INTRINSIC STRENGTH OF MERINO WOOL FIBRES

by

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CHAPTER 5: CELL MORPHOLOGY AND ULTRA-STRUCTURE OF MERINO WOOL FIBRES IN RELATION TO INTRINSIC STRENGTH

ABSTRACT

Several fibre and staple characteristics contribute to phenotypic differences in staple strength. Their relative importance in influencing staple strength is likely to vary depending on complex, but poorly defined, interactions between sheep genotype and a number of nutritional, physiological, and environmental factors. To improve the competitiveness of wool in the textile industry, it is critical that the factors which contribute to tender wool under different circumstances be identified, so that the incidence of tender wool can be reduced by appropriate management and genetic strategies. To this end, the initial purpose of this thesis was to identify the causes for differences in staple strength between young Merino sheep selected for sound or tender wool and managed under a range of nutritional conditions. As wool fibres are also intrinsically weak in comparison to other textile fibres, the second major aim of this thesis was to determine the physico-chemical basis for differences in intrinsic strength. An extensive literature review of the genetic, nutritional, physiological and environmental factors influencing staple strength is presented prior to descriptions of a number of experiments investigating specific aspects of fibre strength

In an initial experiment, the wool growth response to nutrition by Merino weaners selected for high or low staple strength was examined. The hypothesis was that genetic differences in staple strength are mainly associated with differences in 'along-fibre variation' in diameter. While the estimated variation in diameter along and between individual fibres explained most of the variance in staple strength between individual sheep (r^2 = 0.80), the mechanisms responsible for nutritionally induced and genetic differences in staple strength were not the same. Nutrition influenced staple strength by affecting 'along-fibre' changes in diameter, whereas genetic differences in staple strength, at least as far as they are represented by the sheep used here, were largely attributable to 'between-fibre' variations in diameter. Thus, the hypothesis for this experiment was not supported. The practical implications of these findings are that nutritional management and genetic selection can be used concurrently to most effectively reduce the incidence of tender wool production.

In a second experiment, the hypothesis that differences in intrinsic fibre strength (force to break/unit cross-sectional area at point of break) account for the variance in staple strength not attributed to variations in diameter along and between fibres was tested. The average intrinsic strength of individual wool fibres varied between individual sheep by as much as 44%, but

intrinsic strength failed to remove any of the variance in staple strength not already attributed to differences in fibre diameter variation. It was concluded that in this experiment intrinsic strength *per se* did not contribute significantly to the differences in staple strength between sheep, and thus the hypothesis was not supported.

The inherent strength of the keratin material may be an important component of staple strength under conditions where the along-fibre changes in diameter are smaller than in the current experiment, or in wools shorn close to the point of break where the effects of minimum fibre diameter are eliminated. Intrinsic strength should also be more significant in later stage processing, because the contribution of diameter variations to fibre breakage is diminished by this stage. These reasons were justification for a third experiment which tested the hypothesis that differences in intrinsic strength were associated with the proportion of microfibrils relative to the matrix component of the fibre cortex. Wool samples which differed in average intrinsic strength were characterised in terms of the proportion of cortical cell types, total sulphur content and amino acid composition, the relative amounts and composition of keratin proteins by 1D and 2D-PAGE, and the α-helical denaturation enthalpy and transition temperatures by differential scanning calorimetry. Using these analytical techniques, sheep-to-sheep differences in the cellular and molecular characteristics of fibres measured in the region near the point of rupture were, in the main, not significantly related to intrinsic strength. The results therefore did not support the proposed hypothesis.

In a fourth experiment, the hypothesis that differences in the ultrastructure of the microfibril/matrix structure contribute to differences in intrinsic strength was tested. Examination by transmission electron microscopy of 40 fractured ends of fibres revealed that extreme differences in intrinsic strength were not associated with any major disruptions to the normal assembly of the microfibril/matrix composite, or any obvious structural abnormalities or defects within the cuticle or cortical cells. There was also no significant difference in the total volume of microfibrils in the whole cross-sections, but the proportion of paracortical cells (38.0 *vs.* 32.2%) and the density of microfibrils in paracortical cells (57.4 *vs.* 49.4%) were significantly greater in stronger fibres. It was concluded that small changes in density of microfibrils in paracortical cells, which are better aligned with respect to the fibre axis and possibly more stabilised by the surrounding matrix than those in orthocortical cells, have a significant affect on intrinsic fibre strength. The hypothesis was supported. Possible differences

in cortical cell morphology and the proportion of cell membrane complex may contribute to the differences in intrinsic strength reported in this thesis.

In summary, further studies of the complex interaction of genotype and environment with respect to staple and fibre strength are clearly warranted in light of the results presented in this thesis. From an applied point of view, more work is required to develop cost-effective and reliable feeding strategies to minimise the along-fibre fluctuations in diameter. It is proposed that it would also be more desirable to breed sheep with low along-fibre diameter variation, rather than low between-fibre variation in diameter, but for this to occur a technological breakthrough is required to make the measurement of along-fibre variation in diameter commercially viable for use in breeding Merino sheep. The results highlight the complexity of the task of postulating theoretical models on the basis of simple relationships between chemical constitution and mechanical properties, and more basic research is required to clearly define the roles of the microfibril and matrix proteins on the mechanical characteristics of wool fibres.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent of this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Andrew N. Thompson

25/06/98

PUBLICATIONS

Aspects of the work presented in this thesis have been reported elsewhere:

Refereed Journals

Thompson, A.N., and Hynd, P.I. (1998). Wool growth and fibre diameter changes in Merino sheep genetically different in staple strength and fed different levels of nutrition. *Australian Journal of Agricultural Research* **49**; 889-98.

Thompson, A.N., Schlink, A.C., Peterson, A.D., and Hynd, P.I. (1998). Follicle abnormalities and fibre shedding in Merino weaners fed different levels of nutrition. *Australian Journal of Agricultural Research* **49** (in press).

International Conferences

Thompson, A.N., Peterson, A.D., Hynd P.I., and Ritchie, A.J.M. (1995). The failure properties of single wool fibres in relation to staple strength in Merino sheep. *Proceedings of the 9th International Wool Textile Research Conference, Biella, Italy* **2**; 134-42.

Thompson, A.N., Hynd P.I., Peterson, A.D., and Ritchie, A.J.M. (1995). Fibre strength and the proportion of discontinuous fibres in relation to staple strength in Merino sheep. *Proceedings of the 9th International Wool Textile Research Conference, Biella, Italy* **2**; 143-51.

Huson, M.G., Thompson, A. N., Ley, K.J., Bedson, J.B. (1997). The intrinsic strength of wool in relation to its structure. *Proceedings of the 22nd Australasian Polymer Symposium, Auckland (2-5 February, 1997).*

National Conferences

Thompson, A.N., Hynd, P.I., Brownrigg, V.L., and Penno, N. (1996). Protein composition in relation to the intrinsic strength of merino wool fibres. *Proceedings of the Australian Society of Animal Production* **21**; 435.

Thompson, A.N., and Hynd, P.I. (1998). Staple strength and liveweight change of sheep bred for sound and tender wool. *Animal Production in Australia* **22**; 310.

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CHAPTER 1: REVIEW OF THE LITERATURE[1]

1.1. Introduction

The nutritional status and wool growth response of Merino sheep grazing annual pastures in the Mediterranean environments of southern Australia fluctuate markedly throughout the year due to seasonal changes in the quantity and quality of feed available (Rossiter 1966; Bellotti *et al.* 1993). The position of break along the staple for non-reproducing sheep frequently occurs at, or shortly after, the break of season (Rowe *et al.* 1989; Gardner *et al.* 1993; Doyle *et al.* 1995), when there is a rapid transition from low quality dry herbage to highly digestible green herbage. As most sheep in these environments are also shorn during spring (Bell and Ralph 1993; Foot and Vizard 1993), the wool produced is often tender (< 30 N/ktex) and breaks near the middle of the staple (Couchman *et al.* 1993; Baker *et al.* 1994). Both these characteristics adversely influence the processing performance and value of raw wool (Rottenbury *et al.* 1986; Plate *et al.* 1987), and the problem is most evident in young growing sheep (Barton *et al.* 1994).

Staple strength is most commonly expressed as the force required to break the staple divided by the average linear density or thickness of the whole staple. Several fibre and staple characteristics are known to contribute to phenotypic differences in staple strength, such as the variation in diameter along fibres (Bigham *et al.* 1983; Hansford and Kennedy 1990*a*; Orwin *et al.* 1988; Gourdie 1989; Denney 1990; Bray *et al.* 1993), the intrinsic strength of individual fibres (Hunter *et al.* 1983, 1990; Gourdie *et al.* 1992), the variation in length and extensibility between fibres (de Jong *et al.* 1985; Postle *et al.* 1988), and the proportion of discontinuous or shed fibres in the staple cross-section (Schlink *et al.* 1992). Moreover, the relative importance of these components of staple strength is likely to vary depending on complex, but poorly defined, interactions between sheep genotype and a number of nutritional, physiological, and environmental factors (reviewed by Reis 1992 and Hynd and Schlink 1993).

Clearly then, to improve the competitiveness of wool in the textile industry, it is critical that the factors which contribute to tender wool under different circumstances be identified, so that its incidence can be reduced by appropriate management and genetic strategies. To this end, the

[1] This review only includes relevant material published prior to the completion of the experimental work reported in this thesis (December 30, 1995).

initial purpose of the present study was to identify the causes for differences in staple strength between young Merino sheep specifically bred for sound and tender wool and managed under a range of nutritional conditions. Special attention was given to the role of intrinsic fibre strength (force to break/unit cross-sectional area at point of break), because there have been no systematic investigations which have attempted to establish the association between the intrinsic strength of individual fibres and staple strength in Merino sheep of known genetic and nutritional history. The second major objective was to determine the physico-chemical basis for differences in intrinsic strength between different sheep and between individual fibres. The following review of the literature provides background leading to the development of each of hypothesis tested in the experiments reported in subsequent chapters.

1.2. Measurement of staple strength, and its effects on wool processing and marketing

1.2.1. Measurement of staple strength

Staple strength has traditionally been assessed using a subjective method (Roberts *et al.* 1960; Rottenbury 1979). However, staple strength of most wools sold at auction in Australia is now measured objectively using a CSIRO Staple Length and Strength Measurement System (ATLAS) Staple strength is measured under standard conditions (20°C, 65% RH) by determining the *peak force* (Newtons (N); where 1N = 101.9 g) required to break a staple (Fig. 1.1.), normalised for its *average* linear density (ktex); where a ktex is equivalent to 1 g of clean dry wool top per meter of length. Average linear density is estimated from the length of the staple and the calculated clean weight of the broken staple. However, because clean staple weight is estimated from the weight of the broken greasy staple and the washing yield of a bulk wool sample, rather than the yield of the broken staple itself, the staple strength calculated by the ATLAS is corrected according to an adaptation of the regression equation from Kavanagh and Bow (1985). The formula to calculate staple strength (SS) expressed as peak force to break is:

SS (N/ktex) = [peak force (N) to break x staple length (mm) x 100] / [greasy staple weight (g) x yield (%) x 0.71 + 23.42]

Staple strength can also be expressed as the total work (Joules; J) required to break a staple, which is the *area* under the load-extension curve shown in Fig. 1.1, normalised for its *average* linear density (Hunter *et al.* 1983; Gourdie 1989; Gourdie *et al.* 1992; Scobie *et al.* 1994). The linear density of the staple tested is generally estimated from the known gauge length and the actual

weight of clean wool between the jaws. Staple strength (SS) expressed as total work to break is calculated as follows:

SS (J/ktex) = [total work (J) to break x gauge length (mm)] / [clean staple weight (g)]

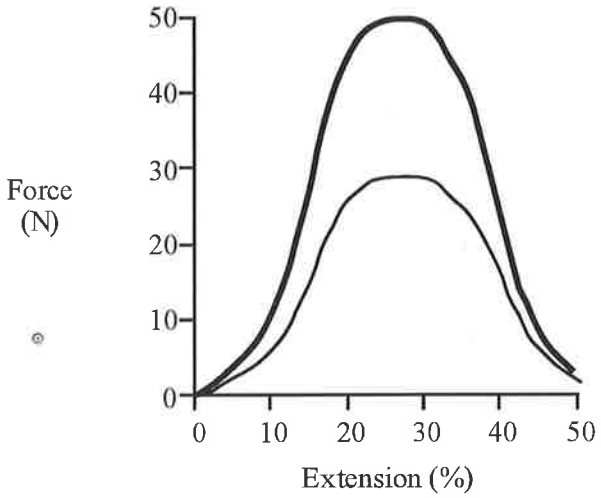

Figure 1.1. Staple force-extension curve for sound (▬▬) and tender (——) wool (adapted from Ross 1985).

As a staple is extended the load borne by the individual fibres increases to a maximum when most fibres are taking the strain, and then declines as fibres begin to break (Fig. 1.1). The precise shape of the curve, and therefore staple strength, is influenced by (*i*) the variation in diameter along the fibres; (*ii*) the average force required to break the fibres within the staple, which depends on their cross-sectional area and intrinsic strength at the point of break; (*iii*) the variability in crimped length and extensibility of fibres within the staple; and (*iv*) the test conditions used (gauge length, extension rate, RH and temperature) (Postle *et al.* 1988). The relative significance of these individual fibre and staple characteristics and test conditions depends on how staple strength is measured and expressed. Work to break a staple is theoretically independent of the variation in length and extensibility *between* fibres within a staple (Caffin 1983; de Jong *et al.* 1985), and for this reason, it has been suggested to be a theoretically more accurate index of the tensile strength of wool fibres than peak force to break. However in Merino wools, peak force is a more accurate predictor of mean fibre length after carding (hauteur), and also a less variable estimate of staple strength, than total work to break (de Jong *et al.* 1985).

Nevertheless, both of the current procedures for measuring staple strength do not identify the specific characteristics of the fibres that determine the tensile properties of the staple. In other

words, wools of similar staple strength may be so for different reasons. The problem is caused by the linear density measurement, which because it is an average value for the staple rather than the minimum, the estimated staple strength is thus a minimum rather than some average across the staple. An alternative method which has been used is to measure the minimum linear density along the staple prior to it being broken, and assume that on average fibres within the staple will break at this point (Baumann 1981). This measurement is referred to as staple tenacity, but it is not known whether it is a better indicator of processing performance of Merino wool than staple strength.

1.2.2. Staple strength and wool processing performance

There is a close association between the characteristics of raw wool and its processing properties, and price relativities should reflect either directly or indirectly the significance of specific fibre characteristics to the processing potential and quality of the yarn and fabric produced (Whiteley and Rottenbury 1990). The value of different wools ($/kg clean) at auction is strongly related to the predicted quantity and quality of the top which can be made from them (Ainsworth 1987). The quantity of top produced is determined by the romaine, which is the amount of wool wasted during the carding and combing processes. The short fibres removed during carding and combing are valued at about 40% of that for the top (Couchman *et al.* 1993). The quality of the top is determined primarily by its mean fibre diameter and hauteur, and to a lesser extent by the coefficient of variation of hauteur (Couchman *et al.* 1993).

The strength of raw wool fed into the card is the second most important fibre characteristic influencing mean hauteur, after staple length (Douglas 1988; Mahar 1990) (Table 1.1). There is a strong positive linear relationship between staple strength and hauteur when staple strength ranges between 20 and 50 N/ktex (Andrews 1979; Rottenbury *et al.* 1986; Plate *et al.* 1987). However, the relationship is influenced by the position of break in the staple (Plate *et al.* 1987). With a break near the tip or base of the staple (i.e. less than 45% middle-breaks), as in the case with most autumn shorn wools, a low staple strength has only a small effect on hauteur, but a significant influence on romaine. When the break is near the middle of the staple, as for most spring shorn wools, a low staple strength has a more significant bearing on hauteur.

Table 1.1. Relative importance of raw wool characteristics for the prediction of mean hauteur (after Douglas 1988).

Raw Wool Characteristic	Relative importance
Staple length (mm)	100
Staple strength (N/ktex)	88
Fibre diameter (μm)	46
Middle breaks (%)	40
Vegetable matter (%)	19

Hauteur (mm) can be predicted using the TEAM equation (r^2 = 0.84; Couchman *et al.* 1993):

$$\text{Hauteur} = (0.52\ SL) + (0.47\ SS) + (0.95\ FD) - (0.19\ M^*) - (0.45\ VM) - 3.5$$

where SL = staple length (mm); SS = staple strength (N/ktex); FD = mean fibre diameter (μm); M^* = adjusted percentage of middle breaks (%). All values of M up to 45% are replaced by a value of 45% as M^*, where as for values of M greater than 45%, the measured value itself if used as M^*; and VM is vegetable matter base (%). These same raw wool characteristics can also be used to predict the coefficient of variation of hauteur and romaine, although the relative importance of each variable is quite different. Staple strength and the percentage of middle breaks are the most important variables influencing the coefficient of variation of hauteur (Douglas 1988). Tops produced from high staple strength wool have a more uniform fibre length distribution than tops made from tender wool (Goddard *et al.* 1975).

$$\text{CV hauteur (\%)} = (0.12\ SL) - (0.41\ SS) - (0.35\ FD) + (0.20\ M^*) + 49.3$$

Vegetable matter content is the most important variable which determines the romaine (%), although staple length, staple strength and mean fibre diameter are also important (r^2 = 0.76; Douglas 1988).

$$\text{Romaine} = -(0.11\ SL) - (0.14\ SS) - (0.35\ FD) + (0.94\ VM) + 27.7$$

The extent of fibre breakage during carding is largely responsible for the effects that staple strength has on hauteur and romaine. While scouring of greasy wool has no significant direct effects on hauteur, the extent of entanglement induced in the scour is the cause of much of the

ensuing fibre breakage (Atkinson *et al.* 1986). About 75% of total fibre breakage occurs during carding (Bownass 1984; Harrowfield *et al.* 1986*a*), and the extent of fibre breakage associated with dis-entanglement and alignment of scoured wool is dependent on: (*i*) severity of the entanglement; (*ii*) fibre-fibre friction; (*iii*) fibre-metal friction; (*iv*) how and where the fibres are pulled; and (*v*) single fibre properties (Harrowfield *et al.* 1986*a*). Increased fibre breakage also decreases top quality due to increased nep formation (Harrowfield *et al.* 1986*b*).

The causes and effects of fibre-fibre and fibre-metal frictional forces are not dealt with in this review, except to say that they are largely dependent on the productivity rate of the card, which determines the pressure on the fibres in the various working zones of the card, and are reduced to some extent by the use of lubricants (Harrowfield *et al.* 1986*a*). The mechanical properties of single fibres such as strength, extensibility and the ability to recover following extension must also affect the extent of fibre breakage during carding, although few attempts have been made to quantify such relationships. While stronger fibres are less likely to break in the card, it is not known if this is entirely due to differences in the amount of material available to bear the load, or whether differences in intrinsic strength are also involved. The mechanical properties of wool fibres are strongly influenced by regain, and there is a trade off between fibre strength and both extensibility and ability to recover from extension (Harrowfield *et al.* 1986*a*); when the RH is raised from 60 to 100%, extensibility increases from about 40 to 60% and recoverability increases 2.5-fold, but strength decreases by about 30%. This large decrease in strength is obviously disadvantageous to carding, and therefore it must counteract any benefits that might arise from the increase in extensibility At present, the optimum regain for minimal fibre breakage during carding is considered to be about 15% (65% RH; 20°C).

Hauteur affects yarn strength and appearance, as well as having an influence on spindle speed and the incidence of yarn breakages ('ends down') during spinning (Plate *et al.* 1987). Fibre breakage during processing from top to yarn can be as high as 15-20% (Goddard *et. al.* 1975), and the effects of hauteur on fibre breakage and yarn properties are more significant at higher spinning speeds (Plate *et al.* 1987). Although high staple strength wools can be spun at a higher speed and with less 'ends-down' (Bastawisy *et al.* 1961), it has not been established whether these effects are due directly to differences in staple strength, or indirectly via effects on hauteur (Rottenbury *et al.* 1986; Plate *et al.* 1987). Similarly, while any effects of staple strength on spinning performance must be attributed to single fibre characteristics, since the structure of the

staple is completely destroyed during scouring and carding, the relative affects of fibre diameter variability, intrinsic strength or other single fibre properties, like extensibility, are not known.

1.2.3. Staple strength and wool market discounts

From the previous section it is apparent that several fibre and staple characteristics affect the quantity and quality of top produced from Merino fleece wool. In recent years the most important fibre characteristics which determine the price received at auction have been mean fibre diameter and staple strength. On average, these characteristics accounted for 48% and 21%, respectively, of the explained variation in the price received for wools sold in Australia during the second half of 1991/92 (Couchman *et al.* 1993) (Fig. 1.2).

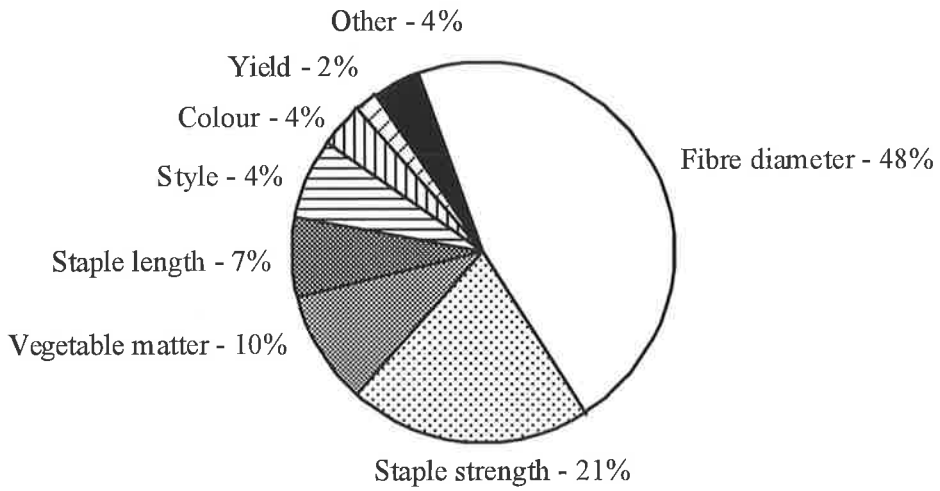

Figure 1.2. Relative contribution of raw wool attributes to prices paid at auction (from Couchman *et al.* 1993).

A significant proportion of the Australian wool clip is tender, especially in the southern states, and the price penalties at auction for tender wool can be substantial. Approximately 25% of all wool produced in Australia during 1991/92 was tender (<30 N/ktex), and about 37% of the wool produced in Western Australia was tender (Australian Farm Journal 1992). The average price discounts for tender Merino fleece wools (20.6 to 22.5 μm; best top making style) that prevailed in the market during the 1991/92 wool growing season were: 4.6% if part tender (26 to 30 N/ktex), 12.7% if tender (14 to 26 N/ktex), and 18.5% if rotten (<14 N/ktex). It is estimated that an additional $40 million per year could be achieved if tender wool was avoided in Western Australia, and that tender wool may cost the Australian wool industry more than $100 million per year.

1.3. Wool follicle structure and fibre growth

Wool follicles in sheep skin are described as either primary or secondary follicles based on their order of initiation and development in foetal skin, and are distinguished histologically on the basis of their associated accessory structures (Auber 1950; Ryder and Stephenson 1968; Montagna and Parakkal 1974; Chapman and Ward 1979; Orwin 1979, 1989; Chapman 1986; Black 1987) (Fig. 1.3). Primary follicles have a sweat gland and a sebaceous gland, and attached to the mid-follicle wall is the *arrector pili* muscle. Secondary follicles may have a sebaceous gland, but no sweat gland or *arrector pili* muscle.

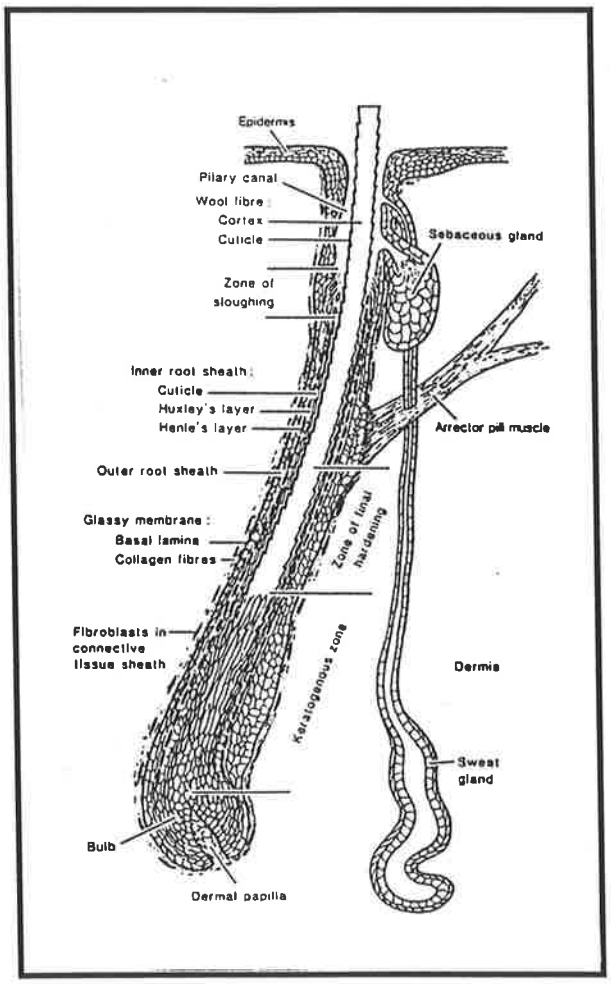

Figure 1.3. Diagrammatic representation of a longitudinal section of a fully developed, active primary follicle, showing the anatomy of the follicle and its functional zones (after Chapman and Ward 1979).

The follicle consists of several cylindrical and concentric cell layers. Surrounding the growing fibre is the inner root sheath, which consists of three layers of cells; inner root sheath cuticle, Huxley's layer and Henle's layer. The inner root sheath is separated from the outer root sheath by

a single layer of companion cells. Surrounding the outer root sheath is 20 nm single layered glassy membrane, and a connective tissue sheath, both of dermal origin. At the base of each follicle there is a bulb enclosing a dermal papilla formed from specialised mesenchymal cells which rarely divide. The dermal papilla has a central function in follicle development and maintenance of fibre growth (Jahoda and Oliver 1990).

Follicles are dynamic organs in which the proliferation, differentiation and migration of cells occur rapidly and simultaneously with the biosynthesis and hardening of the wool fibre and inner root sheath (Chapman 1986). Stem cells originating from epithelium derived cells from the bulb divide rapidly; approximately 20-50 new cells per follicle are produced per hour (Short *et al.* 1965; Fraser 1965; Hynd 1989). In response to the exfoliative effect of new divisions, the new cells are pushed up the follicle shaft as the fibre is formed. Evidence suggests that mitotic activity ends at a height of one cell layer above the apex of the papilla (Fraser 1964, 1965; Short *et al.* 1965; Downes *et al.* 1966), and that all cells in the bulb are capable of division (Wilson and Short 1979; Williams and Winston 1987; Hynd 1989).

Dividing stem cells form up to 10 different cell types, and only a small proportion (9-20%) differentiate into cells forming the fibre itself. The remainder of the cells either stay in the follicle bulb and continue to divide, are resorbed, or differentiate into inner root sheath or the proportion of the outer root sheath which surrounds the follicle bulb. Most evidence suggests that the location of the stem cells with respect to the dermal papilla regulates the fate of the differentiating cell streams (Auber 1950; Epstein and Maibach 1969; Nagorcka and Mooney 1982; Orwin 1989). Each cell line undergoes its own specific differentiation processes, and these have been extensively reviewed (Auber 1950; Rogers 1959*a,b*; 1964; Gemmell and Chapman 1971; Chapman 1971; Orwin 1971, 1989; Chapman *et al.* 1980). Differentiation of cortical cells is the only cell type that will be discussed in more detail in this review.

1.4. Structure and chemical composition of the wool fibre

1.4.1 Cell types, arrangement and ultrastructure

A schematic diagram of a wool fibre showing the different constituent cell types and their internal structure is given in Fig. 1.4. The cuticle forms the outermost layer surrounding the length of the fibre, and consists of one or two layers of flattened cells, approximately 0.3 to 0.5 μm thick, 20 μm long and 30 μm wide (Bradbury and Leeder 1970; Bradbury 1973). These cells overlap each

other both around and along the fibre to produce a ratchet-like profile with the scales pointing towards the tip of the fibre. The cuticle cells consist of three layers: (*i*) epicuticle, a chemically resistant membrane (3 nm); (*ii*) exocuticle, a keratinous layer rich in cystine and representing about two-thirds of the cell; and (*iii*) endocuticle, an inner non-keratinous layer with a low cystine content (Lindberg *et al.* 1949; Rogers 1959*a,b*; Bradbury *et al.* 1968). The cuticle contains few filaments and is rich in proteins that are similar to, but distinct from, the ultra-high sulphur (UHS) proteins of the cortex (Ley and Crewther 1980; Marshall and Ley 1986). Cuticle and cortical cells are bound together by a cell membrane complex about 25 nm thick (Birbeck and Mercer 1957; Rogers 1959*a,b*). The composition of the cell membrane complex is not known precisely, but there is evidence that its proteins are rich in cystine (Leeder 1986; Marshall *et al.* 1991). The integrity of the wool fibre depends on the cellular adhesion provided by this intercellular layer.

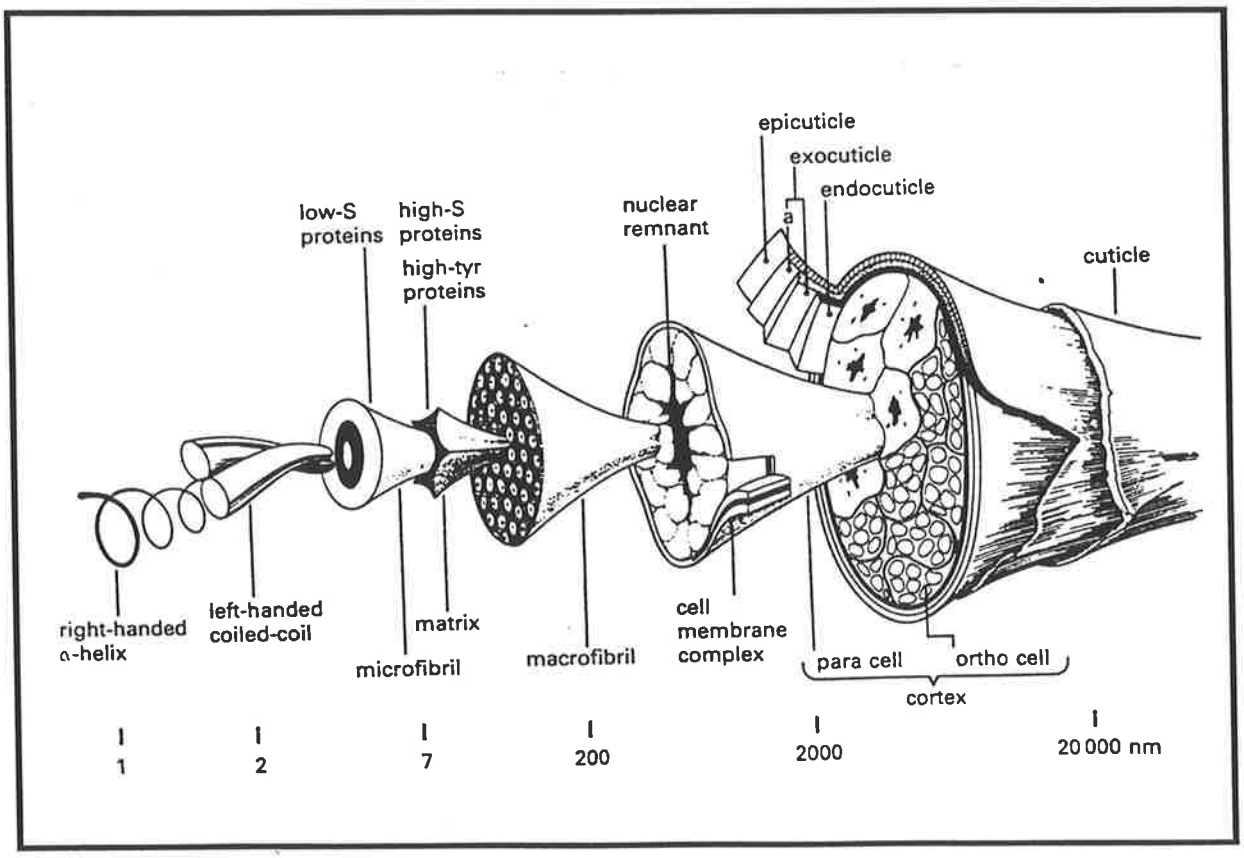

Figure 1.4. Schematic diagram of non-medullated Merino wool fibre showing the different cell types and their internal structure (from MacLaren and Milligan 1981).

The cortex accounts for up to 90% of the wool fibre cellular mass (Mercer 1953; Bradbury 1973). Cortical cells are spindle shaped (length 85-125 μm, width 4.5 to 6.0 μm; Bradbury 1973; Hynd 1989), with their longitudinal axes parallel to that of the fibre. In cross-section the cells are polygonal towards the centre of the cortex, but appear to be flattened adjacent to the cuticle

(Short *et al.* 1965). The ends of the cells are tapered and have several conical processes which interdigitate with similar processes on adjacent cells (Rogers 1959*a,b*). About 90% of the cortical cells consist of filamentous bundles, called macrofibrils. The remaining intra-macrofibrillar material which consists of degraded nuclei and organelles is referred to as nuclear remnants (Bradbury 1973; Orwin and Thompson 1976). The macrofibrils are orientated parallel to the fibre axis and consist of fibrous protein filaments called microfibrils, embedded in a non-filamentous matrix. Microfibrils are considered to traverse the entire length of the cortical cells.

The cortex consists primarily of two different types of cells referred to as orthocortex and paracortex, although some intermediate-type mesocortical cells are sometimes present (Whiteley and Kaplin 1977). Generally, cells of a particular type are clustered into strands running parallel through the cortex. The different cell types can be distinguished by the arrangement and ultra-structure of their macrofibrils, namely the proportion and packing arrangement of microfibrils (Horio and Kondo 1953; Mercer 1953; Fraser and Rogers 1953; Rogers 1959*a,b*; Bones and Sikorski 1967; Dobb 1970; Kaplin and Whiteley 1978) (Fig. 1.5). The microfibrils in the orthocortical cells, which are inclined to the cell axis entwined in a rope-like fashion to produce a whorl-like pattern, are within relatively small well-defined macrofibrils. In mesocortical cells the microfibrillar packing is hexagonal or near-hexagonal, while in paracortical cells the hexagonal arrangement is less regular. The macrofibrils become progressively less well defined in mesocortical cells and are extensively fused in paracortical cells (Rogers 1959*a,b*; Kaplin and Whiteley 1970; Whiteley and Kaplin 1977; Orwin 1979; Orwin *et al.* 1984; Powell and Rogers 1986; Chapman 1990). The average distance between microfibrils is greater in paracortex than orthocortex (Leach *et al.* 1964; Dobb 1970; Dobb and Sikorski 1971). Leach *et al.* (1964) estimated the volumes occupied by microfibrils and matrix by electron microscopy, and reported microfibril/matrix ratios of 0.45 and 0.74 in para- and orthocortex, respectively. Similar values were estimated by Dobb (1970) using electron diffraction (0.33 to 0.48 in paracortex and 0.67 to 0.70 in orthocortex), and Powell and Rogers (1986) reported that the microfibril/matrix ratio was 0.30 in paracortex and 0.60 in orthocortex. The variation in microfibril arrangement between the different cortical cell types undoubtedly reflects the different proportions of microfibrils and matrix, although the actual molecular basis for the differences in packing arrangements is not known.

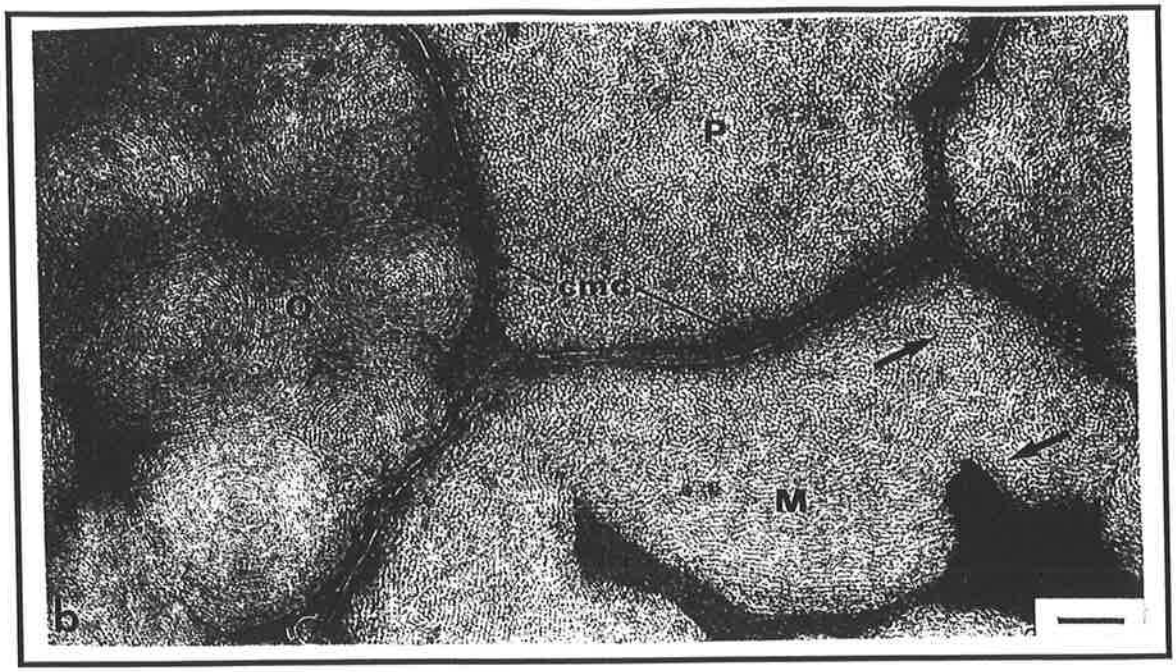

Figure 1.5. Electron micrograph at high magnification of a Romney wool fibre cross-section (OsO_4/Ur-Pb stained) showing different macrofibril definition and microfibril packing arrangements in paracortical (P), mesocortical (M) and orthocortical cells (O). The characteristic whorl-like arrangement of ortho-cortical microfibrils, the random microfibril packing in paracortical cells, and an area of hexagonally packed microfibrils (arrows) are apparent in meso-cortical cells. Scale marker indicates 100 nm (from Marshall *et al.* 1991).

1.4.2. The composition of keratin proteins

Keratin proteins are the main structural proteins of wool fibre cortical cells. There exists a tremendous heterogeneity of proteins and biochemical analyses have established the existence of three major classes: (*i*) low sulphur (LS) proteins, with sulphur contents less than the original wool (1.5-2.0%); (*ii*) high sulphur (HS) proteins, with sulphur contents greater than the original wool (4.0-6.0% S), and; (*iii*) high glycine-tyrosine (HGT) proteins, rich in glycine and tyrosine. Each class of proteins consists of a number of families of characteristic and unique amino acid composition (Table 1.2); reviewed by Fraser *et al.* (1972), Bradbury (1973), Lindley (1977), Crewther *et al.* (1980) and Gillespie (1991).

Table 1.2. Amino acid composition (residues %) of wool and fractionated low sulphur (LS), high sulphur (HS), ultra-high sulphur (UHS) and high glycine-tyrosine type I (HGT_1) and II (HGT_2) proteins.

Amino acid	Wool[a]	Protein family				
		LS[a]	HS[a]	UHS[b]	HGT_1[c]	HGT_2[c]
Lysine	2.7	4.1	0.6	0.9	0.4	0.3
Histidine	0.8	0.6	0.7	1.3	1.1	0.2
Arginine	6.2	7.9	6.2	6.9	5.4	3.8
Aspartic acid	5.9	9.6	2.3	0.6	3.3	2.9
Threonine	6.5	4.8	10.2	11.1	3.3	2.9
Serine	10.8	8.1	13.2	12.7	11.9	5.3
Glutamic acid	11.1	16.9	7.9	7.9	0.6	0.7
Proline	6.6	3.3	12.6	12.8	5.3	3.9
Glycine	8.6	5.2	6.2	4.2	27.9	41.1
Alanine	5.2	7.7	2.9	2.0	1.5	0.8
Half-cystine	13.1	6.0[d]	22.1[d]	29.9[d]	6.0[d]	9.1[d]
Valine	5.7	6.4	5.3	4.3	2.1	0.9
Methionine	0.5	0.6	0.0	0.0	0.0	0.0
Isoleucine	3.0	3.8	2.6	1.7	0.2	0.1
Leucine	7.2	10.2	3.4	1.3	5.5	5.3
Tyrosine	3.8	2.7	2.1	1.9	15.1	18.9
Phenylalanine	2.5	2.0	1.6	0.5	10.4	5.0

[a] Gillespie and Marshall (1980); [b] Gillespie (1991); [c] Marshall *et al.* (1980); [d] Measured as S-carboxymethyl-cysteine

The electrophoretic techniques used to fractionate the proteins in wool have been comprehensively reviewed by Gillespie (1991). The proteins are commonly extracted using reducing agents such as dithiothreitol at pH 8.9 in the presence of high concentrations of urea, and then alkylated using iodoacetic acid to prevent re-oxidation of the unstable cysteine residues and non-specific chemical modifications to the extracted polypeptides. The S-carboxymethylated derivatives are then separated using either: (*i*) one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), where separation of proteins is on the basis of

MW; or (*ii*) two-dimensional electrophoresis, using alkaline PAGE to separate on the basis of charge density in first dimension, and SDS-PAGE to separate on the basis of MW in the second dimension. While no one electrophoretic system separates all the proteins in wool, because of the small differences in size and charge between proteins within a protein family, an appreciation of the complexity can be gained from the 2D-SDS-PAGE pattern of the wool keratin proteins shown in Fig. 1.6. Protein extracts can also be fractionated if required into LS, HS and HGT classes to facilitate closer examination of the proteins within a group.

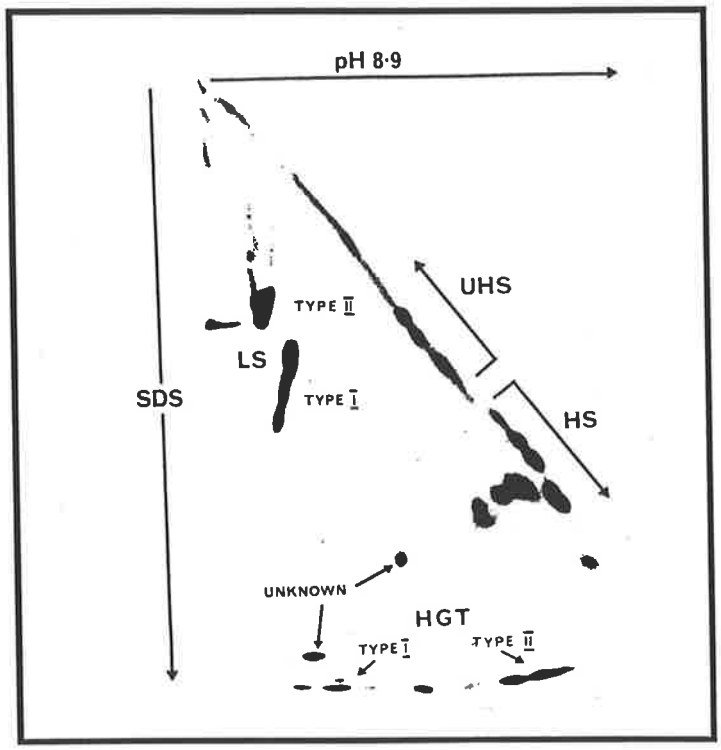

Figure 1.6. Two-dimensional polyacrylamide gel pattern of the wool keratin proteins. The proteins were S-carboxymethylated with [^{14}C]-iodoacetic acid and then separated at pH 8.9 in the presence of 8 M urea in the first dimension and in presence of SDS in the other. The location of the different protein families are shown; low sulphur (LS type I and II), high sulphur (HS), ultra high-sulphur (UHS) on the diagonal, and high glycine-tyrosine (HGT type I and II). Protein positions are revealed by autoradiography (from Powell and Rogers 1986).

1.4.2.1. Low sulphur proteins

The LS proteins belong to the intermediate filament (IF) super family of proteins (Weber and Geisler 1982), and the fine structure of these proteins is shown in Fig. 1.7. The monomeric IF subunits consist of a central α-helical rod domain flanked by non-helical C- and N- regions of

variable size and chemical composition (Steinert and Parry 1985; Steinert *et al.* 1985; Conway *et al.* 1989; Powell and Rogers 1990*a*). Intermediate filament proteins are richest in amino acids which favour α-helical formation; lysine, aspartic acid, glutamic acid and leucine (Table 1.2). The central rod domains, which have an overall length of about 47 nm, are approximately 90% α-helical (308 to 315 residues), and consist of four different size α-helical segments composed of a heptad repeat of the kind $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g)_n$. More than 75% of the *a* and *d* positions are occupied by hydrophobic residues. This heptad sequence favours the formation of a coiled-coil rope structure, in which two right handed-α-helices wind around a common axis in a left-hand manner. The α-helical segments are separated by short non-helical linkers.

Despite their highly conserved structure, the exact sequence of the heptads in the segments differ and permit classification of eight major IF proteins into two subfamilies (Type I, components 8*a*, 8*b*, 8*c*-1, 8*c*-2, and; Type II, components 5, 7*a*, 7*b*, 7*c*) (Marshall and Gillespie 1977; Crewther *et al.* 1980; Powell and Rogers 1986; Conway *et al.* 1989). The Type I IF keratins are generally smaller [MW 45 - 50 kDa (392-416 amino acids) *vs.* 57 - 58 kDa (479-506 amino acids)], and more acidic (isoelectric point; pH 4.5 - 5.5 *vs.* 6.5 - 7.5) than Type II IF keratins (Woods 1979; Lynch *et al.* 1986; Steinert and Roop 1988; Albers and Fuchs 1992; Powell and Rogers 1994*a*). Each type of rod domain is coupled with a specific set of end domains, and a feature of these non-helical "tails" in wool, is that they contain many cysteine residues. Based on known protein sequences, the distribution of cystine residues in IF molecules may be about 3% of the residues in the rod domain, 11% in the N-terminal domain and 17% in the C-terminal domain (Fraser *et al.* 1988). The richness of cystines in the terminal regions of the IF keratins suggest that these residues are involved in disulphide bond formation with other IF proteins and the cysteine-rich proteins of the surrounding matrix.

1.4.2.2. High sulphur proteins

The HS proteins of wool can be resolved into 60-70 minor components which can be grouped into at least four major sub-families (*B2; BIIIA and BIIIB, and UHS*) with MW between 10 and 30 kDa (Crewther 1976; Gillespie 1991). There appears to be considerable intra-family heterogeneity involving loss of segments and point mutations (Swart *et al.* 1976). The cysteine content of HS proteins in wool varies from 17 to 23%, and they are also rich in proline, threonine and serine (Crewther 1976; Marshall and Gillespie 1977; Gillespie 1991; Marshall *et al.* 1991).

The UHS protein family within this group have a MW range from 16 to 28 kDa and about 30% of their amino acid residues as half cystine (Gillespie and Broad 1972; Marshall *et al.* 1991).

The little direct evidence for the structure of the HS proteins suggests that they have a highly convoluted globular form with an average diameter of 2.1 nm (Gillespie 1991). All the components have half-cystine as the C-terminal residue, and except for a few proteins with MW around 16 kDa, have a blocked residue of N-acetyl-alanine (Marshall *et al.* 1991). Repeating units, in which cysteine residues occur as pairs, can be found in some HS proteins (Powell and Rogers 1994*a*). However, there is little sequence homology between the families, with the exception that the 16 and 19 kDa MW families contain a repetitive pentapeptide (Marshall *et al.* 1991). In the major wool protein of the 19000 MW family, about 85% of the molecule is constructed of this repeating sequence (Swart *et al.* 1976). Pentapeptide repeats also occupy 25-35% of the peptide chains of several other HS proteins (Powell and Rogers 1986).

1.4.2.3. High glycine-tyrosine proteins

The HGT proteins, of which over 30 have been identified so far, have MW between 6 and 9 kDa. These proteins are especially rich in glycine (up to 41 residues %), tyrosine (up to 19 residues %) and serine (up to 12 residues %), but contain no methionine, glutamic acid, alanine, valine or isoleucine, and are relatively deficient in lysine and histidine (Table 1.2). They can be divided into two groups (Type I and II) based on their solubility and cysteine to phenylalanine ratio (Gillespie and Frenkel 1976; Marshall *et al.* 1980; Gillespie 1991). The Type I fraction is less soluble and comparatively poor in cysteine (6 *vs.* 9 residues %) but richer in phenylalanine (10 *vs.* 5 residues %) than the Type II fraction. Both classes are heterogeneous, but only the Type I class has been examined in detail. Within this class at least 30 components have been identified with the content of tyrosine ranging from 7 to 20 residues % (Marshall *et al.* 1991). The amino acid sequences of the two Type I class proteins which have been determined show only two small blocks of homology being separated by a region of random sequence (Marshall *et al.* 1991).

1.4.3 . Morphological location of keratin proteins

There is considerable electron microscopic and biochemical evidence which suggests that the LS proteins are the main structural component of the microfibrils (Jones 1976; Crewther 1976; Crewther *et al.* 1976, 1980), and the HS proteins are associated with the matrix (Birbeck and Mercer 1957; Fraser *et al.* 1972; Crewther 1976; Swart *et al.* 1976; Lindley 1977; Powell and

Rogers 1986; Gillespie 1991). Jones (1976) isolated microfibrils and showed by amino acid analysis and gel electrophoresis that they consisted largely, if not entirely of LS proteins, and Fraser *et al.* (1959) showed that the distance between microfibrils, obtained from low angle X-ray equatorial diffraction patterns, increased with increasing cystine content. It has been suggested that a large portion of the non-helical "tails" of the LS proteins project out from the 8-9 nm diameter microfibril core (Steinert and Roop 1988), and indeed it is known from the volume of the LS proteins, that they must, at least in part, occupy space in the matrix. The location of the HGT proteins has been the subject of controversy. However, these proteins have not been found in the cuticle (Bradbury 1973; Marshall and Ley 1986), isolated IFs (Jones 1976) or the cell membrane complex (Bradbury 1973). Moreover, to accommodate their volume which can be as high as 14% in wool, and given the high correlation between the inter-microfibril volume fraction and the mass fraction of the HS and HGT proteins (Fraser and MacRae 1973), the HGT proteins must form part of the matrix. The matrix proteins are known as the intermediate filament-associated proteins (IFAP's).

Given the morphological location of the keratin proteins, and the ultra-structure of the various cortical cell types, orthocortical cells should contain more LS and less HS and HGT proteins than paracortical cells. Amino acid or protein analysis of separated orthocortical and paracortical cells (Fraser *et al.* 1972; Bradbury 1973; Dowling *et al.* 1990), and electron microscopic information (Jones *et al.* 1990), does in fact indicate that the matrix of paracortical cells are enriched with HS proteins. Furthermore, the differences in the sulphur content of the matrix between cortical cells may be due exclusively to the expression and production of UHS proteins in the paracortex matrix (Powell and Rogers 1994*a*). Similarly, the HGT proteins may be located specifically in the matrix of orthocortical cells (Bradbury 1973; Hewish and French 1986; Fratini *et al.* 1994).

1.4.4. Intermediate filament assembly and structure

Keratin IF are obligate heteropolymers, in that they require equivalent amounts of both Type I and Type II IF subunits for their coordinated assembly into filaments (Steinert *et al.* 1985; 1994; Powell and Rogers 1990*a*). IF assembly commences when one Type I and one Type II polypeptide chain line up parallel with their helical portions in register (Parry *et al.* 1987; Conway *et al.* 1989) (Fig. 1.7). Strong hydrophobic interactions between the apolar residues of the *a* and *d* positions of the repeating heptapeptides cause the formation of a coiled-coil rope. The next step in IF assembly involves the association of a pair of coiled-coiled ropes, or dimers, to

form tetramers composed of four IF polypeptide chains. Current evidence suggests that the coiled-coil ropes align in an antiparallel arrangement and a slightly staggered position (Parry *et al.* 1987; Conway *et al.* 1989; Coulombe and Fuchs 1990).

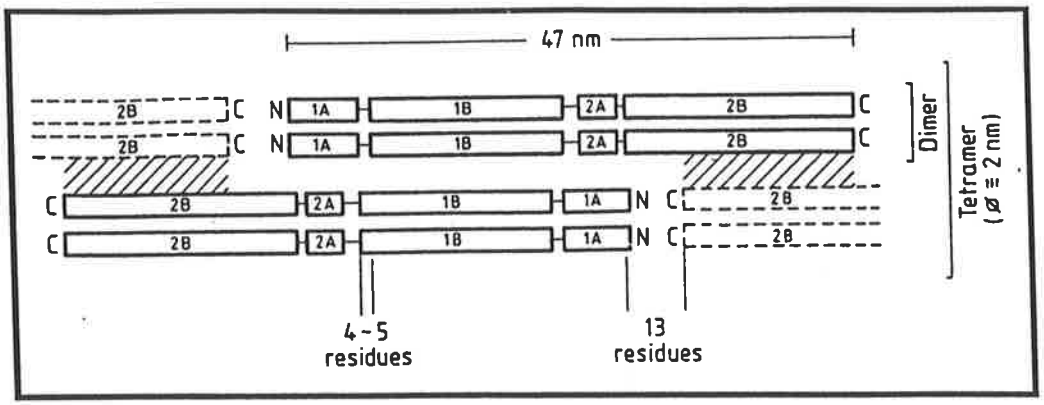

Figure 1.7. Schematic diagram of IF polypeptides (hatching defines segments having α-helical structure), and their arrangement into dimers and tetramers to form the protofilament. The area of interaction of dimers by means of sulphur bonds between 2B segments is shown (adapted from Sparrow *et al.* 1989, by Wortmann and Zahn 1994).

The four chain complexes form 3 nm wide protofilaments, which are largely stabilised by disulphide bonds between the 2B-segments of different dimers (Sparrow *et al.* 1989). An IF is produced when two protofilaments entwine around a common axis to form 4.5 nm protofibrils, and then four protofibrils combine to form the complete IF (Steinert *et al.* 1985; Steinert and Roop 1988). A complete IF therefore consists of 16 Type I and 16 Type II polypeptides.

1.4.5. Disulphide bonds and other crosslinking of keratin proteins

The crosslinking of wool has been reviewed by Ziegler (1977) and MacLaren and Milligan (1981). Wool "normally" has only 20-40 μmoles/g free thiol groups out of approximately 1000 μmoles/g half cysteine (Maclaren and Milligan 1981). This indicates that virtually all (>95%) the cysteine residues are involved in disulphide bonds, and the inertness and insolubility of wool implies that at least some of these disulphide bonds bridge half-cystine residues in different protein chains. Nevertheless, despite the obvious importance of these bonds to the mechanical properties of the wool fibre, it is surprising that relatively little is known about the precise disulphide bonding pattern, although indirect evidence would suggest they occur between neighbouring IF proteins, between IF-IFAP proteins, and also between IFAP proteins.

The large diametral swelling which results from immersion in formic acid (Feughelman 1968), is accompanied by longitudinal contraction up to an acid concentration at which the α-helical structure remains intact. This swelling behaviour is considered to result from the presence of longitudinally spaced linkages between neighbouring microfibrils (Feughelman 1979). In fact, IF-IF and or IF-IFAP disulphide bonds must be present since the IF molecule in wool may contain 55 cystine residues when the total number of unlinked cystine residues maybe only 9 (Fraser *et al.* 1988). Theoretical studies of the limitations imposed on IF structure by disulphide bond formation have shown that intra-chain and intra-rope disulphide bonds are not possible, but inter-rope disulphide bonds, especially between 2B segments of different tetramers, are likely (Fraser *et al.* 1988; Conway *et al.* 1989).

Very little change takes place in the fibre length (Bendit and Feughelman 1968) or microfibril length (MacArthur 1943) with increasing RH, whereas both fibre diameter and the inter-microfibrillar distance increase considerably (Fraser *et al.* 1972). This suggests that the uptake of water is confined primarily to the matrix, and indeed the volume swelling upon wetting is 11% in the microfibrils compared to 53% in the matrix (Fraser *et al.* 1971). This indicates that a high proportion of disulphide linkages must be between cystine residues which both reside in the matrix (i.e. intra-molecular). It has also been argued on theoretical mechanical grounds that at least 80% of the disulphides must be intra-chain, for otherwise the mechanical properties of the wool fibre would correspond to those of a very highly cross-linked non-swelling polymer. Furthermore, each IF molecule can only form 55 linkages whereas about 300 cystine residues per IF molecule are present in the matrix (Fraser *et al.* 1988), and intra-chain disulphide bonds have been detected in incompletely reduced HS proteins (Marshall and Gillespie 1978).

Although cystine residues are responsible for most of the covalent crosslinks in wool, other types of covalent crosslinking are present in small amounts. The isopeptide crosslink is a peptide bond joining the amino group of a lysine residue to the carboxyl group of a glutamic acid residue or, less commonly, to the ß-carboxyl group of an aspartic acid residue (Maclaren and Milligan 1981). Estimates of the amount of the glutamyl-lysine bonds in wool range from 3 to 15 μmol/g (Milligan *et al.* 1971). Tyrosine residues may also participate in the crosslinking of wool, but only in small amounts (2 to 3 μmol/g). Wool fibres are also stabilised by non-covalent bonds, which despite being individually much weaker than the covalent bonds, because of their large number contribute significantly to the mechanical properties of the fibre. The non-covalent bonds, which stabilise the conformation of the α-helical structure of the IF polypeptide chains, are

generally classified as: (i) *hydrogen bonds*; (ii) *hydrophobic bonds*; and (iii) *ionic bonds* or 'salt linkages' (Ziegler 1977).

1.4.6. Cortical cell differentiation and keratin gene expression

The growth of a wool fibre represents the culmination of a differentiation pathway that is accompanied by striking changes in cell morphology, proceeding from the polygonal stem cell in the follicle bulb to spindle-shaped cortical cells overlaid by flattened rectangular cuticle cells. At the cellular level, the formation of the wool fibre involves the expression of genes and synthesis of appropriate proteins, their assembly into the microfibril/matrix complex, their final hardening, and the binding of cells together. The transition from stem cell in the follicle bulb to differentiated cortical and cuticle cells in the fibre occurs in sheep in 2.5 to 4 days (Epstein and Maibach 1969; Chapman 1971; Chapman and Gemmell 1971). During this transition cell volume increases about 3-fold and dry mass increases about 13 fold (Short *et al.* 1965), illustrating the large filling by keratin proteins that occurs.

The events that lead to the terminally differentiated cells in the fully formed fibre have been comprehensively detailed (Bradbury 1973; Montagna and Parakkal 1974; Swift 1977; Orwin 1979, 1989; Powell and Rogers 1990*a*). As cells destined to form the fibre cortex move out of the mitotic zone, there is a sequential and spatial pattern in the synthesis of the keratin proteins which precedes their cross-linking (keratinisation), and the different cortical cell types exhibit distinct differences which are reflected in the time and location of gene activation and expression. Current ideas concerning the sequence of events in the development of the microfibril/matrix composite stem from the histological studies of Rogers (1964), Chapman and Gemmell (1971, 1973) and Orwin (1979), and from the biochemical studies of Downes *et al.* (1963), Wilson *et al.* (1971), Lynch *et al.* (1986) and Powell *et al.* (1991).

Early radio-isotope work of Downes *et al.* (1963), and electron microscopy of Mercer (1961) and Rogers (1964), suggested a two-stage synthesis in which the synthesis of the LS proteins of the microfibrils preceded that of the HS, UHS and HGT proteins of the matrix. This hypothesis was supported by Wilson *et al.* (1971) using radio-labelled methionine to specifically locate LS protein synthesis, but was subsequently modified to incorporate some overlap of the two stages; Chapman and Gemmell (1971) and Chapman (1976) suggested that while synthesis occurs sequentially in the paracortex, it occurs concurrently in orthocortical cells. Electron microscopic

examination of wool follicles revealed that synthesis of matrix commences in the paracortex in the suprabulbar region, approximately 50 μm distal to the level where microfibrils start to form. Above this level and throughout the keratogenous zone there is dual synthesis of microfibrils and matrix. Although the initial orientation of macrofibrils is random, as the macrofibrils increase in size they become orientated parallel to the direction of growth (by upper zone B) and fuse together. In contrast, the macrofibrils forming in the orthocortex do not display distinct microfibrils until a microfibril-matrix complex resolves about halfway up the keratogenous zone. Microfibrils and matrix form concurrently in the distal half of the keratogenous zone until the cells are replete with the microfibril/matrix complex at about two-fifths the distance up the follicle (Chapman 1990). Quantitative measurements indicate that the increase in keratin in the keratogenous zone is attributable to increases in the diameter of existing macrofibrils, rather than the initiation of new macrofibrils (Forslind and Swanbeck 1966).

A similar sequential synthesis has been described in the human hair follicle by Lynch *et al.* (1986), based on immuno-histochemical staining with a presumptive HS protein antibody. They found that co-ordinated synthesis of Type I and II LS proteins occurred 5-10 cell layers above the dermal papilla apex, in an area corresponding to the beginning of the "elongation zone" of the follicle. The HS proteins were first seen 20-30 cell layers above the dermal papilla apex, in an area corresponding to the upper elongation or lower keratogenous zone. *In situ* hybridisation experiments by Powell *et al.* (1991) have also confirmed this sequential and spatial pattern in the expression of the genes coding for the various keratin proteins (Fig. 1.8). They found that the IF keratin genes (Type I and II) are the first to be activated in the development of the wool fibre, and their expression occurs simultaneously in both ortho- and paracortical cells. Subsequently, the genes encoding the HGT keratin proteins are activated in the cells of one half of the cortex, followed a little later by the genes encoding the HS and UHS keratin proteins in the cells of the complementary half of the cortex. Higher up the follicle most cortical cells then produce both HGT and HS keratin proteins, but the expression of the cortical UHS keratin genes is restricted to one half of the wool cortex. The cells that initially show HS and UHS keratin gene expression, and to which the cortical UHS keratin gene expression is restricted, are paracortical cells.

Fraser and MacRae (1980) proposed that after the IFs are assembled, they are arranged in a lattice and spaced about 9 nm, centre to centre, by a bridging polypeptide which may be an IFAP or a projecting segment of the IF polypeptide chain. The hexagonal packing of IF suggests that there must be specific interactions between them (Gillespie 1991). Initially the space between the IF is

filled with an aqueous medium that is progressively replaced by IFAP; the composition and extent of replacement is variable depending on the nature of the keratin and the nutritional and physiological status of the animal.

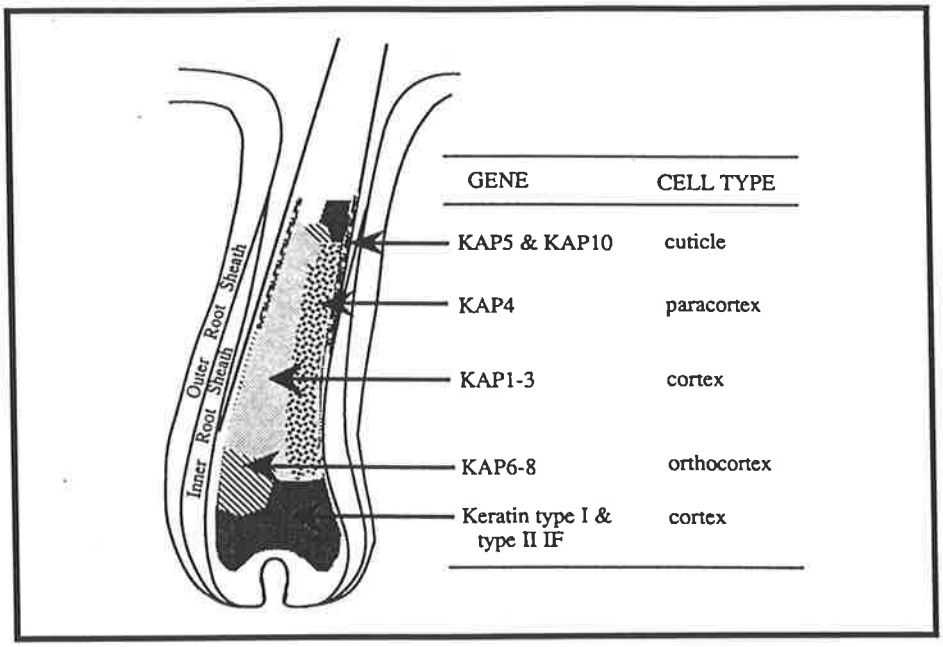

Figure 1.8. Expression of keratin genes in wool follicle differentiation. A schematic summary showing the sequential and spatial pattern in the expression of genes coding for low sulphur (LS), high glycine-tyrosine (HGT), high sulphur (HS) and ultra-high sulphur (UHS) proteins (from Powell *et al.* 1991)

1.5. Fibre and staple characteristics in relation to staple strength

1.5.1. Minimum fibre diameter and along-fibre variations in diameter

Individual wool fibres break under tension when the force at a point along their length produces a local extension which is greater than the breaking extension of the constituent materials of the fibre at that point. As the local extension produced when a fibre is extended longitudinally is likely to be greatest at the thinnest point, most fibres break in this region (Orwin *et al.* 1980, 1984; Fitzgerald *et al.* 1984; Gourdie 1989). Both minimum diameter along the fibre (Smuts *et al* 1981; Hunter *et al.* 1983; Orwin *et al.* 1985), or the actual cross-sectional area at the point of break (Woods *et al.* 1990; Gourdie 1989), are closely correlated with the force required to break individual fibres (r^2 = 0.88 to 0.96).

