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Forensic Examination of Hair

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3.1 Introduction 155
Hairs are a potentially ubiquitous trace material in many types of forensic investigation. Few forensic materials give rise to such differing views on their value as evidence, and these views are often held with a passion. Some believe that hairs provide worthless evidence, while others believe that hairs can provide potentially, and actually, very significant evidence. The application of DNA methods to the examination of hairs has sparked renewed interest in the forensic examination of hairs and the role of microscopic examination.

As editor of this volume, I make no excuse for being a believer in the value of hair examination. However, the purpose of this book is not to take an evangelical stand to persuade the non-believers. The content should provide the balanced reader with enough factual information to enable him/her to form his/her own view.

Chapters deal with the physiology and growth of hair, the microscopic examination of hair, the use of DNA analysis, forensic examination for cosmetic treatments, drugs and elemental analysis, and an assessment of the evidential value and interpretation of hairs as evidence.

In the final analysis, it is for each laboratory system to decide on its own strategy and policy with regard to hair examination. What should be abundantly clear to the reader is that half-hearted commitment to the forensic examination of hairs is dangerous. Laboratory managers and leaders need to decide whether or not they are in a position to make a full commitment. There is also undoubtedly the need for more quality assurance measures, including proficiency testing to ensure the scientific reliability of hair examination.

I thank my authors for their great patience in what turned out to be a very lengthy process. I thank my wife, Margaret, for help with editorial and wordprocessing input. I am glad to report that our marriage has now survived three books!

Finally, this book is dedicated to my parents, John and Jeanetta Robertson.
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John graduated from the NSW Institute of Technology (now the University of Technology, Sydney (UTS)) in 1970 while working as a chemist with the Commonwealth Science and Industry Research Organisation (CSIRO), Division of Mineral Chemistry. In 1971 he joined the Commonwealth Police (now the Australian Federal Police (AFP)) as a graduate chemist, specializing in nuclear science, originally heading the Forensic Analytical Section of the Australian Nuclear Scientific and Technology Organisation (ANSTO) at Lucas Heights, Sydney. Here he utilized Forensic Neutron Activation Analysis to examine and compare samples of forensic origin through their trace element content. From 1990 to 1993 he was Officer in Charge of the Scientific Branch laboratory complex in the Australian Capital Territory. He maintains Forensic Activation Analysis as a general police service for all police forces in Australia and nearby countries. From 1993 to 1997, John was Officer in Charge of the Physical Evidence
Branch, a national branch within the Forensic Services Division of the AFP responsible for the substitution of illicit drugs seized by Commonwealth law enforcement bodies, and coordination of the chemical examination of these drugs by the Australian Government Analytical Laboratories (AGAL). From 1997 to present he has been National Forensic Teams Coordinator of all AFP Regional Forensic Services Teams throughout Australia. John was recently recognized by UTS for his contribution to professional science during a lengthy career.

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Harry graduated in Biochemistry and then gained his PhD researching aspects of hair medulla and inner root sheath proteins with Professor George Rogers at the University of Adelaide, South Australia. He became involved, with Professor Rogers, in forensic hair examination in 1972. In 1975, he established a Forensic Biology Laboratory in Adelaide, conducting blood and body fluid typing and hair examinations. This laboratory subsequently formed part of the Forensic Science Centre in South Australia, where he was a Principal Forensic Scientist until 1996, involved in serology, hair examination and quality management. He is currently a Research Officer in the Department of Microbiology and Immunology at the University of Adelaide.

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1.1 Introduction

Hair is a characteristic of mammalian skin. On some animals it serves to keep them warm, or cool, to protect them from the weather and hazards of the environment, or to camouflage them as protection from attack by their predators.

For the human animal these functions are not really necessary, and hair seems to be a dilemma. If we've got it, it's in the wrong place, or it's the wrong colour or the wrong style. If we haven't got it, we wish we did, or if we have lost it we try to make it look as though we still have it. We worry about the ones that fall out, and the ones that turn grey, or worse yet, white!

And then there are those who worry about whether hair is dead or alive.

‘Living things grow, OK? Hair grows, OK? So hair must be alive, OK?’
‘No,’ came the answer, ‘hair’s not alive, it’s dead!’
‘Well,’ I said, ‘how does it grow, then, if it’s dead?’

(Hackett, 1984)

The short answer to this paradox is that the hair root embedded in the skin is a living tissue and grows to produce the hair we see, which is a dead tissue. It is ‘as dead as rope’ (Montagna, 1963), in spite of what some of the advertising of the cosmetic and hair care companies would try to convince us. The living part, the hair follicle, is an appendage of the skin and develops as an invagination of the epidermis. It is a dynamic and complex organ of considerable interest to molecular biologists as a model for cell differentiation, tissue development, and gene expression and control (Hardy, 1992). The natural growth process of the follicle causes hairs to be shed on a periodic basis, but they are replaced and this cycle is repeated over and over again, although the replacement hair may well be different from its predecessor. It is this periodic shedding that causes hair from a person’s body to be commonly found on clothing and at crime scenes, and which makes them so useful as associative evidence in forensic science.

In comparison to many mammals humans are quite glabrous, sometimes being referred to as ‘the naked ape’. While the number of follicles in humans may be less than in many of the other mammals, we do, however, have as many hair follicles as other primates, and the generally hairless appearance results from the fact that many of the follicles have become so small that the hairs they produce are not easily visible on the surface of the skin. The only truly naked mammals are the whales, which have lost all their follicles (Montagna, 1963).
So what do humans have hair for, especially since there are only a few areas on the body of highly visible growth? It would seem that some is ornamental—perhaps the same as the mane on the lion (Montagna, 1963). Scalp hair undoubtedly comes into this category, and it also has a protective function in shielding the scalp from the sun. The eyebrows and eyelashes, which like scalp hair are present from birth, probably also have protective roles, serving to keep the eyeballs free from dust, rain and sweat (Montagna and Parakkal, 1974; Robbins, 1988). Hairs in other areas may have special functions; those of the outer ear and those of the anogenital regions, for example, provide barriers to the entry of foreign matter such as dirt and insects to these body orifices. The hairs in the nostrils slow down the incoming air and so provide some temperature control, as well as trapping insects and dust and the like. The fact that the hairs at the other main areas of growth (male beard region, pubic region and axillary region) do not fully develop until sexual maturity might suggest that they have an erogenous role (Montagna and Parakkal, 1974).

However, the most important role of hairs in humans may be that of sensory receptors. All hair follicles, and particularly those of the human face and anogenital region, are surrounded by sensory nerves, and these nerves react to pressure on the hair shaft, making them very sensitive to touching (Montagna and Parakkal, 1974).

Hair growth and appearance are under genetic control (Kaszynski, 1987). Hairs originate directly from the person and may be subjected to treatments (such as cutting method and dyeing) which may further aid in forensic comparison. But it must be remembered that each hair is an individual that grows independently of the others, and no two hairs will be exactly alike in all details. To make a reasoned and reasonable forensic examination and comparison of human hair it is therefore necessary to have an understanding of how hairs develop and grow, and of some of the factors which cause variability in these processes, both within individuals and between individuals. It is the aim of this chapter to provide some of that information.

1.2 Embryology of Human Hairs

1.2.1 Development of the Hair Follicle

Hair follicles develop in utero as a downgrowth or invagination of the epidermis into the dermis. Although the development is a continuous process, it is convenient to describe it as a series of stages, namely, pre-germ, hair germ, hair peg, bulbous peg and finally, the hair follicle. These stages have been well described in detail by Pinkus (1958) and are illustrated in Figure 1.1. Other authors use eight stages (Chapman, 1986).

*The Pre-germ Stage*

The first sign of follicle development occurs at about eight weeks after conception as a local clustering of cells in the basal layer of the epidermis. The bulge so formed on the underside of the epidermis is called the pre-germ (Pinkus, 1958) or primitive hair germ (Montagna and Parakkal, 1974). Numerous mitoses occur in the immediate surroundings of the hair germ.

*The Hair Germ Stage*

The second stage follows quickly, and by about eleven weeks after conception the epidermal basal cells elongate perpendicularly to the skin, producing on one side a bulge in the external surface of the epidermis and growing down on the other side into the dermis. Additional cells start to accumulate above the basal layer but beneath the periderm of the skin above the developing follicle (Pinkus, 1958), and at the same time, fibroblasts and mesenchymal cells increase in number below the hair germ to form the beginnings of the dermal papilla (Ebling, 1980). The basement membrane starts to appear at this stage (Pinkus, 1958) and melanocytes (pigment cells) can be seen scattered throughout the epidermis and the developing hair germ (Mishima and Widlan, 1966; Ortonne and
Nerves are prominent in the mesenchyme associated with the developing follicle (Holbrook et al., 1993). The hair germ is initially asymmetric, with one side forming a right angle with the epidermis, the other side slanting and merging gradually with the basal layer. Continued downward growth of the germ is oblique, however, and eventually the column of cells takes up the slanting position of the fully formed follicle (Pinkus, 1958).

**The Hair Peg Stage**

Continued cell division causes the hair germ to grow obliquely down into the dermis as a solid column (or peg) of epithelial cells, the outer layer being continuous with the basal epidermal layer (Holbrook et al., 1993). The cells at the leading edge of the peg are longitudinal and appear to push the cluster of fibroblasts (the developing dermal papilla) further down into the dermis ahead of them. A sheath of mesodermal cells, contiguous with those of the papilla, surrounds the entire peg. Cell division continues
and glycogen appears in the cells, firstly in those of the central core of the developing follicle, and later in the peripheral basal cells (Pinkus, 1958; Montagna and Parakkal, 1974). Melanocytes are distributed almost randomly throughout the peripheral and inner cell layers of the follicle (Mishima and Widlan, 1966).

**The Bulbous Peg Stage**

Tissue differentiation begins at this stage (Pinkus, 1958). The advancing end of the developing follicle broadens and then becomes concave, eventually enclosing the dermal papilla inside its hollow bulb of germinative cells. The papilla remains connected, however, to the dermis via a basal stalk through an opening in the base of the follicle bulb. The melanocytes become localized in the peripheral layer of the outer root sheath above the level of the widest part of the dermal papilla, and also among the germinative cells above the dermal papilla and in the lower part of the bulb (Pinkus, 1958; Mishima and Widlan, 1966; Ortonne and Thivolet, 1981; Holbrook, 1991). At this stage two epithelial swellings or outgrowths appear on the posterior (that is, under the slant) side of the developing follicle. The lower, larger one remains solid, its cells becoming rich in glycogen, together with the rest of the cells of the follicular peg. This swelling is called the bulge, and eventually becomes the site of attachment for the arrector pili muscle. The bulge also marks the point in the follicle above which all follicular components persist throughout the subsequent hair cycles, whereas the parts below are resorbed during the catagen phases (Pinkus, 1958; Montagna and Parakkal, 1974), and it has recently been demonstrated that this region is the location of the pluripotent stem cells that produce the elongation of the follicle in its anagen (growing) phase (Lavker et al., 1993; Kim et al., 1996). The higher swelling is more rounded; its cells accumulate lipid and form the sebaceous gland.

The central cells in the peg elongate backwards towards the epidermis, defining what will become the hair canal, but the developing follicle remains a solid mass of cells and there is no actual canal or channel in the follicle at this stage nor is there an orifice in the skin.

At this point mesodermal cells near the sebaceous gland form into a slender row parallel to the posterior side of the follicle. This row of cells forms the arrector pili muscle (also called the erector muscle), which gradually extends downwards to the bulge (Pinkus, 1958).

An apocrine gland now forms in some follicles from a small knob of cells on the posterior side of the follicle above the level of the sebaceous gland duct. The knob grows into a solid cord with a pointed tip and irregular shape (Pinkus, 1958).

The germinative cells in the hair bulb that are adjacent to the dermal papilla now begin to divide actively. The inner root sheath cells differentiate first, aligning themselves along the follicle and forming the so-called hair cone above the dermal papilla. The hair cone elongates, pushed up by dividing cells from below, forcing its way upwards between the cells of the central core of the hair canal without causing any cell death in this area (Breathnach and Robins, 1981).

When the tip of the hair cone is about halfway up the follicle its outer layer hardens and the central cells inside it start to differentiate to form the tip of the new hair. By the time the hair cone reaches the level of the sebaceous gland, the tip of the hair starts to keratinize and the hair cone fragments (Chapman, 1986). The inner root sheath thus does not extend above the level of the sebaceous gland opening (Pinkus, 1958; Chase and Silver, 1969). The new hair fibre finally breaks through the epidermis and appears above the skin at around 19–21 weeks. This lanugo (foetal) hair is extremely fine with no medulla and the tip is free of pigment (Pinkus, 1958) (see section 1.7.1).

**The Hair Follicle**

It is clear from their origin that hair follicles are an epidermal tissue. The cells of the basal layer of the epidermis which originally extended down into the dermis as the hair peg become the outer root sheath of the follicle (Chase and Silver, 1969), whereas the cells in the follicle core and which form the hair proper are continuous with the intermediate cell layer of the epidermis (Holbrook et al., 1989). (The papilla always remains part of the dermis.) The features of the fully developed hair follicle are described in detail in section 1.3.
1.2.2 Initiation and Growth—Chronology

Follicle development is initiated in the foetus at different times depending on the region. Follicle downgrowths are seen first between the second and third months of development and the activity at this stage occurs mainly on the upper lip and eyebrow region, with some on the chin (Pinkus, 1958). General hair development then begins, starting with the forehead and scalp, at about the fourth foetal month and continues through the fifth month (Szabo, 1958; Montagna and Parakkal, 1974). As the skin of the foetus expands with growth, new follicles form between the original primary ones. The new follicles consist of both primary and secondary (i.e. smaller) types, and they occur initially in groups of three (Montagna and Parakkal, 1974), although in many regions only one of them will persist and go on to form a hair (Holbrook, 1991). Sebaceous glands develop at about the sixth month (Butcher, 1950). The lanugo hairs that are formed initially grow at the rate of 2 mm per week (Baum et al., 1974), but this slows down (catagen phase) and eventually stops (telogen stage) as their follicles go through their first hair growth cycle. The lanugo hairs are shed in utero, beginning with the face and head, between the seventh and eighth months. They are replaced by new lanugo hairs or by vellus hairs (depending on the region) growing from the same follicles. This change continues up to birth or even into the first few months of post-natal life (Butcher, 1950). Thus at birth, follicles will be in all stages of the hair cycle (Montagna and Parakkal, 1974).

New follicles do not develop to any great extent after birth (Pinkus, 1958), and with the further expansion of the skin the foetal follicle distribution pattern tends to be lost, creating the more random arrangement of hairs usually seen for human scalp hair (Ellis, 1958).

Thus the follicles formed in the foetus provide the hairs for the foetus, the child and the adult.

1.3 Structure of the Hair Follicle

The hair follicle is a dynamic organ in which division, differentiation and migration of cells occur in the various tissues of which it is composed. These processes give rise to the growth of the hair fibre, which is formed as the result of the biosynthesis and hardening of the contents of the medulla, cortex and cuticle cells of the hair shaft. This growth, however, is not a continuous process, but one which ceases periodically and then starts again, to be repeated in what is known as the hair growth cycle (Chase, 1954, 1965). The stage of this cycle in which the hair is growing actively is called anagen, when the follicle is full size and maximally biochemically active. This is followed by a quiescent or regression phase (catagen) when metabolic activity (and hair growth) slows down and eventually stops (the resting phase, telogen) (Dry, 1926). This process is discussed in more detail in the context of hair growth in section 1.6.

The main cellular features of the mature, growing hair follicle are illustrated in Figure 1.2. A section of a growing follicle from human scalp is shown in Figure 1.3.

The hair follicle consists of several approximately cylindrical and concentric cell layers. The outer root sheath (ORS), the most external component, encases the other cell layers of the follicle and is contiguous with the epidermis. The inner root sheath (IRS) is composed of three distinct layers, the Henle, Huxley and IRS cuticle layers in that order, which encase the growing hair fibre. The mature fibre contains at least two cell types, the surface layer or cuticle consisting of flattened overlapping cells and the main central cortex of spindle-shaped cortical cells. The hair may include a medulla, a core of condensed cells of a third type stacked in the centre of the cortex. Except for the ORS, the cells of these different layers derive from the germinative cells which reside in the follicle bulb and proliferate during the anagen (growth) phase of the hair cycle. Specialized mesenchymal cells form the dermal papilla which protrudes into the base of the follicle bulb but is separated from the germinative cells by a basement membrane. The basement membrane covers the interfaces of the epidermis and follicles. External to it and around the follicle is the glassy layer consisting of orthogonally arranged collagen fibrils (Rogers, 1957). External to this again is the dermal sheath, a connective tissue layer of a collagen network and fibroblasts.
Attached to the follicle is the arrector pili muscle and, to some follicles, one or more sebaceous glands. The combination of follicle and sebaceous gland is known as the pilosebaceous unit (Pinkus, 1958; Chase and Silver, 1969). Networks of nerves and of blood capillaries (not shown in Figure 1.2) also surround the follicle.

The size of a follicle depends on a number of factors, including the region of the body and whether it is a primary or secondary (smaller) follicle (both of which relate to the type of hair it
Physiology and Growth of Human Hair

produces), and to the stage of the hair cycle. The models developed by Montagna and Van Scott (1958) well illustrate the different sizes and types. A mature anagen follicle of a terminal hair is about 4–5 mm in length (Kligman, 1962).

In structural terms the region of the follicle below the bulge (the attachment point of the arrector pili muscle) is called the lower follicle. This is also known as the transient zone, as it is the part of the follicle which regresses during the catagen phase of the hair cycle. The section between the bulge and the sebaceous gland duct is referred to as the isthmus, and the infundibulum is the region from the isthmus to the epidermis. Thus the isthmus and the infundibulum together make up the permanent zone of the follicle (Figure 1.2) which persists through all stages of the hair cycle.

The follicle is functionally divided into four zones, also shown in Figure 1.2. These zones are based on cellular and biochemical activity and are helpful to aid description of the follicle components and their differentiation. The zones are (1) the cell proliferation and differentiation zone at the base of the bulb, (2) the keratin gene expression zone in the upper bulb, (3) the keratogenous zone in which hardening of the fibre occurs, and (4) the zone of IRS degradation.

The structure and development of various components of the pilosebaceous unit are described in more detail below.

Figure 1.3 Longitudinal section through a growing hair follicle from human scalp, showing various cellular components. Stained with haematoxylin/eosin. Note that shrinkage during the section preparation has caused some separation of layers. Co, cortex; Cu, cuticle; DP, dermal papilla; Ge, germinative cells; IRS, inner root sheath; K, keratogenous zone; M, medulla; Me, melanocytes; ORS, outer root sheath. Bar = 100 µm
1.3.1 The Hair Bulb

The area of active cell division in the follicle is the lower bulb, mostly in the part below what was called the critical level by Auber (1952) (and which is an imaginary line drawn across the follicle at the level of the widest part of the dermal papilla), and also in the area adjacent to and covering the apex or dome of the dermal papilla (Epstein and Maibach, 1969). The epithelial cells of this dividing cell region are undifferentiated and are characterized by a high nuclear to cytoplasmic ratio. The cytoplasm contains a few mitochondria and smooth membranes but no morphologically identifiable filaments (Birbeck and Mercer, 1957a). These cells are now referred to as ‘germinative’ cells and not ‘matrix’ cells as in the past. This not only is a better description of their function but also avoids confusion with the customary use of the term ‘matrix’ to describe the complex of proteins that becomes associated with the keratin intermediate filaments during keratinization.

The germinative cells are several layers deep and are organized so that cell division gives rise to the concentric layers of the follicle and the hair fibre. Up to six distinct cell streams may exist (Figures 1.2 and 1.3) and by continuous division the cells move outwards and upwards to form the tissues of the follicle and the hair fibre. The most central cells give rise to the medulla (when it is present), then, progressing outwards, the next layers respectively give rise to the hair cortex, the hair cuticle, the cuticle of the IRS, the Huxley layer of the IRS and, at the periphery, the Henle layer of the IRS. Differentiation and development in each of the cell streams are distinct from those in adjacent streams (Birbeck and Mercer, 1957a). It has been estimated that no more than 20 per cent of the bulb cells differentiate into the fibre cortex, the rest forming the IRS layers (Wilson and Short, 1979).

Melanocytes (pigment-producing cells) are present among the germinative cells, mostly attached to the basement membrane around the apex of the dermal papilla (Birbeck and Mercer, 1957a; Swift, 1977; Tobin et al., 1995) but some are also found in the ORS (Montagna and Parakkal, 1974; Takada et al., 1992; Tobin et al., 1995). Each melanocyte has a number of dendrites (processes) which extend upwards between the presumptive cortical cells (Montagna and Parakkal, 1974; Swift, 1977).

The germinative cells of the hair bulb are among the most actively dividing cells in the whole body. Cell renewal times reported have ranged from 8 hours (Bullough and Laurence, 1958) to 23–72 hours (Van Scott et al., 1963), but they are difficult to measure and these figures should be taken only as estimates (Epstein and Maibach, 1969). The extensive cell proliferation requires adequate supplies of oxygen and metabolites, such as glucose, fructose and pyruvate, and these are obtained via the blood capillaries (Bullough and Laurence, 1958). Energy for the synthetic activities is provided in the follicle cells through the Embden-Meyerhof and tricarboxylic acid cycles (Adachi and Uno, 1969; Kealey et al., 1991). When follicles become active in the anagen phase the rate of ATP synthesis is doubled as a result of a four-fold increase of activity of the pentose cycle, producing not only increased energy but also essential substances required for the fatty acid and nucleic acid metabolism of the follicles (Adachi and Uno, 1969). Phosphoglucomutase (PGM), an enzyme which catalyzes one of the steps of the breakdown of glycogen to glucose for use in the metabolic cycles, is present in the growing hair root in sufficient quantities to enable the easy detection of its polymorphic forms (Twibell and Whitehead, 1978; Whitehead et al., 1981). The availability of adequate supplies of essential amino acids is vital for normal hair growth. A deficiency in the supply of sulphur amino acids results in lower cell division rates in the follicle germinative cells, a lowered rate of hair growth and a change in the pattern of keratin synthesis (Fratini et al., 1994).

The high mitotic rate of the germinative cells in the growing bulb leaves these hair roots susceptible to anti-mitotic agents used for anticancer therapy. Treatment with these drugs causes the anagen roots to atrophy, and the hairs fall out. This can be quite dramatic (and traumatic), since about 90 per cent of follicles in the scalp are in anagen at any one time (Orentreich, 1969).

There is a highly significant relationship between the hair root diameter and the amount of DNA in the root (Bradfield and Gray, 1975). A single anagen root can yield about 50 ng (von Beroldingen et al., 1989). The genomic DNA in the germinative cells of the bulb can be extracted more easily than that in the differentiated cells further up the follicle and in the hair shaft, and hair roots are thus amenable to DNA typing in a variety of systems following polymerase chain reaction amplification (Higuchi et al., 1988; Westwood and Werrett, 1990; Fregeau and Fourney, 1993).
1.3.2 Medulla Cells

Most of what is known about the differentiation of medullary cells comes from studies of animals other than humans, but the essential stages are similar, regardless of species. Medullary cells arise from the germinative cells of the bulb adjacent to the apex of the dermal papilla (Rogers, 1964). Changes can be seen in this cell stream before they are detectable in the other cell types. At a level about 3–4 cells above the papilla, obvious differentiation into medulla cells begins with the appearance of characteristic electron-dense, amorphous granules. These granules vary in size and are not bound by membrane (Parakkal and Matoltsy, 1964). They contain the protein trichohyalin and stain strongly for arginine (Rogers, 1963). The number and size of the granules increase at later stages of development and large amounts of glycogen are seen in the cells. The mitochondria swell and vacuolate and vesicles appear in the cytoplasm as the cells move higher in the follicle. The nuclei degenerate and the granules tend to become irregular and coalesce.

In a sudden transformation, from one cell to the one above, the granules fuse, giving rise to hardened protein showing a generally granular substructure and which contains citrulline (Rogers, 1963; Harding and Rogers, 1971; Rogers et al., 1977). The hardened protein is not overtly filamentous (Rogers, 1964). It is, however, highly insoluble due to the formation of e-(?-glutamyl)lysine crosslinks (Harding and Rogers, 1972a, 1972b; Rogers and Harding, 1976). When the granules fuse, large intercellular spaces form and the cell contents tend to coalesce at the cell periphery (Parakkal and Matoltsy, 1964). In the mature hair the medulla cells form a central core surrounded by cortical cells, although the cortical cells may interrupt the core on occasion if there has been a pause in the production of medullary cells (see section 1.4.1).

1.3.3 Cortical Cells

The presumptive cortical cells are derived from the central cells in the bulb, surrounding those which form the medulla. Their differentiation and development in the human follicle have been described in detail by Birbeck and Mercer (1957a). The cells initially contain large, roughly spherical nuclei and long, thin mitochondria. The characteristic feature of the cytoplasm is numerous clusters of small dense particles of about 20 nm in diameter. Filamentous material is not seen in the cells at this early stage. The cells are rounded at first but the surfaces become corrugated, with only limited contacts between the cells.

Intercellular gaps of up to 2–3 µm are common, and these persist as the cells move past the melanocytes around the upper part and apex of the dermal papilla in the mid to upper level of the bulb. The gaps allow the dendrites of the melanocytes to penetrate between the developing cortical cells. The melanin pigments synthesized in the melanocytes are packaged into membrane-bound granules which move out along the dendrites. It is believed that the melanin-laden dendrites are engulfed by the membrane of a recipient cortical cell, pinched off and drawn into the cytoplasm in a phagocytic process (Birbeck and Mercer, 1957a; Swift, 1977). Partial digestion of these pieces releases the pigment granules inside the cortical cells.

As the cells move to the upper level of the bulb they rapidly elongate into a spindle shape and the cell contents, including the nucleus and mitochondria, become oriented parallel to the axis of the follicle. Filaments are first seen in the cells at this stage; they are also aligned with the axis of the cell. This is a period of protein synthesis, but the keratin produced is maintained in the sulphydryl state (Rogers, 1959a). As the cells move through the neck of the bulb the bundles of filaments rapidly increase in length and width to form macrofilaments (also called macrofibrils), with the pigment granules lying in rows between them.

Further up the follicle in the keratogenous zone, the macrofibrils become more clearly defined and more filaments (microfibrils), together with amorphous interfilamentous material, are seen. The macrofibrils increase in size and length to fill eventually almost the whole cell, trapping the nucleus and cellular remnants at their interces (Birbeck and Mercer, 1957a). In this process the nucleus becomes
considerably distorted, being converted into roughly a spindle shape like the cells, and located in the centre of the cell surrounded by macrofilaments (Swift, 1977; Rook and Dawber, 1982). The arrangement and composition of the macrofibrils and the keratin microfibrils, which are now called **keratin intermediate filaments** (keratin IF), are discussed in more detail in section 1.4.2.

Finally, at the upper level of the keratogenous zone, in a conversion extending over the length of several cells, the cell contents no longer react for sulphydryl groups. This change in reactivity occurs as the result of almost complete disulphide bond cross-linking of the proteins in the last step of keratinization. During the terminal stages of differentiation the cortical cells harden and dehydrate, reducing the hair fibre diameter by about 25 per cent (Rook and Dawber, 1982). A continuous layer of intercellular material, referred to as the **cell membrane complex** (CMC), appears between the cortical and cuticle cells and at the junctions between cortex and cuticle (Birbeck and Mercer, 1957a, 1957b; Rogers, 1964). From this point on the tissue of the hair fibre is biologically inactive and is considered ‘dead’.

### 1.3.4 Cuticle Cells

Cuticle cells arise from a single layer of germinative tissues outside those that form the cortex (Birbeck and Mercer, 1957b). The first sign of their differentiation occurs at a level between the lower and mid-bulb as a smoothing-out of the cell membrane. The smoothing results from the formation of small areas of contact between successive presumptive cuticle cells. As differentiation proceeds, the number and area of these cell contacts increases until they completely spread over the surfaces between the cuticle cells. This cementing converts the germinative cells into an organized layer of cuboidal cells at about mid-bulb level (Birbeck and Mercer, 1957b). The cells then undergo a slow further change of shape as they move up the bulb and through the neck region, in which they are elongated and flattened, and then tilted to slide over each other to produce a layer of overlapping cells (Swift, 1977). As the cells tilt, their nuclei move to the lower end of the cell and are squashed (Birbeck and Mercer, 1957b).

Granules of about 30–40 nm diameter are initially seen around the periphery of the cells, and these grow in size on the outer side of the cell until granular material fills this half of the cell, compressing the nucleus and cytoplasm into the other half. The granular material rapidly transforms to produce the exocuticle and the ‘A’ layer of the mature fibre cuticle, and the underlying flattened zone of residual cytoplasm condenses and dehydrates to form the endocuticle (Swift, 1977). These aspects are discussed further in section 1.4.3.

### 1.3.5 Inner Root Sheath

The inner root sheath (IRS) tissue comprises three layers of cells which arise as separate but adjacent concentric cylindrical layers from the germinative cells of the hair bulb (Figure 1.2). The layers are each only one cell thick (Birbeck and Mercer, 1957c), but combined they account for about 80 per cent of the cells emerging from the hair bulb (Wilson and Short, 1979; Rogers et al., 1989). The IRS layers are on the outside of the layer that forms the hair fibre cuticle, and indeed the first layer of the IRS is called the IRS cuticle. Outwards from this is the Huxley layer, and then the Henle layer (which is adjacent to the outer root sheath (ORS)).

During hair growth the IRS moves up the follicle inside the surrounding ORS as a result of the division of the presumptive IRS germinative cells. Maturation and hardening of the IRS cells occurs low down in the follicle, with the result that the IRS moulds and shapes the forming hair (Straile, 1965; Kassenbeck, 1981). The upward movement of the IRS cells is more rapid than that of the precortical cells of the hair (Epstein and Maibach, 1969), so that the IRS forms a sleeve that physically supports the forming hair fibre. The support of the hair shaft continues up to the level of the sebaceous gland duct, at which point the IRS cells dissociate and fragment, presumably as the result of proteolytic enzyme activity (Straile, 1965; Fraser et al., 1972), and slough off into the pilary canal (Straile, 1965), releasing the emerging hair (Figure 1.2). The IRS also provides the mechanical function of holding the growing hair in the follicle. This is achieved by an interlocking interaction of the IRS cuticle cells,
which form as scales that point downwards, with the similar but opposing scales on the cuticle of the hair fibre (Straile, 1965). Thus a quick tug on a growing hair can remove the hair with the IRS attached to its root.

Cellular differentiation events are similar in all three layers of the IRS cells but totally different from those that occur in the fibre cortex. The first stage of differentiation is the appearance of amorphous granules of trichohyalin protein (Birbeck and Mercer, 1957c; Rogers, 1964) that is rich in arginine (Rogers, 1963). At the same time, filaments of about 7 nm diameter are found in the cytoplasm (Rogers, 1964). These differentiation processes occur at different rates in each IRS layer (Birbeck and Mercer, 1957c; Fraser et al., 1972; Swift, 1977). In the Henle layer the changes begin immediately above the germinative cells. Similar granules and filaments subsequently appear in the cells of the IRS cuticle and then the Huxley layer (Birbeck and Mercer, 1957c). As the IRS cells move up the follicle the number and size of the granules increase, as does the number of filaments. The final stage of differentiation is a sudden and dramatic transformation, occurring from one cell to the next (Birbeck and Mercer, 1957c; Rogers, 1963, 1964). Granules are no longer visible, and the cells are filled with filaments oriented in the direction of fibre growth (Fraser et al., 1972; Rogers et al., 1991). The cell contents now stain strongly for the amino acid citrulline (Rogers, 1963; Rogers and Harding, 1976). The granule-to-filament transformation occurs at different levels in the three layers of the IRS; the outermost Henle layer hardens first, at about the level of the top of the bulb, whereas the Huxley layer converts last, at approximately the same level as the final events of the hair fibre formation.

The trichohyalin molecule and its gene were first characterized by Fietz et al. (1993) in the wool follicle. The sheep protein is 1549 amino acids long and has a molecular weight of 201,172. It is hydrophilic, and is unusual in that it has one of the highest contents (over 57 per cent) of charged amino acids of any protein. It is further characterized by the four amino acids, glutamic acid, glutamine, arginine, and leucine, constituting over 75 per cent of the protein. Human trichohyalin has also been characterized, and is a protein of 1897 amino acids which is homologous with the sheep protein (Lee et al., 1993b). It is expressed from a single copy gene that maps to chromosome 1q21.1–21.3 (Fietz et al., 1992; Lee et al., 1993b). At the time of transformation and hardening, up to 90 per cent of the arginine residues of trichohyalin are converted to citrulline by the action of a peptidylarginine deiminase (PAD) enzyme (Rogers and Harding, 1975, 1976; Rogers et al., 1977). The formation of uncharged citrulline residues from positively-charged arginine residues causes a change in ionic charge of the protein and results in the protein undergoing a conformational change. It is postulated that the protein disperses as a matrix between the filaments (Fietz et al., 1993). The protein is then insolubilized by the formation of e-(?-glutamyl)lysine cross-links by follicle transglutaminase (Harding and Rogers, 1972b; Rogers and Harding, 1976; Rothnagel and Rogers, 1986; Rogers et al., 1989). The molecular biology of trichohyalin is discussed further in section 1.5.1.

At one time it was believed that in the transformation of the cells the trichohyalin in the granules gave rise to the filaments (Birbeck and Mercer, 1957c; Rogers, 1964), but it is now accepted that the trichohyalin forms a matrix between the intermediate-type filaments (Rothnagel and Rogers, 1986; O’Guin et al., 1992; Fietz et al., 1993; Lee et al., 1993a). This enmeshing of trichohyalin with separately formed intermediate-type filaments in the IRS is an essential difference between the IRS and the medulla, in which the trichohyalin granules fuse together to form a solid mass (Lee et al., 1993a). Definitive identification of the intermediate filaments in the mature IRS cells has yet to be made, but there is some evidence that they do contain keratin IF proteins (Stark et al., 1990).

1.3.6 Outer Root Sheath

The outermost layer of the follicle is the outer root sheath (ORS). Unlike the other layers, the ORS has its own autonomous cell population. The sheath is a single layer at the base of the bulb, but higher up the follicle the number of layers increases. The first increase is to two layers, with the cells on the outer side being elongated and those on the inner layer being flattened (Rook and Dawber, 1982). The ORS cells are attached to each other, and to the cells of the Henle layer of the IRS, by desmosomes (Swift, 1977). At the level of the neck of the bulb the number of layers increases even more (Rook and
Dawber, 1982), so that by the time the cells reach the level of the sebaceous gland the ORS layers are continuous with the various layers of the epidermis (Swift, 1977). Fibrils, and granules which resemble the keratohyalin granules of the epidermis, form in the cells high up in the follicle. Epidermal keratins, rather than hair type IFs, are present in the ORS (Coulombe et al., 1989). It has now been demonstrated that the stem cells which give rise to the follicular germinative cells of the hair bulb at the beginning of each anagen reside in the bulge of the ORS, the swelling located just below the sebaceous gland (Lavker et al., 1993; Kim et al., 1996).

The inner layer of cells of the ORS that lies next to the Henle layer of the IRS is known as the ‘companion cell layer’ (Orwin, 1971). These ORS cells are elongated and flattened, and originate in the bulb near the dermal papilla as a cell type which is distinct from the other cell types in the ORS. They are the first of all types of cells to accumulate keratin-like filaments, and in these cells the filaments have a circumferential orientation. The companion layer cells can be as thin as 1 µm and they are tightly apposed to the hardened cells of the Henle layer of the IRS rather than to the other ORS cells (Rogers, 1964). This has led to the suggestion that the companion cells of the ORS assist in the relative movement of the IRS and the ORS by moving towards the surface of the skin as part of the fibre-IRS complex (Rogers, 1964; Orwin, 1971).

1.3.7 Dermal Papilla

At the base of the follicle and enclosed by the germinative cells of the (anagen) hair bulb is the dermal papilla, a roughly egg-shaped tissue that is derived from fibroblasts in the dermis at the earliest stages of development of the follicle in the foetus. The fibroblasts aggregate ahead of the epithelial plug of the developing follicle as it grows down into the epidermis (Oliver and Jahoda, 1989). During the development of the follicle the developing papilla interacts with the ectoderm to specify the location, timing and type of follicles to be formed (Oliver and Jahoda, 1981).

The papilla is the only dermal component of the follicle; in growing follicles it is attached to a basal plate of connective tissue by a narrow stalk. Although it is eventually surrounded by the germinative cells of the growing bulb, it is separated from them by a basement membrane (basal lamina) (Montagna and Parakkal, 1974).

The dermal papilla is essential for hair growth and is responsible for the control of the hair cycle (Oliver, 1971), but the nature of the signals and signal pathways is not yet known. Removal of the dermal papilla stops hair growth, but after a short lag period a new papilla is generated and hairs of normal length are grown and continue to grow (Oliver, 1971; Oliver and Jahoda, 1981, 1989). Hair growth can also be restored by implantation of a dermal papilla (Cohen, 1965; Oliver, 1971; Oliver and Jahoda, 1981). When the dermal papilla, the hair bulb and up to the lower one-third of the follicle are removed, new smaller papillae regenerate and hairs shorter than normal will grow (Oliver and Jahoda, 1981). It was believed that permanent hair removal by electrolysis or electrodesiccation was achieved when the papillae only were destroyed (Van Scott and Ekel, 1958), but Oliver (1971) and Oliver and Jahoda (1981) have demonstrated that more than the lower third of the follicle must be destroyed as well if the dermal papillae are not to regenerate and stimulate renewed hair growth. The explanation for this lies in the recent discovery that the stem cells that give rise to the germinative cells of the follicle bulb reside in the middle third of the human hair follicle (Kim et al., 1996).

The dermal papilla is the only permanent part of the lower follicle, remaining through the hair cycle while the hair-producing germinative cells disappear and reappear. It does nevertheless have a cycle of its own, in which changes to its structure and synthetic activities occur in phase with the hair cycle (Oliver and Jahoda, 1981). During catagen the papillary cells lose cytoplasmic volume to the point where they contain virtually only a nucleus. The basal lamina shrinks and crinkles and the dermal papilla becomes detached from the bulb (Montagna and Parakkal, 1974). The mucopolysaccharides and alkaline phosphatases that are present for synthetic activity in anagen decrease, and are absent in telogen (Oliver, 1971). The dermal papilla, however, remains as a condensed ball of cells at the base of the shortened follicle throughout telogen. It is believed that the papilla provides the...
stimulus for cell proliferation in the hair germ at the initiation of proanagen and thus the hair cycle may in fact be dependent on a papillary cycle (Oliver and Jahoda, 1981).

As well as controlling hair growth, the dermal papilla also controls the physical characteristics of the hair. Van Scott and Ekel (1958) have shown that there is a direct correlation between hair size and dermal papilla size, and between mitotic activity of the germinative cells of the bulb and the number of cells in the papilla. Hair follicles that grow large (diameter) hairs have large dermal papillae, while smaller follicles with smaller dermal papillae produce thinner hairs (as seen for example in the case of alopecic follicles in the balding scalp). In normal scalp the average volume of the germinative cells of the hair bulb is about ten times the volume of the papilla, and the ratio of the number of mitotic germinative cells in the bulb to the number of cells in the dermal papilla is about 1 : 9 (Van Scott and Ekel, 1958).

Hairs with multiple medullae have multiple apices (one for each medulla) to their dermal papillae (Montagna and Parakkal, 1974). Changes in the shape of the upper surface of the dermal papilla during the growth of a hair can, in conjunction with the inner root sheath, mould the adjacent layers of migrating medulla, cortex and cuticle cells, thus producing a hair of changeable contour (Straile, 1965).

1.3.8 Sebaceous Glands

Sebaceous glands in humans are generally associated with hair follicles, the combination being known as the pilosebaceous unit (Chase and Silver, 1969). The glands are found everywhere on the human skin except the palms, soles and dorsum of the feet (Strauss et al., 1991), and are particularly numerous on the face and scalp (Montagna and Parakkal, 1974).

Sebaceous glands form in utero as an outgrowth of the follicle, the beginning of their development being visible at the bulbous peg stage (Pinkus, 1958). The glands are well formed in the foetus, but after birth they reduce in size and enlarge again at the onset of puberty (Strauss et al., 1991).

Human sebaceous glands consist of multiple aggregates of acini (lobes) which attach to a common duct. The cells within the glands convert their glycogen into lipid within the cellular membrane systems (Holbrook, 1991) and eventually fragment to form sebum (Montagna and Parakkal, 1974). The glands in humans are often attached to the follicular canal by a short duct at a level above the bulge, and the sebum is discharged through this duct and on to the skin via the canal. However, in some regions of the skin the sebaceous glands are not connected to the follicles, and open directly at the skin surface (Montagna and Parakkal, 1974). Thus it would seem that sebum is a phenomenon of the skin and is not essential for hair growth.

The main components of human sebum are squalene, wax esters and triglycerides, and it has been proposed that its function is to waterproof the hair, but this would not be a significant factor in humans (Strauss et al., 1991).

1.3.9 Blood Supply

Hair follicles are well supplied with blood through a network of capillaries and arterioles (Montagna and Parakkal, 1974; Sato et al., 1976). These vascular systems, which form a continuous unit for each follicle, are branches of the dermal plexus or the musculocutaneous arteries (Montagna and Parakkal, 1974). In an active follicle, the capillaries surround the lower third from the base to just above the bulb in a latticework pattern of parallel vessels connected by cross-shunts. There are fewer cross-shunts further up the follicle in the region from the top of the bulb to the level of the sebaceous gland, but a dense network is seen once again around the gland and the pilary canal. Loops of capillaries also form a vascular ring around the orifice of the growing follicle under the epidermis (Montagna and Ellis, 1957).

In large terminal hair follicles the dermal papilla is penetrated in its centre by a large tuft of capillaries, some of which extend to the wall of the inner side of the matrix of the follicle (Montagna and Ellis, 1957). The larger the dermal papilla, the more capillaries it contains (Ryder, 1956). Smaller follicles
with smaller dermal papillae, such as those of vellus hairs and hairs in alopecic skin, are surrounded by a much simpler system of capillaries (Allegra and De Panfilis, 1981), with only a few around the lower part of the bulb and none entering the dermal papilla (Montagna and Ellis, 1957).

During catagen as the follicle bulb atrophies, it moves upwards in the dermis and partially out of the network of capillaries that formerly surrounded it. The dermal papilla also moves upwards and away from its capillary tuft, so that in telogen the dermal papilla is still surrounded by a network of capillaries but not penetrated by any. The blood supply to the permanent (upper) zone of the follicle and the sebaceous gland remains essentially intact. At the next anagen phase, the new bulb grows down through the bundle of capillaries and develops inside it as a new vascular network regenerates (Montagna and Ellis, 1957; Sato et al., 1976). Thus the capillaries are independent of the upward and downward movement of the hair follicle during its growth cycles, and any changes that do occur to the blood vessels follow, rather than precede, changes occurring in the follicle (Allegra and De Panfilis, 1981).

The blood supply to the dermal papilla, the hair follicle and the sebaceous gland form a single functional unit and would therefore seem to be important in the development and nutrition of the follicle. The most densely vascularized part of the follicle is at the level of the keratogenous zone in the upper limit of the bulb, and it is this level which is probably the most important region of exchange of metabolites such as glucose and oxygen necessary for growth (Montagna and Ellis, 1957). Ryder (1956) has shown that when sheep are injected with $^{35}$S-cystine the label appears first in the keratogenous zone and takes only about six minutes to do so. Clearly, this would be a route for the transfer of other materials, including those such as drugs (Baumgartner et al., 1989) and trace elements (Robertson, 1987) which are of forensic and clinical interest, to become incorporated within the hair. Another likely route of transfer would be through the basal germinative cells via the dermal papilla. There is some thought (Zviak and Dawber, 1986) that the follicle capillary network may be a channel for transferring glycogen from the outer root sheath, in which it is plentiful, to the dermal papilla for use in mitotic activity in the hair bulb.

1.3.10 Nerve Supply

A network of nerves surrounds hair follicles from their base to their junction with the epidermis (Montagna and Parakkal, 1974). In particular there is a ring of nerve endings just beneath the branching point of the sebaceous duct (Montagna and Parakkal, 1974; Zviak and Dawber, 1986).

Parallel nerve fibres form a palisade structure around the bulge region of large follicles and around the bulb of smaller vellus follicles. There is great variation in the patterns of the nerve endings, but those around the vellus follicles are always more precise than those of the larger follicles. In quiescent (telogen) follicles the nerve network collapses in the region formerly occupied by the degenerating bulb, but remains intact and active below the dermal papilla (Montagna and Parakkal, 1974). The nerve supply in alopecic skin is a sparse, thin and irregular net of nerve fibres without distinctive characteristics (Allegra and De Panfilis, 1981).

The good supply of nerves to all follicles in the human skin regardless of their size indicates that the nerves have an important function, but since they are not needed for follicle growth it is likely that their role is a sensory one (Montagna and Parakkal, 1974). Montagna and Parakkal (1974) note that the follicles of the hairs in the human face and in the anogenital areas are particularly well supplied with nerves, and suggest that the shafts of hairs act as levers which amplify any movement and so provide a very sensitive response to the slightest touch.
1.3.11 Muscles

Each follicle has a bundle of smooth muscle, known as the arrector pili muscle (Pinkus, 1958) or erector muscle (Ryder, 1963). The lower end of the muscle is attached to the follicle just below the sebaceous gland duct, at the point which represents the lower extremity of the permanent zone of the follicle. The muscle runs at an angle upwards towards the skin, where it is attached to the papillary layer of the dermis.

The follicle is at an angle to the skin surface and the arrector muscle is attached to the posterior side (i.e. the side under the slant—see Figure 1.2). Contraction of the muscle thus causes the follicle to stand more erect and produces the appearance on the skin surface known as ‘gooseflesh’ or ‘goose bumps’. This is a reaction to cold or fright which, while it may be important in many animals, does not appear to serve any useful purpose in humans.

1.4 The Hair

The hair is the major component of the hair follicle commonly examined microscopically in forensic hair comparison. It is a long thin cylinder of keratinized cells and usually has three distinct cellular components:

1. a central **medulla** or core running along the central axis
2. the main component, the **cortex**
3. the **cuticle** (the outer covering).

The general cellular arrangement in a mature hair is shown in Figure 1.4.

![Figure 1.4](image-url) Schematic cut-away section of a mature hair fibre showing the major cellular structures, cortex, medulla cells separated by air spaces, nuclear remnants in the medulla cells, macrofibrils—the aggregates containing the filaments and matrix (see Figures 1.7 and 1.8)
1.4.1 Medulla

The medulla is formed as a column of cells that produce a protein that is distinct from the proteins of the cortex and cuticle in that it contains the amino acid citrulline (Rogers, 1959a, 1964; Harding and Rogers, 1971). During their formation, the cells collapse in such a manner that the medulla appears as a network of cellular connections with spaces and gaps that are filled with air (Auber, 1960).

Figure 1.5 Human hair medulla, (a) A continuous medulla in a pubic hair. Note how the structure is visible where the mountant has replaced the air in the medulla near the worn tip (lower right), (b) Fragmented medulla, and (c) interrupted medulla, in which the cortex replaces the medulla when its formation in the bulb is interrupted, (d) Double medulla in beard hair.
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1952; Rogers, 1964). The network in some mammalian species can be regular and diagnostic of species origin (Hausman, 1920; Wildman, 1954; Brunner and Coman, 1974).

A medulla is not present in all human hairs and this is particularly so for very fine hairs, but when it is present the appearance can be described as irregular globular. In human hair the medulla can be continuous throughout the length of the hair (except for the tip and the root), or discontinuous (Hausman, 1920) (Figure 1.5). In coarser hairs it is generally continuous. When discontinuous, the medulla may be broken transversely at irregular intervals by cortical material (interrupted medulla) or it may only be present irregularly in very small amounts in the cortex (fragmented medulla) (Wildman, 1954). The human hair medulla is not large; it may only be one or two cells in diameter and usually not more than one-third the width of the hair shaft (Figure 1.5). The medulla diameter is generally small in the hair of the child, larger in middle age and largest in old age (Luell and Archer, 1964). Occasionally, hairs with a double medulla (Figure 1.5) are seen (Chowdhuri and Bhattacharyya, 1964; Montagna and Van Scott, 1958).

The medulla may not necessarily be visible by light microscopy, particularly if the mountant used for microscopy has displaced the air from the intercellular and intracellular gaps. When the mountant does not infill, the medulla will appear dark when using transmitted light and very little structural detail will be seen (Stoves, 1957; Ryder, 1963) (see Figure 1.5) and the medulla can be mistaken for pigmentation (Griffith, 1848; Harding and Rogers, 1984). Random infilling of the medulla can produce what appears to be a discontinuous or a fragmented medulla, and this artefact should not be confused with real discontinuities which are generated as the result of periods of non-production of medulla cells in the follicle bulb. Infilling can occur where hairs are damaged (for example, cut) and it can also occur in intact hairs. Swift (1996) has shown that liquids can enter the hair fibre through adventitious holes (of the order of 0.5 nm in size) in the cell membrane complex, and also via exposed edges of the cuticle cells and a network of fine channels. The medulla that is filled with the mounting medium may be better visualized and its structure more clearly delineated using polarized light (Garn, 1951a).

The morphology of the medullary tissue is dramatically different from the rest of the hair shaft components. There are many large intercellular and intracellular spaces and the protein material in the cells appears by light microscopy to be amorphous and unstructured (Matoltsy, 1953; Rogers, 1964). This appearance is the result of the apparent collapse and concentration at the cell periphery of the cell contents on transformation of the trichohyalin granules in the developing cells to the hardened, dehydrated medulla protein (Parakkal and Matoltsy, 1964; Rogers, 1964). Scanning electron microscope studies of human hair medulla clearly demonstrate the very open structure of the medulla (Figure 1.6).

Figure 1.6 Scanning electron micrograph of a longitudinal hand-cut razor section of human beard hair showing the open structure of the medulla. Co, cortex; Cu, cuticle; M, medulla
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Clement et al. (1982) have found that the coalesced protein is present as macrofibrils, oriented randomly within the cells. The macrofilaments are composed of bundles of microfilaments.

Medulla protein has an unusual composition in that it contains the amino acid citrulline in peptide linkage (Steinert et al., 1969). This amino acid is not normally found in proteins, but it is also present in inner root sheath protein (Rogers, 1964; Steinert et al., 1969). The medulla protein is remarkably insoluble, even more intractable than the keratins of the cortex (Matoltsy, 1953; Rogers, 1964), but the disulphide bonds found in keratin are virtually absent (Harding and Rogers, 1972b). In the case of medulla protein the insolubility is the result of extensive cross-linking of its peptide chains by isopeptide links between the side-chains of lysine residues and glutamic acid/glutamine residues (Harding and Rogers, 1971, 1976). This cross-link is the same type as that which forms to insolubilize the fibrin in a blood clot (Pisano et al., 1969). It also occurs in the inner root sheath protein and, in smaller amounts, in keratins of the cortex and cuticle (Rice et al., 1994; Zahn et al., 1994). In contrast again to the keratins, the medullary protein is very susceptible to digestion by proteolytic enzymes (Steinert et al., 1969; Harding and Rogers, 1971) and such enzymes will readily cause loss of medullary ‘structure’, a phenomenon seen in forensic casework with hair that has been subjected to decay conditions (Harding, H.W.J., unpublished observations).

The role of the medulla is unclear. In larger (non-human) hairs where it occupies a large proportion of the hair shaft it may well because of its structure provide some mechanical stiffening, as well as increasing the thermal insulating properties of the hair. Clearly these are not essential functions in human hair (or in wool) which can grow without a medulla. It has been speculated that the medulla forms a channel for waste material. Kassenbeck (1981) has suggested that it provides a path for the evacuation of the water liberated by protein biosynthesis in the dividing cells of the follicle. It may well be that the function of the medulla is to maintain the diameter of the hair with the use of minimum resources and without producing excess weight (Auber, 1952; Ryder, 1973). Be that as it may, the medulla does appear to be the site (or at least a major site) of the ABO grouping ability of hair (Potsch-Schneider et al., 1986).

1.4.2 Cortex

The cortex is composed of cells that are fusiform (spindle-shaped), about 80–100 µm long and 5–10 µm wide at their widest point. The cells are aligned parallel to the axis of the hair fibre and are closely packed in an interdigitating fashion. They are cemented together via intercellular contacts that are referred to as the intercellular membrane complex or cell membrane complex (CMC) (Fraser et al., 1972). The tensile strength of the hair depends in part on these contacts. In the fully formed hair the cortical cells contain some nuclear remnants (Fraser et al., 1972) and also some pigment granules, but they are mostly filled with keratin macrofibrils of about 0.1–0.2 µm in diameter. The macrofibrils are oriented longitudinally in the cortical cells and are thus also parallel to the axis of the hair. Each macrofibril is composed of keratin microfibrils (now called keratin intermediate filaments, or keratin IF) embedded in a matrix of sulphur-rich proteins (now called keratin-associated proteins, or KAP).

The keratin IF proteins and matrix (KAP) proteins in the cortex are not single protein types but are each mixtures of keratin proteins. The proteins are characterized by a high level of sulphur compared to most proteins. In their natural state in the hair they are insoluble in water because they are highly cross-linked by disulphide bonds between adjacent cysteine residues in the protein chains. This insolubility presents a major difficulty in studying the keratin proteins. Solubilization (and hence extraction from the hair) requires the breaking of the disulphide bonds. This is commonly achieved by reduction of the bonds to cysteine residues using mercaptoethanol in 8M urea, followed by alkylation with iodoacetate of the cysteine residues to S-carboxymethylcysteine to prevent disulphide bond reforming. Although much of what is known about hair keratins comes from studies on wool (Powell and Rogers, 1986), considerable work is now being done to characterize the proteins of human hair (see, for example, Baden, 1981; Gillespie, 1991; Yu et al., 1993).
and it is clear that there are similarities and homologies (Marshall, 1983; Gillespie, 1991; Yu et al., 1993).

The sulphur-rich matrix (KAP) proteins are amorphous and comprise about 40 per cent of the protein content of the cell. They have been referred to as ‘high-sulphur’ (HS) proteins and are characterized by a half-cystine content of about 27 mole per cent (Gillespie and Marshall, 1981). The seven protein fractions identified have a molecular weight range by sodium dodecyl sulphate (SDS) gel electrophoresis of 26,500–43,000 (Marshall, 1983).

There are two classes or types (Type I and Type II) of the IF proteins (also known as ‘low-sulphur’ or LS proteins). Eight components have so far been identified (Gillespie, 1991) and as a group they have fewer half-cystine residues (about 8 mole per cent) than the KAP (Gillespie and Marshall, 1981) and a molecular weight range by SDS gel electrophoresis of 55,500–76,000 (Marshall, 1983). They are fibrous proteins with about 50 per cent a-helical content (Baden, 1981). The Type I proteins are acidic whereas the Type II are neutral-basic (Steinert and Freedberg, 1991). The amino acid sequence of one human hair Type I IF protein has been published (Yu et al., 1993). A third group of hair keratin proteins, the glycine- and tyrosine-rich proteins (‘high glycine/tyrosine’, or HT proteins), which is found in the matrix of wool (and mouse hair), has not been found in matrix proteins extracted from human hair (Gillespie and Marshall, 1989).

The electophoretic patterns of extracted proteins from human scalp hair are the same as those for hairs from other body regions of an individual (Gerhard and Hermes, 1987). Polymorphic variations of the proteins are seen between individuals and inheritance of the variants has been demonstrated (Baden, 1976; Yu et al., 1993).

The fundamental unit of the keratin IF is formed from the pairing of the two IF protein types, I and II. Coiled-coils of these proteins are grouped together in the 8–10 nm units of the intermediate filament (microfibril). It has been estimated that keratin IFs contain 32 keratin protein chains within the cross-sectional area of the filament (Steinert, 1993). The helical proteins in the IFs are surrounded by the KAP proteins as a matrix or ‘cement’ (Figure 1.7), to which they are coupled by hydrogen and ionic bonding as well as covalent disulphide cross-links.

It is not known what proportion of the disulphide bonds are intrachain and interchain. It is the cross-linking of each of these IF and KAP families together in the cell that produces the tensile strength and general toughness of hair. (It takes about three times as much force to break a hair as it does to pull it out.) Nevertheless, when hair is wet it is possible to stretch it, especially if it is heated. This comes about by the disruption of some of the chemical bonds (particularly ionic and hydrogen bonds) and the sliding of the polypeptide chains with respect to each other. The deformation will gradually disappear as the structures more or less return to their original positions. More drastic changes to the form of the hair can be achieved if the covalent disulphide bonds are broken (for

Figure 1.7 A diagrammatic representation of the three dimensional arrangement of keratin IFs and the matrix of KAPs. The overall structure is sometimes referred to as the filament-matrix complex or the keratin complex. Modified from ‘The role of keratin proteins and their genes in the growth, structure and properties of hair’ by B.C.Powell and G.E.Rogers, in Formation and Structure of Human Hair, edited by P.Jolles, H.Zahn and H.Hocker, © 1997 Birkhauser Verlag Basel/Switzerland
example, under reducing conditions), the wet hair stretched and the bonds allowed to re-form in new positions. This is the basis of artificial waving (perming) in which the new bonds will more permanently hold the hair in its modified situation.

It is now known that the keratin IF belong to the superfamily of proteins that form 8–10 nm diameter filaments in the cytoplasm of many epithelial cells, where they play the structural role of ‘cytoskeleton’ (Fuchs and Weber, 1994). The keratin IF are an elaborate extension of this role (Steinert and Freedberg, 1991). The manner in which the IFs are packed in the macrofibrils gives rise to two main types of cortical cells in hairs. These cell types are termed the orthocortex and the paracortex (Mercer, 1961; Fraser et al., 1972) and their distribution in the fibre can determine its form; for example, the crimp in wool fibres (Fraser and Rogers, 1955). The orthocortex is always on the outer side of the curve of the crimp. Intermediate types, particularly the mesocortex (or heterotype), are known (Fraser et al., 1972). The different cell types were originally detected by their differential reaction to staining with dyes (Horio and Kondo, 1953). It is known that this is the result of the protein composition and IF packing arrangements, but the reason for it is not understood. In paracortical cells the IFs are arranged mainly in quasi-hexagonal close-packing within the macrofibrils, with the matrix prominent in the interfilamentous space. In orthocortical cells the IFs are closer together and inclined to the cell axis, entwined in a rope-like fashion to produce a cylindrical lattice (Rogers, 1959b; Fraser et al., 1972). The IF packing in orthocortical cells produces a characteristic fingerprint-like ‘whorl’ in electron microscopy cross-sections (Fraser et al., 1972). In the mesocortical cells the IFs are packed in even more regular and extensive hexagonal arrays than are observed in the paracortex (Bones and Sikorski, 1967). The molecular basis for these packing modes is unknown, but it is likely that they are the result of quantitative or qualitative differences in the distribution of hair keratin proteins.

All three types of IF packing can be recognized in electron micrographs of transverse sections of human hair, although the differences between the types are not as marked as seen in wool, and the chemical differences can not be so easily defined (Swift, A.J., personal communication). Fraser et al. (1972) have reported the ‘whorl’ orthocortical pattern in human hair (Figure 1.8), but noted that this was combined with a high proportion of high-sulphur (KAP) proteins (which is more a characteristic of paracortical material). Kassenbeck (1981) has made similar observations and has also reported hairs with mainly mesocortical structure but with orthocortical cells at the periphery adjacent to the cuticle. According to Swift (1977 and personal communication), straight Mongolian hair (which is basically circular in cross-section) is all paracortex, curly Caucasian hair has a core of paracortex with about 5 per cent orthocortex on the perimeter forming a layer about one cell thick and always on the outer side of the curl, and very curly Negroid hair (which is elliptical in cross-section) has a bilateral distribution with the orthocortex also on the outside of the curl, as occurs in wool.

In some hairs small structures called cortical fusi occur in the cortex (Hausman, 1932). The fusi appear dark by transmitted light microscopy and may be mistaken for pigment granules (Harding and Rogers, 1984). That they are not pigment but are small air inclusions is demonstrated by their bright appearance by reflected light (Schwinger and Pott, 1977). A close study of their shape (they are fusiform, i.e. spindle-shaped, and therefore have pointed ends) should also help to distinguish them from the more rounded pigment granules (Noback, 1951). Fusi originally form as small fluid-filled spaces between the developing cortical cells when the cells are soft and pliable in the bulb region. As the cells move up the follicle they harden and keratinize, the hair dries out and the fluid is replaced by air (Hausman, 1932; Noback, 1951). There does not seem to be any genetic basis for the occurrence of fusi. They may occur throughout the length of the hair shaft but they are seldom seen at the tip. Frequently they are found just above the root (Noback, 1951). A possible explanation for this is that the fusi may be formed by mechanical forces as the result of flexing of the shaft at this point (which would correspond approximately to skin level when the hair is still attached to the follicle).

In coloured hairs the cortical cells contain pigment granules in addition to the keratin fibrils. These granules contain the pigment melanin and so are sometimes also called melanin granules. They are ellipsoidal in shape, about 1 µm in length and 0.3–0.4 µm in diameter (Birbeck et al.,
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1956; Swift, 1977), but the size and shape are quite variable (Zviak and Dawber, 1986). Generally they are situated in the inter-macrofibrillar matrix with their long axis parallel to the length of the hair (Swift, 1977). There are two types (and colours) of pigment involved; the black-brown eumelanins, giving dark colours, and the reddish yellow phaeomelanins, giving light colours (Ortonne and Thivolet, 1981). Hair pigmentation is discussed in more detail in section 1.10. The colour of hair is due not only to the colour, density and distribution of the pigment granules in the cortex (Hausman, 1927), but also to the actual amount of melanin polymer within each granule (Swift, 1977). In human hair there tend to be more granules towards the periphery of the cortex than towards the centre (Swift, 1977) (Figure 1.9). They are sometimes also found in the medulla, but not usually in the cuticle of scalp hair (Hausman, 1927; Swift, 1977; Robbins, 1988). There are fewer coloured granules in greyning hair and none in white hair (Szabo, 1965).

Nuclear remnants can still be seen in fully keratinized cortical cells (Birbeck and Mercer, 1957a; Roth and Clark, 1964; Swift, 1977; Seta et al., 1988), but the DNA of the original chromatin is not visible (Rogers, 1969) and there has been some conjecture as to whether DNA is completely removed or broken down (Swift, 1977). The early work of Downes et al. (1966) suggested that the DNA was broken down during keratinization and resorbed, but Kalbe et al. (1988) and Schreiber et al. (1988) have shown that high molecular weight (genomic) DNA (≥20 kb) is present in human hair and can be extracted from it. The studies by Kalbe et al. (1988) on wool cells show that although some of this DNA would come from the cuticle, it is likely that some is also from the cortex since this comprises the major part of the hair shaft. The amount extractable from a single

Figure 1.8 Electron micrograph of a stained transverse section of a keratinized human hair’s cortical cell, a region called the orthocortex. The packing mode of the keratin IF (microfibrils) and matrix into macrofibrils has the appearance of ‘whorls’ that resemble fingerprints. Bar = 0.1 µm. (Courtesy of Dr. L.N.Jones)
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Although the amount of DNA in hair shaft, though, is very small; von Beroldingen et al. (1989) found a level of 0.2–4 pg/cm, an amount which is too small for conventional DNA typing but which can be analyzed for HLA-DQα using polymerase chain reaction amplification (Higuchi et al., 1988). Mitochondrial DNA remains sufficiently intact in hair shaft cells to allow analysis by sequencing after amplification (Vigilant et al., 1989; Hopgood et al., 1992; Wilson et al., 1995).

1.4.3 Cuticle

The outer layer of the hair shaft is called the cuticle. It is composed of flattened, imbricated scale cells (i.e. cells that overlap like tiles on a roof). The cells overlap both longitudinally and laterally to surround the hair completely and hold the cortex together. They slope outwards, their edges pointing towards the tip of the hair. In the follicle these edges interlock with opposing scales of the cuticle of the inner root sheath, helping at least in part to hold the hair in place in the follicle (Montagna and Van Scott, 1958; Straile, 1965). Thus pulling a growing hair with a quick, sharp tug will sometimes yield a hair root with inner root sheath material still attached (Ludwig, 1967).

The cuticle of a human hair forms from a single layer of cells in the follicle. In the mature hair the cuticle cells are roughly rectangular in shape (Kassenbeck, 1981), about 50–60 µm long and about 0.5 µm thick (Swift, 1981). However, they overlap to such an extent that only about one-sixth of each cell is visible on the surface (Ryder, 1963), and the cuticle is effectively multilayered with a thickness of about six cells (about 3–5 µm) and scale edges about 5 µm apart (Swift, 1981).

Cuticle cells form a pattern which can be visualized microscopically on the hair surface and is called the hair scale pattern. The pattern differs between species and can be used for species identification (see Noback, 1951; Wildman, 1954; Brunner and Coman, 1974). Using Wildman’s nomenclature the pattern for human hair is described as ‘close wave’, but the pattern can change with changes to the speed of growth of the hair (Kassenbeck, 1981). In spite of these changes, the scale counts (which are a measure of how far apart the edges are) are relatively constant for scalp hair for an individual providing sufficient counts are made, but they can vary between individuals (Gamble and Kirk, 1940). The scale count also varies on an individual depending on site, the count being significantly smaller (i.e. the scales further apart) for scalp hair than for hairs from nine other body sites, such as pubic, facial and chest. Smaller scale numbers are seen for the scalp hairs of younger people than older, and for facial, axillary and abdominal hairs of females as compared to hairs from the same sites from males (Wyatt and Riggott, 1977).

