

Chapter 2

Chemical Composition of Different Hair Types

Abstract Human hair consists of proteins, lipids, water, trace elements and pigments. The composition of the first four of these components is the focus of this Chapter. About two decades ago the emphasis on the proteins of hair was on its amino acid constituents which provided important information on the relative amounts of different functional groups in different types of hair and in different regions of the fiber. However, as a result of advances in the characterization and classification of the different proteins and genes of keratins and keratin associated proteins the focus today is on the proteins themselves. Several important new contributions to the composition of the surface layers of hair and the proteins of the cell membrane complex have been and are continuing and therefore are summarized in this Chapter. The current state of changes in the amino acids, proteins and lipids of hair by morphological region (including KAP and keratin proteins and where they reside), chemical and sunlight damage, diet, puberty and menopause, and other factors have been and are being made and are summarized here. An expanded section on metals in hair, where in the fiber these metals reside and the functional groups that they bind to and their effects on hair chemistry, toxicity and disorders are included.

2.1 Introduction

Several important new and relatively recent contributions to the structure of the cell membrane complex, the composition of the surface layers of hair, the overall structure of the hair fiber and its follicle have been added to this Chapter. Recent studies revealed details about endogenous and exogenous hair lipids and the critical involvement of proteins and free lipids in the surface layers of hair including lipid contributions to the protective properties of the cuticle and the isoelectric point. Advances in the classification and characterization of the different proteins and genes involved in keratin and keratin associated proteins in human hair are summarized in this Chapter and the analysis of protein fragments from hair

damaged by cosmetic chemicals is a new and exciting area for future research. The effects of menopause on changes in the lipids of scalp hair have been added to this Chapter and the recently found effects of menopause on the diameter of hair fibers have been added to Chap. 9.

Human hair is a complex tissue consisting of several morphological components (see Chap. 1), and each component consists of several different chemical types [1]. Hair is an integrated system in terms of its structure and its chemical and physical behavior wherein its components can act separately or as a unit. For example, the frictional behavior of hair is related primarily to the cuticle, yet, the cuticle, the cortex and its intercellular components act in concert to determine the softness of hair. The tensile behavior of human hair is determined largely by the cortex, yet we have learned that the physical integrity of the fiber to combing and grooming forces is also affected by the non-keratin components of the cuticle and the cell membrane complex. Nevertheless, for simplicity and ease of discussion, the different types of chemicals that comprise human hair are generally described separately in this Chapter.

Depending on its moisture content (up to 32% by weight), human hair, consists of approximately 65% to 95% proteins. Proteins are condensation polymers of amino acids. The structures of those amino acids that are found in human hair are depicted in Table 2.1. Because of the large number of chemical reactions that human hair is subjected to by permanent waves, chemical bleaches, alkaline straighteners and sunlight exposure, many of the proteins are fragmented and several of these amino acids are converted to amino acid derivatives depicted in Table 2.2. The remaining constituents are water, lipids (structural and free), pigment, and trace elements that are generally not free, but combined chemically with side chains of protein groups or with fatty-acid groups of sorbed or bound lipid. These different components of hair: proteins, lipids, water and trace elements are described separately in this Chapter while pigments are described in more detail in Chap. 5.

Studies of the proteinaceous matter of human hair may be classified according to the following types of investigation:

- Studies of individual or several amino acids,
- Analysis of types of amino acids,
- Fractionation and peptide analysis,
- Expression of genes, using in situ hybridization or reverse transcriptase-polymerase chain reaction (RT-PCR) expression by hair follicles or the use of specific protein antibodies or related techniques.

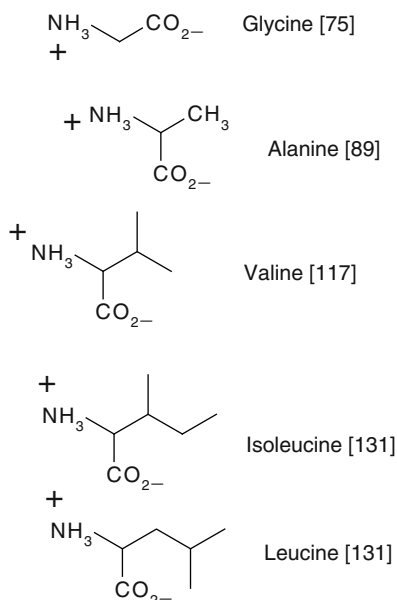
Most studies of individual amino acids of keratin fibers involve the amino acids cystine or tryptophan. Quantitation of cystine can be accomplished by chemical analysis of mercaptan with [2, 3] or without hydrolysis [4] or spectrophotometrically on intact hair [5, 6]. With increasing sophistication in instrumental analysis, ESCA, SIMS, and different absorbance, reflectance and fluorescence techniques, spectrophotometric analysis on intact hair is becoming increasingly important.

Chemical analyses for tryptophan have been described by Block and Bolling [7] and are all hydrolytic procedures.

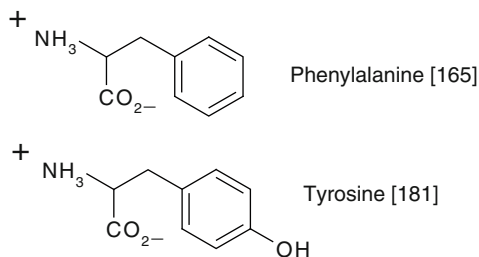
McMillen and Jachowicz [8] based on prior work in the wool industry analyzed tryptophan and its kynurenine reaction products by fluorescence spectroscopy using excitation wavelengths of 290, 320 and 350 nm which provides emission bands at 345, 420 and 465 nm. The emission band with a maximum at 345 nm corresponds to Tryptophan with an absorption maximum at about 360 nm. The emission peak at 465 nm from excitation at 320 and 350 nm matches the emission band of 1-kynurenine which has an absorption maximum at about 360 nm. The emission maximum at 420 nm was ascribed to N-Formylkynurenine and has an absorption

Table 2.1 Structures of amino acids found in hydrolyzates from human hair (molecular weights of these amino acids are listed in brackets)

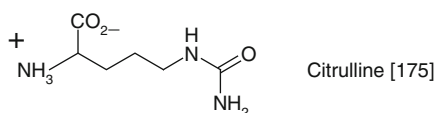
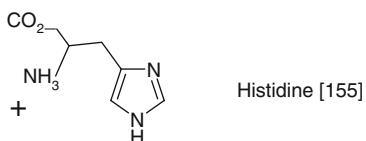
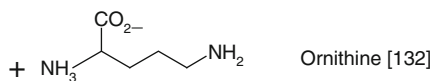
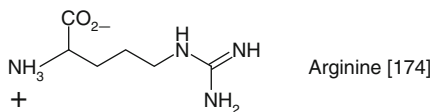
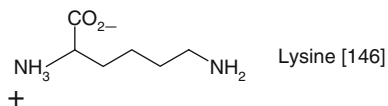
Aliphatic Hydrocarbon R Group



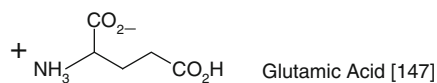
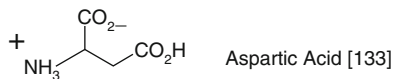
Aromatic Hydrocarbon R group



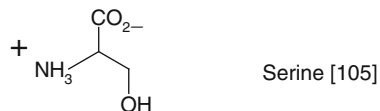
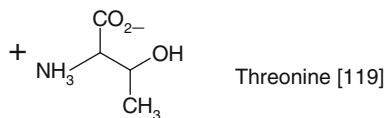
Diacidic Amino Acids



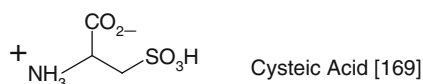
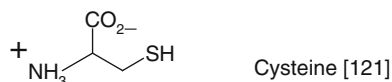
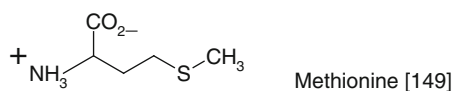
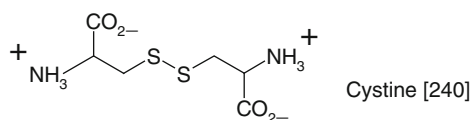
Diacidic Amino Acids



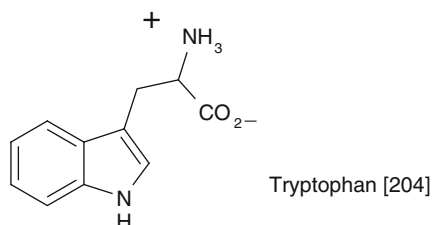
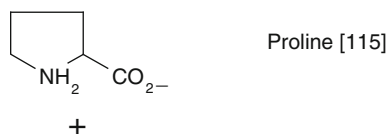
Hydroxyl containing amino acids



Sulfur containing amino acids



Heterocyclic amino acids in hair



Aspartic acid and glutamic acids exist as the primary amides and the free acids in human hair

maximum at 320 nm. In this paper on thermal degradation of hair, the authors claimed that the spectra after thermal exposure indicate a decrease in the emission intensities of all bands, probably related to thermal decomposition of the corresponding chromophores. The largest reduction in the emission intensity is evident for the band at 345 nm corresponding to Tryptophan providing evidence for its photochemical degradation.

Quantitative determination of several amino acids in human hair became increasingly widespread years ago following the development of the ion exchange chromatographic systems of Moore and Stein [9]. But more recently, protein

Table 2.2 Structure of amino acid degradation/derivative products found in human hair^a*Derivatives of cystine*

Cystine oxidation products from peroxide bleaching

–CH–CH₂–S–SO–CH₂–CH– cystine monoxide–CH–CH₂–S–SO₂–CH₂–CH– cystine dioxide–CH–CH₂–SO₃M cysteic acid salt

From sulfite perms and sunlight oxidation

–CH–CH₂–S–SO₃M Bunte salt

From TGA perms

–CH–CH₂–S–S–CH₂–CO₂M

From GMT perms

–CH–CH₂–S–S–CH₂–CO–O–CH₂–CH(OH)–CH₂–OH

Hydrolysis gives the derivative above from TGA perms

From cysteamine perms

–CH–CH₂–S–S–CH₂–CH₂–NH₂*HX

From strong alkalinity as (straighteners, perms, bleaches)

–CH–CH₂–S–CH₂–CH– lanthionine–CH–CH₂–NH–(CH₂)₄–CH– lysinoalanine*Derivatives of amino acids other than cystine*

From strong alkalis (hydrolysis of amides)

–CH–CH₂–CO₂M–CH–CH₂–CH₂–CO₂M

From chemical oxidation

–CH–CH₂–CH₂–SO₂–CH₃ methionine sulfone (sulfoxide not demonstrated)

From TGA perms

–CH–(CH₂)₄–NH–CO–CH₂–SH thioacetylated lysine

From sunlight oxidation

–CO–CO–R alpha keto derivatives and cross-links of these with amino groups

^aDegradation of other amino acids such as tryptophan, lysine and histidine are known to occur from sun exposure, however, identification of the degradation products has not been made

sequencing techniques such as the use of Polymerase chain reaction (PCR) primers, or analysis of cDNA's with sequences that code specific proteins or even digestion to specific peptides and analysis by mass spectrometry or the use of specific protein antibodies or other techniques have become increasingly important. Studies of amino acid types are also used today, but less frequently. These involve determination of a specific functional group where more than one amino acid contains that type of group such as, the titration of basic groups [10] and of acidic groups [10].

Fractionation and peptide analysis is concerned primarily with fractionation into similar peptide types or even fractionation into the different morphological components. Major areas of hair research concerned with the chemical composition of hair and wool fibers, over the last two decades, have involved proteomics or the determination of the total proteins present in a fraction or region of the hair. This definition has been extended by some to include determining the proteins from

which fractions are derived from chemical degradation of hair by perming, oxidation and straightening reactions. Several important papers have been published defining and classifying the types of proteins in human hair fibers. An initial classification of hair proteins was described by Powell and G.E. Rogers. Important additions to this work have been reviewed in papers by M.A. Rogers and Langbein et al. that are described in detail and referenced in Chap. 1 as well as in this Chapter in the section entitled, *Major Protein Fractions of Hair*. In addition, the structure, composition and degradation of the cuticle, the cortex and medulla, the cell membrane complex and the composition of structural hair lipids are the major focus of this Chapter.

2.2 The Amino Acids and Proteins of Different Types of Hair

2.2.1 Whole-Fiber Amino Acid Studies

More than three decades ago, a large number of investigations were described on the analysis of the amino acids of whole human hair fibers. Whole-fiber amino acid analysis has several limitations, because it provides average values for the amino acid contents of the average proteinaceous substances of the fibers. Therefore, for whole-fiber results, cross-sectional and axial differences in the composition of the fibers are averaged.

A second complicating factor is hydrolytic decomposition of certain amino acids. The most commonly used medium for keratin fiber hydrolysis is 5–6 N hydrochloric acid. In studies involving acid hydrolysis of keratins, partial decomposition has been reported for cystine, threonine, tyrosine [11], phenylalanine, and arginine [12] with virtually complete destruction of tryptophan [12].

With the above limitations in mind, the following discussion describes several important factors contributing to differences in the whole-fiber amino acid analysis results of human hair, reported in the literature.

2.2.1.1 Unaltered or “Virgin” Human Hair

Unaltered human hair is hair that has not been chemically modified by treatment with bleaches, permanent waves, straighteners, or hair dyes. Numerous publications [7, 13–28] describe results of the amino acid analysis of unaltered human hair. Table 2.1 depicts the structures for 22 amino acids that have been identified in human hair. Cysteic acid and other amino acids, derived from those amino acids of Table 2.1, are also present in either weathered or cosmetically altered hair, see Table 2.2. Table 2.3 summarizes results from several sources describing quantitative whole fiber analyses of these 22 amino acids. These same amino acids are classified according to functional group in Table 2.4.

Table 2.3 Amino acids in whole unaltered^a human hair (micromoles per gram dry hair)

Amino acid	Reference [13]	Reference [14]	Other references
1. Aspartic acid	444–453 ^b	292–578 ^c	
2. Threonine	648–673 ^b	588–714	
3. Serine	1,013–1091 ^b	705–1,090	
4. Glutamic acid	995–1036 ^b	930–970	
5. Proline	646–708 ^d	374–694 ^d	
6. Glycine	463–513 ^d	548–560	
7. Alanine	362–384 ^d	314	
8. Half-cystine	1,407–1,512 ^d	1,380–1,500	784–1,534 [15] ^d
9. Valine	477–513 ^d	470	
10. Methionine	50–56 ^b	47–67	
11. Isoleucine	244–255 ^d	366	
12. Leucine	502–529 ^d	489 ^c	
13. Tyrosine	177–195 ^d	121–171 ^c	
14. Phenylalanine	132–149 ^d	151–226	
15. Cysteic acid	22–40 ^d	–	
16. Lysine	206–222 ^b	130–212 ^c	
17. Histidine	64–86 ^d	40–77	
18. Arginine	499–550 ^d	511–620	
19. Cysteine	–	41–66	17–70 [15] ^d
20. Tryptophan	–	20–64	
21. Citrulline	–	–	11 [17]
% Nitrogen as ammonia		15.5–16.9%	16.5% [16]

^aHair is assumed to be cosmetically unaltered for Refs. [14, 15, 17]^bNo significant differences among samples analyzed^cThe circled values are results of a microbiological assay by Lang and Lucas [18]^dSignificant differences indicated among samples analyzed^eThese results are a compilation of results from several laboratories and therefore contain no basis for statistical comparison of each individual amino acid from the different laboratories

Note the high frequencies of hydrocarbon, hydroxyl, primary amide, and basic amino acid functions in addition to the relatively large disulfide content. The high frequency of hydrocarbon-containing amino acids confirms that hydrophobic interactions play a strong role in the reactivity of hair toward cosmetic ingredients. Hydroxyl and amide groups interact through hydrogen bonding interactions, while the basic and carboxylic acid groups interact through hydrogen bonding and ionic bonding type interactions.

Of particular note is the fact that most of these functional groups occur at higher frequencies than the disulfide bond in hair. However, these frequencies are whole-fiber frequencies, therein assuming that hair is a homogeneous substrate. This assumption is certainly not the case, as subsequent sections of this Chapter demonstrate.

Table 2.3 shows substantial variation in the quantities of some of the amino acids, notably aspartic acid, proline, cystine, and serine, while considerably less

Table 2.4 Approximate composition unaltered human hair by amino acid side-chain type

Amino acid side-chain type ^a	Approximate micromoles per gram hair
1. <i>Hydrocarbon</i> (except phenylalanine)	2,800
Glycine, alanine, valine, leucine, isoleucine, and proline	
2. <i>Hydroxyl</i>	1,750
Serine and threonine	
3. <i>Primary amide + carboxylic acid</i>	1,450
Primary amide (ammonia estimation)	1,125
Carboxylic acid (by difference)	325
4. <i>Basic amino acids</i>	800
Arginine, lysine, and histidine	
5. <i>Disulfide</i>	750
Cystine	
6. <i>Phenolic</i>	180
Tyrosine	

^aSee Table 2.3

dispersion is indicated for valine, glutamic acid, glycine, alanine, leucine, and arginine.

The following factors can produce differences in whole-fiber amino acid analysis results; genetics, weathering (primarily sunlight exposure), cosmetic treatment, experimental procedures, and diet (not normal diets of healthy individuals, but protein deficient diets).

Marshall and Gillespie [29] proposed special mathematical relationships between cystine and leucine:

- Leucine (residue %) = $-0.31 \times \text{half-cystine (residue \%)} + 11.3$ and between cystine and proline to determine abnormal variations:
- Proline (residue %) = $0.26 \times \text{half-cystine (residue \%)} + 3.8$

These relationships are based on the fact that leucine and cystine are common components of the low sulfur proteins, while proline and cystine are primary components of the high sulfur proteins. They further suggest that the cystine content should be about 17–18% and that large variations beyond the calculated values for these three amino acids indicates some cause of variation such as genetic, environmental (sunlight exposure), cosmetic treatment, diet, etc. Variation from these factors is described next.

2.2.1.2 Amino Acid Composition Related to Genetics

The variation of cystine and cysteine in human hair has been studied extensively. Clay et al. [15] quantitatively analyzed hair from 120 different persons for cystine and cysteine (see Table 2.3). The hair in this study was selected from both males and females of varying age and pigmentation. Analysis was by the hydrolytic

method of Shinohara [30]. These results show a wide spread in disulfide content varying from 784 to 1,534 μmol half-cystine per gram of hair (8.7–17%); substantially different from the cystine level suggested by Marshall and Gillespie for “normal” hair. Significantly more cystine was found in hair from males than females. Also, dark hair generally contained more cystine than light hair. A similar relationship between cystine content and hair color has been reported by Ogura et al. [31].

