1. Skin Structure and Function

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Learning objectives

On completion of this topic you should have:

- an understanding of the development of wool follicles in the skin
- the knowledge of cellular and molecular mechanisms involved in the formation and activity of mature follicles growing wool
- an appreciation of where our knowledge of wool growth is limited

Key terms and concepts

- the epidermis is a vital barrier to the environment
- wool follicles develop from the interaction of epidermal and dermal cells
- the cell layers of a wool follicle are derived from streams of separate cell lineages that are determined by expression of controlling genes
- signalling molecules direct gene expression in the formation and maintenance of wool growth
- the population of follicles of fine wool sheep arises from an abundance of secondary derived follicles.
- the skin has a network of capillaries that provide blood supply to follicles
- nutrients for wool growth cross basement membranes located between dermal cells and the epithelial cells of the epidermis and follicles
- wool quality is related to the ratio of secondary follicles to primary follicles and the ratio can be increased by selective breeding
- follicle stem cells have been located in a bulge region of the outer root sheath and replenish the bulb as required, e.g. in follicle regeneration of the hair cycle
- crimp formation is the result of several follicle events and is not fully understood.

Introduction to the topic

The skin of mammals is the largest of all the organs in the body. If the skin of a typical adult human male were stretched out flat it would cover about 1.5 square meters and weigh about 4 kilograms. Sheep skin would be approximately the same. The skin consists of two main layers, an outermost layer of epithelial cells forming the epidermis and an underlying layer, the dermis that consists of a collagen network produced by fibroblasts. The dermal collagen and fibroblasts surround the hair follicles and beneath the dermis is a layer of fat cells although in sheep this fat layer tends to be minimal. Deeper into the skin layers of sheep is an underlying cutaneous muscle layer that enables the skin to twitch when stimulated.

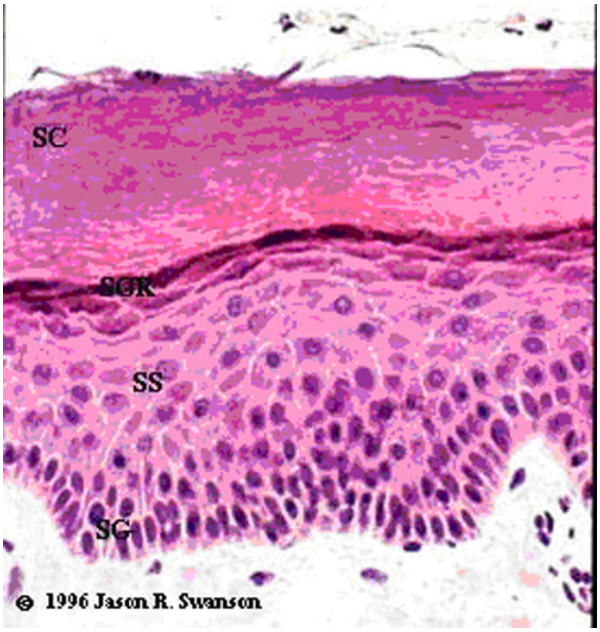
Mammalian skin is a protective organ against micro organisms and other insults that could penetrate from the environment but it also acts as the barrier against water loss. In that context the skin is concerned with the regulation of body temperature through the functioning of the blood supply and sweat glands.

The investigation of the biological mechanisms operating in mammalian skin has been hampered by the fact that the epidermis is very thin and contains cells at different stages of maturation and also hair follicles are very small. However, the past three decades have seen the development of many microscopic and biochemical techniques that enable molecular information to be obtained from small amounts of tissues. Our knowledge of the activities of skin structures has grown enormously. Sheep, mice and humans are the species mainly used by biologists as experimental models and the findings generally apply to the general knowledge of the activity of mammalian skin.

1.1 The epidermis

The epidermis has several layers (Figure 1.1).

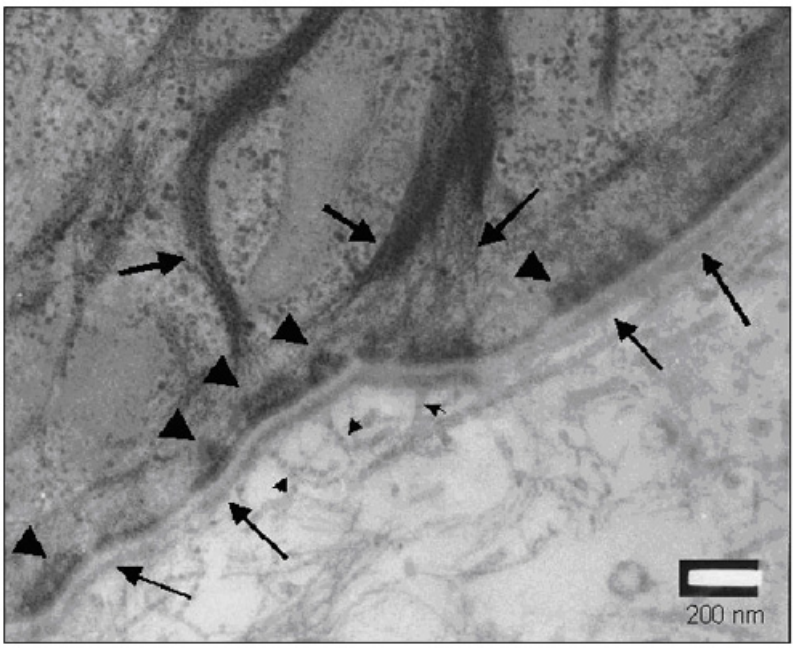
Figure 1.1 The epidermis has several layers including the cornified (SC), granular (SGR), spinous (SS) and basal cell layers (SG). Source: Melton and Swanson (1996).



The basal layer is the proliferating layer and rests on a basement membrane that is situated at the dermal-epidermal junction and is composed of collagen Type IV (Type IV is one of the family of 12 collagens) and mucopolysaccharides such as laminin and fibronectin. The basement membrane can be visualised by transmission electron microscopy (TEM) and the anchoring of the epidermis to the dermis is carried out by structures called hemidesmosomes in the basement membrane (Figure 1.2).

The basal layer of the epidermis contains stem cells from which daughter cells move upwards undergoing differentiation in which the main protein products expressed are the cytokeratins that belong to the keratin intermediate filament superfamily and filaggrin. As the cells differentiate they become the stratum granulosum or granular layer because an important protein, filaggrin, is in the form of granules and the keratin filaments can be seen in the electron microscope as filament bundles. As the cells continue on their outward movement they finally die, the filament bundles aggregate into solid masses with the filaggrin which acts as a “cement” and fill the cells that are now the surface layer called the stratum corneum.

Figure 1.2 TEM of a section of a basal epidermal cell beneath which is the basement membrane (arrows) seen as a diffuse layer with dense patches that are hemidesmosomes (arrow heads) anchoring the epidermis to the underlying dermis. Intermediate filament bundles are attached to the hemidesmosomes.
Source: Mattek Corporation, http://www.mattek.com/pages/products/epidermft and Young (1980).



1.2 Appendages of the skin: Wool follicles and skin glands

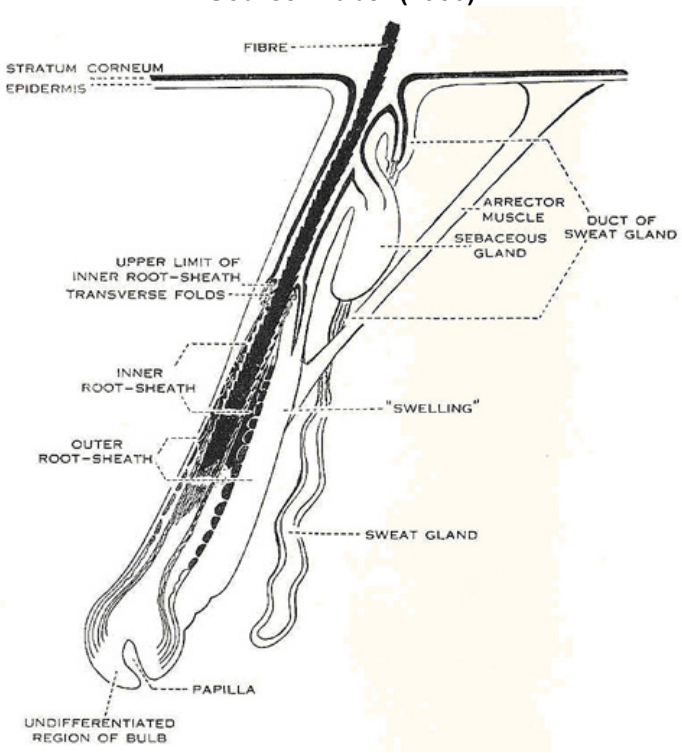
Follicles

There are about 10^8 wool follicles in the skin of fine wool sheep. The adult follicle (Figure 1.3) is a complex of layers of six concentric cylinders of cells and each layer is a lineage of cells (Figure 1.4) established during embryonic development. The histological structure is the same in all mammalian species.