It is not surprising then, that on average, minimum fibre diameter measured along staples has been reported to account for about 50% (range 21 to 85%) of the phenotypic variations in staple strength between sheep (Bigham *et al.* 1983; Hunter *et al.* 1983; Fitzgerald *et al.* 1984; Orwin *et al.* 1985, 1988; Hawker and Littlejohn 1989; Denney 1990; Hansford and Kennedy 1990*a*; Bray *et al.* 1993). The rate of change in fibre diameter along staples has also been found to account for between 17 and 59% of the variation in staple strength between sheep (Hansford 1989; Hansford and Kennedy 1990*a*; K.M.S. Curtis and A.N. Thompson unpubl. data), and importantly, it explained a significant proportion of the variance in staple strength additional to that attributed to co-correlated changes in minimum fibre diameter. It is not known whether the effects of rate of change in fibre diameter on staple strength are simply due to changes in fibre geometry (i.e. average staple linear density), or if there are correlated changes in other fibre characteristics, such as intrinsic strength or extensibility (Collins and Chaikin 1968). Along-fibre changes in diameter are clearly important components of staple strength, and any factor(s) which increase the variation in wool growth and diameter throughout the year will depress staple strength. The relationships are generally weaker under grazing conditions where presumably the effects of the other components of staple strength become more important.

The variation between experiments in the strength of the relationship between changes in fibre diameter and staple strength, may in part, also be a reflection of the accuracy of the techniques used. With the exception of Hansford (1989) who measured fibre diameter at 2 mm intervals along the entire length of the staple, fibre diameter has generally been determined at intervals of 4 weeks or more from wool samples clipped from the mid side region or from dyebanded staples, and yet it is possible that fibre diameter can change rapidly over short lengths of fibre (James 1963). An additional problem is that all these authors have assumed that the staples broke where fibre diameter was lowest, and yet it has already been indicated that this may not always be the case. As the techniques used for measuring the properties of staples will be inherently less precise than those used for measuring single fibres, to accurately determine the factors which contribute to the strength of wool staples, it may be necessary to study the properties of the single wool fibres. Nevertheless, a large proportion of the between-sheep variance in staple strength can not be attributed to changes in fibre diameter, indicating the importance of the other fibre and staple characteristics.

1.5.2. Follicle shut-down and fibre shedding

Wool follicles in the skin of Merino sheep may temporarily become inactive and shed their fibre in response to the "stress" caused by a number of nutritional, physiological and environmental factors (Lang 1945; Lindner and Ferguson 1956; Lyne 1964; Chapman and Bassett 1970; Thwaites 1972; Wilson and Short 1979; Schlink *et al.* 1992). Fibre shedding in Merinos appears to occur independently of seasonal changes in photoperiod, and the sequence of events and associated changes in follicle morphology which precede shedding differ from those described in normally cycling hair and wool follicles (Ryder 1969, 1971, 1973; Chapman 1990; Nixon 1993). Whereas fibres shed during normal catagen are characterised by a capsule-enclosed "brush-end" (Straile *et al.* 1961), and their incidence in Merinos is very low (Ryder 1967), most fibres shed from Merino wool follicles are tapered and often enclosed by a sheath of inner root sheath cells, forming a "club-end" (Schlink *et al.* 1992). The significance of this phenomenon is that shed fibres may adversely influence staple strength. Schlink *et al.* (1992) reported that fibre shedding forms a major component ($r^2 = 0.65$) of staple strength in tender wool (<30 N/ktex), but when staple strength was greater than 30 N/ktex the incidence of shed fibres was on average about 5%. Presumably the effects of shed fibres on staple strength result because such fibres do not span the gauge length between the jaws of the staple breaker, thereby contributing to the tex value but not the force or work required to break the staple (assuming that there is no significant interaction between fibres).

Indications are that follicles producing fibres below 8-12 μm will cease production and shed the fibre (Lang 1945). While the relative sensitivities of sheep from different genotypes to severe environmental conditions causing fibre shedding have not been compared, this result suggests that sheep genotypes producing finer wool may be more susceptible to fibre shedding. Fine wool sheep also have a higher ratio of secondary to primary follicles, and Lyne (1964) reported that under nutritional stress 9.2% of secondary follicles showed evidence of shedding compared to only 1.4% of primaries. Sheep producing wool with a similar mean fibre diameter, but a wider distribution in diameter, may also be more susceptible to fibre shedding, because of the greater proportion of finer fibres. However, other evidence suggests that follicle shut-down in response to hormonal changes may occur without concomitant decreases in fibre diameter (Hynd 1994*c*). It is clear that the relative effects of fibre shedding on staple strength will depend strongly on its association with fibre diameter, however these associations have yet to be adequately determined.

It is expected that if fibre shedding results from nutritional intervention and is associated with a decrease in fibre diameter, then its effects on staple strength additional to those caused by other fibres fining down (but not being shed) will be minimal. If however the fibres are shed in response to a sudden environmental stress and without concomitant reductions in fibre diameter, then the relative effects of shedding on staple strength may be more significant.

1.5.3. Intrinsic fibre strength

Large variations in intrinsic strength have been observed between individual wool fibres from the same sheep, and between fibres from different sheep. Moreover, intrinsic strength, or the stress required to extend a fibre by a specified amount, has been reported to be independent of fibre diameter (Evans 1954; Dusenbury and Wakelin 1958; Burgmann 1959; Roberts *et al.* 1960; Rigby 1962; Campbell *et al.* 1972; Woods *et al.* 1990), to increase (Anderson and Cox 1950; Shah and Whiteley 1966; Collins and Chaikin 1968; Smuts *et al.* 1981) or decrease (Meybeck and Gianola 1955; Gourdie 1989; Gourdie *et al.* 1992) with increasing fibre diameter. O'Connell and Lundgren (1954) found that the sign of the relationship varied depending on sheep breed. Because of these inconsistencies regarding the relationship between fibre diameter and intrinsic strength, there remains considerable debate on whether intrinsic strength does actually vary between fibres and sheep, and if so, do the differences in intrinsic strength contribute to between-sheep differences in staple strength.

The variations between reports can in part be attributed to the inaccuracies of the techniques used to estimate cross-sectional area at the point of break along the fibre. These include: (i) measuring fibre diameter using a short gauge length vibrascope (Evans 1954; Dusenbury and Wakelin 1958; Thorsen 1958; Burgmann 1959; Roberts *et al.* 1960; James 1963), which fails to take account of changes in cross-sectional area over short lengths of fibre; (ii) estimating the point of lowest fibre diameter prior to breaking the fibre, when between 6 and 45% of fibres may not break in this region (Orwin *et al.* 1984; Fitzgerald *et al.* 1984; Gourdie 1989; Gourdie *et al.* 1992); and/or (iii) calculating linear density on the assumption that the fibre is a uniform cylinder (Anderson and Cox 1950; Evans 1954; Meybeck and Gianola 1955; Burgmann 1959; Roberts *et al.* 1960; Rigby 1962; Shah and Whiteley 1966; Collins and Chaikin 1968; Campbell *et al.* 1972; Hunter *et al.* 1983). The limited number of fibres *or* sheep tested may also contribute to the discrepancies between experiments. For example, Anderson and Cox (1950) tested 185 fibres from a single sheep, Burgmann (1959) tested 108 fibre sections from a total of 17 fibres from 8 sheep, Roberts

et al. (1960) tested 24 fibre segments from a total of 6 fibres from 2 sheep, and James (1963) tested 134 fibres from 30 sheep. The genetic and nutritional history of the samples are sometimes unknown or poorly defined (Smuts *et al.* 1981; Hunter *et al.* 1983), despite the fact that genotype and nutrition can have opposing effects on the relationships between fibre diameter and other physical or chemical fibre characteristics, which could be associated with intrinsic strength (Campbell *et al.* 1972, 1975; Smuts *et al.* 1981; Orwin *et al.* 1984; Hynd 1989).

Gourdie (1989), Woods *et al.* (1990) and Gourdie *et al.* (1992) overcame many of the above mentioned technical problems, and estimated the actual cross-sectional area at the position of break by viewing and measuring the area of the fractured ends of the fibres after they had been broken. These authors clearly established that the intrinsic strength of individual fibres from the same sheep vary several fold, and more importantly, that the average intrinsic strength of fibres from different sheep may differ by 30 to 40% (Gourdie 1989; Woods *et al.* 1990; Gourdie *et al.* 1992). These differences in intrinsic strength should theoretically influence the force to break individual wool fibres, and thus staple strength, but the evidence for such relationships is also contradictory. Orwin *et al.* (1980) suggested that differences in intrinsic fibre strength may account for the differences in staple strength between "sound" and "tender" wool, and significant phenotypic relationships between intrinsic strength and staple strength for Merino (Hunter *et al.* 1983) and Romney sheep (Gourdie *et al.* 1992) supported this contention. However, Hunter *et al.* (1990) found that while staple strength was influenced significantly by nutritional and physiological stress, intrinsic strength remained unchanged. Unfortunately these authors did not report individual sheep values for intrinsic strength or staple strength, but a significant relationship between them was unlikely. The precise reasons for the differences between these experiments are not known, but a number of factors may be involved.

It is reasonable to expect that the relative importance of intrinsic strength to staple strength will depend on the strength of the relationships between changes in diameter or other fibre characteristics and staple strength, and also on how intrinsic strength changes with diameter. In the work reported by Hunter *et al.* (1983) and Gourdie *et al.* (1992), it can be calculated that minimum fibre diameter accounted for 25% and 65% of the variance in staple strength, respectively. This implies that other fibre characteristics, like intrinsic strength, were more important determinants of staple strength in the earlier experiment, and this was indeed the case. Hunter *et al.* (1983) found that intrinsic strength of fibres from tender wool (13 N/ktex) was 124 N/ktex compared to 177 N/ktex for fibres from sound wool (42 N/ktex), and 67% of the variation

in staple strength between sheep was accounted for by mean intrinsic strength. It is worth noting that Hunter *et al.* (1983) used different batches of fibres (n=10) to measure force to break and minimum fibre diameter, and consideration of such technical precision may have further improved the strength of the intrinsic strength versus staple strength association. Gourdie *et al.* (1992), in a far more comprehensive experiment, found that only 27% of the variance in staple strength was attributable to differences in intrinsic strength.

It is apparent that provided intrinsic strength can be accurately measured, then it may be an important component of staple strength. What is not known is its relative importance in determining staple strength compared to other fibre characteristics under varying environmental conditions and for different sheep genotypes. However, on *'a priori'* grounds, intrinsic strength should become a more important component of staple strength under less variable environmental conditions (i.e. when the fluctuations in diameter along the fibre are minimised), and may also be more important to staple strength in finer wool sheep, where the rate and extent of changes in fibre diameter are smaller (Jackson and Downes 1979). However, there have been no systematic investigations that have attempted to establish the relationships between the variations in stress-strain properties of individual fibres and staple strength in Merino sheep of known genetic background and nutritional history.

1.5.4. Other fibre characteristics

Ritchie and Ralph (1990) reported that the total coefficient of variation of fibre diameter within a midside wool sample accounted for between 67 and 81% of the variation in staple strength between sheep. While it is not known from this experiment what proportion of the total coefficient of variation of fibre diameter was associated with differences in diameter between staples, along fibres or between fibres, it has been shown previously that about half of the total variation in fibre diameter is probably associated with differences *between* individual fibres (McKinley *et al.* 1976). Much higher estimates of between-fibre variation in diameter have been reported (70-90%; Quinnell *et al.* 1973; Dunlop and McMahon 1974), although they used theoretical or indirect estimates of along-fibres variation which are probably seriously under estimated. As Lewer and Ritchie (1993) reported an equally strong genetic correlation between staple strength and the variation in diameter measured from 2 mm staple snippets, it appears that the variation in diameter *between* individual fibres is indeed an important component of staple strength. While the fibre characteristic(s) *directly* responsible for the effects of fibre diameter

variation on staple strength remain unknown, since fibre diameter is positively related to both fibre length (Hynd 1994*a*) and extensibility (Collins and Chaikin 1965, 1968), wools with a higher total variation in fibre diameter probably have individual fibres with a greater range in length and extensibility. This would influence staple strength by affecting the proportion of fibres which come into tension at the same time. There are currently no published reports on the effects of fibre length variation on staple strength in Merino sheep.

1.6. Physiological factors in relation to wool growth and staple strength

For each animal, the rate of growth, morphology and chemical composition of the wool fibre produced are determined by the interaction between several genetically determined follicle characteristics and the relative availabilities of nutrients to the wool follicles (Black and Reis 1979; Black 1987; Hocking-Edwards and Hynd 1992; Harris *et al.* 1993). The availability of nutrients to the follicle is influenced by the quantity and type of nutrients absorbed form the digestive tract, and the competition for nutrients between wool follicles and other tissues of the body. Key factors in determining the availability of nutrients for metabolism by follicles are therefore the rate of blood flow to the skin, the concentration of nutrients within the blood, and the rate of exchange of nutrients between the blood and the cellular pool of the tissue (Harris *et al.* 1993). Thus, the amount and composition of the diet eaten, the stage of growth and reproductive status of the animal, the environmental conditions, and the presence of parasites and disease may all influence the amount and characteristics of wool grown, and therefore staple strength.

1.6.1. Nutrition

The effects of the amount and quality of feed consumed on wool production are well documented (reviewed by Allden 1979 and Reis 1989). At the follicular level, when the supply of nutrients is limiting, reductions in wool growth occur in response to declines in the volume of the germinative region of the follicle bulb, and a concomitant decrease in the rate of cell division (Hynd 1989). There may also be reductions in the proportion of dividing cells in the follicle which enter the fibre cortex and the volume of the fully differentiated cortical cells (Hynd 1989), but not always (Williams and Winston 1987). Hynd (1989) reported that 96% of the variation in fibre output per follicle was associated with differences in the rate of cell division and the proportion of cells entering the fibre cortex. Clearly then, if staple strength is determined largely by diameter changes, then changes in the supply of nutrients to the follicle for any reason will

have obvious ramifications for staple strength. The implications of variable nutrient supply for staple strength will depend on time of shearing (spring *vs.* summer-autumn), class of stock (weaners *vs.* adults; thin *vs.* fat animals) and time of lambing (autumn *vs.* late winter-spring) (Doyle *et al.* 1994).

In Mediterranean environments, continuous grazing of annual-based pastures at conservative stocking rates is the most common management practice used in sheep production systems (Doyle *et al.* 1993). Under this management, the amount and quality of pasture available varies markedly between regions, seasons and years (Rossiter 1966). This leads to variable nutrient intake by grazing sheep between different seasons, and to large seasonal fluctuations in liveweight, wool growth rate and fibre diameter (Purser and Southey 1984; Thompson *et al.* 1994). Sheep generally lose liveweight during summer/autumn, and the major position of break along the staple for non-reproducing, non-supplemented sheep frequently occurs at, or shortly after, the break of season (Rowe *et al.* 1989; Gardner *et al.* 1993; Doyle *et al.* 1995). The time of shearing can therefore have a direct influence on staple strength. Autumn-shorn wool is generally stronger than spring shorn wool (Baker *et al.* 1994), simply because the major point of weakness along the staple is located within the jaws of the ATLAS staple breaking machine. This means that the strength of summer-autumn shorn wool is determined by other weak regions along the staple.

On the basis of the associations between nutrient intake, liveweight change and wool growth (Allden 1979), management strategies which maintain sheep liveweights at a more uniform level would be expected to increase staple strength. To this end, considerable work has compared the effectiveness of different supplementation strategies during summer/autumn on staple strength. The effects of different types of supplements on staple strength are often inconsistent, probably because of differences between experiments (e.g. climatic conditions, base pasture conditions, class of animal and management practices; Peter *et al.* 1993). While supplementary feeding of young sheep with lupins, oats or barley during the summer/autumn period has been shown to increase the strength of spring-shorn wool (Rowe *et al.* 1989; Thompson and Curtis 1990; Gardner *et al.* 1993), lupins have been reported to be superior to cereal grains in some experiments (Gardner *et al.* 1993), but not others (Rowe *et al.* 1989). Thompson and Curtis (1990) found a significant interaction between type of supplement and feeding level. For spring-shorn wool, the benefits of supplementation to staple strength may only occur if it commences from pasture wilting, prior to senescence, and not after the sheep have started to lose weight (Thompson and Curtis 1990; A.J.M. Ritchie unpubl. data, cited by Peter *et al.* 1993). This is

because the initial declines in wool growth and fibre diameter immediately preceding pasture wilting and senescence occur at a time when liveweights may still be increasing rapidly (Thompson *et al.* 1994). To produce sound wool from sheep shorn in summer-autumn, it may not be necessary to prevent liveweight loss until some time after shearing (Doyle *et al.* 1994).

These results collectively indicate that sheep which maintain liveweight through summer/autumn, with or without the provision of additional supplements, should produce sound wool, irrespective of shearing time. However, feeding for liveweight maintenance does not always guarantee the production of sound wool. The data from 21 supplementary feeding experiments with young sheep, show that slow rates of liveweight gain (<10 g/d) throughout the summer-autumn was necessary for *most* young sheep to produce wool with staple strength greater than 30 N/ktex, however some flocks still produced tender wool (P.T. Doyle, unpubl. data., cited by Peter *et al.* 1993). Clearly other factors must contribute to low staple strength even when these nutritional conditions are fulfilled. Short-term disruptions in nutrient intake following summer rainfall events, in conjunction with the associated hormonal response to the environmental stress (Adams and Wynn 1993), may be a contributing factor. It is also known that the sudden introduction of wheat grain resulting in lactic acidosis (Murray *et al.* 1992), changing the grazing area or the nature of the base feed, or the yarding and transport of animals may all temporarily disrupt nutrient intake.

In the medium to higher rainfall areas of southern Australia, high rates of pasture growth during spring usually mean that its availability and quality does not limit liveweight gain or wool growth, such that 50% or more of the annual wool production may occur between August and the end of November (Purser and Southey 1984; Thompson *et al.* 1994). While increasing stocking rates under 'set-stocking' is likely to accentuate these fluctuations in liveweight and wool growth during the year, and consequently reduce staple strength (Earl *et al.* 1994), these 'normal' seasonal fluctuations can be controlled by appropriate grazing management (Doyle and Thompson 1993; Thompson *et al.* 1994). While Thompson *et al.* (1994) found that nutritional management in spring to control liveweight gain had no affect on staple strength in adult sheep, strip grazing to control intake of dry sheep from the break of season until the end of spring reduced the variation in fibre diameter along the staple in comparison to sheep which were 'set-stocked', and increased staple strength by about 5 N/ktex (Doyle and Thompson 1993). To reduce the along-fibre variation diameter and therefore increase the production of sound wool in Mediterranean

environments, it is apparent that summer/autumn management practices need to be complemented with appropriate winter/spring management practices.

Comparatively little is known about the effects of nutrition on the other components of staple strength, although severe nutritional stress can induce follicle "shutdown" and fibre shedding in Merinos (Lyne 1964; Ryder 1967; Wilson and Short 1979). Dramatic changes in the balance of specific nutrients following the infusion of a lysine-deficient protein (Reis and Colebrook 1972), amino acid mixtures lacking in lysine (Reis and Tunks 1978, 1982), or methionine into sheep consuming a wheat diet (Reis and Tunks 1974; Chapman and Reis 1978; Reis *et al.* 1983, 1986; Reis and Gillespie 1985) all result in the production of weaker staples, as assessed subjectively. Supplementing grazing sheep with a mineral mix containing six macro- and 12 micro-elements also increased staple strength significantly (53 *vs.* 38 N/ktex) relative to a control group (White *et al.* 1992). However, for each of these scenarios, it is not known wether the changes in staple strength resulted from changes in intrinsic strength and or fibre diameter. Zinc and copper deficiency do cause the growth of fibres which are intrinsically weaker (Underwood 1977; Masters *et al.* 1985), but the intrinsic strength of individual fibres does not appear to be influenced by "normal" changes in nutrition in Merino (Hunter *et al.* 1990) and Romney sheep (Woods *et al.* 1990; Gourdie *et al.* 1992). The evidence thus suggests that the differences in intrinsic strength between sheep are genetically determined, and without doubt, the major effects of nutrition on staple strength are via changes in fibre diameter.

1.6.2. Class of sheep

The causes of tender wool in different classes of sheep have not been clearly established, but tender wool is a more common problem in young sheep (Barton *et al.* 1994). Young growing sheep not only have an extra requirement for tissue protein deposition, but they also have limited reserves of fat and protein. The latter also applies for sheep in poorer condition. Consequently, their ability to buffer against seasonal changes in nutrient supply is restricted. Not surprising then, seasonal changes in wool growth and fibre diameter are greater in weaners than in mature dry sheep, although there is only a limited amount of data available (A.N. Thompson unpubl. data). It may be speculated that along fibre variations in diameter may be more significant determinants of staple strength in younger sheep. While this hypothesis is yet to be tested, similar negative correlations have been reported between the total coefficient of variation in fibre diameter and staple strength in hoggets (Ritchie and Ralph 1990) and adults (Hansford 1989).

It was eluded to previously that sudden decreases in nutrient availability, such as that brought about by summer rain or acidosis, may be sufficient to adversely affect staple strength. This would apply especially to younger sheep, since their physiological immaturity would restrict their ability to respond to such 'environmental' stressors. Greater susceptibility to environmental stressors, may also mean that the total cessation of growth by individual follicles plays a greater role in younger animals. A.C. Schlink (unpubl. data) found that up to 40% of follicles in some weaners may shut-down during summer-autumn. The finding that the percentage of wool which is less than 30 N/ktex is independent of mean fibre diameter for hoggets between 18 and 23 μm, whereas it declines with increasing mean fibre diameter of wool from mature sheep (Baker *et al.* 1993), may suggest that follicle shutdown without concomitant declines in fibre diameter is more prevalent in younger sheep. Little is known about the effects of class of animal on intrinsic strength, as the few studies which have given sufficient details regarding the source of the sample used, have used adult Merino (Hunter *et al.* 1990), Romney (Gourdie 1989; Woods *et al.* 1990; Gourdie *et al.* 1992) or mixed-breed (Hunter *et al.* 1990) sheep.

1.6.3. Pregnancy and lactation

Lambing ewes suffer penalties in wool production and therefore staple strength compared to dry sheep (reviewed by Corbett 1979; Masters *et al.* 1993). The depression in wool production during pregnancy and lactation is generally considered to be due to increased competition for nutrients (Williams and Butt 1989), and a decline in the efficiency of use of nutrients for wool growth (Oddy 1985). D.G. Masters and C.A. Stewart (unpubl. data) found that wool growth rates were reduced by 40% at this time compared to dry sheep, despite similar diets, liveweights and changes in liveweight (cited by Masters *et al.* 1993). Similarly, the point of break along the staple for grazing ewes is usually associated with late pregnancy, irrespective of nutritional conditions (Kelly *et al.* 1992). The effects of pregnancy and lactation on wool production and staple strength are more severe when feed availability and quality is limiting, presumably as a result of greater along-fibre changes in diameter (Hansford 1989). In Mediterranean environments, a majority of breeding ewes are joined between November and February, which means that most of the pregnancy occurs during times when paddock feed is low in amount and quality. Supplementation of autumn lambing ewes in mid and late pregnancy to maintain a high condition score can increase wool production and staple strength, but even maintaining a constant conceptus-free liveweight does not always guarantee a staple strength greater than 30 N/ktex (Kelly *et al.* 1992). D. G. Masters and C.A. Stewart (unpubl. data) found that replacing lupins

with protected-proteins for the 6 week period around parturition significantly increased wool growth and staple strength, and Masters *et al.* (1993) suggested that it may be possible to provide specific supplements in critical amounts at strategic times during later pregnancy.

The fibre diameter profile of spring lambing ewes is more uniform and the staple strength higher than for autumn lambing ewes (Foot and Vizard 1993), although significant reductions in wool growth during the latter stages of pregnancy have still been reported (Donnelly 1983). Fibre shedding, possibly induced by the stress associated with parturition itself, may also be a factor contributing to a lower than expected staple strength in reproducing ewes. Corbett (1979) reported that about one-third of the depression in wool growth during pregnancy/lactation was associated with the shedding of fibres from secondary follicles, and Schlink *et al.* (1992) observed that a higher proportion of follicles shutdown during pregnancy in staples which were less than 30 N/ktex. There is no evidence to suggest intrinsic fibre strength is influenced by pregnancy or lactation (Hunter *et al.* 1990).

1.6.4. Other factors

The position of break along the staple is often associated with a particular event, such as summer rainfall, and disease and the presence of parasites can also affect wool production and staple strength (reviewed by Hansford 1989; Besier 1993). While disruptions to nutrient intake following or during such events is likely to contribute to the changes in wool growth, it is also known that circulating levels of many hormones are altered dramatically in response to such stressors (Adams and Wynn 1993). Cortisol increases in response to stress and is the most likely hormone involved with the production of stress-induced low staple strength in grazing sheep. The 'normal' concentration of cortisol in sheep plasma ranges from 1-10 ng/ml, and Chapman and Bassett (1970) showed that increases in plasma cortisol up to 30 ng/ml over 12 weeks progressively inhibited wool growth and increased follicle shut-down, especially when sheep were on a low plane of nutrition. Scobie (1992) found that the mitotic activity in the follicle bulb was depressed when plasma cortisol concentrations were elevated for between 6 to 29 hrs, and that the response to cortisol varied significantly between individual sheep. Administration of ACTH for longer than 7 days to sheep maintained on a constant level of nutrition also depresses wool growth, increases fibre shedding, and produces a break in the staple (Ferguson *et al.* 1965).

The physiological basis for the differences in susceptibility to cortisol induced follicle shutdown is not known, to the extent that there is some disagreement as to whether the effects of cortisol on follicle function are direct or indirect. Nevertheless, it is known that the efficacy of epidermal growth factor, which causes temporary cessation of follicle activity (Hollis *et al.* 1983; Chapman and Hardy 1989; Hollis and Chapman 1989), is increased significantly by ACTH immunisation and physiological stress. This suggests an interaction between stress hormones, epidermal growth factor and follicle function, but this hypothesis is yet to be tested. The effects of the catecholamines on wool growth and staple strength are less well known, although Scobie (1992) suggests that they inhibit the rate of mitotic activity in the follicle bulb within 2-4 hours, possibly due to their vasoconstrictory activity rather than a direct effect on the follicle bulb.

1.6.5. Genetics

Large variations exist between sheep in the rate of growth, morphology and composition of wool fibres. Not surprising then, a considerable range in staple strength has been observed between sheep grazed under similar nutritional and environmental conditions, or given the same diet indoors (reviewed by Reis 1992). Obviously some of this within-flock variability in staple strength reflects genetic differences, although current estimates of the heritability for staple strength in Merino sheep are variable. Rogan (1988) reported a value of 0.17, while studies with two flocks in Western Australia maintained on "high" and "low" planes of nutrition yielded heritability estimates of 0.44 and 0.31, respectively (Lewer and Ritchie 1993). Ponzoni *et al.* (1994) reported heritability estimates of 10 and 16 month old rams of 0.24 and 0.49, respectively. The specific fibre characteristics, and the associated physiological basis, for genetic differences in staple strength are not yet fully understood.

A number of studies have found a strong negative genetic correlation between staple strength and the total coefficient of variation in fibre diameter (Lewer and Ritchie 1993; Ponzoni *et al.* 1994). As the variation in diameter along and between individual fibres make similar contributions to the total variability in diameter (Quinnell *et al.* 1973; Dunlop and McMahon 1974; Sumner and Revfeim 1973; McKinley *et al.* 1976), sheep bred for high staple strength may be less responsive to changes in nutritional conditions, and therefore produce fibres with less variation in diameter along their length. Indeed some sheep are more efficient wool producers on low quality dry feed during summer/autumn, but less efficient wool producers on high quality green feed during winter/spring (N.R. Adams pers. comm). Differences in responsiveness to changes in nutrient

intake may be due to changes in either the partitioning of nutrients to the skin and its follicles, and/or changes in the efficiency in which the available nutrients are utilised by the follicles.

Large genetic differences in the relationship between fibre length growth relative to diameter (i.e. L/D ratio) are evident between Merino sheep managed under similar conditions (10:1 to 25:1; Hynd 1994*a*). Sheep which increase production by altering length growth preferentially to fibre diameter, should produce fibres with smaller and less rapid fluctuations in fibre diameter than those which alter diameter to a greater extent than length. Sheep with high L/D ratio should therefore produce wool with a greater staple strength. Hansford (1989) found no relationship between staple length to diameter ratio and staple strength, however staple length can be a poor indicator of fibre length (P.I. Hynd unpubl. data) due to differences in the arrangement of fibres within the staple (e.g. crimp amplitude, frequency etc.).

Alternatively, or additively, other evidence would suggest that sheep bred for high staple strength have less variation in diameter between individual fibres than sheep bred for low staple strength (Lewer and Ritchie 1993). It is reasonable to expect that differences in between-fibre variation in diameter reflect differences in certain characteristics of the follicle population, like secondary to primary follicle ratio and the diameter of the fibres produced by each of these follicle types. Genetic differences in staple strength could also arise from differences in 'susceptibility' to stress-induced changes in wool growth, and in particular follicle shutdown. The large variation among animals in response to the administration of cortisol or epidermal growth factor, both in terms of the proportion of follicles which ceased production and the extent to which diameter declined in the remaining fibres (Chapman and Bassett 1970; Hollis and Chapman 1989), would suggest a genetic component to follicle shut-down. Similarly, while it is unknown whether sheep which consistently produce high staple strength wool are less susceptible to disease and parasites, the heritability of resistance to a number of diseases is moderate to high (reviewed by Donald 1979; Piper 1987), indicating that differences in susceptibility to infection could be associated with genetic differences in staple strength. Finally, differences in intrinsic strength between sheep also appear to be genetically determined, since most studies have found little or no influence of environment on intrinsic strength (Hunter *et al.* 1990; Gourdie *et al.* 1992), and thus may contribute to genetic differences in staple strength.

1.7. Property-structure relations in wool fibres

1.7.1. Stress-strain curve

The longitudinal mechanical properties of single wool fibres can be measured by comparing their load-extension curves (Speakman 1927; Collins and Chaikin 1965; Bendit and Feughelman 1968; Chapman 1969; Fraser and MacRae 1980; Feughelman 1982, 1987; Hunter *et al.* 1983, 1990; Gourdie 1989; Woods *et al.* 1990; Gourdie *et al.* 1992). However, since the load required to break a single fibre is positively related (r^2 = 0.88 to 0.96) to its cross-sectional area at the point of break (Orwin *et al.* 1985; Gourdie 1989; Woods *et al.* 1990; Gourdie *et al.* 1992), to compare the mechanical properties of different fibres the load applied must be normalised for the cross-sectional area of the fibre at the point of break. The normalised force is referred to as stress, and the extension as strain.

The stress-strain curve for single wool fibres consists of three distinct regions of mechanical behaviour (Fig. 1.9). As the fibre is stretched beyond the de-crimping region, there is a linear increase in load up to about 1% extension and there is little deviation from linearity until an extension of about 2% is reached. This stiff initial region is referred to as the "Hookean" region, and the slope of this region is referred to as the Young's modulus. Beyond about 2% extension is the yield region where the load on the fibre changes relatively slowly as the fibre is extended to about 30%. Up to this point the fibre can completely relax to its original length if the stress is removed. Beyond 30% extension, the fibre stiffens again as it is extended into the post-yield region. The end of the curve denotes the load and extension at which the fibre breaks. Dry wool fibres can be extended by about 30 to 35% before breaking, compared to about 40% at 65% RH, and about 55 to 70% for fibres tested in water. The general form of the stress-strain curve is independent of the water content of the fibre, however the regions of mechanical behaviour are most clearly defined when fibres of uniform cross-sectional area are tested in water at 20°C; where the slopes of the "Hookean", yield and post-yield regions are about 100:1:10, respectively.

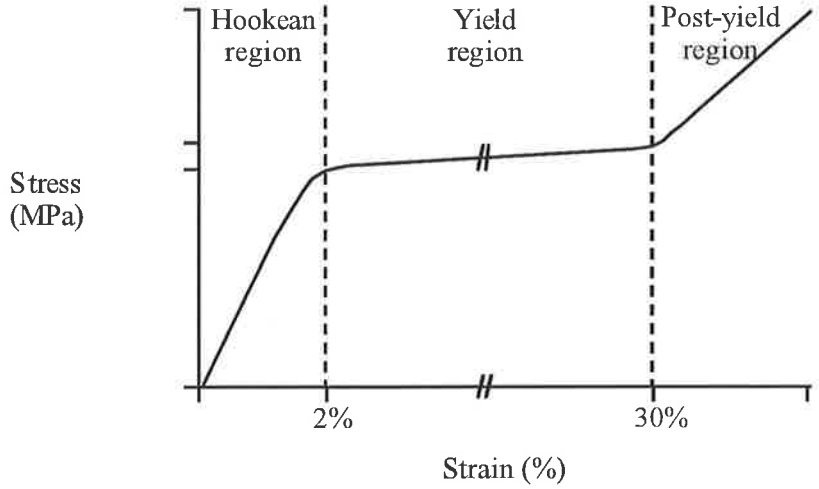

Figure 1.9. Stress-strain curve for a wool fibre tested in water at 20°C showing the different regions of mechanical behaviour (stress and strain are not drawn to scale).

1.7.2. Fibre components in relation to stress-strain properties

It has not been established unequivocally whether or not there are significant differences in the mechanical properties of the cuticle and cortex of wool fibres, but given the vast differences in their chemical composition and structure it would indeed be surprising if there were not significant differences in their stress-strain properties. The small weight fraction of the cuticle, and its lack of molecular ordering (Powell and Rogers 1994*a*), would suggest that its contribution to stress-strain properties of the whole fibre is likely to be limited. Furthermore, when under tensile strain extensive cracking and breakage of cuticle cells occurs before the fibre ruptures (Andrews 1964; Dobb and Murray 1976), suggesting that it may be less extensible than the cortex. While no direct information is available on the mechanical properties of the cell membrane complex between cuticle or cortical cells, its preferential digestion with proteolytic enzymes reduces the strength of the fibre drastically (Elöd and Zahn 1946), indicating that its integrity is at least required for the mechanical coherence of the fibre.

The cortex is the main structural component of the wool fibre, and between 70 and 97% of the fracture surfaces of fibres broken in air (20°C, 65% RH) are relatively smooth and perpendicular or oblique to the fibre axis (Andrews 1964; Hearle *et al.* 1976; Dobb and Murray 1976; Orwin *et al.* 1985; Gourdie 1989). This type of fracture suggests cleavage across individual cortical cells, rather than between the cells along the cell membrane complex, and is typical of breaks in a composite structure with strong interfaces between components (Dobb and Murray 1976). This contention is supported by scanning electron microscopy studies which have revealed that

microfibril diameter and inter-microfibrillar spacing decrease with increasing extension in a manner expected for deformation at constant volume (Dobb and Murray 1976). They found no indication of abrupt changes in the fine structure between adjacent cortical cells, and consequently it is considered that little if any slippage occurs preferentially at the cell boundaries. It is generally believed that the microfibril-matrix complex of the cortex governs the longitudinal stress-strain behaviour of wool fibres.

1.7.3. Molecular structure in relation to stress-strain properties

Considerable work has concentrated on establishing the associations between the shape of the stress-strain curve and the morphology and molecular structure of the wool fibre (e.g. Collins and Chaikin 1965, 1968; Hearle and Chapman 1968; Hearle *et al.* 1971; Feughelman 1979, 1982, 1987, 1994; Fraser and MacRae 1980; Wortmann and Zahn 1994). Despite the complex morphology of wool fibres, from a mechanical view point, the differences between the various components can be reduced to a simple two-phase model, as originally proposed by Feughelman (1959). In this model, high modulus microfibrils form a crystalline, continuous, axially orientated, elastic filamentous phase, which is embedded in an amorphous matrix phase that comprises the non-helical fraction of the microfibrils, the "classical" matrix material, and other non-crystalline viscoelastic components (Wortmann and Zahn 1994). The microfibril/matrix complex is an example of a biological composite in which two components are combined to make best use of the favourable properties of each component, whilst at the same time reducing the influence of some of their less desirable characteristics. For fibre tested in water at 20°C, the primary advantage of this complex is that the easily deformed matrix distributes any applied stress evenly over the filaments, and thus prevents the propagation of cracks from local imperfections or "flaws" (Wainwright *et al.* 1976).

The mechanical properties of a wool fibre change from an isotropic material when dry to a highly anisotropic material when wet (Feughelman 1959). The glass transition of the amorphous matrix phase is about -5°C in water, and increases to about 60°C at 65% RH and 170°C at 0% RH (Wortmann *et al.* 1984). In the dry state the mechanical properties of both the microfibrils and matrix are similar, and the behaviour of the material approaches that of a uniformly hydrogen bonded network. However, the matrix phase is mechanically plasticised and weakened by the presence of water while the microfibrils remain unchanged. Several models have been developed to explain the mechanical behaviour of wet wool fibres in each region of strain in relation to the

molecular state of each of the two phases. There is general agreement as to the factors that control the mechanical relationships in the "Hookean" and yield regions, but there exists major differences in the molecular explanations for the post-yield region (Fig. 1.10).

1.7.3.1. "Hookean" region

Typical values for the Young's modulus for wool fibres tested in water at 20°C are in the range from 1700 to 2000 MPa, depending on rate of extension. At 0% and 65% RH, respectively, the Young's modulus is about 2.7 and 2.0 times greater than that for the same fibre tested in water (Feughelman and Robinson 1969). Most evidence suggests that the longitudinal mechanical behaviour of wet wool fibres at extensions within the "Hookean" region is attributed primarily to the deformation of the α-helices of the microfibrils. X-ray diffraction studies indicate that the coiled-coils are stretched within the "Hookean" region, but there is no change in the proportion of the total length of the protein chains in the α-conformation. Other supporting evidence is that in solutions above 6M concentration of lithium bromide there is a rapid fall in both the optical birefringence, which relates to the presence of organised α-helical material, and the mechanical stiffness of the fibre. Above 6.6 M lithium bromide concentrations, the transition of the α-helical material into a random elastomeric state results in a 20-fold decrease in Young's modulus (Feughelman 1982). Moreover, above 100°C the Young's modulus for a fibre in water begins to drop rapidly, and the Hookean region disappears completely at about 130°C coinciding with the "melting" of the α-helices (Feughelman and Mitchell 1966). The opposition to extension in the "Hookean" region is due mainly to the hydrogen bonds present between the turns of the α-helices (Feughelman and Druhala 1977). Despite conflicting reports on the molecular state of the matrix, it is accepted universally that the matrix contributes little to the Young's modulus. Most argue that the matrix is transformed from a "gel" into a "sol" state at about 1% extension, and thereafter does not stiffen with extension right up to the break (e.g. Feughelman 1959, 1987; Wortmann and Zahn 1994). On the other hand, in the Chapman-Hearle model the matrix is assumed to be a highly cross-linked rubber, which starts with a low modulus but stiffens as the network becomes highly strained (Hearle *et al.* 1971).

1.7.3.2. Yield region

The slope of the yield region varies considerably between fibres due mainly to differences in along-fibre variability in cross-sectional area (Collins and Chaikin 1965, 1968). At the molecular

level, the commencement of the yield region is associated with the initiation of unfolding of the organised α-helical framework of the microfibrils. This view is supported by the observation that extension of fibres beyond 2%, results in a progressive decrease in α-helical content, and a corresponding increase in the ß-keratin configuration as indicated by the high angle X-ray diffraction pattern (Astbury and Woods 1933; Skertchly and Woods 1960). Unfolding of the α-helices is probably initiated in one microfibril, and the lateral transfer of stress results in unfolding of many microfibrils possibly over the complete cortical cell (Feughelman and Mitchell 1966). Lateral striations which propagate rapidly across the fibre have been observed under the electron microscope at the commencement of the yield region (Haly unpubl. data), would support this contention.

The α⇔β transformation proceeds at virtually constant stress, which according to Wortmann and Zahn (1994), can be accounted for by the suggestion by Cifferi (1963) of a thermodynamic equilibrium between the α and β form, which exist in equilibrium under a fixed stress irrespective of their respective proportions. Hearle *et al.* (1971) proposed that a critical stress, much higher than the equilibrium stress, is needed to initiate the crystal lattice transition, and that the stress is transferred to the surrounding matrix. It is reasonable to suppose that the initial breakdown of hydrogen bonds on extension is likely to happen in less stabilised areas of the α-helices. The extension of the yield region unfolds about 30% of the α-helical material present in the fibre, as judged by X-ray diffraction (Skertchly and Woods 1960) and DSC analysis (Spei and Holzem 1991). The α-helical material able to unfold in the yield region does so with no irreversible structural breakdown, so the mechanical properties of the fibre are recoverable at extensions less than about 30% (Speakman 1927).

1.7.3.3. Post-yield region

When fibres are stretched into the post-yield region, there is a very rapid onset of irrecoverability of mechanical properties, the continued decrease in α-helical content, and an increase in free radical formation indicating the rupture of covalent bonds (Pailthorpe *et al.* unpubl. data, cited by Feughelman 1987). Nevertheless, the molecular explanations for the post-yield region remain conjectural (Fig. 1.10). The Hearle-Chapman model, which is a development of the two-phase model of Feughelman (1959), assumes regular covalent linkages between the microfibrils and matrix, such that the fibre stress-strain curve is a combination of the two components (Hearle *et al.* 1971). This model assumes that the rubber-like matrix dominates the slope of the post-yield

region. The explanation is that as more and more microfibrils transfer stress to the matrix, the regions of stress transfer in the matrix eventually join up and the stress rises into the post-yield region. As eluded to, conflict of opinion remains on the molecular state of the matrix phase, but there is little evidence to indicate that the matrix does infact behave as a rubber (Druhala and Feughelman 1974; Feughelman and Collins 1974; Feughelman and Druhala 1976). The independence of post-yield slope to water content of the fibre is a further argument against the relevance of matrix behaviour in the post-yield region. The Chapman-Hearle model also assumes that after nucleation of the crystal lattice transition at about 2% extension, the stress in the microfibrils remains constant, such that their contribution to mechanical behaviour in the post-yield region is minimal. The validity of this assumption is questionable, given the likelihood of major differences in the stability of different segments of the monomeric units of the fibrils.

The series zone model originally proposed by Feughelman and Haly (1960) is an alternative explanation for the post-yield region, which essentially ignores the matrix proteins. In this model, the microfibrils are considered to contain alternating zones (X and Y) of differing mechanical stability. Extension to the end of the yield region results in extension of α-helices in the X zones, which open up reversibly with no structural opposition to the unfolding. Extension into the post-yield region requires opening of the α-helices of the more mechanically stable Y zones, which requires covalent bond breakdown for this unfolding to occur. Evidence to support this model is that the α-helical material in dry fibres exists as two distinct fractions with differing thermal stability (Menefee 1974). The more thermally stable material would correspond to the α-helical material (70%) which unfolds in the post-yield region. Wortmann and Zahn (1994) adapted the series-zone model to the current knowledge of keratin morphology. They suggested that the X zones correspond to the A-segments of the microfibril monomeric units, which unfold without constraint in the yield region, and the Y zones correspond to the more highly crosslinked overlapping 2B regions.

It is worth noting that Feughelman (1994) recently abandoned his series zone model, and now favours a prominent role of the matrix/water complex and its interaction with the microfibrils. In this model, when the fibre is stretched the microfibrils come closer together until they jam up against the matrix proteins at the start of the post-yield region. Further extension beyond the unfolding of the unjammed components of the microfibrils requires the unfolding of the α-helices of the microfibrils and the extension of the matrix proteins (Feughelman 1994). According to Wortmann and Zahn (1994) and J.W.S. Hearle (pers. comm), the key assumption in

this model that water and protein material form separate phases in the matrix, is not valid and is at variance with the observations by Simha and Rowen (1948) that the wool/water system at high regains can be described by the Flory-Huggins solution treatment.

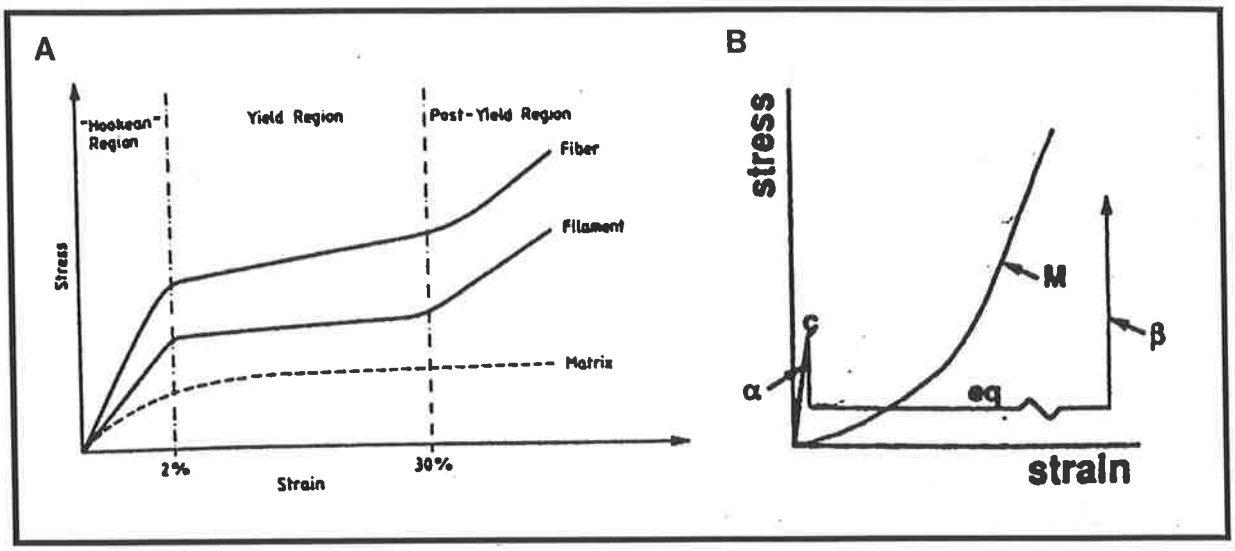

Figure 1.10. The contrast between the Wortmann/Zahn model and Chapman-Hearle model in the roles of the microfibril and matrix phases in determining the stress-strain properties of wool fibres. (*a*) In the Wortmann/Zahn model, the microfibril phase has a stiff Hookean region, a flat yield region and a stiff post-yield region; the matrix yields to an almost constant stress (from Wortmann and Zahn 1994). (*b*) In the Chapman/Hearle model, the fibril has a stiff initial region [α], but at a critical stress [c] the stress drops to an equilibrium value for the α =>β transition; it would increase when the transition was complete [β], but wool does not reach such high extensions before breakage; the matrix [M] is a typical rubber elasticity curve, increasing strongly in stress at high strains (from Hearle *et al.* 1971).

1.7.3.4. Influence of relative humidity on the stress-strain curve

The mechanical properties of keratin fibres are influenced by the RH, temperature, rate of extension and the medium in which the fibres are tested (Speakman 1927; Feughelman and Robinson 1969, 1971; Chapman 1969; Collins and Chaikin 1971), but only the effects of RH are considered in this review. The reason for this is that a majority of the stress-strain measurements on wool fibres have been made in water at 20°C, and comparatively less is known about the stress-strain properties of wool fibres, and how this relates to fibre structure at 65% RH, the conditions that staple strength is measured and wool fibres are commercially processed.

The stiffness of the fibre decreases progressively with increasing RH, and as most of the swelling of keratin fibres occurs between 0 and 50% RH, the effects of changes in RH on the stress-strain behaviour is also greatest in this region. Young's modulus decreases as a fibre is taken from a dry to a wet state, due to changes in the stress at the beginning of the yield region, rather than the length of the fibre at the beginning of the yield region. This implies, that irrespective of RH, the α-helical material of the fibres begins to unfold at the same fibre length. The slope of the yield and post-yield regions decrease with increasing RH, although the effects on the post-yield region are relatively small; the overall change in the range 0 to 100% RH being of the order of 15%. Stress at break, or intrinsic strength, is almost unchanged when RH is greater than 60%, however the strain at the beginning of the post-yield region and at the point of break increases with RH (Speakman 1927).

1.7.3.5. Summary

It is not clear which is the most appropriate molecular model to explain the mechanical behaviour of wool fibres. If we are to have a clearer understanding of wool fibre structure-property relations, it is imperative that the controversy over the different models be settled by further experimental and theoretical research. However, in my opinion, the original series-zone model, most recently adapted by Wortmann and Zahn (1994), is currently the most plausible explanation for the longitudinal stress-strain properties of wool fibres in water. Furthermore, even if the Chapman-Hearle model is closer to the truth, it is still reasonable to suppose that variations in intrinsic strength and other stress-strain properties are due to differences in the quantity and composition of the structural keratin proteins, their physical arrangement (density, packing and alignment of filaments), and the arrangement of the cross-links between them. Whilst the effects of these variations in the molecular characteristics of the fibre on mechanical properties are greater for fibres tested in water, even at 65% relative humidity the matrix is still considered to be much weaker in the longitudinal direction than the microfibrils (Bendit and Feughelman 1968; Feughelman 1968).

1.7.4 Physico-chemical characteristics in relation to stress-strain properties

1.7.4.1. Along-fibre variations in cross-sectional area

Large variations in cross-sectional area occur along the length of all wool fibres, and the effects of these dimensional non-uniformities on their mechanical behaviour has been demonstrated

theoretically and experimentally (Collins and Chaikin 1965, 1968, 1969, 1971). When a non-uniform fibre is extended at a constant rate, it is necessary to consider the fibres as a large number of short sections of fibre. All sections of the fibre are initially extended in the Hookean region, but since the finer cross-sections along the fibre will be extending at a higher rate, as the load increases they will begin to extend into the yield region. As more of the fibre sections begin to extend, the average extension rate for those sections extending in the yield region will then decrease, resulting in a reduction in the rate of load bearing increase. As more and more sections reach the post-yield region, the average extension rate for sections in the yield region will increase, the rate of load change being increased until all sections are extending in the post-yield region (Collins and Chaikin 1969).

The main effects of an increase in the coefficient of variation of cross-sectional area are: (*i*) an increase in yield slope, because fewer sections are extending in the yield region at a particular instant; (*ii*) shorter regions of linearity and loss of sharpness at the transition points between the three regions; and (*iii*) a decrease in breaking extension, because fracture occurs when a large proportion of the fibre being tested is not fully extended. When structural variation is also present along the fibre, then the shape of the stress-strain curve depends on the coefficient of variation of "effective area" (Collins and Chaikin 1969). They also report that stress at break, or intrinsic strength, tended to decrease as the non-uniformity in cross-sectional area increased, but stress at 15% extension was unchanged. It is very difficult to imagine how changes in fibre dimensions along the fibre could influence intrinsic strength, provided that the force to break the fibre is normalised for the cross-sectional area at the point of break, and indeed, Gourdie *et al.* (1992) found no evidence to suggest that intrinsic strength was influenced by along-fibre variations in diameter. The inconsistent effects of the estimated cross-sectional area at the point of break on intrinsic strength, and possible reasons for these discrepancies, have already been discussed in section 1.5.3. of this review.

1.7.4.2. Fibre crimp

Dusenbury and Wakelin (1958) and Smuts *et al.* (1981) found that an increase in crimp frequency was associated with a decrease intrinsic strength. Indeed, Smuts *et al.* (1981) report that intrinsic strength was more sensitive to changes in fibre crimp than diameter, and they concluded that the contradictory trends previously reported between intrinsic strength and diameter may be due to the fact that fibre crimp has not been taken into consideration and that

the effects attributed to fibre diameter were actually due to concomitant changes in crimp. If there is a negative correlation between intrinsic strength and crimp, then any relationship between intrinsic strength and fibre diameter would depend on the relationship between crimp and fibre diameter. Since this relationship may be negative, zero or positive, it follows that the corresponding correlation between fibre diameter and intrinsic strength could also be positive, zero or negative. O'Connell and Lundgren (1954) found that high crimp wools exhibited, in general, a negative dependency of fibre diameter on mechanical properties, whereas low crimp wools showed a positive diameter dependency. These effects were most pronounced within the Hookean region, but were still evident and significant at 30% extension. Others have failed to find a significant relationship between fibre crimp and intrinsic strength for fibres tested at 65% RH or in water (Menkart and Detenbeck 1957).

Barach and Rainard (1950) observed that the introduction of artificial crimp into wools decreased their tensile properties, and suggested that the decrease in intrinsic strength associated with high levels of crimp is due to the presence of kinks and bends which act to increase mechanical stress. However, Huson (1990) showed that while this is probably true for severe kinks, bends *per se* do not reduce tensile failure properties. A more likely explanation for the apparent effect of crimp on intrinsic strength is probably an indirect association between crimp, fibre structure and/or chemical composition. For example, fibre crimp is thought to be a consequence of the bilateral ortho- paracortex arrangement of the fibre. The inter-relationships between fibre crimp, fibre diameter and ortho-para cortical ratios on the tensile properties of wool fibres under standard conditions have yet to be elucidated, as has the effects of fibre structure *per se*.

1.7.4.3. Flaws

Andrews (1964) and Mason (1964) suggested that fracture occurs as a result of crack initiation at natural flaws occurring along the fibre, and that tensile failure properties are unlikely to yield information of fundamental interest. Consequently, the failure properties of wool fibres have received the least attention, and yet they are the fibre characteristics most expected to limit the rate and efficiency at which wool fibres can be processed. Several workers have proposed that stress at 15% extension (Burgmann 1959; Roberts *et al.* 1960; Collins and Chaikin 1965) or 30% extension (Whiteley and McMahon 1965) are less variable and better indicators of intrinsic differences in the properties of the keratin. However, Gourdie *et al.* (1992) found that the between-fibre variation in intrinsic strength was similar to that for stress at 15% extension. Some

fibres have been observed to break at a squashed part of the fibre, which are called nabs, and fibres breaking at these nabs are intrinsically weaker (Woods *et al.* 1990; Gourdie *et al.* 1992).

1.7.4.4. Cortical cell type and arrangement

The number, size and shape of cells entering the cortex, and the proportion and spatial arrangement of each cortical cell type are under genetic control, but are also influenced by environmental conditions and physiological status. This means that the cellular characteristics are not uniform along the length of individual fibres. In Merino sheep, the proportion of paracortex varies from 0 to 60% between individual fibres from the same sheep, and considerable variation exists between individual sheep (Hansford 1989; Hynd 1989). Genetically broader fibres and or low crimp wools tend to contain more orthocortex than genetically fine or high crimp wool (Campbell *et al.* 1972; Orwin *et al.* 1984). Conversely, nutritionally induced broad or low crimp wools contain less orthocortex (Campbell *et al.* 1975; Reis 1979; Hynd 1989). Hynd (1989) reported that the percentage change in the mean proportion of orthocortex when sheep were transferred from low to high nutrition varied between 0 and + 27% for individual Merino sheep. There is also considerable variation between fibres in the arrangement of cell types, and the rate and extent to which these change, along the length of single fibres (Orwin *et al.* 1984). These variations in cortical cell types along and between fibres may have significant effects on staple strength, due to changes in fibre dimensions and/or intrinsic strength.

If along-fibre changes in diameter are an important component of staple strength, then the dimensions of the cells entering the cortex may be important since they influence the length and cross sectional dimensions of the fibre (Hynd 1994*a*). While it is not known whether differences in cortical cell dimensions are due to changes in the ortho:para ratio or to changes in the size of individual cell types, orthocortical cells tend to be about 25% larger than other cortical cell types (Orwin *et al.* 1980, 1984). It follows that sheep whose follicles produce a greater proportion of orthocortical cells may produce fibres with greater relative changes in diameter along their length, and therefore a lower staple strength, than sheep whose follicles produce the same number of cells with a lower proportion of orthocortical cells. However, if intrinsic strength is influenced by the ratio of microfibrils to matrix, and orthocortical cells contain more LS protein microfibrils than paracortical cells, then fibres with higher proportions of orthocortical cells may be intrinsically stronger. Clearly the effects of cortical cell types on staple strength will therefore depend on the relative importance of changes in fibre shape and intrinsic strength. Orwin *et al.* (1985) reported

a positive relationship between the percentage of orthocortical cells and staple strength, which may suggest that under the condition of that experiment, intrinsic strength was a more important component of staple strength than changes in fibre diameter. Hansford (1989) found no relationship between staple strength and % orthocortex in Merino wool.

It is interesting that the relationship between the proportions of orthocortical cells and fibre diameter vary widely between sheep and between fibres of the same sheep (Orwin *et al.* 1984, 1985). These authors found positive curvilinear and linear relationships between the proportion of orthocortical cells and fibre diameter, and suggested that sheep with curvilinear relationships tended to have stronger wool than those with linear relationships, although no quantitative data were reported. Differences in the shape and magnitude of the orthocortex versus fibre diameter relationship may have implications for staple strength, since sheep which are able to maintain higher proportions of orthocortex in their fibres as diameter declines, may produce wool fibres which are intrinsically stronger, and therefore stronger staples. However, in contrast to this speculation, many studies have associated increased intrinsic strength of wool fibres with an increase in the proportion of paracortex (Thorsen 1958; Feughelman and Haly 1960; Chapman 1965; Orwin *et al.* 1980), although the validity of their conclusions is questioned based on the techniques and measurements used. Thorsen (1958) used a staining procedure (sodium plumbite) which degrades cortical cells, and there is little correlation between that method and methylene blue staining which has since been used in most studies for determination of the proportion of paracortical cells. Feughelman and Haly (1960) did not specify if the wool used was from a single sheep or a number of sheep, and Chapman (1965) used only tested 10 fibres from each of 4 sheep. Furthermore, both experiments used stress at 30% extension as an indicator of the mechanical properties of the wool fibre, which may not necessarily be a true reflection of differences in intrinsic strength. In summary, no consistent picture has emerged regarding the association between cortical cell types and either intrinsic strength or staple strength.

1.7.4.5. Protein composition, arrangement and structure

The protein composition of the fibre is subject to variation through genetic, dietary and physiological factors (Frenkel *et al.* 1974; Reis 1979; Gillespie and Marshall 1980; Gillespie *et al.* 1982; Marshall and Gillespie 1989). This variation may stem from alterations in the microfibril to matrix ratio, from changes in the relative proportions of the matrix proteins, and/or from

variations in their constituent protein components (e.g. alteration in the relative proportions of the LS protein subunit polypeptides). Despite the detailed molecular models developed to explain the stress-strain behaviour of the fibre, no clear association has been established between protein composition and either intrinsic strength or staple strength.

The LS proteins are the least variable of the structural proteins in wool (Gillespie *et al.* 1964; Gillespie and Reis 1966; Crewther *et al.* 1976, 1980; Fraser and MacRae 1980; Marshall and Gillespie 1981; Gillespie and Marshall 1983; Marshall *et al.* 1985), and relatively little is known of the physiological mechanisms controlling their gene expression and synthesis of the protein. Woods and Orwin (1987) reported that fibre samples from 64 sheep contained the 8 major LS proteins, and the protein pattern for individual sheep remained qualitatively constant under a variety of physiological conditions (season, age, nutritional status). The protein patterns reported by Woods and Orwin (1987) did not vary despite significant changes in the proportions of the various cortical cells types, which may imply that differences in intrinsic strength are associated with quantitative, rather than qualitative, differences in protein composition. It is interesting however that variable proportions of components 5, 7 and 8 of the LS protein family in wool, horn and hoof appear to be readily accommodated within the microfibril without any apparent effects on physical structure (Marshall and Gillespie 1977; Gillespie and Marshall 1980; Gillespie 1991). Nevertheless, as an equivalent abundance of both Type I and Type II LS proteins are required for their co-ordinated assembly into microfibrils, the loss of a specific LS polypeptide could lead to an imbalance in either Type I or Type II chains, which could presumably inhibit microfibril formation or result in the production of imperfect microfibrils.

In contrast to the LS proteins, dramatic changes occur in the proportion and nature of the HS and HGT protein families. The proportion of HS and HGT proteins in wool may vary from 18 to 34%, and 0 to 12%, respectively, and it is known that both dietary and genetic factors control their synthesis (Gillespie and Reis 1966; Gillespie and Darskus 1971; Campbell *et al.* 1972; Frenkel *et al.* 1974, 1975; Marshall and Gillespie 1981; Gillespie and Marshall 1983). There is a significant relationship between sulphur content of wool and its content of HS proteins (Broad *et al.* 1970). The cystine content of wool varies over a wide range due to differences in its content of UHS proteins, which can reach a level as high as 18% of the weight of wool (Gillespie 1991). When cysteine, methionine or proteins rich in these amino acids are infused into the abomasum of a sheep, the half-cystine content of the wool increases by as much as 35% (3.08 *v.* 4.17% sulphur) due to the synthesis of UHS proteins (Reis and Schinckel 1963, 1964). Smaller but significant

changes in wool composition can be effected by changes in the normal diet (Reis 1979). Nutritionally-induced changes in protein composition result from changes in the proportions of cortical cell types and the composition of these cells (Fratini *et al.* 1994), suggesting an influence on both stem cell differentiation and protein synthesis *per se*.

The amount of matrix proteins, and to some extent their composition, can vary considerably without affecting the physical properties of the wool fibre (Reis and Schinckel 1963; Gillespie *et al.* 1964). The 35% increase in cystine, and presumably disulphide content, following abomasal infusion of methionine (Feughelman and Reis 1967), had negligible effects on the mechanical properties of the fibre. According to the data reported by Crewther (1965), the increase in disulphide content should have produced a 20 to 25% increase in intrinsic strength, based on mechanical measurements on wool fibres differing in disulphide content due to chemical reduction. It was subsequently shown for these sulphur enriched fibres that the increase in cystine was produced in the matrix protein only (i.e. HS and UHS proteins; Gillespie *et al.* 1964), and presumably arranged such that they did not influence the tensile properties of the fibre.

When the cystine in wool fibres is reduced by chemical reduction, there is a linear decrease in the stress required to produce a fixed extension in keratin fibres immersed in water (Harris *et al.* 1942; Sobue 1955; Lindley 1957; Feughelman and Haly 1961; Crewther 1965). Since chemical reduction ruptures the disulphide bonds equally in LS and HS proteins (Springell *et al.* 1964), these results collectively imply that the LS protein microfibrils play a dominant role in the longitudinal mechanical behaviour of wool fibres. Other work shows that the mechanical properties of some α-keratins in water show a progressive stiffening with an increase in HS and HGT content of the fibre (Bendit 1980). This result was explained by a lower water content, and thus a lower plasticising of the matrix, since an increase in matrix proteins into the space between the microfibrils reduces the volume available for water (Fraser and MacRae 1980). It is difficult to separate the effects of changes in protein composition from the direct effects of water on the properties of the matrix proteins.

Several studies have indicated an association between the content of HGT proteins and the strength of wool fibres. Inhibition of HGT protein synthesis, by infusion of zein (Reis and Colebrook 1972), amino acid mixtures lacking in lysine (Reis and Tunks 1978, 1982), or by infusions of methionine into sheep consuming a wheat diet (Reis and Tunks 1974; Chapman and Reis 1978; Reis *et al.* 1983, 1986), all resulted in the production of weak wool as assessed

subjectively. However, supplementation of sheep with a mixture of amino acids lacking methionine weakens wool without a concomitant suppression of HGT proteins (Gillespie *et al.* 1980; Reis and Gillespie 1985), while the omission of phenylalanine from an infusion of amino acids reduces the content of HGT proteins without affecting wool strength (Frenkel *et al.* 1975). Furthermore, the HGT proteins are virtually absent from wool of normal strength grown by Lincoln and felting lustre mutant Merino sheep (Gillespie 1991), and regrowth of wool fibres generated after plucking, and the tips of fibres from lambs and young mice, are of normal strength despite reduced HGT protein content. Hence, these proteins can not be an essential component of the matrix, and it is clear that a reduction in the HGT proteins in wool is not a prerequisite for the production of weak wool.

Over expression of a single hair keratin gene (250 copies of a Type II LS keratin protein) in mice can lead to complex changes in the composition and physical arrangement of the keratin proteins. In the hair cortical cells of these transgenic mice, amorphous protein masses envelope small pockets of orderly arrays of filament and matrix, the opposite of the level of organisation seen in normal hairs. This imbalance of the normal ordered array of hair structural proteins (i.e. microfibril/matrix ratio) has a profound effect on hair strength (Powell and Rogers 1990*b*); similar findings have been described in several disorders of human hair (Price 1990). Trichothiodystrophy is a disease of human hair characterised by an abnormally low cystine content (< 50% of normal), due to a decrease in the proportion of HS proteins, which results in the hair being brittle (Pollit and Stonier 1971; Gillespie and Marshall 1983). The cortical cells also contains large areas with either very sparse or no fibrillar proteins, and an irregular arrangement of the microfibrils relative to the longitudinal axis of the hair shaft (Gummer and Dawber 1985).

Fibres of the same protein composition may be of different intrinsic strength, suggesting that more subtle changes to protein structure and arrangement may be involved in determining intrinsic strength. Although weak wool grown by sheep with a copper deficiency has less UHS proteins and the sulphur content of the HS proteins is also decreased, Gillespie (1964) concluded that the decrease in intrinsic strength was unlikely to be explained on the basis of the observed changes in protein composition, and may be instead be associated with more subtle changes in the proteins within the fibre. The marked decrease in solubility of the steely wool is strong evidence for a change in protein conformation (Gillespie 1964), and there are increases in the concentration of -SH groups (12-30 to 360 μmole -SH/g; Marston 1946). Weak hair from

children with Menkes syndrome contains a set of normal proteins which are incompletely cross-linked (730 *v.* 60 μ mole -SH/g). As human hair in the completely reduced state would contain about 1650 μmole/g of cysteine, it can be seen that oxidation in Menkes hair has progressed only to about 55% of completeness (Gillespie 1991). The mottled mutation in mice also results in incomplete cross-linking (220-270 *v.* 17-66μ mole -SH/g), despite containing a normal set of proteins.

Mice homozygous for the naked (N) gene produce brittle hair (Raphael *et al.* 1982, 1984). The N gene appears to act on the follicle to cause a reduction in the mitotic activity of the follicle bulb and incorrect differentiation of stem cells. A consequence of the latter is the partial loss of cuticle and cortical cells, impairing synthesis of HS and HGT proteins, increased synthesis of LS proteins, and disruptions to the deposition of keratin proteins in the fibre cells (Raphael *et al.* 1982). However, amino acid analyses indicate that simple changes in proportions of proteins have not occurred, but rather new proteins of abnormal composition or HS of low MW have been produced. The results of these protein and amino acid analyses suggest that the weakness of N/N hair probably stems from as much a disruption of fibre structure, as from the absence of or abnormality of one particular structural protein or group of proteins.

1.8. Scope of study

The purpose of the present study was to examine the effects of nutrition on the cellular, molecular and mechanical characteristics of fibres from sheep differing genetically in staple strength. The major objectives were: (*i*) determine which fibre characteristics are most closely associated with staple strength for sheep bred for sound and tender wool, under nutritional conditions typical of that experienced in Mediterranean-type environments; and (*ii*) determine the physical and chemical characteristics of wool fibres in relation to intrinsic strength and other stress-strain properties. The unifying hypotheses tested in this thesis are that intrinsic fibre strength is an important component of staple strength, and that it is determined by the physical structure and chemical composition of the fibre cortex.