No consistent relationship was found between age and cystine content. Although factors such as diet (malnutrition), cosmetic treatment, and environmental effects (sunlight degradation) may have contributed to variation among these samples, such factors were not considered in this study.

With regard to racial variation, nothing has been definitely established. Hawk’s data [23] appears to show subtle differences in the relative percentages of various amino acids found in the hydrolysates of African hair compared to Caucasian hair. Wolfram compiled a more complete set of data from the literature of whole-fiber amino acid analysis of the three major geo-racial groups, showing overlap in the amounts of all the amino acids from scalp hair for these three groups [32]. See the section in Chap. 1 entitled *The Origin of Hair Fiber Curvature* which explains the distribution and composition of different types of cortical cells in hair. Quantitative protein techniques in the section entitled *Major Protein Fractions of Hair* in this Chapter and SNP analysis (Chap. 3) rather than amino acid analysis provides the best means for determining the differences in the proteins of scalp hair of different geo-racial groups.

2.2.1.3 Weathering of Human Hair

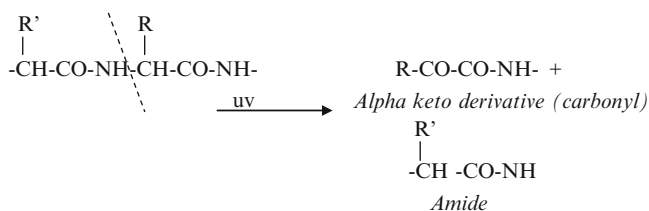
The photochemical degradation of cystine (see Chap. 5) provides a major cause for variation in this amino acid among different hair samples. Weathering effects [33] in human hair may be explored by comparing tip ends (longer exposed) to root ends. In a study by Robbins, the cystine and cysteine contents of tip ends were shown to be lower than in root ends [34]. Complementary to these results, larger amounts of cysteic acid have been reported in hydrolysates of tip ends of human hair than in root ends [13]. Evidence for cysteic acid in weathered wool has also been provided by Strasheim and Buijs by infrared spectroscopy [35] and for some South African Merino wools by Louw [36].

These results suggest conversion of thioester and cystinyl groups in human hair to higher oxidation states by the elements. This conclusion is supported by the work of Harris and Smith [37], who determined that ultraviolet light disrupts the disulfide bond of dry wool. In another study, Robbins and Bahl [6] examined both the effects of ultraviolet light on hair from root and tip sections from several persons using electron spectroscopy for chemical analysis (ESCA) to examine different types of sulfur in hair. Their data suggested that weathering of cystine in hair is primarily a

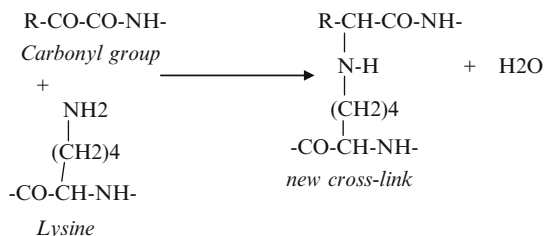
photochemical reaction proceeding mainly through the C-S fission route producing cystine S-sulfonate residues as a primary end product. This reaction also occurs to a greater extent near the fiber surface showing that oxidation of thioester to sulfonate and loss of MEA also occurs by photochemical degradation. McMillen and Jachowicz [8] found that Tryptophan is sensitive to degradation by heat. It is also sensitive to photochemical degradation. Significantly lower quantities of the dibasic amino acids lysine and histidine have been reported in tip ends of human hair compared to root ends [34].

As indicated, for hair damaged by sunlight, in most cases, the amino acids of the cuticle are altered to a greater extent than those of the cortex because the outer layers of the fiber receive higher intensities of radiation. Hair protein degradation by light radiation has been shown to occur primarily in the wavelength region of 254–400 nm. More recent work by Hoting and Zimmerman [38] shows that the proteins of the cuticle are degraded by UV-B and UV-A, but less by visible light and that cystine, proline and valine are degraded more in light brown hair than in black hair. In other words the photo-protective effect of melanin is much better in dark hair than in light hair.

Oxidation at the peptide backbone carbon has been shown to occur from ultraviolet exposure both in wool [39] and in hair [6, 40], producing carbonyl (alpha keto amide intermediates as shown below) which are favored in the dry state reaction more than in the wet state. This reaction is similar to the oxidative damage to proteins and mitochondrial decay associated with aging described by Dean et al. [41] and described in detail in Chap. 5.



The photochemical breakdown of disulfide bridges within structural units of the A-layer and the exocuticle and matrix of the cortex and the establishment of new intra- and intermolecular cross-links via reaction of these carbonyl groups (from uv degradation) with protein amino groups (primarily lysine as shown below) within and between structural units decreases structural definition. These reactions most likely lead to a gradual increase in brittleness and a gradual loss of structural differentiation, see Chap. 5 for details and micrographs that support these conclusions.



2.2.1.4 Experimental Procedures

The inconsistent use of correction factors to compensate for hydrolytic decomposition of certain of the amino acids has already been described. In addition, methods of analysis described in the literature have ranged from wet chemical [20], to chromatographic [13], to microbiological [18]. Reexamination of Table 2.3 with this latter condition in mind shows values for aspartic acid, proline, tyrosine, and lysine as determined by the microbiological assay to be in relatively poor agreement with the other values for these same amino acids determined by wet chemical and chromatographic procedures. In the case of valine, the values for the microbiological and chromatographic procedures are in close agreement. This suggests that for certain of the amino acids (valine) the microbiological assay is satisfactory, whereas for other amino acids (aspartic acid, proline, tyrosine, and lysine), the microbiological method is questionable.

2.2.1.5 Stability of Hair Keratin

Several years ago, a well-preserved cadaver was discovered by archaeologists in the Han Tomb No. 1 near Changsha, China [42]. In the casket, the occupant wore a well preserved hair piece that was more than 2,000 years old. Although this hair was not analyzed for amino acid content, it was analyzed by x-ray diffraction by Kenney [42], revealing that the alpha-helical content had been well preserved. Nevertheless, some minor disruption of the low ordered matrix had occurred owing to reaction with a mercurial preservative in the casket. This suggests that the basic structure of the intermediate filaments of human hair remains unchanged over centuries and its essential structural features are extraordinarily stable but the mercury preservative may be reacting with cystine in the matrix.

2.2.1.6 Cosmetically Altered Hair

Bleached Hair

The whole-fiber amino acid composition of human hair, bleached on the head with commercial hair-bleaching agents – alkaline hydrogen peroxide or alkaline

peroxide/persulfate [43] has been described in the literature [11]. This investigation defines the amino acids found in hydrolysates of hair bleached to varying extents on the head. Data describing frosted (extensively bleached hair using alkaline peroxide/persulfate) vs. non-bleached hair from the same person, bleached on the head about 1 month prior to sampling, are summarized in Table 2.5. These data show that the primary chemical differences between extensively bleached hair and unaltered hair are lower cystine content, a higher cysteic acid content, and lower amounts of tyrosine and methionine in the bleached hair. Mildly to moderately bleached hair shows only significantly lower cystine and correspondingly more cysteic acid than unaltered hair. These results support Zahn's [44] original conclusion that the reaction of bleaching agents with human hair protein occurs primarily at the disulfide bonds. Fewer total micromoles of amino acids per gram of hair are found in bleached than in unaltered hair (see Table 2.5) most likely because of addition of oxygen to the sulfur containing amino acids and to solubilization of protein or protein derived species into the bleach bath [45].

Products of disulfide oxidation, intermediate in oxidation state between cystine and cysteic acid (see Table 2.6), have been shown to be present in wool oxidized by aqueous peracetic acid [46–48]. These same cystine oxides have been demonstrated at low levels in bleached hair [49]; however, disulfide oxidation intermediates have not been shown to exist in more than trace amounts in hair oxidized by currently used bleaching products [50].

The actual presence of large amounts of cysteic acid in bleached hair had at one time been in doubt [51, 52]. It had been theorized that the cysteic acid found in

Table 2.5 Amino acids from frosted vs. non-frosted hair

Amino acid	Micromoles per gram hair		Significant difference for frequencies at $\alpha = 0.01$ level
	Non-frosted fibers	Frosted fibers	
Aspartic acid	437	432	–
Threonine	616	588	–
Serine	1,085	973	–
Glutamic acid	1,030	999	–
Proline	639	582	–
Glycine	450	415	–
Alanine	370	357	–
Half cystine	1,509	731	Yes
Valine	487	464	–
Methionine	50	38	Yes
Isoleucine	227	220	–
Leucine	509	485	–
Tyrosine	183	146	Yes
Phenylalanine	139	129	–
Cysteic acid	27	655	Yes
Lysine	198	180	–
Histidine	65	55	–
Arginine	511	486	–

Table 2.6 Some possible oxidation products of the disulfide bond

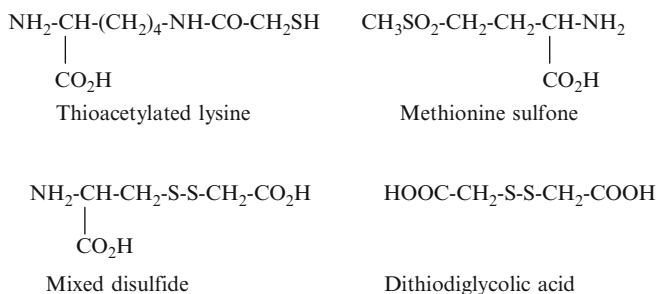
Formula	Name
$R-SO-S-R$	Disulfide monoxide
$R-SO_2-S-R$	Disulfide dioxide
$R-SO_2-SOR$	Disulfide trioxide
$R-SO_2-SO_2-R$	Disulfide tetroxide
$R-S-SO_3H$	Bunte acid or thiosulfonic acid
$R-SO_3H$	Sulfonic acid

bleached hair hydrolysates was formed by decomposition of intermediate oxidation products of cystine during hydrolysis prior to the analytical procedure [51]. However, differential infrared spectroscopy [5] and electron spectroscopy for chemical analysis by Robbins and Bahl [6] on intact un-hydrolyzed hair have conclusively demonstrated the existence of relatively large quantities of cysteic acid residues in chemically bleached hair. Evidence for other sulfur acids, e.g., sulfinic or sulfenic acids, in bleached hair has not been provided. Furthermore, it is unlikely that these amino acids exist in high concentrations in hair, because they are relatively unstable. For details concerning the mechanism of oxidation of sulfur in hair, see Chap. 5.

Permanent-Waved Hair

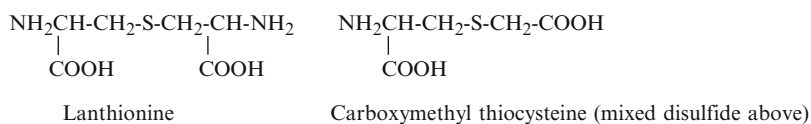
Nineteen amino acids in human hair have been studied for possible modification during permanent waving, that is all of the amino acids of Table 2.1 except tryptophan, citrulline and ornithine. Significant decreases in cystine (2–14%) and corresponding increases in cysteic acid [2, 11] and in cysteine [2] have been reported for human hair that has been treated either on the head by home permanent-waving products or in the laboratory by thioglycolic acid and hydrogen peroxide, in a simulated permanent-waving process.

Trace quantities (less than 10 $\mu\text{mol/g}$) of thioacetylated lysine and sorbed thioglycolic acid have also been reported in human hair treated by cold-waving reagents [2]. Small quantities of mixed disulfide [2, 6], sorbed dithiodiglycolic acid [2], and methionine sulfone [11] have been found in hydrolyzates of hair treated by the thioglycolate cold-waving process.



Methionine sulfone is presumably formed by reaction of the neutralizer with methionine residues; thioacetylated lysine is probably formed by reaction of lysine with thioglycolide impurity in the thioglycolic acid [2]. The mixed disulfide is presumably formed by displacement of thioglycolate on the cystine residues in hair (see Chap. 4 for mechanistic details). One might also expect to find trace quantities of methionine sulfoxide in hair; however, to date this sulfoxide has not been reported.

In alkaline media, the formation of lanthionine residues can also occur [53], see Chap. 4. Zahn et al. [54, 55] reported that thioglycolate can accelerate the rate of formation of thioether residues (lanthionyl) in wool fiber. Therefore, one might expect to find trace quantities of this amino acid in hair permanent-waved in an alkaline medium. Chao et al. [56] demonstrated small quantities of lanthionine and carboxymethyl thiocysteine (see Chap. 4) in hair reduced by thioglycolic acid.



Analytical procedures involving reduction and determination of mercaptan are not accurate determinations of cystine in permanent-waved hair or in hair treated with mercaptan, because mixed disulfide is reduced to mercaptan during analysis, and adsorbed mercaptan can also interfere in the determination. Procedures that do not involve reduction of hair such as ninhydrin detection (alpha-amino group) or dinitrofluorobenzene (DNFB) reaction followed by chromatographic separation [1, 54] discriminate between mercaptans and therefore should be better analytical procedures for detecting the different types of mercaptans and disulfides actually present in permanent waved hair.

Hair Straightened with Alkaline Straighteners

The process of straightening hair with alkaline straighteners is described in Chap. 4 and as shown in that section, relatively large quantities of lanthionine (>100 $\mu\text{mol/g}$) can be found in hair treated with these products that vary from pH 12 to above 13. Relatively large quantities of residues of the diacidic amino acids of aspartic and glutamic acids resulting from the alkaline hydrolysis of the corresponding amide residues would also be expected.

2.2.1.7 Analysis of Acidic and Basic Groups in Whole Human Hair

Both the acid-combining capacity [57, 58] and the acid dye-combining capacity [34, 59] of unaltered keratin fibers have been used to estimate the frequency of basic groups. Similarly, the base-combining capacity can be used to estimate the frequency of acidic groups in hair [57]. The acid-combining capacity of unaltered

human hair fibers is approximately 820 $\mu\text{mol/g}$ [34, 59, 60]. This parameter provides an estimate of the frequency of basic amino acid residues, including N-terminal groups (approximately 15 $\mu\text{mol/g}$) [10, 61] and sorbed alkaline matter, whereas the base-combining capacity provides an estimation of the titratable acidic groups in the fibers, including C-terminal amino acid residues and any sorbed acidic matter.

Alterations to the fibers that affect the apparent frequency of acidic or basic groups, such as hydrolysis, susceptibility to hydrolysis, or the introduction of sulfonic acid groups [25], can affect the acid- and/or base-combining capacity of hair. Therefore, permanent-waving and especially bleaching (oxidation) can affect these titration parameters [11]. The effects of cosmetic treatments and environment on these titration parameters are described in detail in Chap. 6.

2.3 Aging Influences on Hair

As a person ages, hormonal changes contribute to changes in the hair. The more obvious changes are: Hair thickness (hair density or hairs/cm²), hair graying (see Chap. 7), hair diameter (fine-coarseness) and dryness of the scalp and the hair. Hair thinning tends to relate to hair density and therefore to not to be noticeable in women until the mid to late twenties or more commonly a few years later, see Chap. 1 in *Hair Density versus Age for Caucasian Women*. Large increases in hair fiber diameter occur during the first year in life and during the teenage years. Diameter tends to peak at about age 20 for men and in the mid-forties for women, see Fig. 2.1. Figure 2.1 shows a steeper drop for the scalp hair fiber diameter of Japanese males vs. Caucasian males. This effect should be reexamined. Then, with increasing age hair fiber diameter decreases, see Chap. 9 in the section entitled *Fiber Diameter, Cross-sectional Area, Fine-coarse Hair and Age and Hair Growth* and references [62–65].

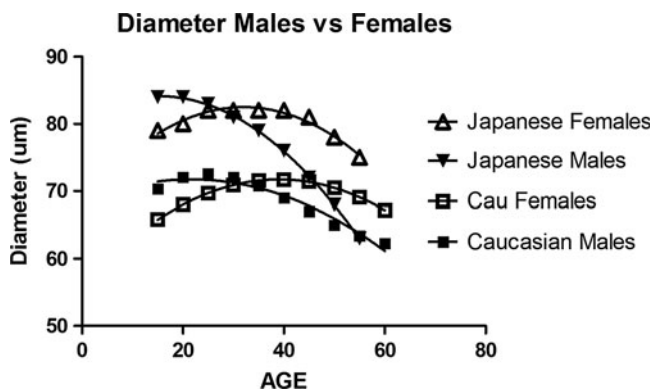


Fig. 2.1 The variation of hair fiber diameter with age and sex

Graying of hair relates to the size and distribution of the melanin granules as well as the types of pigments in the fibers. Gray hair is also dependent on the production of less pigment in the individual hairs with advancing age. It is usually associated with middle age; however, graying can begin in one's early 20s. For more details on the incidence of graying and the age that graying begins, see Chap. 7 in *Gray Hair and Graying of Human Hair*. The formation of hair pigments takes place in the melanocytes in the bulb of the follicle starting with the amino acid tyrosine as described in Chap. 5 in *Hair Pigment Structure and Chemical Oxidation*. Graying occurs when the melanocytes become less active during anagen. The melanin pigments are incorporated into large granules that are transferred into the keratin cells in the zone of keratinization, see Chap. 1.

Hollfelder et al. [66] provided some evidence (from hair from only five individuals) that gray hairs are coarser and wavier than heavily pigmented hairs on the same person. Van Neste [67] found that white hairs are coarser than pigmented hairs and that white hairs have more medulla. However, Gao and Bedell [68] studied gray and dark hairs from only four persons plus one sample of pooled gray hair. They found no significant differences in the maximum center diameter, center ellipticity and cross-sectional areas of gray vs. dark hairs. See Chap. 5 in the section entitled *Hair Pigment Structure and Chemical Oxidation* for additional details.

Less pigmented hairs, such as gray hairs [68], blonde hair or bleached hairs are also more sensitive to light radiation than heavily pigmented hairs. Therefore, lightly pigmented hairs exposed to ultraviolet radiation for a sufficient period will show lower levels of cystine and correspondingly higher levels of cysteic acid particularly in their outer layers when compared to heavily pigmented hairs. In addition damage to the cell membrane complex and tryptophan and other amino acids should occur at a faster rate in gray hair vs. heavily pigmented hairs. Such exposed gray fibers will also provide lower tensile stresses to achieve a given strain level in load-elongation tests and lower bending stiffness see Chap. 5 in *Hair Pigment Structure and Chemical Oxidation*.

