Chapter 1
Morphological, Macromolecular Structure and Hair Growth

Abstract At or near its surface, hair fibers contain a thick protective cover consisting of six to eight layers of flat overlapping scale-like structures called cuticle or scales which consists of high sulfur KAPs, keratin proteins and structural lipids. The cuticle layers surround the cortex, but the cortex contains the major part of the fiber mass. The cortex consists of spindle-shaped cells that are aligned parallel with the fiber axis. Cortical cells consist of both Type I and Type II keratins (IF proteins) and KAP proteins. Coarser hairs often contain one or more loosely packed porous regions called the medulla, located near the center of the fiber. The cell membrane complex, the "glue" that binds or holds all of the cells together, is a highly laminar structure consisting of both structural lipid and protein structures.

Hair fibers grow in cycles consisting of three distinct stages called anagen (growth), catagen (transition) and telogen (rest). Each stage is controlled by molecular signals/regulators acting first on stem cells and then on the newly formed cells in the bulb and subsequently higher up in differentiation in the growing fiber. The effects and incidence of hair growth and hair loss (normal and diseased) for both males and females are described in detail. Molecular structures controlling hair fiber curvature (whether a fiber is straight or curly) and the effects of the different structural units of the fiber on stress–strain and swelling behavior are described in detail.

1.1 Introduction

Since writing the fourth edition, several significant findings have occurred regarding the morphology, the growth and development, and the structure of human scalp hair fibers. Our knowledge of hair growth, development and formation both at the cellular and the molecular levels has continued to increase at a rapid rate and our understanding of the origin of fiber curvature has increased considerably. For example, recent evidence demonstrates more of a bilateral type structure in human hair fibers as curvature increases providing different types of cortical

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structures on the inside of a curl vs. the outside, analogous to wool fiber. Additional details of the surface structure, that is the epicuticle and the cuticle cell membranes have been uncovered providing a better understanding of the surface of hair fibers and the organization and makeup of the three cell membrane complexes that binds all of the hair cells together. Significant findings regarding the lipid composition of hair, its importance to barrier functions, to the isoelectric point and its potential for stress strain involvement have been added.

Important additions to the sections on male and female pattern alopecia have been made including incidence vs. age and affected regions of the scalp. Additional information on hair diameter and hair density (hairs/cm^2) changes with age, hair density in different regions of the scalp and variation by geo-racial group (linking geographic origin and its effects on genetics with race). The effects of pregnancy on scalp hair are also described in greater detail than in prior editions.

Human hair is a keratin-containing appendage that grows from large cavities or sacs called follicles. Hair follicles extend from the surface of the skin through the stratum corneum and the epidermis into the dermis, see Fig. 1.1. Hair provides protective, sensory and sexual attractiveness functions. Hair is characteristic of all

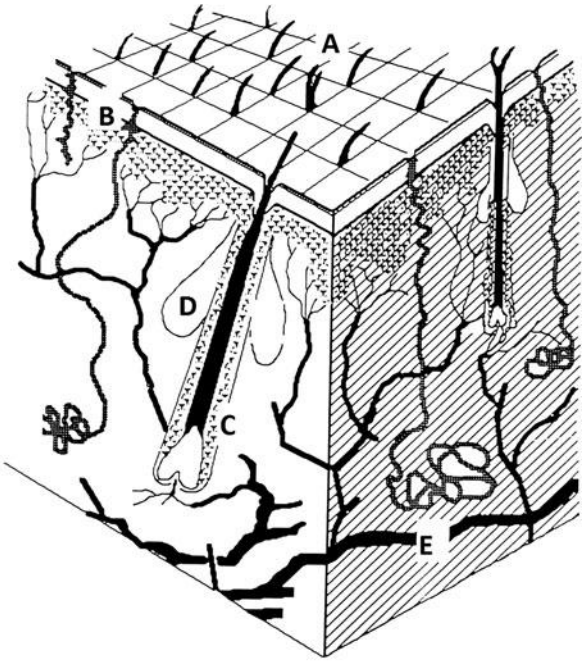

Fig. 1.1 A section of human skin illustrating a hair fiber in its follicle as it emerges through the skin and how it is nourished

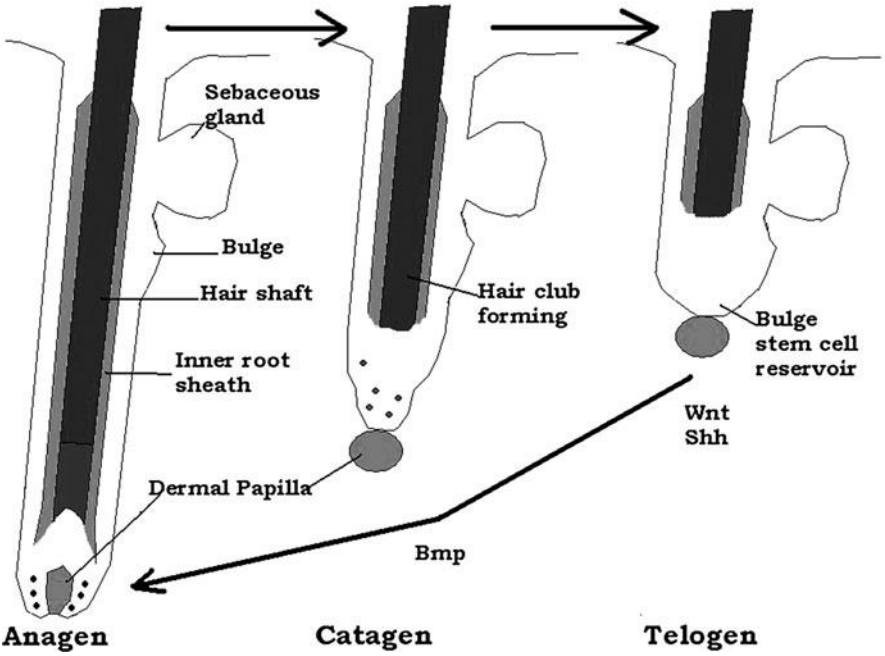

Fig. 1.2 Schematic illustrating the three stages of growth of human hair fibers

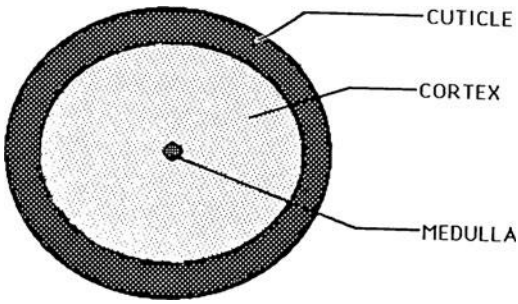

Fig. 1.3 Schematic diagram of a cross section of a human hair fiber

mammals and in humans grows over a large percentage of the body surface. Regardless of the species of origin or body site, human hair grows in three distinct stages and has certain common structural characteristics. These three cyclical stages of hair fibers are called anagen (growing stage), catagen (transition stage) and telogen (resting stage), see Fig. 1.2.

Morphologically, a fully formed hair fiber contains three and sometimes four different units or structures. At or near its surface, hair contains a thick protective covering consisting of one or more layers of flat overlapping scale-like structures called cuticle or scales see Fig. 1.3. The cuticle layers surround the cortex, but the

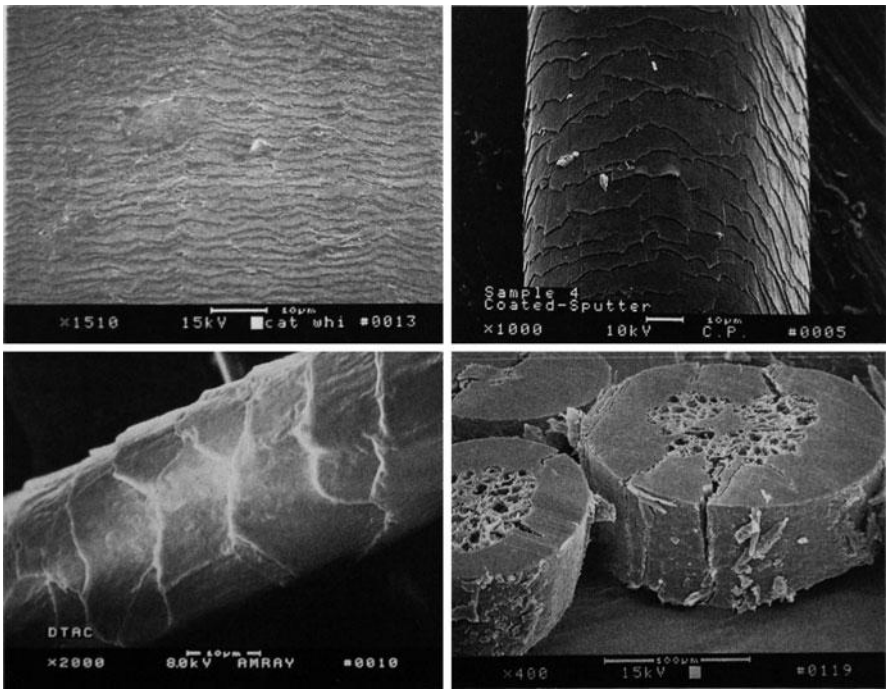

Fig. 1.4 Hair fibers from different mammalian species. *Upper left* is an SEM of a cat whisker (1510 X). *Upper right* is an SEM of a human hair fiber (1000 X). *Lower left* is an SEM of a wool fiber (2000 X). *Lower right* is an SEM of sections of horse tail fiber (400 X)

cortex contains the major part of the fiber mass. The cortex consists of spindle-shaped cells that are aligned parallel with the fiber axis. Cortical cells contain many of the fibrous proteins of hair. Coarser hairs often contain one or more loosely packed porous regions called the medulla, located near the center of the fiber. The fourth important unit of structure is the cell membrane complex the "glue" that binds or holds all of the cells together.

These structures with the exception of the medulla are in all animal hairs, the medulla only in coarser hairs. Figure 1.4 contains scanning electron micrographs (SEMs) of four mammalian species taken at different magnifications. These micrographs demonstrate the cuticle structure of a cat whisker, a wool fiber, a human hair, and a horsetail hair. The cross-sections of the horsetail hair reveal the cortex and the multiple porous channels or regions of the medulla characteristic of coarse hairs, but generally absent from fine animal hairs such as fine wool fiber.

Although this book is concerned with hair fibers in general, the primary focus is on human scalp hair and this chapter is concerned mainly with the morphology, the macromolecular structure and the growth of this unique natural fiber.

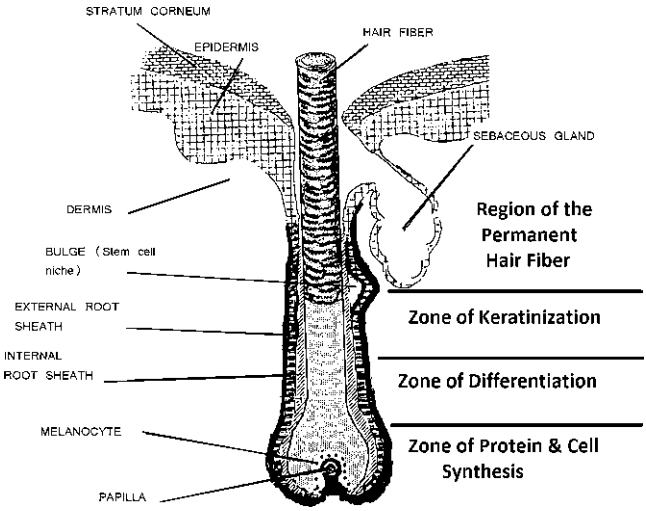

Fig. 1.5 Pilosebaceous unit with a hair fiber in its follicle and the zones of protein and cell synthesis, differentiation, keratinization and the region of the permanent hair fiber as the fiber emerges through the scalp

1.2 General Structure and Growth

The schematic diagram of Fig. 1.5 illustrates an active growing human hair fiber inside the follicle, which is the sac that originates in the subcutaneous tissue of the skin and contains the hair fiber with several surrounding structures involved in its growth. The dermal papilla, located near the center of the bulb is involved in important growth functions during anagen (Fig. 1.2). The basal layer that produces hair cells nearly surrounds the bulb. Melanocytes that produce hair pigment also exist within the bulb close to the dermal papilla. Blood vessels (Fig. 1.1) carry nourishment to the growing hair fiber deep within the skin at the base of the bulb. Figure 1.6 illustrates other important active layers of the growing fiber in the follicle.

The human hair fiber beneath the skin can be divided into several distinct zones along its axis (Fig. 1.5). The zone of biological synthesis and orientation resides at and around the bulb. This zone is sometimes divided into a lower region called cell proliferation or cell matrix. Moving upward in the growing fiber is the region of cell differentiation which leads into the zone of keratinization, where stability is built into the hair structure by the formation of cystine linkages [1]. The next zone that begins below the skin line and eventually emerges through the skin surface is the region of the permanent hair fiber. The permanent hair fiber consists of fully formed dehydrated cornified cuticle, cortical and sometimes medullary cells, but always the cell membrane complex which acts like a natural adhesive, binding the hair cells together.

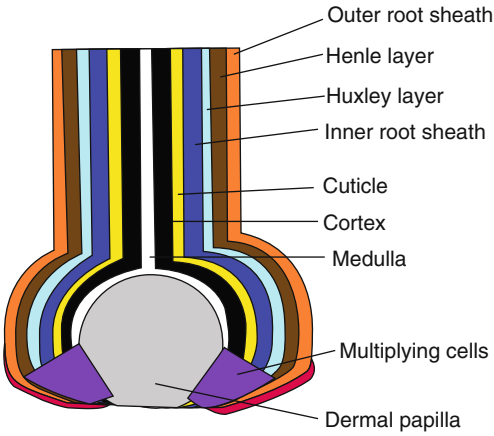

Fig. 1.6 Schematic of an active hair bulb with a hair fiber illustrating the important layers with regard to growth

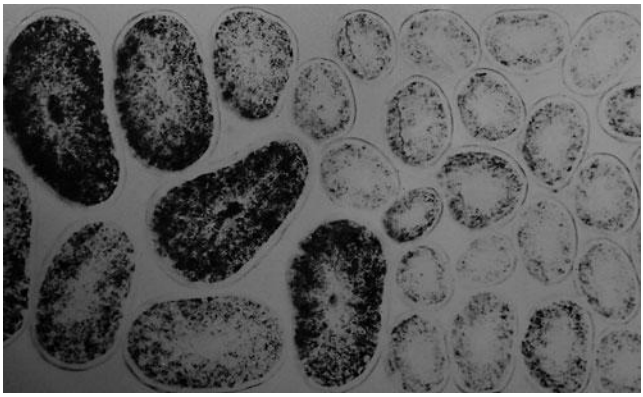

Fig. 1.7 Light micrograph of scalp hair fiber cross sections, illustrating varying fiber cross-sectional size, shape, and pigmentation. Note: lack of pigment in the cuticle

The major emphasis in this book is on the chemistry, structure, and physics of the permanent zone of the human hair fiber and as indicated; the primary focus is on human scalp hair as opposed to hair of other parts of the body.

Randebrock [2] suggested that the diameter of human scalp hair fibers varies from 40 to 120 μm. Others provide a somewhat larger range varying from about 20 to 125 μm. The low values for this latter estimate are undoubtedly due to the inclusion of hair of infants and young children. For adult hair we estimate the variation from means of subjects to be primarily between 45 and 110 μm. The range for individual hairs on individual scalps can exceed these values. Figure 1.7 illustrates the range in fiber diameters and cross-sectional shapes of hairs from five Caucasian adults. For a more complete discussion of hair fiber diameter see the next section and also Chap. 9 and the review by Bogaty [3] and the references therein.

1.2.1 Variation in Fiber Diameter on Different Parts of the Head

Most of the work in the scientific literature is on scalp hair from the vertex or the crown area, although hair from other regions of the scalp is sometimes used. Garn [4] citing others and his own work stated that scalp hair is finest at the temples and most coarse at the sideburns on "normal" scalps. "Normal" scalp usually means pre-alopecia or before the phenomenon of balding begins. The lower sideburns are actually beard hair which is coarser than scalp hair. Tolgyesi et al. [5] demonstrated that beard hair contains a higher amount or higher percentage of hairs with medulla. Beard hair is also more elliptical and it has more irregular cross-sectional shapes and lower disulfide content (cross-link density) than human scalp hair [5].

As indicated, for adults, the mean diameter (from the vertex or crown areas of the scalp) usually ranges from about 45 to about 110 μm and the diameter shows large differences among neighboring hairs on the same head, ranging from a factor of less than 1.4 to more than 2.0 on adult Caucasian women [6]. Garn is essentially in agreement with Yin et al. [6] on these ranges on an individual scalp, claiming as early as 1948 that on the same scalp neighboring hairs may vary by more than a factor of 2. Hair on different regions of the scalp grows at different rates. DeBerker et al. [7] determined that on "normal" scalps, hair grows slowest on the temples (0.39 mm/Day males) and faster on the vertex (0.44 mm/Day males) where it grows coarser. Additional data on growth rates is described later in this chapter.

Three distinct regions (cuticle, cortex and medulla) containing different types of cells are generally apparent in cross sections of fully formed human hair fibers from most parts of the body (see Figs. 1.3, 1.7 and 1.8). After brief discussions on the functions of hair, hair growth/hair loss and treatments for hair loss, the remainder of this chapter focuses on the structures of these three types of cells and the intercellular binding material (cell membrane complex) of human scalp hair.

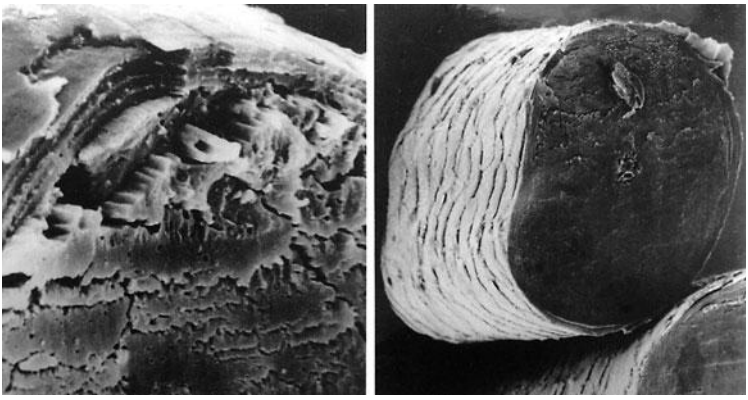

Fig. 1.8 Treated hair fibers cross sectioned with a microtime. *Right*: Note cuticle, cortex, and medulla. *Left*: Note the cuticle layers

1.2.2 Functions of Hair on Different Parts of the Body

Human scalp hair provides both protective and cosmetic or adornment functions. Scalp hair protects the head from the elements by functioning as a thermal insulator. Hair also protects the scalp against sunburn, other effects of light radiation and mechanical abrasion. Hair on parts of the body other than the scalp provides related protective and adornment functions. The adornment function of eyebrows is to the beholder. However, eyebrows also inhibit sweat and prevent extraneous matter from running into the eyes. In addition, eyebrows protect the bony ridges above the eyes, and assist in communication and in the expression of emotion.

Eyelashes are also important to adornment. Eyelashes protect the eyes from sunlight and foreign objects, and they assist in communication. Nasal hairs filter inspired air and retard the flow of air into the respiratory system, thus allowing air to be warmed or cooled as it enters the body. Hair on other parts of the anatomy serves related functions. A general function of all hairs is as sensory receptors, because all hairs are supplied with sensory nerve endings. The sensory receptor function can enhance hair in its protective actions.

1.3 Hair Growth

This discussion on hair growth is considered in two parts:

Follicular/hair apparatus development in the fetus and
Hair follicle cycling or the growth of hairs in the follicle before and after birth

1.3.1 Development of the Follicular/Hair Apparatus with its Essential Structures

Follicular/hair apparatus development in the fetus determines the number and distribution of follicles with their growth structures and the ultimate size of hair fibers thereafter. It includes the length that hair fibers can grow to on all different parts of the body at different stages of life such as the relatively long hairs of the scalp with their long anagen period and the relatively short hairs of the eyebrows with their short anagen. The mesoderm directs the ectodermal cells on how to respond via molecular signals that interact with receptor sites for the normal formation of hair follicles and their contents [8–10]. Several different molecular species have been implicated in the process of hair follicle formation including, Wnt proteins [10–12], noggin [11, 12], lymphoid enhancer-binding factor 1 (LEF-1) [10, 13], sonic hedgehog (shh) [9, 12, 14], beta-catenin [13, 15] and bone morphogenic protein (BMP) [12]. A helpful and concise review describing details of

this information is by Alonso and Fuchs [12]. In 2003, Fuchs and coworkers [11] demonstrated that two molecular signals, Wnt proteins and noggin together can influence immature stem cells to form hair follicles and their internal components. What is so fascinating is that these same stem cells can form either hair follicles or epidermis, but with Wnt protein and noggin signals originating from different parts of the skin and working together, these stem cells produce an activated transcription factor and ultimately hair follicles with their essential structures.

According to Fuchs, this process is multi-step along these lines:

- Wnt protein stabilizes B-catenin increasing its concentration in stem cells
- Noggin inhibits BMP leading to LEF-1 production
- B-Catenin activates LEF-1 (which controls gene activity)
- LEF-1 down-regulates a Gene for the protein E-Cadherin
- Lower levels of E-Cadherin reduce cell adhesion structures and initiates formation of
- Epithelial Buds for follicle formation

Too much E-cadherin (triggers cell adhesion ingredients) can interfere with the downward growth of the stem cells to form a hair follicle. However, with the optimal amount of E-cadherin the stem cells are loosened to the most favorable extent allowing them to grow downward to form a hair follicle with its different cellular structures. At the time of birth approximately five million hair follicles will have been formed over the entire human body, but no additional hair follicles are formed after birth [13]. Research on some of the genes involved in hair loss is described in Chap. 3.

1.3.2 Hair Follicle Cycling and Hair Growth

Generally around the fifth fetal month, the follicles and their growth machinery have been developed, although not entirely mature. Each individual hair after birth is programmed to grow in cycles involving three distinct stages (see Fig. 1.2). These growth stages of the hair fiber are partly controlled by chemical messengers including Wnt proteins (Wnt) [8, 14, 16, 17] and Sonic hedgehog (shh) [9, 16, 18] that stimulate stem cells in the bulge and induce new anagen. Factors that are known to maintain anagen are SGK3 [12, 19] and Msx2 [12, 20]. Androgens (hormones produced by the adrenals and the sex glands stimulate the activity of male sex glands and male characteristics) also play a role in hair development. As indicated in the introduction, the three stages of growth are called anagen, catagen and telogen:

1. The anagen stage, or the actual growing stage, is characterized by intense metabolic activity in the hair bulb. For scalp hair, this activity generally lasts 2–6 years producing hairs that grow to approximately 100 cm in length (~3 ft);

Fig. 1.9 "Three women," by Belle Johnson. Taken about 1900. Hair generally grows to a maximum length of about 3 ft; however, specimens over 5 ft in length have been documented (Reprinted with permission of the Massillon Museum, Massillon, Ohio)

however, human scalp hair longer than 150 cm (~5 ft) is frequently observed in long hair contests (see Fig. 1.9), indicative of a longer anagen period.
Terminal (children or adult) hair does grow at slightly different rates on different regions of the scalp. For example, hair grows at approximately 14-cm/year (~5.5 in./year) on the vertex or the crown area of the scalp of Caucasian females adults; at a slightly slower rate (~13 cm/year) in the temples and generally at even slower rates on other body regions (e.g., ~10 cm/year) in the beard area.

2. The catagen stage or the transition stage lasts for only a few weeks. During catagen, metabolic activity slows down, and the base of the bulb migrates upward in the skin toward the epidermal surface. Molecular regulators that promote the transition from anagen to catagen are: Growth factors (FGF5 and EGF1) and neurotrophins (BDNF, p53, TGFβ1 and BMPRIa) [12].

3. Telogen or the resting stage also lasts only a few weeks (generally 4–8). At this stage, growth has stopped completely and the base of the bulb has atrophied to the point at which it approaches the level of the sebaceous canal.

At the onset of a new growth cycle, a new hair begins to grow beneath the telogen follicle, pushing the old telogen fiber out. The telogen fiber is eventually shed. Sometimes a latency period or a lag time occurs between hair shedding and the subsequent anagen period. This lag time has been called the "hair eclipse phenomenon" [9]. St. Jacques et al. [9] attributed this lag time to a dysfunction involving early shedding and delayed anagen initiation or stunted hair growth between the two anagen phases. The hair eclipse may occur in telogen effluvium (abnormal shedding) associated with new alopecia, post-partum alopecia, seasonal alopecia, alopecia areata or even shedding associated with seborrheic dermatitis or dandruff. St. Jacques et al. [9] suggested that local growth factors or other mediators that are either missing or deficient may be involved in this condition. The effects of antidandruff agents on abnormal shedding are described in Chap. 6 in the section on dandruff.

Kishimoto et al. [17] demonstrated that at the beginning of each growth cycle or new anagen period one or two stem cells that originate in the bulge (Fig. 1.5) are induced by chemical messengers to produce or re-grow the lower portion of the follicle (down to the zone of protein and cell synthesis Fig. 1.5) that ultimately produces hair cells leading to a new hair fiber. Among the more important of these molecular signals or factors essential to follicle induction for hair cycling are Wnt proteins (Wnt) [8, 10, 17] and Sonic hedgehog (shh) [9, 14, 16, 18]. Kishimoto et al. [17] determined that Wnt signaling is essential for maintaining the hair inductive activity of the dermal papilla. Signaling by Wnts and shh is essential for new anagen and these regulators somehow act to initiate formation of the growth region of hair follicles and the production of cells that have the potential to form hair fibers. The cells continue to divide in the matrix of the bulb (zone of protein and cell synthesis) with virtually no differentiation until molecular signals initiate movement upward in the follicle and then differentiation begins in the zone of differentiation see Fig. 1.5. Zhu et al. [21] reported that the concentration of B1-integrin appears to control whether a cell moves upward to differentiate (lower concentration) or continues to divide in the matrix of the bulb. Lin et al. [22] identified notch proteins in differentiating cuticle and cortical cells and suggested these proteins are also involved in differentiation.

The newly formed hair cells near the base of the bulb at the dermal papilla (cell matrix) move upward into the zone of differentiation and the melanocytes in that same region produce the hair pigment or pigments that are incorporated into each growing hair fiber. This pigment is incorporated into the cortical and medullary cells of scalp hair by a phagocytosis mechanism as suggested by Piper [23].

Kulessa et al. [24] found that bone morphogenetic proteins (BMP's) function in differentiation. This fact has been demonstrated by inhibition of BMP's with Noggin which produces an absence of acidic hair keratins (IF proteins) in cuticle and cortical cells and an absence of tricohyalin protein in the medulla.

The expression of several transcription regulators of differentiation (Hocx13, Foxn1, Msx1 and Msx 2) are also reduced to low levels.

Lef1 (lymphocyte enhancement factor) is activated in the initial cortex and is a factor that directly controls the transcription of hair shaft genes in protein production [24, 25]. This activity of Lef1 in hair cycling suggest that Lef1 and BMP's cooperate in hair shaft differentiation and contrasts with the antagonistic action of these two regulators during early follicle development in the embryo as shown by DasGupta and Fuchs [25].

Among the first proteins formed in differentiating cortical cells are the intermediate filament proteins. A single Type 1 (acidic) and a Type II (basic-neutral) intermediate filament protein combine to form helical dimers. These low sulfur dimers aggregate and two dimers combine to form tetramers. The tetramers interact and become connected longitudinally to form sub-filaments sometimes called protofilaments important subunits of the cortex. Human hair has at least 9 Type I and 6 Type II intermediate filament proteins see the section in this Chapter entitled *The Cortex*. Rogers [26] described that after the intermediate filament proteins, the glycine-tyrosine rich proteins, the KAP's (Keratin Associated Proteins) 6, 7 and 8 and finally the sulfur rich proteins are formed. Rogers also suggested that the KAP's 1 and 5 are among the last cortical cell proteins to be expressed.

The cortex forms before the cuticle with the helical dimers forming and aggregating and combining as described to initiate formation of the intermediate filaments. The types and relative amounts of the intermediate filament proteins (IF's) to KAP's help determine the type of cortex that forms, such as orthocortex, mesocortex or paracortex see *The Origin of Hair Fiber Curvature* in this chapter.

The cuticle forms higher up in the follicle and its cystine rich proteins are largely from the KAP5 and KAP10 families [26]. The site and synthesis of 18-methyl eicosanoic acid of the cuticle cell membrane complex is not known, but is believed to be very high up in the follicle during the latter stages of synthesis and differentiation.

After formation of cortical and cuticle cells, the cells remain bound by desmosomes, tight junctions and gap junctions. These will ultimately be replaced by cell membrane complex. During protein synthesis, hair proteins are kept in a reduced state with virtually no disulfide cross-links. During the final stages, the cells move upward into the zone of keratinization (Fig. 1.5) where disulfide and iso-peptide cross-links are formed and dehydration occurs. Disulfide bonds form through a mild oxidative process over a length of several hundred micrometers, and ultimately the permanent hair fiber is completed. Rogers [26] suggested that the keratinization zone is about 1,000 μm long (about ten times the diameter of a coarse human scalp hair fiber). The hair cells must be nearly completely filled with proteins as they are cross linked in about 48 h as they pass through the zone of keratinization [26].

The maturation process (over an individual's life span) for scalp hairs in humans is controlled by androgens and other chemical messengers. Prenatal hairs usually originate in the third or fourth month of fetal life. In humans, prenatal hairs originate from the malpighian layer or the stratum germinativum of the epidermis. Prenatal hairs are sometimes called lanugo and are either lightly pigmented or

contain no pigment. Prenatal or infant hair generally grows to a limit of about 15 cm see Table 1.1. It is very fine and by about 6–7 months after birth is replaced by slightly coarser hair, see Chap. 9 for details. Children's hair or primary terminal hair (pre-pubertal hair), is longer and coarser and generally grows to a length of about 60 cm. Primary terminal hair of children generally begins at about 2–3 years of age.

Soon after the onset of puberty, with its hormonal changes, hair fibers grow longer and coarser; producing what is called secondary terminal hair. In addition to these changes in scalp hair, hair in the axillaries, pubic, and beard areas (for males) becomes longer and coarser at the onset of puberty. Figure 1.10 shows that the maximum diameter of scalp hair for females peaks at a later age than for males (see Chap. 9 for details). The data of Table 1.1 shows that the time-span for anagen is shortest for infants, longer for children and longest from puberty to young adulthood. The data of this table also shows that the maximum attainable length and diameter for human scalp hair also correlate positively.

