Aust. J. Biol. Sci., 1984, **37**, 237–55

Cortical Cell Types and their Distribution in Wool Fibres

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Abstract

A method of determining cell types in wool fibres by methylene-blue staining has been extended to allow relationships between cortical cell type and cortical diameter to be studied in wools from individual sheep. Application of the method to wools from 12 sheep from six breeds showed that orthocortical cells were the predominant cell type produced. The percentage area occupied by orthocortical cells in a fibre cross-section increased with increasing cortical diameter in either a curvilinear (log-linear) or a linear manner. Nutritional stress or season may have affected the relationship in some sheep.

The arrangement of cortical cell type within fibre cross-sections showed less change in linear-type wools as diameter increased than in log-linear-type wools. Proportions and arrangements of cell types sometimes varied along the length of individual fibres, usually in a definite sequence.

Orthocortical cells had larger cross-sectional areas than mesocortical–paracortical cells and were most frequently associated with changes in fibre diameter and shape. Mesocortical cells are believed to be true intermediate cells as they ranged in structure from orthocortical-like to paracortical-like.

Few qualitative differences in wool proteins were found between wools, suggesting that differences in their properties arose mainly from quantitative protein and cellular differences.

Extra keywords: follicles, wool or hair; cell differentiation, wool or hair; fibre diameter, wool or hair.

Introduction

Wool follicles produce fibres with cortices made up of three types of cells: ortho-, meso-, and paracortex (Horio and Kondo 1953; Rogers 1959; Bonès and Sikorski 1967; Kaplin and Whiteley 1978; Wagner *et al.* 1983). Each type is identified by the size and distribution of its aggregated keratin protein bundles (macrofibrils) within the cells and the organization of the 7-nm microfibrils and associated matrix proteins within the macrofibrils. Generally, cells of a particular type are clustered into strands or cords running through the fibre cortex, as in the common bilateral configuration, where half of the cortex is made of orthocortical and the remainder of paracortical cells (Horio and Kondo 1953; Kaplin and Whiteley 1978). Other types of cellular organization have also been reported, especially in fibres of larger diameter (Fraser and Rogers 1955).

The role of cortical cell type in wool characteristics is not well-defined except in crimp. Fibre crimp (changes in fibre direction) is thought to arise from the bilateral configuration of ortho- and paracortical cells. The latter are typically found on the inside of the curve of any crimp, and orthocortical cells on the outside (Horio and Kondo 1953; Fraser and Rogers 1955; Kaplin and Whiteley 1978). While a general relationship exists between staple crimp and fibre crimp, different forms of fibre crimp have been associated with bulk or resistance to compression. In high-bulk wools fibre crimp is believed to be predominantly

0004-9417/84/040237$02.00

helical and in low-bulk wools predominantly uniplanar (Chaudri and Whiteley 1968). Whether the same bilateral configuration of cortical cells gives rise to both forms of fibre crimp has not been clearly established.

Differences in cortical cell type have also been implicated in wool tensile strength. Orwin *et al.* (1980), using pooled data from one sheep breed, showed that cortical cell type and arrangement may differ for wools of differing fibre diameters and staple strengths. As pooled data were used, the relationships may not necessarily hold for individual sheep.

The development of more precise techniques for assessing the amount and arrangement of cortical cell types in wools would seem necessary if a better understanding of their role in wool properties is to be gained.

The purpose of this study was to develop further and evaluate a technique to assess relationships between cortical cell type and fibre diameter of wools from individual sheep and to apply this technique to a limited range of wools as a preliminary to more detailed studies of cortical cell types and wool properties.

Table 1. Background data on samples

Sheep	Age (yr)	Month of wool growth	Mean fibre diam. of fleece (μm)	Mean fibre diam. of sample ± s.d. (μm)	Bulk (cm^3/g)	Sample staple crimps per 25 mm	Body wt (kg)
Romney							
R111	5	July 1981	42·2	34·5 ± 6·6	17·8	2·6	
		Dec. 1981		43·8 ± 6·3			
R381	5	July 1981	37·5	25·5 ± 5·5	21·4	2·5	
		Dec. 1981		42·4 ± 8·6			
R148	5	July 1981	34·2	21·8 ± 4·9	28·5	4·0	
		Dec. 1981		38·5 ± 10·2			58·0
		Feb. 1982		32·0 ± 7·9			52·0
R327	5	July 1981	40·2	33·7 ± 8·1	19·8	2·0	
		Dec. 1981		46·3 ± 10·1			71·5
		Feb. 1982		37·4 ± 9·4			65·5
Feral Romney	2	Mar. 1981	40·4	38·4 ± 7·4	29·5	5·2	
Drysdale	3	Dec. 1981	47·3	46·7 ± 13·0	22·9	2·5	
Lincoln	3	July 1981	—	44·8 ± 11·1	15·7	3·5	
Perendale							
P632	2	July 1978	39·0	35·7 ± 7·5	26·2	3·5	
P715	2	July 1978	34·6	32·4 ± 5·8	27·7	4·7	
Corriedale							
C910	5	July 1981	29·3	27·8 ± 5·9	36·0	14·0	
C340	5	July 1981	28·0	27·8 ± 6·2	26·9	8·0	
Merino	5	Aug. 1979	—	24·1 ± 4·9	29·1	11·0	

Materials and Methods

Sheep and Wool

Twelve sheep of the following breeds were used: Romney (4), feral Romney (1), Drysdale (1), Lincoln (1), Perendale (2), Corriedale (2) and Merino (1). The Romneys and Corriedales were grazed in the same paddock during the period that wool samples were collected. Because of a summer drought and subsequent shortage of grass, these sheep suffered substantial losses in body weight in January and February (Table 1).

Wool samples were clipped from the midside body area and cleaned by double rinses in light petroleum, absolute alcohol, and distilled water, then dried at room temperature (R.T.). Fibre diameter was measured by a standard projection microscope method [International Wool Textile Organization test method, IWTO-8-66(E)]; the degree of fibre crimp by the resistance to compression

or the bulk (Chaudri and Whiteley 1968; Dunlop *et al.* 1974); and staple crimp per 25 mm by measuring the crimps, peak to peak, with a ruler graduated in millimetres. Data on the bulk, staple crimp, and mean fibre diameter of the fleece wool and monthly wool samples, together with the ages of the sheep, are shown in Table 1.

Characterization of Cortical Cell Types

Light microscopy (LM)

Bundles of fibres from four different regions of each wool sample were pre-embedded in JB-4 resin (Polysciences, Inc., Pa, U.S.A.). Enough wool from each bundle was pulled through a plastic tube of internal diameter 1 mm until it was completely filled. This resulted in about 100 fibres lying parallel with each other in each tube. After polymerization of the resin, the plastic tubing was removed and the embedded fibres were cross-sectioned at 2 μm with an ultramicrotome (LKB Ultrotome III, LKB Produkter, Stockholm, Sweden). The sections were dried on glass slides, oxidized with performic acid (35 min at R.T.), stained with 1% (w/v) methylene blue to distinguish areas of ortho- and paracortical cells (Clarke and Maddocks 1965), and mounted in a neutral mounting medium.

The cross-sections of 200–300 fibres, i.e. 50–70 per bundle, were examined from each wool sample. Only cross-sections cut obliquely or buckled were excluded from the analysis. Using a camera lucida attachment and a magnification of ×2900, each cross-section was drawn to record the areas of unstained, lightly stained, and heavily stained cells. These areas were then measured with a digitizing tablet (Summagraphics, Ct, U.S.A.; resolution 0·1 mm). The maximum and minimum diameters of the cortex were also determined. In order to determine if the diameter measurement used altered the results, the diameter of the cortex was expressed as $\frac{1}{2}$(maximum + minimum diameter) or by the minimum alone. The cross-sections were then classed according to diameter into groups of 5 μm, starting at 20 μm, and the mean percentage area of each cell type was calculated for each diameter group. Regression techniques were used to examine the relationship between cortical diameter and orthocortical cell proportions.

At the same time, the arrangement of the stained cells was subjectively classified in order of increasing complexity: 100% unstained; cellular (one or more stained but separate cells apparent); unilobate (a cord of stained cells); bilateral (half the cross-section composed of stained cells); lobate–cellular (a cord plus individual cells stained); bilobate or multilobate (two or more cords); 100% of cross-section stained. Other arrangements were rare and were combined. The diameter range for each sheep was divided into quartiles and the mean percentage of the various cell-type arrangements was calculated for each quartile using contingency tablets.

