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An Electron Microscope Study of Fibril : Matrix Arrangements in High- and Low-crimp Wool Fibres

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Abstract

The crimped structure of wool fibres is generally associated with a bilateral arrangement of ortho- and paracortical cells. The most obvious difference between these cell types is in the arrangement and relative proportions of microfibril and matrix proteins that constitute the fibre cortex.

In the low-crimp fibres examined there is a poorer expression of bilateral cortical asymmetry compared with the high-crimp wools together with a higher proportion of intermediate-staining mesocortical cells. These mesocortical cells exhibit much more regular arrays of microfibrils than paracortical cells. It is suggested that the packing arrangement of microfibrils in all three cell types is basically hexagonal and the variation observed in mature cells is a function of the fibril : matrix ratio.

Introduction

The bilateral arrangements of the ortho- and paracortices in crimped Merino wool fibres is well established. In the orthocortex, whorls of microfibrils are set in a pale-staining matrix, whereas in the paracortex, microfibrils are arranged in hexagonal or pseudohexagonal patterns in a dark-staining matrix (Rogers 1959*a*, 1959*b*).

The appearance of cortical cells exhibiting intermediate staining characteristics has been noted (Rogers 1959*a*; Dobb *et al.* 1961). Bonès and Sikorski (1967), in an examination of the relationship between ultrastructure and mechanical properties, named them mesocortical cells and suggested their possible importance. Support for this contention is provided by Watson (1974), who demonstrated that, apart from the more scattered arrangement of the orthocortical cells in low-crimp fibres in comparison with fibres from the high-crimp group, there also appeared to be a very significant replacement of paracortical cells by mesocortical cells.

Work in these laboratories has been aimed at investigating the magnitude of natural variations in fine structure, chemical composition, mechanical and mechano-chemical properties in a unique group of keratins (Shah 1965; Whiteley *et al.* 1970; Campbell *et al.* 1972, 1975; Watson 1974). These are the high- and low-crimp wools from single character selection groups established at the New South Wales Department of Agriculture's Trangie Research Station (Morley 1951, 1955; Dun 1958).

The wools studied are from the Crimps Plus and Crimps Minus groups selected for high and low staple crimp frequency from Merino stock of common genetic origin maintained under similar environmental and nutritional conditions.

An outstanding feature of the low-crimp wools is their low sulfur content in comparison with that of the high-crimp group, the mean values being 3·18 and 3·69% respectively (Campbell *et al.* 1972). This confirms the observations of Thorsen (1958) and Snyman (1963) that the sulfur content of wool is proportional to the rate

of crimping. Campbell *et al.* (1972) found this conclusion to be valid regardless of whether the crimp differences arose from deliberate selection for differences in crimping rate, indirectly from selection for differences in fleece weight, or as a result of natural variations in crimp frequency within a flock.

These variations in composition have little or no influence on mechanical properties such as stress–strain or stress relaxation, presumably due to the location of most disulfide bonds in the amorphous matrix that makes only a minor contribution to longitudinal mechanical properties at extensions below 30% (Feughelman and Haly 1960; Feughelman and Reis 1967). The swelling (Whiteley *et al.* 1970) and supercontraction (Campbell *et al.* 1972) properties are markedly affected by variations in sulfur content.

In this study, the aim was to extend the earlier work by using the electron microscope to examine the fibrillary patterns of the high- and low-crimp wools with particular reference to the mesocortex which does not appear to have received detailed consideration.

Materials and Methods

Origin and Preparation of Wool Samples

Samples used represented the extreme crimp types from the high- and low-crimp Merino ewes described by Campbell *et al.* (1975).

Staining

Wool fibres were partially reduced in sodium thioglycollate (0·5 M, pH 5·6) for 24 h and after washing, they were stained in an unbuffered aqueous solution of osmium tetroxide (2%, w/v) for 5 days (Rogers 1959*a*, 1959*b*).

Fibres were dehydrated in an ethanol series and embedded in Spurr low viscosity embedding resin (Spurr 1969).

Sectioning

Thin sections (grey-silver interference colours) were cut with a Reichert OM U2 ultramicrotome using a diamond knife, and collected on 483 mesh uncoated grids. They were then stained sequentially in uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) before coating with carbon prior to electron microscope examination.

Electron Microscopy

Sections were examined in a Philips EM300 electron microscope, with an accelerating potential of 80 or 100 kV, and a 30-μm objective aperture.

