

FOLLICLE SHUTDOWN AND WOOL

STAPLE STRENGTH



A thesis

submitted in fulfilment of the requirements for the degree of

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by

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TABLE OF CONTENTS

TITLE PAGE	i
TABLE OF CONTENTS	ii
ABSTRACT	vii
DECLARATION	ix
ACKNOWLEDGEMENTS	x
CHAPTER 1. LITERATURE REVIEW	1
1.1. THE IMPORTANCE OF STAPLE STRENGTH	1
1.2. MEASUREMENT OF STAPLE STRENGTH	4
1.3. DETERMINANTS OF STAPLE STRENGTH.....	7
1.3.1. The role of minimum fibre diameter in determining staple strength	8
1.3.2. The role of rate of change of diameter along a staple and between-fibre variance in fibre diameter in determining staple strength.....	11
1.3.3. The role of intrinsic strength in determining staple strength	14
1.3.4. The role of fibre crimp frequency in determining staple strength.....	14
1.4. FIBRE SHEDDING AND MOULTING.....	16
1.4.1. The effect of genotype on moulting.....	18
1.4.2. The effect of sex on moulting.....	18
1.4.3. The effect of nutrition on moulting.....	19
1.4.4. The effect of light on moulting	19
1.4.5. The effect of temperature on moulting	20
1.4.6. The effect of hormones on fibre shedding.....	21
1.4.7. Histological changes in the follicle and fibre when shedding occurs in response to hormones of hypothalamic-pituitary-adrenal axis	29
1.4.8. The effect of epidermal growth factor (EGF) on follicle shutdown and fibre shedding.....	30

1.5. SUMMARY OF LITERATURE REVIEW AND GENERAL THEME OF THE PROJECTS	31
CHAPTER 2. MATERIALS AND METHODS	34
2.1. <i>IN VIVO</i> EXPERIMENTS.....	34
2.1.1. Selection of animals	34
2.1.2. Feeding.....	34
2.1.3. Cortisol injection	35
2.1.4. Patch wool weight, fibre diameter and staple length	35
2.1.5. Skin sampling and staining	36
2.1.6. Blood sampling.....	39
2.1.7. Plasma cortisol assay	39
2.1.8. Follicle activity (morphology)	40
2.1.9. Statistical analysis	43
2.2. <i>IN VITRO</i> FOLLICLE CULTURE	43
2.2.1. Materials	44
2.2.1. Follicle isolation and length measurement.....	44
2.2.3. DNA synthesis in the cultured follicles	45
2.2.3. Statistical analysis	46
CHAPTER 3. CORTISOL-INDUCED FOLLICLE SHUTDOWN IS A MAJOR DETERMINANT OF STAPLE STRENGTH	47
3.1. INTRODUCTION.....	47
3.2. MATERIALS AND METHODS	49
3.2.1. Selection of animals	49
3.2.2. Design of the experiment	49
3.2.3. Cortisol injection	51
3.2.4. Staple strength measurement.....	52
3.2.5. Staple intrinsic strength measurement.....	53

3.2.6. Measurement of shed fibres.....	53
3.3. RESULTS	54
3.3.1. The effect of cortisol injection on feed intake and live weight	54
3.3.2. The effect of cortisol injection on plasma cortisol concentration	55
3.3.3. The effect of cortisol injection on wool production, staple length and fibre diameter	56
3.3.4. The effect of cortisol injection on the formation of wool break and fibre shedding	61
3.3.5. The effect of cortisol injection on follicle shutdown.....	70
3.3.6. The effect of cortisol injection on staple strength.....	75
3.3.7. The effect of cortisol injection on staple intrinsic strength	76
3.3.8. Relationships between follicle and fibre characteristics and follicle shutdown, fibre shedding and plasma cortisol concentration.....	76
3.3.9. Determinants of staple strength.....	81
3.4. DISCUSSION	82
3.4.1. Design and objectives of experiment	82
3.4.2. Basis of choosing the doses and duration of cortisol injection.....	83
3.4.3. The effect of cortisol injection on feed intake	84
3.4.4. The effect of cortisol injection on plasma cortisol concentration	84
3.4.5. The effect of cortisol injection on wool growth rate	85
3.4.6. The effect of cortisol injection on the formation of wool break and fibre shedding	87
3.4.7. Histological examination of shed fibres induced by cortisol injection	89
3.4.8. The effect of cortisol injection on follicle shutdown.....	91
3.4.9. The effect of cortisol injection on staple strength and staple intrinsic strength	94
3.4.10. Determinants of staple strength.....	96

CHAPTER 4. FLEECE PHENOTYPE INFLUENCES SUSCEPTIBILITY TO CORTISOL-INDUCED FOLLICLE SHUTDOWN IN MERINO SHEEP	97
4.1. INTRODUCTION.....	97
4.2. MATERIALS AND METHODS	98
4.2.1. Selection of animals	98
4.2.2. Design of the experiment	100
4.2.3. Cortisol injection	100
4.3. RESULTS	101
4.3.1. The effect of cortisol injection on feed intake and live weight.....	101
4.3.2. The effect of cortisol injection on plasma cortisol concentration.....	101
4.3.3. The effect of cortisol injection on wool production and fibre diameter.....	103
4.3.4. The effect of cortisol injection on the formation of wool break and fibre shedding	107
4.3.5. The effect of cortisol injection on follicle shutdown.....	109
4.4. DISCUSSION	112
4.4.1. Design and objectives of experiment	112
4.4.2. Basis of choosing the dose and duration of cortisol injection	113
4.4.3. The effect of cortisol injection on feed intake	113
4.4.4. The effect of cortisol injection on plasma cortisol concentration.....	114
4.4.5. The effect of cortisol injection on wool growth rate	115
4.4.6. The effect of cortisol injection on formation of wool break and fibre shedding	117
4.4.7. Histological examinations of shed fibres induced by cortisol injection.....	118
4.4.8. The effect of cortisol injection on follicle shutdown.....	118
 CHAPTER 5. EPIDERMAL GROWTH FACTOR, BUT NOT CORTISOL, SUPPRESSES FIBRE GROWTH IN CULTURED FOLLICLES.....	 121
5.1. INTRODUCTION.....	121
5.2. MATERIALS AND METHODS	122

5.2.1. Addition of cortisol and EGF to cultured follicles.....	122
5.3. RESULTS	123
5.3.1. Preliminary studies on the growth of follicles in culture	123
5.3.2. The effect of cortisol on the growth of follicles in culture.....	127
5.3.3. The effect of EGF on the growth of follicles in culture	129
5.4. DISCUSSION	133
5.4.1. Preliminary studies on the growth of follicles in culture	133
5.4.2. Design and objectives of experiment.	134
5.4.3. The effect of cortisol on the growth of follicles in culture.....	134
5.4.4. The effect of EGF on the growth of follicles in culture	135
CHAPTER 6. GENERAL DISCUSSION	139
6.1. ELEVATION IN PLASMA CORTISOL CONCENTRATION INHIBITS CELL DIVISION IN THE FOLLICLES.....	139
6.2. FOLLICLE SITES SENSITIVE TO INHIBITION OF CELL MITOSIS IN RESPONSE TO ELEVATION OF PLASMA CORTISOL CONCENTRATION	142
6.2.1. Impact of inhibition of cell mitosis on fibre.....	142
6.2.2. Impact of inhibition of cell mitosis on inner root sheath.....	147
6.2.3. Impact of inhibition of cell mitosis on outer root sheath.	148
6.3. APPLICATION OF RESULTS TO COMMERCIAL WOOL-PRODUCING ENTERPRISES	149
6.3.1. Tender wool problem and its impact on wool industry.....	149
6.3.2. Implication of results to development of defleecing agents.	151
6.4. GUIDELINES FOR FUTURE STUDIES.....	152
BIBLIOGRAPHY	154

ABSTRACT

Despite the importance of the low staple strength wool problem, little is known about its causes and prevention and most related studies in this area have concentrated on minimum fibre diameter, variability of fibre diameter and intrinsic strength. Insufficient attention has been paid to other characteristics and one area of particular relevance is whether follicle shutdown has a role in determining staple strength. In this thesis an attempt was made to elucidate the possible mechanisms responsible for, and associated with, follicle shutdown in relation to the occurrence of tender wool. Merino sheep were selected on the basis of either staple strength, or fibre diameter (and fibre diameter variability) and exposed to cortisol to generate sufficient variation in wool growth rate and follicle shutdown to identify possible determinants, mechanisms and cellular events involved in the production of weak wool.

The major determinants of staple strength were follicle shutdown, minimum fibre diameter and staple length. These factors together accounted for 65% of the variation in staple strength. Follicle shutdown accounted for 43% of the variation in midside staple strength ($r^2 = 0.43$, $P < 0.0001$). This study is the first to find a detailed quantitative relationship between follicle shutdown and staple strength and to study minimum fibre diameter concurrently with follicle shutdown and staple length. The relationship between minimum fibre diameter and staple strength did not explain as much of the variation as expected, probably because fibre diameter was not significantly altered by cortisol injection. So stress-induced low staple strength differs from normal low-nutrition effects on staple strength where minimum fibre diameter accounts for up to 60% of the variance (Hunter et al. 1983; Hansford and Kennedy, 1980).

Pronounced difference in susceptibility to shutdown was found not only between primary and secondary follicle types but also between the secondary follicles. To investigate whether the difference in susceptibility is breed related, Finewool and Strongwool sheep were selected and exposed to cortisol. Results indicated that a greater number of follicles stopped producing fibre in Strongwool sheep. This difference in susceptibility to cortisol-induced shutdown may be associated with blood flow rate to the skin, amount of EGF receptor site and the degree of follicle sensitivity.

It was suggested that high plasma cortisol concentration may be involved in wool follicle shutdown, but there is mounting evidence which suggests that other factors may also be involved in this process. Studies *in vitro* indicated that physiological and supraphysiological concentrations of cortisol had no inhibitory effect on cultured follicles, suggesting the *in vivo* effect of cortisol on follicles is induced or requires greater periods of time to operate than the culture system allows. Fibre growth was significantly inhibited when follicles were kept in low concentrations of EGF. It is possible that the cortisol response may be effected through EGF, but this remains to be tested.

DECLARATION

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

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

Hamid Reza Ansari-Renani

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CHAPTER 1.
LITERATURE REVIEW



CHAPTER 1. LITERATURE REVIEW

1.1. THE IMPORTANCE OF STAPLE STRENGTH

Wool fibre strength is an important raw wool characteristic (Hunter, 1990; de Jong et al. 1985), because it greatly influences wool processing properties such as length of the fibres in the top (hauteur) (Hunter et al. 1980). To prevent economic loss and increase income, textile manufacturers have to process fibres through their machinery as fast as possible. In the production of a wool top for spinning into worsted yarn, the raw wool is scoured and then carded. The carding machine takes the scoured and dried fibre and teases it out in parallel strands, at the same time removing most of the vegetable matter. It is during this process plus combing that wool fibres break and wool of low strength tend to break more often than wool of higher strength.

The lower the staple strength, the greater will be the fibre breakage during processing and the shorter will be the hauteur. Andrews and Lunney (1982) indicated that for average staple strength below about 45 N/ktex, there is a significant relationship between hauteur and average staple strength. Hauteur is greatly influenced by the position at which the staple breaks. If breakage occurs at the middle of staple, a short hauteur is expected, on the other hand if breakage occurs towards the ends of the staple, broken fibre will be wasted as noil (the shorter fibre left in carding and combing-Romaine). A formula has been developed in 1988 by AWC, CSIRO and AWTA Ltd to predict the hauteur, coefficient of variation of hauteur and noil, from measurement obtained from the greasy wool (Couchman et al. 1992). The formulae are as follows:

$$Ha = (0.52 \times L) + (0.47 \times S) + (0.95 \times D) - (0.45 \times V) - (0.19 \times M) - 3.5$$

$$CVHa = 0.12L - 0.415 - 0.35D + 0.20M + 49.3$$

$$Rom = -0.11L - 0.14S - 0.35D - 0.51VM - 0.086M + 27.7$$

Ha = Predicted hauteur

CVHa = Predicted coefficient of variation of hauteur

Rom = Predicted romaine

L = Consignment mean staple length (mm)

S = Consignment mean staple strength (N/ktex)

D = Consignment mean fibre diameter (μm)

V = Consignment mean vegetable matter base (%)

M = Consignment adjusted percentage of middle breaks (%)

It has been shown that staple strength is a major value-determining characteristic of Merino fleece wool (Figure 1.1), accounting for 21% of the observed variation in price (Couchman et al. 1992).

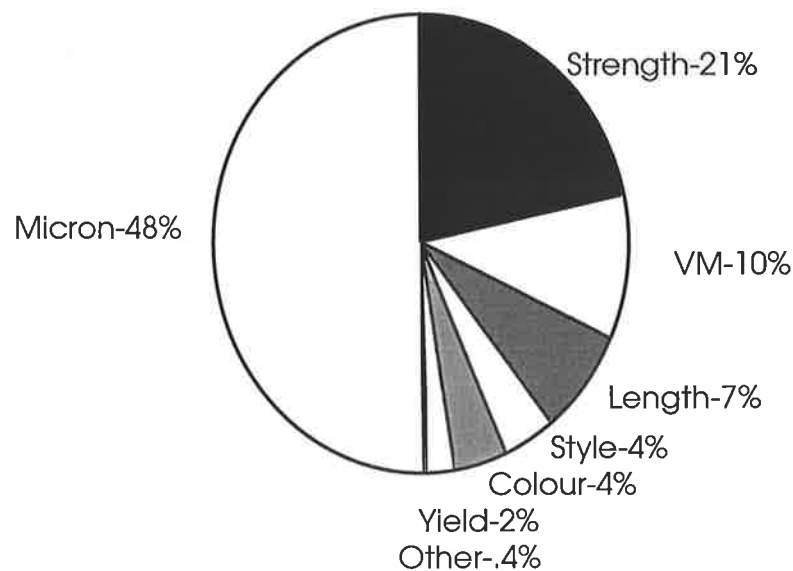


Figure 1.1. Relative contribution of raw wool attributes to prices paid at auction (adapted from Couchman et al. 1992).

Tender wool can be excluded from buying specifications of wool processors and if it is accepted, it is at a considerable price discount. The penalties received by lots at auction in 1992/94 were approximately 3.1% for part-tender wool, 7.5% for tender

wool and 9.7% for rotten wool (see section 1.2). This amounts to an estimated cost to the Australian wool industry of 16 c/kg clean for part-tender wool, 38 c/kg clean for tender wool and 50 c/kg clean for rotten wool (Harrowfield and Kelly, 1994). It is estimated that \$40 million is lost annually in Australia as a result of low wool staple strength. A recent survey of the 1991-1992 National Australian wool clip indicated that more than 25% of overall offerings were classified as tender with 37% wool at the Fremantle selling centre (Australian Farm Journal, 1992). Couchman et al. (1992) indicated that growers in Western Australia alone (with some 35 million sheep, producing some 20% of the national clip) could increase wool returns by some \$12 million per year if they could achieve a staple strength profiles similar to wool sold in Melbourne: The staple strength of wool sold in Melbourne is greater than Adelaide which is greater than Fremantle (Figure 1.2).

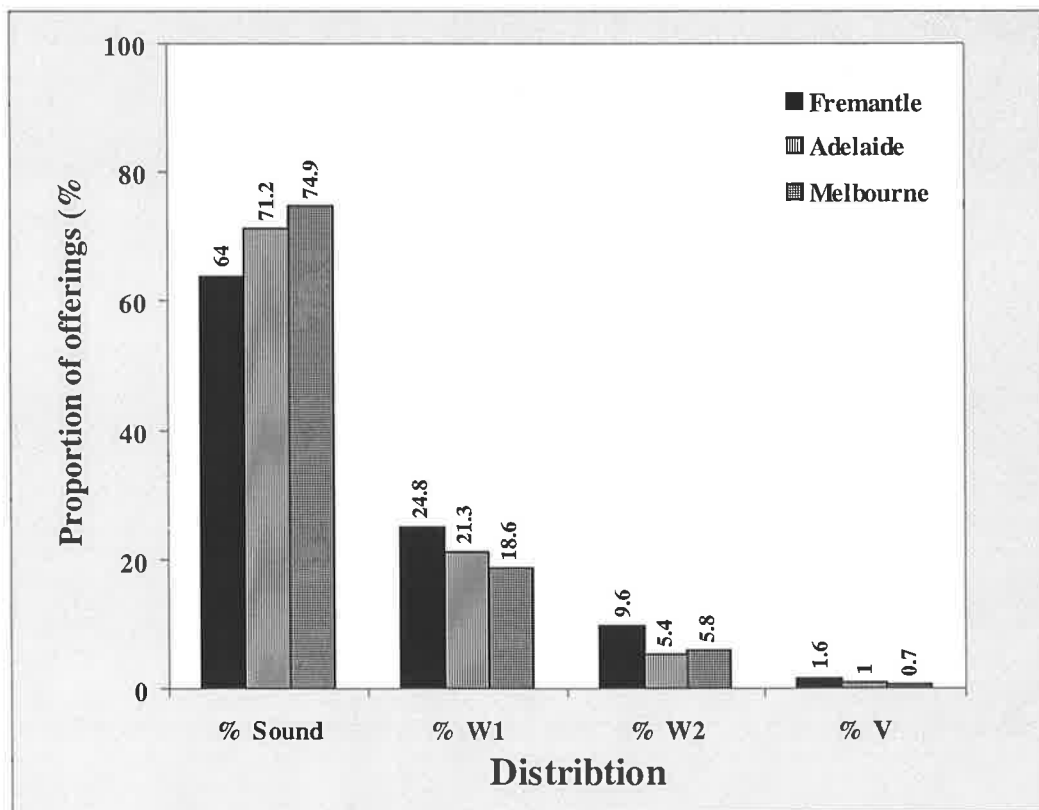


Figure 1.2. Distribution of tenderness fault-1991/1992 (adapted from Couchman, Hanson, Stott and Vlastuin, 1992).

The extent of importance of staple strength is further highlighted by Baker et al. (1993) who indicated that in South-Western Australia up to 60% or more of wool lots less than 22 μm are classified as tender or semi-tender (Figure 1.3). In this study data were collected from a company's (Elders Ltd) clients' sales and classers' specification of sale lots from every second sale during the 1990 calendar year.

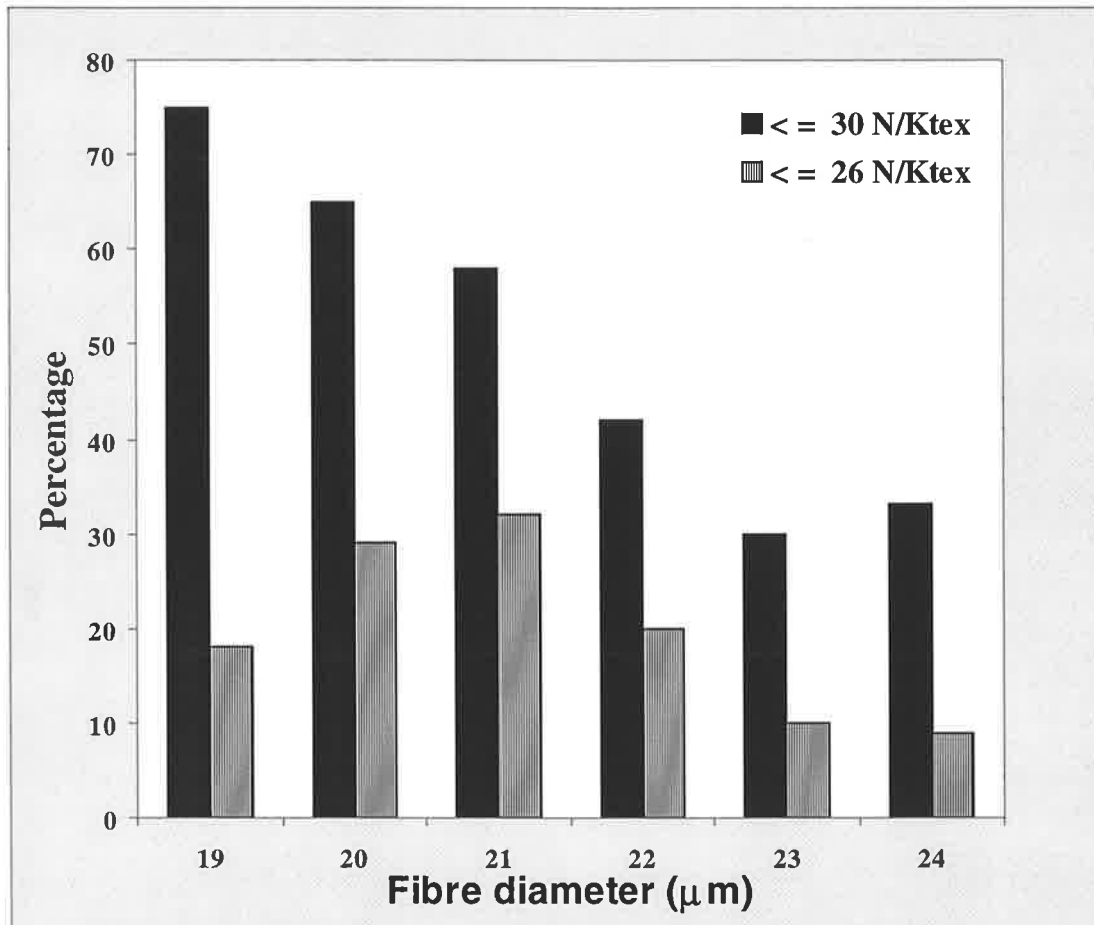


Figure 1.3. Percentage of clean wool that is tender (≤ 30 N/ktex and ≤ 26 N/ktex) (adapted from Baker, Purser, Foley and Wilton, 1992).

1.2. MEASUREMENT OF STAPLE STRENGTH

Traditionally the soundness of a staple is measured by the 'ring' of the staple or the pull required to break a staple. According to this method, staples are classified into two categories. A staple is said to be sound if it can not be broken with the

fingers and if broken without great effort it is considered to be tender. Using this method, it is only possible to measure wool of less than about 25 N/ktex (Heuer, 1979) yet there are varying degrees of staple strength ranging up to 100 N/ktex (Baird, 1984). While this system is cheap and handy, due to differences between classers in evaluating the soundness of wool, there was a need for an objective wool measurement method.

To measure the soundness of wool greater than 25 N/ktex, objective wool measurement techniques have been developed. Automatic ATLAS (automatic test for length and strength) devices were developed (Baird, 1984) and the Australian Wool Corporation commenced objective pre-sale staple strength testing. Wool is classified as sound (wool greater than 30 N/ktex), 'W¹' or part-tender (between 30 and 25 N/ktex), 'W²' or tender (between 24 and 18 N/ktex), and 'V' or rotten (17 N/ktex and below). In 1991/1992 approximately 40% of combing wool offered for sale in Australia was objectively measured for staple length and strength (Couchman et al. 1992).

To measure strength, staples must be conditioned first in a controlled atmosphere (20°C and 65% relative humidity) for 24 hours before testing. This is because the humidity of the air greatly influences physiochemical properties of wool fibres. Testing also must be done in a controlled atmosphere. Clamps are placed on each end of staple which is then broken on a staple breaker system. The unit of measurement of staple strength is grams or Newtons (1kg = 9.8N), or the total energy to break. The Newton is the accepted unit in Australia. However as staples between sheep and within sheep differ in length, diameter and weight, the only practical basis for comparing the strength of wool of different samples would be the strength per unit weight of staple (staple length divided by staple weight) which is expressed as Newton per kilotex (N/ktex). To ensure that staple strength is done properly, it is vital to keep the break of the wool within the clamps (Figure 1.4). As can be seen from the figure, the break of staple B which is considered to be tender is kept within the clamp.

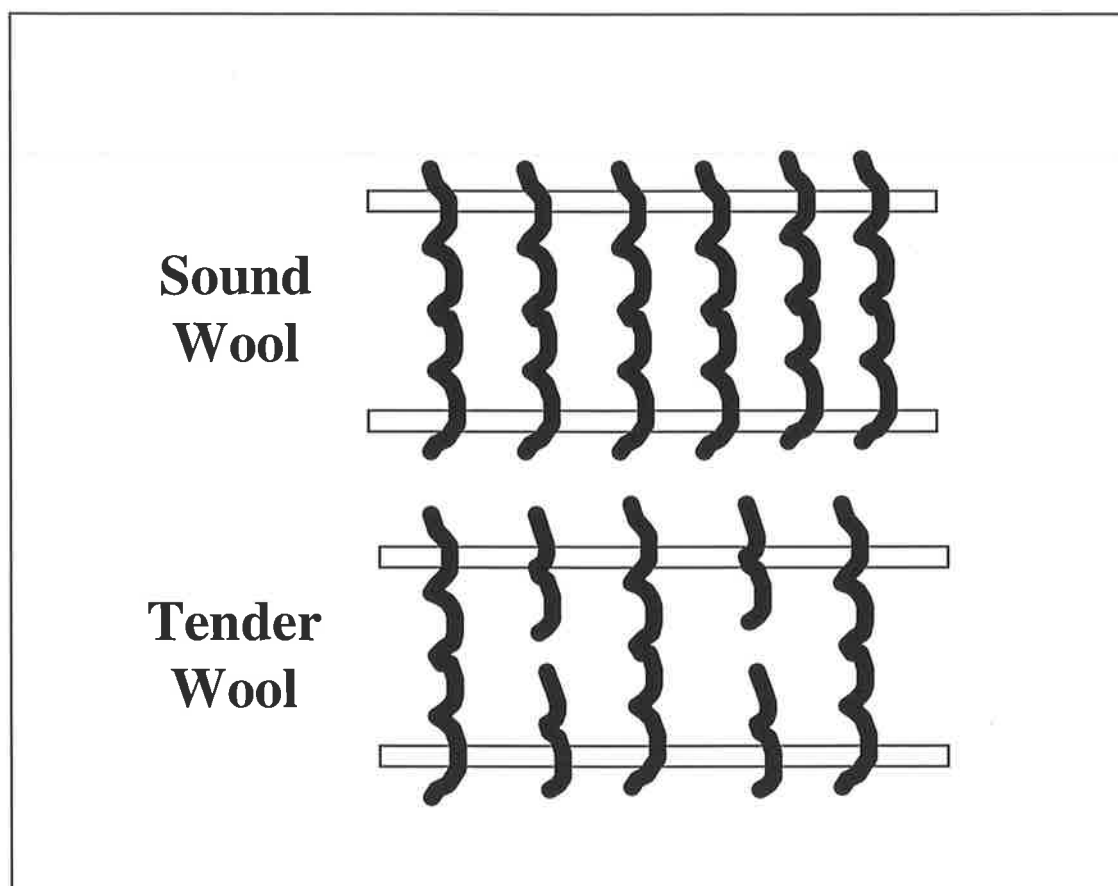


Figure 1.4. Diagrammatic view of sound and tender staples clamped for staple strength testing.

While the breaking graphs of sound staples show little variation in shape, as the staple strength decreases a much wider variety of breaking graphs is recorded. Figure 1.5 shows the stress/strain curves produced by sound staple A-DGH and tender staple B-DIK. As the load increases (DE) the crimp of the fibres (both sound and tender staples) is removed. Distance between DE, when the fibres are essentially straight, have been used to measure the amount of crimp in the staple (Evans, 1954). With increasing load there is a region BC in which the load increases at a constant rate and a small amount of extension occurs. The slope of the steep rise in force has been commonly called the Hookean slope. As the maximum load G is approached some fibres will be breaking. After the maximum load is reached the longer fibres in the staple are broken as the load gradually decreases to GH. The staple breaks completely

at the point H. The extension line DH returns slightly below the starting point as the staple is initially placed on the tester. With a tender staple, there is no defined demarcation between this region and the main staple loading region, as a result such staples have a greater extension at maximum load than a sound staple. The maximum loads of the sound and tender staples is expressed in grams (g) or Newtons.

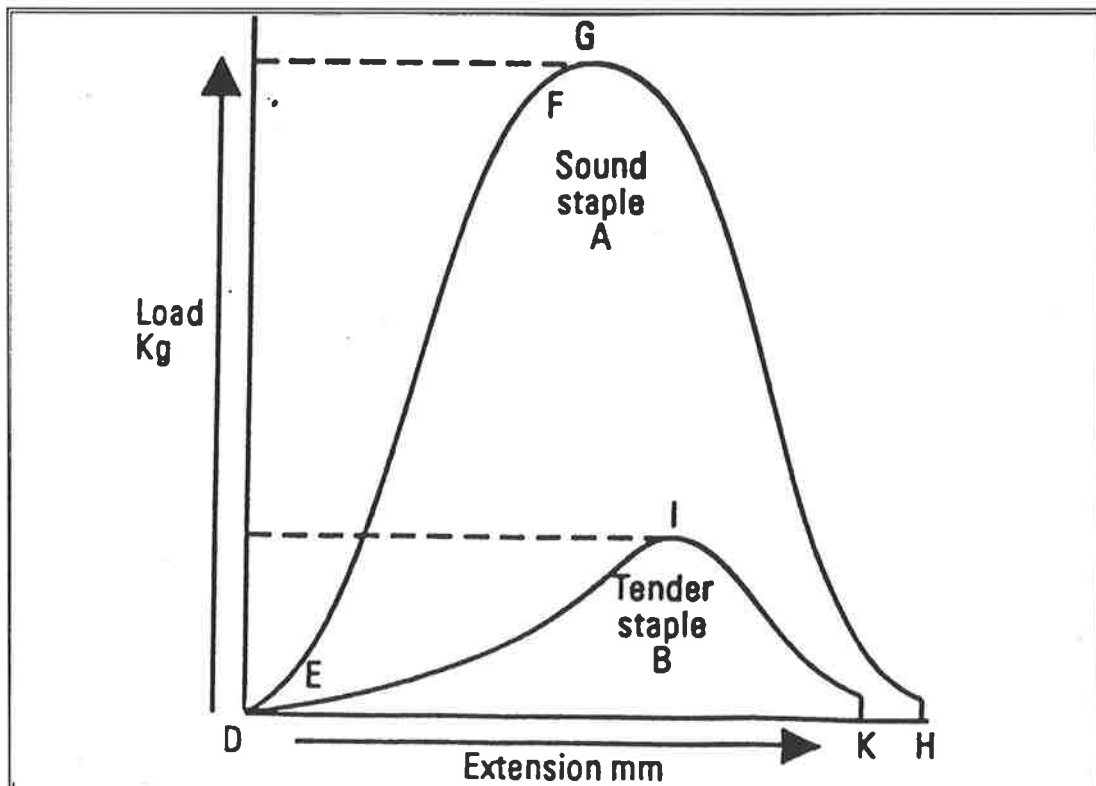


Figure 1.5. Staple strength breaking graphs for sound and tender staples (from Ross, 1985).

1.3. DETERMINANTS OF STAPLE STRENGTH

The concepts of 'tenderness' and 'wool-break' are tied intimately to staple strength. A 'wool breaks' is a fault of fleece wool characterised by low staple strength and a localised zone of staple weakness (Henderson, 1968), ranging from slightly tender to a regions in which there is complete cessation of fibre growth associated with marked reduction in fibre diameter.

1.3.1. The role of minimum fibre diameter in determining staple strength

Minimum fibre diameter is an important determinant of the strength of a fibre (Hunter et al. 1983, Hansford and Kennedy, 1988), as wool fibres are likely to break at the point of lowest diameter (Bigam et al, 1983). Even though minimum fibre diameter is an important determinant, it only accounts for about 20-40% of the variation in staple strength (Hunter et al. 1983; Hansford and Kennedy, 1988) indicating that other fibre characteristics must be involved. Producing fibre of consistent diameter is a problem for the Mediterranean climatic region of Australia. The following factors have been implicated as producing regions of low diameter:

1.3.1.1. Nutrient supply and season. Nutrient supply exerts a major influence on staple strength via effects on fibre diameter (Mata et al. 1990; Earle et al. 1994). The nutrition of each grazing animal is determined by the quantity and quality of pasture feed available (Green et al. 1984; Hawker and Crosbie, 1985), and the number and type of animals per hectare (Belliot, 1993; Earl et al. 1994; Summer and Wickham, 1969; Monteath, 1971; Horton and Wikham, 1979). In a Mediterranean-type environment, four types of herbage availability have been recognised (Purser, 1980). In winter and spring, high quality green feed is available but the quantity of feed is limited. However the feed available in summer and autumn is of poor quality while quantity is limited in autumn. With such a seasonally-dependent nutrient availability, an important factor to consider for staple strength and position of break is the opening of season. The sharp change in pasture quality from a diet of dry feed of low quality to a diet of pasture of high quality is a major cause of variation in fibre diameter and is associated with lower feed intake, fibre diameter and a position of weakness in the staple. The summer/autumn feed deficit results in consumption of feed below maintenance level and loss of weight by the animal and therefore fibre diameter and staple strength are reduced (Figure 1.6).

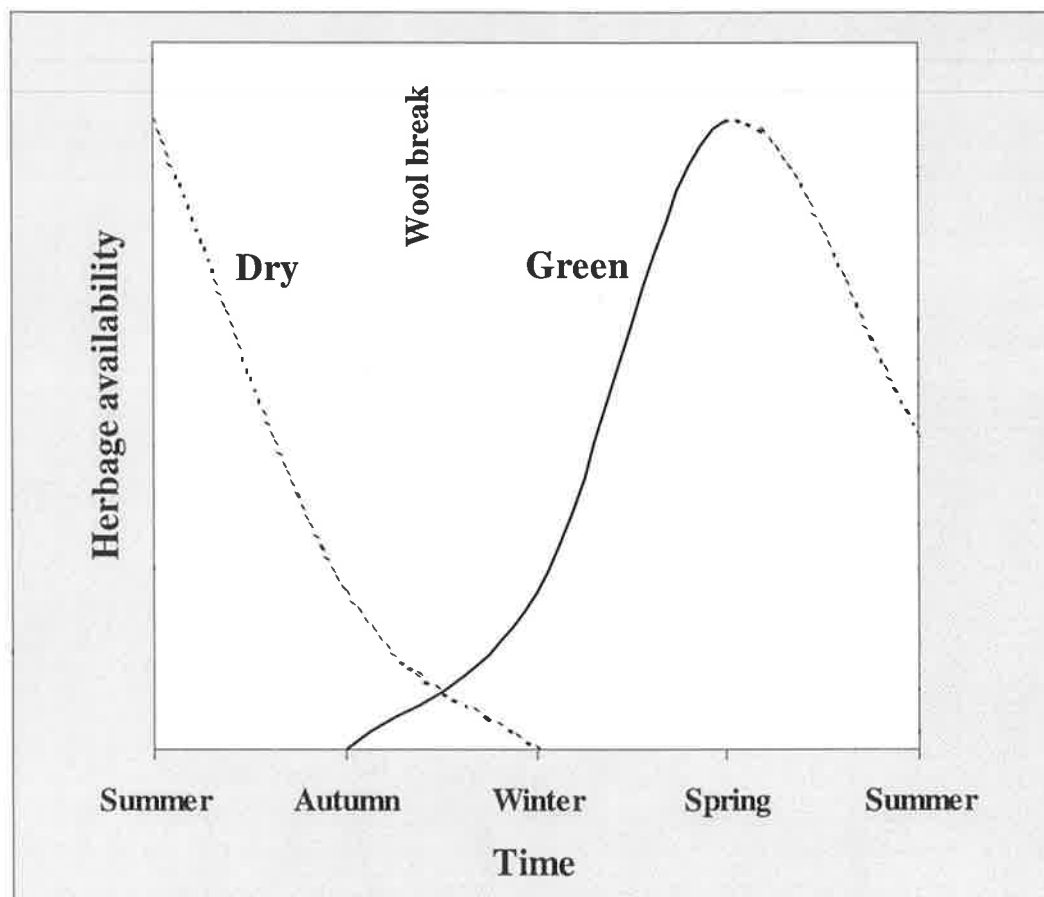


Figure 1.6. Pattern of herbage availability for grazed pastures in a Mediterranean type of environment (adapted from Alden, 1980).

Management can have an important role in improving the staple strength of stock. Increasing stocking rate resulted in decreased fleece weight, staple length and staple strength (Bellotti, 1993). Earle et al. (1994) showed that with increasing stocking rate, staple strength, live weight and minimum fibre diameter all decreased. In order to prevent occurrence of tender wool at the opening of season when poor quality pasture is available it is necessary to try to maintain body weight, particularly of young growing sheep (the most susceptible to tender wool).

1.3.1.2. Pregnancy, lactation and lambing. As a result of a decrease in fibre diameter and length, pregnant and lactating ewes have a lower staple strength than wool from

wether or dry ewes (Bigham, 1983). Hoggets and ewes are two classes of sheep most susceptible to tender wool (Bigham et al. 1978; Bigham et al. 1983). Baker et al. (1993) found that 50 % of fleeces from mature sheep (>1.5 years old) were tender or part-tender, whereas 62% of fleeces from weaner and hogget sheep (<1.5 years old) were tender or part-tender (<30 N/ktex). Lindner (1956) suggested that the development of wool breaks along the staple is associated with the 'stress' of pregnancy. The appearance of fleece tenderness before parturition (Bigham, 1983) implies that the hormonal and/or physiological changes during this process contribute towards the development of this wool fault. Corbett (1979) indicated that wool production of the breeding ewe is depressed by from 10 to 14% compared with that of the non-breeding ewe, with the effects being greater in ewes giving birth to and rearing, twin lambs (Hight et al. 1976; Bigham et al. 1978). Pregnancy in Western Australia takes place in November to February period, at a time when the only feed available is dry, poor quality, pasture residues. The small quantity of available pasture in November and February when most ewes are pregnant (Western Australia) limits nutrient intake of the animals and in turn has a negative effect on staple strength (Masters et al. 1992).

1.3.1.3. Parasites and diseases. Parasites and diseases have been recognised as limiting factors in decreasing feed intake and productivity of sheep. Uncontrolled parasitic infections resulted in decrease wool production, fibre diameter and length (Donald, 1979) which in turn would have drastic effects on staple strength and position of break. The major external parasitic invasion is blowfly strike which contributes to wool breaks and tenderness. The effect of parasites and diseases on staple strength is likely to be stress-related, associated with increased secretion of cortisol. Shutt et al. (1988) reported that in blowfly-affected sheep, the plasma cortisol level is high, due to rapid changes in nutrient supply. It has been indicated that the reduction in the staple strength is due simply to reduction in diameter during part of the growing season (Roberts et al. 1960).

1.3.2. The role of rate of change of diameter along a staple and between-fibre variance in fibre diameter in determining staple strength

Diameter is not constant along a fibre, and fibres in the same fleece vary considerably in mean diameter (Downes, 1971). Therefore if a staple is cut into several segments and the average fibre diameter of each segment measured, the average fibre diameter between the segments will be different. This difference in average fibre diameter is referred to as the rate of change of diameter along staple. Hunter et al. (1983) showed that there is a close association between fibre diameter ($r^2 = 0.41$) and staple strength, however Hansford and Kennedy (1988) indicated that this association is even more ($r^2 = 0.54$) between staple strength with the rate of change of fibre diameter.

Variation in fibre diameter within a staple is due to both variation in fibre diameter along and between fibres of a staple. Ritchie and Ralph (1990) indicated that midside fleece sample collected from two hogget flocks sired by the same group of rams was highly correlated ($r^2 = 0.83$, $P < 0.001$) with coefficient of variation of fibre diameter. This relationship shows that more than 80% of the variation in staple strength can be explained by the coefficient of variation in fibre diameter. James (1963) illustrated that the cross sectional area of wool from Merino sheep on a uniform diet and constant intake is more regular along the length of the fibre than that of wool from field-grown sheep.

In a study with high and low diameter and high and low staple length selection lines Jackson and Downes (1979) found that variation in diameter along the staple might be genetically correlated with mean diameter, but not with staple length. In this study it was also found that sheep within selection lines differed in mean fibre diameter, in variance along the staple and in coefficient of variation along the staple. These findings are important with regards to the position of break along the staple, at which diameter changes occur. What factors, then are likely to influence the rate of diameter change?

1.3.2.1. *The type/extent of stress.* Environmental stresses (pregnancy, diseases, fly strike, injury....) and particularly, nutrition, are important in determining the extent and the rate of diameter change. Reduction in the supply of nutrients will have a drastic effect on the rate of change in fibre diameter (Mata et al. 1990a). Sharp changes in pasture quality from a diet of dry feed of low quality to a diet of pasture of high quality is a major cause of variation in fibre diameter. In Mediterranean climatic regions characterised by rapid change in nutrient supply, sheep that are more resistant to marked fluctuation in fibre diameter, should produce more uniform fibres (James, 1963).