The outermost layer is the outer root sheath (ORS) a cylindrical layer of epithelial cells continuous with the epidermis. Within the ORS in the following order are the three layers of the inner root sheath (IRS), Henle, Huxley and IRS cuticle, the cuticle of the fibre and then the fibre cortex. In coarse wool fibres especially the kempy fibres of wild sheep that have two seasonal coats there is a central core called the medulla. The cells of the medulla are anchored within the surrounding cortical cells and are separated by air gaps and therefore have increased insulating properties. At the base of the follicle is the bulb. It is often bent away from the axis of the follicle. The bulb is where the epithelial cells divide and differentiate to give rise to the fibre and surrounding IRS. The cuticle of the fibre is interlocked with the IRS cuticle and they move up the follicle together as they differentiate, this interaction contributing to the anchoring of the growing fibre in the follicle. At the upper end of the follicle where it narrows near the epidermal orifice (the infundibulum; funnel), the IRS degrades to cell envelopes that are sloughed leaving the fibre to emerge. Cell division in the bulb is a controlled process so that when cells move away there are sufficient germinative cells remaining to maintain growth. There is conjecture as to whether these cells are truly stem cells because in recent years it has been shown from studies of mouse follicle regeneration through the hair cycle, that stem cells reside in the ORS just below the level of the sebaceous gland and can be seen as a “bulge” (see section 1.10). This structure is expected to be present in wool follicles although it has not been directly demonstrated.

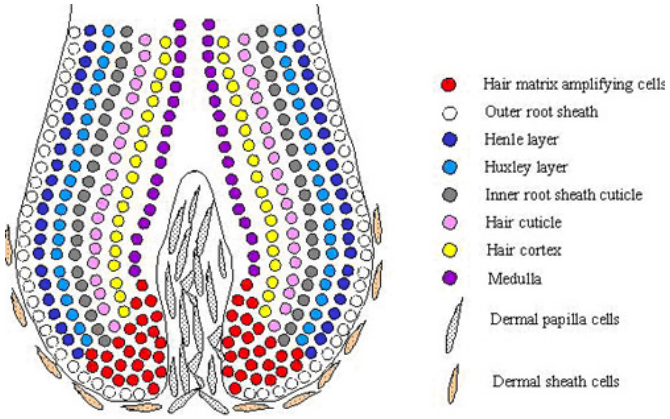
The specialised fibroblastic cells of the dermal papilla are essential for continued hair growth and are continuous with the dermal sheath cells on the external surface of the outer root sheath (see Figure 1.4).

Figure 1.3 A diagrammatic view of the different layers of an adult wool follicle. Source: Auber (1950).



The outermost layer of the follicle, the outer root sheath (ORS), is continuous with the epidermis. The next layer is the inner root sheath (IRS) that consists of three layers of cells, the Henle (He) layer immediately adjacent to the ORS and then the Huxley (Hu) layer and the inner root sheath cuticle (IRSCU). The IRSCU is closely attached to the fibre cuticle as the fibre differentiates and becomes separated from it in the upper levels of the follicle as the IRS degrades. The three layers of the IRS, the fibre cuticle and fibre cortex (and medulla when present) are derived from cell lineages that originate as matrix cells in the bulb and whose differentiation pathways are separately programmed (Figure 1.4).

Figure 1.4 Diagram showing the six cell lineages that derive from the hair matrix cells (or germinative region) and differentiate into the cellular structures of the wool follicle and fibre. Source: Rogers (2004).



The developmental events that lead to a mature wool follicle have been well characterised by histology. Initially, the embryonic skin consists of two layers, the outermost epithelium and underlying dermis. At day 65 (Figure 1.5A) a local accumulation of cells appears as a thickening of the epidermis called an epidermal placode and beneath the placode a concentration of dermal fibroblasts begins to appear. The epidermal cells of the placode grow down into the dermis as a plug (Figure 1.5B) and gradually differentiate into the mature follicle.

Figure 1.5A Histological picture of an early epidermal placode growing from the basal layer of the epidermis where the proliferative and stem cells reside. Beneath the placode some dermal cells are aggregating. Source: Dunn, S (pers. comm. (2005)). Bar =50um.

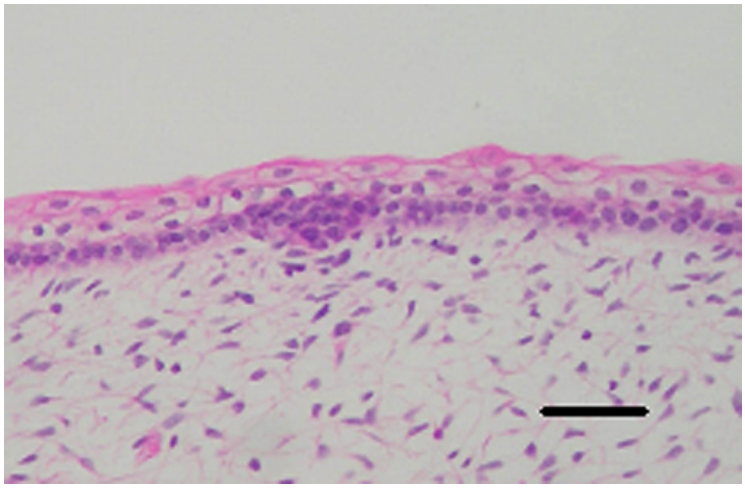
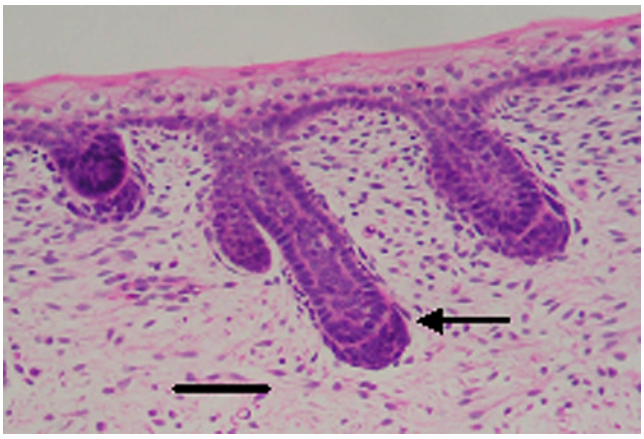


Figure 1.5B The development at 85 days has reached the stage of primary follicle formation and the follicle (arrow) has a prepapilla of densely aggregated dermal cells beneath the follicle base from which the bulb will form and enclose the prepapilla. Source: Dunn, S (pers. comm. (2005)). Bar =50um.



These events were described many years ago and a diagrammatic representation of them is given in Figure 1.6. Analysis of the cellular interactions that are responsible has demonstrated that there are consecutive messages exchanged between the epidermal placode and the underlying dermal cells that will become the papilla of the follicle. These exchanges are represented in Figure 1.7 and have to pass across the basement membrane (Figure 1.8).

Figure 1.6 Diagrams illustrating the major steps in the formation of the wool follicle beginning with the epidermis followed by invagination of a hair plug, aggregation of dermal cells that form the dermal papilla. The appearance of the inner root sheath (IRS) is an early event in the growth of the first fibre that develops with the outward growth of the IRS. The fibre emerges after the formation of a hair canal through which the fibre emerges in the adult follicle. Source: Hardy (1992).

