CRIMP IN WOOL: CORTICAL SEGMENTATION AND TENSILE PROPERTIES OF WELL-CRIMPED AND ABNORMALLY CRIMPED FIBRES OF MERINO WOOL

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Summary

Poorly crimped (or doggy) fibres, produced by follicles with hyperplasia of the outer root sheath tissue, have greater proportions of paracortex than adjacent well-crimped fibres. Associated with this increase in paracortex is an increase in strength, as indicated by significant increases in the stresses in wet poorly crimped fibres at the turnover and breaking points on the stress–strain curve. Use of the stronger mechanical properties of doggy fibres as a means of distinguishing such fibres from the poorly crimped fibres in steely wool, produced by sheep on a copper-deficient diet, is proposed.

The possibility that the state of follicle outer root sheaths can influence the distribution and proportions of the cortical segments in the wool fibres produced is discussed.

I. INTRODUCTION

Staples of normally crimped Merino wool have smaller percentages of paracortex than staples of doggy Merino wool with lower crimp frequencies (Ahmad and Lang 1957; Jones 1961). By contrast, the percentage of paracortex increases with increase in crimp frequency in samples of Merino wool with reversed relationships of crimp frequency to fibre thickness (Snyman 1963). The proportion of paracortex increases also with fibre thickness within breeds (Ahmad and Lang 1957; Thorsen 1958; Snyman 1963) and to some extent between breeds (Thorsen 1958).

Poorly crimped Merino wool fibres, produced by follicles with gross hyperplasia of their outer root sheaths (Chapman, Short, and Hyland 1960; Chapman and Short 1965), have markedly different fibre growth characteristics from adjacent well-crimped fibres (Chapman and Short 1964). For fibres separated on the basis of crimp frequency per unit of fibre length from within staples of Suffolk, Rambouillet, and Navajo wools, Young's modulus and stress at 30% extension in water increase with decrease in crimp frequency (O'Connell and Yeiser 1954). An increase in the stress at 30% extension in water also accompanies an increase in percentage of paracortex (Thorsen 1958). In agreement with this, reduced mechanical stiffness is exhibited after removal by abrasion of the outer para-like portion of Lincoln wool fibres with a radial distribution of ortho- and paracortices (Feughelman and Haly 1960).

It might be expected, therefore, that increased mechanical stiffness would accompany the increase in percentage of paracortex in doggy wool samples (Ahmad and Lang 1957; Jones 1961). This would be in contrast to steely wool, which is

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produced by sheep on a copper-deficient diet, and which also has an abnormal and reduced staple crimp frequency, but a reduced tensile strength (Marston 1946).

The percentage of paracortex and the tensile properties of well-crimped and poorly crimped fibres from within normally crimped and doggy staples of Merino wool have been compared. The results indicate that the state of follicle outer root sheaths possibly has an influence on the distribution and proportions of the cortical segments in the wool fibres produced.

II. Materials and Methods

(*a*) *Wool Samples*

The wool samples examined were from the rump of four fine-wool, non-pregnant Merino ewes. In an endeavour to obtain fibres of lengthwise uniform thickness, these ewes had been fed a constant daily ration in individual pens. The staple crimp was normal on two of these ewes and severely abnormal (doggy) on the other two.

Fibres within each sample were graded as described by Chapman and Short (1964). Twenty well-crimped and 20 poorly crimped fibres were examined for cortical segmentation, and four fibres of these two crimp types were tested for tensile properties from each sample.

(*b*) *Cortical Segmentation*

For the examination of cortical segmentation, the fibres in each crimp type were cut in halves and one portion of the halved fibres was stained directly with methylene blue before cross-sections were cut, while the remaining portion of each crimp type was cross-sectioned prior to being treated with performic acid and stained with methylene blue and eosin.

