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REVIEW ARTICLE

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A discourse on human hair fibers and reflections on the conservation of drug molecules

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Abstract A gross discourse on human hair fibers and their formation is presented stressing the various interdisciplinary aspects, such as the morphological, biological, structural and biochemical data considered to be important in the field of hair analysis. An attempt is made to explain the incorporation of drug molecules during hair fiber formation by using the classical concepts of drug absorption based on lipoid theory and the pH-partition hypothesis as well as a modern biological approach on the permeability of cell membranes. In addition to the physicochemical considerations of the transport properties of a particular drug molecule such as a) the lipophilicity, which determines permeability through the membrane, b) the pKa value, c) the plasma protein binding and d) the molecular size and shape of the drug molecule, drug absorption is thought to be limited by the surface area and the residence time in the hair bulb. The thermodynamic approach according to the Kedem-Katchalsky equations seems even more satisfying. When the principles of biological transport across cell membranes are applied to the cell populations present in the hair root, a hypothesis of extracellular and intracellular drug localizations results. It is speculated that the cell membrane complex (CMC) and the melanin granules present the main sources of incorporated drug molecules within the keratinized hair fibers.

Key words Hair · Drug monitoring · Hair analysis · Ultrastructure · Hair formation · Cell membrane · Biological transport · Nonequilibrium thermodynamic approach

Introduction

After human hair has undergone keratinization, the finished structure is isolated from the continuing metabolic activities of the body and holds the hair constituents in place for a much longer time than most other tissues. The fiber components mainly reflect those metabolic events that occurred during the relatively short time of its formation. Therefore a thorough biological knowledge of hair formation and of the hair structure should be of special interest to forensic toxicologists.

First of all one must distinguish between the composition of the definite keratinized hair fiber and the composition and processes during formation. Human hair is a very complex fiber made up of various morphological components and several chemical species. The fact that hair fibers are cellular tissues was appreciated by early histologists, but was overlooked in times when hair was regarded as more or less a uniform polymer called "keratin". Hair consists of five definite morphological components: cuticle, cortex, medulla, melanin granules and cell membrane complex, each distinct in morphology and chemical composition.

The major components of the hair, such as the cortex cells, cuticle cells as well as the medulla originate from unique matrix cells. The melanin granules are formed by the melanocytes and the cell membrane complex is derived from the plasma membranes of the matrix cells. The strong racial influence in the thickness of hair fibers is well known, with variations of 40–75 μm in Caucasians and of 65–95 μm in hair of Asian origin [31]. It must be stressed that, unlike animal fibers, it is impossible to define a "normal" hair in humans due to the wide variations in the morphological characteristics of hair fibers even from the scalp of a single individual [71]. Ethnic differences in morphological parameters such as color, degree of curliness, shape, thickness and medullation of the hair fibers have been well investigated and show the largest variability in the hair of Caucasians [82, 83].

Briefly, a broad discourse on human hair fiber and its formation is given stressing the various interdisciplinary aspects, such as the morphological, biological, structural and biochemical data considered to be important in the field of hair analysis. In the following, the principles of biological transport across cell membranes are applied to

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cell populations present in the hair bulb which lead to an explanation for the conservation of drug molecules in the hair root.

Morphology and formation of human hair fibers

The hair follicle

The hair follicle is a highly complex skin appendage and may be considered a miniature organ. Histochemical techniques have demonstrated the presence of many enzymes within the hair follicle such as: phosphorylase, aldolase, succinic dehydrogenase, alkaline phosphatase, amino peptidase, arginase, cytochrome oxidase and β-glucuronidase [12].

Most drugs undergo metabolic transformation such as oxidative, reductive, hydrolytic and conjugative reactions in the body. The main site of drug transformation is the liver, but other tissues like the hair follicle may also participate. It was found that there is a mono-oxygenase system operative in the hair follicles which is comparable to the corresponding system in the liver. Vermorken et al. [81] demonstrated that human scalp follicles are indeed capable of metabolizing benzopyrene to dihydrodiols. These findings may provide evidence that biotransformation in drugs of forensic interest may also occur within the hair follicle, which may be considered to be of importance.