Results

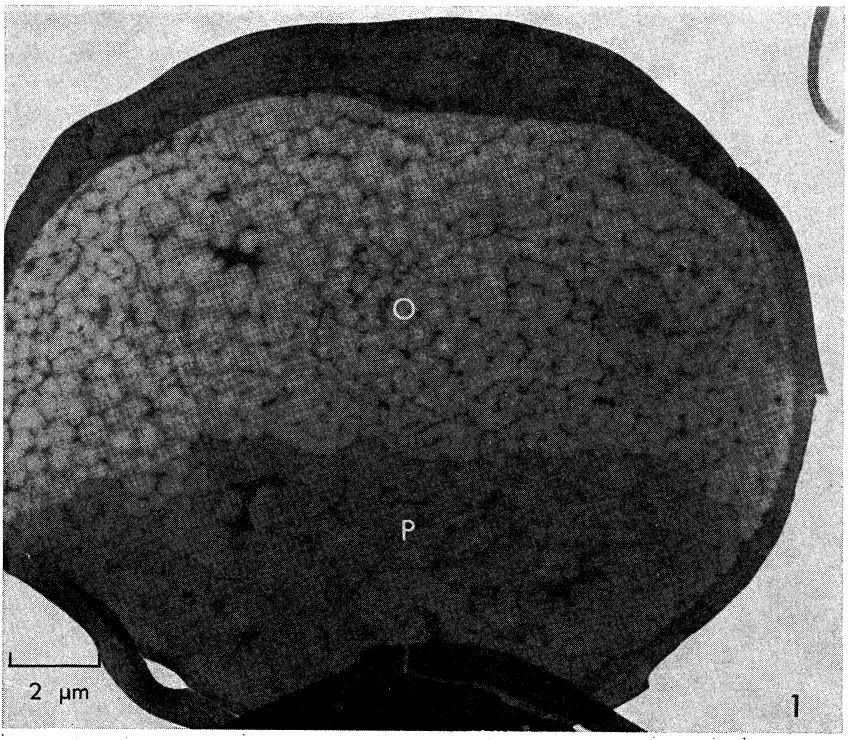
Examination of High-crimp Wools

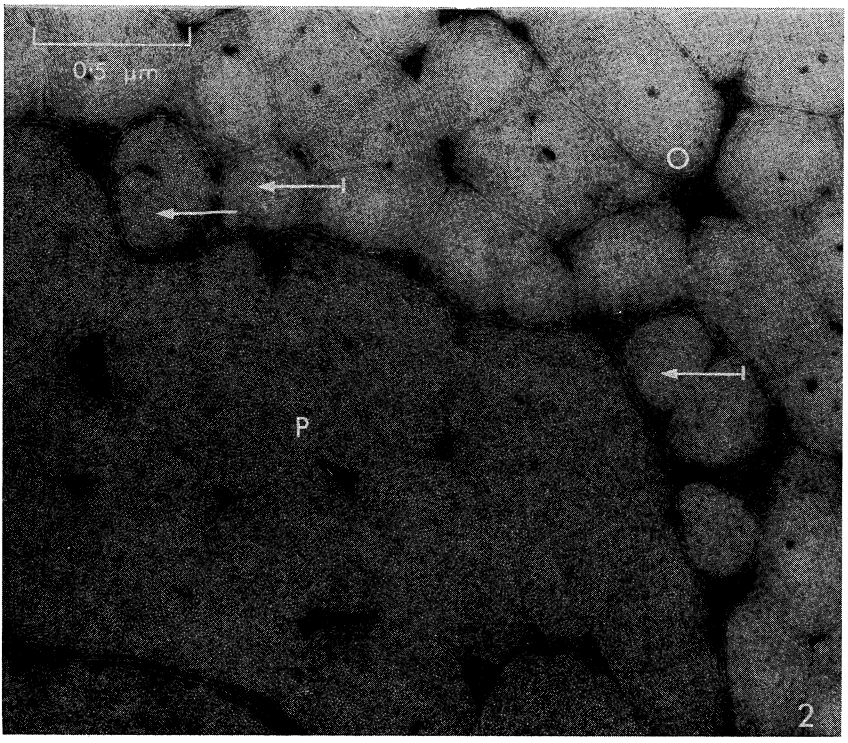
In wools treated by the thioglycollic acid–osmium tetroxide procedure there is a clear demarcation between the orthocortex and paracortex (Figs 1 and 2).