The effect of the staining procedure on the cortical cross-sectional area was measured on 71 cross-sections from four sheep. Drawing errors were assessed by drawing the same 30 fibre cross-sections three times. The effectiveness of the wool-sampling procedure was tested by taking an additional range of wool samples from three of the sheep (R148, July; Drysdale; C340).

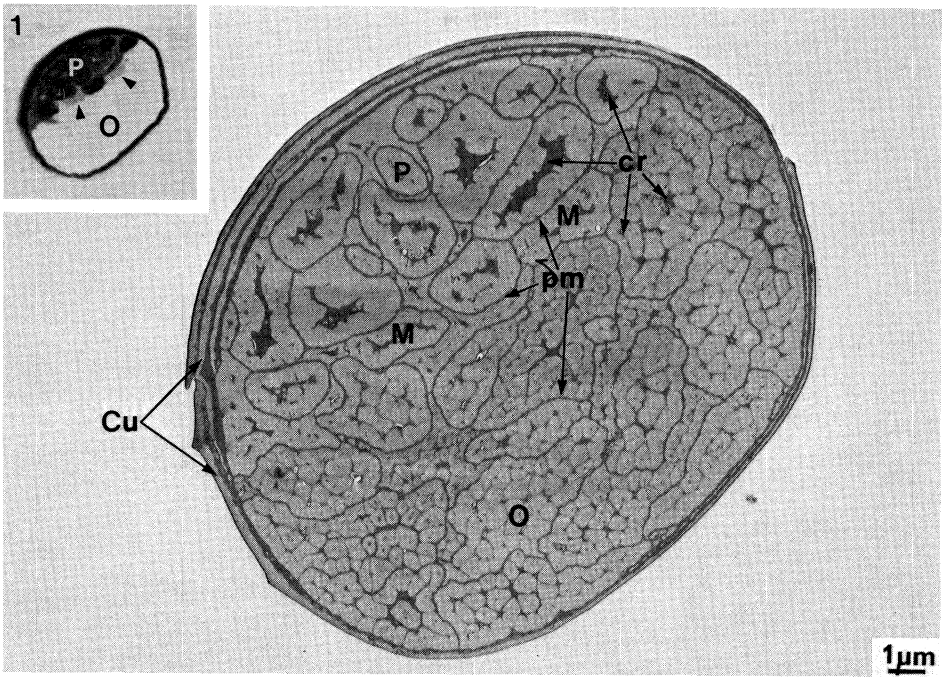
Whole fibres from all the sheep were stained as above, except that performic acid oxidation was carried out for 1 h, the methylene blue staining and differentiation taking up to 1 h longer. Twenty fibres from each sheep were mounted on glass slides and 25-mm lengths were examined at ×160 for each fibre. These lengths covered the growth period from which the fibre cross-sections were sampled. The arrangement of the cell types was assessed as above. The length occupied by each type of arrangement was measured with an eye-piece micrometer, and diameters were measured at the point of change, or at intervals of 5 mm. Particular note was made of the cell-type arrangements occurring in kinks and bumps in the fibres. Some of these kinks and bumps corresponded to the diameter changes called nabs (James and Ward 1965). The data were analysed using the MINITAB statistical package (Ryan *et al.* 1981).

Transmission electron microscopy (TEM)

Three methods of staining with heavy metals were used for correlative TEM-LM studies on wool samples from the Merino (1 sheep), Perendale (2), Corriedale (1), Romney (4), and Lincoln (1) breeds, as follows:

(1) Fibres were embedded in epoxy resins (TAAB Ltd, Reading, England). Thin sections were stained with 2% (w/v) phosphotungstic acid in 50% (v/v) ethanol for 2 h at R.T. (Bonès and Sikorski 1967).

(2) Fibres were stained with 5% (w/v) silicotungstic acid (Dwarte and Vesk 1982), then with 1% (w/v) OsO_4 in 0·05 M phosphate buffer (pH 6) for 1 h at R.T., and finally with 2%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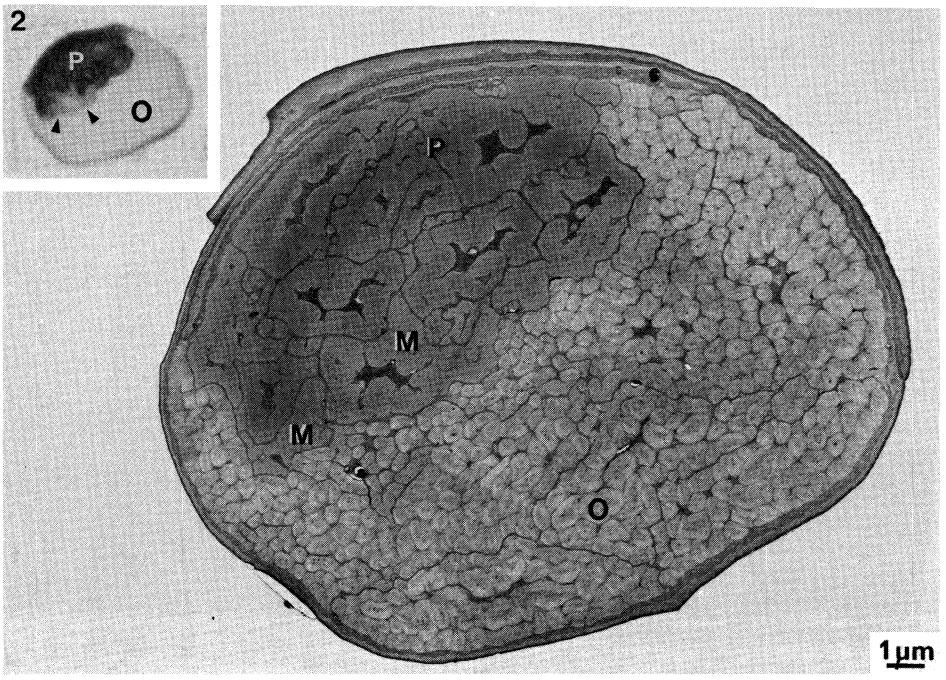
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(w/v) uranyl acetate in 95% (v/v) ethanol. The fibres were dehydrated in ethanol and embedded in acrylic resin (London Resin Co., Basingstoke, England).

(3) Fibres were reduced in 0·05 M 2-mercaptoethanol in 0·05 M phosphate buffer (pH 6·7) at 40°C for 30 min and then immersed in 1% (w/v) OsO_4 in phosphate buffer (pH 6) at 0°C for 1 h, one to three times. After successive washes in 50% (v/v) and 90% (v/v) ethanol, the fibres were stained in 2% (w/v) uranyl acetate in 95% (v/v) ethanol for 90 min at R.T. and embedded in acrylic resin.

For correlative work, adjacent thick (2 μm) and thin sections were processed by the methylene-blue staining procedure above or viewed in a Philips EM300 electron microscope at 80 kV respectively. A total of 101 different fibres, including some from the nutritionally stressed sheep (R148, February sample, and R327, February sample), were examined. The mean cross-sectional area of orthocortical and mesocortical–paracortical cells was determined from 78 of these cross-sections by dividing the number of cells into the area occupied by each cell type.