The scale pattern of human hair corresponds to the edges of the cuticle cells (Kassenbeck, 1981). When the hair emerges from the skin, these edges are relatively smooth, and the cell surfaces are also relatively smooth, although closer inspection may reveal some marks imprinted on them by pressure from the IRS as the cells were keratinizing and hardening in the follicle (Kassenbeck, 1981; Swift, 1981).

Figure 1.9 Transverse section of a human pubic hair showing the higher concentration of pigment granules towards the periphery. Note that no tissue is present in the medulla.
The cuticular surface of the fully developed hair is extremely hard when dry and protects the softer cortex from wear and tear (Swift, 1981). Nevertheless, the cuticle itself is damaged by processes such as weathering, combing, brushing, washing, and abrasion against other hairs, and the scale edges chip away (Swift, 1981). This causes the scale edges to become more irregular and produces the pattern typically seen for human hair (Figure 1.10). This phenomenon becomes more extensive the more the hair is exposed to insult. Since the hair shaft is dead tissue and cannot repair itself, greater damage will be seen towards the tips of hairs as compared with near the root (Bottoms et al., 1972; Swift and Brown, 1972; Montagna and Parakkal, 1974; Swift, 1977). This does not change the overall pattern (Figure 1.11), but it does affect the detail of it (Swift, 1981) and has ramifications with regard to the comparison of patterns (Swift, 1977). If the damage is extensive

Figure 1.10 Light micrographs showing (a) the typical human scale pattern on a (white) scalp hair, and (b) a worn tip end of scalp hair from which the cuticle has been lost and the cortical cells are starting to separate and fray

Figure 1.11 Scanning electron micrographs of human scalp hair showing the difference in the detail of the scale and pattern at different points along the hair shaft. Three different hairs are used for illustration; in all cases the tip end is towards the right, (a) Near the root. (b) Mid-shaft. (c) Near the tip
and ongoing it will reduce the thickness of the overlapping cell layer eventually to the point where there is no longer a cuticle, and the cortex will be exposed (Swift and Brown, 1972). Without the protection of the cuticle the hair feels rough and the cortex frays and falls apart (Figure 1.10), with the formation of split tips and brush ends (Price, 1981).

Three layers are distinguishable by ultrastructural analysis of cuticle cells. They are the endocuticle (on the inner side), the exocuticle (on the outer side), and a narrow layer, called the ‘A’ layer, on the outer edge of the exocuticle. These layers are clearly delineated in electron micrographs by the density of their electron staining (Figure 1.12), with the endocuticle being the least dense and the ‘A’ layer the most (Rogers, 1959c; Fraser et al., 1972; Swift, 1977).

The cuticle cell contents are amorphous; filaments are not visibly prominent but lamella structures resulting from fused granules can be seen (Swift, 1981). The proteins of the exocuticle are cysteine and glycine-rich proteins (Fraser et al., 1972) containing on average 30 mole per cent half-cysteine. There are at least two families of proteins (MacKinnon et al., 1990; Jenkins and Powell, 1994) and the gene for one of these (KAP5) is known for human hair (MacKinnon et al., 1991). The endocuticle also consists mainly of protein, but this contains very little cysteine and large amounts of acidic and basic amino acids (Swift, 1981). Pigment granules are not normally found in the cuticle cells of human scalp hair. Nuclei, or the remnants of them, can be seen in the endocuticle (Swift, 1977; Kassenbeck, 1981). The nuclear DNA is not visible but it is apparently not completely degraded. Kalbe et al. (1988) have

Figure 1.12 Electron micrograph of a stained transverse-section of the cuticle region of a human hair fibre. Note the three overlapping cuticle cells (1–3) external to the cortex (Co). The cell membrane complex ‘CMC’ (arrows) is seen between the cells and is thinner at the junction of the cortex and the cuticle. Each cell is filled with hardened proteins but three layers can be distinguished in the cells, the exocuticle (ex) with the outermost ‘A’ layer and the endocuticle (en). Bar = 0.1 µm. (Courtesy of Dr. L.N. Jones)
shown that high molecular weight DNA (&gt;20 kb) can be extracted from isolated cuticle cells of merino wool and thus presumably at least some of the similar-sized DNA they also extracted from human hair shafts would have been of cuticular origin. The ‘A’ layer (which is about 110 nm thick) contains extremely high levels of sulphur (1 in every 2.7 amino acids is present as a half-cystine), and this results in the unusual hardness of the hair surface (Swift, 1981).

Each cuticle cell is completely surrounded by an epicuticle (Bradbury, 1973). This is a hydrophobic membranous layer about 10 nm thick. It forms the immediate outer surface of the hair. It is resistant to chemical and enzymatic attack, presumably because its protein is highly cross-linked by both disulphide bonds and isopeptide bonds (Zahn et al., 1994). The hydrophobicity is due to a monomolecular layer of a C21 saturated fatty acid, 18-methyl-eicosanoic acid (18-MEA) (Evans et al., 1985) covalently associated with the protein (Zahn et al., 1994). The 18-MEA is also part of the intercellular cement layer (d-band) which binds the overlapping cuticle cells. Damage to the cells causes splitting of this cementing layer and exposes a fresh cuticle cell surface with its 18-MEA layer intact.

The cuticle has functions other than just protecting the cortex. The hair surface shows ‘directional friction’ (friction is less going in the direction from root to tip than in the opposite direction) due to the imbricated arrangement of the scales (Swift, 1977). This phenomenon helps the hairs remove dentritis and irritants from the skin, and it also assists in keeping the hairs aligned (and thus not tangled and matted). The layer of 18-MEA acts as a boundary lubricant in this regard, contributing to the coefficient of friction (Swift, A.J., personal communication).

The cuticle plays a large part in our perception of the ‘feel’ or ‘lift’ of the hair because of its effect on how the hair bends. The multilayering of the cuticle can cause it to be a large proportion of the overall diameter of the hairs, especially fine ones, and it therefore can make a large contribution to the hair stiffness. This effect is particularly seen in hairs of non-circular cross-section (for example, Negroid hairs) where the preferred bending will be in the direction of the minor axial diameter (Swift, A.J., personal communication).

1.5 Molecular Biology of Hair Growth

1.5.1 Structural Proteins of the Hair Follicle and Their Genes

The Complexity of Keratin Proteins and the Nomenclature Problem

The understanding of the complexity of hair keratin proteins as large gene families has increased enormously in recent years through the advent of gene cloning. Most of this advance in knowledge has been in studies of the genes for wool keratins, but the findings are largely applicable to human hair. Human hair genes have been studied directly as well (Yu et al., 1993).

The keratin intermediate filament (keratin IF) proteins consist of two families, Type I and Type II (Figure 1.13); each of these is known to contain four related proteins which are referred to as the low-sulphur protein group in the older literature. As explained earlier (section 1.4.2), there is an obligatory pairing of one Type I and one Type II chain in the structure of the keratin IFs. Eight Type I hair IF sequences are now known from a number of species; five in their entirety and three in large part. These include one complete human sequence (Yu et al., 1993), two complete sheep sequences (Dowling et al., 1986; Wilson et al., 1988) and, for a mouse, three complete (Bertolino et al., 1988; Winter et al., 1994) and two partial (Tobiasch et al., 1992a; Winter et al., 1994) sequences. There are four Type II keratins in hair and there is another minor component in human and bovine hair but apparently not in sheep wool. Four sheep sequences (Sparrow et al., 1989, 1992; Powell et al., 1992; Powell and Beltrame, 1994) and two mouse sequences (Yu et al., 1991; Tobiasch et al., 1992a, 1992b) are known.

The keratin proteins of the hair matrix (keratin-associated proteins, KAPs) are a more complex group and again they have been classified according to amino acid composition (Table 1.1). Part of
this complex group is a very large superfamily with half-cystine contents that vary from 12 to 40 mole per cent. This superfamily is arbitrarily divided into two groups; those with half-cystine contents of up to 30 mole per cent (called high-sulphur proteins, or HS proteins) and those with greater than 30 mole per cent half-cystine content (referred to as ultra-high sulphur proteins, or UHS proteins). Each of these groups consists of several families. In some of the HS and UHS families there are tandem copies of the pentapeptide motif, cys-cys-arg/gln-pro-ser/thr. A family of UHS proteins has been found in the exocuticle region of the hair cuticle (MacKinnon et al., 1990). The other fraction of the matrix is a class of proteins prevalent in merino wool which have high contents of glycine and tyrosine (the high glycine/tyrosine, or HT proteins). These proteins are very low in abundance or absent from human hair (Gillespie, 1991). Two proteins of this class, referred to as Type I, have similar amino acid compositions but are not homologous to each other or with the Type II family, which is a large family of homologous proteins possibly made up of 20–30 members.

Nomenclature for the keratin proteins and their genes is confusing, with new and different names accorded to proteins or genes as research proceeds. The old naming system of low-sulphur, high-sulphur and high glycine/tyrosine proteins originated from wool keratin proteins (Fraser et al., 1972), but it has become restrictive and cumbersome. This became particularly apparent when the epidermal keratin IFs were sequenced and catalogued by Moll et al. (1982). No provision was made to incorporate the wool low-sulphur keratins when it became clear that they are related to the epidermal keratin IF (Hanukoglu and Fuchs, 1982; Weber and Geisler, 1982). The old nomenclature has continued to be applied to the wool keratin IF proteins, and totally different names have been arbitrarily given to the hair IF genes characterized from various species. The system for epidermal keratin IF (Moll et al., 1982) classifies the Type II keratins as K1-K8 and the Type I keratins as...
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K9-K19 (in the genetic databases they are listed under the symbol KRT). New Type I IF keratins can be sequentially added as K20 onwards, but no simple extension is available for the Type II IF keratins. To retain the present Kn format, the Type II numbering would have to jump from K8 to beyond K21, and those in between would be Type I keratin IF, a cumbersome arrangement.

This confusion has led to a proposal (Rogers and Powell, 1993; Powell and Rogers, 1994) for a revised nomenclature still linked to the scheme of Moll et al. (1982) and which incorporates all keratin IF. The families of hair keratin-associated proteins can be classified with a related system.

In the proposed system (Table 1.2), the term Km.nxpL identifies keratin IF (K being replaced with KRT for gene symbols in the databases). The families of high-sulphur, ultra-high sulphur and high glycine/tyrosine hair proteins are distinguished by the term, KAPm.nxpL, indicating keratin-

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wool</th>
<th>IF</th>
<th>HS</th>
<th>UHS</th>
<th>HGT (I)</th>
<th>HGT (II)</th>
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<tr>
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<tr>
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<td>11.1</td>
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<tr>
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<td>12.8</td>
<td>5.3</td>
<td>3.0</td>
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<tr>
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<td>6.2</td>
<td>4.2</td>
<td>27.6</td>
<td>33.6</td>
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<td>22.1</td>
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<td>5.3</td>
<td>4.3</td>
<td>2.1</td>
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<td>0.0</td>
<td>0.0</td>
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<td>1.3</td>
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<td>5.3</td>
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<td>2.1</td>
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<td>0.5</td>
<td>10.3</td>
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</tbody>
</table>

IF, intermediate filaments; HS, high sulphur; UHS, ultra-high sulphur; HGT (I), high glycine/tyrosine (Type I); HGT (II), high glycine/tyrosine (Type II). Data from Marshall et al. (1991).

Table 1.2 A unified nomenclature for keratin proteins

<table>
<thead>
<tr>
<th>Keratin intermediate filament protein Km.nxpL*</th>
<th>Keratin-associated protein KAPm.nxpL†</th>
</tr>
</thead>
<tbody>
<tr>
<td>K – keratin</td>
<td>KAP – keratin-associated protein</td>
</tr>
<tr>
<td>m – either 1 or 2 for Type I or Type II IF</td>
<td>m – a number identifying the family</td>
</tr>
<tr>
<td>n – Moll number or a new number</td>
<td>n – a number identifying the component</td>
</tr>
</tbody>
</table>

x – signifies a variant; use any letter, beginning with ‘a’
p – signifies a pseudogene
L – signifies ‘like’ and refers to a keratin of uncertain identity

* Database gene symbol KRTm.nxpL.
† Database gene symbol KRTAPm.nxpL.
The cataloguing of the keratin IFs and KAPs requires the merging of data from different species into a logical nomenclature. The major criteria for common identity which can reasonably be applied are sequence similarity and pattern of expression. To illustrate the principal features of the suggested new nomenclature, some examples of assignments for the keratin IF and hair KAP genes and proteins are given in Tables 1.3 and 1.4.

### Table 1.3 Keratin intermediate filament proteins

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Old name</th>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Old name</th>
</tr>
</thead>
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<tr>
<td>KRT1.1</td>
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<td>Component 8C1 (sheep)</td>
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<td>K2.1</td>
<td>K1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ha1 (mouse)</td>
<td></td>
<td>K2.2</td>
<td>K2</td>
</tr>
<tr>
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<td>K1.2</td>
<td>47.6 kDa (mouse)</td>
<td>KRT2.3</td>
<td>K2.3</td>
<td>K3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ha3 (mouse and human)</td>
<td></td>
<td>K2.4</td>
<td>K4</td>
</tr>
<tr>
<td>KRT1.3</td>
<td>K1.3</td>
<td>Ha4 (mouse)</td>
<td>KRT2.5</td>
<td>K2.5</td>
<td>K5</td>
</tr>
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<td>K2.6a</td>
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</tr>
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<td>K9</td>
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</tr>
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<td>K12</td>
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<td>K2.9</td>
<td>KII-9, IF type II B (sheep)</td>
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<td>K13</td>
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<td>K2.10</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>type II D (sheep)</td>
</tr>
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<td>K16</td>
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<td>K18</td>
<td>KRT2.13</td>
<td>K2.13</td>
<td>KII-13 (sheep)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endo B (mouse)</td>
<td>KRT2.14</td>
<td>K2.14</td>
<td>KII-14 (sheep)</td>
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<tr>
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<td>K19</td>
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<td>K2.15</td>
<td>KII-15 (sheep)</td>
</tr>
<tr>
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<td>K1.20</td>
<td>K20</td>
<td>KRT2.16</td>
<td>K2.16</td>
<td>65 kDa/HRb-1 (mouse)</td>
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<td>KRT1.21</td>
<td>K1.21</td>
<td>K21</td>
<td>KRT2.17</td>
<td>K2.17</td>
<td>70 kDa (mouse)</td>
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</tbody>
</table>
unusual feature for vertebrate genes, of which there are only a few examples, such as the histone genes, which lack introns. By comparison, the genes of hair matrix proteins are very large families.

In the following discussion, genes are given in *italics* whereas proteins are given in plain text.

**Genes for Keratin Intermediate Filament (IF) Proteins**

The structure of the hair keratin *IF* genes is very similar to that of the epidermal genes. The *Type I* genes contain six introns and are 4–5 kb in size (Figure 1.14), whereas the *Type II* genes contain eight introns and are larger, 7–9 kb in size (Wilson *et al.*, 1988; Kaytes *et al.*, 1991; Powell *et al.*, 1992; Powell and Beltrame, 1994).

### Table 1.4 Intermediate filament associated proteins

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
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</tr>
</thead>
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</tr>
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<td>KRTAP1.2</td>
<td>KAP1.2</td>
<td>B2B</td>
</tr>
<tr>
<td>KRTAP1.3</td>
<td>KAP1.3</td>
<td>B2C</td>
</tr>
<tr>
<td>KRTAP1.4</td>
<td>KAP1.4</td>
<td>B2D</td>
</tr>
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<td>KRTAP2.1</td>
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<td>HS BIIIA1</td>
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<td>ΨBIIIB3A</td>
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<td>UHS K4</td>
</tr>
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</tr>
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<td>KAP7</td>
<td>HGT Type I component C2</td>
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<td>KRTAP8</td>
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<td>HGT Type I component F</td>
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</tr>
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<td>–</td>
</tr>
<tr>
<td>KRTAP11.1</td>
<td>KAP11.1</td>
<td>Hacl-1 (mouse)</td>
</tr>
</tbody>
</table>

Unless otherwise noted, sequences are of sheep proteins, genes or cDNAs. HS, high sulphur; UHS, ultra-high sulphur; HGT, high glycine/tyrosine.
The Type I and Type II epidermal genes map to different chromosomes (Lessin et al., 1988; Romano et al., 1988; Rosenberg et al., 1988; Nadeau et al., 1989; Popescu et al., 1989; Fries et al., 1991; Hediger et al., 1991) except for the Type I gene, K1.18, which maps to the Type II locus (Waseem et al., 1990; Yoon et al., 1994). Linkage between the hair and epidermal keratin genes has been established in mice (Compton et al., 1991) and other data are beginning to reveal the long-range organization of the keratin Type II locus (Powell and Beltrame, 1994; Yoon et al., 1994) in more detail. From these data the locus containing most, if not all, of the Type II IF hair and epidermal genes is possibly 500–600 kb in size, containing 20–30 genes.

A 100 kb segment of DNA from the sheep keratin Type II locus has been characterized (Powell and Beltrame, 1994) in which six Type II genes, three hair and three hair-related, have been mapped. Since genes encoding three of the four sheep wool Type II proteins are clustered within 40 kb and flanked by hair-related genes, it is possible that hair genes are located in a domain that contains all the regulatory controls required for hair gene expression. At least one of the hair-related genes appears from its intact structure to be capable of encoding a functional protein, although no expression was detected in the hair follicle (Powell et al., 1993). The eight known epidermal-type genes, K2.1-K2.8, map to human 12q13 (Yoon et al., 1994) and mapping data have revealed the probable existence of several related genes, possibly hair genes.

The hair and epidermal Type I keratin genes also are linked like the Type II genes (Compton et al., 1991), but information is limited on the size of this group of linked genes. Genomic clones showing linkage of two to three hair and hair-related genes have been described (Powell et al., 1986; Kaytes et al., 1991) and epidermal keratin genes, K1.14 and K1.16, are linked within 10 kb of each other (Rosenberg et al., 1988, Savtchenko et al., 1990) and the K1.13, K1.15 and K1.19 genes are located within a 55 kb segment of DNA (Filon et al., 1994).

Genes for Keratin (Intermediate Filament)-Associated Proteins (KAPs)

In the past few years some interesting features of the KAP genes encoding the proteins of the matrix have emerged. There are at least ten families of KAP genes and each gene is rather small, between 0.6 and 1.5 kb in size. The absence of introns is a rare occurrence in vertebrate genes and the hair KAP genes would constitute one of the largest groups of intron-less genes known.
The human KAP5 gene family that is expressed in the hair cuticle may contain as many as ten genes (Powell et al., 1991) located at two sites on human chromosome 11, at 11p15 and 11q13 (MacKinnon et al., 1991). Of the other KAP gene families that encode cysteine-rich hair proteins (KAP3, 4, 9, and 11), sufficient data have been reported only for the KAP9 family, where three genes are located in an 18 kb piece of DNA (McNab et al., 1989).

The KAP6 gene family that encodes glycine/tyrosine-rich proteins comprises at least nine genes, and in the sheep genome they are located within a 1 Mb segment of DNA, three of them within 40 kb (Fratini et al., 1993). RFLP studies indicate that the KAP8 gene which encodes a different glycine/tyrosine-rich protein is linked to this group (Parsons et al., 1994a) and located on sheep chromosome 1 (Wood et al., 1992). The location of the KAP7 gene is at present unknown.

The findings of Parsons et al. (1994b) suggest that a KAP1 locus is linked to the locus for keratin Type I IF genes (KRT1) on sheep chromosome 11. They discovered the linkage of at least one KAP1 gene to growth hormone by RFLP studies and they deduced the linkage of the KAP1 and KRT1 loci from the linkage between growth hormone and the KRT1 locus. Linked KAP1 and KAP2 genes have been characterized in genomic clones and they are possibly part of a larger locus, several hundred kb in size, in the sheep genome (Powell et al., 1983). There is the possibility of the existence of a supercluster of the KAP1, KAP2 and Type IF (KRT1) gene families. Close genetic linkage is a typical finding for hair genes, and will probably apply to the other unmapped KAP gene families as well. Furthermore, if the results of the cloning and mapping studies that have linked KAP1 and KAP2 loci extend to the other KAP gene families there is the possibility that a supercluster exists containing several KAP families and the Type I (KRT1) family, perhaps totalling more than 60 genes. Such a finding could be significant from the aspect of evolution and regulation of expression. The linkage of a homeobox gene cluster (genes involved in controlling development) to the Type I (KRT1) locus and the mapping of several mouse hair mutants to this region of the chromosome (Nadeau et al., 1989; Compton et al., 1991) indicate that important findings can be anticipated in the future from keratin gene mapping.

Evolution of the IF and KAP Genes in the Genome

From comparative structure and sequence data it has been proposed that over 1000 million years ago the IF superfamily originated from a common ancestor, and that divergence of the progenitors of the Type I and Type II keratin IF genes occurred soon after (Blumenberg, 1989). Conservation of linkage relationships for other non-keratin genes at the keratin Type I and Type II IF loci in the genomes of mouse and human supports the occurrence of extensive duplication involving these homologous gene pairs in the genome (Nadeau et al., 1989; Hart et al., 1992).

The Non-keratin Follicle Protein Trichohyalin

Trichohyalin is the only other abundant structural protein in the hair follicle for which there is information on the structure and organization of its gene. Trichohyalin, as previously discussed, is synthesized in the IRS and the medulla. In the cells of the IRS it becomes a matrix protein between filaments of the IF type, but the proteins of these filaments have not yet been characterized. In the medulla, the trichohyalin turns into several large amorphous aggregates in each medullary cell. The protein sequence of sheep trichohyalin has been deduced from the DNA sequence (Fietz et al., 1993) and reveals that the obligatory 315 residue a-helical domain of heptad repeats characteristic of IFs is not present in the primary structure of the molecule, and hence trichohyalin is not a precursor of an IF but is an IF-associated protein (Fietz et al., 1993). The sequence of 1549 residues of sheep trichohyalin is primarily composed of glutamic acid, glutamine and lysine residues (64 mole per cent) arranged into two separate repeating blocks; the central repetitive region contains 16 tandem repeats of a 28 amino acid motif and the C-terminal region contains 28 repeats of a 23 amino acid motif (Figure 1.15). Both types of repeat contain the substrate amino acids for peptidylarginine

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Figure 1.15 Amino acid sequence of the trichohyalin molecule (sheep) arranged to show the two repetitive regions (central and C-terminal) and the putative calcium-binding region (the E-F hands) at the N-terminal end (Fietz et al., 1993). The amino acids are designated by the conventional one-letter code. The sequence and structure of the human molecule (Lee et al., 1993a) is closely similar.
deiminase (arginine residues) and transglutaminase (glutamine and lysine residues). At the N-terminal end of the protein there are two E-F hands (Fietz et al., 1993). An E-F hand is a region of amino acid sequence in which two α-helices are separated by a section of sequence in which the amino acid residues have oxygen-containing side chains so arranged in space that six oxygen atoms can chelate a calcium ion. Since each E-F hand is capable of binding a calcium ion, as in molecules such as calmodulin, each trichohyalin molecule potentially can bind two calcium ions and functional assays have shown that calcium is indeed bound by trichohyalin (Lee et al., 1993a).

As seen in Figure 1.16, the sheep gene has three exons; a short exon of approximately 50 bp containing 5’ non-coding sequences, a second short exon containing one E-F hand, and a third exon containing the second E-F hand and the rest of the gene (Fietz et al., 1993). The trichohyalin gene then is an exception to the generalization mentioned earlier, that genes for the matrix proteins of the hair structure are without introns. Other proteins that play similar ancillary roles in keratinization, such as profilaggrin and calcyclin, have virtually identical gene organizations (Ferrari et al., 1987; Presland et al., 1992). Trichohyalin, a single copy gene that maps to human chromosome 1q21.1–21.3 (Fietz et al., 1992; Lee et al., 1993b) is clustered with the genes for loricrin, profilaggrin, involucrin, calcyclin and a small proline-rich protein within a 2 Mb segment of DNA (Volz et al., 1993). These genes are expressed in the epidermis and hair follicle and have some structural and functional similarities with trichohyalin (for references, see Volz et al., 1993), raising the possibility that they could have evolved from a common ancestral gene, perhaps by tandem duplication along the chromosome.

1.5.2 The Expression and Regulation of Hair Follicle Genes

With a high level of proliferative activity in the follicle bulb, the hair follicle is one of the most active organs in the body. As the cells move up from the bulb the keratin IF and keratin-associated proteins are expressed sequentially during hair cortical cell differentiation, a finding predicted in broad outline by early electron microscopic and biochemical radiolabelling studies (Mercer, 1961; Downes et al., 1963; Rogers, 1964) but now known in greater detail, as described below.

The Regional and Temporal Expression of Keratin Genes in the Anagen Follicle

Immunolocalization studies using mostly antibodies that recognize all the components of a hair keratin IF family showed that the proteins are present in cells in the middle of the follicle bulb and rapidly accumulate as the cells move up from the bulb and into the lower shaft (Kemp and Rogers, 1970; French and Hewish, 1986, Lynch et al., 1986; Heid et al., 1988a, 1988b; Moll et al., 1988; Bertolino et al., 1990). Studies with cRNA probes for hair Type I and Type II genes have extended the antibody data (Kopan and Fuchs, 1989; Kaytes et al., 1991; Powell et al., 1992; Tobiasch et al., 1992a, 1992b). Figure 1.17 shows the general features of IF gene expression that begins in the upper bulb region.

The Type II genes are expressed sequentially in cortical cell differentiation in separate but closely timed stages (Powell et al., 1992). The K2.12 gene encodes the most divergent protein of
Forensic Examination of Hair

the hair Type II family (Sparrow et al., 1992; Powell and Beltrame, 1994) and its pattern of expression in the middle of the follicle bulb is consistent with the antibody data of Heid et al. (1988a) which showed early and diffuse labelling in mid-follicle bulb cells. From the upper bulb into the lower shaft, two more Type II genes are activated (K2.9 and K2.10) and then a fourth gene (K2.11) is activated (Powell et al., 1992). Given the obligatory pairing of Type I and Type II proteins to form keratin IF, the expression of hair Type I genes would be expected to parallel that of the Type II genes, and a Type I hair IF probe has demonstrated activation of one or more Type I genes in the mid-follicle bulb (Powell et al., 1992).

The complexity of KAP gene expression is much greater than that of the hair IF genes, and the detail of their expression is not as advanced. It mainly shows that the onset of KAP synthesis occurs after the synthesis of keratin IF. The poor antigenicity of the KAP proteins and their content of cysteine-rich motifs are largely responsible for antibody studies having failed to reveal a detailed delineation of KAP gene expression (Lynch et al., 1986; French and Hewish, 1987). The expression is best studied by in situ hybridization with cRNA probes because such probes can be designed to detect either specific genes or highly conserved gene families. At the present time there are in situ hybridization data for the expression in sheep and mouse, but not human, follicles of cortical KAP4, 6, 9 and KAP11 genes (McNab et al., 1990; Fratini et al., 1993, 1994; Huh et al., 1994; Powell et al., 1995).

Figure 1.17 In situ hybridization demonstration of the expression of the IF keratin genes in hair (wool) follicles using specific radioactive gene probes, (a) Longitudinal section and brightfield view of the expression of a Type I keratin IF gene. The expression begins just above the bulb region and extends through the lower third of the follicle length, (b) Expression shown in darkfield. Bar = 50 µm
The patterns of expression of the KAP genes suggest the existence of instructions for cortical cell differentiation acting both temporally and regionally. After the keratin IF genes, the KAP genes are sequentially activated in different patterns in the lower to mid-follicle shaft. The genes encoding the glycine/tyrosine-rich KAPs (KAP6 family, KAP7 and KAP8 proteins) are expressed first (Figure 1.18).

In follicles of merino sheep which, as explained earlier, have a bilateral segmentation of the cortex into orthocortical and paracortical cells, the expression of KAP6 family, KAP7 and KAP8 genes is restricted to the orthocortical cells. Later, cysteine-rich KAP genes are expressed. Initially, the KAP1, 2 and 3 (cysteine-rich) gene families are expressed in the side of the cortex opposite to that expressing the KAP6, 7 and 8 genes but later, all of the cortical cells express the genes. The KAP4 family is expressed slightly later still and only in paracortical cells (Fratini et al., 1994). The distinction between orthocortical and paracortical expression of the matrix proteins correlates with the findings of Jones et al. (1993), who conducted elemental analysis by electron microscopy and demonstrated that proteins rich in sulphur are concentrated in the paracortex and that proteins rich in tyrosine are concentrated in the orthocortex. The expression patterns for orthocortical and paracortical cells in human hair still need to be investigated. In mouse follicles, where the distribution of cortical cell types is more like human than sheep, the expression of the KAP9 and KAP 11 genes seems to occur in all the cortical cells of the lower shaft (McNab et al., 1990; Huh et al., 1994).

The cuticle has a remarkably different ultrastructure to the cortex (Birbeck and Mercer, 1957b; Rogers, 1959b, 1959c; Swift, 1977; Woods and Orwin, 1980) in which lamellar structures resulting from fused granules of cysteine-rich proteins are prominent in the cuticle. Cuticle differentiation was described many years ago by electron microscopy (Woods and Orwin, 1980), but through immunological (Woods and Orwin, 1980; Heid et al., 1988a, 1988b; Stark et al., 1990) and in situ hybridization

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**Figure 1.18** Diagrammatic representation of the temporal and regional expression of different keratin families as revealed by *in situ* studies of the type shown in Figure 1.17. Modified from The role of keratin proteins and their genes in the growth, structure and properties of hair’ by B.C.Powell and G.E.Rogers, in *Formation and Structure of Human Hair*, edited by P.Jolles, H.Zahn and H.Hocker, © 1997 Birkhauser Verlag Basel/Switzerland

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The cuticle has a remarkably different ultrastructure to the cortex (Birbeck and Mercer, 1957b; Rogers, 1959b, 1959c; Swift, 1977; Woods and Orwin, 1980) in which lamellar structures resulting from fused granules of cysteine-rich proteins are prominent in the cuticle. Cuticle differentiation was described many years ago by electron microscopy (Woods and Orwin, 1980), but through immunological (Woods and Orwin, 1980; Heid et al., 1988a, 1988b; Stark et al., 1990) and *in situ* hybridization...
studies (MacKinnon et al., 1990; Jenkins and Powell, 1994; Winter et al., 1994) the genes that are expressed are now being identified. Filaments are conspicuously rare, although antibody studies have suggested that hair and epithelial IF keratins are present at early stages of development (Heid et al., 1988a, 1988b; Bertolino et al., 1990; Stark et al., 1990). Conclusive identification of a Type I hair keratin in the developing cuticle by RNA in situ hybridization was recently reported by Winter et al. (1994). They showed that the gene encoding the mHa2 Type I keratin (K1.4 in proposed nomenclature) was exclusively expressed in the cuticle, with mRNA appearing in cells situated around the periphery of the upper part of the follicle bulb. It is therefore one of the first differentiation-specific genes expressed in cuticle cells and its timing of expression is comparable to K2.12, the Type II keratin gene of the cortex.

Cuticle KAP genes have been isolated that encode a family of cysteine-rich and glycine-rich proteins, the KAP5 family, which are expressed late in hair cuticle differentiation (MacKinnon et al., 1990; Jenkins and Powell, 1994). These proteins consist of repeating cysteine-rich and glycine-rich motifs which probably have highly folded conformations. A new cuticle-specific gene family, KAP10, has been discovered recently and is expressed late in cuticle cell differentiation (Powell et al., personal communication). The KAP10.1 protein is cysteine-rich but has a completely different composition to the cuticle KAP5 proteins, with less than 1 mole per cent glycine, and it will be important to find out where it is located in the ultrastructure of the cuticle cells. Electron microscopic studies have described the appearance of three different kinds of granules in cuticle cells in the upper region of the follicle bulb (Swift, 1977; Woods and Orwin, 1980) and hence it is likely that there are other cysteine-rich keratin genes of the KAP10 family expressed at an earlier stage of cuticle cell differentiation remaining to be discovered.

Molecular Control of Keratin Gene Expression

Relatively little is known about how hair keratin genes are regulated. The clusters of the two keratin IF gene families and five of the KAP gene families suggests that ‘global’ regulatory domains might govern their expression. The promoter (5') regions of hair keratin genes (Figure 1.19) contain potential binding sites for the transcription factor complexes, AP1 and AP2 (Powell et al., 1991, 1992; Fratini et al., 1993).

The involvement of AP1 and AP2 in regulating hair gene expression is supported by the finding that FOS, one of the proteins of the AP1 complex, is expressed in differentiating follicle keratinocytes (Fisher et al., 1991). There are several examples, including a number of epidermal keratin genes (Byrne and Fuchs, 1993; Magnaldo et al., 1993; Casatorres et al., 1994; Lu et al., 1994) of AP1 and AP2 sites intimately associated with the binding sites for other regulatory factors, implicating the involvement of AP1 and AP2 factors in complex control mechanisms.

A major problem in the investigation of hair keratin gene expression and the regulation of the process is the lack of an appropriate follicle cell line. At present, functional assays of potential control sequences of genes require the creation of transgenic mice, an expensive and time-consuming process. Two such studies with hair genes have described the faithful expression of the genes with moderate lengths of promoter sequence; a 400 bp promoter from the hair keratin Type II gene, K2.10, targeted LacZ expression to the hair follicle cortex (Keough et al., 1995) and a 600 bp promoter from the KAP9.1 gene targeted expression to the follicle cortex of transgenic mice (McNab et al., 1989).

Sequences that may be important in regulating gene expression can be identified by comparing promoter regions of related genes, particularly when they can be made between evolutionarily distant species (Gumucio et al., 1992). In such a survey of 15 hair keratin gene promoters, Powell et al. (1991) found highly-conserved DNA sequences grouped with particular gene families. A sequence, CTTTGAAG (then designated HK1), is strictly conserved in all hair keratin IF genes, with a copy located within 180–240 bp of the transcription start site of each gene (Powell et al., 1991, 1992). It is also present in the KAP gene promoters. Recent studies have revealed that HK1 corresponds to the consensus binding site CTTTG(A/T)(A/T) for the protein, lymphoid enhancer factor 1 (LEF-1), which has been shown to bind to the sheep K2.9 and K2.10 promoters (Zhou
Figure 1.19 The DNA sequence of the 5' promoter region of the keratin IF gene *K2.10*. The sequence extends for 350 base pairs distal to the site from which transcription of the mRNA begins. The transcription start site is marked by an arrow head. Several sequences are shown (boxed) which are known to function as promoter regulating elements that bind specific transcription factors (proteins) in other genes.
et al., 1995). These workers suggested that LEF-1 is involved in creating favourable transcriptional domains in the hair keratin gene promoters required for maintenance of hair keratin gene expression, but they have also shown that in mice, LEF-1 mRNA is expressed in different patterns in embryonic vibrissae and postnatal pelage follicles. In vibrissae, LEF-1 is expressed with hair keratin IF genes around the apex of the dermal papilla but in pelage follicles LEF-1 expression occurs throughout the follicle bulb in cells that are not yet expressing hair keratin genes.

In the IRS of the follicle, trichohyacin is an excellent marker for hair follicle differentiatin because its mRNA is expressed in the IRS cells in the bulb much earlier than keratin IF mRNA in the hair cortex. The regulation of this gene has not been studied.

1.5.3 Natural Mutations in Keratins and Diseases of Hair

Mutations that cause defects in the growth and structure of hair in mice have been extensively catalogued by Sundberg (1994a) and are being related to diseases of human hair. Many of the mutations have been mapped but the biological mechanisms by which they affect hair formation are largely unknown, although in some instances the mutations might directly affect hair keratin genes. The naked mutation in mice results in malformed hairs with cuticular defects and a marked reduction in the amount of the glycine/tyrosine-rich proteins (Tenenhouse and Gold, 1976; Raphael et al., 1982, 1984) which are encoded by several genes, but its pleiotropic effects indicate a regulatory gene mutation rather than one affecting structural keratin genes. In sheep, the Felting Lustre mutation also results in a deficiency of glycine/tyrosine-rich proteins (Gillespie and Darskus, 1971) but the genes for them are present in the genome (Rogers et al., 1989). A human equivalent to the naked mouse mutation is hydrotic ectodermal dysplasia (Sundberg, 1994b). This is an autosomal dominant genetic condition in which the scalp hair is sparse and brittle. The fibres show abnormal keratinization (and reduced birefringence) and abnormal structure, including the cuticle which splits easily. The amino acid composition suggests that a matrix protein of the KAP1–3 group is deficient (see Gold and Scrivier, 1972).

Interesting hair phenotypes have appeared in gene knockout mice with defective TGFα (Lieti et al., 1993; Mann et al., 1993) and FGF5 (Hebert et al., 1994) genes. These correspond to the known mouse hair mutants, waved-1 and angora respectively, implicating direct roles for TGFα and FGF5 in hair growth. FGF5 is the gene responsible in part for the initiation of the catagen phase of the hair cycle. A consequence of its disruption is an extension of anagen, resulting in longer fur.

In the nude mutation that maps to mouse chromosome 11, the homozygous mice are born nearly hairless but later develop sparse hairs that undergo normal cycles. Follicle initiation appears to be normal but the nude phenotype seems to arise because the hairs are thin and imperfectly keratinized and break in the pilary canal. Flanagan (1966) found a marked reduction in sulphhydril content in the nude hair follicles and concluded that nu/nu hair follicles were reduced in their content of cysteine-rich proteins, resulting in weaker hair shafts. It is possible that nude directly or indirectly controls the transcription of some KAP genes during hair growth.

Some genetic conditions have been described that affect human hair structure (Goldsmith, 1979; Price, 1990; Gillespie, 1991; Sundberg, 1994a). Two human hair conditions for which there are at least partial causal explanations are the kinked hair of Menkes syndrome (Danks, 1991) and the fragile hair of trichothiodystrophy (Price et al., 1980; Van Neste et al., 1989; Price, 1990). In Menkes syndrome the sulphhydril content of the hair keratin is only partly (~50 per cent) oxidized to disulphide bonds (Gillespie, 1991). This is accounted for by a copper deficiency which is caused by a mutation in a copper transporter protein (Mercer et al., 1993). Copper has long been recognized as vital for normal keratinization of hair through studies on the formation of ‘steely wool’ in copper-deficient sheep. Trichothiodystrophy describes a group of syndromes with similar pathologies affecting hair and nail structure that may be caused by a number of mutations (Price et al., 1980; Van Neste et al., 1989; Price, 1990). Trichothiodystrophic hair is sparse and brittle. Its microscopic appearance is distinctive, showing bright and dark bands by polarizing microscopy under crossed polar (Price et al., 1980). It has about 50 per cent of the sulphur content of normal hair (Pollitt and
Both the cuticle and cortex appear to be affected. The reduced sulphur content is traceable to a deficiency in many of the cysteine-rich proteins, particularly those with the highest cysteine content (Gillespie and Marshall, 1983) and, in this respect, has some similarities with the mouse nude mutation. Presumably this deficiency results in less disulphide cross-linking in the keratin proteins and a reduced structural integrity of the hair cortex and cuticle.

In the rare condition maple syrup urine disease (MSUD), which is caused by a mutation in the branched chain a-ketoacid dehydrogenase enzyme, the hair defect seen involves the structure of the intercellular membrane complex of the hair cuticle (Jones et al., 1996) (Figure 1.20). The consequence of the enzymic abnormality is that the normal surface lipid component, 18-methyl-eicosanoic acid (18-MEA, see section 1.4.3), is largely replaced by eicosanoic acid which has a higher melting point than 18-MEA. This results in greater stiffness and rigidity and possible loss of adhesion between the cells (Jones and Rivett, 1996).

The dystrophic hair disease monilethrix is an autosomal dominant condition of variable alopecia and hair fragility in which many of the hair shafts have a beaded morphology. Recently it has been shown that this disease maps to the Type II keratin cluster on chromosome 12q (Healy et al., 1996). This is the first mapping of a primary human hair disorder. The condition may have its origins in an aberration of filament formation, perhaps the result of a mutation in the coding sequence of the gene for one of the structural proteins.

A gene CGH involved in the pattern of hair growth has been identified in sufferers of congenital

Figure 1.20 An electron micrograph of the cuticle of a human hair from an individual suffering from the genetic disease, maple syrup urine disease. This metabolic disorder leads to a change in the fatty acid composition of the intercellular cell membrane complex (CMC) of the cuticle that causes these layers to become weak and to break down (arrows). Compare Figure 1.12. (Courtesy of Dr. L.N.Jones.) Bar = 0.2 µm
generalized hypertrichosis, a condition characterized by excessive facial and upper torso hair in both males and females (see section 1.9.2). How the gene operates in determining hair distribution is not known, but it has been localized to chromosome Xq24–q27.1 (Figuera et al., 1995).

A mutation in the human homologue of the mouse gene hairless has been found to be associated with the condition alopecia universalis, an extreme form of alopecia in which sufferers lose the scalp hair they were born with, never have eyebrows or eyelashes, and never develop axillary and pubic hair (see section 1.9.3). This is an autosomal recessive condition and the gene has been mapped to chromosome 8p12 (Ahmad et al., 1998). The gene product, a protein of 1189 amino acids, is thought to act as a transcription factor in hair growth. The transition of A-to-G in the nucleotide base sequence at position 1022 of the gene creates a missense mutation leading to the conversion of a threonyl residue to an alanine residue in the defective protein (Ahmad et al., 1998). Although hairless controls hair growth, it is active in both males and females and is therefore unlikely to be involved in common (male pattern) baldness (Knight, 1998).

1.6 The Hair Cycle

Human hair, like the hair of other animals, follows a pattern of growth and rest. The hair grows during the ‘growing’ phase of the follicle, and then it is retained as a dead club hair during the subsequent ‘resting’ phase. A new growing phase follows, producing a new hair which may dislodge the old hair or may grow alongside it. This pattern of growth, loss and regeneration is commonly called the hair cycle (Chase, 1954, 1965). It is through the hair cycle that animals, including humans, are able to adapt their hairs (by replacing them) to suit changes in conditions; for example, a change of hair colour to suit a change in season, or a change of hair type with age, as at puberty in humans. The term ‘cycle’ may, however, be somewhat misleading in relation to humans since it implies a periodic regularity and this is not the case in human hair growth (Chase, 1965).

The hair cycle is a dynamic, continuous process, but for convenience of description and understanding it is divided into three main stages (Figure 1.21). These stages are called anagen (the growing phase), catagen (the transition period between growing and resting) and telogen (the resting phase) (Dry, 1926).

Dry (1926) defined the anagen phase as ‘the largest part of the growing period, lasting from the initiation of growth until, in coloured hair, pigment ceases to pass upwards from the bulb at the base of the hair-root’. Catagen was defined as the phase ‘during which the root is decreasing in size’, and telogen was when the hair had ‘become a club-hair and [was] not growing further’ (Dry, 1926).

The telogen phase can be considered as the mature phase of hair growth (Chase, 1965). Montagna and Parakkal (1974) refer to telogen as the ‘quiescent’ phase. It is at this dormant stage that the hair roots have their least attachment in the follicles and the hairs are most likely to be shed as the result of general wear and tear such as brushing, combing and washing. The stages are illustrated diagrammatically in Figure 1.22.

1.6.1 Anagen

Anagen is a period of high metabolic and mitotic activity. The follicle re-forms after its previous resting phase, in a process similar to its initial development except that the follicle bud (hair germ) is already present and the hair canal to the skin surface is established (Chase, 1954; Chase and Silver, 1969).

Many changes take place in the anagen phase, and to aid description it is helpful to divide anagen into substages. Chase (1954), for example, denoted the substages anagen I to anagen VI, with anagen VI being the stage where the hair has emerged beyond the skin surface. The substages may also be known by the letters A-F, or the terms proanagen (anagen I-IV), mesanagen (anagen V) and metanagen (anagen VI) (Chase, 1965).

Anagen I is the phase in which the hair germ begins its mitotic activity. In anagen II the follicle is growing down around the dermal papilla and the first differentiation of germinative cells into
inner root sheath begins. In anagen III, the follicle attains its maximum length of 4–5 mm (about three to six times that of its resting length) and the bulb surrounds the dermal papilla. Melanocytes can be seen in the bulb, and the inner root sheath has formed. At anagen IV there is high mitotic activity in the cells of the bulb (Bullough and Laurence, 1958) and the formation of the hair cortex and medulla can be seen. Pigment granules are visible. The hair, however, does not yet extend beyond the inner root sheath. By anagen V the follicle has reached its final characteristic onion shape in which the lower part of the bulb encloses the dermal papilla. The point of the new hair has pushed aside the brush-like attachment of the old hair and surrounding cells (Bullough and Laurence, 1958) and has reached the epidermis. The process to this stage takes about 3 weeks (Saitoh et al., 1970). At anagen VI the hair is visible above the skin surface and continues to grow without further changes to the follicle (Figure 1.22) until the catagen phase begins (Chase, 1954).

Hairs plucked at the anagen stage of growth need a strong pull to detach them from the dermal papilla (Ludwig, 1969). The roots will appear fleshy and dark (optically dense), sometimes with pigmentation (Figure 1.23). They may be stretched or broken or both. When anagen hair is removed quickly (even by brushing or combing), sheath tissue may be attached (King et al., 1982; Petraco et al., 1988) (Figure 1.24a). In fact, anagen hairs plucked with a very quick jerk may come away surrounded by all the elements of the lower portion of the follicle, including the papilla.

Figure 1.21 Longitudinal sections of human scalp hair follicles showing different stages of the growth cycle. Note that some separation of the tissue layers has occurred during section preparation, (a) Follicle at anagen. (b) Follicle at catagen. The club root has formed and the follicle has regressed, leaving the dermal papilla at its original depth. (c) Follicle at telogen. The club root has minimal connection with the follicle. Bar = 100 µm
Figure 1.22 Diagrammatic representation of hair follicles at the different stages of the growth cycle. Anagen is the active growth phase during which follicle development takes place and the hair fibre is produced. Catagen is the regression phase in which tissue changes occur as the follicle approaches telogen, the resting phase. The next anagen is thought to be initiated by an interaction between dermal papilla cells and pluripotent stem cells located in the bulge region. B, bulge; APM, arrector pili muscle; SG, sebaceous gland; C, hair cortex and cuticle; M, medulla; DP, dermal papilla. Modified from Cotsarelis et al. (1990), copyright Cell Press.

Figure 1.23 Light micrographs at the anagen stage, (a) The root of a white (non-pigmented) hair showing the medulla and the cavity normally occupied by the dermal papilla, (b) The root of an intensely pigmented hair showing the concentration of melanocytes at the base of the bulb. The hair shaft near the tip end is seen below the bulb. The circular object is an air bubble in the mountant.
Figure 1.24 Light micrographs of hair roots plucked at different stages of the growth cycle. (a) Anagen root, with root sheath material remaining attached to the hair shaft. (b) Catagen/early telogen stage. The root is surrounded by the epithelial sac. (c) Telogen (c) root typical of naturally shed hairs
(Ludwig, 1967). Because the root and sheath tissues are metabolically and mitotically active they are amenable to enzyme typing (Twibell and Whitehead, 1978; Whitehead et al., 1981; Gambel et al., 1987), sexing by fluorescent chromosome analysis (Kringsholm et al., 1977; Mudd, 1984), and DNA typing by the polymerase chain reaction (Higuchi et al., 1988; Uchihi et al., 1992).

1.6.2 Catagen

During catagen the follicle undergoes gradual, orderly morphological and functional changes as it enters its regression phase. The hair gradually stops growing. In human scalp the process is relatively short and probably takes about two to three weeks (Kligman, 1959), although other workers suggest the range is one to four weeks (Ferriman, 1971; Zviak and Dawber, 1986).

As catagen begins, the inner root sheath begins to disintegrate (Chase and Silver, 1969). Melanin production stops. The dendrites contract and no more pigment is injected into the (still growing) cortex which thus becomes pale or white at the root end (Montagna and Parakkal, 1974). Cell division in the bulb decreases and eventually stops. The cells in the upper part of the bulb continue to move up the follicle for some time and to differentiate, but medulla and cuticle formation cease and only the cortex and inner root sheath are formed. Only remnants of the hair bulb remain (Montagna and Parakkal, 1974). Kligman (1959) suggests the process is one not so much of cell dissolution as of de-differentiation.

Vacuoles containing hydrolytic enzymes form in the cells of the lower follicle and the esterases and acid phosphatases break down the follicle cells, allowing the area they formerly occupied to be filled by connective tissue cells. The remains of the follicle cells form an epithelial strand between the dermal papilla and the hair germ (Figure 1.21b). The hair germ forms from the outer root sheath at about the level of the middle of the follicle (at the embryonic bulge, just below the arrector pili muscle attachment). It is from this hair germ that a new follicle will form when growth is initiated. As a result of these processes the follicle reduces to about one-third of its length. The root of the hair eventually becomes a brush-like club consisting only of cortical cells (the hair club). These cells fill with filaments and form keratinous rootlets which anchor the club root in the follicle. The rootlets are firmly attached to cortical cells of the hair shaft on one side and on the other side they interdigitate the two or three layers of surrounding germ cells which form the epithelial capsule or sac (Montagna and Parakkal, 1974).

Hairs at this stage would normally stay attached to the remains of the follicle, but if they are pulled out the roots will often appear non-pigmented and brush-like, sometimes surrounded by the epithelial sac but with no sheath material adhering (King et al., 1982) (Figure 1.24b).

1.6.3 Telogen

Telogen is the mature, stable state of hair growth. The hair is anchored in the follicle by the club root as a result of the processes involved in catagen. The telogen follicle is very short—about one-third the length of an anagen follicle—extending to just below the level of the sebaceous gland. The telogen follicle is relatively simple compared with an anagen follicle; the dermal papilla is separated as a ball of cells located below the epithelial capsule or sac (Figure 1.21c). There are no germinative cells, or cuticle, or inner or outer root sheaths (Montagna and Parakkal, 1974). The cells in the lower region of the follicle are mitotically inert (Bullough and Laurence, 1958) and contain less DNA than the cells of an anagen bulb (Higuchi et al., 1988). Telogen lasts for 3–4 months for human scalp hair (Kligman, 1961; Ferriman, 1971; Zviak and Dawber, 1986). Contrary to popular belief, the mature hairs do not ‘fall’ out. They remain in place until pulled out (for example, by brushing or washing or other forms of friction). Frequently, though, the mature hairs are displaced at the end of telogen by the emerging new hair of the next anagen phase (Kligman, 1961), although sometimes the new hair will grow alongside the old one (Montagna and Parakkal, 1974).

Hairs at telogen stage form the majority of ‘naturally shed’ hairs found on clothing and the like (King et al., 1982), and this is expected as, compared with hairs in anagen phase, they require only a small force to dislodge them. Typically they do not have any root sheath attached, no medulla near the
root and little or no pigment in the root (Figure 1.24c). Not infrequently cortical fusi will be present in the shaft above the club root (Petraco et al., 1988).

Telogen finishes when a new anagen phase commences. Each time a new hair is formed it must be preceded by the formation of an almost entirely new follicle in a regeneration akin to the initial development of the follicle (Chase and Silver, 1969). The first mitotic activity in the production of the new hair occurs in the basal cells of the lower follicle, which grows downwards as a solid column of undifferentiated and dividing cells to surround the dermal papilla (Bullough and Laurence, 1958) and begin the new anagen phase.

1.6.4 Dynamics of the Cycle

In humans, the growth cycles of hairs are not synchronized; each follicle has its own cycle (or rhythm) and is independent of neighboring follicles (Chase, 1954). This lack of synchronization of growth gives a growth pattern described as ‘scattered mosaic’ and in the human scalp, for example, results in a more or less even daily shedding of scalp hairs, although some seasonal variation with increased shedding in the winter can be expected (Orentreich, 1969). On a normal scalp about 90 per cent of the follicles are in anagen and 10 per cent in telogen at any one time. Thus of the approximately 100,000 hairs on the scalp, about 10,000 will be club hairs. With a telogen period of about 100 days, the average daily shedding of scalp hairs is about 100 hairs (Orentreich, 1969). Other workers report similar percentages for anagen and telogen. For example, Pinkus (1981) found 80–85 per cent in anagen, 1–2 per cent in catagen and 10–20 per cent in telogen for the normal scalp, and Zviak and Dawber (1986) report 80–95 per cent of follicles in anagen with less than 1 per cent in catagen and a normal average shedding rate of 100–150 hairs per day. The range, however, is wide and there are too many individual differences for much significance to be attached to these numbers (Montagna, 1976). For example, Kligman (1961) reported a mean of 13 per cent of follicles in telogen, with a range of 4–20 per cent, and noted that each person tends to have their own characteristic telogen count. None the less, in general the percentage of telogen follicles in the scalp tends to be higher in males than in females (Barman et al., 1969) and increases with age (Barman et al., 1965). The percentage of follicles in telogen also varies according to the region on the body (Montagna and Parakkal, 1974).

During pregnancy the proportion of scalp hairs in anagen rises (Montagna and Parakkal, 1974); it may be greater than 95 per cent, so that the shedding rate drops to about 10–15 hairs per day (Zviak and Dawber, 1986).

A minor qualification to the general rule of independence of follicles is the hairs in ‘Meijere’s trio groups’. These groups, originally described by De Meijere (1894) as a basic hair pattern in mammals, consist of three hairs arranged in a group with the longest hair in the centre (Noback, 1951). Hairs grouped in this way are sometimes seen in the human scalp and in other regions, and Saitoh et al. (1970) have found that the three hairs in such groups have almost the same cycle as each other.

Plucking of club hairs stimulates resting follicles to go into anagen, and from there on they will be ‘out of phase’ with their expected growth pattern (Chase, 1954). Although some reports suggest that clipping of hairs will increase the growth rate (Sato, 1976), measurements by Trotter (1928) on beard hair and by Lynfield and MacWilliams (1970) on leg hairs show that shaving does not initiate growth, alter the growth rate or diameter of the hair, nor upset the cycle. Conversely, a reduction in the number of dermal papilla cells in the bulb region (caused, for example, by wounding the dermal papilla) can upset the cycle; some follicles will produce shorter than normal hairs whereas others will produce longer hairs. The increase in length is achieved by increasing the duration of the growing period rather than an increase in the rate of growth (Oliver and Jahoda, 1989).

The lengths of the cycles for hairs at a given region of the human body (such as the scalp) are similar to each other but different from those for hairs at other regions (Saitoh et al., 1970). This is in contrast to animals that have seasonal moulting in which there are one or two cycles per year. In these cases, all hairs are in more or less the same growth phase at any one time and are shed almost
simultaneously (Montagna and Parakkal, 1974). Yet other animals, for example the mouse, exhibit a wave of hair growth in which the growth cycles of the hairs in a particular area are synchronized (Dry, 1926). Thus a wave of moulting develops as this area moves across the body from the throat region towards the back and the sides of the mouse.

Investigators have used a number of methods to measure human hair cycles. The proportion of follicles in anagen, catagen and telogen can be determined by plucking hairs at random and assessing the stage of growth by microscopic examination of the roots (Orentreich, 1969). This method is, however, subject to errors, such as loss of hairs by prior washing or brushing, distortion of the roots if the plucking is not carried out correctly or consistently (Orentreich, 1969; Ferriman, 1971), and the limitation on the number of hairs that can be taken (Ferriman, 1971).

The duration of the anagen and telogen phases can be determined indirectly by dividing the length of an uncut hair by the rate of hair growth (Orentreich, 1969). Alternatively, direct measurement of the phases can be made from the observation of the growth of individual hairs using photography at very close range (Saitoh et al., 1970). When measured this way the ‘growing period’ determined is the time from the appearance above the skin of a new hair to the end of its growth in length, plus three weeks for the (estimated) time for the hair to start growing and reach the skin surface. Thus the ‘growing period’ includes the time of anagen and catagen. The ‘resting period’ (telogen) is the time from the hair reaching its maximum length until it is lost. The duration of catagen is difficult to measure without resorting to histology (Kligman, 1959) and estimates are based on observations of catagen provoked by experimental procedures such as X-ray irradiation.

Estimates of the length of anagen for the normal scalp vary considerably. While no doubt some of this variation reflects differences between individuals, some will also derive from difficulties in measuring (Ferriman, 1971; Montagna and Parakkal, 1974). Saitoh et al. (1970) found a range of 17 weeks to 94 weeks for coarse hairs on a 60-year-old male. Most other workers report longer times; Chase (1954) and Chase and Silver (1969) give two years or more, Orentreich (1969) 1000 days on average, Ferriman (1971) 3 years or more, Montagna and Parakkal (1974) 8 years or more, Pinkus (1981) 5–8 years, Zviak and Dawber (1986) 3–7 years, and Robbins (1988) 4–6 years on average. During these periods of time scalp hairs can grow to a length of 1 metre or more (Robbins, 1988).

In contrast to scalp hair, eyelashes have a very short growing period (30 days) and a relatively long telogen (105 days) (Montagna and Parakkal, 1974). Saitoh et al. (1970) found the following average periods for anagen for different body regions of a 30-year male: moustache, 12 weeks; finger, 7 weeks; arm, 11 weeks; and leg, 17 weeks.

1.7 Hair Types and Morphology

Three types of hair growth are usually recognized in humans, namely lanugo hair, vellus hair and terminal hair. Follicles are not necessarily committed to produce the same type of hair for life, and the type may change depending on age and the region of the body; follicles which in early life produce vellus hairs may well later produce terminal hairs (Garn, 1951b). The terminal hairs develop differently depending on racial background and body site (Danforth, 1926).

1.7.1 Hair Types

Lanugo Hairs

Lanugo hairs are the hairs formed in the embryo as the first product of follicular development, commencing at about five months’ gestation (Zviak and Dawber, 1986). They are fine and soft, and usually not medullated nor pigmented (Pinkus, 1958; Ferriman, 1971; Zviak and Dawber, 1986). They resemble the vellus hairs which replace them in post-natal life (Montagna and Parakkal, 1974) but may be somewhat thicker and longer (Robbins, 1988). Lanugo hairs are shed in about the seventh to eighth month of gestation (Ferriman, 1971).
Vellus Hairs

These replace the lanugo hairs just before birth. They are fine, soft and unmedullated and are usually not pigmented (Ferriman, 1971). They are 1 cm to 2 cm long (Ferriman, 1971), but can be as short as 1 mm with a diameter of 4 µm or less (Robbins, 1988), which explains why they are often not detected. They are commonly called ‘down’ or ‘fuzz’. They are spread more or less uniformly over the body (Danforth, 1926; Ferriman, 1971), even on such apparently hairless areas as the bald scalp and the nose (Robbins, 1988) and forehead and eyelids (Montagna and Parakkal, 1974), but they do not occur on the palms of the hands and palmar surfaces of the fingers nor on the soles of the feet and plantar surfaces of the toes (Zviak and Dawber, 1986). Vellus hairs are found together with terminal hairs on both the trunk and the scalp (Montagna and Parakkal, 1974). Some of the vellus hairs on the ears are replaced by coarse terminal hairs in old age (Montagna and Parakkal, 1974).

Terminal Hairs

Terminal hairs are the hairs that are normally thought of when considering human hairs. They are called terminal in the sense that they represent the final state of differentiation of hair in man (Danforth, 1926). They are long, pigmented coarse hairs, sometimes with a medulla, which replace the lanugo or vellus hairs at specific sites on the body (Ferriman, 1971). They are equivalent to the underhairs, fur and wool of other mammals (Montagna and Parakkal, 1974). Terminal hairs can grow up to one metre or more in length (for scalp hair), with diameters ranging from 30 to 120 µm (Robbins, 1988).

Terminal hairs are classified as primary or secondary hairs (Zviak and Dawber, 1986). An alternative terminology is asexual and sexual hairs, respectively (Ferriman, 1971). Primary terminal hair replaces lanugo hair at the scalp, eyebrows and eyelashes (Zviak and Dawber, 1986) and to a lesser extent on the forearms and legs of both males and females (Ferriman, 1971). In common (male pattern) baldness the follicles producing primary terminal hairs on the scalp (particularly the vertex) revert to their embryonic state and produce vellus hairs again (Montagna and Parakkal, 1974). Secondary terminal hair develops at puberty and replaces vellus hairs in the axilla regions, pubic region and abdomen of both males and females, and in the beard area of males (Ferriman, 1971; Zviak and Dawber, 1986). Increased terminal hair growth is seen at puberty on the forearm and leg as well as other general body sites (Ferriman, 1971). This development of secondary terminal hair is a response to androgens (Ferriman, 1971; Zviak and Dawber, 1986).

About 90 per cent of the hairs of the chest, trunk, shoulders, arms and legs of males are terminal hairs whereas the figure is about 35 per cent for females for the same regions (Montagna, 1976). It is the terminal hairs which most clearly show racial, age, sexual and individual differences (Danforth, 1926).

Not all hairs can be definitively categorized as above and intermediate types will, and do, exist (Danforth, 1926; Ferriman, 1971). Thus wide ranges of pigmentation, thickness, length and extent of medullation can be expected, even within a given body region, and this needs to be kept in mind in any forensic examination and comparison of hairs.

1.7.2 Morphology

The morphology (shape) and length of a hair varies according to body site and racial background. Generalized characteristics of typical hairs from various body regions can be used in forensic work to try to determine their region of origin on the body for comparison purposes (Hicks, 1977; Bisbing, 1982), but it must be noted that there is not necessarily a clear demarcation between regions. The size of the hair grown is directly related to the size of the dermal papilla (Van Scott and Ekel, 1958) (see section 1.3.7) and the shape is influenced by changes in the inner root sheath (Straile, 1965). The length of hairs is genetically determined (Rook and Dawber, 1982) and depends on the growth rate at the site and the length of anagen for hairs at that location (see section 1.6.4). There are six morphological types of hair, based on a combination of macroscopic and microscopic differences using the criteria of length, thickness (diameter), and the size of the root and tip, as well as structural variations (Garn,
There is no detectable difference in hair shape between men and women with respect to race or body site (Rook and Dawber, 1982).

Scalp Hair

Scalp hair can vary enormously in (uncut) length in an individual as well as between individuals. The diameter of scalp hairs shows a relationship to race, and ranges from about 50 µm (for Germans) to over 90 µm (for Vietnamese) (Hayashi et al., 1976). The transverse sectional shape of scalp hair also varies with race. Mongoloid (Chinese) hair is essentially circular in transverse section, Negroid hair shows a flattened ellipse with the major axis being about twice the minor axis; and Caucasian (Western European) and Asiatic Indian hairs (with oval-shaped sections) are intermediate between these extremes (Vernall, 1961). The hair of Australian aborigines is similar to Negro hair in this respect (Trotter et al., 1956; Harding, H.W.J., unpublished observations). Hayashi et al. (1976) have further shown that the degree of curliness of scalp hair depends on the transverse sectional shape, and is therefore race-related. They have shown that the more ellipsoidal the section, the greater is the degree of curliness. Thus Mongoloid hair is essentially straight (and coarse because of its large diameter), whereas Caucasian hairs are straight to wavy with a lot of variation, and Negroid hair is very curly. Menkart et al. (1966) have described the fibre geometry as approximately a cylinder for Caucasian hair and a twisted oval rod for Negro hair; that is, in addition to the wool-like crimp of the Negro hairs they also tend to twist irregularly about their longitudinal axis. This twisting can cause what appear during examination to be fluctuations in the diameter of these hairs, but proper mounting and careful microscopy will avoid confusion with real changes in diameter of hairs. There are no significant differences in the amino acid composition of hairs from different racial groups (Wolfram, 1981).

Scalp hairs of fine diameter may not have a medulla but, when present, the medulla may be continuous or discontinuous. The medulla tends to be more prominent in larger diameter hairs.

Pubic Hair

Pubic hair is generally coarser than scalp hair and shows uniformity among the majority of individuals of the same racial group. The length can range from 10 mm to about 60 mm. The transverse section is irregular and asymmetric, leading to curly, twisted hairs (Garn, 1951b). Pubic hairs usually have a relatively wide, continuous medulla and this is presumably why they tend to be stiff and wiry.

Beard and Moustache Hair

These hairs are also coarse and can grow to 300 mm in length (Garn, 1951b). They generally have a wide, continuous medulla and an irregular or triangular transverse section.

Body Hair

Hairs from the general body areas are generally fine with irregular medullation. All forms of hair and medulla will be found. They can vary in length from 3 mm to about 60 mm (Garn, 1951b).

Eyebrow and Eyelash Hair

Eyelash hairs have an average length of about 1 cm (Garn, 1951b), reflecting an anagen phase of about 30 days (Montagna and Parakkal, 1974). Like eyebrow hairs, they are flattened and curved and their shafts vary between 20 and 120 µm in diameter. They are generally among the darkest hairs on the body (Montagna and Parakkal, 1974). Eyebrow hairs can be longer and curly.
Axillary Hairs

Hairs from the underarm can resemble pubic hairs in terms of their coarseness, medullation and twisting about their axis. They can grow from 10 to 50 mm in length (Garn, 1951b).

