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A hairy story



On your head, you have about 80000 snapshots of your past. Every single hair strand is a storage facility for drugs, medications, nicotine, and even some of the alcohol you have used. The blood nourishes the hair follicle and as the hair grows it encloses the exogenous substances circulating in the bloodstream. When the hair keratinises metabolism stops, the substances get trapped within the protein structure and stay there as long as the hair exists, whether on your head or in your hairbrush.



In Linköping, at the Department of Forensic Chemistry at the National Board of Forensic Medicine, hair is routinely analysed for drugs and medications in a variety of cases. When is hair analysed for drugs interesting? Well, the special thing about hair is its capacity to serve as a calendar of past drug use. Depending on the length of hair, it can stretch as far back as years before the sampling. In cases of death due to drug overdose, it is of importance to find out the pattern of drug use prior to death. Using hair analysis it is possible to estimate any lowered tolerance for a certain narcotic agent or if the deceased recently changed his or her medication, both helping in the interpretation of the cause of death. Another scenario is to control compliance in a patient population. Through hair analysis one can determine the concentrations of drugs and check if the patient is taking the drugs as prescribed by his or her physician. Also, in cases of

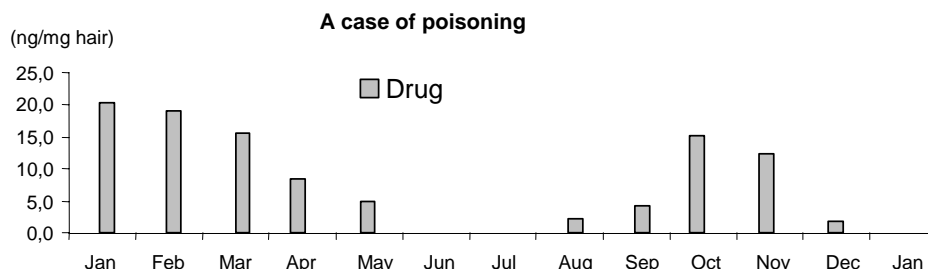


Figure 1. This graph depicts an example of poisoning where hair analysis could confirm exposure to a drug at different occasions during a whole year. The results correlated well with times when the victim showed symptoms of poisoning as well as with periods when the drug could not have been administered.

suspected poisoning the hair analysis might reveal exposure to poisons months and years earlier.

One of the more important issues in hair testing is the sampling. The more controlled sampling the better and the more accurate information can be gained from the analysis. The analysis requires more than a single hair, usually a lock of hair is cut as close to the scalp as possible. Prior to analysis the lock is cut in smaller segments depending on the case. If a "general unknown" analysis is performed the hair is first

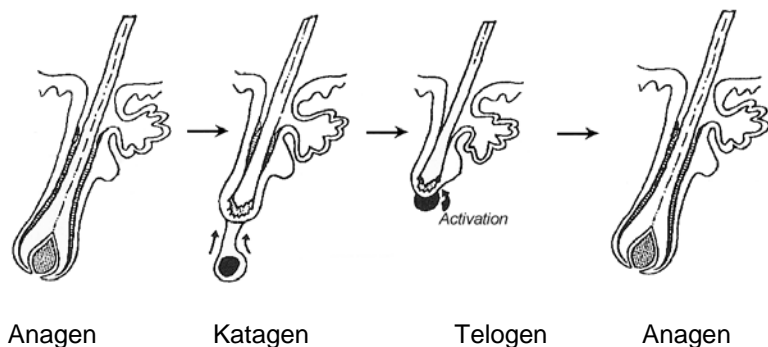


Figure 2. The different stages of the hair growth cycle.

screened for common drugs using LC-MS-MS technique. Any positive result is confirmed by GC-MS where the identity and concentration of the substance is more accurately determined. This chemical analysis is preceded by a "work-up" procedure where the

substances are liberated from the hair matrix, usually by chemical or enzymatic hydrolysis depending on the target drug. After the liberation of the substances, the sample can be extracted in the same way as a blood- or urine samples.

In addition to the routine casework, the National Board of Forensic Medicine also performs research in the field of hair testing to better understand what mechanisms are involved in the binding and storage of different substances in hair.

HOW DOES THE HAIR GROW?