Table 1.1 Approximate human scalp hair length, diameter and anagen vs. age for female caucasians

Approximate		Approximate	
Hair type	Max. length (cm)	Diameter (μm)	Est. anagen (Year)
Infant	~15	30 (N = 26)[a]	~0.5
Children (0.9)	~60	62 (N = 82)[b]	~4 year
Adult (15.29)	~100	74 (N = 98)[b]	~6 year
Adult (30.89)	–	70 (N = 75)[b]	~5 year
Vellus	~0.1	~4	–

[a]Pecoraro V et al. [28], 26 full-term infants; hairs taken within 76 h of birth (13 males and 13 females). The average diameter of dark complexioned newborns was 37 μm while the average diameter for light colored hairs from light complexioned newborns was 22 μm
[b]Calculated from Bogaty [3] and from Trotter and Dawson [27]

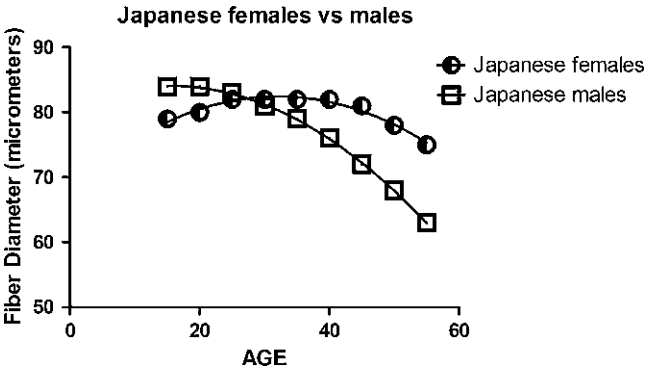

Fig. 1.10 Hair fiber diameter vs. age for males and females (see Chapter 2, section 2.3 "Aging influences on Hair" and Chapter 9 for details and references)

Table 1.2 Differences between primary-terminal and vellus hairs

Primary-terminal hairs	Vellus hairs
Long hairs (~1.0 m or longer)	Short hairs (~1 mm)
Thick hairs (30–120 μm diameter)	Thin hairs (4 μm or less)
Generally (not always) one hair per perpilosebaceous unit	More than one hair pilosebaceous unit
Usually pigmented	Non-pigmented
Longer life cycle (2–6 years in anagen)	Shorter life cycle (in telogen ~90% of time)

As one's age approaches maximum scalp diameter, hormonal changes induce slow gradual shortening of anagen for scalp hair of males. This action causes hair fibers to grow shorter and finer. Ultimately, in many persons this effect results in the transition of terminal hairs to vellus hairs, producing the condition commonly called baldness. Vellus hairs (Table 1.2) grow on those "hairless" regions of the body including the bald scalp, the nose, and many other areas of the body that appear hairless. Vellus hairs do not grow on the palms of the hands, the soles of the feet, the undersurface of the fingers and toes, the margin of the lips, the areolae of the nipples, the umbilicus, and the immediate vicinity of the urogenital and anal openings, the nail regions, and scar tissue.

The phrase, terminal hairs, is normally applied to those long thick hairs that occur during the latter stages of childhood and in adults. Terminal hairs, at some stage of development, grow on the scalp, eyelash area, eyebrow area, axillary and pubic areas, trunk and limbs of males and females, and the beard and mustache areas of males.

1.3.3 Extra Long Hair

As indicated, scalp hair at maturity normally grows to a length of about 3 ft (~90 cm); however, in long hair contests, lengths greater than 5 ft (~150 cm), see Fig. 1.9, are frequently observed and hair of several Guinness record holders have been measured at much longer lengths. Scalp hair length estimates by anatomical site, were made in Florida theme parks on 24,300 "adults" [29]. These hair length estimates by anatomical site were related to anatomical measurements to obtain estimates of free hanging hair lengths in centimeters. A plot of the natural logarithm of the percent population vs. these hair lengths provides a straight line and an equation that with several assumptions permits the estimation of the numbers of persons in the USA and the world with hair lengths up to 183 cm (just beyond ankle length) [30].

Data were also collected via a literature search for even longer hair lengths (ankle length or longer) to provide an equation to estimate the minimum numbers of

Table 1.3 Estimates of hair length in USA and global populations

% population (site)	Approximate Hair length (cm)	Approximate number of persons Calculated from equations A and B[a]
12.04 (Shoulder)	35.5	26.6 million in USA
1.88 (Shoulder blade)	55	4.2 million in USA
0.281 (Waist)	75	620,000 in USA
1.78×10^{-2} (Buttocks)	104	39,300 in USA
8.45×10^{-4} (Knees)	136	1,900 in USA
3.3×10^{-5} (Ankles)	170	73 in USA; 1,500 in world

[a]Numbers rounded off, except where fewer than 100

[b]Population of USA = 270 million, but since approximately 82% of the USA population are age 12 and above use 221 million as the adult population for the USA and since approximately 75% of the world's population are 12 and above use 4.5 billion as the adult population for the world

persons with exceptionally long hair [30]. Estimates of hair length from these studies are listed in Table 1.3.

In March of 1988, Dianne Witt of Massachusetts had the longest scalp hair on record (Guinness Book of Records). Her hair was measured at more than 10 ft long or more than 300 cm. Four years later it was measured at 12 ft (~366 cm) in length, so Ms Witt's hair appeared to be growing at a normal rate of about 6 in. per year (~15 cm). From this estimate of the growth rate at 15 cm/year and actual length, her hair has remained in anagen phase for more than 20 years, (see the section entitled, *A mechanism for hair growth/hair loss and changes in hair size*). So, it would appear that hair that grows to longer than normal lengths does not grow at an excessively fast rate; however it grows for longer time periods (longer anagen phase) than normal length hair.

1.3.4 Excessive Hair Growth

Hypertrichosis is a condition in which an excessive growth of terminal hair occurs usually on the limbs, trunk or face. Hypertrichosis may be localized or diffuse. The most common type is called essential hirsutism or idiopathic hypertrichosis of women. In this condition, terminal hairs grow on women in those areas where hairiness is considered a secondary sex characteristic of males, such as the trunk, the limbs, or the beard or mustache areas. This condition is generally not due to an endocrine abnormality, but is believed to be linked to the transport of testosterone from the endocrine glands to the site of activity (see Fig. 1.11).

Endocrinopathic hirsutism is a rare condition from excessive synthesis of hormones with androgenic properties. This abnormality produces masculinization of females. One symptom of this condition is excessive growth of terminal hairs in regions that are normally "hairless" in females. Classic examples of this disease are oftentimes exhibited in circus sideshows.

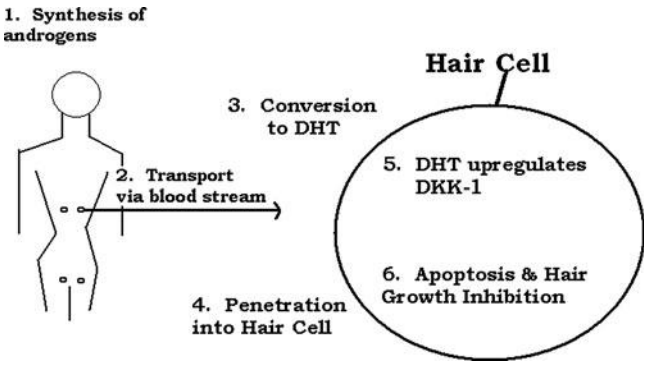

Fig. 1.11 Schematic illustrating how androgens combine with a protein receptor to form an active species that can either stimulate or inhibit hair growth

1.4 Hair Loss (Alopecia)

Hair loss actually involves the transition of terminal hairs to vellus hairs. This condition occurs gradually and at different rates for different persons. This section contains general information on hair loss both for men and women. Immediately following this section are detailed sections on male pattern alopecia and female pattern alopecia. Details and references on the chromosomes and genes involved in these alopecias are described in Chap. 3 in the section entitled *Some Other Hair Traits related to Genetics*.

The phenomenon of androgenetic alopecia tends to occur in a more diffuse pattern among women than men. The term "male pattern baldness" is used for the patterns of balding for men that either begin in the crown of the scalp and move forward or begin in the frontal area of the scalp and recede to create characteristic patterns (Fig. 1.12) which occurs in only a small percentage of women [6] as shown by Venning and Dawber [6].

1.4.1 Hair Density or the Number of Hairs/Unit Area

Barman et al. [31] suggested variation in scalp hair density (hairs/cm^2) between 150 and 300 among normal Caucasians, but current evidence shows this variation is more likely from about 75 to 450 terminal hairs/cm^2. Hair counts on normal scalps generally show less than 10% telogen hairs [32–34]. The considerable variation in hair counts occurs from the following variables: geo-racial group, age, method, scalp region and scalp conditions such as male and female pattern alopecia (later in this chapter) and the menopause (Chap. 2).

The hair density study summarized in Table 1.4 was carried out by Loussouarn et al. [35] on males and females from three different countries with more than 500

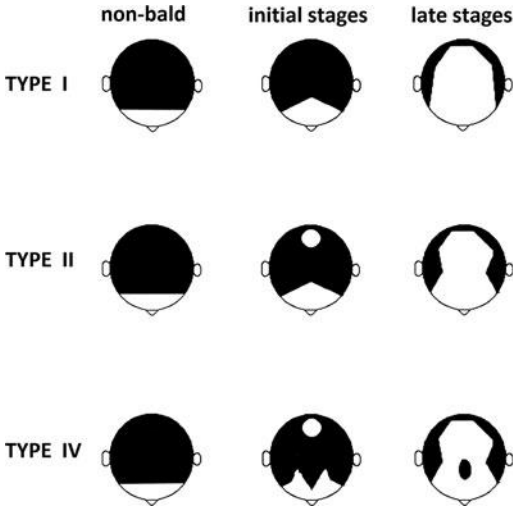

Fig. 1.12 Schematic illustrating different classes and types of male pattern alopecia. Various schemes have been described for classification of male pattern alopecia. This one was adapted from one of Hamilton's

Table 1.4 Comparative hair densities (hairs/cm^2) of different geo-racial groups (panelists 18–35 years of Age) [35]

	Hair density in terms of the number of hairs/cm^2 [a]					
	African (S. Africa + France)		Asian (Chinese)		Caucasian (Paris)	
	Female	Male	Female	Male	Female	Male
	N = 110	N = 106	N = 96	N = 92	N = 51	N = 56
Vertex	199 ± 42	188 ± 46	231 ± 37	217 ± 38	308 ± 68	264 ± 58
Temple	121 ± 38	128 ± 45	117 ± 19	122 ± 27	169 ± 35	151 ± 38
Occipital	167 ± 38	162 ± 41	182 ± 34	179 ± 30	250 ± 49	217 ± 37
Total mean	163 ± 51	160 ± 50	178 ± 57	173 ± 50	242 ± 77	211 ± 65

[a]Values are mean plus or minus standard deviations. Data shows a significant area effect but no significant difference between sexes. Both Asian and African groups provided significantly lower hair densities than for Caucasians

subjects between 1999 and 2003. The Asian subjects were Chinese recruited from Beijing (north China), Shanghai (Central China) and Guangzhou (South China). The Caucasians were from Paris or its suburbs and 98 Africans, living either in Johannesburg, South Africa plus 118 volunteers living in France, but native to West or Central Africa. To address a concern of the few Caucasian women in this study (51), I took hair density data from the parietal region of the scalp kindly provided by Dr Andrew Messenger (for "normal" Caucasian females who came to dermatology clinics with no concerns about hair loss) and analyzed the data for 102 subjects of age 18–35. Distribution analysis provided a normal distribution with a mean hair density of 290 ± 46 which is reasonably close to the hair density value by Loussouarn et al. for 51 female Caucasians of 308 ± 68 between the ages of 18 and 35 and provided reassurance to the data by Loussouarn et al. It also suggested that hair density of the vertex is similar to the parietal region.

This study by Loussouarn et al., with Chinese East Asians, Africans and Caucasians, is consistent with other studies showing that the hair density of Africans [32–34] and of Asians [36] is lower than that of Caucasians. Furthermore, from the data of Table 1.4 the hair density of Asians (vertex and occipital sites) appears to be slightly higher than that of Africans. The data of Table 1.4 suggests that the hair density of Chinese females ages 18–35 is about 25% lower in all three regions of the scalp than that of Caucasian females of the same age.

Loussouarn et al. also analyzed the hair density of males of these same geo-racial groups, see Table 1.4. The hair density of males by geo-racial group shows the same rank order as for females in all three scalp regions. Furthermore, the hair density in the vertex and occipital regions of males (ages 18–35) is significantly lower (matched pairs test) in all three geo-racial groups than for females of the same group. Loussouarn et al. explain this effect by "a difference which may be partially explained by the high prevalence of male androgenetic alopecia in this group".

These data (Table 1.4) also suggest approximately 81,000–121,000 hairs on the scalp (about 242 hairs/cm^2 times 500 cm^2 scalp area for female Caucasians = 121,000 scalp hairs; $178 \times 500 = 89{,}000$ scalp hairs for Asian females; and $163 \times 500 = 81{,}500$ scalp hairs for African females). It is frequently stated that humans lose about 100 hairs/day. For Caucasians assuming 121,000 hairs on the scalp and 7% of the hairs are in telogen phase which lasts about 90 days/year calculates to an average daily fall out of about 94 hairs. For Africans this would be about 63 hairs and for Asians about 69 hairs assuming hair counts as indicated by the data of Loussourarn et al. for females from ages 18 to 35 and the same percentage fall out for each of these three groups. This rate of hair shedding or fall out actually calculates to an average anagen period of about 3.5 years and we normally say it is about 2–6 years. So it is fair to say that adult female Caucasians ages 18–35 lose about 100 (94) hairs/day, Asians about 70 (69) and Africans about 60 (63) hairs/day.

Shedding rates, however, vary to a small degree seasonally and they normally decrease during and increase after pregnancy. Shedding rates also increase with age sometime in adulthood for females (in the mid to late twenties) and sooner for males (as shown later in this chapter). Lynfield [37] determined that the proportion of follicles in anagen increases during pregnancy. Additional details on the effects of pregnancy are described later in this chapter.

With regard to the seasonal effect, in a normal scalp the proportion of follicles in anagen peaks to nearly 90% in the spring (March in the Northern Hemisphere) in temperate climates and falls steadily to a low of about 80% in the late fall (November in the Northern Hemisphere) when the telogen count is highest as indicated by Randall and Ebling [38]. This effect is accompanied by increasing hair fall-out in the fall. As baldness approaches, the anagen time period decreases, thus the percentage of hairs in anagen (normally 80–90 plus percent) decrease as shown by Courtois et al. [39, 40]. The remainder of hairs is in catagen and telogen.

Anagen/telogen ratios are sometimes used as a criterion of the balding condition, that is, as balding progresses the ratio of anagen hairs to telogen hairs decreases. These ratios may be determined by plucking hairs and microscopically evaluating the roots (Figs. 1.13 and 1.14) or even better by the phototrichogram method

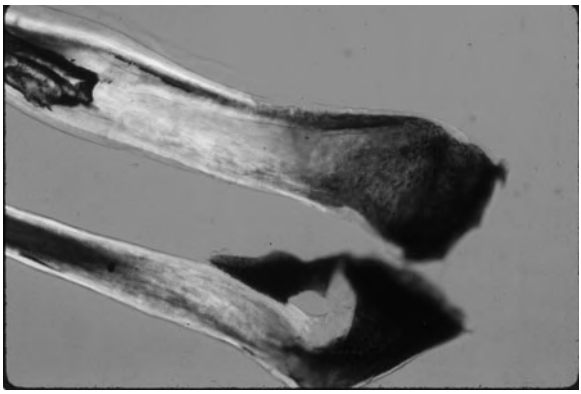

Fig. 1.13 A light micrograph of plucked hair fibers in the anagen stage

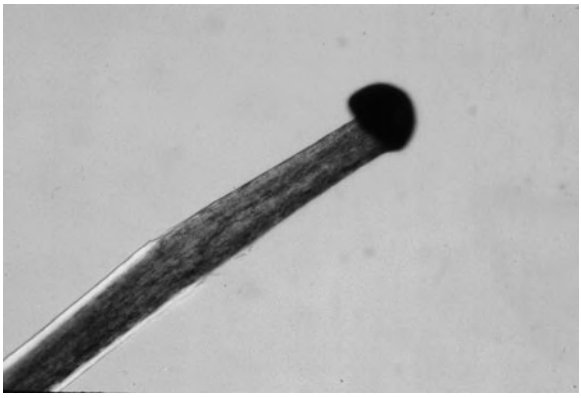

Fig. 1.14 A light micrograph of a plucked hair fiber in the telogen stage

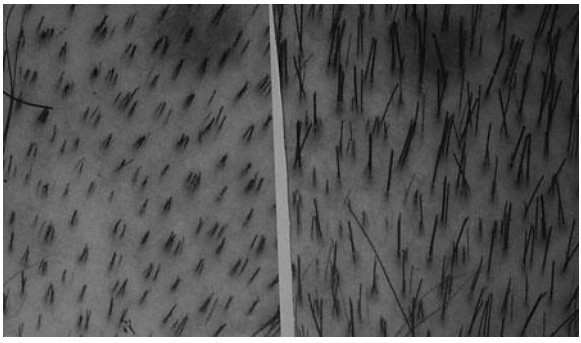

Fig. 1.15 Enlarged photographs of the scalp, *Left*: Immediately after shaving. *Right*: Three days after shaving. Grown hairs are in anagen and non-grown hairs in telogen

(Fig. 1.15) in which a small area of the scalp is shaved, photographed and re-photographed 3–5 days later. Comparison of the two photos reveals those hairs that have grown (anagen hairs, Fig. 1.13) and those hairs that have not grown (telogen hairs, Fig. 1.14) providing a determination of anagen/telogen ratios.

For additional details on hair density including hair density changes with age see the next sections on alopecia entitled *Male Pattern Baldness* and *Hair Loss Among Women*.

1.4.2 Male Pattern Baldness

Male pattern baldness or male pattern alopecia (MPA) is different from female pattern baldness in several ways such as in pattern (compare Fig. 1.12 with Fig. 1.16), incidence (70% of males vs. about 30% of females) and initial age (teens to early 20s for Caucasian males and the late 20s for Caucasian women (data suggests early to mid 20s). Norwood [41] described the incidence of MPA in 1,000 Caucasian males from ages 18 through the late 80s. He classified these males by the Hamilton-Norwood system a similar but more elaborate scheme than that depicted in Fig. 1.12. The data of Table 1.5 summarizes my analyses of these data.

Extrapolation of the data from the equation for Types III through VII (see Table 1.5 for definitions) suggests that Type III MPA begins in some Caucasian males as early as about age 16. The equation for Types III–VII incidence is $Y = -8.986 + 0.8689X.\ 0.01625(X-54.5)^2$ where Y = predicted incidence and X = age. I chose the linear over the quadratic model for types V–VII where: $Y = -10.09 + 0.5679X$ (Y = predicted incidence of types V–VII and X = age) because it was favored by p value and root mean square error. Averaging the extrapolations from the two different models suggests that type V MPA (see Table 1.5) begins in some persons as early as age 19 or 20.

The incidence of MPA Type III has been shown to be lower in both Korean (~14% in a study with 5,531 Korean men) [42] and Chinese men (~20% in a study with 3,519 Chinese men) [43] than in Caucasian men (~70%) [36], in agreement with the findings of Hamilton [44]. See Table 1.6 for additional comparisons at different ages.

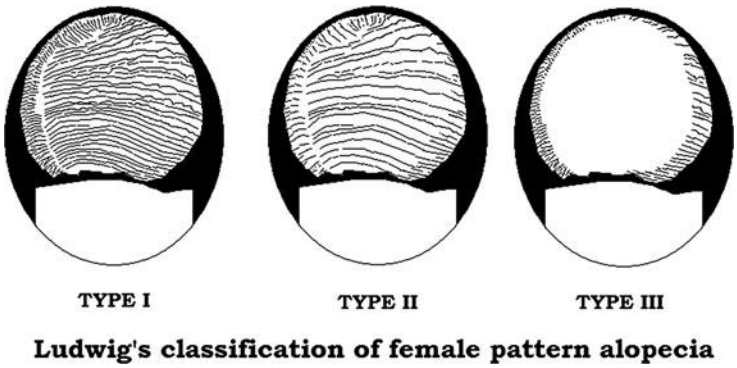

Fig. 1.16 Schematic illustrating Ludgwig's different types of female pattern alopecia

Table 1.5 Incidence of male pattern baldness from calculations of data by Norwood [41]

Age	% Types III–VII[a]	Predicted[b] % Types III–VII[a]	% Types V–VII[a]	Predicted[c] % V–VII[a]
20		7.0		1.3
24.5	12.4	15.7	4	3.8
30		25.3		6.9
34.5	37.6	32.5	9	9.5
40		40.3		12.6
44.5	46.7	46.0	14	15.2
50		52.1		18.3
54.5	53.8	56.3	20	20.9
60		60.6		24.0
64.5	64.4	63.4	31	26.5
70		65.9		29.7
74.5	64	67.2	32	32.2
80		67.9		35.3
84.5	70	67.8	36	37.9

[a]Type III is approximately Type I initial, Fig. 1.12; Type V is approximately initial Types II and IV and Type VII is late stages of Types II and IV of Fig. 1.12
[b]Quadratic model, $R^2 = 0.975$, Root Mean Square Error (RMSE) = 3.877 and $p = 0.0006$
[c]Linear model, $R^2 = 0.972$, RMSE = 2.279 and $p = 0.0001$

Table 1.6 Incidence of male pattern baldness in different geo-racial groups from prediction equations

Percentage showing any MPA Type III[a] through VII[a]				
Age	Caucasian[b]	Korean[c]	Chinese[d]	Asian estimate[e]
24.5	15.7	1.1	0.04	0.57
34.5	32.5	5.5	2.8	4.2
44.5	46.0	12.5	10.4	11.5
54.5	56.3	22.0	22.7	22.4
64.5	63.4	34.0	39.7	36.9
74.5	67.2	48.6	61.6	55.1

[a]Type III is Type I initial of Fig. 1.12 and Type VII is the late stages in Fig. 1.12
[b]From data of Norwood [41], Quadratic model $R^2 = 0.975$; $p = 0.0006$ (Equation above in text)
[c]From data of Paik et al. [42], Quadratic model $R^2 = 0.991$ and $p = 0.0009$
% MPA III–VII Korean = $-29.33 + 0.936\,\text{Age} + 0.01188(\text{Age}-49.5)^2$
[d]From data of Xu et al. [43], Quadratic model $R^2 = 0.993$ and $p = 0.0006$
% MPA III to VII Chinese = $-45.001 + 1.231\,\text{Age} + -0.02382(\text{Age}-49.5)^2$
[e]Average of Korean and Chinese data provides % MPA to represent Asian hair

Clearly MPA begins at an earlier age in Caucasian males than in Koreans or Chinese. Table 1.6 shows an average of the Korean plus Chinese data as an approximation for Asian men. Interestingly, only the last point at the highest age showed a large difference in these two Asian groups. The number for the Korean data point contained the least number of subjects 96 vs. 291 for the Chinese; therefore I would expect the data by Xu et al. for the Chinese subjects to be a more reliable representation of Asian hair for males. I have not been able to find similar extensive data for those of African descent. However, Setty [45] examined

300 Caucasian and 300 Black males at a hospital setting in Washington D.C. and indicated they were chosen randomly. Setty found a lower incidence of balding among Blacks vs. Caucasians.

1.4.2.1 Scalp Hair Density and MPA Versus Age

Among the many useful studies of scalp hair density in MPA was the one by Courtois et al. [40] who studied aging effects on hair cycles, including hair density and lengths of anagen along with an estimate of fiber diameters during an 8–14 year period on the same men (beginning at 25–32 years of age). These panelists are described in more detail in Table 1.7, along with hair densities measured by the phototrichogram method at the beginning and end of the program.

All three groups of subjects showed a reduction in hair density from the beginning to the end of the program with a larger reduction in hair density for the balding groups even though the hair densities were taken in the region of the scalp between the frontal area and crown, a region that is affected by MPA a few years after the frontal region and the crown. The author's described those areas as more prone to alopecia. For example, the frontal or crown areas, would show a more rapid decline in hair density over a shorter period of time. The non-balding group showed a 7.5% reduction in hair density while the 4 most balding members showed a 19.9% reduction in hair density and the 6 balding member group showed a reduction of 15.5% in hair density.

Courtois et al. [40] graphed their data over 3 year periods plotting the percentage of hairs with a growth period greater than X months for each individual on the abscissa vs. time in months (up to 36) on the ordinate. These curves confirmed that the ageing process of hairs in males (beginning at ages 25–32 over approximately a decade) shows a general decrease in the lifetime of hair fibers. This reduction in the lifespan of hairs at this stage of life for males was confirmed by analysis of variance.

Table 1.7 Hair density of men with and without MPA over an 8–14 year period [40]

Group	Hair density in number of hairs/cm^2 [a]			
	Beginning of program	End of program	Delta	Delta/10 years
Non-Balding[b]	288.5 ± 18.4	266.8 ± 12.8	21.7	16.7
Balding (all 6)[c]	219.7 ± 38.2	185.7 ± 21.1	34.0	36.0
Balding (4)[d]	235.8 ± 36.7	188.8 ± 26.1	47.0	49.6

[a]Hair density and telogen density were taken on the vertex, between the frontal area and the crown. This area is affected by MPA after the frontal region and the crown

[b]No signs of alopecia and with telogen density below 15

[c]Two of these subjects showed only fronto-temporal recession with grade III on the Hamilton scale and with telogen density approaching 20; the other four subjects are described below

[d]All four subjects showed more prominent frontal recession and thinning on the vertex than the two above and these four subjects showed grades III to V on the Hamilton scale. The proportion of hairs in telogen of these four subjects was approximately 30%

In addition, the finest hairs displayed the shortest anagen or growth periods, while the coarser hairs showed longer periods of growth.

Courtois et al. [40] pointed out that the average maximum length and the fiber diameters declined as the subjects aged. These scientists approximated hair diameters by comparing them with five groups of calibrated strings that were: very fine <35 μm; fine 35–50 μm; medium 51–65 μm; thick 66–80 μm and very thick >80 μm. Analysis of their data shows that the percentage hairs of the two coarsest diameter groups for each person of the balding group vs. non-balding group was significantly lower at both the beginning and the end of the test. In addition, the percentage difference from the beginning to the end of the test for the very fine diameter hairs of the balding subjects increased more than for the non-balding subjects to a significant degree ($p = 0.0006$). These results suggest that the reduction in fiber diameter with age for males likely appears over a few or several hair cycles and therefore could be different from females in FPA as concluded by Birch et al. [46].

1.4.3 Hair Loss Among Women

Female pattern alopecia (FPA) occurs as a diffuse reduction in hair density of the frontal and crown regions of the scalp; see the schematic of Fig. 1.16 depicting Ludwig's [47] original characterization of FPA. It usually begins just behind the frontal hairline, but in some cases the hairline can also decrease in hair density [48]. At one time it was believed that FPA and MPA were the same disease and both were due primarily to androgens [48]. However, several scientists including Norwood believe that these are two separate diseases. One reason is because the levels of incidence are different (MPA affects up to 70% of Caucasian males while FPA affects a little more than 30% of Caucasian women). In addition, MPA begins in the late teens (sometimes around age 16 for some males) to the early 20s when testosterone levels are high, while for female Caucasians, FPA begins in the twenties and peaks after about age 50 when testosterone levels are low. FPA and MPA also begin and occur in different regions of the scalp, compare Figs. 1.16 and 1.12.

1.4.3.1 The Incidence of Female Pattern Baldness among Caucasians Versus Asians

Norwood [48] determined the incidence of FPA in women by examining a total of 1,006 Caucasian women 20–89 years of age. Birch et al. [46] conducted an important study with two groups of women; one group consisted of 377 women, ages 18–99 that came to clinics for dermatologic reasons other than hair disorders. A second group of 47 women came to the clinic for reasons of hair thinning or FPA. These scientists ran several tests on both these groups of women including the

determination of FPA and hair density. I combined the incidence of FPA of the 377 women from the Birch, Messenger and Messenger study with the 1006 women from the Norwood study and determined best fitting equations. Predicted percentages of FPA from this model equation for the combined data of Norwood and Birch, Messenger and Messenger are summarized in Table 1.8.

By statistical analysis, the combined data of Norwood and Birch, Messenger and Messenger provides a better fit than the Norwood data alone. I believe that the predicted values for the incidence of FPA of Table 1.8 are the best data currently available for the incidence of FPA among Caucasians as a function of age. The incidence of FPA as a function of age of Tables 1.8 and 1.9, and the schematic of Fig. 1.16, define the incidence and region of the scalp that is most affected by this condition.

Table 1.8 Predicted incidence of female pattern hair-loss among Caucasian women from combined data of Norwood [48] and Birch et al. [46][a]

Age	Predicted % female pattern hair-loss
20	2.6
25	5.3
30	8.0
35	10.7
40	13.5
45	16.2
50	18.9
55	21.6
60	23.4
65	27.1
70	29.8
75	32.5

[a]The prediction equation was a linear model with an $R^2 = 0.948$ and $p = 0.001$, providing an equation of $Y = -8.32 + 0.545\ X$ where Y = the predicted incidence of FPA and X = age

Table 1.9 Incidence of FPA among Caucasian and Asian women

Age	Percentage with any female pattern baldness			
	Caucasian[a]	Korean[b]	Chinese[c]	Ave. Korean + Chinese[d]
24.5	3.3 (4.8)	0.2	0	0.1
34.5	14.8 (11.2)	2.3	0.3	1.3
44.5	13.5 (15.8)	3.8	0.8	2.3
54.5	20.8 (20.8)	7.4	1.7	4.6
64.5	26.6 (32.9)	11.7	3.3	7.5
74.5	33.1 (32.9)	24.7	15.4	20.1

[a]Combined data of Norwood [48] and Birch et al. [46] in parentheses from prediction equation $Y = -8.32 + 0.545X$, where Y = incidence of hair loss and X = Age
[b]Data of Paik et al. [42]
[c]Data by Xu et al. [43]
[d]Average of Korean and Chinese hair represents the incidence for Asian hair [42, 43]

1.4.3.2 Incidence of FPA Among Caucasians Versus Asians

The incidence of FPA or extensive hair loss among Asian women is lower than among Caucasian women. For example, Paik et al. [42] studied hair loss in Korean men (5,531) and women (4,601) and found 24.7% of women over 70 years of age have FPA. This value of 24.7% FPA among Korean women that are more than 70 years of age can be compared with 33% among Caucasian women and 15.4% among Chinese women by Xu et al. [24].

