

Practical Connections: Hair Drug Testing

Practical Connections: Drug Testing

Activity Topic:

Drug testing hair samples is a commonly utilized method of determining past illicit drug use. Here we discuss topics including the underlying biology and chemistry of drug deposition in hair, interpretation of laboratory results, racial bias, and ethical decision making.

Prerequisite knowledge needed:

- Physiochemical properties of molecules (Gen. Chemistry and Organic Chemistry)
- Basics of CYP function and drug metabolism (Biochemistry)
- A&P of circulatory system (Gen. Biology, Anatomy and Physiology)
- Basic PK of drug absorption (Organic chemistry, Medicinal Chemistry, Pharmaceutics, Pharmacokinetics)
- Unit conversion calculations (Gen. Chemistry)

Learning objectives:

Students should:

1. Utilize physiochemical information to identify properties of molecules.
3. Describe distribution of drugs in context of circulatory A&P and first pass metabolism.
4. Interpret limitations of laboratory analysis results.
5. Appraise the ethics of drug hair-testing and its use in legal, employment, or sporting decisions.
6. Interpret data from health-science literature.
7. Apply calculations skills.

Student Pre-readings and quiz:



<https://www.thetracker.com/tracking-news/the-nation-proposed-guidelines-for-hair-drug-testing-atr-debate-in-tracking-industry>

Sasaki, G.H. Review of Human Hair Follicle Biology: Dynamics of Niches and Stem Cell Regulation for Possible Therapeutic Hair Stimulation for Plastic Surgeons. 2016 *Clinics in Surgery* 1:Article 1257

Analysis of Hair-Based Drug Testing

Hair-based drug testing is based on the fact that cocaine, opioids, nicotine, and amphetamine can be detected in the hair shaft for months after drug use.¹ However, there are several concerns about these tests.

1. While usually indicating personal drug use, it has been clearly shown that highly volatile agents can be deposited in low levels from chronic exposure to surrounding smoke.²⁻⁴
2. Some OTC and Rx agents may create false-positives.^{5,6} Follow-up with more sensitive techniques such as GC-MS or HPLC should be, but are not always, performed to verify results.
3. Physiological differences in biological sex and race affect test positivity.^{7,8}

This creates several ethical concerns for the use of hair-based drug testing in decisions about employment, probation status, and athletic eligibility. However, positives include that hair samples (1) are easier to collect than blood, and (2) can detect drug use from further in the past than blood or urine.¹

Hair-based drug testing is incorrectly referred to as “hair follicle testing.” These tests actually retrieve samples from the hair shaft, not the follicle.⁹ Otherwise it would require plucking hair directly from the scalp!

The hair shaft grows from the bottom of the follicle (Figure 1).⁹ Capillaries surround the bulb of the follicle, delivering oxygen and nutrients.⁹ The hair shaft includes structural keratin as well as at least two melanin pigments, the amount and combination of which determines hair color.^{7,8} Melanin is deposited as the hair is growing within the follicle, with more melanin present in darker hair.¹⁰

For deposition into a growing hair shaft, a drug must be distributed through the circulatory system and enter cells within the growing hair follicle.⁹ Similarly, for pleasure and rewarding effects, the drug must distribute through the circulatory system and pass through the endothelial cells making up the blood-brain-barrier to enter the intercellular fluid and then the neurons themselves.^{11,12}

