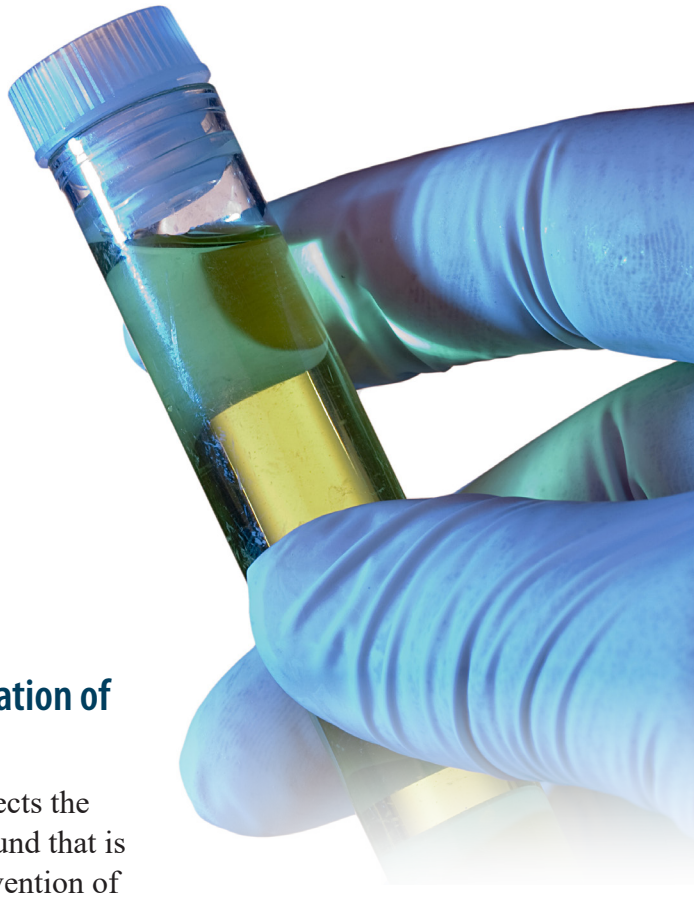
Drug Testing in Urine, Oral Fluid, and Hair

Part 1: Metabolism and Matrix Formation

2018

This is the first of a two-part Drug Testing Matters series on drug testing of urine, oral fluid, and hair. This part provides background information describing the formation of each matrix and how drugs and/or drug metabolites are incorporated into that matrix.



Absorption, Distribution, Metabolism, and Elimination of Drugs

A drug can be defined as a chemical substance that affects the processes of the mind or body or as any chemical compound that is administered as an aid in the diagnosis, treatment, or prevention of disease or other abnormal condition; for the relief of pain or suffering; or to control or improve any pathologic condition.[1] Because all drugs (depending on dose) can be considered poisons, drugs also can be termed toxicants, with the ultimate toxicant being the active form that binds to a specific target molecule and produces a pharmacodynamic effect.

Before a drug can have any effect, it must first be taken into the body. Common routes for the administration of drugs are oral, parenteral (intravenous, subcutaneous, and intramuscular), smoking (air pathway membranes and lungs), insufflation, transmucosal (oral and rectal or suppository), and transdermal. Most drugs have a limited number of absorption pathways, and numerous processes occur contemporaneously to prevent the formation of the ultimate toxicant and its binding to its target molecule (e.g., excretion, detoxication).