Xu et al. [43] studied the incidence of hair loss in Chinese women in Shanghai, China and found a numerically lower incidence of hair loss at all ages than for the study among Korean women, see the data of Table 1.9. The hair loss from these two groups of Asian women is clearly lower at all ages than for the Caucasian women, see Table 1.9. The data was also combined for the Korean and Chinese women providing average values used as estimates for Asian women. Ludwig Type I hair loss was the most common up to the sixth decade for the Korean women. In the sixth decade and at higher ages Ludwig types I and II showed similar occurrence.

1.4.3.3 Hair Density of Men Versus Women and Children Versus Adults

The paper by Birch et al. [46] together with papers by Pecoraro [28, 49, 50] and by Loussouarn et al. [32, 35] provides an entry into hair density as a function of age among women. Only relatively small studies (generally at age 35 or less) were found comparing hair density of men vs. women who were not affected by alopecia. In those cases there were no significant differences in hair density among men vs. women. The study by Loussourarn et al. [35] summarized by Table 1.4 shows lower hair densities for males than females. These scientists attribute part of that difference to male androgenetic alopecia. If differences do exist in hair density between men and women with no signs of androgenetic alopecia, they must be either small and or region or age specific.

Pecoraro, Astore and Barman provide an indication of hair density of children before puberty [49] vs. adults [50] in two of their papers. In their paper on adults from ages 16 through 46 (with only 17 males and 22 females), these scientists found a wide range in hair densities from 175 to more than 300 hairs/cm^2 while Birch et al. [46] found an even wider range from just over 75 to nearly 450 hairs/cm^2 for more than 300 females.

Pecoraro et al. also estimated hair coarseness using 3 coarseness groups: thick (~100 μm), medium (~50 μm) and fine (~25 μm) and noted a decrease in coarseness of hair over the entire scalp, in both sexes, as age advances peaking between ages 16 through age 33 and declining from age group 24–33 to the higher age groups. They also noted that the percentage of telogen hairs increased in all scalp regions with increasing age with the largest change occurring in the coronal region. Note, Pecoraro et al. did not examine the temporal region of the scalp. These scientists also noted decreasing hair density especially in the coronal region with increasing

Table 1.10 Comparative hair densities of children vs. adults in different scalp sites

Region of scalp	Children (ages 3–9) [49]	Adults (ages 16–46) [50]
Crown or coronal	233 highest density	202 lowest density
Parietal	170 lowest density	232 high density
Frontal	196	212
Occipital	193	236 highest density

Table 1.11 Data from quadratic models of "normal" subjects vs. subjects with self perceived hair loss [51]

	Predicted hairs per cm^2 by quadratic model		Instantaneous rates	
Age	"Normal" (N = 315)	Self perceived loss (N = 1,099)	"Normal"	Self perceived loss
25	291.7	272.4	−0.86	−0.65
30	286.9	268.0	−1.05	−1.10
35	281.2	261.4	−1.23	−1.56
40	274.6	252.5	−1.42	−2.01
45	267.0	241.3	−1.61	−2.47
50	258.5	227.8	−1.79	−2.92
55	249.1	212.1	−1.98	−3.38
60	238.8	194.1	−2.17	−3.83
65	227.4	173.8	−2.36	−4.29

age, consistent with data of Birch, Messenger and Messenger. Table 1.10 compares hair densities of pre-pubertal children with those of adults. The hair densities for adults of Table 1.10 appear on the low side (compared with the data of Table 1.11); hopefully the relative differences within the study by Pecoraro et al. are more meaningful. Note the different distribution of hair density on the different scalp regions of the children vs. the adults.

As the data of Table 1.10 show, the children display the highest hair density in the crown or coronal region of the scalp. In direct contrast, adults show the crown to contain the lowest hair density while the occipital and parietal regions contain the highest hair counts. But, the parietal regions contain the lowest hair density of these scalp regions in children. Might this effect in the coronal region vs. the other regions be a sign that the condition of baldness is already beginning because the crown or coronal region of the scalp which has the highest hair density before puberty becomes the lowest hair density after puberty and is the region or part of the region most affected by MPA and possibly by FPA.

1.4.3.4 Hair Density Versus Age for Caucasian Women

Hair densities vs. age in the parietal region of the scalp have been compared for two groups of Caucasian female panelists, one group by Birch et al. [46] and another by Robbins et al. [51]. Both data sets show a highly significant fit for quadratic and cubic models for hair density vs. age with a maximum in hair density in the mid to

high twenties age range. A plot of the data by Robbins and Dawson et al. is summarized in Fig. 1.17. For this study, the site was the left and right parietal region about 3.8 cm from the vertex on each side of the scalp toward the tip of the ear for 1021 Caucasian women from age 18 to 66 (providing more than 2,000 data points). These women believed they had some hair loss. For this figure the data were condensed to 95 data points by ANOVA and then regressed vs. age.

Birch et al. [46] determined hair densities on another group of Caucasian females consisting of more than 300 women age 17–86 who came to dermatology clinics with no complaints about baldness. The site in which hair density was determined was "within a 1 cm diameter circle, about 2 cm lateral to the midline of the scalp, halfway between the vertex and the frontal hair line" in the parietal region of the scalp. Note, the primary difference in the subjects of these two groups of Caucasian women was that the Birch, Messenger subjects came to dermatology clinics with no complaints of hair loss whereas the Robbins and Dawson et al. [51] subjects were enrolled because they perceived hair loss themselves. Therefore the Birch, Messenger group could be called the "normal" or control group.

Best fitting quadratic models were calculated and data from both groups of these panelists are summarized in Table 1.11. These data confirm the findings of others that there is a gradual decrease in hair density with age from near the mid-twenties for female Caucasians which has been shown for other races. Table 1.11 also contains instantaneous rates of hair loss at different ages for the women with self perceived hair loss and those with no complaints of hair loss. These rates were obtained from the first derivatives of the quadratic equations from the regression models and show gradual increases in the rates of hair loss with increasing age. At age 30 and above the differences in these instantaneous rates of hair loss for these two types of panelists become increasingly larger.

This latter effect is illustrated by the rates of change of the rates of hair loss (analogous to acceleration constants) calculated from the second derivatives of the quadratic regression models summarized in Table 1.12 and demonstrates that the instantaneous rates of hair loss from the panelists with self perceived hair loss are increasing at a faster rate than the "normal" panelists.

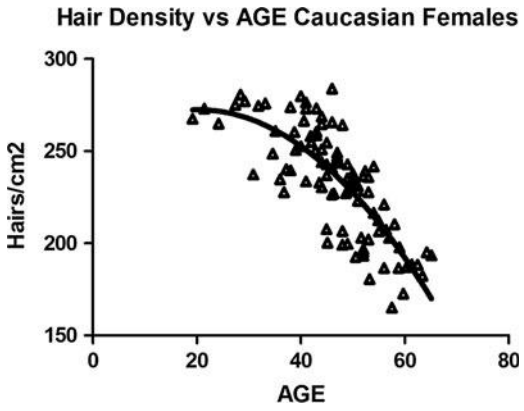

Fig. 1.17 Hair density (hairs/cm^2) vs. age for Caucasian females; in the parietal region of the scalp [51]

Table 1.12 Comparative rates of change of the rates of hair loss of normal subjects vs. those with self perceived hair loss [51]

	"Normal" subjects	Self perceived hair loss subjects
Rate of increase of rates of hair loss	0.038	0.091

Birch, Messenger and Messenger also noted that subjects with high hair density 350 or greater tended to display multiple hairs emerging from single follicles, while those with low hair densities generally less than 200 usually had single hairs rising from most follicle orifices. Others have cited a similar finding [52]. The effects of menopause on hair density and diameters are described in Chap. 2 in the section entitled, *The Effects of Menopause on the Lipids in Hair and on the Hair Fiber*.

Hair loss was studied among Japanese women by Tajima et al. [52] and others [53, 54]. These scientists examined 159 women (46 suffering from hair loss and 113 with little to no hair loss) showing similar effects to the data of Birch, Messenger and Messenger, that is a decrease in hair density with increasing age beyond the mid-twenties. The hair density with respect to age for these Japanese women were slightly lower (5–20% in the different age groups), but otherwise similar to those of Birch, Messenger and Messenger among the Caucasian women.

1.4.3.5 Factors Involved in the Perception of Female Pattern Baldness

Birch, Messenger and Messenger state in this paper [46] that the perception of hair loss is generally determined primarily by decreasing hair density. However, these scientists add that for initial discrimination between Ludwig type I hair loss (Fig. 1.16) and no hair loss, larger hair diameters could weaken discrimination. Another way of saying this is that the subjective impression of FPA is multi-factorial involving hair density, hair fiber diameter and very likely hair fiber curl or the degree of curliness and possibly other factors. The work of Robbins and Dawson et al. [51] support this proposal by demonstrating that both hair density and diameter contribute to the perception of the relative hair amount in a new metric called "relative hair coverage" described in detail in Chap. 10.

Supporting the conclusion that hair curvature should be considered in determining the perception of hair baldness are the facts that increasing hair curvature increases hair volume or body and several small studies show that curly African hair and African American hair has fewer follicles and fewer hairs/cm^2 [32, 33, 35] as compared to Caucasian adult hair and yet the coverage on non-alopecia scalps appears to be at least equivalent.

For women who suffer hair loss such that their hair density is on the low side (150–200 hairs/cm^2), but close to the spectrum of the normal distribution of hair density, other factors such as hair fiber diameter and the degree of curliness will likely enter into their subjective interpretation of FPA. A 25–30% hair density decrease from 400 hairs/cm^2 might be detectable for fine straight hair even though the hair density would still appear rather high at nearly 300 hairs/cm^2. To illustrate

this point, Birch, Messenger and Messenger found a wide range of hair density in subjects who were not concerned with FPA, ranging from approximately 75 to nearly 450 hairs/cm^2. In this study, the clinicians classified more than 50 women (about 15%), among this group not concerned with FPA, as having FPA. The median hair density of that group with FPA was approximately 188 hairs/cm^2, while the median for those not classified as having FPA was approximately 263 hairs/cm^2. Birch et al. [46] also classified three women with a little more than 300 hairs/cm^2 as having FPA. Now, if these high hair density women had very fine and straight hair and they had undergone nearly 30% hair loss from about 430 to 300 hairs/cm^2 then they would likely have been classified clinically as having FPA.

1.4.3.6 Normal or Acceptable Hair Loss

So, what is "normal" hair loss that is acceptable to women? The data of Table 1.11 along with the work of Birch et al. suggests that some women can suffer as much as 25–37% hair loss or decrease in hair density without complaining about hair loss. So, I conclude that a hair density decrease of 30 $\pm$ 5% in the top central area of the scalp just behind the frontal hairline, would be the borderline hair loss at which the factors of hair fiber diameter and hair fiber curl become more and more important with regard to the self determination or perception of a problem with hair loss among women. If the hair is fine and straight then a hair density decrease less than 30% will likely cause concern.

Interestingly, another group of women that Birch, Messenger and Messenger studied was a group of women who came to the clinic with hair loss as their major complaint. The most severe hair loss among this group had hair density less than 125 hairs/cm^2. Assuming a starting point at 290 hairs/cm^2 would provide a hair density decrease of 57%. So clearly a hair density decrease of more than 50% should provide a real hair loss problem for most women regardless of fiber diameter or degree of curl.

1.4.3.7 Hair Miniaturization or Diameter Change in Females Versus Males

Birch et al. [46] examined hair fiber diameter changes and found an extremely weak correlation between hair fiber diameter changes and hair density, $R^2 < 0.03$. This conclusion is consistent with the fact that maximum diameter for women occurs in the early to mid-forties [51] and then decreases with advancing age as most of the literature suggests, see Chap. 9 for additional details. On the other hand, maximum hair density occurs in the mid-to-late-twenties [51].

Birch et al. [46] concluded that if hair miniaturization does occur in FPA it must be different from the balding process in men. Moreover, they suggested that the miniaturization of hairs likely occurs rapidly inside a single hair cycle or over a few years in FPA as opposed to a lengthy gradual process over several hair cycles for

MPA. I could find no confirmation of this suggestion in the literature. Nevertheless, this is an interesting observation that needs to be re-examined, because if it is correct it is very important.

1.4.4 Pregnancy and its Effects on Scalp Hair

One of the clearest non-technical summaries of what happens during pregnancy can be found in the following website of the Mayo Clinic at mayoclinic.com: This website explains that hormone levels during pregnancy inhibit normal hair loss on the scalp. Therefore, during pregnancy one usually observes a "lush head of hair". But, after delivery, the excess hair is shed in a very short timeframe. This effect often provides a shock to the postpartum mother. But, usually within 6 months the hair returns to normal.

As indicated, the mechanism for hair growth involves three stages: A growing period called anagen; a transition period, catagen and a resting period, telogen. At telogen, the "old" hair falls out and is replaced by a "new" hair fiber. The time-span of anagen is normally 2–6 years which determines how coarse and how long scalp hair fibers become. The time-span of anagen is shortest for infants, longer for children and longest from puberty to young adulthood (~ages 13–30). Then sometime in the adult stage of life, anagen becomes shorter with further advancing age, earlier for men (late teens to 20) than for women (mid-to-late-twenties). The percentage of hairs in anagen is normally 85–90% and most of the other hairs are in telogen. The lower the percentage of anagen hairs means more hair fall out and usually signifies alopecia.

Lynfield [37] was the first to provide a general understanding of what happens to scalp hair during and after pregnancy. Lynfield concluded, contrary to the existing view in 1960, that the time of onset and the length of time that hair loss occurs postpartum are highly variable. Lynfield determined that the period of anagen is longer than normal during pregnancy; therefore there is less fallout during that time. However, after delivery there is usually a larger amount of hair loss than normal.

Lynfield [37] began her study with 26 Caucasian women (ages 17–38) and examined the hair roots of different numbers of these women during and after normal pregnancies. She compared her data with a control group of 30 healthy non-pregnant women ranging from age 17 to 40. Lynfield determined anagen and telogen counts by examining hair roots of 50 hairs at a time, in both the frontal and temporal scalp. She focused her results primarily on temple hair since the temporal data provided less experimental scatter, but she indicated that the changes were parallel in both regions of the scalp.

Lynfield's [37] data showed normal anagen percentages, near 85%, in the first trimester of pregnancy for five subjects. These percentages compared favorably with the mean anagen hairs in the non-pregnant control group at $85 \pm 5.6\%$ (mean $\pm$ standard deviation). During the second and third trimester and the first week postpartum, the anagen levels rose to 95%, 94% and 94% respectively.

However, in the sixth week postpartum the anagen levels dropped to 76% and 77% and in the few cases examined at a later time (not specified, but 5 and 8 months from the graph of one subject) the anagen density generally returned to normal. Lynfield noted that contrary to the existing view in 1960, her data indicated that the time of onset and the length of time that hair loss occurs postpartum are highly variable. The time of onset began almost immediately after delivery in two women, 1 month postpartum in 1 and 4 months postpartum in another and it lasted up to 5 months.

Lynfield [37] described one clear exception as a 38 year old woman in her seventh pregnancy whose anagen levels were essentially unchanged and no clinical hair loss was detected. Lynfield speculated that after several pregnancies the hair roots of this woman did not respond to "hormonal fluctuations of pregnancy". She also described five other women whose anagen counts decreased postpartum, but no hair loss could be detected by the clinicians. So, not only are the times of onset and the length of hair loss postpartum highly variable, but the clinical detection of hair loss postpartum is also highly variable.

Pecoraro et al. [55] reported an increase in the proportions of thick to medium and thin hairs during pregnancy. Nissimov and Elchalal [56] confirmed this finding and identified that the mean major-axis diameter of scalp hair was higher in 12 pregnant vs. 13 non-pregnant women. The major-axis diameters of the pregnant women increased (+4.5%), and this increase was first detected at about 2 weeks after conception through the 35th week of pregnancy. On the other hand, the major-axis diameters of the non-pregnant women decreased (−5.2%) toward the scalp over the same 35 week period. This difference is statistically significant at a high level of confidence.

About a little more than a decade ago, Hutchinson and Thompson [57] reported changes in the major-axis diameter of human scalp hairs that they associated with changes occurring inside the follicle. They concluded that hair fibers are not uniform cylinders, but from the distal end toward the scalp there is an increase in size over about a 6–8 cm length (about 3 weeks growth) of fiber, that they associated with the start up of anagen. After that distance the major-axis of the hair fiber decreased progressively through anagen. These effects were confirmed by the work of Nissimov and Elchalal [56]. See the section on hair fiber ellipticity in Chap. 9 for additional discussion on the effect of diameter and ellipticity changes on single hairs over time.

The only data I could find on growth rates during pregnancy are by Pecoraro et al. [55] which indicated 0.0325 cm/day in the first trimester, 0.0315 cm/day in the second trimester and 0.0329 cm/day in the third trimester. Comparing these data with Pecoraro's [50] data in an earlier publication shows adult scalp hair of females grows at 0.0344 cm/day. This suggests that the growth rate slows down during pregnancy. This growth rate effect, if real, could relate to the body compensating for the increased protein demand and number of hair cells required by thicker hair fibers produced during pregnancy and the increase in the number of anagen hair fibers.

The thorough review paper by Ohnemus et al. [58] describes the hair follicle as a target for estrogen, but cautiously states that because of the complex associated

endocrine changes during and after pregnancy it is still not clear whether estrogens or other hormones initiate these effects on hair fibers during and after pregnancy. Also, more data with larger numbers of subjects on hair density of males and females of different geo-racial groups vs. age would be helpful for predicting effects of important cosmetic hair properties and treatments vs. age.

1.4.5 Alopecia Areata, Universalis and Other Forms of Hair Loss

Alopecia areata, another form of hair loss, is believed to be related to the immune system (e.g., autoimmunity). This disease generally occurs as patchy baldness on an otherwise normal scalp, although sometimes hair of other body regions is affected. When the entire scalp is involved, the condition is called alopecia totalis. If terminal hair loss occurs over the entire body, a rare condition, it is called alopecia universalis. Emotional stress has been shown to be one of the initiating causes of areata. See the section in Chap. 7 entitled, *Sudden Graying-whitening of Hair* where alopecia areata has been suggested to be involved. Topical application of steroids is sometimes used to treat areata. However, even when untreated the balding area in time often returns to normal hair growth.

Alopecia induced by physical stress has been termed trichotillomania. This condition occurs from physically pulling or twisting a localized area of hair until noticeable thinning develops. This type of hair loss sometimes occurs in children who unconsciously pull or twist a group of hairs. A similar type of hair loss also occurs in adults.

Telogen effluvium is a term used to describe a sudden but diffuse hair loss that is often caused by an acute physical or psychological stress. This condition usually lasts only a few months and is often reversible. Telogen effluvium has been associated with dandruff and its treatment is described in Chap. 6 in the section on dandruff.

Drugs used in chemotherapy often induce alopecia. However, this type of hair loss is also usually reversible and the "new hair" after chemotherapy can be of a different curvature or a different color than the hair prior to chemical treatment.

1.5 A Mechanism for Hair Growth/Hair Loss and Change in Hair Size

The ratio of anagen to telogen hairs indicates whether hair growth or hair loss is occurring. The length of anagen activity controls the changes in hair size that occur during different stages of the life of mammals. At different ages of humans, such as shortly after birth, at puberty or at maturity, hairs grow to different sizes (different

lengths and diameters). All of these changes generally involve hormones or chemical messengers. See the section in Chap. 2 entitled *Aging Influences on Hair* and the previous section on *Hair Growth* in this Chapter.

Loussouarn et al. [35] described hair growth rates of scalp hair for three different geo-racial groups in these three scalp regions (vertex, temple and occipital). This study showed that the growth rate of the hair of East Asians is higher than that of either Caucasians or Africans, see Table 1.13. The growth rates of the scalp hair for females of these three geo-racial groups' shows that the scalp hair of Africans grows slower than the scalp hair for either Caucasian or Asian females. Furthermore, the growth rate of the scalp hair of Asian females is slightly higher than that of Caucasian females.

The growth rates of males of these same geo-racial groups is summarized in Table 1.13 and parallels the growth rates for females showing slower rates for Africans in all three scalp regions and slightly higher growth rates for Asians than Caucasians.

Over the past five decades, many ingredients have been demonstrated to either inhibit or to promote hair growth, see Table 1.14. More than 40 years ago, Hamilton [59] demonstrated that androgens are a factor in male pattern baldness. For example, long-term injections of testosterone induced a rapid transformation of terminal hairs to vellus hairs in the frontal scalp of stump-tailed macaques [59, 60]. Thus, testosterone, an androgen, produced by the adrenals and the sex glands was shown to play a critical role in controlling the growth patterns of human scalp hair fibers.

Estrogen, a generic term for any substance that exerts biological effects as hormones like estradiol, have been shown to produce positive effects on hair growth when taken internally or applied topically. Systemic estrogen probably prolongs the anagen phase of hair growth by suppressing androgen production [60], and both estrogens and anti-androgens when applied topically have been shown to be capable of suppressing hair loss as shown by Schumacher-Stock [61]. Anti-androgens, substances that are capable of blocking androgen function, include spironolactone, cyproterone acetate, progesterone, finasteride (Fig. 1.18) and dutasteride. These last two ingredients of Table 1.14 are inhibitors of 5-alpha-reductase an important

Table 1.13 Rates of growth of hair of different geo-racial groups (all panelists 18–35 years of age) [35]

	Growth rate in terms of micrometers/day[a]					
	African		Asian (China)		Caucasian	
	Female	Male	Female	Male	Female	Male
	N = 110	N = 106	N = 96	N = 92	N = 51	N = 56
Vertex	294 ± 49	282 ± 52	413 ± 51	430 ± 55	379 ± 51	364 ± 66
Temple	282 ± 46	286 ± 50	393 ± 55	406 ± 46	357 ± 53	368 ± 57
Occipital	274 ± 59	258 ± 48	410 ± 54	417 ± 50	364 ± 56	371 ± 53
Total mean	284 ± 49	275 ± 51	405 ± 54	418 ± 51	366 ± 54	368 ± 58

[a]Values are mean plus or minus standard deviations. Data shows a significant area effect but no significant difference between sexes. These data show that the growth rate for Asian hair is significantly higher than for either Caucasian or African hair

Table 1.14 Some ingredients known to affect hair growth

Retard hair growth	Promote hair growth
Testosterone	Streptomycin
Dihydrotestosterone	Cyclosporin
Retinoids	Diazoxide
Retinoic acid	Estrogens
Retinol	Estradiol
Eflornithine[a]	Progesterone
	Spironolactone
	Minoxidil[b]
	Finasteride[c]
	Dutasteride[c]

Molecular signals essential to follicle induction and growth such as BMP's, sonic hedgehog, several WNT proteins and several receptors such as BMPRIA EGFR, EGRF and TGFR were not included in this table

[a]Chemotherapy drug (known to inhibit polyamine biosynthesis and ornithine decarboxylase)

[b]Potassium channel opener and vasodilator

[c]Inhibits 5-alpha-reductase (conversion of testosterone to dihydrotestosterone)

enzyme in the conversion of androgens to the most active form of testosterone. The topical application of estrogens and anti-androgens probably cause a local inhibition of the androgen function and demonstrate one solution to hair growth, as shown by the proposed mechanism below.

Chemical cures for baldness and the search for a better understanding of the mechanism of this phenomenon often involve androgens, genetic studies and drugs known to be capable of inducing hypertrichosis, such as streptomycin, cyclosporin, diazoxide, tacrolimus (fujimycin), estradiol, oxandrolone, minoxidil, finasteride and dutasteride. Several of these drugs have shown promise in reversing the symptoms of male pattern baldness. Minoxidil and finasteride are currently sold as active ingredients in hair growth products.

Minoxidil (6-amino-1, 2-dihydro-1-hydroxy-imino-4-piperidino pyrimidine) has been shown to re-grow hair with minimal side effects. This drug is a vasodilator and a potassium channel opener. It was originally developed by Upjohn for treatment of hypertension, and has been shown to be capable of reversing male pattern alopecia in clinical trials during treatment periods. However, with minoxidil, best results are obtained under occlusion and in subjects whose condition of balding has not progressed for many years. The re-growth is concentration dependent with a higher efficacy at 5% than 2% active ingredient. Minoxidil is currently sold as a topically applied drug under the trade name Rogaine.

Finasteride (see Fig. 1.18) a drug developed by Merck & Co. for treatment of benign prostate hypertrophy has been shown to inhibit the enzyme 5-alpha-reductase, and thus, to block the conversion of testosterone to the more active 5-alpha-dihydrotestosterone (DHT) [62, 63]. There are two forms of the enzyme 5-alpha-reductase, called Type I and Type II. Finasteride is capable of blocking only the Type II enzyme which is the predominate form of this enzyme in the hair

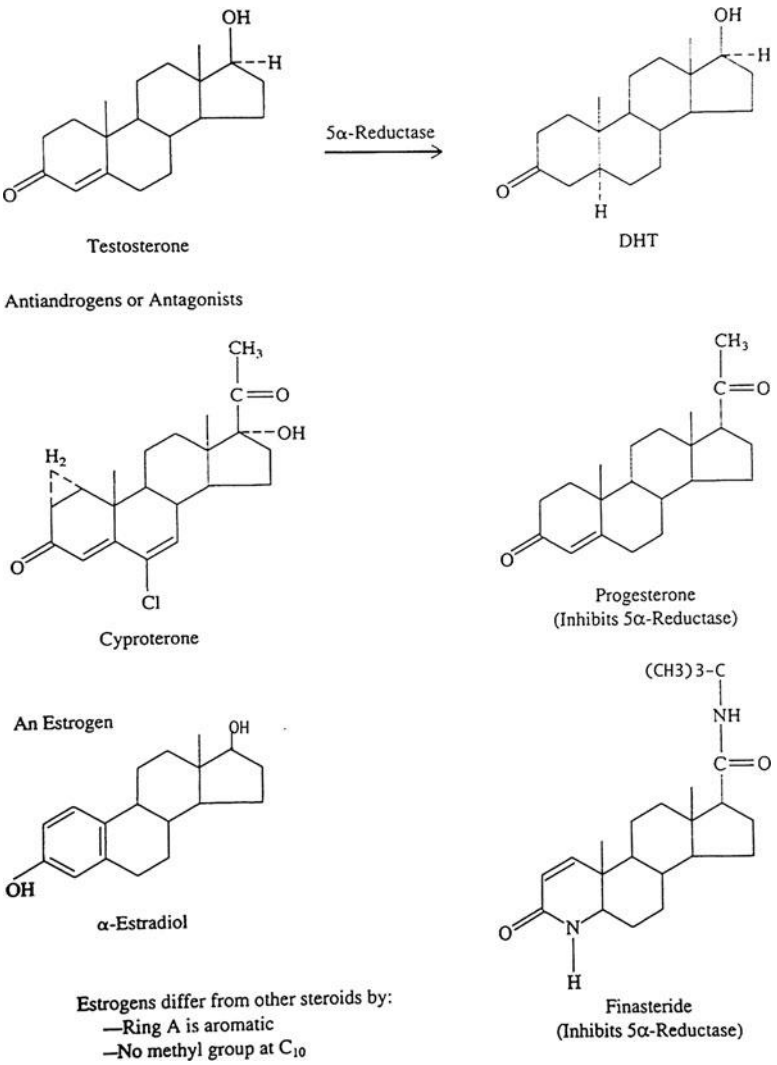

Fig. 1.18 Chemical structures of the active androgens, testosterone and dihydrotestosterone (*DHT*), some examples of antiandrogens, an estrogen and finasteride

follicles and the prostate gland. This action in hair follicles (partially blocks the conversion of testosterone to DHT) suppresses the androgen inhibition of hair synthesis in the hair follicle, thus extending the anagen period to provide longer and coarser hairs [64, 65]. Sung et al. [66] provided evidence that DHT up-regulates DKK-1 from dermal papilla cells thus causing apoptosis in keratinocytes and inhibits hair growth.

Propecia is the trade name for the hair treatment form of finasteride, sold in pill form and taken orally. It is recommended only for males because of potential

problems during pregnancy. Dutasteride (Avodart, a drug for benign prostate hyperplasia) from Glaxo has been touted as an effective hair growth agent that works similar to finasteride, but it has a longer half life (longer residence time in the body), is more active in inhibiting 5-alpha-reductase and more importantly it inhibits both Type I and Type II forms of the enzyme 5-alpha-reductase. Dutasteride is more effective in lowering DHT levels and appears to work faster in the treatment of baldness than finasteride. However, as of this writing the data is not clear that it is a more effective treatment for androgenetic alopecia.

Normal control of the anagen/telogen cycle by the action of androgens (such as the action of 5-alpha-reductase on testosterone), or by anti-androgens and the subsequent alteration of hairs to different sizes is summarized below, also see Fig. 1.11: Molecular signals (Wnt proteins and Sonic hedgehog) most likely from mesenchymal cells called dermal condensate are transported to stem cells in the bulge (Fig. 1.5) to initiate lower follicle formation and anagen [11–22, 67].

Oshima et al. [68] described that stem cells in the bulge move downward in the follicle and divide rapidly to form the follicle matrix. The follicle matrix then begins producing inner and outer root sheath cells and ultimately cuticle, cortex and medullary cells along with the other essential proteins and structures in the lower follicle, see Fig. 1.6.

Cell division continues at a rapid rate and differentiation occurs as the hair shaft cells move upward in the follicle. During various stages of growth, signaling molecules and metabolites are transported between the different cellular layers of Fig. 1.6 to the sites where they exert their activity.

The synthesis or the production of androgens by the adrenals and the ovaries or the testes occurs. These androgenic hormones are transported in the blood stream on carrier proteins such as sex hormone binding globulin (SHBG), to peripheral tissues such as the pilosebaceous apparatus. These hormones then dissociate from the binding proteins.

Testosterone is converted in the hair follicle to the more active hormone DHT.

Testosterone and DHT are transported into hair cells. These steroids act inside the keratinocytes to induce apoptosis most likely involving a receptor protein.

Thus, any agent or process that either enhances or interferes with any of these steps will lead to either greater or less production of longer more coarse hairs. Interference in the transport of androgenic hormones in the blood stream may result in terminal hairs being produced where vellus hairs are normally produced. For example, Barth [69] has shown that the step involving the transport of testosterone on carrier proteins occurs in hirsute women. This effect leads to a reduction in transport proteins (SHBG) and the concomitant increase in the free unbound testosterone level in the blood stream. Thus, the transport mechanism is interfered with and thick terminal hairs are produced in body regions where they are not normally produced.