CHAPTER 2: WOOL GROWTH AND FIBRE DIAMETER CHANGES IN MERINO SHEEP GENETICALLY DIFFERENT IN STAPLE STRENGTH AND FED DIFFERENT LEVELS OF NUTRITION

2.1. Introduction

The available evidence suggests that the relative importance of the various fibre and staple characteristics known to influence staple strength is likely to vary depending on complex interactions between sheep genotype and a number of nutritional, physiological and environmental factors (reviewed by Reis 1992), and that the nature of these interactions is poorly understood at present. For instance, whilst most of the staple strength response to changes in nutrition (Rowe *et al.* 1989; Thompson and Curtis 1990; Gardner *et al.* 1993; Peter *et al.* 1993; Doyle *et al.* 1995) is probably associated with the rate and extent of along-fibre variation in diameter (Orwin *et al.* 1988; Gourdie 1989; Hansford and Kennedy 1990*a*; Bray *et al.* 1993), the response of sheep which are genetically different in staple strength to changes in nutritional conditions is not known.

A number of studies have shown a strong negative genetic correlation between the total coefficient of variation in fibre diameter and staple strength (Lewer and Ritchie 1993; Lewer and Li 1994; Greeff *et al.* 1995, 1997), and that a large component of the overall variation in fibre diameter is that occurring along individual fibres (Quinnell *et al.* 1973; Sumner and Revfeim 1973; Dunlop and McMahon 1974; McKinley *et al.* 1976). It is therefore reasonable to hypothesise that sheep bred for high staple strength are less responsive to changes in nutritional conditions, and produce fibres with less variation in diameter along their length. Differences in responsiveness may be due to differences in the partitioning of nutrients to the wool follicles at certain times of the year and/or the response of the follicles to the available nutrients. To test this hypothesis, young Merino sheep, presumed to differ genetically in staple strength, were fed to produce changes in liveweight typical of those experienced in Mediterranean-type environments[1].

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[1] Data from this Chapter have been published in (*i*) *Proceedings of the 9th International Wool Textile Research Conference, Biella, Italy,* **2**; 143-51 (1995), (*ii*) *Australian Journal of Agricultural Research* **49**; 889-98 (1998); and (*iii*) *Australian Journal of Agricultural Research* **49** (in press) (1998).

2.2. Materials and methods

2.2.1. Sheep and their selection

The sheep used were selected from the first progeny from the staple strength selection flocks (Agriculture Western Australia). Full details regarding the establishment, maintenance and management of these flocks are given by Greeff *et al.* (1997), and only those relevant to the sheep used in the current experiment are given here. The establishment of the selection flocks commenced in 1991 with the screening of 3155 ewe hoggets and 1212 ram hoggets from seven properties running medium wool Merinos in the south west of Western Australia. For each of the sheep screened, liveweight and greasy fleece weight were measured at shearing, and a mid-side wool sample was taken and analysed for yield, mean fibre diameter, staple length and strength, and position of break. Only sheep with a mean fibre diameter less than the average for each flock, and that were within one standard deviation of the flock mean for liveweight, clean fleece weight, mean fibre diameter, staple length and position of break, were considered for selection. A linear regression of fibre diameter (independent variable) against staple strength was then fitted for each flock (average $r^2 = 0.20$), and a total of 50 ewes and five rams per selection flock were selected based on their deviation from the predicted staple strength. Sheep with the largest positive and negative residual terms were selected for the "sound" and "tender" flocks, respectively, and sheep with residuals close to zero were selected for the "control" flock.

The sheep selected were transferred to the Mount Barker Research Station ($38^{o}38'S$, $117^{o}32'E$), where the climate is characteristically Mediterranean, with an average annual rainfall of about 650 mm. They were then shorn and grazed together according to sex until February 1992, when the ewes were single sire mated to rams of similar staple strength. The lambs were born in July, weaned in October and shorn in November. There were 23 wether weaners available from the "sound" flock and 24 from the "tender" flock. Twenty weaners per flock [age 5 - 6 months, liveweight (mean ± s.e.m.) 33.2 ± 0.58 kg] were selected at random. The average staple strength of the parents of the weaners selected were 43.5 ± 1.20 and 11.5 ± 0.74 N/ktex for the "sound" and "tender" selection flocks, respectively. They did not differ significantly in clean fleece weight (2.5 ± 0.17 kg), mean fibre diameter (17.4 ± 0.14 μm) or staple length (90 ± 2.5 mm). Three weeks prior to the start of the experiment (4 January, 1993; day 0), the sheep were transported to the Waite Campus of The University of Adelaide, and housed indoors in individual pens.

2.2.2. Experimental design and nutritional treatments

The experimental design was a 2 x 3 factorial in a completely randomised block; 2 selection flocks x 3 nutritional treatments, with 6 or 7 sheep per treatment. All sheep were offered feed *ad libitum* during the first 56 days, and then sheep within each selection flock were allocated on the basis of liveweight to nutritional treatments designed to produce the following liveweight patterns; (*i*) liveweight maintenance for 287 days (LWM; n = 6); (*ii*) liveweight loss (-50 g/d) for 112 days, followed by liveweight gain (+50 g/d) for 112 days and liveweight maintenance for 63 days (LWL_1; n = 7); or (*iii*) liveweight loss (-100 g/d) for 112 days, followed by liveweight gain (+100 g/d) for 112 days and liveweight maintenance for 63 days (LWL_2; n = 7) (Fig. 2.1).

When sheep were fed to maintain or gain liveweight , the ration consisted of 75% (w/w) oat-based pellets (dry matter digestibility 67.1%, crude protein 13%) and 25% (w/w) lucerne chaff (dry matter digestibility 68.8%, crude protein 21.8%). The oat-based pellets and a low quality wheaten chaff (dry matter digestibility 49.5%, crude protein 8.2%) were offered in equal proportions during liveweight loss. When the quality of the ration was changed, the new ration was introduced gradually over a seven day period. All sheep were offered 25 g Siromin® per day, and water was available *ad libitum*.

The sheep were treated twice for nematode parasites (10 ml Panacur/sheep) and lice (10 ml clout®/sheep; Pittman-Moore Ltd, Sydney, Australia), and given regular intra-muscular injections of vitamins A, D and E (1 ml/sheep; FORTE, Heriot Agvert Pty Ltd, Rowville, Victoria). The sheep were shorn at the start and end of the 343 day experimental period.

2.2.3. Feed intake and liveweights

Feed intake was measured daily, and samples of each ration were stored at -20°C for subsequent analysis of dry matter digestibility and crude protein content as described by Thompson *et al.* (1994). Sheep were weighed once or twice per week prior to being offered their daily ration. If they had gained or lost more than the projected liveweight, the amount of ration offered was adjusted accordingly. Changes in wool-free liveweight with time (g/day) were estimated using linear regression analysis.

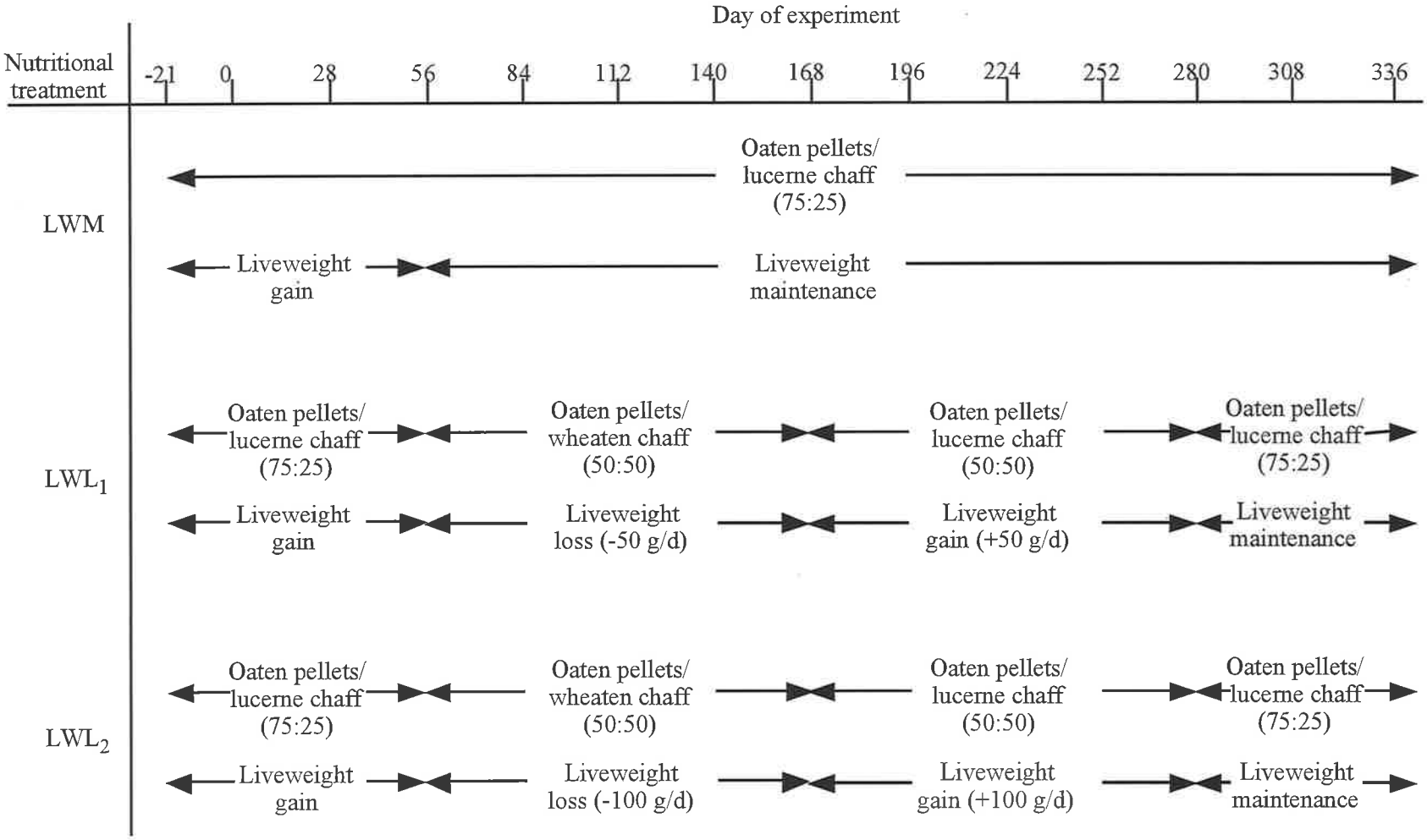

Figure 2.1. Time schedule showing the changes in diet and liveweight for the different nutritional treatments

2.2.4. *Wool growth rate and characteristics*

Wool growth rate per unit area of skin was estimated using the tattoo patch technique (Langlands and Wheeler 1968). The tattooed area (≈ 150 cm^2) was clipped using Oster clippers (no. 40 blade; Model A5-00, Oster Corp., Wisconsin, USA) at approximately 14 day intervals between days 7 and 343. A lag-period of seven days was allowed in all treatments, for the wool produced in the follicle bulb to emerge to a height above the skin which was accessible to the clippers. While the sheep was restrained on its side, the tattooed area was traced onto a transparency sheet and then measured on an image analysis system (Bioquant IV R&M, Biometrics Tennessee, USA).

The harvested wool was exposed to air (20 ± 2°C; 65 ± 2% RH) and weighed 24 h later to determine greasy wool weight. Wool samples were cleaned in hexane (3 x 10 min; Ace Chemical Co., Adelaide, Australia) and hot water (≈ 65°C; 2 x 10 min), dried to a constant weight at 70°C, and re-weighed after 20 min out of a desiccator. The rate of clean wool production (g/sheep/day) was calculated as described by Thompson *et al.* (1994). Wool clipped from the mid-side patch was also used to measure short-term changes in mean fibre diameter, the standard deviation of variation in fibre diameter, and hence the coefficient of variation in fibre diameter. The samples were mini-cored, and the diameter of 2000 fibres was measured using an Optical Fibre Diameter Analyser (Baxter *et al.* 1992).

Dyebands were applied at skin level to wool staples adjacent to the tattoo patch at 28 day intervals between days 63 and 287 (Williams and Chapman 1966). Dyebanded staples were removed immediately prior to shearing, and the length of wool between dyebands was measured on five staples per sheep. Staple length growth (μm/day) was calculated from the distance and time between dyebands. The rate of change in fibre diameter along the staple (μm/mm) was estimated from changes in mean fibre diameter of wool clipped from the mid-side patch at 14 day intervals in relation to staple length, assuming that staple length growth was linear during the 28 day period between consecutive dyebands. The rate of change in fibre diameter was estimated using linear regression analysis between: (i) the first dyeband and the point of minimum fibre diameter; and (ii) the point of minimum fibre diameter and the last dyeband (Appendix Fig. A2.1). It is important to note that the rate of change in fibre diameter estimated using this method was strongly correlated with that estimated from the fibre diameter of consecutive 2 mm staple

snippets ($r^2 = 0.82$, $P<0.001$), and the average changes in diameter along single fibres ($r^2 = 0.72$, $P<0.001$) (A.D. Peterson and A.N. Thompson unpubl. data).

2.2.5. Histology

Skin biopsies were taken under local anaesthetic (1.0 ml 2% Lignocaine hydrochloride with 0.0182 mg/ml adrenaline; Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) at 14 day intervals between days 56 and 280 from the left midside region of each sheep. This procedure ensured that samples were taken in close proximity to the point of break estimated by manually breaking dyebanded staples. The biopsies were taken with a trephine (1 cm diameter), removed with surgical forceps and scissors (Carter and Clarke 1957), fixed in 10% buffered formalin (Appendix, Table A2.1) for seven days, and then stored in 70% ethanol until they were processed.

2.2.5.1. Fibre cross-sectional shape

Fibre shape was measured on days 0, 112, 168, 224 and 280, and at the time estimated to correspond with the position of break along the staple. The biopsies were trimmed of excess wool, placed in individual cassettes (Tissue-Tek III, Miles Laboratories Inc. Naperville, Illinois, USA) and processed as described in the Appendix (Table A2.2) using an automatic tissue processor (Automatic Tissue Processor SE400, Shandon Scientific Co. Pty Ltd., London, England). The samples were embedded in paraffin using a Tissue Tek II Tissue Embedding Centre (Model 4604, Miles Laboratories Inc., Naperville, Illinois, USA), and sectioned on a rotary microtome (Leitz 1512, Ernst Leitz, Wetzlar, GmbH) at 8 μm intervals, transverse to the plane of the follicle at the level of the sebaceous glands.

The sections were floated on water (40°C) and collected onto poly-L-lysine coated microscope slides (poly-L-lysine slide adhesive solution; Sigma Diagnostics, St. Louis, USA). They were then dewaxed at 60°C for 30-60 min and stained with Methylene Blue (Clarke and Maddocks 1965; Appendix Table A2.3). Three replicates of 30 follicles per sample (i.e. 90 follicles/sheep) were selected at random and microscopically examined at 1520 x magnification. Total cross-sectional area (CSA) of the fibre was traced with the aid of a camera lucida attached to a microscope, and the average circularity of the fibre determined utilising an image analysis system (Bioquant IV, R&M Biometrics, Tennessee, USA). Circularity was obtained from the shape factor (SF) as: $SF = 4\pi (CSA/P^2)$, where P is the perimeter of the fibre cross-section; SF ranges from 0 to 1. For the

samples taken at the position of break, the diameter of the major (FD_{major}) and minor (FD_{minor}) fibre axis were measured, and the ellipticity ratio (ER) calculated as follows: ER = FD_{major}/FD_{minor}.

2.2.5.2 Follicle activity state

The activity state of wool follicles was assessed on the basis of their morphology in transverse skin sections (Nixon 1993). Follicle activity was estimated during the period four to six weeks either side of the position of break along the staple; a period which included a well defined peak in the total proportion of inactive follicles. The biopsies were processed and embedded as described in section 2.2.5.1, and were sectioned at 8 μm intervals transverse to the plane of the follicle between the sebaceous glands and the bulbs. After rehydration, every 5th tissue section was then stained using a modification of the SACPIC method (Auber 1950; Appendix Table A2.4).

Slides were then dehydrated and cover-slipped, and three replicates of 300 follicles per sample (ie. 900 follicles/sheep) were selected at random and microscopically examined at 400 times magnification and scored as normal or abnormal. Follicles considered to be normal had circular fibres, regular inner root sheath and randomly arranged nuclei in the outer root sheath (Plate 2.1*a*), whereas abnormal follicles were characterised by: (*i*) nuclei aligned on the periphery of the outer root sheath (Plate 2.1*b*); (*ii*) irregular fibre shape or staining of the inner root sheath (Plate 2.1*c*); or (*iii*) compact hair germ cells and no fibre present (Plate 2.1*d*). The maximum number of abnormal follicles for an individual sheep at any given time was generally observed at a level approximately 80-120 μm below the sebaceous glands.

2.2.6. Total wool weight and characteristics

At shearing, the total weight of greasy wool was recorded, and a wool sample (≈ 150 g) was taken adjacent to the tattoo patch for determination of (*i*) yield; (*ii*) mean fibre diameter and total standard deviation of variation in fibre diameter; (*iii*) staple length; (*iv*) staple strength; and (*v*) position of break. The methods used have been listed by Thompson *et al.* (1994).

Plate 2.1. Photomicrographs of follicles sectioned in the transverse plane, showing the various follicle abnormalities. Follicles considered to be normal (*a*) had circular fibres, regular inner root sheath and randomly arranged nuclei in the outer root sheath. Abnormal follicles were classified as either: (*b*) nuclei aligned on the periphery of the outer root sheath; (*c*) irregular fibre shape or staining of the inner root sheath; and (*d*) compact hair germ cells and no fibre present. Scale bar indicates 20 μm.

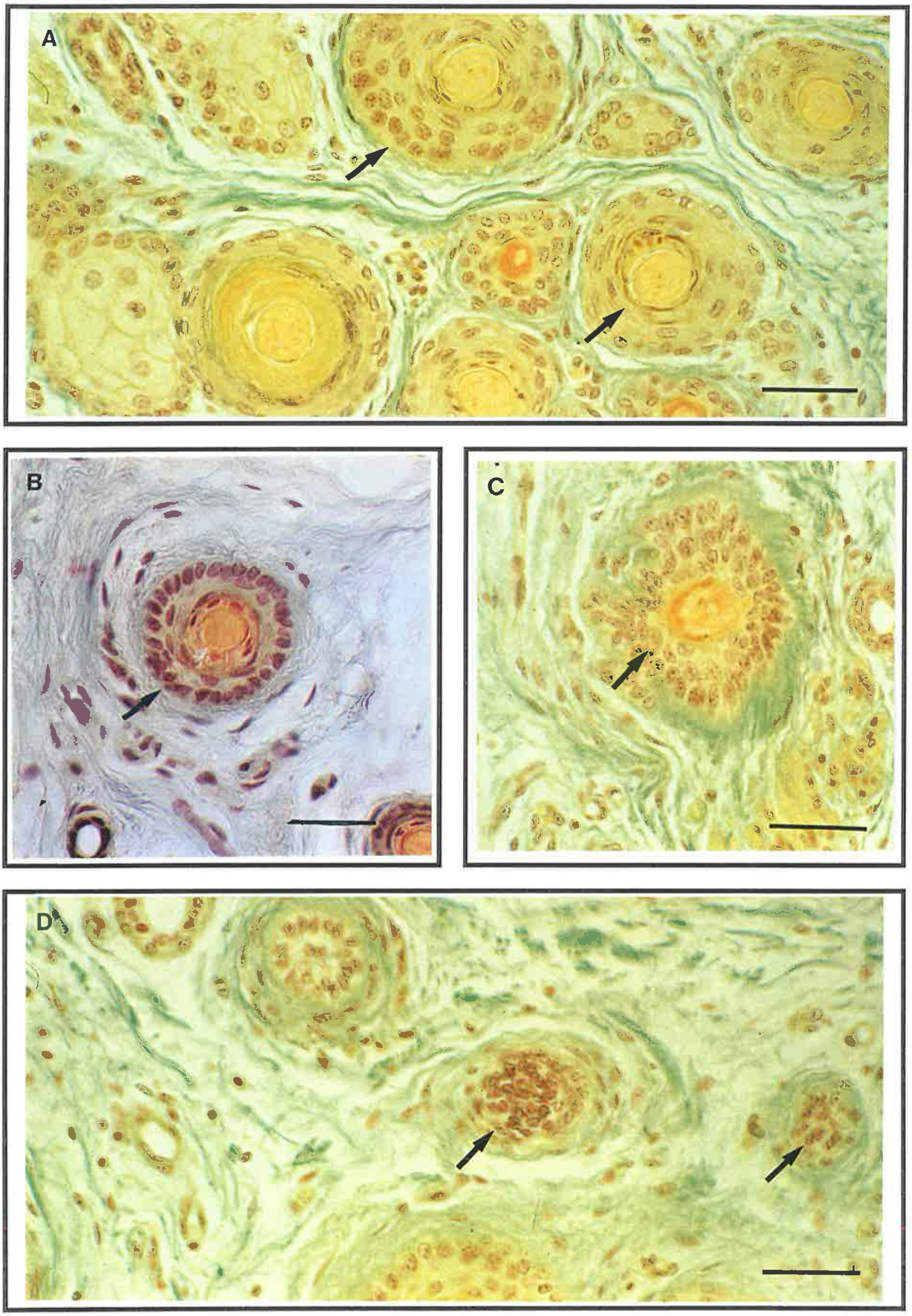

Plate 2.1.

2.2.7. Statistical analyses

Two-way analysis of variance was conducted with selection flock, nutrition and the 'selection flock by nutrition' interaction as the sources of variance. Raw data for follicle abnormalities were log transformed prior to statistical analysis to stabilise the variance; zero proportions were counted as (X+1) (Snedecor and Cochran 1991). The strength of the relationships between the various fibre, follicle and staple characteristics were initially tested using simple linear regression analysis. Relationships within nutritional treatments were not considered. As many of the independent parameters measured were strongly co-correlated, stepwise forward multiple regression analysis was then employed to eliminate these effects. The most highly correlated variable was fitted first, and then individual variables were added one at a time to see which had the greatest effect on the proportion of explained variance (i.e. adjusted r^2 value). All statistical analyses were performed using the statistical package SuperANOVA^{TM} (Type I Sums of Squares; Abacus Concepts Inc., Berkeley, California, USA).

2.3. Results

2.3.1. Feed intake and liveweight change

The changes in feed intake (Fig. A2.2) and liveweight (Fig. 2.1) were as planned, and as they did not differ significantly between sheep from the different selection flocks, the data for the two flocks were combined. After the initial gains in liveweight during *ad libitum* feeding, sheep from the LWM group maintained a relatively constant liveweight throughout the remainder of the experiment, whereas liveweight of sheep from the LWL_1 and LWL_2 groups decreased ($P<0.05$) at 66 and 109 g/day until day 168, and then increased ($P<0.05$) until day 280 at 53 and 92 g/day, respectively. After day 280 there were no significant differences in liveweight between treatments.

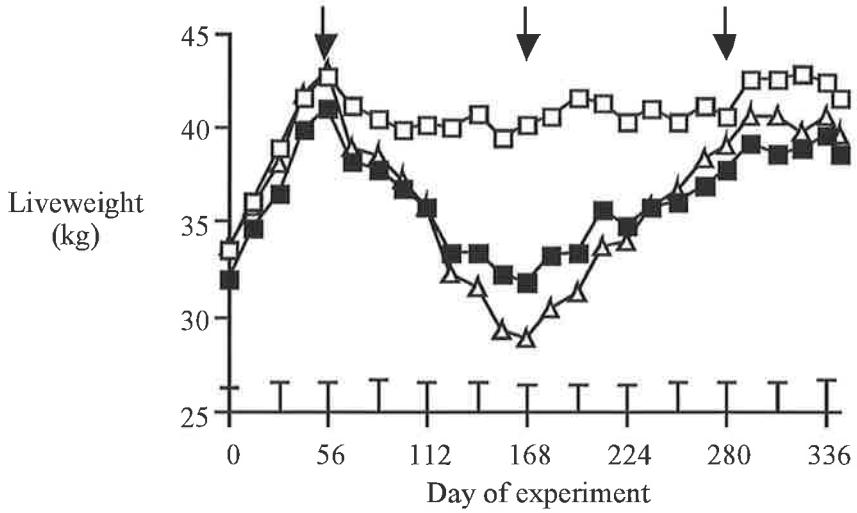

Figure 2.1. Liveweights of sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for the "sound" and "tender" staple strength selection flocks were combined, and points represent treatment means at 14 day intervals. Error bars on the X-axis denote s.e.d, and arrows denote the times when nutrition was altered.

2.3.2. Wool growth rates

During *ad libitum* feeding there were no significant differences in the rate of wool growth between treatment groups. The only difference in wool growth rates between selection flocks thereafter was that sheep from the "sound" flock grew more ($P<0.05$) wool than sheep from the "tender" flock from day 203 onwards (Fig. 2.2*a*). This difference in wool growth rate between selection flocks was due to the response of sheep from LWL_1 and LWL_2 treatments, although the selection flock by nutrition interaction was generally not significant.

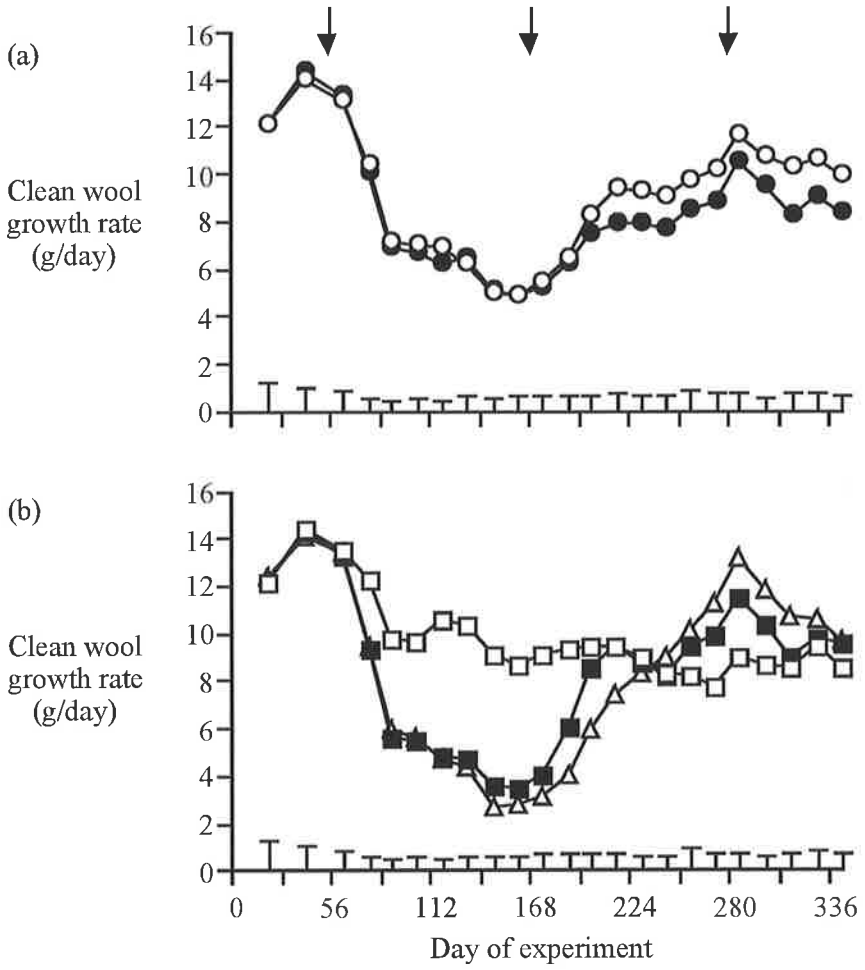

Figure 2.2. Wool growth rates for sheep from (a) "sound" (○, n = 20) and "tender" (●, n = 20) staple strength selection flocks, and (b) nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (△, n = 14). Points represent treatment means at the end of the designated growth interval. Error bars on the X-axes denote s.e.d, and arrows denote the times when nutrition was altered.

Nutritional treatments had large effects on wool growth rates (Fig. 2.2*b*). Wool growth rates for sheep which maintained liveweight declined ($P<0.05$) by 35% over the duration of the experiment, and reached a minimum of 8.6 g/day. In comparison, wool growth rates for sheep from the two liveweight loss treatments declined ($P<0.05$) by 75 to 80%, and reached a minimum near the end of the liveweight loss period of 3.5 and 2.7 g/day, respectively. These sheep responded to the changes in nutrition on day 168 and wool growth rates increased ($P<0.05$) to levels which at times exceeded that for the LWM group. The effects of selection flock and nutritional treatments on staple length growth were similar to those described for wool growth rates.

2.3.3. Along-fibre variation in diameter

Sheep from the "sound" selection flock consistently produced wool with a higher mean fibre diameter than those from the "tender" flock, although the difference was not statistically significant (P<0.10) during the period ± 28 days from the point of minimum fibre diameter (Fig 2.3*a*).

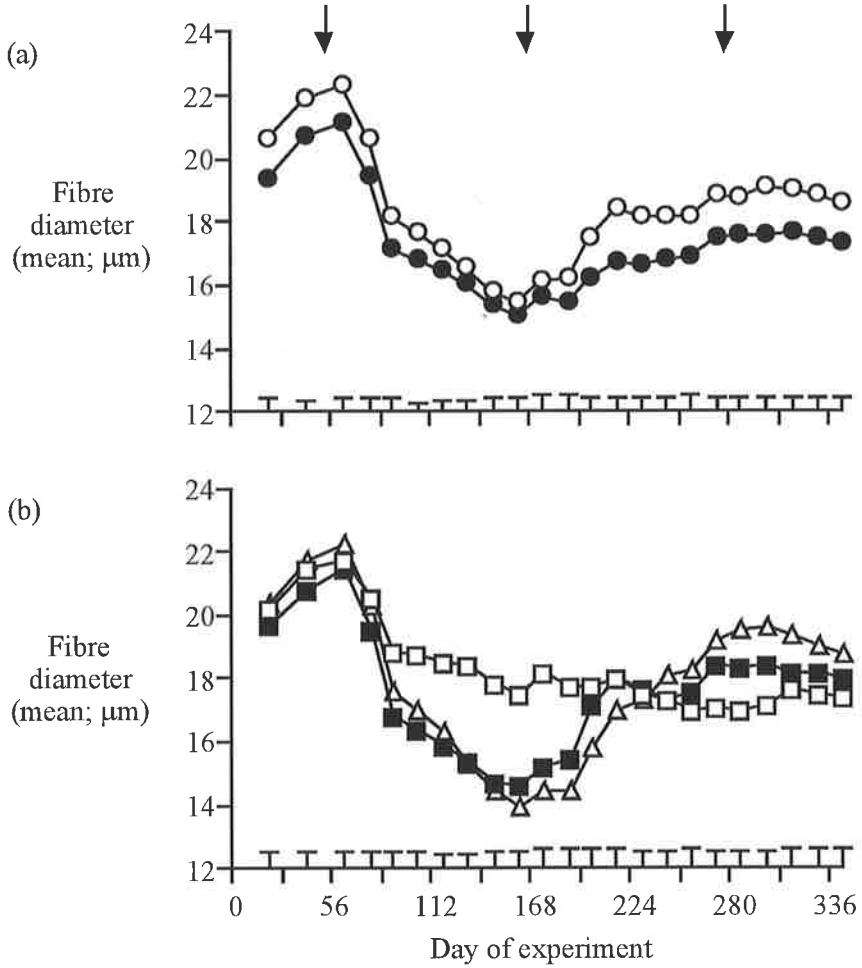

Figure 2.3. Fibre diameter of patch wool produced by sheep from (a) "sound" (○, n = 20) and "tender" (●, n = 20) staple strength selection flocks, and (b) nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). Points represent treatment means at the end of the designated growth interval. Error bars on the X-axes denote s.e.d, and arrows denote the times when nutrition was altered.

The effects of nutritional treatments on changes in mean fibre diameter were similar to those described for wool growth rates (Fig. 2.3*b*). Mean fibre diameter of wool from the LWM sheep declined (P<0.05) by about 23% from the start of differential feeding and reached a minimum of 16.8 μm. This compares to the liveweight loss treatments where mean fibre diameter decreased

(P<0.001) by 35 to 40%, and reached a minimum of 14.5 and 13.9 μm, respectively. The mean fibre diameter of wool from these sheep responded to the increase in nutrition on day 168 and was generally greater (P<0.05) than that for the LWM group from day 273 onwards. There was no significant interaction between selection flock and nutrition.

Taken together, the estimated rate and extent of along-fibre variation in diameter, determined from wool clipped from the midside patch, generally differed significantly between selection flocks and nutritional treatments (Table 2.1). Sheep from the "sound" selection flock produced wool with more along-fibre variation in diameter than those from the "tender" flock, although the differences were relatively small compared to the effects of nutritional treatments. Sheep fed to maintain liveweight produced wool with smaller (P<0.001) and less rapid (P<0.001) changes in fibre diameter than was produced by sheep which lost and then gained liveweight. Minimum wool growth rate and the various measures of along-fibre changes in diameter were strongly correlated (Appendix, Table A2.5). A lower minimum wool growth rate or fibre diameter was associated with more (P<0.05) along-fibre variation in diameter.

Table 2.1. Estimated variation in fibre diameter (FD)† along staples from sheep in the "sound" and "tender" staple strength selection flocks at three levels of nutrition.

a, b, c: different superscripts within selection flock or nutrition comparisons differ at P< 0.05

Characteristic	Selection flock		Nutritional treatment		
	"sound"	"tender"	LWM	LWL_1	LWL_2
$^{A}FD_{range}$ (μm)	7.4^{a}	6.7^{a}	5.5^{a}	7.0^{b}	8.5^{c}
$^{A}FD_{stdev}$ (μm)	2.14^{a}	1.86^{b}	1.53^{a}	1.95^{b}	2.46^{c}
$^{A}FD_{cv}$ (%)	11.1^{a}	10.2^{a}	8.2^{a}	10.5^{b}	12.9^{c}
ΔFD_{dec} (μm/mm)	0.29^{a}	0.25^{b}	0.08^{a}	0.33^{b}	0.40^{c}
ΔFD_{inc} (μm/mm)	0.13^{a}	0.10^{b}	0.06^{a}	0.10^{a}	0.17^{b}

† *Range ($^{A}FD_{range}$) in FD; along-fibre variation in diameter ($^{A}FD_{stdev}$ and $^{A}FD_{cv}$); rate of decrease (ΔFD_{dec}) and increase (ΔFD_{inc}) in fibre diameter.*

2.3.4. Between-fibre variation in diameter

The total variation in fibre diameter within wool samples clipped from the mid-side at 14 day intervals was assumed to reflect the extent of between-fibre variations in diameter, because there

were no differences (P>0.05) in short term diameter fluctuations between the staple strength genotypes (A.D. Peterson and A.N. Thompson unpubl. data). The estimated variation in diameter between fibres was consistently greater (P<0.05) for samples from sheep from the "tender" selection flock than the "sound" flock (Fig. 2.4*a*). The standard deviation of variation in fibre diameter differed significantly between nutritional treatments during the period from days 105 to 190, when it was lower (P<0.05) in wool produced by sheep from the LWL_1 and LWL_2 treatments than in wool from LWM sheep (Fig 2.4*b*).

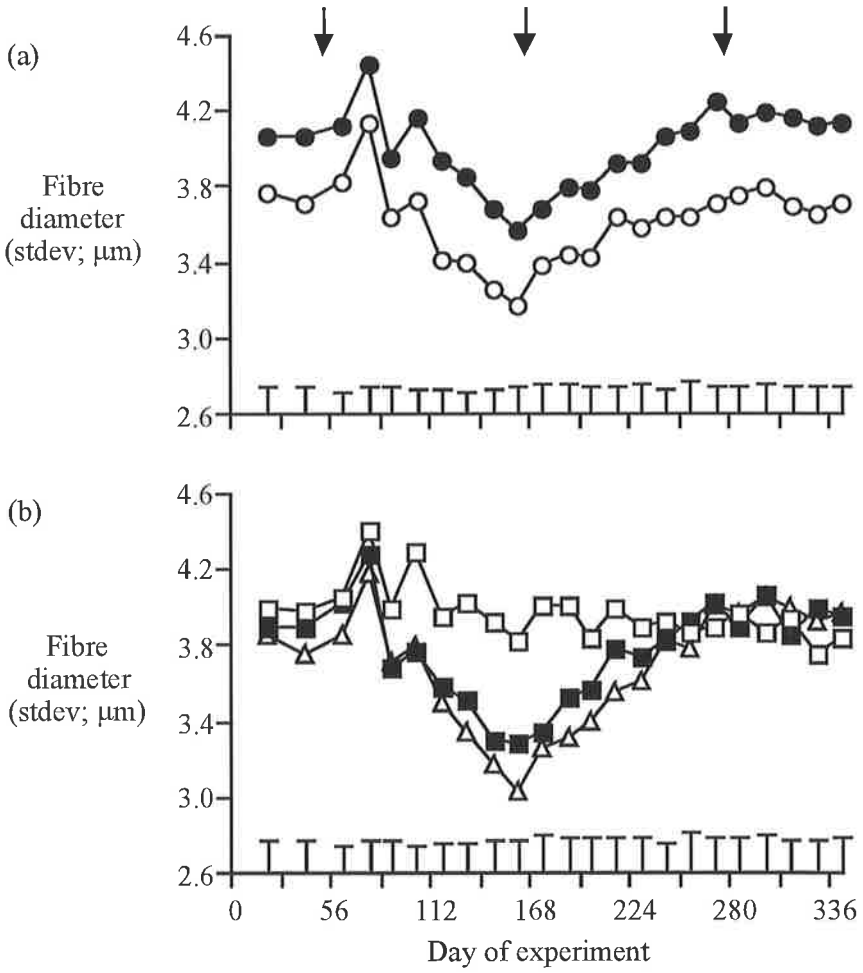

Figure 2.4. Standard deviation of variation (stdev) in diameter of patch wool produced by sheep from (a) "sound" (○, n = 20) and "tender" (●, n = 20) staple strength selection flocks, and (b) nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). Points represent treatment means at the end of the designated growth interval. Error bars on the X-axes denote s.e.d, and arrows denote the times when nutrition was altered.

2.3.5. Fibre cross-sectional shape

There were no significant differences in the average shape factor (0.95 ± 0.001) or ellipticity (1.16 ± 0.006) of fibre cross-sections between sheep from the different selection flocks or nutritional treatments. These values indicate that, on average, fibres were very circular. As a consequence, there was no significant relationship between fibre diameter and shape, either between different sheep or between individual fibres from the same sheep, and no attempt has been made to relate differences in fibre shape to other fibre and staple characteristics.

2.3.6. Follicle activity state

Completely inactive follicles which had already shed their fibre accounted for more than 85% of all follicle abnormalities observed, and for clarity of presentation are hereafter referred to as shutdown follicles. The proportion of shutdown follicles were similar for sheep from both staple strength selection flocks ($P>0.05$), so the data for the two flocks were combined. The proportion of shutdown follicles peaked at the time corresponding to the position of break along the staple, and was significantly ($P<0.001$) affected by nutrition (Fig. 2.5).

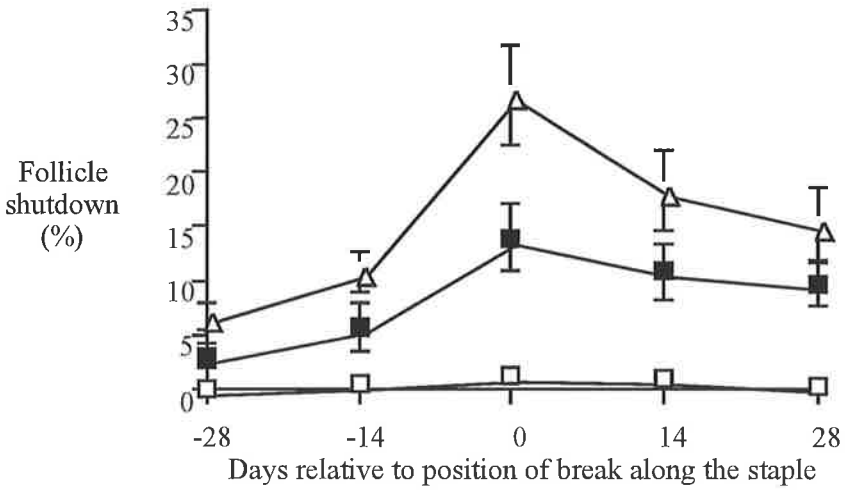

Figure 2.5. Changes in the percentage of shutdown follicles in relation to the position of break along the staple for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined, and error bars denote s.e.m.

The average proportion of shutdown follicles at the point of break was about 27% for sheep which lost liveweight at 100 g/day compared to only 1% for sheep which maintained liveweight. The maximum proportion of shutdown follicles for individual sheep varied from 0 to 53%, and it

is these values which were used as the variable in determining relationships with other parameters. For sheep from the 2 liveweight loss treatments, the proportion of shutdown follicles recovered rapidly after the position of break in response to improved nutrition.

A significant logarithmic relationship existed between fibre diameter measured at the point of break along the staple and the maximum percentage of shutdown follicles (r^2 = 0.45; P<0.001; Fig. 2.6). Follicle shutdown and the variation in response between sheep, increased when fibre diameter was less than about 15 μm.

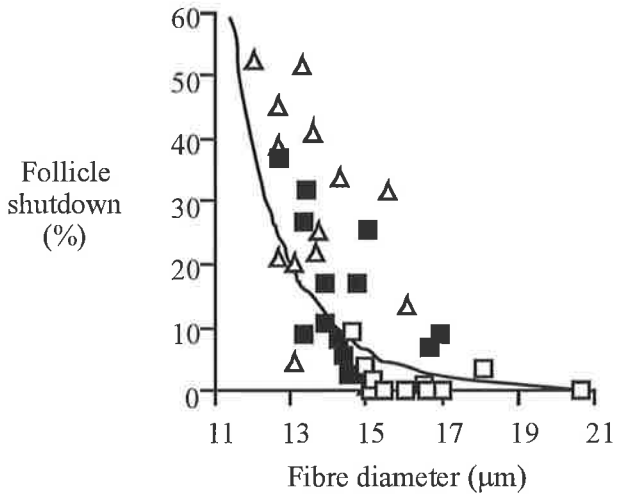

Figure 2.6. Fibre diameter in relation to the proportion of shutdown follicles at the point of break along the staple for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined. Points represent means for individual sheep.

2.3.7. Fleece weight and characteristics

Clean wool weight (P=0.09), yield (P=0.07) and staple strength (P=0.06) all tended to be higher for sheep from the "sound" selection flock than the "tender" flock (Table 2.2). Wool from "sound" sheep also had a higher mean fibre diameter (P<0.001), but a lower total standard deviation (P<0.05) and coefficient of variation in fibre diameter (P<0.001) than wool from "tender" sheep. Staple length and position of break did not differ significantly between selection flocks.

Sheep from the LWM nutritional treatment produced more wool (P<0.01) than those from both the LWL_1 and LWL_2 treatments, and this wool had a higher staple length (P<0.001) and strength

($P<0.001$), and a position of break closer ($P<0.001$) to the butt of the staple. The weight and characteristics of wool produced by sheep from the liveweight loss treatments were similar, and the mean and total variation in fibre diameter did not differ significantly between nutritional treatments. The response of sheep from the selection flocks to the nutritional treatments were variable for yield, staple length and position of break.

Table 2.2. Total clean wool weight (CWW) and characteristics† of mid-side fleece samples taken at shearing from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition

a, b: different superscripts within selection flock or nutrition comparisons differ at $P< 0.05$

Characteristic	Selection flock		Nutritional treatment		
	"sound"	"tender"	LWM	LWL_1	LWL_2
CWW (kg)	3.21^a	2.98^a	3.42^a	2.94^b	2.93^b
Yield (%)	75.7^a	72.4^a	72.5^a	75.1^a	74.3^a
FD_{mean} (μm)	18.8^a	17.5^b	18.4^a	17.9^a	18.2^a
$^TFD_{stdev}$ (μm)	4.1^a	4.4^b	4.2^a	4.3^a	4.3^a
$^TFD_{cv}$ (%)	21.9^a	25.3^b	22.7^a	24.2^a	23.8^a
SL (mm)	98^a	98^a	104^a	96^b	94^b
SS (N/ktex)	26.9^a	21.9^a	34.9^a	22.1^b	16.7^b
POB (% wt from tip)	55.0^a	57.3^a	72.3^a	48.6^b	49.8^b

† *Mean fibre diameter (FD_{mean}); total variation in fibre diameter ($^TFD_{stdev}$ and $^TFD_{cv}$); staple length (SL); staple strength (SS); and position of break (POB).*

2.3.8. Determinants of staple strength

Minimum fibre diameter was the single most important determinant of staple strength, accounting for 66% of the total variance in staple strength generated by selection and nutrition (Fig. 2.7*a*). There were also strong, statistically significant ($P<0.001$) relationships between other measures of along-fibre changes in diameter and staple strength (Figs. 2.7*b* and 2.7*c*), and between the proportion of shutdown follicles and staple strength (Fig. 2.7*d*). While the coefficients for these regressions did not differ significantly between flocks, for similar along-fibre variation in

diameter and proportion of shutdown follicles, there was a tendency for "sound" sheep to produce stronger wool than "tender" sheep.

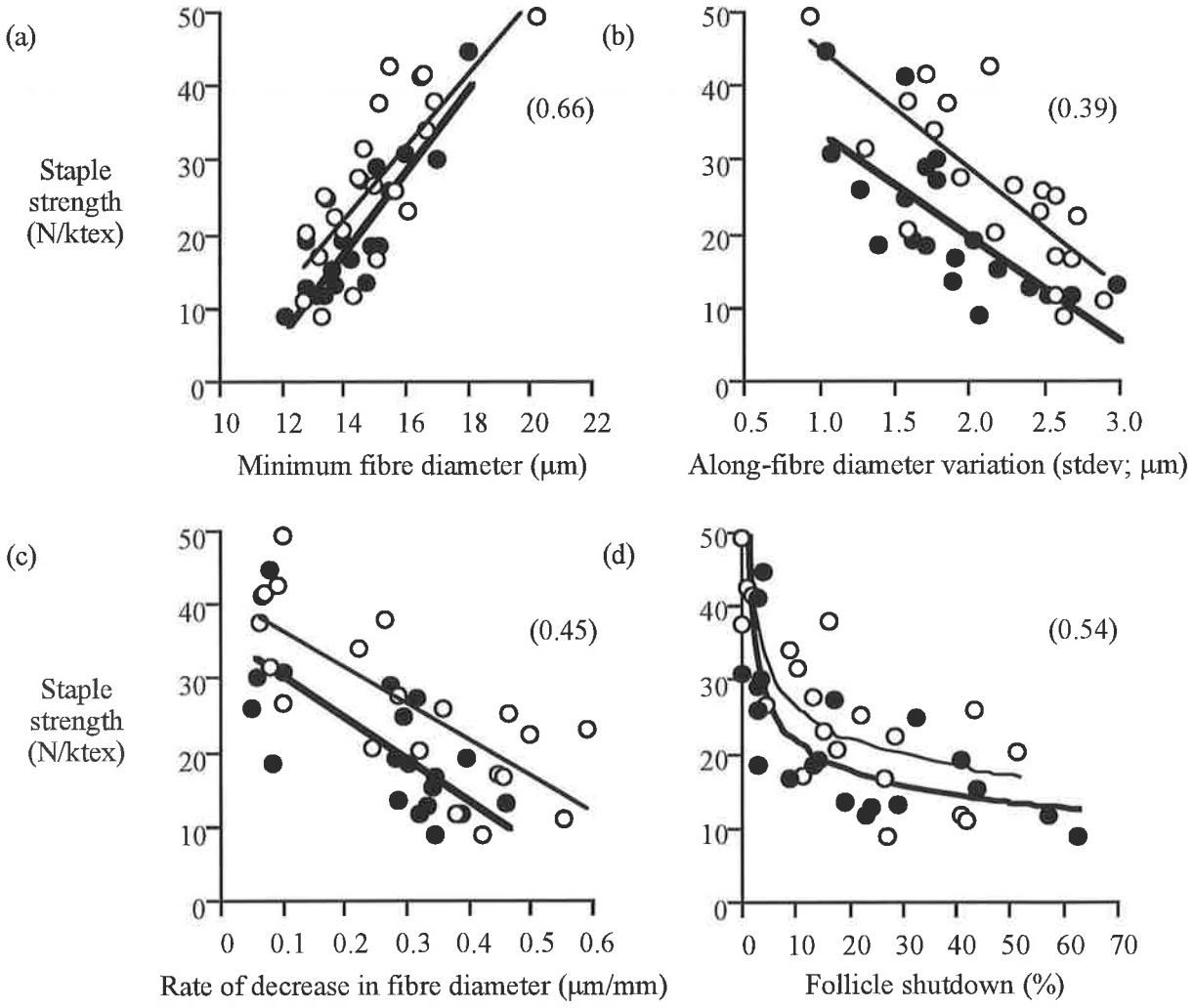

Figure 2.7. Staple strength in relation to (*a*) minimum fibre diameter; (*b*) along-fibre standard deviation of variation in diameter; (*c*) rate of decrease in diameter to the point of minimum fibre diameter along the staple; and (*d*) the proportion of shutdown follicles at the point of break along the staple. Samples were from sheep from the "sound" (○, ——— , n = 20) and "tender" (●, ▬▬ , n = 20) staple strength selection flocks at three levels of nutrition. Points represent means for individual sheep, and adjusted r^2 values for all data combined are shown in parentheses.

Minimum fibre diameter (FD_{min}; μm) and the rate of decrease in fibre diameter (ΔFD_{dec}; μm/mm) in combination explained 72% of the variance in staple strength (SS; N/ktex) between sheep. The subsequent addition of a term for the standard deviation of variation in diameter

between-fibres ($^{B}FD_{stdev}$; μm) measured at the point of break improved the explained variance in staple strength to 80% ($P<0.001$). The following model provided the best fit to the data:

$$SS = -21.7\ (\pm\ 9.81) + 5.0\ (\pm\ 0.63)\ FD_{min} - 18.8\ (\pm\ 6.27)\ \Delta FD_{dec} - 6.5\ (\pm\ 1.72)\ {}^{B}FD_{stdev}$$

Multiple regression analysis revealed that the addition of a term for follicle shutdown after along- and between fibre variations in diameter did not remove any additional variance in staple strength.

2.4. Discussion

2.4.1. Hypothesis

Most of the variance in staple strength between individual sheep in this experiment was explained by the variation in diameter along and between individual fibres ($r^2 = 0.80$), but the mechanisms responsible for nutritionally-induced and genetic differences in staple strength were not the same. Nutrition significantly influenced staple strength by affecting along-fibre changes in diameter, whereas genetic differences in staple strength, at least as far as they are represented by the sheep used here, were largely attributable to between-fibre variations in diameter. There was no evidence to support the hypothesis that along-fibre changes in diameter also contribute to genetic differences in staple strength.

2.4.2. Nutritional effects on the components of staple strength

Sheep fed to maintain liveweight produced stronger wool than sheep which lost and then gained liveweight (Table 5.2). The increase in staple strength in response to more uniform nutritional conditions is consistent with a number of other experiments (Rowe *et al.* 1989; Thompson and Curtis 1990; Gardner *et al.* 1993; Peter *et al.* 1993; Doyle *et al.* 1995), and was most closely associated with a higher minimum fibre diameter (Figs. 2.3 and 2.7). The variance in staple strength explained by minimum fibre diameter in this experiment was within the range (50-75%) reported in other pen experiments where nutrition has been used to manipulate staple strength (Orwin *et al.* 1988; Gourdie 1989; Hansford and Kennedy 1990*a*; Bray *et al.* 1993), despite differences in the breed, genotype and or physiological status of the sheep used, and the nutritional conditions prior to and during the experiments. The association between minimum fibre diameter and staple strength is sometimes weaker under grazing conditions (Bigham *et al.* 1983, Hunter *et al.* 1983; Fitzgerald *et al.* 1984; Hawker and Littlejohn 1989; Denney 1990),

where the effects of environmental factors such as short-term stressors on the other components of staple strength presumably become more important.

The slope of the relationship between minimum fibre diameter and staple strength indicated that an increase in minimum fibre diameter of 1 μm was associated with an increase in staple strength of about 5 N/ktex, and this agrees closely with the 4.8 N/ktex increase reported by Bray *et al.* (1993). From a causative point of view, it seems most likely that minimum fibre diameter influenced staple strength by affecting the amount of material available to bear the load, since the average intrinsic strength of individual fibres had a relatively small effect on the variance staple strength between wool samples from the current experiment (see Chapter 3; Thompson *et al.* 1995). Multiple regression analysis revealed that differences in the rate of change in diameter along the fibre explained an additional 6% of the variance in staple strength not explained by co-correlated changes in minimum fibre diameter. The effects of rate of change in diameter on staple strength were probably due to its influence on the average linear density of the staple.

Follicle shutdown in response to adverse nutritional conditions is consistent with a number of other experiments (Lyne 1964; Ryder 1967; Wilson and Short 1979), as is the association between follicle shutdown and low staple strength (Fig. 2.7*d*) (Schlink *et al.* 1992; Schlink and Dollin 1995; Hynd *et al.* 1998). The maximum levels of follicle shutdown in this experiment were as high as 50-60% for some individual sheep, and this exceeds those recorded previously. This may reflect the severity of the nutritional conditions and the age of sheep used in the current experiment, as all previous experiments have used older animals which may be less susceptible to follicle shutdown because of their greater fat and protein reserves. The results suggest that in this experiment nutritionally-induced follicle shutdown was primarily a consequence of fibre diameter being reduced to an unsustainable level (Fig. 2.6), and they imply that the biological mechanisms governing fibre shedding are likely to be the same as those responsible for changes in diameter. As a consequence, however, fibre shedding failed to remove any variance in staple strength additional to the 80% already accounted for by along- and between-fibre changes in diameter. It is concluded that fibre shedding *per se* did not contribute significantly to the differences in staple strength between sheep in this experiment.

2.4.3. Genetic effects on the components of staple strength

Wool produced by sheep bred for high staple strength had significantly less variation in diameter between individual fibres than wool from sheep bred for low staple strength (Fig. 2.4). This finding is consistent with Bray *et al.* (1995) and Adams *et al.* (1997), even though they measured fibre diameter variability on 3-6 weeks wool growth compared to 14 days in this experiment. Whilst it is acknowledged that even these estimates of between-fibre variation in diameter would include some variance associated with changes in diameter along fibres, recent measurements have shown no differences in diameter fluctuations along 2 mm fibre segments between the staple strength genotypes (A.D. Peterson and A.N. Thompson unpubl. data). As there were also no differences in fibre ellipticity between the staple strength genotypes, it is concluded that substantial differences in between-fibre variation in diameter exist between these flocks of sheep selected for and against staple strength while maintaining mean fibre diameter.

Fleece wool from sheep from the "sound" selection flock also had significantly less total variation in fibre diameter than wool from those in the "tender" flock (Table 2.2). This is consistent with both the Merino (Greeff *et al.* 1997; Adams *et. al.* 1997) and Romney (Bray *et al.* 1995; Woods *et al.* 1995) staple strength selection flocks. While the nutritional treatments we imposed in this experiment generated large along-fibre variations in diameter (Fig. 2.3*b*), this was not reflected in the total variation in fibre diameter of fleece sub-samples. In other words, measurement of fibre diameter variation in midside fleece samples largely reflects between-fibre variations in diameter, and as such may be a useful indirect selection criterion for staple strength (Lewer and Ritchie 1993). However, it may also mask considerable nutritionally induced variations in diameter occurring along fibres.

Fibre diameter and length are both associated with the size of the follicle bulb (Hynd 1994*b*), and tend to change together in response to simple changes in nutrition (Hynd 1994*a*). It follows that high staple strength sheep with a low between-fibre variation in diameter will also have a low fibre length variation. The strength of a staple, expressed as peak force to break, will be greater when the length of individual fibres is more uniform as a greater proportion of fibres will become load-bearing and break at the same time, relative to a staple comprising fibres which are highly variable in length. This work does not discriminate between the effects of variation in fibre length or diameter on staple strength, but length variations are likely to be important. Fibre length variation does account for some of the differences in staple strength between the Romney staple strength

selection flocks (Bray *et al.* 1995). As fibre diameter and extensibility are positively correlated (Collins and Chaikin 1968), it is also possible that wool with a low between-fibre variation in diameter will have a smaller range in extensibility between-fibres, and this would influence staple strength via a similar mechanism to that described for fibre length variation.

A somewhat surprising result in this experiment was that rather than being less susceptible to nutritional changes, if anything the "sound" sheep had slightly more variation in fibre diameter along the staple than "tender" sheep. Whilst differences in feed intake ($P<0.10$) between the flocks during the second half of the experiment undoubtedly contributed to this difference in along-fibre variation in diameter, other factors may also have been involved. For instance, as the differences in wool growth between selection flocks were only apparent for sheep which had previously lost considerable liveweight, and were not associated with any differences in liveweight change, inherent differences in the partitioning of nutrients to the wool follicles and/or the response of the follicles to the available nutrients cannot be discounted. Irrespective of the cause however, these differences in along-fibre variation in diameter were unlikely to explain the 25% differences in staple strength between the selection flocks, since the effects on staple strength of the 0.5 μm differences in minimum fibre diameter would be more than out weighed by the 1.3 μm differences in mean fibre diameter, and thus the average linear density of the staple.

The results presented here appear to be at odds with the findings of Woods *et al.* (1995), Greeff *et al.* (1997) and Adams *et al.* (1997) who found that Romney or Merino sheep selected for high staple strength have a less pronounced seasonal wool growth pattern and/or fibre diameter profile than those selected for low staple strength. In the case of the Romney flocks, this may reflect a greater dependence on differences in mean fibre diameter between the selection flocks (Bray *et al.* 1993), whereas the selection flocks used here had a similar mean fibre diameter. Some of the differences between the present experiment and those reported by Greeff *et al.* (1997) and Adams *et al.* (1997) may be associated with the age of the sheep used, and the nutritional conditions under which the sheep were maintained. They both used older animals and the possibility that there is an interaction for along-fibre variation in diameter between genotype and age or physiological status cannot be discounted. It may be that differences in nutrient partitioning and thus along fibre variation in diameter are less obvious in sheep with a strong demand to grow, but such speculation awaits examination. The sheep in the experiments reported by Greeff *et al.* (1997) and Adams *et al.* (1997) also grazed under 'normal' conditions or had unlimited access to food for most of the time, whereas in the current experiment the availability of

feed was always restricted during differential feeding. The opportunity to express potential differences in feed intake and wool growth between selection flocks would therefore have been eliminated or reduced in this experiment. Despite these inconsistencies, our conclusion that between-fibre differences in diameter, and therefore presumably length and or extensibility, are responsible for most of the differences between sheep selected for and against staple strength, agrees with Woods *et al.* (1995) and Adams *et al* (1997).

There was no evidence to suggest that the sheep used in this experiment which were bred for differences in staple strength differed in their susceptibility to follicle "shutdown" and fibre shedding. This is consistent with the conclusion that differences in fibre properties *per se* are not responsible for the differences in staple strength between the flocks. Schlink *et al.* (1996) also found no differences in the percentage of shed fibres in fleeces from sheep chosen for phenotypic differences in staple strength, and Ansari-Ranani and Hynd (1996) found no differences between sheep selected for extreme differences in fibre diameter variability.

It was interesting that the average rate of wool growth was similar for both selection flocks during declining nutrition, and yet the wool produced by sheep from the "sound" flock was broader than that from sheep in the "tender" flock. This suggests that either the fibre length to diameter ratio and/or the density of fibre-producing follicles in the skin may differ between the selection flocks. While these hypotheses are yet to be adequately tested, Schlink *et al.* (1996) reported that sheep phenotypically selected for high staple strength had a lower average length/diameter ratio than sheep selected for low staple strength. If there are differences in fibre length between the selection flocks, but not staple length, it follows that there may also be differences in crimp frequency and/or definition between the flocks. This would be consistent with Bray *et al.* (1995) who reported that the high staple strength Romney selection flocks had a lower fibre length to staple length ratio, but greater regularity in fibre crimp compared to the low staple strength selection flock.

2.5. Conclusions

It appears that the causes for nutritionally-induced and genetic differences in staple strength are not the same, being differences in along-fibre and between-fibre variations in diameter, respectively. From a practical point of view, as the nutritional and genetic effects on staple strength are additive, this means that they should be exploited concurrently to most effectively

reduce the incidence of tender wool production in Mediterranean environments. However, the data presented here suggest that selection for high staple strength reduces the variability in properties between fibres, rather than the average properties of individual fibres *per se*. Indeed Thompson *et al.* (1995) reported for these same wool samples that the average strength of individual fibres was 92.9 and 92.4 N/ktex for sheep from the "sound" and "tender" selection flocks, respectively, and that there were no significant differences between selection flocks in the total energy to break the staple (1.10 and 1.12 J/ktex for the "sound" and "tender" selection flocks, respectively). Total energy to break the staple is theoretically independent of between-fibre variations in length and extensibility (de Jong *et al.* 1985). It therefore remains to be seen whether genetic improvements in staple strength will result in the same improvements in processing performance which would be predicted from staple strength alone.

The results from this Chapter suggest that between-sheep differences in intrinsic fibre strength are unlikely to be important to staple strength in comparison to changes in fibre diameter. However, very little work has been done on intrinsic fibre strength and its contribution to differences in staple strength in Merino sheep. Moreover, given that fibres with weak regions due to reduced diameter are more likely to break during early-stage processing, this by definition implies that differences in intrinsic fibre strength may influence latter stage wool processing, independent of its effects on staple strength. For these reasons, the remainder of this thesis is dedicated to establishing the potential importance and physico-chemical causes for differences in intrinsic fibre strength in Merino sheep.

CHAPTER 3: INTRINSIC STRENGTH OF SINGLE WOOL FIBRES IN MERINO SHEEP GENETICALLY DIFFERENT IN STAPLE STRENGTH AND FED DIFFERENT LEVELS OF NUTRITION

3.1. Introduction

It has long been recognised that large variations in stress-strain properties exist between wool samples from different sheep, and between different fibres from the same sheep (reviewed by Gourdie 1989). Considerable work has focused on interpreting these differences in stress-strain behaviour in relation to the geometry of the wool fibre and its cellular and molecular structure (reviewed by Feughelman 1987), and while this work has contributed significantly to our understanding of the property-structure relations in wool fibres and other α-keratins, its practical implications are limited by a lack of information on the associations between stress-strain properties of single fibres and processing performance. The properties of wool fibres at the point of failure have received the least attention, and yet they are the fibre characteristics most expected to limit the rate and efficiency at which wool fibres can be processed.

Staple strength is an important determinant of the processing performance of raw wool (Rottenbury *et al.* 1986; Plate *et al.* 1987), and as shown in the preceding Chapter, is closely related to the minimum fibre diameter along the staple. There is contradictory evidence on whether this association can be attributed solely to the amount of material available to bear the load, or whether differences in the average intrinsic strength[1] of individual fibres also contributes to variations in staple strength. Orwin *et al.* (1980) suggested that differences in intrinsic fibre strength may account for the differences in staple strength between "sound" and "tender" wool, and the significant phenotypic relationships found between intrinsic strength and staple strength for Merino (Hunter *et al.* 1983) and Romney sheep (Gourdie *et al.* 1992) support this contention. The hypothesis tested in this Chapter is that differences in intrinsic fibre strength

[1] Intrinsic fibre strength is also referred to in the literature as fibre tenacity (e.g. Hunter *et al.* 1990; Gourdie *et al.* 1992; Scobie *et al.* 1996), or stress at break (e.g. Orwin *et al.* 1985; Gourdie 1989; Woods *et al.* 1990).

account for the variation in staple strength generated by selection and nutrition that cannot be attributed to variations in diameter along and between fibres[2].

3.2. Materials and methods

3.2.1. Wool samples

Stress-strain properties of single wool fibres were measured on samples from the 40 sheep from the experiment described in Chapter 2. The samples were taken at shearing from adjacent to the tattoo patch on the mid-side region of each sheep, and were stored in the dark at 65% RH and 20°C. A small bundle ("staple") of fibres for single fibre tensile tests was removed from the side of the staples prepared for measurement of staple strength (section 2.2.6).

3.2.2. Force-extension curve

A 40 mm staple segment containing the position of break, estimated by manually breaking dyebanded staples, was cut from each of 10 "staples" per sheep. Each bundle of fibres was conditioned at 65% RH and 20° C for at least 24 h prior to tensile testing. Ten single fibres per staple (i.e. 100 fibres per sheep) were then randomly drawn from the butt-end of each fibre bundle, and each fibre was extended to break using a Wira Single Fibre Strength Meter (Type 678, Thorn Automation, Nottingham, U.K.). The gauge length was 20 mm and the strain rate was 150 %/min. Fibres which broke in the jaws were not included in the statistical analysis of the data.

The force-extension data were captured and processed using specifically designed software (Acacia Research, Adelaide, Australia). The analogue signal from the meter was digitised at 100 Hz by a 10-bit IBM data acquisition and control adapter in an IBM 286 microcomputer. Input sampling was set at 55 mS. The force-extension data were written to an internal buffer and displayed on the computer monitor in a graphical form, as shown in Plates 3.1 and 3.2. This enabled a check for fibre slippage in the clamps or other experimental problems. The operator then identified the linear regions of the force-extension curve, and the processing program calculated the slopes and intercepts of the regressions.

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[2] Preliminary data from this Chapter has been published in the *Proceedings of the 9th International Wool Textile Research Conference, Biella, Italy*, **2**; 134-42 (1995).

