1. Skin Structure and Function

George Rogers

Learning Objectives

This topic should provide you with:

- knowing some functions of the skin
- knowledge of development of wool follicles in the skin and some of the mechanisms involved in the formation of mature follicles growing wool.
- the ability to critically assess scientific and other professional literature on wool growth in relation to commercial implications.

Key Terms and Concepts

- the skin forms a barrier to the environment
- wool follicles develop in the foetus from an interaction between the surface epidermal and the underlying dermal cells
- The first follicles to appear as down growths from the epidermis are primary follicles that are the first to appear followed by secondary follicles
- in fine wool sheep breeds, secondary follicles “branch” to give secondary-derived follicles
- the cell layers of a wool follicle are derived from streams of cells that differentiate by expression of specific genes
- the population of follicles of fine wool sheep arises from an abundance of secondary derived follicles
- wool quality is related to the ratio of the secondary follicles to primary follicles and the ratio can be increased by selective breeding
- the skin has a network of capillaries that provide nutrients for growth through the blood supply to follicles
- follicle stem cells have been located in a bulge region of the outer root sheath and replenish the bulb as required, eg. in follicle regeneration of the hair cycle
- crimp formation is the result of several follicle events and is not fully understood.
1.1 Introduction
This topic is titled skin structure and function because the main objective is to describe cellular aspects of the growth of wool. The description of the structure of the skin of the sheep and of its wool follicles not only draws on studies of sheep but also from our knowledge of the similar events in the skin of humans and mice that differ from that of sheep in only minor detail.

All organisms have a skin or surface layer of some kind to protect them from the surrounding environment. In mammals the epithelium (epi= on or around; thelium=nipple) is the name given to that layer of cells that covers the body surface and everything that is derived from it. The skin has an epithelium, as does the lining of the mouth and intestine. In mammals the skin basically consists of two layers, the outer epithelium that lies on top of the dermis (Figure 1.1).

![Figure 1.1: Schematic diagram of the skin epithelium (epidermis) resting on the fibrous tissue of the dermis. Source: G. Rogers 2008](image)

Human skin is the largest of all the organs in the body. If the skin of an adult human male were stretched out flat it would cover about 1.5 square meters and weigh about 4 kilograms. Skin of the sheep 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 network of the fibrous protein, collagen. Collagen is made by cells called fibroblasts.

The skin is a protective organ against microorganisms and other insults from the environment but it also acts as the barrier against water loss. In that context the skin regulates body temperature directly by the blood circulating near the skin surface and the presence of sweat glands. Sweat glands and also the hair follicles, referred to as appendages of the skin, are formed by invagination of cells from the epidermis and a process of differentiation during growth of the embryo.

The investigation of the skin and its appendages is difficult because the epidermis is very thin and the cells are 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.

1.2 The epidermis
The epidermis has several layers (Figure 1.2).

![Figure 1.2: The epidermis consists of several layers including the cornified, granule, spinous and basal cell layers. They are on top of the dermis or connective tissue. Source: G. Rogers 2008](image)
The basal cell layer is the proliferating layer where stem cells divide and their progeny move towards the surface and differentiate as the move toward the surface. The basal layer rests on a basement membrane that forms the dermal-epidermal junction. It is composed a special type of collagen (Type IV) and mucopolysaccharides one of which is called fibronectin. The basement membrane can be visualised in the electron microscope (TEM) as a faint layer (Figure 1.3). The epidermis is anchored to the dermis by structures called hemidesmosomes in the basement membrane (Figure 1.3).

The basal layer of the epidermis contains stem cells from which daughter cells move upwards and filaggrin. As the cells differentiate to finally become the stratum granulosum or granular layer they undergo differentiation in which the main protein products are the cytokeratins that belong to the keratin intermediate filament superfamily. As the cells continue on their outward movement they finally die, the filaments form bundles or aggregates and fill the cells that are the stratum corneum.

**Figure 1.3:** 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. 

### 1.3 Follicles

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 1.4A) a local accumulation of cells appears as a thickening of the epidermis called an epidermal placode. 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 1.4B) and gradually differentiate into the mature follicle.

**Figure 1.4** (A) 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. In (B) 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: Dr. Stephanie Dunn, SARDI Livestock Systems, South Australia. Bar =50um.
Notes – Topic 1 – Skin Structure and Function

These events were described many years ago and a diagrammatic representation of them is given in Figure 1.5.