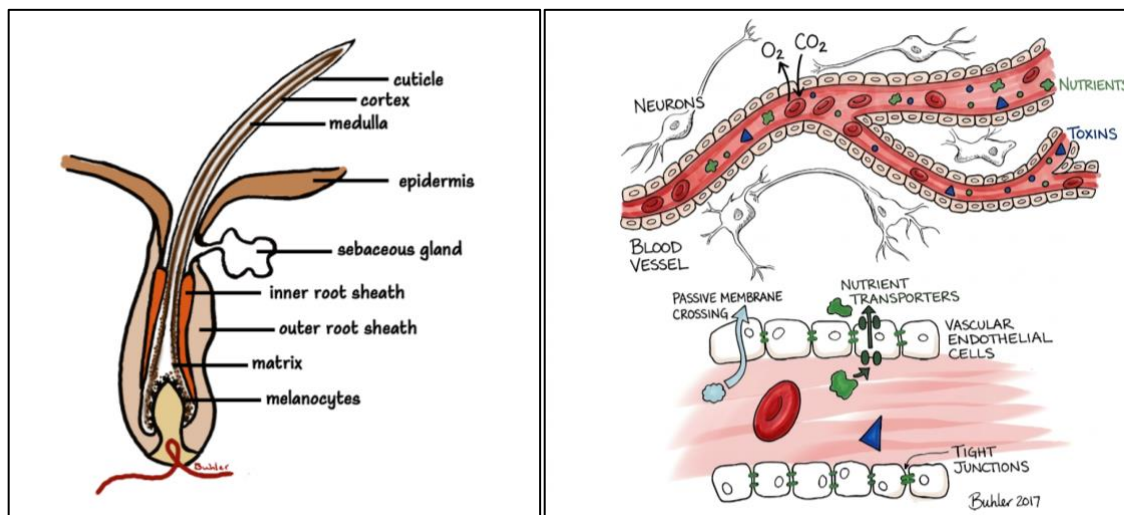


Figure 1. Hair follicle growth and blood-brain barrier

If you are not familiar with the blood-brain barrier watch this 1min 27second video!
<https://www.youtube.com/watch?v=e9sN9gOEdG4> (watch up to 1:27)

The figure (Fig 2) below is an example of the molecular interactions of the antibiotic doxycycline interacting with a section of the melanin polymer chain. A fun fact is that hair testing can be used to monitor long-term antibiotic compliance!¹³

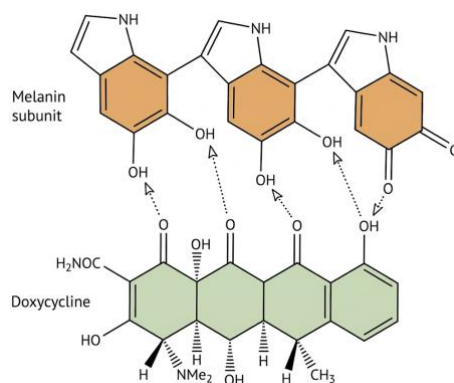


Fig 2. Melanin binding the antibiotic doxycycline

Quiz

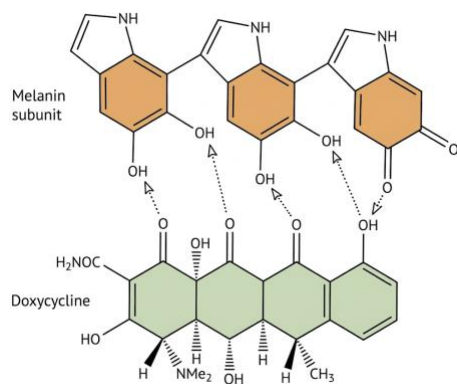
Q1. For each of the following forms of administration, which barriers do each need to pass through to enter circulation? (5pt) pick all that apply- ie, a particular form may need to cross through more than one barrier.

___ PO	a) pulmonary endothelium
___ IV	b) GI endothelium
___ Inhalation	c) oral mucosal endothelium
___ SL	d) cutaneous endothelium
___ Transdermal	e) none (delivered directly to circulation)

Q2. Assuming the desired effect of the drug is within the brain, would metabolism by a P450 usually increase/decrease effectiveness? (2pt)

Q3. Explain above answer: (2pt)

Q3. Based on the figure below, what type of molecular bond/force is involved in binding of doxycycline to melanin? (1pt)



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13. [Venetia A Florou](#), et. al. Human hair as a diagnostic tool in medicine. *Biochem Biophys Rep* 2025 Jul 5;43:102-129.

IN-CLASS Student Worksheet

Hair Drug Testing

Group # _____ Names of students present _____

The majority of background information you will need to complete this activity has been covered in the prerequisites mentioned and in this handout. In the case that there are concepts you are not familiar with, you are encouraged to look them up and then to ask for clarification if needed.