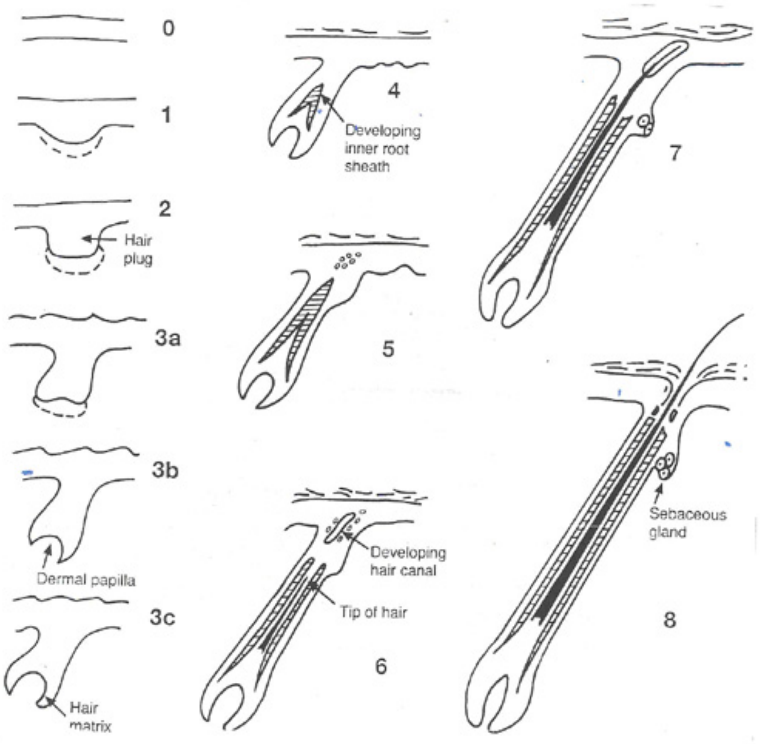


Figure 1.7 Diagrams of the deduced stages of message transfer between epidermis and dermis (epithelial-mesenchymal interaction). The first message is an instruction from the dermal cells (they later constitute the papilla and dermal sheath) to the epidermis and the epidermis instructs the mesenchymal cells to form the dermal papilla. The last step is that the pre-papilla instructs the epidermis to make a hair follicle. Source: Hardy (1992).

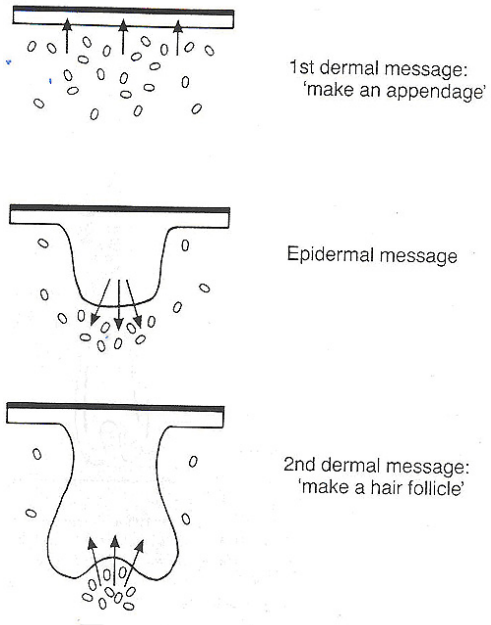
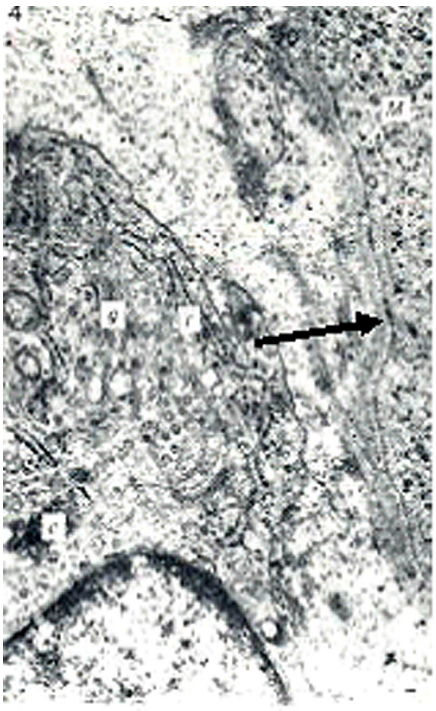


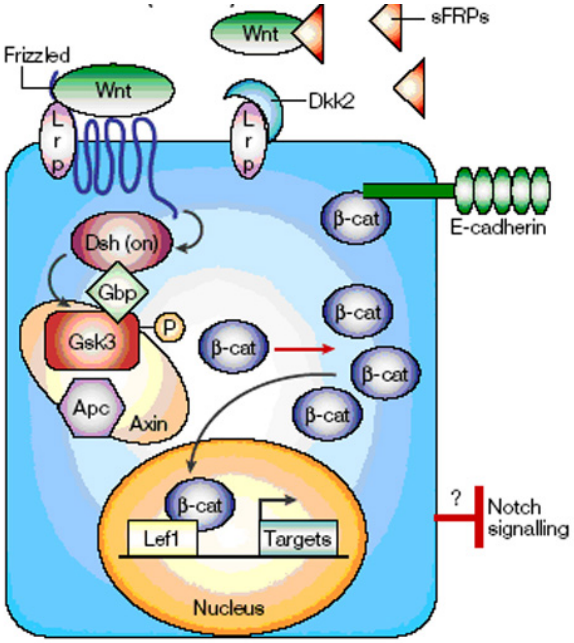
Figure 1.8 TEM of a section through the bulb at the level of the papilla showing an epithelial cell on the right with the basement membrane (arrow) and a dermal papilla cell on the left containing Golgi (g) and membranes of the endoplasmic reticulum (r). Source: Young (1980).



Molecular signals in follicle development and maintenance

There are many molecular signals that have been identified as being involved in initiating the formation of follicles in the skin. There is some evidence that factors associated with nerves play a role. The formation of a nerve plexus beneath the epidermis prior to the formation of the mesenchymal (dermal) condensation has been observed in the development of vibrissae (whiskers) on the snout of the mouse (Exan and Hardy 1980). The distribution of early nerve fibres in the snout might also determine the organised pattern of the vibrissal follicles which is precise and constant between individual mice. It is not known whether neural factors are involved in the formation of wool follicles and their organisational pattern in the skin of the sheep. Factors known as neurotrophins of which there are more than twenty members of this protein family (including nerve growth factor, NGF) and their receptors have been shown to be expressed in the development of hair follicle populations in the mouse although it may have a modulating role rather than a direct inductive role in follicle formation (Botchkarev et al. 1998). The mRNA for NGF has also been detected in the follicle bulb and ORS of wool follicles and may be important in the maintenance of the active (anagen) follicle. Clearly the neurotrophins are important in addition to other growth factors in the formation of wool follicles but the basic cause of their positioning in sheep skin remains unsolved. The establishment of morphological fields involving the interaction of growth factors and receptors is a likely cause and mechanisms of this kind have been analysed (Nagorcka and Mooney 1989) using a theoretical model. Different patterns of follicles can be obtained by varying the threshold of concentration between factor and receptor and triggering a developmental event. For example, a major signalling pathway in follicles is the WNT (Wingless-type) - Frizzled, receptor pathway. The WNT genes encode a family of secreted glycoprotein ligands and Frizzled (Fz) encodes a class of membrane-associated WNT-receptors. The pathway is essential for the maintenance of inducing activity of the dermal papilla (Kishimoto, Burgeson and Morgan 2000). Genes that are turned on in the papilla by the pathway produce proteins that must cross the basement membrane in the papilla to act on the dividing epithelial cells in the bulb. A simplified diagram of the pathway is given in Figure 1.9.

Figure 1.9 A simplified cartoon representation of the activated WNT signalling pathway involved in gene expression in the hair follicle. WNT molecules bind to WNT receptors that set up a cascade of factors that act as a complex and dephosphorylate alpha-catenin (normally phosphorylated and degraded) and then enters the nucleus to activate gene expression. In both normal and the activated state some alpha -catenin is present as part of the cadherin cell adhesion mechanism. Source: Fuchs and Raghaven (2002).