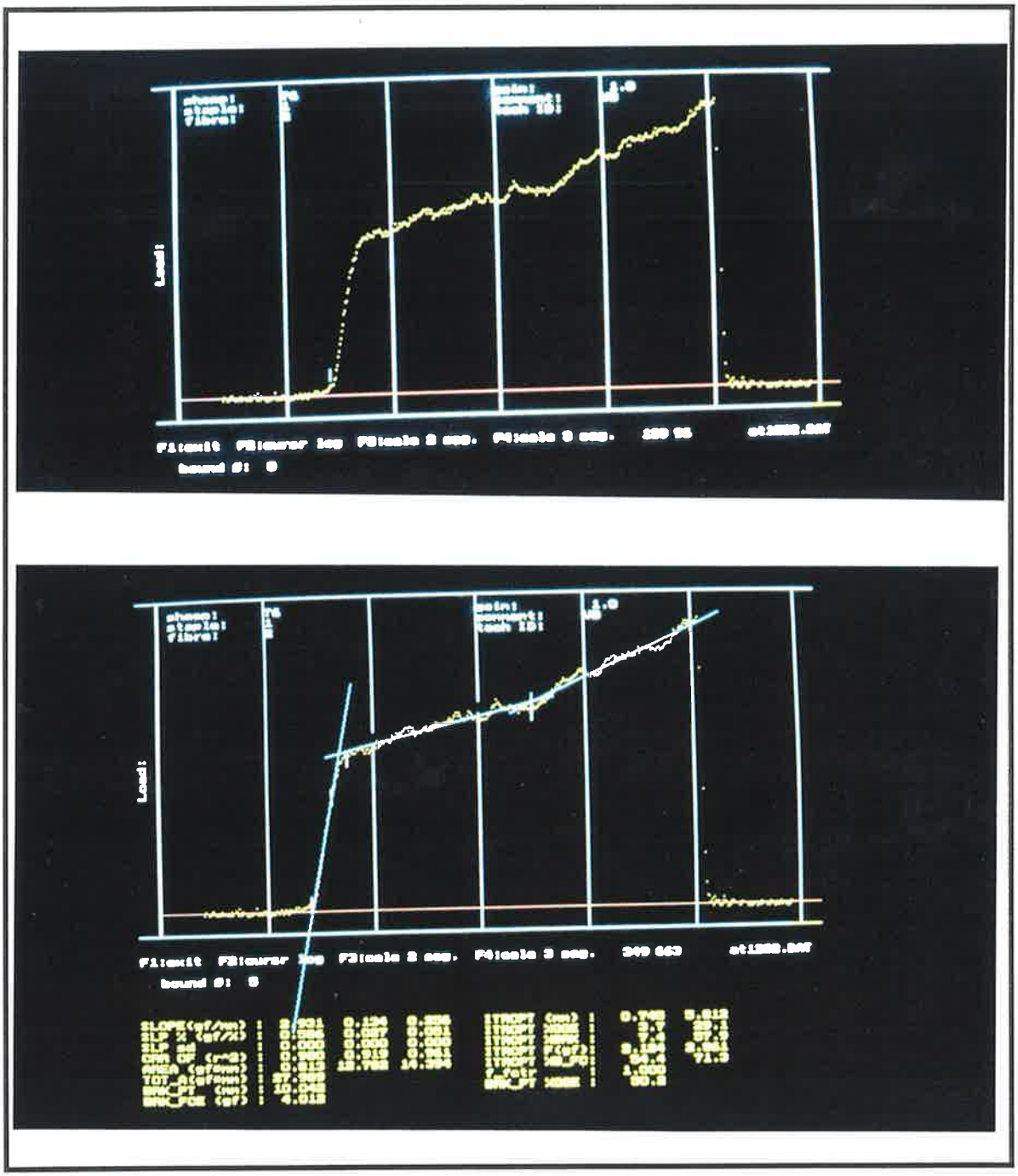

Plate. 3.1. Force-extension curve for a single wool fibre clearly demonstrating the Hookean, yield and post-yield regions. The failure properties of this fibre were: (*i*) force to break (4.5 gf); (*ii*) intrinsic strength (195 MPa); and (*iii*) extension at break (50.1%).

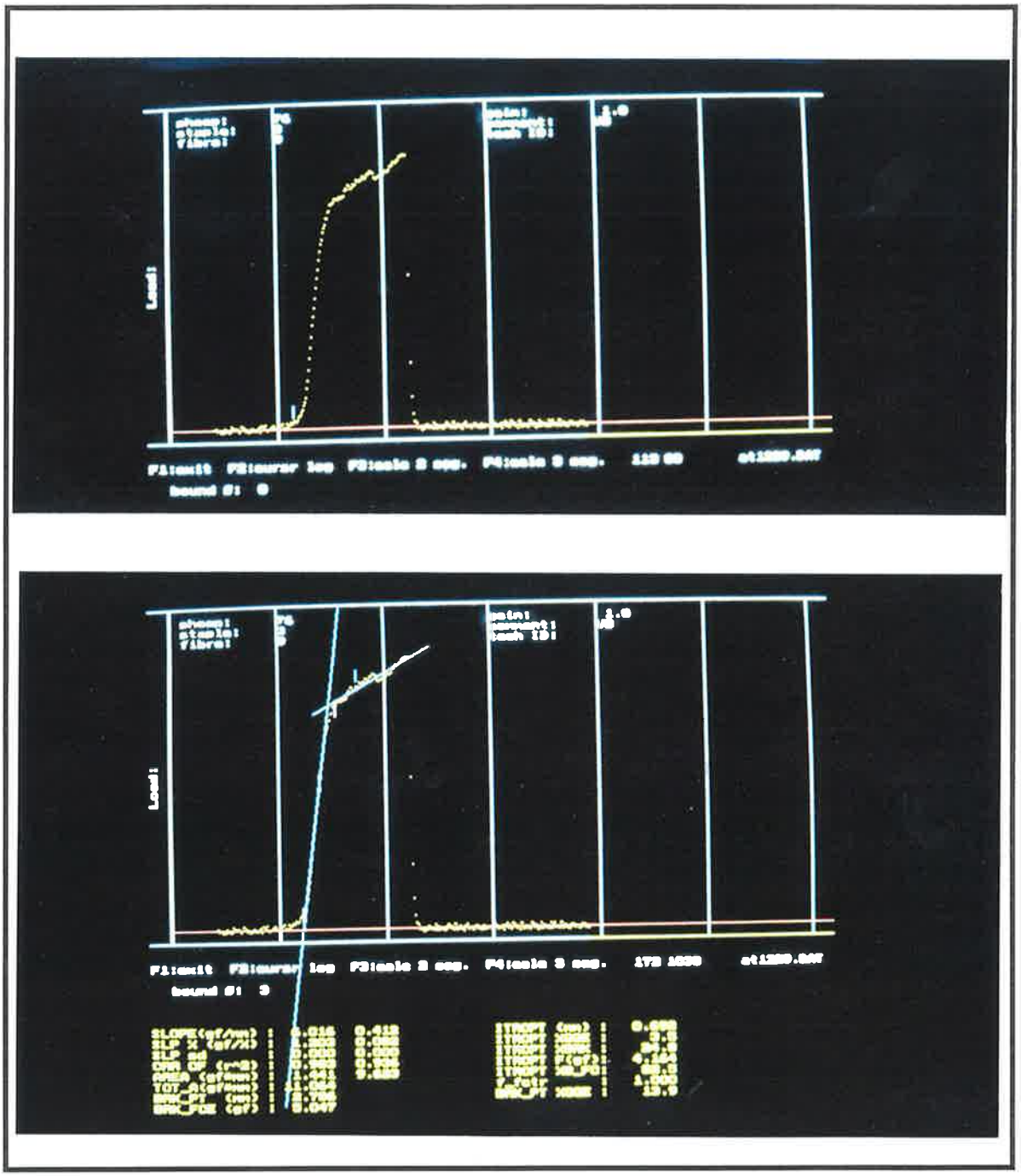

Plate. 3.2. Force-extension curve for a single wool fibre which broke within the yield region. The failure properties of this fibre were: (*i*) force to break (6.5 gf); (*ii*) intrinsic strength (150 MPa); and (*iii*) extension at break (13.8%).

3.2.3. Fibre cross-sectional area at the point of break

The tip and butt ends of the broken fibres were mounted on microscope slides using double-sided adhesive tape (Tesa, BDF Australia Ltd, Smithfield, New South Wales, Australia), such that the broken fibre ends were about 5 mm from the edge of the tape. They were then stained in saturated picric acid for 10 min, relaxed in distilled water (1 h, 20°C), and conditioned for at least 24 h at 65% RH and 20°C. Using this procedure, but without staining with picric acid, Gourdie (1989), Woods *et al.* (1990) and Gourdie *et al.* (1992) all found that there were no significant (P>0.05) differences between pre- and post-break fibre diameters.

The broken fibre ends were then mounted in oil (BDH, Laboratory Supplies, England), cover slipped, and the diameters at the fracture surfaces measured at 1520 x magnification with the aid of a camera lucida attachment and an image analysis system (Bioquant IV, R&M Biometrics, Tennessee, USA.). Five measurements of diameter were made for both the tip and butt-ends of the broken fibres. It was assumed the fibres were circular, since the average correlation coefficient for individual sheep between two random measurements of the fibre diameter (FD; μm) and the cross-sectional area determined from transverse skin sections taken at the time corresponding to the position of break, as described in section 2.3.5, was 0.95 ± 0.003 (Fig. 3.1). Cross-sectional area (CSA; μm^2) was calculated as follows: $CSA = [(FD_{tip} + FD_{butt})/4]^2 * \pi$

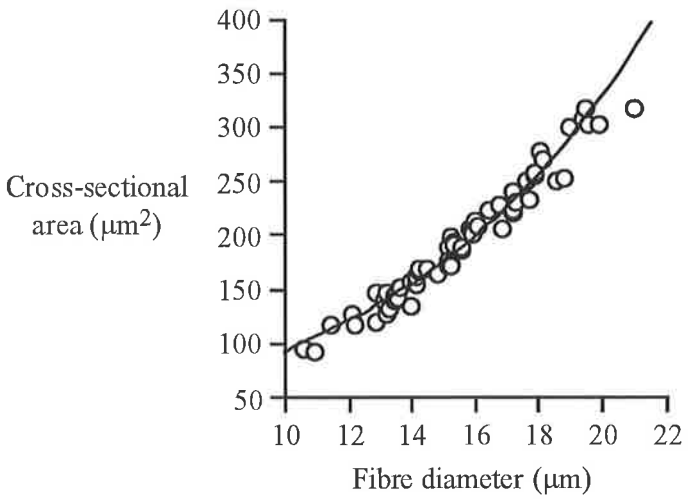

Figure. 3.1. Typical relationship between estimated fibre diameter and cross-sectional area measured from transverse skin sections taken at the time corresponding to the estimated position of break. Points represent single fibres from the same sheep (n_{fibres} = 60).

The morphology of the fracture surfaces of the broken fibres was assessed visually in conjunction with the estimates of diameter. They were classified as either: (*i*) clean break; a smooth fracture surface at right angles to the fibre axis (Plate 3.3*a*); (*ii*) step break; a fracture involving splitting along the fibre axis within the cortex (Plate 3.3*b*); (*iii*) fibrillated break; a fracture occurring between individual cortical cells (Plate 3.3*c*); or (*iv*) bulge break; a fracture occurring at a region of increased diameter (Plate 3.3*d*). The location of the diameter measurement was dependent on the type of fracture, as shown in Plate 3.3.

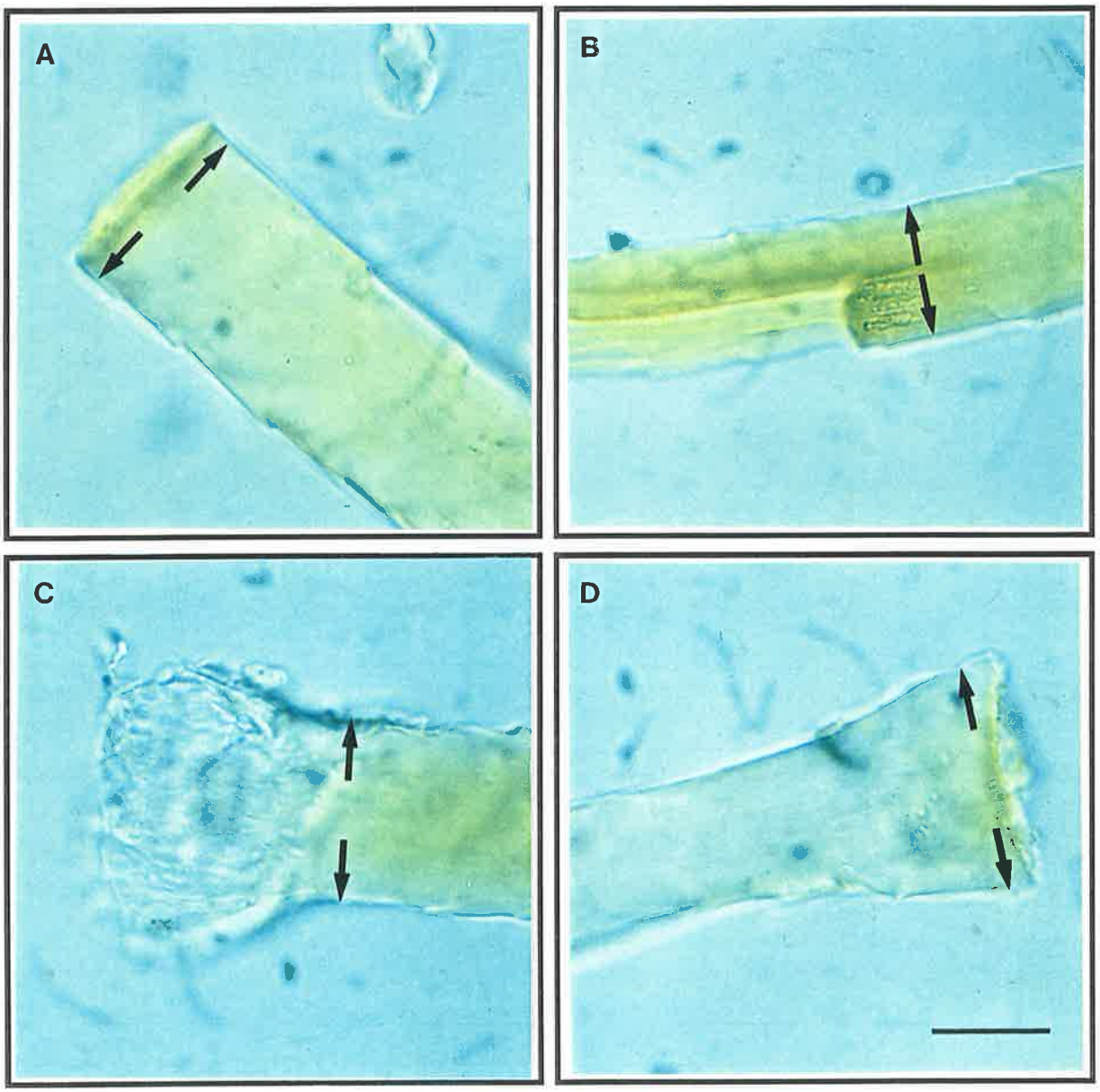

Plate 3.3. Photomicrographs of broken fibre-ends showing the different fracture patterns: (*a*) clean break; (*b*) step break; (*c*) fibrillated break; and (*d*) bulge break. Arrows denote location of diameter measurement, and scale bar indicates 10 μm.

3.2.4. Stress-strain properties

The stress-strain parameters shown in Fig. 3.2. were estimated from the force-extension data and the cross-sectional area at the point of break. Most attention in this thesis is directed towards intrinsic fibre strength, or stress at break, although reference is also made to other key stress-strain properties. Stress-strain parameters were calculated using the following formulae:

Stress (MPa) = Force (gf) x 9810/CSA (μm^2)

Modulus (GPa) = Slope (gf/%) x 981/CSA (μm^2)

Work to break (MPa) = Area (gf/mm) x 490.5/CSA (μm^2)

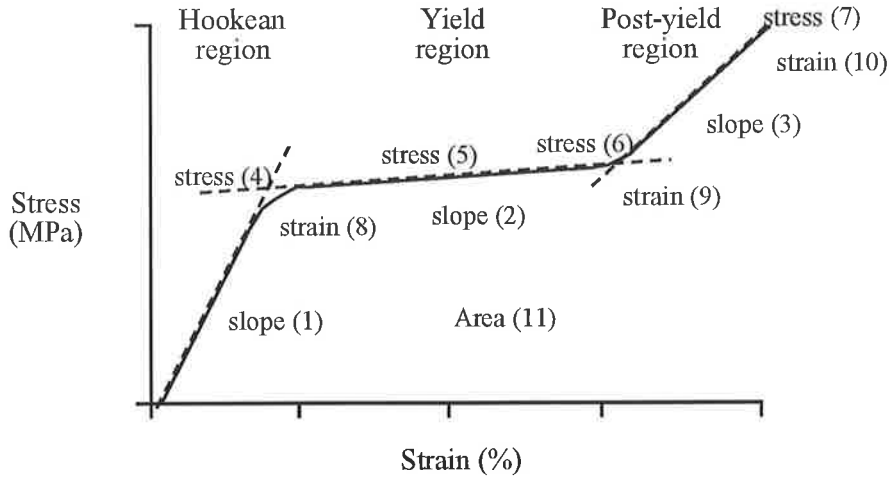

Figure 3.2. Schematic representation of the stress-strain curve of a wool fibre showing the location of stress-strain parameters measured (stress and strain are not drawn to scale). The numbers indicate the positions of features which characterise the stress-strain behaviour of the wool fibre: regression slopes, Young's modulus (1), yield modulus (2) and post-yield modulus (3); stress at inflection points, yield stress (4), stress at 15% extension (5), stress at yield to post-yield turnover (6) and stress at break (7); strain at inflection points, yield extension (8); extension at yield to post-yield turnover (9) and extension at break (10); and area under stress-strain curve, work to break (11).

3.2.5. Statistical analyses

The variance in stress-strain properties between fibres increased with the mean value, thus making the assumption of homogeneity of variance invalid. To nullify this difficulty, all analyses of variance were carried out on the logarithms of the observed stress-strain values, rather than the observed values themselves. Two-way analysis of variance was conducted with selection flock,

nutrition and the 'selection flock by nutrition' interaction as the sources of variance. This analysis was also used to test the effects of fracture behaviour on stress-strain properties. The strength of the relationships between fibre cross-sectional area, stress-strain properties and staple strength were initially tested using simple linear regression analysis. Relationships within nutritional treatments were not considered. Multiple regression analysis was then employed to determine whether differences in single fibre stress-strain properties explained any of the variance in staple strength additional to that attributed to variations in diameter along and between fibres. All statistical analyses were performed using the statistical package SuperANOVA^{TM} (Abacus Concepts Inc., Berkeley, California, USA).

3.3. Results

3.3.1. Measurement of cross-sectional area

The estimate of intrinsic strength and other stress-strain parameters is dependent on the accurate measurement of fibre cross-sectional area at the point of break. For individual sheep, the average cross-sectional area at the position of break for single fibres was strongly correlated ($r^2 = 0.92$, $P<0.001$, Fig. 3.3) with the minimum cross-sectional area along the staple estimated from the diameters of wool clipped from the mid-side patches. The slope of the regression line was not significantly different from one (1.02 ± 0.049; $P>0.05$), but the intercept was significantly less than zero (-26.3 ± 8.78; $P<0.05$).

There was no significant ($P>0.05$) difference in the average cross-sectional area of the tip and butt ends of broken fibres from the same sheep; the average across all fibres being 171 and 176 μm^2, respectively. However, there was considerable variation in the cross-sectional area of the complementary fracture surfaces; the difference in the cross-sectional area between complementary fibre-ends was less than 10% for only 40% of the 4000 fibres tested. The difference in cross-sectional area between fibre ends was negatively ($r^2 \approx 0.10$; $P<0.05$) related to the amount of stress on the fibre at all levels of strain, and thus was used as a covariate in the analysis of these data. The average coefficient of variation in cross-sectional area at the point of break between fibres from the same sheep was 52.4% (range 34.2 to 74.6%).

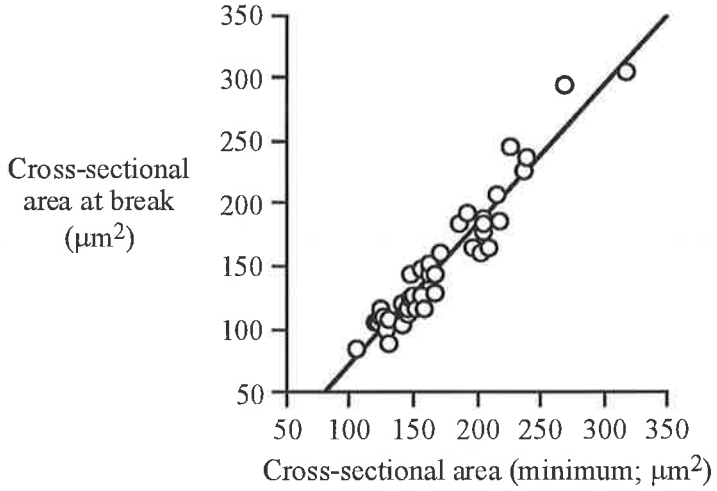

Figure 3.3. The minimum fibre cross-sectional area along the staple estimated from the diameter of wool clipped from the mid-side patch in relation to the cross-sectional area at the point of break along single fibres (n_{fibres} = 100). The fibres were from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition. Points represent means for individual sheep.

3.3.2. Variation in shape of the stress-strain curve

The full complement of stress-strain properties was not measured on all fibres because of variations in extension at break and the extent to which the post-yield region was defined (Appendix, Table A3.1). While more than 98% of all fibres tested broke beyond the Hookean region at about 3% extension, and 94% broke beyond 15% extension, only 38% of fibres had a well defined post-yield region. Moreover, the proportion of fibres which had a well defined post-yield region was significantly (P<0.001) influenced by nutritional conditions (see section 3.3.5), and on an individual sheep basis it varied from 6 to 83% of the fibres tested. Stress-strain behaviour at the yield to post-yield turnover, and within the post-yield region, are therefore not shown because a valid quantitative comparison is not possible.

3.3.3. Sources of variation in stress-strain properties

The coefficient of variation in stress-strain properties between individual fibres from the same sheep, on average, ranged from 20 to 50% (Appendix, Table A3.2). The variation in intrinsic strength between fibres was similar to that for other stress parameters, and was generally lower than that for other stress-strain properties. Fibre-to-fibre variation contributed between 82 and 92% of the total variation in stress-strain properties between all 4000 fibres tested. Of the total

variance remaining, 5 to 15% was due to differences between sheep, and less than 3% to differences between staples.

The total variance in stress-strain properties, and its distribution into the various components, influenced the capacity to detect differences in average stress-strain properties between individual sheep. For the number of fibres tested for each sheep in this experiment (10 staples x 10 fibres/staple), the least significant difference (L.S.D; P=0.05) for the stress-strain properties measured was between 6.4 and 17.2% of the mean value (Table A3.2). The L.S.D. for intrinsic fibre strength and other stress parameters was about 7% of the mean value.

3.3.4. Effects of genotype and nutrition on stress-strain properties

Analysis of variance revealed a highly significant (P<0.001) difference in average stress-strain properties between individual sheep within nutritional treatments (Table 3.1). Across all treatments, the average intrinsic strength for individual sheep ranged from 163 to 235 MPa (44%), the average extension at break from 21.4 to 43.5% (103%), and the average total work to break from 9.4 to 16.8 MPa (79%).

Fibres from sheep from the "sound" flock tended to have a greater average cross-sectional area at the point of break (156 *vs.* 141 μm^2; P=0.10), and required significantly more force to break (3.3 *vs.* 2.9 gf; P<0.05) than fibres from sheep from the "tender" flock. After normalising for differences in cross-sectional area, there were no significant differences in intrinsic strength or other stress-strain properties between the flocks (Table 3.1). There was no significant (P>0.05) interaction between 'selection flock and nutrition' for any of the stress-strain properties measured.

Fibres from sheep fed to maintain liveweight were on average broader at the point of break (210 *vs* 125 μm^2; P<0.001), and required more force to break (4.2 *vs.* 2.7 gf; P<0.001) than fibres from sheep on the restricted diets. There were still significant differences in stress-strain properties between the nutritional treatments after adjusting for the differences in cross-sectional area. Fibres from sheep fed to maintain liveweight were intrinsically weaker (P<0.001) at all levels of strain, but were more extensible (P<0.05) and required more work to break (P<0.05), than fibres from sheep on the restricted diets (Table 3.1).

Table 3.1. Average stress-strain properties of single wool fibres from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition .

a, b,c: different superscripts within selection flock or nutrition comparisons differ at P< 0.05

Stress-strain property	Selection flock		Nutritional treatment		
	"sound"	"tender"	LWM	LWL_1	LWL_2
Young's modulus (GPa)	3.92^a	3.75^a	3.43^a	3.92^b	4.19^b
Yield stress (MPa)	129^a	128^a	123^a	130^b	131^b
Yield modulus (GPa)	0.23^a	0.23^a	0.16^a	0.25^b	0.29^c
Stress at 15% extension (MPa)	157^a	157^a	143^a	161^b	166^b
Intrinsic strength (MPa)	211^a	203^a	193^a	216^b	213^b
Extension at break (%)	33.2^a	31.9^a	36.9^a	32.7^b	28.6^c
Work to break (MPa)	13.2^a	12.6^a	13.7^a	13.2^{ab}	11.8^b

Several other differences in the shape of the stress-strain curve were also apparent between nutritional treatments. The proportion of fibres that had a well defined post-yield region varied significantly ($P<0.001$) from an average of 60% for sheep fed to maintain liveweight to 25% for sheep on the most restricted diet. Moreover, while the stress at the Hookean to yield turnover averaged about 60% of the intrinsic strength at break for all treatments, the average stress at 15% extension for fibres from sheep fed to lose weight was 78% of the break value compared to 72% for sheep fed to maintain liveweight ($P<0.05$). This reflected a greater average yield modulus, but a lower extension at break, for fibres from sheep fed to lose weight (Table 3.1). The subjectively assessed sharpness of the turnover from one region of the stress-strain curve to the next was generally sharper for fibres from sheep fed to maintain liveweight.

The average coefficient of variation in stress-strain properties between individual fibres from the same sheep was consistently greater ($P<0.05$) for sheep from the "tender" flock than the "sound" flock (Table 3.2). However, the effects of nutrition on the between-fibre variation in stress-strain properties were inconsistent. The variation in stress parameters was greater for sheep fed to maintain liveweight, but between-fibre variation in extension and work to break did not differ significantly between treatments (Table 3.2).

Table 3.2. Coefficient of variation (%) in stress-strain properties of single wool fibres from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition .

a, b: different superscripts within selection flock or nutrition comparisons differ at P< 0.05

Stress-strain property	Selection flock		Nutritional treatment		
	"sound"	"tender"	LWM	LWL_1	LWL_2
Young's modulus (GPa)	26.5^a	29.8^b	30.0^a	26.0^{ab}	28.4^b
Yield stress (MPa)	21.1^a	24.5^b	26.6^a	19.8^b	21.8^b
Stress at 15% extension (MPa)	21.1^a	23.9^b	26.2^a	19.8^b	21.5^b
Intrinsic strength (MPa)	20.8^a	25.0^b	27.6^a	19.0^b	22.0^b
Extension at break (%)	26.0^a	29.9^b	25.8^a	26.8^a	31.1^a
Work to break (MPa)	31.4^a	37.7^a	37.0^a	30.5^b	35.7^{ab}

3.3.5. Fibre cross-sectional area in relation to force to break

Fibre cross-sectional area at the position of break (X) was strongly (P<0.001) correlated with the force to break (Y), both between individual sheep (r^2 = 0.96; Fig. 3.4*a*), and on an individual fibre basis (r^2 = 0.69; Fig. 3.4*b*). The log transformed data were described by the formulae: Y = -1.31 (± 0.063) + 0.84 (± 0.029) X; and Y = - 0.96 (± 0.016) + 0.67 (± 0.007) X, respectively.

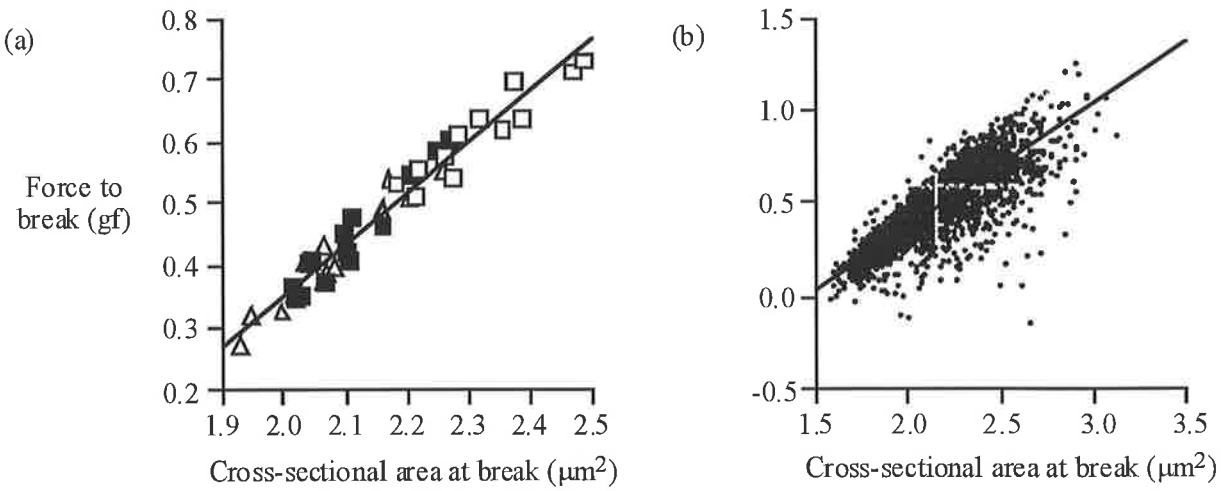

Figure 3.4. Fibre cross-sectional area in relation to force to break: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14); and (*b*) all individual fibres tested($n_{fibres} \approx 4000$). All data were log_{10} transformed.

3.3.6. Intrinsic strength in relation to other stress-strain properties

Intrinsic fibre strength was positively correlated ($P<0.001$) with the modulus of the Hookean and yield regions, and the non-failure stress parameters (Table 3.3). In other words, fibres which were intrinsically stronger at the break were stiffer at all levels of extension. These correlations applied both between individual fibres and between individual sheep, although on some occasions the coefficients for the correlation did differ between individual sheep. The most important relationships in the context of this thesis are also shown graphically (Figs. 3.5 to 3.7).

Table 3.3. Phenotypic correlations (adjusted r^2 values) between stress-strain properties of single wool fibres. Correlation coefficients above the diagonal are between individual sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition ($n_{sheep} = 40$), and below the diagonal are between individual fibres ($n_{fibres} \approx 4000$). All data were $\log_{10}$ transformed, and all regression coefficients were significant and positive ($P<0.001$).

Stress-strain property	Young's modulus	Yield modulus	Yield stress	Stress at 15% extension	Intrinsic strength
Young's modulus		0.48	0.72	0.82	0.62
Yield modulus	0.61		0.27	0.59	0.22
Yield stress	0.69	0.54		0.90	0.78
Stress at 15% extension	0.76	0.87	0.84		0.77
Intrinsic strength	0.61	0.34	0.61	0.52	

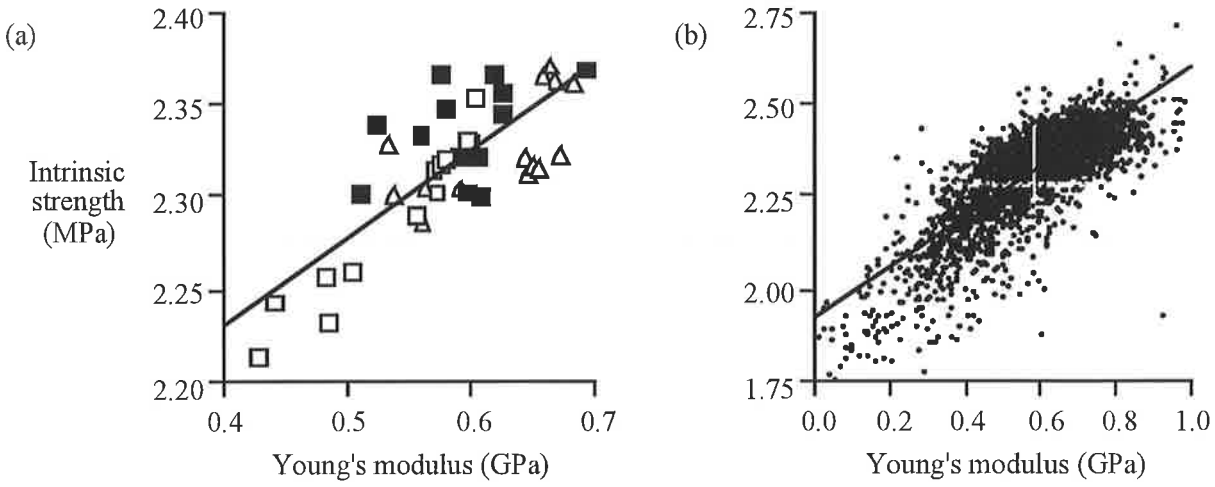

Figure 3.5. Young's modulus in relation to intrinsic fibre strength: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14); and (*b*) all individual fibres tested ($n_{fibres} \approx 4000$). All data were log_{10} transformed.

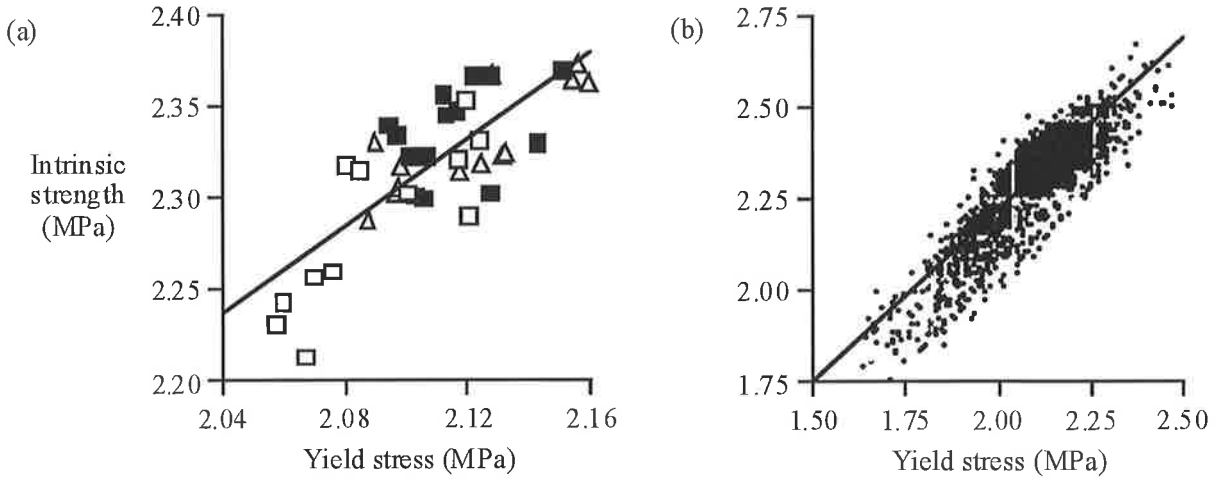

Figure 3.6. Yield stress in relation to intrinsic fibre strength: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14); and (*b*) all individual fibres tested ($n_{fibres} \approx 4000$). All data were log_{10} transformed.

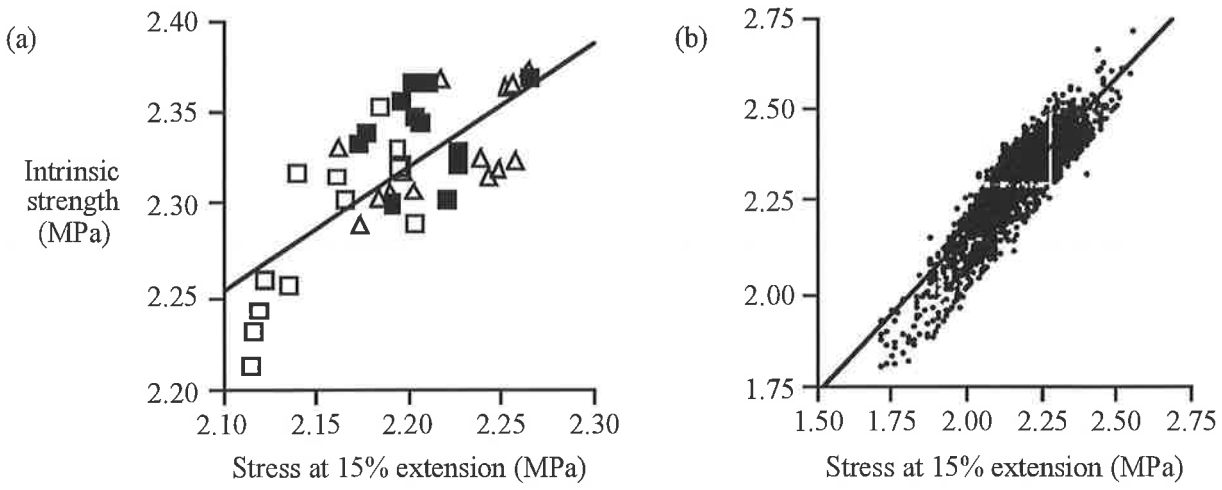

Figure 3.7. Stress at 15% extension in relation to intrinsic fibre strength: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14); and (*b*) all individual fibres tested ($n_{fibres} \approx 4000$). All data were log_{10} transformed.

There was no significant relationship between average intrinsic fibre strength and extension at break between individual sheep ($P>0.05$). On an individual fibre basis, the relationship was weak and positive ($r^2 = 0.12$; $P<0.001$). Fibre extension at the position of break explained between 75 and 85% ($P<0.001$) of the variance in total work to break.

3.3.7. Fibre cross-sectional area in relation to stress-strain properties

Fibre cross-sectional area at the position of break was negatively correlated ($P<0.001$) with intrinsic strength, the modulus of the Hookean and yield regions, and non-failure stress parameters (Figs. 3.8 to 3.11). Fibres with a smaller cross-sectional area at the position of break were intrinsically stronger at all levels of extension, and this was the case regardless of how the data were analysed.

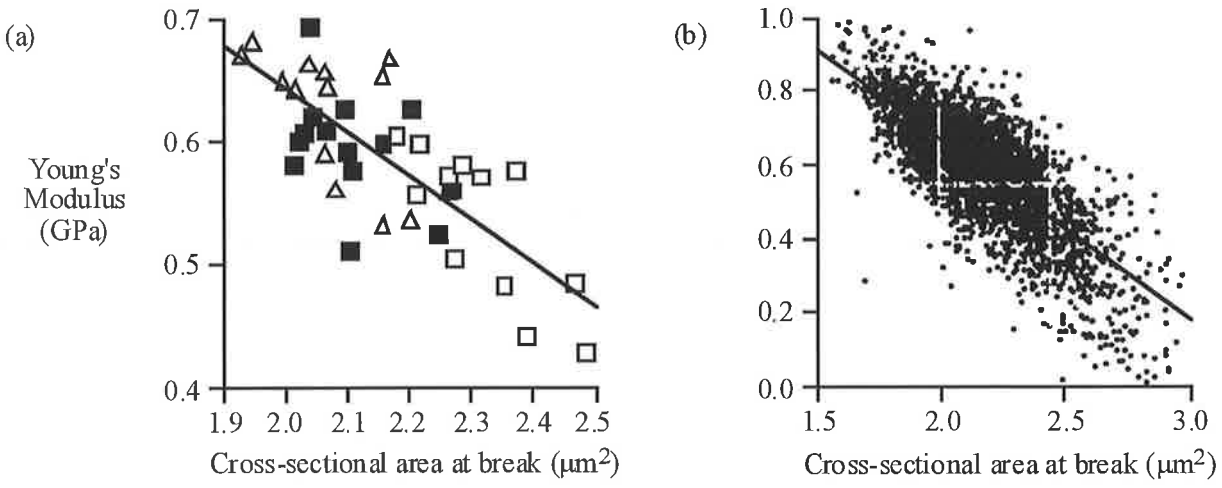

Figure 3.8. Fibre cross-sectional area at the point of break in relation to Young's modulus: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14) (r^2 = 0.69); and (*b*) all individual fibres tested (n_{fibres} ≈ 4000; r^2 = 0.48). All data were log_{10} transformed.

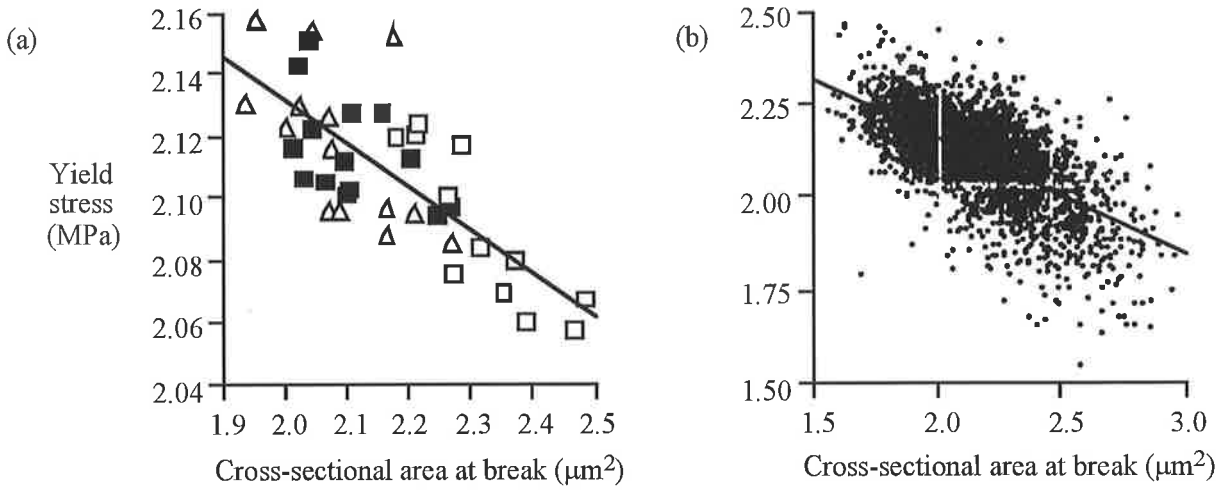

Figure 3.9. Fibre cross-sectional area at the point of break in relation to yield stress: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14) (r^2 = 0.56); and (*b*) all individual fibres tested (n_{fibres} ≈ 4000; r^2 = 0.42). All data were log_{10} transformed.

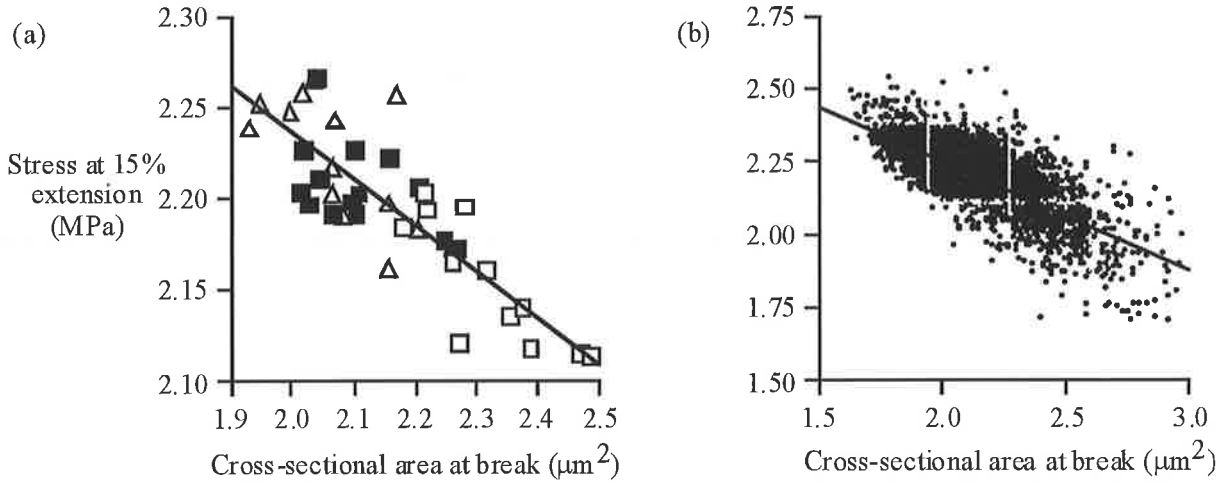

Figure 3.10. Fibre cross-sectional area at the point of break in relation to stress at 15% extension: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14) (r^2 = 0.69) ; and (*b*) all individual fibres tested ($n_{fibres} \approx 4000$; r^2 = 0.54). All data were log_{10} transformed.

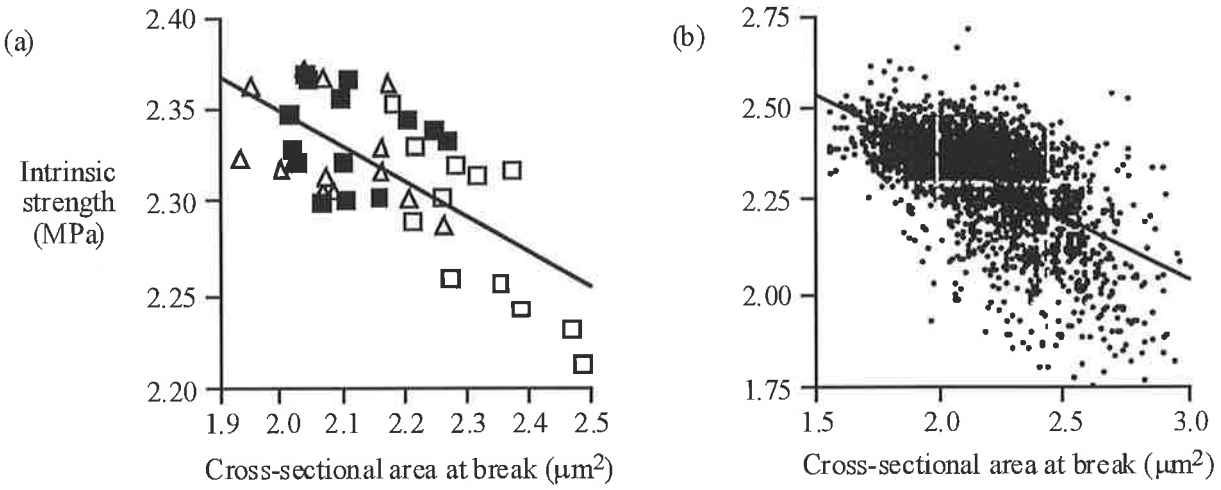

Figure 3.11. Fibre cross-sectional area at the point of break in relation to intrinsic fibre strength: (*a*) mean values for individual sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14) (r^2 = 0.46); and (*b*) all individual fibres tested ($n_{fibres} \approx 4000$; r^2 = 0.35). All data were log_{10} transformed.

Average fibre cross-sectional area at the position of break for individual sheep was positively related to extension at break (r^2 = 0.36; P<0.001) and work to break (r^2 = 0.09; P<0.001). No significant (P>0.05) relationships existed when the data were analysed across all the fibres tested.

3.3.8. Fracture morphology and stress-strain properties

The fracture surfaces of 74% of all fibres tested appeared to be relatively smooth and perpendicular to the fibre axis (Plate 3.3*a*). The proportion of fibres with this break type varied significantly ($P<0.05$) from an average of 68.3% for sheep fed to maintain liveweight to 76.1% for sheep on the restricted diets. Stepped fracture surfaces, where the longitudinal crack varied from less than 5 to more than 500 μm (Plate 3.3*b*), were evident for 21.4% of the fibres, and its occurrence was independent of nutritional conditions. On average, fibres with clean breaks were smaller (152 *vs.* 180 μm^2, $P<0.001$) and broke at a lower force (2.96 *vs.* 3.23 gf, $P<0.001$) than fibres with step breaks. After normalising for differences in fibre cross-sectional area, fibres with clean breaks were intrinsically stronger at all levels of extension ($P<0.001$), were more extensible ($P<0.05$), and required more work to break ($P<0.001$) than fibres with step breaks (Table 3.4). Thus, on an individual sheep basis, a high average intrinsic strength was associated with a greater incidence of "clean" breaks ($r^2 = 0.22$; $P<0.01$).

Table 3.4. Average stress-strain properties of single wool fibres with different fracture types. The fibres were from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition ($n_{fibres} \approx 4000$).

a, b, c, d: different superscripts differ at P< 0.05

Stress-strain property	Fracture type			
	Clean	Step	Bulge	Fibrillated
Young's modulus (GPa)	4.05^a	3.71^b	2.17^c	na
Yield stress (MPa)	132^a	127^b	85^c	na
Stress at 15% extension (MPa)	161^a	154^b	99^c	na
Intrinsic strength (MPa)	217^a	205^b	124^c	99^d
Extension at break (%)	33.0^a	31.6^b	30.7^b	9.3^c
Work to break (MPa)	13.4^a	12.4^b	7.4^c	4.7^d

A small proportion of fibres broke at "bulges" or nabs (3.8%; Plate 3.3*c*), or appeared to have brush-like fibre-ends characteristic of fibres breaking between cortical cells (0.8%; Plate 3.3*d*). The proportion of fibres which broke at nabs was greater for sheep on the better plane of nutrition than their counterparts fed to lose liveweight (10.2 *vs.* 1.3%; $P<0.001$). The proportion of fibrillated breaks was not influenced by nutrition. Fibres with these fracture modes were larger (383 and 270 μm^2 for "bulges" and fibrillated breaks, respectively; $P<0.001$) than either axial or step breaks, but they were also intrinsically weaker ($P<0.001$) and required less work to break ($P<0.001$). The average extension at break of fibres which appeared to break between cortical cells was less than 10% (Table 3.4), and 42% of the fibres broke within the Hookean region. Only failure properties of fibres with this break type are shown in Table 3.4.

The exclusion of fibres with step, bulge or fibrillated breaks did not significantly influence the average stress-strain properties, but it did significantly ($P<0.05$) reduce the variation in stress-strain properties between fibres (Appendix, Table A3.3).

3.3.9. Staple strength in relation to single fibre stress-stain properties

Fibre cross-sectional area at the point of break was most closely associated with staple strength, accounting for 66% ($P<0.001$) of the total variance in staple strength generated by selection and nutrition. As the relationships did not differ for sheep from the "sound" or "tender" staple strength selection flocks, the data for the two flocks were combined (Fig. 3.12). The combined data indicated that, on average, an increase in cross-sectional area of 30 μm^2 was associated with an increase in staple strength of about 5 N/ktex. The relationship between fibre diameter of single fibres at the point of break and staple strength was similar to that between minimum fibre diameter measured on wool clipped from the midside patch and staple strength reported in Chapter 2 (section 2.3.8), although the intercept for the single fibre regression was greater (-29.1 *vs.* -53.2; $P<0.05$).

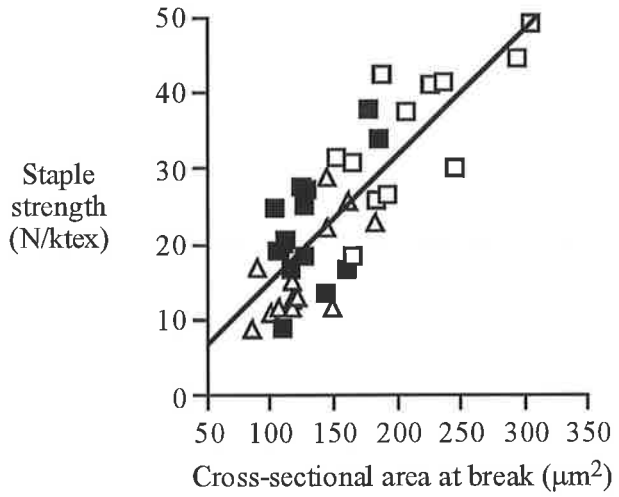

Figure 3.12. Staple strength in relation to single fibre cross-sectional area at the point of break for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined, and points represent means for individual sheep.

Staple strength was negatively correlated ($P<0.001$) with Young's modulus, non-failure stress parameters and intrinsic fibre strength (Fig. 3.13), and positively related to single fibre extension at break ($r^2 = 0.54$; $P<0.001$) and total work to break ($r^2 = 0.24$; $P<0.001$).

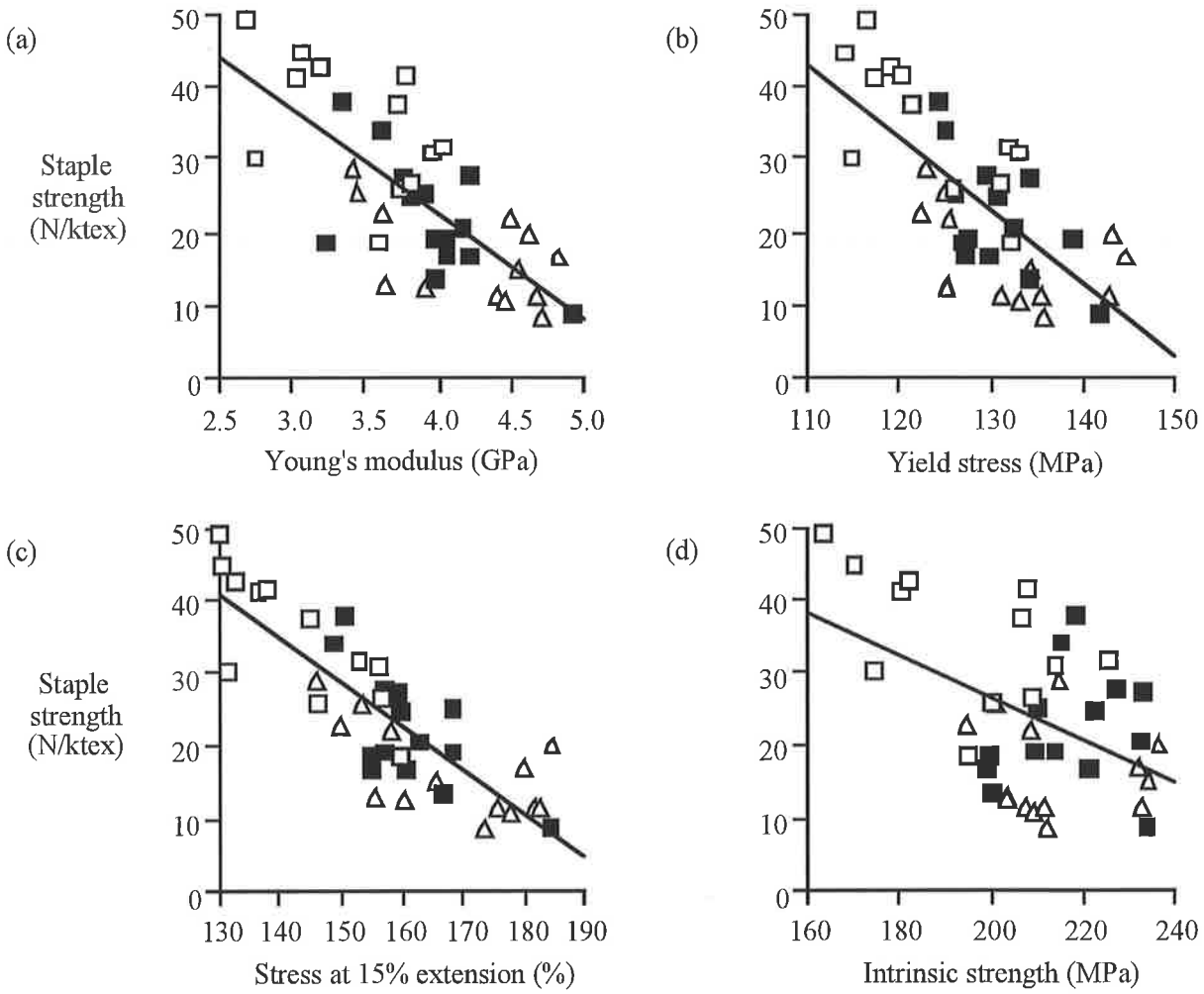

Figure 3.13. Staple strength in relation to (*a*) Young's modulus (r^2 = 0.53); (*b*) yield stress (r^2 = 0.48); (*c*) stress at 15% extension (r^2 = 0.69); and (*d*) intrinsic strength (r^2 = 0.21) of single fibres. The wool samples were from sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14), and the data for sheep from the "sound" and "tender" staple strength selection flocks were combined. Points represent means for individual sheep.

Multiple regression analysis revealed that intrinsic strength, extension at break and work to break did not remove any variance in staple strength additional to the 80% already accounted for by along- and between-fibre variation in diameter (Chapter 2, section 2.3.8). However, stress at 15% extension (P<0.01) and the standard deviation of variation in extension at break (P<0.05) explained an additional 4% of the variance in staple strength. Thus, a total of 84% of the variance in staple strength could be explained by the fibre and staple characteristics measured in this study.

3.4. Discussion

3.4.1. Hypothesis

To my knowledge this was the first experiment which has measured the full complement of stress-strain properties on a large number of Merino wool fibres of well defined nutritional and genetic origin. The average intrinsic strength of individual wool fibres varied by about 25% between sheep within nutritional treatments, and by as much as 44% between all the sheep in the experiment. A surprising result was that intrinsic strength was negatively associated with staple strength, and it failed to remove any variance in staple strength additional to that already attributed to differences in along- and between-fibre changes in diameter. It was therefore concluded that intrinsic strength *per se* did not contribute significantly to the differences in staple strength between sheep in this experiment, and thus the hypothesis was not supported.

3.4.2. Variation in intrinsic strength and other stress-strain properties between sheep

The variation in intrinsic strength, and other stress-strain properties, between individual fibres and sheep in this experiment is consistent with a number of other reports (Anderson and Cox 1950; Evans and Montgomery 1953; Evans 1954; O'Connell and Lundgren 1954; Meybeck and Gionola 1955; Thorsen 1958; Dusenbury and Wakelin 1958; Roberts *et al.* 1960; Whiteley and McMahon 1965; Smuts *et al.* 1981; Hunter *et al.* 1983, 1990; Orwin *et al.* 1985; Woods *et al.* 1990; Gourdie *et al.* 1992; Scobie *et al.* 1996). The magnitude of this variation in intrinsic strength has often been attributed to the technical difficulties associated with the accurate measurement of cross-sectional area at the point of break. It is acknowledged that in the current experiment this factor could have contributed to some of the variance in intrinsic strength between individual fibres from the same sheep. However, as the average cross-sectional area at the point of break for single fibres was strongly correlated ($r^2 = 0.92$) with the minimum cross-sectional area along the staple estimated from the diameter of wool clipped from the mid-side patch, this suggests that measurement error was a minor contributor to the variance in intrinsic strength between sheep. Real and substantial differences in intrinsic fibre strength therefore exist between Merino sheep, and they are similar in magnitude to that found for Romneys (Gourdie *et al.* 1992; Scobie *et al.* 1996).

Intrinsic strength and other stress-strain properties did not differ significantly between sheep from the "sound" and "tender" staple strength selection flocks (Table 3.1). This result is similar to

that reported by Scobie *et al.* (1996), who found no differences in intrinsic strength between groups of Romney sheep genetically different in staple tenacity. It also confirms the findings in Chapter 2 that selection for high staple strength reduces the variability in properties between fibres, rather than the average strength of individual fibres. Indeed, it was shown in this Chapter that high staple strength sheep with a low between-fibre variation in diameter have a lower variation in extension between fibres than low staple strength sheep (Table 3.2). A.D. Peterson (unpubl. data) has found, using these same wool samples, that the high staple strength sheep also have a lower variation in length between fibres than the low staple strength sheep. These findings explain why there is a difference in peak force to break staples between selection flocks, but no significant difference in the total energy to break staples (1.10 and 1.12 J/ktex for "sound" and "tender" flocks, respectively; Thompson *et al.* 1995). Total energy to break is theoretically independent of between-fibre variations in length and extensibility (de Jong *et al.* 1985).

It has been reported previously that both short-term (Woods *et al.* 1990) and long-term nutritional stresses (Gourdie *et al.* 1992), similar to that used in this experiment, have little effect on the intrinsic strength of Romney wool fibres. Hunter *et al.* (1990) reported that intrinsic strength of Merino wool was unchanged despite nutritional or physiological stresses. Burgmann (1959) and Roberts *et al.* (1960) also reported that nutrition did not significantly affect intrinsic strength, however their work was based on a total of less than 30 wool fibres. Collectively, these findings have led to the suggestion that between-sheep differences in intrinsic strength are genetically determined. In the study reported here, fibres from sheep fed to maintain liveweight were intrinsically weaker at all levels of strain than those from sheep on the restricted diets, although the average difference at break was only 5 to 7% (Table 3.1). It is not possible to determine statistically whether the effects of nutrition on intrinsic strength were independent of the concomitant changes in fibre cross-sectional area, because cross-sectional area is a component of intrinsic strength. However, as the relationships between fibres-within sheep were similar to those between sheep, this may suggest that nutritional treatments acted simply by changing fibre cross-sectional area.

Fibres from sheep fed to maintain liveweight were more extensible, and required more work to break, than fibres from sheep on the restricted diets (Table 3.1). This result is consistent with the effects of nutrition on along-fibre variations in diameter (Fig. 2.3), and the strong negative correlation between fibre cross-sectional area variation and extensibility (Collins and Chaikin

1965, 1968, 1969, 1971). When a wool fibre is extended, the degree of extension varies along the length of the fibre being tested, such that the total extension is the cumulative effect of the different levels of extension in short segments of the fibre. While all sections of a non-uniform fibre are initially extended in the Hookean region, sections of the fibre with a smaller cross-sectional area extend more rapidly into the yield and the post-yield regions. Consequently, at the time when the local extension at the thinnest point along the fibre exceeds the breaking extension of the constituent materials of the fibre, causing the fibre to break, the thicker sections of the fibre have not been fully extended. In comparison, when a more uniform fibre is extended, a greater proportion of the fibre sections are fully extended when the fibre breaks. The differences in fibre cross-sectional area variation between nutritional treatments would also explain why fibres from sheep fed to maintain liveweight, on average, had a more well defined post-yield region, and a sharper turnover from one region of the stress-strain curve to the next, than fibres from sheep fed to lose and then gain liveweight.

3.4.3. Intrinsic strength in relation to cross-sectional area and other stress-strain properties

There was a very strong positive correlation between cross-sectional area at the point of break and the force required to break individual fibres ($r^2 = 0.95$), which is consistent with other reports (Anderson and Cox 1950; Roberts *et al.* 1960; Orwin *et al.* 1980; 1985; Dollin *et al.* 1995; Scobie *et al.* 1996). However, after normalising the force-extension data for differences in cross-sectional area, the correlation with intrinsic strength was in fact negative ($r^2 = 0.46$); an increase in cross-sectional area of 100 μm^2 was associated with a decrease in intrinsic strength of about 25 MPa. Woods *et al.* (1990), Gourdie *et al.* (1992) and Dollin *et al.* (1995) reported much smaller decreases in intrinsic strength with increasing cross-sectional area than in this study, and others have shown that intrinsic strength is independent of cross-sectional area (Evans 1954; Burgmann 1959; Roberts *et al.* 1960; Rigby 1962; Campbell *et al.* 1972; Scobie *et al.* 1996) or increases with increasing cross-sectional area (Anderson and Cox 1950; Shah and Whiteley 1966; Collins and Chaikin 1968; Smuts *et al.* 1981). A number of factors have probably contributed to these inconsistencies. For example, a wide variety of inaccurate methods have been used to estimate fibre cross-sectional area at break; the number of sheep used and the number of fibres tested per sheep are often inadequate, especially given the extent of between-fibre variation in intrinsic strength found in this and other experiments (Dusenbury and Wakelin 1958; Gourdie *et al.* 1992); and the genetic and nutritional history of the samples is sometimes unknown or poorly

defined. This is despite genotype and nutrition having opposing effects on the relationships between cross-sectional area and other physical and chemical fibre characteristics (Campbell *et al.* 1972, 1975; Reis 1979; Smuts *et al.* 1981; Orwin *et al.* 1984; Hynd 1989). The effects of these technical difficulties and experimental problems have been minimised in the current experiment and those reported by Woods *et al.* (1990), Gourdie *et al.* (1992) and Scobie *et al.* (1996). Clearly the thicker fibres in this study were intrinsically weaker per unit cross-sectional area than the thinner fibres, and real differences exist between experiments in the relationship between cross-sectional area and intrinsic strength.

It has been suggested that microscopic "flaws" or defects on the surface or within the interior of the fibre (Andrews 1964; Mason 1964), and non-uniformities in cross-sectional area along the fibre (Shah and Whiteley 1966; Collins and Chaikin 1968), are responsible for differences in intrinsic strength. As a result, non-failure properties such as stress at 15% (Burgmann 1959; Roberts *et al.* 1960; Collins and Chaikin 1968) or 30% (Shah and Whiteley 1966) extension, which by definition are independent of so-called "flaws", are often considered to be more appropriate indicators of "non-flaw" structural differences between fibres. Stress at 15% extension is also theoretically independent of variations in cross-sectional area along the fibre, although in practice, significant negative and positive correlations have been reported (Collins and Chaikin 1968). The following results suggest that "flaws" were not the main determinant of the observed variations in intrinsic strength in this experiment: (*i*) intrinsic strength was strongly correlated with non-failure stress parameters, such as Young's modulus, yield stress and stress at 15% extension. In other words, thinner fibres were more resistant to extension per unit cross-sectional area than thicker fibres at all levels up until the point of break; (*ii*) the average coefficient of variation in intrinsic strength between fibres from the same sheep was as low or lower than other stress-strain properties; (*iii*) fibres which were intrinsically stronger were less extensible, and yet premature failure at "flaws" would be expected to reduce intrinsic strength and extensibility at break; and (*iv*) most fibres appeared to break in the region of minimum fibre diameter. Therefore it is likely that real differences in chemical composition and or fibre ultra-structure exist at the point of break between fibres which differed in intrinsic strength.