Dryness/oiliness is another hair property associated with aging. There are two primary sources of hair lipids: The sebaceous glands and the hair matrix cells. Sebaceous glands occur over most of the body where hair fibers exist. These glands excrete their oils through the narrow opening of the hair follicle onto hair and skin surfaces. The output of these glands is related to the size of the gland, age and sex. Prior to puberty, the output of these glands is low and the hair tends to be dry, see the section entitled, *Free Lipids in the Total Hair Fiber* in this Chapter. Sebaceous output increases at puberty through the teenage years and into the second decade. With increasing age beyond the fourth decade, sebum output decreases, but more so in females than in males.

Changes in the composition and amounts of hair lipids on and in the fibers, lower scalp hair density, lower growth rates and lower hair fiber diameters occur with advancing age. These changes affect important hair properties beyond the mid-forties especially for women because these effects are exacerbated by menopause

when hair lipid changes have been shown to make hair on the head less greasy or drier, see *The effects of Menopause on the Lipids in Hair and on the Hair Fiber*, described later in this Chapter. A decrease in softness and smoothness of the hair of post-menopausal women has also been reported.

It is also likely that hair curvature increases with advancing age as has been shown for Japanese women see Chap. 10 in the section entitled *Hair Handle or Feel*. This effect will likely make the hair fibers more prone to frizziness and will decrease hair luster as shown for Japanese hair.

The effects of these age related changes in hair density, diameter or area of cross-section, graying, curvature and hair lipids produce changes in fiber properties which produce changes in important consumer hair assembly properties such as a changes in combing ease, hair body, hair coverage, frizziness, manageability, style retention, etc. which are described in detail in the last section of Chap. 10.

Aging of individual hair fibers on one's head by everyday grooming actions generally results in a gradual degradation of the scales through cuticle fragmentation. This process is related to the actual age or time the individual hair has been on the scalp (or its residence time) rather than to the chronological age of the individual and is described in detail in Chap. 6.

2.4 Chemical Composition of the Different Morphological Components

2.4.1 Cuticle

Bradbury et al. [17] suggested that the cuticle of human hair contains more cystine, cysteic acid, proline, serine, threonine, isoleucine, methionine, leucine, tyrosine, phenylalanine, and arginine than whole fiber. Data calculated from Bradbury's results and those of Robbins [13] on whole human hair fibers are summarized in Table 2.7. Wolfram and Lindemann et al. [69] described comparative cuticle and cuticle-free hair analyses of certain amino acids in human hair and their data are qualitatively similar to those of Bradbury [17] (see Table 2.7). In addition, these authors suggested less tryptophan and histidine in cuticle than in whole fiber.

In general, these results show that cuticular cells contain a higher percentage of the amino acids that are not usually found in alpha-helical polypeptides than is found in whole fiber. Small amounts of citrulline (11 $\mu\text{mol/g}$) have been reported in whole human hair fibers, whereas cuticle is found to be somewhat richer in citrulline (45 $\mu\text{mol/g}$) with only trace quantities of ornithine (5 $\mu\text{mol/g}$) [17].

The three major layers of the hair cuticle, A-layer, the exocuticle and endocuticle, have been separated after enzymatic digestion and analyzed [72]. Their chemical compositions are quite different and are described next.

Table 2.7 Amino acid composition of the different morphological components of hair^a

Amino acid	Cuticle ^b	Whole fiber ^c	Medulla ^d
Aspartic acid	287	449	470
Threonine	524	664	140
Serine	1,400	1,077	270
Glutamic acid	819	1,011	2,700
Proline	994	667	160
Glycine	611	485	300
Alanine	–	374	400
Half-cystine	2,102	1,461	Trace
Valine	634	499	320
Methionine	38	53	40
Isoleucine	184	249	130
Leucine	418	516	700
Tyrosine	132	184	320
Phenylalanine	91	142	–
Cysteic acid	68	29	–
Lysine	–	217	740
Histidine	–	71	100
Arginine	360	529	180
Ammonia	–	–	(700)
Citrulline	45	11	–

^aData are expressed in micromoles amino acid per gram dry hair

^bThe data for cuticle analysis are based on the work of Bradbury et al. [17] who analyzed cuticle and whole fiber from several keratin sources, including human hair, merino wool, mohair, and alpaca. These scientists concluded that there is very nearly the same difference between the amino acid composition of the cuticle and each of these fibers from which it was derived. They listed the average percentage differences used in these calculations. More recent analyses of cuticle and whole fiber of human hair [69, 70] are in general agreement with these data [17]

^cWhole-fiber results approximated by cortex analysis [13]

^dThese data are results of analysis of medulla derived from porcupine quill from Rogers [71]

2.4.1.1 A-Layer

The A-Layer was first discovered and named by Rogers [71] in 1959. It lies immediately beneath the epicuticle cell membrane. It is of relatively uniform thickness (~110 nm). Varying data have been published about its composition due to contamination arising from the difficulty to separate it from adjacent layers of the fiber, the cell membrane and the exocuticle. Swift [73] reported a very high half cystine content for the A-layer at approximately one half-cystine in every 2.7 amino acid residues or about 37 mol% half cystine an exceedingly high cystine content.

The A-layer is the most highly cross-linked region of the hair fiber being cross-linked by both cystine (disulfide) bonds and isopeptide (amide) groups formed between glutamine and lysine by a transglutaminase enzyme [74]. Isopeptide cross-links (sometimes called isodipeptide) are verified by the resistance of the A-layer to solubilization by reaction of a reducing agent (generally a mercaptan) in the

presence of a detergent solution. Generally, most proteinaceous systems that are cross-linked by disulfide bonds alone are readily solubilized by such a medium, but proteinaceous tissues that are cross-linked by isopeptide bonds are not readily dissolved by a reducing medium [75]. Furthermore, isopeptide bonds are also resistant to many enzymes that readily attack peptide bonds.

In this author's opinion, the most reliable estimate for the amino acid composition of the A-layer is the one by Bringans et al. [76] summarized in Table 2.8. Bringans et al. isolated their A-layer by essentially dissolving the rest of the fiber from it. They took Merino wool and scoured it, then solvent extracted it followed by methanolic KOH treatment to remove the MEA layer. The next step was to treat with reducing conditions using tris-2 carboxy ethyl phosphine followed by treatment with pronase E for 7 days. At various stages of treatment the sample was examined by TEM to estimate what structures were remaining in it. The composition of the remaining matter was largely A-layer containing only 3% lysine which at 50% conversion to isopeptide would provide only 1.5 mol% isopeptide bonding. This amount of isopeptide is somewhat lower than the estimate of 2.5 mol% suggested by Zahn et al. [77]. Bringans et al. [76] then digested the remaining A-layer with 2-nitro-5-thiocyano-benzoic acid to produce a large number of small peptide derivatives that were analyzed by mass spectrometry. A large proportion of the peptide derivatives fit the cuticular KAP 5 and KAP 10 families of proteins known to be in hair cuticle in large amounts. In addition, there was also strong homology to the KAP 4 and KAP 12 families of proteins. See the section entitled *The KAP Proteins of Human Hair* in this Chapter.

Table 2.8 Approximate composition of the A-layer from Merino wool by Bringans et al. [76]

Amino acid	A-Layer [76] mole%	Cuticle [76] mole%
Aspartic acid	1.3	4.2
Glutamic acid	5.2	8.1
Threonine	2.7	4.6
Serine	14.2	12.9
Proline	6.6	9.3
Glycine	18.4	9.8
Alanine	3.4	5.5
Valine	6.5	6.6
Isoleucine	2.0	2.7
Leucine	3.9	6.0
Half cystine + cysteic acid	25.1	14.7
Methionine	0.7	0.7
Tyrosine	1.1	3.7
Phenylalanine	1.6	2.1
Histidine	1.2	1.4
Lysine	3.0	2.9
Arginine	3.1	4.9

Table 2.9 Amino acid composition of exocuticle of wool fiber by Bradbury and Ley [78]

Amino acid	Mole percent
Aspartic acid	2.1
Glutamic acid	8.6
Threonine	3.9
Serine	11.9
Proline	12.4
Glycine	8.7
Alanine	6.4
Valine	8.2
Isoleucine	2.9
Leucine	4.6
Half cystine + cysteic acid	20.0
Methionine	0.2
Tyrosine	2.0
Phenylalanine	1.2
Histidine	0.5
Lysine	2.1
Arginine	4.8

2.4.1.2 Exocuticle

Bradbury and Ley [78] provided an amino acid analysis of exocuticle derived from physically isolated cuticle cells of wool fiber after pronase and trypsin digestion. This approach provided 20% cystine, about 10% acidic amino acids, about 7% basic amino acids and about 44% non-polar amino acids, see Table 2.9.

Swift [79] cited approximately 20% cystine in an exocuticle rich fraction from human hair, although the data showed a slightly higher amount which Swift attributes to exocuticle plus A-layer [72, 79]. Swift [72, 79] also indicated that the exocuticle likely contains virtually no isopeptide cross-links because of its rapid digestion in dithiothreitol/papain mixture, a medium that only slowly attacks the adjacent A-layer of hair. The exocuticle has been described by Swift as varying from 100 to 300 nm thick [73, 79] within the same cuticle cell and it averages about 200 nm thick.

The sequences of three mid-cuticle proteins have been described by M.L. Rogers et al. in several different publications and these contain about 20–22% cystine [80].

2.4.1.3 Endocuticle

The endocuticle of human hair has been shown by Swift [79] to be irregular in shape and varies from about 50 to 300 nm thick and averages about 175 nm. Relatively similar amino acid compositions have been reported for endocuticle from wool fiber by Bradbury and Ley [78] and from human hair by Swift and Bews [72], see the data of Table 2.10.

Swift and Bews [72] isolated three chemically distinct protein fractions all of low cystine content. These proteins were all easily digested by protease systems not

Table 2.10 Amino acid composition of hair and wool endocuticle

Amino acid	Endocuticle of hair [72]	Endocuticle of wool [78]	Averages
Aspartic acid	9.3	7.4	8.35
Glutamic acid	12.3	10.3	11.3
Threonine	5.9	5.5	5.7
Serine	10.3	10.7	10.5
Proline	7.3	8.9	8.1
Glycine	6.9	8.2	7.55
Alanine	5.9	6.7	6.3
Valine	6.5	7.5	7.0
Isoleucine	4.1	3.9	4.0
Leucine	8.7	9.3	9.0
Half cystine + acid	5.7	3.1	4.4
Methionine	1.5	0.8	1.15
Tyrosine	3.4	3.6	3.5
Phenylalanine	2.4	3.9	3.15
Histidine	1.0	1.1	1.05
Lysine	4.1	4.2	4.15
Arginine	4.8	5.0	4.9

containing reducing agents suggesting a lack of or relatively low levels of both disulfide and virtually no isopeptide cross-links. The low level of cross-links and the relatively high levels of polar (acidic and basic) amino acid residues suggest that this layer of cuticle cells is the one most prone to a high degree of swelling in water.

Thus, the proteins of the exocuticle and its A-layer are highly cross-linked by cystine (more than 30% combined exocuticle and A-layer) and therefore extremely tough and resilient. In contrast, the proteins of the endocuticle contain very little cystine (~3%) and relatively large amounts of the dibasic and diacidic amino acids.

As a result of these large compositional differences in the A-layer, exocuticle and the endocuticle, the cuticle can be expected to react differently to permanent waves, bleaches, and even to water and surfactants. Roper et al. [81] described a method to determine the cuticle composition from endocuticle of chemically treated wools. Such a procedure should be useful to evaluate changes in the endocuticle of cosmetically modified human hair.

2.4.2 *Proteins of the Cell Membrane Complex*

The structures of the three different types of cell membrane complex (CMC) are described in Chap. 1 in the section entitled, *Structure of the Three Different Cell Membrane Complexes*.

The schematic of Fig. 1.44 depicts cell membrane proteins and multiple layers of proteins in the Delta layer of the cuticle-cuticle CMC analogous to the Delta layer of the cortex-cortex CMC depicted in Fig. 1.45 [82, 83]. The structures and

composition of the proteins of the CMC are still not adequately characterized. The reason for this gap is that it is extremely difficult to isolate proteins from only the cell membranes or only the Delta layer. This difficulty has been the primary obstacle to our understanding the composition and structure of the proteins of this important region of the fiber. Much more scientific attention has been given to the analysis of cuticle cell membranes than to those of the cortex therefore I will begin this discussion on the proteins in the cuticle cell membranes.

2.4.2.1 Proteins in the Cuticle Cell Membranes

The proteins of the cuticle cell membranes are associated with the Allworden reaction [84] as described earlier. The membranous epicuticle supports 18-MEA and is attached to the A-layer on the top of cuticle cells and has been isolated by shaking animal hair fibers during Allworden sac formation and subsequently analyzed for amino acids. Perhaps the most quoted and “reliable” amino acid analysis of the Allworden membrane has been provided by Allen and coworkers [85] and is summarized in Table 2.11. The proteins in the cuticle cell membranes are described in detail in Chap. 1 in the section entitled *The Structures of the Three Different Cell Membrane Complexes*, where these leading references are cited [84, 85, 87–96]. See that section and those references for details.

Table 2.11 Amino acids from the proteins extracted from wool with performic acid [86] vs. Allworden membrane [85]

Amino acid	Allworden [85]	Resistant membranes [86]
Asp	3	5.4
Glu	8.6	10.3
Thr	2.1	5.7
Ser	14.3	10
Tyr	0	0
Pro	4.2	7.1
Gly	23.8	14.2
Ala	3.2	6.5
Val	5.6	4.9
Iso	1.2	2.6
Leu	2.9	4.9
Trp	–	0
Phe	0.4	1.5
His	0.2	1.3
Lys	4.5	8.4
Arg	2.5	4.2
Met	0	0
Cys	21.1	13
Totals	97.6	100

2.4.2.2 Proteins in the Cortical Cell Membranes

Proteinaceous material called “resistant membranes” have been isolated from both the oxidation of wool and or hair with performic or peracetic acid followed by treatment with either ammonia or alkaline urea [86]. The authors of this paper state that this material from the performic acid reaction is similar to their own analysis of Allworden membrane. However, both are clearly different from the Allworden membrane analysis by Allen et al. [85] as summarized in Table 2.11.

Treatment of keratin fibers with either peracetic or performic acid and separation into three fractions according to solubility has been called the “keratose” method by Alexander and Earland [97]. Some additional material on this method is described later in this Chapter in the section entitled, *Other Fractionation Methods*. After oxidation, adjustment of the pH to the alkaline side provides an insoluble fraction called Beta-keratose, about 10% of the weight of the hair. Acidification to pH 4 provides a fraction greater than 50% of the material called Alpha-keratose containing crystalline material, by x-ray diffraction. The third fraction called Gamma-keratose is believed to be largely from the matrix. The Beta-keratose fraction is believed to be proteins derived primarily from cell membrane material; however other proteins are likely present. According to a different workup procedure by Bradbury, Leeder and Watt [86], only 1–1.55% residue is provided. Other workup procedures have been applied to the keratose method [98].

Since the cell membrane lipids of cortical cells are not bound by thioester linkages as in cuticle cells, but by polar and ionic bonds, then no UHSP is necessary for the cell membrane proteins of cortical cells, but proteins with an adequate number of basic sites such as amino and guanidino (for bonding to cholesterol sulfate and to fatty acids) and polar sites such as carboxyl and hydroxyl groups would also be preferred for polar bonding to fatty acids and hydroxyl groups. The cortical cell membranes will most likely be resistant to oxidation, reduction and to acids and alkalies. Therefore, isopeptide bonds will be necessary and these could be formed by proteins such as involucrin and small proline rich proteins that are rich sources of glutamine to react with lysine groups of other proteins as in stratum corneum and in the cuticle cell membranes [85].

The resistant membrane material from the reaction of performic acid on wool fiber by Bradbury, Leeder and Watt [86] provides only about 62% of the amount of cystine as the composition of the Allworden membrane by Allen et al. [85]. It also contains about twice the basic amino acid content and basic amino acids are necessary to form salt linkages to cholesterol sulfate and carboxyl groups of fatty acids to form bilayers for cortical cell membranes. Performic acid derived membrane matter should be richer in cortical cell membranes, since they are a higher percentage of the total membrane matter in keratin fibers. However, other protein contaminants could be from the A-layer and cuticle cell membranes which also contain isopeptide bonds.

A cleaner experimental scheme to isolate pure cortical cell membranes would be to start with pure cortex to exclude cuticle cell membranes and A-layer proteins. Pure cortex from human hair could be provided by the glass fiber method of

Wortmann et al. [99] and then perhaps employ the performic acid reaction or another scheme to provide cortical cell membranes in the absence of cuticle contamination for further workup and analysis.

2.4.2.3 Proteins Extracted from Hair/Wool Believed to be from the CMC

Leeder and Marshall [100] extracted Merino wool with formic acid and also with n-propanol/water (50/50). Proteinaceous matter was removed from the hair fibers with each of these solvent systems. With formic acid, these scientists concluded that the proteins were at least partially derived from the CMC, most likely the Delta layer because the extract contained virtually no cystine. If this proteinaceous material is from the Delta layer it most likely is not from the central proteins, called the contact zone, because Naito et al. [101] provided evidence that the central contact zone contains hydrophilic protein with disulfide bonding.

Leeder and Marshall [100] concluded that the proteins derived from their propanol/water extraction of wool is not entirely from the cell membrane complex, but these proteins also contain high glycine-tyrosine proteins possibly from the cortex. The amino acid compositions of proteins extracted by formic acid, by n-propanol/water and by chloroform/methanol are compared with that of Allworden membrane in Table 2.12.