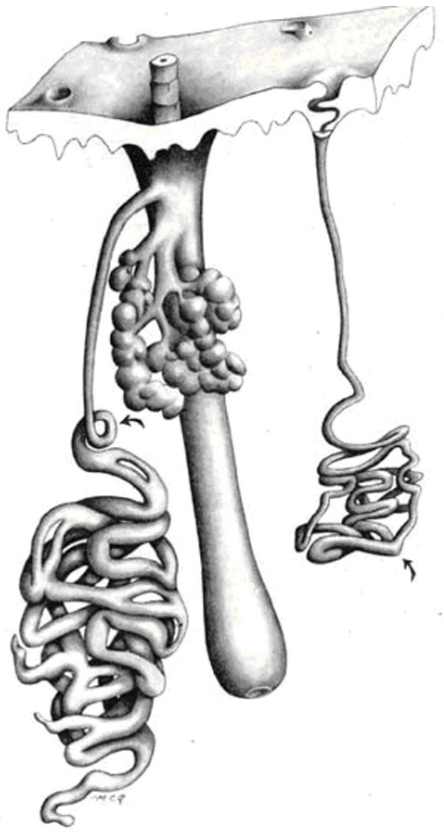
The molecule alpha-catenin is present in the cytoplasm and is a component of cell-cell adhesion. When it is in excess it is phosphorylated by the AXIN/GSK3B/APC complex and destroyed by the protein degradation system. Activation occurs when a WNT ligand molecule binds to the receptor the degradation is shut down and alpha -catenin enters the nucleus where it binds to promoter regions of genes including keratin genes that are then transcribed and expressed.

The central molecule is alpha -catenin which has dual roles. It is part of the cadherin complexes of cell-cell adhesion junctions that hold cells together. Alternatively it acts within the nuclei of cells after transportation from the cytoplasm (DasGupta and Fuchs 1999, Merrill et al. 2001). It forms a transcription complex with the Lef1/Tcf DNA binding family of proteins and this complex activates genes involved in hair follicle development including keratin genes.

Glands

There are two glands in the skin of sheep, the sebaceous glands and the sweat glands. The sebaceous glands are out growths of the outer root sheath and open out into the lumen of the follicles as seen in Figure 1.10. They secrete a wax onto the fibres. The sweat glands in sheep (also called suderiferous glands) are of the apocrine type in which the cells partly disintegrate yielding cytoplasmic contents of lipid and salts. The secretions are known as suint. (In man the sweat glands are of the eccrine type and secrete mainly water and salts).

Figure 1.10 Diagram showing the duct of an apocrine sweat gland (left) connecting to a hair follicle in human skin just above the sebaceous gland. A sweat gland (eccrine type) is represented on the right side. In sheep the sweat glands are of the apocrine type and secrete a fatty secretion onto the skin surface and not necessarily into the neck of the follicle. The human-type sweat glands are not prominent in the skin of sheep. Source: Montagna (1962).



1.3 Blood supply to the skin

The supply of blood to the skin is essential for transporting nutrients to the follicles for the growth of wool. Wool fibres grown from sheepskin in organ culture are smaller in diameter than normal and this was attributed to the lack of a blood supply (Hardy 1951).

The arterial supply to the skin is found as three networks parallel to the skin surface at three main levels (Nay 1966; Ryder 1955; Ryder and Stephenson 1968). Arteries paired with veins enter the skin as a dermal network and branch into mid-dermal and sub-epidermal networks (Figure 1.11).

The sub-epidermal capillaries supply the epidermis but the follicles are supplied with blood via the mid-dermal and dermal networks. The follicles are supplied by two nets of capillary vessels, one is in the dermal papilla (Figure 1.12A) and undoubtedly supplies nutrients and other factors across the basement membrane into the germinative matrix of the bulb that control epithelial cell division in the follicle bulb. The papilla capillaries are increased in amount in larger follicles. The other capillary supply is connected to the papilla and surrounds the lower third of the follicle length (Figure 1.12B) that interestingly is where all the major events of keratinisation occur namely, the intensive synthesis of keratins, keratin cross-linking and removal of water and metabolic products from the completed fibre. In a study of Merino skin Nay (1966) showed that that blood vessels tend to surround and define the groups of primary and secondary follicles (Figure 1.13) discussed in Section 1.5.

Figure 1.11 Cartoon representation of the arterial and venous vessels that supply the three levels of the skin. Source: Rogers, (2006)

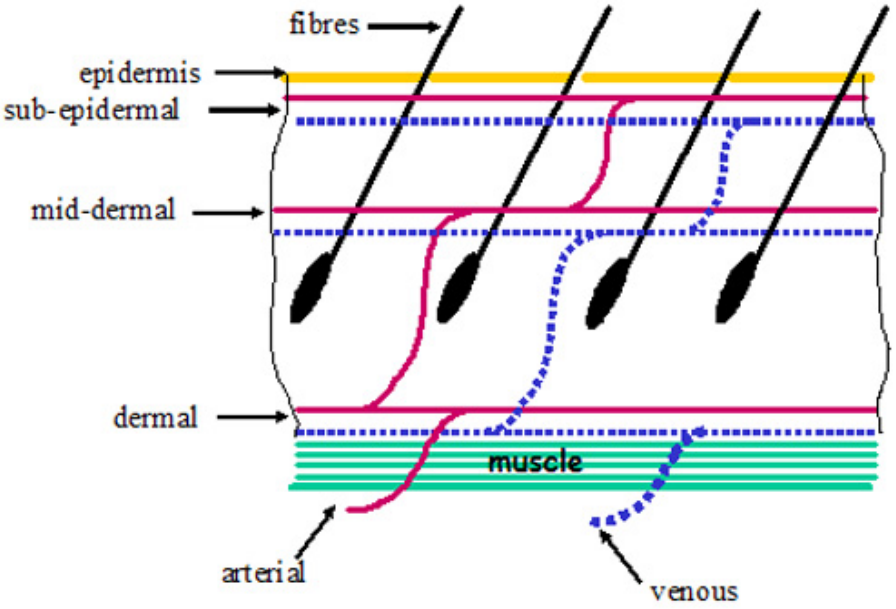


Figure 1.12A Capillaries in the papillae of two follicles. Source: Ryder and Stephenson (1968).

Figure 1.12B The network of capillaries around the lower third of a follicle corresponding to the keratinisation zone where major biochemical events of keratin formation occur. The capillaries are visualised by a colour reaction with haemoglobin of the contained blood in thick sections of skin. Source: Ryder and Stephenson (1968).

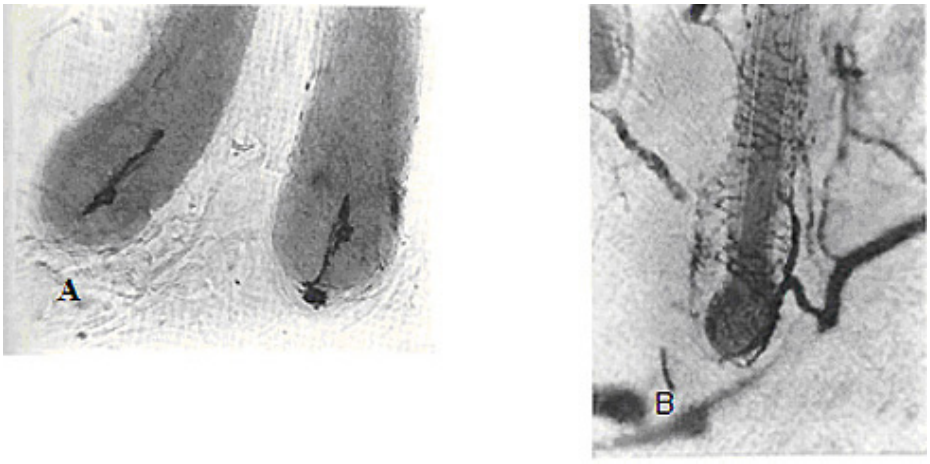
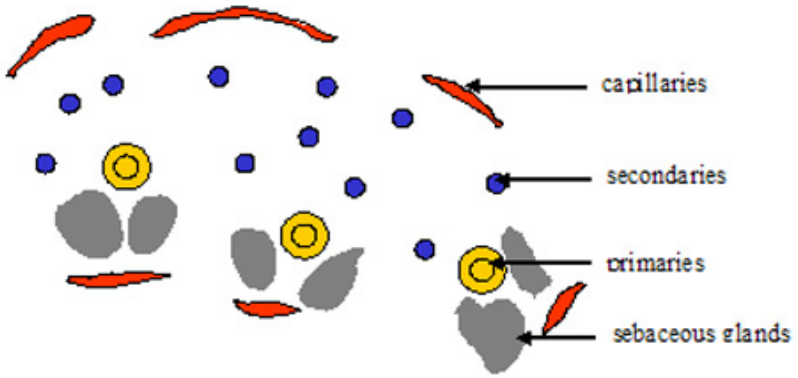


