COMPOSITIONAL STUDIES OF HIGH- AND LOW-CRIMP WOOLS

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Abstract

Merino wools with high staple crimp frequency contained 20% more cystine than poorly crimped wools of the same strain. These differences were reflected in both the quantity and composition of those components of the high-sulphur fraction of wool keratin that are richest in sulphur. It was found that this greater concentration of disulphide cross-linkages in high-crimp wools was associated with a marked reduction in swelling and supercontraction but has no effect on the stress of single fibres at 30% extension in water.

Under special dietary conditions, sheep may produce wool which does not exhibit or fit into this relation between crimp and sulphur content; furthermore, wools without bilateral structure and the accompanying differences in composition between cortices may still show considerable crimp. For these reasons it was concluded that the relation between crimp and cystine content is not one of cause and effect but is the consequence of growth in particular types of follicles.

I. Introduction

The structural basis of the regular crimp waves observed in single wool fibres and wool staples has so far eluded successful identification. These waves have been attributed to a number of factors including the shape and depth of the follicles (Chapman 1965; Nay and Hayman 1969) and the unique bilateral structure of the fibre cortex (Horio and Kondo 1953; Mercer 1953). These factors may not be mutually exclusive. The cortex of crimped fibres consists of two distinct bilateral segments, the para and ortho cortices, situated respectively on the concave and convex sides of the crimp waves. At present, evidence is predominantly in favour of a relation existing between crimp and bilateral structure, largely because of the positive correlation which exists between crimp frequency and the ratio of para- to ortho-cortex (Snyman 1963). It is still uncertain whether this relation is one of cause and effect.

If the relation is one of cause and effect then demonstrable differences should exist in structure and composition between the two types of cortical cells which could be shown to be the causation of the crimp waves. Such differences should also be reflected in differences in composition and properties between low-crimp and high-crimp wool. Evidence is conflicting on differences in composition between ortho- and para-cortical cells. Some electron-microscope studies showed that these cells differed in their relative contents of microfibrils and matrix and hence of low-sulphur

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and high-sulphur proteins (Rogers 1959; Leach, Rogers, and Filshie 1964) and early amino acid analyses tended to support these conclusions (Simmonds and Bartulovich 1958). Other studies have only found small differences in composition between the two types of cell (Haly and Inglis 1964; Bradbury, Chapman, and King 1968). However, the very recent investigations of Kulkarni, Robson, and Robson (1971) showed that ortho- and para-cortical cells differed significantly not only in amino acid composition but also in their relative proportions of high- and low-sulphur proteins.

In the work to be described in this paper the interrelations between the crimping frequency and protein composition of wool has been examined. A previous study using a limited number of wool samples of unknown origin suggested that a positive correlation existed between crimp frequency and the content of high-sulphur proteins and of certain high-sulphur protein subfractions (Gillespie 1965). This relation has now been re-examined using well-defined experimental material drawn from the single-character selection groups for fleece characteristics established at the Trangie Experimental Station in 1951 (Morley 1951; Dun 1958) and from another flock maintained at the University of New South Wales Field Station, Hay, N.S.W. These groups produced by selection from common genetic stock and maintained under similar environmental conditions provide wool with extremes of crimp frequency. In this work a comparison has been made between the mechanicochemical properties of certain of these wool samples, their cystine content, and the proportion of those protein components which are richest in sulphur. The latter proteins have already been shown to be readily altered in amount in wool by genetic and nutritional manipulation of sheep (Reis 1965; Gillespie, Broad, and Reis 1969; Darskus and Gillespie 1969, 1971; Broad, Gillespie, and Reis 1970).

II. Material and Methods

(a) *Origin and Preparation of Wool Samples*

The physical parameters of the three groups of wool used in this study are listed in Table 1. Each group consisted of 10 individual wool samples, five of high-crimping rate and five of low. The tops of the wool samples were cut off and the remainder extracted with ether in a Soxhlet apparatus and washed with two lots each of ether, ethanol, and water. The samples were air-dried.

(b) *Determination of Sulphur and Sulphur-containing Amino Acids in Wool*

Sulphur was estimated by the Earland (1961) modification of the oxygen-flask method of Schöniger (1956). Cystine and cysteine were estimated on conditioned wool by the polarographic method of Leach (1960) employing a manual polarometer (Metrohm type E324). The ASTM procedure (D629-595) was used to determine the moisture content of the wool and the results were expressed as micromoles of each amino acid per gram of dry wool.