Drug delivery: A&P, drug metabolism

Within the bloodstream most drugs of abuse distribute to pleasure and reward centers in the brain. The properties of the drug effect are altered by kinetics and metabolism. The opioid fentanyl is poorly bioavailable, and so is administered by i.v. injection, patch, or buccally. However, PO administration of amphetamine or codeine and intranasal (snorting) of cocaine are common, as is smoking of these and other agents.

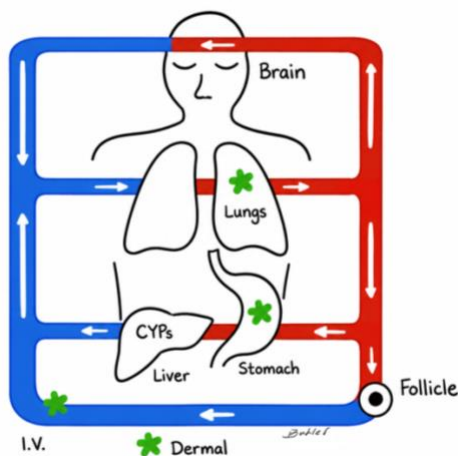


Fig. 1. Common sites of drug administration: inhalation, PO, transdermal, IV (buccal not shown)

Question 1. Rank the above sites of delivery for the shortest to longest delay in reaching the brain.

- 1st (shortest) Lungs. Almost instantaneous. Drug directly enters arterial circulation and is taken directly to brain with shortest trip through the circulatory system.
- 2nd iv. Enters systemic venous circulation immediately but has to go through circulatory system before reaching the brain. A drug delivered this way will reach most

other tissues faster than by inhalation, but this is not the case for delivery to the brain, as the circulatory pathway goes directly to the brain.

- 3rd PO. Must be absorbed in stomach or colon and then into systemic circulation. (Generally 30-60 min for onset of action)
- 4th. Transdermal. Must pass dermal barrier and go through systemic circulation.

Question 2. Categorize the above routes of delivery by which reach the brain before or after hepatic exposure.

Route of administration	liver before brain target	Brain target before liver
Inhalation		X
IV		X
PO	X (only one with first pass effect)	
Transdermal		X

Melanin production and drug deposition: chemistry, biological molecules

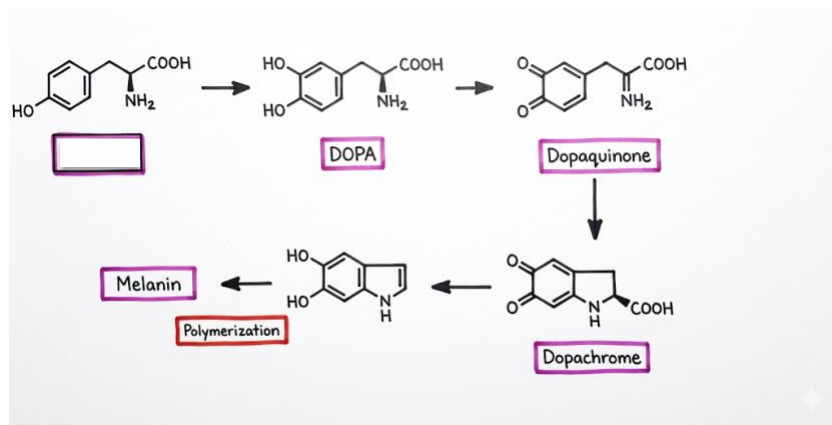


Fig 2. The synthetic pathway of the melanin monomer.

The synthetic pathway of the melanin monomer is shown here (Fig. 2). These precursors of melanin can also go down alternate pathways to produce other important physiological compounds.

Question 3.