1.8 Hair Growth, Distribution and Patterns

1.8.1 Hair Growth Rates

Human hair growth rates are affected by age, sex, race, nutrition, hormonal levels and location on the body (Hamilton, 1958; Saitoh et al., 1969). That being so, it is possible to discuss them only in fairly general terms.

Hairs at the vertex (crown) of the scalp have been most studied and are found to grow at the rate of about 0.35 mm (Myers and Hamilton, 1951) to 0.44 mm (Saitoh et al., 1969) per day, although Chase and Silver (1969) quote a figure as high as 0.5 mm per day. A growth rate of about 0.4 mm per day is equivalent to about one and a half turns of keratin a-helix being made in the follicle every second (Dickerson and Geis, 1969). An entire scalp produces a total of about 1 km of hair in a month (Zviak and Dawber, 1986). If left uncut scalp hair will usually grow to about 60–100 cm. The longest documented length of scalp hair is 4.23 m (Matthews, 1994).

The rate for scalp hair is slightly higher in females than in males (Myers and Hamilton, 1951). The hair in any individual follicle grows at a constant rate. There is no significant diurnal variation and neither shaving nor menstruation have any effect (Saitoh et al., 1969). The rate is, however, slightly lower in pregnancy (Pecoraro et al., 1969). The rate peaks in the age group 50–69 years (Pelfini et al., 1969) and this group also has the greatest proportion of thick hairs in the scalp (Barman et al., 1969).

Taken over the whole body, the average daily rate of growth of hair is greater in males than in females (Pelfini et al., 1969).

Pubic hair grows at the rate of about 0.35 mm per day in both males and females at 15 years of age, and decreases with age (Astore et al., 1979). However, in contrast to scalp hair, there is no change with ageing in thickness or state of the hair cycle. There is also no change with pregnancy.

Pelfini et al. (1969), in their study of ageing and hair growth, found that daily growth rates for hairs at four body regions were greater in men than in women, and decreased in the order pubis (0.40 mm per day), axilla (0.36 mm), scalp (0.35 mm), and thigh (0.29 mm). On the other hand, Myers and Hamilton (1951) give the order as beard (chin) (0.38 mm per day), scalp (0.35 mm), axilla (0.30 mm), thigh (0.20 mm), and eyebrow (0.16 mm). They further note that the growth rate in the axillae is greater in males than females, and that it decreases with age. Saitoh et al. (1969) give a figure of 0.44 mm per day for chest hair and 0.27 mm a day for beard hairs.

There is some evidence of seasonal variation in hair growth rate. Randall and Ebling (1991) have found that the rate of growth of beard hair reached a peak in the summer months after a low in winter, indicating an interaction of endocrine factors in hair growth (Ebling et al., 1991). Thyroid hormone is known to influence the growth of human scalp hair; thyroid deficiency can produce a significant reduction in the mean diameter of hairs (as well as a loss of hairs) (Jackson et al., 1972; Ebling, 1981). Androgens alter the size of the hair follicle and the diameter of the hair fibre (Messenger, 1993). They are required for the development at puberty of pubic, axillary and facial (beard) hair, and later in life are responsible for the changes in hair type characteristic of common balding (alopecia). They are not, however, required for the normal growth and development of androgen-independent hairs such as scalp and eyebrow hairs (Leshin and Wilson, 1981). The role and function of the androgen hormones in hair development is discussed further in section 1.8.3.

The maintenance of hair (together with skin) as the ‘outer boundary’ of an individual even in extremes of starvation results in almost normal hair growth in adults on long-term starvation diets (Desai et al., 1981). In contrast, children suffering from the severe protein-calorie deficiency of kwashiorkor have hair shaft diameters decreased to about a half of normal, and sometimes show no
linear growth at all due to the gross reduction of protein synthesis in all tissues. In one example, the average volume of hair produced per day by well-fed controls was 514 µm³ and that produced by kwashiorkor patients was about 60 µm³ (Sims, 1970).

### 1.8.2 Hair Distribution

It has been estimated that an adult human has a total of about two million hair follicles and about one million of these are on the head (Szabo, 1967). Of the follicles on the head, about 10,000–20,000 occur on the face (Lenihan, 1988) and about 100,000–150,000 are in the scalp region (Ferriman, 1971; Montagna and Parakkal, 1974) with blondes being at the higher end of the range and redheads at the lower end (Rook and Dawber, 1982). About 425,000 follicles occur on the trunk, about 220,000 on the arms and about 370,000 on the legs (Szabo, 1967). There are no significant sexual or racial differences with respect to the numbers of follicles (Szabo, 1958, 1967), but there are differences in the type of body hair produced (Ferriman, 1971). For example, the number of hair follicles of the cheek (about 800–900 per cm²) does not differ significantly between men and women (Szabo, 1958, 1967) but the pilary products are clearly different.

When follicles first develop in the foetus they occur at random but at relatively constant spacing of about 274–350 µm apart, depending on the region (Pinkus, 1958; Szabo, 1958). New follicles form in utero when a critical distance between the first follicles is reached as the foetus enlarges (Pinkus, 1958), but no new follicles are formed after birth and so as the skin area increases with growth, the follicles become more widely separated (Szabo, 1958; Ferriman, 1971).

The number of follicles per unit area is determined by biopsy (Szabo, 1958) and is about 320 follicles per cm² for both balding and hairy scalps (Szabo, 1967). Follicle density estimates are, however, subject to considerable variation between researchers, and Ebling (1980), for example, has reported that the mean number of follicles for scalp skin in normal people aged from 30 to 90 years is 459 per cm², dropping to 306 per cm² for balding persons. The number of follicles reduces significantly with increasing age (Barman et al., 1965). There are about 615 per cm² in adults aged 20 to 30 years, and this falls to 485 per cm² at 30 to 50 years and to 435 per cm² at 80 years of age (Ebling, 1980). The average over the whole body is about 330 follicles per cm², ranging from about 800 on the cheek and forehead to about 45–50 on the arms, thighs and legs (Szabo, 1967). There are no significant sexual differences in the distribution of hair follicles (Szabo, 1967).

The spatial distribution (hair density) of hairs is determined by direct examination, using a magnifier, of a defined area (for example 0.25 cm²) and counting the hairs visible, or by clipping an area, photographing it and counting the hairs in the photograph (Orentreich, 1969). Alternatively, hairs can be plucked from a defined area (Barman et al., 1965, 1969) or counted in biopsy sections (Pinkus, 1981). The measurement of hair density (number per cm²), together with the thickness of hair, rate of hair growth, and hair cycle parameters, is known as a trichogram (Barman et al., 1964, 1965). Automated methods using image analysis are now being applied to these measurements (Chatenay et al., 1996).

Values of hair density obtained for the normal scalp range from about 200 hairs per cm² (Pinkus, 1981) through 250 per cm² (Nataf, 1981) to 175–300 terminal hairs (average 223) per cm² (Barman et al., 1965). These hair numbers are lower than those for follicle numbers because they do not count vellus follicles nor terminal follicles in which no hairs are present or clinically visible (Barman et al., 1965). Hair density in females is slightly less than that of males (Barman et al., 1969) and stays within normal ranges throughout pregnancy (Pecoraro et al., 1969). It decreases with age for all regions of the scalp in both men and women (Barman et al., 1965, 1969).

Pubic hair densities are of the order of 6–31 hairs per cm² for both sexes, reducing to about 10 per cm² at age 65 (Astore et al., 1979).

When considering hair numbers it must be borne in mind that variations seen are a combination of individual variations and of the techniques used for counting; plucking hairs from an area may well miss some, but on the other hand when skin sections are taken they are necessarily small and the numbers counted are generally low.
It is interesting to compare, in the context of solar protection, the hair density of the human scalp to that found on the skin of other animals. The highest figure of 300 hairs per cm\(^2\) quoted above for humans is far short of the 6200 hairs per cm\(^2\) of *Megaleia rufa*, the red kangaroo which inhabits open country in Australia and requires good solar protection, or even the 1920 hairs per cm\(^2\) of the euro (*Macropus robustus*, a type of kangaroo), which shelters from the sun (Johnson, 1981). Merino sheep, commonly farmed in South Australia and bred for high fleece density and fibre yield, typically have about 5000 follicles per cm\(^2\).

### 1.8.3 Hair Growth Patterns

In the foetus all the hairs are lanugo hairs. They begin to be shed *in utero* from the face and the head of the foetus between the seventh and eighth months and are replaced by new hairs growing from the same follicles. Mostly the new hairs are vellus hairs, except for the scalp, eyebrows and eyelashes where terminal hairs appear. This process usually takes place before birth, or shortly thereafter (Butcher, 1950). This second crop of vellus hairs is shed during the first four months after birth, and then for the next ten years there are no gross changes in hair patterns (Rook, 1970), although hair shaft diameters increase, particularly in the scalp in the first three to four years (Duggins and Trotter, 1951). However, patterns of hair growth are transient and change with age, especially during the later teens and twenties (Garn, 1951b).

At puberty, secondary terminal hairs begin to appear, replacing the vellus hairs in the pubic region and the axillae (armpits) in both males and females. The pubic hair appears first, followed by the axillary hair. Terminal hair then also begins to replace the vellus hair at other body sites, proceeding over a period of several years in the sequence: anterior leg, posterior leg, posterior thigh, anterior thigh, forearm, abdomen, buttocks, chest, lower back, arms and shoulders (Reynolds, 1951).

Pubic hair growth is approximately equal in both sexes (Astore *et al.*, 1979). It first appears in girls at between 8.5 and 13 years (average age of 11 years) and takes about three years to develop to an adult pattern (Reynolds, 1951; Marshall and Tanner, 1969). In boys, growth starts about 1.5 years later (at an average age of 12.5 years) and also takes three years to develop (Reynolds, 1951; Marshall and Tanner, 1970).

Axillary hair appears at about 15 years in both boys and girls and reaches its peak between 20 and 30 years of age (Hamilton, 1951a, 1958). It develops to the same hair density in both sexes (Pecoraro *et al.*, 1971). Facial (beard) hair also begins to grow in boys at puberty, starting at about the same time as the axillary hair and replacing the vellus hair on the face in an orderly fashion. It begins at the corners of the upper lip and spreads medially to form the moustache. It then appears on the cheeks and on the chin to complete the beard (Ebling *et al.*, 1991).

In contrast to the development of the sexual hairs, the terminal hairs already present on the frontal hair line of the scalp are replaced at puberty by vellus hairs to establish the adult facial outline in virtually all Caucasian males and most females (Rook, 1970).

The transformation of some hair follicles at puberty to produce terminal hairs characteristic of their body site is related to changes in androgen hormone levels (Randall *et al.*, 1993). Hamilton (1942) first recognized the importance of these hormones in human hair growth from his observation that men castrated before puberty neither grew beards nor went bald unless they were treated with testosterone. The precise mechanisms of how alterations occur in the type of hair produced by a follicle are unknown. The response of a follicle to androgens appears to be intrinsic to the particular follicle and depends on the body site. It is a property that the follicle retains even when transplanted to a different area of the body (Randall *et al.*, 1993) and must therefore be taken into consideration in hair transplant procedures (Orentreich, 1981). The response is presumably determined by alterations in gene expression in the cells of individual hair follicles during embryogenesis by as yet unknown factors (Randall *et al.*, 1993).

Follicles in certain sites of the body (axillae, perineum, face, chest and extremities) have an absolute requirement for androgens in order to produce their terminal hair products (Leshin and Wilson, 1981).
Testosterone secreted by the testes is the principal androgen circulating in the plasma in men, whereas in women the less potent adrenal and ovarian steroid \( ?^\text{4}\text{-androstenedione} \) is the major circulating androgen. The androgenic effects of androstenedione are probably dependent on enzymatic conversion to testosterone in peripheral tissues (by the enzyme \( 17\beta\text{-hydroxysteroid dehydrogenase} \)) (Leshin and Wilson, 1981). Normal axillary and pubic hair growth depends not only on adrenal or ovarian androgen but also on the presence of a specific intracellular androgen receptor in the hair follicle (Leshin and Wilson, 1981); only those hair follicles with androgen receptors can respond to androgens (Messenger, 1993). It has been hypothesized that the hormones act through the dermal papilla of the follicles (Randall et al., 1993), based on the essential role that the papilla plays in differentiation in the hair bulb and its effect on hair size and growth (Oliver and Jahoda, 1989) (see section 1.3.7). Support for the hypothesis comes from the finding that androgen receptors are localized only in the dermal papilla of human hair follicles (Choudhry et al., 1992). It is believed that the hormone becomes bound with high affinity to the receptor protein and the complex is translocated into the nucleus, where it combines with the chromatin and initiates transcription of messenger RNA and, subsequently, protein synthesis (Ebling, 1980; Leshin and Wilson, 1981). The whole system is, however, quite complex, especially in humans (Ebling et al., 1991), and still requires extensive research (Messenger, 1993).

While testosterone and androstenedione are sufficient for the androgenic response of pubic and axillary follicles, the development and growth of the male secondary sexual characteristics of facial (beard), trunk and extremity hair requires adult male levels of testosterone, the specific androgen receptors, and a 5a-reductase enzyme as well (Leshin and Wilson, 1981; Ebling et al., 1991). The 5a-reductase is required to convert the testosterone and \( ?^\text{4}\text{-androstenedione} \) in peripheral tissues to the more potent androgens dihydrotestosterone (DHT) and 5a-androstenedione (Leshin and Wilson, 1981; Messenger, 1993).

Terminal hair patterns change constantly with age and do not reach their peak until the ages of about 50–60 years (Rook and Dawber, 1982), which is about the time that the hairs attain their maximum diameter and length (Barman et al., 1969; Pelfini et al., 1969) (see section 1.8.1). The patterns are determined by genetic factors, including those associated with racial differences (Rook and Dawber, 1982).

As age progresses, hair patterns will change as some follicles are lost (see section 1.8.2). Particularly noticeable changes occur on the scalp in the condition known as common balding (also called male pattern alopecia). This is a condition which affects most people of both sexes to some extent; it is discussed in more detail in section 1.9.3. It is appropriate here, however, to note that balding is initiated in susceptible follicles by the action of the same androgens which cause the development of sexual hair at puberty, but the effects produced are the opposite. Whereas for sexual hair the follicle converts from producing vellus hair to producing terminal hairs, in the case of balding, follicles producing terminal hairs are progressively changed and reduced in size until they are once again producing vellus hairs (Montagna and Carlisle, 1981). It is an exaggeration of the process that normally occurs in ageing (Ebling et al., 1991).

1.9 Hair Growth Problems

Abnormal situations of hair growth do occur, and these can be classed as either excessive growth (hirsutism and hypertrichosis) or reduced hair growth and loss of hair (alopecia).

1.9.1 Hirsutism

Hirsutism is the excessive growth of coarse terminal body hair partly or wholly in the male sexual pattern. Although strictly the definition applies to both sexes, hirsutism is often discussed only for females largely because they are more likely than males to be disturbed by the condition.

There is no absolute measure of what is normal; the distinction between normal and hirsute is subjective and to some extent depends on the individual and their race and culture. The degree of hairiness is not easy to define even clinically, but Garn (1951b) and Ferriman (1971) have attempted to
do this with methods that involve assigning a score to each of a number of body sites. The ratings are still to some extent subjective (Garn, 1951b), so it is difficult to determine the incidence of hirsutism in the population.

Hirsutism is induced by androgenic hormones (Rook and Dawber, 1982), a situation which is not unexpected since the normal transformation at puberty of vellus hairs to terminal hairs on the face, trunk and limbs is also related to changes in the levels of these hormones (Ebling et al., 1991). The interaction of hair follicles with androgens, however, is complex and is not yet completely understood (Ebling et al., 1991). It is not always the case that androgen levels in hirsutism sufferers lie outside the normal range, although in some cases the plasma levels of testosterone and 5-a-dihydrotestosterone may be raised, as can be the level of androstenedione (Ebling, 1980; Ebling et al., 1991). Hormone imbalance, when present, can be traced in some instances to dysfunction of the ovaries, of the adrenals, or of both these tissues, with polycystic ovary syndrome being one of the commonest causes of familial hirsutism (Ferriman, 1971; Rook and Dawber, 1982).

The extent of the hirsutism, and the variation in its pattern, is determined also by genetic factors, including those associated with racial differences (Rook and Dawber, 1982). The pattern of the excessive body hair growth is further influenced by an increased susceptibility to androgen of some follicles in some individuals (Ferriman, 1971; Rook and Dawber, 1982; Ebling et al., 1991). Premature balding of the scalp is often associated with hirsutism in both males and females (Ferriman, 1971) and it is paradoxical that this condition also is promoted by androgens in genetically endowed individuals (Ebling et al., 1991).

Hirsutism can occur in pregnancy, and if it does it starts at about week 20 and usually persists for life, but it may disappear partly or completely after delivery (Rook and Dawber, 1982). Various levels and variations of hirsutism are discussed in some detail by Rook and Dawber (1982).

1.9.2 Hypertrichosis

Hypertrichosis is the growth of hair in any localized or extensive pattern (other than the male pattern) which is excessive for the site and for the age and sex of the subject (Rook and Dawber, 1982). There are many forms and any area of the body can be affected. Likewise there are many causes, both congenital and acquired, but in very few cases are the mechanisms of induction known (Rook and Dawber, 1982).

In one congenital form (hypertrichosis lanuginosa) the subject is excessively hairy from birth, being covered by long silky hair similar to lanugo. This is particularly noticeable on the face. At puberty the pubic, axillary and beard hairs emerge but still retain the characteristics of lanugo. In other congenital forms, however, the hair can be coarse and may be accompanied by increased pigmentation of the skin. Individuals with congenital generalized hypertrichosis (CGH) are born with excessive hair on their face and upper torso and this becomes more dense in the first year of life. CGH is an X-linked condition which is more prominent in males and somewhat patchy in females. The CGH gene has been mapped to the X chromosome (Figuera et al., 1995).

Acquired hypertrichosis can arise as a result of, or subsequent to, a variety of phenomena. In some cases the increased hair growth is transient but this is not always the case. Severe head injury, especially in children, can cause hypertrichosis several months after the event, with the excessive hair growth appearing on the face, the limbs or the body. Injuries which produce scar formation or which result from frequent and repeated friction and abrasion of the skin can lead to localized hypertrichosis. Sometimes hypertrichosis may develop prior to pathological conditions such as cancer and porphyria. Hypertrichosis has been noted on the limbs and trunk of children suffering from malnutrition and is also sometimes seen in women suffering from anorexia nervosa when they suddenly restrict their diet, especially by excluding carbohydrates. Drugs such as streptomycin, minoxidil and cortisone can cause hypertrichosis, usually temporarily, and excessive use of alcohol may also cause it, not only in the adult but also in children born with foetal alcohol syndrome (Rook and Dawber, 1982). Thus the hair population of an individual may exhibit radical changes for a period of time and it is important that this be recognized in forensic work, particularly when comparing hairs some time after an alleged incident.
Alopecia is the term used for loss of hair and baldness. A variety of forms is described clinically, including types in which there is partial loss, localized loss, general loss, temporary loss and permanent loss (see, for example, Rook and Dawber, 1982) but only a few of the more common ones which may have some forensic relevance are discussed here.

A common and essentially permanent form of alopecia is what is now called common baldness (Rook and Dawber, 1982). This condition has previously been known by a variety of names, including male pattern alopecia, but this term is not really appropriate because females are also affected. Baldness is not a disease but rather is an inherent condition of humans and afflicts most males to some extent during their life (and some non-human primates such as chimpanzees, orang-utans and stump-tailed macaques) (Montagna and Carlisle, 1981). It is most common in Caucasoids, less so in Negroids and least common in Mongoloids. Common baldness is recognized by its characteristic pattern of development; recession of the hair line and loss of hair at the lateral frontal region, temples and crown. These areas of hair loss may eventually merge on the male scalp to produce the classical bald head. The various stages of development were defined and described by Hamilton (1951b), and his classification scheme now provides a means of assessment and diagnosis. The condition is common in middle-aged men, but it can also occur in men as young as their late teenage years. In females the condition starts later in life, the developmental patterns are less clear and the final extent is less extreme.

A great deal of folklore and tradition surrounds baldness, its cause and its treatment. Much of this relates to theories that hair is lost as a result of poor blood flow in the skin and consequent lack of nutrition for the follicles, and so many of the suggested remedies have been based on irritants that will generate stimulation of the skin and blood supply (Gerstein, 1986). However, blood and nutrient supply is not the problem, and neither are changes to the skin. The skin on the bald scalp is still capable of growing hair when new (active) follicles are transplanted into it (Montagna and Parakkal, 1974). Common baldness is, in fact, a condition which appears to be inherited as an autosomal dominant trait but which is initiated in the hair follicles in the scalp by the action of androgens (Hamilton, 1942). This requirement for androgens has given rise to the term ‘androgenic alopecia’. In genetically disposed persons affected follicles, under the influence of androgens, diminish in size as they go through a series of hair cycles. At each anagen the follicles become smaller and the growing period becomes shorter, progressively producing hairs which are shorter and thinner. Eventually the follicles become similar in size to those of the foetus, producing fine, colourless vellus-like hairs (Montagna and Parakkal, 1974; Montagna and Carlisle, 1981). A bald scalp is not therefore completely hairless; the follicles have not been destroyed although a small number will be lost due to the balding as well as the ongoing aging process.

The detail of the mechanism by which this process of balding is achieved by androgens is unknown, but it is known that to respond to the hormones the follicle must possess an androgen receptor (Messenger, 1993). Furthermore, follicles from different sites on the body react differently; it is an intrinsic property of the follicle which is determined by gene expression in the cells of each follicle during embryogenesis and which is retained by the follicle when it is transplanted (Randall et al., 1993). There is good evidence that the androgen receptor is in the dermal papilla (Randall et al., 1993) and it is through this tissue, which exerts control over the parameters of the hair and the hair cycle (Van Scott and Ekel, 1958; Oliver and Jahoda, 1989), that the effect is mediated. Testosterone and 5-a-dihydrotestosterone are the main hormones implicated in the balding process (Leshin and Wilson, 1981), but how they act and why they cause diminution of hair growth in the scalp yet stimulate growth at other body sites at puberty and in hirsutism is still not known, and is the subject of much research (Ebling et al., 1991; Messenger, 1993; Randall et al., 1993).

Traditional remedies of irritants, stimulants and nutrients applied to the scalp are not effective in reversing (or stopping) the balding process. It might be expected that anti-androgens would work, but none have been reported (Baden et al., 1989). Several drugs, however, have been found to increase hair growth and have potential in the treatment of common baldness. One of these, minoxidil (6-amino-1,2-dihydro-1-hydroxy-2-imino-4-piperidino-pyrimidine), was originally developed as an
antihypertensive agent and was found to cause increased hair growth when taken internally or applied topically (Devine et al., 1977). Its mode of action seems to be to prolong the growing phase of the cells in the hair bulb, thereby allowing a hair fibre of greater length to develop. The precise mechanisms and the target cells for the action of minoxidil on hair follicles are not known. It is known, however, that minoxidil must be converted to its sulphate to be active, both in smooth muscle vasodilation and in hair growth (Buhl et al., 1990). The localization of a minoxidil sulphotransferase enzyme in the cytoplasm of epithelial cells in the lower outer root sheath (ORS) suggests that these cells are the site of sulphation of minoxidil applied topically for hair growth stimulation (Dooley et al., 1991). Recent studies show that minoxidil (and some other anti-hypertensive drugs) relaxes vascular smooth muscle by opening intracellular potassium channels, and there is some evidence that hair growth regulation is a potassium channel controlled process as well. Since minoxidil can stimulate proliferation of hair cells in culture, it is thought that the drug has a direct effect on hair follicles independent of its effects on smooth muscle and follicular blood supply (Buhl et al., 1993). Minoxidil is available commercially as a topical application and works best in younger individuals in whom the balding process is in its early stages. Continuous and regular application is required, but even so, the reversal is not complete.

Alopecia areata (patchy hair loss) is also a relatively common problem (Rook and Dawber, 1982). Hair loss occurs in either a single, well-defined circular or oval patch, or in multiple patches which may subsequently join up. The loss can be quite rapid and the effect very noticeable as a totally bald smooth skin surface is exposed. However, the hair may regenerate after a few months, firstly as fine colourless hairs and gradually with normal pigmentation and thickness. The condition is known as alopecia totalis if the patches extend to total loss of scalp hair. In these forms of alopecia hairs are shed at two different growth stages, namely anagen and telogen. The mechanism for the disruption of normal hair growth is not known, but clearly the metabolism of the germinative cells of the follicle must be disturbed to cause the anagen shedding (Sato, 1981). The hairs shed at this stage grow progressively thinner before being shed, and are known as ‘exclamation mark’ hairs because of their characteristic appearance (Sato, 1981; Rook and Dawber, 1982). Alopecia areata is often associated with autoimmune diseases such as thyroid disease, but there is as yet no proof of an immune-type mechanism for the hair loss (Sato, 1981). There is, however, some evidence from family histories of genetic factors predisposing to alopecia areata (Rook and Dawber, 1982). Treatment with dinitrochlorobenzene (DNCB) can be effective (Sato, 1981).

Another common alopecia is alopecia diffusa, or diffuse hair loss (Rook and Dawber, 1982). This is a generalized loss of scalp hair in which the end result may not be so obvious as in some other forms of alopecia, but the hair shedding occurs relatively rapidly and is therefore very noticeable (and alarming) to the sufferer. The hairs lost are in the telogen phase and a trichogram (measuring the anagen/telogen ratio) is used to demonstrate that the amount of hair loss is abnormal (Binet et al., 1981). A telogen count of greater than 20 per cent is considered abnormal (Kligman, 1961). Alopecia diffusa affects males and females and can occur in two different forms, although both result in the shedding of telogen hairs. In one form, hairs are lost in normal telogen but there is a delay in the onset of the new anagen phase, and hence a transient reduction in hair numbers. Anaemia and malnutrition can lead to this situation (Rook and Dawber, 1982). In the second form follicles prematurely and suddenly enter telogen and the hairs are shed as normal club hairs in due course. This form, which is also known as ‘telogen effluvium’ (Kligman, 1961), occurs when follicles are stressed as a result of conditions such as fevers (for example, typhoid and scarlet fevers), malnutrition, glandular disorders, some drug therapies (such as anticoagulants and cytostatic drugs) and excessive anxiety. It may also occur in females following childbirth (Kligman, 1961). Hair loss and a reduction of hair numbers occur as a result of the stress on the follicles disrupting their inherent growth cycles so that a larger number than normal of follicles are precipitated into telogen all at the same time. Hairs are shed some two to four months after the stress event, a period which represents the normal length of telogen and the development of a new anagen hair which pushes the club hair out of the follicle (Kligman, 1961). The treatments given for telogen effluvium often appear to be successful, but this is inevitable (provided the stress is not continued) because they are given at the time the hair loss is noticed and by this stage replacement hairs have already started to develop in the follicles (Kligman, 1961).
A mild form of hair loss mostly seen in females is **traction alopecia**. This condition arises from strong and sustained or repeated tension or traction on hair follicles as the result of particular hairstyles, some of which are of traditional national origin (Krstic *et al*., 1981). Frequent use of hair curlers, for example, or the wearing of the hair pulled back tightly and forming a heavy ponytail can damage the hair papillae, causing the follicles to atrophy and the hairs to be permanently lost.

An extreme but rare form of alopecia is **alopecia universalis** in which there is no body hair (Rook and Dawber, 1982). Affected individuals are born with scalp hair, but this is lost at an early age and is not replaced. Only a very few follicles can be detected in the scalp. Sufferers do not have eyebrows or eyelashes and they never develop axillary or pubic hair. The condition afflicts both males and females and is inherited in an autosomal recessive manner (Ahmad *et al*., 1998) (see section 1.5.3).

### 1.10 Hair Colour and Pigmentation

The natural colour of human hair is determined mostly by the presence of pigment granules in the hair shaft (Ortonne and Thivolet, 1981).

#### 1.10.1 Pigmentation

The pigment in hair is present as granules (also called **melanosomes**) and in the scalp hair these are generally found between the macrofilaments of the cortex (Swift, 1977). A few granules may sometimes be found in the medulla, but they are not usually found in the cuticle or IRS (Montagna and Parakkal, 1974). In human hair there is a tendency for the granules to be concentrated more towards the periphery (Swift, 1977) and this can be readily seen in cross-sections (Figure 1.9).

The colour of hair depends on the size, type, number and distribution of the granules. However, the difference in colour between different hairs is due more to the type and amount of pigment in the granules than to their absolute numbers (Swift, 1977). In black hairs the granules are ellipsoidal in shape, 0.8–1.0 µm long and 0.3–0.4 µm in diameter (Swift, 1977). In red and blond hair the granules tend to be smaller and more spherical (Montagna and Parakkal, 1974; Seta *et al*., 1988). The granules may occur singly or they may form groups; they are always singly distributed in dark hair (Jimbow *et al*., 1991).

The pigments in hairs are from the general class of **melanin** pigments. Two main types are recognized in hair: eumelanins, which are dark brown to black in colour; and phaeomelanins, which are reddish yellow (Ortonne and Prota, 1993). All coloured human hair contains various proportions of both eumelanins and phaeomelanins. Brown and black hair results from a predominance of eumelanins, whereas red hair arises when there are more phaeomelanins (Jimbow *et al*., 1991).

#### 1.10.2 Melanin Formation

Hair melanins are polymers produced by melanosomes. The two types can be distinguished by their colour and by their chemical properties; eumelanins are insoluble in acid and alkali and contain nitrogen but no sulphur, whereas phaeomelanins are soluble in dilute alkali and possess both nitrogen and sulphur (Ito and Fujita, 1985). The eumelanins are composed mainly of monomer units of 5,6-dihydroxyindole (DHI) and (to a lesser extent) 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while the phaeomelanins are polymers mostly of 1,4-benzothiazine units (Ortonne and Prota, 1993).

The two types of pigment are biogenetically related, both being derived from the soluble, colourless amino acid tyrosine via an initially common metabolic pathway (Ortonne and Prota, 1993). Tyrosine is oxidized by the action of the copper-containing enzyme tyrosinase, firstly to DOPA (3,4-dihydroxyphenylalanine) and then to DOPA-quinone. The DOPA-quinone can then undergo a series of spontaneous, non-enzymatic cyclization and polymerization reactions to form the eumelanins. If sulphur compounds such as cysteine or glutathione become involved all or part of the DOPA-quinone may be converted to phaeomelanins (Jimbow *et al*., 1986). The details of the interconnection and
interaction of the two diverging pathways are complex and are as yet not completely delineated (Ortonne and Prota, 1993).

It is clear, however, that tyrosinase is a fundamentally important part of the process. Multiple forms of the enzyme have been identified (Ortonne and Thivolet, 1981) and it may be that the polymorphism contributes in some way to the control of the colour produced. There is some suggestion that the type of melanosomes produced may be determined by the level of expression of tyrosinase in the hair follicle, since it is found that follicles producing red hair have either higher levels of activity of this enzyme or larger amounts of it compared with follicles which produce black or brown hairs. Blond hairs also have a high level of tyrosinase activity in their follicles, which suggests that the light colour of the hair is not due to lack of production of melamins, but rather due to the type of melanin formed (Ortonne and Prota, 1993). Follicles producing white hairs, on the other hand, do not demonstrate any tyrosinase activity (Kukita and Fitzpatrick, 1955).

1.10.3 Melanosomes

Melanin synthesis occurs exclusively in melanosomes (Jimbow et al., 1986), the distinctive sub-cellular particles which are the product of melanocytes (Barnicot and Birbeck, 1958; Montagna and Parakkal, 1974). Melanocytes are dendritic cells of secretory cell origin (Birbeck et al., 1956) that are present not only in hair follicles but also in the dermis and glands and other tissues of the body (Jimbow et al., 1986). Where they are in contact with epithelial cells they normally transfer melanosomes to the epithelial cells and are therefore the source of the pigmentation in tissues such as hair and skin (Jimbow et al., 1986). In the hair follicle they are present in all tissue layers but they are not necessarily active in all (Montagna and Parakkal, 1974). Functional melanocytes in the hair bulb are found among the germinative cells at the apex of the dermal papilla (Staricco, 1960; Swift, 1977; Ortonne and Thivolet, 1981). Recent work has indicated that there is a reservoir of inactive melanocytes located in the middle portion of the human hair follicle and from here these cells can migrate upwards or downwards to become active melanocytes in the epidermis or in the germinative cells of the bulb, respectively (Kim et al., 1996). The active melanocytes are dendritic and DOPA positive (Tobin et al., 1995). Their products, the melanosomes, are transferred in a phagocytic type process via the melanocyte dendrites to the forming cortical cells (Birbeck et al., 1956), where they are subsequently fixed in place by the hardening of the cell contents during keratinization higher up the follicle (Swift, 1977), producing a pigmented fibre (Staricco, 1960).

Four stages can be recognized in the development of melanosomes in the melanocyte (Jimbow et al., 1986). Stage I eumelanin melanosomes are spherical vacuoles containing amorphous, proteinaceous materials and a few vesiculoglobular bodies. At stage II, the melanosomes become ellipsoidal with organized lamellae and vesiculoglobular bodies. Tyrosinase activity becomes evident at stage III and some pigmentation of the lamellae begins. The stage IV melanosome containing the final eumelanin product is oval in shape, highly melanized and electron dense (except for the vesiculoglobular bodies). Phaeomelanosomes, on the other hand, are always spherical and do not form lamellae. They contain vesiculoglobular bodies at all stages and melanization occurs both inside and outside of these (Jimbow et al., 1986, 1991). The vesiculoglobular bodies are believed to be key units of melanogenesis, but their exact role is not known (Jimbow et al., 1991).

Melanocytes in the hair follicle actively synthesize pigment only during anagen stages III through VI, based on tyrosinase activity (Fitzpatrick et al., 1958). At the end of the hair cycle, melanin formation and medulla formation stop simultaneously, producing the white, non-medullated root end of the club hair (Montagna and Parakkal, 1974). The fate of the melanocytes when the follicle growth cycle is complete and the process by which they come to produce pigment again at the next growth phase are unclear (Montagna and Parakkal, 1974; Ortonne and Prota, 1993). There is some evidence that they de-differentiate during the catagen and telogen stages, but then proliferate and differentiate during early anagen to populate the new hair bulb region (Sugiyama and Kukita, 1976). It is likely that the melanocyte reservoir recently demonstrated to be located in the middle portion of the follicle (Kim et al., 1996) plays a role in this process.
Forensic Examination of Hair

1.104 Hair Colour and its Variation

The colour of hair to an observer depends not only on the pigmentation in the hair but also on the physical properties of the hair that could affect the way light interacts with it. Thus factors such as the presence or otherwise of a medulla, and the roughness or smoothness (depending on its state of wear) of the cuticle, can modify the manner in which light is reflected and refracted by the hair shaft and thereby have an effect on the perceived colour (Hausman, 1927; Rook and Dawber, 1982). Hence the requirement in forensic comparisons for well-defined, reproducible methods for mounting the hairs and controlling the lighting during examinations.

Hair colour is under genetic control but little is known about its mode of inheritance in humans (Ortonne and Thivolet, 1981; Ortonne and Prota, 1993), although it does appear that at least four genetic loci, which are probably allelic, are involved (Ortonne and Thivolet, 1981). In dark races there is intense selection for dark hair, but in Caucasians there is no strong selection in favour of any particular colour. The inheritance of red hair appears to be dominant to its absence and hypostatic to brown and black (Wassermann, 1974). A major gene for red hair has been assigned to chromosome 4 and there is evidence that a major locus for brown hair is located on chromosome 19 (Ortonne and Prota, 1993).

Dark hair predominates in the world, with black and dark brown hair generally associated with the dark-skinned races (Wassermann, 1974), although blondding among these people, for example the Australian aborigines, is not unknown (Abbie and Adey, 1953). The main races with blond and red hair are the Caucasians of NW Europe (Wassermann, 1974). There have been suggestions that it is possible to determine the racial origin of an unknown hair from a microscopical examination of its pigmentation (see Seta et al. (1988) for a discussion) but there is so much variation within individuals that this is generally not possible (Hausman, 1927).

Like skin, hair colour varies according to body site. According to Wassermann (1974) genital hair is usually lighter than scalp hair and often a reddish tint will be seen even in brown pubic hair. In fair-haired individuals, however, the pubic hair, axillary hair, eyebrows and eyelashes are much darker than the scalp hair (Rook and Dawber, 1982).

The colour of hair also varies with age (Wassermann, 1974). The fair hair of some children will gradually darken as they grow up, and may eventually become brown. Later in life grey hair develops. The grey colour is usually a result of a mix of coloured hairs and white (non-pigmented) hairs, although sometimes individual ‘grey’ hairs with reduced pigmentation are found (Ortonne and Thivolet, 1981). Greying or whitening of the hair is known as canities and occurs in all persons to some extent. It is usually irreversible. The age of onset is variable and hereditary to some extent (Rook and Dawber, 1982). In Caucasians white hair first appears at the age of 34.2 ± 8.6 years (Rook and Dawber, 1982). About 50 per cent of (Australian) people are at least 50 per cent grey at 50 years old, irrespective of sex or hair colour (Keogh and Walsh, 1965). In Negroes the onset occurs on average about ten years later than in Caucasians (Rook and Dawber, 1982). Onset is often noticed earlier in dark-haired individuals because of the greater contrast between the dark and the white hairs. The beard and moustache areas commonly become grey before scalp or body hair. On the scalp, the temples usually show greying first (Rook and Dawber, 1982). White hair has no melanin pigment (Montagna and Parakkal, 1974) and the white colour seen is the result of the light interacting with the keratin protein and the physical structure of the hair. It is important to recognize that visible existing hairs do not change colour in the greying process. Rather, the process can occur only by reducing, or ceasing, the pigmentation in growing hairs while they are still being formed in the follicle, or by the production in subsequent hair cycles of hairs of reduced or no pigmentation (Montagna and Parakkal, 1974). The explanations, therefore, of stories of hair going grey overnight or going white due to worry usually lie in the sudden or rapid loss of dark hairs (see section 1.9.3), thus making the white hairs more visible. Likewise, reports of returning colour most likely result from the growth of new pigmented hairs (Rook and Dawber, 1982). The cellular processes which cause greying have not been elucidated as yet but are the subject of various lines of research. Animal studies suggest that heredity is a significant factor (Ortonne and Prota, 1993).
A number of disorders, some of them also hereditary, produce localized patches of white hair without the onset of greying (Rook and Dawber, 1982), and the possibility of such a situation occurring should be kept in mind when conducting forensic comparisons. Other clinical conditions can lead to more general colour changes. Menkes kinky hair syndrome, an X-linked recessive inherited progressive brain disease, causes (among other defects) the hair to be a generally lighter colour. The lightening of colour is the result of impaired copper absorption which consequently reduces the activity of follicular tyrosinase, for which it acts as a coenzyme, and this affects melanin production (Comaish, 1981). In kwashiorkor, a severe protein-calorie deficiency, the hair is also lightened and may become golden, blond, rusty or light brown, in general or in patches (Desai et al., 1981). These colour changes can be used diagnostically but they are not necessarily seen in all cases (Bradfield, 1981).

1.11 Glossary

‘A’ layer: a narrow layer on the outer edge of the exocuticle of cuticle cells.

Alopecia: a general term for loss of hair or baldness.

Alopecia areata: a form of alopecia in which hair is lost in one or more patches.

Alopecia diffusa: diffuse, generalized loss of scalp hair as may occur as a result of fever, malnutrition, anaemia, glandular problems or the use of drugs.

Alopecia, male pattern: the term sometimes used to denote common baldness, the condition of degeneration and loss of scalp hair seen to some extent in most people of older years. It is a condition which is initiated in the scalp by the action of androgens and is also known as androgenic alopecia.

Alopecia, traction: loss of hair as may arise from the strong and sustained or repeated tension or traction on hair follicles. It usually occurs as the result of hair styles or hair treatments.

Anagen: the active growing phase of the hair follicle. (Greek: aner = up, genesis = mode of formation.)

Androgens: steroid hormones, including testosterone and androstenedione, which are required for the normal development of secondary sexual hair (pubic, axillary, body, and facial hair in males) and the initiation of common balding.

Apocrine gland: a type of sweat gland of uncertain function which forms with some follicles.

Arrector pili muscle: (also erector pili muscle) smooth muscle attached to the posterior side of the hair follicle and which runs up at an angle to the papillary layer of the dermis. Contraction of the muscles due to cold or fright produces ‘gooseflesh’.

Bulge (the): a bulge which develops (in the outer root sheath) on the side of the hair follicle during development and which becomes the site of attachment for the arrector pili muscle. It marks the lowest point of the permanent part of the follicle and contains the stem cells which divide to start the next anagen phase.

Canities: the term used for the greying or whitening of hair.

Catagen: the transitional phase of the hair cycle, between anagen and telogen, when the hair stops growing and the follicle starts to decrease in size. (Greek: cata = down, genesis = mode of formation.)

CMC (cell membrane complex): intercellular contacts that cement the cortical cells together and contribute to the tensile strength of the hair.

Cortex: the main keratin-containing cellular layer of the hair shaft.

Critical level: roughly the widest part of the hair follicle bulb, below which most of the cell division occurs.

Cuticle: the outer cell layer of the hair shaft, sometimes also referred to as the hair scales.

Cycle (hair growth): the repeated growing (anagen), transition (catagen) and resting (telogen) phases of the hair follicle.

Dendrite: a branching cytoplasmic projection of a cell.
Dermal papilla: a projection of dermal tissue into the base of the hair follicle bulb. It controls the physical characteristics of the hair as well as hair growth and is the location of the androgen receptor necessary for the development of sexual hair at puberty.

Dermis: the true skin. It lies beneath the epidermis and contains hair follicles, sebaceous glands, blood vessels, nerves, muscles and so forth. Its basic structure is collagen and other elastic tissue.

Endocuticle: the layer on the inner side of cuticle cells.

Epicuticle: a hydrophobic membranous layer which completely surrounds each cuticle cell.

Epidermis: the outer protective layer of the skin.

Exocuticle: the layer on the outer side of cuticle cells.

Exons: the coding segments of a gene, separated by introns, but which together are transcribed into mRNA which is translated into protein.

Follicle: an invagination of the epidermis which contains the root of the hair.

Fusi (cortical): small spindle-shaped air inclusions that sometimes form between the cortical cells of the hair shaft.

Gene: the unit of inherited material. It is a region of the DNA that produces a functional RNA molecule.

Germinative cells: the dividing cells in the hair bulb which give rise to the various cellular layers of the follicle and hair fibre. They have been known in the past as bulb matrix cells.

Glabrous: free from hair.

Henle layer: the outermost layer of the inner root sheath of the hair follicle.

Hirsutism: excessive growth of coarse terminal body hair partly or wholly in the male sexual pattern.

Huxley layer: the middle layer of cells of the inner root sheath, on the inside of the Henle layer.

Hypertrichosis: the growth of hair in any localized or extensive pattern which is excessive for the site and the age and sex of the subject.

Imbricate: arranged so as to overlap like tiles on a roof.

Infundibulum: the region of the hair follicle between the isthmus and the level of the epidermis.

Inner root sheath (IRS): a sheath of three layers of cells (IRS cuticle, Huxley layer, Henle layer) which surrounds the hair follicle and contributes to the shaping of the hair shaft and to holding the hair in the follicle. The IRS never appears above skin level.

Intermediate filaments (IFs): microfibrils of keratin protein which combine to form the keratin macrofibrils that fill the cortical cells of the hair fibre.

Introns: intervening segments that separate the coding segments (exons) of a gene. They are of unknown function and although transcribed, they do not result in functional mRNA.

Isthmus: the part of the hair follicle between the bulge and the sebaceous gland duct.

Keratin: the complex mixture of specialized proteins produced in epidermal appendages such as hair, horn, nails, claw, scales and feathers. The proteins are characteristically rich in sulphur and are highly insoluble. (Greek: keras = horn.)

Keratin-associated proteins (KAPs): a matrix of sulphur-rich proteins in which the keratin intermediate filaments (IFs) are embedded.

Lanugo: soft, fine downy hair which covers the foetus from about the fifth to the seventh or eighth month of gestation. It is replaced by vellus hairs just before birth.

Matrix: a term used for the sulphur-rich proteins in which the keratin intermediate filaments (IFs) are embedded. It has also been used in the past to describe the dividing cells of the follicle bulb; these cells are now more correctly called germinative cells.

Medulla: the central core of cells present in some terminal hairs.
Melanin: the type of pigments in hairs. The two main types are eumelanin (dark brown to black) and phaeomelanin (reddish yellow).

Melanocytes: dendritic cells in which melanosomes are formed and delivered in the follicle to the developing hair.

Melanosomes: granules which contain the melanin pigment. Also called pigment granules.

Mitosis: the usual process of cell reproduction involving nuclear and cytoplasmic division.

Orthocortex: the part of the cortex on the outer side of the curve of the crimp and in which the keratin IFs are inclined to the cortical cell axis and entwined in a rope-like fashion.

Outer root sheath (ORS): the outermost layer of the hair follicle. It is continuous with layers of the epidermis.

Palmar: relating to the palm, the inner surface of the hand.

Paracortex: that part of the cortex on the inner side of the curve of the crimp and in which the keratin IFs are arranged mainly in quasi-hexagonal close-packing within the macrofibrils.

Pilosebaceous unit: a term used to denote the combination of a hair follicle and a sebaceous gland.

Plantar: pertaining to the sole of the foot.

Primary hair: the terminal hair that replaces lanugo hair at the scalp, eyebrows and eyelashes just prior to birth.

Scale pattern: the pattern formed by the edges of the scale (cuticle) cells on the surface of the hair shaft.

Sebaceous gland: glands of the skin which may be attached to a follicle by a duct. They produce sebum.

Sebum: the product of the sebaceous glands. The main components are squalene, wax esters and triglycerides.

Sexual hair: terminal hair which develops at puberty and replaces vellus hair at the pubis and axillae in both sexes and on the face of males. Also called secondary hair.

Stem cells: cells which divide to regenerate the follicle at anagen.

Telogen: the resting phase of the hair cycle, following catagen. This is the stage when hairs are most likely to be naturally shed. Telogen ends when a new anagen phase begins the formation of a new hair. (Greek: telos = end, genesis = mode of formation.)

Terminal hair: long, pigmented coarse hairs, sometimes with a medulla, which are the final differentiation state for hairs at the particular site (such as the scalp).

Testosterone: the principal male androgen hormone, necessary for the development of sexual hair in males and the development of common baldness.

Trichohyalin: a major protein product of medulla and inner root sheath cells. It is low in sulphur but contains citrulline and is highly cross-linked. (Greek: thrix = hair, hyalos = glass.)

Vellus: soft, fine unmedullated and unpigmented hair which replaces lanugo hair just before birth.

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PHYSIOLOGY AND GROWTH OF HUMAN HAIR


Forensic and Microscopic Examination of Human Hair

JAMES ROBERTSON

2.1 Setting the Scene

I first became interested in the forensic aspects of hair examination in the mid-1970s and first published an article on hairs in 1982 (see Robertson, 1982). It was an exciting period to be a hair examiner, with hair examination experiencing a minor renaissance. A new generation of bright-eyed youngsters, and a few not-so-young scientists like Stuart Kind (apologies to Stuart, but I am sure he won’t mind in the least!) were experiencing ‘reinvention’ and ‘rediscovery’; just maybe there was some value in examining hairs. The work of Barry Gaudette (Gaudette and Keeping, 1974; Gaudette, 1978a) had certainly provided the catalyst for that renewed interest, and along with the studies on the analysis of hair proteins there seemed the very real prospect that we could really advance the cause of hair examination.

There were meetings of a Committee on Forensic Hair Comparison and the formation of a world society of hair examiners—I even have a photograph of its formation at the Oxford meeting of the International Association of Forensic Sciences (IAFS) in 1984!

So what has happened in the years since then, and did anything substantial and lasting emerge from that renaissance? Sadly, I would have to say that little of the promise of the 1980s was realized. For example, protein analysis, in the forensic context, proved to be of academic interest and was not adopted at an operational level. Any flickering ember of interest in protein analysis has died as DNA analysis becomes a reality. The debate aroused by the work of Gaudette has waxed and waned over the years (see Chapter 7).

Following recent rulings in the United States in light of the application of so-called Daubert standards, there may be renewed interest in the studies of Gaudette. Specifically, the case of Williamson v. Reynolds from the US District Court in Oklahoma is of interest to hair examiners. I shall return to the issues raised by this case.

And yet hair examination is about to enter a new and exciting era, as finally the ‘Holy Grail’ of hair examiners (or at least of lawyers and the Courts)—being able to put a statistical frequency estimate on the value of a hair ‘match’—is within our grasp through the analysis of nuclear and/or mitochondrial DNA (mt DNA). Where now for microscopic examination?

In 1982 I contrasted the views on the value of hair examination held on the two sides of the Atlantic. Today, little, if anything, has changed. For those who believe hair examination is a powerful tool mt DNA may arguably even result in less useful information, at least in the immediate future where there is still some uncertainty as to how discriminating mt DNA will be for hairs. For those who viewed hair examination as having only a limited capacity to differentiate, mt DNA, whatever its eventual proven capacity, will be that ‘Holy Grail’. Indeed, some have suggested that there will be no place for the microscope.
In this chapter my aim is to review microscopic examination looking at protocols, individual microscopic features and the process of hair examination and comparison. This has to be evaluated against the changed paradigm imposed by emerging DNA technologies. Other chapters of this volume deal with significant aspects of hair examination, past, current and the future. This chapter will serve as the binder or glue to fill gaps and provide the skeleton, the framework, based on microscopic examination providing the foundation on which other complementary approaches fit.

As Seta et al. (1988) put it, ‘morphological examination should always be considered as the first step of forensic hair comparison...analytical examinations can now be expected to give useful information...thus, a combination of morphological and analytical data can lead to the enhancement of identification probability’.

2.2 Introduction

This book is concerned with the examination of human hair, and hence only a limited treatment of non-human, animal hairs will be given. The examination of human hairs is, nevertheless, only a specialized example of the general examination of hairs. Indeed, the first question which must be considered in any hair examination is whether the hair is of human or non-human origin. This is normally quite straightforward. A knowledge of the basic structure and morphology of hairs is assumed. This is the subject of Chapter 1.

2.3 Non-human Hair

Hairs are composed of three anatomical regions: the cuticle, the cortex and, when present, the medulla. The attempted identification of non-human hairs requires that the fine detail associated with these regions be studied in a systematic way.

With non-human hairs, different types of hair can be present in the fur or peltage. Usually these are visually clearly different, based on their degree of coarseness. While different classification systems exist, the most commonly found types are guard- and under-hairs. Guard-hairs are longer and coarser than the under-hairs, which are usually fine. Guard-hairs also display the widest range of microscopic features, which makes them the most useful for identification. Both guard- and under-hairs should be examined separately if present. Table 2.1 presents the major differences.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Human</th>
<th>Non-human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Relatively consistent along shaft</td>
<td>Often showing profound colour changes and banding</td>
</tr>
<tr>
<td>Cortex</td>
<td>Occupying most of width of shaft – greater than medulla</td>
<td>Usually less than width of medulla</td>
</tr>
<tr>
<td>Distribution of pigment</td>
<td>Even, slightly more towards cuticle</td>
<td>Central or denser towards medulla</td>
</tr>
<tr>
<td>Medulla</td>
<td>Less than one-third width of shaft. Amorphous, mostly not continuous when present</td>
<td>Greater than one-third width of shaft. Continuous, often varying in appearance along shaft, defined structure</td>
</tr>
<tr>
<td>Scales</td>
<td>Imbricate similar along shaft from root to tip</td>
<td>Often showing variation in structure along shaft from root to tip</td>
</tr>
</tbody>
</table>

Table 2.1 Comparison of features of human and non-human hair
Forensic and Microscopic Examination of Human Hair

Table 2.2 Features that should be assessed for animal hairs (from Robertson, 1995)

1. **Profile** (general shape)
   Shield, straight, symmetrically thickened or wavy.

2. **Cuticle or scale features**
   - Scale margin: smooth; crenate (sharp pointed teeth); rippled (indentations deeper than crenate and rounded); or scalloped ( margins with broad rounded teeth).
   - Distance between scales: close; near; or distant.
   - Scale pattern: mosaic (either regular or irregular); wave (simple regular, interrupted regular, streaked, irregular waved, mosaic or regular waved mosaic); chevron (single or double); pectinate (coarse or lanceolate); or petal (irregular or diamond).

3. **Medulla**: note whether present or absent. Where present, it may be continuous, interrupted or fragmented.
   In non-human hairs, it is often continuous with a defined structure. The structure can be of two main classes, ladder or lattice.
   - A ladder medulla is so called because it looks like the rungs of a ladder. Where there is a single row of ‘rungs’, this is a uniseriate ladder; with several rows, a multiseriate ladder.
   - A lattice medulla is so called because it has the appearance of a lattice made up of ‘struts’ of keratin which outline polyhedral-shaped spaces, each of which is continuous with its neighbours.
   A special type of lattice medulla, an aeriform lattice, differs in that the shapes giving the appearance of the lattice have arisen from cell collapse leaving air-filled gaps which are roughly polyhedral in shape.

4. **Colour**: the colour of a hair results from pigment particles deposited in the cortex. Overall, visual and macroscopic colour are important in non-human hair identification, with the detail of pigmentation in the cortex being less important than in human hair. Pigment should be assessed with respect to: amount (sparse or dense); and distribution (along the shaft (even) and across the shaft (denser near centre; denser near cuticle)).

5. **Cross-section**: sectioning is not always carried out because it is destructive. Information that can be gained from cross-sections is three-fold.
   - Good appreciation of pigment distribution across the shaft.
   - The position of the medulla, which can be in the middle (centric) or off to one side (eccentric).
   - The shape of the hair.
   However, these features can also be assessed by optical sectioning when hairs are viewed in a longitudinal plane.

between human and non-human hairs. Table 2.2 gives the features that should be assessed for animal hairs.

Non-human animal hairs should be examined from their root end to their tip end, and variation in the features given in Table 2.2 recorded. In particular, changes in medulla and scale pattern appear to be consistent enough to have value in identification.

It is helpful to use a checklist to record features; this also encourages systematic observation. An example of such a checklist is presented as Appendix 1. Where possible, questioned animal fibres should always be compared with authenticated standards.

Many scientific papers contain descriptions of the microscopy of animal hairs, but few are of real assistance. Usually, they deal with only a very small group of species and take into consideration only those features which have most value in discriminating the members of that group. Often the descriptions also assume that the scientist has a bulk sample, whereas in forensic work each hair often has to be
treated separately. Thus, the identification of all but the more common hairs is often not possible and analysis becomes comparative.

Some examples of common animal hair descriptions follow.

### 2.3.1 Common Animal Hairs

#### Wool-type Fibres

Wool fibres come from different breeds of sheep. Fibres from other animals such as various breeds of goat and llama are also referred to as wool. While an experienced wool grader can distinguish different breeds of sheep by examining unprocessed bulk samples, the identification of breed from single fibres or even very small samples is beyond the expertise of the forensic examiner. Once wool has been processed, there is no accurate way of identifying the breed. Raw wool fibres are classed into four groups based on the degree of coarseness: kemp, outercoat, coarse and fine. In textile end-products, usually only coarse and fine fibres can be recognized. The only features readily assessed are the presence or absence of medulla and scale features. Other fibres are used in similar end-product uses, particularly goat hairs. The scale features of fine wool and goat hairs are compared in Table 2.3.

The most distinguishing feature of wool is its prominent scale margin. Unequivocal identification of fibres as wool is not possible, especially when the fibres transferred during contact situations have indistinct or damaged scales. For this reason, it is safer to refer to them as ‘wool-type fibres’.

#### Cat and Dog Hairs

There is a wide variation in colour, length and profile in hairs from cat and dog breeds. General class characteristics are given in Table 2.4. It must be stressed that these are general characteristics, and there is a great deal of variation within and between cat and dog hairs. As previously stated, the progression of features along the hair shaft provides useful information and, according to Peabody et al. (1983), the following scale progression found in cats does not occur in dog hairs. Dogs show a wider variation in scale pattern progression.

1. **Root**
   - Irregular mosaic: smooth scale margins.
   - Petal: smooth scale margins.
   - Regular mosaic: smooth scale margins.
2. **Tip**
   - Regular mosaic: rough scale margins.

Two further features can be of value in discriminating cat and dog hairs.

<table>
<thead>
<tr>
<th>Table 2.3 Comparison of the scale features of wool and goat hairs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hair source</strong></td>
</tr>
<tr>
<td>Sheep</td>
</tr>
<tr>
<td>Angora goat mohair</td>
</tr>
<tr>
<td>Cashmere goat</td>
</tr>
</tbody>
</table>

* The two features described are the distance between scales and their appearance, respectively.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Whole mount</th>
<th>Cross-section</th>
<th>Scale pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Profile</td>
<td>Medulla</td>
<td>Contour</td>
</tr>
<tr>
<td>Cat</td>
<td>Fine</td>
<td>Ladder</td>
<td>Circular to oval, some angular</td>
</tr>
<tr>
<td>&lt;i&gt;Felis ocreata&lt;/i&gt;</td>
<td>Regular diameter, scale margins fairly prominent</td>
<td>Sparse to dense, even</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td>Continuous fine lattice</td>
<td>Circular to oval</td>
</tr>
<tr>
<td>&lt;i&gt;Canis familiaris&lt;/i&gt;</td>
<td>Regular diameter, scale margins fairly prominent</td>
<td>Sparse to dense, even</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Continuous, sometimes ladder</td>
<td>Varies from none to dense, even or streaky</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Varies from none to dense, even to streaky</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. **Root shape**
   - Cat: elongated, no distinct shape, fibrils often frayed at base of root.
   - Dog: spade- or arrow-shaped.

4. **Medullary index**

A graph of medulla width against hair width shows that the medullary index for cat hairs is, in general, higher than for dog hairs. Not all cat and dog hairs can be correctly identified, but the error rate is small (see Figure 2.1).

### 2.4 Human Hair

#### 2.4.1 Macroscopic Features

In the forensic examination of hairs it is important to start with visual examination followed by macroscopic examination of the morphology of individual hairs. In section 2.5, protocols for the examination of hairs are considered in detail. A complete hair will have a root portion and a shaft portion. A full examination at magnifications of ×10 to ×40 and up to ×60–×100 with incident illumination will enable the observer to record the overall shape of the hair, whether or not a root is present and, where it is present, its shape and appearance, the basic features of the shaft, the appearance of the terminal portion and any gross unusual damage, disease or extraneous features.

#### Hair Length

The length of both known (from an identified person) and recovered (questioned) hairs should invariably be recorded. Hair length may be recorded as actual lengths (Robertson, 1982) or into size categories (Bisbing, 1982; Gaudette and Keeping, 1974; Lee and De Forest, 1984; Strauss,
With a known sample the length of a number of hairs should be measured. Length should be measured where possible by straightening the hair. This can be difficult in practice with tightly curled hair of Negroid origin. Generally, for a meaningful forensic comparison the questioned hair should be within the range of the known hair sample.

Where only a fragment or segment of hair shaft is recovered, the value of a comparison will usually be limited because it will not be possible to compare microscopic features along the entire hair shaft. It would be expected that the variation shown in microscopic features, or the pattern, would be easier to compare in hairs of similar length. In my view it is possible to overstate the importance of comparing hairs of equal length. It is more important that the hairs to be compared show the same overall variation from root end to tip end when examined at a macroscopic level. A meaningful comparison may well be possible with two hairs which vary significantly in length. Conversely, the fact that two hairs are the same length means little. As Gaudette and Keeping (1974) have stated, hair length can be either a major characteristic or a minor characteristic depending on the types exhibited. In my experience the major problem with known hair samples is that the person taking the sample does not consider sufficiently the importance of ensuring that the sample is representative. The tendency with scalp hair is for one site to be sampled. At best a very small number of sites are sampled, and these usually do not include the ‘fringes’ of the scalp. It is the fringes which often show the greatest variation in length and form. The sample should also be relevant to the circumstances of the particular case. For example, where hairs have been left in a vehicle windscreen following an accident, the known hair sample should be taken from as near as possible to any scalp injury from an injured party or from an equivalent area of the scalp or facial hair. Hairs transferred to the inside of a mask or balaclava worn in a robbery may be from different areas of the scalp, or there may be facial hairs. It may be necessary to take a number of hair samples from one individual.

Also, the known sample should be taken at, or as near as possible to, the time of the incident. Hair grows at variable rates depending on a range of factors. As a rough guide, scalp hair grows at about one centimetre per month. A suspect may have had his or her hair cut or cosmetically treated between the commission of an offence and his or her apprehension. It is not unknown for a person to have had their scalp hair removed altogether.

While it should be obvious that it is only possible to compare hairs from the same body origin or location, regrettably it is not uncommon to be asked to examine body hairs or facial hairs and to be supplied with only a scalp hair sample.

In conclusion, the length of recovered hairs, and of a representative number of known hairs, should always be measured. Hair length will usually help to determine the possible body origin or location of a questioned hair. It should not be assumed that two hairs must be of equal length for a meaningful comparison to be undertaken. However, where two hairs to be compared are of significantly different lengths the onus lies on the examiner to explain on what basis it is possible to compare the hairs, and to be aware of potential and real limitations when considering what proper conclusion can be reached.

Hair Shape

Hicks (1977) has listed the general features which can assist in determining body area determination and racial origin—see Tables 2.5 and 2.6. The content of these tables is considered under ‘Hair diameter’ in section 2.4.2. At this stage it is sufficient to point out that the shape and form of the hair is a significant factor in determining body area and racial origin.

Robertson and Aitken (1986) classify shaft profile as being straight, wavy, curly and peppercorn. Seta et al. (1988) have tabulated common terms used to describe general hair form—see Table 2.7. For practical purposes I have found it useful to add curved to my classification. Bailey (personal communication) has proposed the use of a numerical average curvature measure as having value.

In conclusion, hair shape or form should always be recorded. Hair shape will assist in determining body and racial origin although it must be emphasized that caution needs to be exercised in making these determinations. Hair shape has quite limited discriminating power within a single hair
Forensic Examination of Hair

Chapter 1 describes in detail the structure of the hair follicle and the hair growth cycle which result in the structure of human hair roots as seen in forensic examinations. Classically, the hair growth cycle in humans is not cyclical but rather is a continuous process. The average human has in the order of 100,000 hairs, of which about 90 per cent have follicles which are in the growing phase, called *anagen*. Only a very small percentage of hair follicles are in *catagen* growth phase, and about 10 per cent are in the mature resting or *telogen* phase.

Table 2.5 Body area determination (from Hicks, 1977)

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalp hairs</td>
<td>(a) Long with moderate shaft diameter variation.</td>
</tr>
<tr>
<td></td>
<td>(b) Medulla absent to continuous and relatively narrow when compared with its structure in hairs from other body areas.</td>
</tr>
<tr>
<td></td>
<td>(c) Often with cut or split tips.</td>
</tr>
<tr>
<td></td>
<td>(d) May show artificial treatment, solar bleaching, or mechanical damage such as is caused by back-combing.</td>
</tr>
<tr>
<td></td>
<td>(e) Soft texture (pliable).</td>
</tr>
</tbody>
</table>

| Pubic hairs      | (a) Shaft diameter coarse, with wide variations and ‘buckling’.              |
|                  | (b) Medulla relatively broad and usually continuous when present.           |
|                  | (c) Root frequently with follicular tags.                                   |
|                  | (d) Tip usually rounded or abraded.                                         |
|                  | (e) Stiff texture (wiry).                                                   |

| Limb hairs (arm or leg) | (a) Diameter fine with little variation.                                   |
|                        | (b) Gross appearance of hair is arc-like in shape.                        |
|                        | (c) Medulla is broad, discontinuous and with a granular appearance.       |
|                        | (d) Soft texture.                                                         |

| Beard or moustache hairs | (a) Diameter very coarse with irregular or triangular cross-sectional shape. |
|                         | (b) Medulla very broad and continuous.                                     |

| Chest hairs            | (a) Shaft diameter moderate and variable.                                   |
|                        | (b) Tip long and fine, arc-like.                                            |
|                        | (c) Stiff texture.                                                         |

| Auxiliary or underarm hairs | (a) Resemble pubic hairs in general appearance.                             |
|                            | (b) Diameter moderate and variable, with less ‘buckling’ than pubic hairs. |
|                            | (c) Tips long and fine.                                                    |
|                            | (d) Frequently with bleached appearance.                                   |

| Other                     | (a) Eyebrow: some fluctuation. Sabre-like (fusiform) in appearance.        |
|                          | (b) Eyelash: short, stubby with little shaft diameter fluctuation. Sabre-like (fusiform) in appearance. |
|                          | (c) Trunk: a combination of features of limb and pubic hairs. A ‘transitional’ hair. |

In general, a questioned hair should fall into the range of recorded types for a known sample if an inclusionary comparison opinion is to be offered.
A number of authors have estimated the expected daily loss of scalp hairs to be in the order of 100–150 hairs. This is based on hairs remaining in the telogen phase for about 100 days (see Chapter 1). As noted in Chapter 1, it is a misconception that mature hairs fall out. It is more accurate to state that mostly they are removed by force, but the degree of force required is that used during normal grooming, brushing and washing. Hairs lost at the telogen stage will make up the bulk of so-called naturally shed hair.