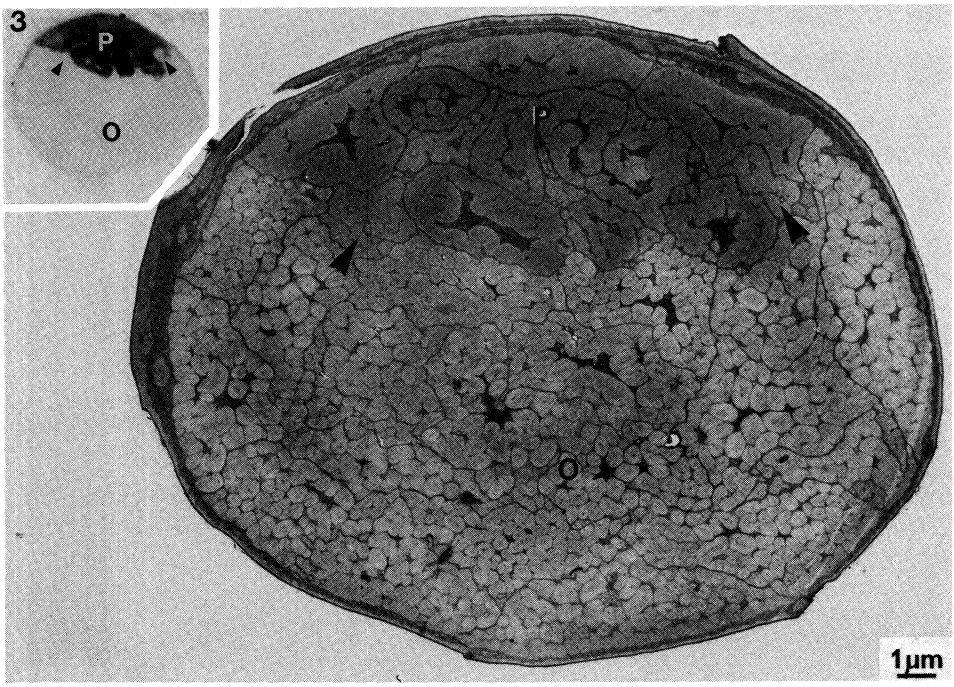
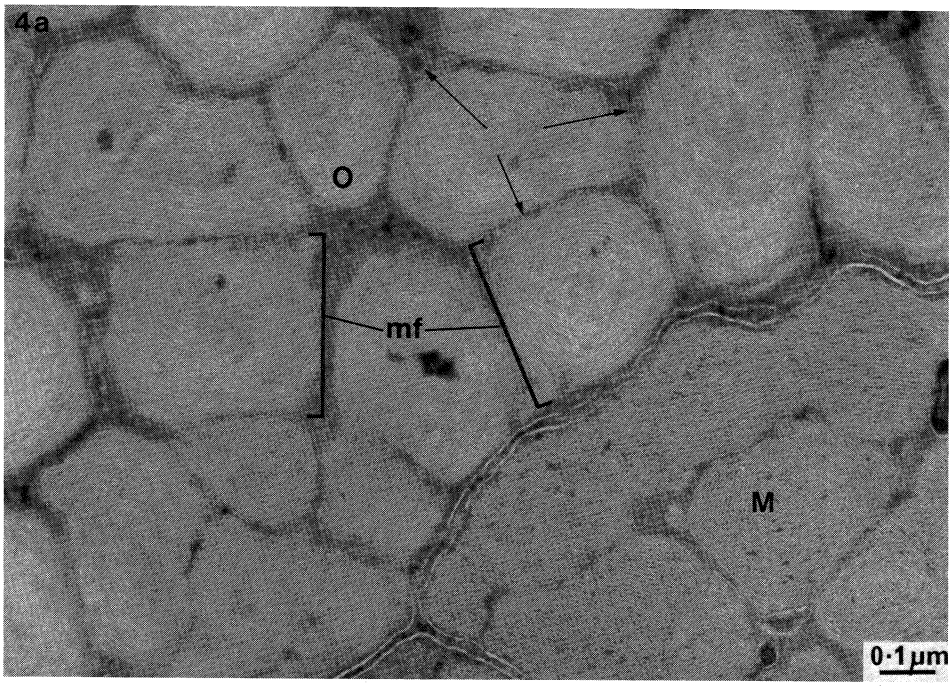
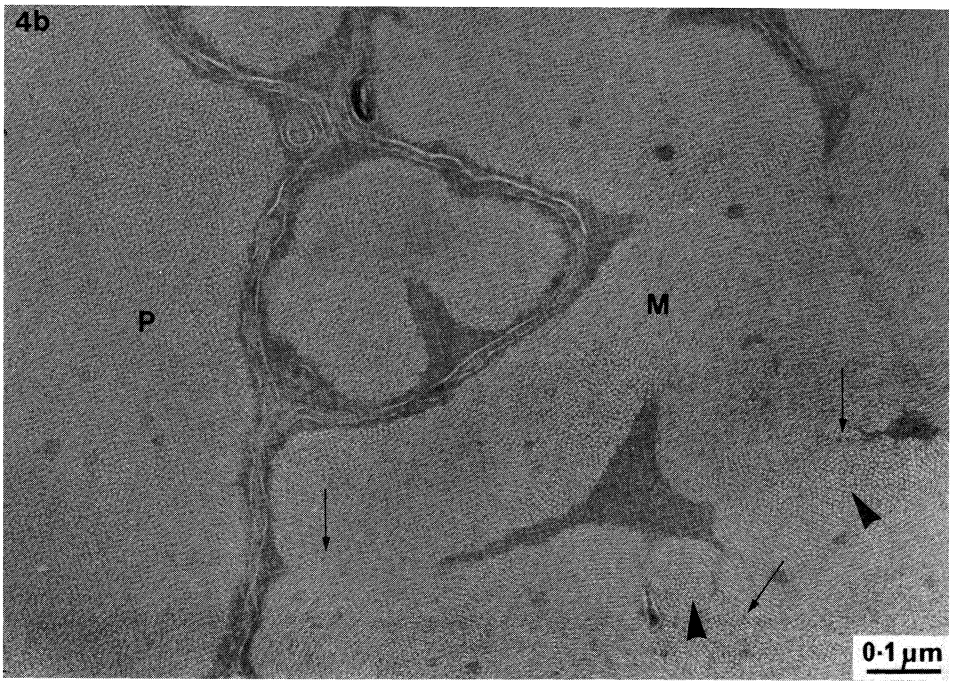


Fig. 3. TEM of a transverse section of a Perendale wool fibre stained as for Fig. 2. Paracortical (*P*) and orthocortical (*O*) cells are clearly differentiated in both this section and the adjacent LM section (inset). Two cells appear to have undergone early stages of mesocortical differentiation but have not stained clearly in the LM (arrowheads).

Fig. 1. Transmission electron microscopy (TEM) of a transverse section of a Merino wool fibre stained by reduction and osmication (see method 3, Materials and Methods, TEM section). Orthocortical (*O*), paracortical (*P*), and two mesocortical (*M*) cells are differentiated. *Cu*, fibre cuticle; *pm*, modified plasma membranes; *cr*, cytoplasmic remnants. Inset shows an adjacent section from the same fibre as seen by light microscopy (LM); the paracortex (*P*) is heavily stained and the orthocortex (*O*) is unstained while two mesocortical cells (arrowheads) are lightly stained.

Fig. 2. TEM of a transverse section of a Perendale wool fibre stained with silicotungstic acid (method 2) showing ortho- (*O*), para- (*P*), and mesocortical (*M*) cells. Inset: the adjacent LM section shows the heavily stained paracortical (*P*), lightly stained mesocortical (arrowheads), and unstained orthocortical (*O*) cells.


4a
O
mf
M
0·1 µm


4b
P
M
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Scanning electron microscopy (SEM)

Twenty-five cleaned fibres from each sheep were slightly extended and attached to double-sided adhesive tape on stubs, coated with gold, and examined in a JEOL-35 scanning electron microscope operating at 15 kV. Prints of fibres magnified ×120 were assessed for changes in shape and diameter where fibre direction changed.

Protein Analysis

Wool proteins were extracted, alkylated, and an aliquot labelled radioactively with [^{14}C]iodoacetic acid (Amersham International, Bucks., England) (Marshall 1981). The labelled proteins were subjected to electrophoresis the same day while the remaining protein extract was dialysed against distilled water and freeze-dried for electrophoresis at a later date. The protein samples were solubilized in 8 M urea containing 0·0625 M Tris (pH 6·9), 2% (w/v) sodium dodecyl sulfate (SDS), and 5% (v/v) 2-mercaptoethanol. After being heated at 100°C for 3 min, the samples were characterized by one-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) according to Laemmli (1970), using 12 or 15% (w/v) slab gels. After electrophoresis the slabs were treated as follows: slabs containing labelled proteins were impregnated with 2·5-diphenyloxazole (PPO) (Bonner and Laskey 1974) and Kodak X-Omat AR film was used to detect the labelled proteins in the dried slabs; slabs containing unlabelled proteins were stained with 0·25% (w/v) Coomassie Brilliant Blue R250 by standard procedures. Molecular weight estimations were made of the separated proteins using bovine serum albumin, ovalbumin, pepsin, trypsinogen, β-lactoglobulin, and lysozyme (Sigma Chemical Co., Mo., U.S.A.) as standards.