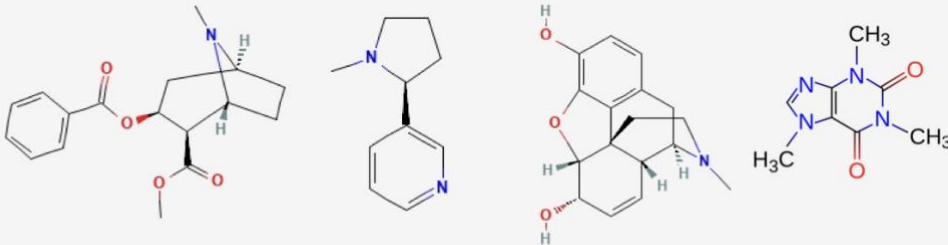
- Identify the **MACRONUTRIENT FAMILY** to which the original precursor molecule belongs. **Amino acid**
- provide the name of the original precursor molecule. **tyrosine**

c) If you had a diet lacking the original precursor molecule, would you still be able to make melanin? Explain your answer.

Yes. Tyrosine is a nonessential amino acid and can be formed by the hydroxylation of phenylalanine in the liver when the intake of tyrosine in the diet is low.

Question 4. Caffeine passes passively through the blood-brain barrier, while the other compounds cannot, and must utilize transporters. Using the information in Fig. 3 below, brainstorm and discuss why this is the case. You should consider a) ionization, b) hydrophilicity, and c) size. (You do not need to do any calculations, but the Henderson Hasselbalch equation is very useful here) Note that clogD is similar to logP but is a pH-dependent measure of lipophilicity and accounts for all forms of a compound at a specific pH (not just neutral compound). That's more detail than you probably care to know, but you can interpret clogD as you would logP: the higher the number the more lipophilic.

	cocaine	nicotine	morphine	caffeine
c logD at pH 7.4	1.22	-0.37	-0.25	0.28
MW	303.35	162.23	285.34	194.19
pKa	8.61	8.1; 3.4	8.21	0.7



logD from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4956512/>

Fig. 3. Molecular properties of drugs.

Example: Cocaine

1. Size: larger, so doesn't pass passively as easily as nicotine and caffeine
2. cLogD: A positive number is more fat-soluble. This drug is hydrophobic, which decreases its solubility in blood, but increases its potential membrane permeability
3. Ionization: mostly charged at physiological pH (tertiary amine), which increases hydrophilicity, and decreases its ability to passively pass through blood-brain-barrier

Nicotine

4. Size: smaller, so passes passively more easily than cocaine and morphine
5. cLogD: this drug is mildly hydrophilic at physiological pH, which increases solubility in blood, while maintaining some membrane permeability
6. Ionization: mostly charged at physiological pH (tertiary amine; the pyridine ring is typically unprotonated at physiologic pH due to its low pKa), which increases hydrophilicity, which decreases ability to passively pass through blood-brain-barrier

Morphine

7. Size: larger, so doesn't pass passively as easily as nicotine and caffeine
8. cLogD: this drug is mildly hydrophilic at physiological pH, which increases solubility in blood, while maintaining membrane permeability.
9. Ionization: mostly charged at physiological pH (tertiary amine), which increases hydrophilicity, which decreases ability to passively pass through blood-brain-barrier

Caffeine

10. Size: **smaller**, so passes passively more easily than cocaine and morphine
11. cLogD: this drug is mildly **lipophilic at physiological pH**, so it travels well in blood and has ability to passively pass through blood-brain-barrier
12. Ionization: **mostly UNCHARGED** at physiological pH (the 5-membered ring component of the xanthine core remains unprotonated due to low pKa), which increases ability to passively pass through blood-brain-barrier

Facilitator's hint: partition is: octanol/water so high partition coefficient = lipophilic
logD: positive= hydrophobic (lipophilic); negative = hydrophilic (lipophobic)

In general, higher logD increases CNS permeation. However, it is great if students realize that highly lipophilic agents will have a hard time being soluble in the blood, and that this can limit actual use of highly lipophilic agents.

The table below by Hitchcock 2006 is not necessary for the question, but is a nice resource to see where several CNS-permeable drugs fit in.