The hair growth cycle consists of periods of growth and dormancy. In humans, each hair follicle has its own cycle independent of its neighbours. The human hair cycle starts with the anagen (or growing) phase during which the follicle develops and the hair is produced. The duration of the anagen phase varies greatly and usually continues for 7 to 94 weeks but may last several years. Catagen is the phase of regression where the activity of the follicle bulb ceases and the dermal papilla contracts as the follicle approaches the resting phase, telogen. See Figure 2. After the telogen phase, yet another growth cycle commences.

HOW DO DRUGS GET INTO THE HAIR?

The pathways for incorporation of drugs into hair and the mechanisms by which they bind to hair constituents have been much debated in the scientific literature. A schematic view of pathways for incorporation of drugs into hair is shown in Figure 3. The relative importance of the different routes is not yet clarified, but several studies have been performed to explain the factors that

influence the incorporation of drugs from the bloodstream.

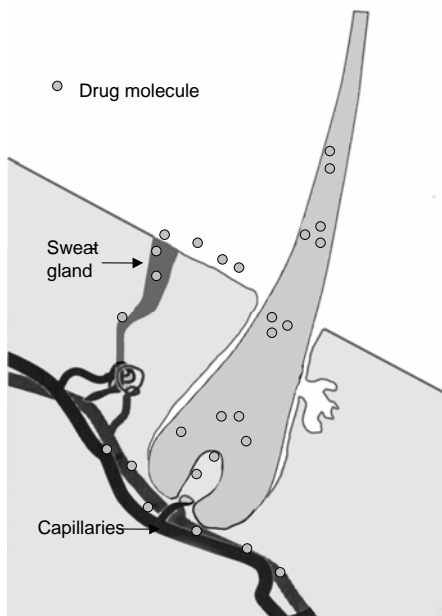


Figure 3. Theoretical model of drug incorporation. Ingested drugs may enter the hair from the bloodstream feeding the dermal papilla as well as by sweat and sebum bathing the mature hair fibre. Modified from Kintz (1996).

HOW DO DRUGS BIND TO HAIR CONSTITUENTS?

The colour of human hair and skin is mostly determined by the quantity and quality of melanin, the major pigment present in epidermal structures of all vertebrates. The Swedish chemist Berzelius, born and raised in the vicinity of Linköping, introduced the word melanin, which is derived from the Greek word melanos meaning dark. Usually one refers to two kinds of melanins even though there are numerous varieties of them. The black to brown eumelanins and the yellow to red pheomelanins. Both have the common precursor, dopaquinone but the resulting macromolecules are chemically distinct. It has been shown that melanins are important binding sites for drugs also in hair.

Actually, the remarkable capacity of melanin to bind various chemicals has emerged as one of the strongest retention mechanisms of the body. The physiological function of this binding is not fully understood. Melanin could function as a local regulator that binds and releases endogenous and exogenous substances, or act as protective chemical filters since they are present in very sensitive tissues such as the eye, ear and brain. The scheme below (Figure 4) shows how codeine molecules diffuse from plasma into the melanocyte where they bind to the forming melanin. Since the pH is low in the melanocyte, the majority of codeine molecules are positively charged enabling interaction with the numerous quinone structures of the melanin. As the drug molecules bind the equilibrium is forced towards the charged form of codeine.