Estimates for the length of the anagen growth phase vary widely, from a matter of weeks to a number of years. This may help to explain why it is possible for human hair to reach lengths of over one metre (Robbins, 1988).

The microscopic appearance of the roots of hairs in anagen and telogen growth phases are characteristic. While the appearance of anagen roots is a little variable, most often they present as a flattened, ribbon-like structure, often with pigmentation present. Catagen roots are usually club-shaped and sometimes have an epithelial sac attached (King et al., 1982).

Anagen hairs do not fall out in the normal course of events and require some use of force to detach them from the dermal papilla. Anagen hairs will sometimes have cellular sheath material attached, but this is not invariably the case. The presence or absence of sheath material appears to
be a characteristic as much of the individual as of the degree of force required to remove the hair (King et al., 1982). It is generally considered that 95 per cent of hairs found in forensic casework will be telogen hairs. These are classically referred to as having club roots.

In the scheme of Robertson and Aitken (1986), the following four features are recorded for the appearance of the root end of the hair (see Figure 2.2):

- absent
- club (bulbous)
- ribbon shape
- sheath present.

Seta et al. (1988) list a number of other schemes for describing hair roots, as detailed in Table 2.8.

Finally, in forensic work hairs from dead bodies are sometimes examined. Seta et al. (1988) have shown that the root end displays a number of post mortem changes three to four days after death. Typically, these include a narrowing of the hair shaft immediately behind the root, internal cell disruption and the root contracting, and sometimes exposure of cortical fibres. A typical atrophied hair may also take on a yellow colouration near the root.

**Tip Appearance**

The appearance of the tip end of the hair shaft may be the result of normal wear of a naturally tapering hair, the result of intervention in the form of hair treatment or the result of damage or an external event such as exposure to heat.
In a newly formed hair the tip end tapers as it emerges from the scalp. It will usually have low pigment density and be non-medullated. In body hairs the tip will become rounded or may show evidence of damage, depending on the habits of the ‘donor’.

Occasionally, body and pubic hair will be cut, and where this is the case it may be a useful forensic feature.

It is with scalp hairs that the widest range of features is usually encountered in forensic casework. As with hair root appearance, a number of authors have reported on schemes used to classify tip appearance. Seta et al. (1988) summarize these as shown in Table 2.9.

These schemes are reasonably consistent, using a range of descriptors to record the physical appearance of the tip. Most include tapered, rounded, frayed, split, various descriptors for cut hairs and a range of other descriptors for various forms of wear and damage. Robertson and Aitken (1986) use the following descriptors for hair tip appearance:

- natural taper
- cut
- rounded
- frayed or abraided
- split
- crushed or broken
- singed.
Examples of some of the above are shown in Figure 2.3.

It has been stated that it is possible to estimate the time since a hair has been cut. This is based on the fact that the edges of a ‘sharp cut’ gradually become rounded with the passage of time (Niyogi, 1962).

With careful examination it may be possible to determine whether or not a particular type of implement has been used to cause damage to a hair or hairs. Some physical crushing can be quite characteristic. Trial experiments are often useful where one tries to simulate a case scenario.

A special case is where hair has been exposed to high temperatures such as in a fire. Some hairs may become blackened and obviously singed or burnt. In some hairs what is left of the tip may have a severely disrupted internal structure in which dark air bubbles are obvious.

**Macroscopic Assessment of Colour**

The colour of a hair as observed at a macroscopic level depends on the pigmentation in the hair shaft and on the physical properties of the hair that affect the interaction of light with the hair (see Chapter 1).
Figure 2.3 Appearance of the tip end of the hair: (a) cut; (b) rounded; (c) frayed (d) split; (e) broken; (f) crushed

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For forensic examinations and comparisons it is essential that colour be assessed under reproducible conditions. My preference is to use a ring light source rather than oblique lighting. The challenge for the forensic hair examiner is that while there are only three basic colours—black, red and blond—in reality hair colour, especially in Caucasians, shows an almost continuous spectrum as a result of small variations in hue and shade, the presence or absence of medulla and, as stated above, the perception by the observer resulting from the interaction of light with the hair. Within an individual, hairs will show a degree of variation, but this is often much smaller than the variation between individuals. Hair colour is known to change with age. This may be the gradual darkening of hairs as a child moves into adulthood, or the development of ‘grey’ hairs to a greater or lesser extent as people age. It is important to note that the overall perception of greyness is the result of a mixture of pigmented hairs and non-pigmented hairs. In fact, ‘grey’ hairs may be entirely devoid of pigment or have severely reduced pigmentation. A number of disease conditions may also result in either localized patches of non-pigmented hairs or an overall change in colour. Chapter 1 discusses these factors in some detail.

Hair colour can also vary within an individual in hairs of different body origin. This can often be seen at its most dramatic at the interface of scalp hair and facial hair. I have seen quite startling changes in the area of ‘sideburns’, where the facial hair has a distinct red colour with the scalp hair being brown. This again demonstrates the importance of representative sampling.

It is important that the forensic examiner record hair colour using an analytical and systematic approach, as hair colour is one of the most useful features to assist in the discrimination of hairs at a macroscopic level. A number of classification schemes are presented in Table 2.10.

It is interesting to note that more recent schemes have tended to define only a limited number of categories using basic colours and some method of shade or intensity assessment. This contrasts with

<table>
<thead>
<tr>
<th>Classifications</th>
<th>Hair Colour Scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trotter (1939)</td>
<td>Pure black, brown black, dark brown, reddish brown, dark blond, light blond, ash blond, red, albino</td>
</tr>
<tr>
<td>Gaudette and Keeping (1974)</td>
<td>Grey, yellow, yellow-brown, red, brown, black</td>
</tr>
<tr>
<td>Bisbing (1982)</td>
<td>White, red, auburn, blond, light brown, brown, dark brown, grey brown, grey, black</td>
</tr>
<tr>
<td>Robertson (1982)</td>
<td>Colourless/translucent, yellow-brown, yellow-red, reddish brown, light brown, mid brown, dark brown, greyish brown, black</td>
</tr>
<tr>
<td>McCrone (1982)</td>
<td>Grey, blond, red, brown, black</td>
</tr>
<tr>
<td>Strauss (1983)</td>
<td>Colour</td>
</tr>
<tr>
<td>Harding and Rogers (1984)</td>
<td>Colour</td>
</tr>
<tr>
<td>Lee and De Forest (1984)</td>
<td>Colour</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
</tr>
<tr>
<td></td>
<td>Distribution</td>
</tr>
</tbody>
</table>

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It is interesting to note that more recent schemes have tended to define only a limited number of categories using basic colours and some method of shade or intensity assessment. This contrasts with
the earlier work of Fisher (reported in Trotter, 1939). Here 30 samples of different hair colours were arranged in a series based on increasing colour intensity. The series was:

- grey-black, ranging from very light grey to absolute black and containing no yellow, brown or red
- yellow-brown, ranging from white-yellow to dark brown-black with many of the colours having a reddish component
- red series.

Robertson (unpublished) has developed a device for use in hair colour assessment. This consists of a microscope slide on which are mounted a series of nine small groups of dyed hairs. Known and questioned hairs can be assessed directly against these colours. The limitation of this device is that it is impossible to replicate the actual colour and variations seen in undyed hairs. Other approaches to classifying hair colour in an objective way have been described (see Seta et al., 1988). Porter and Fouweather (1975) attempted to assess hair colour using Munsell colour charts, but with very limited success.

More recently, hairs have been examined using microspectrophotometers. The conventional wisdom has been that this approach is doomed to failure, since the underlying basis for hair colour is the actual pigmentation. As only two basic pigment types—eumelanin and phaeomelanin—are responsible for hair colour, it is argued that spectra of human hairs will lack discrimination features. The use of FTIR microspectroscopic analysis of human hair followed by chemometric analysis of the spectra has been reported by Panayiotou (1998). The author concludes that this approach can discriminate single, untreated human hairs from different individuals and shows potential to identify the sex and racial origin of hairs.

The scheme I now apply has only five classes (Robertson, 1995). These are:

- colourless
- yellow
- brown
- reddish
- black.

Intensity is recorded as light, mid or dark. Colour is assessed along the length of individual hairs. Where a hair may show, for example, a basic brown hue but with a reddish tinge, it would be recorded as brown with a downward arrow to reddish. Although grey is not included as a category, in my experience many hairs have a grey-brown or grey-black hue. In these instances, a small ‘g’ is added to indicate a grey hue.

Similarly, no category is included for artificially coloured hair. Often, but not always, the presence of artificial colouring can be readily seen at the macroscopic level. In instances where the natural colour of the hair is either a pale shade or colourless, a profound colour change is seen with a marked boundary (see Figure 2.4). This implies there has been some regrowth in the time between when the hair was dyed and when it was examined. With permanent colours, the dye is taken up into the cortex of the hair shaft and the cuticle will not necessarily be coloured. With semi-permanent and short-lasting colourants the colour is mainly deposited on the surface of the hair, and microscopic examination may show the cuticle to be coloured. As this type of treatment is short-term, there is much less chance of seeing a boundary between the treated and untreated areas. Indeed, with quite subtle colourants it may be difficult to detect treatment. Cosmetic treatment of hairs is considered in detail in Chapter 6.

**Disease Condition**

This topic is considered in detail by Seta et al. (1988) in which reference is made to the book by Rook and Dawber (1982). Only a summary is presented here; the reader is referred to the above reference for a more in-depth treatment.
Figure 2.4 Appearance of dyed hairs: (a) profound change in colour; (b) uniform unnatural colour—note that cuticle is coloured
Table 2.11 Relationship between diseases and hair abnormality (from Seta et al., 1988)

<table>
<thead>
<tr>
<th>1. Hair shaft abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Structural defects of the shaft with increased fragility</td>
</tr>
<tr>
<td>(i) Beaded swelling shaft</td>
</tr>
<tr>
<td>(a) Monilethrix (including pseudo-monilethrix)</td>
</tr>
<tr>
<td>(b) Trichorrhexis invaginata (bamboo hair, Netherton’s syndrome)</td>
</tr>
<tr>
<td>(c) Trichorrhexis nodosa</td>
</tr>
<tr>
<td>(ii) Twisted shaft</td>
</tr>
<tr>
<td>(a) Pili torti</td>
</tr>
<tr>
<td>(b) Kinky hair (Menkes kinky hair syndrome)</td>
</tr>
<tr>
<td>(iii) Normal shaft</td>
</tr>
<tr>
<td>(a) Trichoschisis</td>
</tr>
<tr>
<td>(b) Trichothiodystrophy</td>
</tr>
<tr>
<td>(c) Trichoptilosis</td>
</tr>
<tr>
<td>(b) Structural defects of the shaft without increased fragility</td>
</tr>
<tr>
<td>(i) Twisted shaft</td>
</tr>
<tr>
<td>(a) Spun glass hair (uncombable hair, pili trianguli)</td>
</tr>
<tr>
<td>(b) Trichonodosis</td>
</tr>
<tr>
<td>(ii) Normal shaft</td>
</tr>
<tr>
<td>(a) Pili annulati (ringed hair)</td>
</tr>
<tr>
<td>(b) Cartilage hair hypoplasia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Adherent materials to the hair shaft</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Hair casts</td>
</tr>
<tr>
<td>(b) Trichomycosis axillaris (including Trichomycosis pubis)</td>
</tr>
<tr>
<td>(c) Louse egg (Pediculosis capitis, phthiriasis pubis)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Hair root abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Alopecia</td>
</tr>
<tr>
<td>(i) Alopecia areata</td>
</tr>
<tr>
<td>(ii) Alopecia diffusa</td>
</tr>
<tr>
<td>(iii) Alopecia pityrodes</td>
</tr>
<tr>
<td>(iv) Alopecia seborrhoicum</td>
</tr>
<tr>
<td>(b) The effect of drug and radiation on hair root morphology</td>
</tr>
</tbody>
</table>

Seta et al. (1988) have prepared a list of hair diseases and abnormalities and a schematic series of diagrams to assist in the identification of disease conditions. These are reproduced in Table 2.11 and Figure 2.5.

Where seen, these conditions should be recorded as they are powerful discriminators. In over twenty years of hair examination, I would have to confess to rarely having seen most of these disease conditions.

2.4.2 Microscopic Features

Through the systematic examination and recording of macroscopic features, it is usually possible to determine the body origin of a recovered or questioned hair and sometimes to exclude a known hair sample as being the source of a questioned or ‘crime’ hair. However, in many cases it will not be possible at the macroscopic level of examination to differentiate a questioned hair from a known sample, or a number of known samples from each other. Here it is necessary to examine hairs at the microscopic level. In all cases where the examiner seeks to be in any way ‘inclusionary’, microscopic examination followed by direct comparison is essential. This is discussed in more detail in section 2.5.2.
Examination for microscopic features must always include examination of hair shafts along their length from root end to tip end using a brightfield light microscope. Some examiners will also make cross-sections. In general, the features seen at this level of examination are associated with the cuticle, the cortex and the medulla, although information about the root and tip ends will be obtained which complements the descriptions obtained at the macroscopic level.

It is important to look not only at individual features in isolation, but also at their contribution to an overall pattern. Mostly the features to be described are those included in the checklist of Robertson and Aitken (1986) and those agreed by the 1980s Committee on Forensic Hair Comparison (CFHC) (Anon., 1985).

**Hair Diameter**

In Tables 2.5 and 2.6 it can be seen that reliance is placed on hair diameter to differentiate hairs from different body areas and also from hairs of the same body area from persons of different racial groups. For example, Caucasian scalp hair is described as having a moderate shaft diameter, Negroid scalp hair as having a moderate to fine shaft diameter with considerable variation, and Mongoloid scalp hair as having a coarse shaft diameter with little or no variation. It is important to stress that these indicators are generalities. With considerable racial admixtures it is rather simplistic to be anything other than cautious in expressing a view on racial origin, except where very
obvious features are present. It would be entirely unsafe to determine racial origin based solely on hair diameter, as can be seen from the work of Vernall (1961) and Hayashi et al. (1976); see, for example, Vernall’s findings in Table 2.12.

An index of maximum to minimum diameter gives an indication of cross-sectional shape. For example, an index of 1 would be a circle, and an index of 0.5 would be a flattened ellipse. From this index it can be seen that Chinese hairs are the closest to circular, Negroid hairs are the most flattened and Western European and Indian are intermediate in shape.

Kind (unpublished results reported in Porter and Fouweather, 1975) has shown that for Caucasian hairs there is little correlation between hair shaft diameter and the individual. Porter and Fouweather (1975) have also stated that the variation in hair diameter along an individual hair shaft is not a useful feature for hair discrimination.

What, then, can be determined by measuring hair diameter, and where along the hair shaft should the diameter be measured? Gaudette and Keeping (1974) proposed that hair diameter be classified as:

- constant diameter
- slight smooth variation in diameter
- wide smooth variation in diameter
- abrupt variation in diameter.

Shaffer (1982) defined diameter only as fine or coarse, Harding and Rogers (1984) as narrow, wide and variable, while Lee and De Forest (1984) proposed the use of three characteristics: diameter value; diameter variation—none, slight, medium and large; and mode of variation—abrupt change, gradual change.

The diameter of individual hair shafts will vary from root end to tip end as a result of the way in which the hair is produced from the human hair follicle. A complete hair will be narrower at the tip end. Whether or not the shaft diameter is narrower at the root end than the main shaft area would appear to depend on the growth stage. My own observation is that in telogen hairs the root end tends to be narrower but this is not always the situation. With anagen hairs the shaft near the root may be broader. The list of hair comparison characteristics of the CFHC recommends measuring the maximum diameter. The scheme of Robertson and Aitken (1986) also records maximum diameter, although in practice this has now been modified to include range of diameter, excluding natural taper and the shaft immediately behind the root. Clearly, any major variation in diameter should be noted. Care should be taken, especially with flattened twisting hairs from persons of Negroid origin and in some cosmetically treated hairs, not to measure diameter at points where the diameter appears narrow because the hair presents side-on. As some disease conditions result in major diameter fluctuation, profound diameter variation should alert the examiner to this possibility.

In conclusion, overall hair diameter or coarseness is at best an indicator of racial origin. Within a single hair type and especially for hairs of scalp origin, excluding profound variations in hair diameter, for which there will usually be an underlying reason, hairs from different individuals do on occasion display significant differences. It is clear that diameter is not an especially discriminating feature, but it should not be ignored as in some cases it will be a useful feature.

### Table 2.12 Diameter of scalp hairs of different racial groups (from Vernall, 1961)

<table>
<thead>
<tr>
<th>Racial group</th>
<th>Maximum (μm)</th>
<th>Minimum (μm)</th>
<th>Index (max/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian Indians</td>
<td>92.94 ± 1.93</td>
<td>66.49 ± 4.54</td>
<td>0.73</td>
</tr>
<tr>
<td>Chinese</td>
<td>94.28 ± 3.05</td>
<td>76.79 ± 2.13</td>
<td>0.83</td>
</tr>
<tr>
<td>Negroid</td>
<td>98.23 ± 2.45</td>
<td>58.52 ± 1.87</td>
<td>0.60</td>
</tr>
<tr>
<td>Western Europeans</td>
<td>81.94 ± 2.47</td>
<td>56.74 ± 1.33</td>
<td>0.71</td>
</tr>
</tbody>
</table>
Pigment Features

The macroscopic assessment of colour is considered in section 2.4.1. Porter and Fouweather (1975) observed that hair colour as perceived by the human eye was the result of ‘specular reflections from the surface of the hair’. With the aid of low magnification and incident light the overall impression or perception of colour can be assessed. The underlying structural features which contribute to that ‘perceived’ colour can also be seen. Principal among these are the presence or absence of medulla and the density and distribution of pigmentation. It is the latter which determines the hue and shade or intensity of the colour.

The pigment particles found in human hairs are the products of secretory cells or melanocytes which produce melanosomes. Inactive melanocytes are found in the middle portion of the hair follicle. Some of these migrate upwards to become active melanocytes in the hair bulb. From here melanosomes move into the forming cortical cells in a phagocytic process via melanocyte dendrites. The melanosomes finally become fixed in the cells through the subsequent hardening of the cells during the keratinization phase (Jimbow et al., 1986; see also Chapter 1).

There are two chemical forms of pigment present in the melanosomes, eumelanin and phaeomelanin. Properties of these pigments are given in Table 2.13. Chapter 1 presents further detail about melanin formation.

To gain a further insight into the mechanisms whereby pigment particles become distributed through the hair shaft, it is necessary to look more closely at the differentiation and development of the cells which make up the cortex, medulla and cuticle. The reader is referred to Chapter 1 for a detailed consideration of the development processes involved. The precursor cells of the medulla, cortex and cuticle have their origins in the hair bulb. The presumptive cells of the medulla are surrounded by those of the cortex, both in the central area of the bulb.

In the cortex, filaments become aligned with the axis of the cell and develop into bundles which form macrofilaments or macrofibrils. The pigment granules are present between these macrofibrils. Cortical cells are about 3–6 µm in diameter and 100 µm in length. With brightfield microscopy and magnifications of up to ×400, it is not usually possible to see individual cortical cells. There are exceptions in hairs in which the cortex displays so-called cortical texture. Also, when a hair is broken or becomes frayed, the cortical cells can often be seen at the broken ends (see Figure 2.3c). Severe cosmetic treatments can also result in the cortical cell outlines becoming visible.

Prior to the cortical cells becoming keratinized there are spaces between the cortical cells. Another feature of the cortex, cortical fusi, own their origins to these spaces and are the result of air spaces between cortical cells. The term ‘fusi’ appears to have first been used by Hausman (1932), although these microscopic structural elements had been known prior to this time. Cuticle cells develop from a single layer of germ tissue outside those that form the cortex.

Pigment granules are ‘deposited’ as the presumptive cortical cells move past the melanocytes, in the upper part and apex of the dermal papilla. It follows that one would not expect to find pigment granules in the medulla or cuticle. In practice, pigment can be found in both. In particular, pigment granules are sometimes seen in the cuticle of hairs from individuals with densely pigmented hairs.

The processes through which pigment granules become deposited in (mainly) the cortical cells is well established. Much is also known about the chemical composition of hair pigments, and there is some basis at a structural level for these pigments to explain why there should be differences between individuals. However, little is known of the mechanisms which result in pigment distribution between individuals showing variation.

<table>
<thead>
<tr>
<th></th>
<th>Colour</th>
<th>Shape</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eumelanin</td>
<td>Brown, black</td>
<td>Ellipsoidal, oval</td>
<td>0.35 x 1</td>
</tr>
<tr>
<td>Phaeomelanin</td>
<td>Yellow, red</td>
<td>Spherical</td>
<td>0.20 x 0.7</td>
</tr>
</tbody>
</table>

Table 2.13 Properties of eumelanin and phaeomelanin (from Swift, 1977)
The forensic hair examiner has to take a pragmatic and descriptive approach in an attempt to produce a systematic description of the pigment features. A number of authors have published classification schemes in recent years. These are given in Table 2.14. The CFHC has also published a classification scheme (Anon., 1985); this is given in Table 2.15.

The more detailed schemes attempt to classify pigment density and distribution and then focus on the individual pigment granules and how these are sometimes organized into groups, clumps or aggregates. More recent schemes have developed a greater number of descriptors for aggregates, and there now seems to be broad acceptance that it is possible to describe aggregates in terms of being streaked, in round clumps or in oval clumps.

Features which attempt to describe the size of the pigment granules or aggregates have not been defined in any absolute or quantitative sense. It was the intention of the CFHC to produce an Atlas of Hair Comparison Characteristics which would include descriptions of colour photomicrographs. Regrettably, this laudable aim was never achieved.

A number of laboratories have produced series of photomicrographs for their own use (for example, the Landeskriminalampt, Berlin), and Verma has placed a series of micrographs on the Internet. Ogle and Fox (1998a, 1998b) have reported an in-progress study to produce a hair atlas in text and CD-ROM formats.

It has to be recognized that the assessment of pigment features has an element, some might say a large element, of subjective assessment. It is certainly true to say that it is not possible at this time to place meaningful numerical definitions on these features.

Table 2.14 Classification of pigment granules (from Seta et al., 1988)

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>(1) Pigment density: absent, sparse, light, medium, heavy, opaque</th>
<th>(2) Pigment distribution: uniform, peripheral, one-sided, about medulla, cluster, greying</th>
<th>(3) Pigment size: fine, medium, large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaudette and Keeping (1974)</td>
<td>(1) Pigment density: absent, light, medium, heavy</td>
<td>(2) Pigment distribution: uniform, peripheral, one-sided, about medulla, cluster</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3) Pigment size: fine, medium, large</td>
<td>(3) Pigment size: fine, medium, large</td>
<td></td>
</tr>
<tr>
<td>Bisbing (1982)</td>
<td>Absent, liquid, non-granular, granular, multicolour, chain,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3) Pigment size: fine, medium, large</td>
<td>(3) Pigment size: fine, medium, large</td>
<td></td>
</tr>
<tr>
<td>Robertson (1982)</td>
<td>(1) Pigment density: absent, light, medium, heavy</td>
<td>(2) Pigment distribution: uniform, peripheral, one-sided, about medulla, cluster</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3) Pigment size: fine, medium, large</td>
<td>(3) Pigment size: fine, medium, large</td>
<td></td>
</tr>
<tr>
<td>Strauss (1983)</td>
<td>(1) Pigment density: absent, sparse, medium, heavy</td>
<td>(2) Pigment distribution: uniform, peripheral, one-sided, random, medial, cluster</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3) Pigment size: fine, medium, large</td>
<td>(3) Pigment size: fine, medium, large</td>
<td></td>
</tr>
<tr>
<td>Harding and Rogers (1984)</td>
<td>(1) Pigment distribution: uniform, peripheral sparse, central</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2) Pigment size: fine, medium, large</td>
<td>(4) Pigment shape: round, oval, clumpy, streaky</td>
<td></td>
</tr>
<tr>
<td>Lee and De Forest (1984)</td>
<td>(1) Pigment density: light, average, heavy, opaque</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2) Pigment distribution: uniform, random, towards cuticle,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3) Pigment size: fine, medium, large</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4) Pigment shape: round, oval/oblong</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5) Pigment aggregation: streaked, clumped round, clumped oval</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The forensic hair examiner has to take a pragmatic and descriptive approach in an attempt to produce a systematic description of the pigment features. A number of authors have published classification schemes in recent years. These are given in Table 2.14. The CFHC has also published a classification scheme (Anon., 1985); this is given in Table 2.15.

The more detailed schemes attempt to classify pigment density and distribution and then focus on the individual pigment granules and how these are sometimes organized into groups, clumps or aggregates. More recent schemes have developed a greater number of descriptors for aggregates, and there now seems to be broad acceptance that it is possible to describe aggregates in terms of being streaked, in round clumps or in oval clumps.

Features which attempt to describe the size of the pigment granules or aggregates have not been defined in any absolute or quantitative sense. It was the intention of the CFHC to produce an Atlas of Hair Comparison Characteristics which would include descriptions of colour photomicrographs. Regrettably, this laudable aim was never achieved.

A number of laboratories have produced series of photomicrographs for their own use (for example, the Landeskriminalampt, Berlin), and Verma has placed a series of micrographs on the Internet. Ogle and Fox (1998a, 1998b) have reported an in-progress study to produce a hair atlas in text and CD-ROM formats.

It has to be recognized that the assessment of pigment features has an element, some might say a large element, of subjective assessment. It is certainly true to say that it is not possible at this time to place meaningful numerical definitions on these features.
Vernall (1963) has presented data on pigment density counts for groups of individuals from four racial groups. His data reveal Western Europeans to have the lowest pigment densities, but they are also the most variable. There were no significant differences in pigment density between hairs from Negroid, Chinese and Asian Indian individuals.

It is my view that attempting to develop a numerical, quantitative classification of pigment density will not be practical until it is possible to capture images of hairs at magnifications of ×1000 and have these treated with image analysis algorithms. Producing meaningful information and data will still be challenging given that

- at magnifications high enough to see the individual particles, it is only possible to look at a very small depth of field—how many optical sections will be necessary to build up meaningful data?
- it remains to be seen whether image analysis will differentiate and classify the currently defined features or will have the power to look at the holistic image and derive its own pattern
- this data capture and analysis will have to be repeated many times per hair.

### Table 2.15 Pigment features (from Anon., 1985)

<table>
<thead>
<tr>
<th>Pigmentation</th>
<th>Density (value)</th>
<th>Distribution</th>
<th>Aggregation</th>
<th>Aggregate size</th>
<th>Pigment shape</th>
<th>Pigment size</th>
<th>Pigment colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Uniform</td>
<td>Streaked</td>
<td>Large</td>
<td>Round</td>
<td>Fine</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Peripheral</td>
<td>Clumped</td>
<td>Medium</td>
<td>Oblong</td>
<td>Coarse</td>
<td>Brown</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>One-sided</td>
<td>Round</td>
<td>Small</td>
<td>Other</td>
<td>Mixed</td>
<td>Black</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>Random</td>
<td>Oval</td>
<td></td>
<td></td>
<td></td>
<td>Mixed</td>
</tr>
<tr>
<td></td>
<td>Opaque</td>
<td>Central</td>
<td>Banded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gapping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pigment in cuticle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Clearly, the data handling aspect of such an approach will require considerable computer ‘grunt’. While it is probably only a matter of time before these challenges can be answered, the question remains as to whether or not there will be a desire or need to take this approach when DNA testing is available. What will be the role of microscopic examination?

Descriptions of the colour and underlying pigment features are considered to be where most of the discrimination power lies in human hair microscopy. It is important to remember that discrimination comes not from the individual features, but rather from the overall pattern they create and how this pattern varies along the length of individual hair shafts. Checklists of features are important to ensure a systematic and thorough examination. The number of features included needs to balance factors such as the following:

- too many subcategories may lead to increased subjectivity
- too few subcategories may miss useful information
- too many subcategories may lead to examiners being overloaded with ‘potential’ information—they may not record information accurately because it is too onerous—they may simply miss the pattern because they are focused on the detail.

Robertson and Aitken (1986) collected numerous checklists in their survey of hair examiners in the 1980s. The checklists of Robertson and Aitken and of the CFHC attempt to reach an appropriate balance.

Some examples of pigment features are shown in Figure 2.6.

**Medulla Classification**

Section 2.3 discusses the value of the medulla as a feature both to differentiate human hair from non-human hair and to differentiate hairs of different animal origin. The mature medulla in animal
Forensic Examination of Hair
Figure 2.6 Appearance of some pigment features: (a) light density; (b) medium density; (c) medium-heavy density; (d) heavy density; (e) opaque; (f) heavy, streaked distribution; (g) medium, streaked distribution
hairs forms a central core and will often have a recognizable pattern. This results from cell collapse as cell membranes break down and the dead hair cells dehydrate. The index of hair diameter divided by the width of the medulla, the medullary index (MI), is also useful in differentiating human hair from non-human hair.

The MI of human hairs is less than 0.33. There is evidence that the MI of human hair varies with age, becoming gradually larger in older age (Luell and Archer, 1964; Langia, 1966). MI also may be higher in white hairs compared with pigmented hairs (Langia, 1966). Robertson and Walker (unpublished data) have shown that despite considerable variation in MI within one individual, it is possible to show statistically significant differences between individuals. Notwithstanding the latter finding it is generally accepted that MI is not a useful feature to differentiate between individuals for forensic application. MI also does not correlate with the race or sex of an individual (Seta et al., 1988).

By comparison with the medulla of hairs in the wider animal kingdom, the medulla of human hairs is a rather uninteresting structure. The medulla may not even be present, particularly in fine scalp hairs. When the medulla is present it has an irregular globular appearance.

Hausman (1932) produced an early scheme for describing the medulla in human hairs, classifying it as absent, scanty, fragmentary, fractional, broken or continuous. Since then a number of authors have produced classification schemes. Some of the more recent schemes are presented in Table 2.16. The scheme proposed by the CFHC is the same as that of Robertson and Aitken (1986) except that it includes MI and whether or not the medulla is amorphous or patterned.

The many schemes used to describe the human hair medulla have a great deal in common. All describe the medulla in terms of the degree to which it forms a continuous structure. Most schemes also refer to the medulla as opaque or translucent. Here it is important that the observer understand that this may be an artefact. A translucent medulla will result when the air present in the intercellular and intracellular gaps is displaced by the mountant used for microscopy. Where the air is not displaced, the medulla will appear dark when using transmitted light (see Chapter 1).

This process of infilling is not necessarily predictable, and it is possible over a period of storage that the amount of opaque versus translucent medulla may change. It is important that the observer does not miss the degree of presence of the medulla through assessing the easily seen opaque sections but missing the less obvious translucent areas. Examination under polarized light will help one to visualize the full extent of the medulla.

In general, the medulla is more prominent and has a higher MI in non-scalp than in scalp hairs. A very rare event is to see a hair with a double medulla (Montagna and Van Scott, 1958; Chowdhuri and Bhattacharyya, 1964). Examples of medulla types are shown in Figure 2.7.

Cortical Features

The development of the cortex is considered above in relation to how pigment granules are deposited within the cortex. From a forensic viewpoint the organization and distribution of pigment is the single most important feature of the cortex. A small number of other features can be described and assessed. These features fall into two categories: features relating to the structure and appearance of the cortical cells, and what might loosely be described as inclusions.

The only feature which relates to the appearance of the cortical cells is called cortical texture. As with other features, recent authors have developed a number of descriptors and these are listed in Table 2.17.

Some authors attempt to score the degree of coarseness of the texture where present. Other schemes merely record the presence or absence of texture. Lee and De Forest (1984) refer to cortical damage, and in some instances at least this may be a more accurate descriptor.

In normal, healthy and undamaged hair it would be uncommon to see coarse cortical texture. Under normal light microscopy viewing conditions it is rarely possible to see the outlines of individual cortical cells, as the cells are closely packed into a rigid and homogeneous hyaline mass (Seta et al., 1988). It is likely then that where the outline of cortical cells is visible enough to be assessed as coarse, this is the result of some physical or chemical disruption.
Table 2.16 Classification of the pattern of hair medulla (from Seta et al., 1988)

<table>
<thead>
<tr>
<th>Medulla appearance</th>
<th>Medullary index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaudette and Keeping (1974)</td>
<td>Absent</td>
</tr>
<tr>
<td>Fragmental</td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Opaque and translucent</td>
</tr>
<tr>
<td>Continuous</td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Opaque and translucent</td>
</tr>
<tr>
<td>Gaudette (1976)</td>
<td>(1) Medulla distribution</td>
</tr>
<tr>
<td>Trace</td>
<td>&lt;\frac{1}{6}</td>
</tr>
<tr>
<td>Fragmentary</td>
<td>\frac{1}{6} to \frac{1}{4}</td>
</tr>
<tr>
<td>Continuous</td>
<td>&gt;\frac{1}{4}</td>
</tr>
<tr>
<td>(2) Medulla type</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Mainly opaque, some translucent</td>
</tr>
<tr>
<td></td>
<td>Approximately half opaque, half translucent</td>
</tr>
<tr>
<td></td>
<td>Mainly translucent, some opaque</td>
</tr>
<tr>
<td>Bisbing (1982)</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Sparse</td>
</tr>
<tr>
<td></td>
<td>Scanty</td>
</tr>
<tr>
<td></td>
<td>Fractional</td>
</tr>
<tr>
<td></td>
<td>Broken</td>
</tr>
<tr>
<td></td>
<td>Globular</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
</tr>
<tr>
<td></td>
<td>Double</td>
</tr>
<tr>
<td></td>
<td>Cellular</td>
</tr>
<tr>
<td>Robertson (1982)</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Scanty</td>
</tr>
<tr>
<td></td>
<td>Fragmentary</td>
</tr>
<tr>
<td></td>
<td>Fractional broken</td>
</tr>
<tr>
<td></td>
<td>Broken</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td>Strauss (1983)</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>Sharp</td>
</tr>
<tr>
<td></td>
<td>Obscure</td>
</tr>
<tr>
<td>Continuous</td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Opaque and translucent</td>
</tr>
<tr>
<td>Fragmented</td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Opaque and translucent</td>
</tr>
<tr>
<td>Trace</td>
<td>Opaque</td>
</tr>
<tr>
<td></td>
<td>Translucent</td>
</tr>
</tbody>
</table>
Table 2.16 (Cont’d)

<table>
<thead>
<tr>
<th>Medulla appearance</th>
<th>Medullary index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harding and Rogers (1984)</strong></td>
<td></td>
</tr>
<tr>
<td>(1) Category</td>
<td>Width</td>
</tr>
<tr>
<td>Not visible</td>
<td></td>
</tr>
<tr>
<td>Fragmentary</td>
<td></td>
</tr>
<tr>
<td>Discontinuous</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>(2) Type</td>
<td></td>
</tr>
<tr>
<td>Globular</td>
<td></td>
</tr>
<tr>
<td>Ladder</td>
<td></td>
</tr>
<tr>
<td><strong>Lee and De Forest (1984)</strong></td>
<td></td>
</tr>
<tr>
<td>(1) Distribution</td>
<td>Absent</td>
</tr>
<tr>
<td>Medulla absent</td>
<td></td>
</tr>
<tr>
<td>Uniform</td>
<td></td>
</tr>
<tr>
<td>Near tip</td>
<td>Thin, 1–10 μm</td>
</tr>
<tr>
<td>Near middle</td>
<td>Medium, 10–25 μm</td>
</tr>
<tr>
<td>Near root</td>
<td>Thick, &gt;25 μm</td>
</tr>
<tr>
<td>Random</td>
<td></td>
</tr>
<tr>
<td>(2) Configuration</td>
<td></td>
</tr>
<tr>
<td>Medulla absent</td>
<td></td>
</tr>
<tr>
<td>Amorphous opaque</td>
<td>Sparse</td>
</tr>
<tr>
<td></td>
<td>Fragmentary</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Discontinuous</td>
</tr>
<tr>
<td>Cellular opaque</td>
<td>Sparse</td>
</tr>
<tr>
<td></td>
<td>Fragmentary</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Discontinuous</td>
</tr>
<tr>
<td>Amorphous translucent</td>
<td>Sparse</td>
</tr>
<tr>
<td></td>
<td>Fragmentary</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Discontinuous</td>
</tr>
<tr>
<td>Cellular translucent</td>
<td>Sparse</td>
</tr>
<tr>
<td></td>
<td>Fragmentary</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
</tr>
<tr>
<td></td>
<td>Discontinuous</td>
</tr>
<tr>
<td><strong>Robertson and Aitken (1986)</strong></td>
<td></td>
</tr>
<tr>
<td>(1) Medulla distribution</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Medulla &gt; space</td>
<td></td>
</tr>
<tr>
<td>Medulla &lt; space</td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>(2) Medulla type</td>
<td></td>
</tr>
<tr>
<td>Opaque</td>
<td></td>
</tr>
<tr>
<td>Translucent</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.7 Appearance of some medulla types: (a) continuous, translucent; (b) continuous, opaque; (c) medulla greater than non-medullated cortex; (d) medulla less than non-medullated cortex
Coarse cortical texture is often seen towards the tip end of hairs which have been subjected to cosmetic treatment. However, it is possible to get less coarse manifestations of this feature in ‘normal’ hairs. Most often the feature will be seen in less pigmented hairs. Care has to be taken in assessing streaked pigmentation not to confuse it with cortical texture. In pigmented hairs a visual impression of streaking can result from underlying cortical texture.

The origins of cortical fusi are described above. Cortical fusi are spaces between cortical cells which become elongate or fusiform as cortical cells elongate. They are easily seen with normal light microscopy and are usually most abundant near the root end of hairs. Care should be taken not to miss their presence in densely pigmented hairs. They should not be confused with pigment granules. Hausman (1932) describes how fusi and pigment granules may be differentiated by ‘boiling the hair shaft for a few seconds in concentrated sulphuric acid’. Perhaps not a preferred forensic solution!

Most analytical schemes attempt to assess both the abundance and the distribution of fusi. The CFHC scheme lists size, shape, distribution and abundance. Robertson (1995), in common with Gaudette and Keeping (1974) records only whether or not they are present. Robertson argues that it is better then to describe the distribution verbally as only present near the root, or extending some distance up the hair shaft. An assessment can be made of relative abundance without having to classify it as ‘few’ or ‘abundant’. Harding and Rogers (1984) suggest the use of a + to ++++ scale.

The final feature which should be considered in relation to the cortex is the presence or absence of ovoid bodies. Ovoid bodies are well-defined, highly dense clumps of undispersed pigment. Their origin may be as undispersed melanocytes. Their presence in scalp hair is not rare, but neither are they always seen. Where they are present, their abundance can vary widely from very few to widespread. Roe (personal communication) conducted a study aimed at investigating the discriminatory value of quantifying the presence of ovoid bodies. Regrettably, the results of this study have not been published.

Examples of cortical features are shown in Figure 2.8.

Cuticle Features

The outer layer of the hair shaft is called the cuticle and is composed of flattened scale-like cells which overlap like roof tiles (Robertson, 1995). The cells overlap longitudinally and laterally to

---

**Table 2.17** Classification of cortical cell morphology (from Seta et al., 1988)

<table>
<thead>
<tr>
<th>Source</th>
<th>Feature 1</th>
<th>Feature 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaudette and Keeping (1974)</td>
<td>Cortical texture: fine, medium, coarse</td>
<td>Cortical fusi:   present, not present</td>
</tr>
<tr>
<td>Gaudette (1976)</td>
<td>Cortical texture: smooth, medium, fibrous, granular</td>
<td></td>
</tr>
<tr>
<td>Bisbing (1982)</td>
<td>Cortical texture: brittle, damaged, fibrous, cellular, invisible, fusiform, ovoid bodies</td>
<td>Cortical fusi: absent, few, abundant, bunched, linear central, periphery, roots</td>
</tr>
<tr>
<td>Harding and Rogers (1984)</td>
<td>Cortical texture: coarse, smooth, even, blotchy, striated, granular</td>
<td>Cortical fusi: root, tip gradings + to ++++</td>
</tr>
<tr>
<td>Lee and De Forest (1984)</td>
<td>Cortical damage: mechanical, chemical, diseased, crushed, broken, frayed, burnt</td>
<td>Cortical fusi: absent, uniform, near tip, near middle, near root, random</td>
</tr>
<tr>
<td>Robertson (1995)</td>
<td>Cortical texture: not visible or smooth, visible or coarse</td>
<td>Cortical fusi: present or absent</td>
</tr>
</tbody>
</table>
envelop the cortex and protect it from environmental exposure. The scales slope outwards and their free edges point towards the hair tip.

The cuticle of mature human hairs is an average of six cells thick, each overlapping. The shape and arrangement of scales vary between species. For non-human hairs the arrangement or pattern, and how this varies along the length of individual hair shafts, is among the most useful features to identify the species. For human hair the scale pattern does not show significant variation between individuals (Hausman, 1925).

The CFHC (Anon., 1985) produced a comprehensive list for cuticular traits in which features associated with the scales, thickness, margins, sequence and weathering are considered (see Table 2.18).

The shape of the distal margin of scales and the pattern of scales are not generally considered to show natural variation in human hairs, and in normal circumstances would not be assessed. The cuticular margin is subject to weathering and damage, and this is usually assessed.

A number of studies have examined the use of numerical indices of scale features for forensic application. Scale count is a measure of the number of scales over a unit measure. Scale index is a count of the number of scales across the diameter of the hair shaft. Although there has been some debate and disagreement about the value of scale counts (Beeman, 1941; Kirk and Gamble, 1942), Gamble and Kirk (1940) are of the view that scale counts are relatively constant for scalp hairs from one individual and can vary between individuals. However, in order to draw a meaningful conclusion at least 100 counts should be made (Kirk, 1940). The potential value of a scale count as a useful discriminator has not become universally accepted, and I doubt today whether many, or any, laboratory practitioners would assess either scale count or scale index.
The CFHC scheme (Anon., 1985) next assesses how distinct the inner cuticular margin is. The thickness and colour of the cuticular margin are also assessed. The outer cuticular margin is classified as smooth, serrated, ragged, cracked or looped.

Finally, the sequence of traits along the shaft and weathering damage are noted. In fact, the appearance of the outer cuticular margin is in part the result of the latter. As the hair emerges from the skin the cuticular layer is smooth and only becomes noticeably irregular and damaged through a number of processes involving external damage caused by general wear and abrasion, and ‘cosmetic care’ such as brushing, combing and washing (Robbins, 1988). As hair is dead, damage cannot be repaired and it is usual to see more damage toward the tip of hairs (Bottoms et al., 1972). Although cuticle damage is an acquired characteristic, hairs from individuals can show quite systematic wear and damage characteristics.

Where damage becomes extreme the cuticle can be lost completely, exposing the cortical cells to the environment. This can result in frayed ends and split tips (see Chapter 1 and Seta et al., 1988). Rook and Dawber (1982) have defined weathering as the progressive degeneration of hair from root to tip due to a variety of environmental and cosmetic factors.

In the scheme of Robertson (1995) the appearance of the cuticle is assessed only at the outer margins. Figure 2.9 shows typical examples of some of these features. Other classification schemes have been presented by Bisbing (1982), Lee and De Forest (1984) and Strauss (1983). All include assessments of the appearance of the outer cuticle margin.

Robertson (1995), in common with Lee and De Forest, also notes the thickness of the cuticle and its colour. The presence of pigment particles in the cuticle layer should also be noted, as this is generally considered unusual.

All these features can be readily assessed without the need to examine the hair shaft surface either by scanning electron microscopy (SEM) or through taking scale impressions. Seta et al. (1988) outline the various methods by which the latter can be produced if deemed necessary. While scanning electron micrographs provide very attractive and visually compelling evidence of characteristics of the cuticle, it is unlikely that SEM will ever find favour as a routine technique for hair examination. This is because SEM requires quite extensive preparation of hairs prior to examination. The hairs have to be cut into short lengths, and once treated they are not suitable for light microscopy.

### Table 2.18  Cuticular traits (from Anon., 1997)

<table>
<thead>
<tr>
<th>Scale shape</th>
<th>Distal margin</th>
<th>Patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>smooth</td>
<td>level wave</td>
</tr>
<tr>
<td></td>
<td>crenate</td>
<td>arched wave</td>
</tr>
<tr>
<td></td>
<td>rippled</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scale size</th>
<th>Distance between distal edges</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>scale count (scales/unit/measure)</td>
</tr>
<tr>
<td></td>
<td>scale index (scale count/diameter)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inner cuticular margin</th>
<th>Distinct</th>
<th>Indistinct</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Cuticular layer</th>
<th>Thickness</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Outer cuticular margin</th>
<th>Flattened (smooth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serrated</td>
</tr>
<tr>
<td></td>
<td>Ragged</td>
</tr>
<tr>
<td></td>
<td>Cracked</td>
</tr>
<tr>
<td></td>
<td>Looped</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sequence of traits along shaft</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Weathering damage of cuticular surface</th>
</tr>
</thead>
</table>

The CFHC scheme (Anon., 1985) next assesses how distinct the inner cuticular margin is. The thickness and colour of the cuticular margin are also assessed. The outer cuticular margin is classified as smooth, serrated, ragged, cracked or looped.

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In conclusion, features associated with the cuticle layer and scales in human hairs are not normally major contributors to individualization, except where obvious and characteristic damage.

Cross-sections

There has been considerable debate regarding the value of making cross-sections of human hairs for individualization purposes. Those who argue that it is unnecessary point to the fact that:

- cross-sectional shape is variable from hair to hair and along individual hair shafts
- cross-sections yield no information additional to what can be assessed by careful optical sectioning of longitudinal mounts
- cross-sectional shape does not have as much validity as once thought (Seta et al., 1988).

The points in favour of taking cross-sections include the fact that:

- if they are carefully made, very little of the hair shaft is consumed
- variation in cross-sectional shape in human hairs is predictable and consistent provided sections from similar types of hair and of equivalent longitudinal appearance are used
- cross-sections reveal a number of microscopic features more clearly than can be seen in longitudinal mounts
- cross-sectional shape is of value for racial origin determination.
Individual examiners must reach their own conclusion as to whether or not the additional effort in making cross-sections is warranted. Gaudette and Keeping (1974) included cross-sections in their scheme, but later Gaudette (1978a) concluded that it was not essential or even necessary to take cross-sections. He did, however, point out that those individuals who made cross-sections continued to find them useful. In my view it is not necessary to take cross-sections of human hairs unless some exceptional circumstance warrants the additional effort. In general I do not favour any method which essentially destroys the hair in its original condition.

For those who do favour cross-sectioning, Seta et al. (1988) list a number of schemes for classifying cross-section shape and other features which can be observed with cross-sections. The CFHC (Anon., 1985) lists six cross-sectional shapes

- round
- oval
- triangular
- flat
- kidney
- teardrop

and also recommends assessing the degree of departure from a circular cross-section, or so-called eccentricity.

Attempts have been made to assess cross-sectional shape with a numerical index, but this has generally been found to be unsatisfactory and to lead to inaccurate and potentially misleading generalizations (Seta et al., 1988).

2.4.3 Non-microscopy Approaches

The ‘search’ for useful non-microscopy-based approaches has been driven by the inability of hair examiners to place a statistical value on their findings and an underlying uneasiness on the part of some with reaching conclusions based on subjective characteristics. Of course those examiners who ‘believe’ in hair examination argue that it is possible to reach a scientifically sound conclusion. Chapter 7 discusses in detail the evidential value of hairs and these underlying concerns. Suffice it to say that it may be more useful to accept that microscopic features are not usually objective in the numerical sense, but that objectivity in terms of

- the ability of different analysts to reach the same decision
- the ability of one person to reach the same decision, given the same feature or hair to examine on a number of occasions

is attainable when a systematic and thorough examination is conducted (Robertson, 1982).

In fact, attempts to find a numerical basis to assist in the discrimination of hairs using indices such as medullary index or scale count have not proven particularly useful.

The approach taken in this volume has been not to concentrate on historical approaches to hair examination except in so far as they assist in laying the proper foundation for current practice. Hence, many of the non-microscopy approaches which are now consigned to the annals of forensic ‘history’ are not considered. For example, the reader is referred to Seta et al. (1988) for previous work on electrophoretic protein pattern analysis and enzyme analysis of hair root sheath cells in the excellent and comprehensive chapter on forensic hair investigation.

Seta et al. (1988) also deal with a number of chemical instrumental techniques which have been applied to hair analysis. In general, these techniques have looked at some specific aspect of the hair, often some aspect of cosmetic treatment, rather than an inherent feature of the hair. Chapter 6 deals with some of these approaches as they relate to the detection and analysis of cosmetic treatments of hairs.
The even older technique of ABO grouping, which used to be a major topic of debate for forensic hair examiners, has been, once and for all, rendered irrelevant by recent advances in DNA typing.

Topics of potential general applicability which are considered in this volume are elemental analysis and DNA analysis. In specific cases drug analysis and cosmetic analysis may be of value, and these are considered in Chapters 5 and 6. Some would argue that elemental analysis should also be in the latter grouping.

In conclusion, with the exception of DNA, non-microscopy approaches to hair examination seem destined to contribute only in specific and specialized circumstances. The future roles of microscopic analysis and DNA analysis are considered in the next section.

2.5 Examination Process

2.5.1 Underlying Philosophy

In order to design a protocol for the examination of human hair, it is necessary to be quite clear about the purpose of the examination and the conclusions that can be reached. Gaudette (1976) proposes that two possible conclusions can arise from a hair comparison:

(a) the unknown or recovered hair could have come from the same person from whom a known hair sample was obtained

(b) the recovered hair could not have come from such an origin.

(Note that Gaudette used ‘is or is not consistent with’, rather than ‘could have or could not have come from’.) These two possibilities should equate to the real situation, i.e.

1. The hair did originate from the same person as the known sample
2. The hair did not originate from the same person as the known sample.

If the true position is (1) and conclusion (a) is reached, then the examiner is correct. If the true position is (2) and conclusion (b) is reached, then the examiner is also correct. However, if the truth is (1) and the examiner concludes (b), then an error is committed. The examiner has incorrectly eliminated a person as being the source of the recovered hair. This is referred to as a TYPE 1 ERROR. The final combination is that the truth is (2) and the examiner concludes (a). This is a TYPE 2 ERROR and has far more serious consequences, because the examiner has incorrectly included a person as a possible source of a hair.

Because the implications of a type 2 error have potentially serious consequences, hair examiners have always tended towards a conservative approach to minimize this type of error, probably at the cost of an increase in type 1 errors—incorrect exclusion.

As well as type 1 and type 2 errors, the examiner must be aware of the potential for a coincidental ‘match’. Really, nothing has changed since Virchow stated in 1861 that ‘…the hairs found on the defendant do not possess any so pronounced peculiarities or individualities, that no one with a certainty has the right to assert that they must have originated from the head of the victim’ (quoted in Bisbing, 1982).

The CFHC (Anon., 1985) concluded that ‘…it was vital that both the procedures and thought processes involved in a hair comparison be selected by the examiner to minimize type 2 errors’.

I would assert that the starting point in any forensic examination must be to attempt to eliminate the questioned sample, to show that it is different, and not a position of looking for ‘similarities’. This process forces the examiner to focus on reaching the highest level of discrimination. Hair examinations are also conducted against a background where it should be accepted that no two hairs are absolutely identical. Within an individual it is normal to see differences between hairs resulting in a known sample displaying a range of features and, perhaps more importantly, a range of patterns. Concluding that there are no forensically significant differences between a questioned hair and a hair or hairs
within a known sample does mean that the two hairs are necessarily identical in all features along the entire length of the individual hair shafts. The assessment of what is and what is not a meaningful difference goes to the very heart of the skill of the hair examiner. There are no short cuts in hair examination, and there is no substitute for a detailed and systematic examination. The use of a checklist assists in this regard, but it is not in itself always sufficient. Use of written descriptions and observation is also to be encouraged. Neither is a checklist a substitute for the comparison process. Side-by-side comparison of hairs is the only appropriate method to carry out the holistic pattern recognition process which underpins any valid hair examination where the examiner seeks to be in any way inclusionary.

I do not subscribe to the view that this latter process can be conducted without a comparison microscope, unless the examiner is working at a very low level of discrimination. This approach is to be strongly discouraged. There are still conflicting views on the value or role to be played by data sheets or checklists. Robertson and Aitken (1986) summarized the situation in their analysis of a hair questionnaire. As the views expressed at that time probably still reflect the situation today, the section dealing with this subject is reproduced in Table 2.19.

### 2.5.2 Examination protocol

A number of authors have published examination protocols, including Shaffer (1982), Strauss (1983), CFHC (Anon., 1985) and Robertson (1995). The last two schemes mentioned are presented in Tables 2.20 and 2.21 as examples of protocols.

Although there will be variation in the approach used by different examiners, a number of general guidelines should be incorporated in any scheme for it to meet acceptable standards.

It can be expected that these guidelines will be modified, updated, and formalized by the Technical Working Group on Hair Examinations (TWG Hairs) which is currently producing new guidelines.

*There is a strong need for more frequent, realistic and meaningful proficiency tests for hair examiners to support the discipline.*

### Inclusion of Non-microscopic Techniques

As discussed in section 2.4.3, a number of non-microscopic techniques can be applied in hair examination. In general it is preferable to conduct a microscopic examination prior to any other technique. Microscopy is non-destructive and it is often possible to eliminate quickly and accurately hairs which are not of evidential significance. In many cases, especially those involving loci used by numerous people such as car interiors, inside houses and in some public places there will be a background population of hairs for which it will be possible only in exceptional situations to attempt to determine their source. As a full detailed microscopic examination and comparison is a time-consuming task, it is essential to eliminate as much of this background as possible before the comparison stage. Apart from specific situations in which elemental analysis may still have a role, and the very specific situation where hairs are really only a matrix for drug identification, the two usual follow-up techniques will be analysis for cosmetic treatment and DNA testing for either nuclear or mitochondrial DNA. The former is considered in detail in Chapter 6 and the latter in Chapter 3.

A question which is still the subject of debate is the precise role to be played by DNA testing in a hair examination protocol. As DNA techniques, firstly nuclear and now mitochondrial have been applied to hairs, there has been a move of hair examination from specialist fibre and hair groups into biology groups. Nuclear DNA testing should be attempted with relevant hairs where there is any evidence of cellular material near the root, and it is worth attempting with anagen hair roots even in the absence of visible cell material (Linch et al., 1998). This will account for only a small minority of hairs recovered in case situations. mt DNA testing is not yet broadly established in forensic laboratories (Fournery, 1998). Because of the stringent conditions under which such analysis must be conducted, to avoid possible contamination, it may be that full sequence mt DNA
Many respondents had reservations about the use of data sheets, but few were totally opposed to their use. Most expressed the hope that this study would lead to the production of a better and more workable sheet or examination form. The reservations expressed by respondents fell into three broad areas.

**Construction of the data sheet** – A large number of data sheets used in forensic science laboratories were sent to the authors. These varied in complexity from single sheets with a few lines drawn, to complex multifeature punched index cards. Many examiners were not satisfied with their forms finding them either too detailed or not detailed enough!

One person pointed out the difficulty in using his data sheet for both single hairs and a known (control) sample. The major problem associated with the use of detailed data sheets was the time necessary to complete them when, at the end of the day, they could not be used to show whether or not two hairs could have had a common origin. However, the impression was given by some that they were not willing to spend this time completing a data sheet because they considered hair to have such limited evidential value.

There is a fundamental need to define the part to be played by such a form in the examination of hair. Clearly, some examiners consider that the form should be capable of holding sufficient detail to show ‘identity’ of hairs (with or without comparison microscopy?). This could be an achievable aim, but is it desirable? In our opinion, an examination form should play only a minor role in the COMPARISON PROCESS but an important role in the DESCRIPTIVE PROCESS. These two aspects need to be separated and clearly understood. This point will be further emphasized in the concluding section.

**Philosophical objectives** – Many respondents felt that by using a data sheet nonscientists (lawyers) place too great an emphasis on the information contained in the sheet and not on the use of the comparison microscope. Filling in categories for microscopic features, it was felt would imply these features were objective. Others felt that the data sheet was self-limiting and could not adequately represent the continuous variables found in hair. The major concern expressed was that a conclusion regarding the comparability of two hairs could only be made by comparison microscopy.

**Problems with use in court** – A small number of respondents expressed reservations about what would happen if data sheets were available to the defence, who might make spurious use of them either by intent or through a lack of knowledge. Of course this argument could equally apply to the prosecution and displays apparent bias on the part of some respondents. Misuse or abuse of scientific data is not confined to hair examination and in this context is more a problem of the design of the data sheet. It is the job of the forensic scientist to explain to the court what significance and limitations should be placed on the ‘facts’ being presented.

Many respondents expressed the view that there were positive aspects to the use of data sheets, namely, that they

1. encourage systematic observation;
2. produce a written record of the examination; and
3. aid in the training of hair examiners.

However, one respondent went so far as to state ‘...the information that is observed is tabulated in the examiners’ mind and need not be placed on paper’.

In the opinion of the authors, this is a dangerous and ill-conceived stance to adopt, but it does help to emphasize the less outspoken but implied view of many respondents that they do not require extensive, detailed notes of hair examinations. It is difficult to conceive of anyone retaining fine detail of every case under these circumstances. Further, where detailed notes are not taken there is no way in which the results of observations of the examiner can be checked as part of a quality assurance programme or by a defence scientist.

No forensic scientist would comment on ‘matching’ chromatographic data without including the relevant charts and experimental details in case notes, yet some hair examiners would appear to believe it sufficient to comment that hairs give positive comparison without any of the reasons that support that conclusion!
Table 2.20 Examination protocol (Robertson, 1995): Appendix 2 gives checklists supporting this protocol

1. It is essential to receive a sample of known hairs from all relevant individuals in a case. If a hair sample is received from only one party, say a suspect, but not the complainant, then little useful information can be given by the examiner.

   It is desirable also to eliminate a much wider number of people as possible sources of recovered hairs than is often considered. Obvious examples would be hair from a spouse and from children.

   Known samples must be adequate, relevant and representative. For head hair samples, at least 30–40 hairs should be taken from several parts of the scalp and for pubic hairs, 20–30 hairs.

   In determining what is a relevant sample, the case history is important. For example, if the purpose of the examination is to determine who was the driver of a vehicle in a car accident, then hairs should be taken from near the site of injuries to the occupants of the car. This seems only common sense but it does not always happen.

2. Each known sample should be examined using a stereomicroscope equipped to give even illumination. All samples must be examined under standard conditions. On the basis of length and overall hair colour, five to ten hairs are selected to represent the variation present in the bulk sample. It may be necessary to select more hairs where variation is large.

   The shaft profile and length of the selected hairs are then noted using a checklist (see the Hair Examination Record, Sheet 1, p. 134) before single hairs are placed in either temporary or permanent mountant on microscope slides. The colour of the hair and condition of the root and tip are also noted to complete the macroscopic examination.

   It is very important to study the variation in features along the length of the shaft because hair examination involves pattern recognition.

3. At this point, the recovered hairs are treated in precisely the same manner as the known hairs except that (in most cases) all recovered hairs are examined. If anagen roots, with or without sheath cellular material, are present then nuclear DNA typing should be attempted.

   It is important to stress that it is not always possible to describe the features or variation present in a single hair adequately using the discontinuous classifications used in the checklist. It is important to use a combination of written notes and the features list.

4. The extent to which the mounted hairs are then examined for detail using a compound light microscope will depend on the degree of characterization which can be achieved using only macroscopic features.

   For two persons, the possible options are

   (a) it is possible to differentiate all of the hairs within the two known samples;
   (b) it is possible to differentiate some of the hairs from one known sample from some hairs in the other sample; or
   (c) it is not possible to differentiate any of the hairs from the two known samples.

   If the recovered hairs are different from all hairs in the known sample from one person, the recovered hairs are described in detail only if it is important to establish whether or not they have come from the other person, i.e.

   (a) hairs found on clothing of suspect are different from suspect’s and are ‘similar’ to hairs from complainant – a detailed examination is required
   (b) hairs found on clothing of complainant are different from suspect’s – regardless of whether these hairs are ‘similar’ or not to complainant’s, these would not always be examined in detail.

   Clearly, there are exceptions to these guidelines. Each case must be treated on its merits. Where there are more than two known samples, the situation becomes even more complex.
Table 2.20 (Cont'd)

Where it is not possible to differentiate the known hairs on the basis of macroscopic examination, then all must be examined in detail.

Eliminations at this stage will reduce the volume of work and, hence, the time necessary to complete the case.

5. Using a comparison light microscope, features are recorded on the checklist (see Hair Examination Record, Sheets 2 and 3, pp. 135, 136) for all relevant hairs. Magnification should range from nominal objective magnifications of \( \times 10 \) to \( \times 40 \). Again, variation in features along the length of the hair shaft is an important element of the examination.

6. It does not follow that two hairs with the same features recorded on the record sheets cannot be distinguished. The final decision on whether recovered and known hairs could have had a common origin must only be made after the hairs have been compared side by side using a comparison microscope. The results of comparison microscopy are recorded on the Hair Examination Record, Sheet 4 (p. 137).

7. Each recovered hair must be compared with one or more known hairs, selected on the basis of possessing ‘similar’ features to the questioned hair. In order to conclude that two hairs could have had a common origin, these hairs should show the same degree of variation in features along their respective lengths and be indistinguishable at a minimum of one microscopic field of view along their length. There must be no differences which cannot be explained in the context of the overall examination, i.e. forensically significant differences.

8. It is unlikely that any two hairs will be indistinguishable for all features along their entire length.

Table 2.21 CFHC Scheme (Anon., 1985)

PHYSICAL PARAMETERS

1. Known Sample

Because of the variation in microscopic characteristics among different hairs from the same body region of one person, it is important to obtain a sufficient number of hairs in order to represent adequately the ranges of all characteristics present. If the ranges of characteristics are large, it becomes necessary to obtain a large number of hairs. Because they differ in their characteristics, it is important to obtain hairs from different areas of the scalp. Full-length hairs with roots should be obtained for the examiner to examine and compare adequately the variations along the length of a single hair and to determine its growth phase. Since the vast majority of pulled hairs will be in an active growing stage, a combing procedure is also desirable to obtain hairs in the telogen or dead stage.

It is recommended that a known head hair sample consist of at least 20 hairs from each of five different areas of the scalp (centre, front, back and both sides) and that these hairs be obtained by both pulling and combing. The recommended procedure for obtaining combed hairs is to use either a comb packed with cotton or a multibristle brush. The various areas of the scalp should be repeatedly combed over a large sheet of clean paper in a direction opposite to that in which the person usually combs the hair.

From these 100 hairs, a number of hairs (usually 6 to 20, depending on the homogeneity of the sample) should be selected by the examiner as representative of the entire known sample. The selection should be based primarily on gross characteristics such as length, coarseness and colour as observed by macroscopic and stereomicroscopic examination. These hairs should be used for comparison. The remaining hairs are then available for future use if subsequent examinations reveal that, whereas a questioned hair has characteristics close to those of the known sample, a good match to any of the 6 to 20 hairs originally selected cannot be found.
Table 2.21 (Cont’d)

A known pubic hair sample should consist of at least 30 hairs obtained by both pulling and combing from different areas of the pubic region.

For exclusionary purposes, known samples should be requested from all persons who might reasonably be considered a source of a questioned hair. If such samples are obtained and excluded, the significance of any ensuing association is increased.

Some examiners believe that a known sample does not require a large number of hairs and that hairs cut close to the scalp, being easier to obtain, should be used for the comparison process. The committee members, in formulating the recommendation of 100 pulled and combed hairs, believe that 100 pulled and combed hairs are required to guarantee full-length hairs, and that the number chosen should help minimize type I errors. Since an individual loses an average of 100 scalp hairs a day as part of the normal hair cycle, the collection of 100 hairs is not unreasonable. While hairs cut close to the skin line can, if necessary be used for comparison purposes, the root and root end are important parts of the hair and should be obtained whenever possible. It is recommended that research be conducted as to the content and methods of collection of known hair samples.

2. Mounting Medium
To observe adequately the microscopic characteristics of a hair, the hair must be placed in a medium of refractive index similar to that of the hair itself (the isotropic or average refractive index of hair is approximately 1.55). A synthetic semipermanent mounting medium with a refractive index around 1.52 is recommended. Using a mounting medium with a refractive index much different from that of hair will result in excessive shadows and contrast that will tend to mask the internal characteristics of a hair. A semipermanent medium is recommended for convenience. The hair or hairs can then be mounted so as to remain in a fixed location, with no chance of loss from the glass microscope slide. Some of the commercially available semipermanent synthetic mounting media allow mounted hairs to be easily removed from the slide if desired.

3. Comparison Microscope
The use of a high-quality comparison microscope is mandatory when comparing the microscopic characteristics of hairs to distinguish subtle or slight differences that may exist between hairs from different individuals. High-quality objectives are important, but highly corrected planapochromats are not necessary, and a daylight correction filter provides adequate lighting. A comparison microscope can be equipped with several types of stage. The types of stage used, and their placement on the microscope, are a matter of personal preference and should not affect ability to compare hairs.