(i) *Direct Methylene Blue Staining.*—The fibres in each lot were aligned in a bundle, tied near their ends to a glass rod and degreased in diethyl ether (solvent grade). Various staining techniques, including those of Fraser and Rogers (1955) and Jones (1961), proved unsatisfactory. Ultimately the fibre bundles were dyed in a boiling solution of 0·2% (w/v) methylene blue in Sorensen's 0·1M phosphate buffer at pH 7·4 for 5 min with a liquor/wool ratio of at least 10 ml per 1 mg wool. The bundles were then rinsed sequentially in distilled water, in acid–alcohol [1% (v/v) hydrochloric acid in 70% (v/v) ethanol], and distilled water. Different batches of methylene blue powder, even from the same supplier, had markedly different dyeing efficiencies. Each bundle of stained fibres was loaded into a Hardy microtome between small tufts of undyed scoured wool, and a cross-section was cut and mounted under a coverslip on a microscope slide.

(ii) *Staining following Performic Acid Treatment* (Clarke, personal communication).—The fibres in each lot were attached to a U-shaped piece of wire and degreased in petroleum ether. Each bundle was embedded in paraffin wax and cross-sections 10 μ thick were cut and floated on to a microscope slide which had been smeared with albumin. The fibre cross-sections were treated for 1 hr with fresh performic acid solution [prepared by mixing 25 parts by volume of concentrated formic acid with 65 parts of distilled water and adding 10 parts of concentrated (100 vols.) hydrogen peroxide]. The sections were then rinsed in distilled water, immersed for

1–3 min in methylene blue solution at pH 4·6 (prepared by adding 1 g methylene blue powder and 1 g K_2CO_3 to 400 ml water, boiling, cooling, and adding 3 ml glacial acetic acid), rinsed in distilled water and acid–alcohol for 1 min, stained with eosin for ½ min, decolorized in ethanol, cleared in xylol, and mounted with a coverslip. Poorly crimped fibres required up to 3 min in the methylene blue solution before they stained adequately, whereas well-crimped fibres usually required only 1 min.

The patterns of distribution of the cortical segments in the stained cross-sections were examined at a magnification of ×500 on a Reichert Lanameter and classified according to the descriptions of Ahmad and Lang (1957). Subsequently, the boundaries of the cortical segments were traced at the same magnification on good quality tracing paper. Mean proportions of ortho- and paracortices were determined for each fibre bundle by cutting out, bulking together, and weighing on a microbalance the tracings of each type of segment.

(*c*) *Tensile Properties*

The fibres were degreased in diethyl ether and small glass hooks were cemented *c.* 3 cm apart to the fibre ends with collodion solution. Each fibre was attached to a microscope slide with small strips of adhesive cellulose tape, its thickness in air measured at a magnification of ×500 at 2 mm intervals lengthwise, and its mean thickness calculated. Then the fibres were removed from the slides, immersed overnight in distilled water at 20°C, and inserted singly in a Cambridge Extensometer. Each fibre was straightened in water at 20°C by manual operation of the instrument, and the length between the hooks measured to the nearest 0·1 mm with a travelling microscope. Each fibre was stretched in water at 20°C on the Extensometer at a constant rate of loading of 2·4 g/min until the fibre broke. Preliminary trials showed that a lower rate of loading was unsatisfactory. From the stress–strain curve recorded by the Extensometer for each fibre the stresses and strains at the turnover points at the ends of the Hookean and yield regions and at the breaking point, as well as the work done in stretching the fibre, were calculated.

(*d*) *Statistical Analysis*

The percentages of paracortex and the tensile properties of the well-crimped and poorly crimped fibres were analysed by applying *t*-tests to paired comparisons.

III. Results

Of the eight types of cortical segmentation described by Ahmad and Lang (1957) the following were observed in the fibres examined in this study.

A: Clear demarcation along or near the major axis of elliptical fibres or diametrically across circular fibres;

B: Clear demarcation along or near the minor axis;

C: Patterns similar to A or B but with irregular or diffuse demarcation;

F: Two separate segments of paracortex situated peripherally with a central band of orthocortex;

G: A single segment of orthocortex at the periphery.

Fibres with a single segment of paracortex at the periphery, i.e. the reverse of G, were also observed. This type was not described by Ahmad and Lang (1957) and is referred to here as type J.

