pep Pharmacology Education Partnership

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Drug testing: A hair-brained idea!

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A Project Funded by a NIDA Science Education Drug Abuse Partnership Award

Module 2: "Drug testing: A hair-brained idea!"

Description of the module

The ability to detect drugs in hair is becoming a highly accepted method for drug testing in sports, in the workplace and in hospitals. In this module, the distribution of drugs throughout the bloodstream and their accumulation in a specific target (i.e., hair) is described. Several concepts are covered, including 1) the basic anatomy of the circulatory system, 2) the properties of biological membranes that allow drugs to be delivered to hair follicles, 3) the chemical properties of drugs and of hair pigment that allow them to bind to each other, 4) the biological process in follicles to produce pigment (i.e., hair color) and 5) the conversion of drugs to metabolites by enzymes in the liver.

Learning objectives

After participating in this module, students should understand the following:

1. How drugs get transported from the bloodstream to specific cells in the body
2. The nature of capillary membranes that allows drugs to pass through them into cells
3. How hair is made
4. How the color of hair is produced
5. Why drugs that are weak bases bind to hair pigment
6. How volatile forms of drugs in the air can be incorporated into hair

This module integrates information from the following areas:

anatomy, cell biology, chemistry, biochemistry, physics, forensic chemistry, public health

Student Handout

With the advent of very sensitive chemical techniques, scientists have discovered that drugs such as cocaine, morphine, nicotine, and amphetamine can be detected in human hair. Do the drugs get into the hair from inside the body or from the air? The answer is both, depending on the volatility of the drug. This poses a dilemma. For example, drug testing of athletes in sports or job applicants might involve hair samples, which are easier to collect than urine. If the subject has a positive test, how do the testers know if the drug came from the environment or from drug use? These questions must be answered if drug testing in hair is to become a standard procedure. Let's consider both possibilities.

In order for a drug to get into the hair from inside the body, it must be distributed throughout the bloodstream. Drugs like morphine, for example, get into the bloodstream directly by injecting them into a vein. If a person snorts cocaine, smokes a cigarette or swallows an amphetamine pill, the drug must pass first through several barriers to get into the bloodstream.

1. Create a drawing to show how a drug gets into the bloodstream for each of the situations just described; injection, snorting, smoking and swallowing.

Once these drugs reach the bloodstream, they travel throughout the body, wherever the blood goes. As the heart pumps blood, the drugs are carried in the arteries to organs and tissues. The arteries branch off to become very small (these are called arterioles) as they enter tissues. Each cell must be close to the blood vessels in order to receive oxygen and glucose. These 2 substances are required for cells to live. The arterioles branch off again to form capillaries. The capillaries are extremely small so that they can reach all cells. Capillaries deliver nutrients dissolved in the blood such as oxygen and glucose to the hair follicles so that hair will grow. The hair follicle is made up of different kinds of cells that have different functions.

2. Make a diagram of a hair follicle. Show where the different kinds of cells are found in the hair follicle and indicate their major functions.

3. On your diagram, indicate where the capillaries come in contact with the hair follicle. How does the structure of capillaries make them so good at delivering nutrients to cells?

4. How easily can drugs such as morphine, cocaine, nicotine or amphetamine cross the capillary membrane to enter hair follicles?

The hair follicle gives rise to hair, which grows from the bottom of the follicle. Hair is composed of several substances, but mostly of protein.

5. What is the principle protein found in hair? What is so special about this protein that it is an ideal protein for hair?

6. What other biological components are found in hair?

Once drugs such as morphine, cocaine, nicotine or amphetamine get into the hair follicle, they bind to melanin, the pigment that gives hair color. Each of these drugs shares a common chemical property that makes them likely to bind to hair melanin. They are all weak bases. So, they tend to accept a H+ when they are in an acidic environment (where there is a high concentration of H+).

7. Which is more acidic, blood or hair? Why?

8. What kind of molecule is melanin? What is it made from and where is it made?

9. What is the major force that binds these drugs to melanin?

The amount and type of melanin determines the color of hair. More melanin gives rise to darker hair. Blond-haired people have little melanin. People with red hair have a different type of melanin compared to people with brown hair. People with black hair have the most melanin.

10. Would drug-testing of hair from people with different color hair (blonde vs brown or black) or from people of different races reveal different amounts of drugs, even if they all had taken the same dose? What kinds of dilemmas does this pose in cases of drug-testing in sporting events or in the workplace?

It is also possible to detect drugs such as nicotine and cocaine in hair of individuals that do not smoke or do not use cocaine. The drugs do not reach the hair through the bloodstream; instead, they enter hair from the environment surrounding the user or people nearby. This happens when drugs are heated or volatilized so that they can be smoked. Most drugs that are smoked are weak bases. Examples of weak bases that are smoked are nicotine, cocaine and heroin. However, when people smoke drugs, a large portion of the smoke does not enter the lungs. The smoke stays in the air surrounding the smoker and other people nearby. The drugs dissolved in the smoke easily penetrate the hair to bind to melanin.

