CHEMICAL HETEROGENEITY AND CORTICAL SEGMENTATION IN WOOL

by

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Recent investigations[1,2,3] have shown that the cortex of crimped wool consists of two segments which may be differentiated by their differing resistance to alkali swelling, dyeing, peracetic acid-ammonia treatment and enzyme digestion. It has been inferred that the resistant segment (*H*) is the more keratinized by virtue of a greater degree of disulphide cross-linking, although the amino acid compositions of the two segments have not been investigated. It is of interest therefore to recall the earlier isolation and analysis of two protein fractions from wool treated with cetyl sulphonic acid[4]. We have established a relationship between these fractions and the cortical segments by microscopic examination of the action of this reagent on wool fibres.

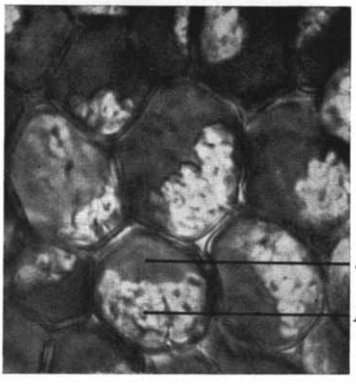

Fig. 1. Cortical cells and scale remnants released by mechanical disintegration of wool fibres treated with cetyl sulphonic acid.

Fig. 2. Cross-section of cetyl sulphonic acid treated wool fibres dyed with toluidine blue.

Merino wool (64's quality) was incubated with 0.05 *M* cetyl sulphonic acid (pH 2) at 65° C. After 6 days there was no apparent change in the histological structure of the fibres apart from the development of striations in the *H* segment and damage to the cuticle as revealed by dyeing tests. Nevertheless, at this stage the fibres could be readily disintegrated mechanically yielding cortical cells and scale remnants, Fig. 1. In cross-sections stained with toluidine blue, Fig. 2, the outlines of cortical cells and nuclear spaces are visible in the *H* segment and the basophilia of the *S* segment is not affected by the treatment. Upon the addition of 0.01 *N* NaOH, the cuticle around the *S* segment ruptures and cortical cells are released, Fig. 3. These cortical cells, which are non-birefringent, swell and gradually dissolve, leaving a strand of cortical cells derived from the *H* segment and adhering scale remnants, Fig. 4.

It would appear from the above observations that the cystine-rich *Aa* residue isolated and analysed by LINDLEY[4] consisted largely of *H* segment and that the alkali soluble fraction *B* was derived from the *S* segment. The high cystine content of the *Aa* residue would appear to confirm the belief that the *H* segment is more highly cross-linked by disulphide linkages, and it should be noted also that the proline content is high. The *B* fraction, in addition to being lower in cystine and proline, has a greater content of glutamic and aspartic acids than *Aa*[5], which probably accounts for the marked basophilia of the *S* segment as well as contributing to its alkali sensitivity.

The bilateral structure of wool can be demonstrated by the unsymmetrical swelling action of dilute ammonia following oxidation of the disulphide bonds with peracetic acid[2]. We have found that in cross-sections of oxidised wool stained with basic dyes, the formerly acidophilic *H* segment now becomes basophilic to a much greater extent than the *S* segment, Fig. 5 and 6. This must be attributed to a higher proportion of cystine in the *H* segment, which is reflected in the analysis

of the *Aa* fraction. It follows that a greater degree of disulphide cross-linking is responsible for the resistance of the *H* segment to alkali swelling and enzymic digestion. The mechanism of the action of cetyl sulphonic acid on wool appears to be one of catalysed hydrolysis, and we have found that a microscopically similar effect is obtained with dilute hydrochloric and oxalic acids in the presence of secondary alcohol sulphate detergents, which greatly accelerate the disintegration of the wool fibre.

It is evident from our microscopical observations that the separation of *H* and *S* segments is by no means complete, the *Aa* residue being contaminated with scale remnants, whilst fraction *B* probably contains inter-cellular material derived from *H*. The fractionation is also open to criticism in view of the work of several authors[6,7,8,9] who have shown that aspartic acid is preferentially

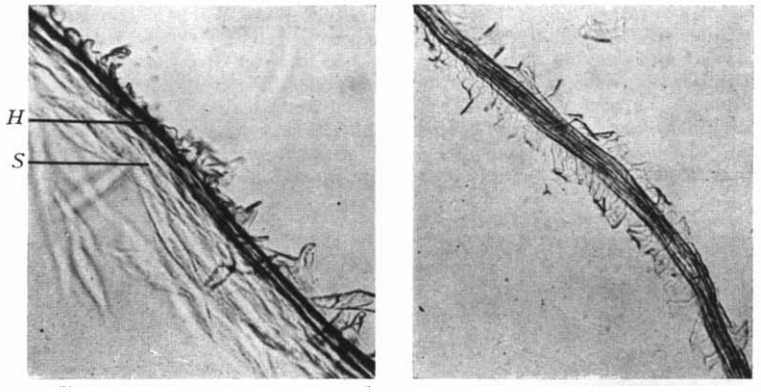

Fig. 3. Cetyl sulphonic acid treated wool fibre, showing dispersion of *S* segment in 0.01 *N* NaOH.
Fig. 4. Later stage showing complete dispersion of *S* segment leaving *H* segment and scale remnants.