Well-crimped fibres exhibit mainly types A, B, and C and an occasional type J. Within doggy staples (sheep 3 and 4), type C is more prevalent among well-crimped fibres. Poorly crimped fibres from within normally crimped staples (sheep 1 and 2) have mainly types A, B, and C with an occasional type G. In doggy staples (sheep 3 and 4), poorly crimped fibres exhibit mainly types C, F, and G. The patterns of cortical segmentation are therefore somewhat different in well-crimped and poorly crimped fibres.

TABLE 1

MEAN PERCENTAGES OF PARACORTEX AND TENSILE PROPERTIES OF MERINO FIBRES, WITH GOOD AND POOR CRIMP

Fibres loaded in water at 20°C

Sheep	Fibre Crimp	Paracortex (%)		"Turnover" Points				Breaking Point		
		Methylene Blue	Performic Acid + Methylene Blue	Extension (%)		Stress (kg/mm²)		Extension (%)	Stress (kg/mm²)	Work (g.cm/mm³)
				At A*	At B*	At A	At B			
1	Good	42	38	3·0	33·5	4·0	6·7	57·1	15·1	425·5
	Poor	44	40	2·4	32·8	4·8	7·2	55·9	16·7	467·9
2	Good	47	35	2·6	32·3	4·2	6·6	52·3	14·7	375·8
	Poor	53	40	2·8	32·7	4·6	6·9	53·6	15·8	413·5
3	Good	46	42	3·3	32·3	3·8	5·7	49·6	12·4	301·4
	Poor	50	46	2·1	31·3	4·2	6·0	53·3	14·8	385·2
4	Good	46	39	2·9	30·8	4·0	5·3	59·0	14·6	428·3
	Poor	56	43	2·1	31·1	4·8	6·2	55·3	15·6	441·8
Mean	Good	45·2	38·5	2·9	32·2	4·0	6·1	54·5	14·2	382·7
	Poor	50·7	42·2	2·3	32·0	4·6	6·6	54·5	15·7	427·1
S.E. of mean difference†		1·7	0·6	0·30	0·35	0·12	0·14	1·60	0·32	14·6
Significance of difference†		$P<0·05$	$P<0·01$	n.s.‡	n.s.	$P<0·05$	$P<0·05$	n.s.	$P<0·05$	n.s

* As in Figure 1. † Poor minus good. ‡ n.s., not significant.

The mean percentages of paracortex and the mean stress–strain data for well-crimped and poorly crimped fibres are given in Table 1, and the mean stress–strain data for each crimp type are plotted in Figure 1. Both staining techniques reveal that poorly crimped fibres have significantly greater proportions of paracortex than well-crimped fibres ($P<0·05$ and $P<0·01$), although the performic acid–methylene blue method appears to give slightly lower percentages of paracortex than does methylene blue alone.

When wet, the two types of fibres do not differ significantly in the percentage extensions at the turnover and breaking points. However, the stresses at these points are significantly higher in poorly crimped fibres than in well-crimped fibres ($P<0\cdot05$). Although more work was required to break the poorly crimped fibres than the well-crimped fibres from each sheep, the overall difference of $44\cdot4$ g.cm/mm^3 (almost 12%)