Results

Characterization of Cortical Cell Types

The uptake of methylene blue by oxidized wool cortical cells was closely related to the type of cortical cell present. Unstained regions of a fibre were composed of orthocortical cells. Cells staining with intermediate intensities usually indicated mesocortical cells, and the most intensely stained cells were usually found to be paracortical cells (Figs 1–3).

Orthocortical cells were defined ultrastructurally by the whorl-like arrangements of microfibrils within relatively small discrete macrofibrils and the occurrence of cytoplasmic remnants between the macrofibrils (Figs 1–4). A cell along the mesocortical–paracortical cell boundary occasionally showed minor but distinct changes in structure as if it has undergone the earliest stages of mesocortical cell differentiation (Fig. 3).

Paracortical cells were defined as those having few but large macrofibrils usually around the periphery of the cells. Cytoplasmic remnants were concentrated in the cell centre while the microfibrillar organization within the macrofibrils was predominantly parallel to the axis of the fibre (Figs 1–3, 4*b*).

Mesocortical cells were found to have a wide variety of forms, ranging from orthocortical- to paracortical-like (Figs 4*a* and 4*b*). The change from orthocortical- to paracortical-like cells involved an increasing size of macrofibrils, localized around the periphery of the cell; localization of the cytoplasmic remnants towards the cell centre rather

Fig. 4*a*. TEM of a transverse section of a Romney wool fibre showing orthocortical (*O*) and orthocortical-like mesocortical (*M*) cells. The macrofibrils (*mf*) of orthocortical cells are composed of whorl-like arrangements of microfibrils and have cytoplasmic remnants (arrows) between them. The macrofibrils of mesocortical cells do not have the whorl-like array of microfibrils, and are not as discretely separated by cytoplasmic remnants.

Fig. 4*b*. TEM of a transverse section of a Perendale wool fibre showing paracortical (*P*) and paracortical-like mesocortical (*M*) cells. Paracortical microfibrils are organized predominantly parallel to the axis of the fibre in large macrofibrils, and the ratio of matrix to microfibrils appears higher than for other cell types. The paracortical-like mesocortical cell has larger macrofibrils and a greater proportion of quasi-hexagonally packed microfibrils (arrowheads) than the orthocortical-like mesocortical cell (cf. Fig. 4*a*). Macrofibrils are often delineated by a change in microfibril direction (arrows) rather than by cytoplasmic remnants.

Fig. 5

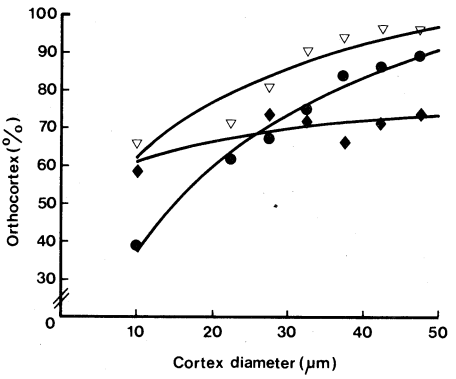


Fig. 6

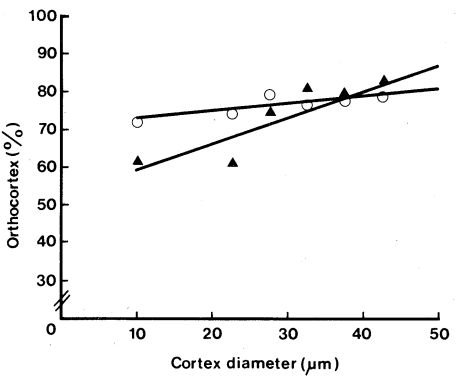


Fig. 5. Fitted curves for the log-linear relationship for three sheep. ▽ R327 (July). ◆ P715. ● Drysdale.
Fig. 6. Fitted lines for the linear relationship for two sheep. ○ C340. ▲ P632.

Table 2. Regressions of percentage of fibre cross-sectional area composed of orthocortical cells (*A*) against fibre cortical diameter (*d*)

Sheep	Sample date	Coeff. of determination (R^2)	β_0 or β'_0 (±95% conf. interval)	β_1 or β'_1 (±95% conf. interval)	s.e.[A]
			(a) Log-linear		
R381	July	85·7	37·6 ± 38·7	16·1 ± 12·1	3·9
	Dec.	79·7	−65·1 ± 68·8	43·2 ± 19·5	12·6
R327	July	89·3	12·0 ± 23·8	21·8 ± 6·7	4·4
	Dec.	97·8	−15·7 ± 26·2	25·4 ± 7·1	1·1
	Feb.	58·2	108·6 ± 12·8	−3·3 ± 3·9	1·6
R148	July	99·4	15·4 ± 6·5	20·7 ± 1·9	1·0
	(repeat)	94·5	7·3 ± 25·4	23·0 ± 7·7	3·2
	Dec.	94·8	−40·5 ± 22·7	32·9 ± 7·7	3·2
	Feb.	81·6	64·2 ± 16·6	9·0 ± 4·9	2·5
Drysdale	Dec.	77·9	−22·3 ± 59·3	27·4 ± 16·8	4·1
	(repeat)	98·7	−41·6 ± 15·1	33·9 ± 4·5	2·2
Lincoln	July	86·9	6·6 ± 24·1	21·6 ± 6·9	5·0
Feral Romney	Mar.	90·8	−49·3 ± 36·2	33·0 ± 10·5	6·0
P715	July	62·4	42·6 ± 24·3	8·0 ± 7·2	4·4
			(b) Linear		
C910	July	70·6	50·5 ± 16·4	0·6 ± 0·5	5·0
C340	July	61·4	71·0 ± 6·4	0·2 ± 0·2	2·0
	(repeat)	81·7	71·3 ± 8·7	0·4 ± 0·3	2·1
P632	July	79·8	52·1 ± 16·0	0·7 ± 0·5	4·9
R111	July	53·8	52·8 ± 17·8	0·5 ± 0·5	6·5
	Dec.	88·3	41·7 ± 10·8	0·5 ± 0·2	1·8
Merino	July	92·5	62·6 ± 51·0	0·5 ± 1·8	1·0

[A] Standard error of estimate of the regression.

than between macrofibrils; and orientation of the microfibrils away from the whorl-like structure of orthocortical cells towards the parallel arrays seen in paracortical cells (Figs 1–4). Macrofibril junctions were often delineated by blocks of microfibrils oriented in one direction, juxtaposing blocks of microfibrils oriented in a different direction. The packing of microfibrils was often hexagonal or quasi-hexagonal (Fig. 4*b*).

Most of the cell-type features were observed regardless of which heavy metal stain was used, except that the reduction and osmication method revealed microfibril–matrix organization more clearly than the other methods.

Of the 101 fibres used to check the accuracy of the methylene blue staining in correlative transmission electron microscopy (TEM)–light microscopy (LM) studies, 62 had diameters below 35 μm and 39 above. TEM was used as the correct indicator of cell type, and the cell types were correlated with unstained (orthocortical) and stained (mesocortical–paracortical) cells. The mean number of mesocortical–paracortical cells mistakenly counted as orthocortical cells was 2% per cross-section and 2·1% per cross-section of orthocortical cells were counted as mesocortical–paracortical. The mean number of cells per cross-section was 71 ± 33. No cells were misidentified in 49% of the fibre cross-sections. The LM method was therefore judged capable of distinguishing between orthocortical and mesocortical–paracortical cells with a good degree of accuracy.

Differentiation between mesocortical and paracortical cells by LM was less satisfactory. Of the cells classed as mesocortical, 2·4% should have been classed as paracortical cells and about 18% of paracortical cells should have been classed as mesocortical cells. Emphasis in this study was therefore concentrated on the orthocortical and the combined mesocortical–paracortical cells.