The morphology of the fracture surfaces appeared to be similar to that reported in the literature, with most (74%) fracture surfaces being relatively smooth and perpendicular or oblique to the fibre axis (Andrews 1964; Mason 1964; Dobb and Murray 1976; Hearle *et al.* 1976; Orwin *et al.*

1985; Gourdie 1989; Dollin *et al.* 1995). While the estimated frequency of clean breaks in this study may be over-estimated because the fibre ends were observed by light microscopy in two dimensions only, Gourdie (1989) also reported that a majority of fracture surfaces viewed end-on were of this type (>80%). Both Gourdie (1989) and the current experiment found that the proportion of clean breaks was greater under low nutrition. This type of fracture suggests cleavage across cortical cells, rather than between the cells along the cell membrane complex, and is typical of breaks in composite structures with a strong interface between components (Dobb and Murray 1976). If it is assumed that the thickness of the cuticle is constant, then the cortex of finer fibres may even be intrinsically stronger than the data presented here suggest. The next most common break was the split-level transverse breaks joined by axial cracks, which may have been short (<5 μm) and poorly defined, or long (>500 μm) and extremely well defined. It has been suggested that the cell membrane complex plays a role in the formation of this type of fracture (Leeder 1986), and indeed, Zimmermann and Höcker (1997) observed that the longitudinal cracks mainly follow the cell membranes or the boundaries of the macrofibrils. In contrast to other reports (Hearle *et al.* 1976; Gourdie 1989; Dollin *et al.* 1995), fibres which broke perpendicular to the fibre axis were intrinsically stronger and more extensible than those with step breaks, although the differences were less than 6%. Zimmerman and Höcker (1990) found that an increase in the proportion of split fractures, which resulted from exposure to artificial sunlight, was associated with a decrease in bundle strength. In agreement with Dobb and Murray (1976), a small proportion of fibres broke between cortical cells, and in the present study these fibres were intrinsically very weak.

3.4.4. Intrinsic strength in relation to staple strength and processing performance

Strong staples comprised individual fibres which were intrinsically weaker per unit cross-sectional area at the point of break. This apparent anomaly is explained by the negative relationship between cross-sectional area at the point of break, the key determinant of staple strength (section 2.3.8), and intrinsic strength. In other words, the effect on staple strength of any increase in intrinsic strength on peak force to break single fibres was outweighed by a much larger decrease in cross-sectional area at the point of break. For example, while nutritional stress increased intrinsic strength by as much as 44%, it decreased cross-sectional area by almost 4-fold (325 *vs.* 87 μm^2), and hence adversely affected staple strength. In contrast to these findings, and despite lacking precision because different fibres were used to estimate the components of intrinsic

strength, Hunter *et al.* (1983) reported that intrinsic strength explained 67% of the variation in staple strength between Merino sheep. Indeed, the average intrinsic strength of fibres from tender wool (13 N/ktex) was 124 N/ktex compared to 177 N/ktex for fibres from sound wool (42 N/ktex). Gourdie *et al.* (1992) also reported that 27% of the variation in staple strength between Romney sheep was due to variations in the intrinsic strength. However, Scobie *et al.* (1996) have shown recently that such a relationship is not always apparent. The precise reasons are not known for the differences between experiments in the relationship between intrinsic strength and staple strength, or why non-failure stress parameters were more closely correlated with staple strength than intrinsic strength in current study.

It is clear that under the conditions of this experiment, where nutritional treatments induced large changes in diameter along fibres, the effects of between-sheep differences in intrinsic strength on staple strength were relatively insignificant compared to the corresponding changes in fibre diameter. If it is assumed that the density of wool is 1.3 g/cm^3 (Postle *et al.* 1988), the calculated average intrinsic strength is 160 N/ktex. As this value far exceeds the average staple strength of these sheep (24 N/ktex), this supports the contention that intrinsic strength is only a minor component of staple strength. It remains to be determined whether intrinsic strength becomes a more important determinant of staple strength under different circumstances. On *'a priori'* grounds, intrinsic strength should become a more important component of staple strength under less variable environmental conditions (i.e. when the fluctuations in diameter along the fibre are minimised). It may also be more important in fine wool sheep which tend to have smaller changes in diameter (Jackson and Downes 1979; Hynd 1994*a*), or in wools shorn close to the point of break where the effects of minimum fibre diameter are eliminated.

Regardless of the possible effects of intrinsic strength on staple strength, it is reasonable to hypothesise that low intrinsic strength will be more significant in later stage processing, because the contribution of diameter variations to fibre breakage is diminished by this stage. Bundle strength in top, measured at a short gauge length (3 mm), is considered to be a measure of the average intrinsic strength of the keratin material (Lamb and Yang 1996). According to Burgmann (1959), Roberts *et al.* (1960) and Gourdie *et al.* (1992), intrinsic strength measured at the point of break is a reasonable measure of the average intrinsic strength along the full length of the fibre. Therefore, the intrinsic strength of raw wool should be closely associated with bundle strength. Bundle strength is known to be strongly correlated with yarn strength (r^2 =

0.54), and therefore spinning and weaving performance; a decrease in bundle strength increases the linear density of the yarn (Bastawisy *et al.* 1961), increases the spinning limit (Townsend *et al.* 1973), and increases "ends-down" in spinning (Gore *et al.* 1990).

An interesting result from this study is the strong positive correlation between fibre extensibility and staple strength. It seems reasonable that the longer a fibre can extend without breaking, the greater the chance of other fibres of variable length and extensibility coming into tension and bearing the total load of the staple. The effects of extensibility on staple strength introduces a new explanation for the effects of minimum fibre diameter and the variation in fibre diameter on staple strength. The association between minimum fibre diameter and staple strength in this experiment is largely due to an increase in the amount of material available to bear the load, and to a lesser extent an increase in fibre extensibility, rather than an increase in the inherent strength of the keratin. Fibre diameter variation influences staple strength via effects on staple linear density and fibre extensibility.

3.5. Conclusion

It is concluded that intrinsic strength was not an important component of staple strength in this experiment. Nevertheless, given that intrinsic strength may be important to staple strength under different circumstances, and that intrinsic strength may be important to later stage wool processing, identifying the causes for differences in intrinsic strength is important. These causes are examined in the following chapters.

CHAPTER 4: CHEMICAL COMPOSITION AND MOLECULAR CHARACTERISTICS OF MERINO WOOL FIBRES IN RELATION TO INTRINSIC STRENGTH

4.1. Introduction

The cortex is the major histological component of the wool fibre, and a number of studies have shown that the number, size and shape of cells entering the cortex, and the proportion and chemical composition of these cells, are influenced by genetic, environmental and physiological factors (Ahmad and Lang 1957; Thorsen 1958; Chapman 1965; Frenkel *et al.* 1974; Gillespie *et al.* 1980, 1982; Orwin *et al.* 1980, 1984, 1985; Hynd 1989, 1994*a,b*; Gillespie 1991; Fratini *et al.* 1994). Considerable variation therefore exists in the cellular and molecular characteristics between fibres from different sheep, and between and along the length of individual fibres from the same sheep, and much theoretical and experimental work has focused on correlating these variations to the shape of the wool fibre stress-strain curve. There is little doubt that the distinct changes in the mechanical behaviour of wool fibres during extension are intimately related to the molecular state of the two-phase microfibril/matrix composite of the fibre cortex (Hearle *et al.* 1971; Feughelman 1987, 1994; Wortmann and Zahn 1994), rather than the properties of the cuticle, cell membrane complex or intermacrofibrillar material.

While there is continuing controversy over the relative roles of the filament and matrix phases in determining mechanical properties beyond 25 to 30% extension (Chapman 1969; Feughelman 1994; Wortmann and Zahn 1994), and it is not clear which theoretical model is most valid, the general consensus is that the longitudinal stiffness of the wet wool fibre at lower extensions is governed primarily by the unfolding of the α-helices within the microfibrils (Feughelman 1979, 1987, 1994; Wortmann and Zahn 1994). The matrix does become stiffer with decreasing water content, but even at 65% relative humidity it is still considered to be weaker in the longitudinal direction than the microfibrils (Bendit and Feughelman 1968; Feughelman 1968). Thinner fibres resulting from nutritional manipulation, were shown in the previous Chapter to be intrinsically stronger than thicker fibres at *all* levels of extension up until the position of break, suggesting that intrinsic strength is *associated* with the same physico-chemical characteristics of the fibre that are directly responsible for the differences in stress at lower extensions. The

hypothesis that differences in intrinsic strength are associated with the proportion of microfibrils relative to matrix was therefore tested.

4.2. Materials and methods

The associations between the average intrinsic strength of wool fibres and their physical and chemical characteristics were established using samples from the sheep used in the experiment reported in Chapters 2 and 3. The following measurements were made at strategic times throughout the experiment: (*i*) the proportion of ortho- and paracortical cells; (*ii*) total sulphur content and amino acid composition; (*iii*) the relative amounts and composition of keratin proteins using 1D and 2D polyacrylamide gel electrophoresis (PAGE)[1]; and (*iv*) the α-helical content and transition temperatures using differential scanning calorimetry (DSC).

4.2.1. Cortical cell type proportions

The proportion of ortho- and paracortical cells was determined from skin biopsies taken on days 56, 112, 168, 224, and 280, and at the estimated position of break along the staple. The biopsies were processed, embedded, sectioned and stained with Methylene Blue after performic acid oxidation (Clarke and Maddocks 1965), as described in section 2.2.5. Three replicates of 30 follicles per sample were selected at random and microscopically examined at 1530 x magnification. The total cross-section of the fibre and the region occupied by paracortical cells were traced with the aid of a camera lucida attached to a microscope, and the area and the paracortex percentage were then calculated using an image analysis system (Bioquant IV, R&M Biometrics, Tennessee, USA). When present, mesocortical cells were included as paracortical cells. Sequential tissue sections (8 μm) from two biopsies sampled on day 56 and identified to have extremes in paracortex percentages, were used as standards and subsequently stained for each of the individual staining runs.

4.2.2 . Sulphur content and amino acid composition

Total sulphur content and amino acid composition was estimated for wool samples clipped from the mid-side patch on days 63, 175, and 287, and at the estimated position of break along the staple. The wool was washed in hexane (3 x 10 min; Ace Chemical Co. Adelaide, Australia) and

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[1] Preliminary data on protein composition in relation to intrinsic strength was published in the *Proceedings of the Australian Society of Animal Production*, **21**; 435 (1996).

hot water (≈ 65°C; 2 x 10 min). The nitrogen content was determined by a combustion method (AOAC 1990), using a LECO FP-428 Nitrogen Determinator, and total sulphur was determined by inductively coupled plasma atomic-emission spectrometry (ICP-AES) after digestion with nitric/perchloric acids, as described in detail by McQuaker *et al.* (1979).

The amino acid composition of wool samples, with the exception of tryptophan, was determined according to the method described by Gillespie *et al.* (1982) and Gillespie and Marshall (1983). In brief, 300 mg of clean wool was weighed into a 25 ml glass screw capped tube, and hydrolysed *in vacuo* by adding 15 ml of 6 M HCl containing 2 mM phenol. After evacuation and replacement of the air with high purity nitrogen, the tube was sealed and heated at 110°C for 24 h. After cooling, 1 ml of 20 μmole/ml nor-leucine was added as an internal standard, the contents were mixed and a 2 ml aliquot freeze dried to remove the HCl. The hydrolysates were then dissolved in 1 ml of 0.1 mM sodium borate, the pH being adjusted if necessary to between 8 and 9 by drop-wise addition of 0.1 M NaOH, and shaken in air for 30 min to oxidise any cysteine produced by reaction between phenol and cystine. The solution was freeze-dried again, and 5 ml of 0.06 M HCl was added. After mixing, the hydrolysate was stored in HPLC vials at -20°C until analysis. All HPLC analysis was performed on a sulphonic acid cation exchange column using citrate buffers of pH 3.25 and pH 6.75 with a post-column ninhydrin reaction. The composition of amino acids was also used to estimate the relative proportions of the HS and HGT proteins, using the experimentally determined regressions reported by Gillespie and Frenkel (1974).

4.2.3. Polyacrylamide gel electrophoresis of keratin proteins

Wool proteins were extracted and characterised by 1D- and 2D-PAGE using the procedures described by Marshall and Gillespie (1977, 1982), but with modifications (Nancarrow 1995). The horizontal gel electrophoresis system used for all protein separations was a Multiphor II Electrophoresis Unit (Multitemp II Thermostatic Circulator; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney, Australia) with a Multidrive XL Programmable Power Supply (Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney, Australia). With this system, the proteins could be separated faster and detected in smaller quantities without the need for radiolabelling. This was due to tighter temperature control and the use of thinner gels with linear polyacrylamide gradients.

4.2.3.1. Wool samples

Twenty staples from each sheep were selected from the midside fleece sample taken at shearing. The position of break along each staple was identified and placed between the jaws of an Agritest Staple Breaker (gauge length 20 mm). After the staples were broken, snippets of wool (< 2 mm) were cut from the fractured ends of fibres from the tip portion of each staple. The fibre snippets (3 to 5 mg) were then placed in 1 ml eppendorf tubes, washed in clean hexane (3 x 10 min; Ace Chemical Co., Adelaide, Australia) and hot water (≈ 65°C; 2 x 10 min), and dried under vacuum (SpeediVac).

4.2.3.2. Protein extraction, alkylation and solubility

Wool proteins were extracted in 8M urea and s-carboxymethylated with iodoacetic acid as described by Nancarrow (1995), and the concentration of protein in the alkylated extracts were estimated using the procedure described by Bradford (1976). The fibre debris remaining after the extraction and removal of the aliquot for alkylation was resuspended in nanopure water and washed thoroughly by vortexing. The suspension was then left at room temperature for 2 h before being centrifuged at 12000 g for 30 min. This procedure was repeated five times. The undissolved fibre residue appeared free of urea crystals and no odour of dithiothreitol was apparent. The residues were then dried for 2 h under vacuum (SpeediVac), and weighed out of a desiccator. Solubility was expressed as the fraction (%) of mass lost during treatment. The results reported are for one test only.

4.2.3.3. One-dimensional polyacrylamide gel electrophoresis (1D-PAGE)

Wool protein extracts for separation by 1-D PAGE were prepared for loading by adding 1 x urea load buffer [3 g urea dissolved in 1 ml 0.5M Tris at pH 6.5 and nanopure water to 8 ml] to each sample to bring the total volume of the sample to 20 µl. This mixture was then vortexed and centrifuged at 12000 g for 1 min (Beckman, Microfuge, USA). The amount of protein loaded onto the gels ranged from 5 to 15 µg (2 to 5 µl), depending on the extraction. Proteins separated in one dimension only were separated on a 0.5 mm discontinuous 12-14% acrylamide, 0.1% SDS gel (ExcelGel XL SDS, gradient 12-14; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney, Australia). The pre-poured gel was placed on the cooling apparatus of the Multiphor II Electrophoresis Unit set at 15°C, and sealed with about 2 ml kerosene (Diggers Kerosene; Ajax Chemicals, Regency Park, SA). Anode (0.3 M Tris, 0.3 M

acetate, 0.4% SDS, pH 6.4) and cathode (0.08 M Tris, 0.8 M tricine, 0.4% SDS, pH 7.1) buffer strips (ExcelGel SDS Buffer Strips; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney) were placed at the appropriate ends of the gel. Loading wells were placed on top of the gel, and gently pressed down to ensure a good seal. The prepared samples, and M_r markers (SDS-PAGE Molecular Weight Standards, High and Low Range; Bio-Rad Laboratories, Hercules, CA), were loaded into the wells and the gel was run at a setting of 20 mA and 1000 Volts for 90 min, and then the current was increased to 40 mA for a further 2.5 h. The gel was terminated when the dye front had migrated to the anode buffer strip. The buffer strips were then removed and the kerosene wiped from the back of the gel. The one-dimensional protein gels were then fixed and stained with Coomassie blue with or without silver, as described by Nancarrow (1995).

4.2.3.4. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)

A full description of the procedure used to separate wool proteins in two-dimensions is given by Nancarrow (1995), and it is only dealt with briefly here. Protein extracts were prepared as they were for 1D-PAGE, and the proteins (30-100 μg) were separated in the first dimension using a 0.5 mm discontinuous polyacrylamide gel, comprising a stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9. Duplicates were run for each sample, and one of the duplicate samples was fixed and stained with Coomassie blue to ensure that the samples had remained in their lanes, and to assess whether the amount of protein loaded was sufficient for a 2-D PAGE. The remaining samples were then thawed at room temperature, and after equilibration, the proteins were separated in the second dimension on a 0.5 mm discontinuous polyacrylamide gradient gel (12-14%) containing 0.1% SDS (ExcelGel XL SDS, gradient 12-14; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney). Upon completion of electrophoresis, the protein gels were fixed and stained with Coomassie blue.

4.2.3.5. Quantification of constituent proteins

Protein groups identifiable on the Coomassie blue-stained gels were quantified using laser densitometry (Molecular Dynamics Computing Densitometer, Model 300A; Pharmacia LKB Biotechnology, AMRAD Pharmacia Biotech, North Ryde, Sydney) and Image Master software. Identification of the major protein families was based upon MW and their position in the gel by

comparison with the well characterised proteins of sheep wool (Powell and Rogers 1986). While the absolute amounts of the constituent proteins in the fibres from different samples is not known, because it was not possible to load the same amounts of protein onto each gel, the relative amounts of the different proteins were compared, assuming that the staining intensity was directly proportional to the concentration of protein.

4.2.4. Differential scanning calorimetry (DSC)

4.2.4.1. Wool samples and their preparation

Wool samples from seven sheep within each nutritional treatment were characterised thermally by DSC (n = 21). The samples used were clipped from the mid-side patch at the time corresponding to the estimated position of break along the staple. Clean wool samples were cut into pieces less than 1 mm long, placed in an open eppendorf and conditioned at 20°C and 65% relative humidity for at least 48 h prior to thermal analysis to ensure constant water content.

4.2.4.2. Denaturation temperatures and enthalpy

Approximately 8-10 mg of the finely-cut wool fibres were accurately weighed and manually compressed into stainless steel capsules. The capsules with a volume of 75 µl can withstand vapour pressure up to 150 atm. Water containing 0.1% non-ionic detergent was added to the wool (150% v/w) by micro pipette. The capsules were then sealed as quickly as possible with a constant torque sealing tool. The use of high pressure sealed DSC capsules eliminates interference from endotherms caused by the vaporisation of water. The sealed capsules were weighed, and the wool samples left to equilibrate for at least 48 h at 20°C. The DSC measurements were conducted on 5-10 replicates per sample. All DSC measurements were carried out on a Perkin Elmer DSC 7 module attached to a Perkin Elmer Thermal Analysis Controller 7/DX. Prior to the analysis of each wool sample, the DSC instrument was calibrated using high purity indium, which has a heat of fusion of 28.45 J/g and an onset of melting temperature of 156.6°C. The capsules were heated rapidly to 50°C, held at 50°C for 30 sec, and then heated linearly at 5°C/min from 50 to 200°C in a nitrogen atmosphere (flow rate equal to 26 ml/min). An empty stainless steel capsule was used as a reference.

The analogue voltage, which reflects the differential heat input into the sample compared with the reference, was recorded as a function of sample temperature. All the thermograms were

generated on an Olivetti M486 computer (Perkin Elmer Thermal Analysis Software Version 2.01), attached to a Hewlett Packard Color Pro printer (Computology Aust. Pty Ltd.). In accordance with conventional practice in calorimetry, endothermic effects, i.e. heat absorption by the sample, are represented by an increase in the ordinate value from the baseline position. The temperatures of the thermal effects were determined from the inflection points of the scanning curve. The "denaturation" point (T_m) was taken as the temperature at the peak of the endotherm curve, and the denaturation enthalpy (ΔH, J/g) was measured from the area under the endothermic curve enclosed by either a linear or sigmoid baseline.

4.2.5. Statistical analysis

The effects of nutritional treatments on cortical cell type proportions, total sulphur content, amino acid and protein composition and thermal characteristics were compared by analysis of variance. The strength of the relationships between the various physico-chemical characteristics and intrinsic strength were tested using simple linear regression analysis. All statistical analyses were performed using the statistical package SuperANOVA^{TM} (Abacus Concepts Inc., Berkeley, California, USA).

4.3. Results

4.3.1. Cortical cell type proportions

Orthocortical and paracortical cells were easily distinguished after staining with Methylene blue, and were bilaterally arranged in most fibre cross-sections examined (Plate 4.1). Considerable variation was observed in the staining intensity of paracortical cells between individual sheep at the same time (Plate 4.1*a*), between sampling times for the same sheep (Plate 4.1*b*), and between individual fibres from the same sheep (Plate 4.1*c*). The variations in the staining characteristics were not staining artefacts, as there were no significant (P>0.05) changes between staining runs in either the proportion (27.7 ± 0.66 and 45.0 ± 0.25) or subjectively assessed staining intensity of paracortical cells in serial sections cut from the same two skin samples.

Plate 4.1. Photomicrographs of transverse skin sections (8 μm) stained with Methylene blue and showing the variation in staining intensity of paracortical cells: (*a*) between individual sheep sampled at the same time; (*b*) between sampling times for the same sheep; and (*c*) between cells within fibres (arrow) and between individual fibres from the same sheep. Scale bar = 20 μm.

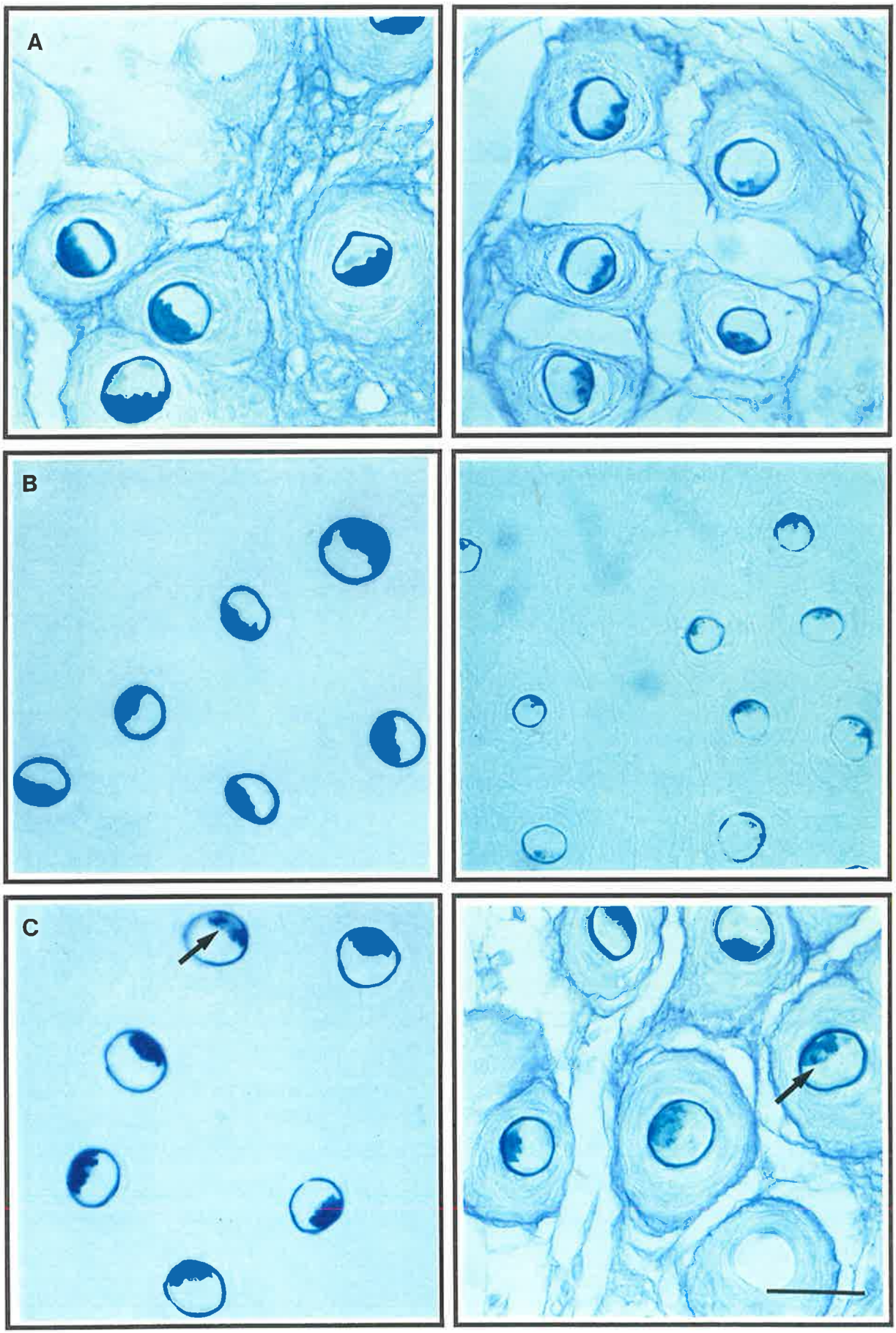

Plate 4.1.

On average, the proportion of paracortical cells decreased ($P<0.001$) from 37% at the start of differential feeding to 30% at the position of break along the staple, and this was the case for all nutritional treatments (Fig. 4.1). The changes in the proportion of paracortical cells to the position of break varied considerably (range -20% to +5%) between individual sheep. A marked decrease in the intensity of staining at the position of break was also apparent for most sheep, although it was not quantified.

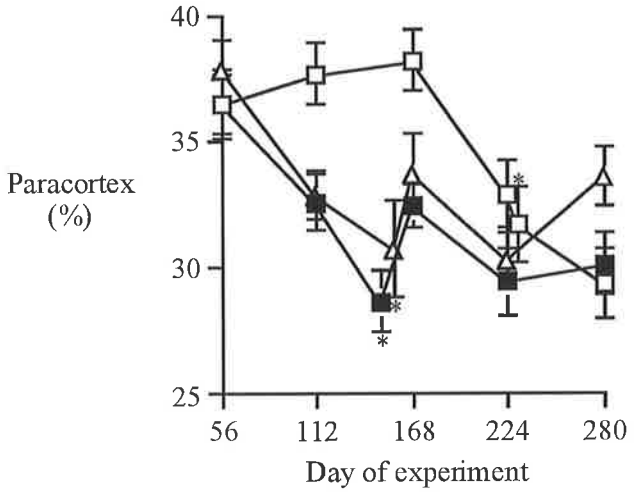

Figure 4.1. The proportion of paracortical cells in the cross-section of wool fibres estimated from Methylene blue-stained transverse skin sections for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined, and points represent treatment means. Error bars denote s.e.m, and * denotes the position of break along the staple.

On most occasions (>80%), there was no significant correlation between fibre diameter and the proportion of paracortical cells, either between individual sheep or between individual fibres from the same sheep. As fibre diameter was closely related to intrinsic strength, it is therefore not surprising that there was no significant ($P>0.05$) relationship between the average proportion of paracortical cells and intrinsic strength.

4.3.2. Sulphur content and amino acid composition

On average, the nitrogen content of wool samples remained constant throughout the experiment, and did not differ significantly ($P>0.05$) between nutritional treatments. The overall average was 15.1% nitrogen (94 to 95% protein). Wool sulphur content decreased significantly ($P<0.001$)

from the start of differential feeding until the position of break along the staple (Fig. 4.2*a*), and the decrease was greater ($P<0.10$) for sheep on the more restricted diet. It was calculated from the nitrogen and sulphur content and the estimated amino acid composition that the percentage recovery of nitrogen and sulphur varied significantly ($P<0.001$) between the four batches of hydrolysates (sampling time), indicating that variable amounts of sulphur and nitrogen were lost during hydrolysis. The values were 93.6 and 95.3% for day 63, 92.6 and 92.3% for day 175, 89.7 and 89.6% for day 287, and 90.0 and 90.5% for the sampling time corresponding to the position of break, respectively. More importantly, there was a considerable range in the amount of nitrogen (74 to 96%) and sulphur (73 to 100%) recovered between individual samples, and unfortunately, this variation was greatest for the batch of samples corresponding to the position of break. To reduce the effects of this analytical error, the percentage recovery of nitrogen or sulphur have been used as covariates in the analysis of the data wherever possible.

The relationships between sulphur content and amino acid composition are shown in the Appendix (Table A4.1). In general, they were consistent with the correlations reported in the literature (Gillespie and Frenkel 1974; Gillespie *et al.* 1980; Marshall and Gillespie 1989, 1990), in that strong (r^2 = 0.43 to 0.85; $P<0.001$) positive correlations existed between amino acids which are concentrated within the HS protein (1/2-Cys, Thr and Pro), the LS protein (Asp, Ala and Leu) and the HGT protein families (Gly, Tyr and Phl). Amino acids more concentrated in the HS proteins were also negatively related to those in the LS and HGT proteins (e.g. Pro *vs.* Leu). Surprisingly, Glu, the most abundant amino acid in the LS proteins, was poorly related to the other amino acids more concentrated within this protein family ($r^2 < 0.24$).

Wool sulphur content and the concentration of most of the amino acids present at higher levels in the HS proteins decreased ($P<0.001$) from the start of differential feeding until the position of break along the staple (Fig. 4.2*a,b*), whereas there were increases ($P<0.05$) in the proportions of most of the amino acids which are predominantly located in the LS (Fig. 4.2*c*) and HGT proteins (Fig. 4.2*d*). At the position of break itself, wool from sheep fed to maintain liveweight tended ($P<0.10$ to $P<0.001$) to have more sulphur, 1/2-Cys, Thr and Pro, but less Asp, Gly, Tyr, Phl and Lys compared to wool from sheep fed to lose liveweight (Table 4.1).

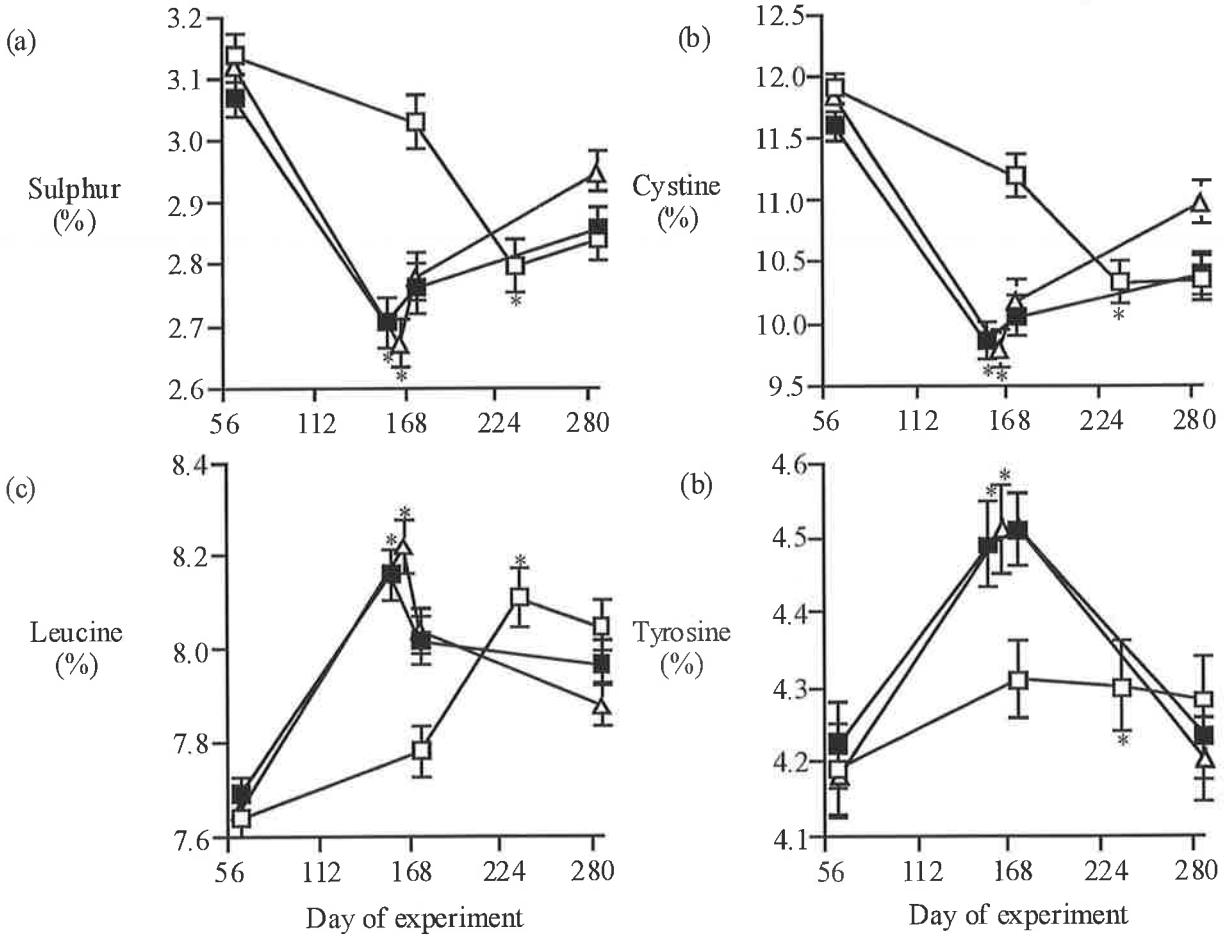

Figure 4.2. (*a*) Percentage sulphur, and the content (residues %) of (*b*) half-cystine, (*c*) leucine and (*d*) tyrosine in mid-side wool samples for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined, and points represent treatment means. Error bars denote s.e.m, and * denotes the position of break along the staple.

After the position of break there were generally no significant ($P>0.05$) changes in the composition of wool samples from sheep fed to maintain liveweight. However, for sheep from the liveweight loss treatments, changes in sulphur content and amino acid composition were in the reverse direction to that which occurred prior to the break. The proportion of some amino acids (His, Arg, Val, Met and Ile) was constant over time, irrespective of nutritional treatment, and changes in the proportion of Ser and Glu over time and between treatments were variable

Table 4.1. Sulphur content (%) and amino acid composition (residues %) of wool clipped from the mid-side patch at the time corresponding to the point of break along the staple for sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition.

a, b: different superscripts within selection flock or nutrition comparisons differ at $P< 0.05$

Chemical composition	Nutritional treatment		
	LWM	LWL_1	LWL_2
Sulphur	2.80^a	2.70^{ab}	2.67^b
1/2-Cystine	10.3^a	9.9^b	9.8^b
Aspartic acid	6.6^a	6.7^b	6.7^b
Threonine	6.6^a	6.4^b	6.4^b
Serine	12.2^a	12.2^a	12.1^a
Glutamic acid	12.4^a	12.2^b	12.2^b
Proline	6.6^a	6.2^b	6.3^b
Glycine	9.2^a	9.6^b	9.6^b
Alanine	5.5^a	5.6^a	5.6^a
Valine	5.8^a	5.7^{ab}	5.7^b
Iso leucine	3.2^a	3.2^a	3.2^a
Leucine	8.1^a	8.2^a	8.2^a
Tyrosine	4.3^a	4.5^b	4.5^b
Phenylalanine	2.9^a	3.0^b	3.0^b
Lysine	3.1^a	3.1^a	3.2^a
Histidine	0.9^a	0.9^a	0.9^a
Arginine	6.9^a	7.1^a	7.1^a

The proportion of paracortical cells in the fibre cross-section was positively (r^2 = 0.25 to 0.35; $P<0.05$) correlated to wool sulphur content (Fig. 4.3*a*), and significant correlations also existed between the proportion of paracortical cells and certain amino acids from the different protein families (Fig. 4.3*b,c,d*). However, the relationships differed significantly ($P<0.05$) between the start of differential feeding and the position of break along the staple.

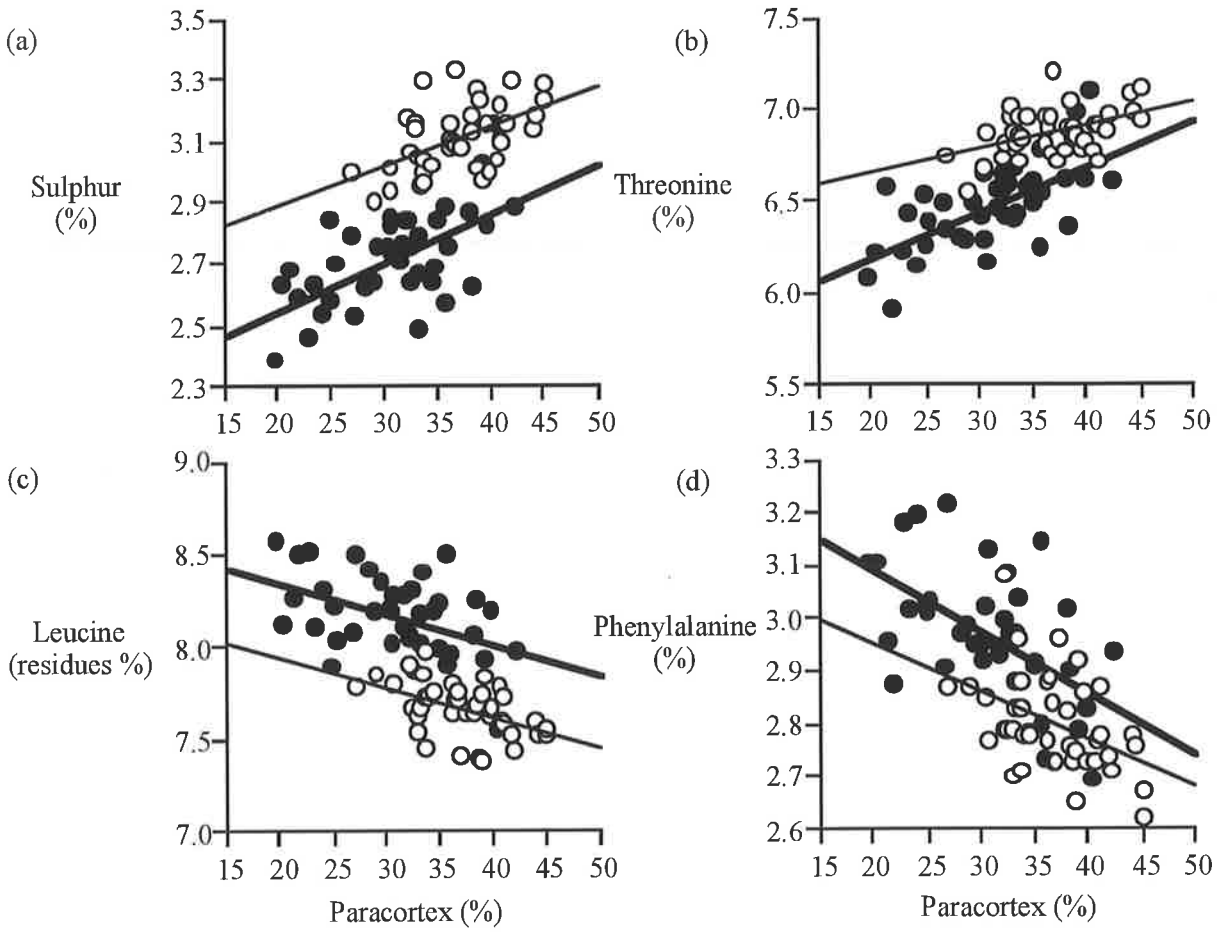

Figure 4.3. The relationship between the proportion of paracortical cells and the content of (*a*) sulphur (%), (*b*) threonine, (*c*) leucine and (*d*) phenylalanine (residues %) of wool samples for sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition. Points represent means for individual sheep, and sampling times are represented by different symbols; day 63 (○; ———) and position of break along the staple (●;———).

On an individual sheep basis, the correlations between fibre diameter and sulphur content or amino acid composition at the position of break were weak ($r^2 < 0.18$), and variable in sign. It was not surprising then that sulphur content or amino acid composition at the position of break were not significantly ($P>0.05$) related to intrinsic strength.

4.3.3. Protein composition

4.3.3.1. Protein composition estimated from amino acid composition

The proportions of HS and HGT proteins were estimated from the composition of amino acids using the experimentally determined regression reported by Gillespie and Frenkel (1974). The assumption implicit in this method, that the composition of the constituent proteins do not differ

markedly from the average (Gillespie 1991), seems reasonable since there was a strong negative correlation between the contents of Pro and Leu (Appendix, Table A.4.1). These amino acids are reliable indicators of the amounts of HS and LS proteins, respectively (Marshall and Gillespie 1990). It appears that sheep fed to maintain liveweight had marginally more ($P<0.05$) HS proteins and less ($P<0.05$) HGT proteins at the position of break than sheep on the restricted diets (Table 4.2). Assuming that the remaining proteins belong to the LS protein family, the results suggest that the proportion of LS proteins was constant ($P>0.05$) across all nutritional treatments.

Table 4.2. Relative proportions of major protein families estimated from the amino acid composition¥. The wool samples were clipped from the mid-side patch at the time corresponding to the position of break along the staple, and were from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition.

a, b: different superscripts within selection flock or nutrition comparisons differ at P< 0.05

Protein family	Nutritional treatment		
	LWM	LWL_1	LWL_2
High sulphur	20.8^a	19.6^b	19.5^b
Low sulphur	67.2^a	67.3^a	67.2^a
High glycine-tyrosine	12.0^a	13.1^b	13.3^b

¥ *HS (%) = 2.5 x half-cystine (moles%) - 5.0; HGT (%) = 5.6 x tyrosine (moles%) - 12.0.*

4.3.3.2. Comparison of wool proteins by 1-D PAGE

The extraction procedure solubilised between 80 and 85% of the fibres, and there were no differences in solubility between treatment groups. This was greater than the level considered adequate for the extracted proteins to truly represent the keratin protein composition of the samples (Gillespie 1991). The extracted wool proteins from the break region of all sheep were characterised by 1-D PAGE after S-carboxymethylation with iodoacetic acid, and subsequent staining with Coomassie-blue (Plate 4.2) or silver (Plate 4.3 to 4.5). The three major groups of proteins corresponding to the LS, HS and HGT families were apparent, and qualitatively, the electrophoretic patterns showed that most of the identified proteins were common to all sheep.

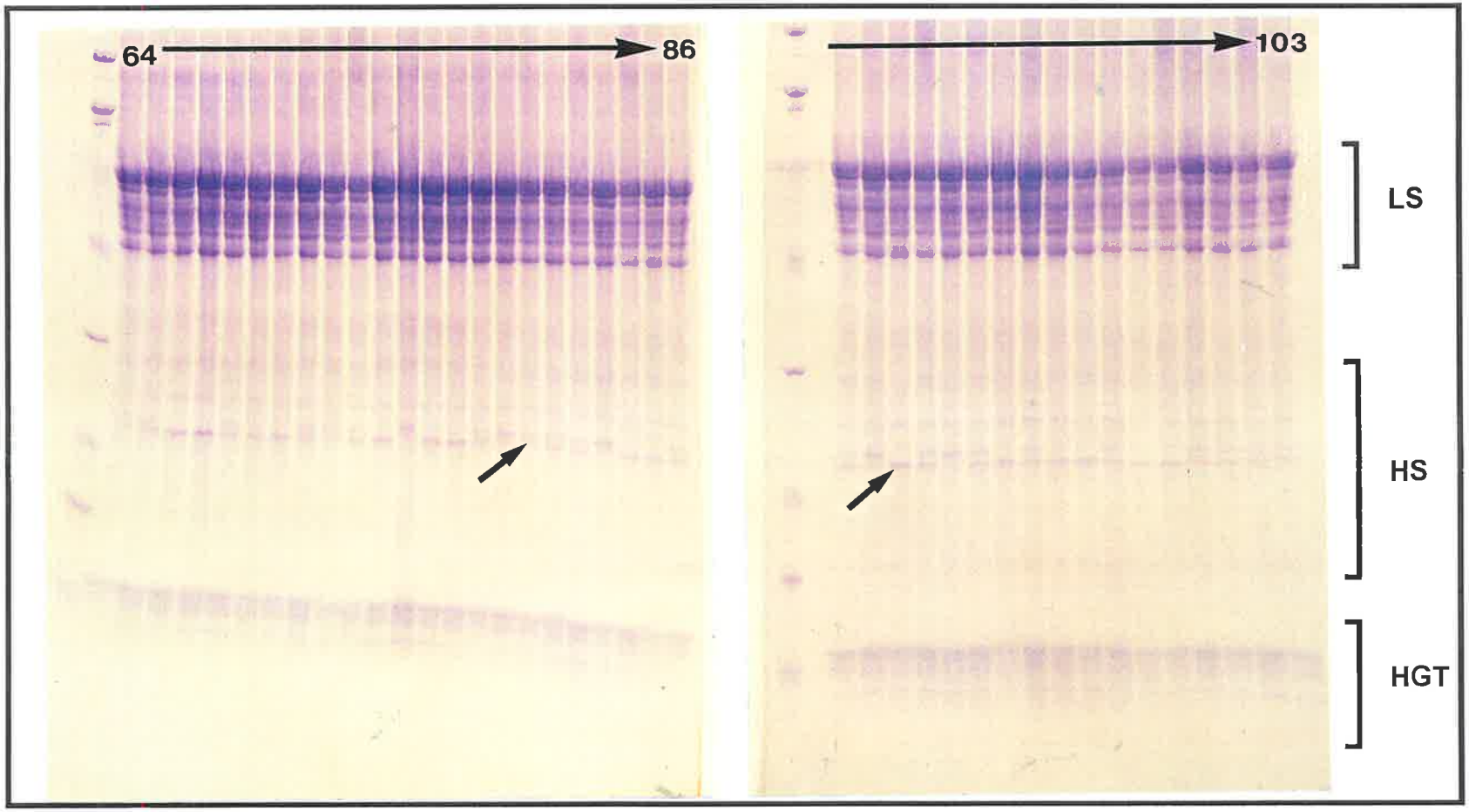

Plate 4.2. Coomassie-blue stained 1D-PAGE patterns of proteins extracted from wool samples differing in intrinsic fibre strength by up to 44%. The average intrinsic strength of the samples are shown in Table A4.2 of the Appendix. The wool samples were cut from the broken tip-end (< 2mm) of staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition, and about 10 μg of extracted protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated, and the arrow denotes the only obvious qualitative difference between the one-dimensional protein patterns.

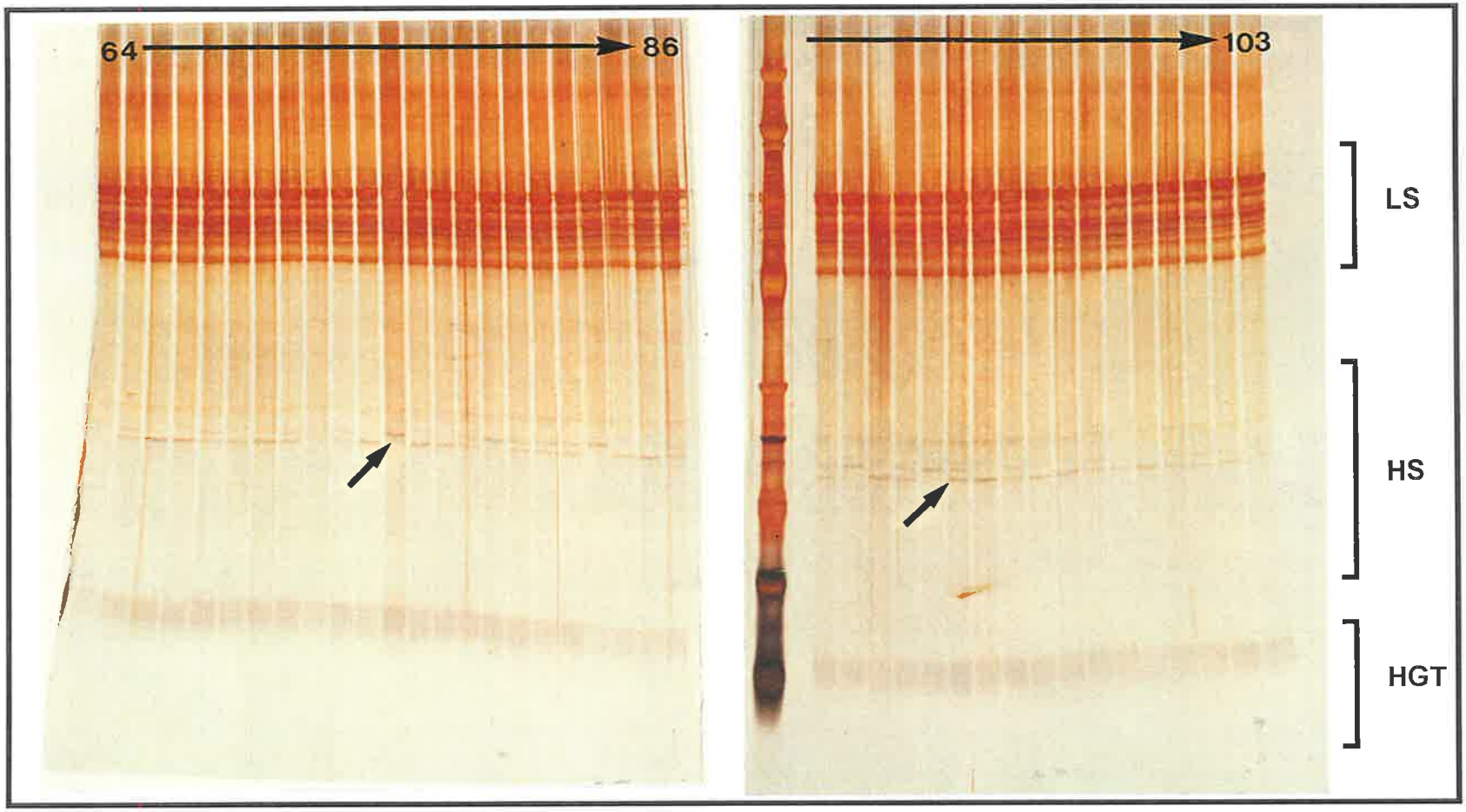

Plate 4.3. Silver-stained 1D-PAGE patterns of proteins extracted from wool samples differing in intrinsic fibre strength by up to 44%. The average intrinsic strength of the samples are shown in Table A4.2 of the Appendix. The wool samples were cut from the broken tip-end (< 2mm) of staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition, and about 0.5 μg of extracted protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated, and the arrow denotes the only obvious qualitative difference between the one-dimensional protein patterns.

Eight different LS proteins with apparent MW from 50 to 75 kDa were identified, and there did not appear to be any observable differences in protein mobility or composition between sheep. This is most clearly evident in the silver stained 1D-gel shown in Plate 4.4. The staining intensity and resolution of the HS, and to a lesser extent the HGT proteins, was relatively poor in comparison to the LS proteins, and it was difficult to determine the precise number and location of these proteins extracted from the different samples. The most obvious detectable variation between samples within the HS proteins was the random occurrence of protein bands with MW ≈ 25 kDa; 50% of the samples had both protein bands, 37.5% only had the low M_r protein band, and 12.5% only had the high MW protein band. As shown in Plate 4.5, a third HS protein of slightly higher MW may also have varied randomly between samples. Three or four HGT protein bands were common to all sheep, and they had MW less than about 10 kDa. Smearing of protein bands was evident in some samples. The location and intensity of the protein bands on the 1D-gels stained with silver appeared to be similar to that for Coomassie blue-stained gels.

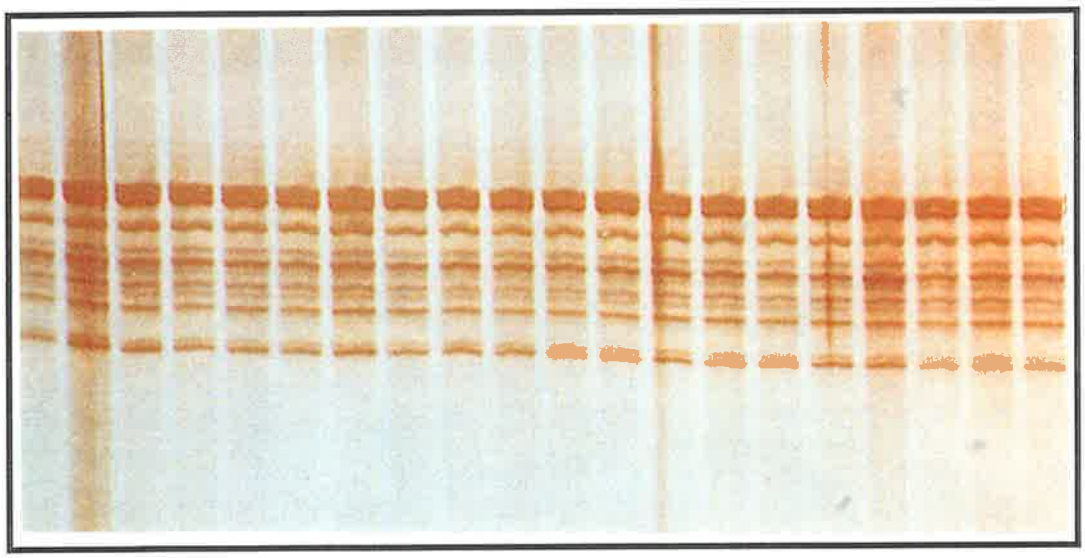

Plate 4.4. Silver-stained 1D-PAGE patterns of the low sulphur proteins extracted from wool samples cut from the broken tip-end (< 2mm) of staples from different sheep. Approximately 0.25 μg of protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. No observable differences in the mobility or composition of the low sulphur proteins were evident.

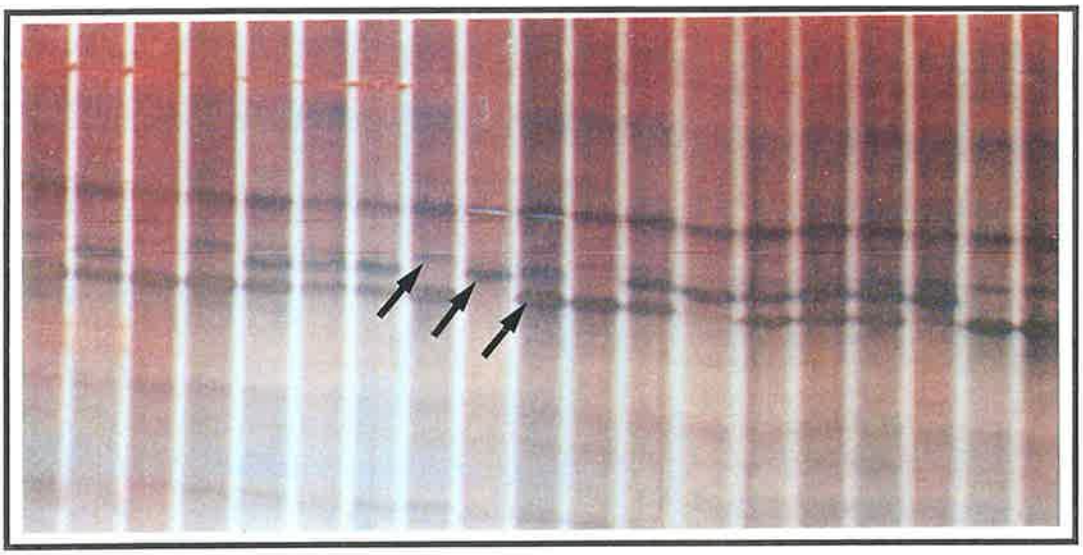

Plate 4.5. Silver-stained 1D-PAGE patterns of the high sulphur proteins extracted from wool samples cut from the broken tip-end (< 2mm) of staples from different sheep. Approximately 5 μg of protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. The random occurrence of three high sulphur protein bands between samples are shown (arrows).

Quantitation of the Coomassie-stained 1D-PAGE pattern shown in Plate 4.2*a* (10 μg protein loaded) revealed that the relative amounts of the three major proteins generally fluctuated within a small range; 50 to 55%, 12 to 18% and 10 to 15% for LS, HS and HGT proteins, respectively. The proportion of LS proteins was not significantly influenced by nutrition, but sheep fed to maintain liveweight had more ($P<0.05$) HS proteins, and tended to have less HGT proteins, at the position of break than sheep on the restricted diets (Table 4.3). This is consistent with the estimates of protein composition based on amino acid composition (Table 4.2). The HS proteins with MW of about 25 kDa mentioned above constituted less than 2% of total protein stained. The high MW smearing was greatest ($P<0.05$) for samples from sheep on the most restricted diet. The silver-stained gels were not quantified.

The effects of nutrition on protein composition were also evident when 5 or 15 μg of protein was loaded onto Coomassie stained 1-D gels (Appendix Plates A4.1 and A4.2). However, differential changes in the staining intensity of individual samples with changes in the amount of protein loaded meant that the correlations between the gels for the different proteins were low (LS proteins, $r^2 < 0.22$; HS proteins, $r^2 < 0.13$) or not significant (HGT proteins).

Table 4.3. The relative proportions (%) of the major protein families estimated from Coomassie-stained 1D-PAGE patterns. The wool samples were cut from the tip-end (< 2mm) of broken staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition. About 10 μg of extracted protein was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h.

a, b: different superscripts differ at P< 0.05

Protein type	Nutritional treatment		
	LWM	LWL_1	LWL_2
High MW smear	17.8^a	18.8^{ab}	20.5^b
LS - type I	27.1^a	26.4^a	26.7^a
LS - type II	26.6^a	26.5^a	26.3^a
LS total	53.6^a	52.9^a	53.0^a
HS total	15.8^a	14.5^{ab}	12.8^b
HGT total	12.9^a	13.8^a	13.7^a

4.3.3.3. Comparison of wool proteins by 2-D PAGE

The 2-D PAGE patterns showed that the separation and resolution of HS and HGT proteins was improved compared to the 1D PAGE patterns. Electrophoretic patterns of S-carboxymethylated wool proteins from samples with extreme differences in intrinsic strength are presented as Coomassie blue-stained gels (Plates 4.5 and 4.6). While there were no obvious qualitative differences in the LS proteins, other than those attributable to small variations in the duration of electrophoresis, a few differences in the HS and HGT proteins between samples additional to those revealed by the 1D-PAGE patterns were apparent. A group of proteins known as the UHS proteins was present in some samples at low amounts, although no individual protein spots could be discerned within the UHS smear. Separation in the second dimension also increased the number of HGT proteins identified to about 10 proteins, but there were no observable changes in the relative proportions of the individual HGT proteins.

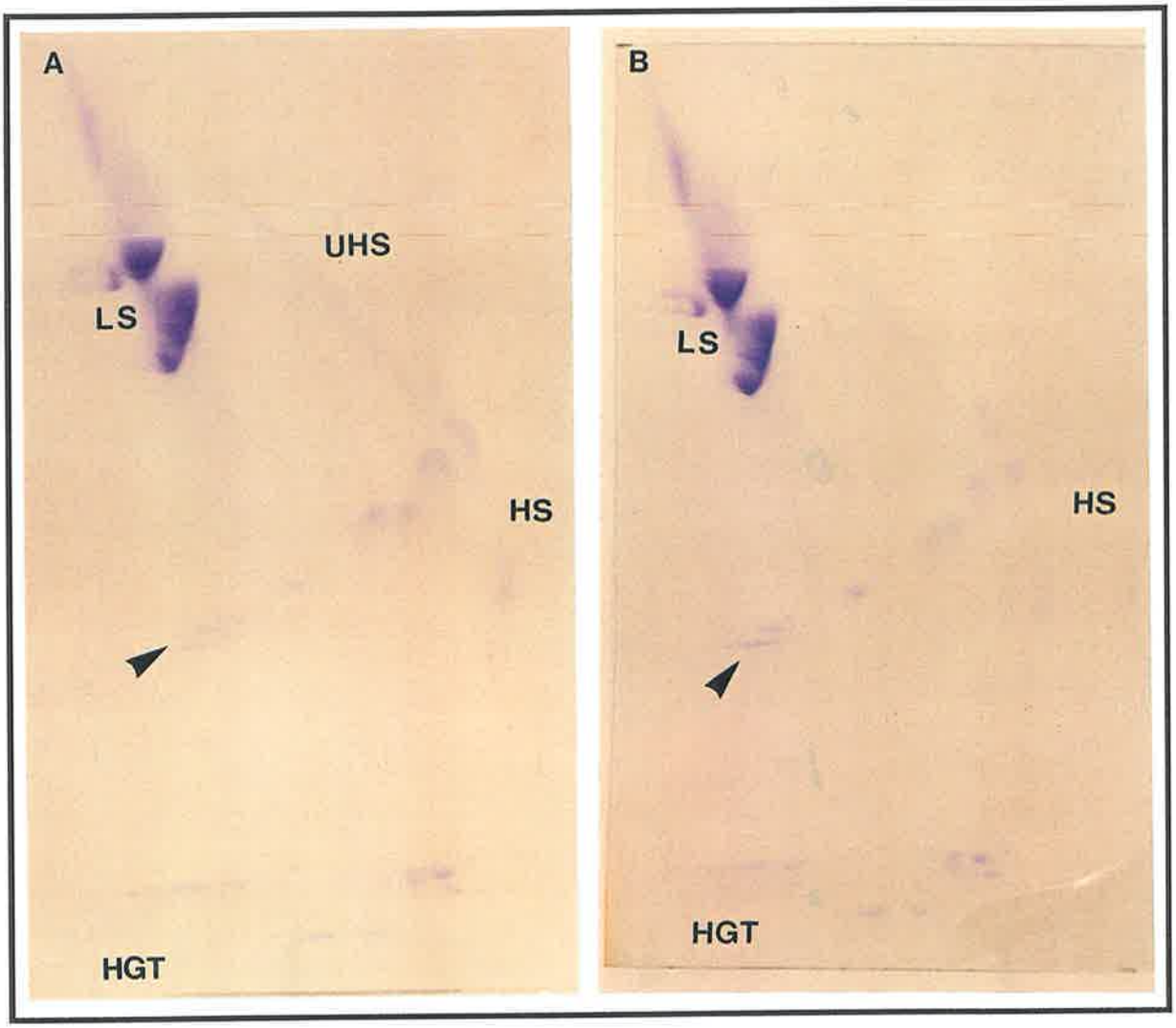

Plate 4.5. Coomassie blue-stained 2D-PAGE patterns of proteins extracted from wool samples cut from the broken tip-end (< 2mm) of staples with differences in average intrinsic strength; (*a*) 163 MPa and (*b*) 235 MPa. The proteins were separated in the first dimension (horizontal) in a discontinuous polyacrylamide gel (stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9) and in the second dimension (vertical) in a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel. The location of low sulphur (LS), high sulphur (HS) and high glycine- tyrosine (HGT) groups of proteins are indicated. Both these samples had two HS protein bands (MW ≈ 25 kD) (arrow heads).

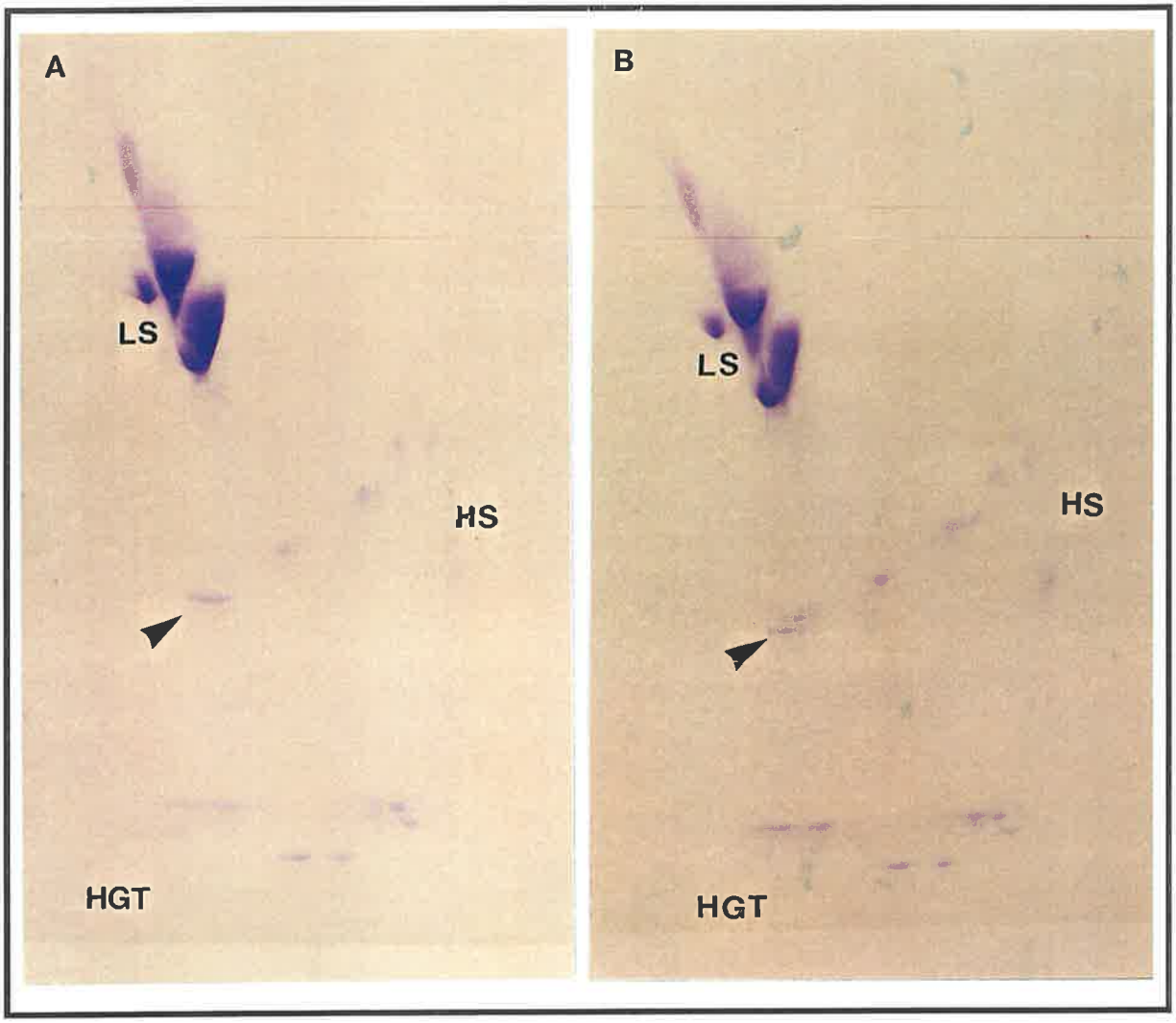

Plate 4.6. Coomassie blue-stained 2D-PAGE patterns of proteins extracted from wool samples cut from the broken tip-end (< 2mm) of staples with differences in average intrinsic strength; (*a*) 208 MPa and (*b*) 233 MPa. The proteins were separated in the first dimension (horizontal) in a discontinuous polyacrylamide gel (stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9) and in the second dimension (vertical) in a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel. The location of low sulphur (LS), high sulphur (HS) and high glycine- tyrosine (HGT) groups of proteins are indicated. The HS protein bands (MW ≈ 25 kD) marked by arrow heads varied between samples, but were not related to intrinsic fibre strength.

Quantification of the 2D-gel patterns were consistent with the conclusions drawn from the amino acid composition and the 1D-gel electrophoretic patterns (Table 4.4). While the LS proteins were not influenced by nutritional treatments, sheep fed to maintain liveweight had more (P<0.05) HS proteins, and less (P<0.05) HGT proteins, at the position of break than sheep on the restricted diets. The effects of nutrition on the content of HS proteins was mainly attributable to differences in the intensity of the UHS smear, and the differences in HGT proteins were due to changes in the proportion of HGT-Type II proteins. Five proteins bands of unknown origin accounted for a total about 3.5% of the proteins stained, and this was the case regardless of nutritional treatment.

