Overview. **Microscopy Techniques**: Scanning Electron Microscopy.

## Further Reading

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# Hair

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## Introduction

Recently, crime has become more vicious and skilful than ever before, so minute substances (trace evidence), which are difficult to hide, are important for the detection of the suspect, especially in cases where he is in contact with the victim or the crime scene. These substances are hair, fibers, soil, etc. Among them hair is noteworthy and can be retrieved easily from the crime scene, because there are ~100 000 hairs on an adult human head and they may be shed one by one every 15 min. The suspect therefore leaves his or her own head or pubic hairs at the crime scene unwittingly. Therefore, hair provides important physical evidence for solving of crime.

Nowadays in the forensic science laboratory, large pieces of human (and animal) hairs collected from the crime scene are examined by morphological methods using macroscope, microscope, and electron microscope, and may be compared with sample hairs of humans associated with the crime. Finally, their origins are determined.

However, it is well known that identification of hair by morphological methods is not always reliable and should be done by a well-trained analyst for maximum reliability. It is difficult to distinguish the suspect's hair from many other hairs left at the crime scene if there is no finding of particular characteristics in the morphology. Therefore, in addition to morphological examination, ABO blood typing from hair can be made, which is helpful in the comparison of hairs. Furthermore, various instrumental methods for hair analysis have been investigated to obtain more objective information and enhance the degree of certainty in forensic hair comparison. These methods, including a new powerful method (deoxyribonucleic acid (DNA) typing analysis), are introduced in this article together with older ones.

One consequence of the application of DNA testing techniques to hair occurring as evidence has been the reexamination of old cases as the new technologies have become available. In the several instances that have been reported, DNA evidence from hairs has contradicted the interpretations based upon the microscopic examination of crucial hairs used in the original trials. In addition, an American Supreme Court decision in 1993, concerning the grounds of the admissibility of scientific evidence, has resulted in a review of many categories of scientific evidence and their use in the American courts. The reliability of hair evidence has been a cause of particular concern and a number of studies to establish the scientific credentials of such examinations have been published. While these issues are primarily of concern to American practitioners the forensic community in general has benefited from these studies.

Another issue that is continually being addressed is the speed and cost-effectiveness of hair comparisons. To this end the systematic codification of microscopic features in order to generate coded categories of variant forms to improve the consistency of hair examination reporting continues to be developed. Potentially, a count can be made of the different codes reported in a laboratory or system of laboratories to enable a numerical evaluation of the commonness of any given variant form to be estimated. This value would assist the court to assess the significance of evidence.

## Structure and Composition of Hair

Hair is an appendage of the mammalian skin produced by an organ known as the hair follicle in the epidermal epithelium. The portion of the hair that lies in the follicle is called the root, whereas the portion above the skin surface is called the shaft. In a cross-sectional view of hair shaft, there is an outer layer of cuticular scales that surrounds the cortex shaft in the center of which a canal-like structure called the medulla is found (**Figure 1**). Pigment granules (melanin) are dispersed usually in the cortex, produced by melanocytes in the follicle. The hair follicle does not produce hair continuously. Hair grows cyclically, with alternate periods of growth and rest. This cycle is divided into three stages (anagen, catagen, and telogen). In the anagen stage, hair grows on, and in the subsequent catagen stage club hair is formed in the follicle. In the next resting telogen stage, hair ceases to grow. After the inactive telogen stage, the follicle goes into another anagen stage and resumes activity. In man, each follicle has its own growth cycle that is independent of the others. During the growth stage, hair grows at a rate of about one centimeter per month. An adult man has ~100 000 scalp follicles, 90% of which are anagen and 10% telogen.

Chemically, hair is mainly composed of particular proteins (keratins), hard and stable to usual physical and chemical treatment. This characteristic of keratin protein is a result of the high degree of disulfide bonding between polypeptide chains in the molecules. Human hair keratin is composed of 21 amino acids. When these amino acids are grouped together according to functional group, there are high content of hydroxyl, primary amide, and basic amino acid functions. Human hair keratin is also characterized by high sulfur content due to high cystine content (17–19% residues as half cystine). The remaining constituents are water (*c.* 13% in 65% relative humidity), lipids (originating from the sebaceous gland in the follicle), pigment (principally eumelanins), and trace elements (calcium, magnesium, potassium, zinc, sodium, chlorine, etc., from sweat deposits and extraneous sources).

## Morphological Examination of Hair

At the crime scene, various fibers and hairs are collected by policemen. They are immediately examined at the forensic laboratory in order to identify the suspect and help solve the crime. The examination needs to establish: (1) whether it is a hair or a fiber (synthetic or plant); (2) its species if it is a hair; (3) its somatic origin if it is a human hair; (4) whether it is similar to the suspect or the concerned persons. Microscopic examination will resolve the first point. It will also allow discrimination between human and animal hair, as the medulla of human hair does not develop well and shows less than 0.30 of medullary index (ratio of medulla diameter to hair diameter). But determining the species of animal requires experience in animal hair examination. Almost every animal hair shows specific shape of cuticule and medulla under microscopical observation. With regard to the somatic origin of human hair, there are some basic features that usually indicate the bodily origin of the hair. For example, head hair has long length, 30–120 μm diameter, tapered tip, little diameter variation, various medullation, sometimes cut tips, and treatment. Pubic hair has a twisted