(c) *Measurement of Stress at* 30% *Extension*

A 4-cm portion of the root ends of single fibres was attached to a microscope slide with collodion flexile and conditioned at 65% R.H. and 20°C. The diameter of each fibre was calculated from measurements made at 1-mm intervals over a 3-cm length of the fibre (magnification × 500). Fibres were then immersed in distilled water overnight at 21°C before extending them in water on a Cambridge Extensometer to 30% extension at a constant rate of straining of 30% per minute.

(d) Measurement of Supercontraction in Concentrated Sodium Iodide Solution

Approximately 4-cm portions of the root ends of single fibres were inserted into supercontraction frames (Whiteley 1962) and their straight lengths measured with a travelling microscope. The frames were then immersed in an 8·3M solution of sodium iodide at 98·5°C for 60 min before straightening the fibres for a second length measurement, the decrease in length being expressed as a percentage of the initial length.

(e) Preparation of Soluble Proteins

Finely cut conditioned wool (5 g) was extracted at 40°C with 150 ml of a solution containing urea (8M), redistilled thioglycollic acid (0·4M) (Harrap and Woods 1965), EDTA (0·001M), Tris (0·1M) adjusted to pH 11 with KOH (I. E. B. Fraser, personal communication). The wool was thoroughly wet with the solution by evacuation and the system continuously shaken. After 2 hr the undissolved wool was filtered off, washed, dried, and weighed to give an estimate of the extent of solubilization.

TABLE 1

ORIGIN AND CHARACTERISTICS OF THE WOOL SAMPLES USED IN THIS STUDY

Group		No. of samples	Mean No. of crimps per inch	Mean diameter (μm)	Sulphur content (%)	Disulphide content (μmoles/g)	Sulphydryl content (μmoles/g)
I*	A	5	17·4	20·5	3·69	474	56
	B	5	6·6	24·3	3·18	396	41
II†	A	5	15·1	21·2	—	443	27
	B	5	6·9	21·2	—	362	28
III‡	A	5	9·6	21·5	—	415	36
	B	5	6·1	24·2	—	398	20

* Trangie: medium Peppin Merino—selected for different crimping rates.

† Trangie: medium Peppin Merino—selected for different fleece weight: A, low fleece weight; B, high fleece weight.

‡ Hay: South Australian Merino—extremes of crimp frequency in a single flock.

The filtrate was treated with an equal volume of a solution containing Tris and iodoacetic acid (20%: 9%, w/v) at pH 8·3. At the completion of the alkylation reaction excess iodoacetate was reacted with 0·1 ml of thioglycollic acid and the solution dialysed. The low-sulphur proteins, precipitated by adding an equal volume of acetate buffer (ionic strength = 1·0, pH 4·5) were removed by centrifugation and the high-sulphur proteins in the supernatant were recovered by dialysis and freeze-drying (Gillespie 1962).

(f) Moving-boundary Electrophoresis

Moving-boundary electrophoresis was carried out in sodium acetate–acetic acid buffer of ionic strength 0·1 at pH 4·5 with protein concentrations close to 1·5%. The protein solution was first dialysed for 16 hr against the acetate buffer and then run in a Tiselius apparatus (LKB) for 210 min at a voltage gradient of about 7 V/cm. The proportion of components was calculated from the relative areas under peaks in the ascending-boundary patterns. On tracings, four peaks, A–D in order of increasing mobility and sulphur content, were arbitrarily defined by vertical lines, the horizontal distance covered under each peak varied very slightly due to the greater mobility of the high-sulphur proteins derived from the high-crimp wools.

(g) Column Chromatography of High-sulphur Proteins

High-sulphur proteins were separated into four subfractions by column chromatography on DEAE-cellulose (EK7392; Eastman Organic Chemicals) (Gillespie 1962; Flodin and Killander

1962). Protein samples (250 mg) were dissolved in and then dialysed against the starting acetic acid–sodium acetate buffer (pH 4·5, ionic strength 0·1), then applied to a 90 by 0·63 cm column and eluted with a linear gradient of sodium chloride (0–0·65M) in the starting buffer. A total volume of 320 ml was applied with a flow rate of 80 ml/hr. The concentration of protein in the fractions (5 ml) was measured spectrophotometrically at 278 nm and protein components were isolated by pooling the fractions eluted between the points indicated by arrows on the elution curve of Figure 5, then dialysing and freeze-drying. After each experiment the column was regenerated by successively pumping through 500 ml each of 1·0M sodium chloride, distilled water, and the starting buffer.