Figure 1.13 Cartoon of the arrangement in cross-section of capillaries (red) around a trio group of primary (yellow) and secondary (blue) follicles. Sebaceous glands are grey (see Coloured Figs on CD). Source: Rogers, (2006)



Wool follicles do not display a coordinated hair cycle (catagen, followed by the dormant state, telogen) as in rodent skin and the lifetime of a wool follicle is believed to be of the order of two years. When they do enter into a stage of inactivity it is a random event and the follicles regress toward the skin surface and the fibres are shed. It has been observed that during this process the capillaries around a follicle become compressed and "tortuous" and remain in that state until the follicle regenerates from telogen into anagen and the follicle actively produces another fibre.

There is reasonably good evidence that blood supply has a direct effect on wool growth. If the blood supply is increased by experimentally interrupting the nerves that control the supply, wool growth increases (Ferguson 1949). Conversely, subjecting clipped areas of a sheep to cold decreases the blood flow and the rate of wool growth is reduced (Setchell and Waites 1965).

1.4 Primary and secondary follicles

There are three kinds of follicles that can be distinguished in the skin of the sheep; primary, secondary and secondary-derived. The first follicles to develop in the skin are the primary follicles and these are arranged in a distinct pattern of trios characterised by a central primary and two lateral primaries (Figure 1.14). At about day 85 secondary follicles (S^O) begin to develop in association with the primaries and together they form a distinguishable group in the skin and by day 105 secondary-derived follicles begin to appear as branches from the secondary follicles. One of the measures used to predict the ability of a sheep to produce fine quality wool is the ratio of secondaries to primaries, called the S/P ratio.

All follicles have sebaceous glands attached at the level of the infundibulum (the funnel shaped neck of the follicle) but primary follicles (P) are distinguished by large sebaceus glands, an arrector muscle attached to the outer root sheath just below the sebaceous gland and are accompanied within the trio by sweat glands. In primitive sheep breeds the differences between primary and secondary wool fibres and their follicles is easily recognised because the primary follicles are relatively much larger, the fibres have a greater diameter than the secondaries and are often medullated. In addition, the coat is seasonal and with change from winter to summer the primary follicles shut down and the outer coat of coarse fibres is lost, leaving the summer coat of finer fibres and a lower fibre density of 10-20 follicles per mm^2.

By comparison, in fine wool breeds such as the Merino, the differences between primary and secondary follicles have been minimised by selective breeding thereby resulting in a fleece of relatively uniform fibre diameter and higher fibre density. The fibres in Merinos are much finer and shorter that in longwool breeds. The crimp in Merinos is even and the staples are square at the tip end whereas in longwools they are tapered. In fine breeds there can be up to about 100,000,000 follicles in the skin of a sheep and as a consequence of this high follicle and fibre density the fleece weight is higher and the fleece has a handle that is "bulky".

In the upper diagram of Figure 1.14 a trio of primary follicles with their associated glands and muscles is illustrated in cross-section. Their associated secondary follicles have only sebaceous glands and the fibres are of smaller diameter. The lower diagram illustrates a primary follicle in longitudinal section with secondaries nearby and the same differences in structure.

The primary follicles in fine wool sheep do not cycle seasonally as they do in primitive breeds and the follicle density is much higher (e.g. 80 follicles per mm^2). The follicle density is the result of increased numbers of secondary-derived (S^D) follicles that develop at around day 105 as branches from the S^O, original secondary follicles at the follicle neck or funnel (infundibulum) with their fibres emerging through the same orifice as the original secondary fibre. This branching can be quite extensive (Figure 1.15).

Figure 1.14 Diagrams of primary and associated secondary follicles. Source: Hardy and Lyne (1956).

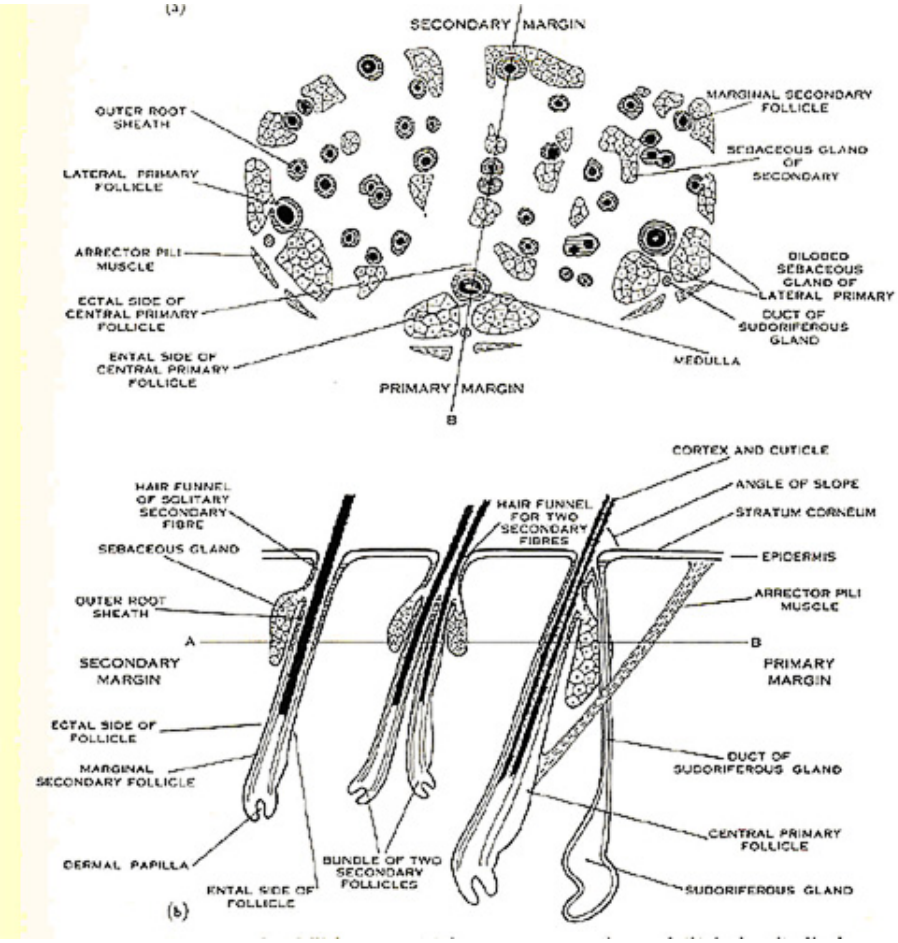
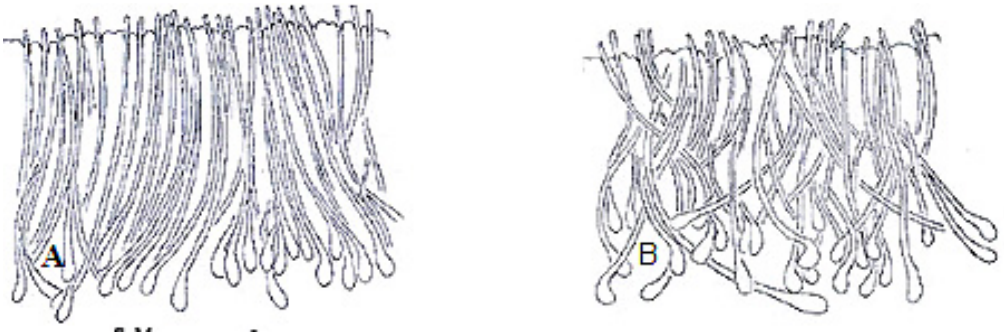


Figure 1.15 Diagram of secondary follicles (S^D)developing from an original (S^O) secondary follicle. The fibres from the secondary-derived follicles will emerge from the same orifice. Source: Hardy and Lyne (1956).