The hair matrix cell populations have a unique ultrastructure [29]. Different events occur in the hair root and in the subsequent segments such as cell division, cell orientation, cell migration, cell differentiation, keratinization and hardening. The differentiation of a bulb cell into a particular cell type is predicted or restricted according to its position in the bulb [56]. About one-third of the mitotically active cells, which move upwards in columns and characteristic layers, form the hair shaft [20]. These cells are localized in a cone-shaped germinative region around the dermal papilla. Morphological development from this level to the completely keratinized hair fiber takes place within a distance of 1200–1500 μm. Assuming that the speed of hair growth is approximately 300–500 μm per day (own data) the cortical cell can undergo keratinization within 3–4 days.

The papilla

The main source for the transport and delivery of blood elements including drug molecules is the vascularized dermal papilla. Considering the enormous amount of amino acids, carbohydrates and lipids required to produce hairs and sheaths, fenestrated vessels for accelerated transport are present [53]. As in many tissues, the common source of energy in the hair follicle is glucose.

Hair growth

Three stages in the hair growth cycle have been recognized: anagen (the phase of active hair growth), catagen (the phase of hair follicle regression) and telogen (the phase of hair follicle rest). The majority (85%–90%) of scalp hair follicles are growing and produce a total of about 25–30 m per day or approximately 800 m of hair fibers per month [23]. This high rate of hair production is the consequence of high metabolic activity and rapid cellular proliferation. Matrix cell kinetics show that the hair matrix cell cycle is probably the most rapid of all normal tissues [43]. In humans cyclical hair growth is asynchronous and has been found to vary in different follicles from 0.1–0.45 mm per day [15]. Much work has been done on the mechanisms that act to increase or decrease the rate of hair growth. The rate of hair growth does show vestiges of seasonal change [64] and is undoubtedly affected by endocrine dysfunction, metabolic and genetic disorders, nutrition, hormones and drugs which target follicular segments and interfere with the cell kinetics of human hair growth [53, 80]. The dynamics of the hair growth cycle vary between different species, different individuals, between different body sites in the same species and between different follicle types in the same body site.

Recent experimental data have revealed a wide variation in the hair growth rate among Caucasians of German origin [60]. This offers the application of a "hair growth window" instead of an average hair growth rate in hair analysis when a particular hair segment has to be correlated to a certain time interval of drug intake. In our study most of the hair growth windows for 28 days were determined to be in the range between 0.75–1.35 cm. In a few individuals extreme values such as a hair growth window of 0.65–0.73 cm and 1.8–2.2 cm per month were observed. In these cases, with increasing distance from the scalp, the application of an average hair growth rate of 1 cm per month would cause problems. Therefore, the determination of the individual hair growth rate should be favored whenever possible as it helps to handle the complexity of human hair growth in hair analysis and to avoid serious errors of interpretation. An easy and simple way to determine the individual hair growth window is by bleaching or coloring a hair strand exactly above the scalp followed by an examination of the clipped hair strand 4 weeks later. Microscopical examination of postmortem scalp biopsies has revealed that the anagen hair follicles are localized in the subcutis at a distance of 3–5 mm, sometimes even at 6 mm from the scalp surface. Our finding is in accordance with the observation of Saitoh et al. [68] on individual uninjured follicles, that new hairs may take about 3 weeks to reach the scalp surface or the point to be cut during the hair sampling procedure for hair analysis. This clearly demonstrates that the first segment of a clipped hair strand is formed more than 4 weeks earlier and does not exactly represent the drug uptake during the month prior to hair sampling.

The hair cuticle

Keratinization of the cuticle cells is distinct from other layers due to their function of forming a resistant shield that protects the material within the hair fiber. Moving in an upwards direction, the cuboidal cells in the hair follicle, that are assumed to differentiate into cuticle cells, show an increase in number and size of trichohyalin granules [7, 63]. These congregate near the lateral cell wall, become confluent and produce an electron dense band, which is termed the exocuticle. The exocuticle is responsible for the structure's resistance to detergents, organic solvents and many other harsh environmental attacks and is structurally and chemically equivalent to the cornified envelope of an epidermal keratinocyte [72, 86]. Each cuticle cell consists essentially of 2 major layers. The inner one-third of the cell is termed the endocuticula and is loosely filled with cellular debris which can be easily hydrolysed by proteases [73, 74]. It is less electron dense than the exocuticle suggesting that less keratinization or protein synthesis has occurred. The amino acid composition of the cuticle clearly differs in many important aspects from that of the cortex [53, 65]. The very extensive cross-linking of the exocuticle by cystine and the high content of proline are indications of non-helical organization of the protein chains. As keratinization proceeds the cell volume is reduced by dehydration and high pressure is applied from the already keratinized inner root sheath, producing extremely flattened cells of a fishscale-like appearance. The cuticle cells are arranged in rings of 6–10 and stacked one on top of the other. In cross sections of hair, the cuticle arrangement always appears multilayered, which is due to overlapping and not because of multiple cell layers. On average a hair fiber is encased by a 4–5 μm thick band of cuticular material in fine and coarse hair. This may be of interest as the fraction of the cuticular material varies with the diameter of the fiber.