![Figure 1.5 Diagrams illustrating the major steps (0-8) in the formation of a wool follicle beginning with the epidermis (0) followed by invagination of a hair plug, aggregation of dermal cells that form the dermal papilla. The appearance of the inner root sheath (IRS, 4) 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 (7) through which the fibre emerges in the adult follicle. Source: Hardy 1992.](image)

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 1.6 and have to pass across the basement membrane (Figure 1.9).

![Figure 1.6 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 in the bulb of the follicle, see Figure 1.7) 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.](image)

There are about $10^8$ wool follicles in the skin of fine wool sheep. The adult wool follicle (Figure 1.7) is a complex of layers of six concentric cylinders of cells and each layer is a lineage of cells (Figure 1.8) established during embryonic development. The histological structure of the follicle is virtually the same in all mammals.

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 the insulating properties of the fleece. 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 (the infundibulum; funnel), the IRS degrades to cell fragments that are sloughed as the fibre emerges. Cell division in the bulb is a controlled process so that when they move upwards to the skin surface there are sufficient germinative cells remaining in the bulb 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” (Figure 1.20). 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 1.8).

Figure 1.7 A diagrammatic view of the different layers of an adult wool follicle. Source: Auber 1960.

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 1.8).
Molecular signals in follicle development and maintenance

The cells that produce wool (and hair and epidermis) are given the generic name of keratinocytes. There are many types of signalling molecules that act on these cells and are involved in initiating the formation of follicles in the embryo and maintenance of hair growth in the adult. Gene activity is regulated both positively and negatively through a large number of transcription factors and other regulatory molecules (Fuchs et al., 2001; Rogers and Hynd, 2001). Most 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 control follicle differentiation into the many cell lineages of the follicles (Figure 1.8) and are becoming increasingly defined.

There was early evidence that factors associated with nerves have an initiating role (Exan and Hardy, 1980). Protein factors of which there are more than twenty members, known as neurotrophins (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 they may have a modulating role rather than direct role in follicle formation (Botchkarev et al., 1998). Just how the skin establishes the arrangement of primary follicles in a trio pattern for example (see Figure 1.14) and follows that by producing secondary follicles is not exactly understood. The establishment of morphological fields or concentration gradients of growth factors and receptors is accepted as the underlying mechanism. Mechanisms of this kind have been analysed (Nagorcka and Mooney, 1989) using a theoretical model in which different patterns of follicles can be obtained by varying the threshold of concentration between factor and receptor that will trigger a developmental event. The molecular basis of the inductive capacity of dermal papilla cells (Figure 1.6) must be
the expression of several genes and the major signalling pathway in these developmental events is the so-called WNT (Wingless-type) - Frizzled, receptor pathway. A simplified diagram of the WNT pathway is given in Figure 1.10. The WNT genes encode a family of glycoproteins that bind to a class of membrane-associated WNT-receptors called Frizzled (Fz). Genes that are turned on in the papilla by signalling pathways produce proteins that must cross the basement membrane in the papilla (Figure 1.9) to act on the dividing epithelial cells in the bulb. This pathway is also essential for the maintenance of inducing activity of the dermal papilla. (Kishimoto, Burgeson and Morgan 2000).

Figure 1.10  A simplified cartoon representation of the activated WNT signalling pathway involved in gene expression in the hair follicle. Source: Fuchs (2002).
It is well established that dermal papilla cells retain their inductive capacity only in the presence of amplifying epithelial cells of the follicle bulb (the ‘matrix’) that are dividing. The expression of several genes in the dermal papilla could be responsible for this and the most highly expressed is a WNT signalling modulator, Wise (O’Shaughnessy et al., 2004). It was found to be expressed in the cortex of the active (anagen) adult follicles of mice and in the bulge region (the site of stem cells, Figure 1.20). 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.

The way it works is that the molecule β-catenin is present in the cytoplasm and one function is as a component of cell-cell adhesion. β-catenin is phosphorylated by an amazing complex of proteins called the AXIN/GSK3β/APC complex and is destroyed by the protein degradation system. Axin is a scaffold protein, GSK3β is glycogen synthase kinase and phosphorylates the β-catenin; APC stands for adenomatous polyposis coli that is encoded by a tumour gene and increases the efficiency of phosphorylation. However, when a WNT molecule (green) binds to the receptor called Frizzle, there is a cascade of factors the central one being Dsh that inactivates the AXIN/GSK3β/APC complex with the result that degradation of β-catenin is shut down and its concentration increases (right hand side of diagram) and enters the nucleus where it binds to promoter regions of genes including keratin genes that are then expressed.

In summary, the central molecule is β-catenin and it has dual roles. It is part of the cadherin complexes of cell-cell adhesion junctions that hold cells together. The other function is 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.