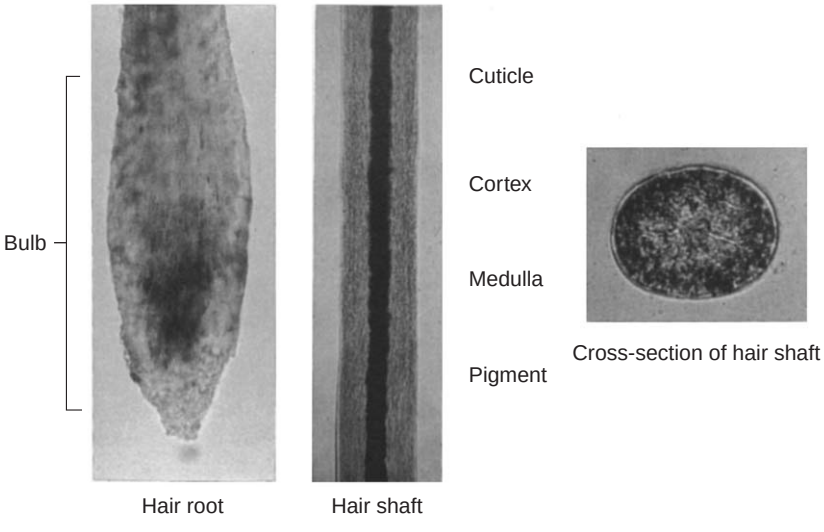


**Figure 1** Microscopic pictures of human hair shed naturally.

form, shorter length, coarse diameter, prominent diameter variation, broad medulla. The morphological association of human hair is carried out by comparing every characteristic of both a suspect's and unknown hair macroscopically and microscopically. The association cannot be done by only one or two characteristics. There are variations within one person from natural growth, but the amount and kind of variation differs between persons. Generally, effective items of macroscopical and microscopical identification of human hair are color, length, diameter, contour, distribution of medulla, and cosmetic treatment. Concerning individuality, there is a limit to the possibility of identifying individual hair among large pieces of hair from many persons by morphological examination.

For some time many forensic laboratories have adopted some form of systematic comparison strategy to improve the consistency of the examinations. One example of this was demonstrated in a study of the efficacy of hair comparisons by microscopic examinations. Fourteen different characteristics were defined as primary and secondary characteristics for comparison. The primary characteristics were the color, cosmetic treatment, texture, pigment distribution, medulla, medullary index (i.e., the width of the medulla as a proportion of the width of the hair), maximum diameter, and presence of cortical fusi (microscopic structures observable within the cortex of the hair). The secondary characteristics were length, cuticular margin, pigment density, pigment size, and shaft diameter variation. By a systematic point-by-point comparison of these characteristics by two examiners working on the same samples in parallel but independently of one another both examiners were able to make correct assignments in a proportion of trials but more importantly did not make any false matches. In other blind trials forensic laboratories could achieve very high rates of correct associations, with no false associations, although some laboratories achieved much poorer success rates. The poorer rates of success have been attributed to the standards of training undergone by the examiners but the studies have provided evidence that, with experienced and well-trained examiners, microscopic hair examination can be a rigorous, truly scientific method of comparison. It is crucial that the hair examiner must be careful that an unwarranted degree of weight is not put on the results.

There are continuing developments of schemes to codify the characteristics by assigning a code to each different alternative form of any given characteristic. Thus, an individual hair specimen may be characterized by a numerical score for each feature defined by the scheme and these can be compiled into an ordered series. In this way hairs can be categorized and a count made of the number of occurrences of a category in a population. Such a count can be made to generate frequency data so that an examiner could draw upon observational data to give a numerical estimate of the rarity or commonness of any of the defined hair types within a population. An important aspect of such scoring systems is the consistency with which the categories are assigned. However, by the use of photographic standards, termed archetypes, published as an atlas of microscopic features, the form, or variate, of each characteristic observed in the hair considered can be categorized consistently by reference to the archetype.

## Chemical Analysis of Hair Components

### Elements

Chemical features determined by analysis of elements in human hair are helpful for hair comparisons. Analysis of elements in the hair has been accomplished by instrumental methods such as atomic absorption spectrometry, neutron activation analysis, energy dispersive X-ray microanalysis (EDX). Among them, EDX equipped with scanning electron microscopy is widely used in forensic hair examination because it facilitates easy analysis of elements whilst observing the hair structure.

Twenty-seven elements are found in human hairs, and the majority of them are due to an accumulation of products from sweat. These elements can be grouped into two classes: physiological and environmental. The former includes sodium, phosphorus, potassium, and sulfur, which are present at high levels. The latter includes arsenic, cadmium, mercury, and lead, which are called toxic elements. As these elements may indicate some particular environment for an individual, and their amounts vary considerably across individual hairs, they can be specific and effective indications for forensic hair comparison. If a particular element was determined in a suspect's hair, it can be easily compared in sample hairs.

Content of elements in hair is closely concerned with hair color, disease, treatment, sex, and weathering. For instance, the amount of chlorine in male hairs detected by EDX is higher than in female ones among 38 adult persons (Japanese) aged from 20 to 50 years (males: $0.67 \pm 0.20\%$ w/w; females: $0.45 \pm 0.12\%$ w/w). Chlorine in hair is considered to come from sweat. Electrothermal atomic absorption spectrometric analysis also shows that calcium is more abundant in permanent waved hair than in untreated hair (waved hair: 2000–4500 $\mu g\, g^{-1}$).

Copper content is low in the hair of infantile pellagra (kwashiorkor) and grey hair of an old man, but is high in trichoschisis and black hair. Content of zinc is low in dwarf and blond hair and also in the hair of pregnant women, but is high in ill-nourished children and black hair. Some elements in hair show significant differences of value according to gender and age, as well as various hair treatments. These variations also occur in different scalp areas and different portions of single hair of a man. If there are some patterns of variation in sample hairs found, it may be helpful to compare them with those of suspect's hair.