The cortex cells

The presumptive cortical cells are derived from the inner part of the undifferentiated region in the hair follicle [9]. When these cells leave the bulb and flow past the melanocyte region, melanin granules are taken up. At this level the cells elongate, fibrous keratin appears as clumps of fine filaments and trichohyalin granules may be found. As the cells move upwards, the filaments increase in length and width. In the zone of keratinization, the filaments condense into larger fibrils, form filamentous bundles of macrofibrils and, together with the interfilamentous proteinous material, fuse to form large masses. The final product of cortical keratinization is aggregates of distinct fibers that run parallel to each other and are separated by dense matrix proteins. In longitudinal section, the cortical cells are irregular in shape and interdigitated. In cross section, the cellular elements of the cortex present very tortuous and irregular outlines.

Confusion and a major problem exists in literature on hair concerning the naming of the different protein families and the naming of the proteins within the families [4]. It should be noted that "keratin" is not a specific substance but a complex category of substances. Recent work [18, 25, 42, 44, 62] has shown that the major differentiation products of the cortical cells are keratin intermediate filaments (IF). Intermediate filaments are the most complex in terms of the number of protein chains.

Human hair contains a number of acidic amino acids, polar amino acids with polar hydroxyl-groups, as well as basic amino acids. When cortex cell differentiation starts, the cells produce acidic and basic protein chains on their way upwards to the keratinization zone. These acidic and basic protein chains interact, starting with the formation of a heterodimer. The final filamentous structure results from further polymerization of tetramers which are the repeat units of the keratin filaments [30]. A common secondary structure is found: a highly converse, central, helical domain consisting of diverse sequences and lengths [24] confirming the findings of Astbury and Woods [3].

As far as known at present the intermediate filaments (IF) of a cortex cell are embedded in a matrix of globular proteins, so-called intermediate filament associated proteins (IFAPs), with interactions of the molecular chains. These IFAPs, which form the matrix, consist of two large families of nonhelical proteins, the "high sulfur group" and the "high glycine/tyrosine" proteins.

The space in the matrix for small molecules is limited by the globular proteins,and it has been demonstrated in the case of water molecules that the microfibrils or intermediate filaments absorb much more water than the IFAPs of the matrix [13, 21, 22]. In the polypeptide chains of the IF and in the IFAPs as in all proteins, a large number of hydrogen-binding sites, ionic-binding sites, amide-binding sites, disulfide-binding sites and electrostatic-binding sites are present. Thus, different drug substances may be easily incorporated during the keratinization process of the cortical cells. Since the cortex makes up the main part of the hair fiber both by weight and by volume, drug binding within the cortex is believed to dominate the analytical results [37], but this opinion has not yet been proven.

The medulla

Medulla formation and its cytology still remain somewhat unknown since the medulla has been less studied. The medulla, if present, forms the central core of the hair fiber and originates from the matrix cells at the tip of the papilla. As these cells move upwards, they start to develop dense spherical granules similar to trichohyalin granules [8]. In differentiating medullary cells vesicles occur and melanin granules from the melanocytes are taken up. By the time filaments which aggregate in bundles are produced, the medullary granules disappear, while the vesicles coalesce into larger vacuoles and an electron-dense trabecular framework is formed. The spaces between and within the mature medullary cells are usually filled with air. The medulla may be formed con-

tinuously or intermittently or may even be absent. In general, the appearance of a medulla in human hair fibers varies. It has been shown that the incidence of hair medulla is closely related to the hair shaft diameter, thus coarse hair fibers such as those of Negroid or Mongoloid origin are almost always heavily medullated. The hair medulla is difficult to isolate and has an insoluble fraction. The presence of ε (γ-glutamyl)lysine cross linkages in medullary protein has been demonstrated [27]. Medullary material seems to be rich in protein and lipids. It contains citrulline, has a very low cystine and low sulfur content and consists of relatively large amounts of acidic and basic amino acids and hydroxyamino acids [65]. Kalasinsky et al. [35] have presented experimental data that reveal that drug substances may be located in the medulla.