1.3.2.2. *Length/diameter (L/D) ratio.* Hynd and Schlink (1992) suggested that fibres with high length growth rate will have smoother diameter changes than those with a low length (if the rate of diameter change is the same in both). It has long been thought that fibre diameter can not be reduced without any reduction in fibre length, several findings suggest that fibre length can be reduced irrespective of any change in fibre diameter. Chapman and Basset (1970) showed that significant reduction in wool weight of cortisol injected sheep was mainly due to reduction in fibre length, irrespective of any change in fibre diameter. Similar results were achieved when the thyroid glands of sheep were surgically removed. Thyroidectomy depressed wool growth (Ferguson et al. 1965) however this reduction appeared to be entirely due to a decrease in fibre length growth (Rougeot, 1965).

In this respect, Hynd and Schlink (unpublished) showed that sheep with low diameter and high fibre length growth rates undergo lower increases in diameter with increased nutrition. This result could be an important finding in improving staple strength. If the rate of change along a staple is an important determinant of staple strength, it may well be that fibre length growth rate plays a crucial role in determination of staple strength. It can be inferred from these results that staple strength can be improved while keeping fibre diameter constant or even reducing fibre diameter. In other words decisions can be made in selecting sheep with longer

fibres while keeping or reducing fibre diameter to improve staple strength. Many physiological-environmental factors have so far been identified which alter L/D ratio:

Epidermal growth factor (Hollis et al. 1983)

Thyroxine status (Ferguson, 1965; Hynd, 1989)

Cortisol status (Chapman and Basset, 1970)

Nutrition (Downes, 1971; Hunter, 1990; Peter et al. 1994; Hynd, unpublished)

Season (Woods and Orwin, 1988)

Temperature (Lyne et al. 1970)

Growth hormone status (Wallace, 1979)

Determining the follicular factors governing L/D ratio is important in understanding mechanisms which directly or indirectly are involved in improving staple strength. Hynd (1989) showed that fibre diameter and length are both determined by rate of cell division and cortical size, but length is most dependent on cortical cell length. This explains why length and diameter often respond together (both being dependent on similar character) but because their dependence on these characters differ in a relative sense, they can respond independently.

1.3.2.3. Average fibre diameter. Average fibre diameter has been implicated to be closely associated with staple strength. In a study with 1400 sale lots in the 1991/1992 wool-selling season in Western Australia, Baker et al. (1993) indicated that there was a strong association between average fibre diameter and staple strength ($P < 0.05$). In another study it was found that average fibre diameter in Western Australia follows a distinct pattern throughout the year as a result of four distinct phases of pasture quality and quantity (Purser, 1980). As fibre diameter decreased the proportion of clean wool within a staple strength of 30 N/ktex or less increased linearly. Also the proportion of wool that was tender (≤ 26 N/ktex) increased as fibre diameter increased (Figure 1.3). On the other hand Hynd (1992) showed that there is a close relationship ($P < 0.05$) between initial diameter and diameter change with increased nutrition. In other words, sheep with low fibre diameter would have lower increases

in diameter with increased nutrition, therefore would have stronger fibres.

1.3.3. The role of intrinsic strength in determining staple strength

This refers to the strength of the keratin material per unit cross-sectional area i.e. independent of the amount of fibre present. It has long been established that fibre strength is largely determined by fibre cross-sectional area (fibre diameter and the rate of change in fibre diameter), however there is mounting evidence which suggests that other fibre characteristics are also important. Several important findings suggest that fibre intrinsic strength may be a determinant of staple strength. The load required to break fibres of the same diameter varies considerably and not all fibres break at the minimum diameter (Orwin et al. 1985). While breaking load increased with increasing fibre diameter (Anderson and Cox, 1950) the load required to break fibres of same diameter (intrinsic strength) varies considerably (Anderson and Cox, 1950; Woods et al. 1990). Even though these findings suggest that weak wool may have low intrinsic strength, there is considerable debate on whether wool fibre intrinsic material has a role in determining wool breaks. Burgman (1959) and Roberts et al. (1960) believe that there is no difference in intrinsic strength between tender and sound wool. On the other hand Orwin et al. (1980) and Bigham et al. (1983) suggest that the cell structure and protein composition of tender wool is different from that of sound wool of the same diameter. Whilst Orwin et al. (1980) proposed that a positive correlation exists between the proportion of orthocortical cell and staple strength, Hansford and Kennedy (1990) found no such relationship.

1.3.4. The role of fibre crimp frequency in determining staple strength

The pattern of distribution of fibre cells has a significant effect on the morphology, physical and chemical characteristics of the fibres. In fine fibres, such as in the Australian Merino, cortical cells show a bilateral segmentation and this is responsible for the crimp, or waviness, of the fibre (Fraser, 1964). There are

variations in fibre crimping, as some fibres within a staple are highly crimped, loss of crimp has been associated with weak wool. Figure 1.7 illustrates the relationship between fibre crimping and staple strength. When fibre length and crimp wavelength are equal the staple crimp would be likely to be clearly expressed and the staple tip would be square (staple A). It is desirable from strength point of view to have fibres of uniform length and crimp wavelength, because in such staples all the fibres present between the jaws of staple breaker system would take the strain applied simultaneously.

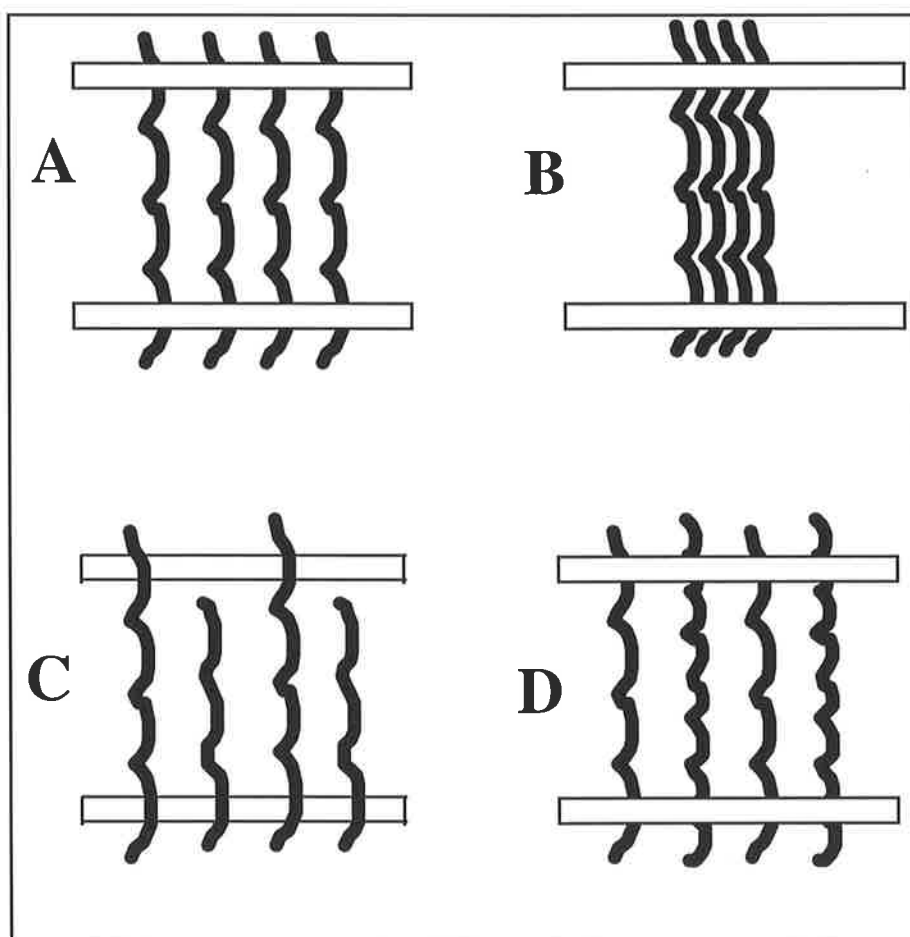


Figure 1.7. Diagrammatic view of different crimped staples clamped for staple strength testing: (A) Length and crimp wavelength of fibres are equal (B) length and crimp wavelength of fibres are equal but the direction of fibre growth is different (C) length of fibres is not equal (D) crimp frequency of fibres are not equal.

If the direction of the fibre growth at the skin level is different, the result is that the crimp tends to be out of phase and a plain appearance may result (staple B), however staple strength may not be affected significantly in such staples. In staples where fibre length is uneven due to unevenness in crimp frequency, felting may occur (Summer, 1969) and as a result the tip of short fibres would not be clamped between the jaws of staple breaker system (staple C). While the short fibres will play no part in the maximum load the staple will sustain, they will be included in the measurement of linear density because of their weight and therefore staple strength would decrease. In a staple where crimp wavelength of fibres is not equal (staple D) because of unevenness in crimp frequency, short fibres would take the strain applied by staple breaker system first, while crimpier fibres are longer than the gauge length and would be the last to be loaded during staple strength measurement. A drastic drop in staple strength of such staples is expected. In staple (IV) adhesion of the tips has occurred, the majority of the fibres will be present in both butt and tip of the staple. Hence, there would be a square tip while the short fibres would be straightened, the longer fibres are forced into convulsions, and the wavelengths of the crimp would not be the same. Consequently shorter fibres are not crimp and would take the strain applied by staple breaker system first, while crimpier fibres are longer than the gauge length and would be the last to be loaded during staple strength testing, therefore a drastic drop in staple strength is expected.

1.4. FIBRE SHEDDING AND MOULTING

Seasonal moulting is common in double-coated ovines such as the primitive Wiltshire and Soay sheep (Slee, 1959 and 1963; Lincoln et al. 1980), feral sheep such as Merino breed in Arapawa Island (Orwin and Whitaker, 1984) and goats (Forsyth et al. 1994; Restall et al. 1994; Rhind, 1994). In contrast, single-coated sheep breeds such as modern Merinos are considered to have a low incidence of seasonal shedding (Ryder, 1962 and 1967). Moulting in Wiltshire sheep follows a sequential, bilaterally-

symmetrical pattern, commencing on the neck, chest and shoulders, and spreading to the back and rump (Slee, 1963). This is essentially the same as found in Mouflan (Ryder, 1960) and Soay sheep (Slee, 1959). There is a seasonal environmental stimulus associated with daylength required to initiate the birth coat moult (Slee, 1965; Ryder, 1969 and 1971). Normally the loss of old fibres in the spring occurs synchronously with the regeneration of new ones; it is minimal near the end of winter and maximal in late summer. Ryder (1966) further observed that follicles within a group shed in the order in which they were developed. Slee (1965) observed lambs born at different times tended to synchronise in onset of shedding so that they were more alike in the dates when they shed than in the ages at which they did so. He interpreted this observation as there is 'ex hypothesis' a seasonal environmental stimulus required to initiate birth coat moult.

A seasonal pattern of coat change is also evident in feral goats and has been retained in the domesticated dairy goat breeds (Forsyth et al. 1994). Primary follicles produce the outer coat (guard hairs) while the undercoat (cashmere) provides the main insulating layer and is derived from secondary follicles. In spring gradual shedding and regrowth in primary follicles plus loss and delayed regrowth of the undercoat leads to a less dense summer coat. Primary follicles are in active growth (anagen) during the summer, becoming inactive (telogen) in winter (Forsyth et al. 1994). Duration of anagen is constant while duration of telogen can be modified by photoperiodic manipulations and hormones (Allain et al. 1994). Through selection for increased production, shedding of the fleece in response to seasonal environmental stimulus has been lost in advanced Merino. However, shedding still seems to occur even in the most advanced breeds under sufficiently adverse environmental conditions.

Although shedding of fleece has been lost in most breeds of sheep, they exhibit a seasonal cycle of fibre growth (Ferguson, 1949, Hardy; 1950; Coop, 1953; Coop and Hart, 1953; Ryder, 1956; Wodzicka, 1960) with a maximum rate in summer and a

minimum rate in winter. Some breeds such as the Merino, however show far less seasonal variation than others (Slee and Carter, 1961; Doney, 1966).

1.4.1. The effect of genotype on moulting

Wild sheep from which domestic sheep were derived (Ryder, 1960) shed their coat completely every spring, however domestic sheep show a wide variation in their tendency to shed from the complete spring moult of the Soay sheep to the continuous wool growth of the advanced Merino breed. Monthly skin samples taken from a wild Mouflon sheep at the age of 3-21 months indicated that the whole coat came away in the spring moult and both primary and secondary follicles showed signs of brush end (Ryder, 1960). Observations made on coat shedding in the Soay sheep indicated that the whole fleece is cast each year, starting about the end of April (Boyd et al. 1964). Burns (1954) found over up to 30% shedding of the primary follicles and up to 60% shedding of the secondary follicles in well-fed sheep of the Suffolk Down breed, and Ryder (1957) found as much as 75% primary follicle shedding and 84% secondary follicle shedding in the Shropshire Down. Cheviot sheep were shedding only 4% of both their primary and secondary follicles. In addition to these breed differences, the tendency to shed varies between individual sheep within a breed and may be determined genetically (Slee, 1959).

There is a genetic effect on the onset of moulting in goats also. The onset of secondary fibre growth is earlier in the Gorno Alti breed of sheep from Southern Siberian than in the Icelandic x Scottish feral (Rhind, 1994). Consequently the mean date of onset of the moult is earlier in Southern Siberia goats.

1.4.2. The effect of sex on moulting

Sex plays an important role in the extent and time of moulting. In a study with 1000 goats (Parry, 1993) animals were classified into five clusters on the basis of three cashmere length measurements in the late spring (November). Cluster one,

which was characterised by no cashmere length growth indicating an early initiation of the cashmere cycle, comprised approximately 60% females, but only 12.5% of males. As the older females in this study would have had kids at foot, this observation would have been influenced by pregnancy and lactation.

1.4.3. The effect of nutrition on moulting

In a nutritional study with Cashmere goats (Restall et al. 1993) a significant difference was found in total fleece and guard hair weight between high and low nutritional groups. In another study with Wiltshire wethers (Slee and Carter, 1961) a significant difference in moulting was found between indoor and outdoor treatment groups. The indoor sheep were entirely on a high-protein pelleted cake, while the outdoor sheep were on lowland pastures with some supplementary winter feed. The indoor sheep as a whole showed a linear rate of shedding with time. Compared with outside sheep, where the curve tended to be sigmoidal, the indoor sheep began to moult earlier but their moulting progressed more slowly.

1.4.4. The effect of day-length on moulting

There is a close association between moulting and day-length in wild mammals (Yeates, 1954 and 1957). It was found that the active growth phase of the short kemps of Limousine breed kept on normal and artificial light rhythms was only weeks in contrast to the longer growing periods of other follicles (Rougeot, 1961). In a study, three groups of Bikaneri ewes were subjected to 8 h light and 16 h darkness, 16 h light and 8 h darkness and normal diurnal photoperiod respectively. The experiment lasted from June until December. The group receiving 16 h light shed in August, and that receiving 8 h shed in July, while the control group did not shed at all. Evidence suggest that change in the light rhythm retarded coat shedding in Black-headed Persian sheep (Symington, 1959). Slee (1965) showed that when Wiltshire lambs were kept in a blacked-out room and exposed to a constant dim light from birth until

6 months of age, the onset of shedding was delayed and the rate of shedding was retarded in comparison with controls kept under natural daylight. However in this experiment, shedding was not prevented altogether in the treated lambs and a slight degree of shedding was remained. This indicates that possibly endogenous factors such as hormonal regulations or ambient temperature were capable of producing a degree of moulting since the lambs could not perceive day-length fluctuations.

Unlike mohair growth, cashmere in the Australian cashmere goat is shed annually (McDonald, 1985) and it responds to photoperiod (Ryder, 1966). McDonald et al. (1987) first described the cycle of cashmere and hair growth in mature female Australian cashmere goats. Cashmere growth commences in December and fibre length increases to a maximum in June-July after which time the fibre is shed from the fleece. They also found that cashmere fibre diameter was variable with minima in February and June-July. These studies clearly indicated that cashmere growth was modified by photoperiod, being initiated around the longest day (Summer solstice), growing through the period of decreasing day-length and being shed after the shortest day (winter solstice). Information about the prevailing photoperiod is relayed to the brain, via the secretion of the pineal hormone, melatonin, during the hours of darkness (Tamarkin et al. 1985).

1.4.5. The effect of temperature on moulting

There have been limited systemic studies on independent effects of temperature on wool growth and fibre shedding. Temperature may increase or decrease wool growth directly by affecting metabolism at the wool follicle, or indirectly by affecting feed consumption (Bottomley, 1979). A recent study by Gebbie et al. (1994) with double-coated Saanen goats, indicated that when day length and temperature were minimal (winter), prolactin concentrations were low and in summer prolactin levels were high. They found that despite maintained summer conditions in winter, plasma prolactin

concentrations declined with temperature and a full winter coat was attained indicating that a positive correlation exists between temperature and prolactin level.

1.4.6. The effect of hormones on fibre shedding

Hormones directly and indirectly affect the process of moulting and fibre shedding. Wool follicle function, development, regression and reactivation is very complex and is controlled by a number of physiological pathways and not all signals controlling these factors have been identified (Weinberg et al. 1992). To understand the role of hormones in regulating wool growth and their impact on the strength of fibres, it is necessary to have a knowledge of the morphology and functional processes of the wool follicle. Figure 1.8 shows a wool follicle. At the base of the follicle bulb, cells are produced and as they move upward, through the narrow channel of follicle, the living spherical cells transform into non living, fibrous, highly elongated cells surrounded by cuticle scales (Auber, 1952; Short et al. 1964).

Two types of follicle have been distinguished by their time of initiation and their appendages. In foetal skin, primary follicles are formed at 60 days post-parturition and 14-20 days before secondary follicles are formed (Hardy and Lyne, 1956) and are associated with sudoriferous glands and the arrector pili muscle. The ratio of secondary to primary follicles (S/P ratio) and follicle density vary considerably between breeds (Table 1.1) (Carter, 1955; Carter and Clark, 1957a; Abosaadi, 1970). These variations are largely breed-related as there is a genetic component to these variations. Sheep with a high ratio of secondary to primary follicles (S/P ratio) will have higher density, less variance in fibre diameter and more even follicle size and depth (Carter, 1955; Carter and Clarke, 1957).

Table 1.1. Representative follicle parameters for selected breeds of sheep.

Wool type	Breed	Follicle density/mm ²	S/P ratio	Mean diameter (µm)
Super fine	Merino	69.7	18.1	20.7
Fine	Merino	82.1	18.7	22.6
Long	Romney	35.7	6.7	37.3
Carpet	Bakhtiari	-	2.7	55.3
Coarse carpet	Karakul	-	3.09	40.47
Felt carpet	Ghezel	-	3.46	51.84

The data are adapted from Meikle et al. (1988) and Abosaadi (1970).

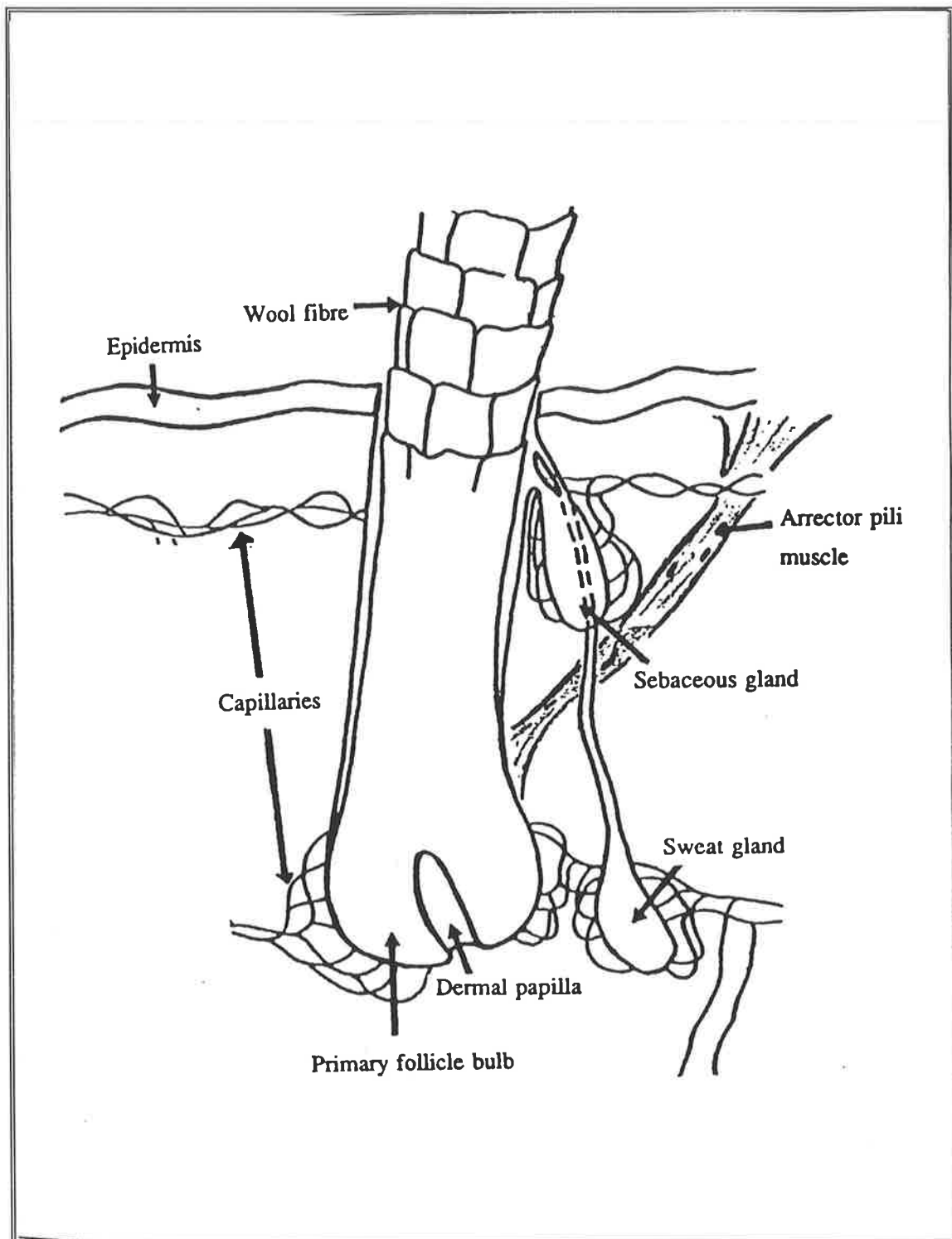


Figure 1.8. General organisation of a primary hair/wool follicle in the skin (from Antonia Holle, 1992).

The hormones known to be secreted by the anterior pituitary are adrenocorticotrophic hormone (ACTH), prolactin, growth hormone (somatotropin), thyroid-stimulating hormone (TSH), and the gonadotrophs, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Figure 1.9). Hormone secretion by the anterior pituitary is controlled by highly specialised neuronal cells in the hypothalamus, which produce hypophysiotropic hormones that reach the anterior pituitary via the hypophyseal-portal circulation and stimulate or inhibit the release of a given trophic hormone. With the possible exception of the pituitary gland, the secretion of the adrenal glands are the most diverse in their effects.

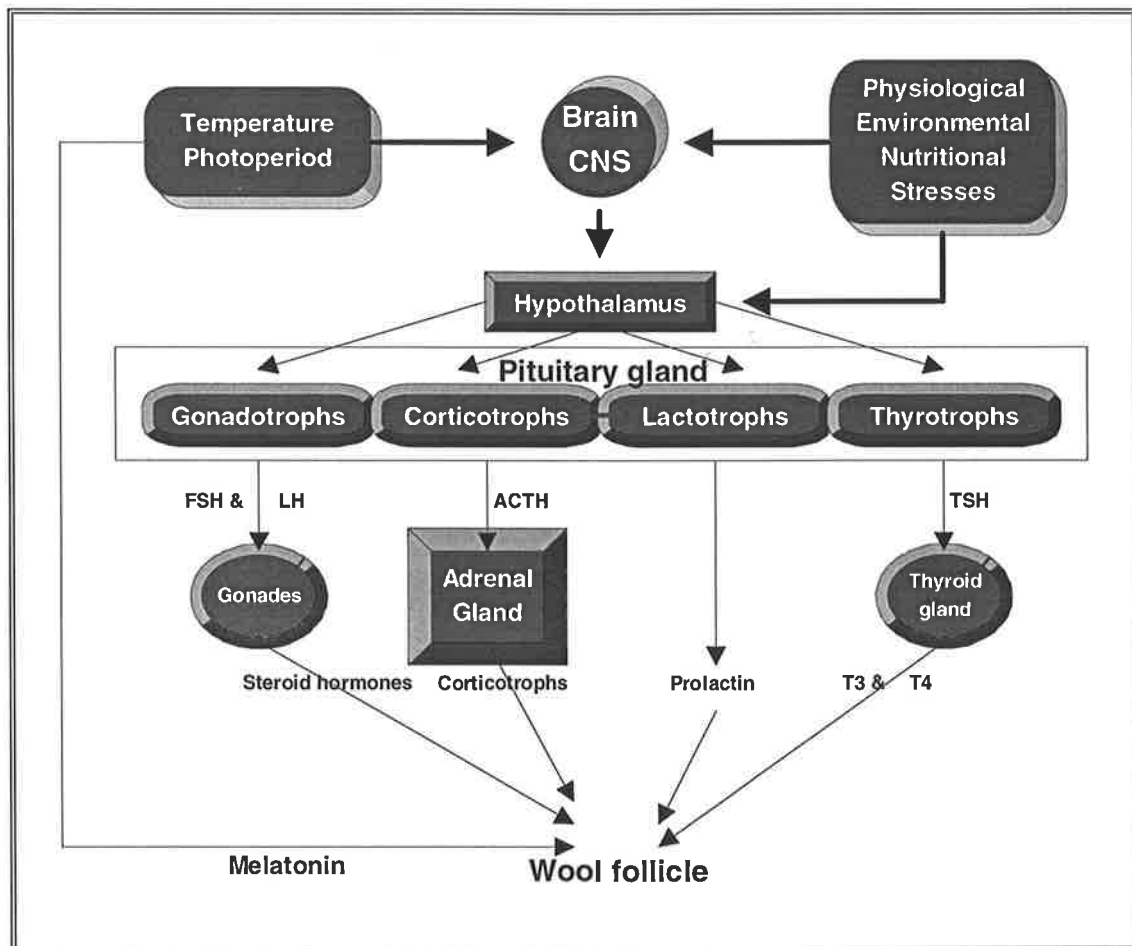


Figure 1.9. Factors implicated in the control of coat moulting cycles. FSH, follicle stimulating hormone; LH, luteinising hormone, adrenocorticotrophic hormone; TSH, thyroid stimulating hormone; T₃, triiodothyronine; T₄, thyroxine. (Source: Gebbie, Forsyth and Arendt, 1994).

1.4.6.1. The effect of Melatonin and Prolactin on fibre shedding

The role of melatonin and prolactin in regulating the timing of the spring moults and the reactivation of follicles has been implicated in double coated goats (Forsyth et al. 1994; Gebbie et al. 1994; Allain et al. 1994; Dicks, 1994). Light treatment (20 h light: 4 h dark) of goats, followed by melatonin implantation resulted in retention of the winter coat, which was then shed in autumn so that a summer-weight coat was present through the winter months (Forsyth et al. 1994). Similarly controls showed actively-growing anagen follicles in August and follicles in telogen in November, while in the experimental group, follicles were in catagen in August and in anagen in November. Prolactin like melatonin, shows marked changes in plasma concentration with season. Dicks (1994) indicated that subcutaneous implants of melatonin, advanced the time of the peak concentration (April v June) and the time of the onset of the peak of prolactin. An increase in prolactin plasma concentration in response to melatonin implants, caused an earlier loss of cashmere associated with the spring moult. Both primary and secondary follicles showed greater activity in the melatonin-treated animals than in controls, indicating an earlier initiation of both guard hair and cashmere growth as a consequence of the treatment. It is proposed that synergistic action of prolactin and melatonin regulate the timing of moult in goats (Gebbie et al. 1994).

1.4.6.2. The effect of Thyroid hormones on fibre shedding

Thyroxine which is the active hormone of thyroid gland is believed to be involved in wool production (Hynd, 1989). In sheep fed a maintenance level ration, thyroidectomy depressed wool growth up to 60% of pre-operative rate (Ferguson et al. 1965). This reduction in wool growth appeared to be entirely to a decrease in fibre length growth rate and fibre diameter remained constant (Theriez and Rougeot, 1962). Follicles can be induced into inactivity by removing thyroid gland. Hynd (1994a) indicated that 25% of the follicles in the hypothyroid sheep were induced into

inactivity. The removal of the thyroid from a newborn lamb prevents the maturation of the secondary follicle, but if the lamb is given L-thyroxine, the follicles will mature (Ferguson et al. 1956). Importance of thyroxine in follicle maturation suggests it would be important in fibre shedding. However it is unlikely that thyroxine plays an important role in determining wool production under field conditions, because even low levels of T_4 in concentration less than 15% of that in intact sheep, resulted in near-normal fibre production (Hynd, 1989). The role of thyroxine in fibre shedding in the field should be examined.

1.4.6.3. The effect of androgens on fibre shedding

These hormones at physiological concentrations has long been thought not to be involved in wool growth regulation (Ferguson et al. 1965). However the possible role of these hormones in regulating wool follicle activity was highlighted recently (Forsyth et al. 1994). In many mammals, hair follicles usually signal adulthood and specific sex. The mane of the lion for example is linked to the hormones of puberty, particularly androgens. Such follicles may also alter with the breeding season, as with the mane of the male red deer (Curlewis et al. 1988).

1.4.6.4. The effect of hypothalamic-pituitary-adrenal axis hormones on fibre shedding

Under natural conditions the position of break is often associated with a particular stress, such as rainfall in summer, availability of feed at the end of summer and the beginning of autumn, and pregnancy. It is likely that hormones at the three levels of regulation are involved in causing low staple strength: Corticotropin-releasing hormone (CRH) from the hypothalamus, ACTH from the pituitary and cortisol from the adrenal gland. High levels of follicle inactivity and fibre shedding have been induced under conditions of ACTH treatment (Lindner and Ferguson, 1956) or cortisol injection (Chapman and Bassett, 1970). ACTH, a hormone secreted by the

anterior pituitary is likely to be involved in the initiation of wool breaks. Daily intramuscular injections of 40 I.U. of ACTH for ten weeks, diminished wool growth progressively until it was completely arrested (Lindner and Ferguson, 1965). These authors observed that coinciding with the resumption of wool growth, a few weeks after cessation of injection of ACTH, a 'break' appeared in the fleece above the surface of the skin. The above experiment led to the suggestion that a temporary hyperactivity of the adrenal cortex may be a cause of 'break' or 'tenderness' of the fleece in the field, since this defect is usually associated with pregnancy, injury, disease or environmental stress.

Administration of high doses of glucocorticoids inhibits wool growth. Chapman and Bassett (1970) showed that successive increases in plasma cortisol level up to 30 ng/ml inhibited wool growth in sheep on a restricted plane of nutrition. However a slight increase in plasma cortisol to about 10 ng/ml in *ad libitum* fed sheep stimulated wool production. Regression of the epithelial and dermal tissues in skin at plasma cortisol concentrations above 30 ng/ml occurred regardless of the amount of food eaten. Administration of 300 mg of hydrocortisone acetate three times weekly, significantly decreased wool production, fibre diameter and fibre length (Spurlock and Clegg, 1962). Downes and Wallace (1965) reported that intradermal injection of low doses of cortisol increased fibre growth rate at the site of injection, but at higher doses caused a decreased wool growth rate.

The major circulating adrenal medulla glucocorticoid in sheep is cortisol and, under normal conditions, plasma concentrations range from 1-10 ng/ml (Bassett, 1963; Basset and Hinks, 1969). There is evidence which suggests that plasma cortisol exhibits circadian fluctuations. Sheep confined to indoor pens exhibited elevated cortisol levels with a definite peak at 0930 hours (McNatty et al. 1972). In another study, Holley et al. (1975) were able to show a circadian rhythm in cortisol levels with a peak at 1600 hours. This evidence suggests that glucocorticoids may be involved in the regulation of wool growth.

Lindner (1959) reported that in ewes with ketosis of pregnancy, a rise in plasma cortisol levels to 43-46 ng/ml occurs, while in healthy animals the cortisol level was of the order of 5-10 ng/ml. In a comment to this effect, he states that "there is little doubt then, that the plasma cortisol concentrations found during ketosis of pregnancy must be regarded as abnormally high". The break in the wool fibre which frequently appears in the course of this disease may be a consequence of the attendant high blood cortisol levels, since this defect can be produced by cortisol administration in well-fed sheep.

Poor nutrition can have a great impact on glucocorticoid secretion into blood. Fasting leads to stimulation of adrenal cortical activity, and can be regarded as a nutritional stress in its own right; the increase in plasma cortisol values during a period of psychological stress is often greater in previously fasted than in fed ewes (Reid 1960a; Reid and Mills, 1962). Reid and Mills (1961) state that "fasting may reduce the rate of cortisol metabolism and excretion thereby leading to greater increase in plasma cortisol during a subsequent stressful situation".

In advanced sheep such as the Merino, primary and secondary follicles respond differently to stress-induced hormones of hypothalamic-pituitary-adrenal axis. When ewes on a constant food intake were injected with 40 I.U. of ACTH for 10 weeks primary follicles shed their fibre to a lesser degree than secondary follicles (Lindner and Ferguson, 1956). In another study Chapman et al. (1982) indicated that when Merino ewes on a maintenance level ration were injected with 50 mg of dexamethasone trimethyl-acetate, more than 70% of the follicles regressed, but relatively fewer primary follicles were affected than secondary follicles. Under such circumstances it is possible that follicle type population and ratio of secondary to primary follicles influence the staple strength of sheep.

An increase in the rate of secretion of catecholamines can cause a drop in wool growth rate. Ferguson (1949) suggested that catecholamine secretion from the adrenal gland acts on wool follicles indirectly through vasoconstriction and reduced blood

supply. Wallace (1979) cites unpublished data which show intradermal injection of both adrenaline and noradrenaline produce a reduction in the uptake of ^{35}S -cystine. Reklewska (1975) has indicated that a positive relationship exists between blood catecholamine levels and wool growth rates. Scobie (1992) suggested that biogenic amines inhibit the rate of follicle mitotic activity within 2-4 hours by either acting as a hormone or as a neurotransmitter being released from sympathetic nerve endings. These studies indicate that vasoconstriction produced by secretion of catecholamines can suppress wool growth.

1.4.7. Histological changes in the follicle and fibre when shedding occurs in response to hormones of hypothalamic-pituitary-adrenal axis

Merino sheep injected with ACTH and cortisol exhibited changes in wool follicles depending on the dose, ranging from a transient disturbance of cuticle formation on some fibres to the induction of catagen of follicles (Lindner and Ferguson, 1956; Chapman and Bassett, 1970). Follicle shutdown proceeds through stages which resemble those described in mammalian catagen hair follicles (Straile et al. 1961; Montagna and Parakkal, 1974). The first signs of follicle shutdown are a reduction in the size of outer root sheath cells which tend to be elongated with the long axis oriented in towards the fibre (Chapman and Bassett, 1970). The outer root sheath cells soon become hyperplastic and accumulation of these cells occur in the follicle followed by loss of crimp (Chapman, 1980). As a consequence of disturbed function of outer root sheath cells, the structure of the inner root sheath is disrupted and in some cases it is totally lost (Chapman, 1988).

A feature of inactivation of follicles is that fibre growth ceases not by a mere cessation of fibre, but with the formation of a distorted-end on the fibre, as normally occurs during catagen of cyclic hair growth (Chapman and Bassett, 1970). Shed fibre carries with it the terminal bulb and sometimes a portion of inner root sheath forming a distorted end structure at the point of break. In a small number of fibres a bulge

structure with dark stained condensed nuclei is formed. Condensation of nuclei inhibits their normal degradation, which would have occurred during keratinization of the fibre (Chapman, 1989) and as a consequence they are retained.

When many follicles cease production a clear window becomes apparent through the wool staple which will contribute to low staple strength as a consequence of reduced load bearing material. It was indicated by Schlink (unpublished) that fibre shedding played a major role in determination of staple strength in fleeces less than 30 N/ktex, while above 30 N/ktex it played no role in staple strength.

Even though the importance of follicle shutdown and fibre shedding has been stressed, there is a lack of detailed quantitative data and the effect of these factors on staple strength has not been recorded. As most wool strength related research has concentrated on minimum fibre diameter and the rate of change in fibre diameter, insufficient attention has been paid to other sources of variation such as follicle shutdown.

1.4.8. The effect of epidermal growth factor (EGF) on follicle shutdown and fibre shedding

EGF, a polypeptide which affects the proliferation of various epidermal and epithelial tissues both *in vivo* and *in vitro*, was isolated from the submaxillary glands of adult male mice (Levi-Montalcini and Cohen, 1960; Cohen, 1962). In fetal animals EGF accelerates several developmental processes including palate formation, incisor eruption, eyelid opening and inhibition of hair growth (Cohen, 1962). It was later found that EGF exerts its effects by stimulating the growth and keratinization of epidermal cells (Cohen and Elliott, 1963). Injection of high doses of EGF in mouse inhibited fibre production (Cohen and Elliott, 1963) and reduced the rate of growth in length and diameter of hair (Moore et al. 1981). This finding was later confirmed in culture by epidermal sheets derived from the chick embryo (Cohen, 1965).

Following treatment of Merino sheep with EGF, wool growth was inhibited and later this compound was developed as a defleecing agent. Subcutaneous or intravenous infusion of EGF in many cases resulted in shedding of the entire fleece (Moore et al. 1981; Moore et al. 1982; Hollis et al. 1983). Animals that received 1-3 mg EGF usually did not shed, but subsequently, the fibres were found to have a zone of weakness 3-4 weeks later. The appearance of wool breaks in sheep treated with EGF resembled that of the break which appeared in sheep wool fibres treated with ACTH (Lindner and Ferguson, 1956) and that of sheep treated with hydrocortisone acetate (Chapman and Bassett, 1970). Singh-Asa and Waters (1983) showed that intravenous infusion of defleecing doses of EGF caused a seven fold increase in cortisol levels up to 70 ng/ml which is more than twice the levels known to produce a break in wool fibre. It was also found that ACTH immunisation increases dramatically the effectiveness of (EGF) as a wool harvesting agent (Adams and Wynn, unpublished). The effect of EGF on staple strength has not been recorded and needs to be investigated, but since EGF injection produced dose dependent changes in wool follicles and fibres ranging from shedding of the entire fleece to appearance of break in the wool, it can be anticipated that an increase in plasma concentration of EGF would affect staple strength.

1.5. SUMMARY OF LITERATURE REVIEW AND GENERAL THEME OF THE PROJECTS

Staple strength is an important raw wool characteristic which is closely related to processing properties of wool. Low staple strength wool has been recognised as a major problem in Merino sheep. A number of physiological and environmental factors including climate, parasites, pregnancy, nutrition and stress have been known to influence the production of high-quality wool. Research to date indicates that a number of wool characteristics such as minimum fibre diameter (Hunter et al. 1983), the variability of fibre diameter (Hansford and Kennedy, 1988) and the intrinsic

strength of fibre (Hunter et al. 1983) are important determinants of staple strength. However insufficient attention has been given to the role of follicle shutdown in determining staple strength, even though a preliminary study by Schlink (unpublished) has highlighted that shedding can be a major determinant of staple strength. Several important questions remain unanswered:

1. To what extent follicle shutdown and fibre shedding are involved in staple strength?
2. What is the quantitative relationship between plasma cortisol concentration, follicle shutdown and staple strength?
3. What are the events in the wool follicle leading to changes in staple strength?
4. Is there a difference between primary and secondary follicles in susceptibility to shutdown?
5. What is the role of cortisol and EGF in follicle shutdown?

To address these issues a series of experiments was initiated. In the first experiment, Merino sheep (Collinsville family group) were selected and split into five groups with similar mean staple strength. To generate large variations in follicle shutdown and to determine the extent to which follicle shutdown is a component of staple strength sheep were exposed to five different doses of cortisol for 4 weeks. Measurements of wool growth rate, percent follicle shutdown, percent shed fibres, plasma cortisol concentration, staple strength and staple intrinsic strength were made and values were compared between cortisol treated groups.