Not only is the transport of testosterone important, transport of other androgens capable of being synthesized into testosterone is also important, because Sawaya et al. [70] demonsrated that the enzyme 3-beta-hydroxysteroid dehydrogenase which converts other androgens into testosterone (see Fig. 1.19) shows greater

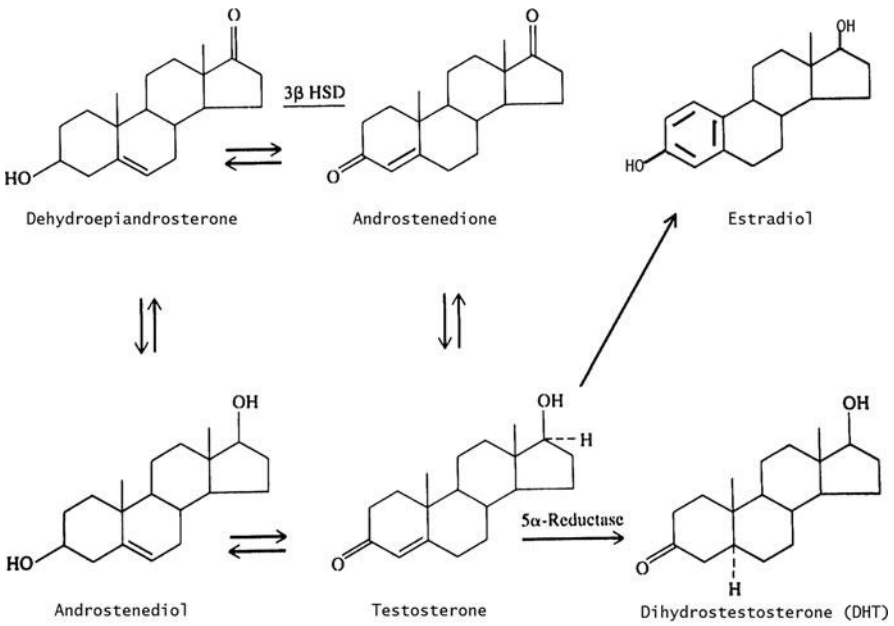

Fig. 1.19 The conversion of androgens to testosterone and DHT

activity in samples of balding scalp as compared to normal hairy scalp. In addition, balding men show increased activity of the enzyme 5-alpha-reductase in the pilosebaceous units and in the skin of the frontal scalp. On the other hand, Griffin et al. [71] concluded that men with a deficiency of this enzyme do not develop baldness. Also see the section entitled *Gene Therapy for Potential Treatments for Hair Loss* later in this chapter.

As indicated, androgens including testosterone are produced by the testes and the adrenal glands [72] and then transported to the sites of activity. Hair root cells contain androgen receptors; however, there is evidence that these receptors are intra-nuclear rather than intracellular, see King and Greene [73] and Welshons et al. [74]. In addition, Sawaya et al. [75] demonstrated a greater androgen binding capacity (DHT) in the nuclei of sebaceous glands taken from patients with bald scalps than from patients with normal hairy scalps. Thus DHT migrates into hair cells in the lower follicle and induces apoptosis to inhibit hair growth and shorten the anagen period.

Consistent with this finding is the one by Orentriech [76] that pilosebaceous units that grow thick terminal hairs when surgically transplanted to a region that is hairless will continue to grow thick terminal hairs. In some cases thick terminal hairs will begin to grow, sometimes in isolation, in regions that are normally hairless, such as the growth of facial hair in women, etc. In other words, the

response to the androgens are dependent on the specific pilosebaceous unit which is often, but not always regionally dependent. These findings suggest that specific pilosebaceous units are somehow programmed to respond to androgens in a way that either induce baldness or grow hair possibly by means of different receptor proteins or another mechanism.

Different receptor proteins for stimulating or retarding hair growth help to explain several apparently disparate facts. In the case of males, at puberty, thick terminal hairs begin to grow in the axilla, the mons pubis and the beard areas. This action occurs in spite of increased levels of testosterone and contrasts to what occurs later (at about age 20 in males) when increased levels of testosterone in the scalp help to cause male pattern baldness. Hamilton et al. [77] also demonstrated that eunuchs when injected intramuscularly with testosterone propionate exhibit an increased growth of coarse sternal hairs and yet, eunuchs when castrated before age 20 show even less growth of beard hair than eunuchs castrated after age 21 [78], see Table 1.15.

The first experiment involving testosterone injection, suggests that this androgen can somehow induce or promote hair growth in the skin of the thorax tissues, as opposed to the scalp, where this same hormone inhibits hair growth. The second experiment among eunuchs shows that a decrease in testosterone level in some body regions, such as the beard area in males decreases hair growth. Additionally, it demonstrates that removal of two of the glands that produce this hormone prior to the maturation of the local tissue (e.g., the scalp) responsible for hair growth, then hair growth will be further inhibited in that tissue. In other words, hair growth is dependent on the local tissue (most likely through specific receptor sites in the tissue) as well as the androgen level.

Epidermal growth factor receptors have been detected in the outer root sheath and in the epidermal papilla by Wollina and Knopf [79] and epidermal growth factors have been linked to the anagen to catagen transformation as shown by Philpott and Kealey [80]. Receptors for thyroid hormone have also been detected in keratinocytes by Mackenzie [81].

In addition to hormonal control and molecular signaling compounds, vitamins and retinoids, and mesenchymal components have been shown to help control the development of follicles and to maintain hair growth. These are fruitful areas of hair growth research. For entries into this literature, see the references by Alonso and Fuchs [12], Reddy et al. [67], Mackenzie [81], Hebert [82], Stenn [83] and Blumberg and Tonnic-Canic [84] and the following references [11–21].

Retinoids including vitamin A, retinol and retinoic acid play an important role in the growth and development of epithelial tissue. In excess, vitamin A and its

Table 1.15 Beard hair growth; before and after castration [78]

Group examined	Wt. Beard hair (mg/24 h) Ave. for ages 30–80
Normal controls	31.6
Castrated after age 21	13.7
Castrated before age 20	7.7

derivatives have been shown to inhibit keratinization [84]. This effect is likely related to DHT production. The sebaceous glands produce sebum that contains DHT. At too high a level, DHT inhibits hair growth and when used with minoxidil it has been shown to increase the effectiveness of the latter. This effect may be related to proper control of sebum production and DHT levels.

Vitamin D3, however, promotes keratinization [84]. On the other hand, there are no scientific studies on healthy subjects demonstrating the effects of dietary vitamins on hair growth. In the case of dietary insufficiency, there are indications that folic acid (a B-complex vitamin) and pyridoxine (a B-complex vitamin, B6) "may" be helpful to hair growth. Reis [85] described a role in cystine metabolism for these vitamins. Panthenol the precursor to pantothenic acid (another B-complex vitamin) has not been demonstrated to affect the growth or development of hair either in a dietary study or through topical application. Other materials known to either inhibit or promote hair growth are listed in Table 1.14. Eflornithine a chemotherapy drug known to retard hair growth has been explored in a joint venture with Gillette and Bristol-Myers Squibb as the active ingredient for a topically applied prescription product to help control facial hair in women.

To summarize hair growth, human scalp hair grows at an average rate of approximately 15 cm (about 6 in. per year). The life cycle of a hair fiber consists of three stages—anagen (growth stage), catagen (transition stage), and telogen (resting stage when the hair is shed). The life cycle of a hair fiber is initiated by chemical messengers that act on stem cells in the bulge. Wnt proteins, Sonic hedgehog and other regulators play a primary role in the anagen phase. Hair growth is partially controlled by androgens and the local tissue most likely through specific receptor sites. Testosterone and DHT are the primary androgens that determine whether hairs increase or decrease in size with age and some other aspects of hair growth and hair loss. During various stages of growth, signaling molecules and metabolites are transported between the different cell layers of Fig. 1.6 to the site where they activate the tissues. In spite of the fact that each follicular unit can function independently, the response by the local tissue tends to be a regional response and it determines whether hairs grow or whether the hair cycle is shortened and ultimately leads to baldness.

Differences in anagen can vary from a few months to up to 8 years or longer. For normal terminal scalp hairs, 2–6 years anagen is an average growth time, producing hairs approximately 1 m long (~3 ft) before shedding. Human hair generally grows in a mosaic pattern, thus, in any given area of the scalp, one finds hairs in various stages of their life cycle. In a normal healthy scalp, the vast majority of hairs are in anagen (about 80–90%); although there are seasonal changes in hair growth, with maximum shedding (telogen) as Autumn approaches (in the Northern Hemisphere, August/September). In all forms of hair loss, there is a more rapid turnover to telogen, thus a larger percentage of hairs are in telogen. In addition, vellus hairs, characterize baldness, although a small reduction in the number of follicles per unit area also occurs. For additional details regarding the biological syntheses and formation of human hair, see references [9–21, 26, 68–70, 75, 79–89]. Different treatments for hair loss are described in the next sections of this chapter.

1.6 Treatments for Hair Loss

Studies are currently underway to identify the genes involved in androgenetic alopecia, alopecia areata and alopecia totalis and alopecia universalis. As of late 2003, only one gene involved in androgenetic alopecia had been identified, that is the androgen receptor (AR) [90]. However, a lot of good work has been accomplished since that time. A summary of the science related to the role of genes and their products in relation to different types of baldness is described in Chap. 3 in the section entitled, *Some Other Hair Traits Related to Genetics*. If you are interested in this area, see references [90–93], the references in that section of Chap. 3 and read current works by the following scientists: Barahmani, N., Botchkarev, V., Brent-Richards, J., Christiano, A., Cotsarelis, G., Cox, G., Dawber, R.P.R., Duvic, M., Ellis, J.A., Fuchs, E., Harrap, S., Hillmer, A.M., Lui, H., Rogers, G., Rothnagel, J.A., Sawaya, M., Shapiro, J., Sinclair, R., Sundberg, J., Tang, L. and Whiting, D.A.

1.6.1 Surgical Treatment of Hair Loss

Several surgical procedures have been used for treatment of hair loss. Although these procedures may be used for most forms of alopecia, they are used primarily for treatment of androgenetic alopecia or for hair loss due to tissue injury such as burns, particularly in cases where extensive baldness exists. These procedures are based on the fact that hairs actively growing in one region of the scalp, such as the occipital region when moved with local tissue to a bald region will continue to grow as they did in the occipital region. These procedures confirm the role of local tissue control in the hair growth process. Current surgical treatments include:

- Hair transplantation
- Scalp reduction
- Transposition flap
- Soft tissue expansion

In the most common form of hair transplantation, small skin plugs containing 15–20 growing terminal hairs each are surgically removed and placed into a smaller cylindrical hole in the balding region of the scalp. Usually several sessions of transplantation are required involving the placement of 50 or more plugs per session. The placement or angling of the plugs is important to the end cosmetic effect. Elliptical grafts or even smaller mini-grafts may be employed and have been described by Shiell and Norwood [94]. Within 2–4 weeks after transplantation, the donor hairs usually fall out and are replaced by new hairs.

Lasers have been introduced into hair transplantation providing several advantages. Erbium and CO_2 lasers have been used. More advantages have been shown by the erbium laser. It allows for a smaller graft and offers the potential to create closer sites for more aesthetic results. Both techniques provide virtually

bloodless surgery and reduce operating times compared with conventional techniques. A diode laser was cleared by the FDA in 1998 and is currently being used for hair removal. This laser functions with 800 nm light and has a cooling device for patient comfort. It provides safe and effective hair removal with virtually no scarring and a decrease in the delay for hair re-growth.

Oftentimes, in cases where the bald area is rather large, scalp reduction is done in conjunction with hair transplantation. This method involves surgical excision of a strip of the bald skin to reduce the total hairless area. Repeated scalp reductions can be performed together with transplantation to provide better coverage for a very bald person.

The transposition flap method [95] involves moving a flap of skin that contains a dense area of hair to a bald area. This method is sometimes employed together with mini-graft implantation along the frontal hairline to provide a more natural appearance.

Soft tissue expansion is another surgical development for treatment of alopecia. In this procedure, soft silicone bags are inserted under the skin in the hair-bearing area of the scalp, usually in the occipital region. The bags are then slowly filled with salt water during a 2–4 month time period. After expansion of the hair-bearing skin, the bags are removed and the bald area of the scalp is excised and flaps are created with the expanded hair-bearing skin.

1.6.2 Hair Multiplication or Hair Induction Treatments for Hair Loss

Some novel and highly technical treatments for hair loss are being explored with encouraging but modest success. Some have referred to these treatments as cloning; however, cloning involves the production of a genetically identical organism. Cloning is clearly not what is being done for the growth of hair fibers (not an organism) on scalps. One successful technique described by Reynolds and Jahoda [96] was first called Trans-gender induction of hair follicles. This method has also been referred to as hair follicle cell transplantation. In this procedure portions of specific cellular structures such as dermal sheath cells are micro-surgically removed from actively growing hairs and injected into the skin of another person. The implanted cells act to promote the formation of new intact hairs. In the case described by Reynolds and Jahoda [96], the donor cells were from a male and implanted into a female, thus the name “transgender” induction. It would appear that some variation on this procedure offers potential as a treatment for hair loss. But, this procedure is only a laboratory curiosity and several steps are necessary to determine if this type of treatment can be brought to fruition. Unger [97] described current concepts, techniques and the future of transplantation in a paper published in the Journal of Investigative Dermatology.

1.6.3 Hair Extensions or Hair Weaves

This technique of adding or attaching hair to your own hair to provide different styles or looks has become popular. Hair extensions originated and were first made popular among African American women such as Janet Jackson, however women of all races today are taking advantage of the styles and looks that weaves provide, if they can afford it. There are several basic techniques for applying hair extensions:

The fusion method sometimes called infusion: This method involves gluing individual hairs; strand by strand or micro-braids to the subject's own strands of hair. Hair strands can be purchased with pre-applied adhesive that must be heated or glue sticks that require heating with a device similar to a glue gun to activate the adhesive. Infusion is the most expensive hair extension method because it takes 8–16 h to manually complete. This process lasts several months allowing contact with water such as shampooing once a week and even swimming.

Bonding involves gluing large strips of hair sometimes called wefts to the roots of the subject's own hair. Bonding glue and remover are sold along with wefts for this process. Bonded hair should not be left in place longer than 1 or 2 weeks because of stress on the roots.

Weaving is a process where a corn row or a track is created around the head with the subject's own hair. Extension hair is then sewn or woven onto the tracks and the subject's own hair lays over the tracks for a natural look.

Netting is where natural hair tresses are braided or woven onto a thin breathable net that fits onto the scalp. Netting can last up to 3 months, however care must be taken to dry the subjects own hair to avoid mildewing.

Because hair extensions provide for a natural look and optional styles they have become popular among female entertainers such as Beyonce, Janet Jackson, Britney Spears, Paris Hilton, Jessica Simpson and many others.

1.7 The Cuticle, Cortex, Medulla and Cell Membrane Complex

1.7.1 The Cuticle

The cuticle is a chemically resistant region surrounding the cortex in animal hair fibers (see Figs. 1.3, 1.7 and 1.8). Geiger [98] described its chemical resistance in the following manner. When isolated cuticle material and whole wool fiber are completely reduced and alkylated, the alkali solubility [99] of the cuticle material is approximately one-half that of whole fiber (85%). Cuticle cells are generally isolated from keratin fibers by shaking in formic acid [100], by enzymatic digestion [98, 101, 102], or by shaking in water [103]. Atsuta et al. [104] successfully applied the method of Taki for removing cuticle cells from wool fiber to remove cuticle

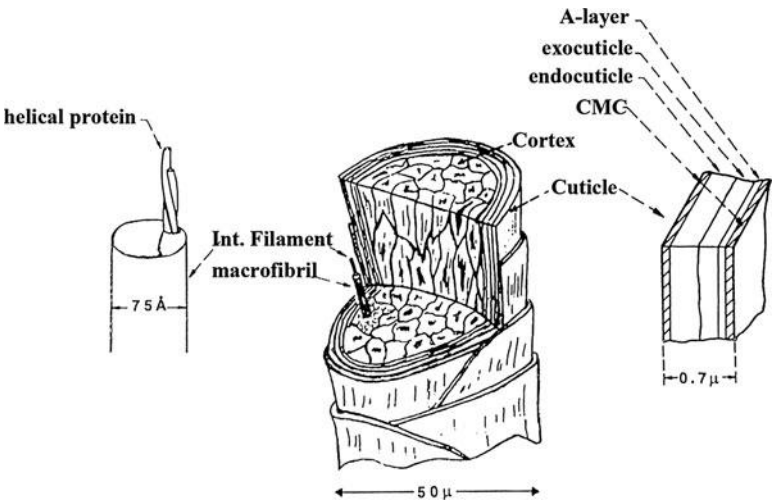

Fig. 1.20 Stereogram of the hair fiber structure, illustrating substructures of the cuticle and the cortex

from human hair fibers. This method involves shaking hair fibers for several hours with 5–6% potassium hydroxide in 1-butanol.

The cuticle consists of flat overlapping cells (scales) that surround the central fiber core (Figs. 1.8, 1.20, and 1.21). The cuticle cells are attached at the proximal end (root end), and they point toward the distal end (tip end) of the hair fiber, like shingles on a roof. The shape and orientation of the cuticle cells are responsible for the differential friction effect in hair (see Chap. 9). Each cuticle cell is approximately 0.5 μm thick, with about a 6–7 μm exposed axial surface or scale interval, and approximately 45–60 μm long. A comprehensive study by Takahashi et al. [105] on about 200 Asians and 200 Caucasians showed an average scale thickness of 0.45 μm for Asians and 0.43 μm for Caucasians, a surface cuticle interval of 6.61 μm for Asians and 6.98 μm for Caucasians within 1 cm of the scalp. The scale interval is in agreement with earlier data by Hardy [91] showing slightly more scales per 0.52 mm for Asians (15.47) vs. Caucasians (15.07) and more scales per unit length for people of East African descent (17.92). See the section in Chap. 9 entitled *Scale Type of Mammalian Hairs is Related to Hair Fiber Diameter*.

The cuticle in human scalp hair is generally 6–8 scales thick for Asians and Caucasians with slightly more scales in Asians as shown in a study by Hardy [106] and Takahashi [105]. Other studies with fewer subjects show variation from about 5 to 10 cuticle layers [100, 101]. The schematic of Fig. 1.22 by Alan Swift [107] illustrates similar dimensions and the layering of the cuticle. Woods and Orwin [108] describe the formation of the single layer of overlapping scales in most wool fibers that is sometimes described as 1–2 scales thick. The number of scale layers can serve as a clue to the species of origin in forensic studies.

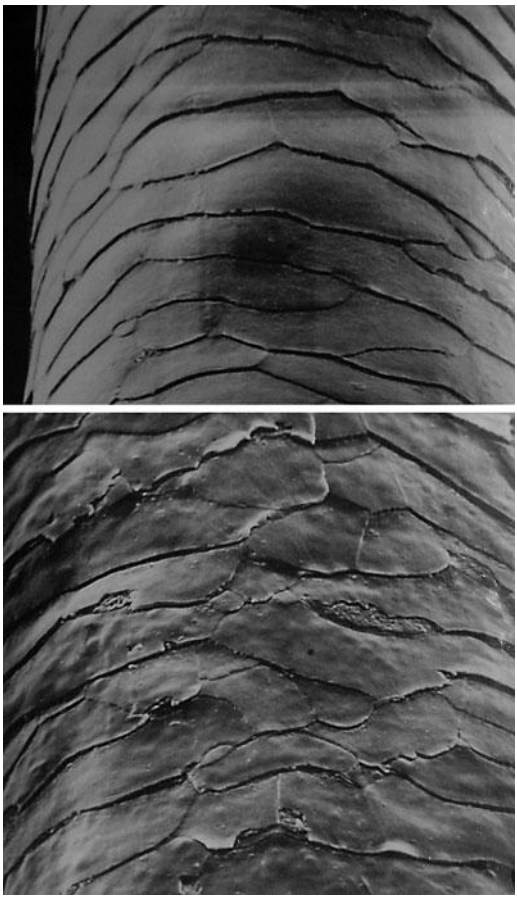

Fig. 1.21 Cuticle scales of human hair. *Top*: Near root end and close to the scalp. Note smooth scale edges. *Bottom*: Near tip end of another fiber. Note worn and broken scale edges

The cuticle of virgin human hair contains smooth unbroken scale edges at the root or proximal end near the scalp (see Fig. 1.21). Cuticle damage evidenced by broken scale edges can usually be observed a few centimeters away from the scalp. Such damage is caused by weathering and mechanical damage from the effects of normal grooming actions, such as combing, brushing and shampooing (Figs. 1.21 and 1.23). In many long hair fibers (25 cm or longer), progressive surface damage may be observed (illustrated by Fig. 1.23). Stage 1 shows intact smooth scale edges and scale surfaces; stage 2 contains broken scale edges; in stage 3, the scales have been partially removed, and in stage 4 the hair splits indicating extensive cortical damage. Garcia et al. described this phenomenon of hair degradation in some detail [109]. See Chap. 6 for additional information and references on this important phenomenon.

The cuticle of both human hair and wool fiber has been shown to contain a higher percentage of cystine than whole fiber [110] and more of the other amino acids that are generally not found in alpha-helical polypeptides [111]. Analysis of the cuticle

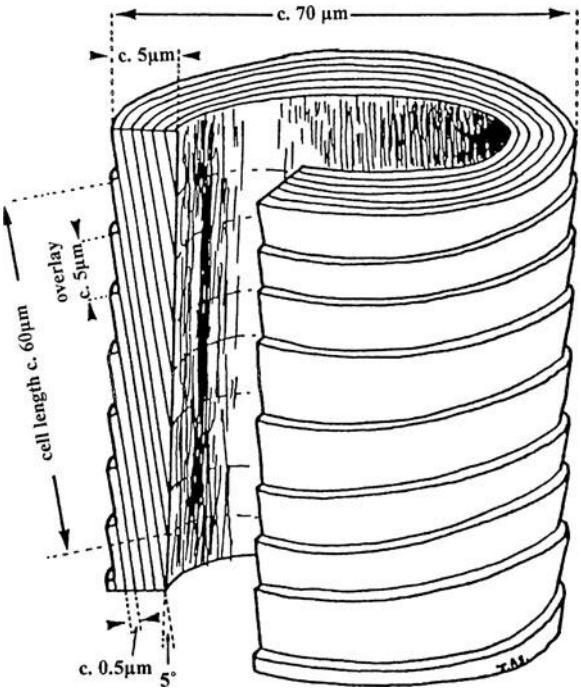

Fig. 1.22 A schematic diagram of the human hair cuticle illustrating its dimensions and layering, by J. A. Swift [107] (Reprinted with permission of the Journal of Cosmetic Science)

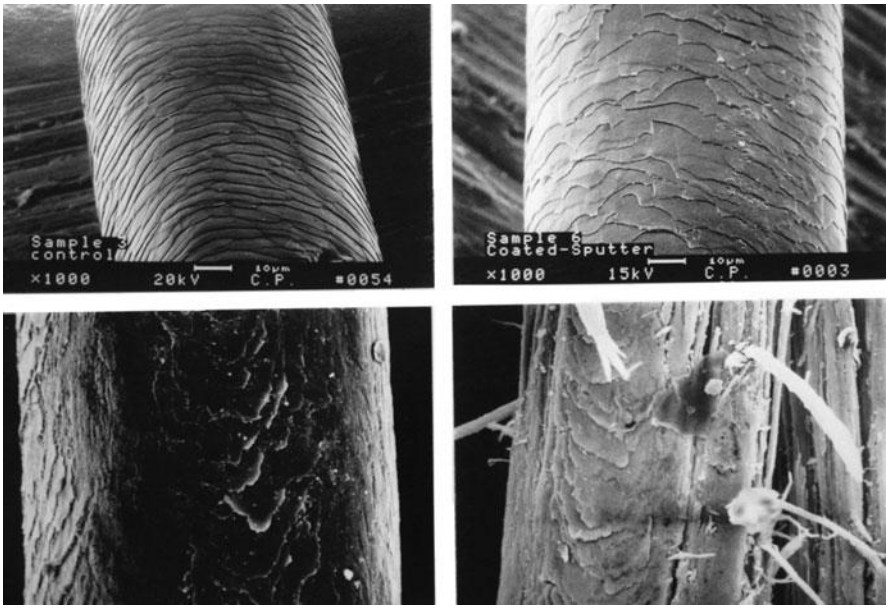

Fig. 1.23 Weathering and cuticle wear. *Top left*: Stage 1, note smooth cuticle edges. *Top right*: Stage 2, note broken cuticle scale edges. *Bottom left*: Stage 3, note complete removal of cuticle (*central area*). *Bottom right*: Stage 4, split hair

of wool fiber by the polarizing microscope shows negligible birefringence [1]. The cuticle of human hair also demonstrates negligible birefringence. Astbury and Street [112] provided x-ray evidence confirming that, in contrast to the cortex; the cuticle of hairs does not contain crystalline domains and as such is not as highly organized at the molecular level as the cortex.

1.7.1.1 The Different Layers of the Cuticle

The schematic diagram of Fig. 1.24 illustrates the internal structure of cuticle cells. The uppermost structure of each cuticle cell contains a thin proteinaceous membrane, the epicuticle that is covered with a lipid layer that includes 18-methyl eicosanoic acid. Different estimates of the thickness of this lipo-protein membrane have been cited [113, 114]; however, 10–14 nm by Swift and Smith [115] is probably the best current estimate. See the section entitled the *Cell Membrane Complex including the Intercellular Matter and the Nonkeratin Regions of Hair* in this chapter and Chap. 2 on this same subject. See also the schematics in that same section in this chapter and the section in Chap. 6 entitled *The Hair Fiber Surface*.

Beneath the cuticle cell membranes are three major layers; the A layer, a highly cross linked resistant layer about 50–100 nm thick (see Fig. 1.24). The A layer contains a high cystine content (>30%) and additional cross links called isopeptide bonds found by Zahn et al. [116]. Isopeptide bonds are created by reaction of glutamine with lysine under the influence of a transglutaminase enzyme. The exocuticle, sometimes called the B layer, is beneath the A-layer. It is also rich in cystine (~15–20%) and highly variable in thickness in each cuticle cell averaging about 150 nm. Underneath the exocuticle is the endocuticle, low in cystine content (~3%) [107] and also highly variable in thickness from about 50 to 300 nm within each cuticle scale [107] (see Fig. 1.24). Figure 1.25 is a transmission electron

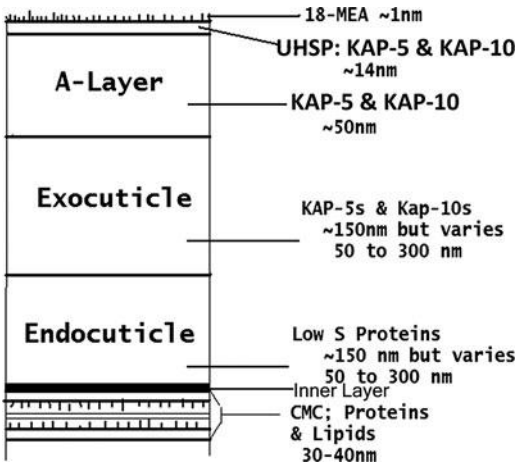

Fig. 1.24 Schematic diagram of the proposed structure of a cuticle cell in cross section

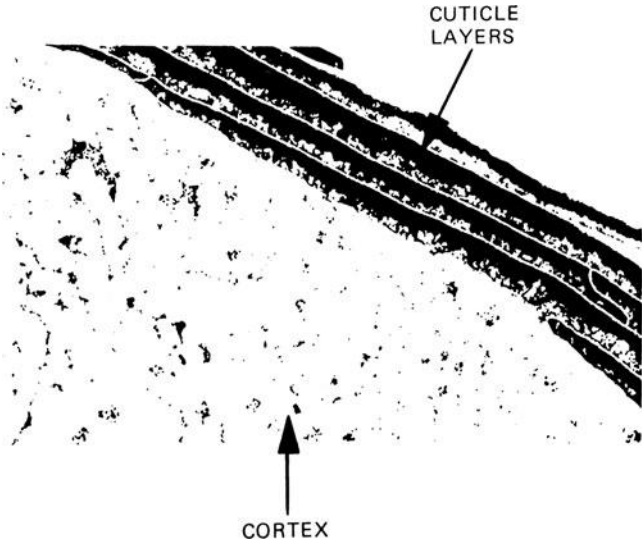

Fig. 1.25 TEM of a cross section of a hair fiber treated with silver methenamine, illustrating high and low sulfur layers of cuticle cells (stained = high-sulfur regions) (Kindly provided by R. Wickett and B. Barman)

micrograph illustrating the high sulfur (cystine) and the low sulfur regions of these layers through a staining reaction with silver methenamine. This stain marker stains the high sulfur regions of the cuticle cells, that is the epicuticle, the A-layer and the exocuticle. For additional details on the chemical composition of these three important layers of the cuticle, see Swift and Bews [117] and Chap. 2.

A portion of the under-membrane of Fig. 1.24 is also epicuticle or "epicuticle-like" matter. The cystine rich proteins of the cuticle are either high sulfur or ultra high sulfur proteins. Structurally different, high sulfur and ultra high sulfur proteins are found in the cortex. See the section entitled *Major Protein Fractions of Hair* in Chap. 2.

The cuticle of human hair is a laminar structure similar to the cuticle of wool fiber. Details of the different layers of the cuticle have been described for merino wool and for human hair in these references [109, 118–121]. Figure 1.24 illustrates the laminar structure of each cuticle cell. Figure 1.26 illustrates the "initial" view of the laminar structure of the cell membrane complex of the cuticle (cuticle-cuticle cell membrane complex) and Fig. 1.27 illustrates the cuticle structure relative to the whole fiber. The cell membrane complex and endocuticle represent vulnerable regions to the chemical and physical interactions of permanent waves, bleaches (including permanent dyes) and to everyday grooming actions. See Chap. 6 for a more complete discussion of this subject. Chap. 2 contains a more complete description of the amino acid and protein compositions of the cuticle and its different component parts.