The melanin granules

Hair pigmentation is due to melanin and depends on the number of melanin granules present, their size and arrangement and distribution in the hair shaft and their melanin composition. When pigment is deficient or lacking the hair appears gray or white. Melanin granules are the cell products of melanocytes, which are specialized cells and are situated at the apex of the dermal papilla. They send out ramifications filled with melanin granules during the anagen phase of the hair cycle [9]. Matrix cells, which are assumed to evolve into cortex cells, phagocytose melanin granules passing by the melanocyte region on their way upwards to the keratinization zone. As the presumptive cortical cells harden during keratinization, the melanin granules are fixed between the keratinous fibrils. They are present in the cortex cells occasionally showing cluster arrangements in the longitudinal fiber axis. They are observed in the medulla, are rarely present in spaces between the cortical cells and are usually absent in the hair cuticle [63].

In human scalp hair three main forms of melanin granules can be observed [46].The size of the melanin granules in black hair is at least twice that of brown hair melanin granules, and is both of oval rice grain shape, of a high electron density and with an internal structure and fleecy surface different from the granules of other hair colors. It is well known that Negroid hair is heavily pigmented and that Mongoloid hair fibers contain the highest quantity and the largest melanin granules, while in Caucasian hair less pigment is found. Blond hairs contain smaller and less numerous melanin granules which are elongated with a layered internal structure and pitted surface. Red hairs contain spherical melanin granules with vesiglobular and proteinous matrices on which melanin deposition is spotty and granular. Sometimes, spherical and oval-shaped granules can be found together [1, 26, 65]. The melanin granules consist of a proteinous matrix and melanin polymers. The macromolecules are formed by condensation on the immature melanin granule within the melanocytes, and they are extremely conjugated [34, 54].

Melanins are high molecular mass polymers. Their formation is controlled by the enzyme tyrosinase which converts tyrosine to dopa and then by a series of reactions to dihydroxyindole and dihydroxyindole-2-carboxylic acid. These indoles are highly reactive and undergo diverse oxidative polymerisation to form the melanin polymer. Hair melanins can be roughly classified into those giving black and brown colors and their derivates, termed eumelanins, and those giving a wide range of light colors, termed pheomelanins. Erythromelanin from red hair is also known. The pheomelanins, the eumelanins and the reddish pigment have different chemical and physical properties, the most obvious being the higher sulfur content of pheomelanins and the low chemical resistance of the polymorphous erythromelanins [53].

The cell membrane complex

In 1957 Birbeck and Mercer [7] gave a detailed description based on electron microscopic observations demonstrating that in fully keratinized hair fibers all cells are enveloped by a modified cell membrane which is derived from the original plasma membrane, and are cemented together by distinctly different intercellular binding material [11]. Similar structural elements are observed in all keratinized tissue and are termed the "cell membrane complex" (CMC). The CMC is built up of chemically resistant cell membrane envelopes, lipid bilayers, intercellular proteinous material and minor components, such as polysaccharides or glycoproteins, and is a continuous phase in the hair fiber [41].

As the matrix cells move upwards and differentiation proceeds, alterations occur in the cell membranes and the intercellular spaces. The intercellular spaces rapidly decrease and the cells become closer together. A typical slightly stained "tram-like" structure of the CMC occurs in electron microscopical sections [66]. It is believed that the lucent bands are due to the presence of opposed hydrophobic ends of lipid bilayers. Analysis of the lipids extracted from human hair reveals that these intercellular structures are composed mainly of free fatty acids, cholesterol sulfate, free cholesterol and ceramides [84, 85]. Only a few investigations have focussed on hair lipids [51] and the CMC has rarely been studied [41]. Experimental data on the transportation of small molecules in human hair fibers has demonstrated that preferential pathways may exist between the cells along the CMC rather than through the keratinized cells [59, 66].