The cylindrical orthocortical macrofibrils are clearly outlined by intermacrofibrillar material while those in the paracortex have a flattened appearance and are much larger and more extensively fused.

Fig. 1. High-crimp Merino wool showing bilateral arrangement of the orthocortex (*O*) and paracortex (*P*).

Fig. 2. High-crimp Merino wool showing irregular arrays of microfibrils in the para-cortex and whorl-like arrangement in the orthocortex. Very regular packing can be seen at the ends of cells (←) and occasionally at the centre of orthocortical macrofibrils (↤).


O
P
2 μm
1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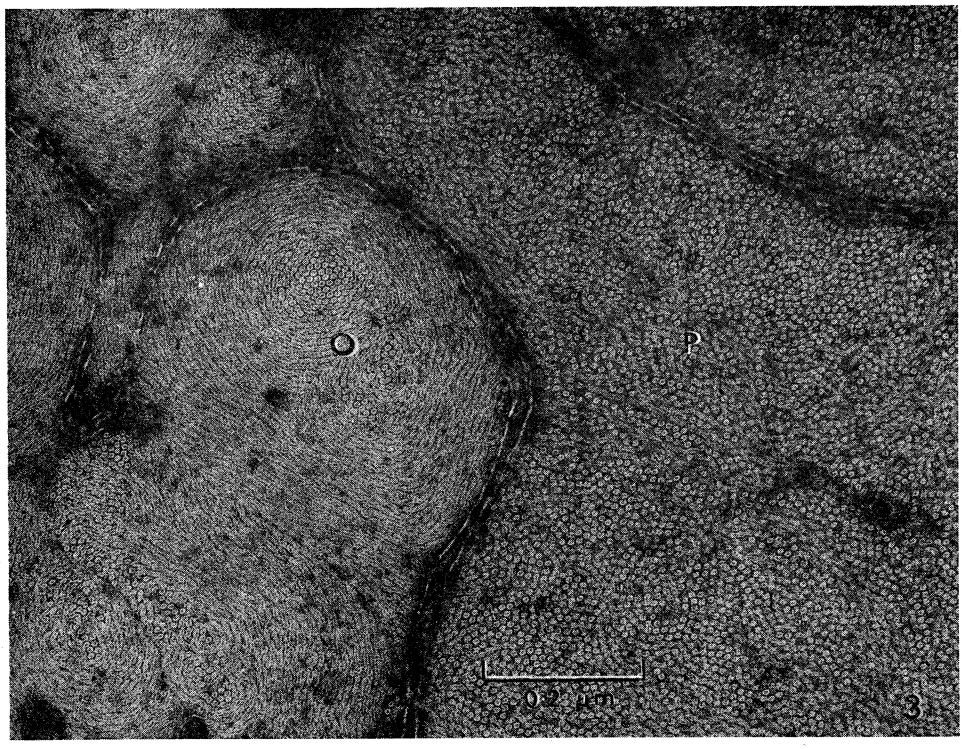
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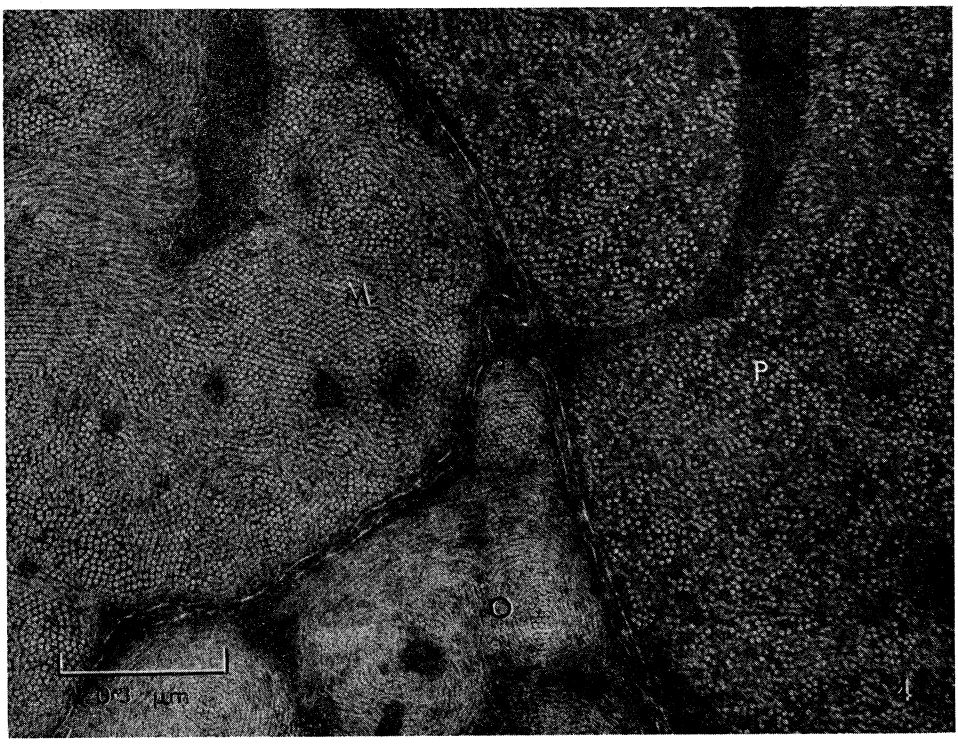
3