In the second experiment, to test the hypothesis that sheep differ in susceptibility to cortisol-induced shutdown, 2 groups of Finewool and 2 groups of Strongwool Merino sheep were selected and exposed to one dose of cortisol (70 mg per day) for a period of 2 weeks. Plasma cortisol concentration, wool growth rate and the percentage of follicle shutdown were measured and compared between groups.

In the third experiment, the direct effects of cortisol and EGF on cultured follicles, were investigated. Anagen primary and secondary follicles were dissected from

Romney and Tukidale sheep skin and maintained with different concentrations of cortisol and EGF.

CHAPTER 2.

MATERIALS AND METHODS

CHAPTER 2. MATERIALS AND METHODS

This chapter covers the general Materials and Methods used throughout chapters 3, 4 and 5. Specific details are given in the appropriate chapters.

2.1. IN VIVO EXPERIMENTS

2.1.1. Selection of animals

Sheep were selected from livestock research stations in South Australia and were tested for either staple strength and or fibre diameter and coefficient of variation of fibre diameter (detail description of the measurements is given in sections 2.1.4 and 3.2.4). Sheep were shorn and brought to the Waite Animal Biotechnology Centre and kept in individual pens in an animal house and offered a maintenance ration (see below). Animals were treated with an anthelmintic prior to the experiments. Water was available *ad libitum* for the entire experimental periods.

2.1.2. Feeding

Chapman and Bassett (1970) indicated that the effects of a slight to moderate increase in plasma concentration of Merino sheep up to about 20 ng/ml are influenced by the amount of food eaten. Only in those sheep whose feed intakes were restricted were successive increases in plasma cortisol progressively inhibitory. When sheep can increase their feed intake, slight increases in plasma cortisol to 10 ng/ml stimulate fibre length growth and wool production (Chapman and Bassett, 1970). To alleviate any feed factor, all sheep were fed a maintenance level ration (containing 15 g N kg⁻¹ dry matter and 9.1 MJ, Australian Feed Services Pty. Ltd.) and 200 g of chopped wheat straw (Australian Feed Services Pty. Ltd.) per day throughout the experiment. The diet was offered once a day at 0800 h. Once a week prior to feeding in the

morning after an overnight fast, sheep were weighed using electronic scales (Ruddweigh beef scales) and their rations adjusted according to live weight.

2.1.3. Cortisol injection

The production rate of cortisol in ewe is 17.3 mg/day or 12 µg/minute (Paterson and Harrison, 1967). Chapman and Bassett (1970) used 75 mg cortisol acetate/day for 12 weeks to cause inhibition and shedding of wool. Practically the question is whether shorter periods of exposure to different doses of cortisol, which are more likely to be experienced in the field, are capable of producing weak wool. Cortisol (Sigma chemical Co., St Louis, Mo. USA) was administered intramuscularly every day during the treatment period. Because cortisol is hydrophobic and is soluble in water only at a rate of 1.0 mg/100 ml, this hormone was suspended in a total volume of 3.0 ml of 80% ethanol and 0.9% saline solution. Sheep of the control group were injected with 3.0 ml of 80% ethanol and 0.9% saline solution.

2.1.4. Patch wool weight, fibre diameter and staple length

Immediately after being brought in to the animal house, sheep were tattooed on the right midside region (Langlands and Wheeler, 1968). A rectangular patch, approximately 120 cm² (10 x 12 cm) in area was tattooed by cutting the anaesthetised (Lignocaine hydrochloride; 20 mg/ml; Apex Lab. Pty. Ltd., St Marys, NSW) skin with a scalpel blade. Every two weeks, wool within the delineated area was closely clipped with Oster small animal clippers (model 40 Oster Corp., Wisconsin, no. 40 blades) and placed in a labelled envelope. The tattooed area was traced onto an overhead transparency and measured using an image analysis system (Bioquant IV, R and M Biometrics, Tennessee). The average of three readings was used to obtain the area of the clipped patch. The patch wool samples were weighed immediately after clipping. To obtain clean patch wool weight, samples were washed in Hexane (10 minutes x 3; Ace Chemical Co. Camden Park, SA) and rinsed in hot water (10

minutes x 2), to remove grease and suint respectively. The washed samples were then oven-dried for 48 hours at 60°C and weighed after cooling for 20 minutes in a desiccator.

The mean fibre diameter of the washed midside patch wool sample was measured by fibre fineness distribution analyser (FFDA) (Lynch and Michie, 1973). This method can measure up to 2,000 fibres in 2 minutes and prints out average fibre diameter, standard deviation (SD) (which measures spread) and coefficient of variation (CV%) (which measures spread in relation to fibre diameter).

The sheep were dyebanded as described by Chapman and Wheeler (1963) on the midside region at known times. To measure staple length, 1% Durafur black powder (ICI/ANZ Pty. Ltd.) was dissolved in 100 ml of tap water and 1ml of hydrogen peroxide (H₂O₂). The Durafur black solution was applied to the skin surface with a 10 ml glass syringe at monthly intervals. Staple length between dyebands was measured using a ruler graduated to 1 mm.

2.1.5. Skin sampling and staining

At the end of the pre-treatment period (stabilisation period), during which the sheep were accustomed to the diet and handling procedures, skin samples were taken to determine the characteristics of the follicles associated with different levels of cortisol. Skin samples were taken from the left midside region of each sheep one week before cortisol administration and every week during the treatment period. Further skin samples were taken 8 to 10 weeks after cessation of cortisol injection. Skin biopsies were taken weekly from the clipped area on the left midside of the sheep. Prior to sampling the skin was injected with lignocaine (0.2 ml), left for 2-3 minutes, and a biopsy taken with a 1 cm diameter trephine. The skin sample was then removed using surgical scissors and tweezers (Carter and Clark, 1957a). The biopsy was fixed in 10% buffered formalin (NaH₂PO₄.2H₂O* [4], Na₂HPO₄ [6.5g], H₂O [900ml], formalin [90%9 (w/w), 100ml]) (AR grade products from Ajax Lab. Pty,

Ltd, St Marys, NSW). The samples were then placed in individual cassettes (Tissue-Tek II, Miles laboratories, Inc. Naperville, Illinois) and dehydrated through a series of graded ethanols and cleared in histoclear (Ajax Chem., Auburn, NSW) using a Citadel tissue processor (Shandon Southern Products, Ltd. England) as follows:

1. 70% ethanol	60 min
2. 80% ethanol	60 min
3. 95% ethanol	30 min
4. 95% ethanol	30 min
5. Absolute ethanol	120 min
6. Absolute ethanol	120 min
7. 1:1 Absolute ethanol : Histo-clear	60 min
8. Histo-clear	120 min
9. Histo-clear	120 min
10. Histological wax (Phoenix Scientific Ind., Norwood, Sa)	120 min
11. Histological wax	120 min

Processed skin samples were embedded in paraffin using a tissue-tek II embedding centre (Tissue-Tek II tissue embedding centre, Model 4604, Miles laboratories, Inc.).

Embedded skin samples were sectioned in the transverse plane at 8 μm using a base sledge microtome. Approximately 60 sections were cut per sample, but only every fifth section was retained. Twelve sections were retained per sample and were floated on a 50°C water bath then transferred to slides treated with poly-L-lysine (Sigma Diagnostics St. Louis, Mo.). Sections were dried in an oven at 60°C for one hour to melt the paraffin. Before staining, all sections were deparaffinised by immersing them in histoclear for 2 minutes and rehydrated in a graded series of ethanols to water. A special tetrachrome stain was used to demonstrate follicular tissue components. This stain 'Sacpic' originally described by Auber (1952) is well

suited for detailed demonstration of the tissue components of the skin as well as revealing different features of keratinisation by differential staining. The staining was carried out as follows:

1. Deparaffinise	30 min
2. Histo-clear	20 min
3. Absolute ethanol	2 min
4. 80% ethanol	2 min
5. 50% ethanol	2 min
6. 30% ethanol	2 min
7. Distilled H ₂ O	2 min
8. Lillie Mayer's Haematoxylin	10 min
9. H ₂ O	5 min
10. 70% ethanol	5 dips
11. Winiwaters Safranin	15 min
12. 70% ethanol	until clears
13. Absolute ethanol	5 dips
14. Picric acid	5 seconds
15. H ₂ O	5 dips
16. Distilled H ₂ O	5 dips
17. Picro-Indigo Carmine	2 min
18. 70% ethanol	until clears
19. 80% ethanol	2 min
20. Absolute ethanol	2 min
21. Histo-clear	5 min

Tissue types are clearly defined in a tetrachrome-like manner and consistent stain intensities are produced. Results are nuclei, black; fibre, yellow; inner root sheath, bright red; outer root sheath, pale green; smooth muscle, green. Sacpic stained

transverse sections are useful for estimating follicle activity because: (A) It accentuates the inner root sheath which accompanies a growing fibre. (B) A large number of follicles can be observed simultaneously and follicle details can be readily obtained.

2.1.6. Blood sampling

Blood samples were taken before and during treatment period; further blood samples were taken two weeks after cessation of cortisol injection. Because plasma cortisol level exhibits circadian fluctuations (McNatty et al. 1972; Holley et al. 1975) collection of blood samples began at 0900 h and 5 ml blood samples were collected every 4 hour until 2100 h. Another blood sample was collected at 0900 h the following day after an overnight fast. Serial blood samples were taken by positioning an indwelling catheter (1.00 mm i.d. 1.5 mm o.d.) in the jugular vein. Blood samples were collected in a 10 ml syringe and were transferred to a heparinized blood tube (Disposable Products, Adelaide, SA) and centrifuged immediately at 3000 r.p.m. for 20 minutes. After drawing a blood sample the cannula was flushed with 0.9% sterile saline containing heparin 100 units/ml (Commonwealth Serum Laboratories, Melbourne, Vic). The plasma detained after centrifugation were stored at -20°C until required for hormone assay.

2.1.7. Plasma cortisol assay

Cortisol-binding globulin (CBG) from animal plasma, as well as antibodies directed at the cortisol molecule, can be used to measure cortisol. The principles of the procedure in outline involves the extraction of CBG by a chemical blocking agent contained in the tracer reagent. The total cortisol in the sample is then free to compete with ¹²⁵I-labelled cortisol for a limited number of binding sites on a sheep anti-cortisol antibody bound to polymer particles. Separation of the antibody-bound complex is effected by centrifugation, followed by decanting of the supernatant. The

amount of tracer bound is inversely proportional to the concentration of cortisol present.

Kodak Amerlex cortisol RIA kit (Kodak Clinical Diagnostics Ltd. Amersham, UK) was used to measure plasma cortisol concentration. The sensitivity of the Kodak Amerlex cortisol RIA kit is approximately 0.1 µg/100 ml. Materials provided by the Kodak Amerlex cortisol RIA kit are:

1. 1 bottle of tracer (^{125}I -labelled cortisol derivative, <370 K bq) in buffer with Antimicrobial Agent (22 ml)
2. 1 bottle of antibody suspension (sheep anti-cortisol, binds $\geq 40\%$ of 1.3 ng cortisol) in buffer with antimicrobial agent (22 ml).
3. 1 set of cortisol standards freeze-dried human serum.

To verify the performance of the assay, controls were assayed in parallel with plasma samples. Aliquots of plasma or standards (50 µl) were pipetted into 12 mm x 75 mm glass tubes in duplicate. Subsequently 200 µl of tracer (^{125}I -labelled cortisol derivative) and 200 µl of antibody (sheep anti-cortisol) were pipetted into all tubes. All tubes were mixed on a vortex mixer and covered with foil and heated for 60 minutes at $37 \pm 2^\circ\text{C}$. The tubes were then centrifuged at 1500 r.p.m. for at least 15 minutes at $18-28^\circ\text{C}$. Supernatant was removed by blotting and the amount of radioactivity was determined by counting on a gamma counter. Results were calculated using an RIA curve fit programme based on logit-log plotting. The percentage of bound ($\%B/B_0$) relative to the zero standard mean (B_0) for each standard and sheep (B) i.e. $(B/B_0) \times 100$ was calculated. The $\%B/B_0$ was plotted against a standard concentration curve. The best fitted line through the mean of duplicate points were drawn and the concentration of cortisol concentration of sheep read from the standard curve.

2.1.8. Follicle activity (morphology)

High levels of follicle shutdown have been induced in sheep injected with hormones of the hypothalamic-pituitary-adrenal axis (Chapman and Bassett, 1970; Lindner and Ferguson, 1956). Under these conditions the wool follicle undergoes substantial histological changes which can be quantified on Saccic-stained sections. Active follicles can be recognised from shutdown follicles in transverse skin sections. The basic criteria for distinguishing an active from a shutdown follicle is the presence of fibre and inner root sheath cells (Chapter 3, page 70; Plates 3.7 and 3.8). In an active follicle sectioned at the sebaceous gland level, a yellow stained fibre is surrounded by a red stained inner root sheath, while in a shutdown follicle these structures are absent or disrupted. In a quiescent follicle where fibre and inner root sheath are absent, the outer root sheath cells are often columnar and radially or spirally arranged in contrast to the rounded shape of this cell type and their random arrangement during anagen. Quiescent follicles can be also identified by their compact hair germ and dermal papilla cells.

These features provide a common and reliable indicator of follicle activity, because follicles can readily be divided into active and shutdown states. Another feature of this method is that a suitable number of follicles can be observed simultaneously. Primary follicles could be easily distinguished from secondary follicles by the positioning of the accessory appendages i.e. sweat gland and arrector pili muscle. The percentage of active and shutdown primary and secondary follicles were determined from the midside cross section of skin samples as described by Nixon (1993). To estimate the percentage of inactive follicles, approximately 300 follicles were counted per skin sample from 10 to 20 randomly selected follicular groups. The key to counting active follicles is the ability to recognise different hair cycle phases in the cross sections of the skin sample at appropriate depth. Figure 2.1 illustrates the relation between hair cycle phases and the transverse section of the follicle. The appearance of the upper follicle neck (Level A) is similar in active and quiescent follicles. Most useful information can be obtained from sections at the level

containing the lower lobe of the sebaceous gland (Level B). Inactive follicles could be identified by the absence of inner root sheath and fibre or the brush end of a fully grown fibre. Follicles in transition (proanagen and catagen) are most difficult to classify from cross sections. Mid to late proanagen follicles can be recognised by the presence of a fine fibre tip. The new fibre is usually located adjacent or distal to an existing brush end. Such follicles can usually be classified as active. In practice, transitional follicles are encountered infrequently due to relatively short duration of these phases.

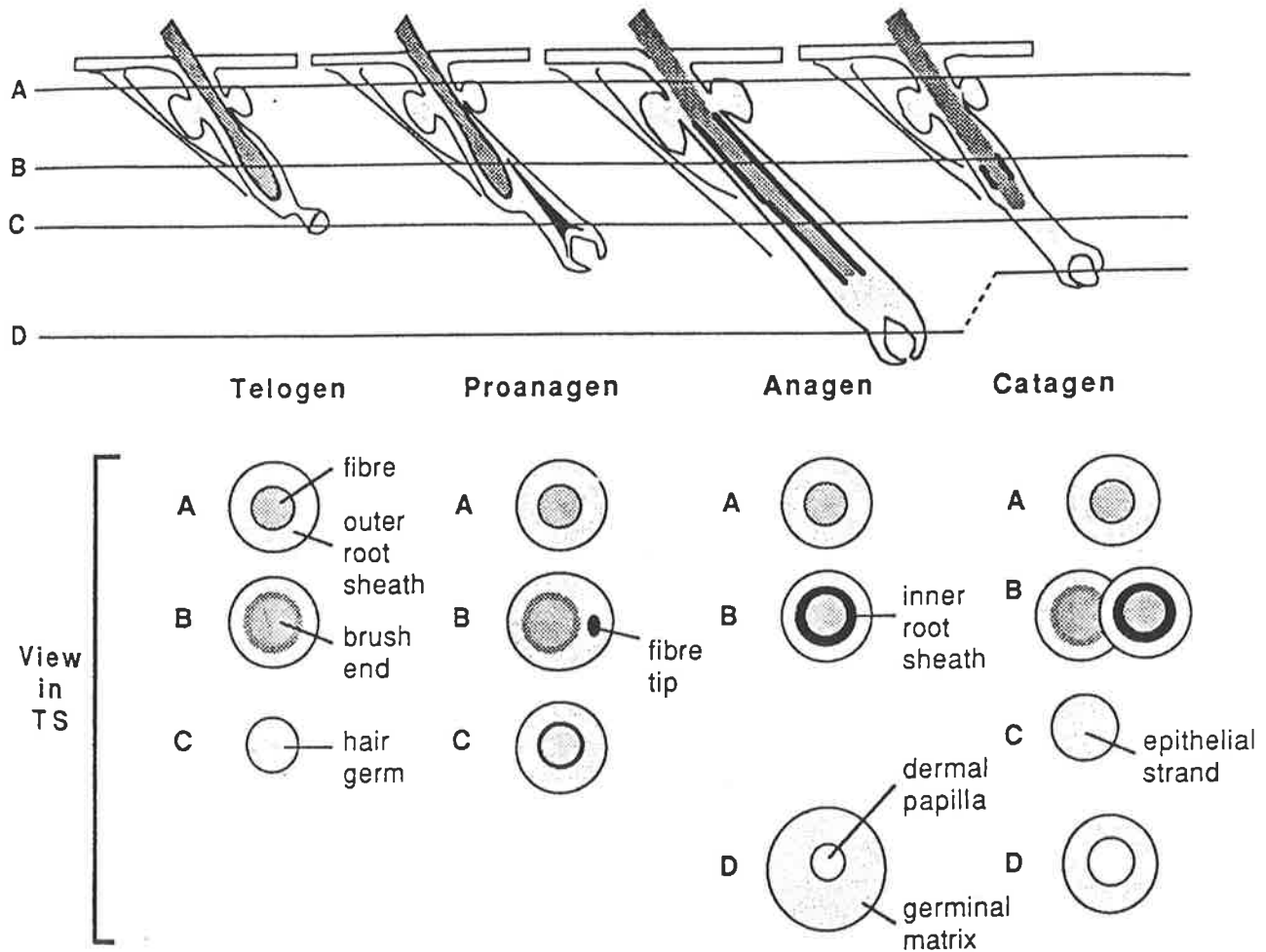


Figure 2.1. Relationship between hair cycle stage and view of the follicles in transverse section. Generalised structure of follicles at the four main stages of the hair cycle are shown (from Nixon, 1993).

2.1.9. Statistical analysis

Analysis of variance statistics were performed using the Super ANOVA Computer package (1989-1990, Abacus concepts, Inc. Berkeley, California) and the means and the standard errors of the means were generated with this program. The measurement of each characteristic was treated independently and the Duncan's new multiple range test was then used to compare the characteristics between groups. Super ANOVA was used to test the effects of time, genotype and cortisol dose on the clean wool weight, fibre diameter, staple length, follicle shutdown and plasma cortisol concentration. Results were considered significantly different when $P < 0.05$.

Simple correlation coefficients were estimated with Super ANOVA. The coefficients were used to analyse wool growth rate and the associated characteristics with follicle shutdown and plasma cortisol concentration.

Stepwise multiple regression was performed with Super ANOVA to derive an association between staple strength and associated characteristics. In this analysis, the parameter estimates of the regression model and their standard errors were examined and the factors with non-significant P values were eliminated until no more parameters could be removed from the equation.

2.2. IN VITRO FOLLICLE CULTURE

Individual microdissected mouse, sheep and human hair follicles have been used to study hair follicle growth in culture. Philpott et al. (1990) demonstrated that human hair follicles can be isolated from a piece of skin using watch makers' forceps. Isolated follicles maintained free floating in supplemented Williams E medium showed a significant increase in length over 4 days. More recently Hynd et al. (1992b) modified this technique using fine needles to isolate follicles and successfully maintained Romney and Tukidale wool follicles in culture with continued fibre production for up to twelve days, at rates approaching those found *in vivo*. In the present *in vitro* studies, follicles from different breeds of sheep have been isolated by

dissection and maintained in individual wells. These follicles show a significant increase in length in time that can be attributed to the production of a keratinized fibre shaft.

2.2.1. Materials

Williams E Medium was obtained from Gibco Laboratories (New York, New York), Phosphate buffered saline (PBS) from Sigma Diagnostics (St. Louis, Missouri) and [methyl-³H]thymidine was obtained from Amersham Australia (North Ryde, NSW). Trichloroacetic acid (TCA) and glycerol (AnalR) were products from BDH chemical (Kilsyth, Vic). The tissue culture wells (3424 Mark II) were products from Apex Laboratories (St. Marys, NSW), 1mM L-glutamine from Sigma Diagnostics (St. Louis, Missouri), Fungizone (2.5 µg/ml) and Penicillin/Streptomycin (100 units/ml) from Gibco Laboratories (New York, New York).

Photographic gel (L-4 Emulsion in Gel form) was obtained from Ilford Scientific Products (Mobberley, Cheshire) and the fixer (Hypam X-Ray Rapid Fixer), Hardner (Hypam X-Ray Hardner) and developer (Phenisol X-Ray Developer) were all products from Ilford (Mt. Waverley, Vic).

2.2.1. Follicle isolation and length measurement

Wool follicles were isolated from skin strips (0.5 mm x 1.0 cm) taken from the locally-anaesthetised (Lignocaine hydrochloride) mid-side of adult sheep. Intact anagen hair follicles were gently removed from the collected skin strips using fine needles and cut at the sebaceous gland level. Isolated hair follicles were maintained in 500 µl of Williams E medium supplemented with 1mM L-glutamine, Fungizone (2.5 µg/ml) and penicillin/streptomycin (100 units/ml). Follicles were maintained in individual wells of 24-well plates at 37°C in an atmosphere of 5% CO₂/95% air. Measurement of the changes in length of the follicles was made at 144 x magnification using an image analysis system (Bioquant IV, R & M Biometrics,

USA). The distance from the base of the follicle bulb to the tip of the fibre was measured at 24 hour intervals.

2.2.3. DNA synthesis in the cultured follicles

Tissue processing and microtomy. Follicles were grown in Williams E medium plus additives for 72 hours, then incubated in 3.7×10^4 Bq (1 μ Ci) of [methyl- 3 H] thymidine (specific activity 0.67 mCi μ mole) for 6 hours. After incubations were completed, individual follicles were washed four times in 5% trichloroacetic acid (TCA). Follicles were then placed in millipore filters and set in micropore cassettes. Follicles were dehydrated through a series of graded ethanols, cleared in histoclear and infiltrated with paraffin wax at 56 $^{\circ}$ C using a tissue-Tek II embedding centre. The samples were then embedded on a tissue-Tek embedding console.

Sections were cut using a base sledge microtome, floated on warm water and mounted on poly-L-lysine slides for radioactivity detection of incorporated [methyl- 3 H] thymidine. Sections were cut at 8 μ m in thickness parallel to the long axis of the wool follicles. Before autoradiography, all sections were deparaffinised by immersing them in histoclear and rehydrated in a graded series of ethanols to water.

Autoradiography. All deparaffinised sections were dried for 12 hours. 14 ml of water was pipetted in a screw-cap tube, and the level was marked with a black texta, then 7.15 ml of it was discarded. To the screw cap tube 140 μ l of 100% glycerol was added to make a 2% solution. In the darkroom under Ilford Safelight F904 the tube containing the 2% glycerol solution was placed in a 45 $^{\circ}$ C water bath. L4 emulsion was added to the water in the screw-cap tube until it just exceeded the 14 ml mark. The screw-cap tube was wrapped in foil and was allowed to melt in the 45 $^{\circ}$ C bath for 30 minutes. The screw-cap tube containing melted emulsion was rotated end over end about 10 times to ensure thorough mixing. The emulsion was slowly poured into the dipping chamber which was also held at 45 $^{\circ}$ C to keep the emulsion at a constant temperature into the dipping. Slides were immersed into dipping chamber for 1-2

seconds and withdrawn in a fluid motion, drained for 5 seconds, then the back of the slide were wiped with a damp wettex and laid on an ice-cooled tray. Dipped slides (wiped-side down) were laid on to the cooled metal tray and left for 10 minutes. Slides were then removed and placed on another tray at room temperature for at least 2 hours. For exposing, the slides were placed in a black light-proof box with silica gel and the lid was fastened with tape. The box was wrapped in 2 layers of foil then a black bag and sealed and kept at 4°C in the refrigerator for at least 7-8 weeks before developing.

Developing. Slides to be developed were allowed to reach room temperature. In the dark room under Ilford Safelight F904 slides were removed from the box and placed into a coplin jar containing kodak D19 developer (undiluted) for 2.5 minutes, distilled water for 10-20 seconds and katafix liquid fixer (diluted 1:4) containing Ilford Hardner (diluted 1:40) for 2 minutes. After fixation the slides were washed in distilled water several times over 30 minutes then air-dried or stained immediately .

Sacpic staining. Serial transverse follicle sections 8 µm thick were stained with Sacpic staining method after deparaffinisation and rehydration (see section 2.1.5).

2.2.3. Statistical analysis

Growth rate was estimated as the slope of regression line relating fibre length to time. The slope of the regression was estimated with the Cricket graph computer software package (Version 1.3.1; Cricket Software, Philadelphia, U.S.A.). This was used to determine the average growth rate of follicles over a 3 day period and to compare the results obtained from different treatments.

Analysis of variance statistics were performed using the Super ANOVA computer package (1989-1990, Abacus Concepts, Inc. Berkeley, California). Duncan's new multiple range test was then used to compare the follicle groups. The effect of EGF dose and follicle type on fibre growth was also determined.

CHAPTER 3.

CORTISOL-INDUCED FOLLICLE SHUTDOWN IS A MAJOR DETERMINANT OF STAPLE STRENGTH

CHAPTER 3. CORTISOL-INDUCED FOLLICLE SHUTDOWN IS A MAJOR DETERMINANT OF STAPLE STRENGTH

3.1. INTRODUCTION

Staple strength is an important raw wool characteristic (Hunter et al. 1990; de Jong et al. 1985), because it greatly influences wool processing properties such as the length of fibres in the 'top' (Hunter et al. 1990). Low staple strength has been recognised as a problem in Merino wool and as a result of price penalties imposed on low staple strength wool, it is estimated that \$40 million is lost annually (Australian Farm Journal, 1992).

Despite the importance of the low staple strength wool problem as indicated in Chapter 1, little is known about its causes or prevention and most related research in this area has concentrated on the minimum fibre diameter (Hunter et al. 1983), the variability of fibre diameter (Hansford and Kennedy, 1980) and the intrinsic strength of the fibres (Hunter et al. 1983). While these factors are important in determining staple strength, they account for only about 50% of the variation in staple strength, indicating that other characteristics must be involved.

Insufficient attention has been paid to these other characteristics and one area of particular relevance is whether follicle shutdown and fibre shedding have a role in determining staple strength. Limited studies by Schlink (unpublished) indicate that staple strength below 30 N/ktex is highly correlated with the incidence of shed fibres, while above 30 N/ktex it plays no role in staple strength. There is however a lack of quantitative data available from studies in which variable shedding incidence is induced.

A role for the hormones of the stress axis in the tender wool problem has been established. High levels of follicle shutdown and fibre shedding have been induced by

cortisol injection (Chapman and Bassett, 1970), resulting in fibre shedding and a 'wool break' in the fleece above the surface of skin. Follicles and fibres underwent substantial histological changes ranging from absence of fibre and inner root sheath to formation of distorted end fibres. However no quantitative relationship has been established between different levels of plasma cortisol hormone, follicle shutdown and staple strength nor has an apparent difference in the susceptibility of primary and secondary follicles to the hormones of stress axis (Lindner and Ferguson, 1956) been studied.

This chapter is directed at identifying the significance of follicle shutdown in determining staple strength and in describing histological changes at the follicular and fibre level induced by cortisol hormone. Establishing a quantitative relationship between plasma cortisol level, the extent of follicle shutdown and subsequent decrease in staple strength would be useful in understanding the wool tenderness problem.

The principal aims of this chapter were:

1. To determine the extent to which follicle shutdown and fibre shedding are related to staple strength in cortisol-injected sheep.
2. To establish the quantitative relationships between plasma cortisol concentration, follicle shutdown and staple strength.
3. To characterise histological changes in the wool follicles and fibres of sheep treated with different doses of cortisol.
4. To quantify the susceptibility of primary and secondary follicles to cortisol administration.

To address these issues, Merino sheep (Collinsville family group) were selected and split into five groups with similar mean staple strength. To generate large variations in follicle shutdown, sheep were exposed to five different doses of cortisol hormone for four weeks. Measurements of wool growth rate, percent follicle shutdown, percent shed fibres, plasma cortisol concentration, staple strength and

staple intrinsic strength were made and values were compared between cortisol treated groups.

3.2. MATERIALS AND METHODS

General Materials and Methods have been discussed in Chapter 2.

3.2.1. Selection of animals

The objective was to select 5 groups of sheep with similar average staple strength values but with a wide variance of staple strength within groups. A flock of 150 South Australian, Strongwool Merino rams (Collinsville family group) grazing at Turretfield Research Centre, were tested for midside staple strength. Staple strength (12 months wool) was used as the basis of selection of two groups of high and low staple strength. A total of 10 staples were carefully selected from midside wool samples (15 g/sheep) taken from sheep and the staple strength value was determined for each sheep (as described in section 3.2.4.). A total of 21 sheep (16 months old) were selected and allocated into 5 groups with similar mean staple strength (Table 3.1).

Whilst it was recognised that selection of sheep was based on phenotypic differences in staple strength, it was considered that such selection would provide a strong indication of where genetic differences may lie because:

(A) the magnitude of the heritability estimate of staple strength is 0.40-0.50 (Li and Lewer, unpublished).

(B) comparisons were made under similar environmental conditions after a long stabilisation period (> 16 weeks) to remove transient environmental effects.

3.2.2. Design of the experiment

A total of 40 weeks was divided into 3 subperiods (pre-treatment, treatment and post-treatment) of 16, 4 and 20 weeks respectively (Table 3.2). At the end of the pre-treatment period sheep were accustomed to diet and handling procedures. During

Table 3.1. Allocation of sheep into five groups on the basis of pre-experimental staple strength measurement (Means with s.e.m.) and the dose of cortisol injection.

Group	Staple strength (N/ktex)	Cortisol dose (mg/sheep/day)
1	37.0 ± 8.9	150
2	37.8 ± 8.4	75
3	40.0 ± 10.8	30
4	37.0 ± 7.3	10
5	38.8 ± 12.1	0

the treatment period, groups of sheep were injected with different doses of cortisol. During the post-treatment period, cortisol injection was stopped and all groups continued to receive a maintenance level of nutrition (refer to diet details in chapter 2, section 2.1.2). This allowed comparisons to be made for wool growth rate and follicle shutdown and regeneration both within and between periods.

Sheep were individually penned in an animal house, weighed once a week and the following samples taken:

___Wool within the tattooed patch area was clipped every two weeks to measure clean wool weight (see chapter 2, section 2.1.4).

___Skin biopsies were taken once a week for measurement of follicle activity and histological studies (see chapter 2, section 2.1.5).

___Serial blood samples were taken every two weeks to measure plasma cortisol concentration (see chapter 2, section 2.1.6).

___At the end of the experimental period, wool samples (10 months growth) were taken from shoulder, midside and rump sites for measurement of staple strength.

Midside wool samples were used to measure staple intrinsic strength (for staple intrinsic strength definition see chapter 1, section 1.3.3; for staple strength measurement see section 3.2.5) and the percentage of shed fibres.

Table 3.2. The outline of the experiment. A total of 40 weeks was divided into 3 subperiods; pre-treatment, treatment and post-treatment period of 16, 4 and 20 weeks respectively. Time sequence of live weight, wool clipping, dyebanding, skin sampling, blood sampling, staple strength, staple intrinsic strength and plasma cortisol concentration measurements is shown.

	pre-treatment	treatment	post-treatment
	1 2 8 9 10 11 12 13 14 15 16	1 2 3 4	1 2 3 4 5 6 7 8 9 10 20
Deworming	*		
Tattooing	*		
Liveweight	*****	****	*****
Wool clipping	* * * *	* *	* * * *
Dyebanding	* * *	*	* *
Skin sampling	* * * *	* * * *	* * * * * * * *
Blood sampling	*	*	*
SS ¹ measurement			*
SIS ² measurement			*
SF ³ measurement			*
Blood RIA ⁴	*	*	*

¹staple strength, ²staple intrinsic strength, ³shed fibre and ⁴radioimmunoassay.

3.2.3. Cortisol injection

It was decided to expose all sheep to cortisol for a period of 4 weeks which is more likely to be experienced in the field (such as the stress of pregnancy or at the

break of the season). Different doses of cortisol ranging from a low dose of 10 mg/day to a high dose of 150 mg/day were chosen based on sheep cortisol production rate (see chapter 2, section 2.1.3). Groups 1 to 5 were injected with cortisol at levels of 2.86, 1.42, 0.5, 0.19 and 0 mg/kg respectively.

3.2.4. Staple strength measurement

To measure staple strength, a staple breaker system (Agritest, Pty. Ltd. NSW) was used which consists of a staple breaker, a control console, thickness gauge and compressor. The principle of the device is as follows:

“A staple is placed in the jaws of the clamps which are then closed. As each staple is stretched the panel on the control console displays the increasing force applied until the maximum value is reached just before the staple begins to break. This maximum force is then ‘frozen’ on the panel and represents the force to break a staple. The stronger the staple, the greater the force applied to break the staple.”

About 15 g of wool sample was taken from each of the shoulder, midside and rump sites per sheep using Oster small clippers. Wool samples were conditioned in a control atmosphere of 20°C (± 2) and 65% (± 2) relative humidity for 48 hours. A total of 10 staples were randomly selected from each of the shoulder, midside and rump sites to measure the staple strength of each sheep. The remaining collected samples were weighed and washed with hexane (3x) and hot water (2x) to get the clean wool production value of each sheep. The washing yield of wool samples was estimated using the following formula.

$$\text{Washing yield} = 117 \times \text{oven dry weight} / \text{greasy wool weight}$$

Each individual selected staple was weighed (g) and the staple length (mm) was measured using a graduation ruler. The ends of each staple were placed in the jaws of the clamps of the breaker system and were stretched at a constant rate until it broke. The force to break the staple was read from the control console panel. The staple strength (SS) of each staple was estimated by using the following formula:

$SS (N/ktex) = [\text{force (N)} \times \text{staple length (mm)}/100] / [\text{staple weight (g)} \times \text{washing yield}/100]$

3.2.5. Staple intrinsic strength measurement

Staple intrinsic strength is estimated by measuring the force (N) required to break the staple divided by the thickness of staple (ktex). In measuring staple intrinsic strength, the aim is not to measure the average cross sectional area of the staple over the region that is held between the clamps, but the aim is to measure the thickness of the staple at the point of the break.

Basically the same procedure is used as measuring staple strength, however the thickness gauge on a staple breaker system is used to measure staple thickness. This gauge gives a thickness measurement in mm that can be converted to kilotex. Each staple at the point of appearance of wool breaks was fed into the rectangular slot on the moveable jaw and compressed to get the thickness reading from the gauge. At least 5 readings were taken over the length of the appearance of wool breaks on each staple. Readings on the gauge were converted to staple thickness in kilotex units using the following relationship:

Staple thickness (ktex) = 3.2 x gauge reading (mm)

For example, a gauge reading of 0.5 mm = 3.2 x 0.5
= 1.6

A total of 10 staples were randomly selected from collected midside sample of each sheep (same sample was used for staple strength measurement). These staples were conditioned as described previously and tested for staple intrinsic strength measurement. The tip and the bottom ends of broken staples were kept for measurement of the percentage of shed fibres.

3.2.6. Measurement of shed fibres

A staining method was used to determine the proportion of shed fibres in a staple. In this staining method the distorted end of the fibre is accentuated, facilitating

detection of shed fibres; distorted end appear red while a normal end appears in yellow.

To measure the percentage of shed fibres, 5 broken staples which were used for the measurement of staple intrinsic strength were selected and the tip portion of each staple was stained as described by Schlinck (personal communication) and mounted on a microscope slide. The staining was carried out as follows:

1. Hexane 3x	10 min/wash
2. Dry using hair drier	1 min
3. Picric acid	3 min
4. H ₂ O 4x	5 dips/wash
5. Dry using tissue paper	1 min
6. Eosin	30 sec
7. H ₂ O 3x	5 dips/wash
8. Absolute ethanol	3 dips
9. Dry in the oven	overnight

Approximately 100 fibres were counted per staple and the average percentage of distorted end fibres of 5 staples were estimated (a total of 500 fibres were counted per sheep). This procedure is a useful method for estimating fibre shedding because a suitable number of fibres can be observed simultaneously. Another feature of this method is that fibres can be readily divided into two distinct 'distorted' and 'normal' or 'shed' and 'unshed' fibres.

3.3. RESULTS

3.3.1. The effect of cortisol injection on feed intake and live weight

There was no significant difference in the average live weight of sheep groups before and after the treatment period (Table 3.3). Since sheep were not fed *ad libitum*

and the feed was kept at maintenance level, no significant weight loss or gain was recorded in any of the sheep groups. All sheep consumed their entire ration and remained in good condition before, during and after cortisol injection.

Table 3.3. Live weights for the groups of Merino sheep; measured before and after treatment period (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

Group	Cortisol dose (mg/kg)	Live weight (kg)	
		before	after
1	2.86	52.5 ± 2.0 ^a	51.0 ± 3.2 ^a
2	1.42	53.0 ± 1.7 ^a	52.0 ± 2.2 ^a
3	0.56	53.5 ± 1.9 ^a	51.0 ± 1.3 ^a
4	0.19	53.0 ± 1.4 ^a	52.5 ± 1.6 ^a
5	0	53.0 ± 3.1 ^a	51.5 ± 3.4 ^a

a = no significant differences between groups or times.

3.3.2. The effect of cortisol injection on plasma cortisol concentration

The average plasma cortisol concentration two weeks before commencement of cortisol injection was very low in all groups of sheep (Figure 3.1). Plasma cortisol increased significantly ($P < 0.0001$) in groups 1, 2, 3 and 4 which received daily injection of 2.86, 1.42, 0.56, and 0.19 mg of cortisol/kg body weight. The maximum mean level of plasma cortisol attained was 10.43 $\mu\text{g}/100\text{ ml}$ in group 1 (approximately 8 fold increase compared with pre-treatment level). There was a significant ($P < 0.0001$) interaction between week and dose. There was an indication

that towards the end of treatment period the plasma cortisol concentration declined in all cortisol treated groups despite receiving a constant dose of cortisol (i.e. compare week 4 with week 2).

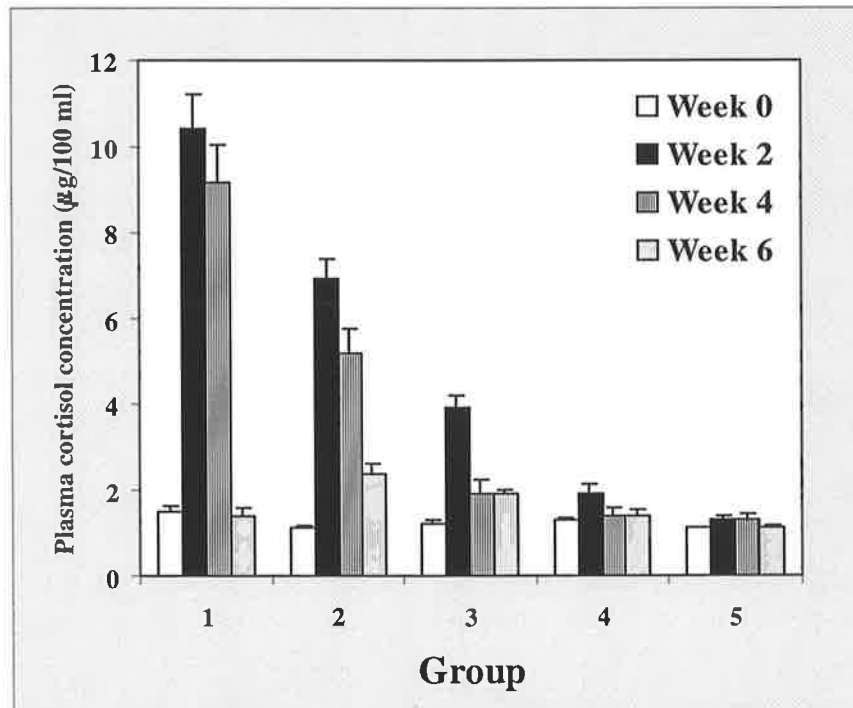


Figure 3.1. Plasma cortisol concentration ($\mu\text{g}/100\text{ ml}$) for the groups of Merino sheep treated with different doses of cortisol; measured 2 weeks before (designated as week 0) during (weeks 2 and 4) and 2 weeks after (week 6) cortisol injection (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

3.3.3. The effect of cortisol injection on wool production, staple length and fibre diameter

Wool production. There was no significant difference in the average clean wool production between groups two weeks before commencement of cortisol injection (Figure 3.2). A significant ($P < 0.0001$) progressive decline in the amount of wool produced in all cortisol treated groups and the control group then occurred. The drop in clean wool production (24, 26, 39, 41 and 63% of pre-treatment) was in proportion

to cortisol dose level, being highest in group 1 and lowest in group 5. Wool production was at the lowest level at the end of the treatment period (4 weeks after cortisol injection started) and it gradually began to recover after this period. Wool production remained depressed even four weeks after cessation of cortisol injection. By week 12 (i.e. 8 weeks after cessation of cortisol injection) all groups had returned to their pre-treatment wool growth rates. There was a significant ($P < 0.05$) interaction between week and dose, indicating that the decline in amount of wool produced differed between groups, the higher dose groups having a greater decline and slower recovery than lower dose groups.