(*h*) *Amino Acid Analysis*

A 30 mg sample from each of the two wools containing the highest and lowest sulphur contents in group I were hydrolysed *in vacuo* at 108° with 6M HCl for 24 hr. The HCl was removed by freeze-drying and the contents of amino acids in the hydrolysate were determined by ion-exchange chromatography with an automatic amino acid analyser (Beckman-Spinco).

III. Results

(*a*) *Relation between the Crimping Rate of Wool and Its Content of Cystine*

It was found that the greater the crimping rate of wool the greater was its content of cystine and this conclusion was valid whether the crimp differences arose from deliberate selection for differences in crimping rates (group I) indirectly from selection for differences in fleece weight (group II), or as a result of natural variations within a flock. The results thus strongly confirm the earlier observations of Thorsen (1958) and Snyman (1963). The significance of the relation between cystine, either measured as –SS– or –SS– plus –SH and crimping rate within each of the three groups was high (0·1% probability level) and this level of significance applied to the three groups when they were treated as a whole. Although there were considerable variations in cystine content within each high-crimp subgroup and each low-crimp subgroup, crimp variations were small and no correlation existed within subgroups between crimping rate and cystine content.

The very variable content of free –SH groups in these wools is noteworthy for the maximum range was from 11 to 60 μmoles/g. As the maximum level in the Trangie group I wools is about three times that usually found in Merino wools it is possible that keratinization in these wools has been incomplete, possibly due to a trace-element deficiency.

(*b*) *Comparison of the Supercontraction Properties of Low-crimp and High-crimp Wools*

Five fibres from each of the wool samples in Trangie groups I and II were supercontracted in 8·3M sodium iodide at 98·5°C. In each case the wools with higher crimp supercontracted less (group I, 30·4%; group II, 34%) than the more lightly crimped wools (group I, 41·7%; group II, 43·1%), results which are significantly different at a 1% probability level (Table 2).

(*c*) *Comparison of the Stress–Strain Properties of Low-crimp and High-crimp Wools*

The stress values at extensions of 30% of five fibres from each wool sample in Trangie group I were measured in distilled water. The mean values for the high-

crimp wools was 5·79 kg/mm², and for the low-crimp wools 5·98 kg/mm². The variations within the classes were quite small and the results do not differ significantly (Table 2).

TABLE 2

STRESS AT 30% EXTENSION AND SUPERCONTRACTION OF WOOLS FROM TRANGIE CRIMP-SELECTION GROUPS AND SUPERCONTRACTION OF WOOLS FROM TRANGIE FLEECE-WEIGHT-SELECTION GROUPS

Five fibres from each wool sample tested

Crimp-selection groups			Fleece-weight-selection groups	
Sample No.	Stress at 30% extension (kg/mm²)	Super-contraction (%)	Sample No.	Super-contraction (%)
	High-crimp wool		High fleece weight	
1	5·88	25·5	1	44·5
2	5·65	28·6	2	36·7
3	5·52	30·8	3	45·6
4	6·12	32·2	4	45·6
5	5·78	35·1		
Mean	5·79†	30·4**		43·1**
	Low-crimp wool		Low fleece weight	
1	5·85	45·6	1	35·0
2	6·24	43·2	2	33·5
3	5·50	42·3	3	31·8
4	6·15	42·1	4	35·8
5	6·17	35·3		
Mean	5·98†	41·7**		34·0**

** Differences significant at 1% level.
† Differences not significant at 5% level.

(*d*) *Relation between the High-sulphur Proteins and Subfractions and the Extent of Crimping of the Wool Fibres from which they were Derived*

A comparison was made of the quantity of high-sulphur proteins in two low-crimp and two high-crimp wools from Trangie group I. The high-crimp wools contained slightly more high-sulphur protein (about 10%) than the low-crimp wools and the proteins from the high-crimp wool were slightly richer in sulphur (Table 3).

Electrophoretic analysis of these high-sulphur proteins revealed differences in the relative proportion of the four principal subfractions (Figs. 1–4; Table 3). As compared with the low-crimp wools the high-crimp wools contained less peak B protein (about 30%), more peak C protein (about 30%), and substantially more peak D protein (about 60%). It was observed that the subfractions from the high-crimp wools, particularly subfraction D, moved slightly faster than the comparable fractions from the low-crimp wools, suggesting that these fractions differed in composition.