11. Weak bases can exist in 2 forms—a charged form (the drug has accepted a H+) and an uncharged form (the free base). Which form is easily volatilized?

12. Why does this form make it easier to penetrate into the hair?

The pattern of drug binding within the hair can help distinguish between a user and a person exposed through second-hand smoke.

13. Is this easier for short hair or long hair? Why?

Another way to distinguish the ways in which drugs enter hair is to analyze the hair for not only the drug but also its metabolites. When drugs enter the bloodstream, they travel to the liver before they go to the rest of the body. There, enzymes participate in chemical reactions to change some of the drug into another form, called a metabolite. For example, some enzymes oxidize the drug by adding an OH group, making the metabolite more polar than the parent compound. Most of the time, these polar metabolites are inactive, although some drugs have active metabolites. The metabolites of drugs travel throughout the bloodstream along with the drugs themselves and can be distributed to some of the same tissues.

14. Where do metabolites go once they leave the liver?

15. What allows the polar metabolites to leave the bloodstream and enter the hair?

16. Can metabolites reach the hair from second-hand smoke?

17. Do you think analyzing the hair for a drug is sufficient, or should analysis also include the metabolites? Why?

Drug administration and distribution

Drugs can enter the body a variety of ways (**Figure 1**; also see Module 1). The easiest way to get a drug into the bloodstream is to inject it directly into a vein. If a drug is ingested by mouth, smoked, or snorted, it must pass several barriers before reaching the bloodstream. (See Unit 1 for a review of the different modes of drug administration). Once in the bloodstream, the drugs can be distributed throughout the body. The route that drugs take follows the circulatory path of the blood. The first pass throughout the body depends on the actual route of administration. Drugs that are smoked go directly with the oxygenated blood from the lungs to the heart. Then they leave the heart through the aorta, the major artery, to travel to the rest of the body. If drugs are injected or snorted, they enter the venous system and get returned to the heart with de-oxygenated blood, before traveling to the lungs and then back to the heart. If a drug is ingested orally, it diffuses into capillaries in the stomach and small intestine that connect to blood vessels that go directly to the liver. So as drugs leave the gut they travel to the liver first (this is called the portal circulation). In the liver, some of the drug is metabolized as it passes through (see discussion of metabolism below). After the drug leaves the liver, it travels through the venous system to the heart, then to the lungs and finally back to the heart to be distributed throughout the rest of the body via the arterial system (**Figure 1**).