Staple length. There was no significant difference in the average staple length between groups one month before initiation of cortisol injection, but there was a significant ($P < 0.001$) decline in staple length in all groups during the treatment period (Figure 3.3). Again the drop in staple length was more evident in groups of sheep which received higher doses of cortisol. Staple length dropped to 38, 48, 64, 61 and 68 percent of the pre-treatment level in groups 1, 2, 3, 4 and 5 respectively. The decline in staple length closely paralleled that in patch wool growth ($r^2 = 0.41$, $P < 0.001$) (Figure 3.4). This suggests that fibre diameter changes in response to cortisol, must have been small. Indeed this was the case (see next section).

Fibre diameter. There was no significant difference in the mean fibre diameter between groups before commencement of cortisol injection (Mean fibre diameter of group 2 was slightly higher than the rest of the groups) but there was a significant decline in fibre diameter in all groups during the treatment period (Table 3.4). At no time did mean fibre diameter differ between treatment groups. No significant relationship was found between fibre diameter and clean wool production.

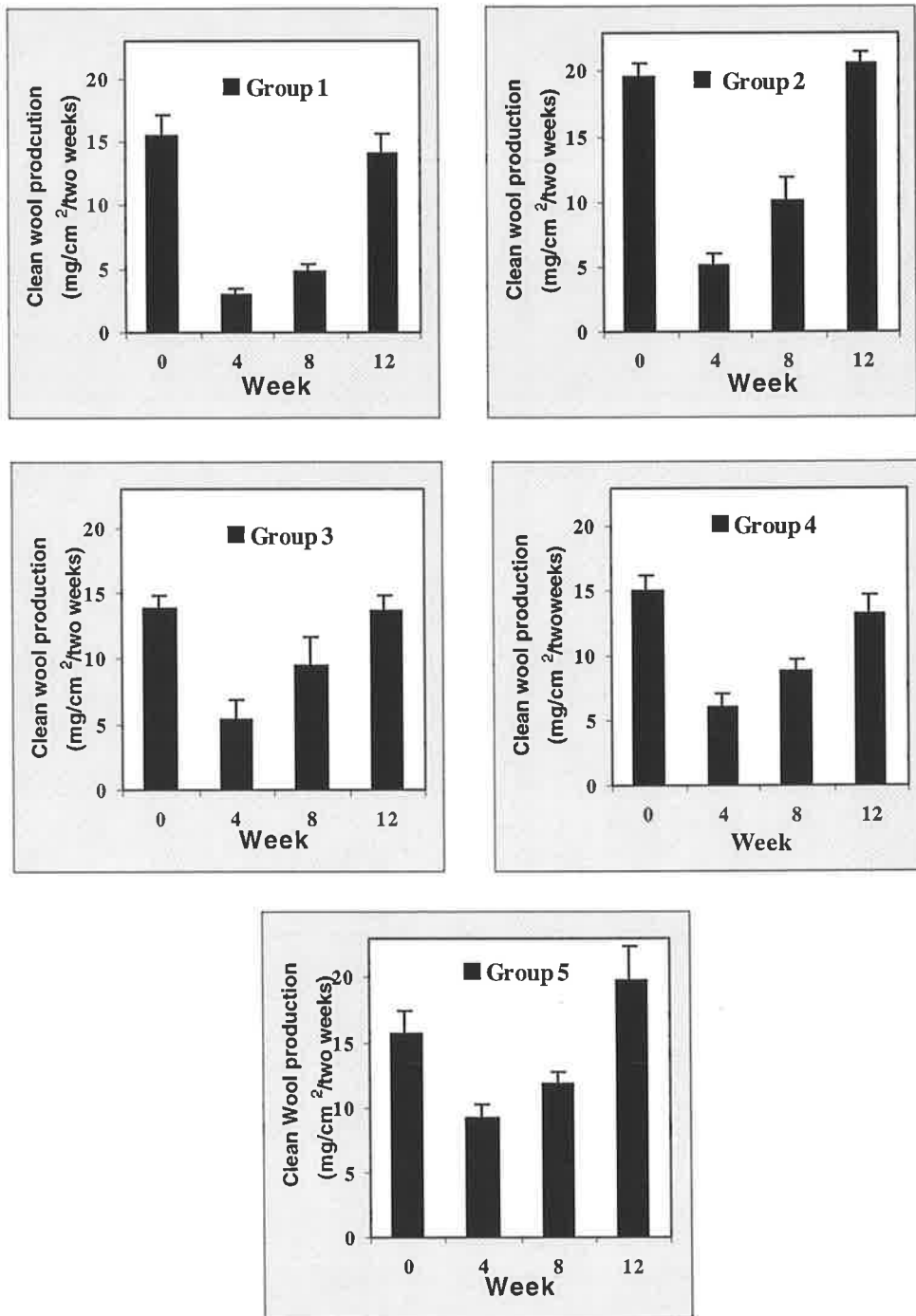


Figure 3.2. Clean wool production per unit area of skin ($\text{mg}/\text{cm}^2/\text{two weeks}$) for the groups of Merino sheep treated with different doses of cortisol; 2 weeks before (designated as week 0), during (week 4) and after (weeks 8 and 12) cortisol injection (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

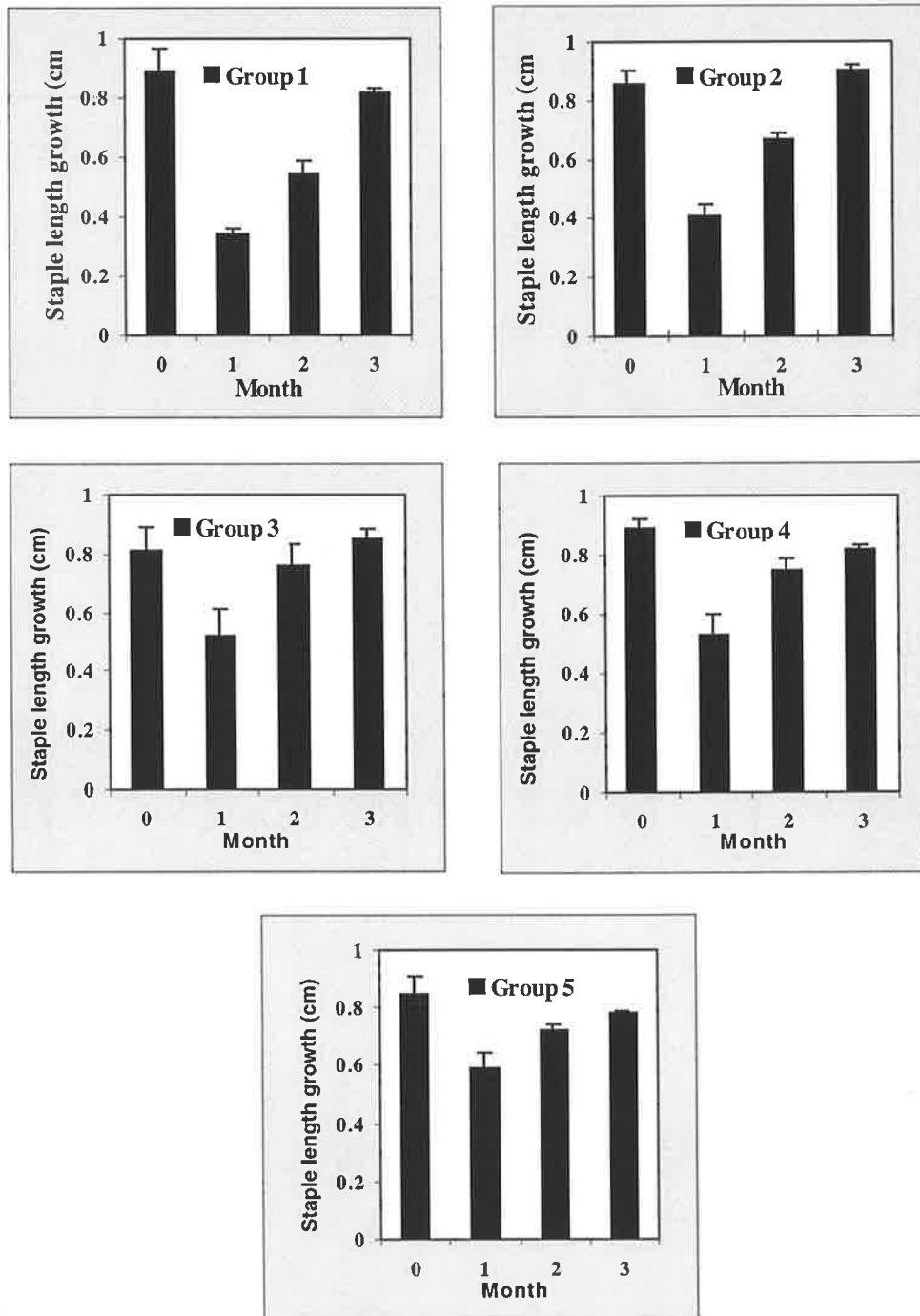


Figure 3.3. Staple length growth (cm/month) for the groups of Merino sheep treated with different doses of cortisol; 1 month before (designated as week 0), during (month 1) and after (months 2 and 3) cortisol injection (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

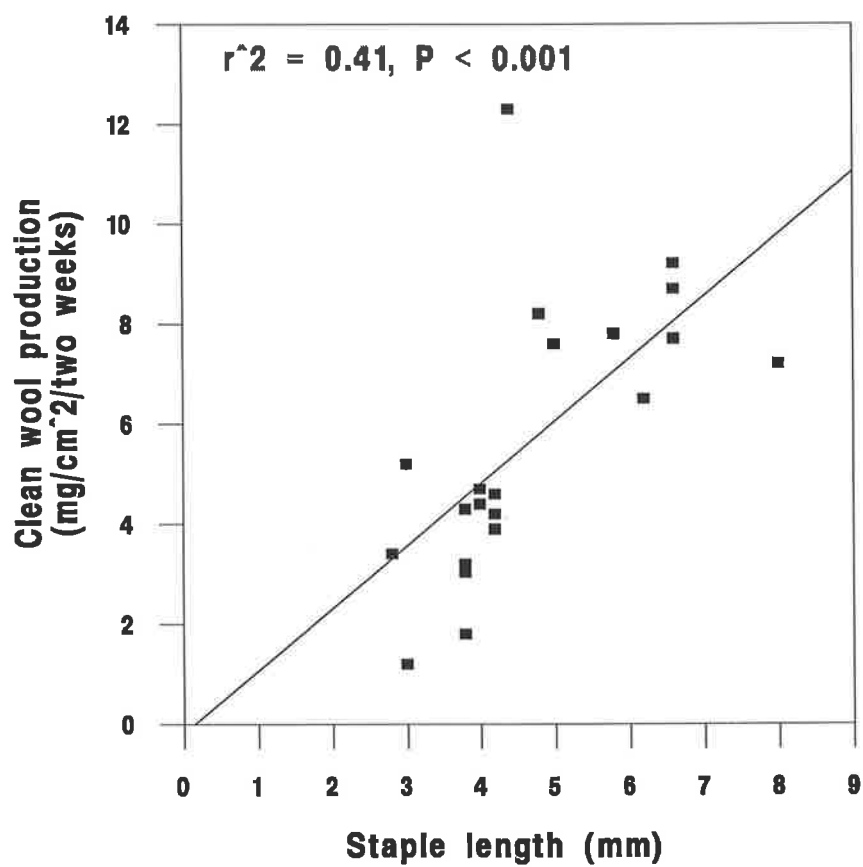


Figure 3.4. The relationship between staple length (mm) and clean wool production per unit area of skin (mg/cm²/two weeks) of groups of Merino sheep treated with cortisol at different doses.

Table 3.4. Fibre diameter (μm) for the groups of Merino treated with different doses of cortisol; 2 weeks before (designated as week 0), during (week 4) and after (week 10) cortisol injection (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

Group	Week		
	0	4	10
1	21.2 \pm 1.0 ^a	18.1 \pm 0.3 ^a	20.2 \pm 0.9 ^a
2	23.0 \pm 1.0 ^a	20.0 \pm 1.1 ^a	23.3 \pm 1.3 ^a
3	21.1 \pm 0.8 ^a	17.3 \pm 0.9 ^a	19.7 \pm 0.9 ^a
4	21.8 \pm 0.6 ^a	17.8 \pm 1.0 ^a	20.0 \pm 0.7 ^a
5	20.8 \pm 0.5 ^a	18.5 \pm 0.8 ^a	20.4 \pm 0.7 ^a

a = no significant difference in fibre diameter between groups within weeks.

3.3.4. The effect of cortisol injection on the formation of wool breaks and fibre shedding

Coinciding with the resumption of normal wool growth 12 weeks after cessation of cortisol injection, a clear 'break' appeared in the wool of cortisol-treated sheep (Plate 3.1). This wool breaks could be described as an opening of a clear channel through the wool sample. The magnitude of wool breaks was proportional to the dose level being greater in groups 1 and 2 and lower in groups 3 and 4. No wool breaks was observed in the sheep of the control group (Plate 3.2).

During the treatment and post-treatment periods, shedding of fibres occurred in response to cortisol injection. It was observed that the extent of fibre shedding varied over the body, however it was more pronounced on the rump and belly and less severe on the shoulders. Local wool casting occurred particularly on the legs and belly areas of individual sheep of groups 1 and 2 (Plate 3.3). As a result of the

formation of a wool break, staples were so tender that they could be easily plucked from the surface of skin.

Histological examinations revealed that at the point of formation of wool breaks, the structure of shed fibres had undergone substantial changes. Some fibres were malformed or degraded and some had holes and cracks in their surface. A small number of fibres exhibited a region of swelling containing darkly-stained bodies just preceding the point of shedding (Plate 3.4). While the majority of fibres which exhibited a region of swelling, in a small number of these fibres disruption was confined to only one side. Under such circumstances, the inner root sheath was carried by the shed fibre forming a distorted end structure which was easily distinguishable from a normal end of an unshed fibre (intact fibre). The distorted end of shed fibres fell into four categories depending upon the structure of the fibre end as follows (Plate 5):

1. Club end fibres. On the ends of these fibres, a thick column of stained inner root sheath was carried by shed fibre forming a club like structure (Plate 3.5).
2. Brush end fibres. Several thin or sometimes two thick columns of stained inner root sheath were carried by shed fibres, forming a brush like structure (Plate 3.5).
3. Step end fibres. A thin stepwise layer of stained inner root sheath was carried by shed fibres forming a step like structure (Plate 3.6).
4. Tapered end fibres. The end of such shed fibres was reduced in diameter and a thin column of stained inner root sheath was carried by shed fibre (Plate 3.6).

Further histological examinations revealed that as a result of elevation in plasma cortisol concentration, fibre cuticle scale pattern and crimp frequency was disrupted in the shed fibres

Elevation of plasma cortisol concentration caused an increase in the percentage of shed fibres. There was a significant ($P < 0.0001$) difference in the mean percentage of shed fibres between the groups (Figure 3.5). On average about 50% of fibres were shed in groups 1 and 2 which was significantly ($P < 0.05$) different from groups 3, 4 and 5. It should be noted however that within cortisol groups there was substantial variation between sheep in shedding percentage with some individuals in the high dose groups having 72% and others only 9%.

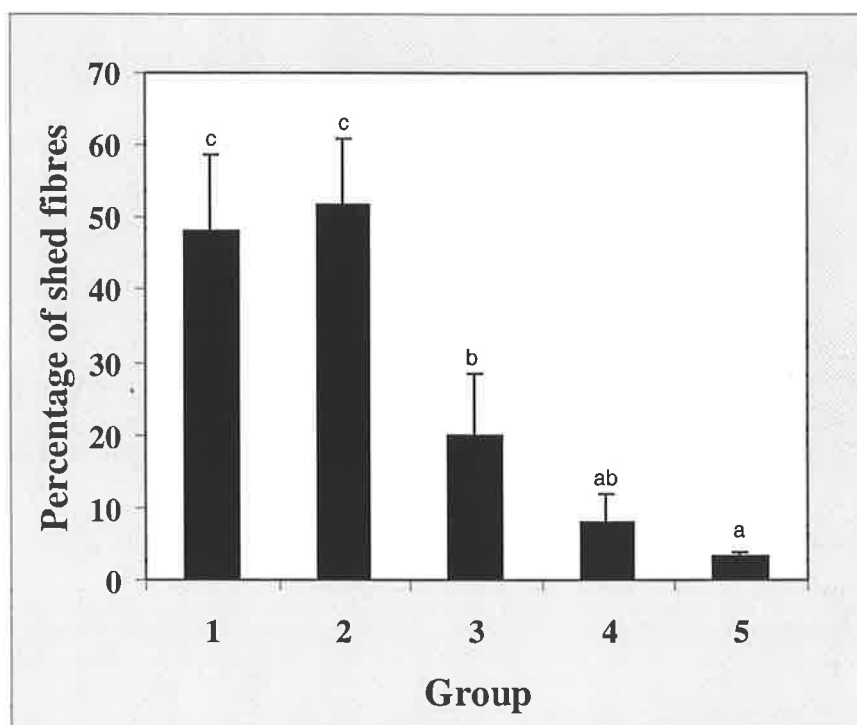


Figure 3.5. Percentage of shed fibres for groups of Merino sheep treated with different doses of cortisol. Measurements were made on staples at the end of the experimental period (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.



Plate 3.1. Coinciding with the resumption of normal growth, a clear break appeared in the wool above the surface of the skin of cortisol-treated Merino sheep.

(a) Midside wool sample from a control sheep (group 5).

(b) Midside wool sample from a sheep which received daily injection of 2.86 mg of cortisol/kg body weight (group 1).

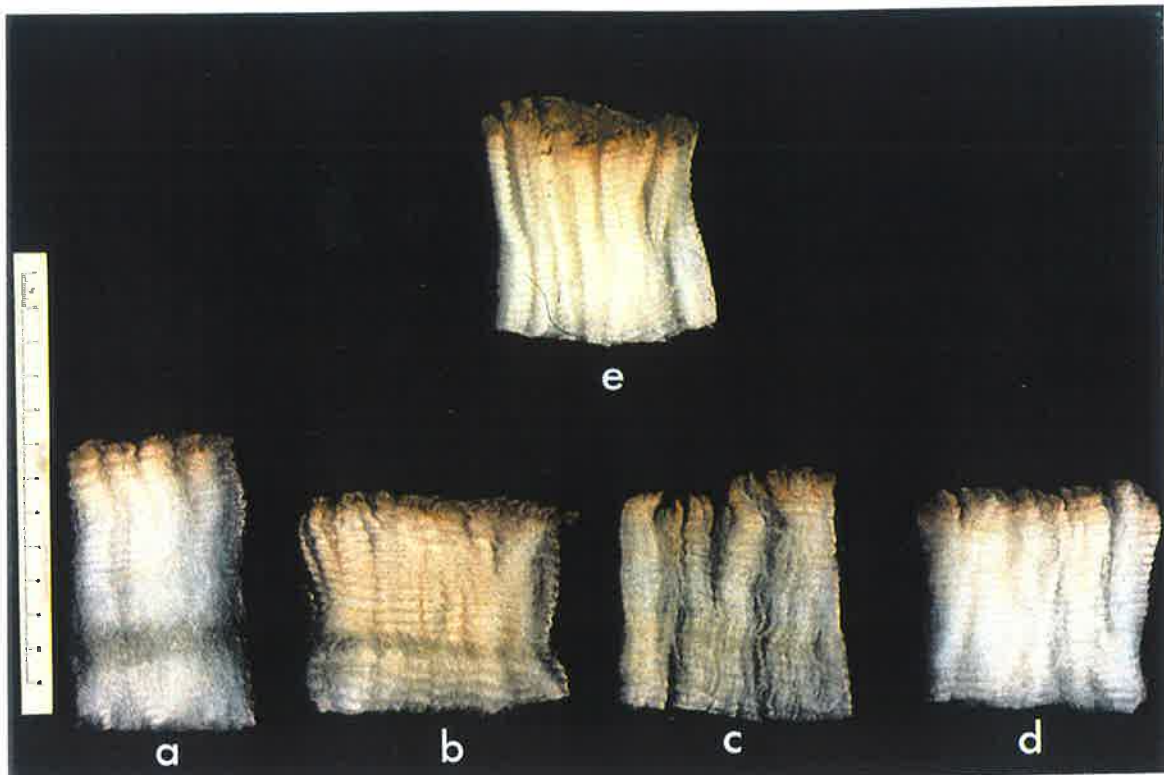


Plate 3.2. The magnitude of wool breaks was greater in groups which received higher dose of cortisol injection. Midside wool samples taken from Merino sheep injected with:

- (a) 2.86 mg of cortisol/kg body weight.
- (b) 1.42 mg of cortisol/kg body weight.
- (c) 0.56 mg of cortisol/kg body weight.
- (d) 0.19 mg of cortisol/kg body weight.
- (e) 0 mg of cortisol/kg body weight.



Plate 3.3. As a result of cortisol injection, complete wool casting occurred at the legs and belly areas of the sheep most affected by cortisol injection in groups 1 and 2. This plate shows a sheep from group 1 which received daily injection of 2.86 mg of cortisol/kg body weight for a period of 4 weeks.

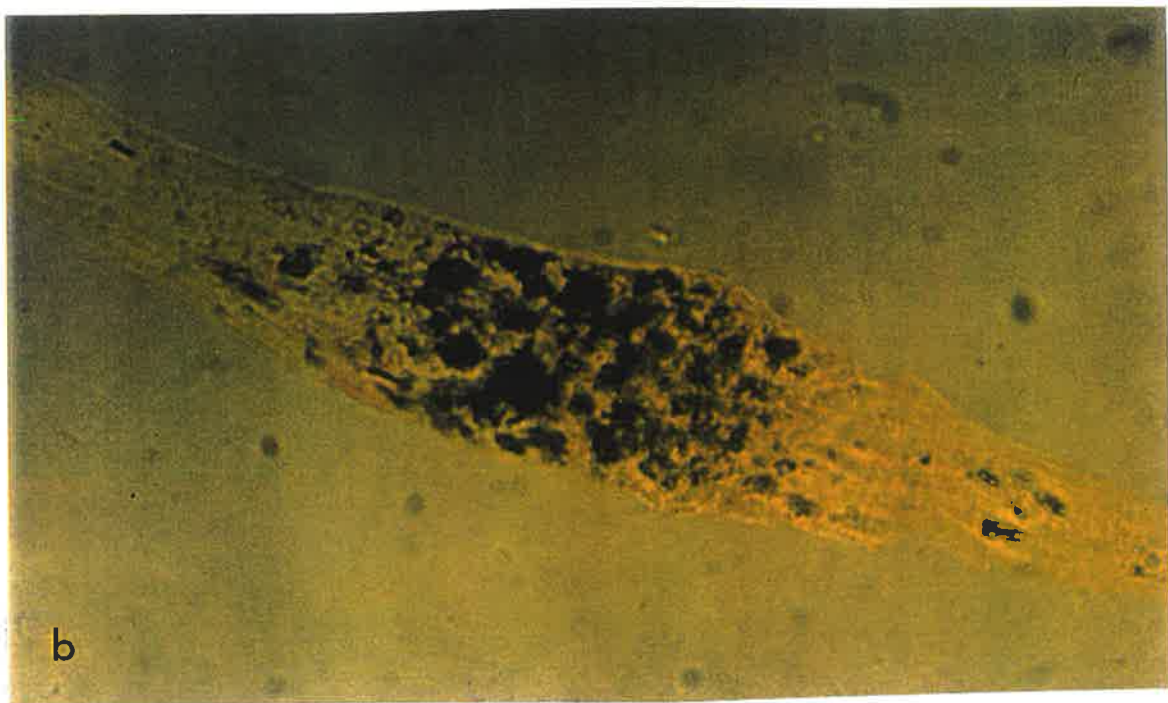
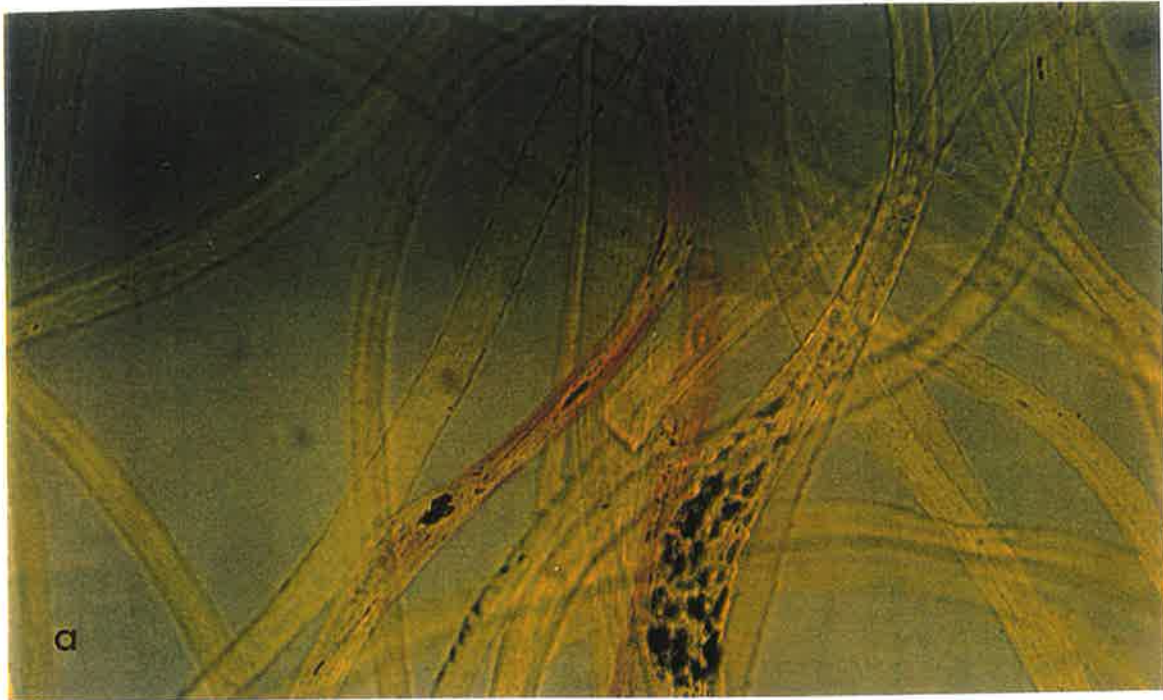


Plate 3.4. Fibres from the weakened region in the fleece of a sheep injected daily with 2.86 mg of cortisol/kg body weight for a period of 4 weeks.

(a) There is a mixture of shed, thickened and continuously growing fibres; Magnification: 1188x

(b) A fibre which exhibited a region of swelling containing darkly-stained bodies. Note the cuticle scale pattern has been disrupted at the point of swelling; Magnification: 1188x.

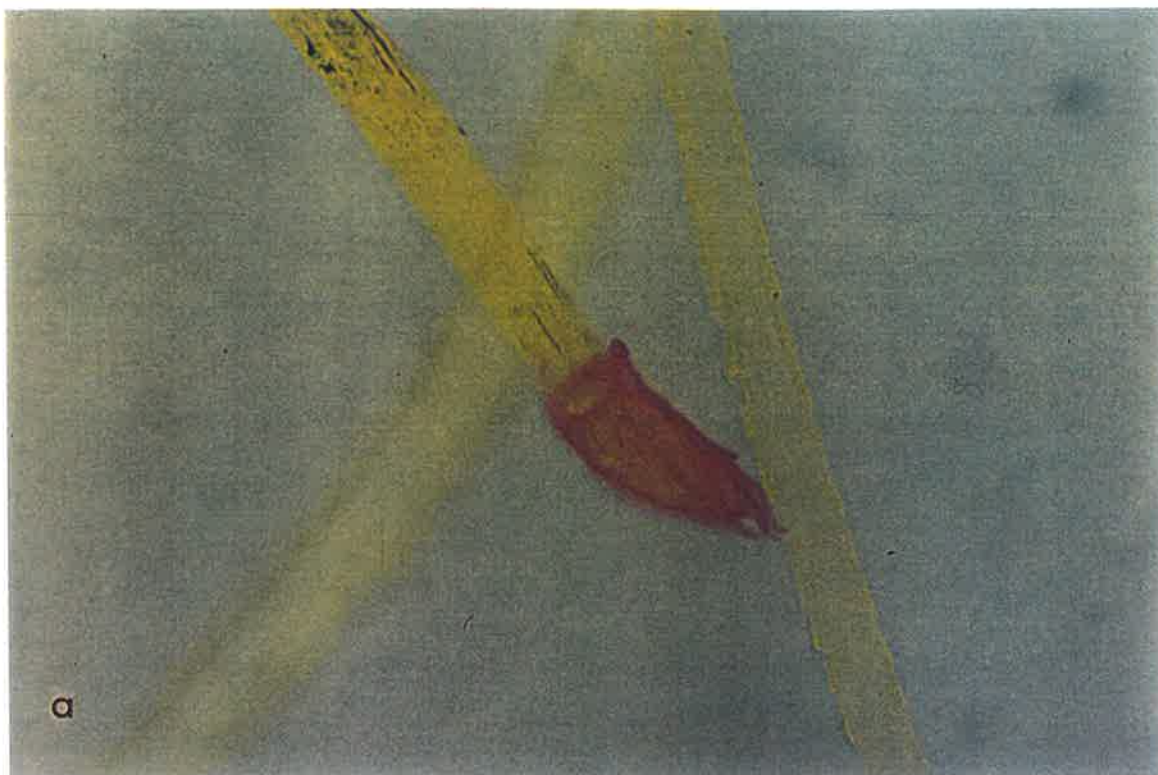


Plate 3.5. Magnification of fibres with (a) club end and (b) brush end from a sheep injected daily with 2.86 mg of cortisol/kg body weight for a period of 4 weeks; Magnification: 1188x.

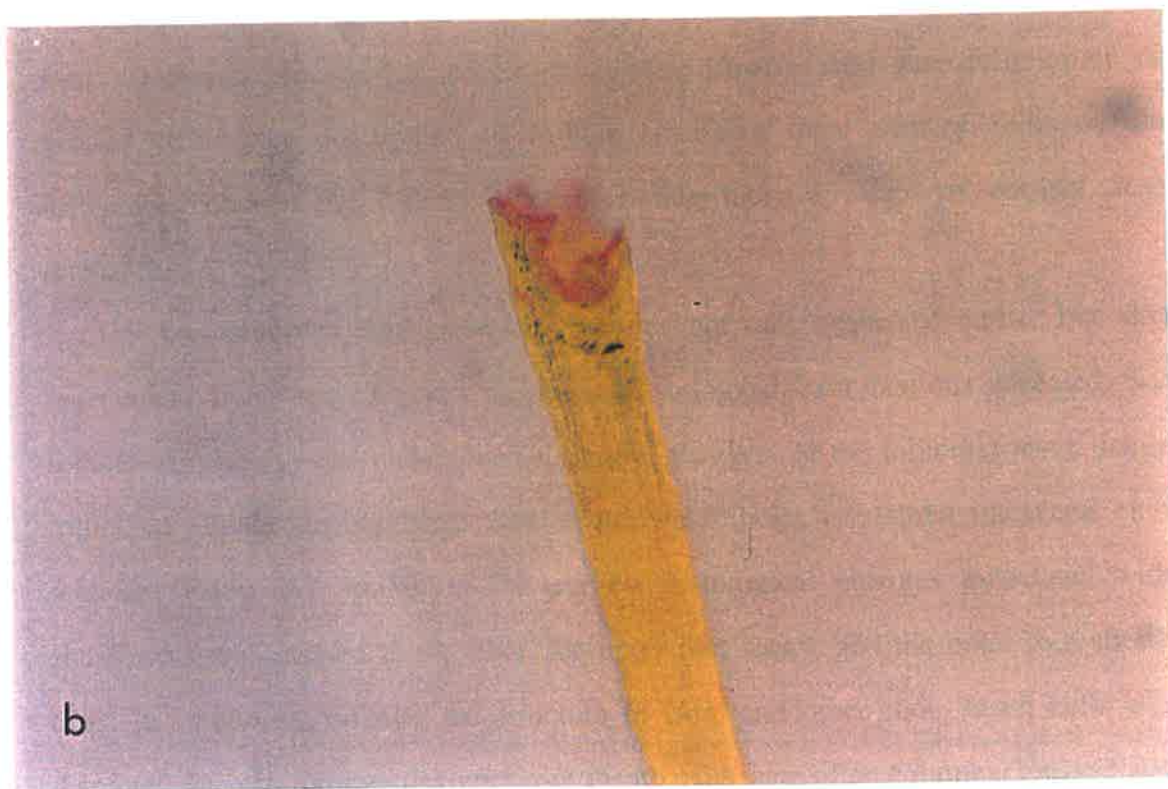


Plate 3.6. Magnification of fibres with (a) tapered end (b) step end from a sheep injected daily with 2.86 mg of cortisol/kg body weight for a period of 4 weeks; Magnification: 1188x.

3.3.5. The effect of cortisol injection on follicle shutdown

There was no significant difference in the percentage of active follicles between groups one week before treatment period. Injection of cortisol was associated with a significant ($P < 0.0001$) progressive increase in the percentage of follicle shutdown (Figure 3.6). Inactivity of follicles started two weeks after commencement of cortisol injection. The maximum level of follicle shutdown occurred 2 weeks after cortisol injection ceased. A significant ($P < 0.0001$) difference in follicle inactivity was found between the groups at this time. It took 9 to 10 weeks before all inactive follicles regenerated. Analysis of variance revealed that there was a significant ($P < 0.0001$) interaction between week and dose.

Primary and secondary follicle shutdown. A summary of the measurement of primary and secondary follicle shutdown of treated groups is shown in Table 3.6. A total of 2520 primary and 47880 secondary follicles were examined from weeks 2 to 9 (in over the period of maximal follicle shutdown). Clearly secondary follicles of all groups were more susceptible to follicle shutdown than primary follicles, the difference between the follicle types becoming more apparent as cortisol dose increased.

It was observed that secondary follicles not only regressed earlier but also regenerated later than primary follicles. Histological examinations revealed that follicle morphology had undergone substantial changes. At the follicular level, active follicles could be distinguished from shutdown follicles in stained transverse skin sections (Plates 3.7 and 3.8). The earliest histological changes associated with shutdown were detected in the fibre, the inner root sheath and the outer root sheath cells. In quiescent follicles the structure of fibre and inner root sheath cells was disrupted. In such follicles the outer root sheath cells were often columnar and radially or spirally arranged in contrast to the randomly-arranged cells in "normal" follicles.

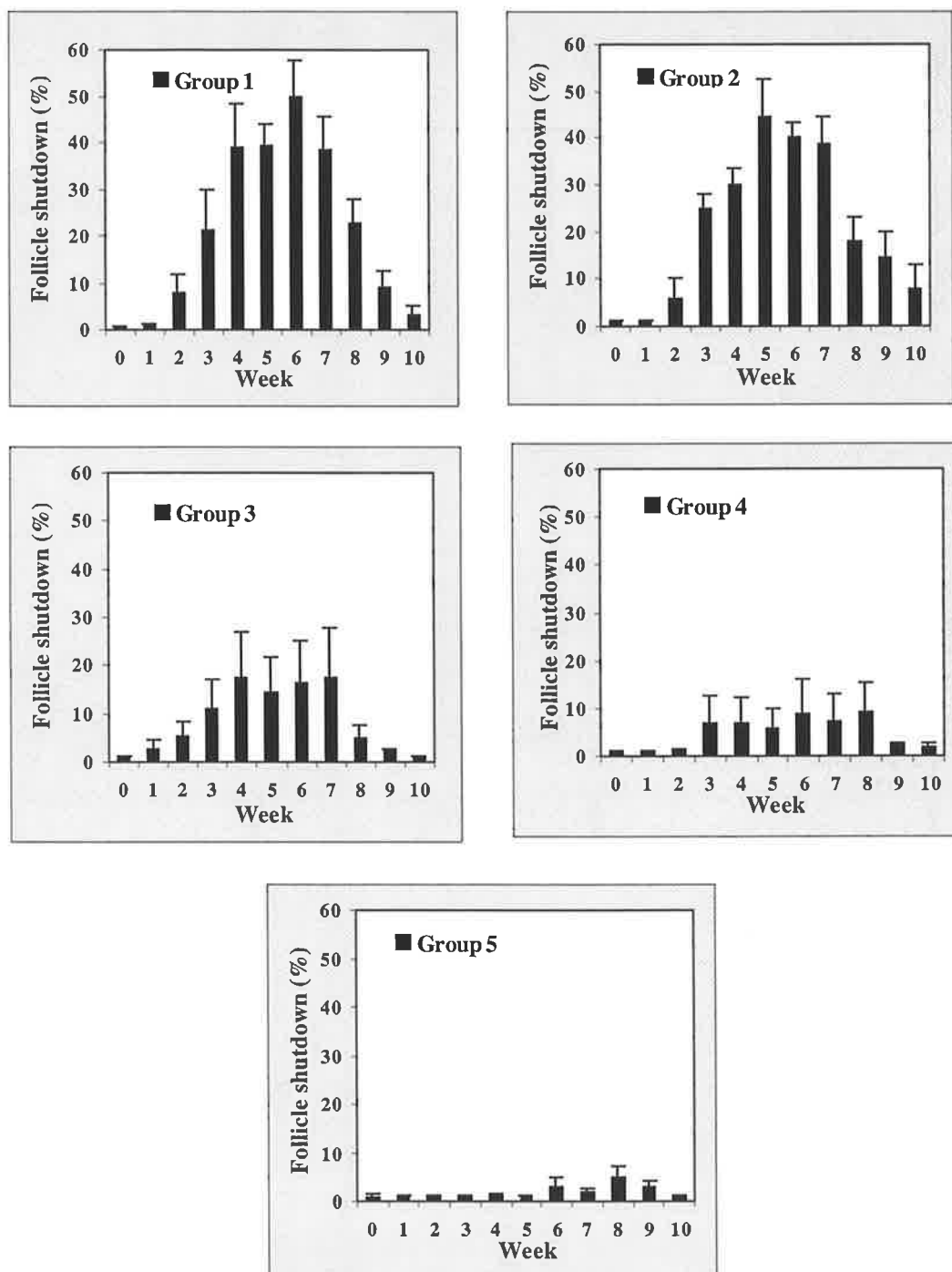


Figure 3.6. Percentage of midside follicle shutdown for the groups of Merino sheep treated with different doses of cortisol; measured 1 week before (designated as week 0), during (weeks 1-4) and after (weeks 5-10) cortisol injection (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

Table 3.5. Average number and percentage of midside primary and secondary follicle shutdown for the groups of Merino sheep treated with doses of cortisol; between weeks 2 and 9 of the experiment. Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

Group	Weeks 2 to 9					
	Primary			Secondary		
	Total	FSD*		Total	FSD*	
		No.	%		No.	%
1	600	37	6.2	11400	3445	30.3
2	480	14	3.0	9120	2840	31
3	480	13	2.7	9120	1061	11.6
4	480	0	0	9120	588	6.5
5	480	0	0	9120	219	2.4

* FSD; follicle shutdown.