TABLE 3

YIELDS OF SOLUBLE PROTEIN, HIGH-SULPHUR PROTEIN, AND HIGH-SULPHUR PROTEIN SUBFRACTIONS OBTAINED FROM FOUR TRANGIE GROUP I WOOL SAMPLES

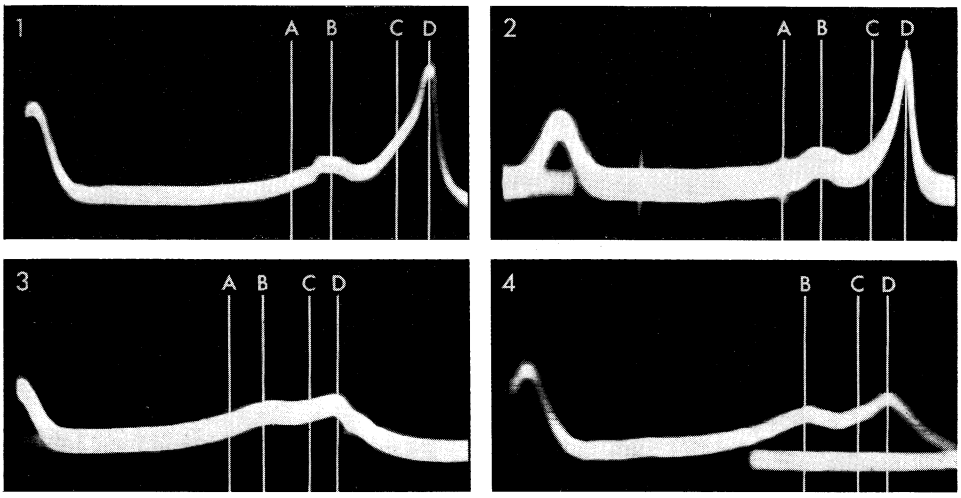
Yields are expressed as grams per 100 grams wool. In addition, protein yields in subfractions are expressed as grams per 100 grams high-sulphur protein. These values are given in parenthesis. The sulphur content of the high-sulphur protein and of the two major subfractions are also given. There were two subsamples in each of the samples tested

Parameter	Sample 1	Sample 2	Sample 3	Sample 4
Crimps per inch	17·1	17·5	7·1	6·4
Soluble protein yield	64	75	74	73
High-sulphur protein				
Yield	25	26	24	22
Sulphur content (%)*	5·85	5·93	5·31	5·61
Subfraction A				
Yield†	1(5)	1(3)	3(11)	2(9)
Subfraction B				
Yield†	5(20)	6(24)	8(34)	8(36)
Sulphur content (%)‡	4·68	4·60	4·22	4·58
Subfraction C				
Yield†	6(23)	6(22)	5(20)	4(9)
Subfraction D				
Yield†	13(52)	13(51)	8(35)	8(37)
Sulphur content (%)‡	6·46	6·87	6·20	5·83

* Mean of five determinations.

† From the electrophoretic runs shown in Figures 1–4; means of duplicate determinations.

‡ From the chromatographic runs shown in Figure 5.



Figs. 1–4.—Moving-boundary electrophoresis patterns (ascending boundary) of high-sulphur proteins run at pH 4·5 in sodium acetate–acetic acid buffer of ionic strength 0·1 at a voltage gradient of 7 V/cm. The sample numbers refer to Table 3: 1, sample 1, 210 min; 2, sample 2, 181 min; 3, sample 3, 188 min; 4, sample 4, 210 min.

To study differences in the composition of these components they were separated by column chromatography on DEAE-cellulose (Fig. 5). The elution patterns confirmed that the four subfractions were present in low-crimp and high-crimp wools in different amounts; this was particularly evident with peak D. Fractions B and D were isolated in sufficient amounts for analysis and it was found that the fractions from high-crimp wool contained more sulphur than those from the low-crimp wools (Table 3).