To test the sensitivity of the separation procedures used, samples shown previously to have significant (P<0.05) changes in chemical composition immediately following the position of break were compared using 1D- (Appendix, Plate A4.3; n = 5) and 2D-PAGE (Appendix, Plates A4.4 and A4.5; n = 2). There were no detectable differences between samples in either the number or location of the proteins identified, or the intensity of the Commassie stained proteins. Furthermore, variations in the proportion of LS and HS proteins between sheep determined using 1D-PAGE were not significantly (P>0.05) correlated to that determined using 2D-PAGE. The correlation for HGT proteins was significant, but weak (r^2 = 0.22; P<0.01).

Table 4.4. The relative proportions (%) of the major protein families and their components estimated by 2D-PAGE and stained by Coomassie-blue. The wool samples were cut from the tip-end (< 2mm) of broken staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition. First dimension separation was in 0.5 mm discontinuous polyacrylamide gel (stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9). Second dimension electrophoresis was in pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel.

a, b: different superscripts differ at P< 0.05

Protein type	Nutritional treatment		
	LWM	LWL_1	LWL_2
High MW smear	8.9^a	7.8^a	7.5^a
High sulphur proteins			
HS #1	1.0^a	0.9^{ab}	0.5^b
HS #2	0.7^a	0.7^a	0.6^a
HS #3	1.2^a	1.0^a	0.8^a
HS #4	2.0^a	1.9^a	1.6^a
HS #5	1.2^a	0.8^b	0.7^b
UHS	2.1^a	0.7^b	0.2^b
HS total	8.3^a	6.0^{ab}	4.4^b
Low sulphur proteins			
Type I	35.4^a	38.7^a	36.8^a
Type II	35.5^a	32.2^a	36.7^a
Total	70.8^a	70.9^a	73.5^a
High glycine-tyrosine proteins			
Type I	3.0^a	4.0^a	3.9^a
Type II	3.6^a	4.8^{ab}	5.3^b
unknown	2.2^a	2.5^a	2.3^a
Total	8.8^a	11.3^{ab}	11.5^b

4.3.3.4. Intrinsic fibre strength in relation to wool protein composition

The amount and composition of extracted proteins characterised by 1D- and 2D-PAGE were generally not significantly (P>0.05) associated with intrinsic strength (Fig. 4.4), or any other stress-strain properties. The exception was a weak negative correlation between the proportion of HS proteins estimated using 2D-PAGE and intrinsic strength (r^2 = 0.22; P<0.01).

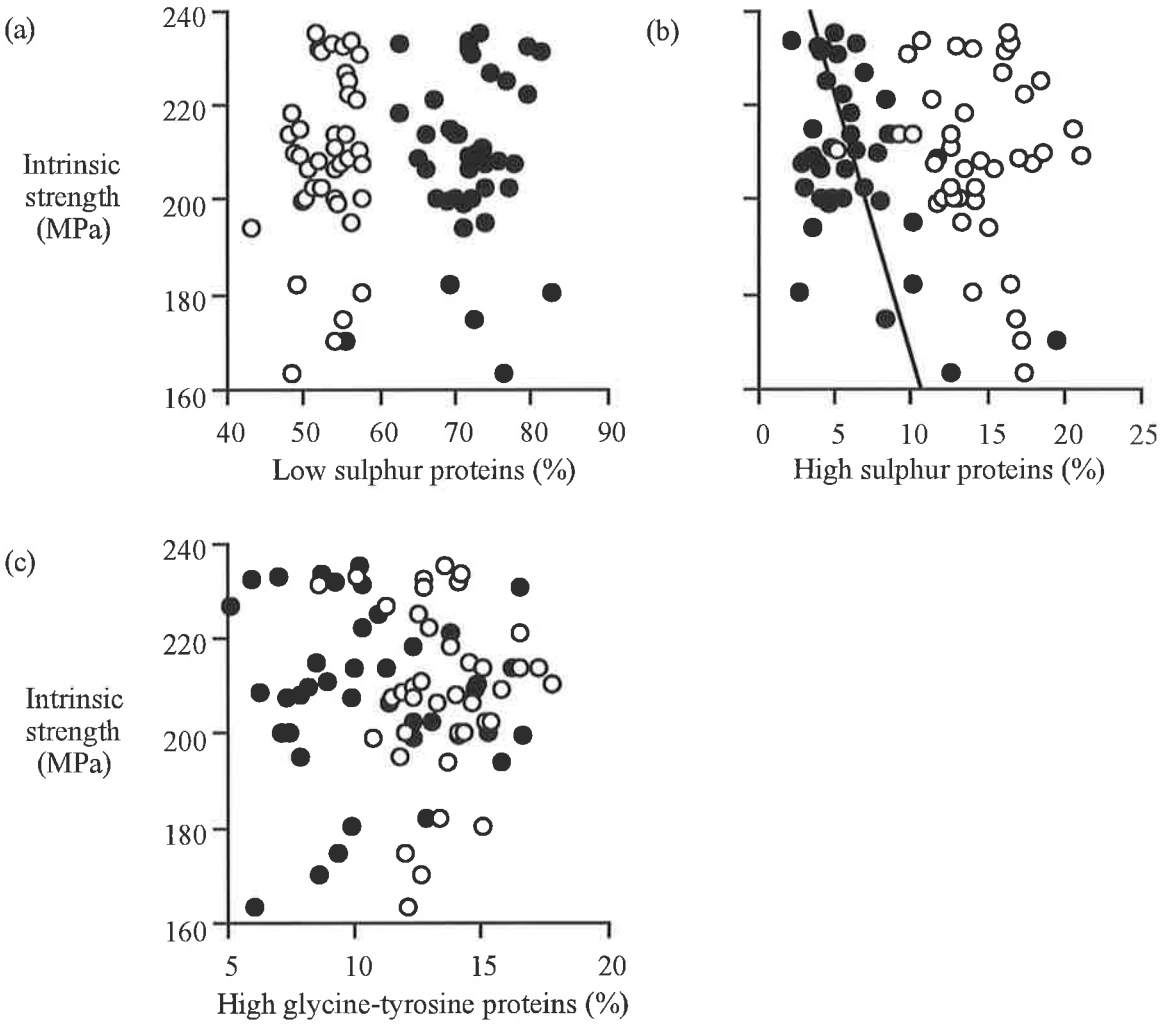

Figure 4.4. The relationship between intrinsic fibre strength and the proportion of (*a*) low sulphur, (*b*) high sulphur, and (*c*) high glycine-tyrosine proteins determined by one-dimensional (○) and two-dimensional (●) polyacrylamide gel electrophoresis. The wool samples were from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition, and points represent means for individual sheep.

4.3.4. Differential scanning calorimetry (DSC)

4.3.4.1. Measurement of thermal properties

The variability in thermal characteristics between replicates of the same wool sample was comparable to, or less than, that reported in the literature (Wortmann and Deutz 1993) (Appendix, Table A.4.3). The variation in denaturation temperatures between replicates were small, such that on average, differences between samples of less than 5% of the mean value were considered to be significant ($P<0.05$). However, the variability in denaturation enthalpy between replicates was much greater, and although it was reduced by using a sigmoid base line to account for changes in the heat capacity of the sample during the denaturation process, it was estimated that differences in mean denaturation enthalpy between samples were only considered to be significant when they exceeded 15 to 20% of the mean values.

4.3.4.2. Variations in the shape and size of DSC thermograms

The DSC thermograms for individual samples (i.e. replicates) differed in size, shape and the location of peaks. Of all the thermograms recorded in this experiment (n = 177), 40 % were classified as having a single peak (Plate 4.6*a*). The remainder had either a single peak with a shoulder (Plate 4.6*b*; 41%), or two separate peaks, or bimodal (Plate 4.6*c*; 19%). Most of the variation occurred between samples from different sheep, although slight differences in thermogram shape were occasionally observed between replicates of the same sample.

On average, wool samples characterised by bimodal DSC thermograms had a higher mean fibre diameter (16.6 *vs.* 14.7 μm; $P<0.05$), more paracortical cells (34.1 *vs.* 27.7%; $P<0.05$), and a higher sulphur content (2.9 vs. 2.7%; $P<0.05$; see Appendix, Table A4.4), than those with a single peak. They also "melted" over a wider (34.2 *vs.* 31.4 °C; $P<0.05$) temperature range and required more (18.5 *vs.* 17.4 J/g; $P<0.05$) energy to "melt" than wool samples with single peaks. For the bimodal shaped DSC curves, the peak temperature of the second peak was 5.1 ± 0.12 °C higher than that of the main melting temperature (145.8 *vs.* 140.7 °C; $P<0.05$). The thermal parameters for samples characterised by a shoulder on the DSC thermogram were intermediate between the two extremes.

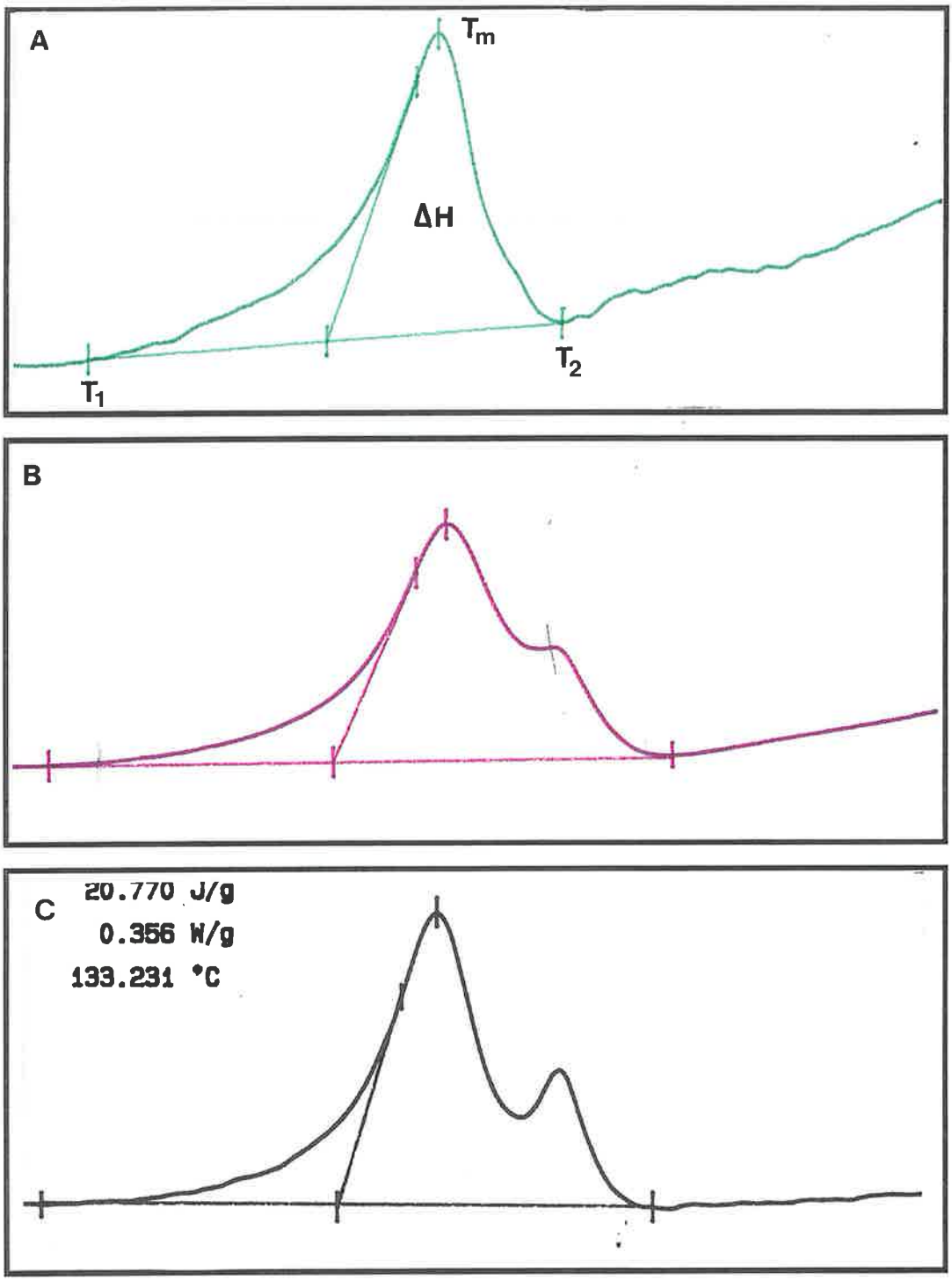

Plate 4.6 (*a*) Single peak, (*b*) single peak with shoulder and (*c*) bimodal DSC thermograms of wool samples heated in 150% (v/w) water at 5°C/min from 50°C to 200°C (curves scanned from originals). The relevant parameters of the curve are identified; T_1 (start of the denaturation peak), T_m (denaturation temperature), T_2 (end of denaturation peak) and ΔH (denaturation enthalpy).

Analysis of variance revealed a highly significant ($P<0.001$) difference in the average thermal characteristics of wool samples from different sheep. Across all samples, the start of the denaturation peak ranged from 116.9 to 126.6 °C (8.3%), the denaturation temperature from 138.4 to 143.1 °C (3.4%), the end of the denaturation peak from 149.1 to 153.7 °C (3.1%), and the denaturation enthalpy from 15.2 to 19.9 J/g (30.9%).

The average thermal characteristics of wool samples from sheep managed under different nutritional conditions are shown in Table 4.5. Wool samples from sheep fed to maintain liveweight had a lower ($P<0.05$) denaturation temperature and a narrower denaturation range ($P<0.05$), but required slightly more ($P<0.05$) energy to melt, than samples from sheep fed to lose and then gain liveweight.

Table 4.5. Mean denaturation temperatures and enthalpy of wool samples from sheep managed under different nutritional conditions. The samples were clipped from the mid-side patch at the time corresponding to the position of break along the staple, and were heated in 150% (v/w) water at 5°C/min from 50°C to 200°C.

a, b: different superscripts differ at P< 0.05

Thermal characteristic	Nutritional treatment		
	LWM	LWL_1	LWL_2
Denaturation temperatures (°C)			
Start (T_1)	120.1^a	120.8^a	120.0^a
Peak (T_m)	140.5^a	142.2^b	142.0^b
End (T_2)	150.6^a	151.7^{ab}	151.8^b
Range (T_2-T_1)	30.5^a	30.9^{ab}	31.7^b
Denaturation enthalpy (J/g)	18.4^a	17.5^{ab}	17.2^b

4.3.4.3. Cortical cell types and chemical composition in relation to thermal characteristics

The proportion of paracortical cells had no significant effects on the denaturation temperatures (Fig. 4.5*a*), but it was positively related to the denaturation enthalpy (r^2 = 0.28, P<0.05; Fig. 4.5*b*).

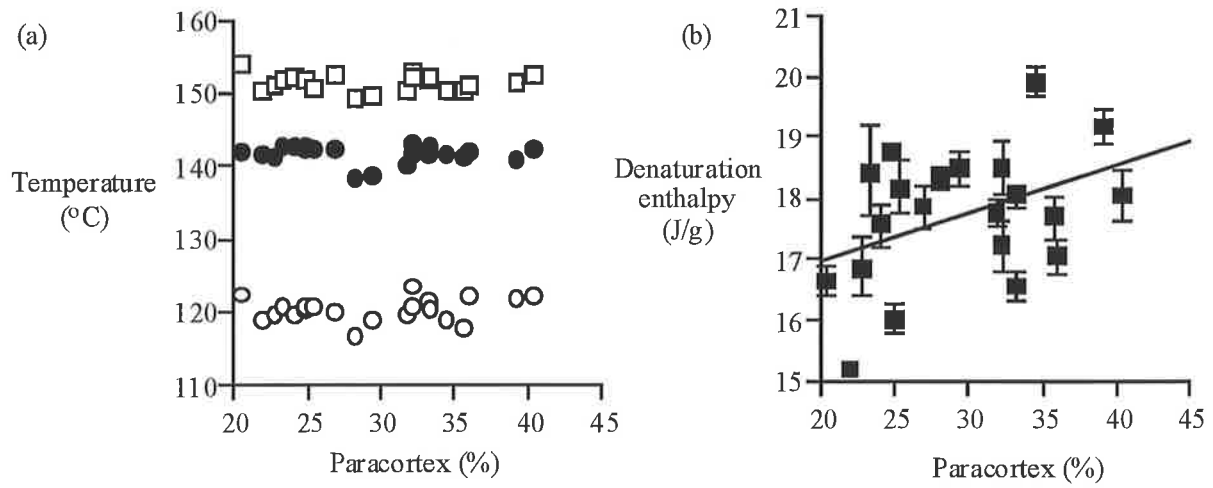

Figure. 4.5. The relationship between the proportion of paracortical cells and (*a*) denaturation temperatures, T_1 (○; n.s.), T_m (●; n.s.) and T_2 (□; n.s.), and (*b*) denaturation enthalpy (■). The wool samples were from sheep managed under three levels of nutrition (n = 7), and were clipped from the mid-side patch at the time corresponding to the position of break along the staple. Points represent means for individual sheep, and error bars denote s.e.m.

The concentration of several amino acids was also correlated with various thermal properties (Appendix, Table A4.4). Certain amino acids present in higher levels in the HS proteins (Thr, Pro, Ser) were positively related to the temperature at the start of denaturation and the denaturation enthalpy, while some amino acids associated with the LS proteins (Asp, Ala, Lys) were negatively related to these thermal parameters.

4.3.4.4. Relationships between thermal characteristics and fibre stress-strain properties

Melting temperature explained 22% of the variance in intrinsic strength (Fig. 4.6*a*). There was no significant (P>0.05) correlation between denaturation enthalpy (Fig. 4.6*b*), or any other thermal properties of wool fibres, and intrinsic strength.

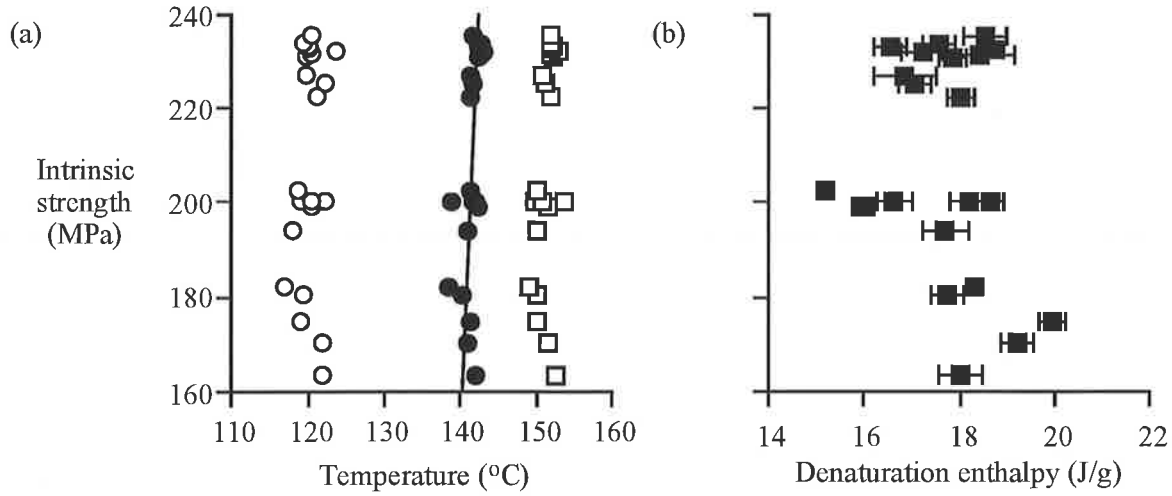

Figure 4.6. The relationship between the intrinsic fibre strength and (*a*) denaturation temperatures, T_1 (○; n.s.), T_m (●; $r^2 = 0.22$, $P<0.05$, n = 21) and T_2 (□; n.s.), and (*b*) denaturation enthalpy (■; n.s.). The wool samples were from sheep managed under three levels of nutrition (n = 7), and were clipped from the mid-side patch at the time corresponding to the position of break along the staple. Points represent means for individual sheep, and error bars denote s.e.m.

4.4. Discussion

4.4.1. Hypothesis

Wool samples which differed in intrinsic fibre strength by up to 44% were characterised in terms of the proportion of cortical cell types, total sulphur content and amino acid composition, the relative amounts and composition of keratin proteins by 1D and 2D-PAGE, and the α-helical denaturation enthalpy and transition temperatures by DSC. The results show that cortical cell type proportions, and the composition and properties of keratin proteins therein, varied significantly between sheep, and in some instances, were influenced by the level of nutrition. However, using the analytical techniques described, these sheep-to-sheep differences in the cellular and molecular characteristics of fibres measured in the region near the point of rupture were, in the main, not significantly related to intrinsic strength. The results therefore did not support the proposed hypothesis that the differences in intrinsic strength observed in Chapter 3 are associated with the proportion of microfibrils relative to matrix.

4.4.2. Cortical cell types

Few attempts have been made to correlate changes in cortical cell types to the mechanical properties of wool fibres from sheep of known genetic and nutritional history, and from those that have, no clear picture has emerged (Thorsen 1958; Chapman 1965; Orwin *et al.* 1980, 1985). In my experiment, there was no evidence to support the contention that finer, intrinsically stronger fibres, contain a greater proportion of orthocortical cells. Literature reports that nutritional stress leads to an increase in the proportion of orthocortical cells (Orwin *et al.* 1984; Hynd 1989), that the microfibril to matrix ratio is higher in orthocortical cells than in paracortical cells (Leach *et al.* 1964; Dobb 1970; Bradbury 1973; Powell and Rogers 1986; Marshall *et al* 1991), and that microfibrils are more resistant to longitudinal extension than the matrix at 65% RH (Bendit and Feughelman 1968; Feughelman 1987), gave every indication that this may be the case.

The average proportion of orthocortical cells in fibre cross-sections increased from the end of *ad libitum* feeding until the position of break along the staple (Fig. 4.1), and the response varied significantly between individual sheep. These changes are consistent with the effects of nutritional stress on cortical cell type proportions for individual sheep reported by Orwin *et al.* (1984) and Hynd (1989). However, because the magnitude of the changes in cell types was similar for all nutritional treatments, there were no differences in the percentage of orthocortical cells between treatments at the position of break, and therefore no correlation between the proportion of orthocortical cells and fibre cross-sectional area. Hansford and Kennedy (1990*b*) found that differences in the proportion of orthocortical cells between sheep were not correlated with FD, and the same can be calculated from the data reported by Orwin *et al.* (1984) and Hynd (1989). On most occasions there was no significant association between cross-sectional area and the proportion of orthocortical cells between individual fibres from the same sheep, which is at variance with Orwin *et al.* (1985) who found strong positive linear or log-linear relationships for individual fibres from Romney sheep.

Intrinsic fibre strength was not correlated with cortical cells type proportions. This differs from other studies which have concluded that the longitudinal stiffness of the fibre decreases as the proportion of orthocortical cells increases, and have attributed this effect to the inferior packing and orientation of the microfibrils within the orthocortical cells (Thorsen 1958; Feughelman and Haly 1960; Whiteley and Speakman 1960; Chapman 1965; Orwin *et al.* 1980). These studies

made no attempt to correlate between-sheep differences in cortical cell type proportions and intrinsic strength, and a closer examination of their data reveals several problems with methodology and inconsistencies in the interpretation of their results. For instance, Thorsen (1958) estimated cortical cell type proportions after staining with sodium plumbite stain, which not only degrades cortical cells but is also only partially correlated with Methylene blue staining (Orwin *et al.* 1984). Indeed, Thorsen (1958) reports that Lincoln fibres consisted almost entirely of paracortical cells (98%), which is clearly incorrect in view of more recent findings (Orwin *et al.* 1984; P.I. Hynd unpubl.). Chapman (1965) found that, on average, poorly crimped, "doggy", fibres had more (12%) paracortical cells and a higher (11%) intrinsic strength than well-crimped, "normal" fibres. However, not only was the experiment based on the stress-strain properties of only 32 fibres from four different sheep, but a comparison of individual sheep means indicates that there was in fact no correlation between paracortex percentage and intrinsic strength. The data reported by Orwin *et al.* (1980) indicate that while paracortex-rich fibres greater than 20 μm do appear to be intrinsically weaker, the most significant changes in cortical cells types between 12 and 16 μm, were not associated with any changes in intrinsic strength. Other data reported by Orwin *et al.* (1985) also suggest that an association between cortical cell types and intrinsic strength was unlikely, thus making the results consistent with the current experiment.

The absence of any consistent correlation between ortho/paracortex ratio and intrinsic strength in this, and other experiments, may be associated with changes in the composition and structure of individual cortical cell types. While Orwin *et al.* (1984) demonstrated a very close correlation between paracortex proportions determined from light microscopy and transmission electron microscopy, several studies have reported that the intensity of paracortical cells stained with Methylene blue differs between individual fibres from the same sheep, and changes in response to nutritional conditions (Thorsen 1958; Chapman 1976; Fratini *et al.* 1994). Subjectively assessed staining intensity of paracortical cells in the present experiment was, in general, more variable and much reduced at the position of break (Plate 4.1). Chapman (1976) found that Methylene blue stained cortical components were very difficult to distinguish following a period of poor nutrition, and that his conclusions drawn by light microscopy proved to be erroneous when electron micrographs were examined. Fratini *et al.* (1994) report that cysteine infusion resulted in a 5-6 fold increase in the mRNA levels encoding a family of cysteine-rich keratin proteins (KAP4 family), and yet the increase in the paracortical cell population was only 1.6 to 2.6 times. Collectively, these results suggest that considerable differences in chemical composition occur

within individual cortical cell types identified by Methylene blue staining, and this appears to be an unavoidable limitation of this staining procedure. It can only be concluded that an estimate of cortical cell types after staining with Methylene blue is an imprecise and highly variable estimate of fibre composition.

4.4.3. Amino acid composition

Wool sulphur content, and that of certain amino acids present at higher levels in the HS proteins, decreased from the start of differential feeding until the position of break along the staple (Fig. 4.2*a,b*), whereas the proportions of some amino acids associated with the LS (Fig. 4.2*c*) and HGT proteins (Fig.4.2*d*) increased. These changes in fibre composition for individual sheep were consistent with the observed changes in cortical cell types along the staple, and the respective differences in chemical composition between these cell types. Ultrastructural (Chapman and Gemmell 1971), autoradiographic (Chapman and Gemmell 1973), X-ray microanalytical (Carr *et al.* 1986; Jones *et al.* 1993), histochemical (Wagner *et al.* 1983), and *in situ* hybridisation (Powell and Rogers 1994*a*) studies, and chemical analysis of separated cells (Chapman and Bradbury 1968; Kulkarni *et al.* 1971; Ito *et al.* 1985; Dowling *et al.* 1990), all indicate that paracortical cells contain higher proportions of HS proteins, and lower concentrations of LS and HGT proteins, than orthocortical cells.

The changes in the composition of certain amino acids along the staple were greater for sheep which were fed to lose liveweight, so unlike cortical cell type proportions, differences existed in amino acid composition at the position of break between nutritional treatments. This supports the suggestion that ortho/paracortical cell ratio is an unreliable estimate of wool fibre composition. It can be seen from Fig. 4.3*a*, that for fibres with an average paracortex percentage of 30%, the sulphur content at the end of *ad libitum* feeding was 3.1% compared to only 2.7% at the position of break. There were corresponding decreases at the position of break in some HS protein amino acids (Fig. 4.3*b*), and increases in some amino acids associated with the LS (Fig. 4.3*c*) and HGT proteins (Fig. 4.3*d*). The composition, and presumably structure, of paracortical cells at the position of break was clearly approaching that of orthocortical cells. If it is assumed that the composition of orthocortical cells was the same for all sheep, and remained unchanged by the level of nutrition, it can be calculated based on the composition of isolated orthocortical cells reported by Bradbury (1973) that this was indeed the case (Appendix, Table A4.6).

One of the problems with amino acid analysis of whole fibres is that the results represent averages of the various components of the fibre. Thus it is not known for certain whether the changes in amino acid composition in this experiment reflect changes in the content of the microfibril and matrix proteins, changes in the composition of these proteins, or both. However, if the composition of the constituent proteins do not differ markedly from the average, and there is evidence to suggest that this was the case, then it is calculated using the experimentally determined regression reported by Gillespie and Frenkel (1974), that sheep fed to maintain liveweight had more HS and less HGT proteins at the position of break than sheep on the restricted diets, and that the proportion of LS proteins at the position of break was constant across all nutritional treatments (Table 4.2). Thus, if the assumption is valid, there was no evidence from the amino acid data to support the hypothesis that intrinsic strength is associated with the proportion of microfibrils relative to matrix.

4.4.4. Protein composition

While no single electrophoretic system can separate all the 50 to 100 keratin proteins in wool (Powell and Rogers 1986, 1997), the three major wool protein families were easily identified in the 1D- and/or 2D-gel electrophoretic patterns. Qualitatively, the electrophoretic patterns showed that most of the identified proteins were common to all sheep. The only detectable differences between samples was the random occurrence of two HS protein bands with MW $\approx$ 25 kDa, and the UHS protein smear was detectable in the second dimension in some samples at low amounts. The lack of variation within the LS protein family has been reported previously (Gillespie 1964; Gillespie *et al.* 1964; Gillespie and Reis 1966; Broad *et al.* 1970; Crewther *et al.* 1976; 1980; Fraser and MacRae 1980; Marshall and Gillespie 1981; Gillespie and Marshall 1983; Marshall *et al.* 1985; Woods and Orwin 1987; Fratini *et al.* 1994; Nancarrow 1995), although Herbert and Rankin (1995) using isoelectric focusing-SDS PAGE recently found significant changes within the LS protein family between samples from different sheep. Other studies have identified more HS proteins, and more variation between individual samples, than the current study (Darskus and Gillespie 1971; Darskus 1972; Gillespie and Marshall 1980; Woods and Orwin 1987). The differences between experiments presumably reflect, in part, differences in the electrophoretic system and detection methods used. Whereas the gels in this study were stained with Coomassie-blue which is a general protein stain, the others have been radio-labelled with iodo-[2-^{14}C] acetic

acid which binds specifically to sulphydryl groups, and thus emphasises differences in the levels of these sulphur-rich proteins.

Quantification of the wool proteins separated by 1D or 2D PAGE indicated that the amount and composition of the LS proteins were not influenced by nutritional treatments, but on average sheep fed to maintain liveweight had more HS and less HGT proteins at the position of break than sheep on the restricted diets. The effect of nutrition on HS protein content was due mainly to changes in the intensity of the UHS smear, and is consistent with other studies (Reis and Schinckel 1963; Gillespie and Reis 1966; Broad *et al.* 1970; Campbell *et al.* 1975; Fratini *et al.* 1994). This is the first report of changes in the proportion of HGT proteins in response to simple changes in nutrition, although it is known that severe dietary imbalances may result in an increase in the level of UHS proteins and a decrease in HGT proteins (Frenkel *et al.* 1974, 1975; Reis 1979).

The effects of nutritional treatments on fibre composition were evident regardless of the analytical technique used. However, on an individual sheep basis, the electrophoretic patterns were not consistent with the amino acid composition results. As there was no evidence that this discrepancy reflects significant changes in the composition of the protein families, or the appearance of major new protein components, this suggests that the PAGE systems used were not sufficiently precise to quantify any differences in protein composition between samples. The findings that the relative amounts of the various proteins in a single extract varied depending on the amount of protein loaded onto the 1D-gel, and that the differences in protein composition between sheep determined using 1D-PAGE were generally not significantly correlated to those determined using 2D-PAGE, confirm that this was the case. As a corollary, no clear relationship was established between protein amounts or composition and intrinsic strength. It is pertinent to note that the results do not necessarily imply that no such associations exist, and indeed more sophisticated separation procedures like that used by Herbert and Rankin (1995), together with more quantitative detection methods, may provide some insight into the associations between protein composition and intrinsic strength. Fractionation of the wool protein extracts into the three main protein classes prior to electrophoresis, and the use of separation parameters most appropriate for each protein family, may also be worthwhile, as would the pre-determined perturbation of composition with single specific keratin genes (Powell and Rogers 1997).

Literature reports on the effects of keratin protein composition on wool fibre stress-strain properties are limited, and the results inconsistent. Feughelman and Reis (1967) found that a 35% increase in wool sulphur and cystine content following abomasal infusion of methionine had negligible effects on the stress-strain properties of wool fibres in water. The explanation for this result was that the increase in disulphide content was confined to the HS and UHS proteins (Gillespie *et al.* 1964), and presumably did not contribute to the stability of the load-bearing microfibrils. Bendit (1980) concluded that the stiffness of wet wool fibres at low extensions was negatively related to the amount of matrix proteins, however the relationship was not significant after removing the data for the single Lincoln wool sample, which had only half the content of matrix proteins (15 *vs.* 29%) and was about 35% stronger than the other wool samples. Indeed, a 12% range in matrix protein content between three Merino wool samples was not associated with any changes in the average stiffness of the fibres. As an alternative to the hypothesis tested, that an increase in matrix protein content would exclude water and thus make the matrix stiffer, Bendit (1980) proposed that the increase in matrix protein must be associated with a decrease in microfibril density.

Several studies have indicated an association between the content of HGT proteins and the strength of wool fibres. Inhibition of HGT protein synthesis, by infusion of zein (Reis and Colebrook 1972), amino acid mixtures lacking in lysine (Reis and Tunks 1978, 1982), or by infusions of methionine into sheep consuming a wheat diet (Reis and Tunks 1974; Chapman and Reis 1978; Reis *et al.* 1983, 1986), all resulted in the production of weak wool as assessed subjectively. However, treatment of sheep with a supplement of a mixture of amino acids lacking methionine weakens wool without a concomitant suppression of HGT proteins (Reis and Gillespie 1985), and the omission of phenylalanine from an infusion of amino acids reduces the content of HGT proteins without affecting wool strength (Frenkel *et al.* 1974, 1975). The HGT proteins are also virtually absent from normal strength wool grown by Lincoln and felting lustre mutant Merino sheep (Gillespie 1991), and regrowth of wool fibres generated after plucking. The tips of fibres from lambs are of normal strength despite reduced HGT protein content. It appears that a reduction in HGT proteins is not a prerequisite for the production of intrinsically weak wool, and indeed, that changes in the composition of matrix proteins are unlikely to be a determinant of differences in intrinsic strength. Bray *et al.* (1995) report that differences in matrix protein composition exist between groups of Romney sheep from the high and low staple tenacity selection flocks, and yet they do not differ significantly in intrinsic strength (Scobie *et al.* 1996).

To adequately test the proposed hypothesis, a more accurate measure of the microfibril volume fraction was needed.

4.4.5. Differential Scanning Calorimetry (DSC)

DSC investigations of wool fibres in water indicated a well-defined endothermic peak at about 140°C, and there is evidence that this results from the denaturation of the α-helical crystalline microfibrils (Schewenker and Dusenbury 1960; Felix *et al.* 1963; Chapman 1965; Bendit 1966; Haly and Snaith 1967; Crighton and Hole 1985; Crighton 1990), and that the area under the curve is a measure of the relative α-helix content of the sample (Wlochowicz and Eder 1983; Wortmann and Deutz 1993; Schmidt and Wortmann 1994). If in the current study the denaturation enthalpy is assigned exclusively to the α-helical material, then despite a 31% range in the α-helix content between samples from different sheep, there was no evidence that it was related to intrinsic fibre strength. While this result does agree with the amino acid and protein composition data, the limitations to the protein data have already been outlined, and it is surprising given other reports that denaturation enthalpy is positively related to wet bundle strength of photo-degraded wool (Schmidt and Wortmann 1994) and wet burst strength of chemically modified wool (Huson *et al.* 1997). Rama Rao and Gupta (1991) also claim that a higher stress at 15% extension and intrinsic strength of Lincoln compared to Merino wool was associated with a higher crystalline content, although denaturation enthalpy was not actually quantified.

A number of studies have found that denaturation temperature is positively related to Young's modulus, yield stress and or intrinsic strength (Rama Rao and Gupta 1991; Schmidt *et al.* 1993), and while this was also the case in the current experiment, the relationship was weak. Differences in the thermal stability of the α-helical fraction have been attributed to the characteristics of the surrounding non-helical matrix, with a higher denaturation temperature being associated with an increase in cystine content (Haly and Snaith 1967; Crighton and Hole 1985; Spei and Holzem 1991; Wortmann and Deutz 1993). Indeed, the differences in cystine content and thus denaturation temperature between ortho- and paracortical cells are believed to be responsible for the bimodal character of some DSC endotherms (Schewenker and Dusenbury 1960; Haly and Snaith 1967; Crighton and Hole 1985; Crighton 1990; Wortmann and Deutz 1993). This theory seems, at first, to fit the current results, as samples with a distinct bimodal DSC thermogram had more paracortical cells that were also more intensively stained by Methylene blue, and a higher

average cystine content, than samples with a single endothermic peak. Most of the DSC thermograms had a single peak with or without a shoulder, which is consistent with the suggestion that the composition of the different cortical cell types at the position of break was similar, or more correctly, not sufficiently dissimilar to lead to denaturation peaks differing enough in temperature to be resolved.

However, while the cystine content did differ between samples with different shape DSC thermograms, it did not explain the differences in denaturation temperature between all the samples. The precise reasons for the differences in denaturation temperature can not be determined from the measurements made. A further surprising result, was that the denaturation enthalpy was positively correlated with the proportion of paracortical cells, which have a lower crystalline content than orthocortical cells, and if anything, was negatively correlated with some of the amino acids associated with α-helical formation. Again the explanation for this is not known, but both Deutz *et al.* (1993) and M. Huson and K. Ley (unpubl. data) have found that the denaturation enthalpy of isolated paracortical cells is greater than that of orthocortical cells. It seems that the denaturation enthalpy represents more than just the denaturation of the α-helices of the microfibrils. Additional thermal processes such as the thermal degradation of the matrix (Cao *et al.* 1997), stress-relaxation or absorption of moisture by the melted α-helices (M.G. Huson, pers. comm.), could be superimposed on top of the melting endotherm. Unfortunately, until it is absolutely clear what denaturation enthalpy is actually measuring, little can be concluded from the DSC work.

4.5. Conclusions

Whilst acknowledging the limitations with the analytical techniques used, there was little evidence to support the hypothesis that the differences in intrinsic strength were associated with the total proportion of microfibrils relative to matrix. As the wool fibres were tested at 20°C and 65% RH, which is approximately 40°C below the glass transition for wool (Wortmann *et al.* 1984), a possible explanation for this result is that the mechanical properties of the matrix and microfibril phases were too similar under these conditions, so changes in their ratio had no detectable affects on intrinsic strength. Cook and Fleischfresser (1990) found that the intrinsic strength of chemically modified wool was only 10-15% less than that of normal wool at 65% RH, compared to 30-40% less at 100% RH. Similarly, Rama Rao and Gupta (1991) found that Lincoln wool was 20% stronger than Merino wool at 65% RH and 33% stronger at 100% RH. However, as the

chemical composition of the samples reported in this Chapter are also not correlated with their intrinsic strength measured in water (M. G. Huson and A.N. Thompson unpubl. data), it is likely that physico-chemical factors other than those considered here are responsible for the observed differences in intrinsic strength.

What then are the causes for the differences in intrinsic strength? Rather than the actual composition of the keratin proteins, one possibility is that intrinsic strength is dependent on the way in which the constituent proteins of the microfibril/matrix structure are arranged, and on the arrangement of the cross-links between them. Whereas no major differences in longitudinal stress-strain properties were evident between wools in which the disulphide and HS protein content had been modified biologically (Feughelman and Reis 1967), chemical reduction of cystine significantly reduces intrinsic strength due primarily to a decrease in yield stress (Lindley 1957; Feughelman and Haly 1961; Crewther 1965; Cook and Fleischfresser 1990). As chemical reduction of cystine ruptures the disulphide bonds equally in LS and HS proteins (Springell *et al.* 1964), and the decrease in stress per disulphide bond broken is much greater at low rather than at high disulphide contents (Crewther 1965), this clearly suggests that the location of the disulphide bonds is critical. Gillespie (1970) found large differences in the degree of swelling in formic acid between wools of similar sulphur content and protein composition, suggesting that differences exist in the distribution of intra- and inter-chain disulphide bonds. It would be expected that interchain disulphide bonds would be more resistant to extension than intrachain disulphide bonds, however no experimental test of this hypothesis has been made. There is a clear need to identify the location of the disulphide bonds in wool more precisely. It is also possible, although less likely, that differences in the degree of cross-linking contributed to the differences in intrinsic strength in the current experiment. Weak hair from children with Menkes syndrome, or mice with the mottled mutation, contain a set of normal proteins which are incompletely cross-linked (reviewed by Price 1990), and intrinsically weak wool grown by sheep with a copper deficiency is also correlated with increases in the concentration of sulphydryl groups (Marston 1946).

The microfibrils in wool are preferentially orientated parallel to the fibre axis, and since it is known from man-made composite structures that small changes in filament orientation drastically reduce intrinsic strength (Wainright *et al.* 1976), differences in molecular orientation may have contributed to the differences in intrinsic strength in this experiment. It may be that the higher

microfibril content in orthocortical cells is counteracted to varying degrees by their lower average orientation in the direction of the fibre axis, and this would explain why no clear picture emerged between amino acid or protein composition and intrinsic strength. While it is not possible to accurately estimate molecular orientation in wool fibres (Rama Rao and Gupta 1992), further insight into the association between fibre structure and intrinsic strength may be gained by quantifying the microfibril/matrix ratio in ortho and paracortical cells. An increase in the density of well-aligned microfibrils in paracortical cells, that is a high microfibril/matrix ratio in paracortical cells, may have a greater effect on intrinsic strength than changes in the density of poorly-aligned microfibrils in orthocortical cells. Changes in the composition and physical arrangement of the keratin proteins in transgenic mice and sheep over-expressing a single keratin gene also has a profound effect on hair and wool strength (Powell and Rogers 1990*b*; Bawden *et al.* 1998). The cortex of hairs from humans with trichothiodystrophy, a disease characterised by the hair being brittle, contains large areas with either very sparse or no fibrillar proteins, and an irregular disordered arrangement of the microfibrils relative to the longitudinal axis of the hair shaft (Gummer and Dawber 1985). It is thus possible that differences in the ultrastructure of the microfibril/matrix structure exist between fibres differing in intrinsic strength.

The discussion thus far has ignored the possible contribution to intrinsic strength by components other than the microfibril/matrix structure. There is strong evidence, however, that the cuticle makes little contribution to intrinsic strength in human hair, and it is reasonable to assume that the same would apply with wool fibres. J.A. Swift (pers. comm.) found that when the human hair is extended, the cuticle undergoes circumferential fracture down to the level of the cortex at many positions along the length of the fibre well before the actual break, so that the load is finally borne solely by the cortex. In contrast, the integrity of the wool fibre does depend on the cell membrane complex, since its disruption by chemical means is known to significantly reduce intrinsic strength (Elöd and Zahn 1946; Baumann 1979). Nevertheless, since it was shown in the previous Chapter that most fibres broke perpendicular to the fibre axis, this suggests that in the current experiment cleavage generally occurred across cortical cells, and that differences in the properties of the cell membrane complex probably did not contribute to the differences in intrinsic strength. The non-keratin components of the fibre may influence intrinsic strength indirectly by affecting the volume fraction of the microfibril/matrix composite. Differences in cell characteristics and ultrastructure in fibres with extreme differences in intrinsic strength are examined in the following Chapter.

CHAPTER 5: CELL MORPHOLOGY AND ULTRA-STRUCTURE OF MERINO WOOL FIBRES IN RELATION TO INTRINSIC STRENGTH

5.1. Introduction

The cortex is the main histological component of the wool fibre, and the distinctive ultrastructure of wool fibre cortical cells arises during differentiation from the association of microfibrils into crystalline arrays separated by matrix (Horio and Kondo 1953; Mercer 1953; Rogers 1959*a,b*; Bones and Sikorski 1967; Dobb and Sikorski 1971; Bradbury 1973; Whiteley and Kaplin 1977; Kaplin and Whiteley 1978; Fraser and MacRae 1980; Orwin *et al.* 1984). Little is known about the precise location of cross-links between the different keratin proteins, however the highly ordered packing arrangement of the microfibrils suggests that there must be specific interactions between the LS proteins of neighbouring microfibrils, and probably between the microfibril proteins and the HS and HGT proteins of the matrix. Variability in the density and packing arrangement of microfibrils between different cortical cell types and individual fibres undoubtedly result from differences in the composition and distribution of the keratin proteins (reviewed by Powell and Rogers 1997).

No attempt has been previously made to relate the ultrastructure of single wool fibres to natural variations in intrinsic strength, although obvious ultrastructural abnormalities of the microfibril/matrix composite, and complex changes in protein composition, are a feature of many human diseases characterised by brittle hair (reviewed by Price 1990), transgenic mouse hair (Powell and Rogers 1990*b*) and Merino wool which is intrinsically weak (Bawden *et. al.*, 1998). While it is difficult to separate the possible effects of protein composition versus ultrastructure on intrinsic strength, there was little evidence in the previous Chapter to suggest an association between protein composition and intrinsic strength. It is therefore reasonable to hypothesise that differences in the ultrastructure of the microfibril/matrix structure contribute to the differences in intrinsic strength observed in Chapter 3. Other non-keratin fibre components, such as the cuticle, cell membrane complex and nuclear remnant material, may also influence intrinsic strength indirectly by affecting the volume of the microfibril/matrix composite in the fibre cross-section. Zahn (1980) reported that the keratin content of different wool and hair fibres varied from 68 to 90%.

5.2. Materials and methods

5.2.1. Wool samples and their preparation

Wool fibres from the experiment reported in Chapter 3 were used for all Transmission Electron Microscopy (TEM) investigations. Stress-strain properties were measured on about 4000 single fibres (40 sheep x 100 fibres/sheep), and cross-sectional area at the point of break and intrinsic strength were used as the basis of selection of four groups of 10 fibres as follows: groups 1 and 2, low cross-sectional area and low (group 1) or high intrinsic strength (group 2); and groups 3 and 4, high cross-sectional area and low (group 3) or high intrinsic strength (group 4) (Fig. 5.1). To be considered for selection, the fibres must have broken perpendicular to the fibre axis, and the cross-sectional area of the complementary fracture surfaces must have differed by less than 10%. Only the tip-end of the broken fibres was examined under TEM.

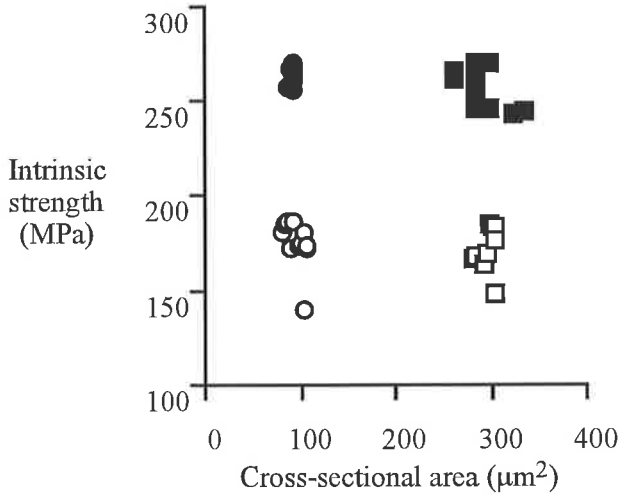

Figure 5.1. Cross-sectional area at the point of break and intrinsic strength of fibres selected for examination by transmission electron microscopy from group 1 (○; 99 μm² and 176 MPa), group 2 (●; 96 μm² and 263 MPa), group 3 (□; 294 μm² and 172 MPa) and group 4 (■; 287 μm² and 254 MPa).

After the cross-sectional area at the point of break was measured for determination of stress-strain parameters, the fibres were stored at 65% RH and 20°C until needed. A small drop of wax was then placed about 5 mm from the broken end of each of the selected fibres to facilitate identification of fibre orientation. The fibres were removed from the microscope slides by cutting through the middle of the drop of wax, and placed in 1 ml labelled eppendorf tubes.

5.2.2. Fibre fixation and dehydration

The ultra-structure of wool fibres is only visible by TEM after several steps of pre-treatment to enable penetration of heavy metals into the hardened structures. The most successful preparative procedure involves partial reduction of the disulphide bonds of the keratins before reacting with osmium tetroxide fixatives. The subsequent embedding in epoxy resins and sectioning is followed by staining with uranyl acetate and lead citrate solutions to intensify the contrast between microfibril-matrix structures, intercellular layers, and the sub-layers of the fibre cuticle (Rogers 1959*a*,*b*).

In this experiment, the fibres were prepared for TEM as described by Powell and Rogers (1994*b*). In brief, a reducing solution (0.4 M thioglycollic acid in 0.2 M sodium acetate pH 5.5) was added to each eppendorf for 24 hr at 20°C. The reducing solution was then removed and the fibres washed twice for 30 min in phosphate buffer solution (PBS; pH 7.2) containing 4% sucrose. The fibres were fixed by agitating in 1% OsO_4 in PBS for 24 h. After removal of the OsO_4, the fibres were dehydrated by sequential immersion in a graded series of ethanol/water mixtures: 70% (v/v) ethanol (1 h); 90% ethanol (1 h); 95% ethanol (1 h); and re-distilled ethanol (2 x 1 hr). The samples were then infiltrated with 50% ethanol/50% Spurrs low viscosity resin (1 hr) (Spurr 1969), 100% resin (2 x 1 hr), and 100% resin (24 hr) using rotational agitation to mix. Spurrs resin was made fresh by mixing 10 g vinyl cyclohexane dioxide (ELR 4206), 5 g Diglycidyl Ether of Polypropylene Glycol (DER 732, Epoxy equivalent 310-330), 26 g of Nonenyl Succinic Anhydride (NSA Epoxy hardener), and 0.2 ml of 2, Dimethylaminoethanol (DMAE). All chemicals for the resin were supplied by ProSciTech, Thuringowa Central QLD.

5.2.3. Fibre embedding and sectioning

The fibre sections were aligned and glued to short lengths of nylon cord, such that the broken end of the fibre was protruding by about 1 mm. After the glue had set, the nylon and attached fibre were suitably orientated in a labelled resin mould. The mould was filled with fresh Spurrs epoxy resin and the fibre manipulated so that it was orientated at about 90° to the end of the mould. The resin was then polymerised by placing the moulds in an oven for 72 hr at 65°C. After polymerisation, the resin moulds were clamped into the microtome chucks and shaped using a stainless steel blade into a four-sided pyramid with a flat top such that the fibre-end was just below the cutting face of the mould. The mould was then positioned in the microtome such

that the end of the embedded fibre was perpendicular to the knife edge. Serial cross-sections of the resin (0.5 μm) were cut using a glass knife and an ultramicrotome (Reichert-Jung, Ultracut E). Glass knifes were made on an LKB knife maker (Type 7801 B). When the end of the fibre approached the cutting edge, the glass knife was replaced with a diamond knife (DiATOME, Leica, Switzerland), and serial cross-sections were cut at 60-80 nm. Several hundred ultra-thin sections were cut per section within 50 μm of the fracture surface. The sections were floated on a water-bath, and picked up and mounted on carbon and Soloidium coated nickel grids (100 μm parallel bars; ProSciTech, Thuringowa Central QLD).

5.2.4. Fibre staining

Sections were double stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) for morphological examination. The grids were floated section-side down on 1% aqueous uranyl acetate containing 0.1% glacial acetic acid for 20-30 min at 20°C, and washed twice with water. The sections were then floated as before for 4 min on Reynolds lead citrate diluted 5x with 0.02M NaOH just prior to use, and washed in water (2x). Reynolds lead citrate was prepared by adding 0.2 ml 5N NaOH and 30 mg lead citrate to 10 ml of recently boiled water, and mixing until dissolved. The stained sections were allowed to dry, and where then re-coated with carbon (≈ 20 nm thick) prior to viewing in the electron microscope.

5.2.5. Transmission electron microscopic examination

Micrographs of fibre specimens were taken using a Phillips CM 100 electron microscope, operated at an accelerating voltage of 40-100 kV and using an objective aperture of 10-20 μm. When a section with few if any wrinkles, and free of other artefacts was identified, a series of images was taken at low (3000 to 6000x) and high (49000 and 72000x) magnification. The electron micrographs were digitised by scanning with a high resolution (1200 d.p.i.) Image Scanner (Reli 9630, Relisys, Milpitas, California, USA). After manipulation of image contrast using Adobe Photoshop 3.0 (Adobe Systems Incorporated, Mountain View, California, USA), the saved image was enlarged to between 10000 and 1000000x magnification and then analysed using a Kontron KS300 imaging system (KONTRON Electronik, GmbH), on an IBM 486 PC.

The following fibre characteristics were measured on most fibre cross-sections:

1. Cortex (including cell membrane complex), cuticle and nuclear remnant cross-sectional area. The number of individual cuticle cells, and the total thickness of the cuticle were also measured at 15 random sites on the periphery of the fibre.

2. Proportional areas occupied by paracortical and orthocortical cells;

3. Number, size and shape of cortical regions enclosed by a cell membrane and containing the microfibril/matrix complex. Every area enclosed by a membrane was considered as representing an individual cell because it was not possible to distinguish between interdigitating processes generally associated with single cells;

4. Total length of cell membrane between cortical cells and between cortical and cuticle cells. The percentage volume of a cross-section of a wool fibre which consists of cell membrane complex was determined from the total cross-sectional area of the fibre cortex, the total length of the cell membrane network and the assumption that the average thickness of the membrane was 25 nm (Rogers 1959*a*,*b*; Bradbury and King 1967); and

5. Density of microfibrils in different cortical cell types (200 x 200 nm in each of 3 cells per cell type).

5.2.6. Statistical analysis

The ultrastructure of wool fibre cross-sections from the different fibre groups was compared using Analysis of Variance, with cross-sectional area at the point of break, intrinsic strength and the cross-sectional area by intrinsic strength interaction as the sources of variance. All statistical analyses were performed using the statistical package SuperANOVA^{TM} (Abacus Concepts Inc., Berkeley, California, USA).

5.3. Results

A well recognised limitation to TEM studies of wool fibre cross-sections is the variable and unknown dimensional changes which may result from the fixation and staining procedures, and from the electron beam of the TEM itself (Fraser *et al.* 1959; Rogers 1959*a*,*b*). In the current study, fibre cross-sectional area estimated from the TEM micrographs was more than 30% greater than that estimated by light microscopy on about 50% of occasions, and the average

increase in cross-sectional area was greater ($P<0.05$) for fibres from the high intrinsic strength groups. It is important to note that these changes in cross-sectional area are not due to an error in the original cross-sectional area estimated by light microscopy, since a re-measure of the cross-sectional area of the complementary butt-ends of the broken fibres agreed closely with their original estimates (overall average 189 *vs.* 179 μm^2; $P>0.05$). While the fibres in this study were sectioned within 50 μm of the fracture surface, along-fibre variations in cross-sectional area and ellipticity may have contributed to the discrepancy between the two cross-sectional area estimates. James (1963) reported variations in cross-sectional area of up to 24% along 40 μm lengths of the same fibre. To minimise the effects of these unavoidable analytical errors, the percentage differences in cross-sectional area between the methods was used as a covariate in the analysis of the ultrastructure data where ever possible. Nevertheless, some caution must be exercised in the interpretation of the TEM dimension measurements reported in this Chapter.

5.3.1. Whole fibre characteristics

The whole fibre cross-sections obtained in this study were mostly free of any wrinkles or other artefacts, or they were insignificant and unlikely to influence the measurements made. Typical low power electron micrographs of transverse cross-sections of Merino wool fibres are shown in Plate 5.1. At this magnification, no obvious structural defects were apparent in the cuticle or cortical cells of any of the fibre cross-sections examined. Other structural features, such as the cell membrane complex between both cuticle and cortical cells, and the nuclear remnant material, also appeared "normal".

In all cases, the ortho- and paracortical cells were bilaterally arranged, and intermediate-type mesocortical cells[1] were sometimes evident at the ortho/para boundary. The cortical cells tended to be polygonal towards the centre of the cortex, but more flattened adjacent to the cuticle. In the orthocortex, the cell boundaries were difficult to distinguish from the intermacrofibrillar material, and the cylindrical, relatively small, macrofibrils were easily identified (Plate 5.2*a*). On the other hand, the cell boundaries between paracortical cells were readily distinguishable, many of the cells had a large central nuclear remnant, and the macrofibrils were closely packed and poorly defined (Plate 5.2*b*).

[1] The area of mesocortical cells was included in the paracortex in calculating ortho/para ratios.

Plate 5.1. Typical transmission electron micrographs (5000x) of whole Merino wool fibre cross-sections showing the segmentation of the cortex into ortho (Or) and paracortex (Pa). A single mesocortical cell (Me) is also evident at the ortho/paracortex boundary. The cuticle (Cu), cytoplasmic and nuclear remnant material (large arrows) and the cell membrane complex (small arrows) are shown. These fibres are examples from the low (*a;* 193 MPa) and high (*b;* 246 MPa) intrinsic strength groups. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 5 μm.

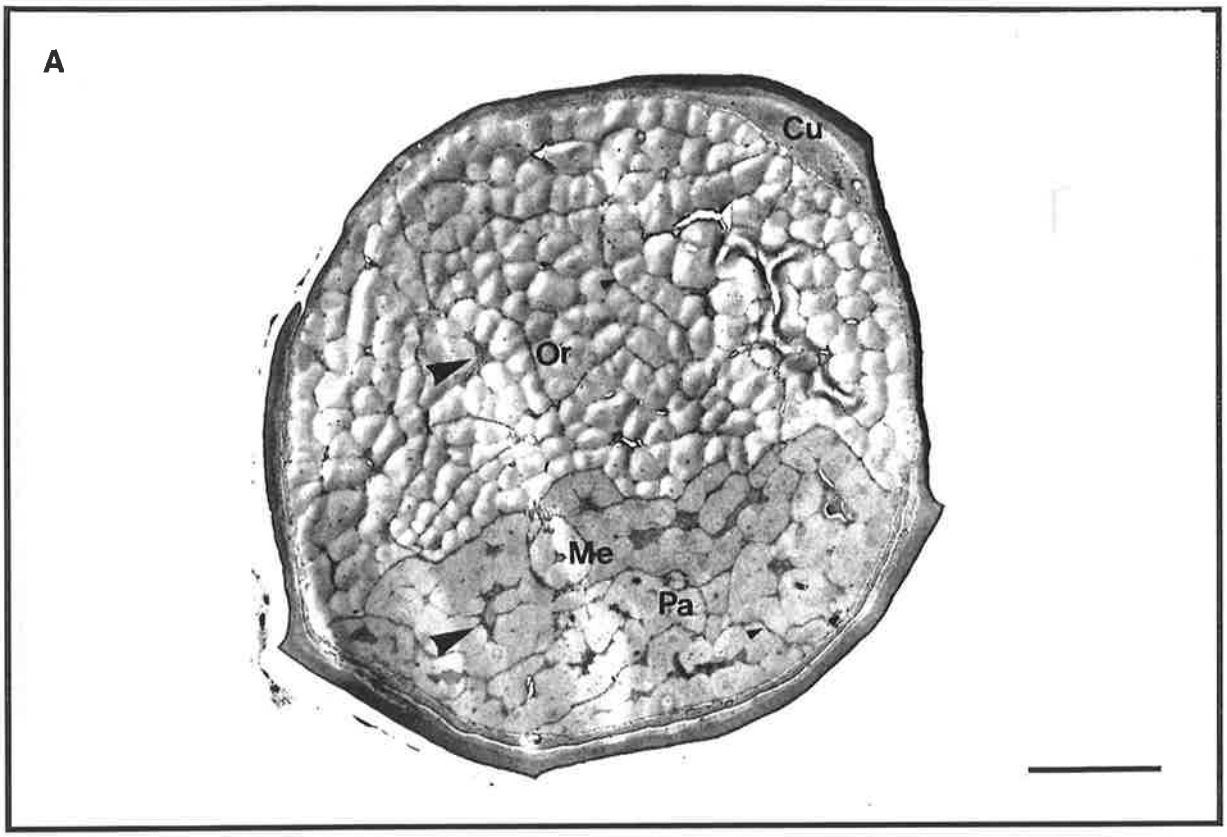

Plate 5.1*a*

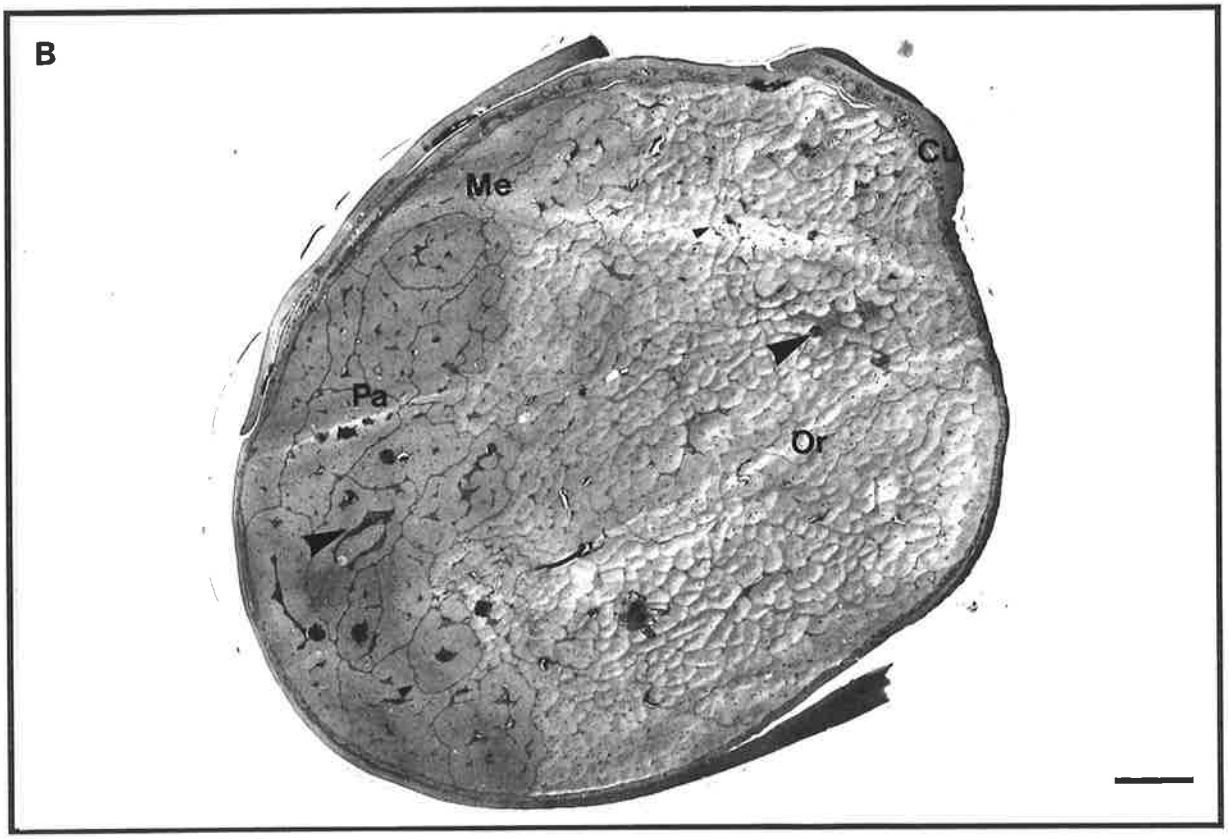

Plate 5.1*b*

Plate 5.2. Transmission electron micrographs of Merino wool fibre cross-sections showing the morphological distinction between orthocortical (*a*) and paracortical (*b*) cells at higher magnification (15000 x). Cu, cuticle. The cell boundaries (cmc) and nuclear remnants (nr) are more readily distinguished in the paracortex, and the differences in macrofibrillar structure between the cortical components are clearly seen. The macrofibrils (mf) in the orthocortical cells are cylindrical and relatively small, whereas in paracortical cells they are closely packed and poorly defined. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 1 μm.