1.3.2 Glands
There are two glands in the skin of sheep, the sebaceous glands and the sweat glands. The sebaceous glands are outgrowths of the outer root sheath and open out into the lumen of the follicles as seen in Figure 1.11. 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 1.11 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.

1.4 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 1.12).

Capillaries near the skin surface (“sub-epidermal”) 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 1.13A) 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 1.13B) 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 1.14) discussed in Section 1.5.
Notes – Topic 1 – Skin Structure and Function

Figure 1.12 Cartoon representation of the arterial and venous vessels that supply the three levels of the skin. Source: G. Rogers 2008

Figure 1.13 A-Capillaries in the papillae of two follicles. B-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 1.14 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. Source: G. Rogers 2008
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 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).

1.5 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 1.15). At about day 85 secondary follicles (S) 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 (Figure 1.15) 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 mm².

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 uniform fibre diameter and higher fibre density. The fibres in Merinos are much finer and shorter that in long-wool 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 is a total of 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 1.15 a trio of primary follicles with their associated glands and muscles is illustrated in cross-section. The secondary population of 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 mm²). The follicle density is the result of increased numbers of secondary-derived (S\text{D}) follicles that develop at around day 105 as branches from the S\text{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 1.16).
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 1.17A) 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 1.17B.

![Figure 1.17](image)

**Figure 1.17** A-follicles can be evenly spaced but are curved with the bulbs flexed in the opposite curvature. B-the follicles are not so evenly spaced and although showing the usual curvature they are twisted in different orientations. Source: Nay 1973.

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

As discussed above the cells of the dermal papilla are vital in the formation and maintenance of hair follicles (Reynolds and Jahoda, 2004). 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).

<table>
<thead>
<tr>
<th>Diameter</th>
<th>Papilla cells per follicle</th>
<th>Follicles per mm²</th>
<th>Papilla cells per mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>90</td>
<td>37</td>
<td>3330</td>
</tr>
<tr>
<td>Low</td>
<td>46</td>
<td>74</td>
<td>3404</td>
</tr>
</tbody>
</table>

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 as discussed in Section 1.3.2, there is an enormous number of proteins and receptors now recognised for their participation in follicle formation.

It is of interest to note that in relation to the formation of initial follicle patterns in skin and later the S⁰ follicles, a signalling protein and its receptor, ectodysplasin-A1 (Eda-1) and EDAR, that might play a role have been characterised. 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 S⁰ follicles in sheep discussed above. Consequently, the authors suggested that Eda-1 could be one of the factors involved in follicle fusion in sheep.
1.7 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 SD 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 visually evaluates wool-growing sheep 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 SD follicle population so that the S/P ratio can be as high as ~50 with ~120 follicles per group (see Figure 17) and 85 follicles per mm² 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 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 1.18 shows an inverse relationship between S/P ratio and fibre diameter.

![Figure 1.18](image1.png)

**Figure 1.18** 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.

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 1.15). The 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 scanning electron microscopy. Fibre bundles from SD follicles emerging from a common opening can be distinguished (Figure 1.19) and counted (Nagorcka et al., 1995).

![Figure 1.19](image2.png)

**Figure 1.19** Several 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 1995.
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 et al., 1990). The stem cells from the bulge can migrate to both the basal layer of the epidermis and the follicle bulb (Figure 1.20) 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.

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 active (anagen) wool follicles into the inactive states (catagen and telogen) and so wool growth shuts down and is characterised by wool fibres when they are plucked, 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 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 appearance of a golf club in the lower part of the wool follicle. 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 result 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 careful studies in recent years have demonstrated that contrary to earlier ideas, the crimp frequency is not a measure of 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 1.21). 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 lost the crimp waves as a result.

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 protein disulphide isomerase that is a ubiquitous enzyme in eukaryotes that catalyses oxidative protein folding (P. Tu and Weissman, 2004). However the enzyme has not yet been demonstrated in wool follicles.
Notes – Topic 1 – Skin Structure and Function

Figure 1.21 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 1995.

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\textsubscript{12}. A single cobalt atom is centrally placed in the B\textsubscript{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. The result is that single follicles in the skin produce a black fibre and although invisible in the bulk of white wool because of such a low abundance the black fibres become evident when wool has been converted to fabric and dyed and hence become a commercial nuisance.

1.13 Implications of basic knowledge for wool production

As research continues 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 10um diameter wool and high quality fleeces but at high cost.

References


Notes – Topic 1 – Skin Structure and Function