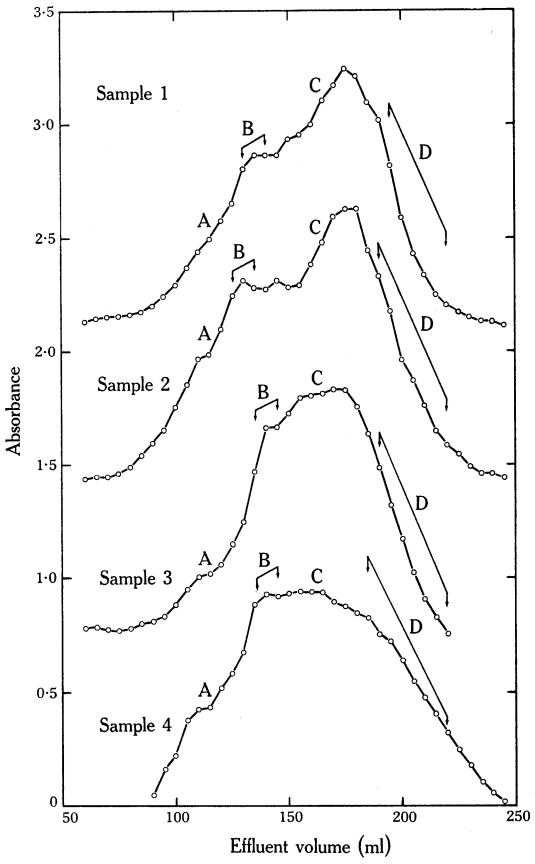


Fig. 5.—Column chromatography of high-sulphur proteins on DEAE-cellulose at pH 4·5. The starting buffer was acetic acid–sodium acetate of ionic strength 0·1 and there was a linear gradient of sodium chloride to a limit of 0·65M. Sample numbers refer to Table 3. The ordinate has been displaced 0·7 units for sample 3, 1·4 units for sample 2, and 2·1 units for sample 1.

(e) *Comparison of the Amino Acid Composition of Low-crimp and High-crimp Wools*

Comparative amino acid analyses of low-crimp and high-crimp wools from Trangie group I are shown in Table 4. If changes of 10% or more are taken as significant then in addition to the expected increase in cystine there were also increases in histidine, tyrosine, and proline. There were decreases in the contents of lysine, aspartic acid, alanine, isoleucine, leucine, and phenylalanine. Table 4 also includes similar results (Watson and Whiteley, unpublished data) derived from an analysis in triplicate, of five high- and five low-crimp wools. These samples did not represent such wide extremes of crimp frequency but the general trend of results is very similar.

IV. Discussion

The results strongly confirm earlier observations that staple crimp frequency is directly related to cystine content in wool fibres (Thorsen 1958; Snyman 1963). In Trangie groups I and II, the average difference in cystine content between high- and low-crimp wool samples was of the order of 20%. The crimp-selection groups from Hay also contained statistically significant differences in cystine although differences in crimp frequency were very much smaller in this case. The very high content of free –SH groups in the Trangie group I wools, almost three times the normal content in Merino wool, might be of some consequence in textile processes which make use of disulphide–sulphydryl interchange reactions.

Table 4

Amino acid composition of low- and high-crimp wools

Results are expressed as the numbers of residues of each amino acid in 100 residues in the protein

Amino acid	Low-crimp wool	High-crimp wool	Percentage difference	Percentage difference*	Amino acid	Low-crimp wool	High-crimp wool	Percentage difference	Percentage difference*
Lysine	2·83	2·53	−11	−4	Alanine	5·84	5·02	−14	−6
Histidine	0·73	0·82	+12	+11	½Cystine	10·50	13·20	+26	+18
Arginine	6·69	6·34			Valine	5·72	5·38		
Aspartic acid	6·48	5·58	−14	−11	Methionine	0·46	0·40		
Threonine	6·14	6·70			Isoleucine	3·26	2·92	−10	−5
Serine	10·10	10·80			Leucine	8·47	7·04	−17	−8
Glutamic acid	12·10	11·50			Tyrosine	3·45	3·82	+11	+5
Proline	6·51	7·36	+13	+1	Phenylalanine	2·64	2·38	−10	−6
Glycine	7·81	8·31							

* These results are derived from triplicate analyses of five high-crimp and five low-crimp wools (Watson and Whiteley, unpublished data).

The structural significance of the higher cystine contents in high-crimp wools is illustrated by the supercontraction data. As it is well established that this phenomenon is extremely sensitive to natural variations in cystine content (Armstrong 1969; Whiteley, Balasubramanium, and Armstrong 1970), the lower equilibrium levels of supercontraction obtained for high-crimp fibres are in line with the increased cystine content of these fibres. This may indicate the existence of additional inter-chain disulphide cross-links in these wools.