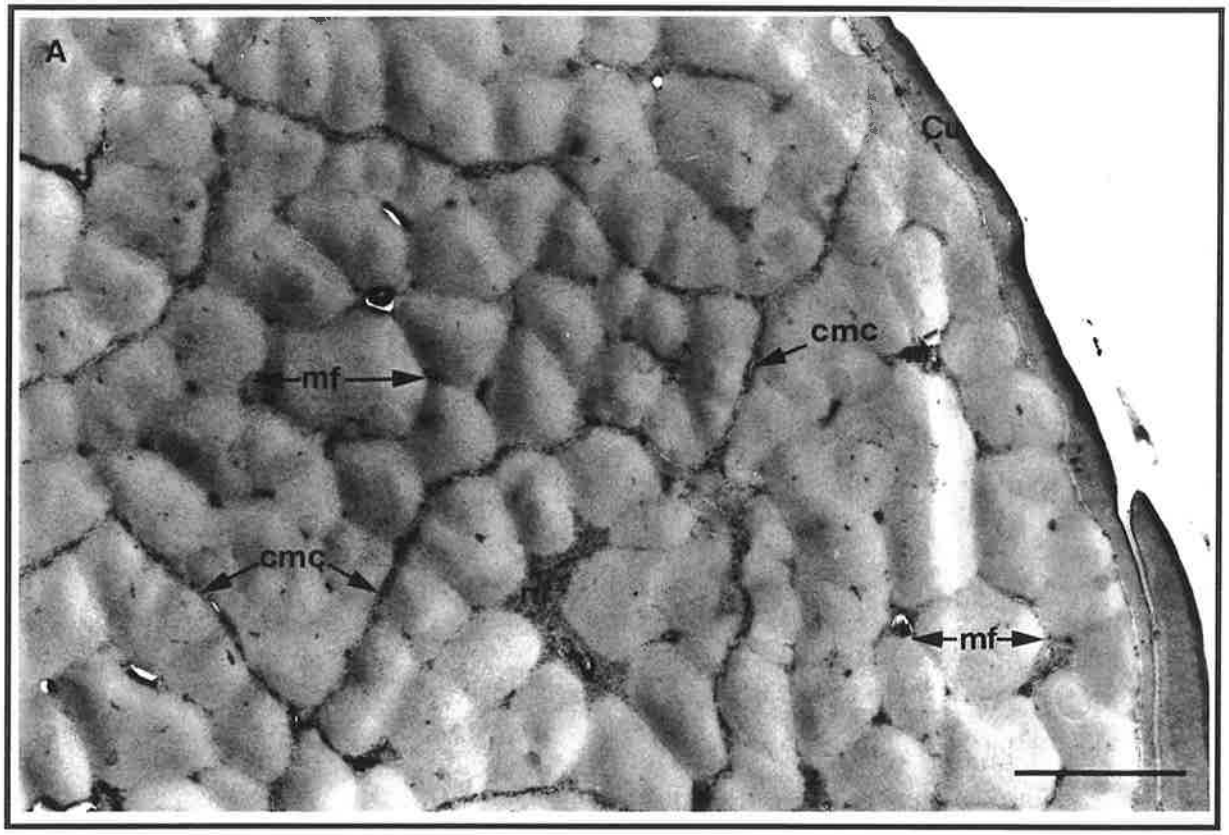

Plate 5.2*a*

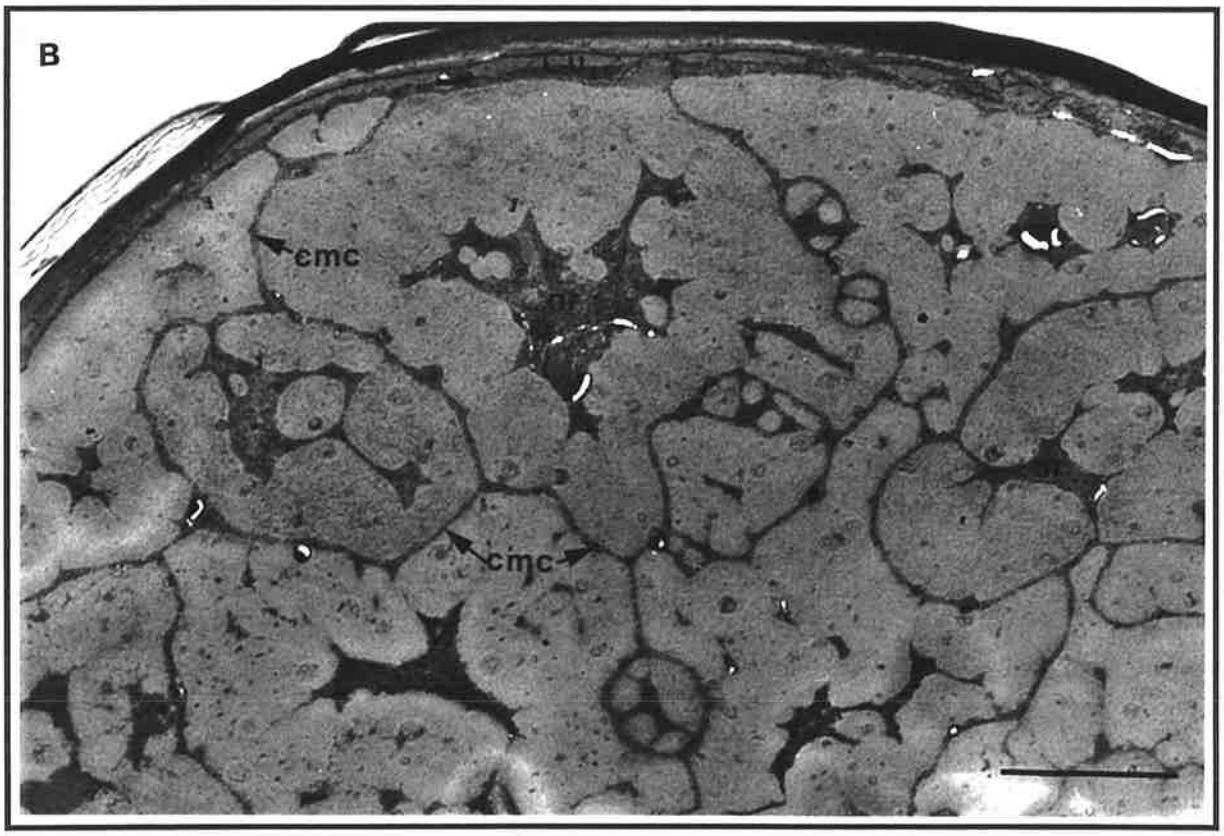

Plate 5.2*b*

The average thickness of the whole cuticle surrounding the fibre cortex was 0.5 ± 0.02 μm (range 0.3 to 0.8 μm), and of individual cuticle cells was 0.34 μm. The average number of overlapping cuticle cells at any point on the fibre surface was 1.45 ± 0.04, and the range was 1.03 to 1.91 (Plate 5.3). This means that some fibres were surrounded by a single layer of cuticle cells, whereas others were almost completely surrounded by two cuticle cells. There were significantly more cuticle cells on the paracortical side of the fibre than the orthocortical side (1.77 *vs*. 1.26; $P<0.001$).

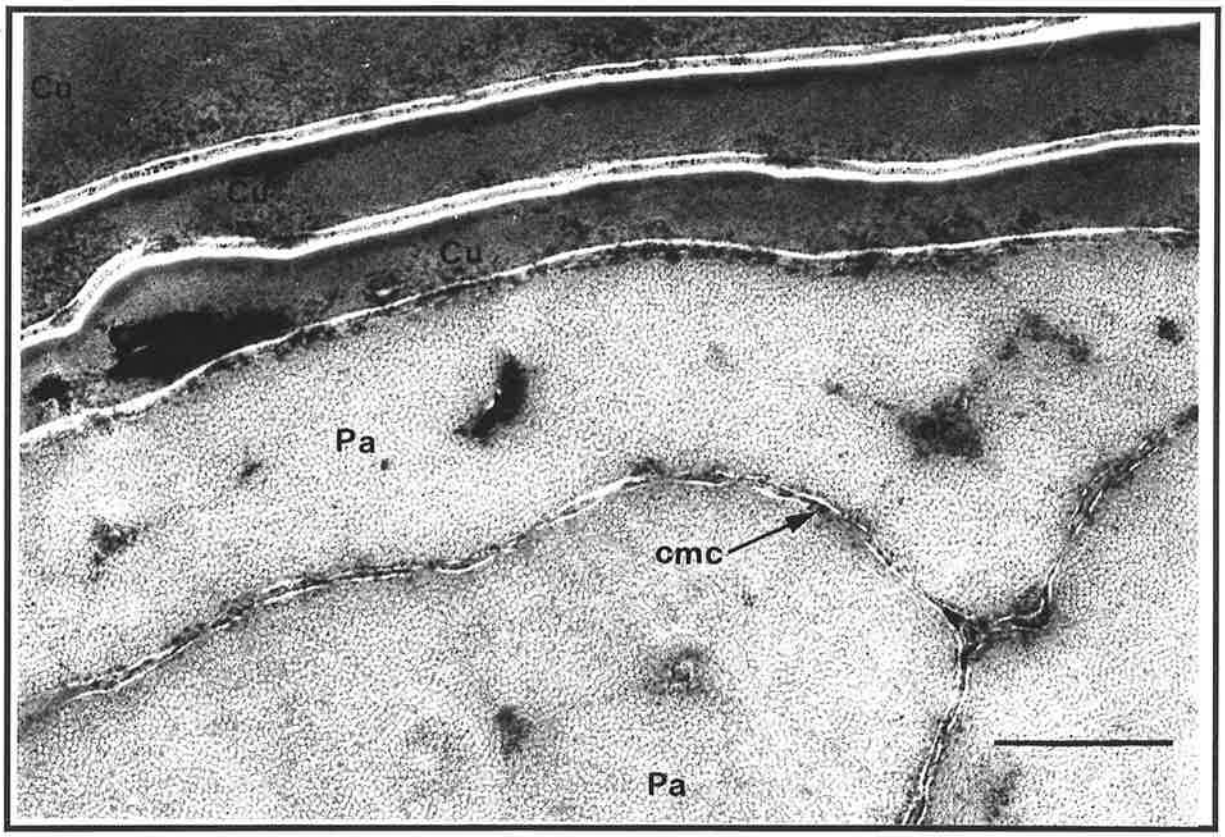

Plate 5.3. Transmission electron micrograph of a Merino wool fibre cross-section at high magnification (46000x) showing three cuticle cells (Cu) overlapping the paracortical (Pa) segment of the fibre cortex. The cell boundaries (cmc) and nuclear remnants (nr) are shown. This fibre was reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 200 nm.

Across all fibre cross-sections examined by TEM, the proportion of the total fibre cross-sectional area occupied by the different histological components varied over a wide range; cuticle 6.4 to 17.2%, cortex 82.8 to 93.6%, cell membrane complex 2.1 to 3.5%, nuclear remnant plus intermacrofibrillar material 1.2 to 6.4%, and macrofibrillar material 73.4 to 88.3%.

On average, fine fibres at the point of rupture had more cuticle and less cortex than broad fibres ($P<0.001$), and the cortical cells of finer fibres had more nuclear remnant material ($P<0.01$) and cell membrane complex ($P<0.001$) (Table 5.1). Finer fibres therefore had a lower proportion of macrofibrils (80.3 *vs.* 85.0%; $P<0.001$). On no occasion was the cross-sectional area by intrinsic strength interaction significant for any of the cellular or ultrastructural characteristics measured at low or high magnification.

Table 5.1. Cell type proportions and non-keratin components for fibres differing in cross-sectional area and intrinsic strength. The estimates were made from electron micrographs of whole fibre cross-sections sectioned within 50 μm of the fracture surface.

a, b: different superscripts within cross-sectional area or intrinsic strength comparisons differ at P< 0.05

Cell type	Cross-sectional area		Intrinsic strength	
	Low	High	Low	High
% of fibre cross-sectional area				
Cuticle cells	13.0^a	9.7^b	12.1^a	10.6^a
Cortical cells	87.0^a	90.3^b	87.9^a	89.4^a
% of cortex cross-sectional area				
Nuclear remnants	4.46^a	3.25^b	4.16^a	3.55^a
Cell membrane complex	3.25^a	2.57^b	2.74^a	3.05^b

Histological examination of ultra thin serial sections cut close to the fracture surface of broken fibres indicated that intrinsically strong fibres tended to have less cuticle and more cortex than intrinsically weak fibres ($P=0.06$), and the cortical cells of the stronger fibres tended to have less nuclear remnant material, but more cell membrane complex ($P<0.01$). Stronger fibres also tended to have a higher proportion of macrofibrils than weaker fibres (81.8 *vs.* 83.3%, $P=0.11$).

5.3.2. Cortical cell characteristics

On average, 65% of the total cortical area was occupied by orthocortical cells (range 38 and 76%), and 35% by paracortical cells (range 24 to 62%). The characteristics of the two major cortical components is summarised in Table 5.2. The average number of ortho- and paracortical cells in the fibre cross-sections was similar, but orthocortical cells were much larger ($P<0.001$) than paracortical cells. The lower shape factor of orthocortical cells ($P<0.05$) suggests that on average they were more convoluted (non-circular) than para-cells, and yet orthocortical cells had less total cell membrane complex ($P<0.05$) because of their greater average size. The other obvious difference was that orthocortical cells had much less nuclear remnant material, and therefore more macrofibrils, than paracortical cells ($P<0.001$).

Table 5.2. Average number and characteristics of ortho- and paracortical cells. The estimates were made from 40 transmission electron micrographs of whole fibre cross- sections sectioned within 50 μm of the fracture surface.

a, b: different superscripts differ at P< 0.05

Cell characteristic	Cortical cell type	
	Ortho-	Para-
Cell number	26.0^a	26.8^a
Cell size (average; μm^2)	6.08^a	3.00^b
Cell size (median; μm^2)	4.59^a	1.82^b
Cell size (% < 1 μm^2)	27.9^a	42.4^a
Cell shape	0.56^a	0.62^b
Cell membrane complex (%)	2.65^a	3.42^b
Nuclear remnants (%)	2.30^a	6.89^b
Macrofibrils (%)	95.1^a	89.7^b

The proportional areas occupied by ortho- and paracortical cells did not differ significantly between cross-sectional area groups (Tables 5.3 and 5.4). The average number and size of both cortical cell types was less in fine than broad fibres ($P<0.05$), but there was no significant difference in median cell size, the proportion of cells less than 1 μm^2, or cell shape. In finer fibres, a greater proportion of the total area of both cortical components was occupied by cell

membrane complex ($P<0.001$), and the paracortical cells in finer fibres had more nuclear remnant material and less macrofibrils than those in broad fibres ($P<0.001$). The proportions of nuclear remnant material and macrofibrils in orthocortical cells did not differ significantly between cross-sectional area groups.

Table 5.3. Characteristics of orthocortical cells for fibres differing in cross-sectional area and intrinsic strength. The estimates were made from transmission electron micrographs of whole fibre cross-sections sectioned within 50 μm of the fracture surface.

a, b: different superscripts within cross-sectional area or intrinsic strength comparisons differ at $P< 0.05$

Cell characteristic	Cross-sectional area		Intrinsic strength	
	Low	High	Low	High
Orthocortex area (% cortex)	64.4^a	65.4^a	67.8^a	62.0^b
Orthocortex cell number	15.3^a	36.9^b	19.6^a	28.9^b
Orthocortex cell size (average; μm^2)	5.16^a	7.00^b	7.02^a	5.15^b
Orthocortex cell size (median; μm^2)	4.09^a	5.08^a	5.99^a	3.18^b
Orthocortex cell size (% < 1 μm^2)	26.8^a	29.5^a	23.7^a	32.5^a
Orthocortex cell shape	0.55^a	0.58^a	0.57^a	0.56^a
Cell membrane complex (% orthocortex))	2.97^a	2.45^b	2.49^a	2.81^b
Nuclear remnants (% orthocortex)	2.33^a	2.25^a	2.49^a	2.09^a
Macrofibrils (% orthocortex)	94.7^a	95.3^a	95.0^a	95.1^a

Intrinsically strong fibres had a greater area occupied by paracortical cells and less orthocortex than weak fibres ($P<0.01$; Tables 5.3 and 5.4). Intrinsically strong fibres tended to have more individual paracortical cells, with less nuclear remnant material and more macrofibrils, than weak fibres ($P<0.10$). These were the only differences in the morphology of paracortical cells between the intrinsic strength groups. As for the orthocortical cells, stronger fibres had significantly more ($P<0.05$) cells which were smaller ($P<0.001$), and a greater proportion of cell membrane complex ($P<0.05$). The total number of cortical cells differed significantly between the low and high intrinsic strength groups, being 43.9 and 57.9, respectively ($P<0.001$).

Table 5.3. Characteristics of paracortical cells for fibres differing in cross-sectional area and intrinsic strength. The estimates were made from electron micrographs of whole fibre cross-sections sectioned within 50 μm of the fracture surface.

a, b: different superscripts within cross-sectional area or intrinsic strength comparisons differ at P< 0.05

Cell characteristic	Cross-sectional area		Intrinsic strength	
	Low	High	Low	High
Paracortex area (% cortex)	35.6^a	34.6^a	32.2^a	38.0^b
Paracortex cell number	15.9^a	37.5^b	24.3^a	29.0^a
Paracortex cell size (average; μm^2)	2.72^a	3.29^b	3.07^a	2.90^a
Paracortex cell size (median; μm^2)	1.85^a	1.80^a	1.83^a	1.82^a
Paracortex cell size (% < 1 μm^2)	39.1^a	45.1^a	40.7^a	43.6^a
Paracortex cell shape	0.61^a	0.62^a	0.61^a	0.63^a
Cell membrane complex (% paracortex)	3.74^a	3.10^b	3.29^a	3.46^a
Nuclear remnants (% paracortex)	8.31^a	5.47^b	7.55^a	6.23^a
Macrofibrils (% paracortex)	87.9^a	91.4^b	89.0^a	90.3^a

5.3.3. Microfibril/matrix arrangement

Macrofibril ultrastructure, consisting of lightly stained circular microfibrils embedded in heavily stained matrix proteins, appeared "normal" at high magnification in all fibre cross-sections examined. There was a considerable range in the packing arrangement and density of the microfibrils between different fibres, and between and within different cells from the same fibre (Plate 5.4 to 5.6). The microfibrils in the orthocortical cells generally appeared to be more closely packed than in paracortical cells, and were arranged in a whorl-like pattern with the hexagonally packed microfibrils only discernible in the centre of the formation (Plate 5.4a,*b*). In paracortical cells the microfibrils were more clearly resolved and the packing arrangement was more random (Plate 5.4*a,c*, 5.5 and 5.6). Most para-cells also included large areas where the microfibrils were arranged in a pseudo-hexagonal manner similar to that seen in mesocortical cells (Plate 5.4*a,d* and 5.5).

Plate 5.4. Transmission electron micrographs of Merino wool fibre cross-sections at high magnification (46000x) showing (*a*) the boundary between orthocortical (Or), paracortical (Pa) and mesocortical (Me) cells; and individual (*b*) orthocortical (*c*) paracortical and (*d*) mesocortical cells. The micrographs clearly show the different macrofibril definition and microfibril packing arrangements in the different cortical cell types; the characteristic whorl-like arrangement of ortho-cortical microfibrils (arrow head), the random microfibril packing in paracortical cells, and the hexagonally packed microfibrils in meso-cortical cells. Paracortical cells also included large areas where the microfibrils were arranged in a pseudo-hexagonal manner similar to that seen in mesocortical cells (arrow head). The cell membrane complex (cmc), nuclear remnant material and residual non-keratin cytoplasmic material (nr) are indicated. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 200 nm.

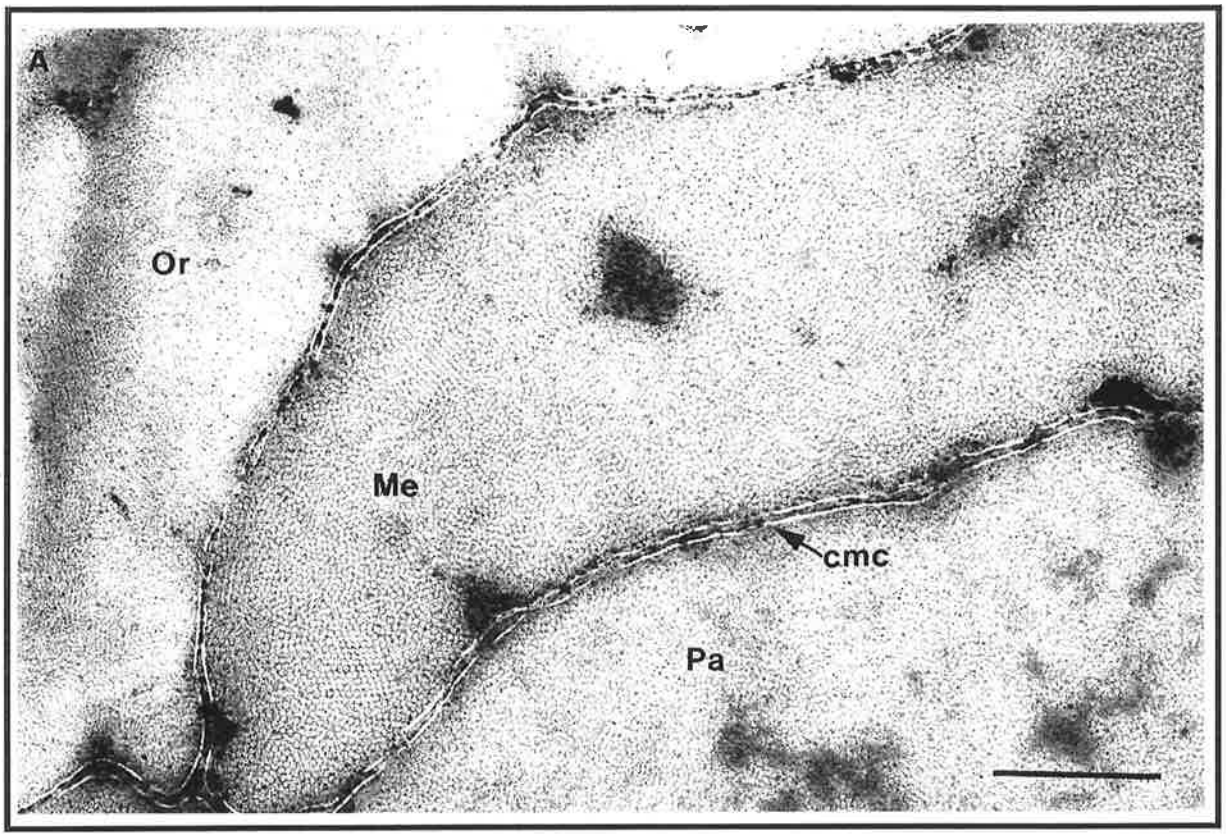

Plate 5.4*a*

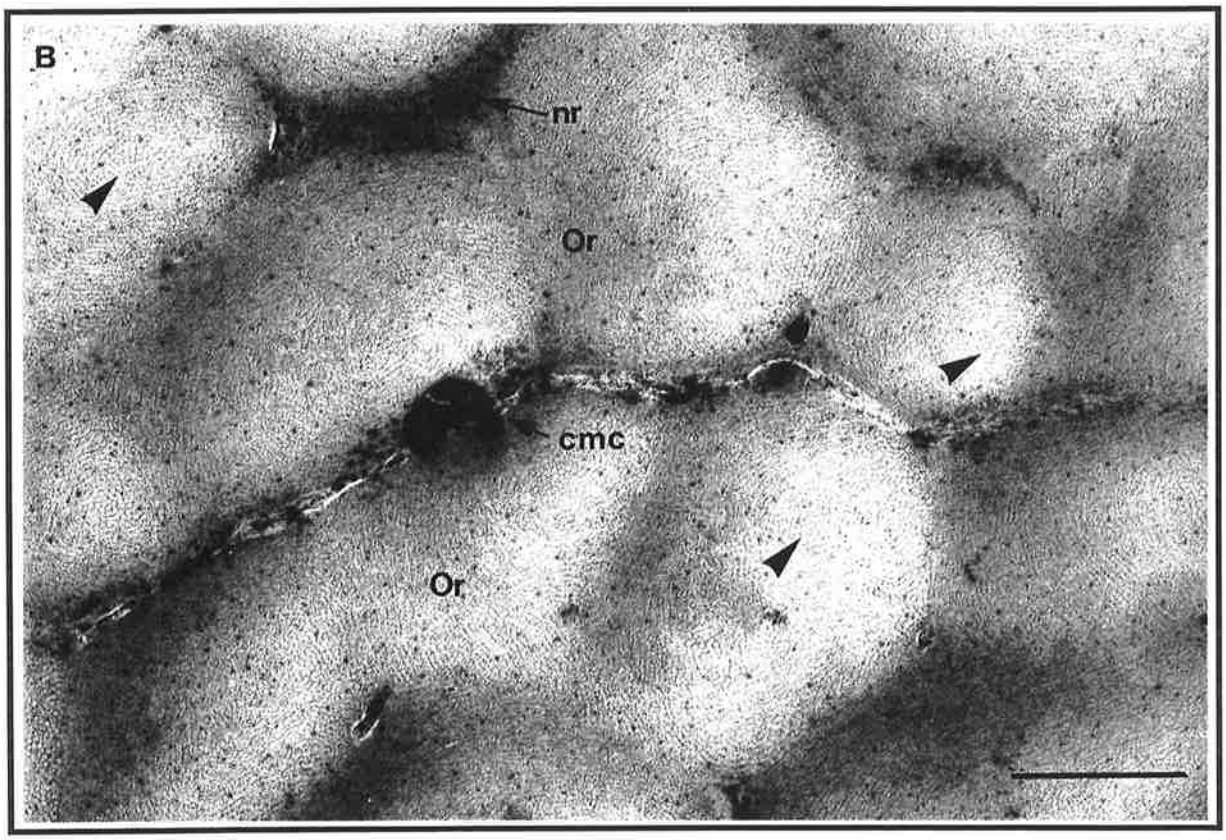

Plate 5.4*b*

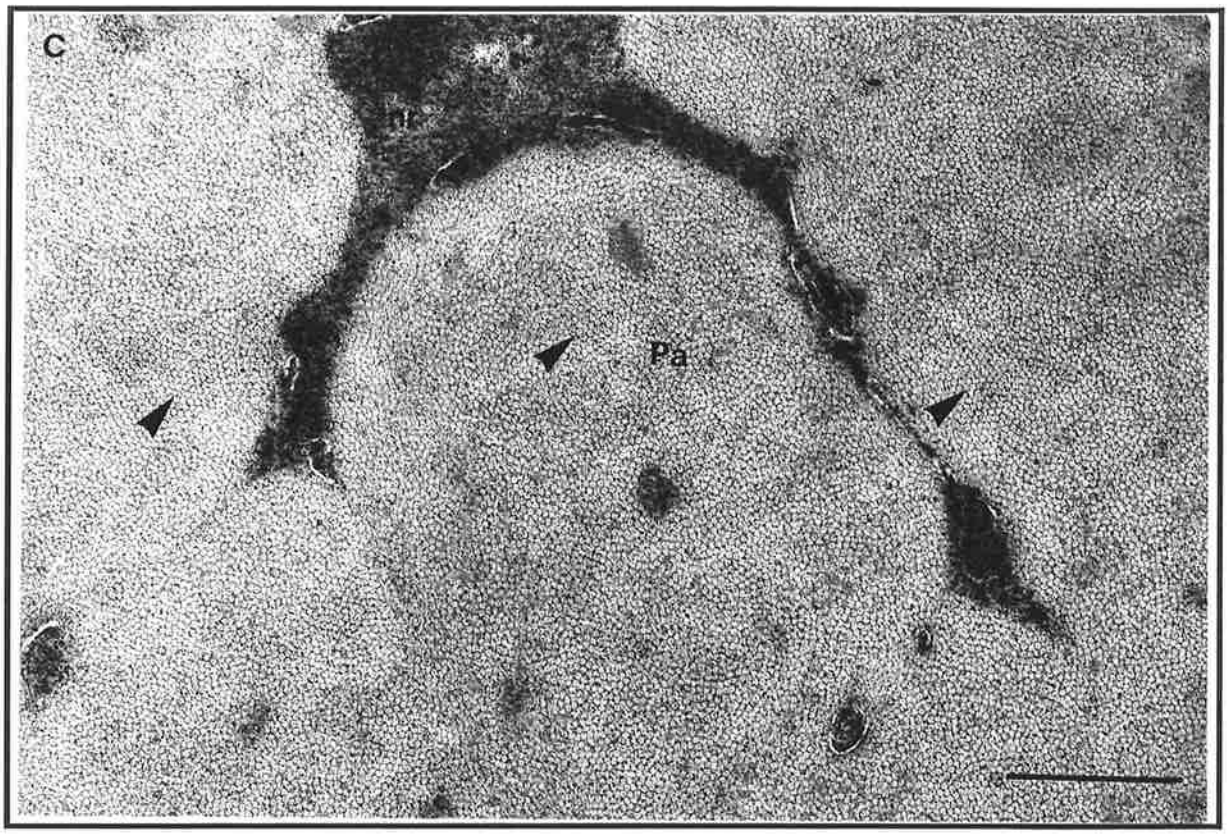

Plate 5.4*c*

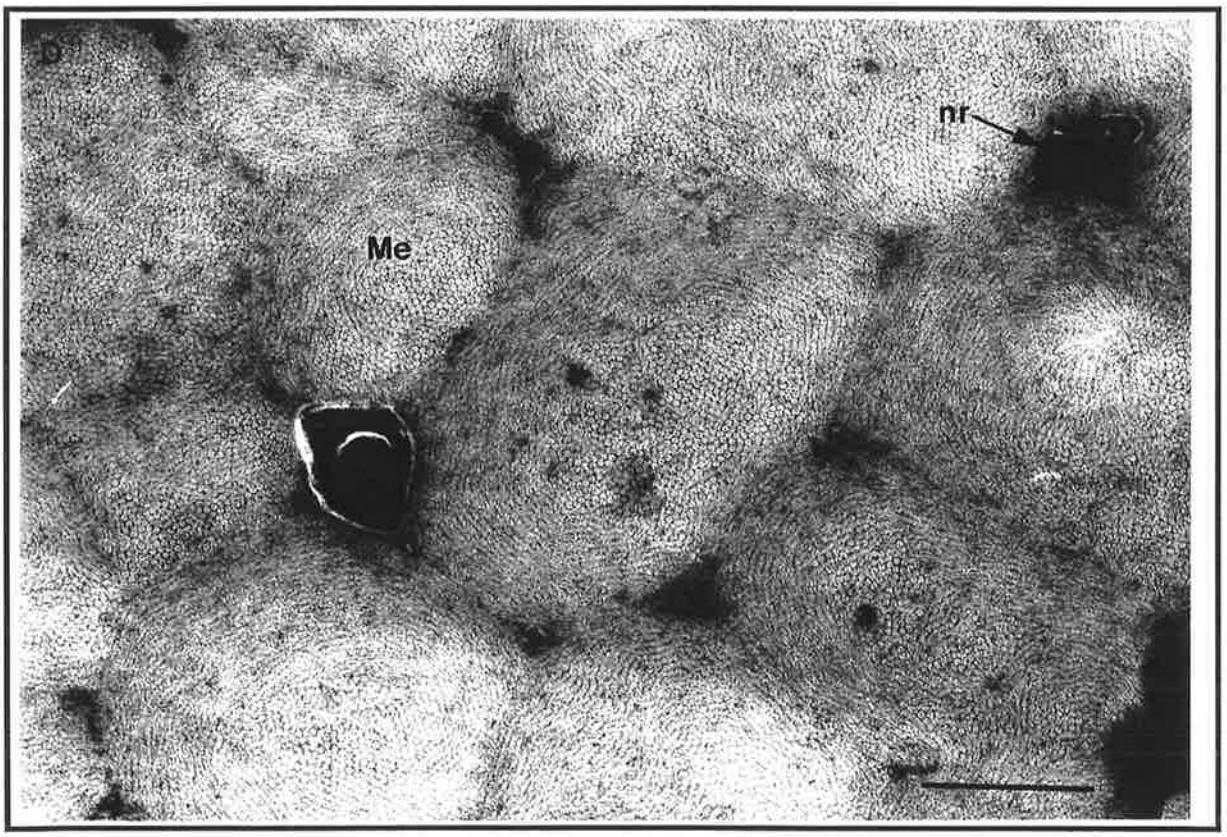

Plate 5.4*d*

5.3.4. Microfibril density and volume

For each wool fibre cross-section, the density of microfibrils was estimated at three or more sites within three ortho- and three paracortical cells. It was assumed for orthocortical cells that the density of microfibrils at the centre of the whorl reflected that of the whole macrofibril. It is pertinent to note that there was no evidence of a relationship between microfibril density in orthocortical (P=0.39) or paracortical cells (P=0.54) and the percentage change in fibre cross-sectional area estimated by light microscopy and TEM. If large changes in fibre cross-sectional area resulted from the fixation and staining procedures, and from the electron beam of the TEM itself, then a negative correlation between microfibril density and the change in cross-sectional area would be expected.

The average density of microfibrils was 25% greater in orthocortical cells than in paracortical cells (1.53 *vs.* 1.22 per 100 nm^2; P<0.001), and the density range within each cortical component was 0.75 to 1.85 per 100 nm^2, and 0.95 to 2.10 per 100 nm^2, respectively (Fig. 5.2). If the diameter of microfibrils was assumed to be 7.5 nm (Fraser *et al.* 1972), the average proportion of microfibrils in the keratin composite was calculated to be 53.3% (range 33.1 to 81.7%) for paracortical cells and 67% (range 42.0 to 92.8%) for orthocortical cells.

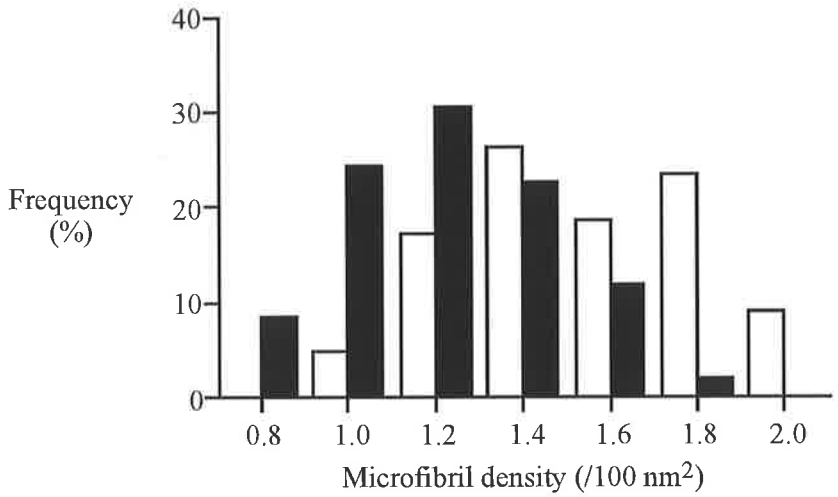

Figure 5.2. Variation in the packing density of microfibrils in orthocortical (□) and paracortical (■) cells. The density of microfibrils was measured at nine or more sites within the ortho and paracortical components of 40 wool fibre cross-sections.

The density and estimated volume of microfibrils tended to be greater in broader fibres, although the difference was not statistically significant in either ortho- or paracortical cells

(Table 5.5). However, because the total proportion of macrofibrils in the fibre cross-section was greater in broader fibres (85.0 *vs.* 80.3%; $P<0.001$), the average density and estimated total volume of microfibrils was also significantly greater ($P<0.01$) in broader fibres.

While there was no significant ($P>0.05$) difference between the intrinsic strength groups in the total volume of microfibrils in the fibre cross-section, the microfibril to matrix ratio was significantly ($P<0.05$) higher in the paracortical cells of fibres which were intrinsically stronger (Table 5.5). Furthermore, because intrinsically stronger fibres also had a greater proportion of paracortical cells (Table 5.3), microfibrils in paracortical cells accounted for 33% of the total microfibril volume in strong fibres compared to only 25% in intrinsically weak fibres ($P<0.05$).

Table 5.5. Microfibril density and estimated volume in ortho- and paracortical cells for fibres differing in cross-sectional area and intrinsic strength. The estimates were made from electron micrographs of fibre cross-sections sectioned within 50 μm of the fracture surface.

a, b: different superscripts within cross-sectional area or intrinsic strength comparisons differ at $P< 0.05$

Cell characteristic	Cross-sectional area		Intrinsic strength	
	Low	High	Low	High
Paracortex				
Density (per 100 nm^2)	1.18^a	1.23^a	1.12^a	1.30^b
Volume (% macrofibrils)	52.3^a	54.5^a	49.4^a	57.4^b
Orthocortex				
Density (per 100 nm^2)	1.47^a	1.57^a	1.50^a	1.54^a
Volume (% macrofibrils)	65.5^a	69.0^a	66.4^a	68.1^a
Total volume (% macrofibrils)	60.9^a	64.4^a	61.2^a	64.1^a
Total volume (% fibre)	48.9^a	54.7^b	50.2^a	53.5^a

The variation in microfibril packing density between different paracortical cells, and between paracortical and mesocortical cells, is clearly shown in Plate 5.5. Several electron micrographs are also shown in the Appendix, including (a) higher magnification (72000x) of the variation in microfibril packing arrangement and density between the different cortical cell types (Plate A5.1 and A5.2), and (b) very high resolution micrographs (600000x) indicated that the microfibrils possessed some resolvable substructure (Plate A5.3).

Plate 5.5. Transmission electron micrographs of Merino wool fibre cross-sections at high magnification (46000x) showing the variation in microfibril packing density within and between different paracortical (Pa) and mesocortical (Me) cells. The micrographs show areas in the paracortical cells where the microfibrils are arranged in a pseudo-hexagonal manner similar to that seen in mesocortical cells (arrow head). The cell membrane complex (cmc), nuclear remnant material and residual non-keratin cytoplasmic material (nr) are indicated. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 200 nm.

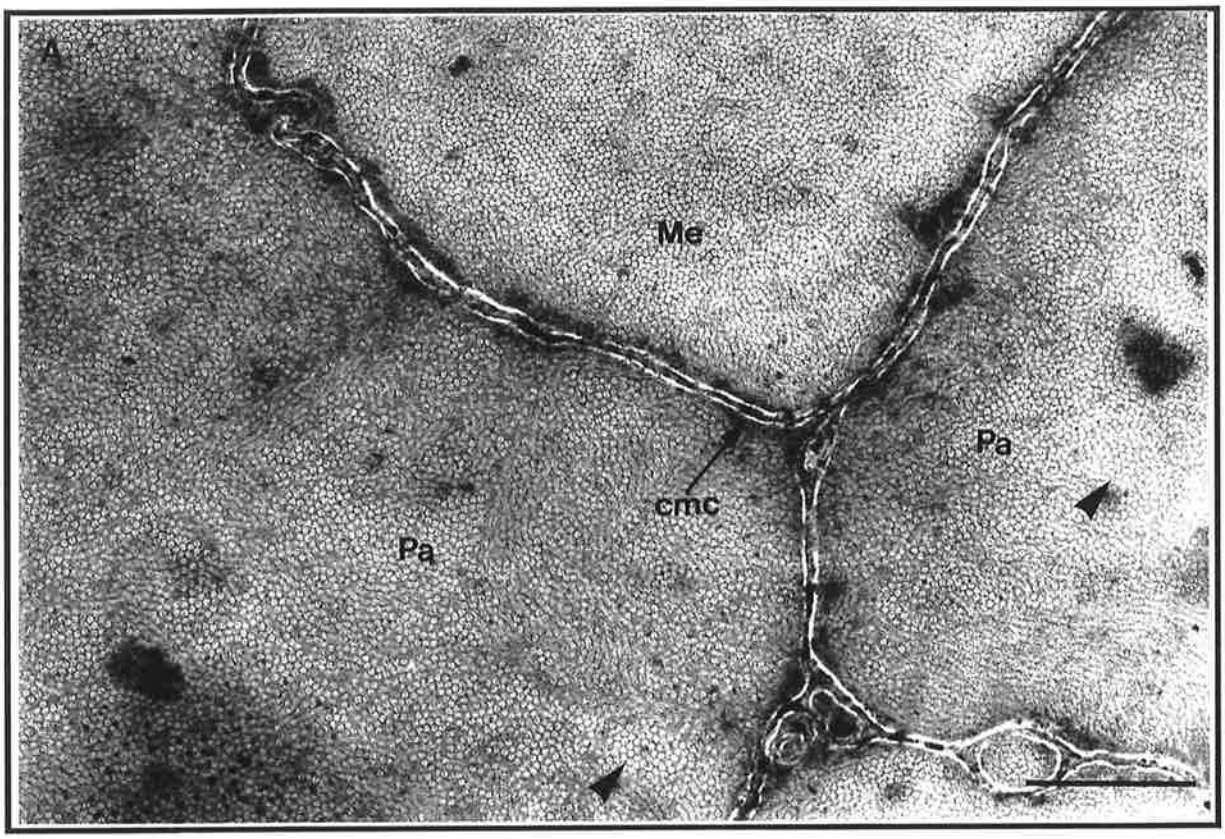

Plate 5.5*a*

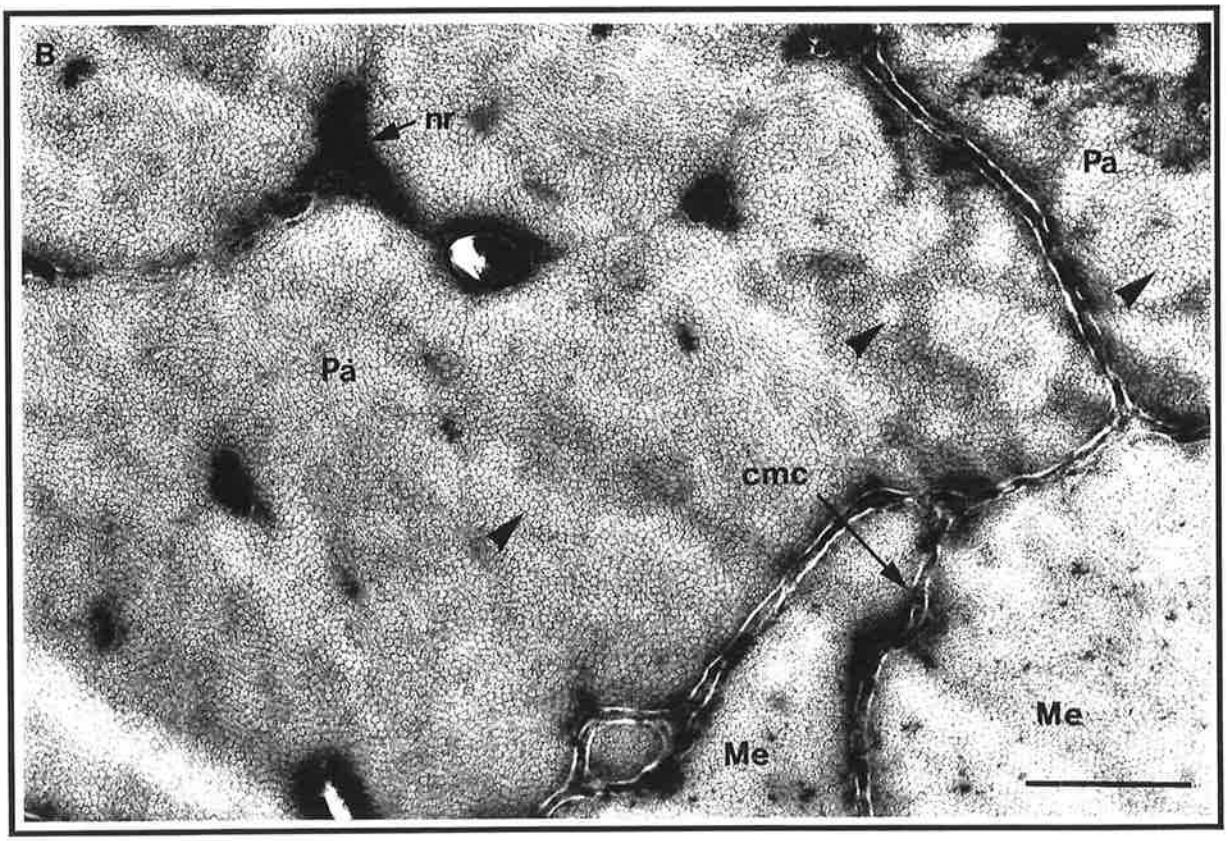

Plate 5.5*b*

5.4. Discussion

5.4.1. Hypothesis

Keratin fibre cross-sections for electron microscopy are notoriously difficult to cut, and it is rare to obtain complete transverse sections of fibres for scrutiny because of tears or folds or because parts of the sections are obscured from view by the bars of the specimen support mesh (Bones and Sikorski 1967; Swift 1977). To my knowledge, this is the first attempt to relate the cellular characteristics and ultrastructure of individual wool fibres to their intrinsic strength, or any other stress-strain property. The experiment is unique in that a large number of fibres with extreme differences in intrinsic strength were examined, the sections were from individual fibres rather than bundles of fibres, and importantly, the fibres were sectioned in close proximity to the fracture surface. This overcomes the potential problem in the previous Chapter, where the composition of the wool samples used may not have accurately represented that of the fibres at the point of rupture.

Electron microscopy studies have provided valuable qualitative information of the general morphological features of wool and other hard keratin fibres at both the cellular and macromolecular level (Rogers 1959*a,b*; Bones and Sikorski 1967; Dobb and Sikorski 1971; Bradbury 1973; Whiteley and Kaplin 1977; Kaplin and Whiteley 1978; Fraser and MacRae 1980; Orwin *et al.* 1984). However, because TEM studies of fibre cross-sections are limited by the dimensional changes which may occur during processing and under the electron beam of the microscope itself, few if any quantitative data have been forthcoming. In the current experiment, statistical procedures have been used to minimise the effects of these unavoidable analytical errors, and the technique used was considered to be sufficiently accurate to detect any obvious differences in cell morphology and ultrastructure between fibres with extreme differences in intrinsic strength (174 *vs.* 259 MPa).

Microscopical examination of 40 fractured fibre ends found no obvious structural abnormalities or defects within the cuticle or cortical cells in any of the fibre cross-sections examined. There was however a considerable range between individual fibres and groups of fibres in the proportions of the different histological components, the number and morphology of individual cuticle and cortical cells, and the packing arrangement, density and estimated volume of microfibrils in the two-phase composite of the fibre cortex. As the fibre cross-sectional area by

intrinsic strength interaction was not significant for any of the cellular or ultrastructural characteristics measured, the following discussion focuses entirely on the differences between the low and high intrinsic strength fibre groups.

Stronger fibres had a higher proportion of paracortical cells in the cortex than weaker fibres (38.0 *vs*. 32.2%), and these paracortical cells had a much higher microfibril/matrix ratio (57.4 *vs*. 49.4%). Indeed, whilst the total volume of microfibrils in fibre cross-sections did not differ significantly between the high and low intrinsic strength groups, being 53.5 and 50.2%, respectively, the microfibrils in the paracortex accounted for 33% of the total volume of microfibrils in intrinsically strong fibres compared to only 25% in weak fibres. The hypothesis that differences in the ultrastructure of the microfibril/matrix structure contribute to the differences in intrinsic strength reported in Chapter 3 was supported, although the observed differences in microfibril/matrix structure are probably not the sole cause for the difference in intrinsic strength. Stronger fibres also had more cortical cells which were generally smaller, and more cell membrane complex, which may contribute to the differences in intrinsic strength.

5.4.2. Cuticle and cortical cell characteristics at low magnification

The well documented cellular arrangement and morphology of Merino wool fibres in transverse section was apparent in all fibre cross-sections examined in this study. Varying numbers of overlapping cuticle cells surrounded the fibre cortex, which consisted of bilaterally arranged ortho and paracortical cells occasionally separated by a few mesocortical cells. The different cortical cell types were clearly distinguished at low magnification on the basis of macrofibril structure, definition of cell boundaries and presence of nuclear remnant material. Orthocortical cells were on average much larger than paracortical cells, which agrees with several other studies (Bones and Sikorski 1967; Orwin *et al.* 1984), and orthocortical cells had less nuclear remnant material and more macrofibril material than paracortical cells. There was a high frequency of small cortical areas (< 1 μm^2) enclosed by cell membrane, which presumably reflects the finger-like processes at the ends of cortical cells which interdigitate with adjacent cells (Rogers 1959*a,b*). The highly convoluted shape of adjacent cortical cells also suggests that these cells interlock in the transverse direction.

The relative proportions of different histological components in wool fibres have been estimated from the non-uniform distribution of specific amino acids in the various components and from

electron micrographs. On average, the cuticle and cortex are considered to account for about 10 and 90% of the fibre cross-sectional area, respectively (Mercer 1953; Bradbury and King 1967; Bradbury 1973), and this agrees closely with the current estimates of 11.6 and 88.4%. In the current experiment the cuticle occupied between 6 and 17% of the cross-sectional area of individual fibres, which is similar to other studies (2-10%, Gralen 1950; up to 20%, Lundgren and Ward 1963). The estimated transverse dimensions of the cuticle cells (Bradbury 1973), and the more extensive overlapping of cuticle cells on the paracortical side of the fibre (Makinson 1978; Phan *et al.* 1995), are also consistent with the literature. The average percentage of the cell membrane complex was 3.0%, which is within the 2.5 to 3.7% range reported by Rogers (1959*a,b*) and Bones and Sikorski (1967). The average proportion of nuclear remnant and inter-macrofibrillar material was 3.5% of the fibre cross-section, which is much lower than the single value of 12.6% obtained by chemical analysis (Bradbury 1973). While the proportion of inter-macrofibrillar material within the orthocortex may be under estimated in the current study, it is likely that a majority of the difference between these experiments is real, since even in the paracortex where the nuclear remnant material is clearly defined, the average proportion was only 7.1% (range 1.7 to 13.8%). These results indicate that, on average, macrofibrils occupied 82.3% of the fibre cross-sectional area, and the range of 73.4 to 88.3% encompasses the 74.1% estimated by Bradbury (1973). If it assumed that the exocuticle contributes 6-7% to the total keratin content of the fibre, then it can also be calculated from the data reported by Zahn (1980) that the macrofibril content of the two Merino samples was between 77.5 and 79.5%. Macrofibrils occupy between 80 and 85% of the entire cross-sectional area of mohair (Zahn 1980) and human hair (Swift 1997).

5.4.3. Comparison of high and low intrinsic strength fibres at low magnification

Intrinsically stronger fibres had a slightly higher proportion of macrofibrils in the fibre cross-section (83.3 *vs.* 81.8%) than intrinsically weak fibres, and this was attributed to less cuticle and nuclear remnant material. A more significant finding was that strong fibres had more paracortex and less orthocortex than weak fibres, which is at odds with the results of Chapter 4 (section 4.3.1), where no correlation was found between cortical cell types and the average intrinsic strength for individual sheep. As the technique used in the current Chapter is more precise than used previously, because the same individual fibres were used for measurement of cell types proportions and intrinsic strength, and cell type proportions were measured at the point of

rupture rather than the region of minimum fibre diameter, it is concluded that real differences exist in cortical cell type proportions between fibres differing in intrinsic strength. While a number of other workers have suggested previously that stronger fibres contain more paracortex (Thorsen 1958; Feughelman and Haly 1960; Whiteley and Speakman 1960; Chapman 1965; Orwin *et al.* 1980), because of the more ordered packing and orientation of the microfibrils within the paracortical cells, their conclusions were based on evidence which in the main was discredited in Chapter 4. To the best of my knowledge, the current work is the most robust indication that intrinsic strength is influenced by cortical cell type proportions.

The number and dimensions of individual cortical "cells", or at least cortical regions enclosed by a cell membrane, in one or both of the cortical components, differed between low and high intrinsic strength fibres. Stronger fibres had 30% more cortical "cells" than weak fibres, and on average, these "cells" were smaller and surrounded by more cell membrane complex. At first these findings suggest that differences in the properties of the cell membrane complex affected intrinsic strength. However, because the measurements made in this experiment were in the transverse direction only, it is not possible to determine whether stronger fibres did actually have more individual cortical cells and cell membrane complex, or whether they had: (i) shorter cortical cells with similar levels of interdigitations (and therefore more chance of sectioning through the interdigitations); and/or (ii) similar length cortical cells with more interdigitations. J.W.S. Hearle (pers. comm.) speculates that the current results could be explained by the extent of slippage between cells. However, microscopic examination of strained α-keratins suggests that the cellular structure conforms to the strain and that cell boundaries are not points of weakness. Dobb and Murray (1976) also found no indication of abrupt changes in the fine structure between adjacent cortical cells, and concluded that little if any slippage occurs preferentially at the cell boundaries. The fibres selected for my TEM work also broke perpendicular to the fibre axis, rather than between cortical cells. No logical explanation can be given for the mechanism via which cortical cell dimensions or the amount of cell membrane complex may influence intrinsic strength, and more accurate information on the morphology of isolated cortical cells in fibres differing in intrinsic strength is needed.

5.4.4. Microfibril/matrix characteristics at high magnification

Macrofibril ultrastructure appeared "normal" at high magnification, and the different cortical cell types were clearly distinguished by the proportion and packing arrangement of microfibrils,

and these differences have been extensively documented (Horio and Kondo 1953; Mercer 1953; Fraser and Rogers 1953; Rogers 1959*a,b*; Bones and Sikorski 1967; Dobb 1970; Whiteley and Kaplin 1977; Kaplin and Whiteley 1978). In orthocortical cells the microfibrils were more closely packed than in paracortical cells, and were arranged in a whorl-like pattern with the hexagonally packed microfibrils only visible in the centre of the formation. This characteristic microfibril pattern in orthocortical cells, reminiscent of fingerprints, is caused by the tilting of the microfibrils from the fibres long axis in a so-called cylindrical lattice. The microfibrils in paracortical cells were packed more randomly, although there were large areas where the microfibrils were arranged in a pseudo-hexagonal manner similar to that observed in mesocortical cells. Besides the hexagonal and less regular types of packing, a distinct layer structure was also seen, and this is attributed to regular arrays of microfibrils being tilted with respect to the axis of the fibre (plane of sectioning).

The volume of microfibrils in the two-phase microfibril/matrix composite was significantly higher in orthocortical cells than in paracortical cells (67 *vs.* 53%), which is consistent with studies which all indicate that orthocortical cells contain more LS microfibril proteins and less HS matrix proteins than paracortical cells (Chapman and Bradbury 1968; Chapman and Gemmell 1971, 1973; Kulkarni *et al.* 1971; Wagner *et al.* 1983; Ito *et al.* 1985; Carr *et al.* 1986; Dowling *et al.* 1990; Jones *et al.* 1993; Powell and Rogers 1994*a*). The volume of microfibrils in ortho- and paracortical cells has been estimated by TEM to be 80 and 50%, respectively (Leach *et al.* 1964), and by low-angle X-ray diffraction to be about 67-70% and 33-48%, respectively (Dobb 1970). The difference in microfibril/matrix ratio between the cell types was less in the current experiment than that determined previously. This is consistent with the evidence presented in Chapter 4, that the composition of paracortical cells at the point of break approached that of orthocortical cells, and the above mentioned finding that the packing of microfibrils in many paracortical cells was closer to that normally seen in intermediate type mesocortical cells. Across all fibre cross-sections, the maximum volume of microfibrils was estimated to be 93 and 82% for ortho- and paracortex respectively, which are both close to the maximum possible packing fraction for parallel fibres is an hexagonal arrangement (90.7%; Nielson 1974).

5.4.5. Comparison of high and low intrinsic strength fibres at high magnification

There was no evidence to suggest that low intrinsic fibre strength was associated with any major disruptions to the normal assembly of the microfibril/matrix composite, which is in contrast to many human diseases characterised by brittle hair (reviewed by Price 1990), and transgenic mouse hair (Powell and Rogers 1990*b*) and Merino wool which are intrinsically weak (Bawden *et. al.*, 1998). There was no significant difference in the total volume of microfibrils in the whole cross-section of individual fibres that differed in intrinsic strength by 50%, which supports the conclusions from the amino acid analysis, protein composition and DSC work reported in Chapter 4. The most striking difference between intrinsic strength groups was that the stronger fibres had a higher proportion of paracortical cells in the cortex than weaker fibres (38.0 *vs.* 32.2%), and these paracortical cells had a much higher microfibril/matrix ratio (57.4 *vs.* 49.4%). Taken together, the microfibrils in the paracortex accounted for 33% of the total volume of microfibrils in intrinsically strong fibres compared to only 25% in weak fibres. It is concluded that small changes in density of microfibrils in paracortical cells, which are better aligned with respect to the fibre axis and possibly more stabilised by the surrounding matrix than those in orthocortical cells, has a significant effect on intrinsic fibre strength.

5.5. Conclusions

The hypothesis that differences in the ultrastructure of the microfibril/matrix structure contribute to the differences in intrinsic strength was supported. The density of microfibrils in paracortical cells was significantly greater in stronger fibres, indicating that intrinsic strength is not so much related to the total volume of microfibrils in the fibre cross-section, but rather the distribution of the microfibrils between the cortical components. Given that the structure of the microfibrils appears to be constant in different cell types, and indeed in different α-keratins (Marshall and Gillespie 1977), it thus seems that factors such as microfibril orientation and stability are important determinants of intrinsic strength. Nevertheless, the observed differences in microfibril/matrix structure are probably not the sole cause of the difference in intrinsic strength, and although their consequences are difficult to predict, possible differences in cortical cell morphology and the proportion of cell membrane complex may contribute to the differences in intrinsic strength.

SUMMARY and CONCLUSIONS

This thesis presents an extensive literature review of the fibre and staple characteristics which contribute to phenotypic differences in staple strength. A series of experiments then tested the unifying hypotheses that intrinsic fibre strength, or the inherent strength of the keratin material of the fibre, would be an important component of staple strength, and that intrinsic strength would be determined by the chemical composition and physical structure of the fibre cortex. The first hypothesis tested was not supported, but there was some evidence that intrinsic strength was at least associated with differences in the ultrastructure of the fibre cortex.

The initial aim of this thesis was to identify the causes for differences in staple strength between young Merino sheep selected for sound or tender wool, and fed to produce differential changes in liveweight typical of that experienced during summer/autumn in Mediterranean-type environments. In the first experiment (Chapter 2), the estimated variation in diameter along and between individual fibres explained 80% of the variance in staple strength between individual sheep, and the mechanisms responsible for nutritionally-induced and genetic differences in staple strength were not the same. Whereas nutrition influenced staple strength by affecting 'along-fibre' changes in diameter, genetic differences in staple strength, at least as far as they are represented by the sheep used here, were largely attributable to 'between-fibre' variations in diameter. As fibre diameter and length tend to change together in response to simple changes in nutrition (Hynd 1994*a*), it was hypothesised from the current results that high staple strength sheep with a low between-fibre variation in diameter will also have a low fibre length variation. Staples comprising fibres which are more uniform in length should be stronger, when expressed as peak force to break, since a greater proportion of fibres will become load-bearing and break at the same time, relative to staples comprising fibres which are highly variable in length. This hypothesis has since been confirmed (A.D. Peterson, unpubl. data).

The practical implications of the findings from Chapter 2 are that nutritional management and genetic selection should be used concurrently to most effectively reduce the incidence of tender wool production. The data indicate that feeding young sheep to maintain liveweight during summer/autumn may be a practical on-farm strategy to reduce the incidence of tender wool production, although its adoption by wool growers may be limited because this strategy does not

guarantee the production of sound wool (Doyle *et al.* 1995; Gherardi *et al.* 1996, 1998), and it is currently not cost-effective. Additional work is required to identify why this strategy sometimes fails, before robust supplementary feeding recommendations can be made for increasing staple strength. While genetic selection for staple strength is a permanent solution to the tender wool problem, the data presented in this thesis, and by Thompson *et al.* (1995), indicate that selection for high staple strength reduces the variability in properties between fibres, rather than the average properties of individual fibres *per se*. It is therefore likely that any benefits in processing performance that result from genetic improvements in staple strength will not be as significant as that which would be predicted from the changes in staple strength alone. Indeed, the similar processing performance of batches of fleeces which differed in staple strength by about 5 N/ktex, due to differences in 'between-fibre' variation in diameter only, supports this notion (A.D. Peterson, P.R. Lamb, A.N. Thompson and C.M. Oldham unpubl. data). In light of these results, it would be more desirable to breed sheep with low along-fibre diameter variation, rather than low between-fibre variation in diameter. However, for this to occur a technological breakthrough is required to make the measurement of along-fibre variation in diameter commercially viable for use in breeding Merino sheep.

In a second experiment (Chapter 3), real and substantial differences of up to 44% in the average intrinsic strength of individual wool fibres were observed between individual sheep, but these differences in intrinsic fibre strength failed to remove any of the variance in staple strength not already attributed to differences in fibre diameter variation. Clearly, intrinsic strength *per se* did not contribute significantly to the differences in staple strength between sheep in this experiment, and this is consistent with several other recent studies (Scobie *et al.* 1996; Masters *et al.* 1998; Peterson *et al.* 1998). Further evidence that intrinsic strength could at best only be a minor component of staple strength in the current experiment was that the average intrinsic strength of individual fibres was 160 N/ktex, and this value far exceeds the average staple strength (24 N/ktex). The results of Chapter 3 indicate that the association between minimum fibre diameter and staple strength reported in Chapter 2 was largely due to an increase in the amount of material available to bear the load, rather than an increase in the inherent strength of the keratin material at the point of break. It was also proposed that both minimum fibre diameter and the variation in diameter along the fibre influence staple strength via effects on fibre extensibility.

While it remains to be determined if intrinsic fibre strength becomes a more important determinant of staple strength under different circumstances, such as when the changes in diameter along the fibre are minimal, it was hypothesised that the effects of differences in intrinsic strength should be more significant in later stage wool processing. The contribution of diameter variation to fibre breakage is diminished by this stage. For this reason, Chapters 4 and 5 of this thesis aimed to identify the causes for the large differences in intrinsic strength between wool samples from different sheep and between different individual fibres, respectively. Evidence from Chapter 3 suggested that "flaws" or defects were not the main determinant of the observed variations in intrinsic strength in this experiment, and that real differences in chemical composition and or fibre ultra-structure should exist at the point of break between fibres which differed in intrinsic strength.

In the third experiment (Chapter 4), wool samples of known intrinsic strength were characterised in terms of the proportion of cortical cell types, total sulphur content and amino acid composition, the relative amounts and composition of keratin proteins by 1D and 2D-PAGE, and the α-helical denaturation enthalpy and transition temperatures using differential scanning calorimetry. Using these analytical techniques, sheep-to-sheep differences in the cellular and molecular characteristics of fibres measured in the region near the point of rupture were, in the main, not significantly related to intrinsic strength. It was emphasised that the results of this study do not necessarily imply that no such associations exist, and indeed more sophisticated separation procedures like that used by Herbert and Rankin (1996), together with more quantitative detection methods, may provide some insight into the associations between protein composition and intrinsic strength. Fractionation of the wool protein extracts into the three main protein classes prior to electrophoresis, and the use of separation parameters most appropriate for each protein family, may also be worthwhile, as would the pre-determined perturbation of composition with single specific keratin genes. A possible explanation for the current results is that the mechanical properties of the matrix and microfibril phases were too similar under the test conditions used, such that changes in their ratio would have no demonstrable effects on intrinsic strength. This is unlikely however, because it has recently been found that the chemical composition of these same wool samples was not correlated to their intrinsic strength measured in water (M. G. Huson and A.N. Thompson unpubl. data), and it is known that in water the mechanical properties of the two phases of the composite structure of the fibre cortex are vastly different.

The results from Chapter 4 clearly suggested that physico-chemical factors other than those considered were responsible for the differences in intrinsic strength. One such factor is the location of the cross-links between the different proteins of the microfibril/matrix structure. It is known that differences must exist in the distribution of intra- and inter-chain disulphide bonds between wools of similar sulphur content and protein composition (Gillespie 1970), and it is inevitable that the location of these disulphide bonds is critical to intrinsic strength, although no experimental test of this hypothesis has been made. Whilst beyond the scope of the present study, there is a clear need to more precisely identify the location of the disulphide bonds in wool. Other factors which may be associated with intrinsic strength include the arrangement of the constituent proteins of the microfibril/matrix structure, since complex changes in the composition and physical arrangement of the keratin proteins in transgenic mice and sheep over-expressing a single keratin gene also has a profound effect on hair and wool strength (Powell and Rogers 1990*b*; Bawden *et al.* 1998), and small changes in filament orientation drastically reduce intrinsic strength of man-made composite structures (Wainright *et al.* 1976). It was thus possible that differences in the ultrastructure of the microfibril/matrix structure existed between fibres differing in intrinsic strength.

In a fourth experiment (Chapter 5), 40 fibres with extreme differences in intrinsic strength were examined by transmission electron microscopy. The experiment was unique in that a large number of fibres was examined, the sections were from individual fibres rather than bundles of fibres, and most importantly, the fibres were sectioned in close proximity (50 μm) to the fracture surface. This avoided the potential problem in the previous Chapter, where the composition of the wool samples used may not have accurately represented that of the fibres at the point of rupture. The electron micrographs revealed that variations in intrinsic strength were not associated with any major disruptions to the normal assembly of the microfibril/matrix composite, or any obvious structural abnormalities or defects within the cuticle or cortical cells. There was no significant difference in the total volume of microfibrils in the whole cross-sections, but the proportion of paracortical cells (38.0 *vs.* 32.2%) and the density of microfibrils in paracortical cells (57.4. vs. 49.4%) were significantly greater in stronger fibres. Small changes in density of microfibrils in paracortical cells, which are better aligned with respect to the fibre axis and possibly more stabilised by the surrounding matrix than those in orthocortical cells, clearly have a significant effect on the intrinsic strength of Merino wool fibres. Possible differences in cortical

cell morphology and the proportion of cell membrane complex may also influence intrinsic strength, although the precise mechanisms are not known. The results of Chapters 4 and 5 highlight the complexity of the task of postulating theoretical models on the basis of simple relationships between chemical constitution and mechanical properties, and more basic research is required to determine the roles of the microfibril and matrix proteins on the mechanical characteristics of wool fibres. It is also essential to define the follicular events responsible for variations in intrinsic strength, and indeed this may be another approach to identifying additional causes for the variation in intrinsic strength observed in this and other studies.

APPENDIX

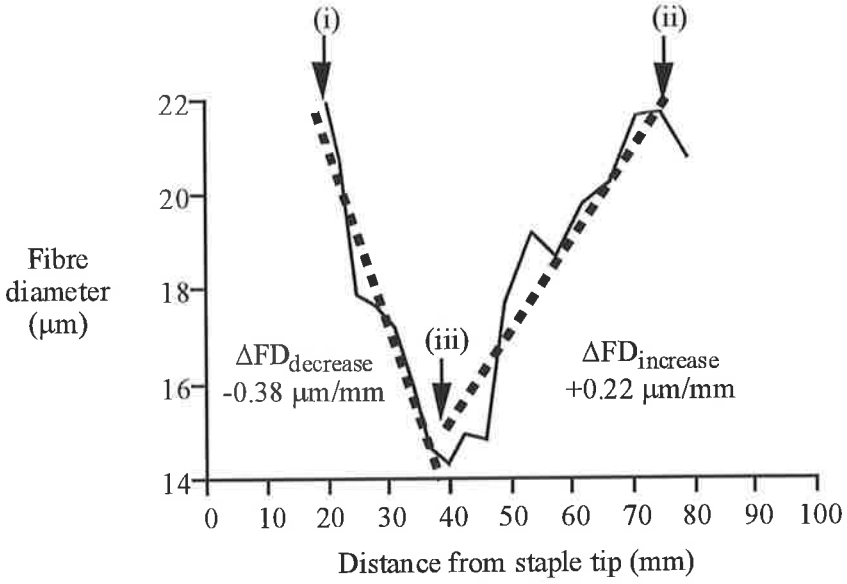

Figure A2.1. An example of a fibre diameter profile constructed from mean fibre diameter of wool clipped from the mid-side patch at 14 day intervals in relation to staple length, assuming that staple length growth was linear during the 28 day period between consecutive dyebands. Linear regression analysis was used to estimate the rate of decrease in diameter between the first dyeband at day 63 (*i*) and the point of minimum fibre diameter (*ii*), and the rate of increase in fibre diameter between (*ii*) and the last dyeband at day 287 (*iii*).