Logan et al. [102] demonstrated that a chloroform-methanol azeotropic mixture provides a very different mixture of proteins than the high temperature propanol/

Table 2.12 Proteins extracted from wool with formic acid and n-propanol water compared with Allworden membrane

Amino acid	Allworden [85]	Formic acid [100]	n-Propanol [100]	CHCl ₃ /MeOH [102]
Asp	3.0	5.7	3.7	6.2
Glu	8.6	7.2	2.4	7.5
Thr	2.1	3.8	3.2	5.1
Ser	14.3	8.1	11.7	13.3
Tyr	0	12.0	16.4	3.0
Pro	4.2	4.0	5.2	6.4
Gly	23.8	19.2	25.0	11.9
Ala	3.2	5.2	2.2	8.0
Val	5.6	4.2	2.8	5.9
Iso	1.2	3.3	0.8	3.5
Leu	2.9	9.2	6.1	8.1
Trp	0	0	—	—
Phe	0.4	5.2	7.8	3.6
His	0.2	1.2	0.7	1.0
Lys	4.5	4.0	0.8	2.7
Arg	2.5	6.2	5.2	4.1
Met	0	0.9	0.2	0.8
Cys	21.1	0.4	5.5	9.0
Totals	97.6	99.8	99.7	100.1

water extraction, see Table 2.12. Could this chloroform-methanol extract be partially derived from cortical cell membranes or part of the outer lamella (outermost layer) of the Delta layer proteins of the CMC? Since Mansour and Jones [103] demonstrated that chloroform/methanol provides large changes to the cortex-cortex CMC in wool, it is likely that the proteins removed by chloroform/methanol are at least partially attached to Beta layers and are at least in part Delta layer proteins of the cortex-cortex CMC.

The method of Swift and Holmes [104] has been used by several different researchers to obtain proteinaceous matter believed to be partially derived from the CMC. This method involves dissolving matter from hair using papain with a reducing agent such as bisulfite or dithiothreitol (DTT). Bryson (Bryson W. Private communication) conducted a series of experiments from which he concluded that the laminated structure observed under the TEM following a 72 h digestion of wool fibers with papain and reducing solution (somewhat standard procedure) is not derived entirely from the CMC. Prolonging the digestion beyond 72 h increased the number of laminated layers beyond what could be accounted for by the number of cortical cells in a fiber cross-section. Bryson concluded that the CMC lipids were rearranging with other proteins and peptides to form these laminated layers.

Mass spectrometric analysis of the proteins of the digestion residue indicated that the majority of the protein component was papain, suggesting that the CMC lipids had rearranged with papain to form the laminated structures. Therefore, Bryson concluded it is not possible to isolate pure proteinaceous CMC by papain digestion. These conclusions by Bryson are consistent with those of Swift and Bews [72] who concluded that although treatments of keratin fibers with enzymes and reducing agents do cause separation of cells they could find no evidence of dissolution of the cuticle CMC via critical electron microscopic examination of treated hair sections. Therefore the value of this method for isolation of CMC proteins is limited because of contamination with papain.

2.4.3 Lipids of the Cell Membrane Complex

2.4.3.1 Methods to Remove Lipids from Animal Hairs for Analysis

To remove external lipids, wool fibers are normally cleaned by scouring with a nonionic agent such as Lissapol and then in scientific studies treated with one or more solvents to remove any remaining external lipids. Non-swelling and/or solvents of bulky molecules (like t-butyl alcohol [105]) have been used to remove external lipids from keratin fibers, that is, lipids that are believed to be soil and not part of the structural lipids of animal hairs. Solvents such as hexane, t-butyl alcohol or heptane and sometimes t-butanol and heptane sequentially [102, 106] have been used to extract external lipids such as wool wax or sebaceous matter from animal hairs. Such lipids are sometimes called external, extrinsic or even exogenous [107] and are not believed to be involved in the intercellular structure of animal hairs.

In the case of human hair, external lipids have been removed by shampoo or sodium lauryl sulfate washing, the safest procedure, or by a combination of shampoo followed by incubation in hexane for only 5 min [107] or in some cases other non-swelling solvents like ether or heptane which most likely do not remove significant amounts of internal hair lipids.

2.4.3.2 Removal of Internal Lipids Not Covalently Bound to Hair

Hair-swelling organic solvents alone or in combination with a second lipid solvent are used to remove internal lipids that are part of the internal structure of hair fibers (of the CMC) but not covalently bonded to hair protein structures. Swelling solvents such as chloroform/methanol [102, 108], methanol [108], ethanol [104], formic acid [109], n-propanol/water [109] or acetone [108] have been used to extract internal matter from animal hairs. The most frequently used solvent for removal of internal lipids has been chloroform/methanol (70/30) although other mixtures have been used. Normally soxhlet extraction is employed; however, multiple room temperature extractions have also been used [107]. Although formic acid and n-propanol/water (generally 1:1) do remove some internal lipids these two solvents also remove some hair proteins of the CMC (most likely from the Delta layers and possibly from other regions of the fibers see the section entitled, *Proteins of the CMC*).

2.4.3.3 Removal of Covalently Bound Hair Lipids Plus Salts Insoluble in Lipid Solvents

Alkaline hydrolysis or methanolic alkali is used to remove covalently bound hair lipids. This technique can be used to remove total hair lipids, but is generally used after extraction of external and internal lipids that are not covalently bound to the fibers. Those covalently bound lipids at or near the fiber surface are generally removed with potassium t-butoxide in t-butanol (bulky cleaving agent in a bulky solvent) [110]. Total covalently bound lipids are generally removed with potassium hydroxide in methanol because alkali in a swelling organic solvent like methanol penetrates well into hair.

In addition to covalently bound lipids, Wertz [111] suggested that salts of cholesterol sulfate bind ionically to cationic groups of the hair proteins and will be insoluble in chloroform/methanol. Therefore these ionically bound hair lipids will remain in the fibrous residue after extraction with organic solvents. Korner et al. [112] used a solution of chloroform/methanol/aqueous potassium chloride to extract CMC lipids from wool and human hair.

2.4.3.4 Total Lipids in Hair Fibers

The total amount of lipid extractable from hair is generally 1–9% of the weight of the hair [107, 113]. Masukawa et al. [107] studied the total hair lipid composition

from 44 Japanese females ages 1 to 81. The lipids were extracted/removed from hair in varying procedures to allow for analysis of several lipids and covalently bound 18-MEA. Total fatty acids and 18-MEA were determined, but other important fatty acids both covalently bound and non-covalently bound were not quantitated such as palmitic, stearic, oleic and palmitoleic acids which have been found in significant quantities in other studies [102, 111, 114–116]. Cholesterol sulfate was also not determined in this effort by Mazukawa et al.

Logan et al. [102] analyzed human hair by extracting it with a chloroform/methanol azeotrope for 5 h after surface lipids had been removed with t-butanol and heptanes. These scientists found 23% palmitic, 25% palmitoleic, 4% stearic, 13% oleic and other fatty acids. These are all non-covalently bound fatty acids with 39% of the total fatty acids being unsaturated (primarily palmitoleic and oleic). Although, it is possible other unsaturated fatty acids were present. Weitkamp et al. [117] analyzed solvent extracted lipids from pooled adult Caucasian human hair clippings and found 51% of the total fatty acids to be unsaturated with palmitoleic and oleic acids as the principal unsaturated fatty acids. However, other unsaturated fatty acids were found in these extracts.

Masukawa et al. [107] initially shampooed the hair and then washed it with hexane allowing a 5 min incubation time. The hexane wash was determined by plotting the amount of lipid extracted vs. the square root of time of the hexane wash. The time that diffusion of lipids from the interior of the fiber began was determined graphically, a reasonable approach to removing external lipid soils from the fibers and leaving most of the internal and structural lipids in the hair.

Mazukawa et al. then removed the hair lipids by extraction with different ratios of chloroform-methanol and separated them into eight groups; their data are summarized in Table 2.13. These data show that approximately 58% of the total lipids removed from hair under these conditions are fatty acids, some are covalently bonded, but others exist as free and non-covalently bound fatty acids. The total fatty

Table 2.13 Lipids in human hair from Masukawa et al. [107] and Wertz and Downing [115]

Type of lipid	mg/g hair	Percentage of total lipid	Source ^a
Hydrocarbons	2.4	9.7	U
Squalene	0.7	2.8	S
Wax esters	4.9	19.8	S
Triglycerides	0.5	2.0	S
Total fatty acids	14.4	58.1 [97] ^b	S
Total covalent F. acids	— (4.0) ^c		M/S
Cholesterol	1.3 (0.6) ^c	5.2	M
Cholesterol sulfate	— (2.9) ^c		M
Ceramides	0.29 (0.5) ^c	1.2	M
18-MEA	0.30 (1.6) ^c	1.2	M
Totals	24.79	100%	

^aSources: U = Unknown; S = Sebaceous Glands; M = Hair Matrix Cells

^bSee Logan et al. [102, 118] for a breakdown of the actual fatty acids in human hair

^cData in parenthesis by Wertz and Downing [115], not in parenthesis by Masukawa et al. [107]

acids found were 14.4 mg/g of hair, but only 0.3 mg/g hair of 18-MEA were found. Wertz and Downing [111] found 1.31–2.1 mg/g of 18-MEA in four different human hair samples (three from individuals and one pooled hair sample presumably Caucasian hair). In a later paper, Wertz and Downing [115] cited 4.0 mg/g total integral (covalently bound) fatty acids with 40.5% as 18-MEA for human hair or 1.6 mg/g 18-MEA. Since most 18-MEA estimates in wool fiber are close to 1 mg/g or higher and human hair contains more cuticle layers than wool fiber one would expect more covalently bound fatty acids in human hair than wool fiber. Masukawa did not list amounts for total covalently bound fatty acids only 18-MEA. Therefore I listed and used the data for Wertz and Downing [115] for total covalently bound fatty acids.

2.4.3.5 Lipids of the Cuticle-Cuticle Cell Membrane Complex

Wertz and Downing [115] examined five different mammalian hairs from sheep, humans, dog, pig and cow and found the percentage of 18-MEA relative to the total amount of covalently bound fatty acids varied from 38% to 48%. Table 2.14 summarizes a tabulation of analyses of the covalently bound lipids of wool and human hair from several different laboratories. These results were all obtained after the fibers had been exhaustively extracted with chloroform/methanol to remove the non-covalently bound fatty acids and then the residue 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.

2.4.3.6 Covalently Bound Internal Lipids of Animal Hairs

Korner and G. Wortmann [119] (Table 2.14), 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 [115] 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 [111] found 41% 18-MEA, 18%, palmitic acid, 7% stearic acid, 4% oleic

Table 2.14 Covalently bound fatty acids in wool and human hair fiber

Fatty acid	Data for wool fiber						Data for human hair [111]
	[114]	[118]	[116]	[115]	[119]	Averages	
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

acid and the remaining small percentages of fatty acids from C16 through C20 with 9% uncharacterized. Negri et al. [116] 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 has been suggested to be related to fiber diameter which determines the number of layers of covalently bound fatty acids in the fibers. However, certainly part of the variance is due to experimental error. The bottom line is that somewhere in the vicinity of $50 \pm$ at least 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. [120] demonstrated with isolated cuticle cells that 18-MEA is essentially all in the cuticle. 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 [121, 122] 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. So, 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 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, Bishop and Jones [123] first found that there are virtually no phospholipids in keratin fibers. This fact was confirmed by Schwan and Zahn [124] and by Rivett [125] casting doubt on whether lipid bi-layers could be involved in the cell membranes of keratin fibers [123]. However, Wertz et al. [126] 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 in the absence of phospholipids if an acid species such as cholesterol sulfate is present with other lipids. Furthermore, evidence has been provided confirming the existence of cholesterol sulfate in human hair by Wertz and Downing [111] and by Korner et al. in wool fiber [112].

The work of Korner et al. [112] builds upon the findings of Wertz et al. on liposome formation and lipids from stratum corneum [126]. Korner et al. [112] demonstrated that cell membrane lipids extracted from human hair and wool fiber with chloroform/methanol/aqueous potassium chloride can form liposomes. This result provided 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 likely that the proteins that these very different lipid layers are attached to are also different. These proteins are the cell membrane proteins and the Delta layer proteins of the cuticle cells and the cortical cells, see the section on *Proteins of the CMC* and the next section of this Chapter.

2.4.3.7 Lipids of the Cortex-Cortex Cell Membrane Complex

Since Mazukawa et al. [107] found 14.3 mg/g total fatty acid, but did not determine the total covalently bound fatty acids, and Wertz and Downing found 4 mg/g total covalently bound fatty acids then the Mazukawa et al. data most likely represents or is closer to the total amount of non-covalently bound fatty acids in human hair. So, if we assume human hair has approximately 14 mg/g of non-covalently bound fatty acids and about ½ the equivalent amount of free lipid in the cuticle relative to covalently bound fatty acid. This provides 2 mg/g free fatty acid in cuticle layers, leaving about 12 mg/g of non-covalently bound fatty acids. If we assume 2 mg/g fatty acid as intracellular lipid that leaves 10 mg/g fatty acids in the cortex-cortex CMC. So, with these approximations, about 10 mg/g of fatty acids will exist in the “bi-layers” of the CMC of the cortex, along with cholesterol, cholesterol sulfate and ceramide (see Fig. 1.45).

Wertz and Downing [115] found cholesterol (0.6 mg/g), and cholesterol sulfate (2.9 mg/g) and ceramides (0.5 mg/g) in their alkaline hydrolysates from human hair after removal of all free lipids by chloroform-methanol extraction. These same scientists also found these same lipid components in hair from sheep, dog, pig, cow and humans varying from (0.3 to 1.4 mg/g) cholesterol, ceramides (0.6 to 1.4 mg/g) and cholesterol sulfate (0.7 to 3.3 mg/g) [115].

Examination of these data from different laboratories suggests the following ingredients in these approximate ratios as the principal components of the bi-layers of the cortex-cortex CMC for human hair:

Lipid component	Approximate amount	Approximate relative amounts
Fatty acids	10 mg/g hair	10
Cholesterol sulfate	0.7–3.3 mg/g	2
Cholesterol	0.6–1.2 mg/g	1
Ceramides	0.6–1.4 mg/g	1

These ratios are clearly not exact, but they show a large amount of fatty acid followed by cholesterol sulfate and smaller amounts of cholesterol and ceramide.

2.4.3.8 Lipids of the Cuticle-Cortex Cell Membrane Complex

If the cuticle-cortex CMC is a hybrid of the cuticle-cuticle CMC and the cortex-cortex CMC the composition of the lipids and the proteins should be essentially a 50/50 mixture of the proteins of the cuticle-cuticle CMC and the cortex-cortex CMC.

2.4.3.9 Lipids of the Surface

These lipids are essentially those covalently attached to the epicuticle such as 18-MEA and those free lipids that are associated with 18-MEA and the epicuticle proteins.

2.4.3.10 After Shampooing an Appreciable Amount of Free Lipid Remains in the Hair Surface

Shaw [127] suggested that washing hair with ether or shampoos in a one-step application leaves virtually the entire hair surface free of lipid and that differences in cleaning efficiencies of surfactants relate to the amounts of internal lipid removed. Recent XPS data show that shampooing does remove some free-lipid from the surface of hair, but even after shampooing an appreciable amount of free-lipid remains in the surface layers, that is in the top 3 nm [128].

2.4.3.11 Free-Lipid in Surface Layers Affects Isoelectric Point of Wool and Hair

Capablanca and Watt [129] examined wool fiber that had been washed with detergent (Lissapol) and extracted with various solvents using a streaming potential method to estimate the effect of free-lipid (including non-covalently bound fatty acids) in the surface layers on the isoelectric point of wool fiber. These scientists found an appreciable effect of free-lipid on the isoelectric point. The surfactant washed wool (containing the most free-lipid) provided an isoelectric point of 3.3. The isoelectric point of wool increased as the effectiveness of the solvent system increased with the most effective lipid solvent providing an isoelectric point of 4.5.

These data show that free fatty acids in the surface layers are an important and essential component of the surface of animal hairs and about half of the free lipid is fatty acid [130]. So, the more free-lipid present in these surface layers, the lower the isoelectric point of keratin fibers. Therefore, all free-lipid is not totally removed and should not be totally removed from the surface layers by shampooing of hair or scouring of wool fiber. In addition, free fatty acids are important to the isoelectric point of animal hair fibers. Furthermore, the amount of free-lipid in the surface of hair fibers will influence hair friction, surface energy and a whole range of important properties including the adsorption of surfactants and other ingredients onto human hair and wool fibers.

Hoting and Zimmerman [38] demonstrated that the cell membrane complex lipids of hair fibers are degraded more by visible light, but also by UV-A and by UV-B light, helping to explain the weakened cell membrane complex and the multiple step fractures observed in sunlight oxidized hair described in detail in Chap. 5. Obvious weak links to photochemical attack on lipid structures are the

allylic hydrogen atoms of unsaturated fatty acids and the tertiary hydrogen atoms of 18-MEA and other species. Hoting and Zimmerman also demonstrated that the cell membrane complex lipids of chemically bleached hair are more readily degraded by physical actions than the lipids of chemically unaltered hair. For example, longer term irradiation does not provide for clean breaks between structural components of hair as was observed for peroxide oxidized hair, see Chap. 5 for details. For more details on the structure of the CMC and the hair surface see the sections entitled *Epicuticle and the Hair Fiber Surface* and *The Cell Membrane Complex Including the Intercellular Non-keratin Regions of Hair* in Chap. 1.

2.4.3.12 Four Different Classes of Human Hair Lipids

There are at least four different but meaningful classifications of hair lipids. Hair lipids are described as free or bound, as endogenous or exogenous lipids, as internal or surface and by chemical functional group or chemical type.

Bound lipids are those that cannot be removed by extracting the hair with lipid solvents because they are covalently bonded to hair proteins. For example, 18-MEA is attached to proteins by thioester linkages, whereas free lipids are extractable from hair using lipid solvents because they are held by weaker bonding forces such as van der Waals attractive forces and sometimes hydrogen bonding or even salt links. Endogenous lipids are those hair lipids that result from biosynthesis in hair matrix cells in the hair follicle, whereas those lipids in the hair that are usually synthesized in sebaceous glands are sometimes called exogenous of an extrinsic source. Internal lipids are those that have either penetrated into the hair or have been incorporated inside the hair fiber as opposed to surface lipids. Chemical groups commonly used for this type of classification are similar to those described in the paragraphs below.

From the comprehensive study of hair lipids by Masukawa et al. [107] hair lipids were described in the section entitled, *Total Lipids in Hair Fibers*. In this study, hair lipids were extracted and analyzed from both the proximal and distal parts of the hair of 44 Japanese females between the ages of 1 and 81 and the composition determined quantitatively. These scientists separated the lipids into four groups by chemical type: Group A: Squalene (SQ), Wax esters (WE), Triglycerides (TG), and fatty acids (FA); Group B: cholesterol (CH) and ceramides (CER); Group C: hydrocarbons (HC) and Group D: 18-methyl eicosanoic acid (MEA). They also classified these lipids by source, for example those from sebaceous glands and those from hair matrix cells. These data are summarized in Table 2.13.