Cross-sectional Areas of Cell Types

The mean cross-sectional area of orthocortical cells in 78 fibres was $8{\cdot}47 \pm 0{\cdot}24$ μm^2, and mesocortical–paracortical cells from 69 of these fibres was $6{\cdot}46 \pm 0{\cdot}21$ μm^2. The difference was highly significant ($P < 0{\cdot}001$).

Relationships between Cell Type and Cortical Diameter

An overall relationship of an increasing proportion of orthocortical cells with increasing fibre diameter was determined for individual sheep. Two distinct types of relationship between the proportion of orthocortical cells and cortical diameter were found: a curvilinear relationship for seven sheep, and linear for the other five sheep. A log-linear regression

$$A = \beta_0 + \beta_1 \ln d ,$$

where A = percentage area of orthocortical cells in a fibre cross-section and d = diameter of cortex, fitted most of the curvilinear data with a good degree of precision (Table 2*a*; Fig. 5). For the wools showing a linear relationship, a linear regression

$$A = \beta'_0 + \beta'_1 d ,$$

also fitted most of the data with a good degree of precision (Table 2*b*; Fig. 6).

The slopes and associated 95% confidence intervals for each of the log-linear relationships are shown in Table 2 and graphically in Fig. 7. Significant differences in log-linear slopes were found between the wools of sheep in different breeds (feral Romney *v.* P715), as well as at different times of the year for the same sheep (R148, Fig. 7). For sheep showing a linear relationship the slopes for C910, P632, R111 and the repeat data for C340 showed a small but significant difference from zero. Some sheep of the same breed showed either linear or log-linear relationships (cf. R111 *v.* R148; P632 *v.* P715). For the wools examined in this study, the extremes in variation in cell type–cortical diameter relationships were: where the proportions of orthocortical cells increased rapidly with increasing cortical diameter to level out at high (>90%) proportions of orthocortical cells (the log-linear

Fig. 7

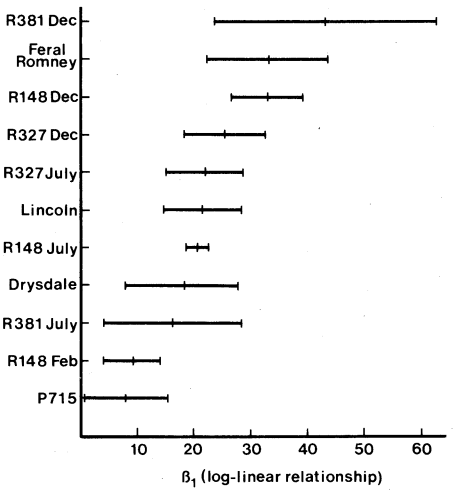


Fig. 8

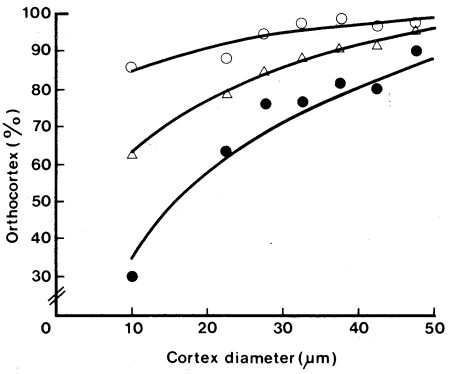


Fig. 7. Slopes (β_1) for the log-linear relationship showing 95% confidence intervals. Bars which overlap indicate non-significance.

Fig. 8. Fitted curves for the log-linear relationship for sheep R148 at different times of the year. ○ February. △ July. ● December.

relationship, e.g. R381); and where only small changes occurred in orthocortical cell proportions as cortical diameter increased, with about 50–55% of cortical cross-sectional area being orthocortex (the linear relationship, e.g. R111).

The log-linear and linear relationships held whether minimum diameter alone or $\frac{1}{2}$(maximum diameter + minimum diameter) of the cortical cross-section was taken as the measure of diameter. The staining procedure and drawing of the cortical cross-sections affected the diameter minimally. The staining procedure increased the diameter by about 1 μm, while errors in drawing cortical cross-sections altered diameter measurements by about $\pm 0 \cdot 8$ μm. Analysis of different wool from the same samples for three sheep verified the relationship identified for the initial samples, and showed that the slopes of the repeat orthocortex–diameter relationships were not significantly different (Table 2).

Sampling the same four Romney sheep at the maximum (December) and minimum (July) wool-growth periods gave the following results. Three of the sheep (R111, R381 and R327) showed no significant difference in the slopes of their orthocortex–diameter relationships for the two samplings (Table 2). The remaining sheep (R148) had significantly higher proportions of orthocortical cells for the same diameters in the July wool (Fig. 8). The February wool of two of these sheep (R148, R327) was also examined as both lost 6 kg of bodyweight after the December sampling because of the drought-induced lack of feed (Table 1). One sheep (R148) had significantly higher levels of orthocortex (Fig. 8), while the other (R327) produced wool with a different orthocortex–diameter relationship, as indicated by its R^2 value (Table 2). The relationship nevertheless showed high levels of orthocortex.

Cell-type Arrangements

The arrangements of mesocortical–paracortical cells in the fibre cross-sections showed considerable variability (Fig. 9). The major type of arrangement varied between samples from the same sheep grown at different times of the year, between samples from sheep of the same breed, and between samples from sheep of different breeds. Different trends in cell-type arrangements were found for the different orthocortex–diameter relationships, and with variation in diameter. The predominant cell-type arrangements in the log-linear samples were, in order of decreasing frequency: bilateral–unilobate; cellular; lobate–cellular; 100% orthocortex. For the linear samples, the bilateral–unilobate and lobate–cellular arrangements were the most frequent (Table 3).

Table 3. Mean percentage of the various cortical cell-type arrangements according to fibre diameter quartiles

Type of cell arrangement	Quartile 1[A]	2	3	4	Overall percentage[B]
	(a) Log-linear relationship				
100% orthocortex	8·3	14·6	15·2	11·1	12·6
Cellular	20·0	28·1	48·6	72·2	28·7
Bilateral–unilobate	52·0	29·9	3·9	5·6	33·4
Lobate–cellular	15·3	15·2	30·7	0·0	22·5
Bilobate–multilobate	2·8	2·0	1·6	11·1	2·2
Other	1·6	0·2	0·0	0·0	0·6
No. of fibre cross-sections	879	1398	381	18	
	(b) Linear relationship				
100% orthocortex	1·8	4·3	2·5	0·0	3·3
Cellular	7·2	8·2	8·0	19·6	8·3
Bilateral–unilobate	81·3	62·6	28·9	2·2	57·9
Lobate–cellular	7·3	19·8	46·5	45·6	23·3
Bilobate–multilobate	0·9	3·2	5·2	19·6	3·3
Other	1·5	1·9	8·9	23·9	3·9
No. of fibre cross-sections	332	854	325	46	

[A] Lowest. [B] Totals for each relationship = 100%.

Log-linear samples had medium frequencies of bilateral–unilobate arrangements in fibres of lower diameter, and the frequency decreased rapidly with increasing fibre diameter (Table 3). This was accompanied by an increase in the frequency of lobate–cellular arrangements and a marked increase in the frequency of cellular arrangements. Relatively constant, but minor, amounts of 100% orthocortical arrangements were found in each diameter quartile. In contrast, linear samples had predominantly bilateral–unilobate arrangements in fibres of lower diameter. As the diameter increased their frequency decreased to reach a minimum in the fourth quartile as compared to the third quartile for the log-linear samples. This decrease was accompanied by a marked increase in lobate–cellular arrangements and a lesser increase in cellular arrangements. 100% orthocortical arrangements were present at low levels only.

Cell-type Variations along a Fibre

Cortical cell-type arrangements showed some variation along the 25-mm lengths of fibre studied. At one extreme, no changes in cell-type arrangement occurred while at the other extreme, an individual fibre could show cell-type arrangements varying through the whole

range (Figs 10*a*–10*e*). The length of any given cell-type arrangement varied considerably and could be as short as 0·1–0·2 mm (Figs 10*b* and 10*e*). However, most fibres had few changes: 45% had 75% or more of their length made up of only one cell-type arrangement and a further 44% had 75% or more of their length made up of two types.