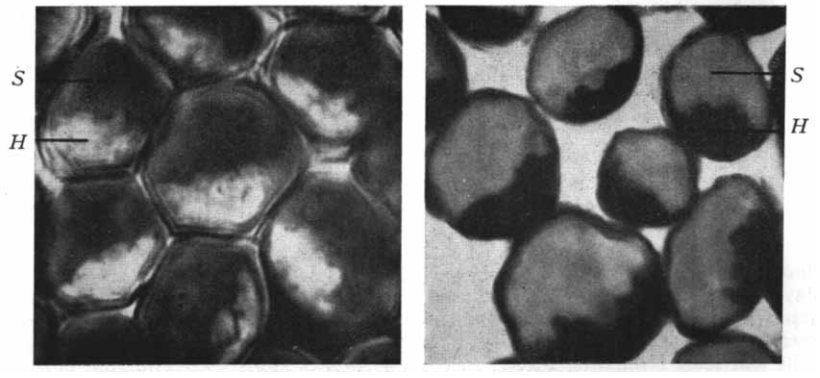

Fig. 5. Cross-section of untreated wool fibres dyed with methylene blue, showing basophilia of *S* segment.

Fig. 6. Cross-section of peracetic acid oxidised wool fibres, dyed with methylene blue, showing greatly increased basophilia in the *H* segment.

released from proteins on mild hydrolysis with dilute acids. It must be recognised, therefore, that in addition to histological differentiation, alterations in the amino acid composition of the segments may occur in our experiments. It is of interest that BISERTE AND PIGACHE[7,8] have shown that initially, soluble peptides of low cystine content are released by prolonged hydrolysis of wool with 0.01 *N* H_2SO_4 yielding a wool residue with an increased cystine content. Also BLACKBURN[10] has reported that the cortical cells remaining after digestion of wool by papain-bisulphite contained more cystine than intact wool. These resistant cells probably correspond to MERCER's *paracortex*[2] remaining after trypsin digestion of supercontracted wool.

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REFERENCES

[1] M. HORIO AND T. KONDO, *Text. Research J.*, 23 (1953) 373.
[2] E. H. MERCER, *Text. Research J.*, 23 (1953) 388.
[3] R. D. B. FRASER AND G. E. ROGERS, *Biochim. Biophys. Acta*, 12 (1953) 484.
[4] H. LINDLEY, *Nature*, 160 (1947) 190.
[5] H. LINDLEY, unpublished results.
[6] S. M. PARTRIDGE AND H. F. DAVIS, *Nature*, 165 (1950) 62.
[7] G. BISERTE AND P. PIGACHE, *Bull. Soc. Chim. Biol.*, 33 (1951) 1379.
[8] G. BISERTE AND P. PIGACHE, *ibid.*, 34 (1952) 51.
[9] S. BLACKBURN, *Biochem. J.*, 47 (1950) xxviii.
[10] S. BLACKBURN, *Biochem. J.*, 47 (1950) 443.

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THE ORIGIN OF SEGMENTATION IN WOOL CORTEX

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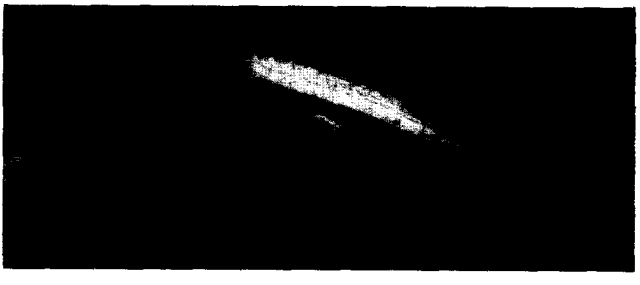
Recently it has been shown[1,2,3,4] that the cortex of crimped wool fibres has a bilateral structure with segments of differing dye-accessibility, alkali sensitivity, cystine content and resistance to enzymic digestion. It is natural to enquire at what stage in the biosynthesis of the wool fibre this differentiation occurs, and we wish to present a preliminary account of our investigations of the origin of cortical segmentation in the follicle.

Fig. 1. (a) Plucked wool root swollen in detergent and alkaline thioglycollate showing segmentation.



(b) The same observed between crossed polaroid screens showing differential loss of birefringence.

The differentiation of cortical cells in the follicle involves a process of elongation accompanied by the deposition of longitudinally oriented fibrils and subsequent hardening or keratinization including the formation of disulphide linkages[5,6]. In freshly plucked roots the unkeratinized portion swells in water and is dispersed by alkali or urea. When the process is observed microscopically