M
P
O
0·3 µm
4

As the magnification is increased (Figs 2 and 3) the two phase microfibril–matrix structure of wool becomes apparent. The matrix is extremely electron-dense and the microfibrils are clearly resolved. Under these staining conditions the increase in weight of the wool was about 45% (dry basis). Rogers (1959*a*) has attributed the greater electron density in the matrix to preferential reaction of the thioglycollic acid with disulfide bonds in this region, reducing them to sulfhydryl groups which react rapidly with osmium tetroxide. The amount of bound osmium exceeds the available sulfur in a molar ratio of about 1·6:1, which indicates that the reaction is not a specific one (Sikorski and Simpson 1959) and that reaction between osmium tetroxide and other amino acid side chain groups must also occur (Litman and Barrnett 1972).

The packing of microfibrils in the paracortex varies considerably and, although near-hexagonal arrays up to about 0·25 μm across can be found, a very irregular arrangement is most common.

The matrix component of orthocortical cells appears to be less in amount and perhaps of lower electron density than the matrix component of paracortical cells. Macrofibrils are whorl-like in appearance, the microfibrils apparently being arranged in concentric layers. Delineation of microfibrils is poor, except in the centre of macrofibrils where microfibrils can sometimes be seen to be packed hexagonally (Figs 2 and 3). The best examples of hexagonal packing are to be seen at the ends of both para- and orthocortical cells (see orthocortical macrofibrils arrowed in Fig. 2).

Along the junction of the ortho- and paracortex individual mesocortical cells whose macrofibrillar appearance closely resembles that of the paracortex, are observed. Their most striking feature is the arrangement of microfibrils which is much more regular than that observed in the paracortex.

It has been suggested (Rogers 1959*b*) that the low fibril:matrix ratio of the paracortical cells contributes to the ordered arrangement of microfibrils whereas in the present work the mesocortical cells appear to contain much less matrix material and yet exhibit more regular packing. The effect is illustrated in Fig. 4 which shows the three cell types lying adjacent to one another. The comparative intensities of staining and macrofibril configurations together with the distinct packing arrangements of the microfibrils are clear.

Examination of Low-crimp Wools

In low-crimp wool the difference in staining density of the ortho- and paracortical components observed in high-crimp wool does not occur (Fig. 5). However, examination at the macrofibril level indicates that bilateral asymmetry is still evident. The orthocortical macrofibrils appear to be similar to those in the high-crimp wools while the remainder of the cortex appears to be composed almost entirely of mesocortical cells.

The frequency of regular packing in mesocortical cells (Fig. 6) is much higher than in paracortical cells.

Fig. 3. Higher magnification of the high-crimp Merino wool shown in Fig. 2.

Fig. 4. High-crimp Merino wool showing junction of orthocortex (*O*), paracortex (*P*), and mesocortex (*M*).

As in the high-crimp wools, many of the mesocortical macrofibrils resemble those of the paracortex, being fused and difficult to delineate clearly, but some also appear to be small and more rounded than those in the paracortex (Fig. 7).

The distribution of orthocortex and mesocortex in these low-crimp wools is usually bilateral, but other distributions, as indicated by Watson (1974), also occur. Some fibre cross sections consist almost entirely of orthocortex with only a small amount of mesocortex; very occasionally the reverse situation occurs.