Changes in cell-type arrangement along a fibre usually followed a sequence. This appeared to be 100% orthocortical through cellular, bilateral–unilobate, lobate–cellular, to bilobate arrangements, or vice versa. Sequential changes in cell-type arrangement were not closely correlated with changes in fibre diameter. Long lengths of fibre with the same type of arrangement could show considerable variations in fibre shape and diameter (Fig. 11). There was no evidence that changes in cell-type arrangement affected all fibres at a given time, except where major nutritional stress had affected wool growth.

Cell Type and Fibre Crimp

Similarities and differences in the two measures of crimp (staple crimp and bulk) used in this study allowed an assessment to be made of the role of the bilateral configuration in crimp for some wool samples. For wools of similar diameter, higher degrees of crimp were associated with higher percentages of bilateral–unilobate fibres in two Corriedale and two Romney samples. Corriedale C910 had higher staple crimp and bulk readings than C340 (Table 1) and 80% of its fibre cross-sections were classified as bilateral–unilobate compared to 58% for C340. The feral Romney also had similar crimp differences from R327 (Table 1) and 56% of its fibre cross-sections were classed as bilateral–unilobate compared to 16% for R327. In contrast, R111, while showing crimp characteristics similar to R327, had double the percentage of bilateral–unilobate forms (32%).

Scanning electron microscopy (SEM) examination of the wool samples indicated that fibre crimp was often (62%) associated with varying degrees of asymmetric changes in fibre shape and diameter, i.e. changes other than those caused by twisting of a fibre with an elliptical cross-section (Figs 12*a*,*b* and 13). The remainder showed no apparent alteration in fibre shape or diameter. Asymmetric changes in fibre shape–fibre diameter were more prevalent in wools of higher bulk. The cell types giving rise to crimp associated with changes in fibre diameter–fibre shape were not rigorously tested in this study. However, most general changes in fibre diameter and shape involved orthocortical cells, even though the number of mesocortical–paracortical cells also varied along the fibre length (Fig. 10). For instance, orthocortical cells alone were involved in 78% of the 100 most extreme type of change in diameter and shape examined (kinks and bumps) (Fig. 11).

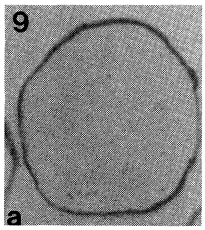
Wool Proteins

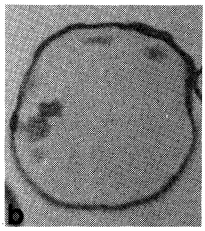
The apparent molecular weight distribution of keratin proteins from all the wool samples showed more similarities than differences regardless of the method used to detect the proteins in the gels (Fig. 14). The most noticeable difference was the presence of a band

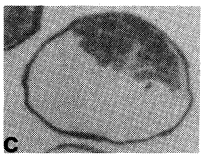
Fig. 9. LM of transverse sections of wool fibres showing different distributions of mesocortical–paracortical cells (methylene blue-stained areas) within the orthocortex (unstained). (*a*) 100% orthocortex, Romney; (*b*) cellular, Lincoln; (*c*) unilobate, Perendale; (*d*) bilateral, Perendale; (*e*) lobate–cellular, Romney; (*f*) bilobate, Romney.

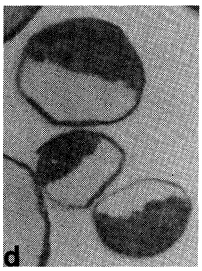
Fig. 10. LM of cortical cell arrangements along wool fibres (mesocortical–paracortical cells stained). (*a*) 100% orthocortex, Romney; (*b*) cellular alternating with 100% orthocortex, Romney; (*c*) unilobate, Drysdale; (*d*) lobate–cellular, Romney; (*e*) cellular–100% orthocortex–unilobate, Drysdale.

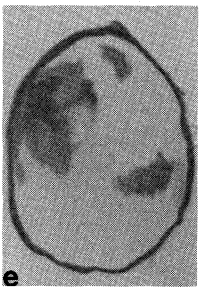
Fig. 11. Variations in cortical cell types in regions of variable fibre shape and diameter: (*a*) amount of orthocortex and mesocortex–paracortex both vary in the 'bump', Romney; (*b*) amount of orthocortex increases in the 'bump' while that of mesocortex–paracortex shows little change, Romney; (*c*) amount of orthocortex changes considerably with changes in fibre diameter while that of mesocortex–paracortex changes very little, Romney.