4. Magnification
Both low- and high-power microscopic examinations are necessary in the comparison of hairs. Stereomicroscopic examinations at magnifications around 10×, of both unmounted and mounted hairs, are important for screening purposes. Observations of mounted hairs with the compound microscope must encompass a variety of magnifications, generally around 50×, 100×, 250× and 400×. The use of high-power (large numerical aperture) objectives allows the examination and comparison of the fine detail present in such characteristics as pigmentation and cuticular scales. These objectives also allow an examiner to view thin sections of the hair. This ‘optical sectioning’ is important because a hair is three-dimensional and certain characteristics vary. Surface appearances of the hair can also be obtained by ‘optical sectioning’.

PROCEDURE
The procedures used by the hair examiner should incorporate the general considerations discussed in this report, proper evidence handling and the correct utilization of proper equipment. As long as these considerations are kept in mind, the committee believes that a considerable amount of leeway should be allowed in choice of procedure to satisfy individual preferences.

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The following procedural differences among examiners should not affect the ability to compare hairs correctly, and are mentioned here only for informational and educational purposes.

1. Number of hairs per slide: some examiners mount all hairs, both questioned and known, one to a slide. Others mount as many as five or more hairs to a single slide, depending on their length. Yet other examiners collectively mount questioned hairs, but singly mount knowns, or vice versa.

2. Method of preliminary screening: some examiners believe that the macroscopic or stereomicroscopic examination of unmounted hairs is of value for screening purposes; others do not, and utilize low-power examinations with mounted hairs only. It is recommended that research be conducted to determine the value of low-power examination of unmounted hairs. In the meantime, the use of such preliminary screening should remain a matter of individual preference.

3. Note-taking: some examiners use preprinted forms while others make personalized notes to describe the characteristics of hairs. Differences of opinion concerning the quality and quantity of note-taking considered necessary, the detail that should be present, and use of notes during the comparison process also exist among examiners. While note-taking should be a matter of personal preference, it is generally believed that notes that accurately reflect the complete results of a case and individually identify the questioned hairs, especially those that are involved in a match, should be taken.

4. Which hairs to examine first: most examiners prefer to examine known hairs first, but some like to begin the examination with the questioned hairs. Again, this is best left to the examiner’s preference and judgement, bearing in mind the circumstances of the case.

The procedure used by the hair examiner must involve a thorough and careful examination of both the gross and microscopic characteristics exhibited by properly prepared hairs while using a high-quality comparison microscope at different magnifications. The questioned hairs should be compared to the known hairs with regard to as many as possible of the characteristics recommended by the committee. The examiner should strive to attain a level of discrimination in hair comparisons such that type 2 errors will be minimized without at the same time incurring an unreasonable number of type 1 errors.

CRITERIA FOR SIMILARITY

In order to conclude that a questioned hair is consistent in macroscopic and microscopic characteristics with a known sample from a particular person, it must first be determined that there are no significant differences in these characteristics or their arrangements. Since no two hairs will ever be exactly the same in all minute details, it is important to determine what differences are significant. It must first be determined that the characteristics exhibited by the questioned hair fit within the range of characteristics present in the known sample. After that, the ideal situation for the hair examiner is to find one or more hairs in the known sample that correspond in all respects (no significant differences) with the questioned hair. If this goal is strived for, the frequency of type 2 errors will be minimized. Depending on the characteristics involved and the circumstances surrounding a case, it may sometimes be possible to utilize several hairs from the known sample to locate characteristics which correspond to those of a questioned hair. However, the ‘duplicate hair’ criterion for a match should be the ultimate goal.

analysis will not be adopted in all laboratories, and certainly not in smaller laboratories. The introduction of less labour-intensive and cheaper approaches to mt DNA such as the minisequencing approach of Tully et al. (1996) may see at least limited mt DNA testing more widely adopted. The issues relating to the use of mt DNA for the forensic analysis of hairs are discussed in detail in Chapter 3.
Allen et al. (1998) have reported success in mt DNA typing with shed hairs by amplifying two small DNA fragments in two separate first PCRs. Jehans et al. (1998) have reported on a method to remove biological contaminants such as saliva and blood from hair prior to mt DNA analysis. Their method used a differential lysis buffer and the authors recommend the routine decontamination of hair shafts where mt DNA analysis is to be conducted.

Key issues which are yet to be resolved are the interpretation of the frequency of occurrence of mt DNA types and the emerging realization that heteroplasmy may be more widespread than previously recognized. It seems clear that mt DNA will be used for the examination of hairs but that it will not be applied to all or even most recovered hairs, at least in the near future, because of cost and other factors. The only debate would appear to be to what extent preliminary microscopic examinations should be conducted prior to DNA analysis. For mt DNA analysis it may well be the case that there will be little if any reduction in the level of microscopic examination as it will be both necessary and desirable to eliminate as many questioned hairs as possible and concentrate mt DNA analysis on only key hairs.

In conclusion, microscopic examination has been criticized as being time-consuming and hence costly. In my view, if a proper protocol is applied, incorporating logical and practical evaluation at each stage of the examination, microscopic examination can be an efficient part of any hair protocol. It will be for each laboratory system to determine whether or not it continues to undertake detailed comparison microscopy in all or only in some case situations. As more rapid methods of mt DNA testing emerge, it is likely that many will make the choice to reduce their commitment to microscopy. Time alone will tell whether or not this creates its own problems. However, for those of us who believe in the value of microscopy the danger is that the discriminatory ability of the analyst will be lost and will not be available in those cases in which DNA results are not obtained. This is not an encouraging scenario.

### 2.6 Reporting Hair Examinations

It is beyond the scope of this section to stray too far into the realm of evidential value of hairs, which is considered by Gaudette in Chapter 7. It is impossible, however, to discuss how to report hair examinations without some reference to the current debate on the evidential value of hairs in light of Daubert versus Merrell Dow Pharmaceuticals and the four ‘foundational predicates’ (Anon., 1997) of Daubert:

- testability
- peer review
- rate of error
- general acceptance.

A casual read of recent editions of *Scientific Sleuthing* will only too readily provide details of several cases in which hair examination has been subjected to scrutiny, focusing on the Daubert predicates or derivatives of them as defined by different courts in the USA.

It would be wise for hair examiners, regardless of the country in which they work, to take notice of the challenges posed by Daubert. Can we prove that hair examination is based on the sound application of scientific method? Can we falsify our hypothesis? Is hair examination inherently reliable and if we believe it to be so, what is the rate of error and can we as a community agree on the standards which are best practice? It will not serve us well to attack those who are in fact the messengers. We must develop coherent, factual, and balanced arguments and proofs.

The value and significance which should be placed on any hair examination clearly depend on all aspects of the forensic process being conducted to the highest standards, from the collection of potential evidence, to the examination and comparison phase, through any special testing and on to the way in which our ‘findings’ are presented in our report or statement for court. This last stage has the potential,
all too often realized, to undo what might otherwise be a well-conducted case. It is to this issue of report writing that I now wish to turn my attention.

2.6.1 Report Writing

Crocker (1991) has stated that ‘the greatest challenge faced by forensic hair examiners is to be able to leave the witness box with a feeling of assurance that members of a jury, or a judge acting alone, have the same appreciation as the examiner does of the proper level of significance to be given to the hair evidence’.

Arguably, the responsibility which lies with the forensic scientist is to go one step further and define what the hair ‘evidence’ does not say and just what are its boundaries and limitations. Clearly, the scientist’s formal report plays an important part in achieving the laudable aim espoused by Crocker. However, the report can only be as good as the weakest link in the chain. This chain includes every step from the recovery of hair to its analysis, interpretation and presentation (see Chapter 7).

Throughout this chapter the need for enhanced quality assurance procedures has been stated. If the ‘science’ in hair examination is to meet the Daubert criteria then there must be improvements in the areas of training, credentialling of practitioners and underpinning of proficiency testing. These issues are considered by Gaudette in Chapter 7. My concern and intent in this section is not to repeat or critique all aspects of the chain, but rather to focus on one particular aspect: the language used to express opinions in hair reports.

The underlying principle which should govern the approach of the hair examiner is that the fundamental hypothesis should be to attempt to exclude an individual or individuals as the donor of recovered or questioned hairs. The language used should reflect this principle. Hence, in my opinion, the use of terms such as ‘similar’ and ‘consistent’ is inappropriate in the hair examiner’s vocabulary.

Crocker (1991) discusses the results of a study to interpret forensic science phraseology. The approach used was to assess the weight placed, by jurors and criminal law professionals, on a series of statements. The positive test statements were

- revealed the presence of
- indicated
- is consistent with
- indistinguishable
- strongly indicates but does not conclusively reveal
- within reasonable scientific certainty
- suggests
- is identical to
- is the same as
- matched
- positive results.

The negative test statements were essentially the opposite of the above, i.e. ‘did not match’ etc. The degrees of certainty were

- positive (certain)—A is B
- positive (probable)—A is probably B
- possible—A could be B, or inconclusive
- negative (probable)—A is probably not B
- negative (certain)—A is not B.
From the results of this survey, the term ‘consistent with’ was considered positive (certain) by 42 per cent of jurors and positive (probable) by 35 per cent of jurors. Eighteen per cent of jurors thought it meant ‘possible’. Professionals were more conservative, with 38 per cent indicating positive (probable) and 62 per cent possible. The term ‘could have’ was taken by 100 per cent of professionals to mean possible; only one juror felt it meant positive (certain) and six per cent of jurors positive (probable).

The critical message which should be clear from this study is that whatever terminology is used by a particular laboratory or an individual, the report (if it is to be meaningful and not potentially misleading) must attempt to spell out what weight or significance is or should be attached to the terms used. Chapter 7 discusses these issues in some depth and canvasses the use of statistical approaches to determining weightings.

If the principle of exclusion is accepted then hair reports should be written from a perspective of what they can say with some certainty, i.e. a particular (questioned) hair could not have come from a particular individual. Even here caution needs to be exercised, as there may be factors which do not allow even this ‘strong negative’ conclusion (Gaudette, 1978b). If it is not possible to exclude a nominated individual as the donor of a questioned hair, the examiner must then attempt to assess the weight and significance which ought properly to be placed on that finding.

I personally dislike the term ‘consistent with’, and at least two major commissions of inquiry into wrongful convictions have criticized the use of this term in the strongest possible way (Shannon, 1984; Kaufman, 1998). While I am not entirely happy with the term ‘could have’, this is the term I have settled on until I am convinced there is a better approach. The use of this term is inclusionary, and I attempt to state in my reports a sense of the strength of that inclusion without using a numerical or statistical estimate.

Some would argue that even the use of ‘could have’ is inappropriate and that the hair examiner should be limited to using ‘excluded’ and ‘cannot exclude’.

An example of my report format is given in Appendix 3. Each laboratory will have formal rules which dictate layout and what must be included in a report or statement. The fundamental principles should remain the same. Section 3.10.3 from the Australian National Association of Testing Authorities (NATA) (Appendix 4) provides guidelines for what must appear in a report issued under NATA’s laboratory accreditation criterion.

2.7 Conclusion

Perhaps in the past too much emphasis has been placed on the perceived and real difficulties in reaching positive inclusionary findings, and too little on the very valuable exclusionary role of hair examination and other forensic examinations. Hair has been viewed by many as a poor investment in time for the return. What this fails to recognize is that in many cases hairs are the only evidence. Can anyone be serious in suggesting that hairs should be ignored when they are the only trace material?

Obviously, in most cases hairs will be only one of a number of trace materials of possible evidential significance. In much the same way as it is appropriate to examine a physical tool mark before embarking on a detailed analysis of a paint chip found on a jemmy, clearly the amount of effort given to hair examination should quite properly be assessed in the context of the overall circumstances. There will be, and are, many cases where hairs will be important and sometimes critical trace materials. In such cases an experienced examiner will often be able to limit very effectively and efficiently the scope of the detailed (and hence time-consuming) examinations necessary.

Much emphasis is placed, by some critics, on the inability of hair examiners to place a numerical figure on an inclusionary finding. Firstly, this fails to recognize that exclusions are often as important, if not more so, than inclusions. Secondly, it fails to recognize that in many case scenarios it is not necessary to comment on the frequency of occurrence of a hair or hairs in the whole population. That is an argument fuelled by the overemphasis on population statistics of the DNA era.

Take, for example, the following real case scenarios.
• A group of hairs recovered from a windscreen of a vehicle involved in a road accident. There are two occupants and neither is willing to admit to being the driver. In this case the hairs clearly did not come from one occupant and were not excluded on the basis of microscopic examination as coming from the second occupant. Note, no roots were present on the recovered hairs.
• A number of body hairs were recovered from tapings found in a waste disposal unit on an international flight. The tapings had been used to secure illicit drugs to a courier. The hairs were not Caucasian and were consistent (in this case the term is appropriate!) with coming from a person from the Pacific Islands. Two Tongan individuals could not be excluded as possible sources of these hairs. They were the only individuals of that ethnicity on the flight in question. Subsequently, DNA testing was able to provide statistical evidence as to the donor of the hairs.
• A pubic hair recovered from inside the vagina of a deceased victim of a sexual assault/murder. The hair was not examined until investigators were directed, by a magistrate at a committal, to have it examined. The defence argued that the hair was left by the offender and did not belong to their client, the nephew of the deceased. Examination of the hair established that the hair could not have originated from the nephew and that the deceased could not be excluded as the source.
• Hairs found in the boot of a vehicle allegedly used to hold the body of a murder victim. Here, the position and condition of the hairs not only were important in helping to establish that the body had been in the boot, but also assisted in reconstructing how the body may have been placed.

In many cases hairs will be only one of a number of trace materials recovered. Often the condition of such hairs is important where, for example, a person has been assaulted by a particular type of implement or weapon. Sometimes the number and location of hairs can be useful in attempting to reconstruct events. As was already demonstrated above, where hairs are not examined, this can leave unanswered questions which make it more difficult for juries to determine the circumstances and facts of a case.

The reality is that hairs are a very common, if not the most common, trace material encountered in forensic work. To ignore them is facile and jeopardizes the proper investigation of crime. The greatest danger is to have them examined by generalists without proper training and competencies.

As the 1990s draw to a close, forensic hair examination is at a crossroads. Its future will be determined by the dual impact of the two ‘Ds’: Daubert and DNA. If hair examiners rise to the challenge of the two ‘Ds’, they will dispel the third ‘D’—the doubters.

If this is to be achieved, much still needs to be done. I believe the following would lay the foundation.

• Hair examiners should take as their starting point the exclusion of individuals.
• Microscopic examination will continue to be the core technique for hair examination.
• Hair examiners must attempt to assess what weight and significance should be attached to any inclusionary finding, no matter how difficult this is.
• There must be formalized training and credentialling for hair examiners. It would be preferable if the latter was based on the development of internationally accepted standards and proficiency testing.
• DNA analysis is already providing useful information which assists in quantifying inclusionary findings, mt DNA will increasingly find application in hair examination. It is still not wholly clear whether or not the latter technique will find broad application in the immediate future.
• Cases will remain where techniques such as elemental analysis may provide complementary and sometimes unique information. Even with the advent of DNA it is important to keep an open mind on emerging techniques which may have a part to play in an overall hair examination protocol.
There remains a need for a concerted research effort to provide a more solid basis for the interpretation of hair evidence. It is also time for laboratory systems and scientists that are either unable or unwilling to embrace hair examination with the necessary investment in time and commitment to withdraw altogether from the field. Half-hearted commitment can only continue to do damage to the credibility of hair examination.

2.8 Acknowledgement

All photographs were taken by Jesse Andrewartha, whose contribution is gratefully acknowledged. All tables from Seta et al. (1988) are reproduced with permission.

2.9 References


Appendix 1: Animal Hair Examination Proforma

AUSTRALIAN FEDERAL POLICE

FORENSIC SERVICES, SCIENTIFIC

ANIMAL HAIR EXAMINATION – SHEET 1

Case Reference:

<table>
<thead>
<tr>
<th>MACROSCOPIC CHARACTERISTICS</th>
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Examined by: _______________________

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OVOID BODIES

OTHER

Examined by: ____________________________

Notes by: ____________________________  Day: ______  Date: ______  Time: ______

135
### AUSTRALIAN FEDERAL POLICE

**FORENSIC SERVICES, SCIENTIFIC**

**EXAMINATION RECORD**

**Case Reference:**

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**Hair Examination Record – Sheet 1**

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**General Description and Comments:**

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1. Assessed with a stereo microscope using standardised illumination
2. Basic colour to be qualified by shade or depth of colour. Light (L), Mid (M) or Dark (D)
3. Note artificial colouring

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**Examined by:**

**Notes by:**

**Day:**

**Date:**

**Time:**
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2 Where present their shape, size and distribution both along and across the hair shaft may have some value when used as comparative features.
Appendix 2: Human Hair Examination Proforma

AUSTRALIAN FEDERAL POLICE
FORENSIC SERVICES, SCIENTIFIC

EXAMINATION RECORD

Case Reference: 

Hair Examination Record – Sheet 3

General Description and Comments\(^3\).

\(^3\)note presence of parasites and disease condition of hair.

Examined by: 

Notes by: 

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Examined by: __________________________________________

Notes by: ____________________________ Day: ______ Date: ______ Time: ______
Appendix 3: Case Report Examples and Hair Protocol

STATEMENT OF WITNESS

Statement of: James ROBERTSON

Age: 47  Occupation: Forensic Scientist

Address: Forensic Services, Australian Federal Police, GPO Box 401, Canberra, ACT, 2601.
Telephone No: 2870468

This statement, consisting of FOUR (4) pages signed by me, is true to the best of my knowledge and belief and I make it knowing that, if it is tendered in evidence, I shall be liable if I have wilfully stated in it anything which I know to be false or do not believe to be true.

Dated the 6th day of March 1998

Signed:

Signature witnessed by:

I hold the degrees of Bachelor of Science (BSc Hons Agric. Botany, University of Glasgow, 1972) and Doctor of Philosophy (PhD, University of Glasgow, 1975).

In 1976 I joined the Forensic Science Unit of the University of Strathclyde, Glasgow, United Kingdom, as a lecturer in forensic science, until moving to State Forensic Science, Adelaide, South Australia, 1985.


I have carried out forensic investigations on behalf of prosecution and defence agencies in Australia and several overseas countries, and presented evidence on numerous occasions. I have also published scientific papers and books relevant to my areas of expertise.

Part of my work has involved the examination and comparison of hairs.

I have examined a number of items in relation to a motor vehicle accident involving X and Y.

A court Report prepared by me is attached. The report consists of four (4) pages, each signed by me, and is dated 6 March 1998. I hereby incorporate that report as part of my statement.

Signed by:  Signature witnessed by:
REPORT OF THE EXAMINATION OF HAIRS

IN THE CASE OF
A MOTOR VEHICLE ACCIDENT
IN VOLVING TWO OCCUPANTS

X
Y

JAMES ROBERTSON BSc PhD

FORENSIC SCIENTIST

PAGE

1 EXAMINATION & OPINION 2
2 CUSTODY OF ITEMS 3
3 HAIR APPENDIX 4

CASE REFERENCE NUMBER SB97/_____

6 March 1998

This report consists of four (4) pages each signed by me

Signed by:  
Signature witnessed by:

141
1 EXAMINATION AND OPINION

1.1 I received SCALP HAIR samples from two individuals, X and Y, and two hairs which I understand to have been recovered from the windscreen of a vehicle involved in an accident.

1.2 I have examined the above samples following the approach and methodology outlined in the attached appendix.

1.3 The known SCALP HAIR sample from X consisted of over 50 hairs ranging in length from about 10 cm to 38 cm. Overall the hairs were a mid to dark brown colour. Individual hairs varied in colour from grey to almost black near the root end becoming a truer brown along the hair shaft. In one hair there was a profound and sharp change in colour from very dark brown to yellow indicative of a dyed hair. Other hairs may have had artificial colour treatment. Ten hairs were prepared for detailed microscopic examination.

1.4 The known SCALP HAIR sample from Y consisted of over 50 hairs ranging from about 16 cm to 29 cm. Overall the hairs were a dark brown colour. Examination at low magnification revealed that there was a profound and sharp change in colour along individual hair shafts from grey to dark brown. The hairs from Y had been dyed. Ten hairs were prepared for detailed microscopic examination.

1.5 Against this background I examined two hairs which I understand were recovered from the windscreen of a vehicle involved in an accident. These hairs were in fact two segments of hair shaft, respectively 7 cm and 6 cm in length. The longer hair had a cut end and a broken end and was a pale to light brown colour. The shorter hair was broken at both ends and was dark brown in colour. Both hair fragments were prepared for detailed microscopic examination.

1.6 After detailed examination of the two known hair samples and the recovered hairs and direct comparisons, I reached the following conclusions.

The pale recovered hair is outside the range of colour of the two known samples and hence I can reach no conclusion as to its origin other than to state it is of human origin and scalp hair.

The darker recovered hair can be distinguished from the hairs of Y and, in my opinion did not originate from this person. The darker hair did not show any forensically significant differences from parts of hairs from X and, in my opinion, she could have been the source of this hair fragment. The recovered hair was a fragment. It did not display the full range of features seen on the complete hairs of X. Under normal circumstances this would increase the chance that someone other than X could have been the source of this hair. However, if the only persons having access to the vehicle were Y and X, then the latter is not relevant. The recovered darker hair had broken ends and is not likely to have been shed during normal activities.

Signed by:                     Signature witnessed by:

142
2 CUSTODY OF ITEMS

2.1 Receipt

I received the following items on 3 February 1998 from _______ ______ of the Australian Federal Police, Forensic Services.

‘1 x yellow top container with hair samples from windsreen’ (on two glass slides marked SB97/______)
‘Scalp Hair............... Y’
‘Scalp Hair............... X’
STATEMENT OF WITNESS

Statement of: James ROBERTSON

Age: 47 Occupation: Forensic Scientist

Address: Forensic Services, Australian Federal Police, GPO Box 401, Canberra, ACT, 2601.
Telephone No: 6287 0468

This statement, consisting of FIVE (5) pages signed by me, is true to the best of my knowledge and belief and I make it knowing that, if it is tendered in evidence, I shall be liable if I have wilfully stated in it anything which I know to be false or do not believe to be true.

Dated the 3rd day of April 1998

Signed:

Signature witnessed by:

I hold the degrees of Bachelor of Science (BSc Hons Agric. Botany, University of Glasgow, 1972) and Doctor of Philosophy (PhD, University of Glasgow, 1975).

In 1976 I joined the Forensic Science Unit of the University of Strathclyde, Glasgow, United Kingdom, as a lecturer in forensic science, until moving to State Forensic Science, Adelaide, South Australia, 1985.

I worked as a senior forensic scientist in Adelaide from 1985 – December 1989 carrying out a wide range of forensic casework. In December 1989 I moved to Canberra to my current position as Assistant Secretary and Head of the Forensic Services Division.

I have carried out forensic investigations on behalf of prosecution and defence agencies in Australia and several overseas countries, and presented evidence on numerous occasions. I have also published scientific papers and books relevant to my areas of expertise.

I have examined hairs relating to a case (Case Ref. SB98/_______) involving ________ (complainant) and ________ (suspect).

A court Report prepared by me is attached. The report consists of four (4) pages, each signed by me, and is dated 3 April 1998. I hereby incorporate that report as part of my statement.

Signed by: Signature witnessed by:
REPORT ON THE EXAMINATION OF ITEMS

RELATING TO ______ (COMPLAINANT)
and
______ (SUSPECT)

by

JAMES ROBERTSON BSc PhD

FORENSIC SCIENTIST

PAGE

1 EXAMINATION & OPINION 2
2 CUSTODY OF ITEMS 3
3 HAIR APPENDIX 4

CASE REFERENCE NUMBER SB98/______

3 April 1998

This report consists of four (4) pages each signed by me
1 EXAMINATION AND OPINION

1.1 I have examined three samples of hair. Two of these were labelled as having been located in a lounge room and a bathroom respectively. The third hair sample was taken from the complainant, ______. The approach and methodology used for my examination is outlined in the attached appendix.

1.2 The known SCALP HAIR from ______ consisted of a good sized clump of hairs from 13 to 18 cm in length. Visually, it could be seen that some of the hairs had been dyed. Examination at low magnification with a stereomicroscope revealed that some hairs were not dyed. These were light to mid brown in colour. In the dyed hairs, there was a sudden and sharp change in colour from a brown hue to a very pale yellow brown or colourless. Along some hairs more than one change in colour was seen. In most hairs, there was also a natural gradual change in colour from a grey black hue near the root end, to a mid brown hue along the hair shaft. Overall, individual hairs, and taken as a group, the known hairs from ______ were distinctive.

1.3 The hairs from the LOUNGE ROOM consisted of a moderately loosely grouped clump of hair with several hundred hairs present. I examined 50 hairs from the clump for the presence of roots and recorded the growth phase of the roots. Each individual loses in the order of 100 scalp hairs per day. Mostly, these hairs are lost as a result of normal grooming and are hairs which are in the so called telogen growth phase. This is when the hair has effectively stopped growing and can be quite readily removed by combing or brushing. Only about 5% of all scalp hairs are in this growth phase. The vast majority of hairs in the human scalp are in an active growth phase called anagen. Hairs in this growth phase have easily recognised root structures. Some hairs in this growth phase can be removed by grooming, however, removal of these hairs would usually require some degree of force.

Of the 50 hairs from the clump in the lounge room, only 2 were telogen hairs. A number of hairs from the clump were removed and prepared for microscopic examination.

1.4 The hairs from the BATHROOM consisted of a tightly grouped clump of hairs with several hundred hairs present. The clump had small pieces of vegetation present and I also observed the presence of reddish colour flakes of material having the appearance of being dried blood. I removed 50 hairs and examined these for the presence of roots, and recorded the growth phase of the roots. Only 4 hairs had telogen roots present. Two hairs had no root present. A number of hairs from the clump were removed and prepared for microscopic examination.

1.5 I examined the selected hairs from both the lounge room and bathroom using a stereomicroscope. At this level, these hairs showed the same range of features as those seen in the known sample from ______. Given the distinctive appearance of the recovered and known hairs, it is likely that the recovered hairs originated from ______. I have requested DNA testing of hair roots from the clumps of hairs from the lounge room and bathroom. I have also requested biological testing of the reddish coloured material from the clump found in the bathroom. The results of the above testing will be the subject of a separate report.

Signed by: ________________________________ Signature witnessed by: ________________________________
1.6 I did not receive a hair sample from the suspect, _____ and hence, I am unable to exclude _____ as a possible source of the two clumps of hair.

1.7 In summary, the clumps of hair found in the lounge room and bathroom could not have been lost as the result of normal grooming or hair loss. Some degree of force would have been required to remove such a large number of hairs in a clumped form.

2 CUSTODY OF ITEMS

2.1 Receipt

I received the following items on 11 March 1998 from _____ _____ of the Australian Federal Police, Forensic Services.

Two sealed yellow manilla envelopes labelled in part respectively ‘Hair Located Loungeroom’
‘Hair from bathroom’

I received a further item on 31 March 1998 from _____ _____ of the Australian Federal Police.

A sealed plastic press seal bag labelled in part ‘Scalp Hair . . . _____’

2.2 Custody

The above items and samples prepared from them have been repackaged and are held in secure storage within Forensic Services.
STATEMENT OF WITNESS

Statement of: James ROBERTSON

Age: 47 Occupation: Forensic Scientist

Address: Forensic Services, Australian Federal Police, GPO Box 401, Canberra, ACT, 2601.
Telephone No: 6287 0468

This statement, consisting of SIX (6) pages signed by me, is true to the best of my knowledge and belief and I make it knowing that, if it is tendered in evidence, I shall be liable if I have wilfully stated in it anything which I know to be false or do not believe to be true.

Dated the 15th day of April 1998

Signed:

Signature witnessed by:

I hold the degrees of Bachelor of Science (BSc Hons Agric. Botany, University of Glasgow, 1972) and Doctor of Philosophy (PhD, University of Glasgow, 1975).

In 1976 I joined the Forensic Science Unit of the University of Strathclyde, Glasgow, United Kingdom, as a lecturer in forensic science, until moving to State Forensic Science, Adelaide, South Australia, 1985.

I worked as a senior forensic scientist in Adelaide from 1985 – December 1989 carrying out a wide range of forensic casework. In December 1989 I moved to Canberra to my current position as Assistant Secretary and Head of the Forensic Services Division.

I have carried out forensic investigations on behalf of prosecution and defence agencies in Australia and several overseas countries, and presented evidence on numerous occasions. I have also published scientific papers and books relevant to my areas of expertise.

I have examined hairs relating to a case (Case Ref. SB98/______) involving _______ (suspect).

A court Report prepared by me is attached. The report consists of five (5) pages, each signed by me, and is dated 15 April 1998. I hereby incorporate that report as part of my statement.

Signed by: ___________________________ Signature witnessed by: ___________________________
REPORT ON THE EXAMINATION OF ITEMS

RELATING TO _____ (SUSPECT)

by

JAMES ROBERTSON BSc PhD

FORENSIC SCIENTIST

PAGE

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2 CUSTODY OF ITEMS  4
3 HAIR APPENDIX  5

CASE REFERENCE NUMBER SB98/_____

15 April 1998

This report consists of five (5) pages each signed by me
1 EXAMINATION AND OPINION

1.1 I have examined the contents of two sealed audit bags — item 5 and — item 6. Item 6 contained a sample of leg labelled as coming from a . Item 5 contained seven items a number of which contained hairs.

The hairs in items 5 and 6 were examined using the approach and methodology outlined in the attached appendix.

1.2 The known LEG HAIR from consisted of twenty hairs or hair fragments ranging in length from about 1/2 cm to 2 cm. Visually, the hairs were a mid to dark brown colour. Stereo microscopic examination revealed that the colour along individual hair shafts varied from light or mid brown hue to a darker reddish brown hue. Individual hairs were in poor condition with hairs being broken, frayed and displaying quite severe wear to the outer cuticle layer. The detailed microscopic features of a selection of these hairs were recorded.

1.3 Item 5 contained seven yellow top sample bottles. Their contents were as follows:

5.2 ‘ON BED (SINGLE BED ON W SIDE OF ROOM)’

A piece of tightly rolled elastoplast fabric type tape and colourless fabric. A number of hairs were stuck to the tape. Five hairs were removed for microscopic examination.

5.3 ‘BENEATH TABLE ON INSIDE OF ROOM’

A piece of used elastoplast fabric type tape. Two hairs were removed from the tape for microscopic examination.

5.4 ‘BEDSIDE TABLE (NEXT TO DOUBLE BED)’

A piece of elastoplast fabric type tape and colourless fabric. No hairs were recovered.

5.5 ‘TISSUE FROM FLOOR NEAR SINK’

A crumpled white tissue. No hairs were recovered and this was not examined further.

5.6 ‘HAIR FROM BATHROOM FLOOR NEAR SHOWER’

A single hair was recovered.

5.7 ‘HAIR FROM FLOOR NEAR SINK – BINSIDE’

Two hairs were recovered.

5.8 ‘HAIR FROM FLOOR NEAR SINK + DOOR’

Three hairs were recovered.

All of the hairs were examined up to the stereomicroscope stage. The results of this examination are as follows:

Signed by: 

Signature witnessed by:
**Forensic and Microscopic Examination of Human Hair**

### Item | Hair no. | Length | Type of hair and brief description
--- | --- | --- | ---
5.2 | 5/2/1 | all about 2 cm to 5/2/5 | - short body type hairs
- visually mid to dark brown
- individual hairs light to mid brown near root end becoming a blackish brown hue
- no significant cuticle damage
5.3 | 5/3/1 | 16.5 cm | - dyed scalp hair
- characteristic orange brown hue
5/3/2 | about 2 cm | | - short body type hair mid brown hue
5.6 | 5/6/1 | 6 cm | - pubic hair
- orange brown hue
5.7 | 5/7/1 | 6 cm | - scalp hair
- orange brown hue
5/7/2 | 4 cm | | - long body type hair
- light brown with grainy appearance
5.8 | 5/8/1 | 4.5 cm | - short scalp hair
- very light brown hue
5/8/2 | 4.5 cm | | - pubic hair
- light to mid brown hue
5/8/3 | 2 cm | | - short scalp hair
- very light brown hue

All hairs were of human origin.

Only those recovered hairs which were body hairs were examined for their detailed microscopic features. These were 5/2/1 – 5/2/5 and 5/3/2.

**1.4** The recovered body type hairs were compared with the known leg hairs from _____ using comparison microscopy. Clear differences were seen between the recovered hairs and the known hairs from _____.

Signed by: ____________________________________________

Signature witnessed by: _________________________________

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The fine hairs from item 5.2 had microscopic features which would indicate they were from a single individual. The single hair from item 5.3 may or may not be from the same individual as 5/2/1 – 5/2/5.

1.5 In conclusion, body hairs recovered from items 5.2 and 5.3 could not have come from the suspect, ______. A number of other scalp and pubic hairs were recovered in items 5.2 to 5.8. It is not possible to comment on from whom these hairs could have originated in the absence of known scalp and pubic hair samples.

2  CUSTODY OF ITEMS

2.1 Receipt

The following items were received within Forensic Services, Weston, ACT on 7 April 1998 from Federal Agent L Joseph of the Australian Federal Police.

A press seal plastic bag labelled in part ‘PS/316 . . . item 5 . . . item 6’ containing two sealed audit bags, ______ – item 6 and ______ – item 5.

Inside audit bag ______, item 6, was a single yellow top sample bottle labelled in part ‘leg hair . . . ______ . . . 6/4/98’

Inside audit bag ______, item 5, were seven yellow top sample bottles labelled in part respectively

2  ‘ON BED (SINGLE BED ON W SIDE OF ROOM)’
3  ‘BENEATH TABLE ON INSIDE OF ROOM’
4  ‘BEDSIDE TABLE (NEXT TO DOUBLE BED)’
5  ‘TISSUE FROM FLOOR NEAR SINK’
6  ‘HAIR FROM BATHROOM FLOOR NEAR SHOWER’
7  ‘HAIR FROM FLOOR NEAR SINK – BINSIDE’

2.2 Custody

The above items and samples prepared from them have been repackaged and are held in secure storage within Forensic Services.
3 HAIR APPENDIX

Protocol for Hair Examination

(1) The known (from a known source) sample(s) is(are) examined and five (or more) hairs are selected to represent the range of hair lengths and colours present. These hairs are placed on microscope slides in semi-permanent mountant, normally one complete hair to each slide. Features such as the profile or shape of the hair, length, colour and condition of the root and tip are then observed using a stereomicroscope and recorded. Using a comparison compound light microscope, detailed microscopic features may be recorded. An important part of this process is to study the variation in features along the length of each hair shaft.

(2) It is not always possible to adequately describe the features or variation present in a single hair using discontinuous classifications. A record sheet or features list is used to ensure systematic and thorough examination but use is also made of written descriptions where appropriate.

(3) After completing an examination of the known hair sample(s), each recovered or questioned hair is examined separately in the same way as the known hair sample(s).

(4) Unless hairs have been excluded at the stereomicroscope stage, each questioned hair is compared with one or more known hairs. These are selected on the basis of possessing similar features to the questioned hair. The comparison process involves looking for differences as well as features in common and comparing pattern of features along the length of the hair shafts being compared.

(5) It is unlikely that a questioned and known hair will be indistinguishable for all features along their entire length. In order to conclude two hairs could have had a common origin, these hairs should show the same degree of variation and be indistinguishable at several points along their respective lengths. Any differences seen should be explicable in the forensic context.

(6) Microscopic examination can exclude hairs as having come from an individual. A conclusion that a hair or hairs could have come from an individual, an inclusion, does not usually mean that this would be the only person from whom the hair(s) could have originated.

The strength of inclusion cannot be given a statistical estimate and can only be evaluated on a case by case basis.
Appendix 4: Section 3.10.3 NATA (National Association of Testing Authorities, Australia), General Requirements for Registration

Endorsed documents shall include:

(a) a title;
(b) the name in which registration is held;
(c) identification of the facility by its registration number, street address or otherwise;
(d) the date of issue of the document;
(e) unique identification of the document (such as by serial number) on each page;
(f) characterization and unique identification of the test item;
(g) the test results, reported either
   – in the manner specified in the test method,
   – accompanied by a statement of uncertainty,
   – with the last figure of a numerical value adopted as a significant figure, and/or a statement
     of compliance or non-compliance with a specification;
(h) any other information specified by the test method or statutory regulation;
(i) a statement of the conditions pertaining to reproduction;
(j) the signature of an approved signatory.

Each page of a multipage document shall bear a statement of the page number and the total number of pages.

In certain circumstances it is important that other information such as the condition of the test item when received, the test time, location, date, environmental conditions, sampling procedure or client’s identity be included. Graphs and other diagrams may also be required. But explanations of results must avoid expressions of opinion, unless written authority has been granted by NATA’s Chief Executive.
Typing of DNA Derived from Hairs

JOSEPH A. DIZINNO, MARK R. WILSON and BRUCE BUDOWLE

3.1 Introduction

The analysis of hair from a forensic viewpoint generally has been limited to microscopic analyses and comparisons. Traditionally, the analysis of protein genetic markers, and sex typing by the presence of sex chromosomes, were additional tools that could contribute to the characterization of the hair, but these analyses could be performed only on forcibly removed hairs. When an anagen phase, or actively growing hair, is plucked from the body it will often have sheath cells or a follicular tag associated with the root. The sheath material is the source of polymorphic proteins (Budowle and Davidson, 1985; Gambel et al., 1987). Unfortunately, the vast majority of hairs encountered at crime scenes do not contain sheath material and therefore genetic marker analysis cannot be performed routinely on hair evidence.

The tools of molecular biology now enable forensic scientists to characterize biological evidence at the DNA level. These DNA typing techniques and their genetic markers are more sensitive, more specific, and more informative than the available battery of protein markers. Currently, the methods available to the forensic scientist include restriction fragment length polymorphism (RFLP) typing of variable number of tandem repeat (VNTR) loci (Wyman and White, 1980; Jeffreys et al., 1985), and amplification of the number of target DNA molecules by the polymerase chain reaction (PCR) (Saiki et al., 1985) and subsequent typing of specified genetic markers (Saiki et al., 1989; Kasai et al., 1990; Budowle et al., 1991, 1995; Comey and Budowle, 1991; Edwards et al., 1992). Any material, including hair, that contains nucleated cells potentially can be typed for DNA polymorphisms. There are a few reports of successful DNA typing of hairs, but these hairs usually contain sheath material (Pilkington et al., 1987; Goedde et al., 1989; Vigilant et al., 1989; Thomson et al., 1992; Uchihi et al., 1992). However, telogen phase hairs contain very little quantity of DNA such that most DNA markers cannot be detected, even with the use of PCR.

Mitochondrial DNA (mt DNA) is a genetic marker which is typeable in telogen phase hairs. Because a cell can contain more than 5000 copies of mt DNA (compared with only two copies of a genetic locus in nuclear DNA) (Bogenhagen and Clayton, 1974), mt DNA analysis is the best approach to the genetic typing of hairs.

Since the majority of evidentiary hairs encountered are in the telogen phase, mt DNA typing is the most suitable DNA typing method and thus will be the main thrust of this chapter. The reader should refer to references on other DNA typing approaches that would be more amenable for typing sheath material (Budowle et al., 1990; Comey and Budowle, 1991; Edwards et al., 1992; Saiki et al., 1989; Vigilant et al., 1989).
3.2 DNA—a Brief Summary

DNA is a polymer composed of four building blocks called nucleotides. A nucleotide contains deoxyribose, inorganic phosphate, and a nitrogenous base (Figure 3.1). Four different nucleotides are found in DNA, and these nucleotides differ in the type of nitrogenous base they contain. Purine nitrogenous bases are adenine (A) and guanine (G). Pyrimidine nitrogenous bases are cytosine (C) and thymine (T) (Figure 3.2). The nucleotides in DNA are bound together covalently via phosphoester linkages between the 5’ carbon of the deoxyribose of one nucleoside and the 3’ carbon of the deoxyribose in the adjacent nucleoside (Figure 3.3). The sequence of the different bases can be in any order along this phospho-deoxyribose backbone. An enormous array of different sequences can be generated with the four different nucleotides within a relatively short DNA fragment. If only ten positions in a DNA chain were considered and each of the positions could be occupied by A, G, C, or T, the number of possible sequence arrays would be $4^{10}$ or 1,048,576. The human genome, in actuality, contains in total a three-billion-nucleotide-long sequence, divided among 23 pairs of chromosomes.

DNA is composed of two polynucleotide chains that form a helical molecule. The two chains associate by hydrogen bonds between specific bases on each strand. An A on one strand will only form hydrogen bonds with a T on the other strand. Similarly, G and C on opposite strands can form hydrogen bonds (Figure 3.4). A single pyrimidine, hydrogen bonded to its complementary purine on the opposite strand, is known as a base pair. If the sequence of one polynucleotide chain is known, then the sequence array of the opposite strand can be deduced. For example, if one strand contains the sequence 5’-ATGATTCAGCCC-3’, then the other strand sequence is 3’-TACTAAGTCGGG-5’. Opposing strands which can bond together are referred to as being complementary. Complementary base pairing contributes to the chemical stability of the duplex DNA molecule.

The phenomenon of complementary base pairing is exploited in all DNA typing methodologies. When a double-stranded DNA molecule is exposed to high temperature or alkali, the hydrogen bonds will be destabilized and the strands will separate. The process of separating DNA into its component single strands is called denaturation. While the two DNA strands are separated during denaturation, the covalent phosphoester bonds that link the nucleotides in a strand are unaffected. Under appropriate conditions complementary DNA strands can bind together (also known as hybridization or annealing).

3.3 Mitochondrial DNA

Most of the DNA in human cells is contained within the 46 chromosomes in the nucleus. On the other hand, human mt DNA is an extrachromosomal, closed circular genome consisting of approximately 16.5 kilobases and resides outside the nucleus (Budowle et al., 1990). The mt DNA genome
Typing of DNA Derived from Hairs

has been completely sequenced (Anderson et al., 1981). It consists of coding sequences for two ribosomal RNAs, 22 transfer RNAs, 13 proteins, and a noncoding region approximately 1123 base pairs long, called the displacement loop (D-loop) or control region. Most of the sequence variation between individuals is found within the control region (Greenberg et al., 1983). The double-stranded mt DNA molecule is composed of one purine-rich strand and one pyrimidine-rich strand, designated the heavy (H) chain and light (L) chain, respectively. Nucleotide positions in the mt DNA genome are numbered according to the convention of Anderson et al. (1981). The origin of replication of the heavy strand begins the numerical designation of each base pair, continuing around the circle for approximately 16,569 base pairs (Figure 3.5).

An important feature of mt DNA, which simplifies DNA sequencing, is its monoclonal nature. With the exception of certain disease conditions where tissue-specific deletions of large segments of the mitochondrial genome have been detected or certain mutations, for practical purposes all copies of an individual’s mt DNA sequence are identical (Monnat and Loeb, 1985; Monnat et al., 1985; Monnat and Reay, 1986). However, a condition, known as heteroplasmic, is more common than previously believed (Wilson et al., 1997). A person is considered heteroplasmic if he/she carries more than one mt DNA type. Sequence heteroplasmic is currently believed to be a transitory

Figure 3.2 The four nitrogenous bases found in DNA: cytosine and thymine are pyrimidines; adenine and guanine are purines
state or an intermediate stage towards homoplasmy within a relatively few generations. Careful analysis and direct comparisons between multiple known samples and a questioned sample should, in most cases, alleviate interpretational difficulties that may arise due to the presence of heteroplasmy.

Nuclear DNA, however, is diploid, consisting of a maternally inherited set of chromosomes and a paternally inherited set of chromosomes. In order to sequence nuclear DNA (with the exception of the monoclonal Y chromosome), these two alleles must first be separated.

The main advantage of typing mt DNA as opposed to nuclear DNA for forensic identification is the high copy number of mt DNA molecules in a cell. While each set of nuclear chromosomes is present in only two copies per cell, mt DNA is present in hundreds or thousands of copies per cell (Bogenhagen and Clayton, 1974). In cases where the amount of extracted DNA is very small or degraded, as in tissues such as bone, teeth and hair, the probability of achieving a DNA typing result from mt DNA is higher than that of polymorphic markers found in nuclear DNA.

Unlike nuclear DNA, mt DNA is maternally inherited (Case and Wallace, 1981; Giles et al., 1980; Hutchinson et al., 1974). Barring mutation, the mt DNA sequence is identical for siblings and all their maternal relatives. This characteristic can be helpful in forensic cases, such as analysis of the remains of a missing person, where known maternal relatives can provide reference samples for direct comparison to the questioned mt DNA type (Ginther et al., 1992; Holland, et al., 1993; Stoneking et al., 1991).

The mt DNA does not undergo recombination. However, the low fidelity of mt DNA polymerase and the apparent lack of mt DNA repair mechanisms have led to a higher rate of mutation in the

![Figure 3.3 A single strand of DNA with three covalently linked nucleotides](image-url)
Typing of DNA Derived from Hairs

Some regions of the mt DNA genome appear to be evolving at five to ten times the rate of single copy nuclear genes (Brown et al., 1982; Cann et al., 1987). It is these regions which are of forensic interest, due to their ability to assist in the differentiation of individuals in the human population. It has been estimated that among unrelated Caucasians, there is an average of one nucleotide difference every 100 bases (one per cent) in the most variable regions of mt DNA (Cann et al., 1987). This average is higher in individuals of African descent, being approximately 2.3 bases differing for every 100 bases (Vigilant et al., 1991).

3.4 mt DNA Nomenclature Issues

The first entire human mitochondrial sequence was described by Anderson et al. (1981). This sequence is commonly referred to as the Anderson sequence and is often used as a standard sequence for comparison with other human sequences. The Anderson sequence is listed as the light
strand sequence, as opposed to the complementary heavy strand of the double-stranded native mt DNA. When a difference between an individual’s sequence and the Anderson sequence is observed, the difference is sometimes referred to as a ‘polymorphism with respect to the Anderson sequence’. For example, position number 16,189 in the Anderson sequence carries the nucleotide thymidine, or T. However, in another individual, that particular position could contain a cytosine, or C. This polymorphism would be designated as 16,189-C, and all other positions in the region sequenced would be identical to the Anderson sequence.

Defined starting and stopping positions for mt DNA sequencing should also be a part of the mt DNA type. For example, in the case of the 16,189-C individual, without defining the scope of the sequence considered, it might be assumed that the entire 16.5 kilobases of sequence exactly matched the Anderson sequence with one exception at position 16,189. However, the majority of polymorphisms are found within two specific segments of the control region (Greenberg et al., 1983). These segments are termed hypervariable region 1 (HV1) and hypervariable region 2 (HV2). HV1 spans the region 16,024 to 16,365 and HV2 is located on the other side of the origin of replication, encompassing positions 73 to 340. The size of each of these regions makes them amenable for amplification by PCR, hence HV1 and HV2 may be useful for identity testing purposes. A possible way to list the mt DNA type of the individual in the earlier example could be to define the starting and ending points of the segment sequenced and then to list the polymorphisms with respect to the Anderson sequence in both HV1 and HV2, as shown in Table 3.1.

As seen in the example in Table 3.1, reporting a mt DNA type as the Anderson sequence (or some other standard sequence) as a reference and listing only deviations from the referenced standard simplifies processing and handling of mt DNA sequence data.
In addition to substitutions, insertions and deletions also are observed as polymorphisms in human mt DNA. Insertions can be indicated by placing a full point after the last base in the Anderson nomenclature, and listing the insertion as the appropriate nucleotide thereafter. For example, length variations are observed to occur in HV2 close to a long stretch of cytosines interrupted by a single thymidine. The stretch of cytosines begins at position 303 and ends at position 315. The Anderson sequence contains an initial stretch of seven cytosines, followed by a single thymidine and five additional cytosines. In some individuals, an extra cytosine is observed in the initial stretch of seven cytosines, resulting in eight cytosines prior to the sole thymidine. The insertion could, by convention, be recorded after the final cytosine in the initial run of seven, and hence be designated as 309.1 C. Should the run of cytosines be longer than eight (for example, nine), prior to the thymidine, the type would be recorded as 309.1 C, 309.2 C. Deletions would simply be recorded as the number of the base or bases missing with respect to the Anderson nomenclature (for example, D315 or D315,316).

### 3.5 Extraction of DNA

The success of DNA typing depends on the purity of the extracted DNA. In the case of PCR, purity refers to the removal of inhibitors that would affect the ability to amplify the target DNA. Ideally, extraction protocols should be simple and inexpensive, and enable the handling of small quantity samples. Procedures that fit these criteria and are suitable for extraction of DNA from small liquid blood samples and bloodstains are based on standard DNA extraction procedures (Maniatis et al., 1982). A number of extraction procedures have been described for obtaining DNA from hair bulbs. These range from methanol washing (Han et al., 1992) to Chelex extraction (Singer-Sam et al., 1989) to rapid digestion procedures (Higuchi et al., 1988) to standard organic extraction protocols (Maniatis et al., 1982). However, the isolation of DNA from telogen hair roots or hair shaft is more involved. The following is a protocol that we have found to be useful for extracting DNA from hair. This protocol enables extraction of DNA from sheath material and telogen hairs.

### 3.6 DNA Extraction from Hair

The criteria for DNA testing to be performed on hairs are as follows.

1. Head and pubic hairs that have been associated through comparison microscopy and confirmed according to operating protocols or as deemed necessary by the examiner.

2. Generally, there should be a known blood and/or saliva sample to be used as a DNA standard. The known sample should be packaged separately from all questioned samples. In some cases hairs may be accepted as known standards.
Based on the microscopic appearance of the hair, an examiner decides whether or not to determine the quantity of DNA in the extract by a slot blot hybridization assay for possible nuclear DNA analysis. If slot blot results demonstrate a sufficient quantity of DNA for nuclear DNA analysis, an examiner should attempt to type nuclear DNA markers. If the slot blot results demonstrate insufficient DNA present for nuclear testing or if no slot blot is requested, mt DNA testing is considered.

Removal of a hair from the microscope slide is critical to any DNA testing of the hair. Precautions should be taken so that the questioned hair is not contaminated with foreign debris and/or fluids. Removal of the hair from the glass microscope slide for DNA analysis should be performed under a flow or dead space hood as follows.

1. Prior to performing any extraction procedures for DNA analysis, a photograph or electronic image of the questioned hair should be prepared or recorded.
2. Clearly circle the root end of the questioned hair to be tested on the cover slip of the slide.
3. Carefully break the cover slip in a circular pattern around the root end of the questioned hair using a carborundum scorer or similar instrument.
4. Place a drop of solvent (Xyless) on the exposed root end area.
5. Carefully remove the cover slip over the root end of the hair. If desired, the entire cover slip may be removed.
6. Being careful not to break the hair, carefully tease out the questioned hair until approximately 2 cm of the root end of the hair can be cut off.
7. Briefly place the cut 2 cm portion of the hair in a sterile 1.5 ml plastic flip-top tube containing solvent (Xyless) to remove any residual adhering mounting media.
8. Remove the cut 2 cm portion of hair and place it in a sterile 1.5 ml plastic flip-top tube containing 100 per cent ethanol. Tightly close the lid of the tube containing the hair.
9. Remount the remaining portion of the hair on a new glass microscope slide. Remount any additional hairs from the slide originally containing the hair on another glass microscope slide.
10. Handle the hair with clean forceps and examine the hair under a stereomicroscope for the possible presence of sheath material, surface debris or bodily fluids.
12. Place the cut 2 cm portion of the hair in a 1.5 ml plastic tube filled with the Terg-a-zyme solution and place the tube in a rack in an ultrasonic water bath. Agitate for approximately 20 minutes. Examine the hair under the stereomicroscope for the presence of surface debris. If necessary, continue to wash the hair in the Terg-a-zyme solution until free of surface debris.
13. Briefly rinse the hair in 100 per cent ethanol, followed by a rinse in dH₂O.
14. Prepare reagent blanks by placing 200 µl of stain extraction buffer into each micro tissue grinder to be used. Briefly simulate grinding. Remove the pestle and proceed to step 18.
15. Place the hair fragment into a micro tissue grinder (Fisher catalogue number K885470–0000) containing 200 µl of stain extraction buffer. (Note: the grinders and pestles are matched sets.)
16. Move the grinder up and down to force the hair into the bottom of the grinder. Grind until fragments are no longer visible.
17. Remove the pestle from the grinder. If liquid is adhering to the pestle head, gently pass the pestle along the inner lip of the grinder cylinder until the liquid flows down into the grinder.
18. Transfer the homogenate liquid or the reagent blank liquid into a sterile 1.5 ml plastic flip-top tube and add 5 µl of proteinase K (10 mg/ml).
19. Vortex on low speed and briefly centrifuge. Place the tube in a water bath and incubate at 56°C for a minimum of 2 hours to a maximum of 24 hours.
20. Remove the tube from the water bath and briefly spin the tube in a microcentrifuge to force any condensate into the bottom of the tube.
22. Vortex for 30 seconds to attain a milky emulsion, then spin the tube in a microcentrifuge for 3 minutes at 10,000 × g.

23. To a Microcon-100 concentrator (Amicon #42413) add 100 µl of ddH O. Carefully remove the aqueous phase (supernatant of approximately 200 µl) of the PCIA extract and place it into the Microcon-100 concentrator. (Avoid drawing any of the proteinaceous interface into the pipette tip.)

24. Add 200 µl ddH O to the plastic flip-top tube containing the aqueous phase of the PCIA extract. Vortex for 30 seconds to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes at 10,000 × g. Carefully remove the aqueous phase (supernatant of approximately 200 µl) of the PCIA extract and place it into the Microcon-100 concentrator. (Avoid drawing any of the proteinaceous interface into the pipette tip.)

25. Spin the Microcon for 5 minutes at 2000 × g.

26. Discard the wash and add 400 µl of filtered ddH O to the top of the Microcon concentrator.

27. Spin again at 2000 × g for 5 minutes and discard the filtrate cup.

28. Add 60 µl of hot (80°–90°C), filtered ddH O to the filter side of the Microcon concentrator and place the retentate cup on the concentrator.

29. Vortex the Microcon device with the retentate cup pointing upwards (do not invert) on medium speed for approximately 30 seconds.

30. Invert the concentrator with the retentate cup and spin in a microcentrifuge at 10,000 × g for 3 minutes.

31. Discard the concentrator. Cap the retentate cup.

32. If DNA quantitation is desired, follow the Slot Blot Protocol (procedure not described here).

33. The sample can then be amplified by PCR. It is recommended that extracted DNA be amplified immediately or stored frozen until amplified.

It should be noted that the tissue grinders (Fisher catalog number K885470–0000) consist of matched sets of grinders and pestles and should be used as such. To facilitate working with the grinders, the grinders are placed in a small plastic tube, such as a 1.5 ml Sarstedt tube. Prior to DNA extraction of a hair, the grinders should be cleaned using the following protocol.

1. When not in use, place 100 µl of 100 per cent ethanol in each grinder containing its matched pestle.

2. Carefully rinse grinders with ddH O and scrub the pestles and the insides of the grinders with cotton tip applicator’s and warm Alconox detergent (Alconox Inc., New York).

3. Rinse the grinders with ddH O and add approximately 100 µl of 4M H SO . (Note: when working with sulphuric acid, proper safety precautions should be observed. Gloves, protective eye wear and a lab coat should be worn and all work should be performed under a fume hood.) Place each pestle in its matching grinder and briefly grind the pestle in the grinder. Allow grinders and pestles to soak in H SO for a minimum of 20 minutes.

4. Remove the pestles and rinse the grinders with ddH O. Place the grinders in a microcentrifuge and pulse spin at high speed to collect the water. Remove the grinders from the microcentrifuge and remove the remaining water with a pipette.

5. Place the grinders with pestles in a non-metallic rack and microwave on high for 2 minutes or until dry.

3.7 Polymerase Chain Reaction (PCR)

The use of the polymerase chain reaction (PCR) (Saiki et al., 1985) to increase the number of DNA molecules in a sample from subanalytical quantities to detectable levels so that routine diagnostic methods can be applied to detect genetic polymorphisms has augmented the capability of the forensic
laboratory. The main quality of PCR is the ability to obtain relatively large amounts of specific DNA sequences from relatively small (picogram or nanogram) quantities of DNA. Thus, PCR should be thought of as nothing more than a sample preparation technique. Additionally, degraded DNA samples (for example, forensic samples, museum samples, biopsy samples, etc.) can be amplified by PCR and subsequently typed. Moreover, PCR analysis takes only a few days for an assay result, and PCR systems are generally amenable to automation. A typical PCR is based on the annealing and extension of two oligonucleotide primers that flank a specific target DNA segment. Generally for PCR to work, information about the target DNA sequence (at least the flanking region) is necessary for primer synthesis. Primers are single-stranded, DNA oligonucleotides usually of 20 to 30 base pairs in length that can be obtained commercially or synthesized in-house. The template DNA to be amplified by PCR is denatured by heating the sample to approximately 95°C using a thermal cycler. After denaturation of the double-stranded DNA molecules, each primer hybridizes by complementary base pairing to one of the separated strands. Primer annealing is accomplished by lowering the thermal cycler temperature to between 37° and 72°C; the choice of annealing temperature generally is dictated by the sequence of the primers. Usually the specific annealing temperature is empirically determined for each primer set/target region combination, although computer programs are available to assist in this determination.

The next phase in the PCR cycle, primer extension, is generally carried out at 72°C, the temperature at which *Thermus aquaticus* (Taq) DNA polymerase, a commonly used thermostable DNA polymerase, can most effectively extend the primers. These three steps (denaturation, primer annealing, and primer extension) represent a single PCR cycle. When the newly synthesized strand extends through the region that is complementary to the other primer, it can serve as a primer binding site and template for a subsequent PCR cycle. Upon repeated cycles of denaturation, primer annealing, and primer extension, an exponential accumulation of a discrete DNA fragment is generated. By repeating the cycle typically 25 to 30 times, millions of copies of target sequence can be obtained.

PCR, in principle, is easy to accomplish. One needs only a DNA template sample, primers, a mixture of four dNTPs, a buffer, and a thermostable DNA polymerase. All ingredients are placed in a reaction tube and inserted into a thermal cycler which enables a programmable change in temperature in the reaction tubes. Routinely, PCR can be carried out in this manner in one to two hours.

Initially PCR amplifications were reported using the DNA polymerase I Klenow fragment from *E. coli* (Saiki et al., 1985). Because of the need to denature the double-stranded DNA template after each PCR cycle at high temperature, the DNA polymerase I Klenow fragment was denatured and could not synthesize DNA in subsequent cycles. Therefore, fresh DNA polymerase had to be added to the PCR after each denaturation step. The discovery of thermostable DNA polymerases greatly facilitated the PCR. The use of thermostable polymerases, such as Taq, eliminated the need to add fresh enzyme after denaturation in the thermal profile and enabled automation of the PCR with thermal cycling machines (Saiki et al., 1988). An ancillary benefit of using thermal stable polymerases is that the primer annealing and elongation steps of a PCR cycle can be carried out at temperatures higher than those used with the Klenow fragment. Higher temperatures significantly increase the sensitivity and specificity of the PCR.

The following are primer sequences that have proven effective for amplification of HV1 and HV2 of the D-loop of mt DNA.

**HV1**

L15997 5'-CAC CAT TAG CAC CCA AAG CT-3'
H16255 5'-CTT TGG AGT TGC AGT TGA TG-3'
L16159 5'-TAC TTG ACC ACC TGT AGT AC-3'
H16395 5'-TGA TTT CAC GGA GGA TGG TG-3'

**HV2**

L034 5'-CAC CCT ATT AAC CAC TCA CG-3'
L047 5'-CTC ACG GGA GCT CTC CAT GC-3'
H285 5'-GGG GTT TGG TGG AAA TTT TTT TG-3'
H408 5'-CTG TTA AAA GTG CAT ACC GCC A-3'
These primers can be used in any combination to amplify portions of the D-loop region, as long as one light and one heavy primer are used in a PCR. It should be noted that smaller size fragments tend to amplify better than large DNA fragments.

To carry out PCR on mt DNA with the above primers, the following conditions can be used.

### PCR reagents
- 10× Perkin-Elmer PCR buffer: 2.5 µl
- Each dNTP (10 mM stock): 0.5 µl
- Each primer (10 µM stock): 0.5 µl
- BSA (1.6 µg/µl): 2.5 µl
- Taq polymerase: 5 units
- DNA template: 10 µl of extract
- qs with H$_2$O to 25 µl

### PCR conditions
- 95°C: 10 seconds
- 60°C: 30 seconds
- 72°C: 30 seconds

Amplify for 36 cycles.

### 3.8 Sequencing

After PCR, the sequence of the amplified product can be determined by the chemical cleavage method (Maxam and Gilbert, 1977) or the chain termination method (Sanger et al., 1977). Since the Sanger method is easier and simpler, it has become the method of choice for sequencing and therefore will be discussed further.

Certain reagents are required to perform Sanger sequencing. These include: a purified, single-stranded DNA template, an oligonucleotide primer, four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), four dideoxyribonucleoside triphosphate analogues (ddATP, ddCTP, ddGTP, and ddTTP), a DNA polymerase, and a buffer. In the process an initiation primer is annealed to a purified single-stranded DNA template (PCR can be used to generate single-stranded DNA templates—for example, see discussion of cycle sequencing below). The primer is extended across the target sequence by the action of a DNA polymerase in the presence of the four dNTPs and one of the ddNTPs. The ddNTP in the sequencing reaction competes with its dNTP analog for incorporation into the newly synthesized DNA strand. However, ddNTPs do not have a 3’ hydroxyl group which is necessary for phosphodiester bond formation, as do dNTPs. Hence, chain elongation is terminated at the point where a ddNTP has been incorporated. Traditionally, the sequencing reaction is carried out in four separate tubes, with each tube containing a different ddNTP. Thus, the fragments in one tube will correspond to a particular base. Generally one of the dNTPs is radiolabelled, and hence all sequencing fragments are internally labelled prior to separation in denaturing polyacrylamide gels. The reaction mixtures are placed in separate adjacent lanes on a high resolution, denaturing polyacrylamide gel and separated by electrophoresis. The result is a ladder of fragments, differing in length by one nucleotide, and after autoradiography the DNA sequence of the amplified target DNA can be determined. This type of sequencing is known as manual sequencing and is time-consuming and labour-intensive, particular for data interpretation.

Instead of manual sequencing, PCR amplified DNA templates can be sequenced through the use of an automated sequencer (Figures 3.6 and 3.7). Although the use of manual sequencing is currently more widespread, automated sequencers provide high throughput and ease of data management through accompanying computer software.

Fluorescently-based automated DNA sequencing was introduced by Smith et al. (1986). In these sequencing reactions, oligonucleotide primers or ddNTPs labelled with a fluorescent dye are
incorporated into the synthesized DNA fragment which is then electrophoretically separated and detected during separation by laser-induced fluorescence. The temporal, sequential passage of these fragments past a detector window enables a determination of the sequence of the template. Since each terminator or primer for each ddNTP reaction can be uniquely labelled (by using different fluorescent dyes), all four termination reactions can be performed together (in the case of labelled ddNTPs), or performed separately and then pooled together (in the case of labelled primers). In either case, the sequencing products are loaded on a sequencing gel in a single lane. Automated sequencing with fluorescent tags allows co-electrophoresis of each of the four termination reactions, thereby reducing the number of lanes which must be run per template from four to one. Moreover, the need for radioisotope labelling, as is done in manual sequencing, is eliminated. For these reasons this approach is attractive to forensic science (Hopgood et al., 1992).

In order to perform sequencing of PCR-generated DNA templates, the DNA template traditionally had to be single-stranded. There are a number of approaches for preparing single-stranded DNA from PCR products. For example, a single-stranded mt DNA template can be generated by modifying the amplification process by asymmetric PCR (Gyllensten and Erlich, 1988). Sullivan et al. (1991, 1992) described a two-round asymmetric PCR procedure for preparing single-stranded mt DNA where, in the first round series of cycles, the entire D-loop is amplified by standard PCR. In the second round, unequal concentrations of nested primers are used during PCR. Because one
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Figure 3.7 Confirmatory sequence information can be generated by directly comparing the light strand (7035-C) of human mt DNA with the reverse complement of the heavy strand (7035-D) of the same region sequenced with a separate primer. Automatic reverse complementary alignment simplifies handling of sequence data, and allows rapid confirmation of template sequence. Differences from the Anderson reference sequence are highlighted by an asterisk of the nested primers is in excess concentration compared with the other nested primer, one of the PCR product strands is amplified preferentially.

However, a technique known as cycle sequencing has more recently been shown to facilitate sequencing using double-stranded DNA templates. Cycle sequencing combines the dideoxy terminator chemistry with the physics and sensitivity of PCR (Carothers et al., 1989). The DNA templates are cyclicly denatured and annealed with a sequencing primer which is extended in the presence of standard PCR reagents. Each fragment is uniquely labelled, as discussed previously. With each cycle, the labelled fragments are denatured and removed from the template, which is available for another subsequent primer annealing, extension and termination cycle. Thus, each template molecule is used repeatedly to generate a new sequencing product with each cycle. The sequencing product is thereby amplified, resulting in a greater quantity of sequence fragments from a limited amount of the DNA template. Because the DNA template is thermally denatured prior to primer annealing, double-stranded DNA can be used as a template in cycle sequencing reactions. This advantage obviates the need to generate single-stranded DNA from amplified PCR products prior to sequencing, thereby simplifying sample preparation. Cycle sequencing can be employed in both manual and automated formats.