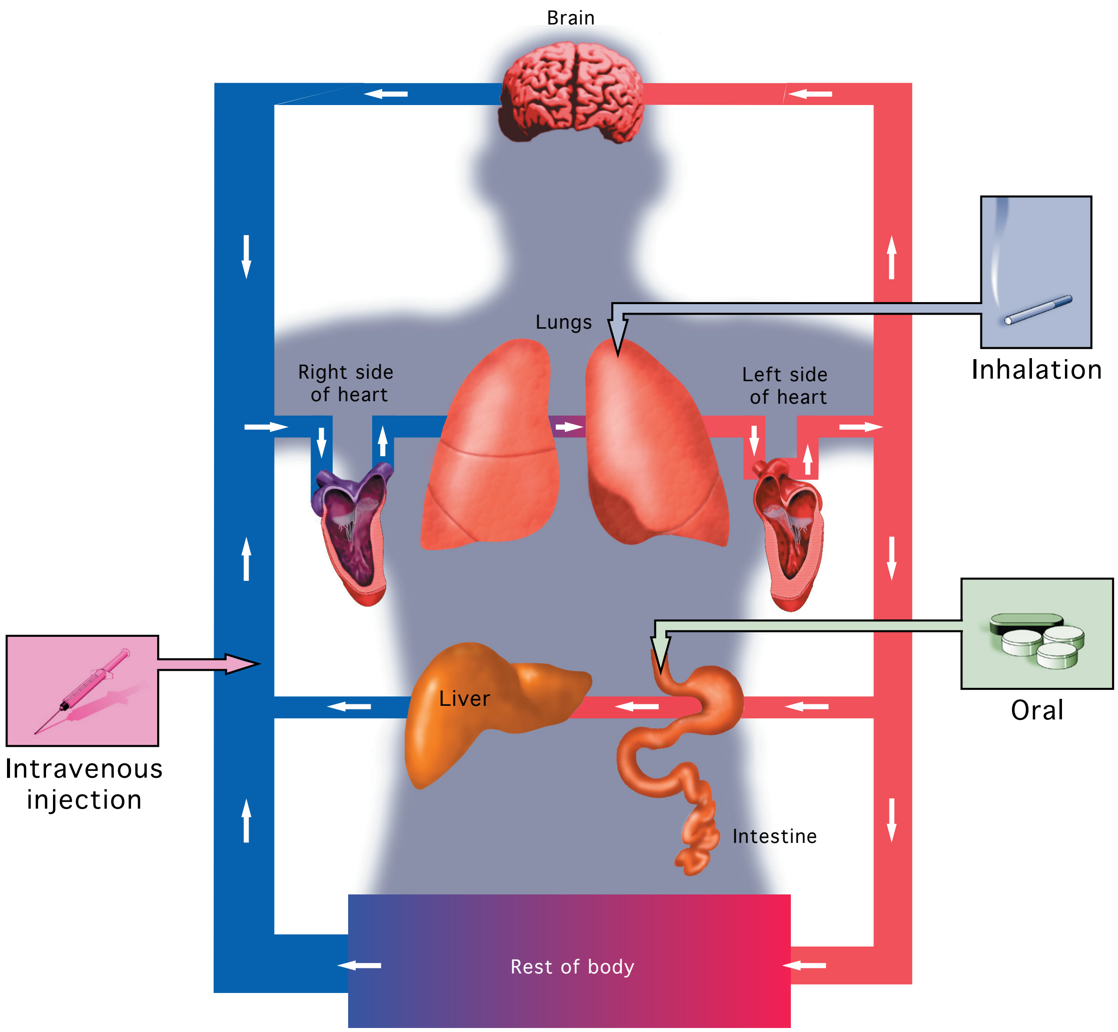

Figure 1. Modes of drug administration & distribution throughout body. Red is the arterial side and blue is the venous side. Adapted from Ray O. and Ksir C. Drugs, Society, and Human Behavior, pg. 154. McGraw-Hill, New York, 2002.

The circulatory system is a very efficient way to distribute drugs throughout the body. As described above, drugs leave the heart by way of the aorta. This main artery branches into large arteries as they travel to various organs. As arteries enter organs, they branch into arterioles, which branch into even smaller units, the capillaries. Capillaries are the smallest form of blood vessels and are very numerous. In fact they are able to deliver nutrients such as oxygen and glucose to every cell in the body. [They also pick up waste such as carbon dioxide and metabolic products.] Unlike membranes of most cells, the membranes of the capillaries (made up of endothelial cells) have numerous pores (or fenestrae) that allow molecules up to 25,000 daltons and charged molecules (see discussion below) to pass through without difficulty (**Figure 2**) (also see Module 1).

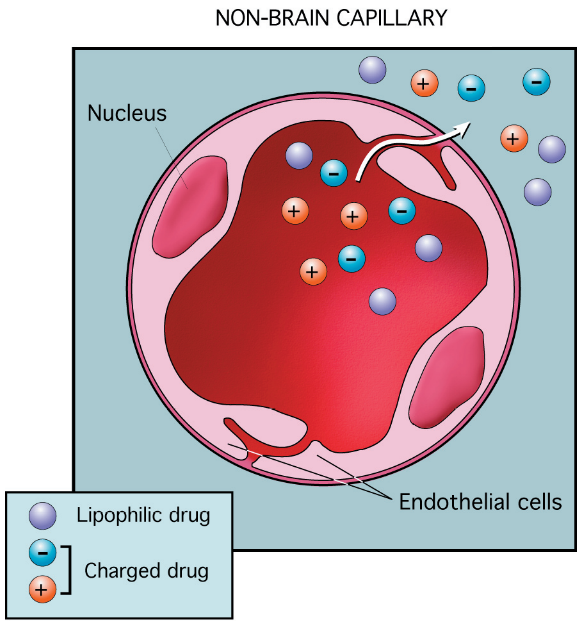

Figure 2. Cross section of capillary showing endothelial cells. In the non-brain capillary fenestrae are present allowing large molecules and charged molecules to pass freely in and out along their concentration gradient.

How do drugs get out of the blood and into hair follicles?

Consider the cells where hair is made. Hair is made in the hair follicles (discussed below). Capillaries come in close contact with the cells of the follicle (**Figure 3**) and provide oxygen and glucose and other nutrients that are essential for the function and survival of these cells. The capillaries also surround sebaceous glands connected to the follicles. These glands secrete sebum (mostly lipids) into the follicle to keep it from drying out. Because the capillary membranes have small pores in them, drugs and other small molecules can leave the capillary easily to enter the follicle. Drugs in the blood also have access to the follicles by traveling through the sebaceous glands.