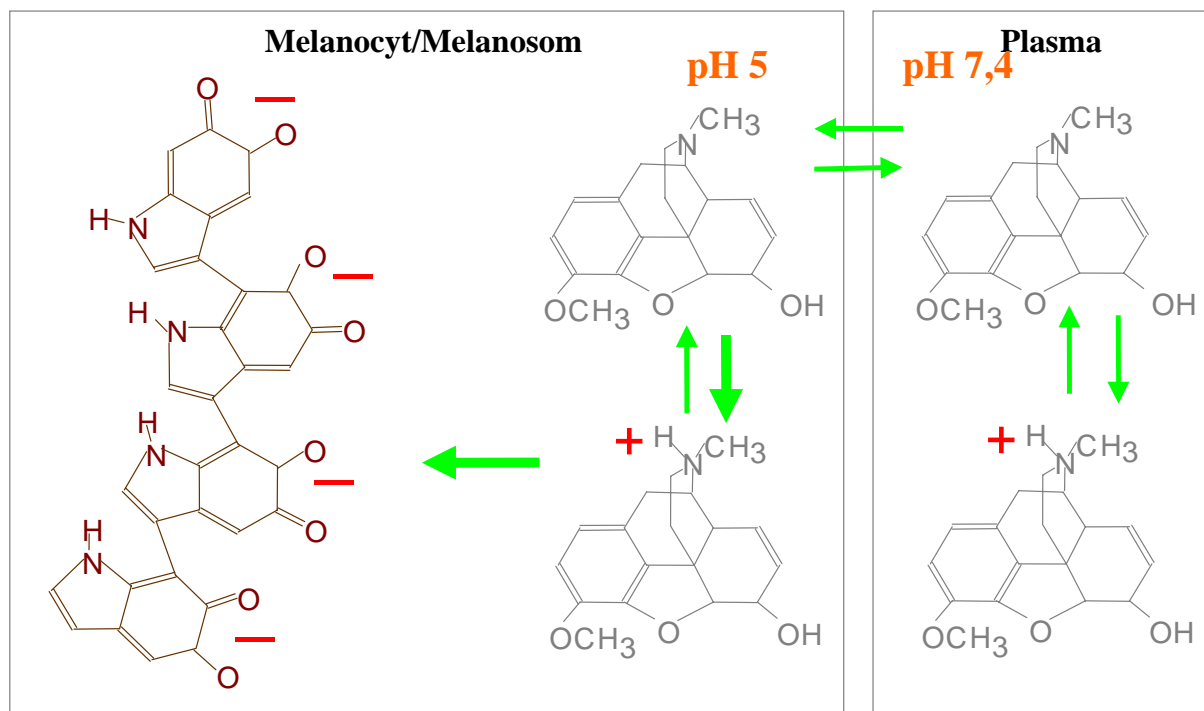


Figure 4. The uncharged codeine molecules enter the melanocyte through the plasma membrane where the synthesis of melanin occurs. The low pH favours the positively charged form of codeine which then interacts with negatively charged functional groups of melanin as well as with the carbon backbone structure through nonpolar interaction.



Figure 5. Sampling of hair in the laboratory.

We have shown that melanin has great impact on the in vivo binding of certain drugs to hair. An excellent example is the incorporation of the CNS stimulant methamphetamine into grey hair. Senile grey hair does not exist more than as an illusion caused by the cessation of melanin production in some of the hair follicles. The pigmented and non-pigmented hairs blend into grey at a distance and the hair colour appears to be grey. When the pigmented strands are separated from the white and analyzed them separately we found that the concentration ratios ranged from 1.8 - 8.0 for methamphetamine. However, because methamphetamine could be detected also in white hair, pigmentation was not the only factor involved. Binding to hair protein or an association with other hair constituents accounts for a up to 35% of drug accumulation in hair.

SOME HISTORIC CASES

The fact that substances are stored in the hair shaft and that they stay there for a considerable time can be illustrated by three cases. Firstly, hair from the English poet John Keats (1795-1821) contained morphine as confirmed by an analysis performed more than 150 years after his death. The morphine is believed to originate from laudanum that Keats administered to ease the pain caused by pneumonia, the disease he

died of. The other case is also from 1821 but concerns Napoleon Bonaparte whose hair was analysed several times during the 20th century to shed light on his death on St Helena. The arsenic found may suggest poisoning but whether it was intentional or accidental remains unsolved. The third case also involves arsenic but is even older. It is a well known anecdote that the Swedish king Erik XIV (1533-1577) was poisoned by arsenic in his pea soup. The rumours of poisoning started immediately after the king's death but no evidence was presented until 400 years later when his grave was opened in 1958. Analysis of hair and bone revealed the presence of arsenic. As for Napoleon, the evidence is not conclusive as arsenic was a lot more common at that time than today. Arsenic was used in wallpapers, paints and not to mention during conservation of bodies.

Facts about the techniques for analyses

LC-MS and GC-MS are two similar techniques that work under different prerequisites. MS is the acronym for Mass Spectrophotometry, a method used to identify and separate molecules according to their mass and electrical properties. The technique can be compared to fingerprinting. The instrument for the separation has an ion source, where molecules are transformed into ions, which are then fragmented into smaller components and accelerated in a variable electrical field where an electron multiplier detect them. The way in which the molecules fragment is unique for each substance and the analyses therefore result in chemical fingerprints.

LC and GC respectively stand for Liquid and Gas Chromatography, both of which are advanced techniques for separation of molecules. When several different substances are present in a sample, they must first be separated before a sample is introduced into the MS instrument. Most often the GC has a much better resolution than the LC, i.e. it can separate many more substances per unit time. On the other hand LC has many more variable possibilities, meaning that it can be used to separate substances with very different physical properties.

More information about these techniques can be found at www.shsu.edu/~chemistry/primers/gcms.html

FURTHER READING

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