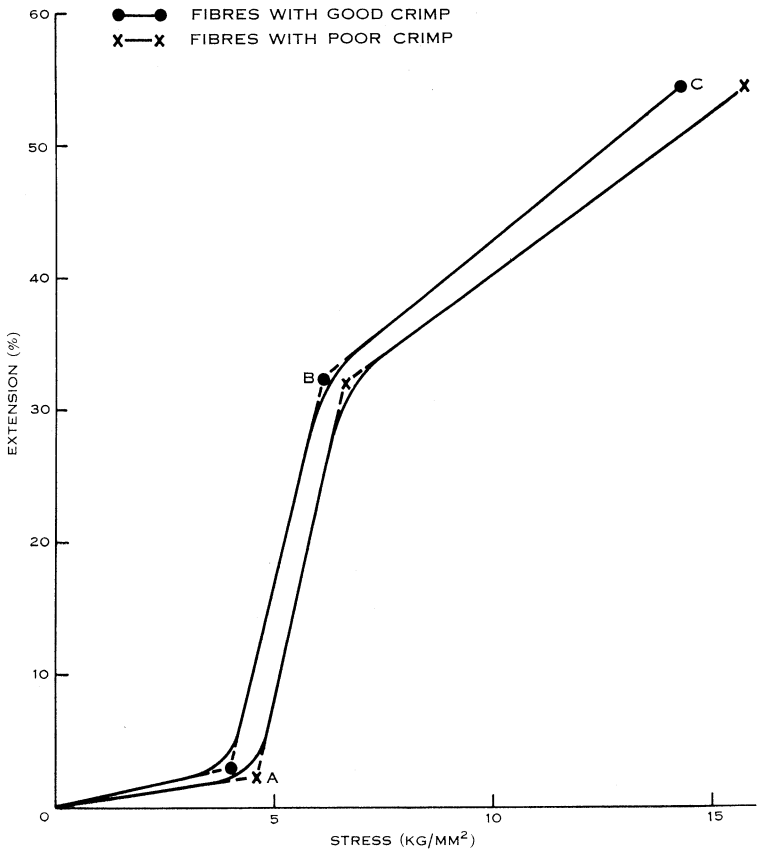

Fig. 1.—Stress–strain curves plotted from the mean data for well-crimped and poorly crimped fibres. *OA*, *AB*, and *BC* represent the Hookean, yield, and post-yield regions respectively.

did not attain significance at $P = 0\cdot05$ because of the wide variability between the four sheep examined in the difference between the amounts of work to break the two types of fibres.

From Figure 1 it can be seen that the differences in the stresses at the turnover and breaking points have resulted in somewhat different slopes of the Hookean and post-yield regions (OA and BC respectively) of the two curves, with only slight difference in the slopes of the yield region (AB).

IV. Discussion

The greater wet strength of poorly crimped (doggy) fibres (Table 1) is in contrast to the reduction in wet strength of the abnormally crimped fibres in steely wool produced by sheep on a copper-deficient diet (Marston 1946). This could be used to differentiate between doggy and steely wools when the possibility of confusion exists in areas susceptible to copper deficiency.

Wools with severe crimp deterioration that are as strong as or stronger than normal could be regarded as doggy with reasonable certainty, while wools that show considerable weakness throughout the length of the abnormally crimped growth could be considered steely. The weakness in steely wool is usually general along its length. However, a weakness (or break) at one particular level can occur in both doggy and steely wools grown during a period of severe stress, e.g. during the last few weeks of pregnancy and at parturition, or during illness. In such instances the wool on each side of the localized weakness would need to be carefully examined.

For wools with only slight crimp abnormality (secondary waves) the differences in strength between the potentially doggy and steely wools might be insufficient to be used as a means of distinction. When both these causes of abnormal crimp occur together, the weakening effect of copper deficiency would be opposed by the strengthening effect of dogginess, and the resultant strength would depend on which effect was the greater.

The abnormal crimp in doggy wool cannot be corrected by dosing the sheep with copper sulphate (Chapman, unpublished data) whereas this treatment almost invariably brings about a rapid improvement in the crimp of previously steely fleeces (Marston 1946). Hence, only in the case of abnormally crimped wool with a general reduction in strength could improvement in staple crimp be expected from copper supplementation, providing the intakes of molybdenum and sulphate were not excessive (Dick 1952, 1953).

The stress at 30% extension is greater in poorly crimped Merino fibres than in well-crimped fibres (Fig. 1), and this agrees with an increase in stress at 30% extension observed with a decrease in crimp frequency in fibres of Rambouillet, Suffolk, and Navajo wools (O'Connell and Yeiser 1954). The relative positions of the curves for poorly crimped and well-crimped Merino fibres (Fig. 1) are similar to those for Lincoln wool fibres which are respectively unabraded (ortho-+paracortices) and abraded (orthocortex) (Feughelman and Haly 1960). The effect of a greater percentage of paracortex in poorly crimped fibres on the wet tensile properties is rather similar to that produced by a decrease in water absorption following treatment of wool fibres with ninhydrin (Feughelman and Watt 1964).