Table A2.1. Protocol for 10% buffered formalin

- 18 L Distilled water
- 2 L 98-100% Formalin (AnalaR®; Hopkin and Williams, Chadwell Health, Essex, England)
- 130 g Di-sodium hydrogen orthophosphate anhydrous (AnalaR®, BDH Chemicals, Australia Pty. Ltd., Kilsyth, Vic, Australia)
- 80 g Sodium dihydrogen orthophosphate (Univar; Ajax Chemicals, Sydney, Australia)

Table A2.2. Protocol for tissue processing

1. 70% ethanol (60 min x 2)
2. 80% ethanol (60 min)
3. 95% ethanol (30 min)
4. 95% ethanol (90 min)
5. Absolute ethanol (120 min x 2)
6. 1:1 absolute ethanol and Histoclear (Histological clearing agent; National Diagnostics, Atlanta, Georgia; 60 min)
7. Histoclear (120 min x 2)
8. Wax (120 min x 2)

Table A2.3. Protocol for Methylene Blue staining (Clarke and Maddocks 1965)

1. Histoclear (20 min)
2. Absolute ethanol (2 min)
3. 80% ethanol (2 min)
4. 50% ethanol (2 min)
5. 30% ethanol (2 min)
6. [a] Performic acid (fresh; 30 min)
7. Distilled water (10 sec)
8. [b] Methylene blue (5 min)
9. Distilled water (10 sec)
10. 1% acid ethanol (1% hydrochloric acid in 70% ethanol) (10 sec)
11. [c] Eosin (1 min)
12. 80% ethanol (10 sec)
13. Absolute ethanol (2 min)
14. Histoclear (2 x 5 min)

[a] Performic acid:

- 250 ml performic acid (90%)
- 100 ml H_2O_2
- 650 ml H_2O

[b] Methylene Blue

- 1% (w/v) Lofflers methylene blue

[c] Eosin:

- 50 ml 1% aqueous eosin
- 390 ml 95% ethanol
- 5 ml 1% aqueous phoxline
- 2 ml glacial acetic acid

Table A2.4. Protocol for modification of the Sacpic staining (Nixon 1993)

1. Histoclear (20 min)
2. Absolute ethanol (2 min)
3. 80% ethanol (2 min)
4. 50% ethanol (2 min)
5. 30% ethanol (2 min)
6. Distilled water (2 min)
7. [a] Lillee Mayer's Haematoxylin (10 min)
8. Rinse in distilled water (2 sec)
9. [b] Winiwater's Safranin (15 min)
10. Differentiate in 70% ethanol
11. Rinse in absolute ethanol (2 sec)
12. Saturated Picric acid (absolute ethanol) (5 sec)
13. Rinse in water
14. Rinse in distilled water
15. [c] Picro-Indigo Carmine (20 sec)
16. Differentiate in 70% ethanol
17. 70% ethanol (2 min)
18. 80% ethanol (2 min)
19. Absolute ethanol (2 min)
20. Histoclear

[a] Lillee Mayer's Haematoxylin:

- 5 g Haematoxylin (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, USA)
- 50 g Aluminium ammonium sulphate (Ammonium alum; AnalaR®, BDH Chemicals, Australia Pty. Ltd., Kilsyth, Victoria)
- 300 ml Glycerol (Ammonium alum; AnalaR®, BDH Chemicals, Australia Pty. Ltd., Kilsyth, Victoria)
- 700 ml Distilled water
- 1 g Sodium iodate (Koch-light Ltd., Suffolk, England)
- 20 ml Glacial acetic acid (AnalaR®, BDH Laboratory Supplies, Poole, England)

Method: Dissolve haematoxylin in a small volume of ethanol and aluminium ammonium sulphate in water with gentle heating. Combine solutions and add remaining ingredients.

[b] Winiwater's Safranin:

- 10 g Safranin O (Gurr, Certistain; BDH Laboratory Supplies, Poole, England)
- 15 ml 95% ethanol
- 145 ml Distilled water

Method: Dissolve the above and add 25 ml of the solution to 80 ml of 50% ethanol and filter

[c] Picro-Indigo Carmine:

- 1 g Indigo Carmine (George T. Gurr, Searle Scientific Services, High Wycome, Bucks)
- 300 ml water saturated picric acid

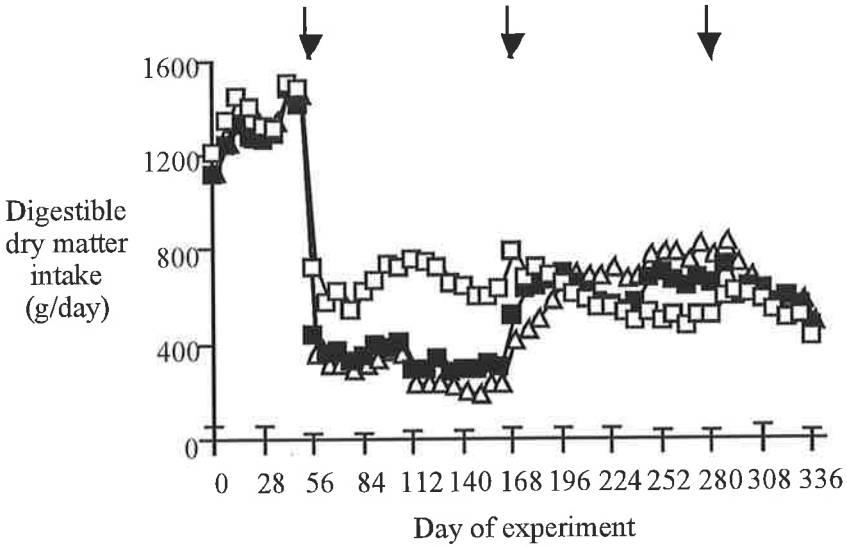

Figure A2.2. Digestible dry matter intake for sheep from nutritional treatments LWM (□, n = 12), LWL_1 (■, n = 14) and LWL_2 (Δ, n = 14). The data for sheep from the "sound" and "tender" staple strength selection flocks were combined, and points represent treatment means. Error bars on X-axis denote s.e.d, and arrows denote the times when nutrition was altered.

Table A2.5. Adjusted r^2 values and slope (in parentheses) of the linear relationships (P<0.001) between estimated changes in fibre diameter (FD)† along staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition .

Characteristic	FD_{min} (μm)	$^{A}FD_{range}$ (μm)	$^{A}FD_{stdev}$ (μm)	$^{A}FD_{cv}$ (%)	ΔFD_{dec} (μm/mm)	ΔFD_{inc} (μm/mm)
WGR_{min} (g/day)	0.62 (+)	0.47 (-)	0.49 (-)	0.61 (-)	0.52 (-)	0.52 (-)
FD_{min} (μm)		0.35 (-)	0.34 (-)	0.58 (-)	0.31 (-)	0.38 (-)
$^{A}FD_{range}$ (μm)			0.94 (+)	0.88 (+)	0.54 (+)	0.65 (+)
$^{A}FD_{stdev}$ (μm)				0.93 (+)	0.59 (+)	0.73 (+)
$^{A}FD_{cv}$ (%)					0.58 (+)	0.72 (+)
ΔFD_{dec} (μm/mm)						0.44 (+)

† *Minimum wool growth rate (WGR_{min}); minimum (FD_{min}) and range ($^{A}FD_{range}$) in FD; along-fibre variation in diameter ($^{A}FD_{stdev}$ and $^{A}FD_{cv}$); rate of decrease (ΔFD_{dec}) and increase (ΔFD_{inc}) in FD*

Table A3.1. The proportion (%) of single fibres from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition that exhibited various key parameters used to characterise their stress-strain behaviour (n_{sheep} = 40, $n_{fibres} \approx$ 4000). The range between individual sheep are shown in parentheses.

a, b: different superscripts within selection flock or nutrition comparisons differ at P< 0.05

Stress-strain property	Selection flock		Nutritional treatment		
	"sound"	"tender"	LWM	LWL_1	LWL_2
Extension > 3-4%	99^a	98^a	99^a (91-100)	99^a (98-100)	98^a (91-100)
Extension >15%	95^a	94^a	95^{ab} (78-100)	96^a (90-99)	91^b (77-98)
Defined post-yield region	40^a	37^a	60^a (40-83)	34^b (6-71)	25^b (6-59)

Table A3.3. Average coefficient of variation in stress-strain properties (%) between fibres with different fracture types. The fibres were from sheep from the "sound" and "tender" staple strength selection flocks and maintained under three levels of nutrition.

a, b: different superscripts differ at P< 0.05

Stress-strain property	Break type(s)		
	All fibres	Clean & step	Clean only
Young's modulus (GPa)	28.2^{a}	26.6^{ab}	26.2^{b}
Yield stress (MPa)	22.8^{a}	21.7^{a}	21.4^{a}
Stress at 15% extension (MPa)	22.6^{a}	21.4^{ab}	21.1^{b}
Intrinsic strength (MPa)	23.0^{a}	21.0^{b}	19.9^{b}
Extension at break (%)	27.9^{a}	27.0^{a}	25.9^{a}
Work to break (MPa)	34.6^{a}	33.0^{a}	31.5^{a}

Table A4.1*a*. Correlation matrix (adjusted r^2 values) for linear regressions between sulphur content (%) and amino acid composition (residues %) of mid-side wool samples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition ($n_{samples}$ = 160).

Amino acid	1/2-Cys	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phl	Lys	His	Arg
Sulphur	0.95 (+)	0.77 (-)	0.68 (+)	0.30 (+)	0.08 (-)	0.66 (+)	0.37 (-)	0.61 (-)	0.06 (-)	0.23 (-)	0.88 (-)	0.18 (-)	0.59 (-)	0.34 (-)	n.s.	0.15 (+)
1/2-Cys		0.80 (-)	0.66 (+)	0.33 (+)	n.s.	0.63 (+)	0.35 (-)	0.63 (-)	n.s.	0.25 (-)	0.86 (-)	0.18 (-)	0.60 (-)	0.39 (-)	n.s.	0.15 (+)
Asp			0.65 (-)	0.39 (-)	0.13 (+)	0.58 (-)	0.20 (+)	0.70 (+)	0.14 (+)	0.31 (+)	0.85 (+)	n.s.	0.41 (+)	0.45 (+)	n.s.	0.15 (-)
Thr				0.39 (+)	n.s.	0.53 (+)	0.42 (-)	0.49 (-)	n.s.	0.11 (-)	0.72 (-)	0.29 (-)	0.54 (-)	0.42 (-)	n.s.	0.18 (+)
Ser					n.s.	0.17 (+)	0.10 (-)	0.30 (-)	0.06 (-)	0.20 (-)	0.34 (-)	0.08 (-)	0.24 (-)	0.29 (-)	n.s.	0.14 (-)
Glu						n.s.	0.31 (-)	0.24 (+)	0.38 (+)	0.09 (+)	0.19 (+)	0.39 (-)	n.s.	n.s.	0.14 (-)	n.s.
Pro							0.48 (-)	0.47 (-)	n.s.	0.14 (-)	0.67 (-)	0.24 (-)	0.43 (-)	0.27 (-)	0.17 (-)	n.s.
Gly								n.s.	0.24 (-)	n.s.	0.28 (+)	0.78 (+)	0.43 (+)	0.07 (+)	0.13 (+)	0.20 (-)

Table A4.1*b*. Correlation matrix (adjusted r^2 values) for linear regressions between sulphur content (%) and amino acid composition (residues %) of mid-side wool samples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition ($n_{samples}$ = 160).

Amino acid	1/2-Cys	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phl	Lys	His	Arg
Ala									0.29 (+)	0.37 (+)	0.72 (+)	n.s.	0.17 (+)	0.40 (+)	n.s.	n.s.
Val										0.30 (+)	0.21 (+)	0.36 (-)	n.s.	n.s.	0.11 (-)	n.s.
Ile											0.45 (+)	0.10 (-)	0.07 (+)	0.12 (+)	n.s.	0.17 (+)
Leu												0.12 (+)	0.48 (+)	0.35 (+)	n.s.	0.15 (-)
Tyr													0.40 (+)	n.s.	0.07 (+)	0.17 (-)
Phl														0.20 (+)	0.13 (+)	n.s.
Lys															0.16 (+)	n.s.
His																0.13 (-)

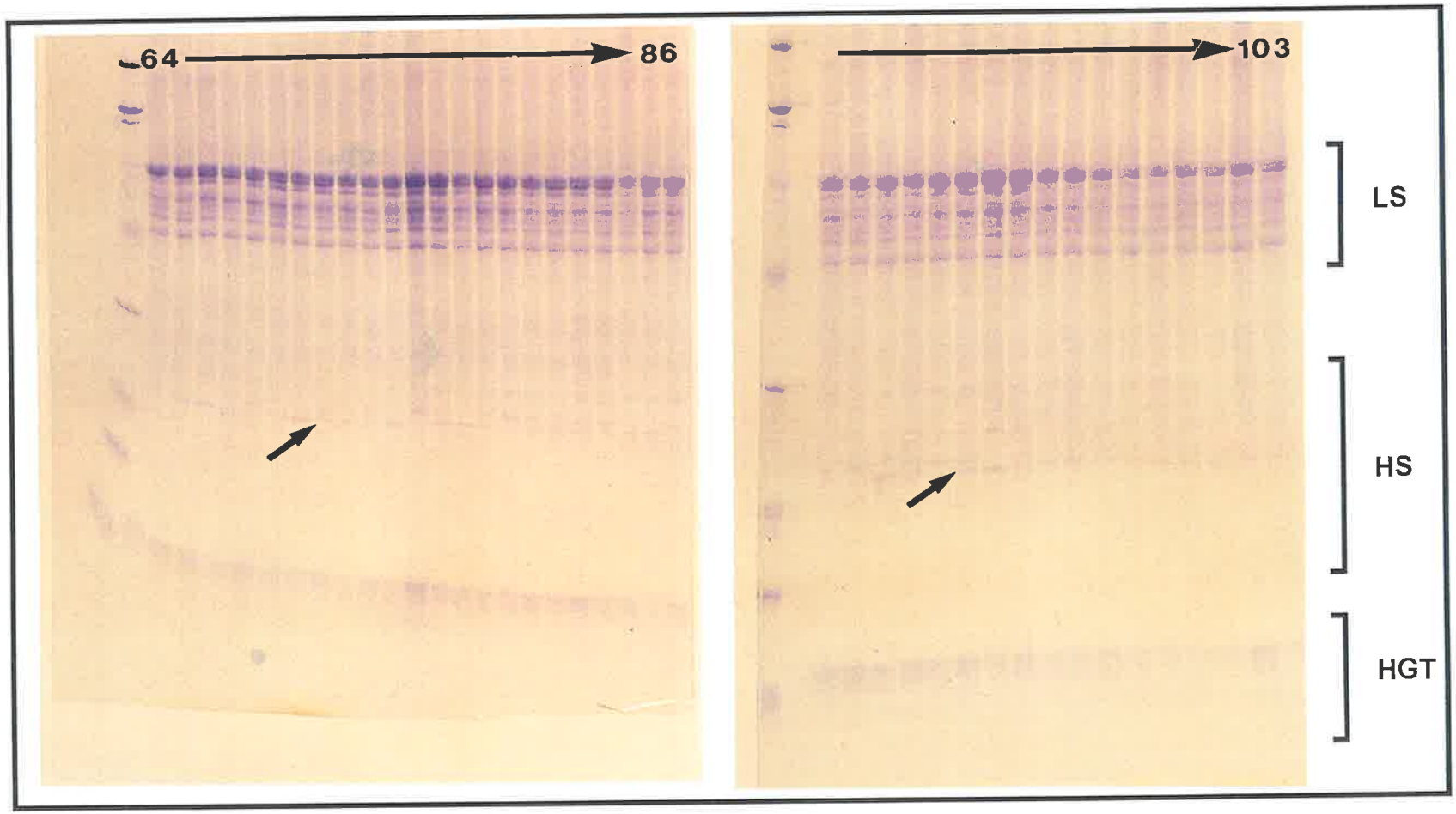

Plate A4.1. Coomassie-blue stained 1D-PAGE patterns of proteins extracted from wool samples differing in intrinsic fibre strength by up to 44%. The average intrinsic strength of the samples are shown in Table A4.2 of the Appendix. The wool samples were cut from the broken tip-end (< 2mm) of staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition, and about 5 μg of extracted protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated, and the arrow denotes the only obvious qualitative difference between the one-dimensional protein patterns.

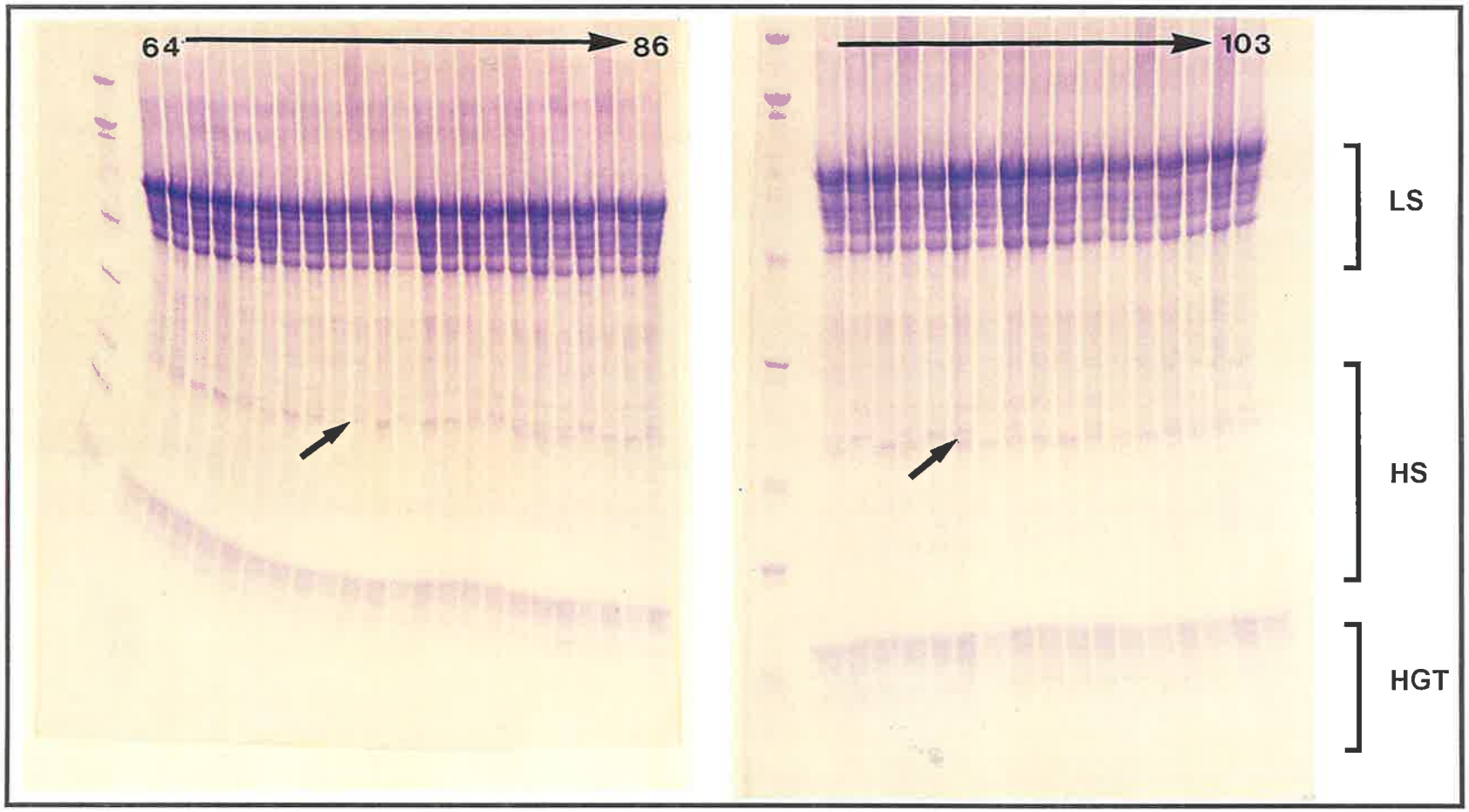

Plate A4.2. Coomassie-blue stained 1D-PAGE patterns of proteins extracted from wool samples differing in intrinsic fibre strength by up to 44%. The average intrinsic strength of the samples are shown in Table A4.2 of the Appendix. The wool samples were cut from the broken tip-end (< 2mm) of staples from sheep from the "sound" and "tender" staple strength selection flocks at three levels of nutrition, and about 15 μg of extracted protein from each sample was loaded onto a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel, and single dimension electrophoresis was run for 4 h. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated, and the arrow denotes the only obvious qualitative difference between the one-dimensional protein patterns.

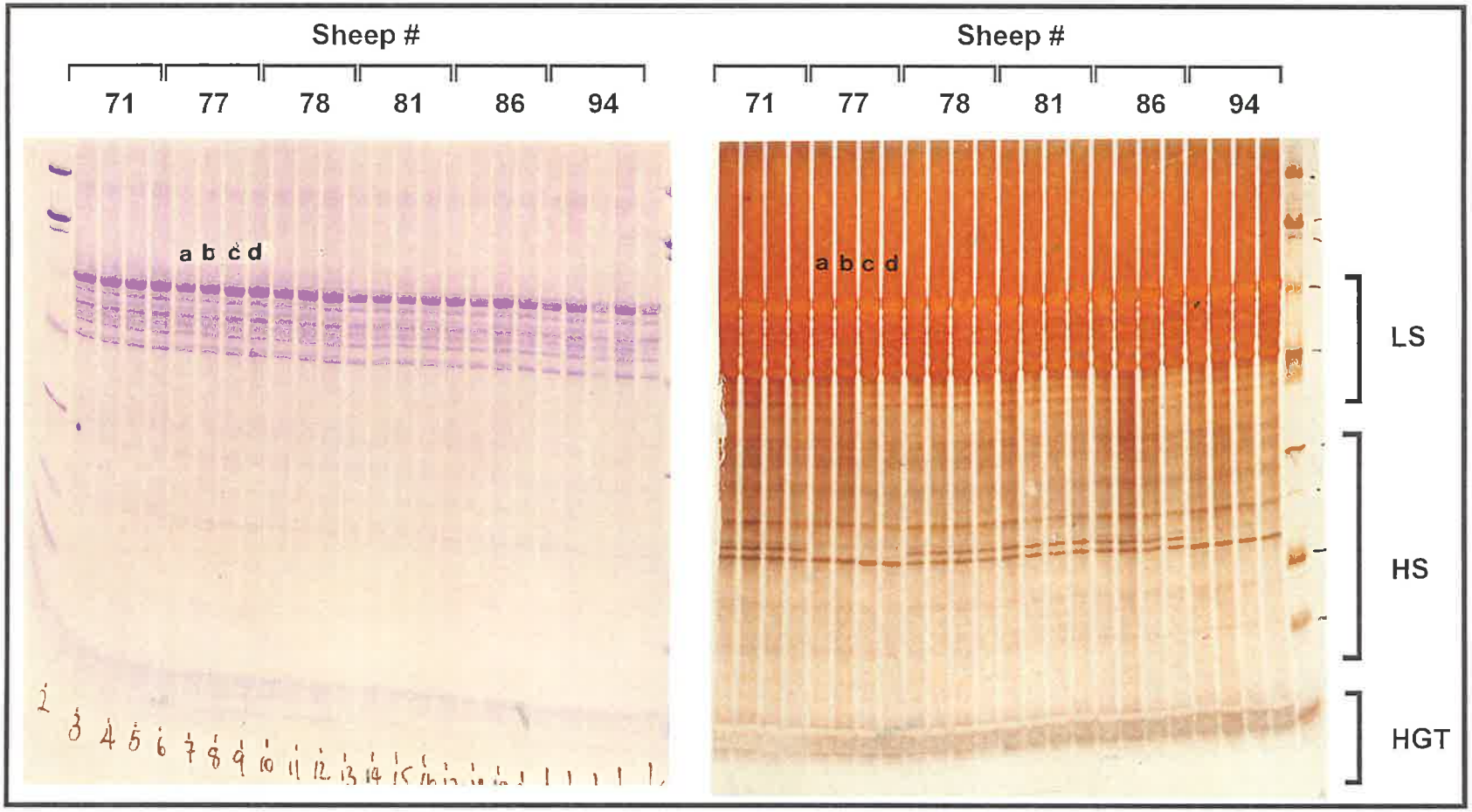

Plate A4.3. (*a*) Coomassie-blue and (*b*) Silver-stained 1D-PAGE patterns of proteins extracted from wool samples cut from different parts of broken staples from six different sheep; (*a*) < 10 mm from broken tip end; (*b*) < 2 mm from broken tip end; (*c*) < 2 mm from broken butt-end, and (*d*) < 10 mm from broken butt-end. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated. Approximately 5 μg and 1.0 μg of protein from each sample was loaded onto the Commassie and silver-stained gels, respectively. Single dimension electrophoresis were run in pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gels for 4 h.

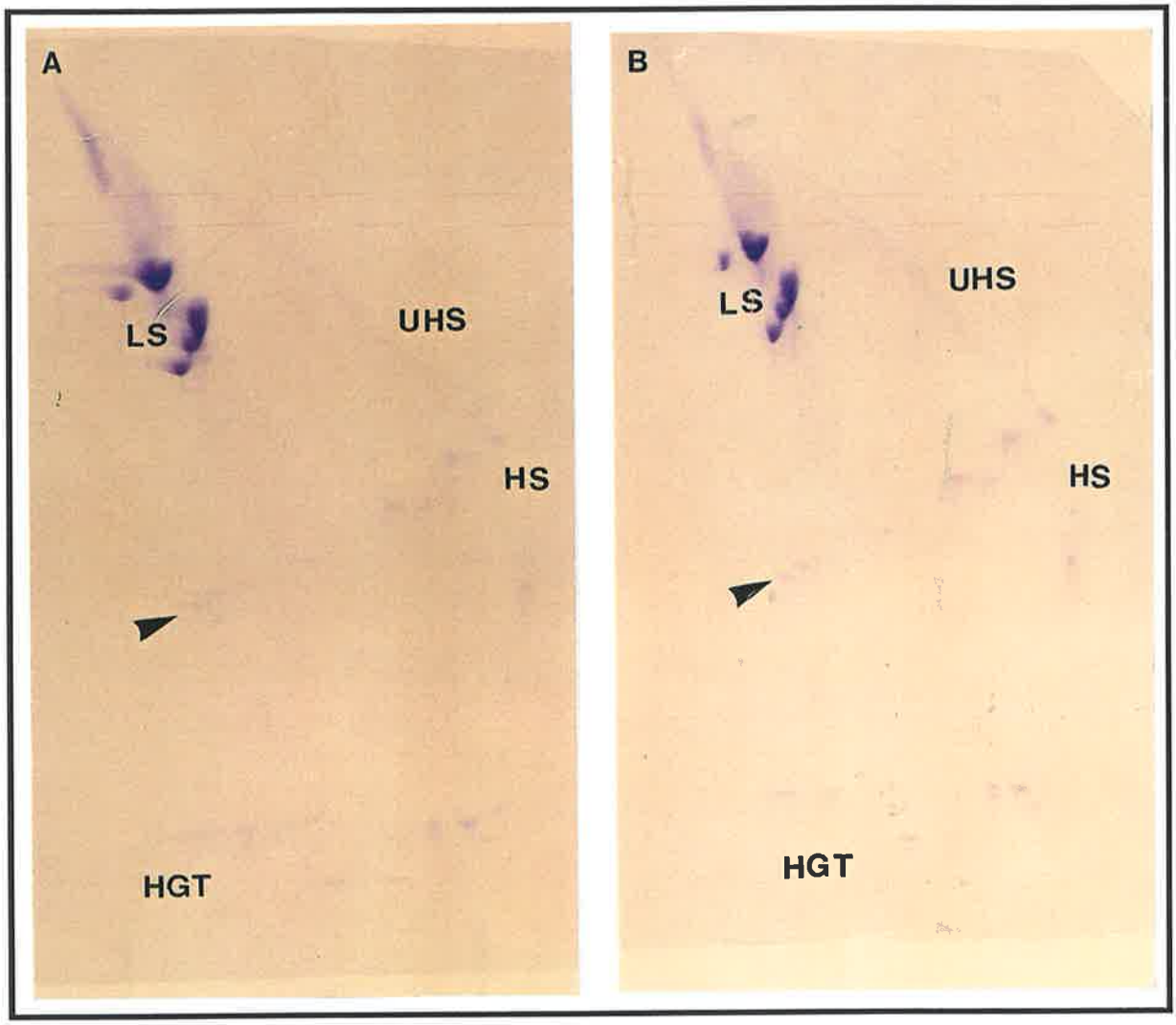

Plate A4.4. Coomassie-blue stained 2D-PAGE patterns of proteins extracted from wool samples cut from different parts of the same staple from a single sheep (#72); (*a*) < 2 mm from broken tip end; and (*b*) < 2 mm from broken butt-end. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated. No detectable differences between the tip and butt samples were evident, including the HS protein bands (MW ≈ 25 kD) (arrow heads). The proteins were separated in the first dimension (horizontal) in a discontinuous polyacrylamide gel (stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9) and in the second dimension (vertical) in a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel.

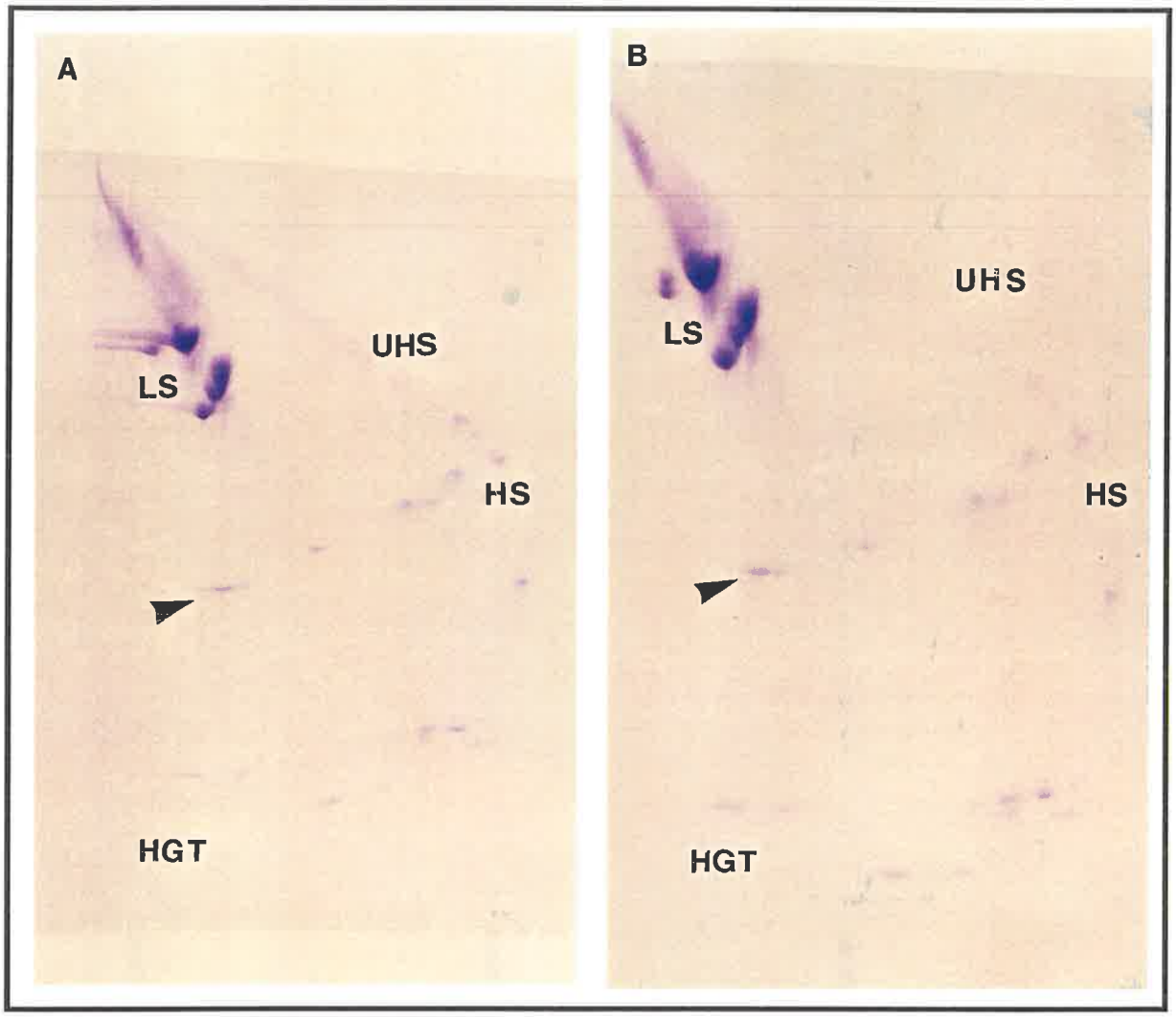

Plate A4.5. Coomassie-blue stained 2D-PAGE patterns of proteins extracted from wool samples cut from different parts of the same staple from a single sheep (#91); (*a*) < 2 mm from broken tip end; and (*b*) < 2 mm from broken butt-end. The location of low sulphur (LS), high sulphur (HS) and high glycine-tyrosine (HGT) keratin protein families are indicated. No detectable differences between the tip and butt samples were evident, including the HS protein bands (MW ≈ 25 kD) (arrow heads). The proteins were separated in the first dimension (horizontal) in a discontinuous polyacrylamide gel (stacking gel of 4% acrylamide, 8 M urea, 0.125 M Tris at pH 6.5, and a resolving gel of 7.5% acrylamide, 8 M urea, 0.375 M Tris at pH 8.9) and in the second dimension (vertical) in a pre-poured 0.5 mm 12-14% acrylamide, 0.1% SDS gel.

Table A4.2*a*. Cross-sectional area (CSA) at the point of break, force to break and intrinsic strength for individual sheep from the"sound" and "tender" staple strength selection flocks and three nutritional treatments. The values are the average of 100 single fibres per sheep.

Sheep tag number	Selection flock	Nutritional treatment	CSA at point of break (μm^2)	Force to break (gf)	Intrinsic strength (MPa)
64	"sound"	LWL_2	150	3.50	231
65	"tender"	LWL_1	143	2.91	200
66	"tender"	LWL_1	118	2.37	199
67	"sound"	LWL_1	122	2.81	227
68	"sound"	LWL_1	107	2.54	233
69	"sound"	LWL_2	146	3.07	208
70	"tender"	LWL_2	114	2.71	233
71	"tender"	LWL_1	127	2.99	232
72	"tender"	LWM	300	5.19	170
73	"sound"	LWM	325	5.40	163
74	"tender"	LWM	186	3.79	200
75	"sound"	LWL_2	107	2.55	235
76	"sound"	LWM	237	4.98	207
77	"sound"	LWL_1	109	2.56	232
78	"tender"	LWL_2	88	1.88	211
79	"tender"	LWL_1	103	2.31	222
80	"tender"	LWL_1	126	2.56	200
81	"sound"	LWL_1	124	2.65	210
82	"sound"	LWL_2	160	3.24	200
83	"sound"	LWL_2	120	2.50	202

Table A4.2*b*. Cross-sectional area (CSA) at the point of break, force to break and intrinsic strength for individual sheep from the"sound" and "tender" staple strength selection flocks and three nutritional treatments. The values are the average of 100 single fibres per sheep.

Sheep tag number	Selection flock	Nutritional treatment	CSA at point of break (μm^2)	Force to break (gf)	Intrinsic strength (MPa)
84	"tender"	LWL_2	120	2.50	202
85	"tender"	LWM	164	3.25	195
86	"sound"	LWM	149	3.41	225
87	"tender"	LWL_2	229	4.19	181
88	"tender"	LWL_2	144	3.12	214
89	"tender"	LWL_2	116	2.39	202
90	"sound"	LWL_1	174	3.87	218
91	"sound"	LWL_2	89	2.08	231
92	"sound"	LWM	190	3.49	182
93	"tender"	LWM	245	4.33	175
94	"tender"	LWL_2	118	2.46	206
95	"sound"	LWL_2	102	2.12	208
96	"sound"	LWL_1	184	4.02	215
97	"tender"	LWL_1	106	2.26	210
98	"tender"	LWL_2	107	2.28	210
99	"tender"	LWM	167	3.61	214
100	"sound"	LWM	206	4.33	207
101	"tender"	LWL_1	105	2.22	214
102	"sound"	LWL_2	183	3.60	194
103	"sound"	LWL_1	158	3.54	221

Table A4.3. Overall mean, and the standard deviation of variation and calculated least significant difference (L.S.D. $P = 0.05$, % mean in parentheses) in denaturation temperatures and enthalpy between replicates of the same wool sample. The samples were heated in 150% (v/w) water at 5°C/min from 50°C to 200°C. The wool samples were from sheep managed under three levels of nutrition and were clipped from the mid-side patch at the time corresponding to the position of break along the staple.

Thermal characteristic	Mean	Standard deviation	L.S.D.
Denaturation temperatures (°C)			
Start (T_1)	120.3	1.71	5.63 (4.68)
Peak (T_m)	141.5	1.91	6.28 (4.44)
End (T_2)	151.3	1.74	5.75 (3.80)
Range (T_2-T_1)	31.1	1.84	6.08 (19.58)
Denaturation enthalpy (ΔH; J/g)			
Linear baseline	17.8	1.22	4.03 (22.65)
Sigmoid baseline	17.7	0.93	3.06 (17.30)

Table A4.4. Sulphur content (%) and amino acid composition (residues %) of wool samples with different shape DSC thermograms. The wool samples were from sheep managed under three levels of nutrition and were clipped from the mid-side patch at the time corresponding to the position of break along the staple.

a, b, c: different superscripts differ at P< 0.05

Amino acid	Thermogram shape		
	Single	Shoulder	Bimodal
Sulphur	2.60 ^{a}	2.76 ^{b}	2.91 ^{c}
1/2-Cystine	9.7 ^{a}	10.2 ^{b}	10.7 ^{c}
Aspartic acid	6.8 ^{a}	6.6 ^{b}	6.4 ^{c}
Threonine	6.3 ^{a}	6.5 ^{b}	6.7 ^{c}
Serine	12.0 ^{a}	12.2 ^{b}	12.4 ^{c}
Glutamic acid	12.3 ^{a}	12.2 ^{ab}	12.2 ^{b}
Proline	6.2 ^{a}	6.5 ^{ab}	6.6 ^{b}
Glycine	9.4 ^{ab}	9.5 ^{a}	9.3 ^{b}
Alanine	5.7 ^{a}	5.5 ^{ab}	5.4 ^{b}
Valine	5.8 ^{a}	5.7 ^{b}	5.7 ^{b}
Iso Leucine	3.2 ^{a}	3.2 ^{b}	3.2 ^{b}
Leucine	8.3 ^{a}	8.1 ^{b}	7.9 ^{c}
Tyrosine	4.4 ^{a}	4.4 ^{a}	4.4 ^{a}
Phenylalanine	3.0 ^{a}	2.9 ^{b}	2.8 ^{c}
Lysine	3.2 ^{a}	3.1 ^{b}	3.0 ^{c}
Histidine	0.9 ^{a}	0.9 ^{a}	0.9 ^{a}
Arginine	7.1 ^{a}	7.0 ^{b}	7.1 ^{a}

Table A4.5. Correlation coefficients (adjusted r^2 value), level of significance and slope (in parentheses of the linear relationships between the composition of individual amino acids (residues %) and thermal characteristics of wool samples (n = 21). The wool samples were from sheep managed under three levels of nutrition and were clipped from the mid-side patch at the time corresponding to the position of break along the staple.

Amino acid	Thermal characteristic			
	T_1 (°C)	T_m (°C)	T_2 (°C)	ΔH (J/g)
1/2-Cystine	0.26**(+)	n.s.	n.s.	n.s.
Aspartic acid	0.42***(-)	n.s.	0.17*(-)	n.s.
Threonine	0.23**(+)	n.s.	n.s.	0.32**(+)
Serine	n.s.	n.s.	n.s.	0.39**(+)
Glutamic acid	n.s.	0.28**(-)	0.14*(-)	n.s.
Proline	0.36**(+)	n.s.	n.s.	n.s.
Glycine	n.s.	n.s.	n.s.	n.s.
Alanine	0.20*(-)	n.s.	0.17*(-)	0.25**(-)
Valine	n.s.	n.s.	n.s.	n.s.
Iso Leucine	n.s.	n.s.	0.17*(-)	n.s.
Leucine	0.43***(-)	n.s.	0.20*(-)	n.s.
Tyrosine	n.s.	n.s.	n.s.	n.s.
Phenylalanine	n.s.	n.s.	n.s.	n.s.
Lysine	n.s.	n.s.	n.s.	0.43***(-)
Histidine	n.s.	n.s.	n.s.	n.s.
Arginine	n.s.	n.s.	n.s.	n.s.

*n.s. = not significant (P>0.05), * = P<0.05, ** = P<0.01, *** = P<0.001*

Table A4.6. Calculated composition of paracortical cells assuming that the composition of orthocortical cells was constant irrespective of nutritional conditions[Ψ]. The wool samples were from sheep managed under three levels of nutrition (n = 7) and were clipped from the mid-side patch at the time corresponding to the position of break along the staple.

a, b, c: different superscripts differ at P< 0.05

Amino acid	Orthocortex (residues %)	Paracortex (residues %)		
		LWM	LWL_1	LWL_2
1/2-Cystine	10.3^a	10.4^a	8.7^b	8.5^b
Aspartic acid	6.7^a	6.3^a	6.6^a	6.8^a
Threonine	6.1^a	7.6^b	7.2^c	6.9^c
Serine	10.2^a	16.6^b	17.1^b	16.6^b
Glutamic acid	12.1^a	13.1^b	12.3^c	12.6^c
Proline	6.3^a	7.2^b	6.1^a	6.3^a
Glycine	8.6^a	10.6^b	11.9^c	12.0^c
Alanine	5.6^a	5.5^a	5.6^a	5.6^a
Valine	5.7^a	5.9^b	5.7^a	5.7^a
Iso Leucine	3.2^a	3.2^a	3.2^a	3.2^a
Leucine	8.4^a	7.6^b	7.6^b	7.9^c
Tyrosine	3.4^a	6.2^b	7.2^c	7.2^c
Phenylalanine	2.7^a	3.3^b	3.9^c	3.7^c
Lysine	2.8^a	3.7^b	4.0^b	4.1^b
Histidine	0.7^a	1.3^b	1.5^c	1.4^c
Arginine	6.8^a	7.2^a	8.0^b	7.6^b

[Ψ] The assumed composition of the orthocortical cells is the average of the values reported by Levau (1959), Chapman and Bradbury (1968) and Kulkarni *et al.* (1971).

Plate A5.1. Transmission electron micrographs of Merino wool fibre cross-sections at high magnification (72000x) showing the different macrofibril definition and microfibril packing arrangements between; (a) orthocortical (Or), (b) paracortical (Pa) and (c) mesocortical (Me) cells. The micrographs clearly show the characteristic whorl-like arrangement of ortho-cortical microfibrils (arrow head), the random microfibril packing in paracortical cells, and the hexagonally packed microfibrils in meso-cortical cells. Para-cells also included large areas where the microfibrils were arranged in a pseudo-hexagonal manner similar to that seen in mesocortical cells (arrow head). The cell membrane complex (cmc), nuclear remnant material and residual non-keratin cytoplasmic material (nr) are indicated. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 200 nm.

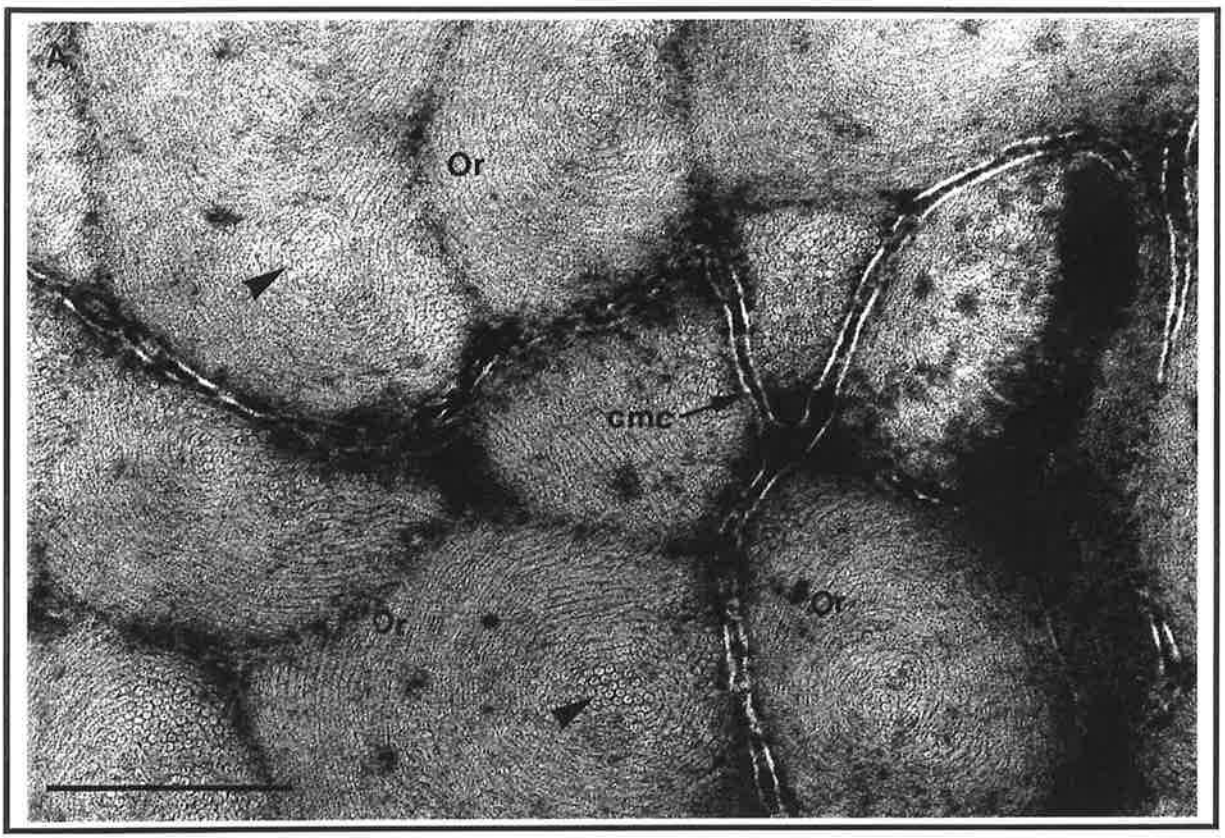

Plate A5.1*a*

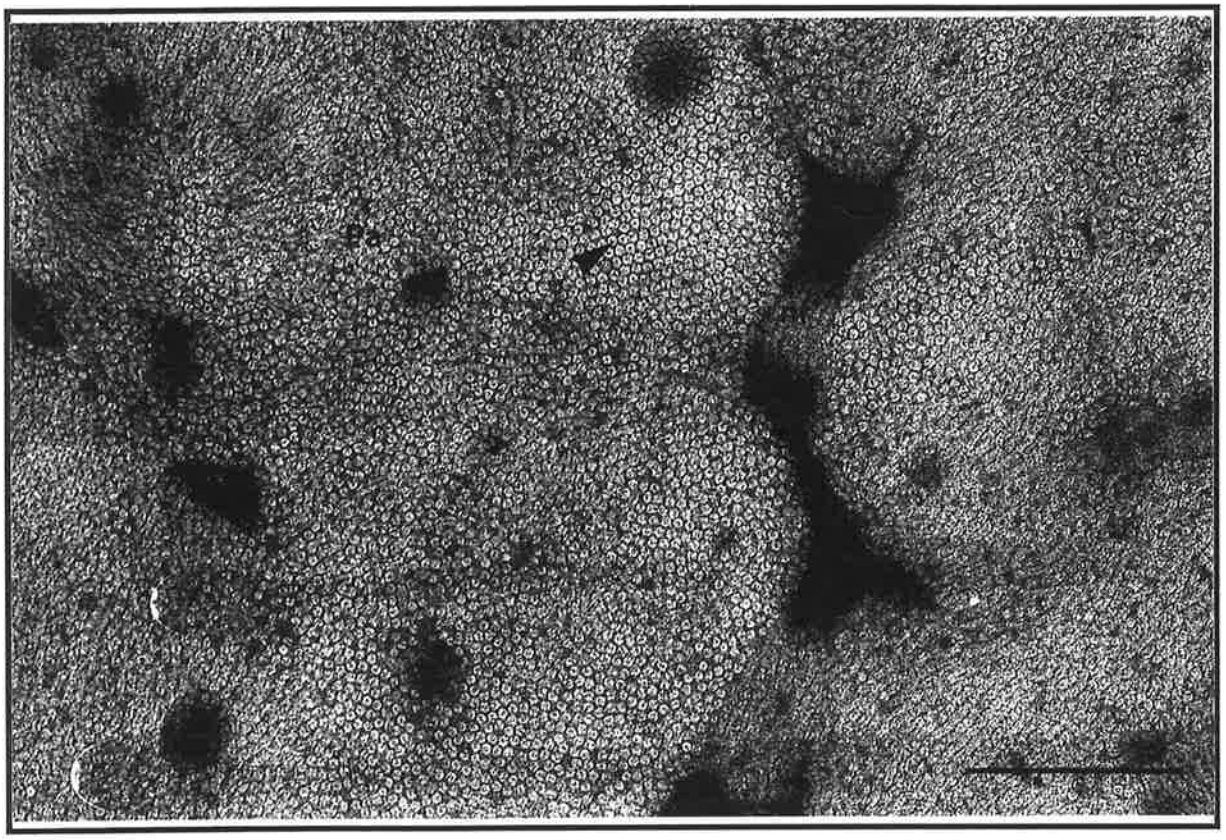

Plate A5.1*b*

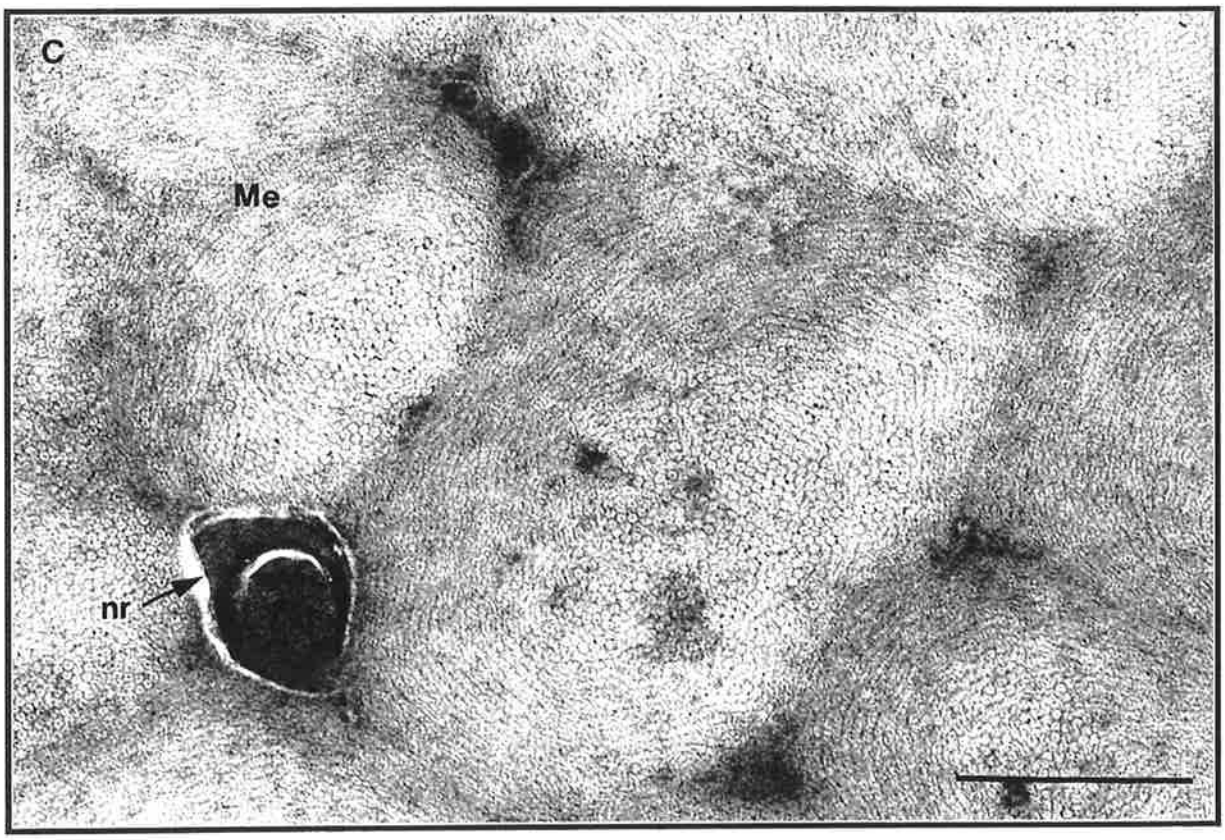

Plate A5.1*c*

Plate A5.2. Transmission electron micrographs of Merino wool fibre cross-sections at high magnification (72000x) showing the variation in microfibril packing density within and between; (a) different paracortical (Pa) cells; and (b) paracortical and mesocortical (Me) cells. The micrographs show areas in the paracortical cells where the microfibrils are arranged in a pseudo-hexagonal manner similar to that seen in mesocortical cells (arrow head). The cell membrane complex (cmc), nuclear remnant material and residual non-keratin cytoplasmic material (nr) are indicated. The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 200 nm.

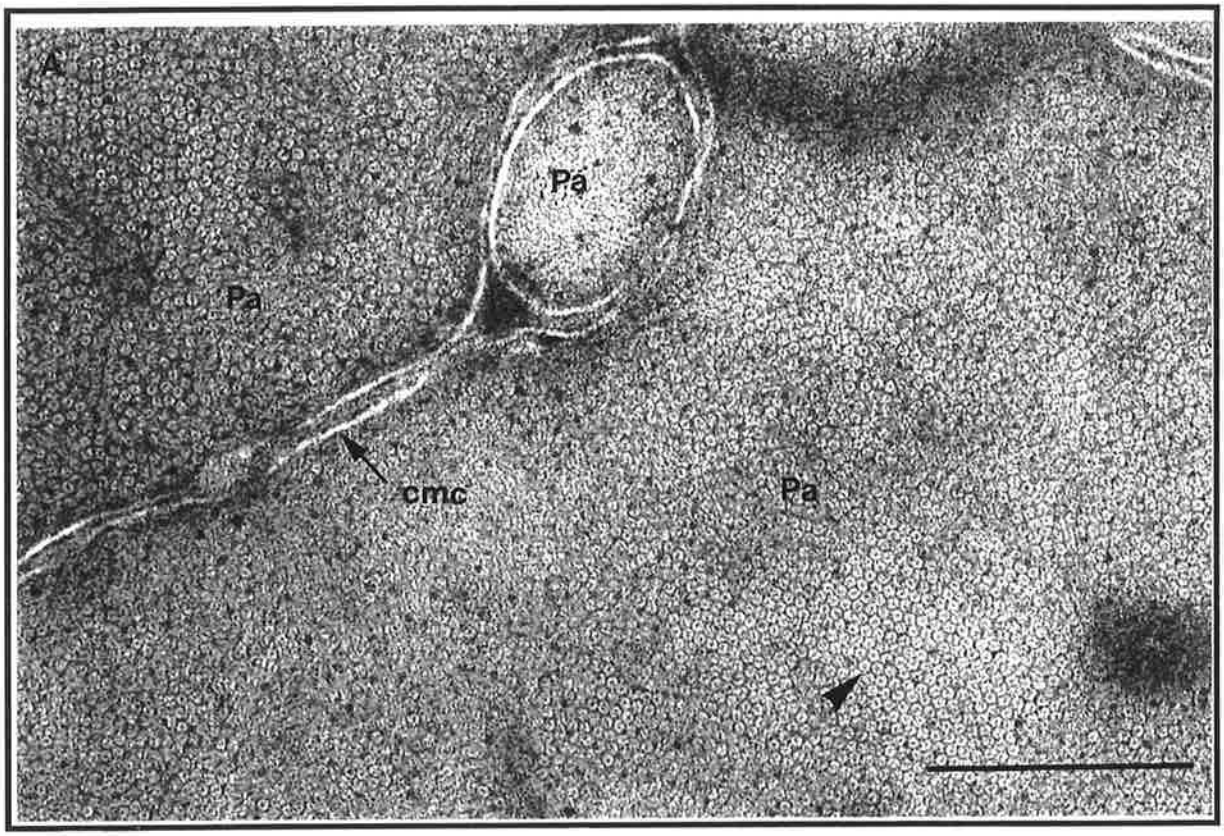

Plate A5.2*a*

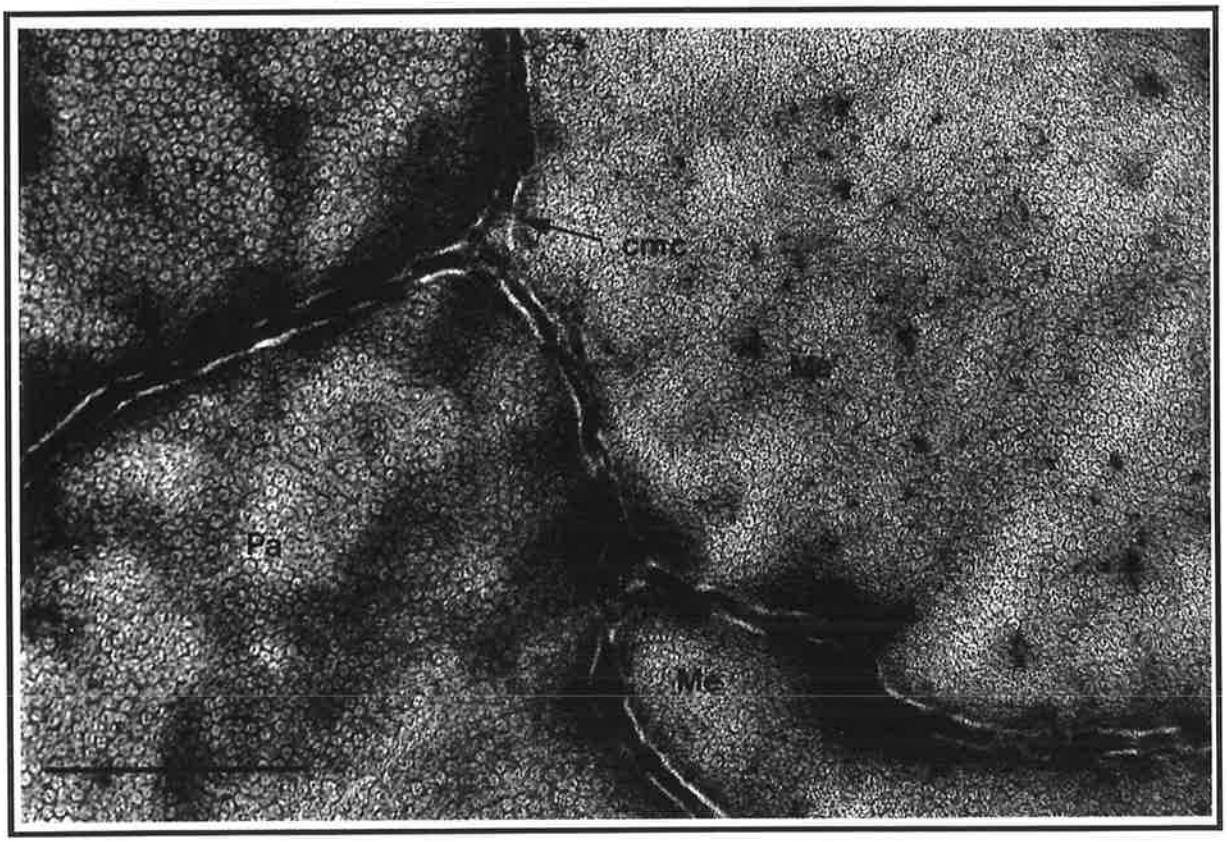

Plate A5.2*b*

Plate A5.3. Electron micrographs of a Merino wool fibre in transverse sections at very high magnification (up to 600000x) showing the apparent substructure of the microfibrils in (*a*) a paracortical cell (Pa); and (*b*) a mesocortical cell (Me). The fibres were reduced with thioglycollic acid and fixed with osmium tetroxide, and after sectioning, stained with uranyl acetate and lead citrate, as described by Rogers (1959*a*,*b*). Scale bar = 100 nm.

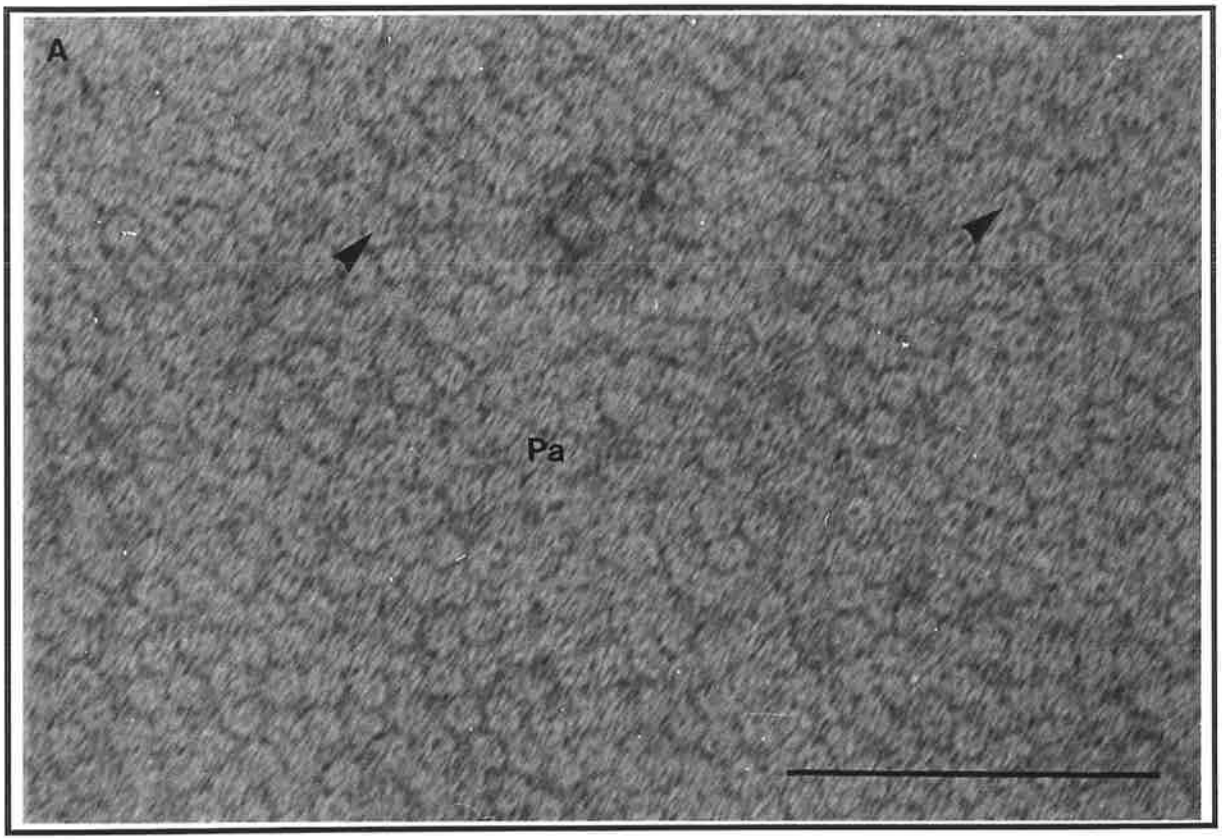

Plate A5.3*a*

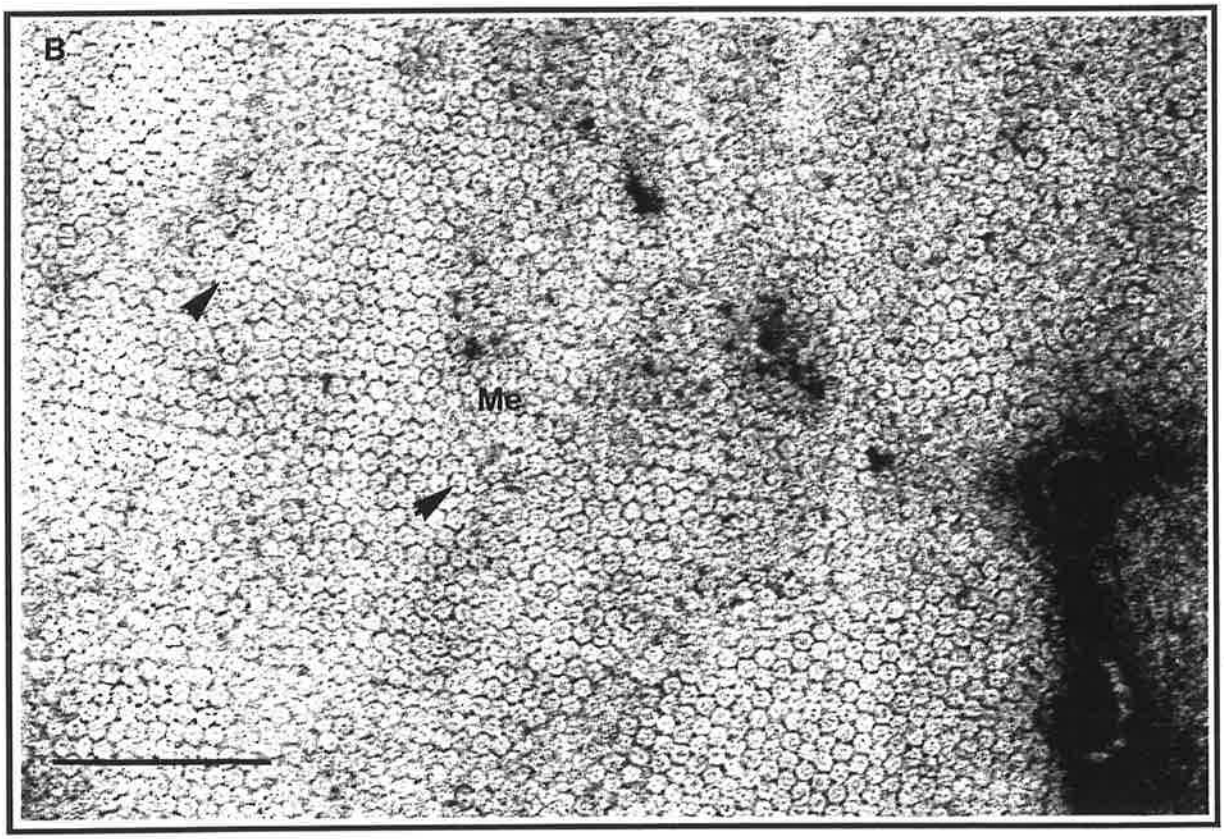

Plate A5.3*b*

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