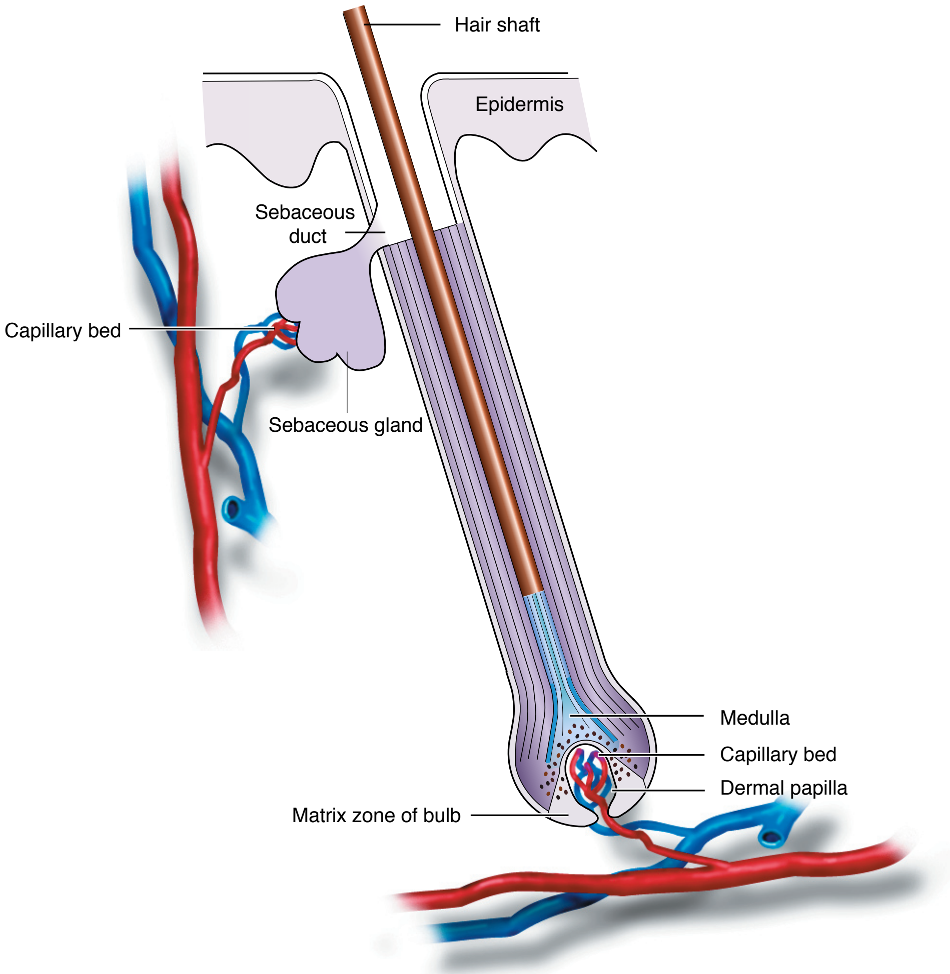

Figure 3. Capillaries surround the bulb of the hair follicle and the sebaceous gland.

The anatomy and composition of hair

The structure and chemical composition of hair provides information about the interactions between drugs that enter the hair follicle and the hair itself. Hair consists of mostly fibrous protein (65-95 %). In addition, it contains a small amount of lipids and water. Hair is made in sac-like structures, called follicles. The follicles are rather simple organs that go through growth and rest phases. They are made of epithelial cells, continuous with the surface epidermis (outermost skin layer) (**Figure 4**). Follicles grow down into the dermis layer and below, forming a canal that holds the hair shaft. Groups of cells in the follicle form a sheath around the hair to help it grow within the canal. Sebaceous glands are connected to most follicles near the skin surface. At the bottom of the follicle there is a bulb containing a bundle of cells. These cells are part of a matrix and they are responsible for making the hair. They require a lot of nutrients because they are constantly going through mitosis to keep up with their synthetic demands. The cells in the matrix include the keratinocytes (“cytes” = cells), which make keratin. Keratin is a fibrous protein that contains many sulphur bonds, which help to give the hair strength and structure. [During a “perm”, the sulphur bonds are broken and reformed to provide the curl.] There are also special cells called melanocytes that make melanin, or hair pigment. Higher up in the bulb, the hair attains a more organized form, consisting of 3 layers of cells. The outermost layer is the cuticle, which is very scaly (easily viewed with a microscope). The cuticle cells help anchor the hair in the follicle and protect the middle layer of cells called the cortex. The cortex forms the major part of the hair and it contains keratinocytes and melanocytes. Higher up the follicle, the keratinocytes become keratinized; that is, they finish making keratin and then they die. The innermost layer is called the medulla. There are large keratinized cells in the medullary layer, along with many air pockets. These air pockets help to determine the sheen and color tones of hair by influencing the reflection of light (see below).