Wool follicles generally are curved with the bulb displaying retrocurvature that is, the flexure of the bulb is opposite to the curvature of the shaft. This feature is shown in (Figure 1.16A) which is from drawings made from sections of skin of different sheep. The follicles are also organised in a regular array with constant depth. However, a range of curvatures is seen in different sheep from the same flock and with varying degrees of "tangle" and one of these is shown in Figure 1.16B.

Figure 1.16A Follicles can be evenly spaced but are curved with the bulbs flexed in the opposite curvature. Source: Nay and Jackson (1973).

Figure 1.16B The follicles are not so evenly spaced and although showing the usual curvature they are twisted in different orientations. Source: Nay and Jackson (1973).



1.5 What is the biological basis of the follicle population and the formation of branching follicles?

Many years of research have clearly shown the importance of the cells of the dermal papilla in the formation and maintenance of hair follicles (Reynolds and Jahoda 2004) and the dermal aggregate beneath the early epithelial plug plays a major role in their cell-cell signalling with epidermal cells to initiate follicle formation and maintain fibre growth. It has been proposed (Moore et al. 1998) that embryonic skin generates a *defined number* of specialised dermal (pre-papilla) cells in skin that induce follicle formation and will finally constitute the dermal papilla. If the pre-papilla cells of the fixed population induce more primaries then the number of secondary follicles induced would be less and the S/P ratio would be lower. Conversely, if a lower number of primaries are induced more secondaries would form and the S/P ratio would be higher. The evidence for this concept is the inverse relationship of follicle density with fibre diameter that has been established by analysis of selection of sheep for breeding that have one or the other of these characteristics. The concept of a predetermined number of follicle initiation sites is strongly supported by the findings that in mature follicles the total population of papilla cells is constant in matched lines of sheep with high and low S/P ratios (Table 1.1).

Table 1.1 Papilla cells counted in the follicle papillae from lines of sheep selected for two levels of fibre diameter. The number of cells per mm^2 of skin is constant. Source: Moore et al (1998).

Fibre Diameter	Papilla cells/follicle	Follicles/mm^2	Papilla cells/mm^2
high	90	37	3330
low	46	74	3404

We are yet to understand how the pattern of trios of primaries is produced. One likely explanation is the presence of diffusible cell factors that set up morphological gradients so that when a threshold of concentration is reached it triggers a developmental event (Nagorcka and Mooney 1989). The molecular nature of the diffusible factors involved in these events are not precisely known but there is an enormous number of proteins and receptors now recognised for their participation in follicle formation (See pages 1-7).

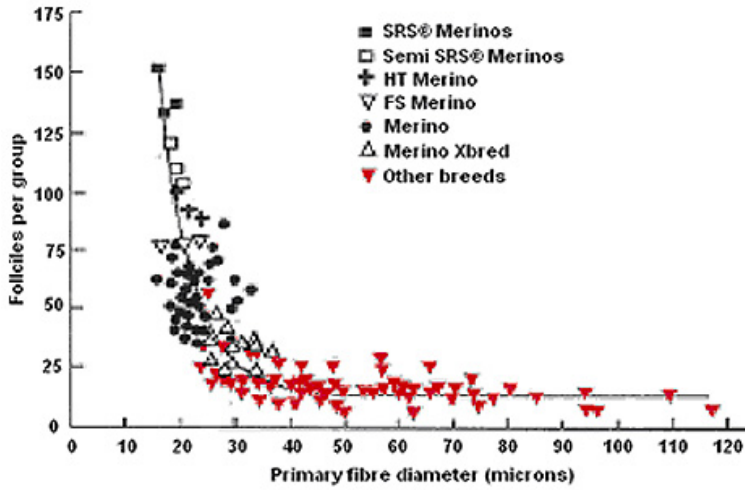
It is of interest to note that in relation to the formation of initial follicle patterns in skin and later the S^D follicles, a signalling protein and its receptor have been characterised, they are ectodysplasin-A1 (Eda-1) and EDAR, respectively. When Eda-1 is over-expressed in transgenic mice there is initiation of many follicles that are fused instead of being separated by interfollicular epidermis (Zhang et al. 2003). The follicles are fused at the level of the infundibulum (the neck of the follicle) and closely resemble the compound S^D follicles in sheep discussed above. Consequently, the authors suggest that Eda-1 could be one of the factors involved in follicle fusion in sheep.

1.6 The soft-rolling skin (SRS) sheep selection method is based on secondary follicles

A method for selecting sheep for producing high quality wool with well-defined crimp, low diameter and long staple has been advocated by Watts (Ferguson 1995). His method is based on the view that S^D follicle populations and the ratio of secondary to primary follicles (S/P) are of prime importance for sheep breeding, a concept that is supported by a limited number of sheep breeders and sheep geneticists. The Watts method evaluates wool-growing sheep visually and chooses those that have dorsal skin that moves easily when manipulated and is thinner without wrinkles. The fleece can be parted into long thin staples about 2mm wide and > 120mm length (one year's growth) with high fibre density, the bundles of fibres emerging from a single follicle group. Analysis shows that the follicle groups have a high S^D follicle population so that the S/P ratio can be as high as 50 with about 120 follicles per group (see Figure 1.17) and 85 follicles per mm^2 skin compared to non-SRS sheep with an S/P ratio of ~24 and ~70 follicles per group.

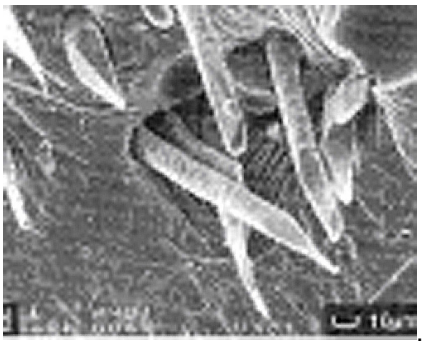
The SRS method is one of several procedures for sheep selection based on quantitative genetics and selection for and against fleece characteristics. At the present time it is claimed to be used by about 30% of woolgrowers and there are livestock scientists who are convinced of its merits for increasing yield and decreasing fibre diameter. The data in Figure 1.17 shows an inverse relationship between S/P ratio and fibre diameter.

Figure 1.17: The data here compares the ratios of secondary to primary follicles with average fibre diameter and demonstrates that the S/P ratios in soft rolling skins have the least diameter. Source: Watts (1995), http://www.srswool.com/.



The method for measuring the S/P ratio is to take a biopsy of the skin and after processing for histological examination, transverse sections are cut at the level of the sebaceous glands for microscopic examination and visual counting of the primary and secondary follicles in the follicle groups (see Figure 1.14). This procedure is expensive. A new method involves shaving an area of a live sheep and applying a layer of silicone dental resin to make a replica of the surface that can be observed using SEM. Fibre bundles from S^D follicles emerging from a common opening can be distinguished (Figure 1.18) and counted (Nagorcka et al. 1995).

Figure 1.18 Seven fibres merging as a fibre bundle from a common follicle orifice in the epidermis of sheepskin. This is a SEM image from a replica of the skin surface using silicone resin. Source: Nagorcka et al. (1995).



1.7 Many signalling factors control wool growth

The cells that produce wool (and hair and the epidermis of the skin surface) are given the generic name of keratinocytes. Gene activity in the keratinocytes of anagen follicles is regulated both positively and negatively through a large number of transcription factors (Fuchs et al. 2001; Rogers and Hynd 2001). Many of the factors are conserved across species and were originally discovered in other organisms such as Drosophila and Xenopus. These regulatory molecules and their networks that control follicle differentiation into the many cell lineages of the follicles (Figure 1.4) are becoming increasingly defined. The WNT (Wingless-type)-Frizzled protein receptor pathway already discussed above in relation to follicle development (see Figure 1.9) is functional throughout fibre growth.