### Instrumental Analysis

New methods in hair analysis using the many remarkable instruments that have now been developed are described in this section. Human hair (40–80 μg: ~1 cm) can be analyzed by pyrolysis capillary column gas chromatography (Pyr-GC). Some main peaks are observed in the pyrograms of hair samples. Components of these peaks are identified by pyrolysis capillary column gas chromatography–mass spectrometry (PyrGC-MS). Toluene and styrene as pyrolysis products are considered to arise from phenylalanine, phenol and cresol from tyrosine, methanethiol from cystine and/or methionine, and carbon disulfide from cleaving free carboxyl groups and disulfide bridges. It is thought that variation in amino acid composition causes differences in pyrograms of the individual hairs. If reproducibility is established, this method is likely to be used in forensic hair comparison. Hair treatments, especially hair bleaching, were reported to have influenced the chemical and physical nature of hair. Infrared (IR) spectra from a single hair can be measured by Fourier transform infrared analysis for detecting the oxidation of hair. There is an absorbance at 1042 $cm^{-1}$ not only in bleached hair, but also in permanent waved hair and dyed hair. This absorbance arises from sulfonic groups that result from the cleavage of disulfide bridges in hair. The oxidation occurs more strongly in the hair tip than in the root. This phenomenon also arises from weathering and ultraviolet irradiation. Though it is usually difficult to establish whether a suspect's hair has been treated or weathered from IR spectra, IR analyses can provide evidential clues for hair comparison without consuming the sample. It is possible to investigate differences between chemical oxidation of a disulfide bond and weather-beaten oxidation in a single hair by electron spectroscopy for chemical analysis, because the bonding energy of sulfur atoms differs under the various conditions. A method of dating samples by electron spin resonance (ESR) spectroscopy has been developed and applied to a wide variety of materials. Red melanin (phaeomelanin), which does not have unpaired electrons, and black melanin (eumelanin), which has unpaired electrons, show different spectra; thus, the intensity of the ESR signal of brown hair is weaker than that of black hair. The ESR signal of cut hair reduces according to the time since cutting. As the radical in the hair of 1 cm long can be detected by ESR analysis, this method will be applied to the field of forensic science. Derivatives such as amino-hydroxyphenylalanine and pyrrole-2,3,5-tricarboxylic acid, respectively, produced from phaeomelanin and eumelanin in human hair (~4 mg) are determined by liquid chromatography (LC). The contents of both melanins can be determined using LC and provide a clue for hair identification.

## Biochemical Analysis of Hair

### Intracellular Enzymes of Hair Root

It has become clear that many intracellular enzymes exist in the root sheath of human hair. They are alcohol dehydrogenase (ADH), esterase D (EsD), α-L-fucosidase (FUC), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), etc. The biochemical properties of these enzymes in hair root are very similar to those in other tissue cells. Some of these enzymes have different proteins with similar enzymatic activity. They are called isoenzymes. These isoenzymes show 3–10 phenotypes. $PGM_I$ isoenzyme is frequently utilized for forensic hair identification, because it shows more phenotypes than other isoenzymes (**Table 1**). Many isoenzymes are detected by means of isoelectric focusing with polyacrylamide gel having a high separating ability. Therefore, polymorphism of these enzymes can be one item of information like ABO blood groups for hair identification if the activity of the hair root enzyme is observed.

### Electrophoresis of Hair Keratin Protein

Forensic hair identification has been investigated using the electrophoretic patterns of hair keratin

**Table 1** Representative isoenzymes in human hair root

| *Isoenzyme system* | *Numbers of phenotype* | *Methods of electrophoresis* |
|---|---|---|
| ADH | 3 | Isoelectric focusing |
| EsD | 3 | Isoelectric focusing |
| FUC | 3 | Isoelectric focusing |
| G6PD | 4 | Starch gel or polyacrylamide gel |
| 6PGD | 3 | Starch gel |
| $PGM_1$ | 10 | Isoelectric focusing |

protein, which have a variety of composed peptides and a genetic polymorphism. Hair keratin, a polymer of amino acids, has a firm structure and is not dissolved in water, common buffers, or organic solvent. In order to solubilize hair keratin, the bridge linkage of disulfide bond is cleaved by reductant (e.g., 2-mercaptoethanol), and sulfydryl groups produced by the reduction are alkylated by iodoacetate for the purpose of preventing reoxidation. As a result of that, a soluble derivative of *S*-carboxymethylkeratin corresponding to native protein in hair is obtained, and becomes a more soluble substance suited for electrophoresis by adding urea (protein-denaturing reagent).

Keratin proteins in human hair are composed of low-sulfur proteins (mol. wt. ~50 000–76 000) of cortical fibrils and high-sulfur proteins (mol. wt. ~25 000–43 000) of matrix among fibrils. Phenotype frequency of low-sulfur protein variant is ~4% in Caucasian hairs detected by 10% polyacrylamide gel electrophoresis, but its variants have not been detected in Mongoloid (Japanese) hair. On the other hand, high-sulfur protein variants have been detected in ~30% of Japanese. These variants are considered to be inherited in autosomal dominant fashion. Furthermore, a two-dimensional electrophoresis system has been developed for obtaining detailed information of these hair proteins. More different patterns can be observed by this analysis than by the one-dimensional system, and are said to arise from nutritional, physical, and environmental factors and disease in individuals. For example, a two-dimensional system using acidic (pH 3) polyacrylamide gel–sodium dodecyl sulfate provides eight variable protein groups or spots, in which some additional spots or their absence are related to those factors. These electrophoretic analyses of hair proteins are also effective in the identification of animal species.