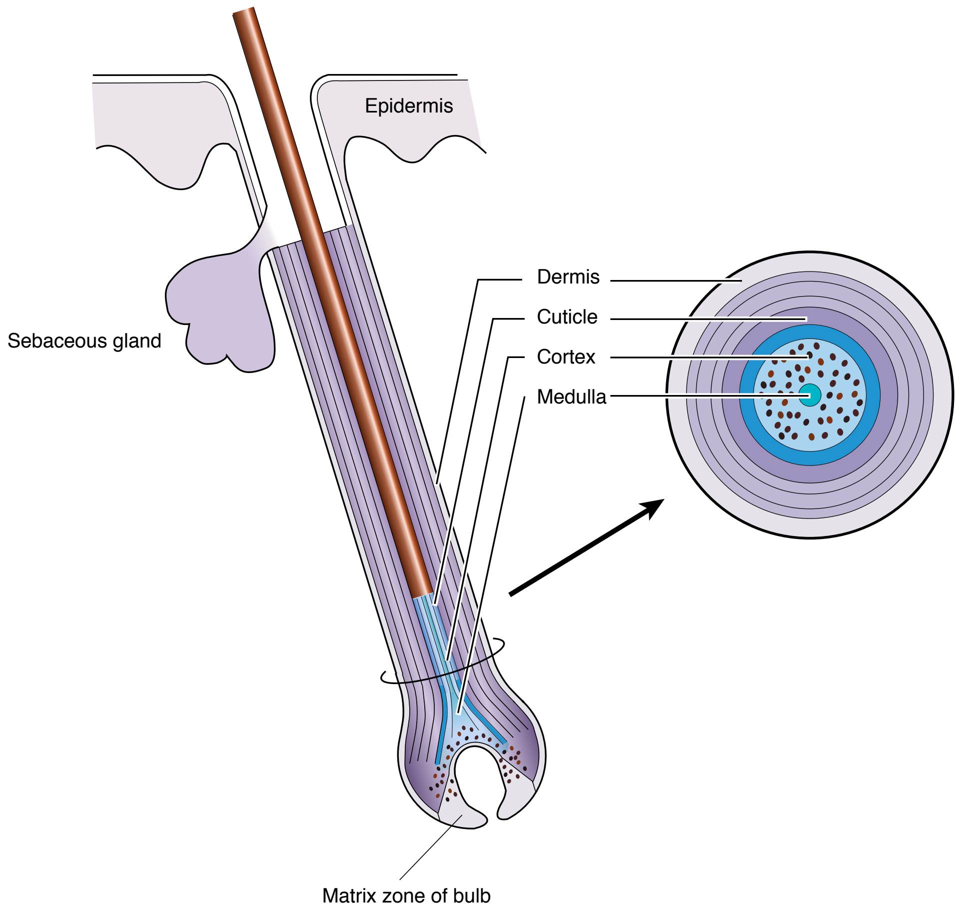

Figure 4. Detailed view of a hair follicle. A cross section of the hair shaft shows the 3 layers of cells. The small dark spots in the cortical cell layer are the melanin granules.

The chemistry of hair and hair color

The color of hair can be explained by a little bit of biology, chemistry and physics. Hair color is determined by the absorption and reflection of light and it is based on the amount and the type of melanin present (this is genetically determined). For example, when light hits white or blond hair, which contain little melanin, almost all of the light is reflected, and this reflected light contains all parts of the visible spectrum. When light hits brown hair, which contains moderate amounts of melanin, some of the light is absorbed, and the reflected light appears colored. The actual color depends on the type of melanin present in the hair. Black hair absorbs almost all light that hits it because it has a lot of melanin. Little light is reflected and the hair appears black.

Melanin is contained in granules in the melanocytes, but it can not provide any color when sequestered in this way; it must be transferred to the keratinocytes. The melanocytes are scattered about, mostly in the bulb and in the cortical layer of the hair, sandwiched between the numerous keratinocytes. The melanocytes have long tendrils or dendrites that make contact with the keratinocytes. The keratinocytes actually phagocytize the tips of the melanocyte dendrites, stealing a portion of melanocyte cytoplasm that contains the melanin granules. Once inside the keratinocytes, the melanin is able to absorb light and give the hair color.

Melanins are the most widely occurring pigments in nature. In addition to giving hair its color, melanins give color to eyes, freckles, skin, scales and feathers. They are also responsible for the browning of plants. However, melanin is important for more than just hair color. This biopolymer is able to bind drugs just as receptors bind to drugs. In order to understand how this happens, it is helpful to understand how melanin is made and its basic structure. Within the melanocytes, melanin is synthesized from tyrosine, a naturally occurring amino acid that is also found in neurons where it participates in the synthesis of the neurotransmitter, dopamine (see Unit 3). Different enzymes are found in melanocytes and in dopamine neurons to synthesize melanin or dopamine from the same starting material (tyrosine). For example, with the help of the enzyme tyrosinase and some copper, tyrosine is oxidized by O_2 eventually into special dopaquinones that have color (**Figure 5**).