Table 1. Suggested Physicochemical Property Ranges for Increasing the Potential for BBB Penetration.

property	top 25 CNS drugs mean values	suggested limits	% of top 25 CNS drugs in suggested range	preferred range	% of top 25 CNS drugs in preferred range
PSA ($\text{\AA}^2$)	47	<90	96	<70	76
HBD	0.8	<3	100	0–1	92
cLogP	2.8	2–5	68	2–4	52
clogD (pH 7.4)	2.1	2–5	61	2–4	61
MW	293	<500	100	<450	100

Stephen A. Hitchcock, Lewis D. Pennington; Structure–Brain Exposure Relationships. *J. Med. Chem.* 2006; 49 (26): 7559–83. <https://doi.org/10.1021/jm060642i>

Hair-based drug testing and personal drug use: ethics, calculations



<https://diversity.social/latest-visible-minority-news-today/>

Recalling your pre-reading, hair-based drug testing is based on the fact that many drugs can be detected in the hair shaft for months after drug use. However, there are several concerns about these tests. These concerns include that physiological differences in hair composition and growth affect test positivity.

Question 5. How does hair color and length impact the sensitivity of hair to testing?

Support your answer scientifically.

Drugs would bind at higher concentrations to darker hair. Darker haired people would be more likely to test positive, depending on the sensitivity of the test. Persons with very short hair would not be able to prove anything about past use. This could allow a person to avoid a positive test for past use, or result in a person's inability to prove they were not a chronic user. Other factors include hair growth rates; bleaching hair; etc

Bias could also occur when testing is done on samples of hair from persons with varied hair growth rates. Persons with faster hair growth would “clear out” drug-containing hair faster. Because there are racial differences in hair growth rates, groups with slower hair growth rates would be more likely to test positive. Whole scalp growth rates have been reported as:

African Ancestry	280 ± 50 um/day
Asian Ancestry	411 ± 53 um/day
Caucasian Ancestry	367 ± 56 um/day
Diversity of hair growth profiles. G. Loussouam, C. El Rawadi, G. Genain. <i>Int J Dermatology</i>. 2005 44(1)6-9. Table 4 Hair Growth Rates. (As for many health and clinical topics, racial ancestry-based data is highly limited. I could not find information about drug binding in Native American, Austronesian, or Polynesian hair.)	

Figure 4. hair growth rates

LabCorp, a corporate employee drug testing business, stated that a 1.5 inch sample from the root will demonstrate drug use within the past 3 months. However, more precise data on hair growth is presented by Loussouam et. al. in Figure 4 above.

Question 6. Using the Loussouam data, how many months of hair growth is included in a 1.5 inch sample for each racial group? (assume there are 30 days/month) SHOW ALL CALCULATIONS

Step 1. Calculate inches/month from the um/day units:

The Int. Journal of Dermatology says whole scalp average is

African: $280 \text{ um/day} = 8.4 \text{ mm/month} = 0.33 \text{ inches/month}$

Asian: $411 \text{ um/day} = 12.3 \text{ mm/month} = 0.48 \text{ inches/month}$

Caucasian: $367 \text{ um/day} = 11 \text{ mm/month} = 0.43 \text{ inches/month}$

Step 2. Calculate how many months an 1.5 inch sample would ACTUALLY indicate for each group:

African $1.5 \text{ inch} \times 1 \text{ month} / .33 \text{ inches} = 4.5 \text{ months}$

Asian $1.5 \text{ inch} \times 1 \text{ month} / .48 \text{ inches} = 3.1 \text{ months}$

Caucasian $1.5 \text{ inch} \times 1 \text{ month} / .43 \text{ inches} = 3.4 \text{ months}$

(Facilitators' hint: students may get wildly wrong answers based on incorrect unit conversion. For example they may get 400 months! Ask them whether that number realistically makes sense. Suggest that if it just doesn't appear real-world then they should double check their conversions.) Some groups may need some reminders about cross-multiplication.

These numbers represent averages, but we can still use this data for a rough prediction of whether a person within a larger group may test positive after a set amount of time. You have a company that requires no drug use within the past 120 days (4 months) for hiring, and takes a 1.5 inch hair sample for verification.