Principles of biological transport across membranes applied to the matrix cells and melanocytes in the hair bulb

The plasma membrane of the matrix cells in the hair follicle is the primary barrier between the cytoplasm and the extracellular fluids such as blood and lymph, provided that the basal lamina that surrounds the hair bulb, does not

act as a selective filter. The most widely accepted model of cell membrane organization is the mosaic fluid model established by Singer and Nicolson [69], in which the proteins are embedded in a discontinuous lipid bilayer. The distribution of the lipids is asymmetrical since the hydrophobic interior of the bilayer is populated by long hydrocarbon chains while the polar ends of the lipids are exposed to the aqueous surroundings of the membrane. There is a fluidity present in the lipid portion of the membrane. The cellular membranes are in a constantly changing state as both the lipids and the proteins move laterally along the surface of the membrane and molecules can move in and out of the membrane. Highly lipophilic substances will essentially dissolve in the bilayers and generally the rate of diffusion of lipophilic solutes is proportional to their lipid solubilities and diffusion coefficients in lipids [2]. The permeability to ions due to channel forming proteins is many orders of magnitude greater than that of the bilayers, but these membrane pores are limited from 5 Å–10Å in diameter [39]. Although being mostly polar molecules at physiological pH, either weak electrolytes or weak organic bases, drug substances are not assumed to cross the plasma membrane of the matrix cells via the relatively narrow pores. It also seems unlikely that facilitated transportation by carriers could occur.

Nowadays the classical concepts of drug absorption based on lipoid theory [16] and pH-partition hypothesis [33] must be considered first approximations rather than unifying models, but both still form a useful basis and may be applied to drug absorption during hair fiber formation. Almost 80 years ago Overtone [55] demonstrated that a number of organic compounds penetrate cells at rates roughly related to their lipid/water coefficients. For different classes of substances, suitable models for the permeability of biological membranes have been established by using the octanol/water and n-hexadecane/water partition coefficients [70] for the particular pH milieu. Organic anions or cations appear to cross the cell membranes much more slowly than their corresponding non-ionic species, and will not diffuse at a significant rate and might even be neglected in the following considerations. The interest is focussed on the undissociated drug molecules, which are known to cross cell membranes preferentially.

At physiological pH the likelihood for basic drugs to cross the cell membrane is much higher than for acidic substances. Indeed hair analysis data suggest that the pKa value of a particular substance seems to influence the incorporation as basic drugs like opiates, amphetamines or cocaine can be detected more easily in keratinized hair fibers than drug molecules with acidic character like lysergic acid diethyl amide (LSD) or diazepam. For a given pH milieu the ratio of non-ionised to ionised drug molecules with known pKa value can be calculated from the Henderson-Hasselbalch equation [32]. In a particular environmental milieu the pH-dependence of dissocation and lipid solubility of a substance may result in an unequal distribution of a drug substance in compartments with different pH milieus favoring the accumulation of undissociated basic drug molecules in the compartment with higher H^+ concentration. The isoelectric point of keratinized hair fibers is close to pH 6 and indicates the acidic nature of hair [65]. Although data on the pH conditions during hair fiber formation is not available as far as is known, the preferential incorporation of basic drugs into hair could already be explained.

Furthermore it is known, that the larger the molecule the less it is likely to pass through the cell membrane, therefore the plasma protein binding and the molecular size and shape of the drug molecule influence its transmembrane transport and may present some of the limiting factors for incorporation. These reflections match experimental data on the effect of structural factors on the incorporation of drugs in hair, recently presented by Nakahara et al. [50]. Two principal factors which are not considered in the classical absorption theories are surface area and residence time. The anatomical size of the hair bulb, the speed of cell migration and the time period of keratinization of the matrix cells are considered to be directly related to bioavailability and incorporation of the drug molecule. The hair bulb may present a cone-shaped tube with decreasing drug concentration in the extracellular fluids from the area next to the dermal papilla upwards to the keratinization zone. The drug absorption pattern may depend upon longitudinal drug concentrations as well as on changes in the permeability of the cell membranes, which occur as soon as keratinization has started. It might be speculated that only the lower part of the hair bulb and the local flow rate of the extracellular fluids may determine the effective absorption area and the residence time for drug incorporation in the hair root.

The chemical factors such as polarity, lipid solubility and solute ionisation (lipid/water partition coefficient, pH and pKa effects) and the principles of mass transfer processes such as passive diffusion according to Fickian law seem useful tools to approximate the phenomena of drug uptake during hair fiber formation, but they cannot describe the processes of biological transport sufficiently. The approach to biological transport processes is thought to require more sophisticated methodologies. These concepts also assume, that the route taken by most of the drugs and drug metabolites, is to enter the matrix cells by dissolving in the lipid bilayer and crossing by free diffusion similar to nonelectrolyte transportation [75, 79].