### Amino Acids

Remarkable amino acid analyzers have been developed and can analyze one piece of hair. Variation of amino acid composition in human hair is caused by nutrition, disease, and treatments (permanent waving, bleaching etc.). The effect of cosmetic treatments such as bleaching, dyeing, or permanent waving on the amino acid composition is to decrease the content of cystine and increase the competent of cystic acid depending on the extent of the treatment. Thioacetylated lysine, carboxymethylthiol cystine, dithiodiglycolic acid, and methionine sulfone have been found in permanent waved hair. This information on variation in amino acid analysis of a single hair may be helpful for forensic hair comparison.

### DNA Analysis

DNA fingerprinting was developed for individualization system in 1985. Analyses using DNA profiling with variable numbers of tandem repeat polymorphism has been carried out for identification of forensic samples such as bloodstains and sectional stains. Concerning forensic hairs, several DNA analyses of hair root sheath cells have been reported. However, DNA analysis of a hair shaft has not succeed to date, because DNA recovery from a hair shaft is in order of tens of picograms and only low molecular weight DNA (below 200 base pairs (bp)) is left in the hair shape. Recently, microsatellite DNA polymorphism has been detected by the polymerase chain reaction technique. This polymorphism can be applied to DNA analysis of hair shaft. If DNA analysis is combined with microscopic and instrumental analysis in forensic examination, hair individualization can be more accurate.

### Mitochondrial DNA Analysis

Human identification by testing the nuclear DNA within cells has become a powerful and sensitive tool. It can be used with those hairs that possess some tissue, usually in the form of cells from the hair root or from sheath cells that adheres to forcibly removed anagen hairs. The presence of these tissues often requires microscopy to find them and in some instances a result can be obtained from the hair shaft. However, in many instances such tissue is not present, such as the case of telogen hairs that have been shed naturally, and there is insufficient material for tests of nuclear DNA to be productive. An alternative strategy is to test the DNA content of cell organelles called mitochondria. These are present in the cytoplasm of the cells and take part in the cellular respiratory mechanisms to provide energy for cellular processes. They are present in high numbers within the cells in tissues that are dividing rapidly. Each particle contains multiple copies of the mitochondrial DNA, or mtDNA, with the result that the copy number of the mtDNA can be several thousand-fold greater than the nuclear DNA.

The dermal material in the hair root where the hair shaft is produced is an example of such tissue. As the shaft is created and subsequently keratinized the mitochondrial particles are trapped in the shaft and persist in the hair. The mtDNA thus presents a more promising target for DNA testing and indeed the exploitation of this feature of hair has become an important part of forensic hair analysis.

### Haplotypes and Databases of mtDNA

The mtDNA genome occurs as a single, double-stranded, DNA loop. The bases making the loop are

numbered one to 16 569, representing the entire loop. Two parts of the loop have been found to possess greater variety in the DNA base sequence than the rest of the loop. These two regions are called hypervariable regions I and II or HVRI and HVRII, respectively. The mtDNA sequence has been entirely determined and a widely recognized reference sequence, such the Cambridge Reference Sequence (CRS), can be used as a standard against which the sequence of any portion of the mtDNA of a sample is compared. There is no opportunity for recombination of the base sequences present on the mtDNA loop from generation to generation but mutations do occur within the mtDNA, especially within the hypervariable regions, so that the base sequence changes at certain sites. These changes can be in the form of transitions, transversions, or insertions or deletions. A transition is a change of one purine to another purine, for example, an adenine base changed to a guanine, or a pyrimidine changed to another pyrimidine, e.g., a cytosine to a thymidine. A transversion is a change of a purine to a pyrimidine, or a pyrimidine to purine. Insertions or deletions of base sequences are called indels. The mtDNA loop hypervariable regions HVRI and HVRII are rich these variations. Many forensic laboratories typically sequence positions corresponding to the HVRI and HVRII regions.

A nomenclature system has been developed for mtDNA typing. It has been adopted as a convention within various bodies of the forensic science community. Simply put the rules are that the bases determined from a sample of mtDNA are compared to that of a reference sequence, for example, the CRS, at corresponding base positions. Where transitions or transversions have occurred, the change is denoted using the mtDNA base position number followed by the initial letter of the base found in the sample at that position. Only those positions that differ from the reference sequence are stated. The CRS is based upon a human mtDNA sequence that has been found subsequently to possess uncommon base types at certain positions. For example, a specific position of the CRS may be an A while most people possess a G at that position. Therefore, many sample mtDNA determinations, should they include this position, will include the recorded variation of a G at that position because it differs from the reference sequence. Where there is concordance between the sample bases at a given position with those of the reference sequence then there will be no specific designation on the assumption that the base type of the sample is the same as reference sequence.

Indels are denoted by citing the base position at the 5′ side of the indel and using a decimal point to indicate an insertion; for example, specifying the base type as a capital letter of the base name following the position number, 85.1C or 85.1A. Where there is a mixture or an ambiguity then the letter N is used; however, the International Union of Pure and Applied Chemistry letter convention can be used. In the case of deletions again the 5′ base position number is used, followed by the letter d; so, for example, a deletion might be denoted '85d'.