2.4.3.13 Bound and Free Lipids

The bound lipids are those lipids of the cell membrane complex that are covalently bonded to proteins including the 18-MEA attached to the epicuticle at the surface, described earlier in this Chapter and in Chap. 1. 18-MEA is part of a lipid monolayer surrounding each cuticle cell. 18-MEA is bound to the top of each cuticle

cell (and part of the scale edge) through thioester linkages [87]. 18-MEA forms the outer surface layer of the virgin hair surface as well as the top layer of each cuticle cell. The bottom of each cuticle cell and part of each scale edge is covered primarily with straight chain fatty acids that are mainly palmitic, stearic and other fatty acids including some oleic acid. These fatty acids are bound either through ester or thioester linkages to the underlying proteins. All other lipids that have been described in the literature are believed to be free lipids, that is, lipids that are not covalently bonded to hair proteins and they exist on and in the cuticle and the cortex. Additional details of covalently bound fatty acids are described in the section on the Cell Membrane Complex in this Chapter and in Chap. 1.

2.4.3.14 Surface Lipids of Human Hair

If we define the hair surface as the top 3–5 nm of the hair fiber, we find that 18-MEA is the primary lipid of that surface, but there is also free lipid in this surface too and there will likely be more free lipid in the surface the longer the time interval between shampooing and when lipid analysis is made. Evidence to support free lipid in the surface of hair stems from defining the hair surface as the top 3–5 nm and using x-ray photoelectron spectroscopy (XPS) to measure the C/N ratios in that surface. Estimates appear in Table 2.15 for the percentages of free lipid, 18-MEA, protein and total lipids in the outermost 3–5 nm of the fiber.

Some of the assumptions in these calculations are that the epicuticular proteins consist of 16.7% nitrogen and 45.3% carbon as calculated from the composition of CE proteins by Zahn et al. [88]. For 18-MEA, the carbon content is represented by

Table 2.15 Estimates of lipids and protein in the surface layers of hair fibers as a function of washing and treatment

Treatment	% Protein	% MEA	% Free lipid	Calc C/N	Found C/N	Total lipid
Wool Soxhlet CHCl ₃ :MeOH [131]	53.7	46.3	0 ^a	6.9	6.9	46.3
Roots dei water ^b 18" cut at scalp [6]	16.2	35.6	48.2	26.8	26.7	84
Tips dei water ^c 18" cut at scalp [6]	23.2	18.1	58.7	17.9	18.0	77
Hair rinsed ^d dei. Water [6]	35.3	44.2	20.5	11.4	11.4	65
Hair washed ^e SLS [6]	44.0	44.2	11.8	8.8	8.8	56

^aSulfur VI assumed to be 20% Ward et al. scoured their wool with surfactant and then Soxhlet extracted it with a 2:1 CHCl₃: MeOH solvent therefore the free lipid content was assumed to be 0% and the Sulfur VI was assumed to be 20%, close to the value found by Carr et al. [132]

^bSulfur VI = 38.6%

^cSulfur VI = 68.8%

^dSulfur VI = 23.7%

^eSulfur VI = 23.7%

the acyl group without the sulfur because the sulfur is part of the protein structure. Therefore, the % carbon of 18-MEA is 81.6% and 75% carbon formerly used for the free lipids as suggested by Carr, Leaver and Hughes [132] from the work by Rivett et al. [133].

Carr, Willis St. John and George [132, 134] examined wool fiber by XPS in which the fibers had been Soxhlet extracted with chloroform/methanol. These scientists calculated that 18-MEA is approximately 1 ± 0.5 nm thick. This estimate is smaller than the length of the 18-MEA molecule which approaches 3 nm. Therefore, Zahn et al. [77] concluded that the 18-MEA chains fold back on themselves on the surface of keratin fibers to achieve the measured thickness of the lipid layer.

The data of Table 2.15 suggest that some free lipids are bound within the 18-MEA layer and as free lipids can be removed and then the 18-MEA chains fold back on themselves as suggested by Zahn et al. [77]. However, at higher free lipid levels there is less nitrogen and therefore less protein in the top 3–5 nm. Thus as more free lipid is incorporated within the 18-MEA layer it allows the 18-MEA chains to straighten out to accommodate the free lipid and to approach the expected length of 18-MEA (2.7–2.8 nm calculated by this author) and to occupy a higher percentage of the top 3–5 nm of the surface.

The data of Table 2.15 compares root ends vs. tip ends of hair more than 12 in. long (~30 cm) and cut directly from the scalp. This hair was not shampooed prior to XPS analysis, but only rinsed with deionized water. These data show very high free lipid levels in spite of the high oxidation level revealed by the SVI data showing 38.6% oxidized sulfur at the root ends and 68.8% oxidized sulfur at the tip ends. In spite of the high oxidation levels of this hair the free lipid levels are also high because of the accumulation of free lipids in or on the surface and the lack of shampooing of the sample prior to analysis. This conclusion is confirmed by the data of this same Table showing the effects of shampooing on the free lipid content of another hair sample.

The data of Table 2.15 also shows the effects of washing the hair with sodium lauryl sulfate on the surface lipids. Note, this hair was sampled near the root ends from another person who had a lower level of oxidized sulfur compared to both root and tip ends of hair described by the data in this same table. Washing the hair with sodium lauryl sulfate removed free lipids from the hair surface, but still left about 12% free lipids in the top 3 nm of the hair.

Capablanca and Watt [129] demonstrated that free lipids in the keratin fiber surface serve to lower the isoelectric point of wool (hair) and thereby affect the charge character of the surface. This free lipid could affect the binding of conditioner ingredients on and in the surface layers. Free lipids are also very difficult or virtually impossible to completely remove simply by shampooing or by means that are available to consumers. Thus, free lipids should be viewed as vital components of the hair fiber surface that are important for protection of the hair and to the interactions of conditioners and shampoos rather than as simply soil. For more details on the surface structure of human hair see the section in this Chapter entitled *Epicuticle and the Hair Fiber Surface*.

2.4.3.15 Free Lipids in the Total Hair Fiber

The data of Mazukawa et al. [107] (Table 2.13) demonstrated that fatty acids (~58%), wax esters (~20%) and hydrocarbons (~10%) comprise the major part of the free lipids in hair, almost 90% of the total lipid (free plus bound) in hair from a population of 44 Japanese females. These data are generally consistent with the types of free lipids found in the hair of Caucasian adults showing the largest amount as free fatty acids and the second largest amount as wax esters [135, 136].

A few years ago, Hussler et al. [137] isolated and identified low levels of ceramides in human hair lipid from Caucasians. Masukawa et al. identified these same ceramides as free lipids (~290 $\mu\text{g/g}$ hair) at levels similar to those of 18-MEA by Masukawa et al. [~300 $\mu\text{g/g}$ hair average at the proximal ends (470–220 $\mu\text{g/g}$ variation among individuals)], but slightly higher than the levels found by Hussler and co-workers (~100 $\mu\text{g/g}$).

According to data by Nicolaides and Rothman [138] lipid extracted from human hair is similar but not identical to the composition of scalp lipid. However, cell membrane complex lipid is also partially removed by extraction of hair with lipid solvents or surfactants. In a sense, the scalp serves as a lipid supply system for the hair, with sebum being produced continuously by the sebaceous glands [139, 140]. Sebum production is controlled hormonally by androgens that increase cell proliferation in the sebaceous glands. In addition, seasonal and even daily variations in the rate of sebum production do occur [139].

The aging of the sebaceous glands in man is controlled primarily by endocrine secretions [139]. For children, sebaceous secretion is low until puberty, when a large increase in sebaceous activity occurs (see Fig. 2.2). Note, the data of Fig. 2.2 did not permit a plot of the entire age curve for females; however, the same general effect of low sebaceous activity for males and females before puberty does exist.

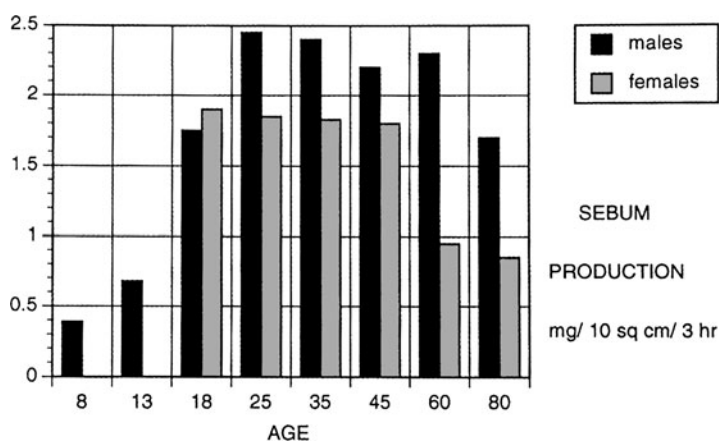


Fig. 2.2 Variation of sebum production on foreheads with age. Data are from Pochi et al. [141, 142]

For all ages, sebaceous-gland activity is lower for women than for men [139], and shortly before menopause (generally decreasing substantially in the mid-forties age range), there is a distinct decrease in sebum secretion, to even lower levels, see Fig. 2.2 and, *The effects of Menopause on the lipids in hair and on the hair fiber*. For males, there is more of a gradual reduction in sebum secretion with age beyond about 30 years. Strauss and Pochi [140–141] concluded that in both males and females, androgenic secretions are of primary importance for sebaceous-gland development and activity.

Extraction of human hair with “fat solvents” removes approximately 1–9% lipid matter. Ethanol, a solvent that swells hair, removes more material from hair than non-swelling solvents like benzene, ether, or chloroform. Hair consists of surface and internal lipid. In addition, part of the internal lipid is covalently bound and part is not covalently bound but both types of lipid can be cell membrane complex lipid. The cell membrane complex is laminar in structure and is composed of both protein and lipid layers; however, this structural lipid is not phospholipid [143, 144] like the lipids normally associated with bilayers of cell membranes (see the sections on *The Structures of the Three Different Cell Membrane Complexes* in Chap. 1 and additional parts of this chapter).

The data (1–9% extracted hair lipid) represent total matter extracted from hair clippings of individual men and women. Although the conditions for extraction can influence the amount of matter extracted from hair (Crawford R. Private communication), the values here represent “approximate” maxima and serve to indicate the variation in the amount of solvent extractable material from hair among individuals. Presumably, the principal material in these extracts consists primarily of free fatty acids (FFA) and neutral fat (esters, waxes, hydrocarbons, and alcohols). Gloor [145] classified the different components of sebum into six convenient groups: free fatty acids (FFA), triglycerides (TG), free cholesterol (C), cholesterol and wax esters (C & WE), paraffins (P), and squalene (S). These classes are similar but not identical to the classification groups by Masukawa et al. [107].

Spangler synthetic sebum (Table 2.16) provides a working formula to represent an imitation of average sebum. It contains lipid compounds to represent each of the six components of Gloor’s classification for sebum. Nicolaides and Foster [146] examined ether extracts of pooled hair clippings from adult males and found 56.1% as FFA and 41.6% as neutral fat. In contrast, daily soaking of the scalps of adults

Table 2.16 Spangler synthetic sebum

Lipid ingredient	Percentage
Olive oil (TG)	20
Coconut oil (TG)	15
Palmitic acid (FFA)	10
Stearic acid (FFA)	5
Oleic acid (FFA)	15
Paraffin wax (P)	10
Squalene (S)	5
Spermaceti (WE)	15
Cholesterol (C)	5

Table 2.17 Composition of FFA in human hair lipid

Chain length	% Total FFA	% Unsaturated FFA of this chain length
7	0.07	—
8	0.15	—
9	0.20	—
10	0.33	—
11	0.15	—
12	3.50	4
13	1.40	3
14	9.50	15
15	6.00	25
16	36.00	50
17	6.00	67
18	23.00	80
20	8.50	85
22	2.00	—
Residue	4.00	—
Total	100.80	

(males) in ether provided 30.7% FFA and 67.6% neutral fat. Nicolaides and Rothman [138] suggested that this apparent discrepancy is likely from lipolytic hydrolysis of glycerides in the stored hair clippings.

Analysis of the FFA extracted from pooled hair clippings of adult males was conducted by Weitkamp et al. [117]. Their study did not contain data concerning the effect of lipolysis on the structures of FFA in hair fat. Saturated and unsaturated fatty acids ranging in chain length from 5 to 22 carbon atoms were found in human hair fat [117, 147]. Location of the double bond in the unsaturated acids is suggested to occur at the 6, 7 position, with some 8, 9 and other isomers. Data from the study by Weitkamp et al. [117] are summarized in Table 2.17. In addition to the acids reported by Weitkamp et al. [117], Gershbein and Metcalf [147] examined the total fatty-acid content (following saponification) of human hair fat and found traces of C5 and C6 carboxylic acid and small quantities of C19 and C21 acids, as well as branched-chain isomers of several other fatty acids [147].

Comparison of the FFA content [117] with the total (hydrolyzed) fatty acid content [148] is summarized in Table 2.18. This comparison assumes that data from different laboratories are comparable. With the exception of the C16 and C20 acids, the data in columns A and B of Table 2.18 are very similar for each corresponding acid. Equivalence suggests that the relative amounts of each acid in ester form would be the same as the relative ratios of the free acids, and that hydrolysis may occur on standing (or other conditions) to increase the ratio of FFA to esters. The noteworthy exceptions are the C16 saturated acid that must exist in ester form to a greater extent than suggested by the relative ratios of free acids and the C20 unsaturated acid, that was found only in trace quantities by Gershbein and Metcalf [147]. A further conclusion from these studies is that the principal acyl groups present in human hair lipids are from the C16 fatty acids.

Table 2.18 Comparison of FFA content of human hair with total fatty acid content

Chain length	% Total FFA [117]	% Total fatty acids [147]
12	3.36	2.19
13	1.36	–
14	8.10	8.40
15	5.50	6.70
16	18.00	24.90
17	2.00	2.30
18	5.00	4.60
20	1.30	–
Chain length	Relative ratio to C14 FFA	Relative ratio to C14 total fatty acids
12	0.4	0.3
13	0.2	–
14	1.0	1.0
15	0.7	0.8
16	2.2	3.0
17	0.3	0.3
18	0.6	0.6
20	0.2	–

Only those acids above 1% are listed

Analysis of some of the neutral material from human hair lipid, for example, triglycerides, cholesterol or wax esters, and paraffins provides a mixture as complex as that of the fatty acids [117, 138, 146, 149]. Although not all of the compounds of these different components of hair lipid have been fully analyzed, it is obvious from the discussion on fatty acids and the literature on wax alcohols in human hair lipid [117, 147, 149, 150] that the variation in chain length and isomer distribution of all of these esters must be extremely complex. The data of Table 2.18 compares the free fatty acid content of human hair with the total fatty acids from hydrolysis. These data show that C16 fatty acids are at the highest levels consistent with other data, but do not contain 18-MEA because these data are more than 40 years old.

It is well known that the amount of sebaceous secretion increases with age near puberty [139, 140]. The composition of the sebaceous secretion also changes at that time [151]. Nicolaides and Rothman [151] demonstrated that the paraffinic hydrocarbon content of sebum is highest in children (boys), lower in men, and lowest in women. These same two scientists also showed that the squalene content of the hair lipid of children is approximately 1.35% of the total lipid content and about one-fourth that of adults. Sebum from boys' age 6 to 12 was examined in this study and compared to that from both men and women. In addition, the cholesterol content of the hair lipid of adults is lower than that from children: 3.7% vs. 12.2% [151].

Nicolaides and Rothman [138] determined with small sample sizes that hair from African-Americans contains more lipid than hair from Caucasians. Gershbein and O'Neill [149] examined the distribution of fatty alcohols of human hair lipid to determine the relative amounts of fatty alcohols and sterols with regard to sex, race, and scalp condition. Samples originated from Caucasians and African-Americans,

both full haired and balding, and from Caucasian women. The data indicated essentially no differences among these parameters between the two racial groups or between the sexes. Kreplak et al. [152] examined lipid profiles in transverse cuts across hair using synchrotron infrared micro-spectrometry and determined that Caucasian hair often contains lipids localized inside the medulla and the cuticle, but it occurs to a lesser degree inside the cuticle. Further, the African-American hair that was analyzed does not show these same hair lipid effects across the section and is insensitive to solvent extraction.

Several other factors relevant to differences in sebum composition on the scalp have been described in the literature. Anionic surfactants or ether extraction of the scalp does not stimulate the rate of re-fatting [153, 154]. Selenium disulfide in a shampoo increases sebum production [154] and it alters the ratio of triglycerides to free fatty acids found in sebum. Presumably, this latter effect involves reducing the microflora responsible for lipolytic enzymes on the scalp that hydrolyze triglycerides to free fatty acids. Zinc pyrithione appears to behave similarly and has been shown to increase hair greasiness [155], presumably in an analogous manner. However, ketoconazole (another antifungal agent) behaves in the opposite manner. Pierard-Franchimont et al. [156] confirmed the increase in sebum excretion rate for selenium sulfide and further demonstrated that ketoconazole decreases sebum excretion.

Several studies demonstrated significant differences in the lipid composition of oily vs. dry hair. Perhaps the most comprehensive study in this regard was by Koch et al. [144], who examined hair surface lipid from 20 dry- and oily-haired subjects, 3 days after shampooing, and found the following correlations with increasing hair oiliness:

- An increasing percentage of wax esters in the lipid,
- An increasing ratio of unsaturated to saturated fatty acids,
- An increasing amount of monoglycerides, and
- A decreasing percentage of cholesterol esters with increasing oiliness.

The quantity of total lipid was not found by Koch to correlate with hair oiliness. However, this is not surprising (in a several-day study), because the quantity of lipid on hair tends to level after a few days from shampooing because of partial removal of excess lipid by rubbing against objects such as combs or brushes and even pillows and hats.

Koch et al. [144] explained oily vs. dry hair by the rheological characteristics of the resultant scalp lipid. For example, increasing the ratio of unsaturated to saturated fatty acids should decrease the melting point of the sebum, making it more fluid and thereby more oily. Monoglycerides are surface-active and therefore should enhance the distribution of sebum over the hair [144]. Factors such as fiber cross-sectional area or hair curliness were kept constant in Koch's experiments and thus not considered; however, one would expect the degree of oiliness to affect straight, fine hair the most and to have the least cosmetic effect on curly coarse hair [157].