Three major steps are discussed, any of which involves an equilibrium of the solute between two states and a variety of thermodynamic and kinetic states:

1. the solute must leave the aqueous environment and enter the membrane after the shell of water molecules has been stripped off,

2. the solute must cross the membrane,

3. the solute must leave the membrane to enter the cell after a shell of water has been regained.

This opinion is supported by the findings of basic membrane research which provide strong evidence for equivalent nonelectrolyte diffusion across the lipid bilayers [2]. In these studies a good correlation was found between the

permeabilities of some nonpolar solutes and their lipid solubilities or lipid/water partition coeffients. It was shown that the permeability of membranes to certain classes of nonelectrolytes is much more sensitive to solute size or molecular weight than diffusion coefficients of the solutes in aqueous solution. Similar to observations on diffusion in polymers, the more rod-like, less spherical molecules are found to cross the plasma membrane more rapidly, when comparing transmembrane diffusion rates of nonelectrolytes having similar molecular weights [70, 79]. Davson and Danielli [19] postulated that the way for the solute through the fluctuating hydrocarbon chains of the bilayer presents a sequence of transitions between energy wells in the bilayers. A more convenient and attractive model for drug substances is represented by the nonequilibrium thermodynamic approach of passive transport. The equations referred to as the Kedem-Katchalsky equations characterize the coupled flux of solute and solvent through a membrane permeable to both species [36]. As the solute flux is a linear function of the volume and exchange fluxes, a linear dependence on the hydrostatic and osmotic pressure differences exists across the membrane. The processes can be expressed by transport coefficients, terms of solvent drag and of diffusion driven by the solute concentration difference.

During cell migration out of the hair bulb, statistically any of the matrix cells can come into contact with drug molecules, if present in the extracellular fluids. Provided that drug metabolization does not occur in the hair follicle or is of minor importance, the extracellular concentration of the drug solute during this time period is mainly given by the actual blood concentration of the free, non-protein-bound and undissociated portion. Presumably equilibrium is established between the drug concentration in the extracellular fluids and in the plasma membrane related to the lipid solubilities or lipid/water partition coefficients of the particular drug substances. Transmembrane transport of the drug substances may arise. Providing a physiological intracellular and extracellular pH milieu exists, the drug concentration in the matrix cells can never exceed the equilibrated concentrations between the plasma membrane and the extracellular fluids. Inside the matrix cells the uncharged molecules will dissociate according to pKa value. If an intracellular pH lower than that of the extracellular fluids is discussed, in the case of basic drugs a greater number of molecules may cross the membrane and an intracellular accumulation of the drug molecules may occur.

When cell differentiation proceeds, the situation is thought to become highly complex. The level at which the differentiating matrix cells are partially or completely cut off from the systemic circulation is unknown. After the cells have left the bulb region a release of the membrane associated drug solutes and the diffusion back to the systemic circulation is unlikely to occur, primarily due to the increasing dehydration processes. The fate of the intracellular drug substances is completely unclear. In principle three pathways for the intracellular drug molecules can be discussed:

– Increasing cell dehydration may lead to an intracellular concentration of the drug substances which start to move outward according to the altered concentration gradients. As this process is thought to be relatively slow and alterations of the plasma membrane itself occur during this time period, the main part of the drug molecules might remain in the cell membrane.

– As the matrix cells undergo dramatic transition and all of the cytoplasmic organelles vanish, the intracellular drug molecules may be partially or totally eliminated from the cell by excretion. This might result in an increasing concentration of the drug molecules in the cell membrane and outside the cells along the intercellular spaces leading to drug accumulation in the CMC.

– During cell differentiation the intracellular drug molecules may be captured by the cell differentiation products. For example in the cortex cells preferentially charged drug molecules may be bound to/or associated with the IF and/or the IFAPs and additionally might accumulate on the surface of melanin granules which are known to act as biological adsorbers. As already mentioned, the velocity and the mode of cell dehydration and differention of the matrix cells varies. The matrix cells differentiate into highly specialized cells distinct in morphology, chemical composition and structure, thus the fate of the intracellular drug molecules may also show wide variation. The questions, whether the distribution of the drug substances in a transverse plane of a single hair fiber is homogeneous or hetereogeneous, or whether the amount of drug substances present may or may not be influenced by morphological parameters of the hair fiber, cannot yet be answered.