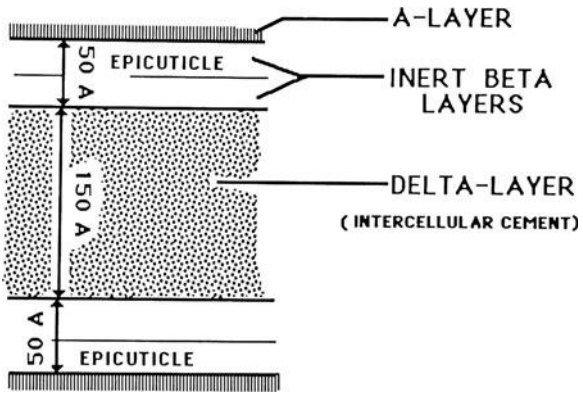

Fig. 1.26 Schematic illustrating cell membrane complex in animal hairs (Adapted from Fraser et al. [124])

1.7.1.2 Epicuticle and the Hair Fiber Surface

Negri et al. [122] demonstrated that the outer surface of hair fibers consists of about 75% of a heavily cross-linked protein and about 25% fatty acid that is predominantly 18-methyl eicosanoic acid. These authors proposed a model wherein the fatty acid layer (lipid layer) is connected to the underlying fibrous protein layer through thioester linkages involving the cysteine residues of the underlying epicuticle proteins [122, 123], see Fig. 1.28.

Negri et al. [123] also demonstrated that alcoholic alkali and chlorine treatments remove the fatty acid layer from the cuticle. These scientists concluded that the attachment of 18-methyl eicosanoic acid is through thioester linkages because chlorine water should not remove this lipid layer if it were attached through an ester or amide linkage; however chlorine water will readily cleave thioester bonds. Related layers exist between cortical cells that are unstained with protein stains, but these are removed by soxhlet extraction with chloroform-methanol. Extraction with this lipid solvent suggests that covalent attachment is not involved in the complex lipid layers between cortical cells [123]. See the section in this chapter entitled, the *Cell Membrane Complex including the Intercellular Nonkeratin Regions of Hair* and Chap. 2 on this same subject for a more complete description of the cell membrane complex.

So, the surface of mammalian hairs is covered with a thin covalently bound lipid layer of 18-methyl eicosanoic acid that is bonded to a proteinaceous cell membrane called epicuticle [124]. Jones and Rivett [125] concluded that Sims and XPS "indicate the surface of wool fibers is almost exclusively hydrocarbon" consisting of 18-methyl eicosanoic acid and free lipids (see Chap. 2 for details). This protein membrane is approximately 13 nm thick [114] (see Figs. 1.24, 1.25, 1.26, 1.27). In Fig. 1.24, the surface cell membrane consists of the epicuticle (proteins) and the 18-methyl eicosanoic acid which is sometimes called the upper or outer Beta layer. Since the attachment of 18-methyl eicosanoic acid to hair is through thioester linkages and the cell membrane protein is cross linked by cystine linkages, the methyl eicosanoic acid must be attached to an ultrahigh sulfur protein.

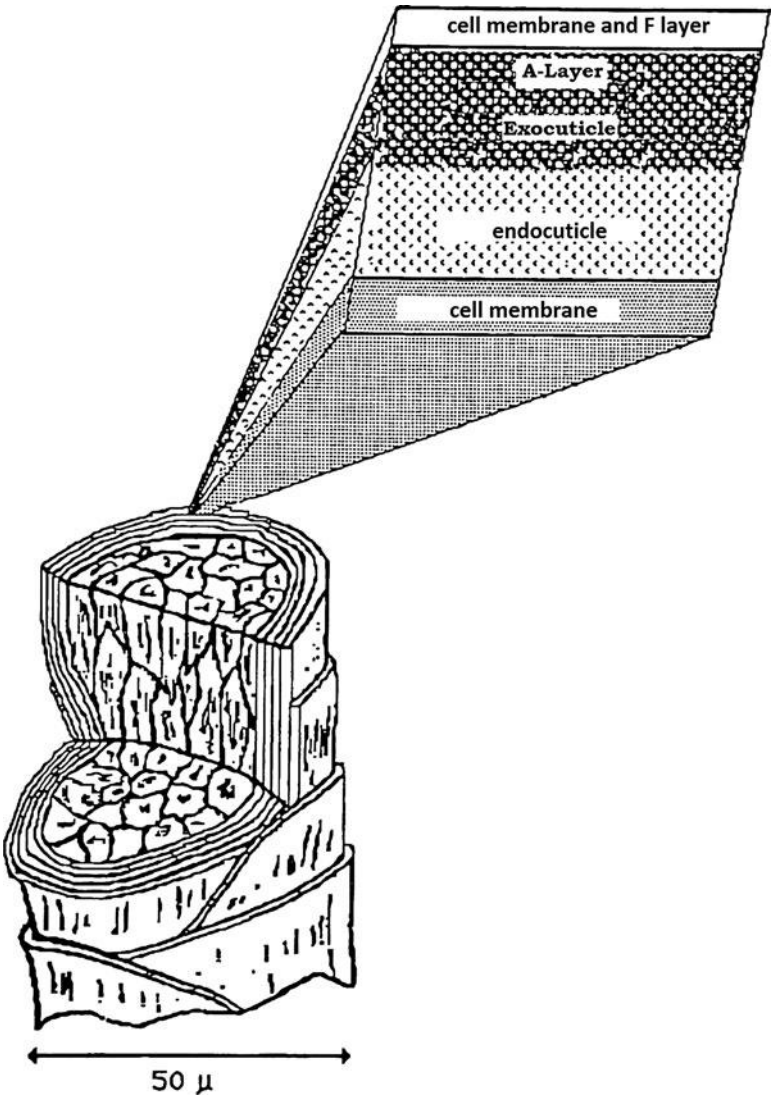

Fig. 1.27 Schematic diagram illustrating cuticle layers with respect to the whole fiber

As hair is exposed to repeated washing, drying and rubbing actions and to sunlight, changes occur in these surface layers leading to the formation of sulfur compounds and acids such as mercaptan, sulfinate and sulfonate groups. These actions lead to a decrease in the free and bound lipid content of the surface thereby converting the virgin hair surface from a hydrophobic, entity with little surface charge to a more hydrophilic, more polar and more negatively charged surface, see Chap. 2 and the discussion in Chap. 6 for more details.

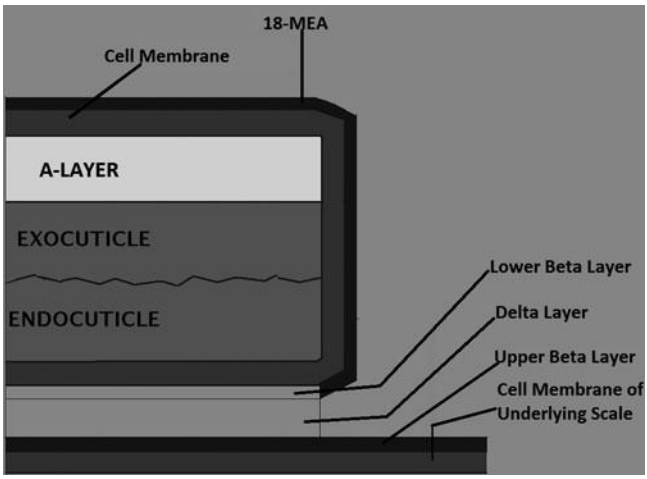

Fig. 1.28 Schematic illustrating lipid structures in the surface and CMC of the cuticle of hair

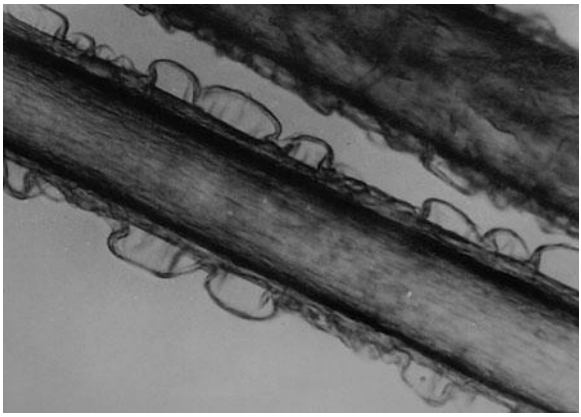

Fig. 1.29 Allworden sacs formed at the surface of hair fibers during reaction with chlorine water

More than 90 years ago, Allworden [126] observed that sacs or bubbles form at the surface of the fibers during treatment with chlorine water (see Fig. 1.29). Chlorine water diffuses into cuticle cells and attacks thioester linkages removing the lipid layer from the hair surface. It further degrades the proteins beneath the epicuticle by attacking the disulfide bonds cleaving the protein cross links and oxidizing them to higher sulfur acids producing water-soluble species too bulky to diffuse out of the semipermeable membrane. Swelling then results, due to osmotic forces, producing the characteristic Allworden sacs [122, 127].

After removal of the surface lipids, wool fiber still undergoes the Allworden reaction [122]. This fact confirms that these surface lipids are attached to the cell membranes of the cuticle, and furthermore their removal alters but does not destroy

the cuticle cell membranes. At this stage the primary integrity of the membrane is most likely due to the resistant isopeptide cross-links in the cuticle cell membranes.

Leeder et al. [128] provided evidence that the cell membrane lipids of wool fiber do not consist of phospholipids that normally form bilayers in living tissue. It was suggested at one time that the epicuticle is a continuous membrane covering only the very fiber surface [129]. However, Leeder and Bradbury [100] isolated single cuticle cells from wool fiber and demonstrated that single cuticle cells undergo the Allworden reaction, thus proving that this membrane surrounds each cuticle scale. What has been described as a continuous epicuticle may be cell membrane complex, consisting of epicuticle and intercellular binding material that could produce the appearance of a continuous sheath.

Of the methods described for isolation of epicuticle, first and foremost is the method by Lindberg et al. [129]. This method involves treatment of intact fibers with chlorine water or bromine water followed by neutralization and shaking and is a modification of the Allworden reaction. Another method, by Langermalm and Philip [113] involves dissolving the bulk of the fiber from the membrane with dilute sodium sulfide. Neither of these procedures produces pure epicuticle, but they probably provide cell membrane material and part of the underlying A-layer.

Swift and Holmes [114] described a relatively nondestructive method involving extraction with hot ethanol for removing some epicuticle material from human hair fibers. These same scientists concluded that the epicuticle of hair contains both lipid and fibrous protein layers, and is cell membrane material but does not have sufficient contrast with its surroundings to allow microscopic identification. Hair fibers, when extracted extensively with hot ethanol, are less resistant to enzymatic degradation than ether-extracted hair and do not undergo the characteristic Allworden reaction with chlorine water. It has been suggested therefore that extraction of hair with hot ethanol removes either a portion of or degrades the epicuticle membrane sufficiently to prevent the Allworden reaction from occurring. Chemical analysis of epicuticle-like substance removed from hair by hot ethanol extraction shows that both protein and fatty acids are present (20–30%) [130, 131]; qualitatively similar results have been reported by Leeder and Bradbury for analysis of epicuticle isolated from merino wool [100].

Allworden membranes have been isolated and analyzed quantitatively for amino acid content by Allen et al. [132] who found approximately 21% half-cystine in these isolated membranes. Additional details of the amino acids and their quantities found by Allen et al. and by others are described in Chap. 2.

Zahn et al. [133] used Allen's amino acid data for Allworden membranes and known compositions of loricrin, involucrin and an ultra high sulfur protein in a multiple regression analysis. This statistical procedure provided indirect evidence for 51% ultra high sulfur protein, 42% loricrin and 7% involucrin in Allworden membranes. Zahn et al. suggested the possibility of a relationship between the membranes of hair and the cellular envelope of skin which also contains loricrin and involucrin. But, more recent evidence by Rogers and Koike [134] rules out loricrin and involucrin in the epicuticle suggesting strongly that 18-methyl

eicosanoic acid is attached to an ultrahigh sulfur protein most likely of the KAP 5 or 10 types which are cross-linked via cystine and also by isopeptide bonds.

Leeder et al. [135] defined the epicuticle as a chemically resistant proteinaceous membrane that remains on keratin fiber surfaces after strongly bound lipids have been removed with potassium t-butoxide in anhydrous butanol. Thus, the epicuticle is a proteinaceous layer about 130 Å thick covered by a strongly bound structural lipid that Leeder called the F layer (18-methyl eicosanoic acid). The F layer is not a frequently used term today, but it represents the outermost lipid layer of the fiber surface.

Several different laboratories have analyzed the outer surface of wool and human hair via XPS examining the outer 2–3 nm of the hair surface [136–139]. Ward et al. [136] estimated the thickness of the lipid layer of 18-methyl eicosanoic acid at 1 ± 0.5 nm. From Carbon/Nitrogen analysis, assuming XPS examines the top 3 nm, Carr et al. [139] estimated 60% protein and 40% lipid in the top 3 nm of soxhlet extracted wool. This estimate provides for 36% 18-methyl eicosanoic acid (at 1.1 nm thick) and 4% Free Lipid in the top 3 nm of this wool sample. A similar estimate using data of Robbins and Bahl [137] provided 12% free lipid remaining after shampooing human hair. These data suggest that free lipid is an integral part of the surface of hair (most likely between 18-methyl eicosanoic acid molecules) after and between normal shampooing and hair treatments. See Chap. 2 for additional details.

Capablanca and Watt [140] examined wool fiber that had been washed with detergent and extracted with various solvents using a streaming potential method. These scientists found an appreciable effect of free lipid on the isoelectric point with surfactant washed wool having an isoelectric of 3.3 while the most effective lipid solvent extracted wool provided an isoelectric of 4.5. These data show that the true isoelectric point of the surface hair proteins is close to 4.5. Furthermore, free lipid which contains fatty acids is an important and essential component of the surface of animal hairs, especially for hair in good condition that has only been cleaned with shampoos. Furthermore, the more free lipid (fatty acid) in the surface layers, the lower the isoelectric point of the fibers.

We know that the surface of hair contains 18-methyl eicosanoic acid attached to a fibrous ultra high sulfur protein and the source of this surface is the cuticle-cuticle cell membrane complex. Furthermore, we know that the thickness of the upper Beta layer in the cuticle-cuticle cell membrane complex is about 3 nm [141–143]. However, the thickness of the lipid layer on the surface of wool fiber measured on exhaustively extracted hair by Ward et al. [136] using XPS was about 1 nm. Zahn et al. [116] proposed a model to explain this smaller than expected thickness of the MEA in which the surface chains of MEA fold back on themselves. Recently, Natarajan and Robbins [144] through computer modeling calculated an MEA layer on a KAP-5 ultra high sulfur protein backbone to be 1.08 nm thick in excellent agreement with the calculations by Ward et al. [136].

XPS shows that shampooed hair and scoured wool contain more lipid at the surface than can be accounted for by MEA alone. Furthermore, wool extracted by different solvents provides different isolectric points suggesting that free fatty

acids/lipids are in the top 3 nm of the hair surface. Therefore it is reasonable to conclude that in the cell membrane complex of the cuticle and in the virgin most surface there is free fatty acid in between MEA molecules causing it to stretch out to approach its full length of about 2.75 nm at an angle of 72° and with additional assumptions provides a lipid layer thickness close to 3 nm, see the section in this chapter entitled *Thickness of the Cuticle Beta Layers*. This value is in agreement with the thickness of the upper Beta layer [141, 142] that ultimately becomes the major part of the new hair fiber surface when hair is deformed and abraded in the dry state [107].

1.7.2 The Cortex, its Cells, Macrofibrils, Matrix and Intermediate Filaments

The cortex constitutes the major part of the fiber mass (70–90%, the lower percentage in fine hair) of human hair and consists of cells and intercellular binding material. The intercellular binding material or the cell membrane complex is described later in this chapter.

1.7.2.1 Cortical Cells

Randebrock [2] found that cortical cells of human hair fibers are generally 1–6 μm thick and approximately 50–100 μm long (see Figs. 1.20 and 1.30), although considerable variation in their size and shape has been reported. Figure 1.30 is an SEM of a split hair with separated cortical cells appearing like splintered wood. Figure 1.31 is a high magnification image of the same split hair illustrating the macrofibrillar structures inside cortical cells. Straight to wavy Caucasian hair contains a more symmetrical cortex, like straight mohair fiber, and most (but not all) of the cells are of the same general type with regard to the ratio of fibrillar to nonfibrillar matter (highly crystalline = fibrillar; less organized = nonfibrillar).

Many wool fibers contain two or even three types of cortical cells that are sometimes segregated into distinct regions (Fig. 1.32) that can be observed in cross section [145]. These cell types are called orthocortex, paracortex, and mesocortex. Orthocortical cells contain less matrix material between the intermediate filaments and lower sulfur content (~3%). Kassenbeck [146] indicated that paracortical cells are smaller in diameter, and they have smooth and rounded borders and higher sulfur content (~5%) [146]. Mesocortical cells contain intermediate cystine content [147].

Morphologically, the cortical cells of human scalp hair of Caucasians are similar but not identical to those of wool fiber. Kassenbeck [146] determined that cortical cells adjacent to the cuticle in human hair are more flat and contain less sulfur than the remaining cortical cells that comprise the bulk of the cortex. Kassenbeck calls

Fig. 1.30 SEM of a split hair. Note cortical cell fragments

these heterotype cortical cells. Leon [148] several years ago noted in his review on hair that "Negro" hair contains a higher proportion of orthocortex cells than Caucasian hair. Swift [149] more recently provided evidence on a limited sampling of Nigerian hair for a higher percentage of orthocortical type cells (roughly 50/50 para to orthocortex) than in straight hair of Caucasians which he classified as predominately paracortex with a small arc (about 1 cell thick) of orthocortex at the periphery somewhat similar to Kassenbeck's description of Caucasian hair.

Thibaut et al. [150] and Bryson et al. [151] investigated the different types of cortical cells and their structures in more detail and identified different distributions of different cell types for straight vs. curly hair. Their findings are summarized in this chapter in the section entitled *The Origin of Hair Fiber Curvature*.

Kassenbeck [146] suggested that the biological function of crimped animal hairs is to trap large volumes of air in the hair coat to provide thermal insulation. For animals with both summer and winter fur: Summer fur—begins to grow rapidly in the spring, producing long and coarse hairs that are less crimped to inhibit the formation of air pockets and to permit cooling.

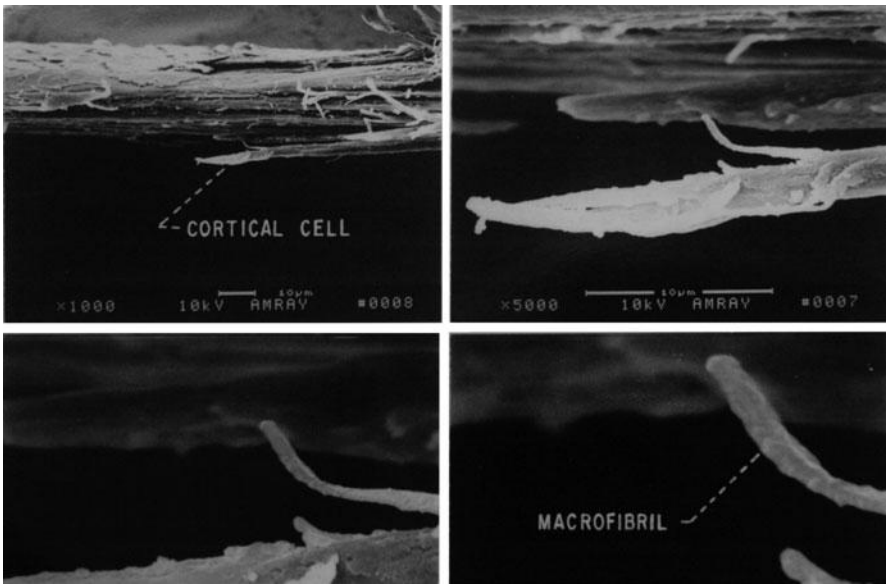

Fig. 1.31 Scanning electron micrograph of a split hair showing details of cortical structure

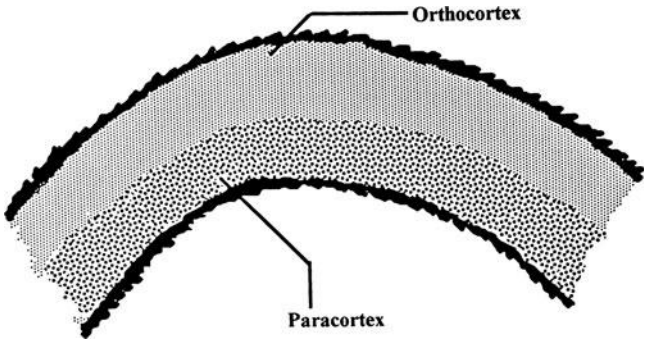

Fig. 1.32 Schematic of a wool fiber, illustrating orthocortex and paracortex regions of the cortex in relation to crimp

Winter fur—begins to grow in the autumn, yielding short, stiff, crimped hairs to trap large volumes of air in the coat for thermal insulation. Perhaps the seasonal effect on anagen/telogen ratios for human scalp hair is related to the summer/winter effects on hair growth in fur bearing animals.

Kassenbeck [146] further explained that the growth rate of animal hair and the morphological structures of both cuticle and cortex are relevant to the hair shape and to the cooling and insulation functions. Cortical cells also contain pigment granules and nuclear remnants. The nuclear remnants are small, elongated cavities near the center of the cells. The pigment granules are small, oval or spherical

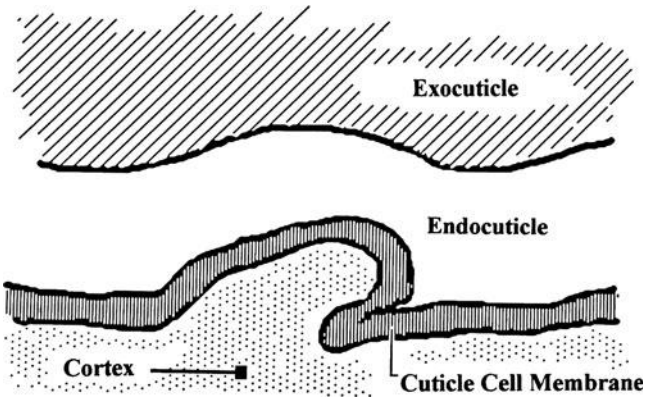

Fig. 1.33 Schematic illustrating Piper's interlocking scheme for linking cuticle to cortex

particles of approximately 2,000–8,000 Å units (0.2–0.8 μm) in "diameter" [152] that are dispersed throughout the cortical and medullary cells. Both these structures comprise only a small fraction of the cortex. Generally, pigment granules do not occur in the cuticle of scalp hair; however, pigment granules have been observed in the cuticle and the medulla of beard hair, especially in heavily pigmented hair [5].

Birbeck and Mercer [153] suggested that pigment granules enter the cortical cells by a phagocytosis mechanism in the zone of differentiation and biological synthesis. Piper [154] presented evidence that cortical cells are linked to adjacent cuticle cells via complex interlocking structures occurring through a mechanism involving phagocytosis (see Fig. 1.33).

Cortical cells may be isolated from human hair by procedures involving either shaking in formic acid [100, 155], or other solvents (Erhardt H, Private communication), or enzymatic digestion [98, 101, 102]. Another procedure involves shaking hair fibers in water in the presence of glass beads by Wortmann [103] to strip the cuticle cells from the hair to provide cortex with intact cell membranes free of cuticle. In addition to nuclear remnants and pigment granules, the cortical cells of human hair contain highly important spindle-shaped fibrous structures called macrofibrils or macrofilaments (see Figs. 1.20, 1.31 and 1.34).

1.7.2.2 Macrofibrils

Randebrock [2] followed up on the pioneering studies of George Rogers on wool and other hair fibers and found that the spindle-shaped macrofibrils in human hair are approximately 0.1–0.4 μm in width or diameter. The macrofibrils comprise a major portion of the cortical cells (see Figs. 1.34 and 1.35). Each macrofibril consists of intermediate filaments originally called microfibrils (highly organized fibrillar units) in a matrix, a less organized structure that surrounds the intermediate

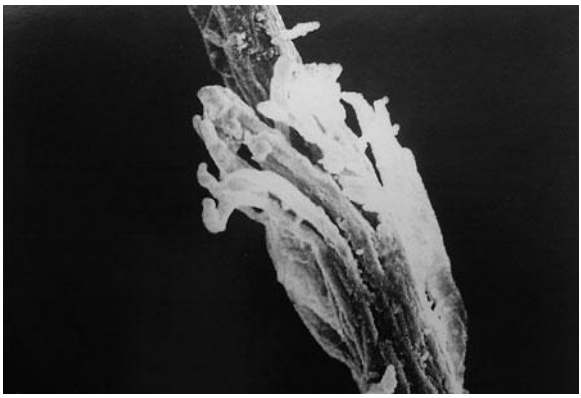

Fig. 1.34 Scanning electron micrograph of cortical cells of a human hair fiber. From a split hair

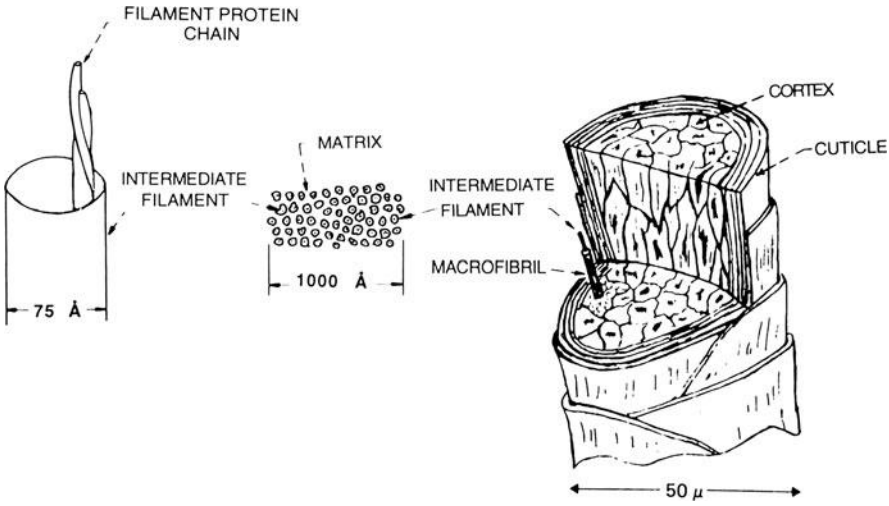

Fig. 1.35 Stereogram of a human hair fiber including intermediate filament-matrix structures

filaments. For more details on the intermediate filament structures see the section entitled *Intermediate Filaments* in this chapter.

1.7.2.3 Matrix

Various estimates of the relative quantities of matrix to intermediate filament protein (amorphous to crystalline proteins) have been made for both wool fiber and human hair [156, 157]. Although the relative quantities vary [158], the matrix-to-intermediate filament ratio in human hair is generally greater than 1.0. Protein derived primarily from matrix (gamma keratose) can be isolated from keratin fibers by the method of Alexander and Earland [159]. See Chap. 2 in the section entitled

Other Protein Fractionation Methods. This method involves oxidation of hair using peracetic acid followed by alkaline treatment. Analysis of the gamma keratose from human hair indicates a higher proportion of sulfur compared to the other keratose fractions or to whole fiber [87]. Corfield et al. [160] isolated matrix material from merino wool by this procedure. Chemical analysis gave a relatively high proportion of sulfur and a correspondingly greater proportion of cystine compared to the other fractions or to whole fiber [87].

Electron microscopy takes advantage of the high cystine content of matrix and the ability of cystine to react with osmium tetroxide to reveal the fine structure of hair in the following manner. Reduction of the fibers followed by treatment with osmium tetroxide, prior to sectioning, produces a heavily stained matrix revealing the relatively unstained intermediate filaments [161].

Matrix comprises the largest structural subunit of the cortex of human hair fibers. It contains the highest concentration of disulfide bonds of the cortex and the majority of these are probably intra-chain bonds rather than inter-chain bonds, because the matrix swells considerably when wet with water. Mechanically the matrix resembles a lightly cross-linked gel [162] rather than a highly cross-linked polymer. Matrix is often referred to as the amorphous region; although evidence suggests that it does contain some degree of structural organization [163]. A spacing of 28 Å has been demonstrated in mohair matrix. Spei attributed this spacing to structural repeat units of the matrix [164].

Proteins of the matrix are sometimes referred to in the literature as keratin associated proteins (KAP's) or as inter-fibrillar associated proteins (IFAP). Rogers et al. [165] suggested that there are essentially three classes of KAP's based on amino acid composition. The high sulfur KAP's (containing about 20% to about 30% cystine), the Ultra high sulfur KAP's (containing approximately 30% or more cystine and about 20% or more serine) and the KAP 6–8 which are tyrosine/glycine rich KAP's, see the section entitled *The KAP Proteins of Human Hair* in Chap. 2 for more details about the KAP proteins in human hair.

1.7.2.4 Intermediate Filaments

As indicated above, the macrofibrils in human hair contain subfilamentous structures called intermediate filaments (IF) (formed from intermediate filament proteins or keratins) and originally called microfibrils (microfilaments). The macrofibrils are arranged in spiral formation in the cortical cells. The radius of each spiral of the macrofibril, is approximately 4,000-Å units [166], and the width or diameter of an intermediate filament is close to 75 Å (see Fig. 1.20).

Two of the six known Types of IF proteins are in keratin fibers. The exact organization within the IFs of hair fibers is still being determined, although several basic structures were proposed back in the 1980s [167, 168] and improved upon since then. The filamentous polypeptides of human hair fibers are classified as Type I and Type II and these differ by their amino acid sequences resulting in acidic (Type I) and neutral to basic (Type II) proteins. Crewther et al. [167] in 1983

concluded that the IFs contain precise arrays of the low-sulfur proteins, containing short sections of alpha-helical proteins in coiled coil formation, showing a heptad repeat unit. The coiled coils are interrupted at three positions by non-helical fragments and are terminated by non-helical domains at both the nitrogen (N) and carbon (C) termini of the chain (Fig. 1.36). The individual filament-like protein chains of Fig. 1.35 are arranged into coiled coil dimers each containing one strand of type I and a second strand of type II chains (Fig. 1.36). These coiled coil dimers are then coiled around other dimers forming tetramers and higher ordered tubular type structures with very complex molecular associations head to tail forming longer filaments and lateral associations across coils forming complex IF structures which ultimately produce the different protein domains of orthocortex, mesocortex and paracortex, etc.