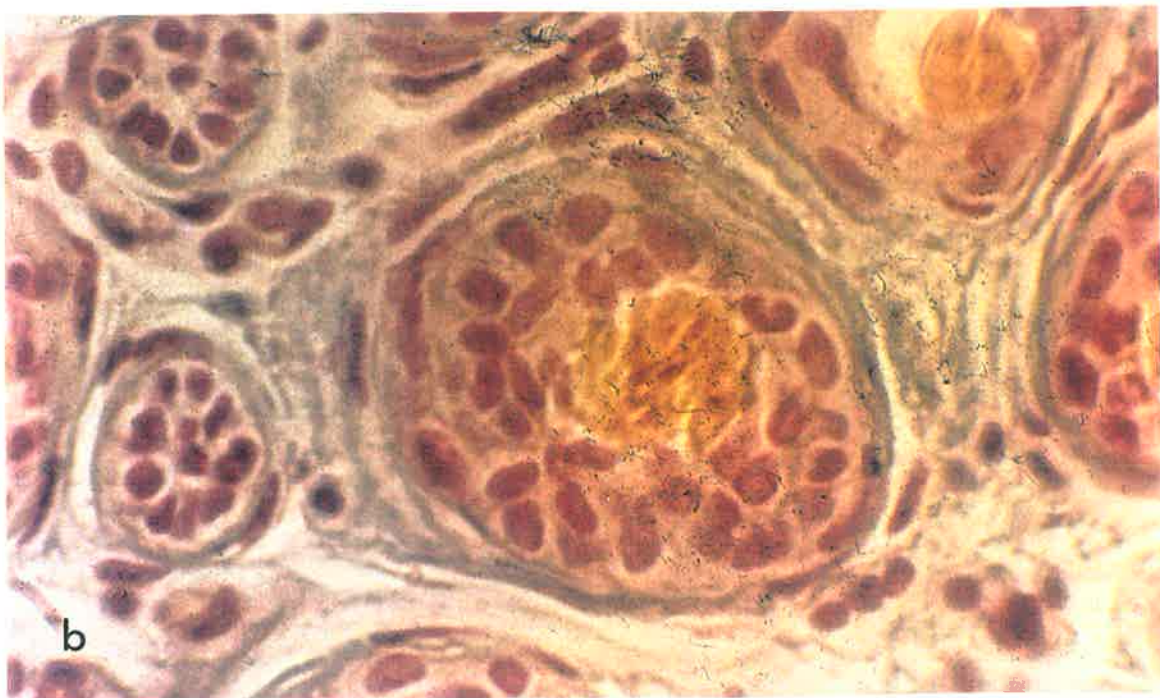
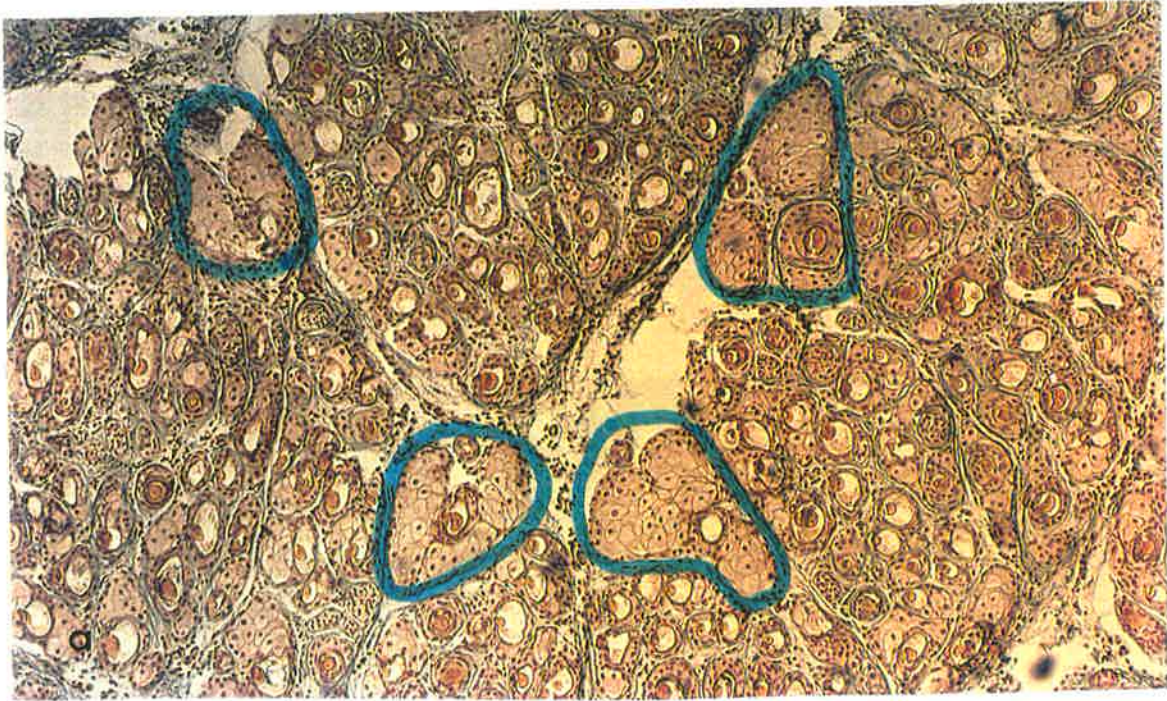


Plate 3.7. Transverse section through the skin sample (at the sebaceous gland level) from an adult Merino sheep after daily administration of 2.86 mg of cortisol/kg body weight for a period of 4 weeks. Delineated follicles are primary follicles associated with arrector pili muscle, sebaceous and sweat glands. All other follicles are a mixture of active and inactive secondary follicles; Magnification: (a) 43x (b) 425x.



Plate 3.8. Inactive secondary follicles in transverse section through the skin sample (at the sebaceous gland level) from an adult Merino sheep after daily administration of 2.86 mg of cortisol/kg body weight for a period of 4 weeks. In an inactive follicle, fibre and inner root sheath which respectively take up yellow and red stains are absent or disrupted. Note the hyperplastic outer root sheath cells of quiescent follicles are often columnar and radially or spirally arranged; Magnification: 1188x.

3.3.6. The effect of cortisol injection on staple strength

As a result of treatment, staple strength was significantly ($P < 0.0001$) reduced at the end of the experiment (Figure 3.7). The extent of reduction in staple strength was in proportion to the dose level, being greatest in group 1 which received 2.86 mg of cortisol/kg body weight.

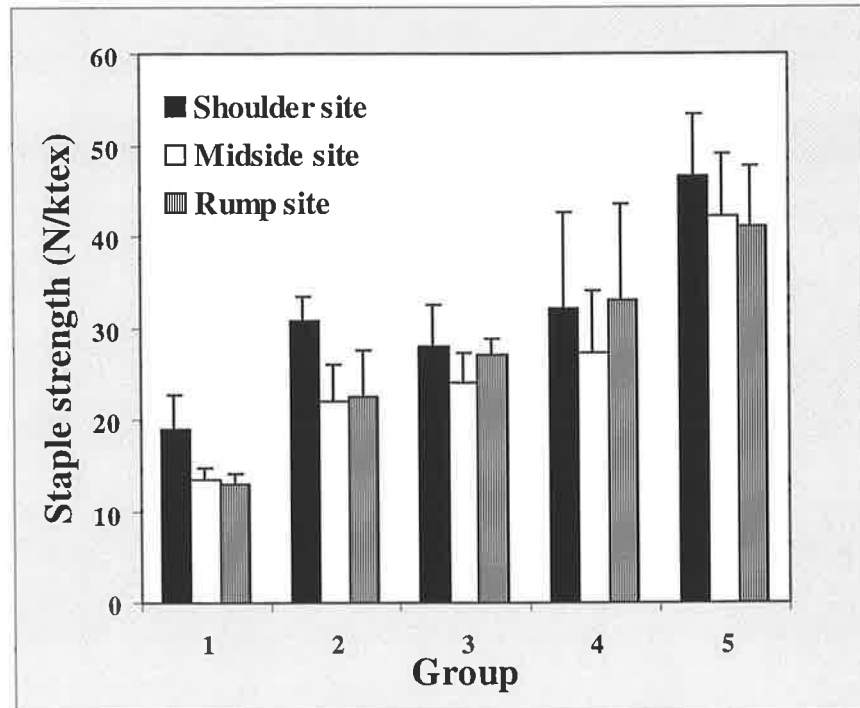


Figure 3.7. Staple strength values at shoulder, midside and rump areas for the groups of Merino sheep treated with different doses of cortisol; measured at the end of the experimental period. Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

There was no significant difference in staple strength between sites although the values for shoulder were slightly higher than midside and rump areas.

3.3.7. The effect of cortisol injection on staple intrinsic strength

There was no significant difference in the staple intrinsic strength of groups at the end of the experiment, however the control group had slightly higher values than the combined cortisol treated groups (Figure 3.8).

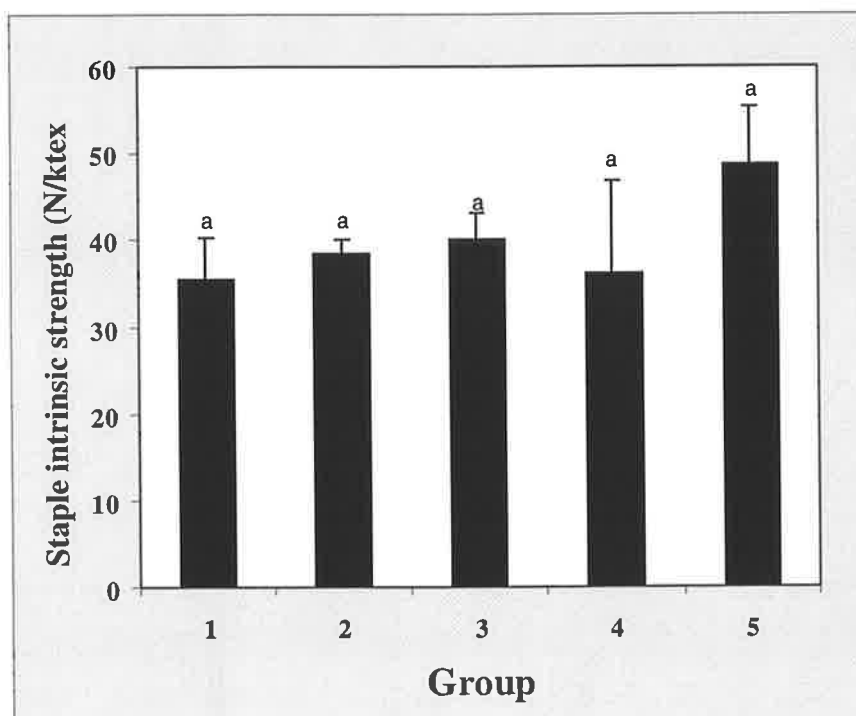


Figure 3.8. Midside staple intrinsic strength (N/ktex) for the groups of Merino sheep treated with different doses of cortisol; measured at the end of the experimental period (Means with s.e.m.). Groups 1, 2, 3, 4 and 5 were respectively injected daily with 2.86, 1.42, 0.56, 0.19 and 0 mg of cortisol/kg body weight for a period of 4 weeks.

a = no significant difference between groups.

3.3.8. Relationships between follicle and fibre characteristics and follicle shutdown, fibre shedding and plasma cortisol concentration

Variation in midside staple strength in different groups which was generated by cortisol injection was related to follicle shutdown ($r^2 = 0.43$, $P < 0.0001$) (Figure 3.9), (Table 3.7) and fibre shedding ($r^2 = 0.43$, $P < 0.005$) (Figure 3.10). A highly

significant relationship was found between follicle shutdown and fibre shedding ($r^2 = 0.78$, $P < 0.0005$) (Figure 3.11). Follicle shutdown was closely associated with plasma cortisol concentration ($r^2 = 0.60$, $P < 0.0001$) (Figure 3.12).

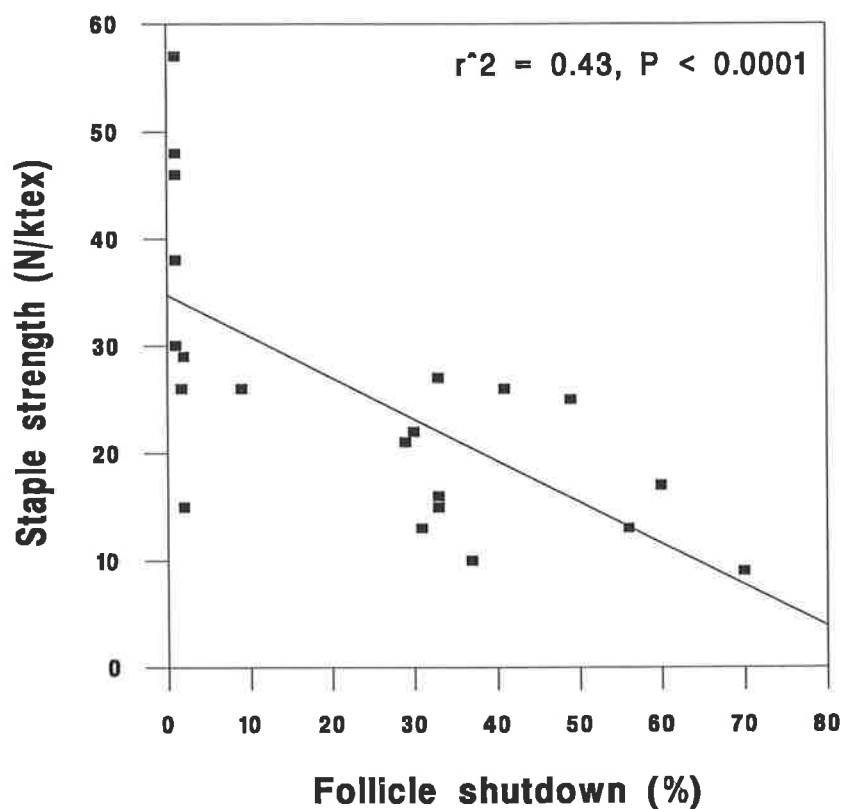


Figure 3.9. The relationship between follicle shutdown (%) and midside staple strength (N/ktex) of groups of Merino sheep treated with cortisol at different doses (Note the initial high variance of the 0 group due to phenotypic selection for divergent staple strength).

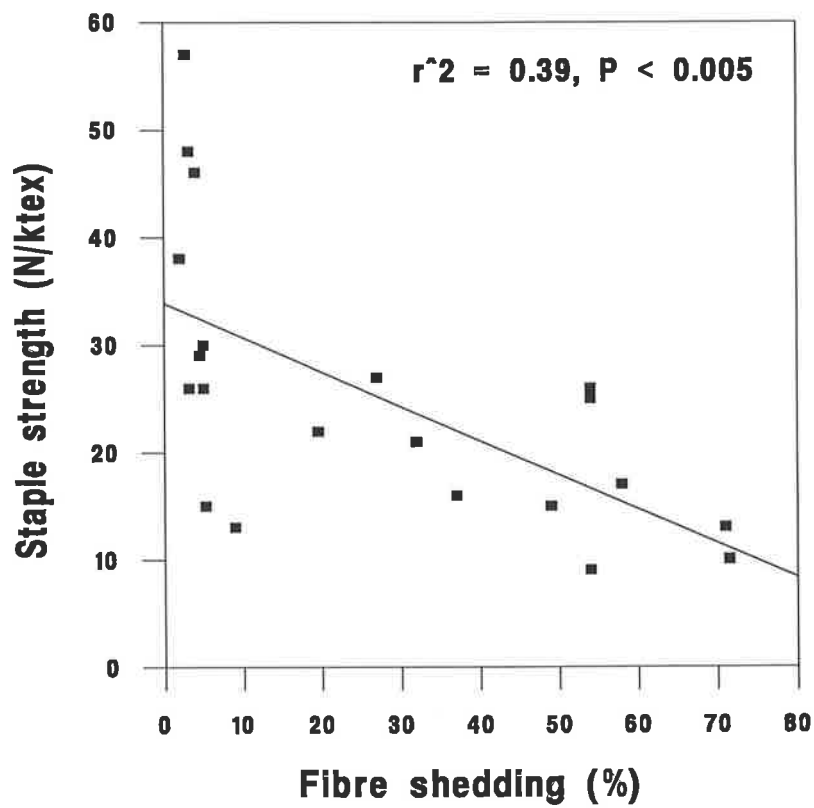


Figure 3.10. The relationship between fibre shedding (%) and staple strength (N/ktex) of groups of Merino sheep treated with cortisol at different doses (Note the initial high variance of the 0 groups due to phenotypic selection for divergent staple strength).

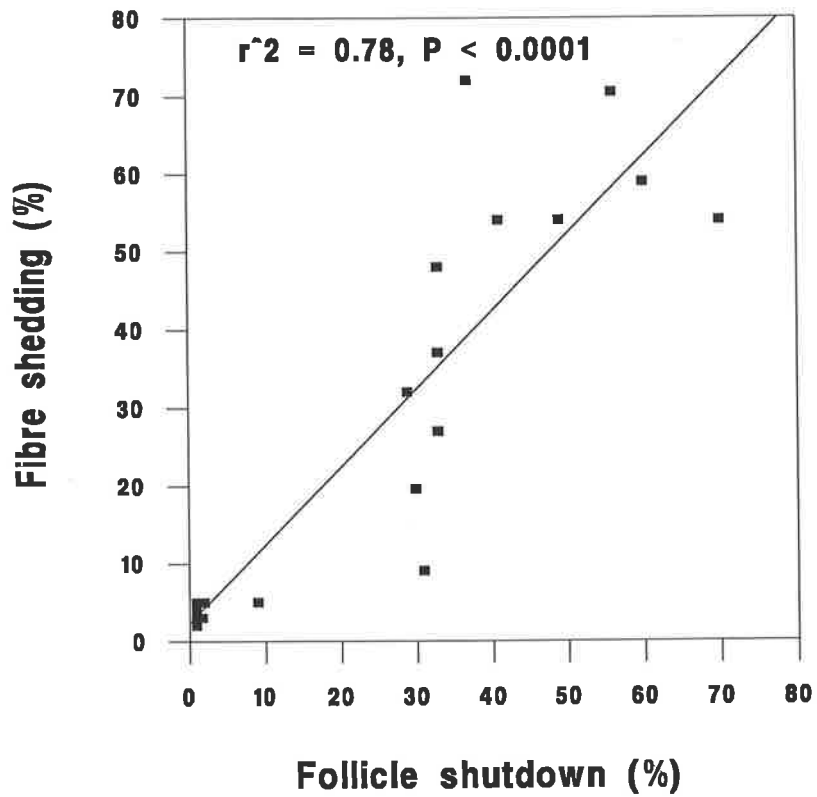


Figure 3.11. The relationship between follicle shutdown (%) and fibre shedding (%) of groups of Merino sheep treated with cortisol at different doses.

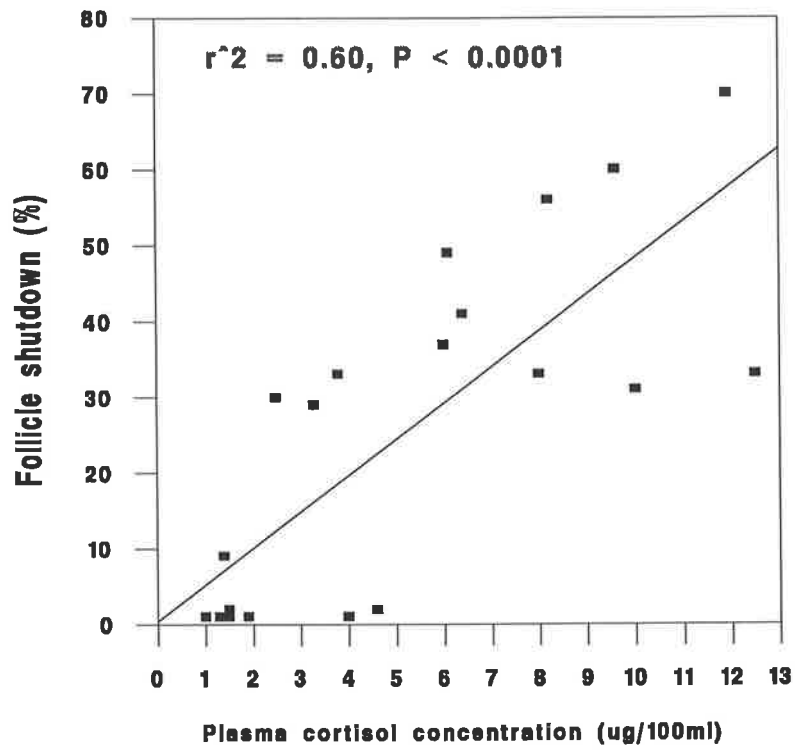


Figure 3.12. The relationship between plasma cortisol concentration ($\mu\text{g}/100$ ml) and follicle shutdown (%) of groups of sheep treated with cortisol at different doses.

Table 3.6. Table of r^2 values for the relationships between various follicle and fibre characteristics including staple length (SL), minimum fibre diameter (MFD), clean wool production (CWP), and follicle shutdown (FSD), fibre shedding (FS), staple strength (SS), staple intrinsic strength (SIS) and plasma cortisol concentration (PCC).

Characteristic	SIS	PCC	CWP	SL	MFD	SS	FSD	FS
Staple intrinsic strength	1.00							
Plasma cortisol concentration	0.01 ^{NS}	1.00						
Clean wool production	0.11 ^{NS}	0.36 ^d	1.00					
Staple length	0.27 ^e	0.35 ^d	0.41 ^c	1.00				
Minimum fibre diameter	0.30 ^e	0.01 ^{NS}	0.13 ^{NS}	0.09 ^{NS}	1.00			
Staple strength	0.50 ^b	0.40 ^d	0.48 ^b	0.54 ^b	0.20 ^e	1.00		
Follicle shutdown	0.01 ^{NS}	0.60 ^a	0.54 ^a	0.49 ^b	0.01 ^{NS}	0.43 ^a	1.00	
Fibre shedding	0.01 ^{NS}	0.45 ^a	0.51 ^b	0.46 ^b	0.01 ^{NS}	0.39 ^d	0.78 ^a	1.00

^a $P < 0.0001$, ^b $P < 0.0005$, ^c $P < 0.001$, ^d $P < 0.005$, ^e $P < 0.01$, ^{NS} Not significant.

3.3.9. Determinants of staple strength

In the analysis of all measured characteristics associated with staple strength, it was possible to include the percentage of follicle shutdown, diameter of fibres of a staple, length of staple and production of clean wool (it was not possible to include staple intrinsic strength, because its unit of measurement was similar i.e. N/ktex). It was found that follicle shutdown, minimum fibre diameter and staple length accounted for 65% of the variance in staple strength. The equation which best predicted the staple strength was:

$$SS = -25.67 - 0.064 \text{ FSD} + 2.19 \text{ MFD} + 37.03 \text{ SL} \quad (R^2 = 0.65)$$

where SS is staple strength.

FSD is follicle shutdown.

MFD is minimum fibre diameter.

SL is staple length.

3.4. DISCUSSION

3.4.1. Design and objectives of experiment

The present study is the first study undertaken to successfully establish a relationship between follicle shutdown, fibre shedding, plasma cortisol concentration and staple strength. Related studies with hormones of the hypothalamic-pituitary-adrenal axis reviewed previously in chapter 1, provided evidence to suggest that follicle shutdown can be induced by these hormones. Lindner and Ferguson (1956) were the first to show that administration of pituitary ACTH to sheep, suppressed wool growth by inducing follicle shutdown, however plasma cortisol concentration was not measured nor were detailed histological studies made. Chapman and Bassett (1970) showed that high plasma cortisol concentration caused follicle shutdown, but they made no attempt to correlate this with the strength of staple. In this chapter an attempt was made to elucidate the possible mechanisms responsible for, and associated with, follicle shutdown in relation to the occurrence of tender wool.

Merino sheep were selected on the basis of staple strength and exposed to different doses of cortisol to generate sufficient variation in wool growth rate, plasma cortisol concentration, follicle shutdown and fibre shedding to identify possible determinants of staple strength and mechanisms operating in dysfunctional follicles. Whilst it was recognised that selection of sheep was phenotypic, it was considered that such

selection would provide a strong indication of where genetic differences may lie because:

(A) The magnitude of the heritability estimate of staple strength and fibre diameter is 0.40-0.50 (Li and Lewer unpublished).

(B) Comparisons were made in similar environmental conditions after a long stabilisation period (≥ 16 weeks) to remove transient environmental effects.

3.4.2. Basis of choosing the doses and duration of cortisol injection

The basis of choosing the doses of cortisol was the endogenous rate of sheep cortisol production. Patterson and Harrison (1967) indicated that the rate of cortisol secretion is about 12 μg per minute or 17.3 mg per day. All the treatments in the literature were of high doses of cortisol, whereas in this experiment a range of low, intermediate and high doses were used. To generate large variations in follicle shutdown sheep were exposed to five different doses of cortisol injection. Groups of sheep received 0, 0.5, 1.5, 3.9 and 7.8 times the normal level of cortisol production per day, the logic being that values of 5 to 10 times the normal plasma cortisol concentration have been frequently observed in sheep under stress and in ewes with pregnancy toxaemia (Lindner, 1959; Reid, 1960a).

Previous studies have used a long duration of exposure to cortisol to cause a break in the wool. Chapman and Bassett (1970) used a treatment of 12 weeks duration to inhibit wool growth, while Lindner and Ferguson (1956) used 8 weeks duration. Spurlock and Clegg (1962) used a duration of 15 days at a cortisol dose of 300 mg per day to cause wool breaks and this was not successful. Intravenous infusion of 0, 5, 10, 20 and 40 mg of cortisol for five consecutive three day periods had no effect on follicle mitotic activity (Scobie, 1992). In the present experiment a duration of 4 weeks was used because such a duration is a reasonable time for sheep to experience elevated cortisol in response to pregnancy toxaemia, disease or blowfly strike.

There is evidence that the nutritional state of sheep would influence cortisol response. Thwaites (1972) showed that poorly-fed sheep had a greater weakness in

the fibre of sheep treated with cortisol than well-fed sheep. To eliminate the feed factor in the present experiment all groups of sheep were fed at a maintenance level. The fact that all sheep consistently consumed all their daily ration and maintained constant liveweight throughout the experiment means that wool responses were entirely associated with cortisol treatment and not due to a secondary nutritional effect.

3.4.3. The effect of cortisol injection on feed intake

In this experiment, the average live weight of groups was not significantly different before and after treatment period. No loss of feed intake was recorded in any of the sheep. This is in disagreement with the result of Bassett (1963) who found that large doses of cortisol injection depressed feed intake. This discrepancy may be explained by the fact that in the present experiment sheep were fed at maintenance level, while in Bassett's experiment sheep were fed *ad libitum*. While a large dose of cortisol injection is associated with depressed feed intake (Bassett, 1963), at lower doses, feed intake may increase (Spurlock and Clegg, 1963). In man, cortisol over-production is associated with increased appetite (Thorn et al. 1959), while cortisol under-production is associated with loss of appetite (Reichlin and Brown, 1960). It is not just the dose, but also the duration of cortisol injection that is important in the depression of feed intake (Chapman and Bassett, 1970).

3.4.4. The effect of cortisol injection on plasma cortisol concentration

The average pre-treatment plasma cortisol concentration of sheep groups was very low which compares favourably with those found by Bassett and Hinks (1969). The plasma cortisol concentration in the high dose groups showed a marked increase two weeks after cortisol injection started compared with pre-treatment level. The elevation in plasma cortisol concentration was in proportion to the dose level. Plasma cortisol concentration reached a peak two weeks after commencement of injection. The Maximum plasma cortisol level attained (10.43 μg per 100 ml) in group 1 was high

in comparison with the pre-treatment level (1.5 μg per 100 ml), but values over 5 μg per 100 ml have been frequently observed in sheep under psychological stress and in ewes with pregnancy toxaemia (Lindner, 1959; Reid, 1960a). Hence such stresses are sufficient to impair the normal productivity of sheep due to hyperadrenocortical activity.

There is an indication that 4 weeks after the commencement of injection, the plasma cortisol concentration declined in comparison with the plasma cortisol concentration at two weeks after commencement of cortisol injection. This result suggests that cortisol is being metabolised and removed from the circulatory system at an accelerated rate (Bassett, 1963), as with a moderate increase in plasma cortisol, dilation of dermal capillaries occur (Chapman and Bassett, 1970).

The site of cortisol injection could have a marked impact on the degree of response of animals. In the present experiment, intramuscular injection of cortisol in aqueous suspension form caused a significant ($P < 0.0001$) increase in plasma cortisol concentration for a long period of time. In sheep the plasma half life of cortisol is about 20 minutes (Reid, 1960b) and intravenous injection would result in faster metabolism of this hormone. Percutaneous applications of cortisol in rats inhibits the growth of hair, however this inhibition is limited to the area treated (Downes and Wallace, 1965).

3.4.5. The effect of cortisol injection on wool growth rate

Increase in plasma cortisol was inhibitory to wool growth. Chapman and Bassett (1970) also found that an increase in plasma cortisol caused a progressive decline in the wool production of sheep on restricted food intake. However they indicated that at the same concentration of cortisol, wool production increased in *ad libitum* fed sheep. Therefore the nutritional state of the animal affects the wool production response of sheep to cortisol.

The drop in wool production was more pronounced in the group which received the highest dose of cortisol injection, indicating that reduction in wool growth was in

proportion to the dose level. The major part of the decline during an increase in plasma cortisol resulted mainly from a marked increase in the number of inactive follicles and a reduction in staple length. The contribution of fibre diameter to reducing wool production in response to cortisol was almost zero, because fibre diameter was not significantly affected by cortisol. Analysis of variance revealed that there was no significant interaction between week and dose indicating a similar, low level of change in fibre diameter in all groups. The decrease in staple length without any appreciable change in fibre diameter raises the question "what follicular events are responsible for this pattern of response?" The answer probably lies in the histological events that take place in the keratogenous zone of the fibre. Due to delayed hardening of the layer of the inner root sheath in response to the injection of depilatory compounds, normal activity of inner root sheath cells in the keratogenous zone is disrupted (Chapman, 1989). Likewise sheep infused intravenously with depilatory doses of epidermal growth factor over a period of 24 hours, exhibited delayed hardening of the layers of the inner root sheath which is detectable soon after the start of the infusion (Hollis et al. 1983). This disruption is accompanied by gross dilation of the endoplasmic reticulum with both intra- and inter-cellular accumulation of fluid and flocculant material (Chapman, 1988). The fluid accumulation frequently causes distortion of the adjacent fibre. In the majority of affected follicles the fibre and inner root sheath hardens without any change in fibre diameter. On the other hand elongation of fibre cells, which normally commences in the suprabulbar region of anagen follicles is absent in affected follicles (Hollis et al. 1983). Consequently fibre length decreases irrespective of any change in diameter (see chapter 6, section 6.3.2). Thyroidectomy also depresses wool growth (Ferguson et al. 1965; Hynd et al. 1989), due almost entirely to a decrease in fibre length growth rate (Theriez and Rougeot, 1962). The characteristics of cortisol in depressing wool growth rate without an appreciable change in fibre diameter mimics the effects of thyroxine hormone. One might be tempted to postulate that the suppression of length growth rate by increased plasma cortisol concentration had resulted from a concomitant decrease in thyroxine

production. However, such a postulation would not account for localized stimulation of fibre-length growth by very small doses of cortisol injected intradermally (Downes and Wallace, 1965). Therefore, the suppression of length-growth by cortisol appears to be an effect peculiar to an increase in plasma cortisol concentration. The drop in wool production without any change in fibre diameter has also been induced in sheep treated with cortisol hormone (Chapman and Bassett, 1970).

The drop in clean wool growth rate without any change in the percentage of active follicles in control sheep which did not receive any cortisol injection is intriguing. It can not be attributed to cortisol because the plasma cortisol concentration did not increase significantly in control sheep during the experiment. Therefore the drop in wool growth rate in control sheep is the result of another mechanism. The effects could have been due to slowing of the rate of mitosis resulting from injection-induced vasoconstriction. Vasoconstriction during short term sudden stresses is a well-established phenomenon (Slee, 1966) which causes a reduction in blood supply (Reklewska, 1975) in response to increased secretion of catecholamines (Ferguson, 1949). It is possible that the drop in wool production in control sheep is the result of increased catecholamine secretion from the adrenal gland (see detail in chapter 6, section 6.3.1).

3.4.6. The effect of cortisol injection on the formation of wool breaks and fibre shedding

Coinciding with the resumption of the normal wool growth 12 weeks after cessation of cortisol injection, a 'break' appeared in the wool. The formation of a wool breaks in the course of this experiment can be described as the appearance of a clear window through the wool sample characterised by a zone of weakness. This zone of weakness is attributed to cessation of normal fibre production by a large number of follicles.

The results of this experiment suggest that the formation of wool breaks in treated sheep was associated with high plasma cortisol concentration. This indicates that even in the most advanced breed of sheep such as the Merino which has a low incidence of shedding in response to seasonal environmental stimuli (Ryder, 1962), fibre shedding can be induced. Lindner and Ferguson (1956) also indicated that daily intramuscular injection of 40 I.U. of ACTH for ten weeks diminished wool growth and coinciding with the resumption of wool growth, a few weeks after cessation of injection of ACTH, a break appeared in the fleece. Hence it is likely that hormones at three levels of hypothalamic-pituitary-adrenal (HPA) axis are involved in the formation of wool breaks; corticotropin releasing hormone (CRH) from the hypothalamus, ACTH from the pituitary and cortisol from the adrenal gland. Occurrence of wool breaks can also be induced in the non-shedding breeds when treated with defleecing doses of EGF and cortisol analogues such as dexamethosone trimethyl-acetate (Chapman et al. 1982) or EGF (Hollis et al. 1983).

There is a close resemblance between the wool breaks caused by cortisol and EGF (Hollis et al. 1983). EGF, developed as a defleecing agent, has diverse biological activities including palate formation, incisor eruption and inhibition of wool growth. There is an indication that EGF and cortisol may have an involvement in the formation of wool breaks. Singh-Asa and Waters (1983) indicated that infusion of defleecing doses of EGF caused a seven fold increase in plasma cortisol concentration, which would be sufficient to inhibit fibre production and initiate wool breaks. It is more likely given that EGF receptor sites in the inner and outer root sheath and the bulb of Merino wool follicles (Wynn et al. 1989) (see chapter 6, section 6.3.1) that EGF has direct effects independently of cortisol (see *in vitro* studies).

Disturbance of fibre growth by cortisol can result from reduced cell production in the follicle bulb and impaired protein synthesis in fibre cells in the keratogenous zone (Chapman, 1989). The administration of depilatory dose of cortisol causes an inhibition of mitotic activity (Chapman, 1989), but reduced production of fibre cells

may also result from an increase in apoptosis. Apoptosis is a process involving fragmentation of scattered, single cells and subsequent phagocytosis of the cell fragments by adjacent cells (Kerr et al. 1972). Follicles which shutdown exhibit apoptosis and enter catagen with cessation of fibre growth and eventually fibre shedding takes place (see chapter 6, section 6.2).

Fibre shedding in cortisol treated sheep started on the hairy areas of the hind legs and belly region extending to the rump and the rest of the areas of the body. This sequence of shedding in response to cortisol injection differs from the sequence of natural shedding in the primitive ovine. Natural shedding follows a sequential, bilaterally-symmetrical pattern, commencing on the neck, chest and shoulders, and spreading to the back and rump (Slee, 1959). This suggest that natural shedding is under the control of different hormones. The role of prolactin and melatonin hormones in regulating the timing of shedding and the reactivation of follicles has been implicated in the primitive ovine (Forsyth et al. 1994; Gebbie et al. 1994; Allain et al. 1994).

3.4.7. Histological examination of shed fibres induced by cortisol injection

Histological examination revealed that at the point of wool breaks, numerous distorted end structures (club end, brush end, step end and tapered end) were formed by shed fibres. A feature of fibre shedding is that fibre growth ceases not only by a mere cessation of fibre growth, but with the formation of a distorted end on the shed fibre. A possible explanation for formation of distorted fibre ends induced by cortisol is that when a follicle stops producing a fibre, the inner root sheath and possibly some outer root sheath cells slough into the follicle lumen and subsequently are carried away by the shed fibre. This explanation may not be true for shed fibres in response to seasonal environmental stimuli, since there is a difference in the fibre end structure between naturally shed and induced shed fibre. Inner root sheath cells which are normally absent from the shed fibres of primitive sheep (Lang, 1946) remain attached to regressed follicles of cortisol-treated sheep.

A small number of fibres exhibited a region of swelling; the nuclei in these fibres became condensed and darkly stained (pycnotic). The pycnotic nuclei which developed in the fibres of affected follicles are similar to those reported in fibres of sheep dosed with ACTH (Lindner and Ferguson, 1956), glucocorticoid analogues (Chapman et al. 1982), N-[5-(4-Aminophenoxy)pentyl]phthalamide (Chapman and Rigby, 1980), cyclophosphamide (Brinsfield et al. 1972) and mimosine (Reis et al. 1975). The presence of these pycnotic nuclei appeared to inhibit the normal hardening process in this portion of the fibres, because a weakened, poorly keratinised length of fibres was subsequently present. Condensation of nuclei in response to cortisol injection inhibits their normal degradation, which normally occurs during keratinization of the fibre (Chapman, 1980) and as a result they are retained. Just what role the degradation of the nuclei in fibre cells plays in the keratinization of wool fibres is unknown.

Formation of small pycnotic nuclei occurred in conjunction with disruption of the cuticle scale pattern. Loss of cuticle has been induced under conditions of ACTH (Lindner and Ferguson, 1956) and EGF treatment of sheep (Hollis, 1983). Due to disruption of the activity of inner root sheath cells in the keratogenous zone and accumulation of fluid adjacent to the fibre, disruption of cuticle formation occurs (see chapter 6, section 6.3.2). Even though the disruption of cuticle scale pattern may not influence the longitudinal mechanical properties of fibres significantly (Reis, 1992), it would greatly influence the dyeing property of fibres, because the cuticle acts as a barrier to the entry of dyestuff into the fibre.

In Finewool sheep breeds such as Merinos, cortical cells show a bilateral segmentation and this is responsible for the crimp, or waviness of the fibres (Auber, 1952). A condition which became prevalent in treated sheep was that at the site of formation of wool breaks, crimp frequency was disturbed and in some cases it was lost. Loss of crimp is common in sheep with age (Chapman et al. 1960). This condition is developed when accumulation (hyperplasia) of outer root sheath cells around the keratogenous zone of the follicles occurs (Chapman et al. 1960).

Hyperplasia appears to be triggered by delayed hardening of inner root sheath cells and accumulation of fluid adjacent to the fibre (see chapter 6, section 6.3.3). Loss of crimp will cause an unevenness in fibre length. This condition will lead to reduction in staple strength. Such staples are made up of a mixture of poorly and well crimped fibres, the former being shorter than the later. While the majority of the fibres will be present in both bottom and tip of the staple clamped for strength testing, short fibres would take the strain applied by the staple breaker system first, while crimpier fibres are longer than the gauge length and would be the last to be loaded during staple strength measurement. A large drop in staple strength would result.

3.4.8. The effect of cortisol injection on follicle shutdown

The average pre-treatment follicle shutdown of the sheep was very low, and only about 1% were in an inactive stage. Through selection for increased production, wool-growing modern sheep breeds such as the Merino have become “non shedding” and only a small percentage of follicles shed fibre during normal cyclic wool growth (Ryder, 1962). Nevertheless shedding of the fleece still seems to occur even in the most advanced breeds of sheep under adverse environmental conditions (see chapter 1, section 1.4).

Elevation in plasma cortisol concentration was inhibitory to normal follicle activity, and follicle shutdown was cortisol dose responsive. The question is whether similar results are achieved in the field under natural conditions. No detailed study has been conducted so far to examine the extent and the importance of follicle shutdown in sheep in general and in Merino sheep in particular. With the Mediterranean type of climate which is characterised by marked seasonality in the quantity and quality of available pasture (Purser, 1980), it would be expected that follicle shutdown would play an important role in the quantity and quality of wool produced in such environments. Indeed evidence suggests that the position of wool breaks under field conditions is closely associated with the break of season, but the

relative importance of temperature, rainfall, pasture changes etc have yet to be resolved.

Follicle shutdown was so pronounced amongst the sheep on high cortisol dose groups (i.e. groups 1 and 2) that more than 30% of follicles had stopped activity. Similar results were achieved when groups of sheep were put on adverse nutrition. Lyne (1964) indicated that as many as 30% of follicles stopped activity when sheep were fed a poor diet (roughage containing 2.6% crude protein, *ad libitum*) for 6 to 9 weeks. The result of this experiment suggests that under field condition adverse nutrition may cause follicle shutdown.