Bore et al. [158] found that the structures of the C18 fatty acids of oily and dry hair differ. For subjects with dry hair, Bore et al. found the predominant isomer as octadecenoic acid (oleic acid), whereas for subjects with oily hair 8-octadecenoic acid was the predominant isomer. Thus oily hair is different from dry hair in its chemical composition and in its rheological character. Hair lipid plays a critical role in shampoo evaluation (Crawford R. Private communication) and in surface effects of hair, such as frictional effects [159]. See Chap. 6 for discussion of the removal of hair lipid by shampoos.

2.4.4 The Effects of Menopause on the Lipids in Hair and the Hair Surface

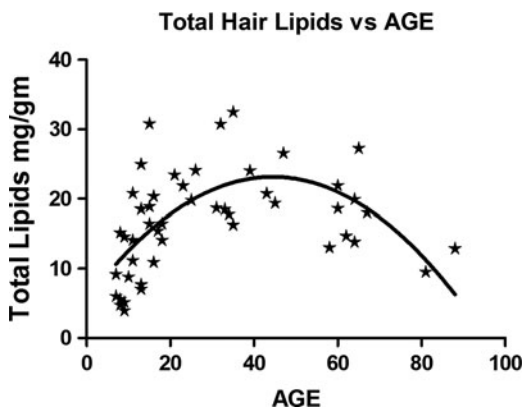
Wills et al. [160] showed that the cholesterol and ceramide (both matrix cell origin) content of the hair of pre-menopausal women was significantly higher while wax esters and squalene contents (both primarily from sebum) were significantly lower in post-menopausal women. These same authors noted that their analytical procedure could not distinguish between wax esters and cholesterol esters; however the wax ester levels are much higher in adult human hair than cholesterol esters as shown by the work of Pochi, Strauss and Downing [161].

Wills et al. [160] also found that the hair of pre-menopausal women ($N = 80$) was significantly greasier than the hair of post-menopausal women ($N = 47$). These scientists used expert visual assessment for this determination. In this same study, the hair of post-menopausal women on hormone replacement therapy ($N = 39$) was intermediate in greasiness and all three groups' scores were significantly different from each other. In addition, the amount of lipid found on the forehead of these same subjects was significantly higher in the pre-menopausal group than both of the other groups and the post-menopausal group not on hormone replacement therapy had the lowest amount of lipid (-57% vs. the pre-menopausal group). This effect is consistent with that of Pochi and Strauss [141, 161] who showed that hair on the foreheads of women decreased significantly in the mid-forties and has been attributed to the menopause.

Analysis of the amount of actual hair lipid by Wills et al. was on only 20 selected subjects of each group and proved to be not significantly different between the pre- and post-menopausal groups. These authors speculated that perhaps other factors such as "permeability of hair to sebum changes with menopause" or that the manner that the groups were balanced interfered with this determination.

The ages of these three groups were: pre-menopausal, mean age 30, range 24–34; post-menopausal, mean age 60, range 50–76; and post-menopausal with hormone replacement therapy, mean age 57, range 48–68. So, the prime variables are menopause and hormone replacement therapy, however there is also an additional factor of age especially between pre- and post-menopausal groups.

Fig. 2.3 Total hair lipid levels as a function of age. Data are from the P&G Wella Hair Research Group



Data for lipids on and in hair by age from an unpublished study by P&G/Wella Hair Research Group (see Fig. 2.3) shows a corresponding relationship to that found on foreheads by Pochi and Strauss [141] and by Wills et al. [160]. In this study, hair samples were collected from 51 Caucasian females varying in age from 7 to 88. This hair was extracted and analyzed by GC/MS (at the German Wool Research Institute (DWI)) for lipids. A Box plot of the Wella data revealed two outliers and the data was not normally distributed by the Shapiro-Wilk test (Shapiro-Wilk $W = 0.942$ and $p = 0.0147$). But, when these two outliers were rejected the data provided a normal distribution with a Shapiro-Wilk W of 0.978 and a $p = 0.5$ (very good). These normally distributed data when regressed vs. age provided a quadratic model with $p < 0.0001$, root mean square error of 5.623 and an r^2 of 0.387. From the model equation, the maximum for hair lipids was at age 45 corresponding to about where the steep drop occurs for sebum production on the foreheads of women by Pochi and Strauss [141] of Fig. 2.2. The age 45 peak appears to be influenced by the peri-menopause and is consistent with the work and conclusions of Wills et al. [160].

It is very clear from all of this work on hair lipids and age that the lipid levels in hair change with age. Large changes occur both at puberty and around ages 45–55. These changes at middle to advanced age are greater for women than for men. The changes that occur at both of these stages of life involve not only lipid levels, but also composition changes in the hair lipids. Wills et al. [160] in their study on pre- and post-menopausal effects determined that these changes affect hair greasiness, hair shine, hair softness and smoothness. All four of these properties decrease significantly with menopause and age.

In addition to these effects, Mirmirani and Dawson et al. [162] determined that post-menopausal women have significantly lower frontal scalp hair density, lower growth rates and lower hair fiber diameters than pre-menopausal women. A phototrichogram method was used to quantitate these hair parameters. Two studies were conducted by these scientists; an initial study included 44 women, 20 in the post-menopausal group and 24 in the pre-menopausal group. The second study included 177 women (ages 40–60) with 54 in the pre-menopausal, 33 in a

peri-menopausal group (irregular periods or cessation of periods for less than 12 months.) and 90 in the post-menopausal group. Hair growth rate was significantly lower in frontal than occipital regions. Growth rates were also significantly higher in pre-menopausal vs. post-menopausal women in both frontal and occipital sites. Hair density in the occipital site was not affected by menopause; however, hair density in the frontal site was significantly lower in post-menopausal vs. pre-menopausal women.

In both frontal and occipital sites pre-menopausal women had higher anagen percentages than in post-menopausal sites. Average hair fiber diameters were significantly higher in pre-menopausal vs. post-menopausal women in the frontal site, but not in the occipital site (no significant difference). In the expanded study on the frontal site, average fiber diameters were significantly higher in pre-menopausal vs. post-menopausal and peri-menopausal sites. However, there was no significant difference in hair fiber diameters in peri-menopausal and post-menopausal sites. The data suggest that the fiber diameter effect is independent of age. Mirmirami and Dawson et al. concluded that clinical observations support the effects of estrogens on hair biology; however, the current evidence is not adequate to attribute specific hair changes to hormonal effects of menopause.

This decrease in hair fiber diameter with menopause will decrease tensile and bending stiffness of hair fibers. The effects on fiber diameter in combination with the hair density decrease in the frontal region should produce changes in important consumer assessments in that scalp region. For example, hair body will decrease. This effect should appear immediately after shampooing; however the decrease in hair greasiness that will appear after a day or two or longer will tend to partly offset the hair body effect except for the everyday shampooer. I would anticipate related effects on combing ease, that is, as Robbins and Reich [163] have shown the decrease in stiffness will tend to make the hair more difficult to comb while the decrease in hair density/area will tend to make the hair comb easier. Which of these effects are stronger is too difficult to say. Nevertheless, the decrease in hair greasiness will also tend to make the hair more difficult to comb. But, the greasiness effect will take a day or longer after shampooing to take effect. So, the net effects on combing ease are more difficult to predict than on hair body without actual combing data.

2.4.5 The Composition of the Cortex

Since the cortex comprises the major part of the hair fiber mass, results of whole-fiber analysis of hair may be considered to be a good approximation of the composition of the cortex (see Table 2.7). The largest errors resulting from this approximation will be in those amino acids occurring in smaller quantities in the cortex.

Average cortex is rich in cystine (although there is less cystine in cortex than in cuticle). The cortex is also richer in diacidic amino acids and lysine and histidine

than is cuticle. However, the two main components of cortex, the intermediate filaments and the matrix, are very different in chemical composition. The intermediate filament proteins are rich in leucine and in glutamic acid and those amino acids that are generally found in alpha-helical proteins. Although small quantities of cystine (~6%), lysine, and tyrosine are also regularly arranged in the intermediate filaments [143], for additional details see the section entitled, *Type I and II Keratin Proteins (IF Proteins) of Human Hair* in this Chapter. On the other hand, the matrix is rich in cystine (about 21%, calculated from the sulfur content of gamma keratose of human hair) and proline and those amino acids that resist helix formation such as the KAP Proteins in *The KAP Proteins of Human Hair* in this Chapter. For additional information on the composition of the intermediate filaments and also the matrix, see the section on fractionation and peptide analysis of hair in this Chapter. Also see the section in Chap. 1 entitled *The Origin of Hair Fiber Curvature* which explains the distribution and composition of proteins of different types of cortical cells in human hair.

2.4.6 The Composition of the Medulla

Studies of the medulla of human hair are complicated, because it has poor solubility and is difficult to isolate, see the photomicrographs of the medulla in Chap. 1. In fact, most of the experimental work on medulla has been on African porcupine quill, horse hair, goat hair, or human beard hair medulla rather than medulla of human scalp hair fiber. Rogers [164] described the amino acid composition of medullary protein isolated from porcupine quill, and his results are summarized in Table 2.7 showing very low levels of cysteine and high levels of basic amino acids such as lysine and acidic amino acids such as glutamic acid.

Blackburn [165] determined some of the amino acids from medulla of wool fibers. Most wool fibers do not contain a medulla; however, some coarse wool like kemp or mohair does contain this porous component. Although Blackburn's results are more qualitative, they agree in general with the data of Rogers, suggesting low cystine content compared to whole fiber, and relatively large amounts of acidic and basic amino acids.

Langbein et al. [166] demonstrated 12 hair keratin proteins and 12 epithelial keratins that are potentially expressed in medullary cells of human beard hair medulla. The genes that form these keratins are located on the type I KRT18 gene along with genes located on chromosomes 17 and 12. These scientists also found a few cortical cells in this same beard hair medulla. This cortical cell effect may be exclusive to human beard hairs because this same pattern has not been reported in other highly medullated animal hairs. Langbein et al. concluded that medulla cells are distinct from all other hair follicle cells in keratin expression profile and keratin number.

If one assumes that medullary protein of porcupine quill is representative of medullary protein of human hair, some interesting comparisons can be made of the

three morphological regions of human hair. Among the gross differences is the fact that cuticle has even higher cystine content than whole fiber while medulla has only trace quantities of cystine. Medulla also appears to have relatively small amounts of hydroxy amino acids and relatively large amounts of basic and acidic amino acids compared to the other two morphological components of animal hairs. These facts suggest that medulla will be more susceptible to reactions with acids and alkalis and to ion exchange reactions such as reactions with anionic and cationic surfactants, ionic dyes and metals. But medulla will be less sensitive to reaction with reducing agents. One must also consider that since medulla is located at the core of the fiber, it is protected by both the cuticle and the cortex and by the slow rate of diffusion through these two morphological regions.

2.5 N-Terminal and C-Terminal Amino Acids and SCMK Fractionation

2.5.1 N-Terminal Amino Acids

Kerr and Godin [167], used the dinitrophenylation method of Sanger [168] and identified valine, threonine, glycine, alanine, serine, glutamic acid, and aspartic acid as N-terminal amino acids in human hair. Quantitative data by Leon [61], Speakman [169] and Hahnel [170] for N-terminal amino acids of human hair are summarized in Table 2.19. All of these references identify the same seven amino acids as N-terminal residues in human hair. In addition, there is agreement for the relative quantities of glycine, alanine, serine, glutamic acid, and aspartic acid as N-terminal groups. However, the quantitative data for valine and threonine are in discord. The apparent disagreement of these data may be due to differences in the relative ratios of the different proteins in the different samples caused by

Table 2.19 N-terminal amino acids in human hair (relative ratios)

Amino acids	Micromol/Gm hair	Relative ratios of amino acids	
		Reference [61]	Reference [170]
Valine	4.0	8	4
Threonine	4.0	8	6
Glycine	3.9	8	8
Alanine	1.0	2	2
Serine	1.0	2	2
Glutamic acid	1.0	2	2
Aspartic acid	0.5	1	1
Total	15.4		

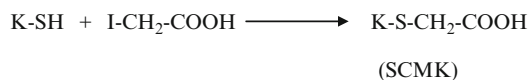
either sampling or experimental procedures. Hahnel [170] reported these same seven amino acids as N-terminal residues in calluses, psoriasis scales, nails and hair fiber.

2.5.2 *C-Terminal Amino Acids*

The C-terminal amino acids in human hair have been identified by Kerr and Godin [167] using the hydrazinolysis method of Niu and Fraenkel-Conrat [171]. These amino acids are threonine, glycine, alanine, serine, glutamic acid, and aspartic acid. Interestingly, all six of these amino acids also serve as N-terminal residues. These same six C-terminal amino acids have been identified by Bradbury as C-terminal residues in wool fiber [172].

2.5.3 *Fractionation Procedures*

More extensive peptide investigations of keratin fibers generally consist of solubilizing the keratin; separation of the resultant mixture by means of solubility, chromatography, or electrophoresis; and analysis of the resultant fractions. A commonly used method for preparing keratins for sequencing or peptide analysis consists of solubilizing the keratins with strong reducing solutions, usually salts of dithiothreitol or thioglycolic acid [173] or by using enzymes or mixtures or sequential treatments of reducing agents and enzymes [76]. With the S-carboxy methyl keratin procedure, the reduced keratin is reacted with iodoacetic acid, forming the S-carboxy methyl keratin (SCMK) derivatives [174] to enhance the solubility of the proteinaceous matter and to prevent reoxidation of the thiol groups. Radiolabelled iodoacetic acid is often used to tag the fractions and gel electrophoresis to separate the different protein fractions.



A relatively large amount of effort has gone into the fractionation of wool fiber into its major protein components and the characterization of the resultant fractions. Thus, the mysteries underlying the detailed structures of the major proteins and polypeptides of wool and human hair fibers are gradually being unraveled. The following papers by Bringans et al. [76], Crewther et al. [175, 176], Gillespie [177], Corfield et al. [178], Cole et al. [179], Chaps. 2 and 3 in the book by Fraser et al. [180], Fraser's paper [181], the book by Rogers et al. [182], and the papers by Swift [183] and by Powell and Rogers [184] and Langbein [185] and are leading entries into this work.

2.6 Major Protein Fractions of Hair and Gene Expression

During the past decade, a considerable amount of work has been done on the fractionation and amino acid sequencing of some of the major proteins of human hair. In addition, expression of genes, using in situ hybridization or reverse transcriptase-polymerase chain reaction (RT-PCR) expression by hair follicles or the use of specific protein antibodies or other techniques have been useful in helping to elucidate where and when the follicle genes are expressed.

The phrase major protein is used in the sense of the highest concentration proteins in the fibers or a specific region of the fibers. In this field, the following abbreviations are commonly used to describe the more important protein types under investigation:

KAP, keratin associated proteins [formerly IFAP (intermediate filament associated proteins)]

IF, intermediate filament proteins, now referred to as keratins [1]

HS, high sulfur proteins

UHS, ultra high sulfur proteins

HT, high tyrosine proteins

HGT, high glycine tyrosine proteins

Rogers [1] described the terms keratin and keratin-associated proteins explaining that the term keratin today generally refers to the intermediate filament proteins of the fiber, a clear distinction from the past. On the other hand, many of the KAPs are the high sulfur and ultra high sulfur proteins that commonly occur in the cuticle as well as in the matrix of the cortex.

For one procedure of analysis, Rogers et al. [182] suggested extracting the hair with dithiothreitol in alkali and 8 M urea, labeling with C14 iodoacetic acid at pH 8 and separation by polyacrylamide gel electrophoresis (PAGE) in sodium decyl sulfate solution. This procedure provides a separation into two major fractions for human hair consisting of high sulfur proteins that are from the matrix and classified as KAP proteins and a second fraction of low sulfur proteins that are IF material. A third fraction from wool fibers, but not present in human hair, consists of HGT proteins that are also matrix or KAP proteins. The status of the research concerned with differentiation into cuticle and cortical KAP proteins and the genes that correspond to these various proteins is summarized in the papers by Powell and G.E. Rogers [184] and in the review by G.E. Rogers [1] and in these two excellent reviews by Langbein and M.A. Rogers [185, 186].

2.6.1 *The KAP Proteins of Human Hair*

The keratin associated proteins include those that form the matrix of the cortex and the high cystine containing proteins of the cuticle. These proteins were discovered

more than three decades ago and some of their sequences are described in this reference by Powell and G.E. Rogers [184]; however the more recent review by M.A. Rogers and Langbein [187] covers the literature over the three decades leading up to 2006 and is extremely helpful to anyone interested in this area of research. Clusters of genes on at least five chromosomes 17q12-21, 21q22.1, 21q23, 11p15.5 and 11q13.4 are involved in the production of more than 80 different KAPs [80, 159].

The paper by Rogers and Langbein et al. [187] contains a helpful diagram that I have modified and used for the schematic of Fig. 2.4. This diagram illustrates several of the important KAP proteins in human hair cuticle and cortex. Rogers and Langbein suggested that the KAP 5, KAP 10, KAP 17.1 and KAP 12 occur in the largest amounts in human hair cuticle whereas the KAP 1, KAP 2, KAP 3, KAP 4, KAP 9, KAP 7, KAP 19.1 and KAP 19.2 occur in the largest amounts in the cortex. Anyone interested in the KAP's of the human hair cuticle and the cortex, their sequences, the domains that these proteins are found in and their genomic expression should read this review paper by Rogers and Langbein [187].

G.E. Rogers further explained that the KAP proteins of the matrix are a large group of perhaps as many as 100 different proteins. Rogers described the order of expression of genes and thus the synthesis of many of the proteins of the different parts of the hair fiber. The Ultra high sulfur proteins including those of the cuticle are among the last KAP's that are expressed. There are at least two unique families of proteins the KAP5 and KAP10s of the hair cuticle which are major components

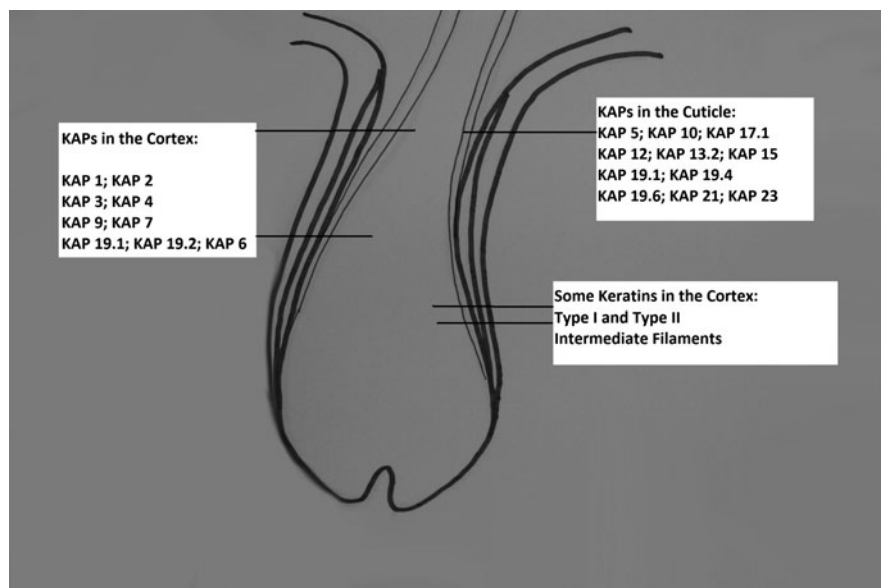


Fig. 2.4 Schematic indicating some KAP proteins of human hair cuticle and cortex, patterned after a schematic by Rogers and Langbein [159]

of the exocuticle [1], the A-layer [76] and the epicuticle membrane of the hair surface and of cuticle cells [95].