The schematic of Fig. 1.36 shows a general structure for the initial formation of dimeric units of IF structures. In this schematic, at the end N terminus, E1 is the end domain, V1 is a variable sequence and H1 is a high sequence region. At the C terminus, E2 is the end domain, V2 is a variable sequence and H2 is a high sequence region. The cystine content, of the low sulfur region of an IF is about 6%. It is not uniformly dispersed between domains of an IF chain. The rod domain contains about only 3% half-cystine, which is about one half-cystine residue, while the N terminal domain contains about 11% half-cystine and the C terminal unit about

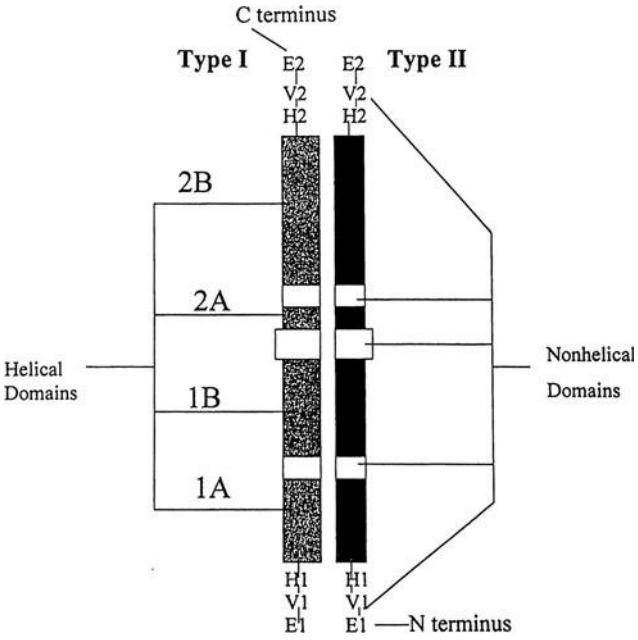

Fig. 1.36 Schematic illustrating the structure of an intermediate filament protein (type I-type II dimer). E are the end domains (E2 the C terminus and E1 the N terminus), V a variable sequence region and H is a high sequence region

17% half-cystine. Fraser [168, 169] suggested that these half-cystine residues are involved in disulfide linkages and that most of the disulfide residues exist in the matrix rather than in the IFs.

These dimeric units aggregate in an anti-parallel arrangement to form structural units composed of four protein chains or tetramers [167, 168]. Tetramers are connected end to end forming subfilaments called protofilaments which interact together to form the IFs of the cortex. Seven to ten of these tetramer units are believed to combine or aggregate into a larger helical structure, forming the IFs of the cortex of animal hairs. It would appear that the most favored structure is still the one proposed by R.D.B. Fraser et al. [168] in the 1980s. Fraser's concept contains a total of 8 protofilaments and consists of a single core protofilament surrounded by seven protofilaments [168]. For a more complete discussion of IF structures of keratin fibers, see the paper by ErRafik, Doucet and Briki on IFs of human hair [170], the paper by Powell and Rogers [89] and the references by Langbein and M. A. Rogers et al. in this chapter including [171, 172] and the papers by R.D. Bruce Fraser [173] and R.D.B. Fraser [174].

Langbein and M.A. Rogers et al. [171] reported that there are nine members in the human Type I subfamily that can be divided into three groups where H = hair, a = acidic, b = basic, and the number corresponds to a two dimensional staining spot. The names in parentheses are newer names as summarized in Table 1.16. Group A: hHa1 (K31), hHa3-I (K33a), hHa3-II (K33b), hHa4 (K34) and Group B: hHa7 (K37), hHa8 (K38) and Group C: hHa2 (K32), hHa5 (K35), hHa6 (K36). This latter group represents structurally unrelated hair keratins. Langbein and Rogers

Table 1.16 Nomenclature for the keratins found in the hair fiber and the hair follicle

Type I acidic		Type II basic to neutral	
Former name	Newer name	Former name	Newer name
Keratins found in the hair fiber itself			
hHa1	K31	hHb1	K81
hHa2	K32	hHb2	K82
hHa3-I	K33a	hHb3	K83
hHa3-II	K33b	hHb4	K84
hHa4	K34	hHb5	K85
hHa5	K35	hHb6	K86
hHa6	K36		
hHa7	K37		
hHa8	K38		
Ka35	K39		
Ka36	K40		
Hair follicle Keratins found in the root sheath			
K25irs1	K25	K6irs1	K71
K25irs2	K26	K6irs2	K72
K25irs3	K27	K6irs3	K73
K25irs4	K28	K6irs4	K74
		K6hf	K75

et al. [172] described that the Type II keratin subfamily contains six members divided into two groups and designated as: Group A: hHb1 (K81), hHb3 (K83), hHb6 (K86) which are structurally related and Group C: hHb2 (K82), hHb4 (K84) and hHb5 (K85) which are distinct. The sequence in which these keratins are expressed in the follicle is also described by Langbein and Rogers et al. in these papers. For additional details on these important hair proteins see Chap. 2 in the section entitled *Type I and II Keratin Proteins (IF Proteins) of Human Hair* and references [168–174].

1.7.2.5 Helical Proteins of the Intermediate Filaments

The subunits that constitute the IFs of hair fibers are polypeptide chains of proteins see Fig. 1.36 that are combined together as described in the section above. The coiled sections or the helical domains of these protein chains are approximately 10 Å in diameter, including side chains, and are believed to approximate the form of an alpha helix, first proposed by Pauling and Corey [175–177] (see Figs. 1.37 and 1.38).

Pauling and Corey proposed the alpha helix from the x-ray diffraction analysis of keratin fibers pioneered by Astbury et al. [178–180] and MacArthur [181, 182]. Wide-angle x-ray diffractions (up to approximately 15 Å repeating units) of unstretched human hair and other keratin fibers (wool and porcupine quill) show several related spacings, among which are an equatorial spacing (perpendicular to

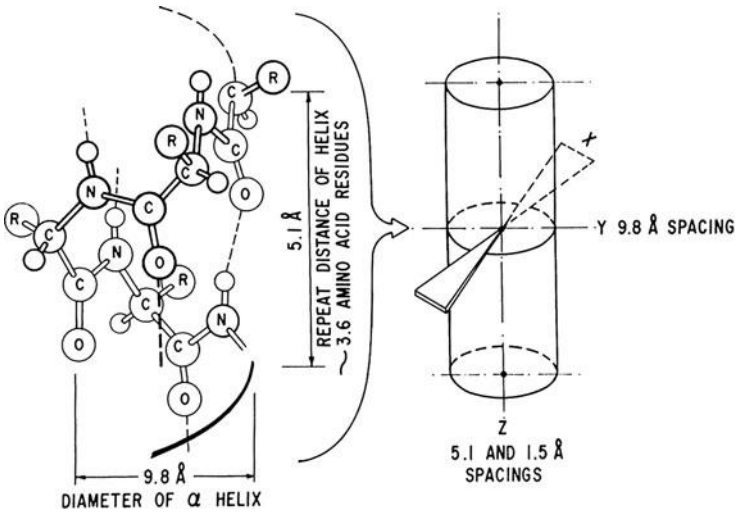

Fig. 1.37 Structure of an alpha-helix proposed by Pauling and Corey

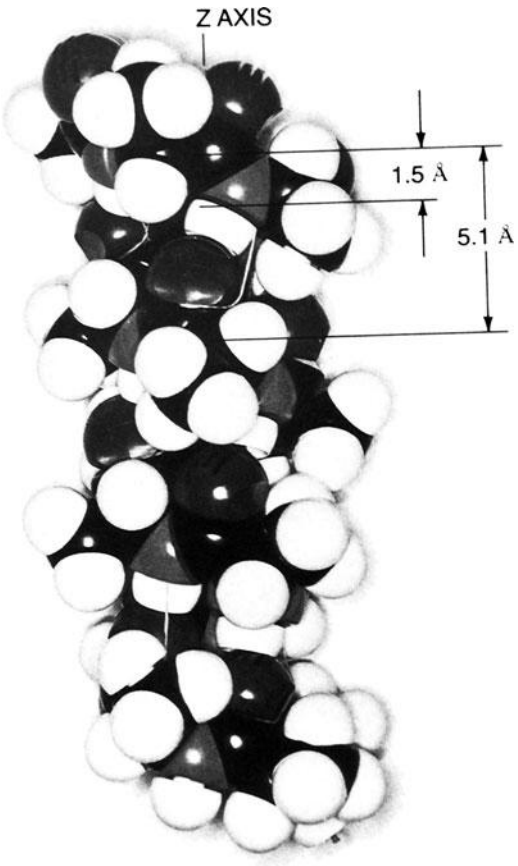

Fig. 1.38 Molecular model of a left-hand helix of polyalanine. A right-hand helix (spiraling in the other direction) is the pattern found in most proteins, including animal hairs (see Fig. 1.37)

the fiber axis) of 9.8 Å and meridional spacings (parallel to the fiber axis) of 5.1 and 1.5 Å (see Figs. 1.37 and 1.38).

Pauling and Corey interpreted the 1.5-Å spacing to represent the distance between each amino acid residue. The 5.1-Å spacing was assigned the repeat distance for coiling; corresponding to 3.6 amino acid residues and the 9.8-Å spacing represented the center-to-center distance between each alpha helix. This latter spacing approximates the thickness of the alpha helix. A linear polypeptide alpha helix would have a repeat distance of 5.4-Å units. Therefore, coiling of each helix [183] was proposed to account for the shorter 5.1 meridional spacing. Furthermore, it was originally suggested that two- or three-strands of polypeptides were coiled about each other analogous to a twisted rope [184–186]. This structure has been routinely referred to as the "coiled coil" model. The model that is now accepted for animal hairs is the two-strand rope polypeptide described in the previous section entitled, *The Intermediate Filaments*.

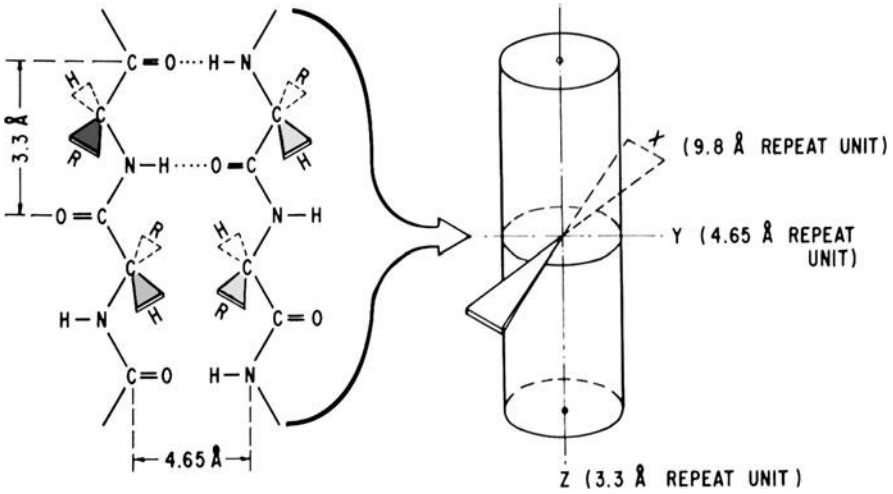

Fig. 1.39 Portions of two polypeptide chains in the beta configuration. The cylinder represents a hair fiber, and the axis identifies the orientation of the proteins in the fiber. See corresponding molecular models in Fig. 1.40

1.8 Stretching Hair and Stress Strain Models

Stretching hair can produce splits or cracks in the endocuticle and the cell membrane complex. Transverse cracks also occur in the cuticle layers as well as damage to the cortex. Nevertheless prior to this past decade, most of the scientific attention relating to stretching hair has been concerned with cortical effects; see Chaps. 6 and 9 for details related to damaging effects by stretching hair.

Astbury [179] found that water produces negligible effects to the wide-angle x-ray diagram of keratin fibers. However, extension in water diminishes the intensities of the reflections corresponding to the α helix and produces a pattern called β keratin represented by a 3.3-Å reflection along the fiber axis (the Z axis in Fig. 1.39), a 4.65-Å reflection at right angles to the Z axis (along the Y axis), and a 9.8-Å reflection at right angles to the Z axis (along the X axis) [167]. The molecular model of Fig. 1.40 describes the interpretation of these reflections in terms of molecular structure. Most explanations of this phenomenon invoke an α to β transformation, that is the transformation of molecules of the α-helical structure into the pleated sheet arrangement of the β structure.

To explain the stretching behavior of hair, many scientists consider hair consisting of only two components, IFs and matrix; however, to explain the fracture behavior of hair we must also involve the cell membrane complex. Some swelling models also consider only the intermediate filaments and matrix; however, Swift [187] provided some consideration to the non-keratin components for explaining the swelling behavior of keratin fibers. See the next section entitled *Swelling Behavior of Hair*.

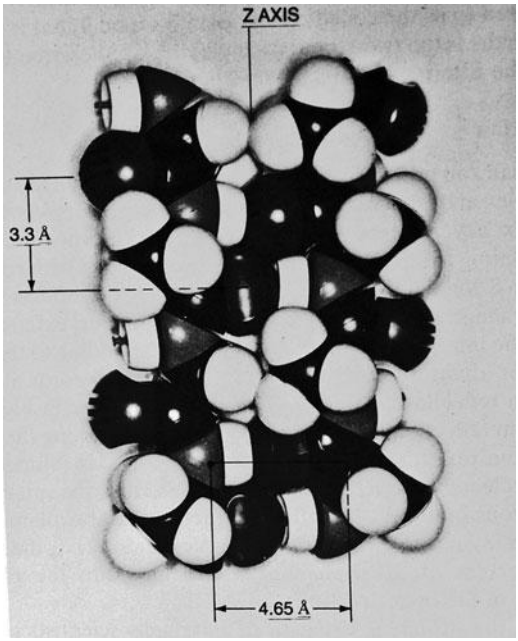

Fig. 1.40 Molecular model of two polyalanine chains in the beta configuration

To refine our understanding of the mechanical properties of keratin fibers, models involving the intermediate filament-matrix level of structural organization have been employed by Feughelman [188–190], Bendit [191], Hearle [192], Chapman [193], Wortmann and Zahn [194], and Kreplak et al. [195] and others. Only three of these models will be described along with some more recent relevant work.

1.8.1 Feughelman's Two Phase Model

Feughelman [189, 196] nearly 50 years ago proposed a two phase model in the cortex of animal hairs involving a relationship between mechanical properties and molecular configuration. At about the same time, Bendit [191] considered the α to β transformation to explain part of the stress strain curve of animal hairs. Feughelman's two phase model [188] consisted of water-impenetrable rods (IFs) oriented parallel with the fiber axis embedded in a water-penetrable matrix. This two-phase model is useful for helping to explain the mechanical properties of virgin keratin fibers including extension, bending, and torsional properties and also the swelling behavior of unmodified keratins. Feughelman explained the initial part of the stress strain curve, which is the "Hookean" region, by suggesting that the alpha helices of the IFs are strained, and hydrogen bonds of the globular proteins of the matrix are involved. Feughelman suggested that upon further extension into the

yield and postyield regions, the α to β transformation occurs in the coiled proteins of the IFs producing a loss of helical structure, which is recovered on relaxation.

Feughelman's model explained that the globular proteins of the matrix act in parallel with the IFs. The matrix phase is weakened by the presence of water. On the other hand, the crystalline regions of the IFs are virtually inert to water over the entire load-extension curve. In 1994, Feughelman modified and updated his model to what is called an X/Y zones model [190]. For additional details of the extension behavior of keratin fibers see the remaining discussion in this Chapter and Chap. 9 and for effects of extension on the cuticle see Chaps. 6 and 9.

1.8.2 Wortmann and Zahn's Model

Wortmann and Zahn [194] in 1994, proposed a different model placing more emphasis on the structures of the IFs and less emphasis on the matrix which they considered a gel to sol system. These scientists suggested that the opening up of two different parts of the IF monomer is responsible for the yield and post yield parts of the stress strain curve. Furthermore the increased slope in the post yield area is due to the sulfur bonds in one of the monomer segments of the IFs and that disulfide-sulfhydryl interchange occurs in the post yield region.

This model by Wortmann and Zahn [194] does seem to address many of the concerns of others [195, 197, 198]. For example, Wortmann and Zahn calculated that about 20% of α helices have opened up at the end of the yield region [27, 31]. Furthermore, they suggested that 48–56% of the α helical material has been converted to β segments upon the breaking stress in water (60–70%) strain. These calculations by Wortmann and Zahn are consistent with the findings of Kreplak [195].

The structural model by Wortmann and Zahn [194] places less emphasis on the matrix proteins and explains the fiber tensile behavior in terms of the molecular structure of the IFs and bonding within and to these important structures. This model suggests that the yield region arises by the α-helical chains uncoiling because they are not restricted by disulfide bonds. In this model the post yield region occurs in the domains restricted by disulfide bonding and the disulfide bonds continue to inhibit stretching until the fiber breaks. However, even this model fails to explain the stress.strain behavior of chemically oxidized or sun oxidized keratin fibers.

1.8.3 Other Models/Modifications and Some Concerns

More recently, slight modifications or neuances to the above models (or a new model as suggested by the authors) were proposed by Kreplak et al. [195] with supporting evidence by small-angle x-ray scattering on stretched α-keratin fibers. Interpretation of the data of these scientists suggested that the mechanical stretching

of hair fibers involves both stretching and sliding of keratin molecules inside the IFs. Furthermore, the water content of the fibers determines the relative importance of sliding vs. stretching. For example, when stretching hair fibers in water, the molecular sliding process is dominant up to about 40% strain where it is believed that stretching becomes more important. However, at 45% RH both stretching and sliding occur and the end result is unfolding of the α-helices to β-sheets as originally proposed by Bendit [191].

Two papers by Cao [197, 198] provided additional information to consider with respect to these structural models. In the first paper, Cao [197] emphasized that the assumption of a one to one micro.macro (molecular to fiber) relationship has been made by most structural models for explaining the stress.strain behavior of keratin fibers. Cao suggested that the macroscopic 40% extension of the fiber is not actually matched by 40% extension of polypeptide chains throughout the fiber, but intermolecular slippage can occur that can cause rearrangement of the lattice structure.

Cao examined hair and wool fiber using x-ray analysis examining the 5.1 meridional fraction characteristic of the α-form and the 4.65 equatorial diffraction characteristic of the β-form. Cao concluded that a 40% stretch of wet hair that is held at 40% elongation does not display any evidence of a β-form, but only α-form crystallites, however the same 40% stretch followed by steam setting for 20 min shows a β-form crystalline pattern. So, Cao concluded that the α to β transition occurs only while a stretched hair or wool fiber is being set using steam, not while it is being stretched. To my knowledge no one in this field has either addressed, found or reproduced this finding by Cao.

In Cao's second paper on this subject [198], he demonstrated irregular multiple necking or narrowing deformations along the length of the hairs rather than a continuously uniform elongation along the length of the fibers. Cao drew an analogy to the same phenomenon that occurs in the stretching of synthetic polymers. X-ray analysis showed β-crystallinity in the necked sections and α-crystallinity in the non-deformed un-necked sections of the fibers. Furthermore, the greater the percentage of stretching the more β-crystalline form resulted.

Kreplak et al. [199] in a more recent publication sheds additional light on the stretching behavior of horse hair fibers. These scientists showed by wide angle x-ray scattering and high spatial resolution infrared microspectroscopy that the α to β transformation occurs near 20% strain for wet hair and not before. This is close to the end of the yield region, not near the beginning such as 5% strain as suggested by earlier investigators. The data is consistent with the unfolding of α helical coiled coils below 20% but not transitioning to the β confirmation until above 20% strain. Kreplak et al. [199] also suggested that their data is consistent with the α to β transition occurring from 20% to 50% strain and that the transformation occurs first in the fiber core and then moves slowly to the fiber periphery. They suggested that this transition from the fiber core to the periphery is related to differences in crystallinity (the IFs) in the fiber core vs. the periphery.

These experiments by Kerplak et al. were with horse hair, a very straight hair fiber. But we know that there are different crystalline distributions between straight hair and highly coiled hair fibers. For example, both straight human hair and wool

fiber have been shown to possess an annular type cortex with para type cortical cells in the core, meso type cells in between and orthocortical type cells at the periphery of the cortex [150, 151]. On the other hand, highly curled hairs have been shown to contain a bilateral type cortex with more para type cells in the concave side of the curl and ortho type cells in the convex side. Therefore, with Kreplak's model, I would anticipate that stretching straight hairs of all types would originate first in the core and move to the periphery as found by Kerplak et al. [199]. However, for highly curled hairs, I would conclude that the alpha to beta transition would first begin in the most concave side of curls and then move to the other side.

1.8.4 Fractographic and Damaged Hair Concerns with These Models

To explain the load elongation behavior of the most virgin parts of the fibers these models appear to be reasonably sufficient. However, to explain load elongation behavior up to and including catastrophic failure for certain types of hair damage, these models do not explain fractographic results and consequences of certain types of hair fiber damage. For example, as hair is damaged especially by free radical oxidative treatments fractures are propagated along the axis through the cell membrane complex [200, 201] and the medulla [200] providing step fractures, fibrillated ends and split hairs [200, 201]. Feughelman [202] in his book explained that the stronger cortical cells dominate the bulk properties of the cortex until the CMC fails. He stated further that the CMC does not have "any effect on the measurement of the mechanical properties of the cortex until failure is approached." Furthermore, he did not describe any instances where damage occurs to the CMC or produces effects on the mechanical properties.

Fractures can occur in the CMC before catastrophic failure in hair fibers on live heads with sunlight exposure and/or normal oxidative cosmetic treatments and on African hair with twists. I arranged with a local hair dresser to collect hair clippings from a few of his selected customers; those he believed to have split hairs. A questionnaire was devised and completed by the hair dresser in collaboration with the customer on the type of hair and the different treatments and conditions that the hair had been exposed to. Hair cuttings were collected from eight female Caucasians. Prior to cutting, all hair samples measured approximately 35–55 cm long and the amount of hair cuttings varied from a total of 2–12 g. A total of 272 split hairs were found and classified into six different types of splits. The highest frequency and most severe splits were from four panelists who frequently treated or exposed their hair to peroxide-persulfate or to two or more of these products/exposures known to involve free radicals: peroxide-persulfate, sun bathing, oxidation dyes and hot irons (straightening or curling). From these hairs, several examples were found indicative of CMC fracturing before catastrophic failure. Two of these

examples will be described and several are illustrated in Chap. 10 in the section entitled, *Split Ends, Types, and their Occurrence & Formation*:

- Split hairs NOT Split ends. This type of split can occur as far as 4 cm from the tip end, and it occurs before catastrophic failure.
- Broom type effects resulting from fractures in the CMC several cm from the tip end. This effect is caused by oxidative cosmetic damage and grooming actions and is analogous to the fractures at the nodes of the hair abnormality in trichorrhexis nodosa. This type of effect occurs before catastrophic failure. These effects illustrate that fractures can occur in the CMC before catastrophic failure and they should produce changes in the tensile curves in some cases prior to the post yield region. However, if such effects are observed in testing, the data could lead to premature failure for some fibers and will often be omitted as outliers. So, hairs that have been exposed to free radical cosmetics and/or sunlight and taken directly from live heads can have extensive damage to the CMC. Therefore, I conclude that hairs treated similarly but fractured less extensively in the CMC should produce some detectable changes in their mechanical behavior prior to catastrophic failure.

Kamath and Weigmann [200] determined that more smooth fractures are formed than axial fractures at high humidity or in the wet state. Furthermore, at high humidity or the wet state crack initiation tends to be near the cuticle-cortex boundary, and then the crack propagates toward the center of the cortex [200]. Kamath and Weigmann concluded that when the hair is wet or at high humidity the swelling pressure of the cortex on the cuticle is involved in crack initiation. In addition, more axial splitting was obtained from hair fibers in which the cuticle had been damaged and partially removed by abrasion than non-abraded hair permitting Kamath and Weigmann to conclude that a strong intact cuticle inhibits axial splitting of hair fibers [6].

Robbins [203] in his review of the CMC described its sensitivity to free radical reactions as demonstrated for both human hair and wool fiber and that this sensitivity leads to an increase in step fractures, split hairs and fibrillated ends.

Brown and Swift [201] tested root sections and weathered tips of long (more than 50 cm) human hair fibers from six Caucasian females in a tensile tester at room humidity and temperature. These scientists observed more smooth fractures in the root sections and more longitudinal and circumferential splitting (axial fracturing) in the more weathered tip ends.

The stress.strain models (described above) do explain load elongation in the Hookean and yield regions for virgin hair, but they are less effective in the post yield region particularly in damaged hair when fractographic studies and other evidence suggest that the CMC is involved [200, 201].

For example, undamaged virgin hair roots generally provides a smooth fracture in water, usually beginning near the cuticle-cortex boundary [200] (in the post yield region) and it continues across cortical proteins and appears to be consistent with the stress.strain models. However, for sun damaged hair or highly weathered tip ends, the CMC can be sufficiently damaged primarily by free radical oxidation to

weaken the cortex CMC [203] and with it the cell membrane proteins; so axial fracturing occurs and the CMC is involved.

In this case, a fracture may begin in the cortical proteins in the post yield region as suggested by either the Feughelman model (in the amorphous proteins) or the Wortmann-Zahn model (in the intermediate filaments). After the crack has propagated to where it encounters the weakened cell membrane complex it is diverted and propagated through the CMC and the medulla (if present) and then diverts once again to another region of the cortical proteins to provide a step fracture. On the other hand, if the CMC is extensively damaged, once the crack first diverts into the CMC it can spread in the CMC to provide a split hair or a fibrillated end depending on how badly the CMC has been damaged. So, to explain the stress.strain behavior of these types of damaged hair fibers the intercellular regions of the hair fiber must also be involved.

If we have a hypothesis or a model that explains the tensile properties of undamaged virgin hair, but it does not explain the stress.strain behavior of many common types of hair damage or even damage to the weathered tip ends of hair and African type hair, it is of limited value. So, to increase the value of these models they should be extended to explain common forms of hair damage in addition to simply explaining undamaged virgin hair. It would appear that the weakest links in "virgin" hair fibers to tensile extension are the structures inside cortical cells. However, with most types of hair damage, as strain or extension continues to higher levels, fracturing extends to and involves the cell membrane complex by forming step fractures, fibrillated ends or split hairs. Furthermore for catastrophic failure that forms a step fracture to occur a crack inside a cortical cell must extend across and axially through the CMC. Therefore the CMC must be involved in any hypothesis or model to fully explain the tensile properties of hair especially when considering damaged hair fibers.

I conclude with Hearle [192] that we need a better understanding of the molecular organization of what is called the "amorphous" matrix and the keratin associated proteins to better understand the mechanical properties of hair and wool fiber. But, in addition we need a better understanding of the molecular organization of the cell membrane complex too.

1.9 Swelling Behavior of Hair

The organizational level believed to control the swelling behavior of keratins is the secondary and tertiary structure of the IFs and the matrix [204]. As indicated previously, the IFs consist of proteins containing alpha-helical segments embedded in the less organized matrix of high cystine content.

In keratin fibers like human hair and wool fiber, the helical proteins of the IFs are oriented parallel with the axis of the fiber (see Fig. 1.41). The IFs help to maintain the structural integrity of the fibers while most of the volume swelling takes place in the matrix proteins [204, 205]. This is consistent with Feughelman's [188] two-phase model of water-impenetrable rods (IFs) in a water-penetrable matrix. As a

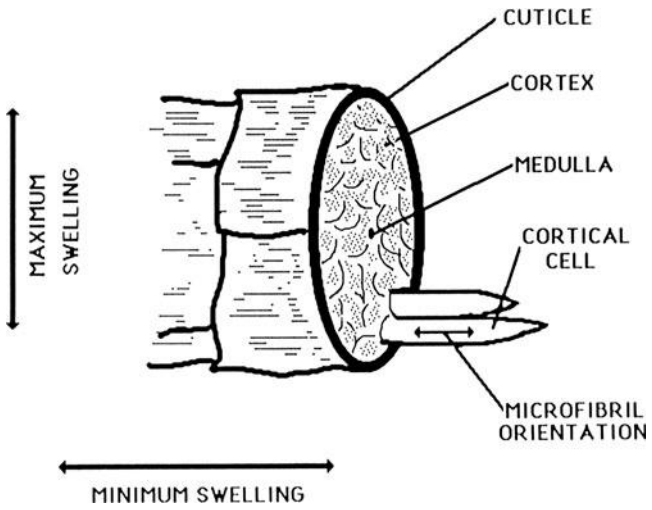

Fig. 1.41 Schematic illustrating the directional swelling of human hair

result, maximum swelling occurs between the IFs and minimum swelling occurs along the axis of the IFs. Therefore, maximum swelling occurs in the diametral dimension of hair, and minimum swelling occurs in the longitudinal dimension that is along the axis of the fibers (see Fig. 1.41). For example, Stam et al. [206] has shown that from 0% to 100% relative humidity, hair increases nearly 14% in diameter, but less than 2% in length. Other reagents such as sodium lauryl sulfate, formic acid, and thioglycolic acid produce more swelling than water but dimensionally they swell hair similarly; that is they produce greater swelling in the diametral dimension than in the fiber length [204].

Swift [187] and others suggested that the non-keratin portions of hair are also important to fiber swelling. For example, Swift demonstrated by the penetration of fluorescent labelled proteins in water that a large order swelling occurs in the non-keratin regions of hair. The diametral swelling of hair by water from the dry state is about 14% to 16%. On the other hand, Spei and Zahn [207] using x-ray diffraction measurement of inter-IF separation distances indicates that swelling of only 5.5% occurs. Swift, therefore, proposed that part of the difference can be explained by swelling that occurs in the non-keratin regions of the cuticle and the cortex. For additional details on swelling of human hair, see Chap. 9.

Protein from the IFs of human hair can be isolated by oxidation with peracetic acid according to the method of Alexander and Earland [159]. One fraction obtained by this procedure is called alpha keratose. See Chap. 2 in the section entitled Other Protein Fractionation Methods. The alpha keratose fraction amounts to about 45% of the fiber mass, containing a substantially lower proportion of sulfur than the other two keratose fractions (see Table 2.16). The low sulfur content suggests a relatively low proportion of the amino acid cystine in the IFs and therefore a low proportion of cystine in the alpha-helical proteins. This conclusion

is consistent with the amino acid analysis of alpha keratose isolated from merino wool by Corfield et al. [160], showing a relatively low percentage of cystine and a high percentage of the other bulky amino acids. This keratose fraction is in all probability not purely IF in origin and likewise not pure alpha-helical protein. However, the fact that it can produce an x-ray pattern similar to that of alpha keratin [159], and the other two-keratose fractions cannot, suggest that its origin is the IFs.