### Mitochondrial DNA Sequence Alignment

The comparison of sequence data with a reference sequence requires careful attention to sequence alignment due to the possibility of discrepancies between sequences of the samples and that of the reference sequence arising from indels. The convention for aligning sequences for comparison is given here in general terms but there are a number of special circumstances that are dealt with more thoroughly in the literature. The conventions propose that sequences should be aligned so as to minimize the number of differences between the sample sequence and the reference sequence. In cases where there are equally good alternative means of aligning the sequences the priorities should be to align by the indels first so that the displacement of portions of the sequence arising from deletions or insertions are allowed for, after which come translations and then transversions.

Once the alignment has been made the combination of variations occurring at different places on the mtDNA strand can be identified and sequences can be compared with the reference sequence and cross-comparisons between different hair samples made. In Caucasians, there appear to be an average of around eight positions within the HVRs where the nucleotides differ between unrelated people and around 15 differences between people of African descent. As there is no recombination of the mtDNA the combination of variations in the HVRs is inherited as a single entity called a haplotype. The mitochondrial databases therefore count the frequency of the different haplotypes.

The HVR regions possess the greatest number of variable sites in the mtDNA and the base sequence of the variable regions, or the major parts of them, of the questioned hair are compared to the corresponding sequences determined from the reference hairs. Although there may be potentially many different haplotypes the maternal inheritance of the mitochondrial genome will restrict the diversity observed in a population, as many individuals will possess coancestry by the maternal line. Nonetheless, in studies carried out of the haplotypes observed in different populations the number cooccurring in different

individuals is small. As databases grow and more mtDNA sequences are determined there will be continual adjustments to the frequencies observed for different haplotypes. Population databases consisting of the sequences from the mtDNA from unrelated individuals of different populations have been established.

Certain haplotypes share a high proportion of the types of nucleotides found at the variable sites. Phylogenetic studies can be used to identify groups of haplotypes that show a high degree of commonality. Such groupings are termed haplogroups and in Caucasians it has been found that 10 haplogroups account for 99% of the population and 90% of this proportion of the population are represented by five haplogroups.

### Heteroplasmy

The sperm head possesses no mitochondria and upon fertilization only the contents of the sperm head enter the egg cell. A consequence of this is that, in principal, at least, no paternal mtDNA is passed to the fertilized call and only the maternal mtDNA is transmitted to the next generation so that in theory all the mtDNA sequences in a person should be the same, i.e., homoplasmy. However, in practice different populations of mtDNA may coexist within one person, i.e., heteroplasmy. In the course of embryonic development the number of mtDNA molecules is greatly reduced, perhaps to less than 10, so that the chance of a variant mtDNA haplotype, present in the original pool in only a small proportion of the total mtDNA, being passed on is reduced. This therefore acts as a brake, or bottleneck, on the accumulation of heteroplasmic variations in the pool of mtDNA types. As the reductive process does not select for particular mtDNA haplotypes heteroplasmic variants may be missing from some cells after the bottleneck. If different variants were present in the original pool then some cells may possess one type of variant and others a different variant. In most tissues there will be a mixture of mtDNA from different cells so that an analysis of the mtDNA haplotypes will reflect the heteroplasmic mixture if any is present. It is possible that one haplotype predominates to the extent that a different haplotype present may not be detectable but this is a separate issue. However, the analysis of the mtDNA from the shaft of a single hair in a forensic context may not represent a sampling of the different heteroplasmic haplotypes present in the donor.

The hair shaft is produced from a collection of rapidly dividing cells in the hair bulb matrix of germinal cells. These cells divide rapidly and contain many mitochondria. The new cells produced do not divide again but swiftly differentiate into cuticle cells, cortical cells, and medullary cells. One member population of the germinal cell matrix is the melanocytes. These are pigmented cells that produce melanin and store it in melanosomes. These cells produce the pigment granules that give the color to the hair and have large energy requirements.

The melanocytes come from the neural crest, a group of cells in the neural tube of embryo. These cells move out to different places in the embryo to develop into a variety of types of adult cells. The melanocytes are distributed throughout the skin at random and constitute a different population of cells from the rest. They become a part of the germinal cell matrix.

The melanocytes divide slowly, unlike the other cells of the germinal matrix of the root bulb, but they produce dendritic arms that are assimilated by endocytosis into the precortical cells that are moving out of the germinal matrix. These precortical cells are undergoing keratinization as a part of the process of the formation of the cortex of the hair and that involves the intensive production of keratin protein filaments. Therefore, there are two sources of the mtDNA molecules present in the cortex of the hair, one from the germinal cells of the matrix of the root bulb and the other from the melanocytes. The proportions of each contribution of mtDNA molecules are not known; however, there will be very little opportunity for daughter mtDNA molecules to be produced in the differentiating cortical cells while the melanocytes are continuously producing more pigment and mitochondria. Consequently, there can be genetic drift in the proportions of mtDNA molecules from the two sources so that haplotypes of mtDNA observed along the shaft of the hair can be different in different places. Also, different hairs that have come from the same follicle can potentially have different haplotypes. Therefore, while mtDNA analysis is undoubtedly a powerful tool for the forensic analyst there are special considerations that have to be taken into account in any interpretation made.