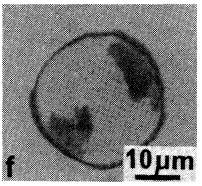

9
a

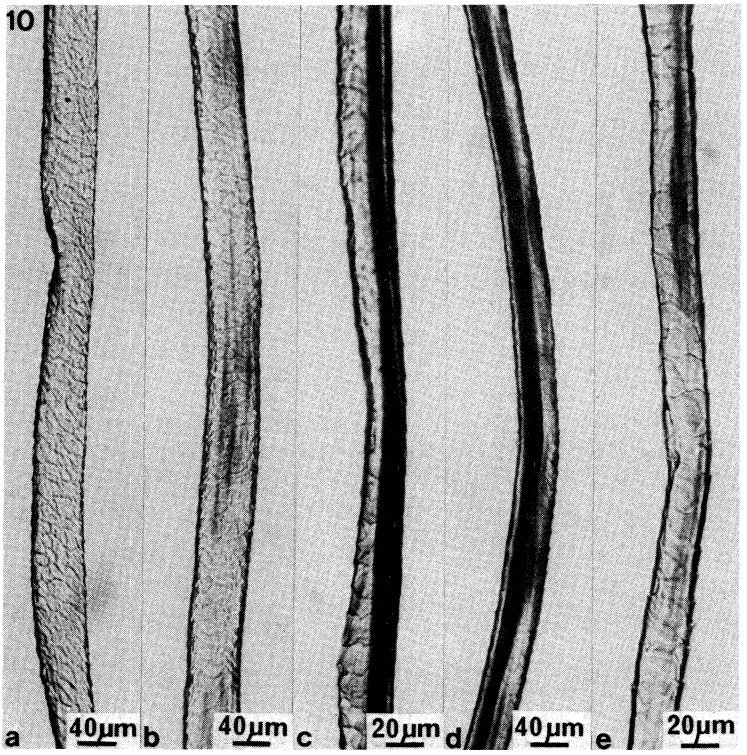

b

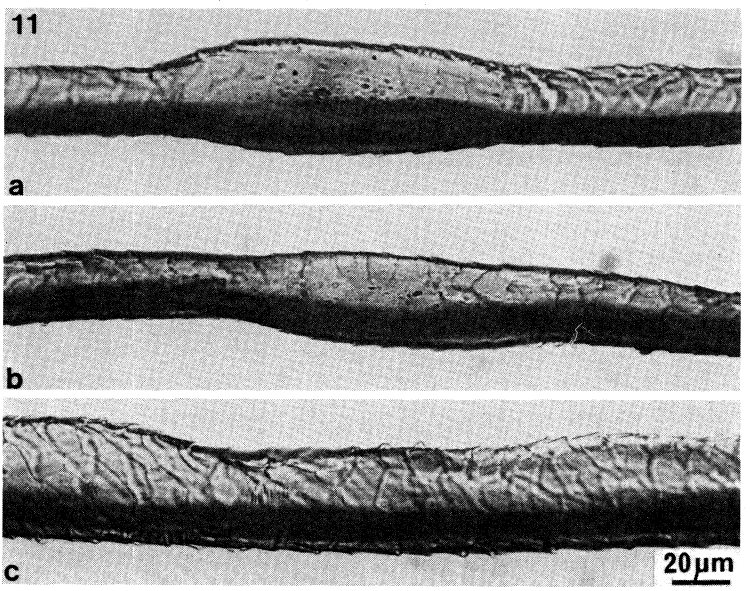

c

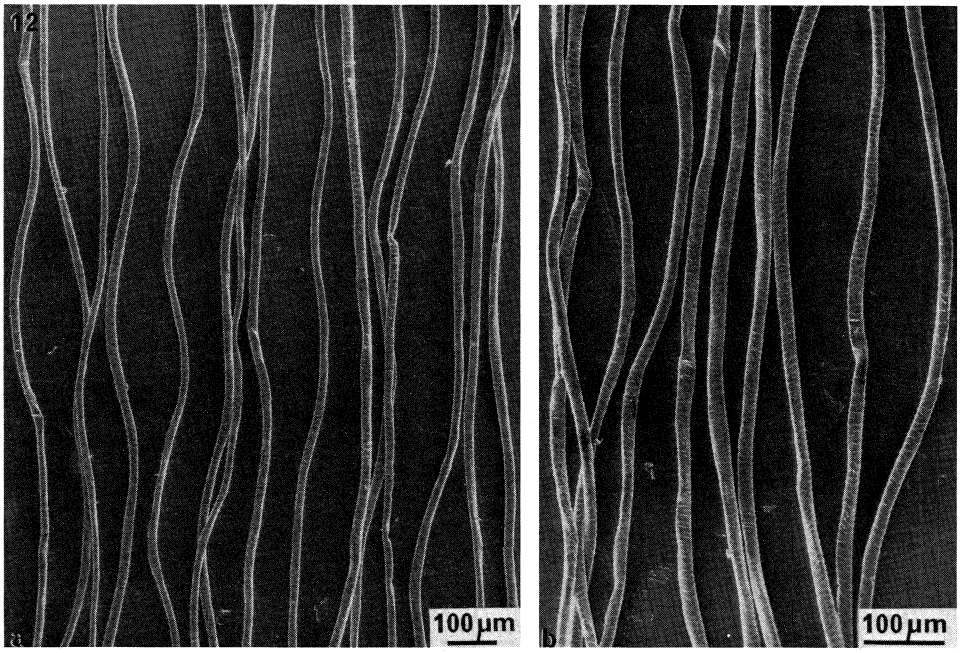

d

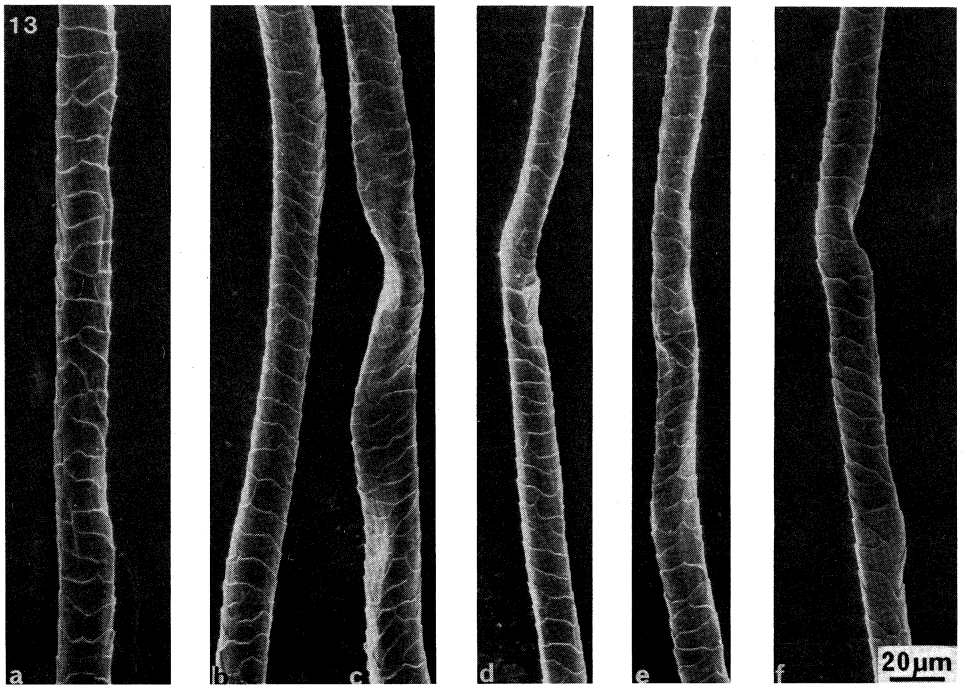

e


f
10µm


10
a
40µm
b
40µm
c
20µm
d
40µm
e
20µm


11
a
b
c
20µm


12
a
100 µm
b
100 µm


13
a
b
c
d
e
f
20 µm

of protein of apparent molecular weight 20 000 in some sheep (Fig. 14). The sheep which showed significant differences in its proportions of orthocortex at different times of the year (R148, Table 2*a*) did not show any qualitative protein differences between samples (Fig. 14*b*).

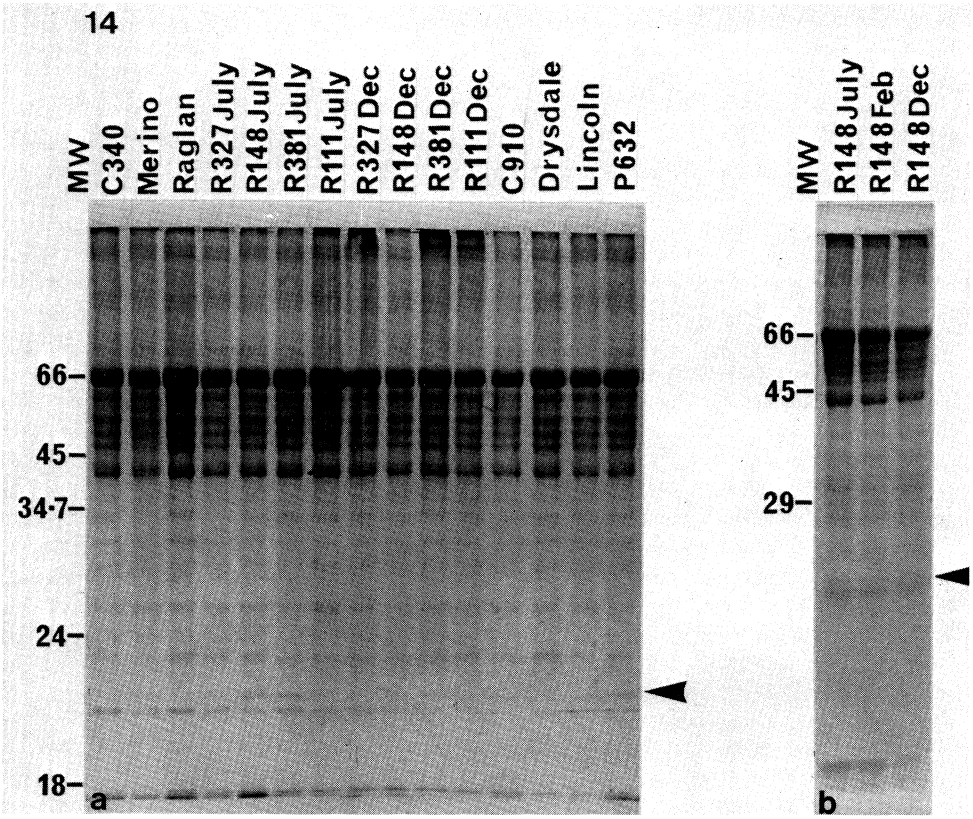


Fig. 14. SDS–PAGE of wool proteins stained with Coomassie Brilliant Blue: (*a*) 12% gel; (*b*) 15% gel. Note the presence of a band of apparent molecular weight 20 000 from some sheep (arrowhead). Numerals on the left-hand side of each figure are molecular weights of standards $\times 10^{-3}$.

Discussion

This study points to orthocortical cells as the predominant type of cortical cell present in wool fibres. The development of a technique of sufficient accuracy to quantify orthocortical cells separately from mesocortical–paracortical cells in wools from individual sheep has allowed several features of wool fibres and their differentiation to be examined.

The overall relationship of increasing proportions of orthocortical cells with increasing fibre and cortical diameter, determined in this study for individual sheep, in general could be described mathematically by either log-linear or linear regressions. As wool from a wide

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Figs 12*a*,*b*. SEM of wool fibres from Corriedale C910, showing changes in fibre direction frequently associated with changes in fibre shape and diameter.

Fig. 13. Higher-power SEM of wool fibres from Corriedale C910, showing (*a*) changes in fibre diameter not associated with changes in fibre direction; (*b*) gradual changes in fibre diameter and fibre direction; (*c*)–(*e*) marked changes in fibre shape and diameter associated with varying degrees of change in fibre direction.

range of breeds was examined and both types of relationships were found in sheep from the same breed (Romneys and Perendales), these relationships may hold for the wools of most, if not all, breeds. The relationships found for individual sheep also provide a basis for interpreting earlier results based on pooled data from several sheep of one breed (Orwin *et al.* 1980; Orwin and Woods 1980). In the earlier study (Orwin *et al.* 1980) two curves were presented, one associated with low and one with high staple strengths. The log-linear relationship established in the present study can be equated with the low-strength curve, the linear relationship with the high-strength curve.