1.10 The Origin of Hair Fiber Curvature

We frequently refer to hair fibers as being round, however most hair fibers are actually oval shaped. In addition, many hairs defy a single word or measurement for their cross-sectional shape because they are often twisted and indented with very irregular cross-section and surface appearances; see Chap. 9 for a more complete discussion of hair fiber shape including illustrative micrographs of hair fibers.

More than 40 years ago, Mercer [208] proposed that the shape of hair fibers is determined by the shape of the hair follicles in the zone of keratinization. Lindelof et al. [209] concluded from 3-D computer-aided reconstruction of serial sections of human hair follicles from ten patients of three biological races that the shape of the follicle is a primary factor in determining the final hair form or shape. For example, Lindelof et al. reported that the African follicle had a helical form, while the Asian follicle was essentially straight and the follicle of Caucasians varied between these two extremes.

Orwin [210] found that breeds of sheep that bear fine-wool tend to have follicles of narrower diameters and longer follicles correlate with longer wool fibers. These facts suggest that the size and shape of the follicle could play a role in the final shape of hair fibers and that the growing fiber takes the shape of the mold where hardening or keratinization occurs. Thus, if the follicle or sac that the fiber is formed in is highly curved in the zone of keratinization, the emerging hair fiber should be highly curled, but, if the follicle is relatively straight, the emerging hair will be straight. This mechanism to explain hair fiber curvature is analogous to the shape that is formed for an extruded monofilament for synthetic polymers.

An alternative explanation considers the bilateral structure of some keratin fibers like wool. A helical fiber will arise if opposite halves of the fiber grow at different rates or if opposite halves contract to different extents during drying or with moisture changes. This conclusion is analogous to the way a bilateral thermostat bends with changes in temperature and considers protein composition and distribution as important factors for fiber shape.

In the 1950s Mercer [211] and Rogers [212] independently identified two types of cortical cells in merino wool fibers. These two types of cells were named orthocortex and paracortex (see Fig. 1.32). Later, Kaplin and Whiteley [213] were able to distinguish between three different types of cortical cells in high-crimp and low-crimp merino wool. Cells on one side of the cross section contained

whorls of IFs. These were called orthocortical cells, while the names paracortical cells and mesocortical cells were used for those cells without whorls of microfibrils that were arranged opposite to the orthocortical cells in wool fibers of high crimp and low crimp, respectively [214, 215].

Highly crimped wool fibers like merino wool, camel, vicuna and guanaco hair have all been shown to contain bilateral cortical structures with nearly an equal amount of ortho-cortical and para-cortical cells [215, 216]. On the other hand, non-bilateral structures have been described for relatively straight animal hairs such as mohair and alpaca [215].

In 1972 Leon [148] described both orthocortical and paracortical cells for Negro hair with a "higher proportion of ortho type cortical cells than for Caucasian hair". Swift [149] more recently reported that highly twisted hair from a Black man from Nigeria was asymmetrically divided and contained about 50% paracortical and 50% orthocortical cells. On the other hand, straight hair from Japanese contained only para-cortical cells and thus did not contain bilateral structures of ortho and paracortical cells. For curly Caucasian hair, Swift observed mostly paracortex with a layer of only one cell thickness of orthocortical cells at the periphery of the cortex, but not a bilateral structure.

Horio and Kondo [217] found in fine high crimp wool fibers that the bilateral arrangement of ortho- and paracortical cells occurs with the orthocortex on the outside of the curve or curl and the paracortex on the inside of the curl. This arrangement was confirmed by Fraser and Rogers [218].

Campbell et al. [219] described the effects of diet on the shape of wool fibers. Campbell worked with two types of sheep, sheep that provided high crimp wool and sheep that provided low crimp wool. These scientists demonstrated that when both groups of sheep were placed on a low nutrition diet, the number of crimps/cm of wool increased see Table 1.17. However, when these same sheep were placed back on the normal nutrition diet the number of crimps/cm of wool decreased once again see Table 1.17.

Campbell explained these results by suggesting that wool fibers produced on a low nutrition level moves more slowly through the follicle than fibers produced at a normal nutrition level. Furthermore, curved follicles with a faster growth rate should move the soft unhardened fiber through the zone of keratinization faster and the faster the fiber moves through the zone of hardening the fewer crimps produced. But, an alternative explanation is that on a low nutrition diet sheep are not capable of producing the required amount of the specific proteins that are

Table 1.17 The effect of nutritional levels in sheep on wool fiber crimp, from Campbell et al. [219]

	High crimp wool			Low crimp wool		
Nutrition level	Normal	Low	Normal	Normal	Low	Normal
Crimps/cm	7.0	9.0	6.7	1.7	3.8	2.0
% S	4.08	3.17	4.08	3.26	2.75	3.22
% High S protein	32	22	29	24	17	20

necessary for producing the required bilateral content for a high degree of crimp and curl, i.e., specific IF or KAP proteins. Note, the percentage of high sulfur proteins also decreased and increased with the crimp, see Table 1.17. This effect of producing less high sulfur proteins would produce a lower paracortical cell content resulting in less bilateral content and therefore less crimp or curl.

1.10.1 Structures in the Cortex Associated with Curvature

The cortical cells of human hair are composed of fibrillar components called macrofibrils that are connected by inter-macrofibrillar material, cytoplasmic remnant and melanin granules. The macrofibrils consist primarily of filamentous proteins that form IF's that are held together laterally and in orientation by amorphous type proteins called keratin associated proteins (KAP's). It has been shown that the distribution of different cortical cell types with their corresponding IF arrangements are related to hair fiber curvature in wool, camel, alpaca and mohair fiber [215–217] and in human hair fibers [150, 151].

Thibaut and coworkers [150] studied hair from six persons of Caucasian, North African and African descent. This hair was described as straight (Curl type I), wavy (Curl type II or III) and curly human hair (Curl types IV–VII). These STAM (Chap. 9) curvature types are based on my estimates from the photographs in the Thibaut et al. paper. Thibaut et al. found evidence for three types of cells in the cortex of these hairs. These scientists indicated that the cell types were similar to the orthocortical, mesocortical and paracortical cells found in wool fiber [217, 218, 220, 221]. In straight hairs, a core of paracortical type cells were generally surrounded by mesocortical and orthocortical type cells in an annular type arrangement.

The amount of mesocortical type cells decreased with increasing fiber curvature. Only orthocortical and paracortical type cells were identified in tightly coiled African hair and these were distributed asymmetrically with the orthocortical type cells predominately on the convex side of the curl and the paracortical type cells on the concave side, see Figs. 1.32 and 1.42.

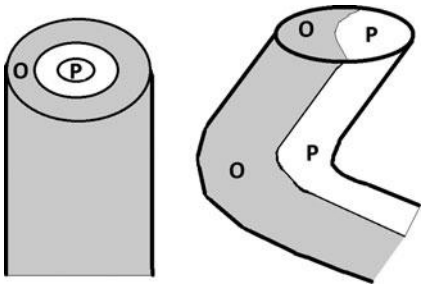

Fig. 1.42 Schematic of a straight and a curled hair illustrating orthocortex type and paracortex type distributions in the fiber

Thibaut et al. [150] indicated that the distribution of the keratin protein hHa8 (a building block of specific IF's) was found to be associated with the amount of curliness. As the degree of curvature increased the amount of hHa8 keratin accumulated more to the concave side of the curl. In tightly curled hair it was almost exclusively on the concave side along the length of the fiber [150]. Since hHa8 keratin is a component of IF protein and its location in the fiber cross-section is curvature dependent one can conclude that the organization and likely the orientation of the IFs are most likely related to hair fiber curvature.

Kajiura et al. [222] studied a wide range of hair fiber curvatures (Curl types I, III, IV, and V–VIII, which I classified from the curl radii provided) of human hair (African American, Asian and Caucasian) and wool fiber by small angle x-ray scattering (SAXS). These scientists found that the gap between IFs is larger on the concave side of a curl in human hair and smaller on the convex side. This suggests more matrix material between IFs on the concave side of a curl in human hair and is consistent with the observation in wool fiber of more paracortex (more matrix material than orthocortex) on the concave side of a curl or curve and more orthocortex on the convex side of a curl [218, 221], see Table 1.18 and Figs. 1.32 and 1.42.

Bryson et al. [151] examined hair fiber curvatures from Curl type I, III and IV of Japanese hair (I calculated STAM Curl types from curl diameters in that paper) and they described four different types of cortical cells. These scientists labeled these cell types as A, B, C and D and found all four cell types in straight and curved hairs. The cells were more symmetrically distributed in straight hairs consisting mainly of annular bands of cell types around the center of the fiber analogous to the arrangements in straight Caucasian and Asian type hair [150].

Curved hairs (Curl type IV) in fluorescent stained and TEM hair sections showed strong bilateral symmetry with respect to the distribution of cell types with mainly B type cells and some C or D type on the convex side and primarily C type with a

Table 1.18 Some properties of orthcortical and paracortical type cells in keratin fibers

Orthocortical B or D[a] cells	Paracortical and C[a] cells
On convex side of curl [151, 212, 218]	On concave side of curl [212, 218]
Lower: Matrix/IF's [222–224]	Higher: Matrix/IF's [222–224]
More crystalline	Less crystalline
IF's: Helical whorl-like [151, 220]	IF's: Parallel arrays [220][b]
Less cystine rich proteins [225][228][c] fewer cross-links	More cystine rich proteins [225][228][c] more cross-links
Lower sulfur content [227]	Higher sulfur content [227]
Acidic: Binds more Cationic dyes	Basic: Binds more anionic dyes
More extensible and flexible [151]	Less extensible and flexible [151]
Lower water binding (all RH's)[d]	Higher water binding (all RH's)[d]

[a]The properties of this table have been demonstrated for orthocortical and paracortical cells in wool and I conclude they are directionally similar for B and C cells in hair
[b]Bryson et al. [151] show C cells more of a hybrid between ortho- and paracortical
[c]Shown concave vs. convex side of curl and assumed to be in cell types as indicated
[d]I conclude these effects for water absorption based on crystalline (IF) content.

few B type cells on the concave side similar to the findings of Thibaut et al. [150] who called the cells types ortho-, meso-, and paracortical types.

Table 1.18 describes some important properties of orthocortical and paracortical cells in wool fiber. These properties are assumed to generally (but not entirely) correspond to the properties of B type and C type cortical cells in human hair respectively.

Bryson et al. [151] suggested that the cortical cell types of wool fiber are easier to differentiate and are more distinctly separated bilaterally than in high curvature Japanese hair. These scientists [151] also suggested that type B cells of human hair are similar but not exactly the same structurally to orthocortical cells of wool fiber see Table 1.18. The macrofibrils of type B cells contain "helical/whorl-like IF arrangements" similar to orthocortical cells [151]. Type C cells although similar structurally to paracortical cells of wool fiber are more of a hybrid between orthocortical and paracortical cells with respect to their arrangement of IF's [151]. The macrofibrils of type C cells contain IF's in both "helical/whorl-like" arrangements and "parallel arrays" [151].

The type B cells of Japanese hair [151] and orthocortical cells of wool fiber [212, 218] are primarily in the convex side of fiber curls while the type C cells of Japanese hair [151] and paracortical cells in wool fiber [212, 218] are largely in the concave side of curls. The orthocortical cells [223, 224] and the B cells (on the convex side of curls) contain more IF material [151, 223, 224], less matrix [151, 224, 226] and a lower cross-link density [225] and therefore are believed to be more flexible and more extensible than paracortical cells [151] or C type cells [151] on the concave side of curls.

Bryson et al. [151] suggested when hair or wool fiber is wet with water and dries out the convex fiber side (less cystine, therefore more extensible and flexible) should extend more longitudinally than the concave fiber side causing the fiber to bend toward the region of highest type C cell or paracortical cell concentration. This suggestion is consistent with the cystine composition of these different cell types, see Table 1.18.

Thibaut et al. in another publication [228] provided an explanation for this type of asymmetric cell production and growth by demonstrating that in curly follicles, both the outer root sheath (ORS) and the connective tissue sheath lack symmetry along the follicle. Where the follicle is convex, the ORS is not as thick and the rate of differentiation by the inner root sheath is decreased. Therefore, these scientists concluded that an asymmetric distribution of proteins in curved hair follicles relates to and is controlled by this lack of symmetry in the ORS and connective tissue sheath around the follicle.

Furthermore, Thibaut et al. [228] demonstrated that when curly hair fibers (African or Caucasian) are dissected and removed from the scalp biopsy and the lower part of the hair follicle placed in in-vitro media the curvature of the emerging fiber appears to be retained as if it were growing in the follicle. From these experiments these scientists concluded that hair curl is "programmed from the bulb" and is related to or controlled by "asymmetric differentiation" as the fiber moves up the follicle.

I conclude that the primary factor controlling hair fiber curvature is programmed from the bulb by the symmetry of protein distribution. However, whether hair follicle shape in the zone of keratinization affects hair fiber curvature in some way analogous to the production of a synthetic filament as it is extruded or whether curvature is controlled entirely by programming from the bulb by the symmetry of the distribution of proteins in the final fiber awaits further research.

1.11 The Structure of the Cell Membrane Complex

The cell membrane complex (CMC) consists of cell membranes and adhesive material that binds or "glues" the cuticle and cortical cells together in keratin fibers. G.E. Rogers from his seminal high resolution transmission electron microscope (TEM) studies of animal hairs provided evidence for the general structure of the CMC. The CMC consists of a central Delta layer approximately 15 nm thick sandwiched by two lipid layers called Beta layers each in the vicinity of 5 nm thick [212, 229], see Fig. 1.26 adapted from Fraser, MacRae and Rogers [223]. Jones et al. [230] described the uncertainty of the composition of the Delta layer because of the difficulty of isolating it without changing it.

Questions still exist about the relative thickness and composition of the Beta layers between cuticle cells vs. the Beta layers of cortical cells (see Figs. 1.43, 1.44 and 1.45) and between the upper Beta layer vs. the lower Beta layer of cuticle cells. Although, most authors quote the thicknesses of the Beta layers between 2.5 [114] and 5.0 nm, 6.0 nm has also been cited [130]. In addition the upper Beta layer

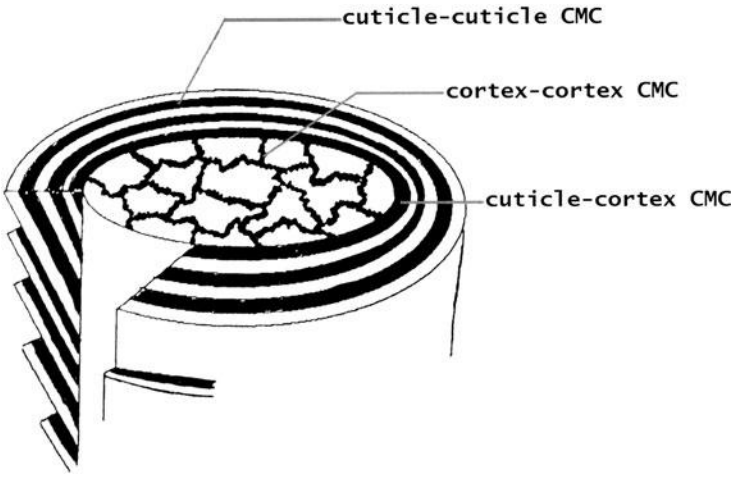

Fig. 1.43 Schematic diagram illustrating the location of the three types of CMC in hair fibers (Reprinted with permission of the Journal of Cosmetic Science [203])

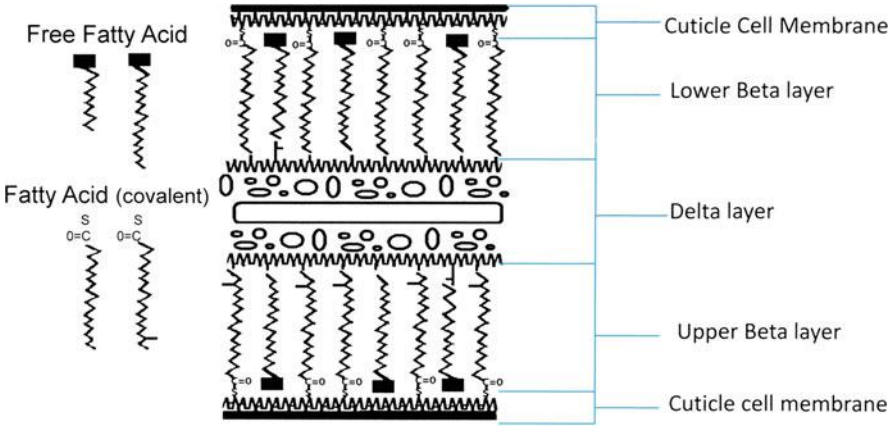

Fig. 1.44 Schematic proposed for the cuticle-cuticle CMC [203] (Reprinted with permission of the Journal of Cosmetic Science)

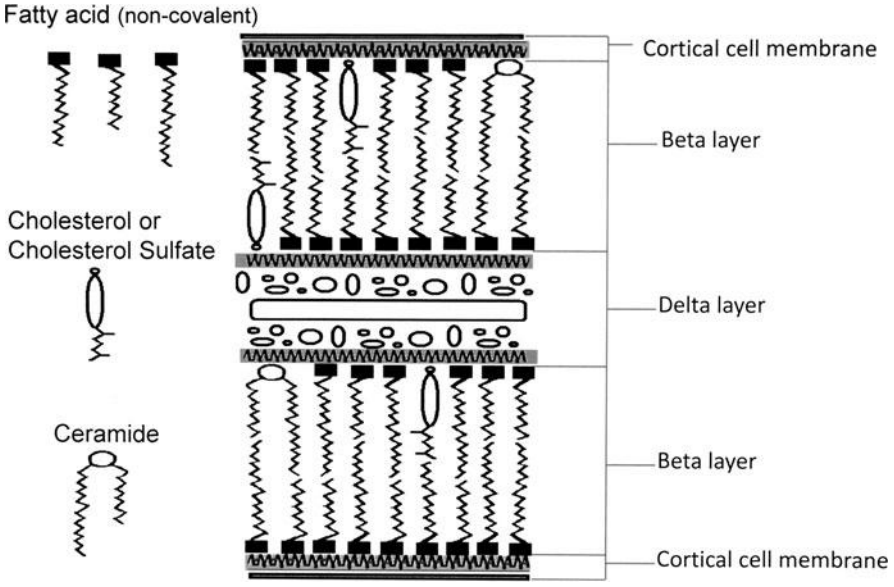

Fig. 1.45 Schematic representing the cortex-cortex CMC [203] (Reprinted with permission of the Journal of Cosmetic Science)

appears to be thicker than the lower Beta layer [229, 231]. Swift [107] in his review of the human hair cuticle described the difficulty of obtaining accurate measurements of the Beta layers in the high resolution TEM. Swift's explanation clarifies the uncertainty that exists in ascribing mono-layers or bi-layers to these lipid strata on the basis of TEM measurements alone. From his TEM studies, Swift

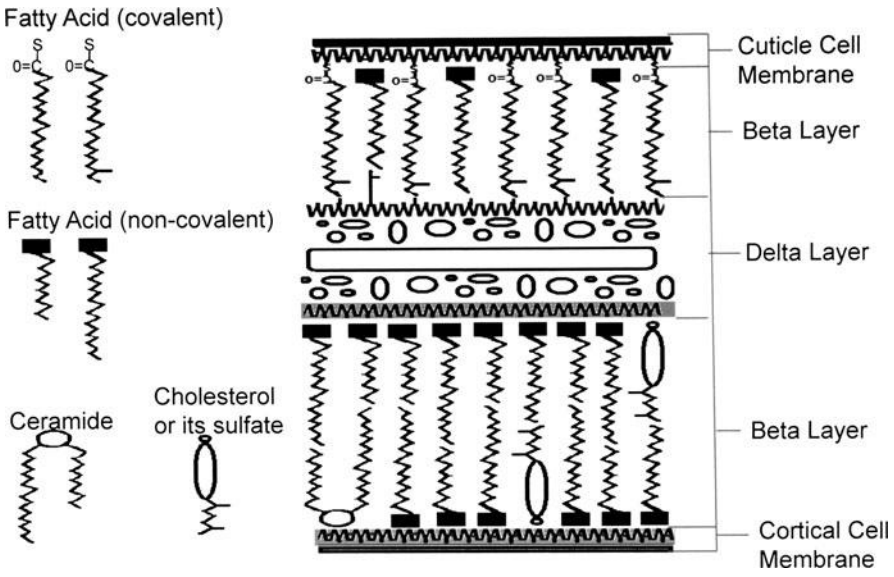

Fig. 1.46 Schematic representing the cuticle-cortex CMC [203] (Reprinted with permission of the Journal of Cosmetic Science)

[232] cited 3 nm thicknesses for the cuticle Beta layers. Relatively recent analyses by microbeam diffraction [142, 143] also cite 3 nm thicknesses for these same layers between cuticle cells, see the section entitled, *Thickness of the Cuticle Beta Layers* in this chapter.

Three types of CMC have been described in the literature [233]: cuticle-cuticle CMC representing CMC between cuticle cells, cortex-cortex CMC representing CMC between cortical cells and cuticle-cortex CMC representing CMC at the cuticle cortex boundary see Fig. 1.46. Since Rogers' [212, 229] initial description of the CMC and his additional work demonstrating that the Delta layer of the cortex consists of five sub-layers [223] several additional important developments have occurred that will be described in the next section adding details to this important structure in animal hairs.

1.11.1 General Differences for Cuticle-Cuticle CMC Versus Cortex-Cortex CMC

Jones and Rivett [125, 234] provided evidence that the CMC of the cuticle contains 18-methyl eicosanoic acid (18-MEA) in its upper Beta layer. 18-MEA has never been shown to be in the CMC of the cortex. The facts strongly suggest that the CMC of the cuticle has monolayer lipids that are attached by covalent bonds (primarily thioester) [122, 235] with some ester or amide linkages [235] to proteins of the cell

membranes on one end and attachment by van der Waals attractive forces to proteins of the Delta layer on the hydrophobic end of the fatty acids (Fig. 1.44). The evidence shows that the CMC between cortical cells consists of lipid bi-layers that are not attached by covalent bonding to protein layers. The lipid bi-layers of the cortex are bound by salt linkages and polar bonding to the cortical cell membrane proteins on one side and similarly attached to the Delta layer on the other side of the bi-layer, see Fig. 1.45.

1.11.2 The Cuticle-Cuticle CMC

In 1916 Allworden [126] discovered that Chlorine water reacts with the cuticle cells of wool fiber to produce large bulbous sacs on the fiber surface. Chlorine water degrades proteins beneath the cuticle cell membranes, (most likely cleaving and oxidizing disulfide bonds between the epicuticle and the A-Layer [236]) producing water soluble species too large to diffuse out of the semi-permeable cuticle cell membrane. Swelling results from osmotic forces and the cuticle membrane stretches producing the Allworden sacs (Fig. 1.29) that separate from the underlying proteinaceous cell layers.

The epicuticle membrane was first isolated and named by Lindberg et al. in 1949 [237, 238]. Nineteen years later, Leeder and Bradbury [100] defined the epicuticle as the "thin outer membrane which is raised on the surface of fibers as sacs by treatment with chlorine water" in the Allworden reaction. The epicuticle (uppermost cuticle cell membrane) provides the supporting structure for fatty acids in the cuticle, see Fig. 1.44. It is also attached to the A-layer of cuticle cells of wool and human hair and together with 18-MEA is perhaps the most thoroughly studied part of the CMC. Leeder and Rippon [239] in 1985, suggested that the epicuticle was proteinaceous and covered with a strongly bound lipid layer that could not be removed by lipid solvents, but could be removed with alcoholic alkali. They called this covalently bound lipid layer the F-layer.

The F layer together with the cuticle cell membranes (essentially the epicuticle) is analogous to the cornified envelopes or the cellular envelope of stratum corneum. In 1945, Weitkamp [240] reported 18-MEA in wool wax (degras). Forty years later, in 1985, Evans et al. [241] demonstrated that 18-MEA is covalently bonded to the keratin fiber surface by reacting wool fiber with anhydrous alkali after solvent extractable lipids had been removed. The cleavage of 18-MEA with chlorine water by Negri et al. [122] and by hydroxyl amine at neutral pH by Evans and Lanczki [235] support its attachment by a thioester linkage rather than an ester or amide link. In addition, Evans and Lanczki [235] and Korner and G. Wortmann [242] provided evidence for ester and/or amide attachment of some fatty acids (primarily palmitic, stearic, oleic and others) mainly in the lower beta layer on the bottom of cuticle cells.

Jones et al. [243] demonstrated that essentially all of the 18-MEA is in the upper Beta layer of the cuticle-cuticle CMC. Maple syrup urine disease (MSUD) is a

genetic defect in humans and Poll Herford cattle [244] involving 18-MEA. MSUD is caused by a deficiency in the enzyme involved in the synthesis of 18-MEA. Isoleucine is a precursor in the biosynthesis of 18-MEA (an anteiso-fatty acid), involving the branched chain 2-oxo acid dehydrogenase, the enzyme that is deficient in this genetic defect [234]. Anteiso- fatty acids in skin are synthesized from the amino acid, isoleucine [245]. Jones and Rivett in their TEM studies of MSUD [234, 243] found that the structural defect of MSUD in human hair occurs only on the upper surface of cuticle cells (upper Beta layer) where 18-MEA is replaced by straight chain C18 and C20 fatty acids. But, the undersides of cuticle cells (lower Beta layer) are not affected in MSUD. These facts confirm that 18-MEA is attached to the top surface of cuticle cells (upper Beta layer) and not to the underside.

The proteins in the cuticle cell membranes are described in detail in this Chapter in the section entitled *Epicuticle and the Hair Fiber Surface*. In 1993, Negri et al. [122] proposed a model for the keratin fiber surface consisting of a monolayer of 18-MEA covalently bonded to an ultra high sulfur protein through a thioester linkage. These three scientists proposed this attachment at approximately 1 nm spacings. Furthermore, they suggested that the protein support was in the beta configuration and it might be attached to the Allworden membrane.

Although widely varying estimates of the thickness of the epicuticle have been made from 5 to 14 nm, one of the more recent and reliable estimates is by Swift and Smith [115]. These two scientists examined wool fiber, human hair and several other mammalian hairs using high resolution TEM. They identified that the epicuticle is approximately 13 nm thick and is rich in cystine. Swift's estimate of the epicuticle thickness is consistent with the maximum thickness reported by several other workers [129, 132, 246].

Leeder and Bradbury [100, 247] discovered that the Allworden reaction takes place with isolated cuticle cells from several different animal hairs including wool and human hair fiber, proving that this proteinaceous material completely surrounds each cuticle cell and is not a continuous external membrane on hair fibers. In this important scientific effort, cuticle cells were isolated by shaking animal hairs in formic acid. The isolated cuticle cells were then exposed to chlorine water. Formic acid is known to solubilize some proteins believed to be largely from the delta layer of the cell membrane complex. These effects on hair fibers will be discussed later in the section entitled *Proteins of the CMC*.

In the intact fiber Allworden sacs form over the top of cuticle cells (the exposed surface). Leeder and Bradbury suggested that "the sac always occurs on only one side of the cuticle cell" that is the top of cuticle cells and not the bottom [100, 236, 247]. They explained that this effect occurs because the connecting bonds on the top of cuticle cells are between the epicuticle and the A-layer and therefore are most likely through disulfide cross-links that are vulnerable to chlorine water oxidation [236]. Furthermore, they suggested that the connecting bonds on the underside of cuticle cells are between the membrane and the endocuticle (actually the inner layer, a layer about 10–40 nm thick [107] between the endocuticle and the cell membrane and similar in composition to the exocuticle). The bonding on the

underside of cuticle cells is resistant to chlorine water oxidation [236] and therefore could be amide linkages.

Negri et al. [122] determined that the Allworden reaction is an effect of the membranous proteins around cuticle cells. Furthermore, 18-MEA is not required for the formation of Allworden sacs because the sacs can be produced from cuticle in which 18-MEA has been removed by prior treatment with either methanolic KOH or potassium t-butoxide in t-butanol. Because of the bulky nature of the t-butoxide anion, it removes only covalently bound fatty acid at or near the fiber surface. Furthermore, Negri et al. [122] demonstrated that removal of the covalently bound fatty acid facilitates the formation of Allworden sacs because the rate of formation of the sacs increases with prior removal of the covalently bound 18-MEA.

Zahn et al. [133] proposed from indirect evidence using multiple regression analyses for the amino acids from Allen's Allworden membrane data that loricrin, involucrin and an ultra-high sulfur protein were in the epicuticle. These scientists were relating the cell envelope of keratin fibers to the cell envelope of human stratum corneum and the work of Steinert and Marekov [252], Jarnik et al. [253] and Steven and Steinert [254]. See Table 1.19, describing the amino acid analyses

Table 1.19 Amino acids (in mole%) of Allworden membrane vs. calculated values for Wool CE by Zahn et al. [133] and proteins at one time believed to be part of this membrane

A. Acid	Wool CE	H. Loricrin	H. Involucrin	H. UHSP	H. SPRP	Allworden
Asp	2.7	0.3	2.8	3.4	0	3
Glu	9.8	4.4	45.8	8.2	28	8.6
Thr	2.2	2.2	1.6	10.3	2.4	2.1
Ser	15	22.8	1.6	10.9	0.4	14.3
Tyr	0.2	2.5	0.8	1	0	0
Pro	4	2.9	5.7	9	31.2	4.2
Gly	24.5	46.8	6.7	5	0	23.8
Ala	3.2	1	1.5	1.4	0	3.2
Val	3.5	3.5	3.7	3.8	9.6	5.6
Iso	1.1	1.6	0.4	1.6	0	1.2
Leu	2.4	0	14.6	2.4	1.6	2.9
Trp	0	0.3	0	0	0	
Phe	0.8	2.9	0.6	0.8	0	0.4
His	0.9	0.3	4.7	0.7	0.8	0.2
Lys	5.3	2.2	7.4	3.7	12.8	4.5
Arg	1.7	0	0.7	5.6	0	2.5
Met	0	0	0.9	0	0	0
Cys	22.7	6	0.3	32.2	11.2	21.1
Totals	100	99.7	99.8	100	98	97.6

Wool CE calculated by Zahn et al. [133]
Human loricrin from Hohl et al. [248]
Human involucrin from Eckert and Green [249]
Human SPRP from Marvin et al. [250]
Human UHSP from Tezuka and Takahashi [251]
Allworden membrane from Allen et al. [132]

of these and other important proteins adapted from the paper by Zahn, Wortmann and Hocker.