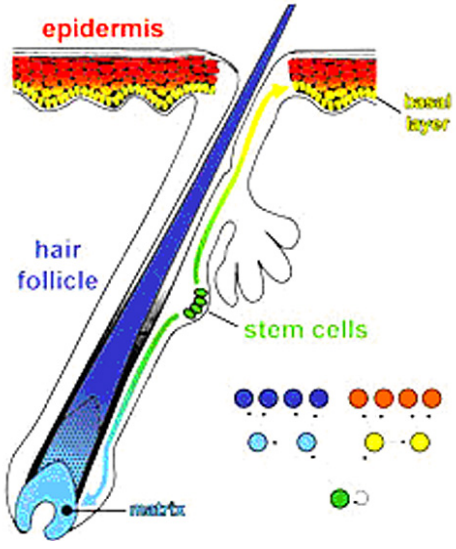
It is well established that dermal papilla cells retain their inductive capacity only in the presence of epithelial cells of the follicle bulb that are dividing (matrix cells, see Figure 1.4). The expression of several genes in the dermal papilla could be responsible for this and the most highly expressed is a WNT signalling modulator called Wise (O'Shaughnessy et al. 2004). It is expressed in the cortex of the active (anagen) adult follicle and in the bulge region. It has been suggested that the "fine tuning" of signalling pathways might be controlled by activity of such modulators rather than variations in the levels of the protein factors of the pathways.

1.8 The hair cycle and the follicle bulge of stem cells

Hair follicles do not grow constantly but undergo cyclic changes of growth, regression and dormancy, events that are known respectively as anagen, catagen and telogen (Dry 1926). Another term exogen has been suggested to describe the loss of the dormant hair from the follicle that occurs after telogen. In mice the anagen phase continues for about 20 days and then the follicle progresses into catagen and telogen and then anagen begins again. These changes occur as waves of activity and inactivity over the body (Chase 1965). In sheep, the anagen phase appears to be maintained continuously; some catagen and telogen follicles can be found but they are relatively low in abundance.

When follicles move out of telogen they regenerate from a store of stem cells that is referred to as "the bulge region" that is visible histologically in mice as a localised swelling of the outer root sheath just below the sebaceous gland (Cotsarelis, Sun and Lavker 1990) (Figure 1.19). The stem cells from the bulge can migrate to both the basal layer of the epidermis and the follicle bulb when it regenerates from telogen. It is presumed that bulge-type stem cells are present in sheep although experiments have not been published showing them in a bulge-type region.

Figure 1.19 Cartoon of a follicle illustrating the location of stem cells in a bulge of the outer root sheath below the sebaceous gland. The stem cells (green) can give rise to two lineages one destined for the epidermis (yellow) and another for the follicle bulb (blue). (see Coloured Figs on CD) Source: ISREC, http://www.isrec.ch/research/groups/research_groups_detail_eid_1682_lid_2.htm.



The process of follicle regeneration from catagen is dependent on the activity of the dermal papilla that still resides beneath the catagen ("club") hair as a small group of cells. In response to signals that are presently uncertain, the papilla induces proliferation of the residual epithelium that grows down into the dermis and then moves into the anagen phase again to produce a new hair.

1.9 Effect of hormones, growth factors and nutrition on wool growth, follicle "shutdown" and the strength of wool fibres

Many physiological and environmental factors can influence wool growth (Reis 1992) by affecting the rate of cell proliferation and cell death (the process called "apoptosis") in the follicle bulb. For example increased circulating thyroxine or growth hormone will stimulate wool growth whereas the adrenal glucocorticoid, cortisol and related drugs such as dexamethasone and flumethasone, inhibit it. Their action is to drive the anagen wool follicles into catagen and so wool growth shuts down and is characterised by plucked wool fibres having frayed ends or brush-ended fibres. In sheep that are stressed by environmental conditions including low nutritional levels, the circulating cortisol is increased and that gives rise to follicle shutdown.

The seasonal effect of nutrition when there is less abundant pasture after summer causes gradual "fining" of the wool fibre and if the decline in fibre diameters is low enough in severe cases of under-nutrition there can be a temporary shutdown of the follicles. This thinning of the growing fibre along part of its length causes a decrease in staple strength, a condition called "tenderness". A wool staple that is tender is a major cause of short hauteur and is a problem for wool processors. The variation in diameter along a staple, and the position of the thinnest region has the major impact on whether or not wool is tender. If there are large fluctuations in diameter throughout the year and the thinnest portion is in the middle of the staple, the staple is more easily broken. However, if fibre diameter is relatively constant and the thinnest point is close to an end, the wool is likely to be classified as sound.

The induction of a "break" in growing wool can be achieved by the injection of milligram amounts of several drugs that inhibit wool growth and one that has been extensively tested for harvesting wool instead of shearing is the growth factor, epidermal growth factor (EGF). This growth factor is found in the submaxillary gland and has a pronounced effect on developing tissues including an inhibition of hair growth. The EGF is now produced by genetic engineering from soy bean and is currently used commercially as a defleecing agent to replace shearing. The shutdown activity of EGF appears to be caused by the induction of apoptosis (cell death) in the follicle bulb (Hollis and Chapman 1987).

1.10 Origin of crimp

Wool follicles usually curve and spiral in the skin and the bulbs are bent to one side giving the lower part of the wool follicle the appearance of a golf club. These features were well described by Auber (1950) and give rise to wool fibres with either a spiral or a more linear wave. Staple crimp results from the fibres growing out together with their curvatures in register. Individual wool fibres are always curly to some extent and in fine wool sheep where the fleece density is high (because the secondary follicle population is high) they emerge from the skin as a staple or column of fibres. The staples have the form of uniplanar waves that are evenly spaced and relatively close compared with strong-wool sheep. This characteristic is one of the factors in the commercial evaluation of raw wool quality although crimp frequency is not fibre diameter. However, a relationship between staple length and crimp has been demonstrated, the longer the staple the lower is the crimp frequency.

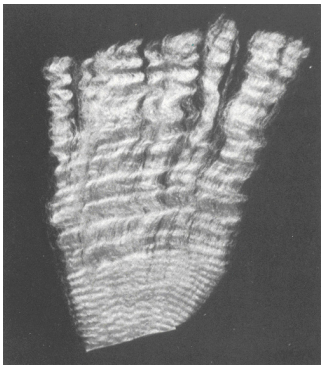
The cause of curliness and crimp is not understood. One causative factor that was proposed was a periodic contraction of the musculature attached to hair follicles (Chapman 1965) however one problem with this proposal is that the muscles are found only on primary follicles. A more likely explanation is that it is the result of local signals from the dermal papilla causing the overlying epithelial cells, the germinating cells of the matrix, to divide asymmetrically in the bulb; there is

evidence for this (Fraser 1964) and also for the earlier keratinisation on one side of the growing fibre (Fraser and Rogers 1954). Presumably the signals vary in their location so that the bulb flexes as a result and the curvature of the growing fibre is fixed in space at a given moment by protein synthesis and chemical cross-linking. The surrounding sheaths of the follicle especially the inner root sheath and the outermost collagenous sheath (glassy layer) act as constraints in the process forcing the cells to move upwards and not outwards. In fine wool with a well-defined bilateral structure the orthocortex follows the convex or outer aspect of a curvature whereas the paracortex is on the concave or inner side The function of the bilateral organisation of two types of cortical cells as a causative factor in the process is still not clear because it is absent in less-crimped coarser wools even though the crimp is still present.

1.11 Trace metal deficiencies

There are two metals, copper and zinc, that are known to have marked effects on wool growth and another, cobalt, is important for sheep but its deficiency does not affect wool growth directly. Copper deficiency in sheep causes a loss of pigmentation in black wool sheep but more importantly a lack of copper in the diet produces wool with distinctly less crimp and is referred to as “steely wool” (Figure 1.20). Analyses of the wool from copper deficient sheep suggested that the cross-linking of sulfhydryl groups to disulfide bonds is incomplete and moreover Marston (1955) claimed that histochemical tests indicated a delay in the process during keratinisation in the follicle. His interpretation was that during fibre growth the delay in cross-linking allowed the fibre to remain in a “plastic” state and loss of the crimp waves is a result.