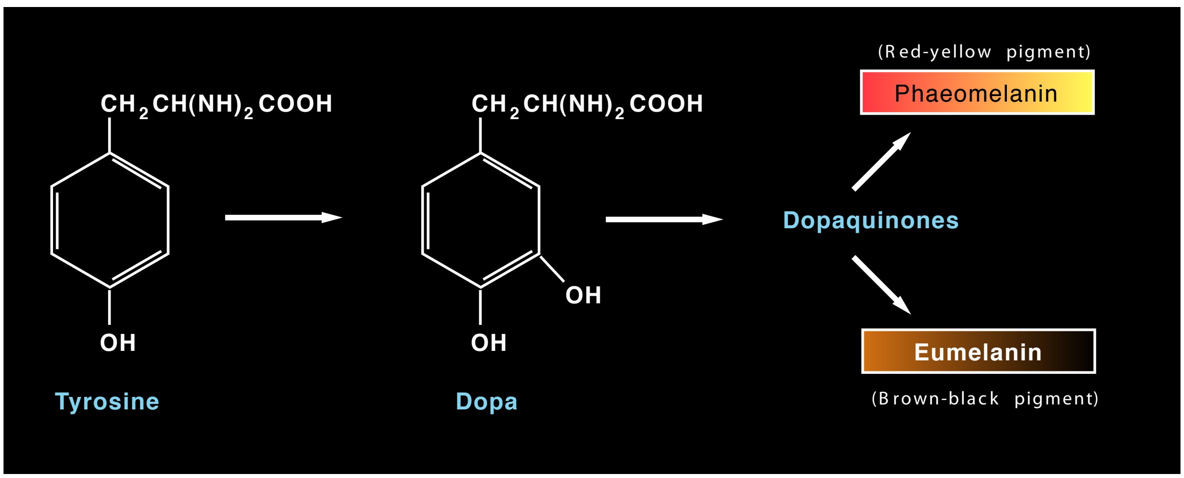

Figure 5. Melanin is synthesized (oxidized) from tyrosine with the help of the enzyme tyrosinase.

The dopaquinones are then bound together in a long strand (polymerized) to form melanin (**Figure 6**). These polymers contain numerous carboxyl groups (COOH) giving melanin an acidic character. However, at normal cellular pH of 7.4, the COOH tends to donate a H+ leaving an overall negative charge (COO-) on melanin. (Many shampoos are “pH-balanced”, i.e., they are acidic to match the pH of the hair itself.)

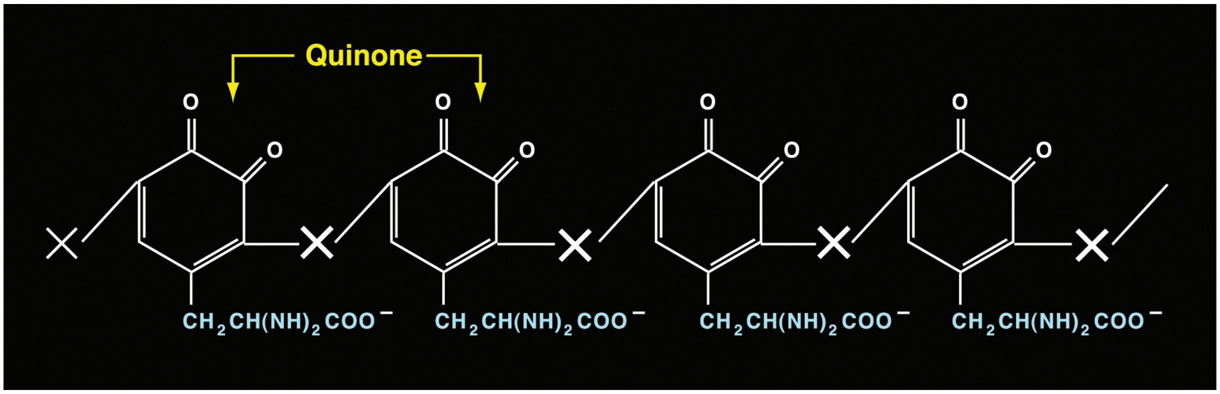

Figure 6. Melanin is a polymer of dopaquinones. The X indicates additional groups containing COOH. The COOH groups tend to lose the H atom leaving them with a negative charge.

Binding of drugs to hair

In order for drugs to become incorporated into the hair, they must enter the follicle. This occurs by two routes. One route of entry is from the capillaries surrounding the follicle. The other route of entry is through the sebum secreted by the sebaceous glands connected to the follicle (drugs can enter the follicle from sweat as well). Most drugs will have little trouble leaving the capillaries since there are many pores or fenestrations that allow their passage. Once the drugs get into the follicles and the hair cells, the pH of the hair cell environment will determine if the drugs can get out. For example, when drugs that are weak bases are in an acidic environment, they tend to exist in a predominantly charged form (they accept a H+ from the donor) (see Module 1). This is the case for drugs such as nicotine, morphine, cocaine and amphetamine. These drugs are weak bases and they bind to the melanin because it is acidic. The nature of the bond between oppositely charged atoms is electrostatic. Electrostatic forces are very common in the formation of bonding between drugs and proteins (either enzymes or receptors) (see Module 5). When drugs bind to receptors on neurons, they change the electrical activity of those cells. However, in hair, the binding of drugs to melanin does not affect the cell's function. Instead, the weak bases become trapped inside the hair cells because they exist mainly in their charged form. As charged molecules, they have difficulty diffusing through the lipid membranes of the hair cells to reach the capillaries. The more melanin present in the hair, the more binding of weak base drugs. So, for the same dose of drugs such as cocaine and morphine, higher levels of these drugs are present in black and brown hair compared to blond hair. Non-Caucasian races with black hair have higher concentrations of these drugs in their hair compared to Caucasians. After a single dose, the drugs remain in the hair from 2-8 months (**see Figure 7**), depending on the individual. Drugs such as cocaine can even be detected in the hair of newborn babies after use of cocaine by the mothers during the 2nd and 3rd trimesters of pregnancy. Washing of the hair does not eliminate the drug, although anything that destroys the pigment will decrease the binding of the drug.