Follicle regression started two weeks after and reached a maximum level 6 weeks after commencement of cortisol injection. Almost all follicles regenerated 5 to 6 weeks after cessation of injection. This time sequence of anagen-telogen-anagen differs from the natural cycle in the primitive ovine such as the Wiltshire Horn (Slee, 1963). In Wiltshire sheep, follicle inactivity occurs in spring synchronously with the regeneration of new follicles in response to an environmental stimulus (light). It is proposed that in double-coated ovines a synergistic action of prolactin and melatonin determines the retention of old follicles and regeneration of new ones (Gebbie et al. 1994).

Different follicle types underwent varying degrees of regression; fewer primaries stopped producing fibre than the secondary follicles in response to cortisol injection. Between weeks 2 to 9 of the experiment, on average 30% and 31% of secondary follicles had shutdown, while only about 6% and 3% of primaries had stopped activity in groups 1 and 2 respectively. Similar results were achieved when groups of sheep were fed adverse nutrition. Lyne (1964) indicated that when 5 groups of Merino sheep were fed different diets ranging from poor to good quality feed for a period of 6 to 9 weeks, primary and secondary follicles underwent varying degrees of regression. In this nutrition experiment of total follicles counted, 1.41% of primaries and 9.22% secondaries had shed fibres; a difference of 6.5 fold in responsiveness. No study of Merino primary and secondary follicle shutdown has been conducted under

field conditions, but a study with coarse wool sheep has indicated that these follicles respond differently. Doney and Smith (1961) concluded that the winter drop in wool production was due to a 50% shedding of fine fibres. There was also a total loss of kemp fibre, but they represented a small proportion of the primary fibres. Most of the fibres presumed to be growing in primary follicles, that is, the coarse fibres, showed no change in number. Differences in susceptibility of primary and secondary follicles to shutdown have also been reported under conditions of ACTH (Lindner and Ferguson, 1956) and dexamethosone trimethyl-acetate (Chapman et. 1982). These results indicate that primary follicles are less susceptible to shutdown under adverse conditions such as poor nutrition and exposure to cortisol and ACTH injection.

It was observed that secondary follicles regressed earlier and regenerated later than primary follicles. Hollis et al. (1983) also observed that the last follicles to be regenerated were mostly small secondary follicles when sheep were treated with EGF. This raises the question 'why do follicles differ in susceptibility to shutdown'. This difference may be due to variability in the number of EGF receptor sites (see chapter 6, section 6.3.1). It is also possible that the difference in susceptibility is associated with surface area, arrangement of blood vessels, uptake of hormones from the blood and extracellular fluids, efficiency of utilisation of hormones within the follicle or a combination of these factors (see chapter 4, section 4.6.8).

A close relationship ($r^2 = 0.78$, $P < 0.0001$) was found between follicle shutdown and fibre shedding, indicating that fibre shedding was a major feature of the reaction of the follicle population to attendant high plasma cortisol concentrations. Coinciding with the histological changes that took place in the fibre, follicle shape and morphology also underwent major histological changes. Microscopic observations of follicles sectioned at the sebaceous gland level using the 'Saccpic' staining method, revealed that cortisol injection caused disruption of the structure and finally disappearance of the inner root sheath and fibre from the follicle. Disappearance of the inner root sheath has also been induced by injection of EGF (Hollis et al. 1983). In the Merino, the layers of inner root sheath harden in the following sequence,

Henle's layer, cuticle and Huxley's layer (Gemmell and Chapman, 1971). Before inner root sheath cells start to disintegrate, Henle's layer flattens and fuses into a homogenous single stratum while cuticular cells retain their individuality but widen their intracellular spaces (Auber, 1952). This involves dilation of endoplasmic reticulum with both intra- and eventual inter-cellular accumulation of fluid and flocculent material (Chapman, 1989) and subsequent disruption and loss of inner root sheath cells and fibre (see chapter 6, section 6.3.2).

Disruption of inner root sheath cells and fibre is accompanied by disruption and accumulation (hyperplasia) of outer root sheath cells. Histological examinations revealed that the outer root sheath cells of affected follicles were often columnar and radially or spirally arranged in contrast to the rounded and randomly-arranged cells in normal follicles. The irregularity of outer root sheath cells also occurs in sheep fed whole wheat grain and supplemented abomasally with methionine (Chapman and Reis, 1978). The cause of the outer root sheath hyperplasia is unknown, however it appears to be triggered by distortion of poorly formed and improperly keratinised fibres in the distal part of the follicles (Chapman, 1980) (see chapter 6, section 6.3.3).

3.4.9. The effect of cortisol injection on staple strength and staple intrinsic strength

As most of the wool strength related research has concentrated on minimum fibre diameter and the rate of change in fibre diameter, insufficient attention has been paid to other potential sources of variation in staple strength such as follicle shutdown. Only a limited study by Schlink (unpublished) suggested that fibre shedding plays a major role in determining staple strength in fleeces less than 30 N per ktex. The results of the present experiment indicate that indeed follicle shutdown is an important determinant of staple strength when sheep are injected with cortisol. Follicle shutdown was closely ($r^2 = 0.43$, $p < 0.0001$) related to staple strength, which means that more than 40% of the variation in staple strength could be accounted for by follicle shutdown. The present study is the first study undertaken to establish a

detailed quantitative relationship between follicle shutdown and staple strength in sheep.

As a result of follicle shutdown and fibre shedding, staple strength was significantly ($P < 0.0001$) decreased in cortisol treated sheep. The extent of reduction in staple strength was in proportion to the cortisol dose level and degree of follicle regression. Groups with a higher percentage of follicle shutdown had a greater reduction in staple strength. The drop in staple strength was so pronounced that in some individual sheep in the high dose groups, staples could be easily broken by the pull of fingers. Under such circumstances wool would be classified as 'V' or rotten (17 N per kilotex and below). This result suggests that follicle shutdown is a major factor underlying weak wool when sheep are injected with cortisol.

There was no statistical difference in the staple strength of different regions of the body, but the shoulder region was slightly stronger than the midside and rump regions. This is in agreement with the previous finding that fibre shedding was more common at the belly and rump area than the shoulder and neck regions of the sheep. It has been found that wool from the neck region has the highest and the belly region the lowest staple strength (Ross, 1982). It is possible that the composition of the keratin material grown on the belly wool is different from that grown on the midside and neck regions. An alternative explanation is that belly wool is more likely to be affected from contact with the ground and hence more subject to wear. This factor may affect the keratin material and enhance its degradation. The variation in staple strength could be also attributed to the level of wool production at different regions of the body. It has been found that there is a dorso-ventral and anterior-posterior gradient in wool growth rates (Young and Chapman, 1958) in agreement with the finding that staples from shoulder region are stronger than midside and rump regions.

There is a debate as to whether wool intrinsic material has a role in determining wool breaks. While Burgman (1959) and Roberts et al. (1960) believe that there is no difference in intrinsic strength between tender and sound wool, Orwin et al. (1980) and Biggam et al. (1983) suggest that the cell structure and protein composition of

tender wool is different from that of sound wool of the same diameter. When depilating doses of cortisol are given, not all follicles exhibit a similar degree of apoptosis and reduced cell mitosis and only those follicles that are mostly affected enter catagen with cessation of fibre growth. Such doses can induce a partial break resulting from cessation of growth and shedding of some of the fibres while the remainder are less affected. This is probably why the staple intrinsic strength between sheep groups was not significantly different.

3.4.10. Determinants of staple strength

The stepwise regression analysis indicated that follicle shutdown, minimum fibre diameter and staple length best predicted staple strength. A close association was found between staple strength and follicle shutdown ($r^2 = 0.43$, $P < 0.0001$). Minimum fibre diameter has been found to be associated with staple strength (Hunter et al. 1983), but has never been studied concurrently with follicle shutdown and staple length. While the relationship between minimum fibre diameter and staple strength did not explain much of the variation ($r^2 = 0.20$, $P < 0.01$), a close relationship ($r^2 = 0.54$, $P < 0.0005$) was found between staple length and staple strength. The reason for such a relationship is that cortisol reduces wool production mainly from a marked reduction in staple length irrespective of any change in fibre diameter (see sections 3.3.3. and 3.4.5). Greater reduction of staple length without any change in fibre diameter would reduce length/diameter ratio. Hynd and Schlink (1992) stated that fibres with high length growth rate will have smoother diameter changes than those with a low length growth rate, suggesting that staples of cortisol treated sheep undergo greater diameter changes than the staples of control sheep and consequently cortisol treated sheep would have a greater reduction in staple strength.

CHAPTER 4.

**FLEECE PHENOTYPE INFLUENCE
SUSCEPTIBILITY TO CORTISOL-INDUCED
FOLLICLE SHUTDOWN IN MERINO SHEEP**

CHAPTER 4. FLEECE PHENOTYPE INFLUENCES
SUSCEPTIBILITY TO CORTISOL-INDUCED FOLLICLE
SHUTDOWN IN MERINO SHEEP

4.1. INTRODUCTION

The results of the preceding chapter suggest that an increase in plasma cortisol inhibited wool growth mainly by inducing high levels of follicle shutdown and fibre shedding. Not only was there a difference in the susceptibility to shutdown between primary and secondary follicles, but also considerable variation was evident between the secondaries. This raises the question ‘why do follicles differ in susceptibility to shutdown’? This differential susceptibility may be related to the shape, size, depth, density and primary to secondary ratio of follicles or to the surface area, arrangement of blood vessels, uptake of hormones from the blood and extracellular fluids, efficiency of utilisation of hormones within the follicle or a combination of these factors.

To test the hypothesis that sheep differ in susceptibility to cortisol-induced shutdown, 2 groups of Finewool (LL and LH) and 2 groups of Strongwool (HL and HH) Merino sheep were selected as follows:

LL = Low fibre diameter, low coefficient of variation of fibre diameter.

LH = Low fibre diameter, high coefficient of variation of fibre diameter.

HL = High fibre diameter, low coefficient of variation of fibre diameter.

HH = High fibre diameter, high coefficient of variation of fibre diameter.

All 4 groups of sheep were exposed to one dose of cortisol (1.42 mg/kg/day) for a period of two weeks. Plasma cortisol concentration, wool growth and follicle shutdown were measured and compared between groups.

Whilst it was recognised that selection of sheep was phenotypic, it was anticipated that these groups would differ markedly in follicle size and arrangement and would provide a strong indication of where genetic differences may be because:

(A) The magnitude of the heritability estimates of fibre diameter and coefficient of fibre diameter is 0.4 and 0.8 respectively (Williams, 1991).

(B) Comparisons were made in similar environmental conditions after a long stabilisation period (>10 weeks) to remove transient environmental effects.

4.2. MATERIALS AND METHODS

General Materials and Methods have been described in chapter 2.

4.2.1. Selection of animals

The objective was to select 2 groups of sheep from each of a Finewool Merino flock (n = 150) and a Strongwool Merino flock (n = 150) such that 2 groups within each genotype differed phenotypically in coefficient of variation of fibre diameter. The sheep, 9 months of age at the time of selection, grazed at different farms in livestock research stations in South Australia. About 50 grams of wool was sampled by Oster small animal clippers from the midside area. Fibre diameter and coefficient of variation of fibre diameter was measured on 2000 fibres on a fibre fineness distribution analyser (FFDA) (see detail in chapter 2, section 2.1.4). A total of 40 sheep (20 from each flock) were selected and were divided into 4 groups (n = 10) based on fibre diameter and coefficient of variation of fibre diameter (Table 4.1) as follows: finewool groups, similar low fibre diameter but low (LL) or high (LH) coefficient of variation of fibre diameter; Strongwool groups, similar high fibre diameter but low (HL) or high (HH) coefficient of variation of fibre diameter. These 40 sheep were then individually-penned in an animal house and offered a maintenance ration (see detail in chapter 2, section 2.1.2) adjusted according to liveweight.

Table 4.1. Fibre diameter (FD, μm), coefficient of variation of fibre diameter (CV, %) of Merino sheep differing in fleece phenotype (LL, LH, HL and HH) before commencement of treatment period. Groups of sheep were injected daily with 1.42 mg of cortisol/kg body weight for a period of 2 weeks.

Group	Sheep No	FD (μm)	Average FD (μm)	CV (%)	Average % CV
LL*	69	14.8	16.2 ^a ± 0.25	18.6	18.4 ^a ± 0.18
	101	15.0		18.6	
	82	16.1		18.3	
	70	16.1		20.3	
	91	16.4		18.4	
	96	16.4		18.2	
	95	16.5		17.8	
	97	16.5		18.8	
	85	17.1		17.9	
	66	17.2		17.0	
LH**	74	15.8	16.7 ^a ± 0.17	23.2	23.1 ^b ± 0.19
	68	16.0		22.6	
	93	16.1		22.5	
	103	16.5		22.5	
	79	16.8		22.9	
	86	16.9		23.8	
	84	17.0		23.1	
	75	17.2		22.7	
	71	17.2		23.4	
	100	17.2		24.3	
HL***	87	22.7	24.0 ^b ± 0.36	20.2	20.7 ^c ± 0.23
	102	22.9		19.9	
	99	23.0		19.8	
	77	23.2		21.6	
	92	23.2		21.6	
	72	24.4		21.3	
	73	24.8		19.9	
	65	25.3		20.9	
	88	25.3		20.3	
	81	25.4		21.2	
HH****	80	22.7	23.6 ^b ± 0.22	29.1	26.4 ^d ± 0.36
	76	23.1		26.6	
	90	23.2		25.6	
	89	23.2		25.7	
	67	23.3		25.6	
	98	23.7		26.6	
	83	23.8		26.3	
	94	23.9		25.8	
	78	24.7		27.2	
	64	24.8		25.3	

Superscript within groups significantly differ at $P < 0.0001$.

*Low fibre diameter, low coefficient of variation of fibre diameter (LL); **low fibre diameter, high coefficient of variation of fibre diameter (LH); ***high fibre diameter, low coefficient of variation of fibre diameter (HL); ****high fibre diameter, high coefficient of variation of fibre diameter (HH).

4.2.2. Design of the experiment

A total period of 26 weeks was divided into 3 subperiods; pre-treatment, treatment and post-treatment of 10, 2 and 14 weeks respectively (Table 4.2). At the end of the pre-treatment period sheep were accustomed to diet and handling procedures. During the treatment period sheep of all groups were injected with cortisol hormone to induce follicle shutdown. Sheep were individually penned in an animal house, weighed every other week and the following samples were taken:

__Wool within the tattooed patch area was clipped every two weeks to measure clean wool weight (see chapter 2, section 2.1.4).

__Skin biopsies were taken once a week for measurement of follicle activity and histological studies (see chapter 2, section 2.1.5).

__Serial blood samples were taken every two weeks to measure plasma cortisol concentration (see chapter 2, section 2.1.6).

Table 4.2. The outline of the experiment. A total of 26 weeks was divided into 3 subperiods; pre-treatment, treatment and post-treatment period of 10, 2 and 14 weeks respectively. Time sequence of live weight, plasma cortisol concentration, wool clipping and follicle shutdown measurement is shown.

	pre-treatment										treatment		post-treatment														
	1	2	3	4	5	6	7	8	9	10	1	2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Deworming	*																										
Tattooing	*																										
Live weight	*			*		*		*		*		*	*		*		*		*		*						
Wool clipping											*		*		*		*										
Skin sampling	*								*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Blood sampling													*														
Blood IA¹													*														

¹Radioimmunoassay

4.2.3. Cortisol injection

In chapter 3, different doses of cortisol ranging from 0 to 2.86 mg of cortisol/kg body weight were injected for 4 weeks to cause follicle shutdown. In the current experiment an aqueous suspension of hydrocortisone acetate at 1.42 mg/kg body



weight was administered intramuscularly to all individual sheep on daily basis for a period of only two weeks. It was considered that this would be sufficient dose and time to allow different groups to show differential responses if there were any.

4.3. RESULTS

4.3.1. The effect of cortisol injection on feed intake and live weight

There was no significant difference in the average live weight between sheep groups before and after the treatment period (Table 4.3). During different periods of the experiment, all animals consumed their ration and no significant loss of weight was recorded.

Table 4.3. Live weights for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH); measured before and after treatment period (Means with s.e.m.).

Group	Live weight (kg)	
	Before	After
LL	49.9 ± 1.6 ^a	48.2 ± 1.7 ^a
LH	48.6 ± 1.2 ^a	47.1 ± 1.2 ^a
HL	49.9 ± 1.1 ^a	50.0 ± 1.1 ^a
HH	48.5 ± 0.9 ^a	47.0 ± 1.4 ^a

a = no significant difference in live weight between groups or times.

4.3.2. The effect of cortisol injection on plasma cortisol concentration

Groups differing in fibre diameter and coefficient of variation of fibre diameter.

There was no significant difference in the average plasma cortisol concentration between groups two weeks before commencement of cortisol injection. Plasma cortisol concentration increased significantly ($P < 0.0001$) two weeks after commencement of cortisol injection (Figure 4.1).

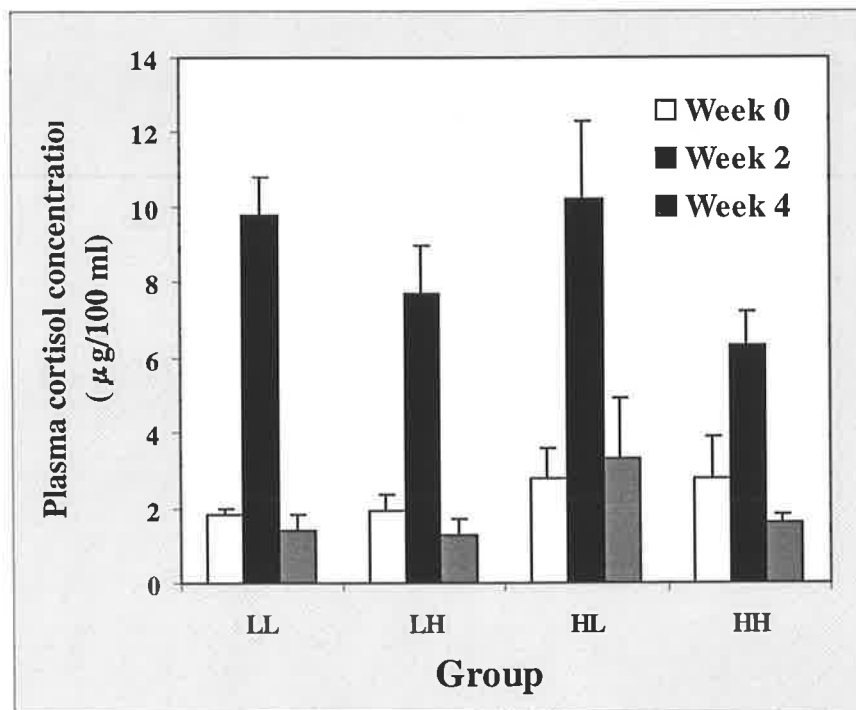


Figure 4.1. Plasma cortisol concentration ($\mu\text{g}/100\text{ ml}$) for the groups of sheep with different fleece phenotype (LL, LH, HL and HH), measured 2 weeks before (designated as week 0), during (week 2) and after (week 4) cortisol injection (Means with s.e.m.).

Analysis of variance revealed that there was no significant interaction between week and group indicating that all 4 groups had a similar pattern of elevation in plasma cortisol concentration. Two weeks after cessation of cortisol injection, plasma cortisol concentration decreased and no significant difference was found between groups in this period.

High and low coefficient of variation of fibre diameter groups. Analysis of variance revealed that combined high coefficient of variation groups (LH + HH) had significantly ($P < 0.05$) lower plasma cortisol concentration than the combined low coefficient of variation groups (LL + HL) two weeks after commencement of cortisol injection (Table 4.4)

Table 4.4. Plasma cortisol concentration ($\mu\text{g}/100\text{ml}$) for sheep groups combined based on high (LH + HH) and low (LL + HL) coefficient of variation of fibre diameter; measured 2 weeks after commencement of cortisol injection (Means with s.e.m.).

Group	Plasma cortisol concentration ($\mu\text{g}/100\text{ml}$)
LH x HH	69.7 ^a (± 7.9)
LL x HL	99.8 ^b (± 11.6)

a, b: Superscripts within group comparisons significantly differ at $P < 0.05$.

4.3.3. The effect of cortisol injection on wool production and fibre diameter

4.3.3.1. Wool production

Groups differing in fibre diameter and coefficient of variation of fibre diameter.
 There was a significant ($P < 0.0001$) difference in the average clean wool production between groups before commencement of cortisol injection (Figure 4.2). Increase in plasma cortisol concentration was associated with a significant ($P < 0.0001$) progressive decline in wool production in all groups of sheep. Wool production was at the lowest level 2 weeks after cessation of cortisol injection (week 4); it gradually started to recover and took 8 to 10 weeks before it returned to pre-treatment level.

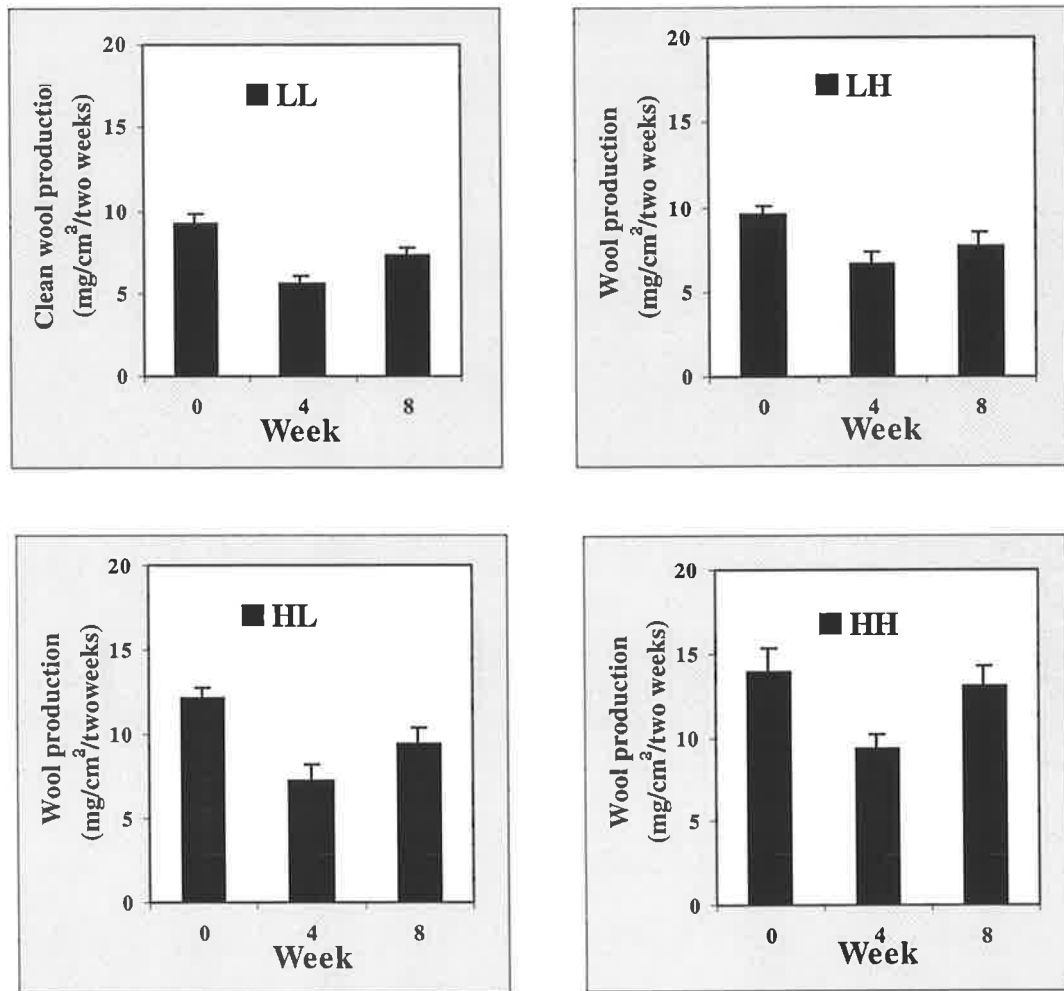


Figure 4.2. Clean wool production per unit area of skin ($\text{mg}/\text{cm}^2/\text{two weeks}$) for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH), measured 2 weeks before (designated as week 0) and 4 and 8 weeks after cortisol injection (Means with s.e.m.).

There was no significant interaction between week and group, indicating that the level of the drop in clean wool production was similar in all groups of sheep (Table 4.5).

Table 4.5. Analysis of variance of clean wool production for groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH).

Source	df	F	P
Week	4	61.40	0.0001
Group	3	27.30	0.0001
Week x group	12	0.43	0.95
Residual	180		

High and low fibre diameter groups. On average, Strongwool groups (HL + HH) produced about 1.4 times more wool than the Finewool groups (LL + LH) throughout the experiment (Table 4.6).

Table 4.6. Clean wool production per unit area of skin (mg/cm²/two weeks) for Finewool (LL + LH) and Strongwool (HL + HH) Merino sheep, measured 2 weeks before (Designated as week 0) and 4 and 8 weeks after commencement of cortisol injection (Means with s.e.m.).

Group	Week		
	0	4	8
Finewool	9.4 ± 0.40 ^a	6.1 ± 0.43 ^a	7.5 ± 0.41 ^a
Strongwool	13.1 ± 0.73 ^b	8.3 ± 0.65 ^b	11.2 ± 0.80 ^b

a, b: Superscripts within group comparisons significantly differ at $P < 0.0001$.

4.3.3.2. Fibre diameter

Groups differing in fibre diameter and coefficient of variation of fibre diameter. Analysis of variance revealed highly significant effects of week ($P < 0.0001$), group ($P < 0.0001$) and week \times group interaction ($P < 0.0002$). There was a significant difference ($P < 0.0001$) in pre-treatment fibre diameter between groups two weeks before cortisol injection started. Fibre diameter dropped to its lowest level four weeks after commencement of cortisol injection, and gradually started to recover and took about 8 to 10 weeks before it reached the pre-treatment level (Figure 4.3).

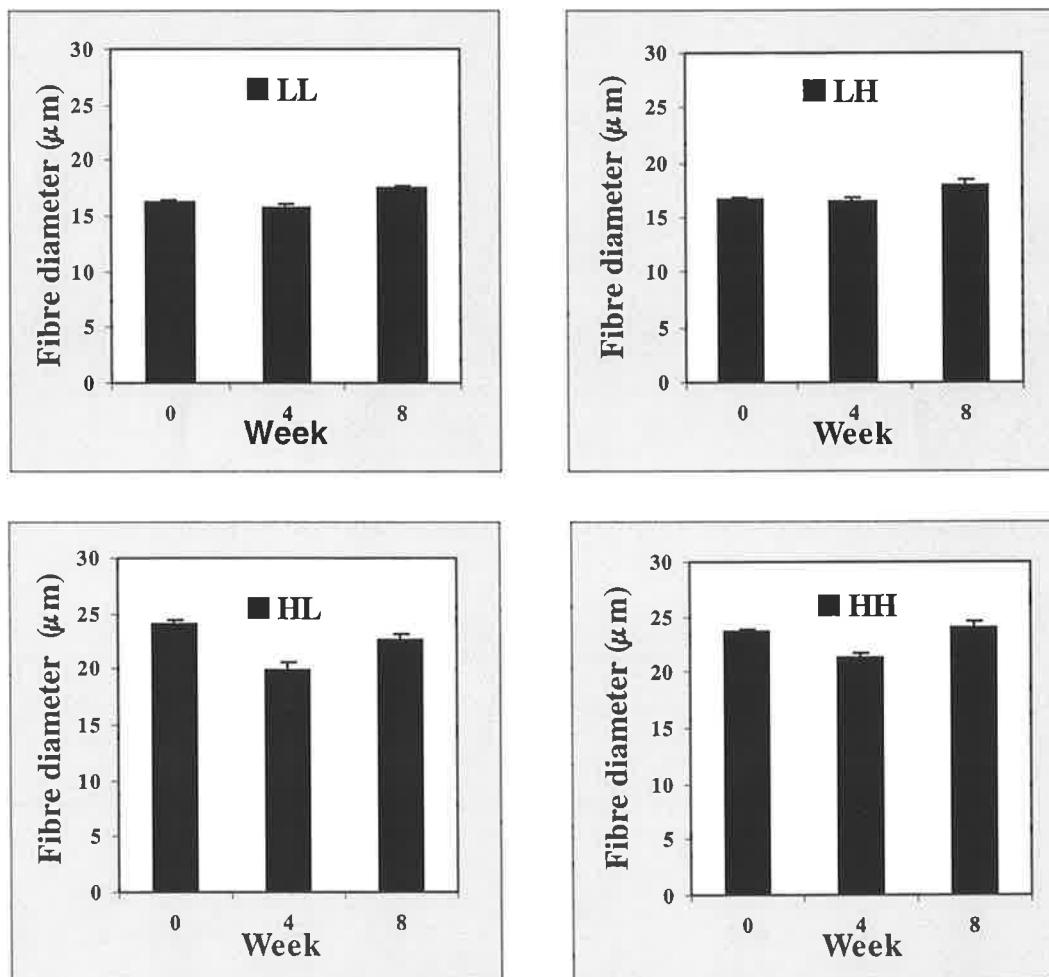


Figure 4.3. Fibre diameter (μm) for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH); measured 2 weeks before (Designated as week 0) and 4 and 8 weeks after commencement of cortisol injection (Means with s.e.m.).

High and low fibre diameter groups. Fibre diameter underwent varying degrees of change in Finewool and Strongwool groups. While Strongwool groups (HL + HH) on average had a drop of 3.2 μm , the drop in the Finewool groups (LL + LH) was only 0.28 μm two weeks after cessation of cortisol injection (week 4) (Table 4.7).

Table 4.7. Fibre diameter (μm) of Finewool (LL + LH) and Strongwool (HL + HH) Merino sheep measured at 2 weeks before (designated as week 0) and 4 and 8 weeks after commencement of cortisol injection.

Group	Week		
	0	4	8
Finewool	16.4 \pm 0.15 ^a	16.1 \pm 0.28 ^a	17.8 \pm 0.25 ^b
Strongwool	23.8 \pm 0.27 ^a	20.6 \pm 0.41 ^b	23.4 \pm 0.40 ^a

a, b: Superscripts between week comparisons significantly differ at $P < 0.0001$.

4.3.4. The effect of cortisol injection on the formation of wool breaks and fibre shedding

Coinciding with the resumption of normal wool growth a few weeks after cessation of cortisol injection, a clear 'break' appeared in the wool of both Finewool and Strongwool sheep. The magnitude of the wool breaks was observed to be similar in all groups of sheep but differences were found between and within groups. At the site of formation of the wool breaks, the density of fibres had substantially dropped as a result of fibre shedding. Finewool and Strongwool sheep shed fibres all over the body, but it was more pronounced at the rump and belly areas. In severe cases complete local wool casting occurred particularly at the rump site (Plate 4.1).



Plate 4.1. As a result of cortisol injection, complete wool casting occurred at the belly and rump areas of Finewool and Strongwool sheep. This plate shows a Finewool Merino sheep injected daily with 1.42 mg of cortisol/kg body weight for a period of 2 weeks.

Histological examinations revealed that shed fibres of Finewool and Strongwool sheep were malformed or degraded, some having holes and cracks (these changes were similar to those found in chapter 3; see section 3.3.4).

4.3.5. The effect of cortisol injection on follicle shutdown

Groups differing in fibre diameter and coefficient of variation of fibre diameter.

Analysis of variance revealed that follicle shutdown was significantly ($P = 0.0012$) dependent on fibre diameter while coefficient of variation of fibre diameter had no effect ($P = 0.17$). There was no significant difference in the percentage of inactive follicles between groups before initiation of cortisol injection (Figure 4.4). Elevation of plasma cortisol concentration was associated with a significant ($P < 0.0001$) increase in the percentage of inactive follicles two weeks after injection started. It should be noted however that within Finewool and Strongwool groups there was substantial variation between sheep in follicle shutdown percentage with some having 60% and others only 3% at week 4 when maximum number of follicles had shutdown.

High and low fibre diameter groups. While there was no significant difference in the maximum level of follicle shutdown (about 20% for all groups) on average high fibre diameter groups (Strongwool sheep; i.e. HL + HH) had significantly ($P < 0.0001$) higher percentage of follicle shutdown than low fibre diameter groups (Finewool sheep; LL + LH) (Table 4.8).

4.8. Percentage of shutdown follicles averaged over time (weeks 1 to 8) for Finewool (LL + LH) and Strongwool (HL + HH) Merino sheep (Means with s.e.m).

Group	Percentage of follicle shutdown
Finewool	9.8 ± 0.9^a
Strongwool	13.5 ± 0.9^b

a, b: Superscripts between group comparisons significantly differ at $P < 0.0001$.

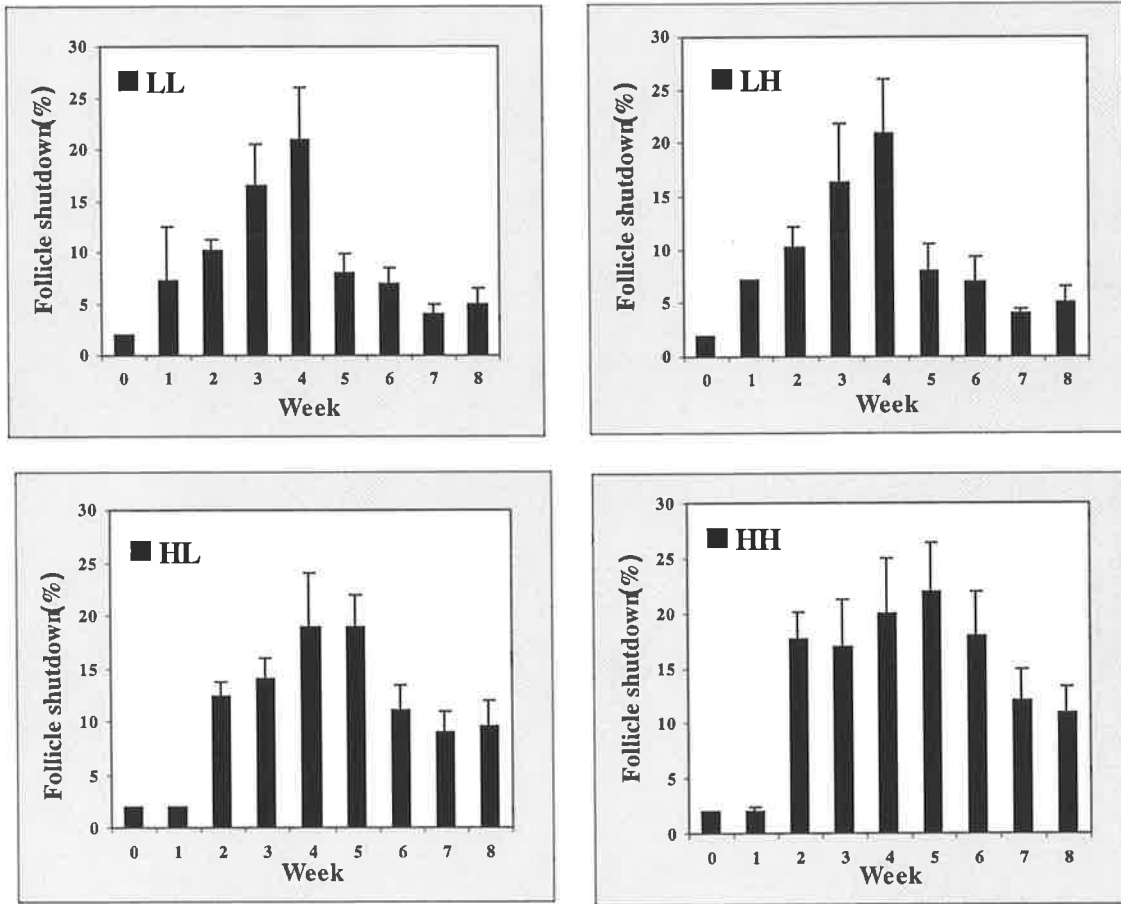


Figure 4.4. Percentage of shutdown follicles measured at 1 week before (Designated as week 0), during (Weeks 1 and 2) and after (Weeks 3, 4, 5, 6, 7 and 8) cortisol injection (Means with s.e.m.); for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH).

Histological examinations revealed that follicle morphology had undergone similar changes as in chapter 3 (see section 3.3.5).

Primary and secondary follicle shutdown. A summary of the measurement of primary and secondary follicles in each group is shown in Table 4.9. A total of 6300 primary follicles and 77700 secondary follicles were examined from weeks 2 to 8 in all 4 groups of sheep (at week 2 follicle shutdown started and at week 9 most of the follicles had regenerated). Shutdown of primary follicles was more pronounced in Finewool (LL + LH) than Strongwool (HL + HH) sheep. Less than 1% of primary follicles and 17% of secondary follicles stopped activity in Strongwool sheep, while in Finewool sheep, 7% of primary follicles and 11% of secondary follicles regressed.

Table 4.9. Average number and percentage of primary and secondary follicle shutdown for the groups of Merino sheep differing in fleece phenotype (LL, LH, HL and HH). Table shows total number of follicles counted and the number and percentage of follicle shutdown between weeks 2 to 8.

Group	Weeks 2 to 8					
	Primary			Secondary		
	Total	FSD*		Total	FSD	
		No	%		No	%
LL	1050	45	4.3	19950	2132	10.7
LH	1050	101	9.6	19950	2194	11.0
HL	2100	5	0.2	18900	2782	14.7
HH	2100	3	0.1	18900	3483	18.4

*FSD; follicle shutdown.

4.4. DISCUSSION

4.4.1. Design and objectives of experiment

The present study is the first undertaken to compare the extent of follicle shutdown in groups of sheep differing phenotypically in fleece characteristics. In chapter 3 it was revealed that follicle shutdown is an important determinant of staple strength and that pronounced differences in susceptibility to shutdown exist not only between primary and secondary follicles but also between the secondaries. This raises the question ‘why do follicles differ in susceptibility to shutdown?’ It is possible that the variability in size, shape, depth, uniformity and density of follicles is associated with follicle shutdown. To answer this question four groups of sheep differing in fibre diameter and coefficient of variation in fibre diameter were selected and exposed to a moderate dose of cortisol hormone for a relatively short period of time to determine if phenotype (genotype?) influenced susceptibility to follicle shutdown. The differences in fleece characteristics between groups were phenotypic but since the heritabilities of fibre diameter and coefficient of variation of fibre diameter are about 0.4 and 0.8 respectively (Williams, 1991) it would be reasonable to assume that a large proportion of the phenotype is genetic in origin.

There are two reasons to suspect that follicle morphology influences staple strength:

1. Finewool sheep have lower staple strength (Baker et al. 1993).
2. High coefficient of variation wools have lower staple strength (Hansford and Kennedy, 1988).

Given that fibre diameter is closely associated with follicle dimensions (Hynd et al. 1989) it seemed reasonable to assume that selection based on fibre diameter and diameter variabilities would provide groups differing in follicle characteristics. Low average fibre diameter of Merino sheep is associated with high secondary to primary ratio with a high density of fibres per unit area of skin (Turner et al. 1986), low ratio of diameter of primary to secondary fibres and more uniformity in fibre diameter (Jackson et al. 1975). Therefore the phenotypic differences in fleece characteristics of

each of the sheep groups in the present experiment would be likely to be reflected in follicle morphology differences as follows:

Genotype	Sheep group	Likely follicle morphology
Finewool	LL	High density, high S/P ratio, uniform follicle size and depth, high paracortical cell %, low dP/dS* ratio.
	LH	High density, high S/P ratio, irregular follicle size and depth, high paracortical cell %, low dP/dS ratio.
Strongwool	HL	Low density, low S/P ratio, uniform follicle size and depth, low paracortical cell %, high dP/dS ratio.
	HH	Low density, low S/P ratio, irregular follicle size and depth, low paracortical cell %, high dP/dS ratio.

*dP/dS; diameter of primary follicle/diameter of secondary follicle

4.4.2. Basis of choosing the dose and duration of cortisol injection

The choice of injection of 1.42 mg of cortisol/kg body weight for all groups of sheep was on the basis of results of chapter 3. In that chapter it was indicated that at this dose and higher dose of 2.86 mg/kg per day, cortisol injection caused a similar elevation in plasma cortisol concentration as stressed sheep under field conditions (Lindner, 1959; Reid, 1960). In the present experiment the duration of cortisol injection was two weeks; a period more likely to be experienced in the field by sheep under short periods of stress. The regime successfully induced a significant percentage of follicles to shutdown without complete wool casting, thereby allowing group comparisons.