The small numbers of paracortical cells in low-crimp fibres usually occupy only about 10% of the total cross-sectional area of the cortex and are readily demonstrated by staining unreduced fibres with osmium tetroxide alone (Fig. 8).

Discussion

These results, together with Watson's (1974) findings on the ratio and distribution of the ortho-, para- and mesocortical cells, shed new light on the structure of the cortex of wool fibres. In comparison with the uncertainties involved in use of the light microscope to study cortical differentiation in wool, the electron microscope with its high resolution enables the different cortical-cell types to be more readily identified. Reservations should be made, however, concerning the possibility that the reduction procedure may alter the structure of the untreated wool together with the fact that the reaction of heavy metal with embedded tissue is non-stoichiometric.

In the paracortex of high-crimp wool the fibrillary pattern differs from the generally accepted description of hexagonal or pseudohexagonal packing. These packing arrangements are rare compared with a more irregular configuration of the microfibrils. Only small amounts of mesocortex are found in these wools.

Although the staining of mesocortical cells resembles that of the orthocortex, the mesocortical macrofibrils are morphologically more akin to those observed in the paracortex, being large and irregular in shape, extensively fused and lacking the intermacrofibrillar material so common in the orthocortex.

In the low-crimp wools the cortex appears to be stained more uniformly whereas examination at the macrofibril level indicates the presence of bilateral segmentation. The orthocortex is similar to that found in the high-crimp wools. The unique feature of these fibres is the high content of mesocortex. They contain an average of 41·6% mesocortex compared with 2·7% in high-crimp wools (Watson 1974). Other reported values range from 1 to 4% (Bonès and Sikorski 1967). It is probably because of the difficulties inherent in recognizing the mesocortex under the light microscope that this component has hitherto not always been considered to be a distinct entity. Watson (1974) found that low-crimp wools had to be stained for much longer periods (20 min) than high-crimp wools (10 min) to reveal the distribution of cortical cell types.

The present study confirms Watson's finding that the segmentation pattern in low-crimp wools is much more complex than the simple bilateral arrangement exhibited by high-crimp fibres. In addition, the packing of microfibrils has been

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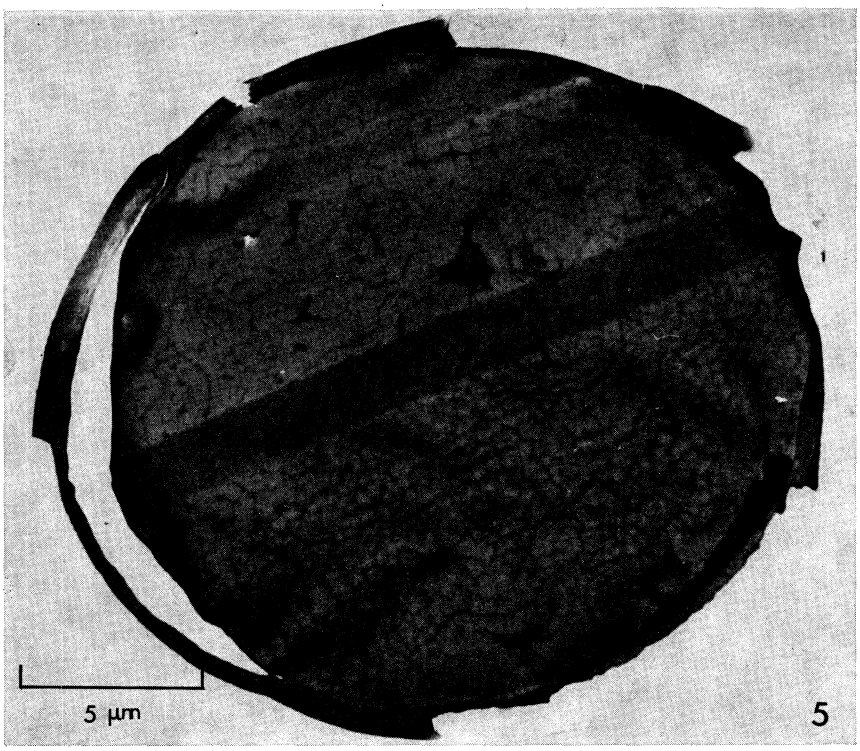
Fig. 5. Low-crimp Merino wool. The clear cortical segmentation which occurs in the high-crimp wool is lacking.