Other studies of relationships between cortical cell type and fibre diameter have produced conflicting results. An increase in paracortical cell proportions with increasing diameter has been reported in some breeds (Thorsen 1958). However, as the stain used (sodium plumbite) was degradative and correlated only partially with methylene blue staining and no ultrastructural basis of the technique was provided, it is difficult to compare Thorsen's results directly with those from this study. Four other studies have reported mean percentages of orthocortical cell areas higher than 50% for wools of various mean diameters (Ahmad and Lang 1957; Snyman 1963; Chapman 1965; Bonès and Sikorski 1967), without defining relationships between orthocortical cell proportions and the diameter of the individual fibres in the samples. One of these studies (Ahmad and Lang 1957) reported that an anomalous Merino wool type averaged 55% paracortical cell area. There is some question about the specificity of the methylene blue-staining method used, however (Fraser and Rogers 1955).

The limited evidence from repeat samplings of the four Romney sheep indicates that the type of relationship between orthocortical cell proportions and fibre diameter (i.e. log-linear or linear) may be basic (genetic?) to individual sheep, as it holds true at different times of the year. Nutritional stress appeared to disturb the normal relationship at one sampling for one sheep. In view of the known effects of nutritional stress on wool cell and protein production (Schinckel 1962; Reis and Schinckel 1963; Gillespie *et al.* 1969; Reis 1979; Wilson and Short 1979; Chapman and Black 1981), this is not surprising. The reversion to mainly orthocortical cell production under nutritional stress indicates that this is the basic cortical cell produced by the wool follicle.

An annual cycle of wool production involving changes in diameter and length growth occurs in the Romney (Story and Ross 1960). Our study has shown that for three of the four Romney sheep sampled at the maximum and minimum wool-growth periods, the relationships between orthocortex and fibre diameter did not differ significantly at these times. The fourth sheep did show significant differences, indicating that caution is necessary if general conclusions are to be drawn from wool sampled at one time.

These preliminary results indicate that the technique developed in this paper is useful in allowing mathematical descriptions of the relationship between cortical diameter and cortical cell proportions to be made for wools from individual sheep. The technique seems to be applicable to a wide range of breeds and appears to be best suited for comparing wools from sheep kept under known nutritional conditions.

Changes in cell-type arrangement with increasing fibre diameter have been reported (Fraser and Rogers 1955; Orwin and Woods 1980). This study has confirmed that the common bilateral–unilobate cell-type arrangements become less prevalent at higher diameters. However, at greater fibre diameters, these changes differ for wools showing log-linear or linear relationships. Linear wool types retain higher percentages of the combined bilateral–unilobate and lobate–cellular arrangements at larger fibre diameters than log-linear wool types. The basic difference between the two types of wool seems to lie in the ability of the follicles of sheep with linear-type wool to form more mesocortical–paracortical cells in their larger fibres, and in the arrangements of these cells in the fibre.

The study of individual fibres showed some paradoxical differences from the cell type–cortical diameter relationships obtained by analysing cross-sections. Changes in arrangement and proportions of cell types were found at different places along the fibre,

and these changes did not necessarily occur when fibre diameter increased or decreased. However, the changes were typically sequential, and usually favoured one or two arrangements so that, provided an adequate number of fibres (200–300) was examined, a statistically useful average proportion of orthocortical cells for a given diameter range could be obtained from cross-sections of different fibres.

Cell-type arrangement, its variation along the fibre length, and the variations in mesocortical cell type have implications for cortical cell differentiation in the follicle during fibre formation. Our results indicate that a single follicle can produce varying numbers of any cortical cell type and organize them in a variety of ways within a fibre. The variety of mesocortical cell forms has led us to define them as a true intermediate cell type covering the full range of features between ortho- and paracortical cells, rather than a unique cell type having one set of intermediate features (Bonès and Sikorski 1967; Kaplin and Whiteley 1978; Wagner *et al.* 1983). The variable nature of mesocortical cells may indicate that the differentiation path followed by a cortical cell is not irrevocably determined in the follicle bulb, but is subject to other physiological influences during differentiation. As earlier work has shown that, under adequate nutritional conditions, ortho- and paracortical segmentation arises in the follicle bulb (Fraser and Rogers 1955; Fraser 1964; Chapman and Gemmell 1971) further work is required to establish the means by which cortical cell-type arrangements may vary along the same fibre.

Crimp is the wool property most strongly correlated with cortical cell type and its organization (Horio and Kondo 1953; Fraser and Rogers 1955; Kaplin and Whiteley 1978). High crimp is associated with a bilateral arrangement of ortho- and paracortical cells, although a negative relationship between fibre diameter and crimp frequency must also be taken into account (Whiteley and Charlton 1975). Our results indicate general support for the bilateral or unilobate cell-type arrangement being associated with greater crimp for two Corriedale and two Romney wools. However, another Romney wool did not confirm to this pattern, raising the possibility that other factors play a role in crimp.

Another possible mechanism of crimp formation is suggested by the changes in fibre shape and diameter associated with fibre crimp. Fibre shape and diameter changes are likely to be determined by alterations in the numbers and arrangements of the cells in the region where the fibre direction changes, irrespective of the cell types involved. Our study indicates that orthocortical cells may play a major role in this type of crimp, but further work is needed to verify these hypotheses. Changes in fibre shape and diameter need not necessarily be directed by changes in cell division in the follicle, but could also occur by relative cell slippage during fibre formation (Orwin and Woods 1982).

In general, fibre diameter and shape changes were found to be predominantly due to changes in numbers of orthocortical cells. In part, this dominance of orthocortical cells is the result of the larger (about 30%) cross-sectional area of individual orthocortical cells compared to mesocortical–paracortical cells. This difference was recognized earlier (Kassenbeck and Leveau 1957; Bonès and Sikorski 1967) and was measured directly in this study. The mean cross-sectional area of the same cell type varies considerably in different fibres. For fibres with equal numbers of orthocortical and mesocortical–paracortical cells, a greater area of the fibre cross-section will be composed of orthocortical cells, and changing the numbers of orthocortical cells will affect fibre shape and diameter to a greater extent than a similar change in mesocortical–paracortical cell numbers.

The separation of carboxymethylated wool proteins according to apparent molecular weight, even though these may differ considerably from true molecular weights (Marshall 1983), has allowed qualitative differences between wools to be assessed. In general, the wool protein patterns of the various wools studied here showed many similarities. This was to be expected from previous work on Dorset Horn and Merino wool (Marshall and Gillespie 1977). Many chemical and physical differences in cortex-derived properties in the wools used in this study would, therefore, most likely arise from differences in quantities of various proteins associated with differences in cortical cell-type numbers and

arrangement in a fibre rather than with major qualitative differences in wool proteins. For example, Merino wools of different crimp frequency exhibit different proportions of high-sulphur proteins (Campbell *et al.* 1975) and mesocortical cells (Kaplin and Whiteley 1978). The Romney sheep which had significantly different proportions of orthocortical cells at three different samplings in this study also showed no qualitative protein differences. Nevertheless, the significance of the small qualitative differences (the presence or absence of a protein of apparent molecular weight 20 000) found in this study for wool properties has yet to be determined.

Acknowledgments

The authors appreciatively acknowledge the assistance of Dr B. L. Thomas in the development of the digitizer program; Miss K. Knight and Mr S. Parsons for technical assistance; Dr R. C. Marshall and Dr J. M. Gillespie, Division of Protein Chemistry, CSIRO, for advice on the protein analysis; the staff of the Templeton Research Station, Ministry of Agriculture and Fisheries, for assistance with some of the sheep; and Mrs J. Orwin and Dr L. F. Story for editorial assistance.

The financial assistance of the Vernon Willey Trust for part of this study is also gratefully acknowledged.

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Manuscript received 7 November 1983, revised 17 April 1984, accepted 25 June 1984