Opinions have been expressed that microscopic examination of hairs should be regarded as a presumptive step to act as a screening procedure prior to mtDNA analysis. However, many reports also emphasize the importance of the combination of the two approaches because the contribution the microscopic comparison can make. The microscopic examination gives an independent comparison of different characteristics. The analysis of mtDNA remains an expensive procedure, and not all hair specimens yield a usable result. Microscopic examination can eliminate obvious mismatches such as many nonhuman hairs, it can be used on fragments

possibly too small for DNA testing, and it can yield other information such as an association with racial, Caucasian/European, African, or Asian, ancestries. It is necessary to use microscopic examination to obtain information about the possible body areas and it can also yield other information such as cosmetic treatment. The microscopist can also identify if cellular tissue is adhering to the hair. This is a potential source of material for DNA testing using the same system of combined tests of independent short tandem repeats, or multiplex STR analysis, as used with other biological materials. This test can produce very powerful evidence of association between a hair and a donor if there is a match of DNA types.

## Immunological Analysis of Hair

Keratin proteins and blood group substances such as antigens are identified in human hair, whereas immunoglobulin has not been detected in it. Blood group substances (A, B, and H) are contained mainly in the hair cortex. These substances have the chemical property of glycolipids. An absorption–elution technique for determination of ABO blood group from forensic hair sample has been developed. Using this technique it is possible to determine the ABO blood group from one single hair $\sim$6 cm in length. Furthermore, the unlabelled antibody immunoperoxidase (peroxidase–antiperoxidase) method is utilized for determining the blood group of more minute hair. The ABO blood grouping is used for discrimination in forensic hair comparison, especially when the analyst cannot distinguish the suspect's hair from the control sample hair with similar morphological features.

## Chemical Analysis of Components Remaining on or in Hair: Hair Shampoo, Hair Care Products, and Oxidative Dyes

People use hair shampoo, hair rinse, hair care products, and coloring preparations regularly for the purpose of hair beauty, and seldom change the brands of these products. Therefore, forensic hair identification becomes easier if these components remaining on or in human hair can be detected. The main components of hair shampoo are anionic (for instance, lauryl sulfate triethanolamine, sodium lauryl sulfate) or nonionic substances. Other components are amphoteric detergent, conditioner, agents increasing viscosity or foaming, dye, and perfume. Shampoo components remaining in 5–10 cm long hair are extracted with a solvent of acetonitrile and water, and these extracts are analyzed by LC. The liquid chromatograms for four individuals shown in **Figure 2** are different from each other. Because quantitative ratios of various components of shampoo products differ from ones of the hair's extracts, it is difficult to identify the brand of shampoo used. But LC

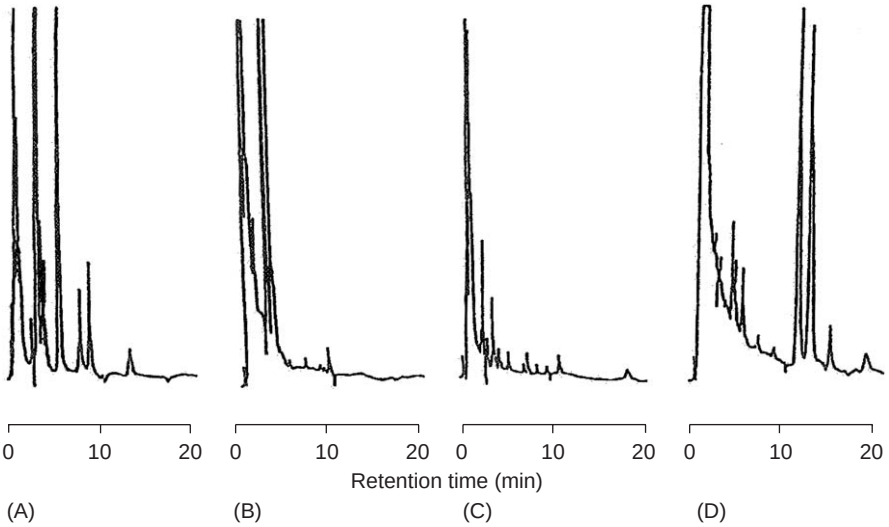


**Figure 2** Liquid chromatograms of hair extracts from four different persons. Extracting solvent: acetonitrile and water (70:30, v:v); equipment Varian model 5000 liquid chromatograph with Waters Associates fixed-wavelength absorbance detector (model 440) operating at 254 nm; column: 20 cm, 5 μm Nucleosil C-18, 4.6 mm i.d. (Skandinaviska Gene Tec AB); mobile phase: acetonitrile and water (50:50); flow rate: 1.5 ml $min^{-1}$. (Reproduced with permission from Andrasko J and Stocklassa B (1987). HPLC analysis of shampoo residues in human head hair. *Proceedings of the Eleventh Meeting of the International Association of Forensic Science*, vol. 20, pp. 249–255.)

nondestructive analysis can give more objective and helpful information in individualizing of target hair among forensic hair samples. Identification of the brand of hair care products (men's and women's hair care products, oxidative dyes, hair sprays, and hair growth promoters) by components remaining on or in human hair is investigated. These components are identified mainly by GC, GC–MS, and thin-layer chromatography (TLC) analysis after extraction with ether or methanol. The main components of men's hair care products (hair liquids) remaining on human hair are identified as 2-hexyldecanol (emollient), isopropyl myristate (emollient), diethyl phthalate (perfume reserver), benzyl benzoate (perfume), methyl dihydrojasmonate (perfume), pentapropylene glycol (moisturizer), oxybenzone (sunscreen), etc. In the experiments, hair liquids of 57 brands produced by 17 companies are grouped into 21 classes on the basis of main components remaining on human hair, and among them hair liquids of 11 brands are identified by particular components (docosamethyldecasiloxane (polisher), isostearyl alcohol (emollient), diisopropyl sebacate (emollient), benzyl salicylate (sunscreen, perfume reserver), etc.). The major components of hair liquids remaining on human hair can be detected as long as 5 days after treatment. Other hair care products are also grouped similarly.