Fig. 6. Mesocortex of low-crimp Merino wool. Microfibrils are arranged in hexagonal or near-hexagonal array and the macrofibrils are fused.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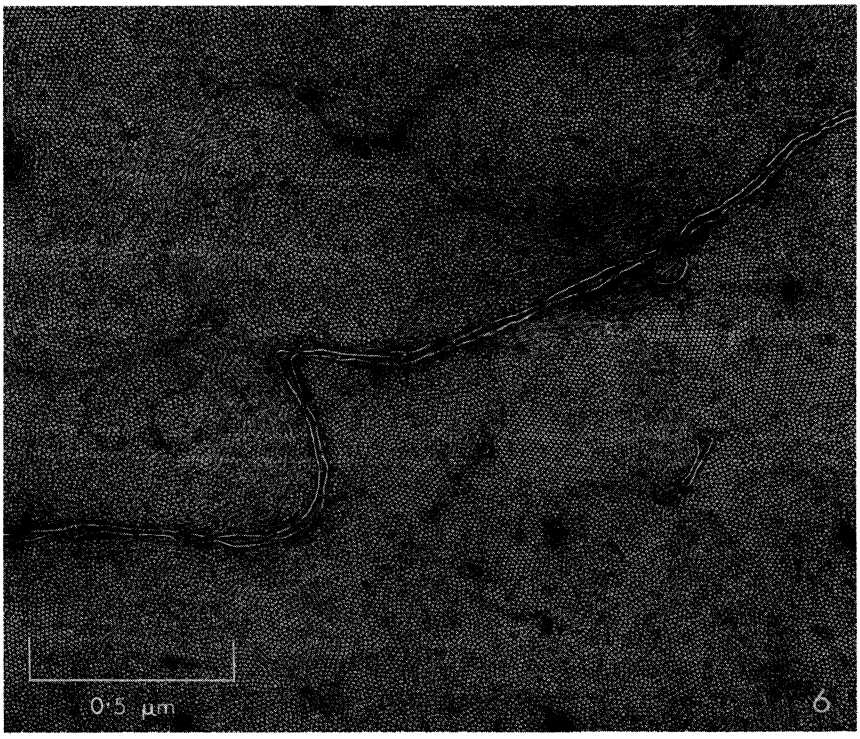
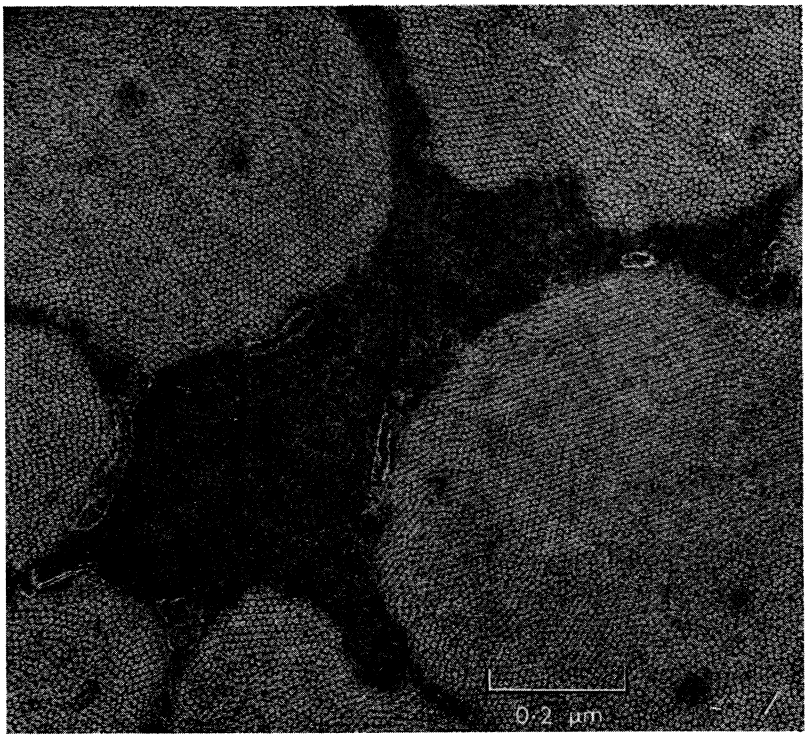
5