4.4.3. The effect of cortisol injection on feed intake

There is evidence that the nutritional state of sheep influences the effect of cortisol on sheep. Thwaites (1972) showed that poorly-fed sheep had greater weakness in the

fibre than well-fed sheep when cortisol was administered. To eliminate the feed factor in the present experiment, all sheep groups were fed at a maintenance level. Bassett (1963) indicated that cortisol injection at a dose of 1.42 mg/kg (on average) for a duration of 12 weeks depressed feed intake. No information is available on the relative effect of a depilatory dose of cortisol injection on the feed intake of Finewool and Strongwool sheep. The results of the present experiment indicated that feed intake was not affected by cortisol injection and no significant loss of weight was recorded in either the Finewool and Strongwool sheep groups. Any difference between groups, then, must reflect effects of cortisol dose.

4.4.4. The effect of cortisol injection on plasma cortisol concentration

There was no significant difference in average plasma cortisol concentration between Finewool and Strongwool groups. This result supports the result of Williams et al. (1986) who found that fleece-plus and fleece-minus sheep did not differ in measured plasma cortisol concentration. This finding is important in terms of wool production, since a large amount of variation is found in wool production within strains of Merino sheep (Turner, 1977), and that a slight increase in plasma cortisol concentration could increase wool production (Chapman and Bassett, 1970). The difference in productivity of Strongwool and Finewool Merino sheep (Hocking Edwards and Hynd, 1994) therefore can not be attributed to differences in plasma cortisol concentration.

Plasma cortisol concentration was however significantly ($P < 0.05$) higher in the low coefficient of variation of fibre diameter groups (LL + HL) than high coefficient of variation of fibre diameter groups (LH + HH) two weeks after commencement of cortisol injection. The difference in plasma cortisol level between groups of sheep may reflect the difference in adaptability of these groups to external stimuli. Wool growth in Scottish blackface sheep which is characterised by having highly variable fibre diameter was more sensitive to the depressing effect of cold exposure than in Merino x Cheviot sheep which are more uniform in fibre diameter (Slee and Ryder,

1967). From this study it is possible to conclude that Merino sheep with low coefficient of variation of fibre diameter may be more adaptable to external stimuli. This result is confirmed by the fact that Strongwool sheep with high coefficient of variation of fibre diameter had significantly ($P < 0.0001$) higher percentage of follicle shutdown (see section 4.3.5). The difference in susceptibility to cortisol injection could be due to differences in systemic factors such as the rate of metabolism and utilization of cortisol (see chapter 6, section 6.3.1).

Although plasma cortisol concentration was much higher in Finewool and Strongwool groups two weeks after treatment compared with the pre-treatment level, no sign of physical or psychological stress was observed in these sheep. Plasma cortisol concentration values of 5 to 10 times the normal level have been frequently observed in sheep under stress and in ewes with pregnancy toxaemia (Lindner, 1959; Reid, 1960a and c) which is similar to the elevation of plasma cortisol concentration in the sheep of present experiment. The dose chosen was therefore appropriate and reflective of "real world" values.

4.4.5. The effect of cortisol injection on wool growth rate

The elevated plasma cortisol concentration two weeks after commencement of cortisol injection was inhibitory to wool growth in all groups of sheep. The major part of the decline in wool growth occurred two weeks after cessation of injection (week 4) when wool growth was substantially reduced to almost 50% of the pre-treatment level in both Finewool and Strongwool groups. The reduction in clean wool production was due to the combined effect of a reduction in fibre diameter and increase in the percentage of inactive follicles and possibly a reduction in staple length (no measurement of staple length was made in present experiment). Given the hypothesis that cortisol prevents elongation of fibre cells and delays hardening of inner root sheath cells (Chapman, 1989) staple length must have decreased in both Finewool and Strongwool sheep (see chapter 3, section 3.4.5). Due to the fact that the level of decline in wool production was similar in both of these groups of sheep,

despite a higher percentage of follicle shutdown (see section 4.6.8) and a greater reduction in fibre diameter in Strongwool sheep; Finewool sheep must have a greater reduction in staple length in order to have a similar level of reduction in wool production. The finding that cortisol alters the transcription process by the activation of different gene sets (Ward, 1980), and that a fine control exists in keratin gene expression between ortho- and paracortical cells (Powell and Rogers, 1986) suggest that Finewool and Strongwool sheep could have varying decline in staple length.

Analysis of variance revealed that there was a significant ($P < 0.0002$) interaction between week and group, indicating that fibre diameter underwent varying degrees of change in Finewool and Strongwool groups. While Strongwool groups (Groups 3 and 4) on average had a drop of about $3.2 \mu\text{m}$, the drop in Finewool groups (Groups 1 and 2) on average was only $0.28 \mu\text{m}$ two weeks after cessation of cortisol injection. The point for discussion are: what systemic factor could account for the effect on fibre diameter; secondly, can the difference in response between genotype be explained? The drop in fibre diameter could not have been due to elevation in plasma cortisol level, since the results of the previous experiment indicated that cortisol reduces wool production without any change in fibre diameter. Chapman and Bassett (1970) also showed that the contribution of fibre diameter in reducing wool production in cortisol-treated sheep was almost nil. The effect could therefore be due to other systemic changes. It is highly possible that injection-induced vasoconstriction could have been responsible for this change. Increased catecholamine secretion have been linked with a drop in blood flow (Ferguson, 1949) and inhibition of follicle activity (Scobie, 1992).

In the present experiment, however, fibre diameter in Strongwool sheep was more sensitive to the depressant effects of injection-induced stress than in the Finewool Merino sheep. It is possible that the genotype difference in response may arise from systemic factors. It has been demonstrated that there is a greater blood flow to the skin of Strongwool than Finewool sheep (Hocking Edwards and Hynd, 1991). If it is assumed that increased catecholamine secretion was the stimulus involved in

reduction of fibre diameter, consequently greater blood flow to the skin of Strongwool sheep would cause greater supply of these hormones to the follicles and hence greater drop in fibre diameter in these sheep is expected.

4.4.6. The effect of cortisol injection on formation of wool breaks and fibre shedding

After wool production returned to normal levels, a clear break appeared in the wool of sheep most susceptible to cortisol. This wool break resembled that of the break which occurred in the previous experiment (see chapter 3, sections 3.3.4 and 3.4.6). The intensity of wool breaks in both Finewool and Strongwool groups was similar but variation was found between and within sheep of the groups. This difference in the intensity of wool breaks in individual sheep may be due to differences in susceptibility of sheep to cortisol injection.

The pattern of fibre shedding was similar in Finewool and Strongwool groups. Shedding started from the hairy areas of the hind legs and belly region extending to the rump and the rest of the areas of the body. This pattern of shedding is in agreement with the finding that there is a presence of a dorso-ventral and anterior-posterior gradient in wool growth rate (Young and Chapman, 1985). The sequence of shedding in response to cortisol differs from the sequence of natural shedding. In primitive sheep, natural shedding follows a sequential, bilaterally symmetrical pattern, commencing on the chest and shoulders and spreading to the back and rump (Slee, 1963). Casting of the fleece in the Soay sheep (Boyd et al. 1964) proceeds across the body in similar sequence to that observed by Slee (1959) in the Wiltshire and Ryder (1960) in the Mouflon. In seasonal moulting, adjacent follicles are in the same stage of the growth cycle at the same time (Ebling, 1964) while in cortisol induced shedding each follicle has its own cycle independent of that its neighbours.

4.4.7. Histological examinations of shed fibres induced by cortisol injection

Histological examinations of shed fibres revealed that similar changes took place in the shed fibres of sheep treated with different doses of cortisol in chapter 3. Detailed description of these changes has been discussed in the previous chapter (see chapter 3 sections 3.3.4, 3.3.5, 3.4.5, 3.4.6, 3.4.7 and 3.4.8 and chapter 6).

4.4.8. The effect of cortisol injection on follicle shutdown

The average pre-treatment follicle shutdown of Finewool and Strongwool groups was very low, with only about 2% inactive follicles. Through selection for increased production, Finewool and Strongwool Merinos have become 'non shedding' and only a small percentage of follicles shed fibre during normal cyclic wool growth (Ryder, 1960).

Elevation in plasma cortisol concentration was inhibitory to the normal activity of follicles. A significant ($P < 0.05$) difference was found in follicle shutdown averaged over time between Finewool and Strongwool Sheep. Strongwool sheep (HL + HH) had a significantly higher percentage of follicle shutdown than the Finewool sheep (LL + LH). Greater susceptibility of Strongwool sheep to cortisol may be associated with (A) the degree of follicle sensitivity to shutdown (B) rate of blood flow to the skin (C) the number of EGF receptor sites. Each of these factors are discussed below.

As follicle shutdown was found to be significantly ($P = 0.0012$) dependent on fibre diameter and that follicle dimension is closely associated with fibre diameter (Hynd, 1989) it seems reasonable to assume that cortisol injection would induce a higher percentage of follicle shutdown in Strongwool than Finewool sheep. Strongwool sheep are characterised by having lower follicle density and larger follicle size; such follicles are likely to have a greater level of uptake of hormone because they occupy greater surface area, hence they are more sensitive to shutdown. This result indicates that genetic differences in the percentage of follicle shutdown may arise from differences in the sensitivity of follicles to exogenous cortisol.

The microvasculature of the skin of sheep in respect to higher blood flow and percentage of follicle shutdown of Strongwool sheep may be of significance. Ryder (1956) observed anatomical differences in the arrangement of cutaneous capillaries between breeds of sheep. Nay (1966) reported that the presence of a regular vascular arrangement associated with straight follicles in high-wool producing Merinos in contrast to an irregular vascular arrangement associated with tangled follicles in Merinos selected for low levels of wool production. These studies indicated that the area of vascular tissue per unit volume of skin may have a role in the rate of blood flow to the skin and therefore determine the genetic differences in the percentage of follicle shutdown between Strongwool and Finewool sheep.

Given the possible association between EGF and cortisol in follicle shutdown (see *in vitro* studies) it is possible that genetic difference in the number of inactive follicles may be associated with the number and affinity of EGF receptor sites. Concentration of EGF receptor sites may be determined genetically and may be related to the time of initiation of follicle types in foetus skin; primary follicles are formed at 60 days post-parturition and 14-20 days before secondary follicles are formed (Hardy and Lyne, 1956). Due to the larger follicle size of Strongwool sheep and the finding that the number of EGF receptor site is related to the rate of cell proliferation (Martin et al. 1983) it is reasonable to assume that Strongwool sheep have a greater percentage of follicle inactivity (see detail in chapter 6, section 6.2.1).

The results of the present experiment indicate that the primary follicles of Finewool sheep are more susceptible to shutdown than the primary follicles of Strongwool sheep in response to cortisol injection. The difference in susceptibility to shutdown may be associated with surface area, arrangement and a number of blood vessels around the follicles. Nay (1966) reported that networks of blood vessels were never seen around every follicle in the skin of sheep.

Substantial variation in follicle shutdown was found between sheep within Finewool and Strongwool groups. A possible reason for this variation is that individual sheep may metabolize or utilize cortisol at different rates. The rapidity

with which cortisol is removed from the circulatory system or the efficiency with which cortisol is utilized within the animal may play an important role in determining the susceptibility of individual sheep to follicle shutdown.

CHAPTER 5.

**EPIDERMAL GROWTH FACTOR, BUT NOT
CORTISOL, SUPPRESSES FIBRE GROWTH IN
CULTURED FOLLICLES**

CHAPTER 5. EPIDERMAL GROWTH FACTOR, BUT NOT CORTISOL, SUPPRESSES FIBRE GROWTH IN CULTURED FOLLICLES

5.1. INTRODUCTION

The hormonal basis of follicle shutdown and fibre shedding has not yet been fully elucidated. High levels of follicle shutdown and fibre shedding leading to formation of wool breaks have been induced in Merino sheep injected with cortisol (see chapters 3 and 4), suggesting that high plasma cortisol concentration may be involved in the tender wool problem, but there is mounting evidence (Moore et al. 1982; Singh-Asa and Waters, 1983; Chapman and Hardy, 1988; Philpot et al. 1990; Behrendt et al. 1993; Wynn and Adam, unpublished) which suggest that other factors may also be involved in this process.

Epidermal growth factor (EGF), developed as a defleecing agent, would be a potential physiological candidate in the formation of wool breaks. Sheep injected with low doses of EGF, shed fibres and bore a zone of weakness or wool breaks (Moore et al. 1981). When human hair follicles were maintained with EGF *in vitro* at a concentration of 10 ng/ml, there was a striking change in the morphology of follicles (Philpot et al. 1990) but the effect of low concentrations of EGF is unknown.

It has been indicated that intravenous infusion of defleecing doses of EGF caused a seven fold increase in plasma cortisol concentration (Singh-Asa and Waters, 1983), which is sufficient to induce follicle shutdown and initiate wool breaks (see chapter 3; Chapman and Bassett, 1970). Localization of receptors for EGF in the wool follicle of Merino sheep (Wynn et al. 1988) and the finding that ACTH immunization increases the efficacy of EGF (Behrendt et al. 1993; Adam and Wynn, unpublished) suggest that there may be a possible association between these two depilatory substances.

The key objectives of this chapter are:

1. To examine the role of cortisol and EGF in follicle shutdown.
2. To investigate the effect of different concentrations of EGF and cortisol on primary and secondary wool follicles.

To achieve these objectives, it is necessary to study the action of EGF and cortisol at the follicular level. This, together with the fact that concentrations at the level of wool follicles *in vivo*, are unknown, points to the need for and investigations of the response of wool follicles *in vitro*. Studies *in vitro* have the advantage of allowing direct effects of substances on follicle function to be determined. An *in vitro* technique which involved microdissection of anagen follicles from human scalp skin has been reported by Philpott et al. (1990). More recently, Hynd et al. (1992) have modified this technique using fine needles to isolate follicles from skin strips and successfully maintained Romney wool follicles in Williams E medium.

In this chapter the wool follicle culture system was used to identify the follicular responses of Romney and Tukidale primary and secondary follicles to exogenous cortisol and EGF at low and high concentrations.

5.2. MATERIALS AND METHODS

Dissection, maintenance and DNA synthesis of follicles *in vitro* have been discussed in chapter 2, section 2.2.

5.2.1. Addition of cortisol and EGF to cultured follicles

To prepare a stock solution, tissue culture tested cortisol and EGF (Sigma Diagnostics, St. Louis, Missouri) were reconstituted in a solution of 1.0% bovine serum albumin (BSA) in phosphate buffered saline (PBS) obtained from Sigma Diagnostics (St. Louis, Missouri).

To examine whether cortisol inhibits fibre growth *in vitro*, the response of isolated follicles to physiological and supraphysiological doses of this hormone was studied. Tukidale (primary and secondary) and Romney follicles (n = 12/dose) were incubated

in medium containing 0, 10, 50 and 1000 ng/ml of cortisol for a period of 3 days. Evidence suggests that fibre growth is largely regulated by autocrine/paracrine mechanisms (Moore, 1989). Hynd et al. (1992) in an *in vitro* study using Romney wool follicle observed that fibre production was maximal in a nutrient medium lacking hormones and fetal calf serum. They concluded that autocrine or paracrine production of growth factor is occurring in the follicle bulb cells or dermal papilla cells. To examine whether autocrine or paracrine factors in the dermis influence the efficiency of cortisol on follicles, fibre growth in Tukidale primary follicles with dermis was compared with the fibre growth of Tukidale primary follicles without dermis, maintained with cortisol at 0 and 50 ng/ml ($n = 12/\text{dose}/\text{treatment}$) over a period of 6 days.

Studies *in vitro* indicated that isolated human hair follicles with 10 ng of EGF/ml show considerable morphological changes (Philpott et al. 1990) however it is not known whether at lower concentrations of EGF would induce similar effects. To examine the effect of different doses of EGF on Tukidale (primary and secondary follicle types) and Romney follicle growth *in vitro*, comparison was made between controlled follicles ($n = 12$) and follicles ($n = 12/\text{dose}$) maintained with 0.125, 0.25, 0.5, 1, 25 and 50 ng of EGF/ml.

The basis for distinguishing Tukidale primary from the secondary follicles was their size; Tukidale primary follicles are much larger in size compared with the secondary follicles.

5.3. RESULTS

5.3.1. Preliminary studies on the growth of follicles in culture

Intact anagen wool follicles were removed from skin strips using fine needles and maintained in Williams E medium successfully. Fibre length significantly ($P < 0.05$) increased *in vitro* with time (Figure 5.1). The average growth rate of Tukidale primary and secondary and Romney follicles cultured in Williams E medium was 406

(range 146-764), 173 (108-307) and 255 (81-546) $\mu\text{m}/\text{day}$ respectively (Figure 5.1). Examples of the responses in wool follicle length are shown in plate 5.1 at 0 and 3 days of incubation in culture medium. Photographs of cultured follicles revealed that the length growth was due to normal growth of fibre and was not associated with damage during dissection or after maintenance in culture medium (Plate 5.1). In addition, the pattern of [methyl- ^3H] thymidine uptake by cultured follicles showed that the typical pattern of DNA synthesis is taking place (Plate 5.2), an indication of normal mitosis. Autoradiography observations revealed that the majority of cell division takes place in the bulb cells around the dermal papilla.

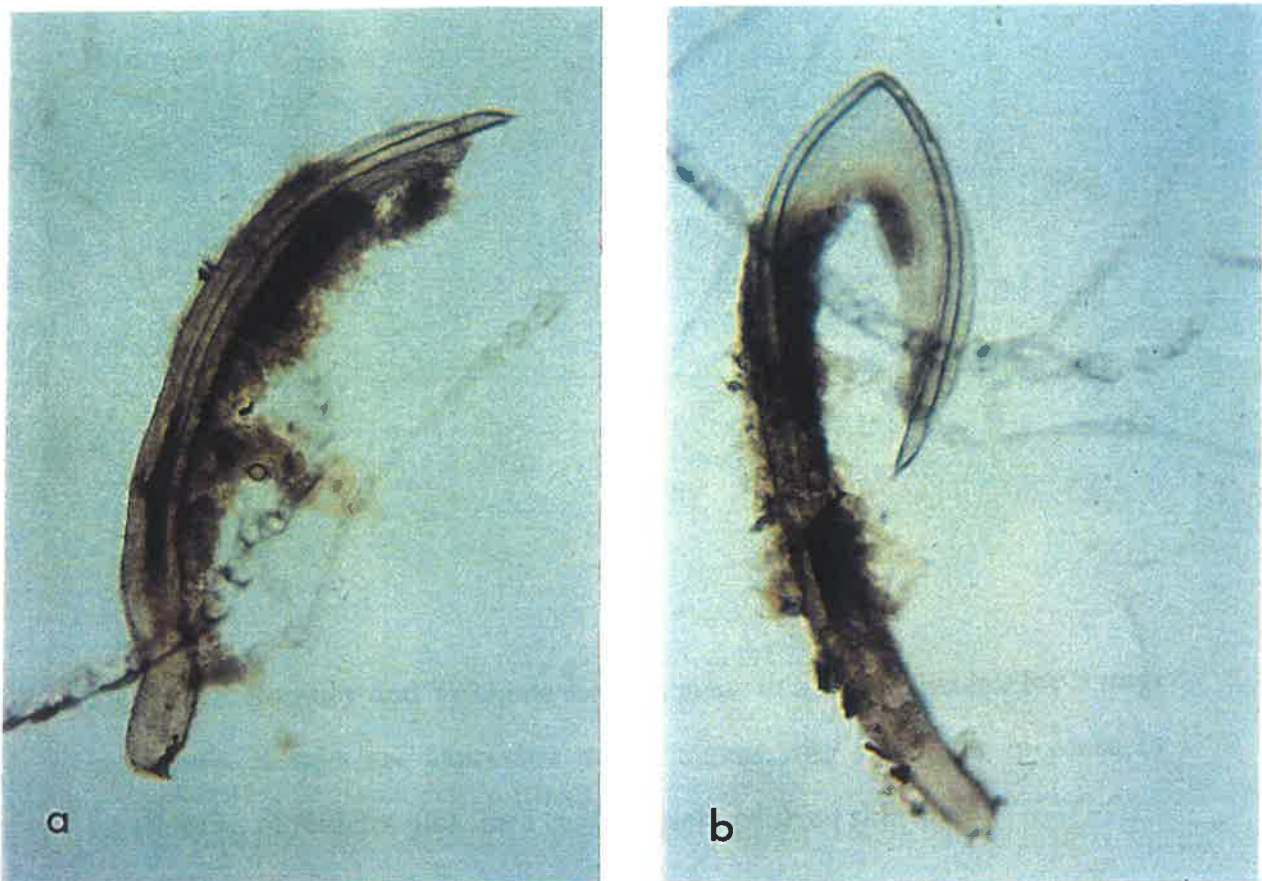


Plate 5.1. Light micrographs taken under an inverted microscope showing Romney wool follicle growth from 0 to 72 h after isolation. (a) Freshly isolated follicle (b) after 72 h maintenance in culture medium.

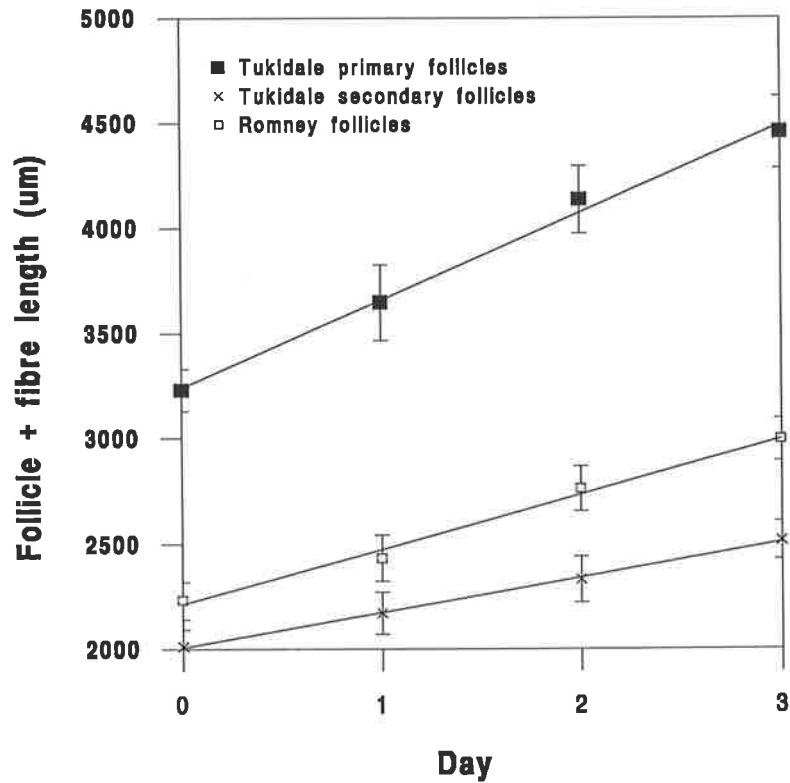


Figure 5.1. Romney and Tukidale follicles grow in a linear fashion for 3 days in culture. Results are expressed as the means with s.e.m. for increase in length (μm) of follicles plus fibre ($n = 12$) isolated from skin strip.

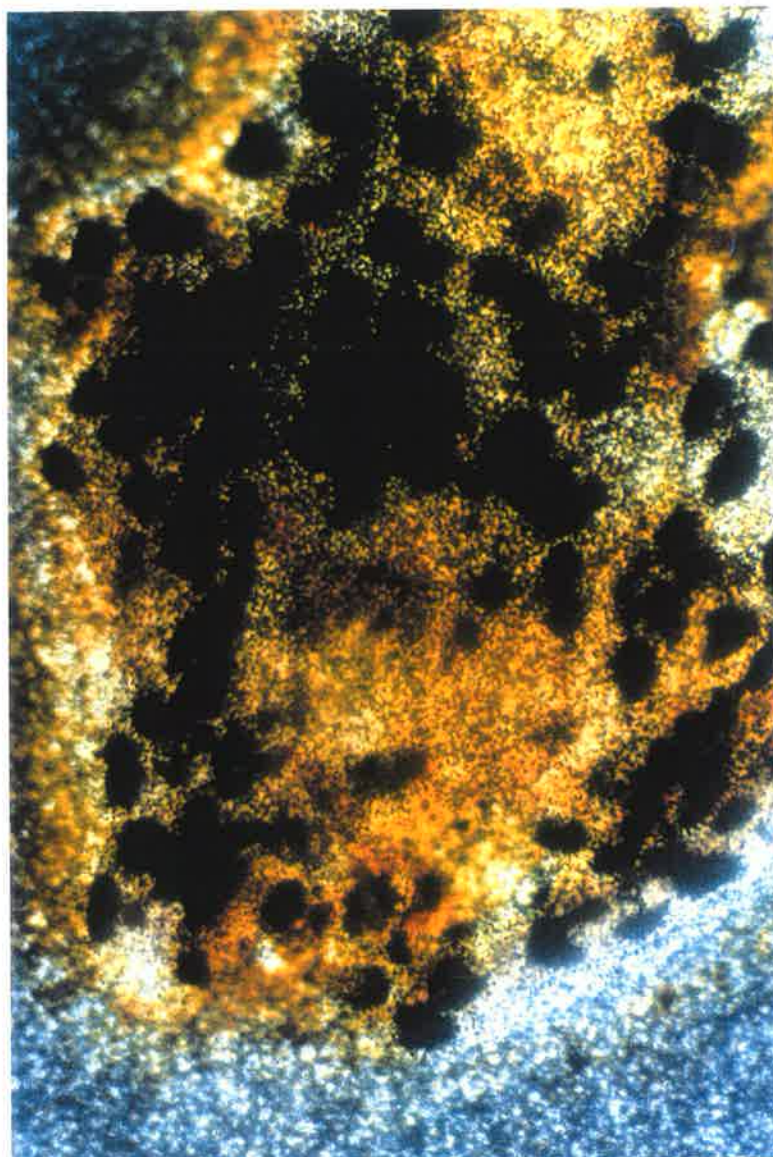


Plate 5.2. Tritiated thymidine autoradiograph of isolated Romney wool follicle. Isolated follicles in culture show the normal pattern of DNA synthesis is taking place, an indication of normal mitosis. Follicles were grown in culture for 72 hours, then incubated in 3.7×10^4 (1 μ Ci) of [methyl- 3 H] thymidine for 6 hours. Under Ilford Safelight F904 longitudinal sections of the follicles were then exposed to L4 emulsion, developed and stained.

5.3.2. The effect of cortisol on the growth of follicles in culture

Physiological and supra-physiological concentrations of cortisol had no effect on the growth of Tukidale primary and secondary follicles over a 3 day period (Figure 5.2). Similarly at the same concentrations, cortisol had no effect on fibre growth from Romney follicles (Figure 5.3).

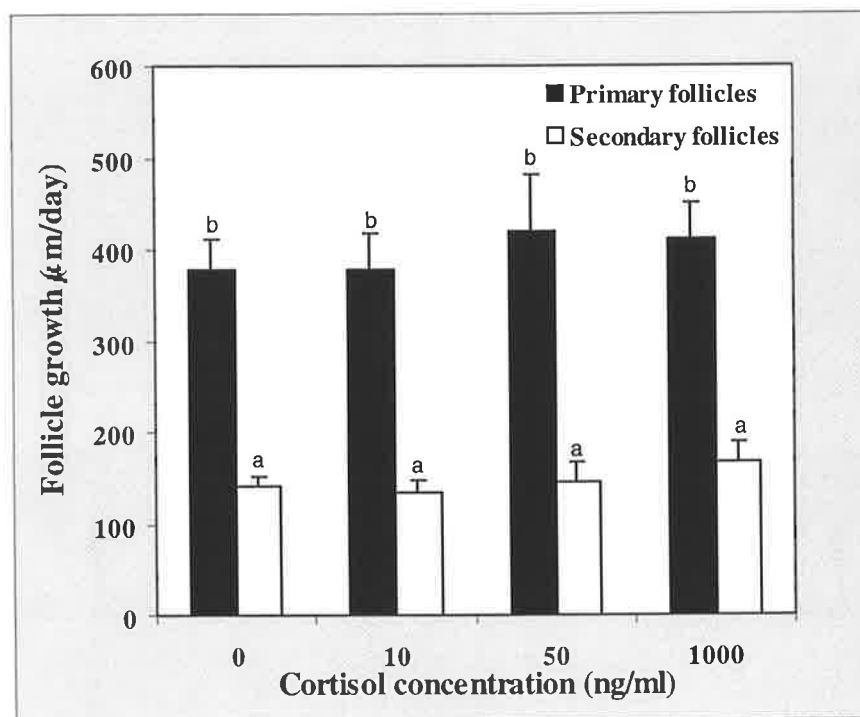


Figure 5.2. Cortisol has no effect on the growth of Tukidale primary and secondary follicles *in vitro*. Follicles were maintained *in vitro* over a 3 day period in the presence of 0, 10, 50 and 1000 ng of cortisol/ml ($n = 12/\text{concentration}$). Results are expressed as means with s.e.m.

a, b: bars with different superscripts differ in follicle growth at $P < 0.05$.

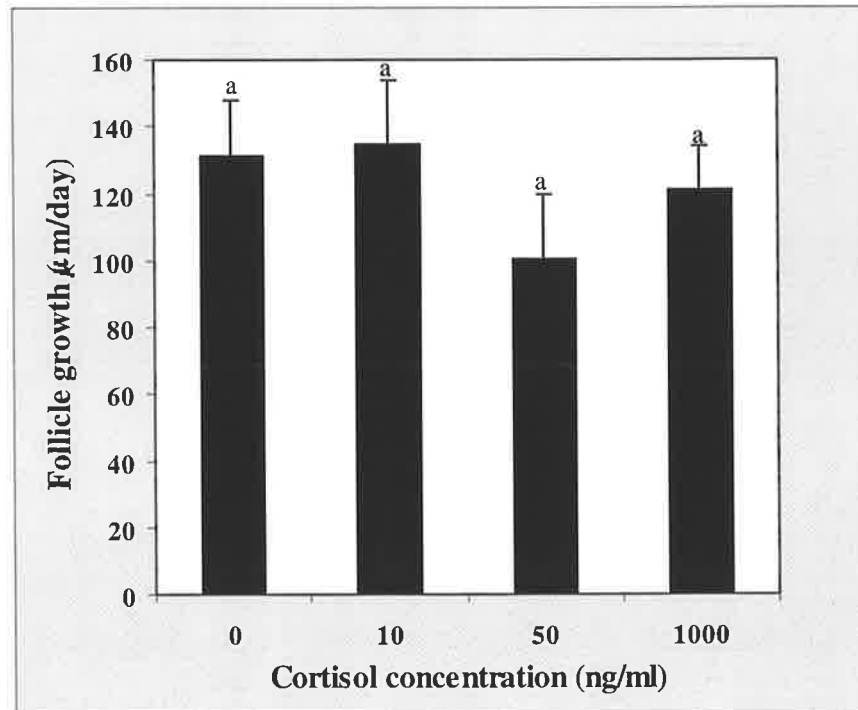


Figure 5.3. Cortisol has no effect on the growth of Romney follicles *in vitro*. Follicles were maintained *in vitro* over a 3 day period in the presence of 0, 10, 50 and 1000 ng of cortisol/ml ($n = 12/\text{concentration}$). Results are expressed as means with s.e.m.

a = no significant differences between cortisol concentrations.

Different concentrations of cortisol had no effect on the growth of Tukidale primary follicles with or without dermis over a 6 days period (Figure 5.4). Unlike the effects observed in sheep treated with cortisol, where follicles underwent substantial histological changes, no morphological changes were observed in cultured follicles.

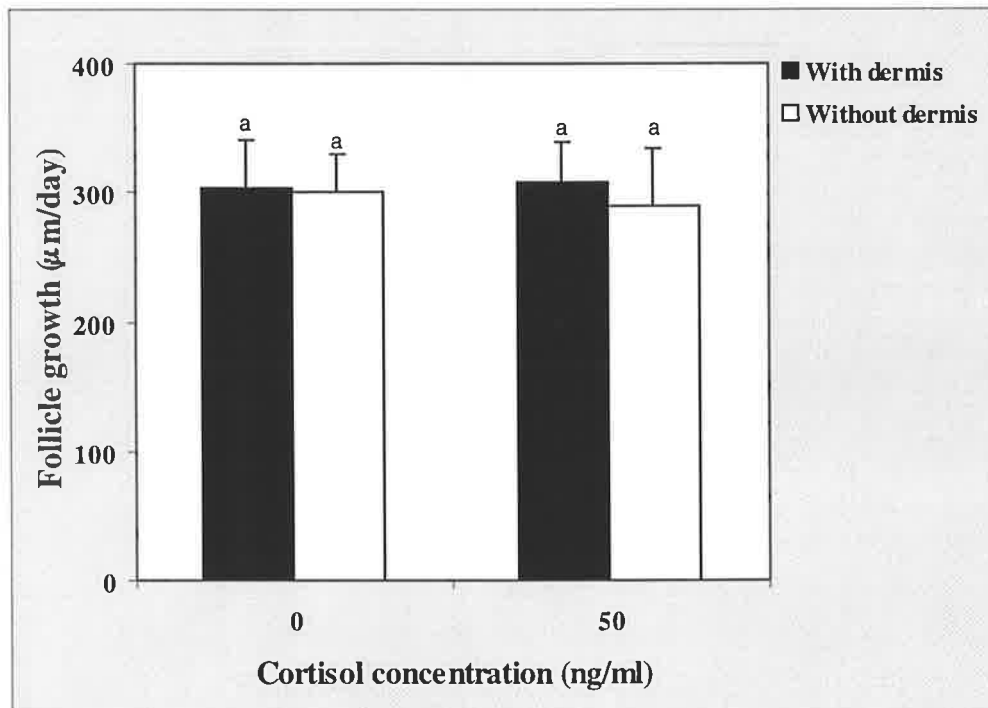


Figure 5.4. Cortisol has no effect on the growth of Tukidale primary *follicles in vitro*. Follicles were maintained *in vitro* for a 6 day period in the presence of 0 and 50 ng of cortisol/ml ($n = 12/\text{concentration}$). Results are expressed as means with s.e.m. a = no significant differences between cortisol concentrations.

5.3.3. The effect of EGF on the growth of follicles in culture

All concentrations of EGF (≥ 0.125 ng/ml) significantly ($P < 0.05$) reduced the fibre growth of Romney wool follicles (Figure 5.5). Since primary and secondary follicles responded differently to cortisol injection *in vivo* (see section 3.3.5) and no study has yet been done with EGF in this respect, the effect of different concentrations of EGF on the fibre growth of Tukidale primary and secondary follicles was studied. EGF at concentrations of 1, 10 and 50 ng/ml significantly ($P < 0.05$) reduced fibre growth of primary and secondary follicles (Figure 5.6).

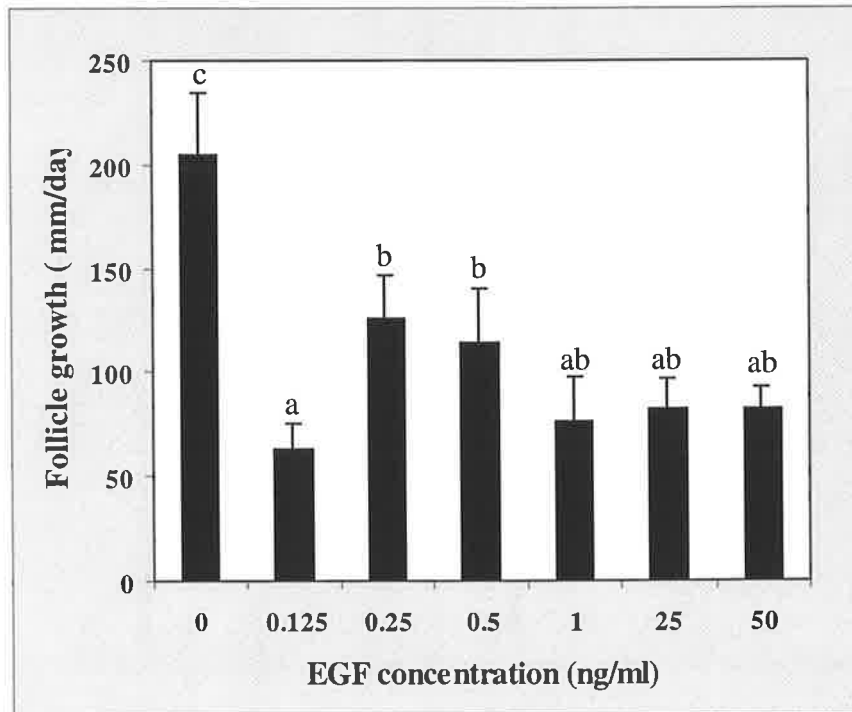


Figure 5.5. EGF has a significant effect on the growth of isolated Romney wool follicles *in vitro*. Follicles were maintained *in vitro* over a 3 day period in the presence of 0, 0.125, 0.5, 1, 25 and 50 ng/ml of EGF ($n=12/\text{concentration}$). Results are expressed as means with s.e.m. Different superscripts significantly differ at $P < 0.05$.

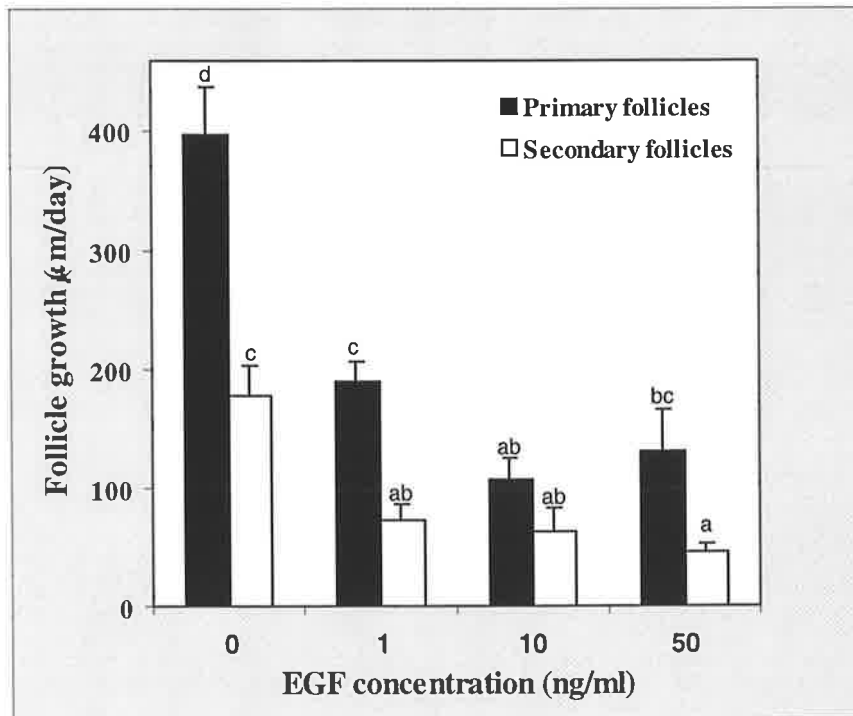


Figure 5.6. EGF has a significant effect on the growth of isolated primary and secondary wool follicles *in vitro*. Follicles were maintained *in vitro* over a 3 day period in the presence of 0, 1, 10 and 50 ng/ml of EGF (n = 12/concentration). Results are expressed as means with s.e.m. Different superscripts differ significantly at $P < 0.05$.

Analysis of variance revealed that there was no significant interaction between follicle type and EGF dose, indicating that follicle types had similar susceptibility to EGF *in vitro*.

The morphology of wool follicles maintained with $\text{EGF} \geq 0.125$ ng/ml was substantially changed with a subsequent cessation of fibre production. Observations made on follicles maintained with EGF over a 72 hour period revealed that changes were particularly noticeable in the follicle bulb around the dermal papilla. Cultured follicles formed distorted fibre ends which differed from those observed *in vivo*. The fibres of cultured follicles firmly attached to the inner root sheath forming a distorted fibre end and, this structure extruded from the

follicle bulb (Plate 5.3), whereas *in vivo* distorted end fibres move upward toward the surface of skin.