The HS proteins generally contain about 20% of their residues as half-cystine and the UHS proteins usually contain between 30% and 35% residues of half-cystine. These latter proteins in wool have been shown to be affected by the cystine/cysteine level in the wool follicle [1] which is determined by the cystine/cysteine level in the plasma. Proline generally occurs in the high sulfur proteins at a relatively high level (about 7–9%) and has been suggested as an indicator for the HS proteins. Marshall and Gillespie [29] suggested that the half cystine content of normal human scalp hair should be in the range of 17–18% and should not vary with age, but should vary only from sunlight, cosmetic treatment and biochemical abnormalities.

Although, the HS and UHS proteins are rich in half-cystine they contain very little to no methionine. Methionine, in the diet, is important to these proteins because it can be converted into cysteine [184]. The important role of cystine/cysteine in protein synthesis and to hair growth in the follicle is summarized well by Powell and Rogers [184] who described this subject in great detail. In the late 1990s there were at least eight families of KAP's ranging from 12 to 41 mol% cystine. As indicated before, there are also glycine-tyrosine rich KAP's, high sulfur cuticle KAP's and high sulfur cortex KAP's among others.

Jenkins and Powell [188] examined five proteins expressed by the KAP5 family of genes in sheep that encodes cysteine rich/glycine rich keratins in the cuticle. All of these proteins are high cysteine (~30%), high glycine (~27%) proteins of the cuticle and in humans this gene family (KAP5) is from chromosome 11. Rogers in his lucid review paper described this complex area even detailing some of the signals that regulate cell specificity and gene expression. For further details of this area of research see the excellent reviews by Professor George Rogers [1] and the outstanding reviews by M.A. Rogers and Langbein [185–187].

2.6.2 Type I and II Keratin Proteins (IF Proteins) of Human Hair

Two of the six different Types of IF Proteins are found in human hair. Type I and Type II keratins are distinguished by their isoelectric point, the Type I proteins being acidic and the Type II being basic or neutral. The nomenclature for the hair keratins is explained in Chap. 1 in Table 1.16. Important references on these important proteins are [185–189]. Langbein and M.A. Rogers et al. [185, 186] and Langbein and Schweitzer [189] described that the human hair keratin gene families have nine members in the Type I family and six members in the Type II family. The genes are on human chromosomes 17q12-21 and 12q13 and there are 15 functional genes, 9 for the Type I and 6 for the Type II families. The highest expressed keratins of the cuticle are: Type I hHa5 (K35) and hHa2 (K32) and for

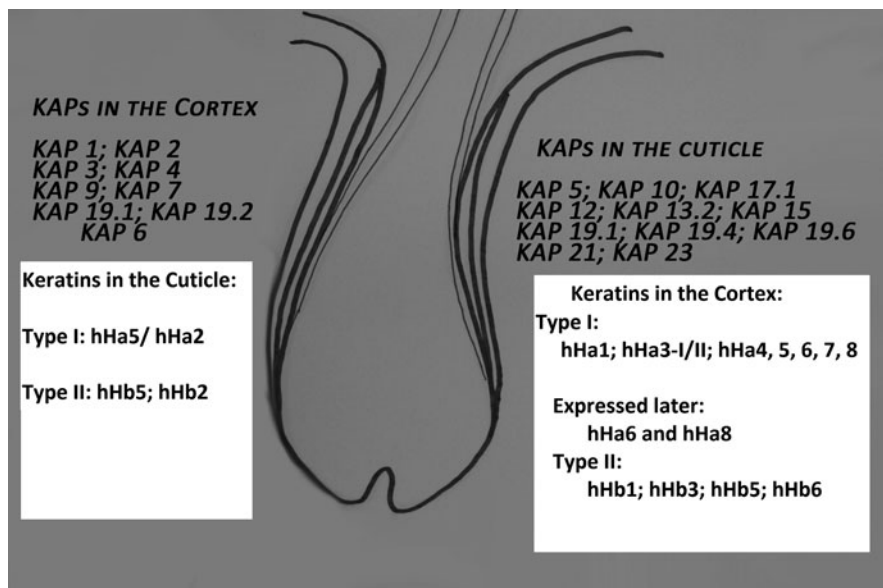


Fig. 2.5 Schematic summarizing the IF keratins of human hair; Data from Rogers and Langbein [157, 158]

Type II are hHb5 (K85) and hHb2 (K82). Three of these same keratins are also in the matrix of the cortex: hHa5 (K35), hHa2 (K32) and hHb5 (K85). The Type I keratins of the middle to upper cortex are hHa1 (K31), hHa3-I (K33a)/II (K33b), hHa4 (K34), hHa5 (K35), hHa6 (K36), hHa7 (K37) and hHa8 (K38). Type II keratins of cortex are hHb1 (K81), hHb3 (K83), hHb5 (K85) and hHb6 (K86) [185, 186] see Fig. 2.5. The names in parenthesis are a newer nomenclature.

The intermediate filaments in different tissues show some similarity in form; see Fig. 1.36 in Chap. 1 in the section entitled *Intermediate Filaments* for a discussion of these structures; however these structures differ considerably in their exact composition and configuration [186–189]. The common structural feature among this class of proteins is the central helical rod [188]. On the other hand, a primary difference is in the amino and carboxyl domains. These domains vary in both amino acid sequences and size [189]. The end domains contain many cysteine residues and these can even form cystine cross-links with cysteine residues of KAP proteins of the matrix.

As described above, the intermediate filament protein molecules in keratins are composed of two different types of polypeptides, Type I (acidic side chains) and Type II (neutral to basic side chains). Equimolar quantities of a Type I and Type II are necessary to form an Intermediate filament (Fig. 1.36). These two chains initially coil about each other forming a two strand coiled-coil rope, thus the initial formation of each filament requires one acidic polypeptide that coils about a basic

polypeptide partner or mate. Additional coiled coils join together end to end and laterally. For additional details and references see Chap. 1 in the section entitled *Intermediate Filaments*.

The cystine content of the low sulfur intermediate filament regions is about 6% and is not uniformly dispersed between domains of an intermediate filament chain. The rod domain contains about only 3% half-cystine or as little as one half-cystine residue, while the N terminal domain contains about 11% half-cystine and the C terminal unit about 17% half-cystine [190, 191]. It would appear that these half-cystine residues are involved in disulfide linkages but that many more disulfide residues exist in the matrix.

The acidic Type I intermediate filament proteins of human hair represent a class of proteins that are about 44 and 46 K in molecular weight, while the basic-neutral Type II proteins are about 50, 59, and 60 K [192]. Langbein and Schweitzer [189] described the IFs of the Medulla, the inner root sheath, the outer root sheath and the companion layer and discussed even newer nomenclatures for this important class of hair proteins. Amino acid sequences for the intermediate filament polypeptides from several proteins including wool fiber were initially described by Crewther et al. [176]. For a more comprehensive discussion of the IFs, see the manuscript by Powell and Rogers [184] and the references therein; also see the more recent reviews by Rogers [1] and the important papers by Langbein and M.A. Rogers and especially the review by Langbein and Schweitzer [189] and the discussion and references in Chap. 1 in the section entitled *Intermediate Filaments*. Intermediate filaments are involved in a large number of diseases. For a lead into that subject see Chap. 3 and the discussion on *Hair Abnormalities*.

2.6.3 *Tricohyalin Protein*

Tricohyalin is a granular, proteinaceous material found in the cytoplasm of cells of the inner root sheath that envelopes the growing hair fiber; see Fig. 1.6 of Chap. 1. It is a major protein synthesized during hair growth and can also be found in the matrix of the cortex and in the medulla of fully formed hair fibers. However, its role in the growth of human hair fibers is not fully understood at this time. The amino acid composition of tricohyalin protein found in sheep, guinea pig and human hair follicles has been reported by Rogers et al. [193]. Tricohyalin contains citrulline resulting from arginine conversion through the enzyme peptidyl arginine deiminase [192]. It also contains many repeat units and is larger in human hair than in wool fiber (1,897 vs. 1,549 amino acid residues). Its sequencing studies show that tricohyalin is not a precursor of IF proteins. For more details on this unique protein, see the review by Powell and Rogers [184].

2.7 Other Protein Fractionation Methods

An older method of fractionation of keratin fibers, the method of Alexander and Earland [97, 194, 195], consists of oxidation of the disulfide bonds of the hair to sulfonic acid groups, using aqueous peracetic acid solution, and separation of the oxidized proteins, generally by differences in solubilities of the different components of the mixture. The primary three fractions in this separation are called keratoses. The amino acid composition of these three fractions isolated from merino wool has been reported by Corfield et al. [195].

Fractionation of human hair into keratoses by the method of Alexander and Earland [97] as modified by Corfield et al. [195] has been reported for human hair by Menkart et al. [25] (see Table 2.20). This procedure consists of oxidation of the fibers with aqueous peracetic acid and solubilization in dilute alkali. The insoluble fraction is called beta keratose and is believed to consist of proteins derived primarily from cell membranes and similar matter. See the previous section in this Chapter entitled, *Proteins in the Cortical Cell Membranes*, describing an amino acid analysis of an extract of this fraction. Acidification of the solution to pH 4.0 produces a precipitate called alpha keratose that is believed to originate primarily in the crystalline or fibrillar regions of the cortex. The material remaining in solution has been labeled gamma keratose. It is the fraction containing the largest percentage of sulfur (see Table 2.20) and is believed to consist of proteins derived primarily from the amorphous regions of the fibers (primarily from the matrix of the cortex). Of special interest is the significantly larger gamma keratose fraction from human hair compared to merino wool (see Table 2.20). This is consistent with the higher cystine content in human hair.

Using a similar procedure, Crounse [196] examined a portion of the alpha keratose fraction by quantitative amino acid analysis. He found similar quantities in the amino acids of this fraction obtained from human hair and from fractions of nails and epidermis, except for cystine, cysteine, and glycine.

A modified version of this procedure has been described by Wolfram and Milligan [197]. Their procedure involves esterification of the carboxyl groups that are believed to reside primarily on the alpha-helical proteins and proteins of the hair surface. Esterification decreases the solubility of these proteins, allowing the non-esterified proteins (of the matrix) to be extracted more easily. The soluble fraction of this procedure is called gamma*keratose; it resembles gamma keratose but provides a higher yield. The insoluble residue exhibits birefringence and is called the alpha-beta*keratose fraction.

Table 2.20 Percent keratoses in human hair [25]

Fiber type	Alpha-keratose	Beta-keratose	Gamma-keratose	Total
Merino wool	56 (1.88) ^a	10 (2.13)	25 (5.84)	91
Caucasian hair	43 (2.38)	14 (4.00)	33 (6.60)	90

^aPercent sulfur in parentheses [25]

Other fractionations of human hair have been reported by Vickery et al. [26] and Andrews and deBeer [135] and by Lustig et al. [136]. The former paper describes a hydrolytic separation and the latter a fractionation by sulfonation followed by reduction [19]. These procedures have not been pursued to a great extent because of the inherent amino acid degradation in the initial solubilization reaction.

2.8 Diet and Hair Composition

Ultra high sulfur protein production appears to be very sensitive to the amount of cysteine that is present in the diet of sheep [198] or available in the follicle. The same phenomenon most likely exists for human hair. Other than malnutrition, hair proteins have not been shown to be influenced by diet. Campbell et al. [198] demonstrated via nutritional studies on sheep that crimp frequency can be explained by considering fiber growth rates as influenced by diet; see the data of Table 2.21. These data show that crimp counts increase at low nutritional levels where the growth rates are slower for both high crimp and low crimp producing sheep. The percentages of sulfur and of high sulfur proteins also decrease at the low nutritional levels where growth rates are slower. So protein composition as influenced by diet (malnutrition vs. normal nutrition) affects the ratio of high to low sulfur proteins in sheep and it plays a role in determining hair fiber curvature. It is likely that this same effect exists in humans, because of the results described (below) on malnutrition effects on hair composition and growth.

Studies of the effects of diet in persons suffering malnutrition such as protein deficiencies show that diet supplementation can influence the protein composition of human hair. However, such effects have only been demonstrated among persons suffering from severe malnutrition and never among healthy persons on a normal diet. For example, the cystine, arginine, and methionine contents of human hair have been reported to be influenced by diet that is insufficient in protein content. Koyanagi and Takanohashi [142] conducted a study among eight- to nine-year-old Japanese children who had been fed millet and very little animal protein. Analysis of the hair from these children revealed cystine contents as low as 8.1% (675 μ mol half cystine per gram of hair) rather than 17–18% as suggested by Marshall [29] as the normal half cystine level in humans. Diet supplementation with shark liver oil produced a significant increase in the cystine content of the hair among these children. Diet supplementation with skim milk for 6 months produced an even larger increase in cysteine, most likely from an increased synthesis of the Ultra high sulfur proteins.

Table 2.21 Effect of nutrition on high S Proteins & Crimp from Campbell et al. [198]

Nutritional level	High crimp wool			Low crimp wool		
	Norm	Low	Norm	Norm	Low	Norm
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 Prot.	32	22	29	24	17	20

Although, the HS and UHS proteins are rich in half-cystine they contain virtually no methionine. Methionine in the diet is important to these proteins because it can be converted into cysteine and cystine [184]. The important role of cystine/cysteine in protein synthesis and to hair growth in the follicle is summarized well by Powell and Rogers [184].

Cystine, methionine, and sulfur contents of the hair of children suffering from kwashiorkor have also been reported to be lower than that of normal children [199]. The arginine content of hair has been reported to decrease as a result of kwashiorkor [200]. In fact, Noer and Garrigues [200] reported arginine contents of human hair in severe cases of kwashiorkor, as low as one-half the normal level. By analogy with the effects of diet and sulfur enrichment on the high-sulfur proteins in wool fiber [201, 202], these effects of a lower arginine content in hair are probably a result of a decreased synthesis of the sulfur-rich proteins that likely contain arginine too.

Cosmetic advertisements abound with the suggested or implied nutrient or health-benefit claims provided by proteins or vitamins or even provitamins in cosmetic products. Marshall and Gillespie [202] offer the following conclusion with regard to nutrition and hair. "In healthy humans, it is unlikely that any significant variation in the proteins of hair will result from normal changes in nutrition." Therefore, it is much less likely that such changes could ever be induced from these same ingredients or their precursors when applied topically in a shampoo or a hair conditioner.

In fact, there have been no systematic studies of the effects of nutrients like vitamins on the rate of wool or hair growth or structure. However, there are some indications that in dietary insufficiencies, supplements of folic acid (a B complex vitamin) or pyridoxine (a B complex vitamin, B6) could be helpful to hair growth. The logic behind these indications is that these vitamins play a role in cysteine metabolism. However, cysteine metabolism does not take place in the non-living part of the hair fiber. On the other hand, panthenol, the precursor to pantothenic acid (another B complex vitamin) has never been demonstrated in any published scientific study to affect the nutrition or growth of hair. In a review on nutrition and hair, Flesch [148] reported, "There is no objective evidence available to support the assumption that pantothenic acid has a biochemical role in the production of hair." Thus, there is no current objective evidence to support a nutritional benefit to hair by this vitamin precursor.

Among sheep with dietary insufficiencies, the minerals copper and zinc when supplemented to the diet have been shown to be important to wool fiber growth. Their effectiveness is attributed to the important roles these minerals play in sulfur amino acid metabolism; copper serves to catalyze the oxidation of cysteine to cystine cross-links during fiber synthesis [203]. A related effect has been shown to occur in African children with a deficiency in riboflavin and pantothenic acid wherein the hair grows with no or minimal pigment and is straight. This effect has been associated with a copper deficiency and is explained in Chap. 3. Zinc is required for cell division to occur and it also appears to play a role in liver disease and protein metabolism [204].

2.9 The Analysis and Origin of Protein Fragments from Damaged Hair: Useful Methodology for the Future

Partial removal and analysis of proteinaceous matter from damaged keratin fibers can be traced back to the alkali solubility test. The alkali solubility test involves exposing a weighed amount of hair to a fixed volume of 0.1 N sodium hydroxide solution at 20°C for a fixed time [205, 206] and isolating, drying and weighing the hair. The loss in weight provides the amount of protein loss from the keratin which is almost always greater in damaged hair than undamaged hair. For example, Dubief [40] examined undamaged hair, the same hair exposed to visible light and hair exposed to UV plus visible light and found 1%, 1.6% and 3.5% alkaline soluble matter respectively. Inglis and Leaver [45] were one of the first to show that proteinaceous derived fragments were removed or dissolved into the bleach bath by treatment of wool fiber with aqueous alkaline hydrogen peroxide treatment.

Various schemes to solubilize and isolate the proteinaceous fragments or matter from damaged hair have subsequently been tried. Oku et al. [207] analyzed total proteinaceous fragments from the hair dissolved in permanent wave solutions and recommended this as an assay for hair damage. Sandhu and Robbins [208] shook chemically damaged and control hair fibers in water or detergent solutions and analyzed the total dissolved and insoluble proteinaceous matter separately and together by the Lowry test. Inoue, Ito and Kizawa [209] extracted the hair with different reducing solutions and analyzed the extracted proteinaceous matter which they called Labile proteins by the BioRad Protein assay. The amount of extracted proteinaceous matter from the hair of three different permed treatments by these scientists appeared to relate to the reduction in the tensile breaking force. Ruetsch, Yang and Kamath [210] extracted bleached hair, UV treated hair, permed hair and bleached/UV treated hair and bleached/permed hair with 0.05 M dithiothreitol, 8 M urea and Tris buffer for 24 h and then sonicated the extract for 30 min. They then derivatized the reduced hair with 20% iodoacetamide to prevent reoxidation. These extracts were then separated and analyzed by electrophoresis. Some of the conclusions from this study were:

- Chemical bleaching with alkaline peroxide, fragments the matrix and the intermediate filament proteins,
- Permanent waving hair produces soluble fragments from the matrix proteins,
- Multiple perms and permanent waving followed by UV treatment decreases the Intermediate filament extractable protein fragments, but the matrix protein fragments can still be extracted. Multiple perming and UV treatment can render IF proteins less extractable possibly by producing higher molecular weight proteins via cross-linking or fusion reactions see Chap. 5 in the section entitled *Long Term Irradiation Produces Fusion Reactions Across Structural Boundaries*. Shorter term UV treatments were not examined.