Figure 1.20 The effect of copper deficiency on crimp. In the earlier growth on a copper deficient diet the wool crimp is present but the frequency is much reduced and this affects the “feel” that is described as “Steely”. The later growth is with a diet that is supplemented with copper and the wool that has grown has a distinct crimp. Source: Marston (1955).



The activity of copper is unknown but the oxidation of SH groups to SS bonds might be by copper alone or as a component of an enzyme that has sulfhydryl oxidation activity (Gillespie 1990; Marston 1955). A more likely candidate for the process of oxidation is the protein disulphide isomerase that is a ubiquitous enzyme in eukaryotes that catalyses oxidative protein folding (Tu and Weissman 2004). However the enzyme has not yet been demonstrated in wool follicles.

Merino lambs on zinc deficient diets (4mg/kg feed) produce wool fibres that are improperly keratinised. Using electron microscopy it was found that the wool follicles contained a higher proportion of apoptotic bodies (indicators of cell death) in the follicle bulb but the rate of bulb-cell production was not decreased. It was concluded that the reduction of wool growth in zinc deficiency was possibly a direct effect on mechanisms of protein synthesis (White et al. 1994).

Cobalt is a trace metal of importance because it is a component of Vitamin $B_{12.}$ A single cobalt atom is centrally placed in the B_{12} molecule (otherwise known as cobalamin) and one of its functions is as a cofactor in the activity of an enzyme called methionine synthase. This enzyme participates in the synthesis of methionine from homocysteine and a derivative of folic acid. Cobalt and hence cobalamin are vital for the normal health of sheep although a deficiency of it does not directly affect wool growth.

1.12 Pigmentation

The pigment-forming cells in skin are melanocytes that are dendritic cells with cytoplasmic projections called dendrites on their surfaces. These cells are present in the basal layers of the epidermis but are not present in the basal layers of the follicle bulb of Merino sheep. The melanocytes synthesise the black pigment melanin in membrane-bound particles called melanosomes. They are injected into adjacent epithelial cells by transient fusion of the membrane of the melanosome dendrites with the epithelial cell membrane. In certain circumstances such as ageing or exposure to sunlight epidermal melanocytes can migrate into the follicle bulb and become located and active around the boundary between the dermal papilla cells and the overlying epithelia cells (see Figures 1.1 and 1.2). The result is that single follicles in the skin produce a black fibre. Although invisible in the bulk of white wool because of their low abundance, the black fibres become evident when wool has been converted to fabric and dyed and hence they become a commercial nuisance.

1.13 Implications of basic knowledge for wool production

As research continues (Sheep Economics Programme) it is likely that the signalling factors that control formation of secondary derived follicles will be identified and possible ways of increasing follicle population and decreasing diameter could be devised. For example the gene(s) responsible could be introduced into the sheep genome by transgenesis and directed to express in the follicles. The transgenesis process involves DNA containing a gene or genes being microinjected into the nuclei (actually the pronucleus from the sperm head) of fertilised sheep ova. The DNA integrates with the genome and can be transcribed. Several of the treated eggs are usually deposited in the uterus of a ewe and when they have developed into a lamb or lambs they may express the phenotype of the injected gene. The alternative would be to develop a drug that can stimulate a gene activity or substitute for it and administer it to sheep. Another possibility in the future is the discovery of genes that control the rate of wool growth and to manipulate them in sheep.

Established methods of managing wool growth by nutritional supplementation can already minimise the effect of seasonal changes on follicle shutdown. Furthermore, selective breeding combined with careful control of nutrition and of the environment in which the sheep grow their wool already have produced 10µm diameter wool and high quality fleeces but at a high cost.

Readings [3]

The following readings are available on CD:

1. Reis, P.J. 1992, 'Variations in the strength of wool fibres – A review,' *Australian Journal of Agricultural Research, v*ol. 43, pp. 1337-1351.
2. Rogers, G.E. 1990, 'Improvements of wool production through genetic engineering' *Tribtech,* January 1990, vol. 8.
3. Rogers, G.E. 2004, 'Hair follicle differentiation and regulation,' *International Journal of Developmental Biology,* vol 48, pp. 163-170.

Activities	@ Available on WebCT
Multi-Choice Questions	@ Submit answers via WebCT
Useful Web Links	@ Available on WebCT
Assignment Questions	@ Choose ONE question from ONE of the topics as your assignment. Short answer questions appear on WebCT. Submit your answer via WebCT

Summary

Summary Slides are available on CD
The skin is the largest organ in the mammalian body and consists of two layers. The outermost epithelial layer, the epidermis, sits on the dermis a connective tissue layer separated from the epidermis by a specialised layer called the basement membrane. The skin is a vital barrier to the environment. Wool follicles and sweat glands are derivatives of the epidermis and develop in the foetus and newborn animal in response to biochemical signals from the dermis and also the epidermis. There is evidence that the follicle density of the adult skin is predetermined by a population of pre-papilla cells that induce the primary and secondary follicles to develop and then secondary-derived follicles that branch from secondaries. A high abundance of secondary-derived follicles appears to be a strong determinant of wool fibre quality in terms of fibre diameter, crimp and staple form.

The dividing cells of the follicle bulb if they are depleted during a hair cycle or follicle shutdown from other causes, are probably restored by the niche of stem cells located in the outer root sheath below the sebaceous gland. Nutrients and oxygen are supplied to the skin by an elaborate network of fine blood vessels that penetrate into the dermal papilla and also present as a network around the outer root sheath of the lower third of the follicle. There are many nutritional, hormonal and environmental factors that can influence the rate of wool growth. Inadequate protein intake can lead to follicle shutdown as can growth factors such as epidermal growth factor, a property that has been developed as biological harvesting and alternative to conventional shearing. Trace metals such as copper, zinc and cobalt are important in wool growth and the biochemical basis of their activities is essentially understood.

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Glossary of terms

Apocrine	Sweat glands in the skin that secrete mainly lipids
Artery	A blood vessel that conveys blood to a tissue or organ
Cell lineage	The specific pathway of differentiation that a group of cells follows
Dermal cell	Fibroblast cells that synthesise collagen, the fibrous protein that composes the deep layer beneath the epidermis and that surrounds hair follicles
Dermal sheath	The layer consisting of a type of fibroblast, adjacent to the outer root sheath of the follicle, continuous with the dermal papilla and makes collagen Type IV and mucopolysaccharides
Eccrine	Sweat glands in the skin that secrete mainly inorganic salts and water
Epithelium	The layer of cells of ectodermal origin that makes up a tissue surface
Hair cycle	The different stages of follicle activity of hair production (anagen), partial regression of the follicle (catagen) and a dormant stage (telogen). The stages vary greatly between different species. In sheep, anagen can last for 2 years whereas in mice it is a few weeks
Involucrin	A protein found in cell envelopes that modifies the plasma membrane of keratinocytes to become a physically tough membrane
Isopeptide bond	Found as a cross-link in some proteins and connects the epsilon amino group of a lysine side chain with he gamma carboxyl group of a glutamic acid side chain. The normal peptide bond of proteins connects amino acids via the alpha carboxyl group of any amino acid to the alpha amino group of another
Keratinocyte	The differentiated cells of skin and related tissues that A protein found in cell envelopes that modify the plasma membrane of keratinocytes to become a physically tough membrane produce keratin intermediate filaments and keratin associated proteins as their major cytoskeleton products

Loricrin	A cystine-rich protein found in cell envelopes with involucrin that modifies the plasma membrane of keratinocytes to become a physically tough membrane
Matrix cells	The dividing cells in the bulb of hair follicles. The term matrix is also used to describe the proteins that occur between the intermediate filaments of hair and epidermis
Molecular signal	A protein or smaller molecule that binds to a receptor on or in a cell and affects a cellular process
SEM	Scanning electron microscopy
Stem cell	A cell that can proliferate and renew cells in a tissue when they are lost. They can partially retain the property of pluripotentiality. That ability, to differentiate into any type of tissue cell, is the characteristic of embryonic stem cells
TEM	Transmission electron microscopy
Transgenic animal	An animal that has developed as the result of the introduction of a gene into the genome of the animal by microinjection of DNA into the nucleus of a fertilised ovum
Vein	A blood vessel that conveys blood from a tissue or organ