In the application of this method to an actual crime involving injury, components of hair care products remaining on hair adhering to a suspect's clothing were analyzed by GC and GC–MS, since morphological investigation had shown that some of these hairs were different from the victim's head hairs. The five main components remaining on hairs from both the victim and the suspect's clothing was found to be exactly the same. These gas chromatograms are shown in **Figure 3**. Two of these components were identified as dibutyl phthalate (perfume reserver or emollient) of peak 1 and the unidentified component of peak 4 originated from hair sprays which the victim used.

Hair coloring preparations are classified into four types: temporary dye, metallic (progressive) dye, ionic (acidic and basic) dye, and permanent (oxidative) dye. The oxidative dye is the most popular hair dye because of its color durability and easy application. Oxidative dyes are usually composed of dye compounds (A) solution and a hydrogen peroxide (B) solution. Both solutions are creamy. The A solution also contains strong alkaline ammonia to open hair cuticles. Dye components polymerize into indophenol-like trimers, etc., in the presence of hydrogen peroxide, primarily in the cortex of the hair. Hairs 3 cm long dyed by oxidative dyes of 34 brands are dissolved in the solution of methanol plus 3 mol $l^{-1}$ sodium hydroxide and sodium hydrosulfite dithionite), and these methanol extracts are analyzed by TLC. As shown in **Figure 4**, detectable amounts are present in hair dyed by 16 brands. These brands are classified into three groups from the identified components; i.e., in the first group only *p*-phenylenediamine (PPDA) is detected, the second group only 2,5-diaminotoluene sulfate and the third group PPDA and an unidentified compound that may be formed in the dyeing process are detected. Furthermore, after neutralization of the alkaline solution with 3 mol $l^{-1}$ acetic acid, it is extracted with ether. From the extracts, other dye components, *o*-, *m*-, and *p*-aminophenols are identified by GC–MS after trifluoroacetylation. The identification of brands of oxidative dyes used has been no more successful than that of brands of shampoos used. But it is possible in

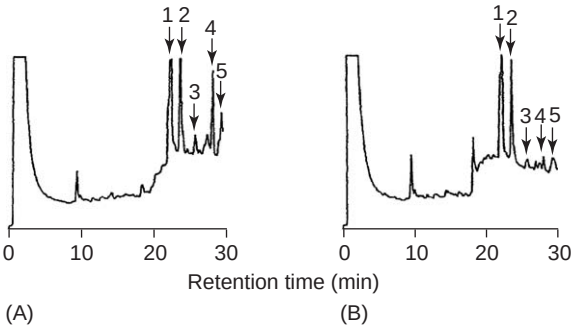


**Figure 3** Gas chromatograms of components remaining on human hair adhering to suspect's clothing (A) and victim's head hair (B). Equipment: Shimadzu GC-4CM with a flame ionization detector column: 3 mm i.d. × 2 m, 1% Silicone OV-17 on Chromosorb-W (AW-DMCS, 80–100 mesh); column temperature: program from 100 to 280°C at 5°C $min^{-1}$; carrier gas: nitrogen, 40 ml $min^{-1}$.

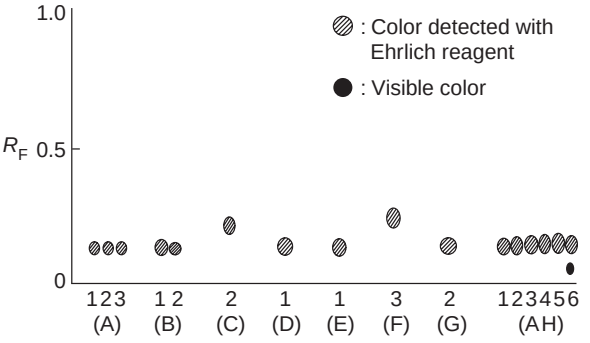


**Figure 4** Thin layer chromatograms of oxidative dyes remaining in human hair. Capital letters (A–H) are assigned to indicate the companies of dye products. Numbers (1–6) indicate brands of products. TLC plate: Silica-gel 60 (20 × 20 cm, 0.25 mm thickness, purchased from Merck Co.); developing solvent chloroform–ethyl acetate–methanol (6:3.5:0.5, v/v).

some cases to ascertain whether target hair is dyed or not by analysis of remaining components of hair coloring preparation.

### Drugs

If amphetamines are detected in a suspect's head hairs, the suspect may be arrested under stimulant control law and the hairs can be used as evidence in a trial. Furthermore, various drugs contained in human hairs not only provide evidence of crime under drug control laws but also important clues to hair comparison for other criminal investigations. Many cases of drug abuse have been established by using human hair. These drugs are stimulants (amphetamines), heroin, phencyclidine, cocaine, barb rates, etc.

It is generally thought that blood capillaries break in bulb of hair root and drugs are taken into the hair while matrix cells produced in hair papilla grow and are keratinized. The drugs are contained mainly in hair cortex and amount to 50–100 ng mg$^{-1}$ single hair (equivalent to $\sim$10 cm long). The drugs can be detected by radioimmunoassay and GC–MS. In the GC–MS analysis of amphetamines in human hairs, sample hairs of 10 mg are washed with 0.1% sodium lauryl sulfate and water three times under supersonication, and are dried. These hairs are dipped in the solvent mixture (2 ml) of methanol and 5 mol l$^{-1}$ hydrochloric acid (20:1) under supersonication. Then it is left to stand overnight at ambient temperature. The solvent mixture is separated, and is dried under a stream of nitrogen. The resulting residues are dissolved in the solvent mixture (200 µl) of trifluoracetic anhydride and ethyl acetate (1:1), and are heated at 55°C for 20 min. The reaction mixture is dried under a stream of nitrogen. The residue dissolved in ethyl acetate is subjected to GC–MS analysis. A mass spectrum of trifluoroacetyl derivative of methamphetamine extracted with methanol from suspect's hairs in a stimulant offences case is shown in **Figure 5**.