3.9 Dot Blot Typing

An alternative method for typing mt DNA can be accomplished by using specific oligonucleotide hybridization assays (Stoneking et al., 1991). This approach is a simple assay based on a dot blot format. The amplified mt DNA is immobilized onto a nylon membrane and the presence or absence of a particular sequence is determined by hybridization with a sequence-specific oligonucleotide probe. Under appropriate conditions, the probes hybridize only to mt DNA sequences which contain their exact complement. Thus, a different probe is required for each variant that will be detected in the mt DNA fragment. The presence of the allele in the amplified product is determined via an identifier molecule (or tag) on the 5’ end of the probe after hybridization to the immobilized PCR product.

More recently, this dot blot system has been improved to accommodate a large number of probes by converting it to a reverse dot blot format (Saiki et al., 1989). In this situation, the different sequence specific probes that detect each variant of a particular locus are fixed to a nylon membrane strip, and
the amplified product is allowed to hybridize with the immobilized probes. The types carried in the sample are identified via a tag on the 5’ end of one of the primer sequences used for PCR. The cumbersome nature of a test with a number of probes is greatly reduced because the amplified material is hybridized with all the probes in a single hybridization.

3.10 Forensic Applications

Kalbe et al. (1988) and Schreiber et al. (1988) demonstrated that high molecular weight DNA can be recovered from multiple hair shafts. However, very little DNA is contained within a single hair shaft (Higuchi et al., 1988).

Mitochondrial DNA typing, using a variety of methods, has proved to be valuable as a means for human identification of forensic-type samples (Ginther et al., 1992; Higuchi et al., 1988; Holland et al., 1993; Hopgood et al., 1992; Örrego and King, 1990; Pääbo, 1989; Pääbo et al., 1988, 1989; Stoneking et al., 1991; Sullivan et al., 1992). In fact, sequencing of amplified mt DNA has been sought and been found successful on such items as 7000-year-old brain tissue (Pääbo et al., 1988), 5500-year-old bone (Pääbo et al., 1989) and 4000-year-old mummified tissue (Pääbo, 1989). Higuchi et al. (1988) have sequenced amplified mt DNA from a single hair root. Additionally, Sullivan et al. (1991, 1992), have been able to type successfully mt DNA in hair, bone fragments, and necrotic skin.

At many crime scenes, hairs may be the only forensic evidence to associate the perpetrator with the crime. The incorporation of DNA typing into the forensic analysis of hairs can augment the probative value of hairs as forensic evidence.

Due to the large number of hairs routinely found at a crime scene, it will still be necessary to conduct a microscopic examination of the hairs from the crime scene. After a hair from a crime scene is examined microscopically, and it is determined to have similar characteristics as a known hair, DNA typing of the hair(s) can be performed.

In a major case the forensic scientist may still be faced with the daunting prospect of having to determine the mt DNA ‘type’ of many hairs. Tully et al. (1996) argue that mt DNA analysis is labour-intensive and hence expensive, and as an answer have developed multiplex solid-phase minisequencing as a less expensive tool for exclusionary testing in forensic analysis. They estimate that for samples excluded by a minisequencing screen the cost savings would be in the order of 60 per cent, with an 80 per cent improvement in turnaround time.

The advent of DNA typing of hairs also may provide opportunities to characterize hairs that previously were unsuitable for substantial microscopic examination. Microscopic examination and comparison of hair characteristics usually has been limited to head and pubic hairs. Hairs from other parts of the body were not amenable to characterization. For example, in a hit-and-run accident limb hairs from the victim might be found on the bumper of the perpetrator’s car. Generally, microscopic examinations on these hairs will result in limited associative value. However, DNA typing of these hairs is possible.

Generally, in humans mt DNA is considered homoplastic. However, with better technology and more samples being typed, examples of heteroplasmy in humans have been observed. For example, heteroplasmy was observed at position 16,169 of the mt DNA control region in the putative remains of Tsar Nicholas II of Russia (Gill et al., 1994). The mt DNA sequence analysis of the remains of the Tsar’s brother, Grand Duke of Russia Georgij Romanov, also demonstrated heteroplasmy at position 16,169 (Ivanov et al., 1996). Comas et al. (1995) detected heteroplasmy at two positions (16,293 and 16,311) in the mt DNA from a plucked hair from an anonymous donor. Wilson et al. (1997) observed a family that carried an mt DNA heteroplasmic state at position 16,355. In this family of a mother and two children blood and buccal swab samples demonstrated the heteroplasmy, and in some cases individual hairs carried either a C or a T at position 16,355. Thus, some hairs appeared homoplastic. It may be that heteroplasmy in humans is not as rare as previously believed. One might suggest that the presence of heteroplasmy should invalidate the use of mt DNA as a DNA marker for forensic comparisons. However, this suggestion would be inappropriate. Most known samples will be blood or buccal swabs, and the cells in these samples are multiclonal in origin. Thus, heteroplasmy most likely will be detected in the known sample. If a hair sample contains one of the two heteroplasmic lineages, then an
interpretation of an exclusion would be incorrect. Although it is not anticipated to be as likely an occurrence, the same interpretation holds true for a heteroplasmic hair and a homoplasmic blood or buccal swab. If hairs are used for both the known and questioned samples and a difference of, for example, one nucleotide is observed in the mt DNA sequences, then failure to exclude would still be the proper interpretation. However, it might be desirable to type additional known hairs or to obtain a known sample more likely to demonstrate the heteroplasmic, if present.

mt DNA sequencing is a viable technique for human identification testing. As with all forensic identification techniques, issues related to reliability and reproducibility must be addressed. Automated sequencers offer high throughput and ease of data handling, but also introduce issues related specifically to this technology. Replication of sequencing runs, which independently confirm nucleotide designations, is a good way of overcoming sequence ambiguities. As many different methods exist to sequence DNA, templates should be shared among laboratories, and the results should be independently assessed for reliability.

Validation studies must be conducted on any new forensic technique. As the chemistry of DNA is very well understood, these studies should be confined to examining the direct influence of common environmental agents (e.g. dyes and bleaches) on human hairs.

Statistical interpretation of mt DNA sequence information is straightforward. A calculation of the frequency of a sequence is simply the number of times the particular sequence has been observed within a specified region, divided by the number of samples in the appropriate database. Where sequencing ambiguities arise, these positions can be considered non-fixed, and all possibilities can be included in the statistical calculation. However, meaningful frequency estimates require that reference databases containing DNA sequence information be free from ambiguities within the region of comparison.

We propose some basic guidelines, as follows.

1. A common nomenclature should be adopted which simplifies recording sequence polymorphisms by listing only deviations from a reference sequence and assumes all other positions are identical to the reference.

2. Extracts should be amplified in duplicate whenever feasible.

3. Amplicons should be sequenced in duplicate, either by sequencing the complementary strand of the duplex DNA molecule or by additional sequencing of the same strand.

4. A common nomenclature for primer designation should be adopted. At present, mt DNA primers are listed by the strand (H for heavy and L for light) followed by the numeric designation from Anderson et al. (1981) of the 3' ending base of the primer.

5. Laboratories should share template DNAs for interlaboratory comparison of sequence information. This will facilitate validation of numerous sequencing techniques, ensuring that quality control can be maintained regardless of a laboratory’s methodology. Independent, non-forensic laboratories should be included in this effort to implement consensus quality control measures.

6. A human mt DNA database from HV1 and HV2 of the D-loop should be compiled from existing published sequences and ongoing research. When feasible, DNA samples from contributors of published mt DNA control region sequences found in the literature should be obtained and sequenced by forensic laboratories to ensure interlaboratory quality control and fidelity of the forensic mt DNA database.

It should be stressed that mt DNA sequencing is an analytical procedure which the forensic scientist must understand in its proper context. In routine forensic cases where nuclear DNA is extracted from a sample, polymorphic nuclear markers such as DQα and short tandem repeat loci can and should be
utilized. These markers should take precedence over mt DNA analysis. Not only are such markers currently more statistically informative than mt DNA, they are also less susceptible to contamination. However, mt DNA can and should be the method routinely used with certain classes of biological evidence which by nature have very little DNA, such as telogen hairs, hair shafts, bones and teeth.

In conclusion, molecular biology tools have revolutionized the way the forensic scientist can analyze biological evidence. The PCR now makes it possible to characterize at the DNA level samples that contain small quantities of DNA, such as hair shafts. It can be anticipated that further advances in molecular biology will be used to facilitate the analysis of forensic biological evidence.

This is publication number 94–13 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

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Elemental Analysis of Hair for Forensic Application—a Personal Journey

JOHN GOULDING

4.1 Introduction

The promise that ‘sometime in the future we shall not only be able to say that hair found at the scene belonged to X but that it left his head on a certain date’ might with the benefit of hindsight seem to have been a somewhat overstated assessment of the potential of the application of elemental analysis for forensic hair examination when it was made by Stuart Kind in 1963 (Cornelius, 1973). Certainly, by 1985, Gaudette was of the view that ‘the future of trace element analysis in forensic hair comparison is somewhat limited’.

The literature dealing with elemental composition in human hairs, especially as it relates to non-forensic applications in environmental and health studies, is extensive. There have been numerous reviews and books in which specific aspects of the topic have been considered. It is critical that a clear distinction be drawn between the application of elemental analysis in forensic science and in other fields of endeavour, especially medical science. While some of the issues are of common interest, much of the scepticism about the value of elemental analysis would seem to stem from its use as an indicator for disease conditions. For example, in a comprehensive review prepared on behalf of the Trace Element Laboratories Sub-committee, UK Supra-regional Assay Service, Taylor (1986) drew attention to a number of difficulties and limitations including:

- problems with inter-laboratory studies in achieving reliable and consistent analytical measurements
- difficulties with the interpretation of analytical results as a result of variation resulting from factors such as age and sex of subjects, colour and growth site
- difficulties in controlling and evaluating the influence of external contamination
- lack of correlation between elemental concentrations in hair and in metabolically important tissues.

Taylor concluded that with few exceptions, such as extreme exposure to methyl mercury, trace element analysis of hair is not a useful procedure and may even provide misleading data. Dormandy (1986) has stated that ‘much of what is “known” about biological trace elements is a mixture of improbable fact and plausible nonsense’. He concluded that ‘in general, just as the feasibility of a surgical operation is no indication for its performance so the availability of an atomic emission spectroscope is no justification for feeding it with hair digests!’ Regrettably, application of elemental analysis in the field of commercial hair analysis has attracted such scathing criticism as to bring the whole area of the use of elemental analysis under serious doubt (Barrett, 1985).
Given this somewhat depressing backdrop, is there any value in considering elemental analysis for forensic application? Having spent the past 22 years as the sole forensic activation analyst in Australia and having applied elemental analysis in over 150 hair cases, I am perhaps not an unbiased observer, but then, is there such a species? What I shall attempt to do in the remainder of this chapter is to present some of the available information which will assist the reader to reach his or her own conclusion.

4.2 Occurrence of Elements in Human Hair

In the order of 27 elements have been identified in hair (Valkovic, 1972), ranging in concentration from less than 1 ppm to several hundred ppm. Pankhurst and Pate (1979) detail 46 elements in human hair. Elements which have a physiological role are often present in the highest concentrations. These include sodium, phosphorus, potassium and sulphur. Cadmium and zinc are also present at high levels. Some of the remaining elements may be considered non-essential and are acquired by the body as environmental contaminants, particularly toxic elements such as arsenic, cadmium, mercury and lead. There is considerable variation in the levels of trace elements from even ‘normal’ healthy human beings. This variation exists within individuals (both in hairs from different areas of the scalp and within single hairs) and also between individuals (Robertson, 1987). The variation between hairs holds out the opportunity and basis for individualizing hairs from one person. If the overall variation in hairs from different individuals was of the same order as that found within a single person, then no meaningful conclusion would be possible. That this is not the case is demonstrated by the work of Pillay and Kuis (1978). Early studies by Perkons and Jervis (1966) and Jervis (1966) using bulk hair samples also support the conclusion that there are sufficient differences in trace elemental composition to provide individuality. Obrusnik et al. (1973) examined segments of single hairs and showed that of nine elements measured, six showed variation between hairs comparable in magnitude to that found in the population at large. However, with cobalt, zinc and mercury, the variation in concentrations between hairs from individuals was much smaller than in the general population. These workers concluded that although the basic criterion for the application of elemental profiling for individualization had been met for these three elements, further verification was required. A similar conclusion was reached by Dybczynski and Boboli (1976) where often elements studied, only copper and zinc showed acceptable variation from one person to another. Renshaw et al. (1973) confirmed that for copper and lead, variation within hairs from a single person was about half that found in the population in general.

Perhaps not surprisingly, Seta et al. (1988) found that variation in the level of ‘biological elements’, phosphorus, potassium and sodium, did not appear to provide much promise of individualization.

In general it may be safe to conclude that there is at least the basis to support the fundamental requirement that variation between individuals exceeds that found within an individual for some elements. These are likely to be in the non-essential category. However, the story is unfortunately much more complex than merely meeting this fundamental requirement. A number of factors have the potential to influence results. Some of these factors relate to the way in which elements enter the hair (endogenous elements), while others relate to external or exogenous elements. Each needs to be considered in turn.

4.2.1 Factors Affecting Endogenous Elements

According to Taylor (1986), factors such as age, sex, site of growth and colour of hair are all associated with variations in the concentrations of trace elements in hair.

The effect of the sex of an individual is not at all clear. Some studies have indicated that sex can be a determining factor. Creason et al. (1975) found that there were greater concentrations of many trace elements in the hair of female donors than of males. They proposed that this might be explained by the
higher inorganic content of female hairs. Other studies have either supported the above finding (Schroeder and Mason, 1969; Nomoto and Sunderman, 1970; Spruit and Bongaarts, 1977; Ryan et al., 1978) or found no differences based on the sex of the donor (Taylor (1986) and references cited therein).

Across a number of elements no clear picture emerges, and the work of Creason et al. (1975) is, in general, not supported by other studies.

The situation with regard to the age of donors is even more complex. It is beyond the scope of this chapter to deal with the considerable literature relating to this factor. The reader is referred to Taylor (1986). Suffice it to say that from a forensic perspective, there is no clear trend for a wide range of elements that age is, in itself, a major determinant. Any changes which take place appear to be over quite lengthy time periods involving years. Few studies appear to have been carried out specifically aimed at looking at short-term variations. It is this type of change that would be of most interest and perhaps concern to forensic scientists. The forensic scientist needs to be aware that changes in elemental composition over short time frames cannot be discounted, and the most meaningful comparative analysis will be where samples have been taken or recovered within a relatively short time of each other. Where this is not the case, caution should be exercised in interpreting differences.

Schroeder and Mason (1969) first reported on the relationship between hair colour and trace element content. Samples were collected from 181 subjects ranging in age from 10 months to 102 years. Seven elements were measured. The relationship between colour and elemental composition was quite complex, with blond hair having higher levels of zinc than those found in brown and black hair, but lower levels of other elements than brown and black hair. There were other differences between brown, black and red hair. For copper and chromium, colour was complicated by the interacting influence of other factors such as the age and sex of the donors.

Sky-peck and Joseph (1983) eliminated the influence of sex of the donor by examining hairs from women only. Samples were taken from 458 subjects between the ages of 3 and 70 years. Colour appeared to have no influence on a number of elements including arsenic, bromine, calcium, chromium, manganese, rubidium, strontium and sulphur. Iron, nickel, copper and zinc were high in red hair compared with brown, whereas mercury levels were lower. Differences were also seen between blond and brown hairs. Taylor (1986) also reported on the results of a number of more limited studies in which the influence of hair colour on the levels of elements was seen.

The literature relating to the possible influence of racial origin in elemental levels is also dealt with by Taylor (1986). Whether or not racial origin has any real cause-effect relationship is not at all clear. In one study (Sky-peck and Joseph, 1983), significant differences in a number of elements were reported where hairs from Caucasian, black and oriental subjects were tested. For example, lead levels in black subjects were 5 and 18 times those of samples from Caucasian and oriental subjects. A number of authors have proposed that apparent racial variations may in fact be related to different hair type and colour (Cherry, 1981; Sky-peck and Joseph, 1983).

It would appear that it may be overly simplistic to conclude that racial origin is the real cause of variations in elemental composition between individuals.

In conclusion, from a forensic perspective, the actual cause-effect relationship which may result in variations in elemental composition in hairs from different individuals is not, in itself, of prime importance. What is clear is that there are a number of quite logical reasons why it might be expected that variations will occur in the human population. Although it would be preferable to have a better understanding of the underlying cause-effect relationships, our limited understanding should not inhibit us from having confidence that the variations we see are real. In the field of genetics, we do not allow ourselves to be put off from relying on what we see, variations in phenotype, just because we do not always know the precise underlying genotypic reasons. None the less, more studies aimed at elucidating the underlying causes of variations in endogenous elements would be highly desirable.

In this section, it has been assumed that the elements being measured are indeed endogenous. The next section deals with the question of whether this is a valid assumption.
4.2.2 Factors Affecting Exogenous Elements

Dormandy (1986) comments that ‘an additional difficulty in interpreting analytical results is the tendency to find ambient rubbish; it clings to metals with a special tenacity’.

The ability to determine whether or not the elemental composition of a hair is the result of endogenous elements or includes a component of exogenous elements is fundamental in assessing the validity of the application of elemental analysis for forensic purposes. It is this question more than any other, and the inability to answer it satisfactorily, that has undermined, and continues to undermine, the credibility of elemental analysis for broadly based applications.

In order to attempt to answer this question, it is necessary to understand the factors which may contribute to the trace element composition of human hair. In Chapter 1, a very detailed coverage of hair growth and its physiology is given. The growing hair follicle has an abundant supply of blood vessels and the hair produced within the follicle should mirror the components present in the blood. From a purely forensic standpoint, it is not of great concern whether or not there is a meaningful correlation between blood or other tissue levels and those seen in the hair. This is the concern of clinical medical practitioners (Taylor, 1986) who might wish to use hair as a test ‘tissue’ because of the ease of collection.

From a forensic perspective, hair has an additional advantage in that analysis over the length of a hair will reflect changes in the elemental status of the donor over a time period of weeks and months. Scalp hair grows at about 1 cm per month. Elements accumulated during growth will remain in situ at their point of deposition.

So far, so good!

The problem which arises is that hair is on the external surface of the human subject and, as such, it is exposed to environmental factors. These may relate to the grooming regime used by the individual involving everything from shampoos to hair gel, e.g. elements such as zinc, mercury and selenium as found in anti-dandruff formulations or heavy metals from hair tints and colourings.

In addition, trace elements may arise from external contamination from soils and dust in the air which, when mixed with perspiration, settle on and lodge under the microscopic scales of the hair shaft. These external elements do not necessarily remain on the surface of the hair shaft. Pötsch and Moeller (1996) have shown that rhodamine B, applied to hairs as either aqueous solution or methanol/ethanol solution, enters hair fibres at the scale edges between the cuticle cells. Further penetration into the cortex occurs and this is greater for aqueous than for alcoholic solutions. Movement into and out of the hair fibre is facilitated by ultrastructural damage to the hair fibre such as might be caused by cosmetic treatment of hairs. Pötsch and Moeller (1996) conclude that reliable interpretation of drug monitoring of distal hair segments far away from the scalp or hair samples, or both, with cosmetic treatment seems to be even more difficult. What is true for drugs and for dye interactions with hairs will also be true for the movement of elements in solution, or carried in sweat, into and out of human hairs.

Maes and Pate (1976) found increasing levels of copper away from the root, and have suggested that the level of copper reflects increasing contributions from external sources and not from dietary copper entering the hair from the follicle. Seta et al. (1988) have shown elevated calcium levels in hairs which have been subjected to permanent waving. Andrasko (1984) has further investigated calcium levels along the hair shaft, and shown increases corresponding to treatment times. Studies by Bate (1966) and Van der Berg et al. (1967) have shown that manganese and sodium exchanged in both directions, while other elements did so at reduced reaction rates. Taylor (1986) discusses a number of other papers in which longitudinal variation in elemental composition in human hairs has been found. These have included cadmium, chromium, lead, mercury and zinc. Taylor (1986) concludes that such longitudinal variation may reflect anatomical changes which develop naturally along the shaft of the hair. However, it is considered more likely that the variations are the consequence of general environmental exposure and contamination.

Clearly, it would be unsatisfactory for forensic applications if elemental profiles were a mixture of endogenous and exogenous elements with no way of, at least, reliably standardizing their relative contributions. It is probably fair to conclude that regardless of the procedure or protocol adopted, it
will be impossible to remove completely and reliably all exogenous influences. Indeed, it may be that the exogenous elements are forensically significant and their removal may be undesirable in some circumstances. The aim of any forensic protocol must be to produce an analyte (treated hair) which will give reproducible and reliable measurements. Absolute levels of elements will be less important.

The remainder of this chapter deals with the approach used by the author, with an assessment of instrumental techniques applied to elemental analysis.

4.3 Procedures and Protocols

4.3.1 Selection of Hairs Suitable for Analysis

Before a chemical profile can be carried out, the questioned hair’s growing stage must first be identified.

This chapter will not describe in any detail the growth cycle of human hair, except to say that hair has basically three growth phases: anagen, catagen and telogen. The first phase is the growing stage of the hair and is easily identified from its root morphology. This hair type has been found to be the most reliable for trace element analysis, as it most accurately reflects the current trace element profile of a person’s hair.

Anagen hairs can be identified by close examination of the root. A bulbous, club-like end indicates that the hair was not growing at the time of sampling. Hairs in the anagen growth phase will have roots of flattened appearance. In many cases, these hairs will also have a sheath of cellular material close to the root.

Since hairs from different parts of the body have different growth cycles and are subject to different conditions, each hair type can exhibit a different trace element profile. Head hair is generally longer, subjected to increased outside contamination (hair spray etc.) and has a different growth cycle to those growing in the pubic region. Pubic hairs are less likely to have hair treatments applied, and this hair is generally protected from its environment by clothing. It is essential that only like be compared with like (pubic with pubic, head hair with head hair, etc.).

To recap: hairs grow and thus will change with time. Head hair is subject to cosmetic treatments, haircuts, specialist shampoos and the like, all of which will affect its trace element content. Thus it is important to determine where on the body the hair originated and whether it was growing at the time.

4.3.2 Collection and Storage of Hairs

Under ideal conditions, the hairs to be analyzed should be sampled under conditions laid down by the IAEA Advisory Group on Applications of Nuclear Methods in Environmental Research (IAEA, 1977)—see Appendix 3. However, these conditions are laid down for ideal analytical conditions where the chemist has full control over the samples to be analyzed. The forensic chemist rarely has this luxury.

The correct collection and storage of hairs is critical to the final analysis. Known hairs must be plucked and the samples need to have been taken within a week of the alleged event (preferably the same day), since hair can be significantly altered in an intervening period and thus preclude a meaningful comparison. The hairs sampled should be of similar length (therefore will have a similar exposure/growth period on the body) to recovered or questioned (crime) hairs.

Since each individual hair has its own growth cycle it acts as a unique entity for the accumulation of its trace elements. Thus a representative sample must be collected to reveal the range of concentrations present over the region sampled. The author’s experience has shown that a minimum of 20 hairs must be sampled.

The recovered, questioned or evidence hairs must likewise be complete and in the anagen growth phase.
All hairs should be air-dry and stored under moderate conditions, preferably within a sealed polyethylene bag. All hairs to be compared must be stored under comparable conditions in comparable containers. Hair sealed in an airtight container has been found to remain reasonably constant in trace element concentration. However, hair stored for an extensive period, particularly in paper envelopes, has been found to lose weight (due to moisture loss): as a consequence, the trace element mass, in relation to the sample, increases, yielding a more concentrated trace elemental result in that hair. Since hair submitted for analysis has often been collected by different persons, not necessarily following a standard approach, it is important that the analyst immediately ensures that all samples are transferred to identical sterile containers.

In summary:

- hair must be sampled complete with the root
- the hair must be in the anagen (growing) phase
- hairs of similar length from the same area of the body must be chosen
- sampling must be done as soon after the event as possible, since the trace element content of hair may alter with time and conditions.

4.3.3 Pre-analysis Preparation

Sample Washing

Having received acceptable samples of hair, a decision has to be made whether to analyze the hair for its endogenous content only or the whole sample, including any contaminants that may be present. Often a person’s profession can influence the type of contamination present (they might work in a gold mine, or live near a lead smelter). Chittleborough and Steele (1980) supported a no-wash policy for environmental and forensic studies. However, most authors in the past have chosen to wash the hair in order to attempt to remove contamination. This raises the question of how and with what the sample should be washed.

Table 4.1 lists some of the various washing processes published.

There are many more ‘recipes’ throughout the literature. Taylor (1986) considers some of these.

The International Atomic Energy Agency (IAEA) recommends for neutron activation analysis that the hair be washed using 10 minute contacts with 25 ml portions of acetone, water, water, water and acetone successively. Other workers have tried ionic and non-ionic detergents, chelating agents such as EDTA and organic solvents (Salmela et al., 1981). McKenzie (1978) has suggested that a short, cold wash with 0.1 mol/l EDTA removes only external contamination. However, a more generally held view is that EDTA is too aggressive and will lead to the removal of endogenous

<table>
<thead>
<tr>
<th>Washing process</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether followed by detergent and water</td>
<td>Gordus (1973)</td>
</tr>
<tr>
<td>Benzene/water</td>
<td>Dybczynski and Boboli (1976)</td>
</tr>
<tr>
<td>Soak with water, then methanol and rinse</td>
<td>Edds and Lambdin (1973)</td>
</tr>
<tr>
<td>Ether reflux</td>
<td>Coleman et al. (1967)</td>
</tr>
<tr>
<td>Hexane/ethanol/water</td>
<td>Hambridge et al. (1972)</td>
</tr>
<tr>
<td>Carbon tetrachloride wash</td>
<td>Schroeder and Mason (1969)</td>
</tr>
<tr>
<td>Acetone/ether/water</td>
<td>Petering et al. (1973)</td>
</tr>
<tr>
<td>Water/acetone</td>
<td>Verghese et al. (1973)</td>
</tr>
<tr>
<td>Triple distilled water</td>
<td>Valkovic (1977)</td>
</tr>
<tr>
<td>One per cent non-ionic detergent/water</td>
<td>Weiss et al. (1972)</td>
</tr>
</tbody>
</table>
elements (Taylor, 1986, and references therein). All washing procedures are likely to extract varying quantities of the endogenous elements, particularly the soluble ones such as sodium and potassium (Taylor, 1986). Thus a strict regime of empirical washing times and styles must be employed to minimize the variation in the extraction rate of the mobile elements.

The author has always used a sequence of two 1 minute washes with a non-ionic detergent followed by seven 1 minute washes with ultra-pure distilled water. This is strictly adhered to. Detergent is preferred because of the premise that normal hair shampoo is detergent-based and is the normal treatment used by the population to wash their own hair. Remembering that the analysis being carried out is generally a comparison analysis, the suspect and source hairs must be treated identically.

Sample Drying

Following this washing technique, it is important that the hair be dried in a non-invasive manner. It has been found that the best reproducibility is obtained when the hair is allowed to dry at ambient temperature and in an area adjacent to the balance used to weigh the sample mass prior to analysis. If dried too vigorously, such as in a dessicator, or a warm oven, hair will accumulate moisture while its mass is being estimated. This results in a long and tedious weighing exercise, with each sample of hair continuously increasing in mass until an equilibrium is reached with the surrounding atmosphere.

Cleaning of Laboratory Containers

The probability of introducing unwanted contamination to trace elemental analyses is considerable, and thus the purity of reagents and the cleanliness of all laboratory plasticware and glassware must be taken into consideration. Some of the most sensitive laboratory procedures (ICPMS, Carbon Furnace AA, etc.) require the prior dissolution of the sample and the subsequent analysis of that resultant solution. To ensure that there is minimal contamination, the following cleaning procedures are applied for all reaction/dissolution vessels.

All plasticware is first soaked in methyl isoburyl ketone (MIBK) for 24–48 hours to leach out miscellaneous organic and chelated materials (if any). This is followed by 48 hour immersion in 5M hydrochloric acid solution to dissolve trace metals adhering to the surfaces of the containers. Following this, another immersion in MIBK is carried out and the item is rinsed thoroughly with double distilled water. If the containers are small, they must be stored under double distilled water until use. Larger vessels must be stored full of the pure water. When one is about to use the container, it is emptied and immediately used for the laboratory function required.

Glassware often contains surface contaminants, thus a 1 hour soak in dilute 5 per cent hydrofluoric acid may be required prior to the normal cleansing method to remove physically the ‘contaminated’ outer layer of the glass.

Glassware is routinely soaked for 12 hours in contact with aqua regia (1:3 HNO₃:HCl) followed by total immersion in 5M HCl for 48 hours. Glassware is then thoroughly rinsed in double distilled water and stored in such a way as to be totally immersed in the ultra-pure water or completely filled with the water until it is ready to be used.

Practice has shown that with all containers (glassware in particular) it is always wise to check thoroughly for a blank reading of the target element prior to use in actual analyses. It has been found that certain beakers, flasks, etc. maintain an unacceptably high blank in spite of thorough cleansing, while others, having been treated identically, produce excellent blank values.

4.3.4 Analysis

Reference Levels for Elements in Human Hair

The general occurrence of elements in human hair is considered in section 4.2, but the question of what could be considered to be the normal range of concentration for elements in human hair is not addressed there: see Table 4.2.
It is not possible to predict a ‘normal’ concentration of any particular element. There are a number of reasons why it is difficult to define normal ranges for elements in human hair. These include the fact that there is no standardization of pre-treatment protocols applied by researchers in this field. Hence, it is impossible to know the relative contribution of endogenous and exogenous levels. Some idea of the average concentrations expected can be gleaned from the ‘standard hair’ prepared by Okamoto et al. of the National Institute for Environmental Studies (NIES) Japan Environment Agency (1985).

In that work, human hair (20 kg) was collected from three barber shops in Tsukuba and Tokyo. It was washed with 0.3 per cent non-ionic detergent in an ultrasonic cleaner, well rinsed with distilled water and dried overnight at 80°C. The dried hair was pulverized in an agate ball mill, sieved, blended and packaged as individual 2 g units which were in turn sterilized by gamma radiation.

The values of sodium and potassium in particular are somewhat lower than those observed by the author over the years. This may reflect the extensive aqueous pre-treatment.

### Table 4.2 Analytical values of human hair (from Okamoto et al., 1985)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean (μg) certified values</th>
<th>SD (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>728</td>
<td>30</td>
</tr>
<tr>
<td>Iron</td>
<td>225</td>
<td>9</td>
</tr>
<tr>
<td>Magnesium</td>
<td>208</td>
<td>10</td>
</tr>
<tr>
<td>Zinc</td>
<td>169</td>
<td>10</td>
</tr>
<tr>
<td>Potassium</td>
<td>34</td>
<td>3</td>
</tr>
<tr>
<td>Sodium</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Copper</td>
<td>16.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Manganese</td>
<td>5.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Mercury</td>
<td>4.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Strontium</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Nickel</td>
<td>1.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.20</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Reference values
(Mean, μg/g)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>250</td>
</tr>
<tr>
<td>Aluminium</td>
<td>240</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>165</td>
</tr>
<tr>
<td>Bromine</td>
<td>90</td>
</tr>
<tr>
<td>Titanium</td>
<td>22</td>
</tr>
<tr>
<td>Lead</td>
<td>6.0</td>
</tr>
<tr>
<td>Barium</td>
<td>3.2</td>
</tr>
<tr>
<td>Selenium</td>
<td>1.4</td>
</tr>
<tr>
<td>Rubidium</td>
<td>0.19</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.10</td>
</tr>
<tr>
<td>Antimony</td>
<td>0.07</td>
</tr>
<tr>
<td>Scandium</td>
<td>0.05</td>
</tr>
</tbody>
</table>

It is not possible to predict a ‘normal’ concentration of any particular element. There are a number of reasons why it is difficult to define normal ranges for elements in human hair. These include the fact that there is no standardization of pre-treatment protocols applied by researchers in this field. Hence, it is impossible to know the relative contribution of endogenous and exogenous levels. Some idea of the average concentrations expected can be gleaned from the ‘standard hair’ prepared by Okamoto et al. of the National Institute for Environmental Studies (NIES) Japan Environment Agency (1985).

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The values of sodium and potassium in particular are somewhat lower than those observed by the author over the years. This may reflect the extensive aqueous pre-treatment.

### Analysis in the Forensic Context

Can hairs be compared on the basis of trace element content? As mentioned above, opinions on this question are extremely varied. In the author’s experience, the answer has been a qualified ‘yes’. When the many conditions governing the receipt of suitable samples have been fulfilled, the presence of unusual elements, or concentrations of common elements, can be used for valid comparison purposes.
Generally, samples taken from the victim/suspect will yield more elements than those taken from the scene, since the sample size is commonly larger than the scene sample.

Comparing hair on a multielemental basis is not a simple task. As previously stated, each hair is an individual with an individual trace element content. In Appendix 1, a number of case histories are presented to attempt to illustrate some of the challenges and complexities faced in this type of comparative analysis. Upon close examination of the examples displayed in Appendix 1, quite varied concentration levels can be seen to exist in the hairs of the five cases.

Often, qualitative results will suffice to exclude the hair in question. Should, however, the qualitative results point to an inclusionary finding, quantitative results may have to be evaluated to support that result (see cases 3–5, Appendix 1).

Single element analysis can be a more straightforward exercise. When considering this aspect of trace element analysis in hair, the question being considered is usually whether or not an alleged victim has suffered some form of heavy metal poisoning. This may have occurred as a result of deliberate administration of the toxin in the case of poisoning (commonly such elements as arsenic), or by ingestion from polluted surroundings, e.g. lead from leaded petroleum products, or flaking paint in old buildings.

The main advantage of hair analysis over other sample types is the durability of hair, which allows it to remain intact long after the softer body tissues have degraded. Depending on the element in question, the hair shaft can reveal such facts as whether the ingestion was acute or chronic, and if acute, sometimes, depending on conditions, how many doses were administered.

In order to evaluate the results of these analyses, one needs to have a knowledge of what constitutes a ‘normal’ concentration level for that element for that individual. This is particularly a problem when environmental pollution rather than deliberate poisoning is being considered. In the case of pollution, levels are often elevated by factors of two or so. With deliberate poisoning, levels can be 10 to 50 times the values commonly encountered. Some examples of single element analysis follow.

**Arsenic Poisoning**

Arsenic can often be found in hair, its concentrations depending on the environment, scalp treatments and diet of the individuals involved. Levels higher than 10 µg/g can be found in affected populations (Jervis and Tiefenbach, 1978). Generally speaking, normal levels are below 3 µg/g. Values above this limit should be viewed with suspicion. The author (Goulding, 1979) has analyzed hair for arsenic in over 20 cases in Australia, and has found that when the victim had been hospitalized (prior to death, often), hair has been an excellent medium to discern the history of arsenic ingestion. If, on the other hand, the victim had received a massive dose of arsenic and died some time before discovery, experience has shown that the exogenous contamination of the hair by the dried, arsenic-rich perspiration completely masked any meaningful pattern that may have been contained within the hair shaft. In spite of rigorous and repeated washing of the hair, this external contamination could only be reduced slightly and could never be removed to the degree that would expose the endogenous arsenic pattern. (See case of P.P.O. in Appendix 2).

By analyzing only anagen hairs, and sectioning the hairs in sections of 0.5 cm, it is possible to determine the history of arsenic ingestion using the approximation of 1 cm hair growth per month and 3 µg/g as a ‘normal’ concentration. (Examples of these analyses are detailed in Appendix 2.)

**Mercury Poisoning**

Chronic mercury poisoning is often found in communities where occupational exposure is high, or where there is an ingestion of contaminated foods such as fish. Hair and tissue can be tested to estimate the exposure of individuals to this toxin.

Within hair, mercury can be found at levels 230–350 times higher than in blood (Skerfving, 1974; Hansen et al., 1983). However, because people can tolerate very high levels up to 300 ppm without exhibiting any symptoms, 200–800 ppm is usually associated with mild to moderate symptoms and severe symptoms are reported in people with 400–1600 ppm (Al-Shahristani and Al-Haddad, 1972). A firm relationship is known to exist between values in the hair and those in the blood.
One must be careful with the interpretation of mercury levels, as external contamination such as dust and creams can be significant. Up to 288 ppm was found for dental assistants and dentists (Lenihan et al., 1973) and levels up to 9220 ppm were found in Kenyans who used a mercury compound as a skin ointment (Dale et al., 1975).

As a toxin, mercury exhibits an average in vitro half-life of 72 days (it varies between 35 and 189 days) (Al-Shahristani and Shihab, 1974).

**Lead Poisoning**

Lead is often requested in a forensic context. It can be related to arsenic poisoning, since a common poison is lead arsenate. It can be a result of environmental poisoning from the flaking of old paint in buildings or from the ingestion of high lead levels in petroleum products. The forensic chemist can be called upon to determine the lead levels in hair in order to confirm or deny claims of this nature in court cases.

Lead occurs in hair frequently at two orders of magnitude higher than that found in the blood of a victim (Habercam et al., 1974; Chattopadhyay et al., 1977); however, lead does not appear to be fixed in the hair shaft in a manner similar to arsenic. Instead, it can be quite variable along the shaft, increasing with distance from the root end (Kopito and Shwachman, 1975).

**Thallium Poisoning**

Thallium detection in hair has been reported (Henke and Bohn, 1968; Kijewski, 1984). (The author has yet to find this element in hair when dealing with suspected poisoning.) Thallium affects the formation of keratin leading to alopecia (Metier and Vock, 1984). This may help explain why little success has been reported in the correlation of the hair and blood levels.

It has been found with low-level exposures such as atmospheric pollution. Brockhaus et al. (1981) studied the environmental load carried by a community living in close proximity to a cement plant that emitted thallium-rich dust. The possibility of external contamination by absorption onto, then into, the hair shaft in this context is significant.

**Methods of Analysis**

A variety of methods of analysis exhibit the necessary sensitivity to detect and measure the trace element content of hair. *No one method is the best suited for hair analysis.*

Such techniques include graphite furnace atomic absorption spectroscopy (GFAAS), emission spectroscopy (ES), X-ray fluorescence (XRF), micro XRF, inductively coupled argon plasma emission spectrometry (ICAP), spark source mass spectrometry (SSMS), energy dispersive X-ray microanalysis (EDX), inductively coupled plasma arc mass spectrometry (ICPMS), and the nuclear-based analyses of neutron activation analysis (NAA), particle induced X-ray emission (PIXE) and particle induced gamma ray emission (PIGME).

The technique used could be expected to reflect what is available to the laboratory, though some researchers have attempted to compare techniques (Ryabukhin, 1980; Seta et al., 1988; Fardy and Warner, 1992).

The choice of elemental analysis should be determined by the analysis technique which is best suited to the purpose.

If a small area of the hair is to be analyzed, then a sensitive, highly specific analysis such as micro XRF, micro-beam PIXE/PIGME, ICPMS with laser ablation of the sample, or scanning electron microscopy/EDX analysis should be considered. These techniques offer the analyst the ability to examine a specific area or cross-section of the hair in a specified location.

If, on the other hand, the element being sought is suspected to be in small concentrations, then the technique which offers the greatest sensitivity for the hair matrix should be chosen. Such techniques as ICPMS (Fardy and Warner, 1992), NAA (Guinn and Hoste, 1978), PIXE/PIGME (Baptista et al., 1981; Chen et al., 1981), GFAAS, or even anodic stripping voltammetry (ASV) (Chittleborough and
Steele, 1980) are suitable. Here the most cost-effective analytical tool is arguably the graphite furnace atomic absorption spectrometer. With a wide array of sensitive elemental lamps available, excellent sensitivity is obtained for the elements of forensic importance (As, Pb, Hg, etc.).

This technique has been used in the past, by the author, to augment neutron activation analysis. Following the analysis for arsenic in hair by NAA, more hair is then submitted to GFAAS for lead determinations to confirm the possibility that lead arsenate was used.

For simultaneous multi-elemental determinations and comparisons, the techniques that seem to offer the best solutions are ICPMS, NAA, PIXE/PIGME and SSMS.

Although it is possible to analyze hair non-destructively by some of the techniques (XRF, NAA), in general, hair is so easily damaged that any exhaustive analysis will inevitably lead to the destruction of, or severe damage to, the specimen. A comparison of many of the available techniques is presented in Table 4.3.

Data Analysis and Interpretation

Once determined, the pattern displayed by the trace element content has to be successfully interpreted. Many techniques have been applied over the years to carry out pattern recognition. These include the early attempts by Parker which are comprehensively described by Coleman et al. (1967), and the ‘minimal spanning tree’ approach described by Boulle and Peisach (1977) and Pillay and Peisach (1981). Because the trace elemental content of individual hairs varies with the individual follicle, the statistical matching has to be carried out on a mean value of the determination. Here, it is preferable to utilize a ‘weighted mean’ calculation as described below. These means become the comparator values.

The weighting factors are set equal to the inverse square of the standard deviation. Thus a series of measurements, \( x_i \), each with a standard deviation \( s_i \), are averaged to produce a weighted mean \( \langle x \rangle \) and a standard deviation of the mean \( \langle s \rangle \), as follows:

\[
\langle x \rangle = \frac{\sum_i (x_i/s_i^2)}{\sum_i (1/s_i^2)}
\]

\[
\langle s \rangle = \sqrt{\frac{1}{\sum_i (1/s_i^2)}}
\]

When one is comparing real forensic samples, however, the sample from the scene is often smaller in quantity than the control sample taken from the victim/suspect, hence certain elements are often not detected in that smaller sample. There is no statistical way of evaluating this probability, and one has to decide when comparing one sample with another which parameters to use. This decision is based on the experience and knowledge of the examiner. The scientist must be able to substantiate the conclusions to the satisfaction of the court.

4.4 Conclusions

Valkovic (1972) has stated that ‘poor sampling, improper preparation of samples and inappropriate statistical analysis are easily recognized in the majority of papers’ dealing with trace element analysis. Taylor (1986) concluded that much of the experimental and investigative work has failed to take into account a range of important factors, resulting in many studies being of doubtful validity. Indeed, it would not be an exaggeration to conclude that the use of elemental analysis by commercial laboratories for applications in the ‘health industry’ have brought elemental analysis to the verge of disrepute.
Table 4.3  Common instrumental techniques for qualitative and quantitative elemental analyses (Willard et al., 1988; Skoog and Leary, 1992)

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutron activation methods are based on the measurement of radioactivity that has been induced in samples by irradiation with neutrons. Nuclear reactors are a source of thermal neutrons, which react efficiently with most elements of analytical interest, and are therefore widely used for activation analyses. The radioactive decay of the isotopes produced in the sample leads to the emission of a number of products including alpha particles, beta particles, gamma photons, and X-ray photons. These emissions can be detected and counted to produce a qualitative and quantitative profile of the sample.</td>
<td>Neutron activation methods offer several advantages, including high sensitivity, minimal sample preparation, and ease of calibration. The high neutron densities produced by nuclear reactors lead to detection limits that for most elements range from $10^{-3}$ to $10 \mu g$. The procedures are often non-destructive.</td>
<td>The major disadvantages are the need for large and expensive equipment and special facilities for handling and disposing of radioactive materials. Another handicap is the long time required to complete analyses when long-lived radioactive isotopes are being used.</td>
</tr>
<tr>
<td>X-ray fluorescence (XRF) methods are commonly based on the excitation of a sample with a beam of X-rays from an X-ray tube or a radioactive source. Under these circumstances, the elements in the sample are excited by absorption of the primary beam and emit their own characteristic fluorescence X-rays. The X-rays emitted from a sample can be analyzed by either a wavelength dispersive or an energy dispersive system. XRF is one of the most common analytical methods for the qualitative analysis of elements having atomic number greater than oxygen (&gt;8); in addition, it is often employed for semiquantitative and quantitative elemental analyses.</td>
<td>XRF spectra are relatively simple and easy to interpret, with limited spectral line interference. The technique is non-destructive and analyses can be performed on samples ranging from barely visible specimens to relatively large objects. (Micro-XRF systems can operate with beam sizes down to about 10 ( \mu m ) in diameter.) Multielement analyses can be performed in a few minutes with good accuracy and precision.</td>
<td>XRF methods are generally not as sensitive as the other techniques available. In the most favourable cases, concentrations of a few parts per million can be measured. More commonly, the concentration range will be from about 0.01 to 100 per cent. XRF methods tend to be ineffective for lighter elements, with difficulties in detection and measurement becoming increasingly worse as atomic numbers become smaller than 23 (vanadium).</td>
</tr>
<tr>
<td>Bombardment of materials by ions such as protons can also lead to the emission of fluorescence X-rays. One such technique is known as particle-induced X-ray emission (PIXE). Protons of several million electron volts of energy from an ion accelerator have a flux density about two orders of magnitude greater than that of a standard X-ray tube. Protons do not penetrate deeply into matter and thus the use of protons or other ionized particles is particularly useful for very thin samples such as particulate samples collected on a thin substrate. Elements heavier than magnesium ($Z = 12$) can be detected using PIXE. (Elements lighter than silicon ($Z = 14$) can be detected via particle-induced gamma ray emission (PIGME).)</td>
<td>The ultimate limit of X-ray fluorescence (XRF) in absolute terms is about $10^{-8}$ g, whereas that of the PIXE method is around $10^{-12}$ g. The technique is non-destructive and relatively rapid, with little sample preparation required. Ion beams can be focused using magnetic quadrupole lenses in order to analyze microscopic particles. The focused beam can be scanned across the sample to produce elemental maps.</td>
<td>An ion accelerator is required to produce the high-energy particles required for these techniques. Only samples capable of withstanding the target chamber vacuum conditions are suitable for analysis.</td>
</tr>
</tbody>
</table>
With both flame atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES) samples in solution are introduced into a flame using a process called nebulization. The flame converts analytes in solution to atoms in the vapour phase. In AAS, the free atoms absorb characteristic radiation focused on the flame from an external cathode lamp. Absorption of light at a wavelength characteristic of a particular element can be related to the concentration of that element in the original sample. In AES, the flame also supplies the energy necessary to move the electrons of the free atoms from the ground state to excited states. The nature (wavelength/intensity) of radiation emitted by these excited atoms provides the basis for analytical determinations.

Electrothermal atomic absorption spectroscopy (EAAS) relies on the same principles as flame AAS; however, atomization occurs in what is commonly referred to as a graphite furnace (GFAAS). The production of free analyte atoms by an electrically heated carbon atomizer is more efficient than with a flame atomizer. The entire sample is atomized in a short period and the background ‘noise’ produced by a flame is avoided. However, electrothermal atomizers can maintain the relatively high concentration of free atoms for only a brief time (a second or two).

Emission spectroscopy based on plasma, arc, and spark atomization offers several advantages over flame and electrothermal atomization due to the more energetic processes involved. In the argon plasma employed for emission analyses (e.g. ICP-AES), temperatures as great as 10 000 K are encountered with good stability and low noise. The sample is carried into the hot plasma by a flow of argon. The sample may be an aerosol, a thermally or spark-generated vapour, or a fine powder. In arc and spark sources, sample excitation occurs in the gap between a pair of electrodes. Passage of electricity from the electrodes through the gap provides the necessary energy to atomize the sample and excite the resulting atoms to higher electronic states.

Flame AAS and AES procedures have been successfully applied to the determination of over 70 elements with sensitivities that fall in the ppm to ppb range. In addition, atomic spectroscopic methods are among the most selective of all analytical methods. They also offer the advantages of speed and convenience.

The replacement of the flame by an electrothermal furnace in AAS results in improved detection limits for many elements. Absolute detection limits typically lie in the range of $10^{-10}$ to $10^{-13}$ g of analyte. Materials can be atomized directly, thus avoiding the solution step required in flame AAS.

Lower inter-element interference is encountered as a direct result of the higher atomization temperatures. Good spectra can be obtained for most elements under a single set of excitation conditions; spectra of dozens of elements can be recorded simultaneously. Due to high atomization efficiency, lower detection limits are possible. Plasma sources permit the determination of non-metals such as chlorine, bromine, iodine and sulphur.

Flame AAS and AES require that the sample be dissolved in solution in order to undergo nebulization. Some substances interfere with the absorption or emission measurements and must be removed or masked. AAS methods require an individual cathode lamp for each element (or sometimes for a limited group of elements). Flame atomization is a complex process and flame stability is critical. Flame AES requires a particularly high degree of operator skill.

As for flame AAS, individual cathode lamps are required for each element (or sometimes for a limited group of elements). Graphite furnace atomization can also suffer from sample matrix effects and poor reproducibility due to short analysis times (compared with flame AAS where average signals can be recorded).

Instrumentation for plasma-based techniques is more expensive than flame AAS systems by a factor of two to five. The cost of argon is also a major operating expense. Quantitative arc and spark analyses demand precise control of the many variables involved in sample preparation and excitation. A set of carefully prepared standards is required for calibration and an internal standard is generally employed.
Table 4.3  (Cont’d)

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With inductively coupled plasma–mass spectrometry (ICP-MS),</strong> the sample is introduced into the ICP unit by conventional ICP sample introduction methods, which include nebulization, hydride generation, electrothermal vaporization, and laser ablation. A portion of the ionized gas from the tail flame of the ICP is introduced into the vacuum system of the mass spectrometer. Most instruments use a quadrupole mass analyzer and yield essentially unit mass resolution over a mass range up to m/z = 2000.</td>
<td>Detection limits in ICP-MS are generally better than in ICP emission spectrometry, especially for elements near the centre of the periodic chart that have very rich emission spectra. Under ideal conditions, detection limits of a few tens of parts per trillion are possible for most elements using 10 second integrations.</td>
<td>The major disadvantage is the high purchase and running costs associated with the technique, coupled with the necessity to dissolve samples in ultra-pure media being ever mindful not to overload the resulting solution due to its ultra-sensitivity. Laser ablation of samples has overcome this, but brings the additional problem of selective, rather than representative, sampling.</td>
</tr>
<tr>
<td><strong>With the scanning electron microscope–energy-dispersive X-ray analyzer (SEM/EDX),</strong> electron bombardment excites the characteristic X-ray fluorescence of the elements present in the sample, with the X-ray emission being detected and measured via an energy-dispersive system. In obtaining an electron microscopic image and in performing an electron microprobe analysis, the surface of a solid sample is swept in a raster pattern with a finely focused beam of electrons. The SEM provides morphological and topographic information about the surface of the sample, while the scanning electron microprobe furnishes qualitative and quantitative information about the elemental composition of various areas of the surface.</td>
<td>Since electrons cannot penetrate deeply into solid materials, electron microprobe methods characterize the surface layers of solids and are useful for analyzing surfaces. SEM/EDX systems offer excellent spatial resolution (from &gt;1 to 0.01 μm for the electron image – to 0.1 μm chemically for the EDX image) and allow the operator to examine the physical structure directly and obtain elemental information from selected areas of interest simultaneously.</td>
<td>Since electrons quickly lose their energy by colliding with molecules, methods that involve electron bombardment (or detection of emitted electrons) must be carried out in a vacuum. As a result, only solid samples can be analyzed. XRF typically has 10x to 100x better sensitivity than SEM/EDX. (Elemental detection with SEM/EDX is generally in the concentration range of 0.1 to 100%.)</td>
</tr>
</tbody>
</table>
Does it follow that such a poor prognosis should exist for application in the context of forensic investigation? In the opinion of this author, the answer is an unequivocal ‘No’. Clearly, single element analysis for elements such as arsenic or mercury is a valid technique, and hair is an excellent sample as it allows interpretation of not only whether or not a poison has been ingested, but also when, and how often. Although such cases are rare, there is a need for expertise to be retained for this type of application.

With respect to multi-element analysis and comparisons, there are clearly more difficulties. These need not be terminal, but it has to be accepted that elemental analysis is not a routine technique to be applied in all cases. Many of the problems which bring into question the use of elemental analysis for non-forensic applications are not necessarily problems for forensic applications. For example, the problem of establishing normal reference levels for elements for clinical applications is at least of less significance for forensic applications. This is because forensic applications place more emphasis on qualitative and comparative analysis. Certainly it is important to know what might be expected ranges for elements. Also, the variation in elemental composition arising from factors such as age, sex, racial origin and hair colour are not a problem for forensic applications, as they are the underlying basis for some of the population variance which determines the level of discrimination. The limitations imposed by the uncertainty resulting from the quality and reliability of available data are two-fold. First, it is less than satisfactory that many unanswer-able questions remain regarding the fundamentals of elemental concentrations in human hairs. Secondly, and partly because of the former, it is not possible to construct population databases which can be relied upon for strategic interpretations. Hence, for multi-element applications in forensic casework, each case has to be considered largely on its own merits at a tactical level.

In conclusion, elemental analysis does have some specific applications in poisoning cases or environmental exposure to provide unique information. Multi-element analysis should be considered where other techniques are not appropriate or have not produced a result. A variety of analytical techniques are now available, and it is not the case that elemental analysis need be restricted solely to those who have access to neutron activation analysis. Perhaps further research looking at the difficult cases from a microscopic perspective could define where elemental analysis could fit into an overall protocol for hair examination in the future.

4.5 Acknowledgements

My thanks go to my colleagues Dr Chris Lennard and Dr James Robertson for their assistance in preparing this chapter.

4.6 References


Appendix 1: Case Histories—Multi-element Applications

Comparisons of hair using trace element compositions are not as straightforward as comparisons of inert material such as glass, metal or soils. Hair is variable in its trace elemental content from follicle to follicle on the same head, hence a great deal of experience and judgement is required on the part of the analyst. Thus these types of comparison have to be approached with an appropriate degree of conservatism.

The first two cases described are those where qualitative results were sufficient to exclude the hairs in question, a factor that can quite often be as important as inclusions. One such incident (Case 1) occurred early in the experience of the author.

Case 1: Alleged Rape of K.A.C.—Mudgee, NSW, Australia (1971)

A young girl, K.A.C., in the NSW town of Mudgee was raped one night in her own bed while her parents were away from the house. Upon their return, the mother slept with her in the same bed that night to comfort her. The investigating officers, who had not been contacted till the following morning, found a single pubic hair in the bed that they had both occupied. Thus the hair could have originated from one of three sources: the victim, the rapist or the mother. Not long after, they detained a suspect and forwarded samples of his pubic hair, the mother’s pubic hair and that of the victim for comparison analysis (all individuals were blond).

Quantitative analysis was not proceeded with in this case since the qualitative results were sufficient to exclude the suspect as a possible donor of the recovered hair. The hair was comparable with those from the mother, particularly so with the unusual presence of europium (see Table A1.1). Other evidence in this case led to the subsequent conviction of the suspect.

Case 2: Alleged Rape of P.M.B.—Queensland, Australia

The woman was reportedly raped in the suspect’s vehicle. Hairs were collected from the vehicle, her clothing and the suspect, in an attempt to place her within the suspect’s vehicle. Qualitative results showed that the hair on the victim’s clothing was her own while the hair from the rug in the car and the suspect’s clothing originated from him. A single hair was found on the floor of the
Case 3(5,11),(993,988): Murder of S.Z.—Sydney, NSW, Australia

This young girl was found murdered in the back yard of the suspect in the Sydney suburb of Guildford. As with most cases dealt with by the author, the evidence submitted was corroborative, and a small part of the overall prosecution case. In this case, single hairs were extracted from various locations in and around the house. Many of these were eliminated by visual comparison, but hairs submitted from a rug off a bed where she was allegedly assaulted before her murder, and hairs taken from the trousers that the suspect was wearing at the time were analyzed.

The hairs were all individually examined by neutron activation analysis. Trace element contents were found to be quite variable. From qualitative results none of the tested hairs could have originated from the suspect (see Table A1.2). One hair in particular (number five) qualitatively and quantitatively matched the hair taken from the victim’s body (see Table A1.4). The presence in the victim’s hair of the unusual element uranium was a significant factor. The other hairs in Table A1.3 were quite similar in quantitative concentrations of the elements detected, which indicated that they originated from the victim, but the highly variable nature of these individual hairs made their origins less than conclusive.

The suspect was convicted of murder on this and other evidence, and jailed for life.

Case 4: Murder of V.A.—Sydney, NSW, Australia

This was a case where no suspect was involved and the detectives were attempting to determine where the victim had met her death. Her body was found some distance from her house and it was believed that she had been murdered in her bathroom. A considerable number of hairs were found near and on her body, including hair taken from her hand. Hair was also found in her bathroom shower recess. She had been strangled and there was no sign of a struggle or blood.

The hair from the victim and that found in the bathroom was forwarded for comparison. Qualitative analysis proved promising, with all hairs containing the same trace elements. The hairs in her hand were also the same. (There was a hope that the murderer’s hairs might have been pulled out during the struggle.)
Table A1.2  Alleged rape of P.M.B. – qualitative results only

<table>
<thead>
<tr>
<th>Hair from victim</th>
<th>Hair from victim’s clothing</th>
<th>Hair from victim’s coat</th>
<th>Hair from car</th>
<th>Hair from suspect’s tee-shirt</th>
<th>Hair from car-rug</th>
<th>Second hair from car-rug</th>
<th>Hair from suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold</td>
<td>Gold</td>
<td>Gold</td>
<td>Scandium</td>
<td>Gold</td>
<td>Gold</td>
<td>Gold</td>
<td>Gold</td>
</tr>
<tr>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
<td>Scandium</td>
</tr>
<tr>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
<td>Mercury</td>
</tr>
<tr>
<td>Bromine</td>
<td>Bromine</td>
<td>Bromine</td>
<td>Bromine</td>
<td>Bromine</td>
<td>Bromine</td>
<td>Bromine</td>
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<tr>
<td>Manganese</td>
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<td>Manganese</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Cadmium</td>
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<tr>
<td></td>
<td>Calcium</td>
<td>Calcium</td>
<td>Calcium</td>
<td>Calcium</td>
<td>Calcium</td>
<td>Calcium</td>
<td>Calcium</td>
</tr>
</tbody>
</table>

The following elements were common to all hairs: sodium, chlorine, antimony, zinc, potassium.
<table>
<thead>
<tr>
<th>Hair from victim</th>
<th>Hairs from suspect’s rug</th>
<th>Hairs from suspect’s trousers</th>
<th>Suspect’s hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>Aluminium</td>
<td>Aluminium</td>
<td>Aluminium</td>
</tr>
<tr>
<td>Copper</td>
<td>Copper</td>
<td>Copper</td>
<td>Copper</td>
</tr>
<tr>
<td>Mercury</td>
<td>Mercury</td>
<td>Gold</td>
<td>Mercury</td>
</tr>
<tr>
<td>Gold</td>
<td>Gold</td>
<td>Lanthanum</td>
<td>Gold</td>
</tr>
<tr>
<td>Lanthanum</td>
<td>Lanthanum</td>
<td>Uranium</td>
<td>Uranium</td>
</tr>
<tr>
<td>Uranium</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following elements were common to all hairs: sodium, chlorine, zinc, manganese.
The quantitative results were quite confusing, and the results were left open to speculation (see Table A1.5). Hairs found in and around the victim were quite definitely her own. Conversely, the hairs found in the suspected murder room were quantitatively quite different. One can suggest reasons for this, such as the environmental contamination of the hairs from the body, the washing effects of the hairs found in the shower recess, or the fact that they could have been some other person’s hair (though it is felt that this last scenario is unlikely). Whatever the reason may be, the results were not considered suitable for court presentation. The case illustrates the far from simple nature of multi-elemental comparisons of hair.

**Table A1.4** Elemental concentrations of hair no. 5 (values in μg/g unless indicated, with an overall error of ±20 per cent)

<table>
<thead>
<tr>
<th>Element</th>
<th>Hair from deceased</th>
<th>Hair no. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>4.1%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.5%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Bromine</td>
<td>900</td>
<td>800</td>
</tr>
<tr>
<td>Zinc</td>
<td>900</td>
<td>900</td>
</tr>
<tr>
<td>Magnesium</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Aluminium</td>
<td>550</td>
<td>400</td>
</tr>
<tr>
<td>Manganese</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Lanthanum</td>
<td>90</td>
<td>60</td>
</tr>
<tr>
<td>Uranium</td>
<td>120</td>
<td>17</td>
</tr>
<tr>
<td>Copper</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Mercury</td>
<td>16</td>
<td>2.4</td>
</tr>
<tr>
<td>Gold</td>
<td>2.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Table A1.5** Murder of V.A. (all values are in μg/g with an error of ±20%)

<table>
<thead>
<tr>
<th>Element</th>
<th>Victim’s hair</th>
<th>Hair found near body</th>
<th>Hair found on victim’s stomach</th>
<th>Hair found in victim’s hand</th>
<th>Hair found in suspected murder room</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>51</td>
<td>56</td>
<td>65</td>
<td>58</td>
<td>108</td>
</tr>
<tr>
<td>Mercury</td>
<td>6.0</td>
<td>8.0</td>
<td>5.9</td>
<td>6.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Cerium</td>
<td>2.0</td>
<td>1.5</td>
<td>1.9</td>
<td>1.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Scandium</td>
<td>6.0</td>
<td>6.0</td>
<td>5.1</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Chromium</td>
<td>21</td>
<td>23</td>
<td>25</td>
<td>20</td>
<td>2.0</td>
</tr>
<tr>
<td>Gold</td>
<td>0.106</td>
<td>0.110</td>
<td>0.121</td>
<td>0.151</td>
<td>0.001</td>
</tr>
<tr>
<td>Antimony</td>
<td>6.0</td>
<td>5.8</td>
<td>6.3</td>
<td>5.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Silver</td>
<td>1.1</td>
<td>1.5</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Iron</td>
<td>3.0</td>
<td>3.1</td>
<td>3.1</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Cobalt</td>
<td>3.0</td>
<td>3.5</td>
<td>3.7</td>
<td>4.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Lanthanum</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>25</td>
</tr>
</tbody>
</table>

Sodium, potassium, bromine and chlorine were not estimated due to the preliminary washing of all hairs prior to analysis.

The quantitative results were quite confusing, and the results were left open to speculation (see Table A1.5). Hairs found in and around the victim were quite definitely her own. Conversely, the hairs found in the suspected murder room were quantitatively quite different. One can suggest reasons for this, such as the environmental contamination of the hairs from the body, the washing effects of the hairs found in the shower recess, or the fact that they could have been some other person’s hair (though it is felt that this last scenario is unlikely). Whatever the reason may be, the results were not considered suitable for court presentation. The case illustrates the far from simple nature of multi-elemental comparisons of hair.

**Case 5: Rape of Two Women in New Zealand (1985)**

The two young women were tied up and raped by a very strong man (a member of a biker gang). Following the event he fled, leaving behind a hat he had been wearing and a scarf which he had
used to tie one of the victims. Head hair was removed from these items along with head hair from a small face washer (the New Zealanders referred to it as a ‘flannel’), and pubic hairs were found on the sheets and bed where the girls were raped.

Microscopic examination of the hair enabled the victims’ hair to be eliminated, and the trace element comparison was only carried out on the hairs of similar appearance.

Qualitative analysis of these hairs was quite interesting (see Table A1.6). Microscopic examination of the samples showed very dirty hair. Since the hair seemed to be consistent with that taken from the suspect, it was decided that this “contamination” could be as definitive as the endogenous elements. Hence the hair was not washed prior to the analysis.

The hairs yielded a very large number of elements (26 elements were found in the large head hair sample supplied from the suspect).

There were uncommon elements present, namely europium, ytterbium and lanthanum, with high concentrations of sulphur.

The hair from the ‘flannel’ was distinctly different. However, the hairs taken from the hat and the scarf left at the scene compared well with the head hairs of the suspect, particularly with the presence of those unusual elements mentioned above. The pubic hairs from the bed were comparable with the pubic hairs taken from the suspect (see Table A1.7).

When presented with this evidence, the suspect confessed to the crime and was subsequently jailed for the offence. This precluded an expensive trial (and consequently a trip to New Zealand for the author).
Appendix 2: Case Histories—Single-element Application

Case 1: The Case of W.W.

The premise that the arsenic-rich hair segment progressed along the hair with time was adequately demonstrated by a poisoning case that occurred in Brisbane, Australia, in 1978.

W.W. consumed arsenic-laden beer on 4 April 1978, was hospitalised and survived. The beer was analyzed and found to contain arsenic trioxide. His hair was submitted for analysis some two weeks after the incident to confirm that it was indeed a single dose of arsenic.

Though the arsenic content was slightly elevated all along the hair shaft, the first two 0.5 cm segments (root inclusive) yielded the majority of the arsenic load.

Subsequent hair samples were sampled 23 weeks and 29 weeks following the ingestion. The former (23 weeks) was sectioned and analyzed with the expected result; however, the final sample of hair was taken after the victim had had his hair cut—this resulted in only two shafts being long enough to still display the arsenic. By a stroke of good fortune, the resultant histogram demonstrated that the arsenic was concentrated in a very tight band (segment 14), indicating that the individual hairs had been sectioned in the same space, either side of the ‘arsenic-rich’ segment.

Results are shown in Figures A2.1, A2.2 and A2.3. Dating of the hair growth showed a good approximation to two weeks per 0.5 cm section.

Case 2: The Case of K.P.

K.P., of South Australia, was suspected of receiving a toxic metal poison: he had previously been hospitalized for suspected lead poisoning in October 1978 and had recently been treated once more.
Figure A2.1 Hair of W.W. 2 weeks after ingestion

Figure A2.2 Hair of W.W. 23.5 weeks after ingestion
in hospital (March/April 1979) for similar symptoms. This time arsenic was found and his hair was forwarded for sectional arsenic analysis by NAA.