How do drugs get into hair from the environment?

When drugs are smoked or inhaled as a vapor, a portion of the smoke or vapor escapes into the air surrounding the user or others nearby. To be dissolved in the smoke or vapor the drug must be heated to a high temperature in its uncharged (non-polar) form. This is especially the case for weak bases (See Module 5). For example, nicotine and cocaine are smoked in their uncharged or free base form (see Module 1). The same is true for heroin or opium smokers. Because the uncharged forms of these drugs are lipophilic, they can easily penetrate the hair shaft (that part of the hair above the skin). Once in the hair shaft, the drugs can bind to melanin as described above. One way to distinguish between drugs entering the hair from the body vs the environment is to look at the pattern of drug binding along the hair itself. With very recent drug use, the drug will be present in the hair root (they can be found in the hair root as early as 8 hours after drug use), but not in the hair shaft above the skin. With past drug use, the drug will be located in a small section of the hair at a distance from the root proportional to the number of months after the drug use (hair grows at 0.35 mm/day or about 1 cm/month). Experimental subjects given low doses of cocaine show this pattern of cocaine binding to hair (Figure 7). In contrast, environmental exposure will produce no real pattern. The length of hair that is exposed to the air during the time of exposure will uniformly bind the drug.

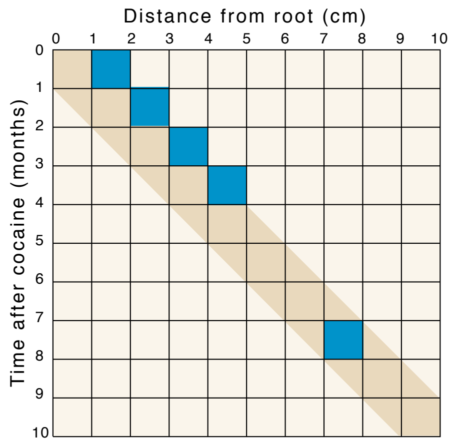

Figure 7. Pattern of cocaine binding to hair from a subject that snorted 1 low dose of cocaine.
From: Henderson et al. J. Analytical Toxicology, 20:1-12, 1996.

Another way to distinguish the route of entry of a drug into the hair is by analyzing the hair for the drug metabolites. All drugs must go through the liver as they circulate through the bloodstream. In the liver, most drugs are metabolized by enzymes to inactive products. Their structure is changed into a little more polar (charged) forms so that it is easier to eliminate them in the urine (charged compounds get trapped in the urine and can't get back into the bloodstream). As the metabolites circulate throughout the bloodstream some of them stay in the kidney to be excreted, and some of them reach other parts of the body. They are able to enter hair follicles, just as the parent drug. So cocaine and its metabolites can be found in hair and produce the same pattern of accumulation. If the cocaine were to enter the hair through a vapor from the environment, there would be no metabolites present in the hair.

Glossary

endothelial cells—cells that line the blood vessels and capillaries

epidermal cells—cells that constitute the outer layers of skin

epithelial cells—cells that line all free surfaces such as the skin, nasal passages and the gut

fenestrae—small pores in the capillaries, probably spaces between endothelial cells, that allow solutes (whether charged or uncharged) to move through the capillaries

follicle—a small sac; hair follicles are internalized structures of epithelial cells in which the hair is synthesized and grows

keratin—the major protein found in hair; it is very fibrous and contains many sulphur bonds

keratinocytes—the cells in which keratin is synthesized within the follicle

lipophilic ('lipid loving')—high lipid solubility. Lipophilic compounds dissolve readily in oil or organic solvent. They exist in an uncharged or non-polar form and cross biological membranes very easily.

melanin—the pigment found in hair, skin, feathers, etc.. It is a biopolymer found in granules within melanocytes and transferred to keratinocytes to provide hair color.

melanocytes—the cells in the hair matrix and in the cortex that synthesize melanin.

metabolite—usually an inactive form of a drug or other substance that is more polar (charged) than the parent compound. Drugs are metabolized by enzymes primarily in the liver.

mucosal cells—a special type of epithelial cell that can secrete mucous. This protects the cells lining the tissue.

non-polar—a chemical property of a substance that indicates an even distribution of charge within the molecule. A non-polar or non-charged compound mixes well with organic solvents and lipids but not with water.

polar— a chemical property of a substance that indicates an uneven distribution of charge within the molecule. A polar substance or drug mixes well with water but not with organic solvents and lipids. Polar or charged compounds do not cross cell membranes (lipid) very easily.