However, more recently, Rogers and Koike [134] used laser capture microscopy to dissect the cuticle, cortex and inner root sheath of human hair fibers. In this manner, these scientists isolated RNA which was subjected to PCR analysis with specific primers to identify mRNA's encoding the surface proteins. No evidence was found for either loricrin or involucrin in the cuticle cell membrane sections, but evidence was found for KAPs 5 and 10 proteins that were likely cross-linked by both disulfide and isopeptide bonds.

Therefore, the proteins of the cuticle cell membranes are associated with the Allworden reaction [126] and are related to the epicuticle and from the work of Rogers and Koike [134] contain KAP's 5 and 10 ultra high sulfur proteins. Since the attachment of 18-MEA to hair proteins is through thioester linkages and the cuticle cell membrane protein is cross linked by cystine bridges, Negri et al. [122] proposed that the lipid layer must be attached to an ultrahigh sulfur protein (UHSP) that can provide attachment sites at approximately 1 nm spacings along the top of its folded chains. This attachment is likely to the KAP's 5 and or 10 proteins.

1.11.3 Bilayers Versus Monolayers in the Cuticle-Cuticle CMC

Whether or not the covalently bound lipids of the cuticle-cuticle CMC are bonded to another lipid layer on their hydrophobic end forming a bi-layer or they are bonded to a hydrophobic protein in the Delta layer is still debated, but this author believes the evidence clearly favors the monolayer model [107, 255] for the following reasons:

- If the Beta layers are mono-layers then 18-MEA is linked to the Delta layer through hydrophobic bonds making the upper Beta layer susceptible to failure at the Delta layer where it has been shown to fail [107, 255–257].
- Swift [107] pointed out that a monolayer model fits better from the point of view of CMC measurements, see the section below entitled, *Thickness of the Cuticle Beta Layers*. Free lipids are very likely in the cuticle Beta layers and the distribution and orientation of these will help determine the thickness of Beta layers.
- If bi-layers exist in the cuticle, there are two options for bonding of the second fatty acid layer to the Delta layer. One is for fatty acids to be covalently bonded to the Delta layer, but this option is not plausible because in human hair and wool fiber 40–50% of the covalently bound fatty acids are 18-MEA [258–260]. Therefore, there are insufficient covalently bound fatty acids in human hair and wool fiber to account for all these fatty acids. The other option is bonding of the second layer of fatty acids through hydrophobic linkages to the covalently bound fatty acids and bonding to the Delta layer through polar and ionic

bonding. However, this type of bonding would provide Beta-Beta failure and not Beta-Delta failure and allow for solvent removal of the non-covalently bound lipid layer which has been shown to occur in cortex-cortex CMC but not in cuticle-cuticle CMC [261, 262]. To provide Beta-delta failure from this bi-layer model, the new hair surface would form a bi-layer consisting primarily of hydrophilic acid groups at the very surface, so this bi-layer model is also not plausible.

- Negri et al. [263] noted that formic acid removes proteins more readily from the cortex-cortex CMC and it modifies CMC junctions of the cortex more than those of the cuticle which is consistent with covalent and hydrophobic bonding of the cuticle-cuticle CMC as shown by the monolayer model of Fig. 1.44, rather than a bi-layer model.

1.11.4 Thickness of the Cuticle Beta Layers

Much confusion exists about the actual thickness of the CMC monolayers as mentioned previously. Swift [232], from a TEM study cited 3 nm thickness for the cuticle Beta layers. Relatively recent analyses by microbeam diffraction [142, 143] also cite 3 nm thicknesses for these same layers between cuticle cells. Part of the confusion about the actual thickness and bi-layers vs. monolayers exists because many consider only the fatty acyl group of 18-MEA as the Beta layer. However, as the following discussion will show that view is not possible.

To measure the thickness of the beta layers Swift [232] used a uranyl acetate/lead citrate stain that stains only carboxylic acid groups. So he measured the unstained part of the TEM images for the Beta layers which he found to be approximately 3.0 nm thick [232]. Thus, only part of Swift's measurement was actually the length of the hydrocarbon portion of 18-MEA fully stretched out (from the thioester group to the end of the hydrocarbon chain which is about 2.39 nm long (for MEA attached at a 90° angle and fully stretched out), see Fig. 1.47. Assuming that the fatty acids of free lipids in between MEA chains are oriented so that the carboxylic acid groups are associating with the thioester groups which is the most stable orientation, then that is where the staining begins on one side because the stain reacts with carboxylic acid groups.

But, 18-MEA is attached to the protein membrane at an angle of approximately 72° (from molecular modeling). So, this angle provides a length of 2.28 nm for the acyl group of 18-MEA alone for the upper Beta layer. Now, since free lipid structures are in between these 18-MEA chains then 18-MEA will be fully stretched out from that angle of 72°.

Now, for the entire unstained part of the Beta layer we must also consider the fatty groups of the Delta layer proteins that the 18-MEA is bonded to all the way to the nearest carboxylic acid side chain; because that is where the staining begins on the other side of this lipid layer. Assuming that this outer protein of the Delta layer is in the Beta configuration and the Van der Waals bonding of 18-MEA is to a

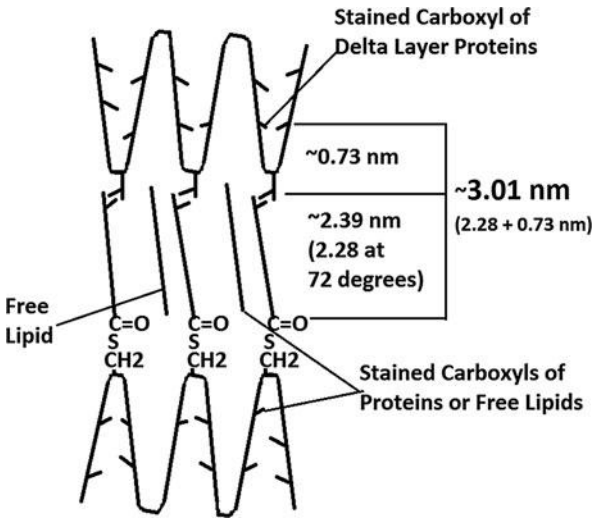

Fig. 1.47 Schematic illustrating the thickness of the cuticle upper Beta layer

hydrocarbon containing amino acid (which it must be), and the nearest amino acid contains a carboxylic acid unit, then this length is approximately an additional 0.73 nm.

Therefore, the hydrocarbon groups of the Delta layer proteins together with the hydrocarbon groups of 18-MEA form what is actually the unstained lipid Beta layer. So, the total calculated thickness of the Beta layer by TEM would be approximately 2.28 + 0.73 = 3.01 nm. Swift [232] found 3.00 nm in excellent agreement. Now if the fatty acid groups are oriented so that their carboxylic groups are near the terminal hydrocarbon end of 18-MEA, then the thickness would be even larger. But, this should be a less stable orientation and not consistent with XPS data showing that the surface of virgin keratin fibers is hydrocarbon-like [125].

1.11.5 Globular Versus Glycoproteins in the CMC

In formic acid extracts, Allen et al. [264] found evidence for glycoproteins in several different animal hairs which they suggested could be from the CMC. However, they suggested that these ingredients might also be remains of cell membrane glycoproteins from the follicle or they could be functional adhesive materials from the CMC. I believe the current evidence favors globular protein in the Delta layer as functional adhesive materials for these reasons:

- The Delta layer resists solubilization by aqueous reducing or oxidizing agents or by acids and alkalies [265]. If the CMC contains globular proteins like many other membranes, then they contain large domains of hydrophobic amino acids on their surfaces [266]. Such a surface is ideal for the hydrophobic ends of the

covalently bound fatty acids to adhere to. Furthermore, this type of globular protein should be resistant to aqueous reagents as Bryson found.

- Bryson et al. in 1995 [265] isolated lipid soluble lipoproteins from the Delta layer of cortex-cortex CMC and not glycoprotein.
- The Delta layer stains with Phosphotungstic acid (PTA). This is either a reaction of hydroxyl groups of a polysaccharide or a primary amine function. Swift [107] explained that this reaction is blocked with dinitrofluorobenzene (DNFB); therefore it is more likely a reaction involving primary amine groups, consistent with a globular protein.
- The Delta layer reacts with periodic acid/silver methenamine [107] a method for polysaccharides, however, Swift [107] also pointed out that since cystine interferes with this reaction, it is still consistent with a globular protein in the Delta layer.

Thus, the globular protein model is consistent with the currently known reactivity of the cuticle-cuticle CMC and the proposed structure of Fig. 1.44. Therefore the glycoproteins that Allen, Ellis and Rivet found were most likely remains of cell membrane material from the follicle.

1.11.6 The Cortex-Cortex CMC

Wertz and Downing [259] found that the percentage of 18-MEA relative to the total amount of covalently bound fatty acids varied from 38% to 48% in five different mammalian hairs including sheep, humans, dog, pig and cow. Table 1.20 summarizes a tabulation of analyses of the covalently bound lipids of wool and human hair from several different laboratories. These results were obtained after the fibers had been exhaustively extracted with chloroform/methanol to remove the non-covalently bound fatty acids and then the residue was saponified with methanolic alkali showing that 18-MEA accounts for about 50% of the covalently bound fatty acids in these wool fibers and about 40% in human hair.

Table 1.20 Covalently bound fatty acids in wool and human hair fiber

	Data for wool fiber					Data for human hair	
Fatty acid	[235]	[267]	[268]	[269]	[242]	Averages	[258]
16:0	8	11	8	17	20	12.8	18
18:0	8	12	6	10	25	12.2	7
18:1	7	8	5	5	0	5	4
MEA	51	43	72	48	55	53.8	41
Others	26	26	9	20	trace	16.4	30

Data are expressed in percentages_and references are in brackets

1.11.7 Covalently Bound Internal Lipids of Animal Hairs

Korner and G. Wortmann [242] (Table 1.20), analyzed covalently bound fatty acids in isolated wool cuticle and found 55% 18-MEA, 25% stearic and 20% palmitic acid with "only traces of other straight and odd number carbon chain fatty acids." For wool fiber Wertz and Downing [259] found 48% 18-MEA and 17% palmitic acid, 10% stearic acid, 5% oleic acid and the remaining covalently bound fatty acids ranged from C16 through C20 with 6% uncharacterized. For human hair, Wertz and Downing [258] found 41% 18-MEA, 18%, palmitic acid, 7% stearic acid, 4% oleic acid and the remaining small percentages of fatty acids from C16 through C20 with 9% uncharacterized. Negri et al. [268] found 72% 18-MEA, 8% palmitic acid, 6% stearic acid and 5% oleic acid in wool fiber.

The variation in these data from different laboratories is quite large. Part of the variance must be related to fiber diameter and the number of layers of covalently bound fatty acids in the fibers. However, certainly part of this variance is due to experimental error. The bottom line is that somewhere in the vicinity of 50 $\pm$ about 10% of the covalently bound fatty acids in most keratin fibers is 18-MEA and that hair fibers from sheep, humans, dog, pig and cattle and likely most keratin fibers contain palmitic, stearic and oleic with other fatty acids as the remaining covalently bound fatty acids.

In 1990, Kalkbrenner et al. [269] demonstrated with isolated cuticle cells that 18-MEA is essentially all in the cuticle. Since 18-MEA represents more than 40% of the total covalently bound fatty acids in human hair and about 50% in wool fiber, 18-MEA is confined to the upper Beta layer of the cuticle [243, 244] while most (essentially an amount equal to the 18-MEA) of the other covalently bound fatty acids are confined to the lower Beta layer. Therefore, most of the covalently bound fatty acids in wool and hair fiber must be in the cuticle-cuticle CMC with some in the cuticle-cortex CMC (to be described later) and virtually none in the cortex-cortex CMC. Therefore, if most of the covalently bound fatty acids are in the cuticle-cuticle CMC, then most of the lipids of the cortex-cortex CMC must be bound to the membranes on one side and to the Delta layer on the other side by non-covalent bonds. The fact that most of the remaining lipids can be removed by solvent extraction confirms that this is the case.

Leeder et al. [128] were the first to report that there are virtually no phospholipids in keratin fibers. This fact was confirmed by Schwan and Zahn [270] and by Rivett [271] casting doubt on whether lipid bi-layers could be involved in the cell membranes of keratin fibers [128]. However, Wertz et al. [272] demonstrated that liposomes (lipid bi-layers and a presumed precursor to the formation of lipid bi-layers in the CMC of keratin fibers) can form from lipids in the absence of phospholipids if an acid species such as cholesterol sulfate is present. Furthermore, evidence has been provided confirming the existence of cholesterol sulfate in human hair by Wertz and Downing [258] and by Korner et al. in wool fiber [273].

The work of Korner, Petrovic and Hocker [273] builds upon the findings of Wertz et al. on liposome formation and lipids from stratum corneum [272]. Korner et al. [273] demonstrated that cell membrane lipids extracted from human hair and wool fiber with chloroform/methanol/aqueous potassium chloride can form liposomes. These findings provide evidence for a bi-layer structure of the internal lipids of the Beta layers of the cortical CMC in wool fiber and in human hair, see Fig. 1.45. Such extracts must come primarily from the cortex-cortex CMC because covalently bound MEA and the other covalently bound lipids of the cuticle CMC are not removed with this solvent system.

Therefore, if the Beta layers of the cuticle cells are primarily covalently bound fatty acids with some free lipids (see Fig. 1.44) and the Beta layers of cortical cells consist primarily of lipid bi-layers (Fig. 1.45), then it is highly probable that the proteins that these different types of lipid layers are attached to, that is the cell membrane proteins and the Delta layer proteins of the cuticle cells and the cortical cells, are also different.

1.11.8 Differences in Cuticle-Cuticle, Cortex-Cortex and Cuticle-Cortex CMC

As early as 1975, Nakamura et al. [233] provided evidence from staining reactions that the disulfide content in the Delta layer in cuticle-cuticle CMC is lower than the disulfide content of the Delta layer in either cuticle-cortex or cortex-cortex CMC. In addition, Nakamura et al. added that the Delta layer of the cuticle-cuticle CMC stains similar to the endocuticle.

In 1983, Leeder et al. [128] used TEM to study the effect of solvents on wool fibers and found that formic acid treatment of wool modified the CMC of the fibers. This effect was only observed between adjacent cortical cells and not between cuticle and cortical cells. These scientists suggested that these results are consistent with differences in the CMC between cuticle cells vs. the CMC between cuticle and cortical cells.

Peters and Bradbury [274] observed that formic acid treatment of wool modified the cell membrane complex of the cortex “but that of the cuticle appears unchanged”. They also analyzed “resistant membranes”. These membranes were isolated by shaking wool fibers in formic acid and then oxidized with performic acid. This treatment produced an oxidized cell membrane material; however, the amino acid analysis produced considerably lower values for cystine than the analysis of Allworden membranes by Allen et al. [132]. Peters and Bradbury concluded that the “CMC of the cuticle differs from that of the cortex”.

Leeder et al. in 1985 [275] described differences in the staining characteristics of the cuticle-cuticle CMC, the cuticle-cortex CMC and the cortex-cortex CMC. After dyeing the fibers with a Uranyl dye these scientists found a layer of dye around each cuticle cell that was restricted to the CMC of the cuticle and not in the CMC of the

cortex. They found only one dye layer at the cuticle-cortex junction and none in the cortex-cortex CMC, but two layers of dye in the cuticle-cuticle CMC. In their paper, these scientists referred to the observations of Nakamura [233] on differences in the staining characteristics of these three types of CMC.

Mansour and Jones in 1989 [261] treated wool by Soxhlet extraction with chloroform/methanol for 5 h and subsequently in boiling water for 15 min. They examined the fibers by electron microscopy after each stage of treatment. After the initial solvent extraction, the cuticle-cortex CMC appeared unmodified, while the staining intensity of the Beta layers between cortical cells were changed and appeared "intermittent". After solvent extraction for 5 h and hydrolysis for 15 min significant structural changes were observed. The cortex-cortex CMC showed an overall reduction in definition in the Delta layer and the Beta layers displayed a lack of clear definition. These scientists suggested that solvent extraction of intercellular lipids makes the hair more vulnerable to hydrolytic damage with the largest changes occurring in the cortex-cortex CMC. These scientists related this effect to a reduction in tear strength of wool fiber by solvent extraction and hydrolysis. These results show that the cuticle-cortex CMC behaves differently from the cortex-cortex CMC to solvent extraction. The cuticle-cortex CMC is damaged by solvent extraction and subsequent hydrolysis, but not as severely as the cortex-cortex CMC.

Logan, Jones and Rivett [262] in 1990 examined wool fibers by TEM after extraction with chloroform/methanol and found that the cuticle-cuticle CMC appeared unchanged compared to untreated fibers. On the other hand they found that the Delta layer in the cortex was smaller and displayed variable staining intensity in most regions which they deduced as "incomplete or preferential extraction". These scientists examined fiber sections after chloroform/methanol extraction followed by treatment with formic acid. They noted large changes in the Beta and Delta layers of the cortex-cortex CMC which were "rarely observed" in the cuticle-cuticle CMC. They concluded that these results show "inherent differences exist between CMC's of cuticle and those of cortical cells".

Negri and Rivett et al. [263] in a paper in 1996 referred to the work of Leeder et al. [275] and cited the work of Leeder et al. [128] who showed that the unstained Beta layers of the cuticle and cortex react differently to formic acid treatment. Leeder and Marshall [276] demonstrated that formic acid removes proteins from the cortex-cortex CMC and it modifies the CMC junctions of the cortex but not the cuticle-cuticle CMC junctions and they referenced Nakamura [233], and Leeder et al. [128] and Peters and Bradbury [274] on these effects. They concluded that these observations suggest that only the Beta layers of the cuticle-cuticle CMC contain covalently bound lipids while the Beta layers of the cortex contain lipids and a "stain-resistant membrane protein" that is "likely to be of a different structure than the cuticle membrane".

Inoue et al. in 2007 [277] analyzed human hair by microbeam x-ray diffraction after extraction with polar organic solvents (methanol or chloroform/methanol) at 37°C for 6 h. These treatments remove some material from the Delta layer of the cuticle-cuticle CMC, but the Beta layers were unaffected. On the other hand, the

Beta layers of the cuticle-cuticle CMC appeared to be affected by hexane extraction under the same conditions. The observation that changes in the Delta layer of the cuticle-cuticle CMC by chloroform/methanol extraction could be detected suggests this method is more sensitive than TEM [262]. The fact that Inoue et al. observed changes in the Beta layers of the cuticle-cuticle CMC by hexane extraction could result from removal of free lipids between the covalently bound fatty acids of the cuticle-cuticle CMC resulting in folding back of the covalently bound fatty acids in the Beta layers accounting for the differences found.

The above discussion shows clearly that both the lipid Beta layers and the proteins of the cell membranes and those of the Delta layer of the cuticle-cuticle CMC differ from those of the cortex-cortex CMC, with evidence for differences from the cuticle-cortex CMC also.

1.11.9 The Structure of the Cuticle-Cortex CMC

The following proposal for the cuticle-cortex CMC (Fig. 1.46) is based on logic and the following supporting evidence. The work of Nakamura [233] suggested that the cuticle-cortex CMC differs from both the cuticle-cuticle CMC and the cortex-cortex CMC. The work of Leeder et al. [128] and of Mansour and Jones [261] demonstrated that the cuticle-cortex CMC is more resistant to solvents than the cortex-cortex CMC. But, the most convincing evidence for this model (Fig. 1.46) is the Uranyl dye study by Leeder et al. [275]. Treatment of wool fiber with Uranyl dye showed two layers of dye in the cuticle-cuticle CMC, one layer of dye in the cuticle-cortex CMC and no layers of dye in the cortex-cortex CMC.

Since the cuticle-cortex CMC bridges cuticle and cortical cells, it is logical to assume that it is a hybrid based partly on the cuticle-cuticle CMC and the cortex-cortex CMC. Therefore, the membrane on the cuticle side would be the cuticle cell membrane which supports covalently bound fatty acids that are bonded either through thioester, ester or amide linkages and these covalently bound fatty acids are connected on their hydrophobic end to a hydrophobic protein in the Delta layer.

The membrane on the cortex side is a cortical cell membrane that supports fatty acids bound through polar and salt linkages as illustrated in the schematic of Fig. 1.46 and these fatty acids form a lipid bi-layer. The Delta layer of the cuticle-cortex CMC then should contain a hydrophobic protein on one side (bound to the Beta layer on the cuticle side) and a hydrophilic protein on the opposite side bound through polar and salt linkages to the lipid bi-layer. Leeder et al. [275] in their TEM study on dyeing and diffusion suggested that either the cuticle Beta layer or the resistant membrane surrounding cuticle cells has an affinity for the uranyl dye whereas the cortical cell membrane or the Delta layer between cortical cells does not. The models of Fig. 1.46 (for the cuticle-cortex CMC), Fig. 1.44 (for the cuticle-cuticle CMC) and Fig. 1.45 (for the cortex-cortex CMC) are consistent with the results and explanation by Leeder et al. [275] of the uranyl dye binding in the different CMC's.

1.11.10 The Formation of the CMC in Developing Hairs

The following description of the formation of the CMC in the developing hair fiber was taken from the work of Rogers [26], plus from the early work by Orwin and coworkers [278] along with more recent work by Jones and coworkers [279]. For more details of the formation of the CMC in developing hair fibers, I refer you to the review by Jones and Rivett [125] and this paper by Jones, Horr and Kaplin [279].

In the latter stages of development of the hair fiber, desmosomes or intercellular bridges, gap junctions (where cells exchange molecules) and tight junctions (intercellular junctions where cell membranes fuse) are established between differentiating keratinocytes of the hair fiber and the inner root sheath to varying extents as they move upward in the hair follicle. Orwin et al. [278] described that gap junctions and desmosomes cover about 10% of the plasma membrane of cortical cells in the bulb region and then they gradually degenerate.

Tight junctions are established between Henle's outermost layer of the inner root sheath and Huxley's layer of the inner root sheath and between Henle cells and the close companion layer of the outer root sheath. These junctions are replaced with a new cell membrane complex that gradually develops as a continuous complex between the cells. Similar events should occur for cuticle-cuticle CMC, cuticle-cortex CMC and cortex-cortex CMC with appropriate distinctions.

1.12 The Medulla

Fraser et al. [169] suggested that fine animal hairs such as merino wool—consist only of cuticle and cortex, but with increasing fiber thickness, a third type of cell, the medulla, is usually found (see Figs. 1.3, 1.4 and 1.48). In thick animal hairs such as horse tail or mane or porcupine quill, the medulla comprises a relatively large percentage of the fiber mass. However, in human hair, the medulla—if present—generally comprises only a small percentage of this mass. The medulla may be either completely absent, or highly variable [280], for example, it may be continuous along the fiber axis, or discontinuous. In some instances, a double or divided medulla may be observed (see Fig. 1.49).

Medullary cells are loosely packed, and during dehydration (formation), they leave a series of vacuoles along the fiber axis see Figs. 1.48 and 1.49. At higher magnification medullary cells appear spherical and hollow inside and are bound together by a cell membrane complex type material (see Fig. 1.50). Menkart et al. [156] suggested that the medulla contributes negligibly to the chemical and mechanical properties of human hair fibers. Therefore, for human hair, medulla is of greater importance to forensic science (for hair comparison identification) than to cosmetic science.

Das-Chaudhuri and Chopra [281] compared medulla with scalp hair fiber diameters for 12 different populations from different geographical regions. These

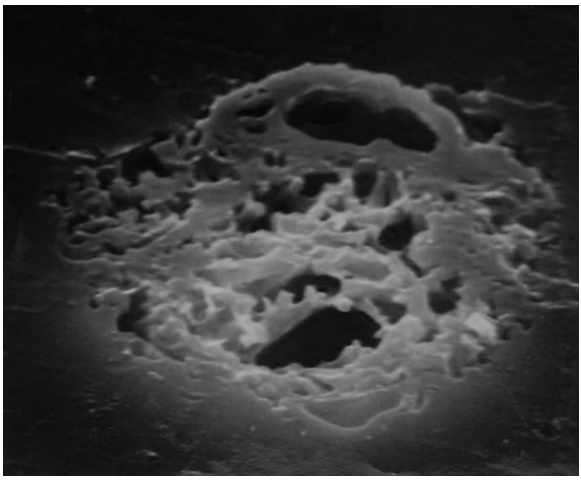

Fig. 1.48 Scanning electron micrograph illustrating the porous medulla of a hair fiber cross section

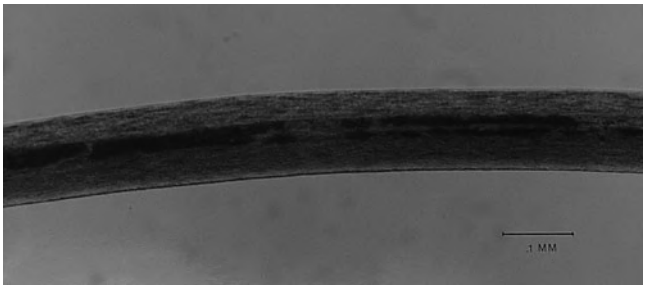

Fig. 1.49 An optical section of a light micrograph illustrating a hair fiber with a divided or double medulla. Multiple medullas seem more common in facial than scalp hair (Kindly provided by John T. Wilson)

scientists considered only hairs with and without medulla and considered a medullary ratio of P/Q where P represents the total number of medullated hairs and Q the number of total hairs. One hundred hairs per individual were examined. Their data provided a significant correlation between hair diameters and medulla with a correlation coefficient of 0.58 and an index of determination of 0.34.

Banerjee [282] collected data from 12 different populations in India where he considered hair fiber diameter and three medullation types: hairs with a continuous medulla, a discontinuous medulla and hairs with no medulla. Examination of the means of the different diameters of these three medulla classes by the matched pairs test shows a highly significant relationship with $p > t = 0.02$. The hairs with no medulla were the finest, those with a discontinuous medulla were medium in diameter and those with a continuous medulla were the coarsest. Hardy [106] also found a positive correlation between human scalp hair fiber diameter and medullation.

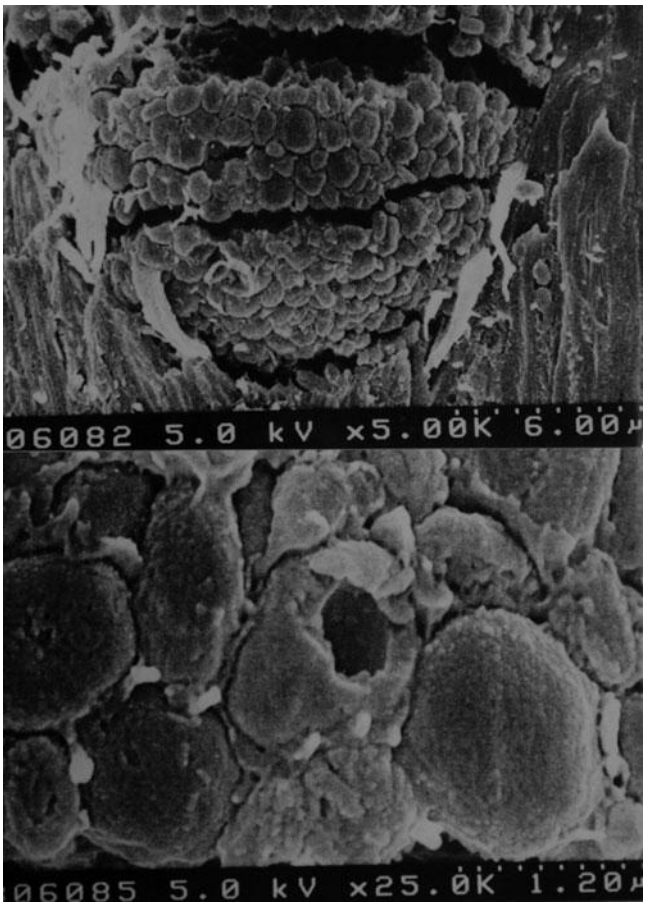

Fig. 1.50 Scanning electron micrograph illustrating the hollow sphere-like structures of the medulla (Kindly provided by Sigrid Ruetsch)

Wynkoop [283] classified hairs according to four different medulla types: absent, scanty, broken and continuous. She considered age and fiber diameter vs. medulla type. Wynkoop concluded that the amount and type of medulla are not related to age, but the amount of medulla is related to hair fiber diameter and that the finest hairs generally do not contain a medulla, medium-sized hairs generally contain a broken medulla and the thickest hairs generally contain a continuous medulla. So, there is a strong positive relationship between hair fiber diameter and the amount of medulla; thus fine hair of children generally does not contain a medulla [49], but coarser hairs of adults generally contains either a discontinuous or continuous medulla.

Tolgyesi [153] demonstrated that beard hair is coarser than scalp hair and it contains a higher percentage of medulla than scalp hair. There is generally more medulla in the coarser hairs of Asians than Caucasians; however, many Caucasian

hairs do contain medulla. The more fine the individual hair fiber, the lower the probability that medulla is present and the lower percentage of medulla mass.

At one time the presence of keratin proteins in the medulla was questioned [284]. In addition, Dobb [285] indicated that medulla of many hairs is difficult to isolate and therefore has received little scientific attention, however, Langbein et al. [280] demonstrated that several keratin proteins and a few cortical cells can be found in human beard hair medulla. These scientists found that 12 hair keratins and 12 epithelial keratins are potentially expressed in medullary cells and the keratin arrangement is very irregular in each medulla cell. The chemical composition of medullary protein derived from African porcupine quill has been reported by Rogers [205] and is described in Chap. 2 along with additional details on the composition of medulla from human beard hair and the medullary proteins by Langbein et al. [280].

The medulla does seem to play a role in gray hair, as suggested by Nagase et al. [286] by scattering light through a change in refractive index at the air to hair interface of medullary "pores". This effect is analogous to the effect in the genetic abnormality of pili annulati also known as ringed hair. Pili annulati appears as bands or rings of silver/gray and dark regions along the fiber axis. These bands are not associated with pigmentation. Musso [287] working with guidance from RDB Fraser observed that ringed hair contains bands or areas with air spaces in the cortex along the axis that correspond to the silver or gray bands. The air spaces are believed to be caused by a defect in the synthesis of the microfibril-matrix complex in the cortex, most likely with less being produced. This effect creates cavities or air spaces in the hair [287], see the section entitled *Hair Abnormalities* in Chap. 3. The medulla may also be involved in the splitting of hairs since in addition to the CMC it provides a pathway or an area of weakness for the propagation of cracks along the axis of the fiber as described by Kamath and Weigmann [200].

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