The initial study on hairs that were 5–6 cm in length displayed a strong arsenic peak, corresponding to his most recent hospitalization.

When we were asked to determine the previous poisoning, his existing hair was not long enough to cover the intervening period. Nevertheless, he sported a fine moustache which was duly (pain-fully) sampled and analyzed. The resultant histogram confirmed the belief that this, too, had been a case of arsenic ingestion. The dating of the moustache hairs was slightly different to the scalp hair growth; however, the peak was close enough to confirm the police suspicions: *two distinct poisoning episodes*.

Results are presented in Figures A2.4 and A2.5.
Case 3: The Case of G.M.

G.M. was admitted to hospital in an outback town of NSW, Australia. He was suffering many and varied symptoms, making diagnosis extremely difficult. He appeared to improve and was discharged, only to return some weeks later with worse symptoms than before.

He deteriorated, and was subsequently transferred to a major city hospital where he continued to falter and finally died in January 1975. Just prior to his death in his weakened condition (from pneumonia), small lines on his fingernails caused the medical staff to suspect arsenic poisoning. His hair was subsampled and sent for sectional analysis. The resultant histogram confirmed the presence of arsenic prior to his final hospitalization in August 1974—see Figure A2.6.

Case 4: The Case of P.P.O.

This victim was found near to death and died on arrival at hospital in Perth, Western Australia (June 1978). His hair was sampled some time later and sent for arsenic analysis.

It was obvious from the samples that he had perspired profusely and this perspiration had dried in situ prior to the samples being taken. The hairs were routinely washed with the mild detergent, but the results displayed an overwhelming load of arsenic, too great to make any definitive estimation as to the number of doses and the timing of those ingestions (Table A2.1). It was obvious that the dried, arsenic-rich perspiration had contaminated the whole of the hair shaft.

This was confirmed when the hair was vigorously washed ultrasonically several times. The arsenic load, though much reduced, was still far too large to allow an accurate assessment of the ingestion history. The assumption was made that his death was the result of a single massive dose of arsenical weed killer.
Figure A2.6 G.M. scalp hair analysis

Table A2.1 P.P.O.’s scalp hair analysis

<table>
<thead>
<tr>
<th>Hair segment</th>
<th>Arsenic (µg/g)</th>
<th>Hair segment</th>
<th>Arsenic (µg/g)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>229.2</td>
<td>1</td>
<td>46.8</td>
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<td>107.4</td>
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<td>39.1</td>
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<td>128.9</td>
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<td>122.7</td>
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<tr>
<td>20</td>
<td>107.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Case 5: The Case of W.M.A.

This gentleman died in a West Australian Hospital in 1977. He had been suffering for some considerable time prior to hospitalization, and his hair was sampled immediately following his death. It was known to be arsenic poisoning but it was not known how long he had been ingesting it.

Sectional analysis yielded a long and continuous dosage of arsenic. It might have been due to some external contamination, but the long history of his symptoms and the fact that he had been sponged regularly during his final hours seemed to indicate that the load of arsenic was indeed the result of chronic poisoning. It was revealed that he was a keen gardener and regularly dusted his salad vegetables with arsenic weedicides—see Figure A2.7.

Case 6: The Case of K.S.

K.S. died in New Zealand in 1970 under suspicious circumstances. She had not been sick previously and died suddenly. Her hair, when analyzed, yielded one acute dose of arsenic immediately prior to her death—see Figure A2.8.

Case 7: The Case of S.I.D.

This 57-year-old woman died suddenly in Victoria, Australia in April 1987. Enough suspicion was aroused following her death to enable her body to be disintered two months later (June).

Her hair was sent for NAA analysis, and following the necessary detergent washing, it was analyzed for arsenic.

Figure A2.7 W.M.A. scalp hair analysis
The results yielded a massive single dose of arsenic, the actual concentrations being in the vicinity of 1000 ppm. It must be realized that the hair had been buried for two months: though it had not yet come into contact with the surrounding soil, it had atrophied to some extent and, as a consequence, its overall density had been reduced, thus increasing the relative proportion of the arsenical load. Nevertheless, the results were indicative of a single dose of arsenic immediately prior to her death.

The homicide squad discovered a drum of herbicide containing sodium arsenate solution on her property—see Figure A2.9.

Appendix 3: IAEA Advisory—Sampling Conditions for Hair

1. Considerable care should be exercised that the hair samples collected from a given population group constitute a statistically random sampling. Studies on a national scale may involve the collection of specimens from 1000 or more individuals.

2. Each hair should be clipped close to the scalp (within a few millimetres). The distal and proximal ends should be identified. It is recommended that, as a rule, 10 cm of the strand should be left for routine analysis, cutting off the distal end. However, a length of 5 cm may be used for individuals with short hair. In order to make measurements for 10 cm long hair comparable with those for 5 cm long hair, the 10 cm strands should be cut in two halves to be analyzed separately. Similar considerations are valid for strands of 15, 20 etc. cm in length. The hair should be cut with clean plastic scissors. If a metallic tool has to be used, oxides must be carefully removed and a few millimetres must be cut off from both ends of each strand, using a plastic or quartz tool under laboratory conditions. From the head of each individual not
fewer than 100 strands should be collected, 5–10 from each of 10–20 different 2–4 mm² spots, evenly distributed over the head. The total weight of the 5 cm samples is typically between 10 and 100 mg. But if the technique, time, labour and expenses make this impossible, separate determinations for the different spots (1–5 mg for each sample) would be desirable.

3. Each sample should be numbered with an anonymous code and identified during analysis and in reports by this number. Full particulars should be obtained from the donor on sex; race; age; natural colour of hair; occupation; nationality; present and earlier places of residence; cosmetic, medical, and other treatments of the hair; characteristics of diet; material of tableware and other kitchen utensils; smoking and drinking habits; medicines taken; and especially on any cases of suspected and actual contamination or poisoning. The hair samples must be sealed in a clean polyethylene bag or in paper envelopes and kept in a clean dry location.

4. Before proceeding to the analysis of collected hair samples, each laboratory should thoroughly test out its entire analytical procedure. This includes careful preparation of the various elemental standard solutions, blank determinations on the cleaned polyethylene and quartz containers, and blank determinations on the analytical grade acetone and distilled water to be used to wash the hair samples. Analysis should also be performed on suitable biological reference materials (i.e. NBS Orchard leaves or bovine liver).

The remainder of the recommendations go on to detail the irradiation conditions and counting equipment to be used under nuclear-based methods of analysis, and can be found in the literature (Ryabukhin, 1980).
5

Drug Analysis Using Hair

IAN R. TEBBETT

5.1 Introduction

Urine has become the most widely accepted specimen used for the detection of drug abuse. The popularity of this matrix for drug testing is probably due to the fact that, unlike blood, the collection of a urine sample does not require medical supervision. However, to ensure that the urine sample is authentic, it is often necessary to observe the subject producing the specimen. This invasion of privacy may cause embarrassment to some individuals and result in resistance to participation in a drug testing programme. Since most drugs of abuse are eliminated from the body within two to three days after use, urine analysis is also limited in its ability to detect drug exposure. If it is known that a drug screen will be required on a certain day, as a condition of employment for example, a few days’ abstinence prior to the urine being collected will result in the drug user going undetected. It is because of these problems associated with urine analysis that alternative specimens have been investigated for their potential as reliable indicators of drug exposure. To date, the most promising matrix for the detection of drug abuse appears to be hair.

5.1.1 General Considerations—Hair

It has been known for decades that trace metals in the body accumulate in the hair and can be detected weeks or even months after the original exposure. Numerous articles have been published describing the detection of heavy metals in hair, and some of this work will be discussed later from a historical perspective. More recent investigations have focused on the use of hair as a sample for the detection of pharmaceuticals and drugs of abuse. The sample preparation and analytical techniques have been developed and refined to allow analysis for a range of drugs in as little as a few milligrams of hair.

In theory, the analysis of hair for drugs of abuse offers several advantages over the use of body fluids.

• A hair sample can be collected without the need for special facilities or equipment, or medically trained personnel.

• The collection of a small hair sample is relatively non-invasive and, unlike urine, the hair sample is not readily altered by dilution or the use of other drugs prior to the test.
• The stability of drugs in the hair matrix has been illustrated in a celebrated case in which cocaine and its metabolites, benzoylecgonine and ecgonine methyl ester, were found in hair samples from ancient Peruvian coca leaf chewers dating back to AD 100 (Springfield et al., 1993).

• Once collected, the hair sample needs no special storage requirements and takes up much less storage space than urine samples.

• Since hair grows at approximately 1 cm/month, the sample offers a long-term record of drug use, with even relatively short hair holding several weeks or even months of information (Figure 5.1). Abstinence from drug usage for a few days will not therefore avoid detection.

• It is generally recommended that 50–100 hairs be used for analysis, although recent publications describe the use of as little as 5–10 mg of hair and even beard shavings have been used successfully. Most workers have described the vertex, or crown, of the head as being the most uniform sample of hair for analysis, although hair from other body areas has been used. While pulling the hair will result in the most representative sample, this is obviously painful to the subject and most samples are collected by cutting. It should be stressed that the hair should be cut as close to the scalp as possible in order to obtain the most recent growth.

The model generally proposed to explain the incorporation of drugs into hair is one in which the drugs enter the hair by passive diffusion from the bloodstream into the growing cells at the base of the hair follicle (Figure 5.2). Human hair grows in cycles, known as the anagen, catagen and telogen phases (Bost, 1993). Anagen is the growth phase, lasting two to three years, and the rate of growth is generally 0.3–0.4 mm/day or 0.9–1.2 cm/month. During this phase, the capillary blood supply around the follicle provides nutrients and also delivers any extraneous materials which might be in the bloodstream such as drugs or metals. These trace compounds become incorporated into the hair shaft and are carried along its length as the hair grows. The catagen phase is a transition between active growth and a resting phase. The root of the hair becomes keratinized, forming a club end, and begins to separate from the bulb or papilla of the hair follicle. The hair then goes into a no-growth phase known as telogen. This lasts two to three months before growth of a new hair shaft begins to occur in the dermal papilla. As it grows, the old hair will be forced out. A fuller treatment of the growth of human hair is given in Chapter 1.

More recent findings, however, suggest that drugs may enter hair from multiple sites via different mechanisms and different times of the hair growth. Henderson (1993) proposes a more complex model of drug incorporation into hair:
Drug Analysis Using Hair

during formation of the shaft
- during diffusion from blood to the actively growing follicle
- after formation via secretions of the apocrine and sebaceous glands
- after hair has emerged from the skin, from the external environment.

Pötsch et al. (1997) have proposed a biochemical concept for the endogenous incorporation of drug molecules which helps to explain some of the apparent contradictions and inconsistencies in hair drug research. This model is based on the principles of biological transport across cell membranes and biotransformation, and considers the effect of drug melanin affinity.

Pötsch and Moeller (1996), in studying the uptake of rhodamine B into hair, have shown penetration to be much greater from aqueous solution than from methanol/ethanol solvent. They have proposed that rhodamine B is a suitable model for studying the uptake of basic drugs from the external environment.

The effect of cosmetic treatment on the interpretation of endogenous drugs has been studied by Skopp et al. (1997). They concluded that the cosmetic history of a hair sample should be considered but that the effect of cosmetic treatments such as bleaching or perming on the endogenous-exogenous shunt was small.

Drugs can be transferred to hair from multiple body compartments located in tissues surrounding the hair follicle (Figure 5.3). These mechanisms could also be drug-specific, increasing the probability of detecting certain drugs while making others more difficult to detect. A more precise understanding of the incorporation of drugs into the hair is critical to interpretation.

The critics of hair analysis for drugs claim that there are too many unanswered questions concerning the reliability of the technique for it to be accepted as a routine method for drug testing. Although this technique is in its infancy and subject to a certain amount of criticism, the use of hair as a sample for forensic toxicologic examination is by no means new. The detection of heavy metal poisons and environmental contaminants in human hair has been well documented for many years. Before discussing the relative merits of hair analysis for drugs, perhaps we should examine what has already been established with regard to the deposition of heavy metals in this sample. Many of the issues, such as age, sex, disease state and contamination, which may have an influence on the

Figure 5.2 Diagram of a hair follicle (Harkey, 1993)
incorporation of drugs into hair, have already been considered as influencing heavy metal incorporation into this specimen.

5.1.2 Lessons from Heavy Metal Studies

The use of maternal hair samples as a means of estimating exposure of the foetus to metal toxins was described by Marsh et al. (1981) in a study of 84 Iraqi mothers and their infants who had been exposed to methylmercury during pregnancy. The methylmercury had been ingested as a fungicide, and peak maternal hair mercury concentrations were found to be related to the frequency of maternal toxic symptoms during pregnancy and to neurological effects in the infants. A dose-response relationship between tissue blood and hair accumulation of cobalt was also shown in rats (Nation et al., 1983). Adult rats fed daily rations of laboratory chow laced with cobalt chloride were tested for behavioural effects and these effects were correlated with cobalt levels determined by atomic absorption. Analyses revealed a dose-response effect regarding tissue accumulations of cobalt in blood, various tissues and hair. A subsequent study compared hair concentrations and behavioural effects of nickel in rats (Nation et al., 1985). Adult male rats were fed 0, 10, or 20 mg Ni/kg body weight via a 10 g daily food ration. Following 14 days of exposure, animals were trained over a period of 61 days to lever-press for food while continuing to experience the daily doses of nickel. Those rats treated with 20 mg/kg Ni lever-pressed at a significantly lower rate than controls. Atomic absorption spectrophotometric analysis revealed a dose-response accumulation of nickel in the kidney, but analyses of other specimens including hair did not show differential agent accumulations. A similar lack of correlation between blood and hair levels of lead was reported by Grandjean (1984). A woman who had been maliciously poisoned by frequent oral doses of lead nitrate over an extended time period was not diagnosed as such until approximately five months after the first symptoms appeared. Analysis of hair taken at two different times was used as evidence in court. The hair lead concentrations exhibited rapid changes and normalized shortly after chelation treatment had been instituted. This finding suggests that the hair lead level may reflect a current absorption level rather than the lead content of whole blood which, in this case, changed much more slowly. Further evidence of the lack of correlation between lead concentrations in blood and hair was found in a study of hair of workers exposed to high levels of airborne lead. Despite

![Figure 5.3 Proposed multi-compartment model for drug incorporation into hair (Henderson, 1993)](image-url)
extremely high blood lead levels and severe clinical lead poisoning, lead concentrations in hair were relatively low.

Head hair was shown to be unsuitable for the determination of aluminium for the purpose of estimating renal function (de-Wolff, 1985). Several dialysis-related diseases can be ascribed to an increased body burden of aluminium. Determination of this metal in clinical samples is therefore of great assistance in the diagnosis, treatment and prevention of aluminium intoxication. de-Wolff, however, pointed out that there are many pitfalls in the estimation of aluminium in biological fluids, and recommended that the performance of these assays should be restricted to specialized laboratories participating in an external quality assessment programme. The lack of correlation between exposure and hair aluminium concentrations prompted de-Wolff to state that scalp hair appears to be of no use for the assessment of the body burden of aluminium in the individual patient.

Although hair and urinary zinc concentrations were found to be low in children of short stature, or after prolonged upper respiratory infection, hair analysis for zinc was also found to be unreliable for the diagnosis of such conditions because of excessive variability in individuals (van Wouwe et al., 1986). The only observation which could be made was that compared with controls, hair zinc was significantly low after respiratory infection and high in short stature.

The possibility that interaction between different compounds in the subject’s body may influence the incorporation of a drug into the hair has been raised. Similar concerns over the interaction between dietary and toxic trace metals have been investigated in the rat (Elsenhans et al., 1987). Exposure to toxic and essential metals is thought to be reflected by corresponding metal concentrations in tissues. However, these compounds may influence each other in regard to their retention in the body. In order to test this hypothesis, a basic diet containing 20 ppm each of four toxic metals (arsenic, cadmium, nickel and lead) and adequate amounts of essential metals was fed to rats for two weeks. Test groups received the basic diet with increasing concentrations of one of the toxic metals (up to 90 ppm arsenic, 180 ppm cadmium, 365 ppm nickel, and 394 ppm lead). Arsenic, cadmium, nickel, lead, copper, iron, manganese and zinc were determined by atomic emission spectroscopy in liver, kidney, intestine, brain, muscle, bone, skin, blood and hair. A linear relationship between diet and tissue concentration was observed for arsenic and nickel in the kidney, for cadmium in the liver, and for lead in the bone. In other tissues saturation was observed. While cadmium-iron interactions were common to most of the tissues, other interactions were detected only in specific tissues, for example: arsenic-copper in the kidney, cadmium-zinc in the liver, and arsenic-manganese, cadmium-manganese or nickel-copper in the intestine. Increases of renal lead and intestinal cadmium by dietary nickel, and a decrease in bone arsenic by dietary lead, were the most pronounced interactions between the toxic metals. The results demonstrated that potential target organs for the evaluation of metal exposure need to be carefully analyzed for interfering metal-metal interactions. It is possible that similar interactions between dietary elements and/or of pharmaceutical products with drugs of abuse may influence the uptake and accumulation of the latter into an individual’s hair. Such an interaction would be an additional factor to be considered in the interpretation of drug concentrations in hair.

Since illicit drugs do not generally consist of standardized preparations, the question has been raised as to whether different formulations of the same drug would influence its incorporation into the hair, in the same way as different formulations of pharmaceuticals have different bioavailabilities. Again, this question has already been considered with heavy metals. The effect of different formulations of aluminium on the incorporation of the metal into various tissues including hair was studied (Winterberg et al., 1987). Two groups of dialysis patients who had received equal amounts of the different aluminium-containing phosphate-binders Aludrox and Antiphosphat were studied. It was found that aluminium levels were significantly higher in the plasma, bone, and hair of patients who had received Aludrox as phosphate binder. It is therefore possible that the type of preparation and also the diluents present in an illicit substance may influence its incorporation into the hair.

Antimony (Dorea et al., 1989), germanium (Obara et al., 1991), mercury (Kinjo et al., 1993), selenium (Clark et al., 1989), cadmium (Stewart-Pinkham, 1989) and other trace elements have all been determined in hair. The lesson to be learned from these studies with heavy metals is that hair concentrations are extremely variable, affected by different dosage forms and interaction with other
trace metals, and most importantly show little correlation with blood levels or physiological effect. *If the same is true for drugs of abuse*, the interpretation of hair drug concentrations in most cases will be impossible.

In addition to the potential problems associated with hair analysis mentioned above, the following concerns have been raised.

- Unlike urine, there are no generally accepted procedures for the analysis of hair for drugs of abuse and there is no information on the accuracy, precision, sensitivity, and specificity or appropriate cut-off levels that define potentially false positive or false negative results for either screening or confirmation procedures.
- No acceptable reference material is available to standardize the analytical methods.
- The details of procedures for the analysis of forensic samples must be validated and be available for peer review before hair testing can become accepted as an alternative drug-testing procedure.
- The relative abundance of drugs and their metabolites in hair is unknown.

We may not therefore be targeting our analytical efforts towards the appropriate compounds. In fact, the amount of drug incorporated into hair, especially for cannabinoids, may be below the minimum detectable limits for routine GC/MS procedures. What are the mechanisms by which drugs and metabolites are incorporated into hair? What is the relationship between the amount of drug used and the concentration of drug in the hair? What is the minimum dose required to produce a positive result? What is the minimum time between drug use and detection in the hair shaft? How are drug incorporation and retention in hair biased by race, age, sex or other individual differences? To what extent is externally applied drug in sweat, secretions or the environment retained in the hair? What is the effect of various washings and hair treatments on the drug concentration in the hair? Questions have been asked concerning different types of hair retaining drugs for longer periods than others. While some argue that duration of retention is irrelevant, a positive result indicating drug use, if the drug is retained longer by a certain racial group, testing will show positive in this group while negative in others. The issue of discrimination against ethnic minorities and women, who tend to have longer hair than men, has thus already been raised. Only one study has addressed this issue: Mieczkowski and Newel (1993), in a study of black and white arrestees, found that although blacks test at a higher positive rate than the white group, this is consistent with usage as indicated by self-report data. None the less, without doubt the issue of race and sex bias will be used by defence lawyers in cases where hair analysis has been used. What follows is an outline of techniques used at present for the determination of drugs in hair, together with the present level of knowledge concerning the above questions.

5.2 Drugs in Hair

The detection of heroin/morphine by radioimmunoassay techniques in the hair was first described in 1979 (Baumgartner et al., 1979); radioimmunoassay of hair (RIAH) was subsequently extended to include phencyclidine, cocaine, marijuana and benzodiazepines. These studies showed that hair analysis and urine analysis were complementary techniques. Urine analysis shows recent drug use, whereas hair samples can be used to determine the individual’s long-term drug use history. Moreover, in contrast to qualitative information from urine analysis, it has been claimed that hair analysis provides quantitative information on the severity and pattern of an individual’s drug usage.

The accumulation of drugs in hair has been studied in human subjects and animal models, the latter offering the advantage of providing carefully controlled conditions. In addition, the ratio of body weight to hair is much greater in animals than in humans, consequently the fraction of the ingested drug which accumulates in animal hair is proportionately greater. It is also more acceptable to take multiple samples from animals for analysis. There are, however, certain difficulties associated with the
use of animal models, in particular the possible contamination of animal fur by drug-containing urine or faeces. An attempt to minimize this problem has been made by housing animals in individual cages, on wire mesh to minimize contamination from excrement, and by collecting hair only from the back of the animal. Before analysis of human or animal hair, the hair must be carefully cleaned from possible contamination. For this purpose, wash steps have been specially designed.

5.2.1 Decontamination Procedures

Baumgartner and Hill (1993) described the following procedure for decontamination of human hair: the sample, approximately 5–10 mg, is washed in ethanol for a period of 15 minutes at 37°C, followed by three 30 minute washes with pH 7 phosphate buffer also at 37°C. It was claimed that if external contamination is found, repeated washing rapidly removes the contamination, i.e. it is present in the first and second washings but not the third and fourth. This group argued that continued washing of hair in such cases does not remove any further material deposited in the hair from the bloodstream during the synthesis of the hair fibre. However, even these authors agree that in some cases there are exceptions to this rule, and extensive washing is required. To identify such cases, the drug content of the last wash is assayed and compared to the content of the final hair extract. If the ratio is not at least 10 to 1, the analysis is repeated on another sample of the same specimen until analysis of the washes show that a plateau has been reached. These elaborate washing procedures have been developed to avoid the possibility of a hair sample testing positive as a result of external contamination. Baumgartner and Hill (1993) recommended that an appropriate wash solvent be chosen on the basis of porosity of the hair, as indicated by its uptake of methylene blue. Non-porous to slightly porous hair is washed with phosphate buffer 0.01M at pH 5.6, porous hair with varying mixtures of ethanol and water. Prior to washing with phosphate, the hair is washed with ethanol at 37°C in an oscillating tube for 15 minutes to remove oils, grease and cosmetics. With the extended wash procedure, phosphate buffer is withdrawn at time points and analyzed until the concentrations reach a plateau (Figure 5.4). In an alternative procedure, referred to as truncated, the hair is washed for 15 minutes in ethanol then three times for 30 minutes each in phosphate buffer. Drug concentrations in the wash are compared with drug concentrations in the extract. Hair is digested using an enzymatic digestion as follows. To 10 ml of 0.5M Tris buffer (pH 6.2) 60 mg of dithiothreitol, 200 mg sodium dodecyl sulphate and 200 units of Proteinase K are added. A 2 ml aliquot of this solution is added per 20 mg of hair, which is sufficient for five drug screens and confirmation. Digestion is allowed to proceed at 37°C overnight with oscillation after which the digest is centrifuged and the supernatant analyzed by gas chromatography-mass spectrometry. Other workers, however, argue that even with the wash steps described above, the possibility of external contamination cannot be excluded.

Cone et al. (1991) evaluated two washing procedures for their efficiency in removal of cocaine from environmentally contaminated hair. In one experiment, dry cocaine hydrochloride was powdered and shaken in a vial with pieces of hair. In a second study, hair was suspended in a large flask while free base cocaine was vaporized. Both methods resulted in a very high level of contamination. Hair treated with cocaine powder was washed with duplicate washings of 0.1N HCl, ethanol, methylene chloride or aqueous dodecyl sulphate. Although this resulted in 90 per cent of the cocaine being removed, detectable quantities of cocaine were left in all cases and none of the washes completely removed cocaine from hair contaminated with cocaine vapour. The combination of a detergent wash followed by methylene chloride washes with shaking resulted in the most efficient removal of the drug but also resulted in some extraction of the drug from inside the hair matrix.

These results suggest that washing procedures alone are not sufficient to distinguish hair with external contamination from that of cocaine users. False positives may therefore result from environmental contamination.
Analysis of the methanolic wash of the hair of cocaine users also revealed the presence of cocaine metabolites, indicating that washing removes cocaine from the interior as well as from the exterior surface of hair during decontamination procedures. In the same study, two unique metabolites of cocaine, cocaethylene and norcocaine, were identified by GC-MS in the hair of cocaine users. Their presence cannot be explained by environmental contamination and, together with cocaine, provide convincing evidence of cocaine abuse. The amount of cocaine in hair predominated over all metabolites generally by a factor of 5 to 10. Given the possibility of external contamination, however, it would appear that the detection of metabolites in the hair is the only unequivocal way of proving drug use. A similar finding, that cocaine can be removed from the hair shaft during the washing process, was made by Henderson et al. (1992). Hair samples obtained from South American Indians who were identified as daily chewers of coca leaves were analyzed by a sensitive GC-MS method for cocaine, benzoylecgonine, and ecgonine methyl ester. The mean cocaine concentration in the hair of five subjects was 15.2 ± 11 ng/mg hair with a range of 1.0–28.9 ng/mg, mean benzoylecgonine concentration was 2.8 ± 1.6 ng/mg hair, range 0.3–4.4 ng/mg, and mean ecgonine methyl ester concentration was 1.6 ± 1.7, range 0–4.4 ng/mg hair. The finding that cocaine was present at approximately five times higher concentration than benzoylecgonine and approximately twelve times higher than ecgonine methyl ester is surprising given the much longer plasma half-lives of these metabolites, and suggests that factors other than the drug concentration in blood may be important in determining the amount of drug incorporated into hair. Washing the hair before analysis with 1 per cent dodecyl sulphate, methanol, and distilled water reduced the concentration of cocaine in the hair but also reduced the concentrations of the metabolites.

5.2.2 Extraction Procedures

Having washed the hair sample in an attempt to eliminate or at least reduce external contaminants, any drugs present must be extracted from the matrix. For this purpose, several extraction procedures have been described. These are generally solvent-based extraction procedures utilizing boiling solvent for
extended time periods, acid/base extractions, or alkaline digestion of the hair sample. Baumgartner
(personal communication) has used a proprietary method in which the hair is digested and an antibody
subsequently added which acts as a specific extracting agent. Details of this procedure are, however,
available. Enzymatic procedures have been described as a possible alternative for the digestion of
hair. Enzyme digestion techniques primarily involve the use of a proteinase, although Offidani et al.
(1993b) reported that the enzyme pronase together with dithiothreitol resulted in a more efficient
extraction with less interference to RIA than proteinase. A typical enzyme digestion procedure is as
follows. A solution of 3 mg of proteinase K, 20 mg of dithiothreitol and 100 µl of a non-ionic detergent
in 0.01M solution of Tris buffer at pH 6.2 is prepared. The solution is incubated with the hair sample
at 37°C for up to 72 hours. The analytes of interest are then extracted from the hair digests using
standard solid phase or liquid-liquid extraction. Enzymatic digestion provides a high recovery in excess
of 80 per cent for cocaine metabolite, while alkaline hydrolysis causes its chemical breakdown.

Enzyme digestion may have some advantages over chemical methods for certain drugs (Offidani
et al., 1989), although it has been reported that this procedure is irreproducible. For these reasons, acid
digestion has been suggested as being the most useful. Extraction of a hair sample with 0.1N HCl at
45°C overnight resulted in a high recovery of cocaine and benzoylecgonine, although the hair structure
remained intact. Re-extraction of the same sample a second time showed negligible amounts of the
drugs, indicating that the first extraction was efficient. Time studies have indicated that the amount of
drug extracted from the hair with this technique reaches a plateau at about 20 hours. Other acidic
extraction procedures using different heating times and acid strengths have also been reported (Valente
et al., 1981; Niwaguchi et al., 1983; Marigo et al., 1986; Franceschin et al., 1987).

5.2.3 Analytical Methods

Despite the present lack of widespread acceptance of the technique of hair analysis for drugs, many
reports are available in the literature which describe the use of human and animal hair for the detection
of drugs of abuse.

Once the drug is extracted from the hair sample, the same analytical procedures as used for urine
and other biological fluids may be used. A review of many of these procedures is presented in an
article by Moeller (1992). This article reviews the analysis of 31 drugs and drug metabolites in human
hair by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas
chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and mass spectrometry (MS).
By far the most accepted technique for this purpose is gas chromatography-mass spectrometry. Other
techniques have however been described for the analysis of hair for the presence of drugs, and these
include Fourier transform infrared spectroscopy (FTIR) of microtomed sections (Kalasinsky et al.,
1993), HPLC and capillary electrophoresis (CE) (Tagliaro et al., 1993). The use of direct probe tandem
mass spectrometry has been reported for direct analysis of hair for drugs of abuse without sample
preparation (Kidwell, 1993). However similar experiments report that when several short segments of
hair were placed in the end of a direct insertion probe and placed in the mass spectrometer, it became
increasingly difficult to distinguish between hair of drug users and non-users, largely as a result of
interference from the hair matrix itself. Methods which have been described for the determination of
various drugs of abuse in hair are described below.

The use of fluorescence polarization immunoassay (FPIA) has been applied to the screening of hair
samples for the presence of drugs of abuse (Kintz et al., 1992b). Hair was decontaminated in 5 ml of
ethanol for 15 min at 37°C and then incubated in 3 ml of 1M sodium hydroxide for 1 hour at 100°C.
The aliquots were neutralized and analyzed using an Abbott ADx for benzodiazepines, barbiturates,
antidepressants, opiates, cocaine, amphetamine, and cannabis. All the positive samples were confirmed
by GC-MS. Only one false positive was detected which was apparently caused by interference of a
phenothiazine with the antidepressants’ antibody. The results demonstrated the capability of ADx for
toxicological screening of human hair. The same authors described a GC-MS procedure utilizing a
DB-5 capillary column for the separation of barbiturates, antidepressants, benzodiazepines, beta-
blockers, nicotine, opiates, benzoylecgonine, cannabis and amphetamines, after decontamination in
ethanol, solubilization in sodium hydroxide at 100°C for 10 min and extraction in chloroform/isopropanol/n-heptane (50:17:33 v/v) (Kintz et al., 1992c).

5.2.4 *Illicit Drugs*

**Cocaine**

Several authors have described the analysis for cocaine and metabolites in hair. Smith and Liu (1986) described the detection of low nanogram and picogram quantities of benzoylecgonine, the major metabolite of cocaine in extracts from perspiration stains, menstrual bloodstains, and hair using RIA. In this case the hair samples were subjected to mechanical pulverization as part of the extraction procedure. Balabanova and Homoki (1987) described a similar method for the determination of cocaine in human hair by RIA and GC-MS. The gas chromatography-mass spectrometry assay was fairly standard, with helium as carrier gas and a 30 m bonded phase fused silica OV-1 capillary column at 290°C oven temperature. Chemical ionization GC-MS has also been described for the simultaneous analysis of cocaine, benzoylecgonine, and ecdongine methyl ester in human hair (Harkey et al., 1991).

Hair samples were cut into 1 cm sections, washed with 1 per cent sodium dodecyl sulphate, rinsed with deionized water and methanol, and then digested overnight in a solution containing Tris buffer, sodium dodecyl sulphate, Proteinase K, and dithiothreitol. Digested hair samples were extracted with Bond Elut Certify (TM) solid-phase extraction columns, derivatized with N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA), and analyzed by GC-chemical ionization mass spectrometry using isobutane as reagent gas. The method was quantitative, did not cause degradation of cocaine, and requires as little as a 5 mg sample of hair. Full scan spectra were used for identification of cocaine and its metabolites. The use of ion-trap mass spectrometry, which tends to offer greater sensitivity than quadrupole instruments, has also been described for the identification of morphine and cocaine in hair extracts of drug addicts (Curcuruto et al., 1992).

RIA and GC-MS confirmation for cocaine and metabolites in hair samples extracted by acid digestion was described by Martinez et al. (1993). This study compared the effectiveness of hair analysis versus urine for the detection of cocaine use. 55 per cent of the hair was found to contain cocaine or benzoylecgonine, as compared with only 4.3 per cent of the urine samples, illustrating the advantage of hair analysis in detecting drug use over an extended period. Most hair samples contained cocaine or benzoylecgonine in the range of 25–100 ng per sample which consisted of 100 mg hair. Consistency was seen between the results of analysis by GC-MS and RIA. All hair samples testing negative for benzoylecgonine by radioimmunoassay also tested negative by GC-MS, and four samples containing the highest amounts of cocaine and benzoylecgonine by RIA were similarly found to contain the highest amounts by GC-MS.

The main advantage of hair analysis, namely to determine long-term exposure, has led to this technique being used to determine the gestational cocaine exposure of the foetus during pregnancy (Graham et al., 1989; Marques et al., 1993). Neonatal hair from seven infants whose mothers were known cocaine users averaged 5430 mg of benzoylecgonine per gram of hair, with a range of 200 to 27,500 mg/g of hair. Cocaine metabolite was still detectable in the hair of infants two to three months after birth, but was negative for infants 1 year and older, corresponding to loss of foetal hair in the few months after birth. The use of hair analysis was also described as part of a case study on the distribution of cocaine in a stillborn foetus (Klein et al., 1992).

In order to utilize hair measurements of cocaine as a biological marker of systemic exposure, both animal and human investigations on the dose-response characteristics of this phenomenon have been performed (Forman et al., 1992). This study concluded that both maternal and foetal accumulation of cocaine and benzoylecgonine follow a linear pattern within the clinically used doses. Similarly, a good correlation was observed in animals between maternal dose and foetal hair accumulation.

Analysis of the newborn’s hair has been purported as a method of evaluating *in utero* drug exposure (Kintz et al., 1992a). Hair samples were collected at the time of delivery from 63 neonates whose mothers were known to be heroin (nine cases), nicotine (forty cases), benzodiazepines (eleven cases),
cocaine (two cases) and amphetamine (one case) users. In all cases, the corresponding drug was found in neonatal hair from the infants, with concentrations in the range 0.61–3.47 ng/mg morphine, 0.15–11.80 ng/mg nicotine, 3.36–17.55 ng/mg diazepam, 0.78–31.83 ng/mg oxazepam, 0.71–2.47 ng/mg benzoylecgonine, and 1.21 ng/mg amphetamine. A significant correlation was established between nicotine concentration in the hair of the neonates and in the hair of their mother.

Hair analysis for cocaine has also been used in the investigation of a case of accidental cocaine poisoning (Martz et al., 1991). A 25-year-old man consumed an entire bottle of an imported Colombian soft drink which was later found to contain a large quantity of cocaine as part of a smuggling scheme. The subject was hospitalized for acute cocaine intoxication but died 24 days later. On admission to hospital, the patient’s blood was found to contain 2.3 mg/l of cocaine and 4.5 mg/l of benzoylecgonine. Subsequent segmental analysis by GC-MS of hair samples taken at autopsy revealed a peak cocaine concentration in the segment corresponding to the time he ingested the cocaine. This case illustrates the use of hair analysis as an adjunct to traditional analytical techniques and may help document the cause of death even when the subject dies some time after ingestion of the toxic substance. Hair analysis should be considered in cases where for whatever reason blood and urine specimens have not been collected in a timely manner.

The application of hair analysis for cocaine as a tool in basic clinical research has been demonstrated. In the following example, chronic cocaine use was verified by hair analysis in a study concerning the immunotoxic effects of the drug (Chen et al., 1991). Peripheral blood lymphocytes from 47 poly-drug users with a history of cocaine abuse were analyzed for in vitro production of interleukin-1, interleukin-2, gamma-interferon and plasma levels of soluble IL-2 receptor. Cocaine use was confirmed and quantified by analysis of hair and urine samples, and subjects were grouped into three based on the extent of cocaine metabolites detected. Although no significant differences in interleukin-1 and interferon production were seen between the three groups, subjects with higher levels of cocaine in hair also showed higher levels of interleukin-2. This study shows not only the possible interaction of cocaine with the immune system, but also the use of hair analysis to assess the extent and frequency of drug use in such an investigation.

As previously mentioned, in addition to unchanged cocaine and its major metabolites benzoylecgonine and ecygonine methyl ester, norcocaine and cocaethylene have also been detected in hair (Landry, 1992). Cocaethylene is produced as a result of transesterification of cocaine when used in the presence of alcohol. It has recently received a great deal of interest, being a psychoactive analogue of cocaine which has been shown to be more toxic than the parent compound. Not a natural alkaloid of the coca leaf, cocaethylene can be identified in the urine, blood, hair, and neurological and liver tissue samples of individuals who have consumed both cocaine and alcohol. GC and GC-MS analysis have been used to detect cocaine and cocaethylene in hair extracts of mice injected with 20 mg/kg cocaine hydrochloride (Pirozhkov et al., 1992). In addition to the cocaine, some mice were fed liquid diets containing ethanol (26 per cent of total calories). The average concentrations of cocaine in different experimental groups were in the range of 0.9–2.4 ng/mg of hair. There were no significant differences in hair concentrations of cocaine among groups receiving cocaine treatment. However, in hair extracts of mice treated with cocaine and ethanol, levels of cocaethylene were below the limit of detection. While it has previously been suggested that detection of cocaine metabolites in hair is the only sure way of identifying cocaine use, this study illustrates that, even in a controlled experiment involving chronic administration of cocaine, the incorporation of metabolites into the hair is so low as to avoid detection. It has also been shown that cocaine is more readily taken up into the hair shaft than are its metabolites benzoylecgonine and ecygonine methyl ester, in spite of predominantly higher levels of the metabolites in the blood. These findings suggest that until further work has been performed to characterize the relationship between hair drug/metabolite concentrations and the corresponding blood levels, interpretation of the results of hair analysis will be difficult (Nakahara et al., 1992a).

The potential influence of disease states on the incorporation of drugs into the hair was investigated in a study in which the effect of murine retroviral infection on hair and serum levels of cocaine and morphine was examined (Poet et al., 1992). LP-BM5 retrovirally infected female mice were administered cocaine, morphine, or both, by daily intraperitoneal injection for nine weeks. Drug concentrations
were measured by radioimmunoassay in serum and in hair extracts. Hair samples obtained from all drug-treated mice were positive for the drug injected, while none of the saline-treated mice had detectable drug levels in hair or serum. The average morphine concentration obtained from non-infected mice was 11 ng/mg hair whereas the amount found in the LP-BM5 infected mice was significantly higher (20 ng/mg hair). Mice injected with both morphine and cocaine were given 50 per cent of the regular dose of each drug and drug levels in the hair of these animals were approximately half those of mice injected with the full dose of the single drug, illustrating a dose-hair concentration relationship for these two compounds. Non-infected mice treated with both drugs had a mean value of 7 ng morphine/mg hair and 374 ng cocaine/mg hair, while retrovirus-infected mice had significantly higher concentrations, 10 ng morphine/mg hair and 1160 ng cocaine/mg hair. Serum concentrations of cocaine and morphine were significantly higher in the retrovirus-infected animals from 40 min to 1.5 h. The increased concentrations of cocaine and morphine in serum during retrovirus infection are accompanied by a significant increase in the amount of drug incorporated into the hair matrix. This study illustrates that disease states, or any factor which elevates blood concentrations of cocaine or morphine, will affect the degree to which these drugs are incorporated into the hair shaft. This phenomenon again must be taken into account for a meaningful interpretation of hair drug levels to be made.

**Amphetamine**

As well as for cocaine, GC-MS has been widely described for the determination of amphetamines in hair. Methamphetamine in hair after oral administration to rats was identified and determined by mass fragmentography (Niwaguchi et al., 1983). Rat hair was washed with HCl/methanol, followed by methanol and water. The hair was crushed in 0.6M HCl, the suspension was alkalized with sodium carbonate, and extracted with chloroform/isopropanol. The extract obtained was purified by column chromatography on aluminum oxide and derivatized by trifluoro-acetylation. The movement of methamphetamine along the hair shaft with several months’ hair growth was investigated by Nakahara et al. (1992b) using methoxyphenamine as a model compound. Five healthy subjects were administered 50 mg of methoxyphenamine orally once a day for seven days. Scalp hairs from the posterior vertex were collected every two to eight weeks after the initial dose, the hairs were cut into 1 cm sections and extracted with acidified methanol. Methoxyphenamine in the extract was determined by GC-MS. The drug moved along the hair shaft at the rate of 2.8–3.2 mm/week, according to hair growth, without diffusion. The band of hair containing the drug was found to be approximately 5 mm wide. In the case of identical doses, the drug level was highest in the root side and lowest in the distal side. It was found that the drug level in hair had decreased approximately 50 per cent five months later. It was noted in this study that drug concentrations in the hair were significantly affected by damage to the hair and/or decomposition of the drug.

Japanese workers also developed a rapid and sensitive high-performance liquid chromatographic method for the determination of low concentrations of the stereoisomers of methamphetamine and amphetamine in hair (Nagai et al., 1989). The acetyl derivatives of the stereoisomers of methamphetamine and amphetamine extracted from the hair of drug abusers were separated on two stereoisomer analytical columns connected in series at 50°C. The mobile phase was a mixture of n-hexane and isopropanol (9:1) and detection was by UV absorbance at 220 nm. The practical limit of detection for the analysis was 62.5 ng of the stereoisomers of methamphetamine and amphetamine.

Nakahara et al. (1991) described a method for the determination of methamphetamine and amphetamine in hair by GC-MS using the stable isotope-labelled internal standards, 2-methylamino-1-phenylpropane-2,3,3,3-d4 and 2-amino-1-phenyl propane-2,3,3,3-d4. Extraction of hair was achieved with methanol/5M hydrochloric acid (20:1) using ultrasonication, and the limit of detection was 0.5 ng/mg. Sectional analysis of monkey and human hair after methamphetamine ingestion suggested a good correlation between the duration of drug use and drug distribution in the hair. In further work, however, it was concluded that sectional analysis of hair may be useful in determining past drug history but is subject to a degree of variability (Nakahara et al., 1990). Ion mobility spectrometry (IMS) also has been used to screen hairs for the presence of methamphetamine (Miki et al., 1997).
The development of a simple and rapid method using IMS for the detection of 3,4-methylendioximethamphetamine (MDMA) (ecstasy) and 3,4-methylendioxiethamphetamine (MDEA) (Eve) in human hair has been reported by Keller et al. (1998). In early studies of MDMA in hair (Moeller et al., 1993; Kintz et al., 1995), GC-MS was used.

**Opiates**

Using a radioimmunoassay technique, hair samples of opiate abusers, drug-related fatalities, cancer patients receiving morphine and experimental guinea pigs receiving codeine were investigated for opiates (Puschel et al., 1983). Using 50 mg samples of hair, it was found that no correlation existed between administered doses of opiates and their concentrations in hair of both humans and experimental animals. By sectioning the hair, the approximate period of drug use in humans could be detected. However, these findings could not be confirmed by the animal experiments. The growth rate of the hair, diffusion and adhesion processes may influence the transport of drugs along the hair. As previously described, this study also showed that external contamination and washing procedures resulted in a concentration effect on the drug content of the samples.

Unique metabolites of heroin have also been targeted in the hair of opiate abusers in an effort to verify use of this substance. Hair samples from 20 documented heroin users were shown to contain 6-acetylmorphine, in all samples (Goldberger et al., 1991). Heroin was identified in smaller amounts in seven of these samples. The identity of 6-acetylmorphine and heroin was established by comparison of full scan spectra of extracts to standard reference materials. The presence of 6-acetylmorphine generally predominated over heroin, morphine, and codeine and its detection can be used to differentiate heroin users from other types of opiate exposure such as ingestion of poppy seeds, or the use of pharmaceuticals containing morphine and codeine. In addition to morphine and 6-acetylmorphine, indicative of heroin usage, codeine and dihydrocodeine have been detected in the hair of opiate addicts (Sachs et al., 1993). The opiates were extracted after dissolving the hair samples in sodium hydroxide and hydrolysis with HCl. The quantitative determination of dihydrocodeine was achieved by derivatization with heptafluorobutyric anhydride and analysis by GC-MS. A similar approach was taken by Nakahara et al. (1992c). A reliable analytical method for total morphine in hair was established by GC-MS with single ion monitoring. The hydrolytic extraction of the morphine analogues in hair with 10 per cent HCl for 1 hour at 100°C gave quantitative recovery of morphine.

The distribution of opiate concentrations in hair from different body regions was investigated (Kintz and Mangin, 1993) by determining the concentrations of morphine and codeine in head hair and hair from the axillary and pubic regions obtained from twelve fatal heroin cases. Hair was prepared by a decontamination procedure in dichloromethane at 37°C for 15 min, solubilization in sodium hydroxide at 100°C for 5 min, neutralization with hydrochloric acid and centrifugation. After extraction in chloroform-isopropanol-n-heptane (50:17:33; v/v) at pH 9.2, drugs were derivatized and separated on a 12m BP-5 capillary column. Quantitation was achieved by GC-MS using selected ion monitoring. The highest morphine concentrations were found in pubic hair (0.80 to 41.34 ng/mg), followed by head hair (0.62 to 27.10 ng/mg) and axillary hair (0.40 to 24.20 ng/mg). It has been suggested that the higher concentrations of drugs in pubic hair may be associated with its lower rate of growth compared with head hair or due to sweat or urinary contamination. Offidani et al. (1993a) found similar results for morphine, cocaine and benzodiazepines, with highest drug concentrations being found in pubic hair, followed by head, then axillary.

The time course of appearance of morphine and codeine in beard hair after single dose administration in two human subjects was monitored by RIA and confirmed by GC-MS (Cone, 1990). Both morphine and codeine appeared in beard hair approximately seven to eight days after drug administration at a time when drug levels in urine, plasma, and saliva were not detectable and drug-induced effects had disappeared. Drug levels in beard hair appeared to be dose-related in this case.
Phencyclidine and Cannabinoids

The detection of phencyclidine (PCP) and cannabinoids in hair has been described. PCP can be detected in human hair with commercially available radioimmunoassay reagents (Baumgartner et al., 1981), and a study investigating the presence of tetrahydrocannabinol in head, pubic and axillary hair has been described. The concentrations were determined by RIA followed by GC-MS (Balabanova et al., 1989). However, the detection of both these drugs in hair samples is hampered by the extremely low levels found in this matrix. The suitability of hair as a sample for the detection of cannabinoids and phencyclidine is therefore questionable. Recent studies by Kauert and Röhrich (1996) and Cirimele et al. (1996) have detected THC, cannabinol and cannabidiol, the last two compounds using GC-MS analysis.

5.2.5 Pharmaceuticals

In addition to the recent interest in hair as a specimen for long-term detection of drugs of abuse, and its historical use for the determination of exposure to heavy metals, many other drugs have been detected in hair. Most studies involving the determination of pharmaceuticals in hair have primarily focused on drugs given chronically for the treatment of psychiatric disorders. Growing hair was shown to continue to reflect the individual dosage history of haloperidol in patients receiving long-term treatment with the drug (Uematsu and Sato, 1990). In patients in whom the drug had been discontinued, the portion of hair that reflected the change of dose continued to move upwards. A 50 per cent change in the dose of haloperidol was also reflected in the hair of patients, and the hair portion representing the change was also shown to move upwards. These results indicated the potential usefulness of hair for assessing the individual past dosage history of haloperidol. Profiles of the steady-state concentrations of haloperidol in plasma versus time were determined in ten Japanese patients whose schizophrenic symptoms were clinically controlled by fixed, oral maintenance doses for greater than four months (Uematsu et al., 1992). These data were used to determine the pharmacokinetic factors that correlate best with haloperidol concentrations in hair. When the concentration of haloperidol and reduced haloperidol in hair was compared with the daily dose and respective AUC, Cmax, or trough concentration in the plasma, the parameter that best correlated with the concentration of haloperidol in hair was the area under the plasma concentration time curve (AUC). Therefore, the concentration of this substance in hair was considered to be representative of its mean amount in the body.

A study was conducted on 60 psychiatric patients to evaluate the reliability of a drug-exposure screening test based on GC-MS analysis of hair samples for amitriptyline, and the possibility of using the hair concentrations of amitriptyline to monitor patients’ therapeutic compliance (Tracqui et al., 1992b). Hair levels of amitriptyline ranged from 0 to 17.21 ng/mg. A significant relationship was found between the hair concentrations and the cumulative intake of amitriptyline over the studied period, but was not considered reliable enough to estimate one individual’s therapeutic compliance. The same authors also detected amitriptyline in the hair of a victim of a fatal poisoning involving the drug (Tracqui et al., 1992a).

Other studies have investigated the incorporation of antimicrobial drugs into hair. Using TLC and GC-MS, chloroquine and its major metabolite monodesethylchloroquine were identified in hair samples of numerous patients who received this antimalarial drug for several months (Viala et al., 1983). Sequential concentration of chloroquine in hair was shown to correlate with ingested dose and duration of therapy (Runne et al., 1992). Hair samples were also used for the measurement of another antimicrobial quinolone and results used as an indicator of drug exposure (Uematsu et al., 1993).

Scalp hair samples were obtained at 1 month intervals up to 5 months from healthy male volunteers after administration of the investigational drug. Hair was sectioned into 1 cm lengths from the scalp end, dissolved in 1 N sodium hydroxide and assayed by HPLC. In all subjects, the drug was detected in the portions of hair corresponding to the administration period, assuming a hair growth rate of 1 cm/month. No significant axial diffusion of the agent along the hair shaft with time was detected.
The quantitation of ofloxacin in human scalp hair by HPLC with a fluorescence detector was described by Miyazawa et al. (1991). A highly significant positive correlation was observed between the total dose and the concentration of ofloxacin in the 2 cm long hair segments. Such a positive correlation was also revealed in rat hair sampled after repeated intraperitoneal administration of ofloxacin over a 5-week period. In a subsequent study (Miyazawa and Uematsu, 1992), the distribution of ofloxacin along a single hair shaft was analyzed in detail for use as an index of hair growth and as a time marker for drug analysis in hair. A single hair obtained from each of seven subjects, who had taken ofloxacin for 1 to 4 days 2 to 5 months before hair sampling, was cut into 1 cm long portions successively from its scalp end. Ofloxacin in each hair portion was measured by HPLC and the distance from the scalp end of the hair portion containing ofloxacin was determined. Ofloxacin was observed to distribute only in one to three consecutive 2 mm long pieces of hair, showing no large diffusion along the hair shaft with time.

Terbinafine levels were determined in hair as well as in serum, stratum corneum, dermisepidermis (without stratum corneum), sebum and eccrine sweat before, during and after 250 mg doses orally to healthy volunteers once daily (Faergemann et al., 1991). Terbinafine is concentrated rapidly in stratum corneum (up to 9.1 µg/g of tissue), primarily by diffusion from the vascular system through the dermis and epidermis. It also reaches high concentration in sebum (up to 45.1 µg/ml) after several days and continues to concentrate in sebum for up to 2 days after discontinuation of the drug. Hair concentration reaches levels of 2.6 µg/g, indicating high drug levels in and around the hair follicle.

5.3 Conclusions

The advantages of hair as a matrix for drug testing have been outlined, and include ease of collection, stability of the sample, and fewer storage and transportation problems than with urine. Above all, hair allows a long-term estimation of drug usage. The superior overall performance of hair analysis for the detection of drug abuse has been demonstrated in a number of field studies with cocaine, heroin and PCP compared with confidential self-reports and the results of urine analysis. Hair analysis identified virtually 100 per cent of the self-reporting users, as compared to 0, 12 and 30 per cent respectively for urine analysis. In another study of 47 newly hospitalized psychiatric patients, hair analysis showed that eleven patients were regular users of PCP. In each case urine analysis was negative. These data were useful in that they indicated that the psychiatric disorders were likely to be chemically induced rather than pathological.

Questions over the reliability of hair analysis are raised primarily over two issues: the possibility of external contamination and interpretation of drug concentrations in the hair. Baumgartner and Hill (1993) list six reasons why they can eliminate passive contamination as a source of drugs in hair: the weak binding of drugs to the hair matrix, the resistance of hair to penetration of exogenous drugs, the existence of inaccessible domains in the hair, the presence of metabolites in the hair, the positive correlation of drug use and amount present in the hair, and decontamination of hair by normal hygiene. Blank and Kidwell (1993), however, discuss alternative interpretations of each of these points. The incorporation of drugs in hair may involve a complex series of events which may quite conceivably differ between individuals (Henderson et al., 1992). Drug concentrations in hair do not closely reflect the blood concentrations at the time of use, thereby suggesting that a more complex mechanism than simple entrapment is occurring. Drugs can bind tightly to the hair surface by an ion exchange mechanism, and it has been shown that hair readily absorbs drugs from aqueous solution (Cone et al., 1991). The presence of metabolites such as norcocaine and cocaethylene, which can only be produced as a result of ingestion of the drug, is a good indication of drug use if they can be detected. However, amphetamines, PCP and marijuana metabolites are very low or not detectable in hair, and benzoylecgonine, the major urinary metabolite of cocaine, is a hydrolysis product which could occur on the surface of the hair after external contamination with cocaine.
Repeated shampooing has been found to have no effect on the drug content of the hair, although drug levels have been found to be affected by other treatments such as bleaching. The degree of this effect is dependent on a number of factors including the nature of the drug, and the hair type. None of the treatments which have been investigated, however, would have caused a positive hair sample to have tested negative. Baumgartner and Hill (1993) advocate that the hair be first examined for chemical damage by treating with methylene blue, the degree of uptake by the hair of this dye being indicative of hair damage. ‘Correction factors’ are apparently being developed to enable a relationship between drug use and concentration in the hair to be established. The basis of these correction factors is cause for concern, and it would probably be wiser simply to report a positive or negative result than to attempt to give quantitative data based on an assessment of the degree of uptake by a hair sample.

Quantitation of drug levels in hair is difficult because drug levels are not uniform throughout the hair, along the hair shaft, or between hairs of an individual. As reported above, several workers have illustrated a difference in drug concentrations in hairs from different regions of the body. Good correlation between drug use and hair concentrations has only been shown in animal fur, which is not a good model for human hair. There is, however, a much better correlation between hair concentrations of pharmaceuticals, dose and physiological effect than with hair levels of drugs of abuse, usage and effect. This may reflect a more controlled and regular use of the pharmaceutical product rather than illustrating the variability of drug incorporation into the hair.

Other problems with quantitation of drugs in hair include lack of reproducibility in extracting the drugs from the hair and the lack of an adequate reference standard. However, the National Institute of Standards and Technology (NIST) in Maryland, USA (Welch et al., 1993) has gone some way towards the development of adequate spiked control hair samples as potential quality control samples. NIST, using this approach, organized an interlaboratory comparison of the analysis of hair for drugs. Eleven laboratories were sent hair samples from known drug users, drug-free hair and hair which had been soaked in a drug solution. The laboratories correctly identified cocaine at levels above 1 ng/mg with one exception. No false positives were reported for cocaine and benzoylecgonine. Four false positives involved the detection of opiates when none were present. Three of the false positives were reported by the laboratory using MS-MS. This laboratory also apparently generally reported concentrations an order of magnitude lower than those reported by other laboratories. Neither acid digestion nor enzymes resulted in a consistent precision. Five false negatives were reported.

Interestingly, in an unrelated study, five test specimens were submitted to a commercial hair testing laboratory (Anon., 1990). Hair from a heavy marijuana smoker was correctly identified and hair from a poly-drug user was correctly noted to have marijuana present, although methadone and PCP, which were also used, were not detected. Hair from a non-drug user that had been soaked in a solution of PCP was found to be ‘too contaminated to establish use’, while drug-free hair which had been exposed to crack cocaine vapours tested positive for cocaine. Hair from a 7-year-old girl that had been soaked in cocaine metabolite tested positive for cocaine, while hair from a marijuana smoker who smoked about once a week was found to be drug-free.

These test cases and the preceding discussion illustrate the fact that a great deal of further work is required before hair analysis can be accepted as a routine method for drug detection. Even if external contamination can be excluded by suitable washing of the hair or the detection of metabolites, the concentrations of drugs incorporated into the hair are subject to so many variables that interpretation would seem impossible. Factors influencing the incorporation of the drug into hair include the nature of the drug itself, the presence of other drugs or nutrients, the type of preparation used, the route of administration, the condition of the hair and the influence of hair treatments, disease, race and sex. It would appear that at this time, the results of hair analysis should be used with caution as an adjunct to traditional methods of drug analysis.

However, the subject of drug testing using hairs will not go away. There have been five major international conferences on hair analysis for drugs and forensic toxicology in the period 1992 to 1996, and several research groups (Baumgartner, Kidwell, Pötsch, Skopp and others) are actively
pursuing many of the issues which require answers. An international Society of Hair Testing (Anon., 1997) was initiated in 1996 with the objectives of finding answers to three basic issues of:

- legal aspects of hair analysis, including sample collection and reference materials
- criteria for obtaining a positive result
- the relationship between drug dose and hair concentration.

A primary aim of this society is to ensure quality in hair testing, and to this end members of the society have been active in developing interlaboratory quality control tests (Kintz and Cirimele, 1997; Sachs, 1997). The results of these tests indicate that much work still needs to be done to improve the reliability and consistency of testing methods and protocols. There is much to be positive about in the steps being taken by the society. Perhaps in the years to come it may be possible to adopt a less cautious and more positive approach to the use of hair as a medium for drug analysis.

5.4 References

ANON., 1990, Drug free workplace conference: speaker rebuts common drug testing myths and severely criticizes hair testing. Forensic Drug Abuse Advisor, 2, 81–84.


6.1 Introduction

From the physical evidence perspective, hair falls primarily within the category of associative evidence. The value of this type of evidence is primarily for comparison purposes in an attempt to associate individuals with objects, scenes, or other people. In general, hair makes good forensic evidence for several reasons: it is often lost unwittingly, it is relatively chemically and biologically stable, and the average human loses approximately 100 head hairs daily. The most useful hair comparison technique currently is morphological examination using light microscopy. However, while unequivocal exclusions are possible, when an adequate and representative known sample is available, our ability to associate a questioned hair with a known sample is limited with this approach. Evidence of cosmetic treatment is noted, when present, during the microscopic examination of a hair sample. On occasions in which the questioned and known samples exhibit similar treatments, detection and comparison of the nature of the treatment could be a useful adjunct to microscopic comparison. Similarly, a questioned hair can be excluded as coming from the donor of the known sample if the treatment histories are discernibly different.

6.2 Cosmetic Treatments

Cosmetic products used on hair, broadly categorized, include short-term, topical treatments as well as long-term whole-fibre treatments (Robbins, 1985). The extent of chemical and structural modification will depend on the nature of the treatment applied. Surface treatments tend to interact on or near the hair surface or cuticle. These include such products as shampoo, conditioner, temporary hair dye, styling sprays and gels. Products used for their long-term effects necessarily alter hair chemistry at and within the cuticle layer as well as deeper within the hair fibre. These whole-fibre treatments include permanent waves, relaxers, bleaches and certain dyes.

One of the overriding chemical effects of most whole-fibre cosmetic treatment on hair is oxidation. Oxidation of cystine disulphide bonds has its most pronounced effect on areas that are cystinerich, such as the a-layer; exocuticle of the cuticle cells, the matrix of the cortex; and pigment granules. During hair bleaching the primary consequence of the treatment is oxidation of the melanin. The initial step in permanent waving and relaxing calls for reduction of the disulphide bonds to facilitate slippage and longitudinal displacement of the protein strands to reshape the hair filament. This is followed by
Forensic Examination of Hair

re-oxidation to reform disulphide linkages in order to set the hair in its new configuration. This can be accomplished by slow oxidation in air or by the use of mild oxidizing agents. Oxidative treatments cause changes in hair keratins. The cystine disulphide bond is irreversibly cleared, giving rise to two sulphonic acid groups in the form of cysteic acid residues.

6.3 Hair Dyes

Hair dyes can be categorized in several ways: by chemical type or by expected retention and degree of penetration into the hair fibre. Generally the following scheme should speak to each characteristic:

- temporary dyes/surface coatings/acidic dyes
- semi-permanent/limited penetration/disperse dyes
- permanent/deeper penetration/oxidative (reactive) dyes.

Temporary hair dyes or rinses are designed to be removed with one subsequent washing. Semi-permanent colourants are expected to persist through several washings, and permanent dyes should retain their colour indefinitely (McLaughlin, 1963). With the permanent dyes, re-dying the hair is only necessary to dye the proximal portion as the hair grows out.

6.3.1 Temporary Hair Dyes

Commonly used temporary hair colourants are acidic or anionic dyes in which the dye molecule is negatively charged. These form reversible ionic bonds with the free NH\(^+\) groups on basic amino acid residues in hair (Bell and Whewell, 1963). Since the rinses must be readily removable, the temporary dyes are essentially water-soluble surface coatings which rinse off with an aqueous washing. The relatively large molecular size of the acidic dyes make them well suited for this purpose since they are effectively prevented from penetrating the hair fibre. The common acidic dye preparations are aqueous solutions of species bearing an azo group (-N=N-) and a solubilizing group (e.g. SO\(_3\)Na) in a slightly acidic medium (Anstead, 1963). The weak surface binding of these molecules produces no permanent chemical or structural change in the hair fibre.

The product may also contain a variety of other ingredients such as surfactants, thickeners and an acidic buffer. Robbins (1994) lists prototype hair rinse formulations—see Table 6.1. A range of hair colouring ingredients used in modern hair colour rinses are shown in Figure 6.1.

Temporary hair colour rinses will usually contain from two to five colour ingredients in order to reach the desired shade. Tints for grey hairs may have only two dyes, while red, brown and black colouration will usually have four or five dyes (Robbins, 1994). Typical formulations are given in Table 6.1.

6.3.2 Semi-permanent Dyes

Hair dyes that are designed to persist through several washings are the so called semi-permanent dyes. These colourants are made of pre-formed dye molecules that diffuse into and bind within the hair fibre to a limited degree without the need for subsequent interaction with hydrogen peroxide (Anstead, 1963; Bell and Whewell, 1963). Primarily consisting of neutral aromatic amines, nitro aromatic amines and anthraquinones (Robbins, 1994), these polar substances diffuse slowly into the shaft where weak polar and van der Waals binding occurs between the dye molecule and the hair.

Examples of dyes used in semi-permanent products are shown in Figure 6.2. According to Robbins (1994), as many as twelve dyes may be required to achieve a desired colour. The product may also include water and glycols, surfactants, fragrance, amide and acid or alkali to achieve the desired pH.
Table 6.1 Prototype hair rinse formulations (reproduced with permission from Robbins, 1994, p. 255)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage of ingredients for desired hair colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brown</td>
</tr>
<tr>
<td>Nonoxynol-9</td>
<td>1.0</td>
</tr>
<tr>
<td>Hydroxyethylcellulose (HHR)</td>
<td>0.7</td>
</tr>
<tr>
<td>Cetyltrimmonium chloride</td>
<td>0.6</td>
</tr>
<tr>
<td>Neodol 91-2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Citric acid trihydrate</td>
<td>0.5</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td></td>
</tr>
<tr>
<td>Direct black 51</td>
<td>0.05</td>
</tr>
<tr>
<td>Acid violet 43</td>
<td>0.04</td>
</tr>
<tr>
<td>Direct red 80</td>
<td>0.03</td>
</tr>
<tr>
<td>Acid orange 24</td>
<td>0.04</td>
</tr>
<tr>
<td>External D&amp;C violet 2</td>
<td></td>
</tr>
<tr>
<td>D&amp;C red 33</td>
<td></td>
</tr>
<tr>
<td>FD&amp;C yellow 6</td>
<td></td>
</tr>
<tr>
<td>D&amp;C yellow 10</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>96.54</td>
</tr>
</tbody>
</table>

Some other dyes used in this type of product are direct black 51, direct red 80, acid black 2, D&C yellow 10, and other FD&C and D&C dyes.

Figure 6.1 Some hair colour ingredients used in colour rinse products (reproduced with permission from Robbins, 1994, p. 254)
Since the dye molecules are only slightly soluble in the aqueous medium used in these preparations, they are dispersed in a suspending agent, accounting for their designation as ‘disperse dyes’ (McLaughlin, 1963). In light of the solubility of these dyestuffs their application to the hair relies on the following equilibrium (McLaughlin, 1963):

\[
\text{Dyestuff (solid)} \rightleftharpoons \text{Dyestuff (in sol.)} \rightleftharpoons \text{Dyestuff (on hair)}
\]

Figure 6.2 Examples of dyes used in semi-permanent products (reproduced with permission from Robbins, 1994, p. 251)
Because of this relationship, subsequent aqueous washings push the last equilibrium to the left and the dye gradually washes out. The limited water solubility of these chromophores is essential for the colourant deposition, since this is a diffusion-controlled mechanism (Wong, 1972). Anything that increases the solubility of the molecules would upset the partitioning favouring the hair and in effect ‘hold back’ the dye from the hair. Apart from the presence of the weakly bound dye molecules, there is no significant alteration of the hair fibre with the use of these non-reactive dyes.