The greater proportion of paracortex in staples of doggy wool than in staples with normal crimp (Ahmad and Lang 1957; Jones 1961) is the combined effect of more paracortex in poorly crimped fibres (Table 1) and a greater frequency of poorly crimped fibres in doggy staples (Aiken and Ryder 1962). Snyman's (1963) finding of an increase in the percentage of paracortex with increase in crimp frequency is in contrast to the results of Ahmad and Lang (1957), of Jones (1961), and of the present study. Incidentally, Snyman listed incorrectly the counts based on crimp frequency and/or

fibre thickness for two of his samples, A60 and A7. According to Snyman's method of selection, A60 should have been included in his low-crimp group of samples, and A7 should not have been chosen, since the counts based on its crimp frequency and fibre thickness agree.

However, Snyman's high- and low-crimp groups can be considered alternatively as groups with large and small fibre thicknesses respectively. The larger percentages of paracortex in the group with the larger fibre thicknesses are then in agreement with Ahmad and Lang (1957), with Thorsen (1958), and also with the present study in which the poorly crimped fibres are thicker than the well-crimped fibres (Chapman and Short 1964). From these various studies it becomes apparent that the percentage of paracortex is dependent on fibre thickness rather than on crimp frequency.

Disposition of the cortical segments, on the other hand, is related to crimp form (Fraser and Rogers 1953; Horio and Kondo 1953; Mercer 1953; Ahmad and Lang 1957; Glynn, Lang, and Wardle 1960; and present results), and to some extent to fibre thickness (Fraser and Rogers 1955; Ahmad and Lang 1957; Thorsen 1958). Poorly crimped fibres exhibit segmental distributions not observed in well-crimped fibres (see Section III), and are produced by follicles with gross irregular enlargements of the outer root sheaths adjacent to the supra-bulbar and keratogenous zones of the fibres (Chapman, Short, and Hyland 1960). These features in conjunction with the larger percentage of paracortex in poorly crimped fibres suggest that the state of the outer root sheath has an influence on cortical segmentation.

An increase in the proportion of paracortex with increased amount of outer root sheath tissue is in apparent conflict with the allocation of the "presumptive paracortex" in a bilaterally segmented fibre to the thin side of its follicle (Mercer 1954). This allocation was apparently based on the unstated concept that follicles are static, and was made by extrapolation down the follicle from a level where keratinization is complete, and where the paracortex is adjacent to the thinner part of the inner root sheath (Fraser and Rogers 1954). Likewise, Fraser (1964) by extrapolation down the follicle concluded that "in the case of the deflected bulbs, the cells on the ectal side of the germinative region (i.e. those maintaining mitotic 'activity') correspond in follicular position to the paracortex of the fibre on the thin side of the follicle, whilst those on the ental side (i.e. differentiating cells) correspond in follicular position to orthocortical cells."

The situation, however, needs to be reconsidered in the light of the changes in the angles of emergence of fibres during crimp formation, which indicate that the orientations of follicles change during crimp formation (Chapman 1965). In this dynamic state, the configuration of a follicle at the time of mitosis of a cortical cell would be different from that during subsequent keratinization of that cell. The extent of the difference would depend on the time for the cell to pass from the region of cell division in the bulb to the level where keratinization is complete and on the time for a crimp to form.

The outer root sheath around the lower part of the follicle is rather flaccid, and in a follicle with a deflected bulb is generally thicker on the same side as the concavity of the bulb flexure. If the period between the final division of a cell and completion of its subsequent hardening approaches half the time of formation of a crimp, the

changes in follicle configuration would be such that a cell which hardens on the inside of a crimp, i.e. in the paracortex (Fraser and Rogers 1953; Horio and Kondo 1953; Mercer 1953), and apparently on the thin side of the follicle would have formed on the concave side of the bulb flexure adjacent to the thickened portion of the outer root sheath.

However, it remains to be determined whether the differences between ortho- and paracortical cells are initiated during cell division or during subsequent cell growth prior to fibrillation. Likewise, it is unknown whether cortical cell type is predetermined or is dependent on the environment (at or subsequent to the time of cell division) due to the follicle configuration, which so markedly affects the patterns of mitotic activity and of cortical segmentation (Fraser 1964).

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