In view of these results it is rather surprising that the stress–strain properties of single fibres drawn from high- and low-crimp samples do not differ. It has been shown, for example, that decreases in the cystine content of wool arising from undernutrition are accompanied by a decrease in fibre strength (Middleton 1971). On the other hand, large increases in cystine content produced as a result of infusions of sulphur-containing amino acids into the abomasum have little effect on mechanical properties (Feughelman and Reis 1967; Armstrong and Feughelman 1969; Whiteley, Balasubramanium, and Armstrong 1970). In both situations, however, variations in cystine content induced by dietary means are closely related to both swelling and supercontraction behaviour (Gillespie 1970; Whiteley, Balasubramanium, and

Armstrong 1970). These observations would appear to indicate that the additional cystine linkages are located in the high-sulphur proteins of the matrix in such a way that they do not interfere with the unfolding of the helices of the microfibrils, at least up to extensions of 30%.

The causes of these differences in cystine content between low-crimp and high-crimp wools have been examined for four wools from Trangie group I. These differences in cystine content seem to be entirely due to changes within the high-sulphur group of proteins, the high-crimp wools containing not only more of these proteins (10%) but also proteins which are richer in sulphur (11%) than comparable proteins from the low-crimp wools. Moving-boundary electrophoretic analysis showed that the high-sulphur proteins of the high-crimp wools contained slightly less of the proteins of peak B, slightly more of peak C, and considerably more of peak D. The latter peak contained the proteins of highest sulphur content.

The increased sulphur content of the proteins of peak D from the high-crimp wools indicated that they differed in composition, at least partially, from the proteins of the low-crimp wools. On the basis of the studies of Gillespie, Reis, and Schinckel (1964) and Gillespie and Reis (1966), it appears very likely that the major part of these differences is due to the presence in the high-crimp wools of larger amounts of the very-sulphur-rich proteins of fraction D2 (Gillespie and Reis 1966). The molecular basis of the changed sulphur content of fraction B is not known although Gillespie, Broad, and Reis (1969) also found changes in the composition of the proteins in this peak following sulphur enrichment.

These differences between the protein composition of low- and high-crimp wools are so similar to those observed between control and sulphur-enriched wools that with some confidence it can be concluded that we are dealing with the same phenomenon and that the differences in composition between high-crimp and low-crimp wools are nutritionally induced (Gillespie and Reis 1966). This conclusion is supported by the amino acid analyses of Table 4 which indicate that the differences in composition between high- and low-crimp wools are similar, although smaller in magnitude, to the differences which have been observed between control and sulphur-enriched wool (Gillespie, Broad, and Reis 1969; Broad, Gillespie, and Reis 1970). The key factor in this situation may lie in the inverse relation which exists between fleece weight and crimp frequency, the high-crimp wools coming from low wool-producing sheep and hence are slow growing whereas the reverse is true for the low-crimp wools (Morley 1955). Gillespie and Reis (1966) showed that if two sheep of different wool-producing capacity were fed equal amounts of food, the high producer of wool grew wool of low sulphur content (3·0%) whereas the low producer provided wool of comparatively high sulphur content (3·7%). The latter wool contained more high-sulphur protein (25% compared with 20%) and more of the proteins in peaks C and D than did the wool of lower sulphur content. Electrophoretic analysis showed that the wool from the high producer contained almost none of peak D2 proteins whereas wool from the low producer contained a substantial amount. It seems therefore that the differences in sulphur content between high-crimp and low-crimp wools results from their growth in different follicles subjected to different environmental and nutritional conditions at the follicle level. The different shapes of these follicles has already been well established (Nay and Hayman 1969).

Further light on the relation between crimp and cystine content comes from experiments in which sheep were maintained on a starvation diet, during which wool grew slowly and with a minimum sulphur content and yet retained a high crimp. Conversely during the sulphur-enrichment studies of Reis (1965) in which rapidly growing wool of very high sulphur content was obtained there was no evidence for an increase in crimping rate. It seems likely therefore that the relation between crimp and cystine content is not one of cause and effect but is rather a fortuitous one resulting from the particular growth characteristics of the wools.

What of the relations between ortho- and para-structure and crimp? Undoubtedly there is a positive correlation between the ratio of para to ortho and crimping rate but there is abundant evidence that many of the wools at the lower end of the range of crimping rates show little or no bilateral symmetry and thus would be classed as having zero crimp potential and yet in fact have a staple crimp frequency of 5–7 crimps per inch. Therefore the ortho–para cortex relationships and the compositional variations associated with them are not the underlying cause for the existence of crimp although they may, however, play some part in determining the extent.

V. Acknowledgments

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