6.3.3 Permanent Dyes

The class of permanent dyes owes its persistence to the fact that these colouring formulations utilize precursors (small molecules capable of diffusion into the hair shaft) or primary intermediates which undergo oxidation reactions once inside the fibre or form larger molecules containing the chromophores. The larger size of these molecules once formed effectively prevents their diffusion out of the hair. The common oxidative dye precursors are difunctional derivatives of benzene such as ortho- and para-aminophenols and para-phenylenediamines (Tucker, 1971)—see Figure 6.3 for examples.

The precursor molecules are oxidized (usually by hydrogen peroxide) to active intermediates which are capable of condensing with unoxidized precursors (self-coupling) or coupling agents included in the formulation. The new dinuclear, trinuclear or polynuclear indo dye can undergo further reaction with dye precursor or coupler to polynuclear species which infrequently undergo intramolecular cyclization to phenoxazines or phenazines (Corbett, 1972; Robbins, 1994). The utility of the couplers lies in the subtlety of shades produced through their addition. The common couplers (resorcinols, m-aminophenols and m-phenylenediamines—see Figure 6.4 for examples) have strong electron donating groups and, as such, react with the electrophilic intermediates.

Hydrogen peroxide is the preferred oxidizer since the oxidation reaction of the precursors is slow relative to the condensation with the couplers, therefore the latter reaction is preferred. Further, the only residual species following oxidation with hydrogen peroxide is water. Corbett (1972) reports the active intermediate of these reactions to be the diiminium or quinoniminium ions of the precursor as appropriate. In addition to the presence of the dye molecules in the hair shaft, the oxidative process of these hair products can cause changes in the keratins.

The reader interested in a fuller treatment of oxidation dyes or permanent hair dyes is referred to the comprehensive coverage in Robbins (1994).

6.3.4 Metallic Dyes

A type of hair colourant that is used to a limited extent today is the metallic dye. Almost exclusively, the only metallic dye in use currently is composed of 1 to 2 per cent solutions of lead acetate and surface active agents. This process involves reaction of the metallic salt with sulphur groups in the surface of the hair to produce insoluble lead sulphide and lead oxide (McLaughlin, 1963). This type of dye is marketed primarily as a colour restorer for men with grey hair. The hair tends to appear dull with this treatment since the hair shaft can become coated with the reaction products.

6.4 Bleach

Another category of products designed to change the colour of hair is bleach. The effect sought is lightening of the hair through destruction of the chromophore of the pigment. Human hair colour is manifest through a combination of factors such as pigment granule content and density, cuticle transparency and reflectivity as well as the presence or absence of air in the medulla. Pigment granules contain melanins, protein-bound polymers of indole quinone units (Henderson et al., 1962; Bisbing, 1982). Commercial products marketed for lightening hair have been formulated to dissolve and disintegrate the melanin granules. The common bleaching products are alkaline (pH 9–11) solutions of hydrogen peroxide, salts of persulphate and stabilizers. The action of the oxidizers, however, is
not limited to their effects on the pigment granules. A decrease in the cystine content of human head hair following moderate bleaching has been found. This change is concomitant with an increase in cysteic acid residues. Extensive application of bleaching products may additionally produce a small decrease in the methionine, threonine, lysine, histidine and tyrosine content (Robbins, 1994). The shift in cystine content is a result of irreversible oxidative cleavage of the disulphide bonds which Robbins (1994) reports occurs primarily through S-S fission, producing two cysteic acid residues per disulphide bond oxidized. Intermediates of the oxidative cleavage of cystine to cysteic acid such as monoxides and dioxides have been detected during wool oxidation. These species have been demonstrated only in very low concentrations in human hair oxidation (Robbins, 1994).

6.5 Permanent Waves and Relaxers

Cosmetic treatments for hair include those intended to alter permanently the three-dimensional configuration of the hair fibres. Modern preparations include both cold and acid permanent wave (perm) products as well as ‘straighteners’ or relaxers. Although the end product sought with
permanent wave treatment and straighteners is different (curl v. lack thereof), the chemical and physical steps are comparable. The process includes softening, rearrangement and hardening. The softening process is designed to free cross-linking in adjacent polypeptide chains so that they may be shifted longitudinally relative to each other. Only the protein chains are free to slide past each other unencumbered, allowing the hair to be manipulated into the desired style. Reshaping is followed by reformation of the cross-linking bonds between the proteins to give the new shape permanence. To achieve the degree of freedom between the polypeptide chains necessary, the softener must be capable of breaking several types of attachment. The forces responsible for the helical configuration of the protein chains are van der Waals attractions, hydrogen bonding, salt linkages (cross-links between acidic and basic amino acids) and disulphide bonds (Gershon et al., 1957). Changing the pH of the environment will disrupt the salt linkages. Hydrogen bonds will be extended and therefore inactivated by providing an aqueous environment. The van der Waals forces are too weak to be a significant concern. The disulphide bonds between the polypeptide chains are of major concern, in both relaxers and permanent waves. These are cleaved reversibly by reducing agents to form sulphydryl groups. Commercial permanent wave products primarily utilize one of two reducing agents: mercaptans or sulphites. Preparations for home permanents usually employ the cold wave process in which thioglycolic acid (a mercaptan) in alkaline medium (pH 9) or sodium sulphite in a slightly acid solution (pH 6) serves as the reducing agent. Some professional salon permanents utilize glycerylmonothioglycolate (GMT) in acidic medium; however, this process requires an external heat application to achieve the desired effect. Relaxers are formulated either to exploit the mercaptans and sulphites for reduction or to use an alkaline medium to accomplish this step. Hardening or reformation of the disulphide bonds may be accomplished through the application of acidic hydrogen peroxide (or similar mild oxidizer), mild alkaline (for sulphite reduction), or water rinsing followed by drying and air oxidation (relaxers using alkaline reduction). It has been suggested (Robbins, 1994) that reduction of the sulphide bond with thiol proceeds in two nucleophilic displacement reactions producing cysteine residues, while sulphites react by double decomposition producing a mercaptan and a bunte salt (organic thiosulphate) per reacted bond (Gershon et al., 1957).
An ideal permanent wave or relaxing preparation would be specific in its reaction with the disulphide bonds and hence result in no secondary chemical changes within the hair fibre upon bond reformation. However, amino acid analysis of whole hair samples treated with relaxers and permanents reveals minor alterations in amino acid residue levels in relation to untreated hair. The most significant changes detected were decreased cystine and proline residues as well as an increase in arginine content for both treatments. Proline levels decreased in permed hair and increased in relaxed hair relative to virgin samples (Chao et al., 1979). Robbins (1985, 1994) further reports the possible formation of lanthionyl residues, disulphide, mixed disulphide, carboxymethyl thiocystine, and thioacetylated lysine. Also, physical changes occur due to the harsh nature of these treatments. Among the effects on hair from chemical treatment are fibre swelling, tensile and torsional strength decrease, and fibre friction increase (Robbins, 1985).

### 6.6 Shampoos, Conditioners and Polymers

The most common—and, at least in many parts of the world, ubiquitous—treatment for hair is the use of shampoos, often along with conditioners. The interaction of these treatments with hairs and their effect on hairs is core to any consideration of hair cosmetics. However, in the context of forensic endeavour the ubiquitous nature of their use makes them, arguably, of less interest. This is partly because, after rinsing, hair will often show little detectable trace of the product used. Current fashion has seen increased use of polymer-based treatments including styling gels, and such treatment may be detectable by microscopic examination. Robbins’ (1994) book, *Chemical and Physical Behavior of Human Hair*, is very much the definitive text in the field of hair cosmetics, and the interested reader is referred to it for a more in-depth consideration of this topic.

The primary role of shampoo is to remove dirt and soils from the hair and scalp. Secondary functions may include dandruff control and, increasingly conditioning with the introduction of so-called ‘2-in-1’ shampoos. Shampoo will contain a number of ingredients including:

- primary surfactant for cleaning and foaming
- secondary surfactant for foam/viscosity enhancement
- viscosity builders: gums, salt, amide
- solvents to clarify the product
- conditioning agents
- opacifier for visual effects
- acid or alkali for pH adjustment
- colours
- fragrance
- preservative
- UV absorber
- speciality active ingredients such as anti-dandruff agents (Robbins, 1994).

The primary role of conditioners is to make hair easier to comb. This is done through the sorption or binding of lubricating and conditioning ingredients to the surface of hairs. According to Robbins (1994), hair conditioners are usually composed of the following types of ingredient:

- oily or waxy substances including mineral oil, long-chain alcohols or triglycerides or other esters, including true oils and waxes, and silicones or fatty acids
- cationic substances consisting of monofunctional quaternary ammonium compounds or amines or polymeric quaternary ammonium compounds or amines
• viscosity builders
• acid or alkali for pH adjustment
• colours
• preservative.

As previously stated, shampoos and conditioners can be expected to interact mainly at the surface of the hair fibre. However, as the cuticle at the surface may be damaged, the possibility exists for these substances to interact with the cortex. Notwithstanding the earlier observation that most residues would be expected to be removed by rinsing, some residues may remain. This is the basis on which detection of these residues may be worth pursuing in the forensic context.

Polymers have been used in cosmetic products for a number of decades; however, the introduction of 2-in-1 shampoos in the past decade has seen increased use of polymers. Polymers are found not only in shampoos and conditioners but also in styling products (lotions and gels), mousses and hair sprays (Robbins, 1994). The formulation of these products has changed throughout the 1990s driven by environmental considerations to reduce volatile organic compounds (VOCs).

Robbins (1994) considers polymers in hair products in terms of:

• binding interactions
• chemical nature
• in situ polymerization reaction mechanisms
• rheological or flow properties
• film formation and adhesive properties.

Much of this treatment relates to product performance and is not strictly relevant in a forensic context, although it may impact on the way in which the product is deposited or retained on the hair and hence the likelihood that it will be detected in a forensic protocol.

Robbins (1994) reports that limited penetration into the hair of lower molecular weight polymers (10 kDa) can take place. With higher molecular weight polymers (up to 500 kDa) there may still be some limited diffusion into the cuticle of relatively undamaged hair, and intracellular diffusion is possible in damaged hair.

The chemical composition of polymers includes a very wide range of compounds formulated in neutral, anionic and cationic products. Polyvinylpyrrolidene alone and as a co-polymer is a common component.

A recent trend in hair products, especially popular with teenagers, has been the use of styling gels. These are aqueous or alcohol/water-based and are applied to wet hair before styling.

Table 6.2 gives a typical example of a styling gel formulation (Robbins, 1994).
6.7 Detection and Characterization

6.7.1 Dye Treatments

Cosmetic treatments can be categorized, for detection purposes, into those that produce visible changes in the hair shaft (dyes), and those that are detectable only through their chemical effects on the fibre. Through a careful light microscopic examination, dying or bleaching is usually detectable visually. If the hair follicle was in the anagen stage (undergoing cell proliferation) when treated, natural hair colour will be observable between the root and the portion of the hair above the scalp line at the time of treatment. When this is the case, additional associative information may be gleaned from a comparison of the length of untreated hair in the questioned and known samples. However, this can only have meaning if the comparison is conducted on two hairs that were in the anagen growth stage from treatment through shedding. The importance of an adequate representative known sample cannot be overemphasized.

The potential exists for comparing the colourant used on dyed hairs that appear similar microscopically. One technique used for comparison of hair dyes is extraction followed by thin layer chromatography (TLC) of the extract. Several effective solvents have been reported (Macrae and Smalldon, 1979; Roe et al., 1985) for the extraction of hair dyes from hair and wool for each of the major classifications: reactive, metallic and acid. Additionally, solvent mixtures for TLC plate development have been explored (Macrae et al., 1979; Roe et al., 1985). No single system will be adequate for every circumstance; therefore, when faced with a sample that has been dyed, the appropriate chromatographic conditions should be explored on the known sample prior to extraction of the questioned hair. This method of dye comparison is relatively inexpensive and simple, but the extraction is somewhat destructive. Some workers have experienced difficulties with streaking of the spots after development. Towards the same end, Tanada et al. (1991, 1994) successfully detected five of the most common constituents of oxidative hair dyes by gas chromatography-mass spectrometry (GC-MS), and GC-MS utilizing selected ion monitoring (SIM), of the alkali degradation products of hairs treated with these dyes.

Sample preparation for these techniques is relatively complex. Initially, the sample must be washed and decomposed with alkali and heat. Extracts of the decomposition are then trifluoroacetylated and injected into the gas chromatograph (GC). This procedure is a destructive one and, as such, consideration should be given to the priority of other non-destructive analysis.

In the earlier approach by Tanada and co-workers (1991), some 50 mg of hair was required. In the later technique using SIM, between 1 mm and 10 cm of hair was required depending on the component. The five components of oxidation dyes were p-phenylenediamine, toluene-2,t-diamine, o-aminophenol, m-aminophenol and p-aminophenol. The authors indicate that it may be possible to identify which brand of oxidation hair dye has been used based on a comparison of the ratio of aminophenols versus diamines (Tanada et al., 1994).

6.7.2 Oxidative Treatments

Hair treatments that do not result in a visibly detectable alteration of the fibre may be indirectly discernible by examination of the altered hair chemistry that results from the cosmetic treatment. The products commonly used currently that have an oxidative effect on the hair include bleaching, permanent waving, relaxing, and some permanent dyes (reactive). It has been reported (Chao et al., 1979) that each of these treatments alters, to some extent, the cystine/cysteic acid content by oxidative cleavage of the disulphide bonds. Caution should be used, however, when relying on sulphonic acid residue content for determination of prior cosmetic treatment. The cystine/cysteic acid content of human hair displays both inter- and intra-sample variability which can arise from different causes (Chao et al., 1979).
Techniques that have been advocated for the detection of the products of oxidative cleavage of the disulphide bonds include FTIR, methylene blue staining, and electron spectroscopy for chemical analysis (ESCA). Some techniques using infrared absorption for the analysis of oxidized hair and wool have assigned 1040 cm\(^{-1}\) to the S=O stretching vibration of the sulphonic acid function in the cysteic acid residues (Tumosa and Brenner, 1985). Brenner et al. (1985) used FTIR to determine the presence of oxidative treatment on head hair from several volunteers. The workers used the technique as a qualitative analysis, monitoring the presence or absence of the characteristic peak. The possibility of naturally occurring sulphur-oxygen bonds, such as those produced through the weathering of hair, complicate this determination. In a population study including 135 males and females with both treated and untreated hair, a peak at 1040 cm\(^{-1}\) appeared in the spectra of four (three per cent) people with untreated hair. However, among the individuals with treated hair either a peak or a shoulder was apparent in 100 per cent of the spectra. Further, caution should be exercised with regard to sampling location within the hair. Several studies report the natural increase in oxidative cleavage products toward the distal end of hairs. This is most likely due to the increased environmental exposure due to the fact that distal portions of longer hairs are appreciably older.

In more recent studies (Panayiotou, 1998) the application of chemometrics techniques to FTIR spectra has indicated new promise for this approach to the detection of oxidative treatments. The authors were able to predict such treatment from analysis of hairs from individuals who had and who had not undergone such treatment.

Robbins (1994) suggests the use of electron spectroscopy for chemical analysis (ESCA) for the detection of oxidative treatment. Though ESCA is a surface analytical method it is reported that the cuticle is affected to the greatest extent during treatment of hair due to the limitations of diffusion (Chao et al., 1979). Robbins (1994) points out that formation of the cystyl residues proceeds via different mechanisms for weathering and artificial treatment. Therefore, the binding energy spectrum for hair naturally bleached by ultraviolet light should be distinguished from that treated with one of the oxidative cosmetic treatments.

Roe et al. (1985) have reported successfully detecting chemical treatment in human head hair utilizing methylene blue staining. Since methylene blue, a basic stain, has a substantial affinity for the electron-dense sulphonic acid, it is taken up by the hair in the region subjected to oxidative treatment. The stained hair is examined microscopically for a demarcation in colour between the stained portion and unstained portion, indicating treated and untreated portions of the hair. The untreated portion of the hair is generally present due to hair growth between treatment and shedding or loss. Staining with methylene blue is insensitive to the source of oxidative cleavage of cystine, therefore artificial treatment and weathering damage would not be differentiated with this technique. Additionally, any hair that had been subjected to an oxidative treatment would not respond to the staining. Therefore, the type of treatment applied to a given hair would not be determinable solely with this technique. The value of this information for comparison purposes is limited given the general nature of the determination. Further, while this is a quick and simple technique, it has the drawback that the staining results in a permanent alteration of the hair.

The use of scanning and transmission electron microscopy has shown that bleaching results in pigment particle destruction at the surface of the cortex. There is a loss of pigment granule fine structure, followed by the granules forming clumped groups or conglomerates and eventually decomposition of these structures to leave large optically empty spaces. Minute granules sometimes seen on the surface of bleached hairs are thought to be pigment debris (Seta et al., 1988).

### 6.7.3 Other Cosmetic Treatments

Andrasko and Stocklassa (1983) have reported on the use of high performance liquid chromatography (HPLC) for the analysis of shampoo residues in human head hair. Their technique used reverse phase chromatography with two detection wavelengths. They found it could be applied to single hairs and was non-destructive. Despite the authors’ reporting very promising results for distinguishing over 20
shampoos and correctly assigning shampoos used by test individuals, this work does not appear to have progressed further.

Roe et al. (1985) report success using micro Fourier transform infrared spectroscopy (FTIR) of hair extracts to detect lacquers which are a common ingredient in styling preparations. The absorption peak at 1240 cm\(^{-1}\) was found to correspond to the acetate group of the polyvinyl acetate (PVA) used as a copolymer with polyvinyl pyrrolidone. Of course this is only useful for those products containing PVA. The author acknowledges the limitations of the technique in the case where the hair is contaminated with blood, as frequently happens, since blood gives a broad peak in the region encompassing 1240 cm\(^{-1}\).

6.7.4 Cuticle Damage

Scanning electron microscopy (SEM) can produce very attractive images of the surface of human hairs. Undoubtedly, the depth of focus achieved with SEM dramatically illustrates the type of damage to the cuticle which can result from weathering and applied influences such as grooming. According to Robbins (1994), actions such as combing and brushing, shampooing (during both the lathering and drying steps, including towel drying or blow drying of hair), rubbing hairs during styling, such as curling, braiding or clamping hairs in a bun or a knot, cause damage to the cuticle. This results in gradual chipping of the cuticle and, in extreme cases, removal of large pieces of scales and even exposure of the underlying cortex.

It would appear that hairs that are treated continually with bleaching, dying or permanent waving will show cuticle damage (Swift and Brown, 1972; Seta et al., 1988). The degree of such damage may reflect the cosmetic/treatment history of the individual. The health and nutritional status of an individual may also contribute to the potential for hairs to become damaged.

Thus, while damage to the cuticular layer may indicate the influence of cosmetic and grooming treatments, caution needs to be exercised in assigning a precise cause for such damage. In the normal forensic examination of hairs the surface scale pattern is not normally visualized under light microscopic conditions as mountants are chosen with the aim of looking inside the hair. However, gross scale damage can be assessed at the outer margins. It would be an exaggeration to suggest that some limited damage, especially at the proximal end, would be a highly distinguishing feature. However, significant damage should certainly be noted and may be a useful comparative feature. SEM is not usually necessary, nor are scale casts (impressions) usually made.

Finally, it is sometimes possible to detect deposits on the surface of hairs caused by the application of hair treatments such as gels. Once again SEM will produce attractive pictures of such deposits, but they can normally also be seen with routine light microscopic examination.

6.8 Conclusions

At least in the developed world, nearly everybody will apply some form of cosmetic treatment to their hair. The range of products, formulations and variables in use of cosmetic treatments holds out the potential for the forensic scientist to differentiate between individuals. The reality is that little of that potential is being realized. Most protocols for the forensic examination of hairs will include an assessment of whether or not a hair shows evidence of being dyed. This will usually involve the use of light microscopic examination noting colour changes, whether or not the change is profound and clear-cut, and perhaps measuring the approximate time since an individual has had his or her hair dyed. However, caution should be exercised when interpreting results based on these determinations. Intact hairs in the anagen phase will experience growth. The dynamic nature of actively growing hair may result in larger intervals of untreated hair at the proximal end than would be exhibited by a shed hair from the same individual. This will result in a disparate appearance of hair originating from a common source. The need for adequate, contemporaneous and representative known samples cannot be overstated.

It would be rare for any follow-up chemical analysis of the dyestuff to be conducted. It would be even rarer for any attempt to be made to analyze other cosmetic effects caused by shampooing, conditioning or the application of hair treatments such as the use of mousse or gel. Deposits left by the latter and damage to the cuticular layer would be noted.
There are a number of possible reasons for this. At a technical level there are problems with sample size where the amount of substance or chemical left on a single hair shaft may be below detection limits. Increasingly, however, this is unlikely to be the case as detection limits for many analytical techniques are routinely effective in the picogram and sub-picogram level. Perhaps a contributory reason for the non-application of such techniques is that hair examiners are few and far between and will often have a background not in analytical chemistry, but rather in the biological sciences. Indeed, with the emergence of DNA testing, there may be even fewer reasons in the future to further investigate cosmetic treatments beyond what can be seen with low power light microscopic examination.

Undoubtedly, there is useful information which could be obtained in many cases currently being worked on in forensic laboratories; the techniques exist. There is a need for a considerable research effort if any of the potential is to be achieved. This effort needs to be focused on the interpretation of the analytical information from a forensic application perspective aimed at producing quite simple techniques which can be incorporated into a forensic protocol. The approach should be to eliminate recovered hairs which are different from known hair samples, to reduce the number of hairs subjected to DNA analysis. Where a number of recovered hairs appear to be from a single source, there may still be occasions where more discriminating techniques should be applied, assisting in reaching an inclusionary finding.

Finally, the totality of the physical evidence in each case needs to be considered. When hair evidence is found it must be evaluated in the context of the case. Focusing on inherent features of the hair to the exclusion of other factors, such as cosmetic treatment and environmental damage, can be a serious mistake.

6.9 References


Evidential Value of Hair Examination

BARRY D.GAUDETTE

7.1 Introduction

The body of a young woman was found in a remote field in Western Canada. She had been stabbed and her throat was slashed. There were indications that she had been sexually assaulted. Faced with investigating a homicide for which there were no suspects or eye witnesses, the police started searching for physical evidence. They recovered several hairs, most of which were found clutched in the victim’s hands. One hair was found in her mouth. In the laboratory the hairs were all identified as human pubic. Through comparison to a known sample of the victim’s pubic hair, it was determined that the hairs in question were not her own, and that they had several unusual microscopic features.

The police interviewed numerous suspects during their investigation. Each time, known samples of the suspect’s pubic hair were obtained and submitted for comparison to the questioned hairs from the young woman’s body, and each time they did not match. Finally, about five months later, a pubic hair sample was obtained from a known sex offender. Examination of this hair sample revealed the same unusual characteristics as the questioned hairs. When told of the match, the police re-interviewed the suspect. He still denied all knowledge of the murder. However, once informed that hairs similar to his had been found on the victim’s body, the suspect gave the police a full confession, and subsequently entered a guilty plea in court.

Of course, not all cases involving forensic hair examination are that spectacular. Forensic hair comparisons, which are most frequently conducted in crimes of violence such as murders and sexual assaults, generally provide only corroborative evidence which helps to establish associations between combinations of the following: an accused person, a victim, the crime scene, or a weapon.

In discussing the evidential value of forensic hair comparisons, we will look at each of the five stages of the physical evidence process: the occurrence of hair as evidence, its recovery, the analytical process of forensic hair comparison, interpreting the significance of the evidence, and report writing and court presentation of hair comparison evidence.

7.2 Occurrence of Hair as Physical Evidence

How do hairs come to occur as physical evidence? The answer lies in the Exchange Principle, first formulated by Edmond Locard (1930), who stated that: ‘The microscopic debris that cover our bodies are the mute witnesses, sure and faithful, of all our movements and all our encounters’. This principle means that whenever two objects are in contact, there will always be a transfer of material from one to the other, although in some instances the amount of material transferred may be too small to detect, or may be rapidly lost subsequent to transfer.
The original source of all transferred hairs is the human or animal body area from which they grew. As part of the normal hair cycle humans have an average daily hair loss of 75–100 scalp hairs. Of course, this loss is not evenly distributed; most occurs during hair grooming (combing, brushing and washing). A struggle involving hair pulling (as often occurs during crimes of violence) can greatly accelerate hair loss.

Two mechanisms of hair transfer exist: direct transfer in which hairs direct from the original source are transferred; and indirect transfers which involve one or more intermediaries such as clothing, bedding, and household or automotive upholstery. Direct transfer is always primary transfer (defined as that transfer which occurs whenever a person transfers his or her own hair to an object, place or other person). Indirect transfer can be either primary or secondary. Secondary transfer arises when a person transfers hair that is not his or her own to an object, place or other person. Unlike primary transfer, secondary transfer does not imply direct association, and can lead to falsely incriminating evidence.

Gaudette and Tessarolo (1987) conducted several experiments on hair transfer. The goal was to obtain information on hair transfer mechanisms and learn about the role of some of the variables involved, as well as to obtain preliminary data on the extent and importance of secondary hair transfer. The authors found indirect hair transfer mechanisms to be much more common than direct ones. They also demonstrated that secondary transfer of human scalp hair can and does occur in casework situations. The extent of secondary transfer was found to be extremely variable, being dependent on such factors as texture and fibre type of clothing worn, grooming habits of persons involved, and whether or not horizontal objects (such as upholstered chairs or car seats) used by several people are involved.

Peabody et al. (1985) investigated the shedding of hairs into several types of headgear. They concluded that the number of hairs shed into headgear varies with the type of headgear and the individual, and that hairs from headgear are most similar (with respect to stage of the hair cycle) to hairs from known samples obtained by combing. It is important to consider hair growth phase in studying the occurrence of hair evidence.

Quill (1985) collected hairs recovered from his clothing at the beginning and end of each working day for a 30-day period and compared them with known samples from himself, his family, and his co-workers. He found that the few hairs recovered from his clothing during the day were all contributed by himself and those in his immediate environment.

Since it is rare for suspects to be apprehended immediately after hair is transferred in a crime, the concept of hair persistence on clothing becomes important. Gaudette and Tessarolo (1987) found the persistence of hairs on clothing to be quite similar to that previously reported for fibres (Pounds and Smallldon, 1975). A typical decay curve is shown in Figure 7.1. Robertson et al. (1987) obtained a similar decay curve. They found movement and loss of hairs on clothing to be influenced by many variables, and concluded that ‘it would be quite unjustified to attempt to predict accurately the number of hairs likely to be present in a specific case or to attach great significance to where these hairs are found’.

Although most human hairs are removed from items during laundering, Simons (1986) found that some can still be found on items after laundering, and that hair transfers can occur during laundering.

Mann (1990) investigated scalp and pubic hair transfer and persistence through study of casework received over a 6-year period. Her results for scalp hair were consistent with those from the previously mentioned studies. She found, however, that forensically significant pubic hair transfer occurred quite infrequently in the cases she examined.

In addition to its ubiquitous nature, hair has two other properties that make it a frequently occurring form of physical evidence. First, unlike some other types of biological evidence, hair is remarkably stable to most environmental conditions, and will not easily break down. Secondly, since it is relatively unnoticeable to the untrained eye, a criminal is not likely to make a special effort to destroy hair evidence.
7.3 Recovery of Hair Evidence

To have evidential value, a hair must not only be transferred to a forensically significant location and persist there; it must also be successfully recovered as evidence. Recovery of hair evidence can occur in two locations—at the crime scene and in the laboratory. As much hair recovery as possible should be conducted in the specialized facilities of the laboratory. Provided that it is at all portable, a crime scene item suspected of having adhering hairs should be seized and brought in to the laboratory. Each exhibit item should be packaged immediately in its own separate container. Clean bags with no exposed inside seams are the best containers for clothing and other large items. Clear plastic vials or metal ointment tins are recommended for small objects. To prevent accidental contamination, exhibit items in an unpackaged state should not be handled by more than one person. By placing clear cellulose tape in contact with the area in question, hairs can be recovered from items which cannot practicably be brought to the laboratory.

In the laboratory, the simplest method of recovering hairs is to search items visually under oblique lighting at different angles, using tweezers to pick off any readily visible hairs. The examination table should be of a size sufficient to accommodate large items, have a smooth non-glossy white finish (to assist in finding hairs that fall to the table), be carefully cleaned prior to each use, be located near a good source of natural lighting, and be equipped with a movable light source capable of providing illumination to the entire area.

Particularly with dark coloured and rough textured items, visual searching should be supplemented with a second method of hair recovery, taping. Although it was originally designed as a method to recover textile fibres, experience has shown that taping can recover many hairs which are not readily visible.

To tape an exhibit item, place a strip of transparent cellulose tape adhesive side down on the item, and rub the back surface of the tape with your thumb or forefinger. Systematically repeat the process until the entire item or area of interest has been taped. Then place the tape strips between two layers of clear plastic (such as a clear acetate document protector) for preservation.

Figure 7.1 Hair persistence on clothing with wear
Under most circumstances, vacuuming is not recommended as a method of hair recovery because, as discussed by Moreau (1987), it has some serious disadvantages which could greatly reduce the evidential value of hairs recovered by this means.

Since hairs are readily transferable, it is important to follow a rigid programme of contamination prevention similar to that recommended for textile fibres (Gaudette, 1988).

### 7.4 The Process of Forensic Hair Comparison

As discussed in Chapter 3, forensic DNA analysis is beginning to play an increasingly important role in hair comparison. However, these tests are not always successful, and are not universally applied. Even when they become so, macroscopic and microscopic methods will remain useful for screening purposes. For these reasons, and because forensic DNA analysis has already been discussed in Chapter 3, this section will concentrate on macroscopic and microscopic hair comparison. A knowledge of the process of forensic hair comparison should also be of value to those whose primary duties involve DNA analysis since some of the concepts to be discussed also apply to DNA analysis, which also has a significant subjective component. As well, forensic DNA specialists are well advised to familiarize themselves with universally applicable basic concepts of forensic science, such as: ‘a visual match occurs whenever there are no unexplained, forensically significant differences between a questioned sample and a known’.

The forensic hair examiner’s role is to evaluate the points of comparison and determine, on the basis of his or her training and experience, whether or not a questioned hair is consistent with a known sample. To be deemed to match a known sample, a questioned hair must fit within the range of characteristics of the known sample, and also be similar in all major characteristics to at least one hair within that known sample, with its characteristics varying in a similar manner along the length of the shaft and across the diameter. If not, the hairs are dissimilar. (Conclusions and interpretations of these results will be discussed in the subsequent sections.)

Forensic hair comparison is a complex field that is in many ways as much an art as a science. There is no substitute for gaining experience through conducting a large number of hair comparisons. This is one of the steps towards obtaining what Jackson (1975) has termed ‘visual literacy’ and setting a proper level of discrimination.

Several findings from studies of visual literacy have important implications for forensic hair comparison. In a study of student radiologists looking for pathological features on X-ray film, Thomas (1969) found that instead of examining the entire film, each radiologist had a distinctive pattern of eye search movements, and that all examiners did not look at the same areas of the film. The same is likely true of hair examiners. This is why use of forms to record hair characteristics is recommended. Such forms make the examiner look more carefully, and such forced looking helps improve visual literacy.

A second finding on visual literacy is that a person interested and enthusiastic about seeing will see more than one not so inclined (Tuddenham, 1962). Motivation, self-confidence, fatigue, boredom (and certain medications) can also affect the capacity to see. For this reason a hair examiner should arrange his or her work day in such a manner that hair comparisons are conducted only when the examiner is fresh, and that they are discontinued when fatigue sets in.

A third finding is that a great deal of information arriving at the brain from the retina fails to obtrude on the consciousness. Everyone has had the experience of suddenly being able to ‘see’ something once someone points it out to them. In fact they had been looking at it all along but not seeing it. A study by Kind and Owen (1977) demonstrated that it is important to have special training in hair comparison and not just microscopy. Other studies have shown that different hair examiners note different characteristics in hairs, and that one examiner will make an incorrect association of a hair that another examiner sees as being obviously dissimilar (Gaudette, 1978; Bisbing and Wolner, 1984). Therefore, it is advisable for a hair examiner to have important hair comparison results confirmed by another examiner.
Jackson (1975) stated that: ‘…all of what is seen by the retina may not be transmitted centrally. This means that something has to be added based on our index of suspicion, given history, or past experience. In other words, what is perceived is in part constructed by the observer’. On the basis of personal preferences, training received, and past experience, different examiners place emphasis on different characteristics, and use different systems in comparing hairs. It also may be that people have different natural discriminating abilities.

The final finding on visual literacy relevant to forensic hair comparison is that prior information affects seeing. This means that once one questioned hair from a given exhibit item has been found to match a known sample, an examiner must work hard to resist the urge to lower his or her level of discrimination when comparing the second, third, fourth, etc. questioned hair recovered from that same item.

Thomas’ study (1969) found that most students tended to fall into two groups—a small group able to see quickly and effortlessly, and a larger group that were only able to see slowly and with difficulty. This is likely because the process of comparison is a pattern recognition process. Psychologists have recognized that there are two sides to the human brain—one that works in a creative analogue fashion and one that works in a logical step-by-step fashion. The creative side is able to make great leaps to produce a gestalt. Hence its workings are much quicker and more difficult to comprehend than the logical slower workings of the other side. To be truly visually literate in a comparison process, a person must develop creative gestalt ability—hence the ‘art’ in forensic hair comparison.

When we recognize the face of a friend we do not do so by going through a logical set of characteristics (blond hair, blue eyes, big nose, etc.). Rather we do so instantly by a little understood creative pattern recognition process. Forensic hair comparison is somewhat analogous except that, since a hair examiner is hampered by the constraint of not being able to see the entire hair in any one field of view, he or she must conduct pattern recognition comparisons serially, bit by bit. That hair comparison is more of a pattern recognition process than a logical step-by-step one was shown in a study (Gaudette, 1985a) where two groups of examiners both arrived at the same level of discrimination even though one group felt they required comparison of hair cross-sections to attain that level. The author concluded that ‘an experienced hair examiner immediately gets a feeling for whether two hairs are similar. Even though the examiner may then try to logically justify the feeling through a step by step examination of each of the hair characteristics, the first feeling remains the dominant basis of his or her discriminating ability. Thus, beyond a certain minimum, the exact number and type of characteristics specifically examined is immaterial. Crosssections could therefore be considered as extra characteristics’.

An examiner able to develop the ability to recognize patterns innately to a higher degree than the ability to evaluate characteristics logically may appear to be merely making good guesses when conducting hair comparisons. However, when the same examiner is consistently able to make such ‘good guesses’, it must be recognized that he or she has developed the art of forensic hair comparison, and has been able to fine-tune his or her discriminating ability to a level that minimizes incorrect associations without incurring an unreasonable number of incorrect eliminations.

The process of macroscopic and microscopic hair comparison has a strong subjective component. Accordingly, the training, experience, and general competency of the hair examiner can have a significant impact on the evidential value of the comparison.

It is important that all of these be up to the highest standards and periodically checked through proficiency testing and other quality assurance measures.

### 7.5 Interpreting the Significance of Hair Comparison Evidence

Unlike blood, the mere presence of hair on an exhibit item is usually not by itself of any evidential value. Comparison to a known sample is generally required. Once such a comparison is made, the next (and most important) step is to assess its significance. This involves not only using the results to develop a conclusion, but also interpreting that conclusion to form an expert opinion to be expressed in a report or court testimony. We will begin this section with a general discussion on evaluating associative
forensic science evidence. Next, we will look at studies on probabilities and human hair comparison and the criticisms that have been made of them. We will then examine some other studies on the value of macroscopic and microscopic hair comparison evidence. We will also discuss the proposed use of frequency of occurrence data. Finally, we will consider the impact of forensic DNA analysis on the significance of forensic hair comparison evidence.

7.5.1 Evaluating Associative Forensic Science Evidence

Despite a growing consensus among statisticians that the best approaches to the evaluation of evidence involve the use of Bayesian inference (Evett, 1983; Aitken and Stoney, 1991), many forensic scientists have continued to use classical statistical methods because of perceived difficulties in getting the courts to understand and accept the Bayesian approach. For the most part, the following discussion is designed to be of use whether or not a Bayesian perspective is taken. Since development of the Bayesian approach to evidence evaluation is beyond the scope of this chapter, I will simply refer the reader to the excellent discussions of this topic given by Robertson and Vignaux (1995) and Evett (1990).

The fundamental question to consider when evaluating associative forensic science evidence is ‘What is the value of the evidence in establishing a particular association?’ (Gaudette, 1986). To answer this question we need to know the answer to at least three other questions:

1. What is the probability that the association (or elimination) was due to coincidence?
2. What is the probability that the association (or elimination) was due to examiner error?
3. What is the probability that there is an alternative explanation for the evidence such as secondary transfer, contamination or deliberate planting?

From a Bayesian perspective, the important questions are:

(a) What is the probability of the hair evidence if there was association?
(b) What is the probability of the hair evidence if there was no association?

These two questions are related to the previous three questions. If we subtract the answers to the ‘elimination’ forms of the first three questions from a probability of one, we have an estimate of the probability sought by question (a). The ‘association’ forms of questions 1, 2 and 3 relate to the probability in question (b).

At this point, let us note that there are two possible states of nature with regard to association. Either there was some form of association (A), or there was not (N). Ignoring inconclusive results, there are two possible outcomes of a forensic scientist’s examination—either the evidence indicated association (E), or it did not (E). If the state of nature is A and the forensic scientist gives an opinion indicating E, the forensic scientist is correct. Similarly, if the state of nature is N and the forensic scientist says E, he or she is also correct. However, if the state of nature is A and the forensic scientist says E, a type I error or incorrect exclusion has occurred. If the state of nature is N and the forensic scientist has given an opinion indicating E, there was a type II error or incorrect association.

An analogy can be made to a fire alarm. Type I errors correspond to the fire alarm not ringing when there is a fire. Type II errors correspond to the fire alarm ringing when there is no fire. Depending on the decision we are making and the constraints involved, knowledge of the probability of type I or type II errors or both can greatly assist in making the decision. With the fire alarm, a type I error would be more serious than a type II error; with hair comparison, a type II error would be the more serious since it could result in falsely incriminating a suspect. (It is for this reason that hair examiners should set a level of discrimination that minimizes type II errors without incurring an unreasonable number of type I errors. It is interesting to note that when the role of macroscopic and microscopic hair comparison changes from providing stand-alone evidence to screening evidence prior to DNA analysis, the level of discrimination should also change. With a screening technique, it is the type I errors which are the
more serious. The hair examiner’s rule of thumb then changes from ‘if in doubt, throw it out’ to ‘if in doubt, include it.’) In report writing and court testimony, once a questioned hair has been found to be indistinguishable from a known sample, it is only the probability of type II errors that is important in evaluating the evidence, just as once a fire alarm has rung it is the probability of type II errors, and not the probability of type I errors, that influences our decision as to whether or not to leave the building. In attempting to determine the value of forensic hair comparison in establishing associations, we primarily need to know the probability of type II errors due to coincidental matches, examiner errors, and alternative explanations for the evidence.

### 7.5.2 Gaudette and Keeping’s Studies of Probabilities and Human Hair Comparison

A lay person might observe that there are quite a few differences in the gross appearance of people’s hair. Some people have long hair, some short; some have curly hair, some straight; some have dark coloured hair, some light; some people bleach and dye their hair, some do not. A lay person would not, however, have any idea of the large intrapersonal variation in hairs, or of the large number of hair characteristics that can be observed microscopically, and the number of variables each characteristic can have. Accordingly, a lay person would not have any intuitive feel for the average value of forensic hair comparison evidence.

In an attempt to rectify this situation, Gaudette and Keeping (1974) published the results of a study in which, with the aid of a card-coding system, they conducted 366,630 comparisons on 861 hairs from 100 individuals (an average of 8.61 hairs per individual were chosen as being representative of the range of characteristics present in known samples of about 100 hairs per individual). In these comparisons, nine pairs of hairs were found to be macroscopically and microscopically indistinguishable. They calculated that if a single scalp hair selected at random from individual A was found to be consistent with a single hair selected at random from individual B, the chance that the match was due to coincidence was about 9/366,630 or 1/40,500. If a single hair selected at random from A was found to be consistent with a representative known sample from B (consisting in the study of an average of about nine mutually dissimilar hairs), on average the chance of a coincidental match was 9 × 1/40,500, or about 1 in 4500.

In a similar study with pubic hairs, Gaudette (1976) conducted 101,368 comparisons of 454 hairs from 60 individuals. Sixteen pairs of pubic hairs were found to be macroscopically and microscopically indistinguishable. Therefore, if a single pubic hair selected at random from person X was found to be consistent with a single pubic hair selected at random from individual Y, an estimate of the average probability of a coincidental match would be about 16/101,368 or 1/6336. If the single hair selected at random from X was found to be consistent with a known sample of pubic hairs (which in the study consisted of about eight mutually dissimilar hairs), an estimate of the average probability of that one hair having originated from someone else would be 8 × 1/6336, or about 1 in 800. The greater likelihood of a coincidental match for pubic hair may be due to the variation in characteristics of pubic hairs throughout the population being less than that for scalp hairs.

An interesting finding of the pubic hair study was that hairs from one individual were involved in three matching pairs of hairs, while hairs from seven other individuals were involved in two matching pairs. This shows that certain hair types and certain individuals are more likely than others to be involved in coincidental hair matches.

The Gaudette and Keeping results refer to the situation where a single questioned hair is found to be consistent with a known sample. The finding of two or more questioned hairs to be consistent with the known sample will greatly reduce the probability of a coincidental match. A probability estimate cannot be obtained by simply multiplying 1 in 4500 by 1 in 4500, however, since independence cannot be assumed.

It should be emphasized that the Gaudette and Keeping probability results are average values made up of the sum total of all hair types—from unusual hairs (where probability of a coincidental match would be virtually 0) to hairs of average commonness (where the probability of a coincidental match
would approximate 1/4500), to common featureless hairs (whose probability of a coincidental match would be considerably greater). The Gaudette and Keeping results can provide a good estimate of the average value of hair comparison evidence in establishing associations when the following conditions are met:

- the probability of examiner error is very low (this condition should be met when a well-trained qualified examiner carefully conducts the examination)
- the probability of secondary transfer, contamination, or deliberate planting of evidence is very low
- Caucasian hairs are involved.

Ever since their first use in a study of house paint (Tippett *et al.*, 1968), the forensic application of average probabilities has aroused controversy. Several authors have discussed the theoretical limitations of average probabilities (Aitken, 1987; Aitken and Robertson, 1987; Aitken and Stoney, 1991), and cautioned against their blind and indiscriminate use (Gaudette, 1978; Aitken and Stoney, 1991). However, for practical reasons (as will be discussed in section 7.5.5), despite their limitations, average probabilities are likely all that will ever be available with forensic hair comparison. As pointed out by Gaudette (1987), when used properly (as will be discussed in section 7.6), average probabilities can provide a good aid to decision-making, and have the added advantage of not conveying a false impression of exactness. The importance of careful wording in probability statements was discussed by Gaudette (1982) and Stoney (1984).

Barnett and Ogle (1982) claimed that the Gaudette and Keeping studies contained defects in experimental design, and had improper statistical treatment of the data. Gaudette (1982) and Wickenheiser and Hepworth (1990, 1991) offered rebuttals of much of this criticism. Furthermore, although Barnett and Ogle criticized Gaudette and Keeping’s work, it should be noted that they offered no studies of their own to refute Gaudette and Keeping’s results, nor did they claim that hair comparison evidence is not good evidence.

### 7.5.3 Other Studies on the Value of Hair Comparison Evidence

A wide range of opinions as to the value of hair evidence has appeared in the literature. Some authors take a disparaging view of hair evidence. The following quotation is typical: ‘There is nothing about hair comparable to the specificity of fingerprints, and at best the probability of establishing identification from hair is perhaps no greater than the probability of determining identification using the ABO blood group system in blood smears’ (Camps, 1968). On the other hand, the following quotation is typical of those authors who consider hair comparison evidence to have a high value: ‘From research studies, it has been shown that hairs from two individuals are distinguishable and that no accidental or coincidental matches occurred, and would, therefore in actual casework be a relatively rare event’ (Strauss, 1983). The generally prevailing view of the value of hair comparison evidence lies between these extremes. These two quotations are representative: ‘Through hair comparison it is presently only rarely possible to determine that a questioned hair did or did not originate from a particular person. In the vast majority of cases it can only be stated that a questioned hair is or is not consistent with having originated from a particular person. Accordingly, hair comparison evidence is generally only of value when used in conjunction with other evidence’ (Gaudette, 1985b), and

1. ‘So far, a hair or hairs have not been shown to have any features exclusively confined to an individual.
2. Any indication of identity based on an examination of hair can therefore only be established in terms of probability.
3. The probability is increased, under certain circumstances, if all the characteristic elements are considered and is increased to an even greater extent when unusual features such as uncommon colours, disease, etc. are present’ (Martin, 1957).

Seta et al. (1988) provide a good review of some of the historical views on the evidential value of forensic hair comparison. Although a large number of individuals have expressed opinions as to the value of hair comparison evidence, actual research studies on the topic have been more limited. In addition to the work of Gaudette and Keeping, the following experimental studies have been reported.

Kirk (1940) reported that a group of his students were, without exception, able to match one questioned hair to the correct known sample in a group of 20, all of similar colour and from individuals of similar age.

Gaudette (1978) discussed two additional experiments on the value of hair comparison evidence. In the first experiment, 100 randomly selected questioned hairs were compared in a blind trial to one known sample. This experiment was repeated three times with three trainees, each near the end of a one-year training period. Two of these trainees correctly chose the one and only hair that was consistent with the known sample. The third trainee first concluded that four of the questioned hairs were consistent with the known sample. After examining the hairs more closely and consulting with other examiners he was easily able to identify one of his choices as being incorrect, leaving three hairs he thought to be consistent with the known sample—the correct one and two others. When the author examined the hairs, he stated that one of the two others could be eliminated, but the remaining one was indistinguishable from hairs in the known sample. Another experienced examiner then studied the hairs and also concluded that one of the two others could be eliminated. This time, however, it was the one opposite to that picked by the author! All examiners did agree that the correct hair was consistent with the known sample. The hairs that caused the type II errors in this experiment were common featureless hairs.

In the second experiment, the author compared 100 known hair samples to one questioned hair. He repeated the experiment three times using different sets of hairs. Twice the one and only correct known sample was picked as being consistent with the questioned hair (i.e. no type I or type II errors were made). In the third trial, a common featureless hair was chosen as the questioned hair. This hair was found to be consistent with two of the known samples, the correct one and one other (i.e. one type II error was made).

Strauss (1983) conducted a series of seven experiments in which ten questioned hairs were compared with ten known samples. In each of the seven experiments, the known and questioned hairs were selected by a neutral party from a hair pool so that differing numbers of questioned hairs actually matched the known samples. Each time Strauss correctly matched all questioned hairs to their correct known samples (i.e. no type I or type II errors were made).

Bisbing and Wolner (1984) reported on a study in which each of seven questioned hairs was compared with several known samples. Results are given in Table 7.1. Hairs in this study were from twins, the majority of whom were below the age of six. The majority of the subjects were blond. Most of the hairs were common featureless types and cut samples were used, thereby reducing the number of comparative features.

Wickenheiser and Hepworth (1990) repeated the Gaudette and Keeping study with experimental design modifications aimed at overcoming some of the criticisms Barnett and Ogle levelled at the original work. They collected representative hair samples of at least 100 hairs from each of 97 Caucasian individuals, including some closely related individuals from several generations. From each sample, they macroscopically selected five to 13 hairs to represent the range of characteristics present. The main variation from Gaudette and Keeping’s procedure was that they had an independent person randomly number the mutually dissimilar hairs and add 53 additional hairs randomly chosen from the original known samples of the 97 individuals. Therefore, since several duplicate hairs were included in the study, when Wickenheiser and Hepworth encountered hairs they could not distinguish, they would not be biased by the knowledge that the hairs had to have originated from different sources.
With the assistance of a personal computer database to eliminate unnecessary microscopic comparisons of obviously dissimilar hairs (examiner #1 still required 749 one-to-one microscopic comparisons, and examiner #2 required 2006), they each were able to conduct 431,985 hair comparisons. Examiner #1 found seven pairs of hairs to be indistinguishable, and examiner #2 found six. In all cases these matches were between duplicate hairs. Neither examiner found any hairs from different individuals which coincidentally matched.

This study led to several interesting conclusions, as follows.

• If a one-to-one microscopic match between two hairs is found, the probability that it is a coincidental match is remote.

• As reflected in the differences in the number of direct microscopic comparisons required by the two examiners, the classification of hairs varies greatly between examiners.

• The classification of hair is inconsistent because of variations over time. This made the sorting procedure used susceptible to error.

• Macroscopic selection of five to 13 hairs to represent a known sample is frequently inadequate. This is why they did not find more matches between duplicate hairs (38 of the 53 duplicate hairs were found to be unique in that they had no matching hair in the known sample selected). Experimental work aimed at determining the optimum composition of a representative known sample is therefore warranted.

Apart from the pubic hair study discussed previously (Gaudette, 1976), no study on the value of non-scalp human hair comparisons has been published.

In conjunction with a celebrated American murder case (State of Georgia vs Wayne Williams), the author conducted a study of the significance of dog hair comparison by comparing hairs from the suspect’s German Shepherd dog with hairs from twelve other German Shepherd dogs. The hairs from the suspect’s dog were divided into ten types depending on colour and whether they were guard or intermediate hairs. Eight of the twelve comparison dogs had no hairs matching any of these ten types. Three of the comparison dogs each had one type of hair that was macroscopically and microscopically indistinguishable from one type of hair on the suspect’s dog. The remaining dog had two hair types

Table 7.1 Results of simulated forensic comparisons (reproduced with permission from Bisbing and Wolner (1984)).

<table>
<thead>
<tr>
<th>Questioned specimen number</th>
<th>Hair colour</th>
<th>Number of known specimens</th>
<th>Number of matches*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Examiner 1</td>
<td>Examiner 2</td>
</tr>
<tr>
<td>1</td>
<td>Brown</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Blond</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Blond</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Brown</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>Brown</td>
<td>7</td>
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<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Blond</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Blond</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* All matches are incorrect (type II errors). No type I errors were made because none of the known pools contained the true source.
indistinguishable from the suspect’s dog. It should be noted that the twelve comparison dogs were not selected at random from the population of all dogs, but were deliberately chosen so that their coats closely matched the suspect’s dog. Had they been randomly chosen, an even smaller number of coincidental matches would have been found.

In a blind study involving comparison of 15 questioned hairs with known hair samples obtained from 25 purebred German Shepherd dogs, Suzanski (1988) made no type II errors and correctly assigned six of the 15 questioned hairs to their known sample of origin. In a later extension of the study, Suzanski (1989) compared 25 questioned hair samples of about ten hairs each with known samples from 100 mixed breed and purebred dogs of various types. He was able to assign all 25 correctly with no incorrect associations.

From these studies of various types of hair, we can conclude the following about the value of macroscopic and microscopic forensic hair comparison evidence.

- With a few isolated exceptions, the macroscopic and microscopic characteristics of hairs are not unique to an individual. Accordingly, it is possible for type II errors due to coincidental matches to occur in forensic hair comparison.
- Type II errors are a relatively rare event in forensic hair comparisons conducted carefully by qualified, well-trained examiners. Accordingly, macroscopic and microscopic hair comparison evidence is generally good corroborative evidence.
- There are several factors which can increase or decrease the probability of type II errors in a given case. Accordingly, each case must be considered on its own merit.

### 7.5.4 Use of Frequency of Occurrence Data

Some forensic scientists have proposed setting up a computerized database of hair comparison characteristics which they would then use to state frequency of occurrence data in court (Aitken and Robertson, 1986; Hoffmann, 1991). In theory, this approach is very attractive. However, practical problems make such an approach both unattainable and undesirable. First, presentation of frequency data on their own can lead to a distorted picture of the value of evidence, along with a false sense of exactness (Gaudette, 1986). Secondly, there is the difficulty of characterizing the hairs for a database. It requires examiners to adopt a checklist approach rather than the more natural pattern recognition approach. Two hairs described alike can be markedly different microscopically (Gaudette and Keeping, 1974; Strauss, 1983). Two examiners are likely to describe hairs in slightly different ways (Gaudette and Keeping, 1974; Podolak and Blythe, 1985). The same examiner will even vary his or her description from day to day (Wickenheiser and Hepworth, 1990). Ogles (1991) suggested that such difficulties can be overcome by using hair characteristic reference standards or archetypes. However, as Wickenheiser and Hepworth (1991) pointed out, classification based on archetypes would be limited to one field of view, thus forcing an examiner to decide subjectively which of the hundreds of possible fields of view best represents the hair characteristic. Finally, setting up such a database would be extremely time-consuming. Accordingly, results-oriented research (such as the previously described studies) is much preferable to the database approach. This is not to suggest, however, that information from databases would not be valuable. On the contrary, it could be quite useful in helping examiners to decide which characteristics, and combinations thereof, are unusual and which are common.

### 7.5.5 The Impact of Forensic DNA Analysis

Although macroscopic and microscopic forensic hair comparison provides good evidence, its evidential value is dwarfed by that of forensic DNA analysis. With matches in four or five probings, RFLP analysis can provide probabilities of coincidental matches that are less than one in millions or billions, and newer approaches involving PCR of short tandem repeat (STR) multiplexes provide even stronger
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evidence. In the long term, the ability to apply highly probative DNA technologies to virtually all hairs can be expected to relegate macroscopic and microscopic hair comparison to the role of a screening technique. Indeed, it is possible that, as forensic DNA analysis techniques become much quicker and less expensive, microscopic analysis of hairs could cease to be routinely practised. However, in the short to medium term, a gulf will likely remain between the potential of DNA analysis as a hair comparison method and the practical realization of this potential. RFLP analysis of single hairs is possible only if root sheaths are present, and this occurs in only a small percentage of hairs encountered in casework. PCR extends the range of candidate hairs to include any hair with a root. However, amplification is not successful with all hairs, and some PCR-based techniques (such as HLA DQ Alpha) have evidential values that are lower than those of microscopic analysis.

How should the DNA results be integrated with the macroscopic and microscopic results to provide a common statement of evidential value? Three possible situations can be identified: (1) when DNA analysis is not possible or provides no or inconclusive results; (2) when DNA evidence provides results of high probative value; and (3) when DNA evidence provides results of intermediate probative value. The first two present no particular problems. In the first the macroscopic and microscopic hair comparison evidence is evaluated in the usual way, and in the second the forensic DNA analysis evidence is evaluated and presented with the macroscopic and microscopic hair comparison being either ignored or mentioned only in passing. In the third situation, examiners might be tempted to multiply the probative value of the DNA evidence by that of the macroscopic and microscopic match. There are two reasons why this temptation should be resisted. First, since the probative value of the DNA evidence (which is usually based on frequency of occurrence data from a reference population database) is calculated in a different way to the evidential value of the macroscopic and microscopic match, multiplying the two would be like ‘multiplying apples and oranges’. Secondly, even when the evidential values of the two methods of obtaining a match are determined in similar ways, it has not yet been demonstrated that the two are independent. Accordingly, interpretations of each should be presented separately, with a statement that the results of the two methods substantiate each other, leading to an overall increase of the evidential value by an indeterminate amount. The only way to arrive at a more definite number would be to repeat some of the hair comparison experiments with extra categories for the DNA results.

Forensic DNA analysis has had another important impact. It has brought about an animated and extensive debate over statistical assessment of evidential value. Many of the issues raised in this debate are the same ones previously encountered, in a much lower profile form, in the discussion of probabilities and hair comparison. The following are among the questions being asked (Lempert, 1991; Weir, 1992): ‘What is the relevant reference population?’; ‘Is the probability of false positives due to laboratory error not more relevant than the probability of a coincidental match, and, if so, how can the probability of laboratory error in a given case be reliably estimated?’; ‘How is quantitative forensic evidence to be combined with other non-quantitative evidence in the case?’; ‘Are juries likely to confuse frequencies of coincidental matches with probability of guilt?’; ‘Does presentation of numerical probabilities invade the province of the jury?’; ‘Should numbers or quantitative statements or both be used in reports and court testimony?’; ‘How valid are independence assumptions?’; ‘Should a frequentist or Bayesian approach be taken?’; and ‘What is the optimum degree of conservativeness in assumptions?’ Despite numerous scientific publications and much courtroom argument, there is no definitive answer to most of these questions. Indeed, it is doubtful whether there ever will be; many of them depend on subjective personal values (such as degree of conservativeness) and not on objective scientific facts. Furthermore, as Stoney (1992) has stated:

‘...the disciplines of population genetics and statistics will not lead us through an objective process to absolute identification. They cannot do this because as we approach very rare frequencies of events, or very rare alleles and genotypes, the strength of these disciplines wanes and their objectivity, so well-developed for populations and multiple events, gives way to subjectivity. The focus changes from general laws acting on populations or multiple events to one on specific events and the question of individual identity.... Eventually, we are faced with a choice between pseudoobjective calculations, made by extrapolating well beyond the provable, or accepting the reality of subjective certainty'.

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The added perspective of the forensic DNA analysis experience has served to substantiate the wisdom of the approach I have advocated for determining the evidential value of macroscopic and microscopic hair comparison evidence. Indeed, some publications (Berry et al., 1992; Risch and Devlin, 1992; Herrin, 1993; Sudbury et al., 1993) have even proposed the use of average probabilities, based on studies of pairwise comparisons, for assessing the evidential value of DNA evidence.

Forensic DNA analysis has had another impact on the more traditional forensic science disciplines—it has caused the courts to look more closely and stringently at areas such as microscopic hair comparison. One American court (Williamson v. Reynolds, 904 F. Supp. 1529 E.D.Oklahoma, 1995) even went so far as to deem forensic hair comparison inadmissible according to Daubert criteria. Robertson and Vignaux (1995) provide a good discussion of these criteria and their limitations. They also give an excellent explanation of why forensic scientists should be considered the ‘relevant scientific community’, and provide a good counter-argument to the Williamson v. Reynolds court’s finding that general acceptance of forensic hair comparison ‘seems to be among hair experts who are generally technicians testifying for the prosecution, not scientists who can objectively evaluate such evidence’. Another questionable aspect of the decision in Williamson v. Reynolds was the finding that there is a ‘scarcity of scientific studies regarding the reliability of hair comparison testing’. It is hoped that the studies mentioned in this chapter will provide ammunition to counter this argument in future cases. The Williamson v. Reynolds decision is something of an isolated incident, since the great majority of legal opinion has held forensic hair comparison evidence to be admissible. Nevertheless, it should serve as a wake-up call that the courts will be viewing our field with higher powered microscopes when determining the weight and admissibility of hair comparison evidence. It will be incumbent upon practitioners to provide the research and quality assurance necessary to ensure that forensic hair comparison can successfully withstand this closer inspection.

7.6 Report Writing and Court Testimony

On the basis of the results of an examination, a forensic scientist must draw a conclusion which he or she then interprets in giving an expert opinion as to evidential value. Conclusions and expert opinions are given in report writing and court testimony.

Exact wording of conclusions will depend on examiners’ preferences and their laboratory’s policy. In the past (Gaudette, 1985b), I have recommended the following symmetrical spectrum of conclusions. (A positive conclusion is defined here as one drawn from a finding of similarity between a known sample and a questioned hair. A negative conclusion is one arising from a finding of dissimilarity.)

**Strong positive:** the questioned hairs originated from the same person as the known sample.

**Normal positive:** the questioned hairs are consistent with having originated from the same person as the known sample.

**Inconclusive:** no conclusion can be given as to whether the questioned and known hairs have a common origin.

**Normal negative:** the questioned hairs are not consistent with having originated from the same person as the known sample.

**Strong negative:** the questioned hairs could not have originated from the same person as the known sample.

The great majority of macroscopic and microscopic hair comparisons will result in normal positive or normal negative conclusions, with the other three being rarely encountered. Where forensic DNA analysis is possible, a strong positive conclusion will often be appropriate.

The normal positive and normal negative conclusions cover a wide range of evidential value. Accordingly, it is important that they be further interpreted in reports and court testimony. First, the examiner should mention that hair comparison is not usually a positive means of personal identification. This should be followed by an estimate of the average value of forensic hair comparison evidence. This can be based either on personal experience or on some of the published...
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Table 7.2 Some factors which tend to weaken positive hair comparison conclusions (reproduced with permission from Gaudette (1985b))

1. The presence of incomplete hairs.
2. Questioned hairs which are common featureless hairs.
3. Hairs of non-Caucasian racial origin.
4. A questioned hair found in conjunction with other unassociated hairs.
5. Known samples with large intra-sample variation.

Table 7.3 Some factors which tend to strengthen positive hair comparison conclusions (reproduced with permission from Gaudette (1985b))

1. Two or more mutually dissimilar hairs found to be similar to a known sample.
2. Hairs with unusual characteristics.
3. Hairs found in unexpected places.
4. Two-way transfer – for example, a victim’s hair found on an accused’s clothing and an accused’s hair found on the victim’s clothing.
5. Additional examinations such as forensic DNA analysis.

Table 7.4 Some factors which tend to weaken normal negative hair comparison conclusions (reproduced with permission from Gaudette (1985b))

1. Deficiencies in the known sample:
   (a) not enough hairs
   (b) not representative
   (c) contains incomplete hairs
   (d) large time difference between offence and procurement of known sample.
2. Incomplete questioned hairs.
3. Questioned hair has macroscopic characteristics close to those of the known sample.

Table 7.5 Some factors which tend to strengthen normal negative hair comparison conclusions (reproduced with permission from Gaudette (1985b))

1. Known sample has more than the recommended number of hairs.
2. Known sample shows little intra-sample variation.
3. Questioned hair has macroscopic and microscopic characteristics very dissimilar to those of the known sample.
4. Two or more questioned hairs found together in a clump are dissimilar to the known sample.

studies described in section 7.5. The examiner should then discuss factors weakening or strengthening the evidence in the particular case. Some factors which can weaken hair evidence in a particular case are given in Table 7.2. Some factors which tend to strengthen normal positive hair comparison conclusions are given in Table 7.3. Factors weakening negative conclusions are given in Table 7.4, and factors strengthening them can be found in Table 7.5.
Evett (1990) has proposed a novel approach to forensic science report writing and court testimony. This approach, which has recently been put into extensive use in Britain, has the advantage of being readily comprehensible to client groups such as police investigators and court officials. It involves assignment of a verbal convention to the degree of support that evidential value (in the form of a likelihood ratio) offers to a particular hypothesis (C). C is formulated to answer directly a question of interest to the police or court, e.g. that person X was in association with person Y, or that a particular knife was used to stab person X. With a likelihood ratio of 1 to 10, it would be stated that the findings provide limited support for C; for 10 to 100, ‘the findings provide moderate support for C’; 100 to 1000, ‘the findings provide strong support for C’; and for more than 1000, ‘the findings provide very strong support for C’. The forensic scientist’s report or written statement would then include the following components:

- a summary of the scientist’s perception of the circumstances surrounding the crime and the arrest of the suspect
- a list of the competing hypotheses or explanations for the evidence
- an assessment of the probability of the evidence under each of the alternatives
- an opinion which summarizes the extent to which the evidence supports one of the alternatives.

The approach outlined earlier in this section can be readily adapted to fit Evett’s approach. By using the average value of hair evidence coupled with the factors strengthening and weakening the evidence, a forensic scientist can determine which of the four categories (very strong support, strong support, moderate support, or limited support) is appropriate for a particular case. With DNA evidence, a fifth category (provides extremely strong support) may often be warranted. When using this approach, careful wording of hypotheses is extremely important, as is consideration of all the facts and evidence surrounding a case. Great care must be taken in interpretation of negative hair comparison results, since it must be borne in mind that ‘absence of evidence is not necessarily evidence of absence’. For example, the finding of a few hairs on a suspect’s shirt which matched neither his own nor the victim’s known sample would have no probative value. On the other hand, it would be highly significant if a clump of hairs found clutched in a murder victim’s hand matched neither her own nor a suspect’s known sample.

By taking advantage of collective knowledge and experience, we can calibrate and improve interpretation, report writing, and court testimony. Two useful vehicles for this are the workshop approach (Cook et al., 1993) and the intelligent knowledge-based systems approach (Evett, 1993), both of which could be adapted for use in forensic hair comparison. These approaches, which are based on pooling, structuring, and testing knowledge, offer the exciting possibility of being able to apply the best knowledge to all aspects of every case, thereby improving report writing and court testimony concerning forensic hair comparison.

As we move towards the twenty-first century, forensic science must transform itself to better meet the needs of our clients. Hair evidence is no exception. Although DNA and other advances will make analysis more technically complex and bring some exciting changes to forensic hair comparison, it must be remembered that the evidential value of hair evidence depends on all five stages of the physical evidence process. A forensic hair examiner must be concerned with the occurrence of hair as evidence, its recovery, analysis, interpretation, and presentation. There is plenty of scope for research in all of these areas.

### 7.7 Acknowledgements

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