J. (1965). Replacement kinetics of integumental epithelia. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 399–408. (Angus and Robertson Ltd: Sydney.)

Van Wyk, C. M., and Nel, H. A. M. (1940). Studies on the basic characteristics of South African Merino wool. II. Specific gravity. *Onderstepoort J. Vet. Sci. Anim. Ind.* **15**, 325–32.

Vseboldov, E. B., and Prusova, L. S. (1966). Some quantitative interrelations between the layers of the sheep wool follicle and their association with the growth of wool. (In Russian.) *Inst. Eksp. Biol., Alma-Ata* **3**, 112–24.

Ward, W. H., Binkley, C. H., and Snell, N. S. (1955). Amino acid composition of normal wools, wool fractions, mohair, feather and feather fractions. *Text. Res. J.* **25**, 314–25.

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