Attention should also be drawn to the second cell population present in the hair bulb, the melanocytes. These highly specialized cells produce melanin polymers and melanin granules and are located around the apex of the papilla. In contrast to the hair matrix cells these cells are differentiated, are in very close contact with the blood supply from the dermal papilla and do not migrate. Although a common organization of the cell membranes of living cells is assumed, specializations which meet the requirements of the specific cells are known. It was reported that the protein/lipid ratio and the distribution of the lipid fraction among the major lipid classes of the cell membranes may vary considerably [2, 79]. Thus the permeability of the melanocyte membrane for drug molecules might be different from the transport barrier of the matrix cells. It is known that interactions of particular drugs with melanin polymers and the melanin granules occur [28, 40, 57, 58, 61], but the molecular binding mechanism is not known in detail. The phenomenon is believed to be a type of adsorption in which forces occur that may be due to charge transfer reactions, the melanin being the electron receptor, as well as to electrostatic forces. Hydrophobic interactions as well as ionic attraction also may occur between positively charged molecules and the anionic carboxyl groups of the melanin polymer [10, 45]. Being en-

trapped by the melanins and during melanin granule formation inside the melanocyte, a permanent concentration gradient will result for the drug substances with high melanin affinity and the influx into a melanocyte will be higher than into a matrix cell. The results of the few studies on both human and animal hair fibers have already indicated that the interaction of drugs with the melanin granules may have implications in assaying clipped scalp hair [48, 52]. With haloperidol, ofloxacin and methadone the measured concentrations were higher in pigmented than in non-pigmented hair [5, 77, 78, 87]. Buhl et al. [14] demonstrated the uptake of a radio-labelled drug by autoradiography and found an accumulation in the differentiating matrix cells superior to the dermal papilla with a distribution similar to that of pigment. Drug uptake was significantly less in unpigmented follicles. All these data demonstrate a definite link between differential uptake of various drugs and hair melanins, therefore the measurement of drugs with high melanin affinity in hair samples is biased by pigmentation [47]. However, the results of the above studies also prove that the melanins/melanin granules are not the only and probably not the main source of the drug substances found in hair samples. The principles of biological transport and hair analysis data provide evidence that one of the minor components of hair, the CMC, may be of greater importance for the conservation of the drug molecules than has hitherto been assumed.

Concluding remarks

The phenomenon of the conservation of drug molecules during hair fiber formation can be explained by biological principles and the physico-chemical approach of transport mechanisms. The incorporation of a particular drug substance during hair fiber formation may be mainly linked to the number of undissociated, free (pKa value, non-protein bound) drug molecules and to the solute lipophilicity (lipid/water partition coefficient), molecular size and shape as well as to the melanin affinity of the drug molecule.

Provided that external environmental factors have neither removed nor added drug substances to the hair [6, 17, 38], the presence of a drug substance at a particular short distance from the scalp reflects that the drug was available to the hair follicle at the time of formation and keratinization of that hair section. Thus, analysis of hair segments as recording filaments at different distances from the scalp provides retrospective information. However, a complex situation is obvious. Only if sufficient information on the hair growth of a person is available and the existence of a delay due to intradermal growth is taken into account, can a rough correlation be made between the distance of a section from the scalp and the time interval since that section was formed in the hair follicle. Indeed a note of caution seems necessary. Recent experimental data indicate that the incorporation of a drug substance correlates to its plasma concentration time curve (AUC) [49, 50, 67]. Therefore, it must be stressed that for some particular drug substances the time period of drug incorporation during hair fiber formation may decisively differ from the time of drug consumption.

One important aspect that should not be forgotten is that alterations in ultrastructure and chemical composition, such as considerable decomposition, or loss of drug substances may occur after the hair fiber has been expelled from the skin surface. It is obvious that the constituents which the drug molecules are thought to be mainly associated with, are known to be primarily affected by all sorts of environmental factors [65].

For all the reasons mentioned above, the biology of hair, the ultrastructure, the biochemistry and the physicochemical properties of the hair sample under investigation as well as at least the pharmacological knowledge of the specific drug substance, seem indispensable for a valid interpretation of the results in hair analysis [76].

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