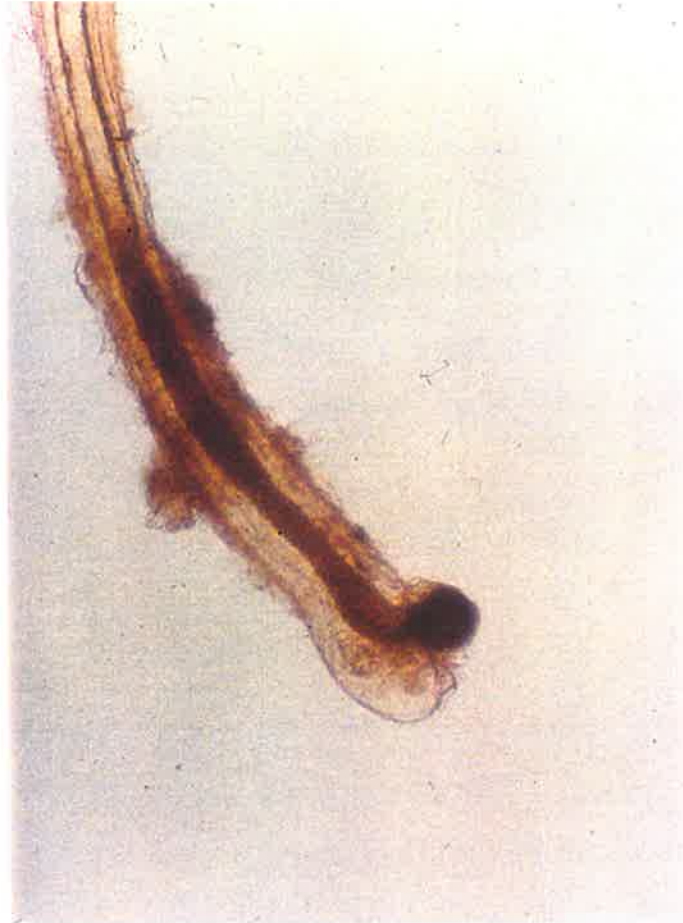


Plate 5.3. Light micrograph taken under an inverted microscope showing the effect of epidermal growth factor (EGF) on the Romney wool follicle maintained in culture over a 72 h period. Normal activity of the follicle was disrupted in the keratogenous zone where inner root sheath cells joined with the fibre and formed a distorted end structure. Later the distorted end structure collapsed and was extruded from the follicle bulb; Magnification: 43x.

5.4. DISCUSSION

5.4.1. Preliminary studies on the growth of follicles in culture

As previously shown (Hynd et al. 1992) wool and hair follicles can be successfully maintained *in vitro*. Growth was linear for at least 3 days, and approached 50% of “*in vivo*” rates. The average fibre length growth of Tukidale primary and secondary and Romney follicles cultured in Williams E medium over a 3 day period was 406 (range 146-764) and 173 (range 108-307) and 140 (85-270) $\mu\text{m}/\text{day}$ respectively, which is at about half the average *in vivo* rate (Woods and Orwin, 1988). Considerable variation in fibre length growth was observed between follicles. Damage to the architecture and physical properties of wool follicles during skin collection, follicle dissection and measurement may contribute to this variation. Depletion or absence of a particular growth factor or essential nutrients could be another possible reason for this variation.

Considerable variation was also found in the average fibre length growth of control Tukidale primary and secondary and Romney follicles maintained in culture over a 3 day period in different experiments in a range of 303-406, 139-173 and 103-255 $\mu\text{m}/\text{day}$ respectively. Since *in vitro* experiments were conducted in different seasons using different adult sheep, the variation in fibre length growth could be due to seasonal and between sheep differences in fibre production. Seasonal variation in wool growth in sheep is a well established phenomenon (Coop and Hart, 1953; Ryder, 1956; Hutchinson and Wodzicka-Tomaszewska, 1961; Slee and Carter, 1961) with the greatest length of wool grown in summer and early autumn (Hardy, 1950).

Photographs taken for growing follicles *in vitro* indicated that the growth of follicles were associated with the growth of both follicle inner root sheath and fibre. Furthermore the pattern of cell division and DNA-synthesis in cultured follicles was investigated by incorporating [methyl- ^3H] thymidine into culture medium. The results of [methyl- ^3H] thymidine autoradiography demonstrated a typical pattern of DNA-synthesis is taking place in the freshly isolated follicle. Even though the

observation that the majority of cell division takes place in the follicle bulb around the dermal papilla suggest that follicle culture is a valuable system for the understanding of follicle activity, however further investigation is needed to improve the system in order to decrease high variation in fibre growth of follicles.

5.4.2. Design and objectives of experiment.

Despite the presence of large variations in fibre growth rate, it was demonstrated that cultured follicles can be maintained and produce a fibre *in vitro*. The present study is the first undertaken to examine the effect of very low concentrations of EGF and cortisol on primary and secondary follicles *in vitro*. Previous *in vivo* experiments (Chapters 3 and 4) indicated that cortisol injection indeed causes a high level of follicle inactivity, but because the effect of the low concentrations of cortisol at the level of wool follicles *in vivo* are unknown, points to the need for investigating this response *in vitro*. The *in vitro* model allows statistical comparison to be made between treatment effect of depilatory substances. Since Merino sheep were used for the *in vivo* follicle shutdown studies (Chapters 3 and 4) the intention was to use Merino follicles for the *in vitro* studies also, but due to the small size and intertwining of follicles of this breed, it was extremely difficult to dissect large numbers of intact and viable Merino follicles. Consequently it was decided to use Romney and Tukidale breeds of sheep for this purpose.

5.4.3. The effect of cortisol hormone on the growth of follicles in culture

The results of *in vitro* experiments indicated that unlike the effects observed *in vivo*, addition of different concentrations of cortisol in the medium had no significant inhibitory effect on fibre growth over a 3 days period. Wallace (1979) also stated that unpublished results of Ward suggest that cortisol has no effect on wool follicles under conditions imposed on *in vitro* cultures of wool follicles within 6 hours. Culture of skin slices taken from an adrenalectomised sheep and placed in media containing cortisol at concentrations of physiological level had little effect on [³H] thymidine

uptake (Hynd and Applebee, unpublished). In another study, Scobie (1992) showed that over the range of doses used, 4-6 hours after exposure, cortisol had little effect on the functioning of wool follicles *in vitro*. While these studies indicate that cortisol does not suppress cell division in the short term, longer periods of exposure of cultured follicles to cortisol have not yet been studied. To examine whether a longer period of exposure to cortisol would inhibit fibre growth, follicles were maintained with supraphysiological concentrations of cortisol for 6 days. The results indicated that the growth rate of follicles maintained with cortisol was not affected. The possibility that some locally-produced inhibitory factor associated with dermis (Moore, 1989) may inhibit the action of cortisol *in vitro*, was examined by removing dermis from dissected follicles. The results indicated that there was no significant difference in fibre growth with or without dermis, maintained with supraphysiological concentrations of cortisol for a period of 6 days. Furthermore, the morphology of follicles was not affected with addition of different concentrations of cortisol to the medium. The *in vitro* results are in contrast to the results observed *in vivo*, where fibre growth was substantially inhibited and follicles underwent considerable morphological changes (see sections 3.3.5 and 4.3.5), suggesting the *in vivo* effect of cortisol on follicles is induced or requires greater periods of time to operate than the culture system allows or it is also possible that cortisol response may be effected through another factor.

5.4.4. The effect of EGF on the growth of follicles in culture

If cortisol response is effected through another factor, a review of literature revealed that a potential depilatory substance which has a close association with cortisol was EGF. In fact Singh-Asa and Waters (1983) indicated that infusion of defleecing doses of EGF caused a seven fold increase in plasma cortisol concentration, which is sufficient to inhibit fibre production and initiate wool breaks. Sheep injected with EGF shed some fibres and had a zone of weakness or wool breaks (Hollis et al. 1983). The appearance of wool breaks in EGF treated sheep

resembled that of the break in sheep treated with cortisol (see sections 3.4.6 and 4.4.6) and ACTH (Lindner and Ferguson, 1956). The imposition of psychosocial stress and the ACTH immunisation increased the efficacy of EGF as a wool harvesting agent, resulting in 60% weaker staples than the unstressed non-immunised sheep (Behrendt et al. 1993; Behrendt, 1994). The study of Adams and Wynn showed that cortisol administration actually suppressed EGF receptor numbers without altering receptor affinity. However in this study EGF defleecing was not attempted. These studies and the localization of receptors for EGF in the skin of sheep (Wynn et al. 1988) suggest that a strong association exists between EGF and cortisol in inhibiting wool production.

The present *in vitro* study is the first to show that very low concentrations of EGF inhibit fibre growth. All concentrations of EGF ≥ 0.125 ng/ml significantly ($P < 0.05$) inhibited fibre growth. EGF inhibition of fibre growth *in vitro* at concentrations of ≥ 0.125 ng/ml compares favourably with *in vivo* concentrations. Moore et al. (1983) demonstrated that intravenous infusion of Merino sheep with 3 to 5 mg of EGF in many cases resulted in shedding of the entire fleece, but at lower doses of 1 to 3 mg, the fibres were found to have a zone of weakness.

Inhibition of fibre growth of EGF treated culture follicles is probably due to absence of elongation of fibre cells which normally commences in the suprabulbar region of anagen follicles (Hollis et al. 1983). This may come about by reduced cell proliferation in the follicle bulb and impaired protein synthesis in the fibre cells (Chapman, 1989) or from an increase in apoptosis (Kerr et al. 1972).

In all concentrations of EGF ≥ 0.125 ng/ml, individual follicles were induced into a premature anagen-to-telogen transformation. Philpot et al. (1990) indicated that when follicles were maintained with EGF at a concentration of 10 ng/ml, there was a striking change in the morphology of the human hair follicle, but they did not attempt to determine the effects of EGF on follicles at lower concentrations. The *in vitro* EGF action of follicle shutdown mimics the *in vivo* action of cortisol (see sections 3.3.5 and 4.3.5) and EGF (Hollis et al. 1983). The histological changes that take place in

wool follicles when sheep are injected with depilatory compounds are discussed in sections 3.4.5, 3.4.6, 3.4.5 and in chapter 6. When sheep are injected with EGF over a period of 24 hours, delayed hardening of the layers of the inner root sheath is detectable within 6 hours of the start of the infusion and lasts for more than 48 hours (Hollis et al. 1983). This is accompanied by further disruption of the activity of the inner root sheath cells and the adjacent fibre in the upper part of keratogenous zone. Similarly *in vitro* cessation of fibre growth by EGF was accompanied by the characteristics of follicle shutdown. The earliest changes were detectable within hours of the addition of EGF. During this period, normal activity of follicles was observed to be disrupted in the keratogenous zone, where inner root sheath cells joined with fibre and formed a distorted end structure which resembled those found *in vivo*. Unlike the effect observed *in vivo*, formation of distorted end structure was not accompanied by the characteristics of follicle shutdown where the distorted end fibre regress towards the skin surface, but rather this structure collapsed and extruded from the follicle bulb. The extrusion of distorted end fibre is probably due to delayed hardening and disruption of inner root sheath cells with both intra- and inter-cellular accumulation of fluid and flocculent material in the follicle (Chapman, 1989).

Statistical analysis revealed that there was no significant interaction between follicle types and EGF dose, indicating that primary and secondary follicles had a similar decrease in fibre growth. Since EGF is a potent depilatory substance, it is not unexpected to observe that follicle types respond similarly to this compound. Hollis et al. (1983) indicated that fibre and inner root sheath of sheep injected with 4.5-4.7 mg of EGF were partially disrupted within the first 6 hours of infusion and all follicles stopped producing fibre within 6-8 days, suggesting that all follicles had similar degree of susceptibility to EGF.

Similar susceptibility of Tukidale follicle types to EGF may be breed related. It was indicated (see chapter 4, section 4.3.5) that Finewool and Strongwool follicle types responded differently to exogenous cortisol; fewer primary follicles regressed in Strongwool than Finewool sheep. This breed difference in susceptibility may be due

to arrangement of networks of blood vessels around the follicles (see section 4.6.8) or to the concentration of EGF receptor sites (see sections 3.4.8 and 6.3.1).

CHAPTER 6.
GENERAL DISCUSSION

CHAPTER 6. GENERAL DISCUSSION

This chapter is directed at discussing the morphological and cellular changes in the wool follicle and fibre, follicle sites sensitive to inhibition of cell mitosis and factors which influence the degree of elevation in plasma cortisol concentration and cessation of cell division following treatment of sheep with cortisol. The potential implications of this study for commercial wool production are also discussed.

6.1. Elevation in plasma cortisol concentration inhibits cell division in the follicles

The potential sequence of events occurring in the follicle in response to elevated cortisol levels is diagrammatically represented in Figure 6.1. Wool follicles in anagen are highly organised structures, in which occur cell division and migration, synthesis of macromolecules, controlled degradation of organelles in the fibre and of cells in the inner root sheaths and eventually sloughing of inner root sheath and some outer root sheath cells (Chapman, 1989). The process of fibre formation is complex involving a highly-integrated differentiation pathway which is subject to influence by hormones, cell regulators and inhibitors of normal cell function. Therefore, it is a simple task to disrupt this process in such a way that fibre production is ceased. Elevation in plasma cortisol concentration in response to external stimuli can impair follicle activity by inhibition of mitotic activity (Chapman, 1989) or increase in apoptosis (Kerr et al. 1972). The most obvious method of halting cell division is to prevent DNA synthesis, since the genetic material of the cell must be replicated before cell division can occur. Synthesis of cells can be stopped by depilatory compounds, such as EGF (Hollis, 1983) and N- [5-(4-aminophenoxy-pentyl)]phthal-amid (Chapman, 1980). Cell division inhibition results in follicle shutdown and fibre shedding within a few days of the inhibition occurring, because the supply of keratinocytes, essential for continued fibre growth, is disrupted.

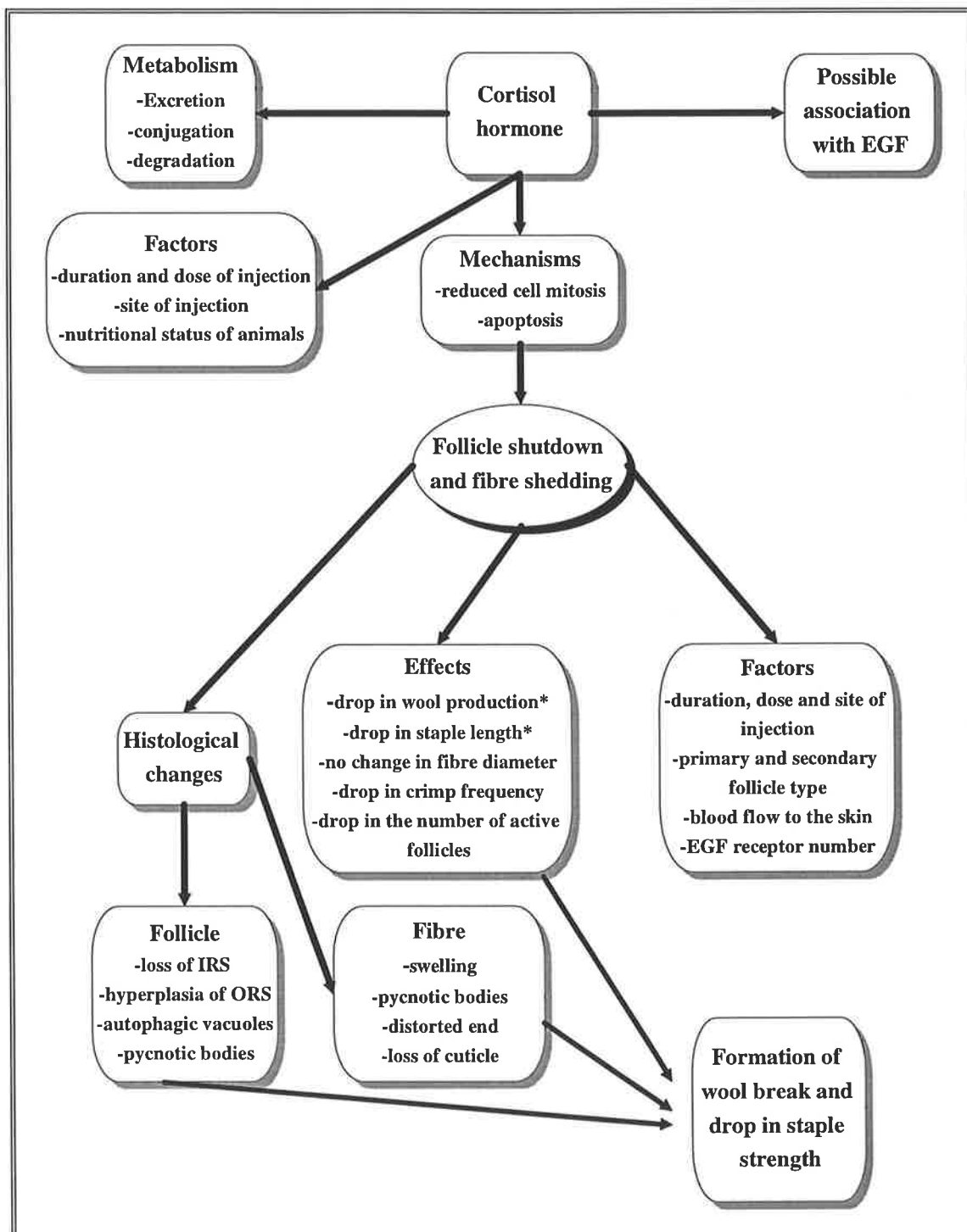


Figure 6.1. Diagrammatic representation of the potential sequence occurring in the follicle in response to elevated cortisol levels.

The rate and degree of synchrony of cessation of mitotic activity in primary/secondary and in secondary follicles of different size, shape and depth is of considerable importance with regard to the structure of the ends of depilated fibres. In spite of some similarities, the ends of the shed fibres exhibit appreciably different structures, which may be related to the degree of cessation of mitotic activity following cortisol injection. For instance, ends with tapered structure may possibly be produced by rapid, synchronous cessation of cell division; ends with step-like structure may be the result of slightly slower, less synchronous inhibition, while brush and club ends may involve much slower cessation of cell division.

Along with the inhibition of mitotic activity, reduced cell production may result from an increase in apoptosis, a process involving a mechanism of controlled cell deletion, which appears to play a complementary but opposite role to mitosis in the regulation of cell dynamics (Kerr et al. 1972). Apoptosis characteristically affects scattered single cells and is manifested histologically by the formation of small, roughly spherical or ovoid cytoplasmic fragments, some of which contain pycnotic remnants of nuclei. Structural changes in apoptosis take place in two discrete stages: the first comprises the formation of apoptic bodies, the second their phagocytosis and degradation by other cells. Little is known of the factors that initiate apoptosis, however in certain circumstances it is an inherently programmed event, determined by an intrinsic "clock" specific for the cell type involved. Some degree of environmental control is evident; hormones are known to affect apoptosis (Menkes et al. 1970). The formation of dark-staining bodies or autophagic vacuoles are of particular importance in apoptosis. Autophagic vacuoles have been reported in follicles of milk-fed lambs with a suspected vitamin deficiency (Chapman and Black, 1981), sheep fed poor-quality roughage for prolonged periods (Chapman, 1989), artificially reared lambs (Chapman and Black, 1981), zinc-deficient lamb (Masters et al. 1985), sheep dosed with cyclophosphamide (Brinsfield et al. 1972), mimosine (Reis et al. 1975), EGF (Hollis et al. 1982) and glucocorticoid analogues (Chapman et al. 1982). However, vacuoles are not only produced by exogenous chemicals, but also occur during the

catagen phase of normal cyclic hair growth in rats and mice (Parakkal, 1970) and seasonal shedding in Wiltshire Horn sheep (Chapman, 1980b). Regardless of how they are induced, formation of these vacuoles is associated with increased activity of proteolytic enzymes and breakdown of tissue proteins (Mortimore, 1982), and exhibit acid phosphatase and acid esterase activity (Pearson and Malkinson, 1969; Parakkal, 1970; Brinsfield et al. 1972). As such, they would promote autolysis of cell contents during regression of the follicles (Chapman, 1980).

6.2. Follicle sites sensitive to inhibition of cell mitosis in response to elevation of plasma cortisol concentration

Elevation in plasma cortisol concentration in response to exogenous cortisol injection disrupts follicle activity. The various sites where such disruption can take place and have an impact are discussed below:

6.2.1. Impact of inhibition of cell mitosis on fibre. The follicle bulb is the part of follicle most sensitive to cortisol because it is the region where cell division occurs. In the suprabulbar region of the follicle, cell division is complete and the events involved in specific keratin biosynthesis take place. These can be divided into transcription of the keratin specific genetic information into keratin messenger RNA, and its subsequent translation into the various keratin proteins. Cortisol and its synthetic derivatives, are known to alter the transcription process by the activation of different gene sets, and these compounds may have part of their effect at this point in the follicle (Ward, 1980).

It is likely that protein synthesis in cells of the fibres in the keratogenous zone of the follicles is affected rapidly by a reduction in keratin material resulting in distortion of fibre architecture of various kind such as the formation of cracks, thickened fibre ends (club ends), continuously growing fibres with very thin regions, formation of pycnotic bodies and finally shedding of wool (Chapman et al. 1983). Consequently, the time taken for disturbed protein synthesis to be manifest as impaired keratinisation and distortion of fibres in the upper parts of follicles reflects the time required for cells in

the fibres to migrate through the keratogenous zone and thence to the distal part of the follicle (Chapman, 1971). Disappearance of the distortion of fibre after cessation of cortisol injection is slower than its development, possibly as a result of the time required for the accumulated outer root sheath cells (hyperplasia) to be exfoliated and the follicles to resume their normal architecture.

Impaired keratinisation of the wool fibres would result in breakage of the fibre (shedding) at the weakened stretches. The distal portions of the broken fibres slough into the fleece causing formation of wool breaks; and the degree of mitotic activity of follicles following cortisol injection may be related to these structures (as discussed previously). However it appears that as a consequence of reduced cell mitosis in the follicular region of the follicle and impaired protein synthesis in the keratogenous zone, inner root sheath and outer root sheath cells are attached and carried by the distal portion of the shed fibre.

The development of pycnotic nuclei in shed fibres appears to have occurred in the follicle bulb (Chapman, 1971). The pycnotic change in these nuclei apparently inhibits their normal degradation, which would have occurred during keratinization of the fibre, and as a consequence these nuclei are retained (Chapman, 1980). Whether the synthesis of keratin proteins in the fibres is affected and the reason for the impairment of keratinisation of the portion of the fibre with retained pycnotic nuclei is unknown.

The degree of elevation in plasma cortisol concentration and cessation of cell division in response to the exogenous cortisol is likely to be influenced by a number of factors including:

- (1) The site and the dose of injection.
- (2) The metabolism of cortisol.
- (3) The nutritional status of the recipient.
- (4) Primary and secondary follicle type.
- (5) The size and shape of follicles.
- (6) Blood flow to the skin.
- (7) EGF receptor site number and affinity.

These factors will be discussed in different sections of this chapter.

The site of administration of cortisol could have a marked impact on the amount and the length of time for which cortisol is circulating in the blood. In sheep, the plasma half life of cortisol is 20 minutes (Reid, 1959a) and intravenous injection would result in faster metabolism of cortisol. Percutaneous application of cortisol in rats inhibits the growth of hair, however this inhibition is limited to the area of treatment (Downes and Wallace, 1965). As the effect of elevation of plasma cortisol concentration on wool follicle is confounded with the stress of injection, it would be of interest to study other means of administration of cortisol, such as oral dosage. This would limit the number of complicating factors in such studies.

The level of elevation in plasma cortisol concentration can be influenced by the amount of food eaten. Chapman and Bassett (1970) indicated that plasma cortisol concentration of sheep which were initially fed *ad libitum* was lower than in sheep on restricted intakes. This was later supported by Thwaites (1972) who showed that poorly fed sheep had a greater weakness in the fibre after a 14-day treatment with cortisol than well-fed sheep. These results indicate that the availability of nutrient material could alter the efficacy of cortisol in inhibiting cell division.

The variation in the degree of inhibition of cell mitosis may be due to variation in blood flow to the skin and utilisation of the depilatory factor within the follicle. As blood flow to the skin has been found to be different in different breeds of sheep (Setchell and Waites, 1965) and in different strains of Merino breed (Hocking Edwards and Hynd, 1991), it is expected that the degree of impairment of follicle activity would increase with greater transport of depilatory factor to the follicles with increasing blood flow to the skin. It was indicated that Strongwool sheep had a higher percentage of follicle shutdown than the Finewool sheep (see section 4.3.5) when exposed to cortisol, which may reflect greater blood flow to the skin of Strongwool sheep.

The degree of elevation in plasma cortisol concentration could be related to genetic differences and fleece characteristics of sheep. It was shown that plasma cortisol concentration was significantly higher in sheep with a low coefficient of variation of

fibre diameter. In Canada, which is characterised by temperatures well below zero, the results of a study during a 4 year period involving fleeces of 338 mature ewes and 251 yearlings of four different breeds revealed that breed was the most important factor contributing to variation in fibre diameter (Vesely et al. 1965). The authors concluded that the difference in fibre characteristics between the breeds may reflect a difference in adaptability of sheep to Canadian range conditions. Slee and Ryder (1967) indicated that wool growth in Scottish blackface sheep characterised by having highly variable fibre diameter was more sensitive to the depressing effect of cold exposure than in the Merino x Cheviot sheep which is more uniform in fibre diameter. The authors do not state whether the genetic difference in response arose from systemic factors or from a difference in follicle sensitivity to similar stimuli. The results of the present experiment show that such differences in susceptibility could be due to (A) systemic factors such as the rate of metabolism and utilisation of cortisol, as the elevation in plasma cortisol concentration of high coefficient of variation of fibre diameter sheep was significantly lower than low coefficient of variation of fibre diameter sheep; (B) differences in follicle sensitivity to cortisol, as high fibre diameter sheep (Strongwool) had a significantly higher percentage of follicle shutdown over time. In breeding for resistance to harsh environmental conditions, coefficient of variation of fibre diameter, which in the present study was related to an elevation in plasma cortisol concentration and fibre diameter which was related to percentage of follicle shutdown, are important factors to consider.

The degree of inhibition of cell mitosis and protein synthesis in the keratogenous zone may be the result of a difference in follicle sensitivity to similar systemic stimuli. Studies *in vitro* suggest that a possible association exists between cortisol and EGF in inhibition of cell division in the follicle bulb and cessation of protein synthesis in the keratogenous zone. It has been indicated that infusion of defleecing doses of EGF caused a seven fold increase in plasma cortisol concentration (Singh-Asa and Waters, 1983) and that imposition of psychosocial stress and ACTH immunisation increases dramatically the efficacy of EGF (Behrendt et al. 1994). These findings may be

important in terms of difference in susceptibility of primary and secondary follicle types, secondary follicles differing in size and shape and between breeds and strains of sheep. Such differences may be associated with differences in the amount of follicle EGF receptor sites. Studies have shown that the biological action of EGF requires that the molecule binds first to specific cell surface receptors (Carpenter and Cohen, 1979). Binding is followed by clustering of receptors and endocytosis of the ligand receptor complex (Haigler et al. 1979) which depletes the surface of receptors giving rise to the phenomenon of down regulation (Carpenter and Cohen, 1979). The extent of the biological responses to EGF appears to partially depend on the concentration of the growth factor and receptor down regulation (Adamson et al. 1981; Adamson and Warshaw, 1982). Green et al. (1983) indicated that EGF receptor distribution is correlated with epithelial regions which have proliferative capacity and that the number of epidermal basal receptors is related to the rate of epidermal cell proliferation. Wynn et al. (1989) localised EGF receptors in undifferentiated cells of the inner and outer root sheath and the bulb of Merino wool follicles. They also observed differences in receptor concentration between follicles *in vivo* perfusion of ^{125}I -labelled EGF. This difference in EGF receptor concentration may be the cause of the existence of varying degrees of susceptibility to shutdown between follicles. The localisation of receptors in these regions is consistent with the morphological changes in follicle response to the depilatory action of cortisol and EGF (Hollis et al. 1983). In view of these studies, and the recent investigation into the changes by intradermally injected EGF (Chapman and Hardy, 1988) and the localization of receptors for EGF in the follicle bulb, it is possible to postulate that cortisol inhibits follicle bulb cell division indirectly via the effects of EGF on wool follicle.

Along with cortisol, reduced cell division and DNA synthesis in the follicle bulb results from an increase in the rate of secretion of catecholamines, as this change could account for the drop in wool growth rate of control sheep which were not administered with cortisol (section 3.3.3). Observations on the follicles in skin sections confirm the conclusion that follicle shutdown is restricted to cortisol-induced sheep,

and that drop in wool growth rate of control sheep is the result of another mechanism involving slowing of the rate of mitosis. Scobie (1992) suggested that biogenic amines inhibit the rate of mitotic activity within 2-4 hours by either acting as a hormone or as a neurotransmitter being released from sympathetic nerve endings. Ferguson (1949) suggested that catecholamine secretion from the adrenal gland acts on wool follicles indirectly through vasoconstriction and reduced blood supply. Injection-induced vasoconstriction therefore seems likely to have been responsible for the decreased wool production in sheep.

6.2.2. Impact of inhibition of cell mitosis on inner root sheath. Although cortisol affects cell mitosis in the bulb, its effect is apparent in the inner root sheath higher up in the follicle. Histological examination of skin from cortisol-treated sheep revealed that the structure of inner root sheath of inactive follicles was disrupted and in some cases this cell type was totally lost. Although this structure does not become incorporated in the final assembly of keratin proteins, it has a key role in supporting and shaping the hardened wool fibre. The inner root sheath, a highly differentiated part of the follicular wall (Auber, 1952), immediately surrounds the fibre. Its three components, peripheral Henle's layer, middle Huxley's layer and axial cuticular layer originate from the proliferating bulb matrix cells. In the Merino, the layer of inner root sheath harden in the following sequence; Henle's layer, cuticle and Huxley's layer. Henle's layer normally hardens in the suprabulbar region, whereas the inner root sheath cuticle and Huxley's layer, harden in the upper part of the keratogenous zone (Gemmell and Chapman, 1971). An important target in the inner root sheath is the formation of the amino acid citrulline in the trichohyalin protein moiety (Harding and Rogers, 1976). The impairment of inner root sheath sloughing may be due to inhibition of trichohyalin formation. Consequently the cells of Henle's layer do not harden in the suprabulbar region (Chapman, 1980) and follicles lose their shape.

The major part of decline of wool production in cortisol-treated sheep resulted mainly from an increase in the number of inactive follicles and a decrease in staple length irrespective of any change in fibre diameter. The drop in wool production in

cortisol-treated sheep without any change in fibre diameter could be related to disruption of hardening of inner root sheath cells. Delayed hardening of the layers of inner root sheath is accompanied by gross dilation of the endoplasmic reticulum with both intra- and inter-cellular accumulation of fluid and flocculant material (Chapman, 1989). The fluid accumulation frequently causes distortion of the adjacent fibre and induces disruption of cuticle formation. In the majority of affected follicles, the fibre and inner root sheath harden without any change in fibre diameter. On the other hand elongation of fibre cells, which normally commences in the suprabulbar region of anagen of follicles is absent in affected follicles. (Hollis et al. 1983). Consequently fibre length decreases irrespective of any change in diameter. In another study Hynd (1989) showed that diameter is determined by the rate of cell production (which in turn is associated with bulb area, papilla area and papilla shape) and the size of the cortical cell post-keratinization. Length is also determined by the rate of cell division and cell size but is most dependent on cortical cell length (Hynd, 1989). This explains why length and diameter often respond together (both being dependent on similar character) but because their dependence on these characters differ in a relative sense, they can respond independently.

6.2.3. Impact of inhibition of cell mitosis on outer root sheath. Wool growth can be disrupted by events occurring in the outer root sheath. The function of this structure is not clear, but its distal half has been implicated in removing the inner root sheath from the hardened wool fibre and the follicle (Chapman, 1980). Therefore the impairment of inner root sheath cells could be a consequence of disturbed function of outer root sheath cells (Hollis et al. 1982). A consistent feature associated with the partial degradation of fibre in cortisol-treated sheep was the accumulation (hyperplasia) of outer root sheath cells and consequent loss of crimp. The reason for hyperplasia is unknown but it may result from retardation of the process of elimination of outer root sheath cells into the follicle lumen, which occur in this region of normal wool follicles (Gemmel and Chapman, 1971).

Among sheep with different degrees of staple crimp abnormality (or “dogginess”) as the frequency of follicles with gross hyperplasia of outer root sheaths increases the proportion of fibres lacking crimp increases (Chapman et al. 1960; Aiken and Ryder, 1962). It has been indicated that wool samples with staple crimp abnormalities, fibre lacking crimp are shorter than crimped fibres (Aiken and Ryder, 1962; Chapman and Short, 1964). Therefore, hyperplasia of the outer root sheaths of follicles also has an effect on fibre growth characteristics in addition to crimp. Loss of crimp or unevenness in fibre length will lead to reduction in staple strength as a result of a reduction in load bearing material.

6.3. Application of results to commercial wool-producing enterprises

6.3.1. Tender wool problem and its impact on the wool industry. The importance of hormones in control of wool fibre synthesis was indicated unequivocally in the initial studies of Ferguson et al. (1965), who demonstrated that hypophysectomy resulted in the cessation of wool growth. The results of present experiments indicate that high quality wool production in sheep can be impaired by elevation in plasma cortisol concentration with a formation of a break in the wool. The term “break” is used to refer to weakness at one point along the staple of wool and can be described as the appearance of a clear window through the wool sample. This zone of weakness is distinguished as a level in the staple at which many fibres in fact have a break, owing to cessation of fibre production by follicles. In the Mediterranean environment due to large variation in feed supply throughout the year, the position of break in wool staple under field conditions is often associated with a particular long term stress, such as rainfall in summer and availability of feed at the end of summer and the beginning of autumn.

The disadvantage of tender wool, and occurrence of a break in the staple is that it results in increased fibre breakage during processing particularly in combing, and in decreased Hauteur. As a consequence price penalties are imposed on such wool by processors. Management practices are effective in preventing the occurrence of wool

breaks. At the opening of the season or following a heavy rain in summer, one can attempt to avoid the formation of wool breaks in young growing sheep (the most susceptible to the tender wool) by beginning to feed with supplements. Nutritional stress in the late pregnancy often causes a break before lambing. Spring lambing and autumn shearing reduces the incidence of tender wool problem, as the stress of late pregnancy coincides with availability of sufficient green feed. The nutritional stress at the break of the season may produce the weaker, fine section of the fibre near the end of staple, instead of in the middle as with autumn lambing and spring shearing.

Evidence indicated that the nutritional state of the animal affects the level of response to cortisol (Chapman and Bassett, 1970; Thwaites, 1972), therefore the level of availability of feed during an increase in plasma cortisol concentration can have an impact on the degree of susceptibility of sheep to shutdown. Increasing stocking rate results in decreased fleece weight, staple length and staple strength (Bellotti et al. 1992). Earle et al. (1994) showed that with increasing stocking rate, staple strength, live weight and minimum fibre diameter all decreased. Hughes et al. (1996) showed a strong effect of stocking rate on the percentage of follicle shutdown. Increased stocking rate and overgrazed pastures not only increases the risk of damage to pastures and soils, but also depletes the animal from reserves against stress; consequently the chance of occurrence of follicle shutdown and wool breaks increases. It is of great importance to minimise the chance of occurrence of wool tenderness especially at the opening of the season when poor quality pasture is available.

The results of the present experiments indicate that sheep differ in susceptibility to shutdown. This difference may be due to a number of factors such as: differences in efficiency of utilisation of cortisol and number of EGF receptor sites and the degree of follicle sensitivity to external stimuli. Under field conditions, sheep are exposed to a variety of environmental stresses and attempts should be made to select sheep which are more tolerant to such stresses in order to produce better quality wool. On the other hand incorporation of staple strength into breeding objectives would have benefits to

improving staple strength as this fleece characteristic is moderately to highly heritable (Howe et al. 1991).

The result that wool production dropped in control sheep which did not receive any cortisol suggests that wool production can be impaired by increased secretion of catecholamines. With respect to commercial sheep farming for wool production, frequent excitement associated with rounding up, yarding, transport and handling animals may induce stress and the subsequent release of catecholamines is likely to reduce wool growth rate. Also natural short term stresses such as transiently reduced environmental temperature depress wool growth (Slee and Ryder, 1967) possibly as a result of release of catecholamines and reduced blood flow. The amount and the frequency of such short term stresses which are created under different conditions should always be a major concern and attempts should be made to minimise these excitements and stresses.

6.3.2. Implication of results to development of defleecing agents. As keratin is the primary structural component of wool and contains disulphide linked polypeptides, splitting the disulphide bonds by reduction increases the alkali solubility of wool and allows fibre weakening or dissolution (McDonald et al. 1980). Cortisol injection causes a dramatic drop in wool production, staple length and the number of active follicles. A substantial number of fibres are shed but the entire fleece does not cast since shed fibres are retained by continuous fibres which are not affected by cortisol. Weakened wool could be harvested by manually breaking the continuous fibres at the weakened zone. The use of cortisol as a defleecing agent offers a possible solution for avoiding time consuming and expensive mechanical harvesting. However a number of aspects need to be considered on the defleecing action of cortisol.

Following dosing sheep with cortisol for the purpose of inducing a complete break in the wool, more than 50% of follicles must stop producing fibre for a period of time. As such, considerable growth of wool can be lost, because it takes at least 10 weeks before all the follicles resume normal activity. However, the extent to which induction

of wool breaks would make up for the loss of wool due to follicle regeneration would depend on what dose and how long cortisol injection would persist.

Although fibres produced after cortisol injection are of similar nature to those induced to shed, repeated dosing may lead to a reduction in follicle number and acitivity with a consequent broadening of the remaining fibres. A similar observation has been made by Short (1955).

Variability to treatment is evident within as well as between animals. When fibre shedding occurs, it is from the belly and ventral trunk regions and the wool on the dorsal thoracic region is less likely to be shed. To be acceptable as a practical defleecing agent, cortisol must have similar effect in all sheep and body sites. These widely differing responses, occurring with cortisol at the same level, highlight one of the main problems associated with defleecing characteristic of this compound. The variation in the form and degree of weakening produced within and as well as between animals may arise from systemic and non systemic factors such as the proportion of follicles affected, the degree to which follicles are affected, the length of time and the level of concentration of cortisol circulating in the blood, blood flow to the skin, the extent of supply of hormone to the follicle and efficiency of hormone within the follicle.

As intramuscular injection of insoluble hydrocortisone acetate for a long period of time is impractical under field conditions, other alternatives such as subcutaneous or oral dosage need to be considered.

6.4. Guidelines for future studies

Present studies indicate that hormones and growth factors are involved in coordinating wool growth. However, further investigation must be carried out under natural grazing conditions to identify factors affecting wool quantity and quality with respect to the occurrence of the tender wool problem. Identification of factors involved in staple strength reduction and fibre shedding should allow for the correct timing of supplementary feeding to prevent tender wool. The timing of supplementation should

be such that the sheep is fed prior to initiation of the wool follicle changes leading to fibre shedding. As a potential source of variation in staple strength sufficient attention must be paid to follicle shutdown, as this factor is found to be a major determinant of staple strength in cortisol treated sheep. With the prevention of fibre shedding and an improvement in staple strength there should be an increase in Hauteur and a decline in noil.

Because of limitations of *in vivo* studies under field conditions associated with care and handling of animals, it is necessary to develop the *in vitro* systems to examine various influences of cortisol and other depilatory substances on the growth of follicles. The pattern of cell mitosis which was observed in culture follicles appeared to be representative of that which would be expected in the lower portion of the follicle *in vivo*. The observation that shutdown follicles have a different behaviour than that of the control follicles suggest that a culture system could be valuable for the understanding of the natural mechanisms involved in periodic fibre shedding which is observed in sheep. However further investigation is needed to improve the system in order to increase the longevity and decrease the high variation in fibre growth rate of cultured wool follicles.

Considerable effort is required to be obtain on a detailed understanding of follicle activity. Studies of the natural shedding phenomenon are particularly relevant. However a technique is required to get answers in the shortest possible time. The rapid advances in gene analysis, together with new techniques for follicle and cell culture, will enhance the understanding of the processes involved in cell mitosis and keratin biosynthesis in the near future, and it is with this understanding that it will be possible to prevent wool tenderness or to induce wool breaks either biologically or chemically.

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