0·5 μm
6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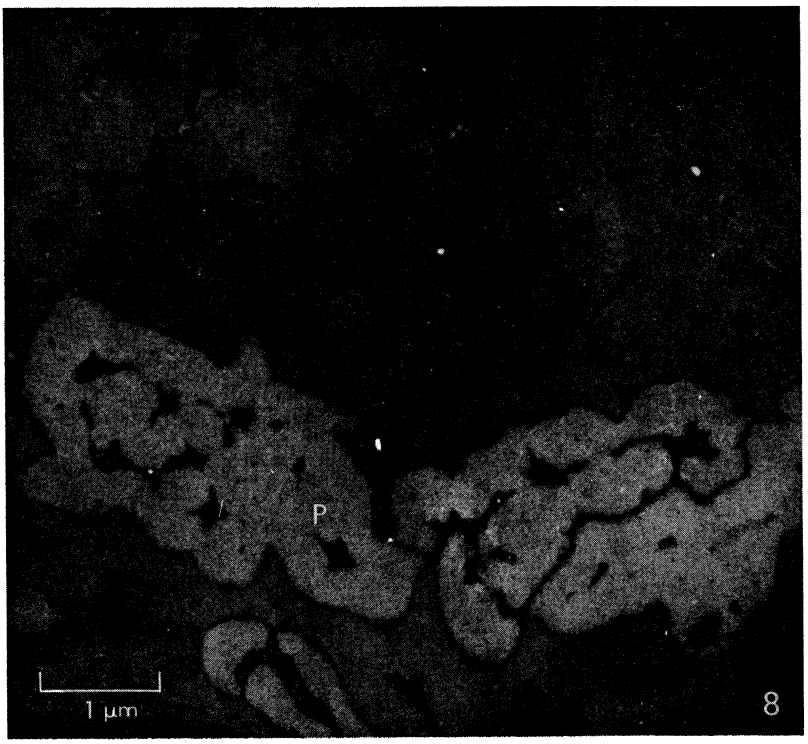
P
1 µm
8

shown to be much more regular in meso- than paracortical cells. The stained appearance of the mesocortex resembles the orthocortex, suggesting that it contains less matrix than paracortical cells, which are known to be richer in matrix material (Leach *et al.* 1964; Dobb 1970), sulfur, and high-sulfur protein (Simmonds and Bartulovich 1958; Kulkarni *et al.* 1971) than orthocortical cells. Compositional studies by Campbell *et al.* (1972, 1975) on the wools used in the present work taken in conjunction with Watson's (1974) observations support these conclusions.

The fine structure of the three cortical cell types can be summarized as follows:

Paracortex: Largely random or, occasionally, hexagonal arrangements of microfibrils. Microfibril:matrix ratio low. Macrofibrils poorly defined and extensively fused.

Mesocortex: Extensive areas of microfibrils in a clearly resolved hexagonal or quasihexagonal arrangement. Microfibril:matrix ratio intermediate. Macrofibrils similar to those in paracortical cells.

Orthocortex: Poorly resolved microfibrils grouped together in a 'fingerprint' pattern of discrete and regular macrofibrils. Microfibril:matrix ratio high. Tilting of the specimen reveals a twisted hexagonal arrangement of microfibrils.

These results are somewhat at variance with currently accepted views of microfibril packing. As the mesocortical cells lie adjacent to, or enclosed within, the paracortex in well-crimped fibres, their high degree of order may have been assumed to typify the paracortex. It is also interesting that the random arrangement observed in the paracortex partly resembles the description of paracortical cells produced on a sulfur-rich diet which results in a greater proportion of matrix material than in normal wool (Gillespie *et al.* 1964).

The results of both Bonès and Sikorski (1967) and Watson (1974) suggest that the percentage of orthocortex remains constant whereas the amounts of paracortex and mesocortex, and ultimately the sulfur content of the wool, are dependent on the amount and composition of the matrix protein.

These results are in full agreement with the compositional studies of Campbell (1972) who demonstrated an increased content of high-sulfur (matrix) protein in the high-crimp wools and a correspondingly higher content of low-sulfur protein in low-crimp wools. This latter protein fraction comprises the well ordered microfibril phase which displays only slight variation in composition between keratins (Crewther *et al.* 1966; Fraser *et al.* 1971). The basic difference between the two groups of wools appears to be, therefore, in the matrix proteins of the paracortex–mesocortex complex.

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Fig. 7. Mesocortex of low-crimp Merino wool in which the macrofibril profiles are approximately circular.

Fig. 8. Paracortical cells (*P*) within the mesocortex of low-crimp Merino wool. Osmium tetroxide stain only.

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