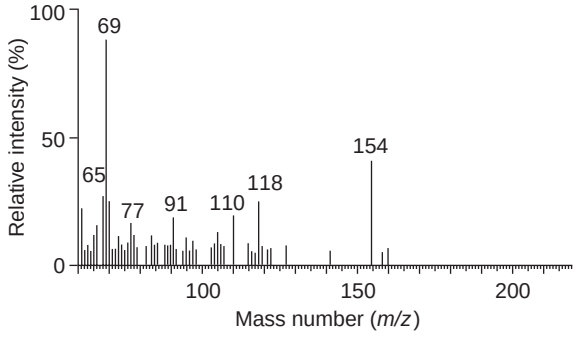


**Figure 5** Mass spectrum of trifluoroacetyl derivative of methamphetamine extracted from suspect's head hairs. Sample hairs: 100 pieces of 1 cm long hair; extracting solvent of hairs: methanol–5 mol l$^{-1}$ hydrochloric acid (20:1); equipment Shimadzu GCMS-OP 1100EX; column: Shimadzu capillary column HiCap-CBP10-W25-100, 0.53 mm i.d. × 25 m cyanopropyl (thickness 1.0 µm); column temperature: 140°C; carrier gas: helium 20 ml min$^{-1}$; ion source temperature: 270°C; electron energy: 20/70 e/V.

Compared with urine, hair samples have many advantages as an evidential material, because of easy sampling and handling, long stability time (approximately 3 days–1 year after intake), and high sensitivity (detectable at the nanogram per milligram level). Moreover, an individual history of drug intake can be worked out by analyzing each segment along the hair shaft from hair root to hair tip.

*See also*: **Activation Analysis**: Neutron Activation. **Amino Acids**. **Atomic Absorption Spectrometry**: Principles and Instrumentation. **Cosmetics and Toiletries**. **Electron Spin Resonance Spectroscopy**: Biological Applications. **Electrophoresis**: Isoelectric Focusing; Polyacrylamide Gels; Two-Dimensional Gels. **Elemental Speciation**: Overview. **Forensic Sciences**: DNA Profiling. **Gas Chromatography**: Pyrolysis; Mass Spectrometry; Forensic Applications. **Immunoassays, Applications**: Forensic. **Polymerase Chain Reaction**. **Proteins**: Physiological Samples. **Surfactants and Detergents**. **X-Ray Fluorescence and Emission**: Energy Dispersive X-Ray Fluorescence.

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# Illicit Drugs

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## Introduction – Types of Drugs

Drugs of abuse can be divided into different types according to their method of production or isolation. Natural drugs are active constituents and/or secondary metabolic products, of plants and other living systems, which are isolated by some method of extraction. Morphine is one such example. Semisynthetic drugs are products from natural sources, which have undergone some chemical refinement such as diamorphine, which is the diacetylated reaction product of morphine. Synthetic drugs are artificially produced substances for the illicit market, which are almost wholly manufactured from chemical compounds in illicit laboratories (for example amphetamine). Some forms of synthetic drugs are also called designer drugs and are substances whose molecular structure has been modified from the original in order to optimize their effect on the one hand and bypass laws and regulations governing the control of substances on the other.

## Natural and Semisynthetic Drugs

### Opiates

Opium is a natural product obtained from unripe poppy capsules. There are over 100 species of the genus Papaver (or poppy) known, however only two, *Papaver somniferum* and *Papaver setigerum* D.C., are the main plants cultivated in the production of opium.

An incision is made in the poppy capsule and the latex, which oozes from the incision, is scraped off and collected to produce a raw opium gum. When fresh, the material is a sticky tar like brown substance with a liquorice like odor which becomes brittle as it dries. Raw opium is also obtained from poppy straw. Opium is a complex mixture containing sugars, proteins, lipids, and water as well as the active alkaloid compounds (10–20%). About 40 alkaloids are known and fall broadly into two categories:

- Phenanthrene alkaloids such as morphine, codeine, and thebaine.
- Isoquinoline alkaloids such as papaverine and noscapine.

The relative amounts of these alkaloids varies and is dependent on factors such as climate, soil fertility, altitude, available moisture, and age of the plant. Raw opium can be used for smoking but must first be extracted most commonly with water. Morphine can still be detected in the left over dross that remains in the opium pipe.

**Morphine production** Several purification processes can be used in the preparation of crude morphine from raw opium, two of which are highlighted below. Not all production laboratories may use these methods.

1. The lime method involves the addition of calcium hydroxide (lime) to opium in water. This completely extracts morphine and codeine and partially extracts thebaine and papaverine. Ammonium chloride, alcohol, and ether are added and the solution filtered. Morphine precipitates while thebaine and papaverine remain in the ether layer. The crude morphine is purified by refluxing in dilute sulfuric acid in the presence of charcoal. The final morphine base is precipitated as an off white material by addition of ammonium hydroxide.
2. Raw opium is dissolved in water and the pH adjusted to 6.5 to precipitate noscapine and papaverine, which is removed. The solution is then made alkaline to precipitate morphine and extracted