More recently Sinclair, Davis and Flagler et al. [211] analyzed water solutions and suspensions from shaking hairs in water similar to the method of Sandhu and Robbins, but Davis and Flagler took this method to a higher level by determining some of the hair proteins from which the fragments/matter were actually derived. Davis and Flagler [211, Davis MG, Flagler M. Private communication] analyzed the proteinaceous matter in solutions and suspensions from bleached and undamaged hair via Matrix assisted laser desorption ionization-Time of flight mass spectrometry (MALDI-TOF) and also by 2 Dimensional Gel Electrophoresis (2DGE) and demonstrated significant decreases in chemically bleached hair in these cortex proteins: acidic keratins 31, 33a, 33b and basic keratins 81, 83, 85 and 86. K32 of the cuticle was also decreased by bleaching. This assay currently is not as amenable to KAP proteins as it is to keratins which may be due to the small fragment size requirements for mass spec analysis. More creative extension to this type of procedure should expand its utility and reveal very useful information in the future for hair research.

2.10 Water: A Fundamental Component of Human Hair

Table 2.22 summarizes the effects of relative humidity on the water content of human hair (Anzuino G. Private communication). Additional data are described in Chap. 9 in the section entitled *Water (RH), pH and Solvents and the Dimensions of Hair*. Obviously, the determined moisture content of keratin fibers depends on the conditions selected as the state of dryness [142] as well as on the RH. The amount of moisture in hair also plays a critical role in its physical and cosmetic properties, as described in Chap. 9. The data of Table 2.22 were obtained by dehydration of the fibers in a dry box over calcium chloride and determining the regain at increasing humidity. Chamberlain and Speakman [212] reported the moisture content of human hair by moisture regain from the dry state and by way of dehydration from 100% RH. Their data show a hysteresis wherein the moisture contents at intermediate humidity are slightly lower by the hydration method than by dehydration. This hysteresis phenomenon is described in more detail in Chap. 9.

Similarly, hair dried with heat can exhibit lower moisture content than hair dried at room temperature [213]. After heat-drying, hair absorbs moisture but does not

Table 2.22 Water content of hair at different relative humidities

RH ^a	Approximate moisture content ^b (%)
29.2	6.0
40.3	7.6
50.0	9.8
65.0	12.8
70.3	13.6

^aTemperature = 74°F

^bEach value is an average of five determinations on dark brown Italian hair from DeMeo Bros. reported to be undamaged chemically. The hair was not extracted with solvent

return to the room temperature dried moisture level until it is either rewet with water or conditioned at a higher relative humidity. Thus, a hysteresis exists between heat-dried hair and room temperature dried-hair similar to that from absorption vs. desorption of moisture.

Hysteresis phenomena in the water sorption by high polymers [214] and by other proteins such as wool fiber [215] and casein [216] have also been described. Smith [214] suggested that hysteresis is a result of differences in the ratio of “bound” to “free” water in the substrate, with a larger amount of bound water present on desorption than on absorption because more water binding sites are accessible from the wet state than the dry state.

Undoubtedly, the several hydrophilic side chains (guanidino, amino, carboxyl, hydroxyl, phenolic, etc.) and peptide bonds of keratin fibers contribute to water sorption, although there is controversy over the primary water-binding groups. Leeder and Watt [216], in a very interesting study involving water sorption of unaltered and deaminated wool fibers, concluded that the binding of water by amino and guanidino groups is responsible for a large percentage of the water sorption capacity of keratin fibers, especially at low humidities. On the other hand, Breuer concluded that the peptide bonds are preferential sites for hydration [60].

The conclusions of Leeder and Watt are supported by Pauling [217], who described the negligible attraction of water by the polypeptide nylon, and the apparent agreement between the number of molecules of water initially sorbed by several proteins and the number of polar side-chain groups in those proteins.

Spectroscopic studies of the nuclear magnetic resonance (NMR) of both human hair [218] and wool fiber [219] indicated that the protons of water in keratin fibers are hydrogen-bonded and are less mobile than in the bulk liquid. At relative humidity, below 25%, water molecules are principally bonded to hydrophilic sites of the fiber by hydrogen bonds and can be described by Langmuir’s fundamental theory for the absorption of gases on solids [220]. As the humidity increases, additional water is sorbed, producing a decrease in the energy of binding of water already associated with the protein. At very high RH, above 80%, multi-molecular sorption (water on water) becomes increasingly important.

Feughelman and Haly [220] and Cassie [221] suggested two different models for estimating the amounts of bound “un-mobile” and mobile “free or liquid” water present in keratin fibers. Feughelman and Haly defined bound water as water associated with the keratin structure and mobile water as water not associated directly with the keratin structure. This model considers the decrease in energy of binding of water molecules already associated with the keratin structure with increasing water content. King [222] discusses two- and three-phase adsorption theories to explain the adsorption of moisture by textile materials. His conclusions and cautions are pertinent to this same phenomenon in human hair. King suggested that it is relatively easy to derive a sorption isotherm that fits an empirical relation using two or three adjustable coefficients, and he cautioned others in keratin science to make sure the theory they consider does not contradict accepted physical principles. The effects of water on swelling, friction, tensile, and other properties of human hair are described in Chap. 9.

2.11 Trace Metals in Human Hair

There are a number of studies describing the quantitative determination of various elements of human hair other than carbon, hydrogen, nitrogen, oxygen, and sulfur. In particular, the inorganic constituents of human hair appear to be receiving some attention because of their potential in diagnostic medicine as described by Maugh [223], and to a lesser degree in forensic science. However, the fact that certain transition metals such as iron and copper can catalyze the formation of free radicals in oxidative reactions has picked up interest in cosmetic science too.

The mineral content of human hair fibers is generally very low (less than 1%). It is sometimes difficult to determine whether this inorganic matter is derived from an extraneous source (which much of it is) or whether it arises during fiber synthesis. In addition, many metals of human hair exist as an integral part of the fiber structure, such as salt linkages or coordination complexes with the side chains of the proteins or pigments, although the possibility of mineral deposits or compound deposits as in soap deposition also exists.

Pautard [224] reported the total ash content of human hair to be as low as 0.26% of the dry weight of the fibers. But, Dutcher and Rothman [225] reported ash contents to vary from 0.55% to 0.94%. Among the trace elements reported in human hair are Ca, Mg, Sr, B, Al, Na, K, Zn, Cu, Mn, Fe, Ag, Au, Hg, As, Pb, Sb, Ti, W, V, Mo, I, P, and Se. The actual origin of most of these elements in human hair is due to a variety of sources that are described below. However, from a study involving quantitative analysis of 13 elements in human hair and in hair wash solutions, Bate et al. [226] concluded that a large portion of the trace elements in the hair originate from sweat deposits.

In the case of metals, the water supply generally provides calcium and magnesium to hair. Smart et al. [227] reported that oxidation dyed hair washed multiple times in tap water accumulates high concentrations of metals in the sulfonate rich exocuticle of the hair. This nano-scale ion mass spectrometric study provides evidence that calcium binds to the sulfonate groups produced by oxidation. Common transition metals such as iron, manganese and copper also deposit in hair from the water supply. Copper from swimming pools has been reported by Bhat et al. [228] to turn blond hair green at low concentrations. Other sources of metals in hair are sweat deposits, diet, air pollution, and metabolic irregularities. Metal contamination can also arise from hair products that provide zinc or selenium (antidandruff products), potassium, sodium, or magnesium (soaps or shampoos), and even lead from lead acetate-containing hair dyes.

2.11.1 Transition Metals and Free Radical Reactions

The Transition metals Fe, Cu, Mn, Co and V are very active and can participate in one electron transfer reactions and thereby participate in free radical reactions.

Of these metals, Fe and Cu are the most likely to be found in human hair and will be the focus of this discussion. As described in Chap. 5, trace quantities of these metals can participate with either hydrogen peroxide or hydroperoxides formed in hair to form hydroxyl radicals through the Fenton reaction. In addition, free radicals can be formed by direct photolysis of hydroperoxides. Some iron complexes or phenolics [229] or even the excitation of dyes or fluorescent whitening agents in the presence of one electron donors (Fe, Cu, Mn, Co and V) as described by Millington [230] can produce superoxide radical by a variety of reactions including autooxidation of mercaptans such as cysteine. Transition metals in hair can be endogenous or exogenous. Exogenous sources are:

- The water supply used for bathing and washing hair
- Swimming pool water
- Airborne pollutants

Kempson et al. [231] cited a study by Trunova et al. [232] who concluded that Cu and a few other elements are reliable indicators of endogenous consumption, but Fe and Ca are not. This citation suggests that Fe and Ca contents of hair are more readily affected by environmental influences than Cu. Cu is involved in two important metabolic processes; one in the keratinization of human hair fibers (oxidation of thiol to disulfide) and in the oxidation of tyrosine to melanin involving the enzyme tyrosinase which also requires Cu [233]. Therefore, Cu is endogenous [234] to hair fibers. However, Cu can also arise in hair from exogenous sources such as swimming pool water producing the green hair phenomenon [228]. Ca is primarily exogenous in origin. Although Fe and Cu are also exogenous metals, the study by Trunova et al. (above) suggested that the Fe content of hair is influenced more by exogenous sources than the Cu content of hair [232, 233]. As indicated earlier, this study by Trunova suggested that Ca and Fe will compete more effectively (not exclusively) than Cu for acidic sites on hair including sulfonate and carboxylic acid sites.

2.11.2 Functional Groups that Bind Specific Metals

Kempson et al. [231] reviewed the existence in human hair for metals like Cu and Zn with some data on Fe and Ca and other metals. They suggested that Ca has a higher affinity for carboxylic acid and sulfonate groups than Cu. These same scientists suggested that Cu(II) has a preference for binding with primary amine groups $\{-\text{NH}_2\}$ and Cu(I) has a higher affinity for thiol groups $\{-\text{SH}\}$. Kempson et al. also stated that “perhaps. . . Cu and Zn do not form soaps with lipids” in hair, however, Cu is known to form water insoluble soaps in-vitro when reacted with lipids such as butter or with oils such as cottonseed oil or soy bean oil as described by Berry [235]. It may be that Ca has a higher affinity for carboxylic acids and sulfonic acids in hair, but Cu does actually react to form “soaps” with carboxylic acids, but perhaps to a lesser degree in the presence of Ca.

2.11.3 Regions of the Fiber that have a High Affinity for Metals

Regions of high carboxylic acid content are the endocuticle, the cell membrane complex (especially the CMC of the cortex) and the medulla. These areas of the fiber are likely to have a high affinity for divalent and trivalent metals. Therefore, these areas are likely to absorb Ca and Fe with smaller amounts of Cu if the metals can diffuse to those regions. In the case of oxidation dyed or bleached hair the high cystine containing regions of the fibers (A-layer, exocuticle, cuticle cell membranes, and the matrix of the cortex) will also contain large amounts of sulfonate and will attract Ca and Fe and also Cu. Calcium has been shown by Smart et al. [227] to accumulate in the sulfonate rich exocuticle of oxidation dyed hair.

2.11.3.1 Pigments of Hair Contain High Metal Content

The pigments of human hair are described as containing more metals than other regions of the fibers. Dutcher and Rothman [225] reported that the iron content in red hair is higher than in hair of other colors, while Kosla et al. [236] in Warsaw Poland found that hair of schnauzer dogs contains more Fe than hair of humans, but found no effect of color. Furthermore, Liu et al. [237] determined that significant amounts of Cu and Zn are bound to both black-hair and red-hair melanosomes, however, the Fe content is four times higher in red-hair melanosomes. The pigments of human hair are also capable of producing hydroxyl and other free radicals as shown by Qu et al. [238] and also by Haywood [239].

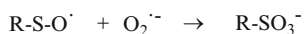
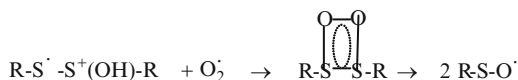
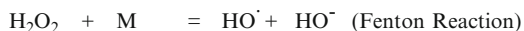
2.11.4 Simulated Swimming Pool and Copper Binding to Hair

Rhamachandran Bhat et al. [228] in an attempt to simulate Cu sorption from swimming pool water containing copper based algacide concluded that natural white or lightly bleached blonde hair will absorb Cu from 10 ppm Cu solution as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ at pH 5.9 in chlorox containing water after 1 h. Bleached hair absorbed more Cu than non-bleached hair, the non-bleached hair turned light green and the bleached hair darker green. In both cases the absorbed Cu was in the periphery of the hair as shown by EDXA-SEM cross-sections. Interestingly, pre-treatment of the hair with a quaternary ammonium conditioner inhibited the color formation in the hair, probably by competitive inhibition. Nevertheless, small amounts of Cu were still found in the hair with about three times the amount in the lightly bleached hair.

2.11.5 Metals that Bind to Hair do so Specifically

As indicated above, metals like Cu and Fe can bind to polar groups in hair to participate in oxidation-reduction reactions by generating active oxygen compounds such as in the Fenton or other reactions. They can also bind to groups in hair such as sulfur groups and participate in electron transfer from the metal to sulfur or the reverse. For example, Maletin et al. [240] studied the mechanism of the oxidation of copper(I) ions with thiuram disulfide and determined that the rate of the reaction occurred by one electron transfer from the Cu⁺ ion to the disulfide in a complex of this type [Cu^I(disulfide)]⁺ to form the anion radical of the disulfide complex. This anion radical then dissociated leading to the formation of a Cu⁺⁺ adduct of the dithiocarbamate. Simpler disulfides (simpler than thiuram disulfide) will form a complex of the [Cu^I(disulfide)]⁺ type and then by one electron transfer will form the anion radical of the disulfide complex (or cation radical of the disulfide) which will then dissociate to form the thiyl radical and thiolate anion or the sulfinyl radical (in the case of the cation radical). The thiyl radical or radical ions produced will participate in oxidation reactions as described in this paper to form cysteic acid or other products. Thiuram disulfide was selected for this study because of its specific spectroscopic properties.

2.11.6 A Proposal for Free Radical Oxidation of Disulfide in Hair by Alkaline Peroxide



The disulfide cation radical is involved in this reaction scheme because it involves oxidation by superoxide anion radical for which some evidence has been provided in the oxidation of cystine and another disulfide with hydrogen peroxide in aqueous solution by Katritzky et al. [241]. Misra [229] determined that superoxide

can be generated by the autoxidation of a large number of compounds including thiols [229], some iron complexes [229] and quinines [229]. Millington determined that some dyes [230] are capable of generating this reactive oxygen species. In addition, Bruskov et al. [242] generated superoxide anion radical by heating (40°C) aqueous buffers saturated with air containing transition metal ion impurities (copper and iron) which serve as electron donors. Molecular oxygen could also oxidize the sulfinyl radical to cysteic acid. Other free radical mechanisms along with more details on oxidation of the disulfide and thioester bonds in hair are described in Chap. 5 in the section entitled *Mechanisms for Free Radical Reactions in Human Hair*. If this scheme is involved in the oxidation of keratin by alkaline peroxide it could explain why alkaline peroxide is more damaging than peroxycarbonate.

2.11.7 Heavy (Toxic) Metals in Human Hair

Although heavy metals occur at low concentrations in human hair, they sometimes accumulate at concentrations well above those levels present in blood or urine. Concentrations of metals such as cadmium, arsenic, mercury, and lead in hair tend to correlate with the amounts of these same metals in internal organs [223]. This is one of the reasons why hair is being considered as a diagnostic tool. Wesenberg et al. [243] found a positive correlation between cadmium levels of hair and target organs (femur, kidney, liver, spleen, heart, muscle tissue, and adrenal glands of Wister rats). Fowler [244] indicated that the highest levels of arsenic in humans are normally found in hair, nails, and skin. Furthermore, it is well known that human hair serves as a tissue for the localization of arsenic during arsenic poisoning.

Hasan et al. [245] reported significantly higher levels of nickel, arsenic, cadmium and mercury in the hair of children living in urban vs. rural areas of the United Arab Emirates. Conclusions were that heavy metal contamination could be due to industrial activity and that hair analysis has the potential of being an effective tool for evaluating toxic elements in humans. Heavy metals such as lead can also arise from air pollution. For example, Milosevic et al. [246] showed significantly higher concentrations of lead in hair of 200 persons living within 5 km of a lead smelter plant than in a control group of 200 persons living at a distance more than 10 km from that same pollution source.

2.11.8 Other Disorders Related to Accumulation of Metals in Human Hair

Analysis of hair can often serve as an indication of even more complicated disorders. For example, a study by Capel et al. [247] indicated significantly higher concentrations of cadmium in hair from dyslexic children than in a normal control group. These scientists suggested that cadmium analysis of hair may be used in

early detection and that excessive cadmium may be involved in this type of learning disorder.

Dankes [248] described Menkes syndrome as being linked to a copper deficiency resulting in abnormal keratinization because copper is involved in the oxidation of cysteine to cystine during keratinization. In this genetic disorder, kinky hair is symptomatic of this disease. This kinky hair results from an unusually high mercaptan level of cysteine, wherein only about 50% of the cysteine is oxidized to disulfide bonds during keratinization.

Children with cystic fibrosis have been found to contain several times the normal level of sodium in their hair and considerably less than normal calcium [223]. Persons suffering from phenylketonuria (phenyl ketones in the urine) contain less than average concentrations of calcium and magnesium in their hair [223]. Victims of kwashiorkor have higher than normal levels of zinc in their hair [223] and low levels of sulfur and the cystine rich proteins [171]. Hair analysis has also been considered as a screening tool for diabetes, because low levels of chromium in the hair have been demonstrated in victims of juvenile-onset diabetes [223]. Hair analysis offers possibilities for diagnosis of several other maladies or disabilities. For more information on this subject see the review by Maugh [223] and the book edited by Brown and Crounse [249].

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