Question 7. You have three potential employees who were all at a party together 4 months ago and used a recreational drug. None have used any recreational drugs since. The three candidates are of different descent: Caucasian, Asian, and African ancestry. Based on this information, for whom might LabCorp come up with a "do not hire" recommendation? Explain your reasoning.

In Caucasian or Asian ancestry, it would likely come up negative for drug use, while in African ancestry it would likely come up positive for drug use, and therefore result in a "do not hire" recommendation. This is because with slower growing hair, the drug deposit would still be present, while in faster growing hair it would not.

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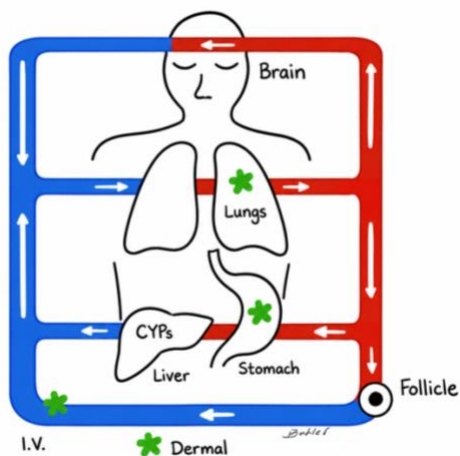


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PO		
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Melanin production and drug deposition: chemistry, biological molecules

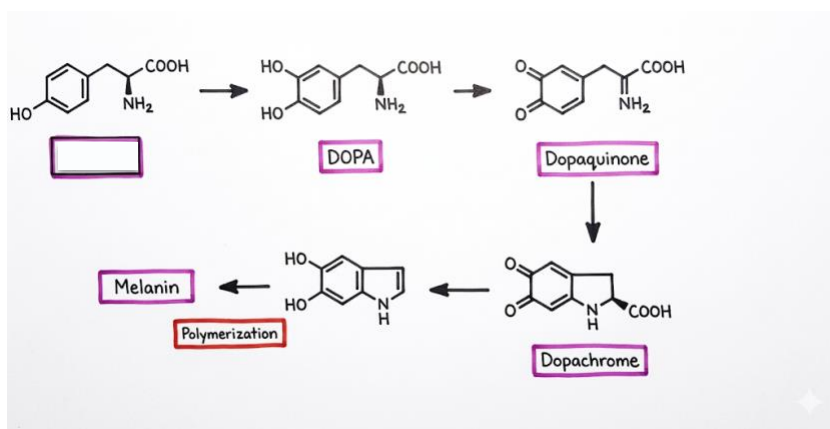


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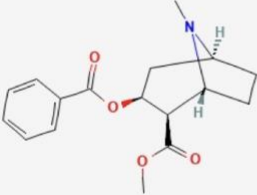
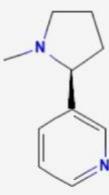
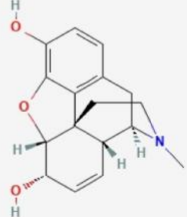
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Nicotine

4. Size:
5. cLogD:
6. Ionization:

Morphine

7. Size:
8. cLogD:
9. Ionization:

Caffeine

10. Size:
11. cLogD:
12. Ionization:

Hair-based drug testing and personal drug use: ethics, calculations



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Figure 4. hair growth rates

LabCorp, a corporate employee drug testing business, stated that a 1.5 inch sample from the root will demonstrate drug use within the past 3 months. However, more precise data on hair growth is presented by Loussouam et. al. in Figure 4 above.

Question 6. Using the Loussouam data, how many months of hair growth is included in a 1.5 inch sample for each racial group? (assume there are 30 days/month) SHOW ALL CALCULATIONS

These numbers represent averages, but we can still use this data for a rough prediction of whether a person within a larger group may test positive after a set amount of time. You have a company that requires no drug use within the past 120 days (4 months) for hiring, and takes a 1.5 inch hair sample for verification.

Question 7. You have three potential employees who were all at a party together 4 months ago and used a recreational drug. None have used any recreational drugs since. The three candidates are of different descent: Caucasian, Asian, and African ancestry. Based on this information, for whom might LabCorp come up with a “do not hire” recommendation? Explain your reasoning.

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