receptor—a protein to which hormones, neurotransmitters and drugs bind. They are usually located on cell membranes elicit a function once bound.

sebum—a fatty substance made in and secreted by sebaceous glands attached to hair follicles

tyrosine—an amino acid found in melanocytes and in neurons that is the starting material for the synthesis of melanin and dopamine, respectively.

tyrosinase—the enzyme found in melanocytes that is required to help oxidize tyrosine to make melanin. Without tyrosinase, there is no hair pigmentation.

weak acid—a compound that tends to give up a H+ when placed in an alkaline solution

weak base—a compound that tends to accept a H+ when placed in an acidic solution

Module 2: Supplemental Classroom Activities

“You Be The Judge”

Objective(s):
1. To demonstrate understanding of drug analysis using hair and urinalysis
2. To explore the ethics of drug testing and its implications
3. To understanding binding (chemical bonds) and binding sites

Standards and Skills:
AD2, AE1, CB3, CC5, CE2, CF1, CF6

For many employers today, job applicants must undergo drug testing. One company’s drug policy states that all applicants must be drug free for at least 6 months prior to application. Applicants will be subjected to hair sample and urine analysis tests.

Based on what you have learned about how drugs enter and exit the body and how drugs bind to the melanin in hair, study each applicant’s profile and identify applicants who ***could be proven*** ineligible for employment. Each group should discuss the facts and prepare answers to the four questions listed below.

Facts:

Hair grows approximately 1 cm / month
Metabolites found in hair denote drug use at that time.
Overall binding to the entire hair shaft denotes secondary exposure.
Eating poppy seeds may give a false positive result because they contain trace amounts of opiates that can be detected in a urinalysis.

Profiles:

a) White male, 30, alopecia (no hair at all), eats poppy seed muffins almost every day.

b) Asian male, 24, black hair, 3 cm long, overall binding of entire hair shaft.

c) Black male, 27, shaved head, eats poppy seed bagels every day.

d) Hispanic female, 22, auburn hair, 40 cm long, metabolites found 3 cm from root.

e) White female, 36, dark brown hair, 36 cm long, metabolites found last 20 cm of hair.

f) White female, 37, prematurely fully gray hair, 22 cm long. Negative urine test.

g) Black female, 31, albino, hair 28 cm long, eats poppy seed muffins everyday.

h) White male, 29, blond hair 30 cm long. Smokes cigarettes.

1. Who (if any) were proven drug users during the past 6 months?

2. What should policy makers know about these tests’ results in order to make fair interpretations?

3. What are the ethical implications of mandatory drug testing?

4. If you were the employer making the hiring decisions would you eliminate anyone else on this list? (If so, is it legal for you do so?)

Teacher Notes:
Make available copies of pages 6-11 from the teacher guide for each group. Each group should answer each of the four questions above and be prepared to defend their conclusions in a class discussion.

The assessment item may be used as a group assessment or individual. The truth table application may be used for those students who have difficulty keeping trace of several facts at one time.

Assessment:
Consider the following information and re-examine your applicant list and determine if you would eliminate any additional persons from your hiring list. For each individual, A-H, write a statement of available for hire or not to hire and give reasons for those not to be hired based on drug usage.

The positive test for opiates after eating poppy seeds should last about 3-5 days. The applicant could be asked to avoid eating poppy seeds and return in five days for re-testing.

Hair for drug testing is usually taken from the head but can be taken from any part of the body.

Additional results:
A, C, E were re-tested. A and C were negative urinalysis and G tested positive

Optional:
When you have numerous facts to consider it is sometimes easier to draw conclusions based on a truth table. This is just a way to organize ones thinking. You should list all the fact you know, then draw conclusions.

Truth Table

Individual	Hair	Urinalysis	Hair Results	Preliminary conclusion	Actions Taken	Results	Conclusions
A	Alopecia	+ poppy seed			Retest 5 days	- urinalysis	
B	Black hair 3 cm long	-	+ Overall binding				
C	Shaved head	+ poppy seed			Retest take hair from body	Trace in hair, - urine	
D	Auburn hair	?	+ 3cm from root				
E	Brown hair 36 cm long	?	+ last 20 cm				
F	gray	-	-				
G	albino	+ poppy seed			retest	+ urine	
H	blond			smokes			

Resources

The following resources provide supplemental information that pertains to the topic in this module.

RR Levine, CA Walsh and RD Schwartz. *Pharmacology: Drug Actions and Reactions* , Parthenon Publishing Group, New York, 2000. Chapters 4-6.

MR Harkey, (1993) *Anatomy and physiology of hair.* Forensic Science International 63:9-18.

CE Orfanos and R Happle. *Hair and Hair Diseases*, Springer Verlag, New York, 1990. Chapters 6 and 8.

GL Henderson, MR Harkey and C Zhou. (1996) *Incorporation of isotopically labeled cocaine and metabolites into human hair: Dose response relationships.* Journal of Analytical Toxicology 20:1-12.