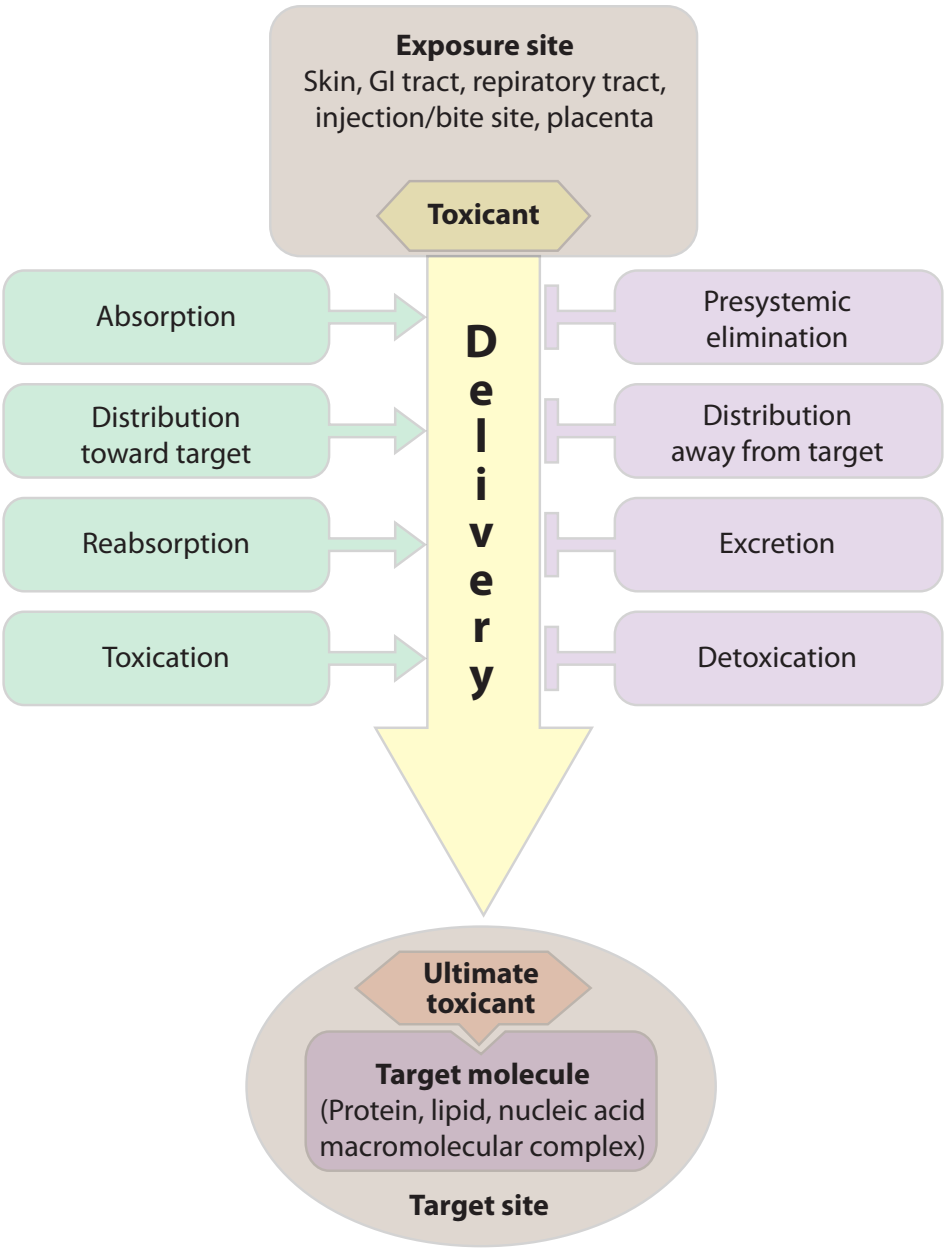


Figure 1. Absorption, Distribution and Excretion of Drugs as Toxicants (p. 52, Reference 2).

An example of a specific drug that is administered for pain relief is codeine. Compounds produced during the metabolism of codeine are shown in Figure 2. If administered orally, codeine is absorbed and taken into systemic circulation. If administered intravenously (parenterally), codeine is taken into systemic circulation instantaneously ("Absorption" in Figure 1). Intramuscular and subcutaneous administration delay codeine entry into systemic circulation. Some of the codeine in systemic circulation is eliminated as the parent drug into urine and, possibly, bile, thus removing the codeine from being available to interact with an opiate receptor or convert into the more active morphine metabolite ("Presystemic Elimination" in Figure 1). Although the parent drug codeine shows approximately 10% of the analgesic activity of

its active metabolite morphine, in CYP2D6-competant individuals, codeine is converted partially into the more active morphine ("Toxication" in Figure 1; also called "metabolic activation"); in this article, morphine is considered the major ultimate toxicant.[2,3] In the context of long-term use, roughly 10% of administered codeine is also converted into active hydrocodone ("Toxication" in Figure 1). Some codeine is deactivated by conversion into codeine glucuronide in the human liver ("Detoxication" in Figure 1). Distribution is a two-way street in most drug delivery processes, including that for codeine. Some codeine (along with any formed morphine, *vide supra*) is distributed to locations in the body where it can interact with an opiate receptor and/or be metabolized into morphine, and some is transported to areas where receptor interaction and/or conversion cannot take place, as denoted by "Distribution toward target" and "Distribution away from target", respectively, in Figure 1. The elimination of codeine glucuronide, morphine and its glucuronides and sulfates, and the small amounts of hydrocodone and hydromorphone formed through renal and biliary processes falls into the category of "Excretion" in Figure 1. Counterbalancing "Excretion" in Figure 1, eliminated drugs and their metabolites can be reabsorbed either by the renal tubules or, in the case of morphine glucuronide, through the intestinal wall and converted back into active morphine by intestinal glucuronidases (a combination of "Reabsorption" and "Toxication" in Figure 1, commonly referred to as enterohepatic circulation). In the context of chronic morphine or codeine use, a small amount of the morphine may be metabolized into active hydromorphone (again, "Toxication" in Figure 1). Notably, the overall distribution and metabolism depend on protein status, the presence or absence of mutant forms of metabolic enzymes and transporters, and the presence of small molecules other than the drugs/drug metabolites of immediate interest that may positively or negatively influence metabolism and transport. Renal and hepatic elimination depend on the overall status and health of the respective organs.

Although parent codeine has some analgesic activity, the ultimate toxicant is morphine, with the 6-glucuronide conjugate and minor amounts of hydrocodone and hydromorphone also serving as ultimate toxicants (see Figure 2). After drug exposure, a random sample of blood, blood product, or oral fluid (*vide infra*) drawn from the body and analyzed for the drug and its metabolites will be a snapshot of all the processes illustrated in Figure 1. Using codeine as the example drug, the extent to which each of the target analytes in Figure 2 is detected and resulted on a toxicology report is a function of both the time between drug exposure and sample draw and the analytical capabilities of the laboratory. The drug and metabolites that an analytical toxicologist reports from a urine drug test are similar to those reported from tests of blood, blood products, and oral fluid, but the relative concentrations are averaged over the time that the urine was being formed and collected in the bladder (minutes to hours). Blood, blood products, and oral fluid tend to be better matrices for finding active parent substances. As an excretory product, urine is better for finding metabolites, although metabolites and parent substances can usually be found in blood, blood products, and oral fluid. Unless a technique such as segmentation is employed, the results of hair testing usually demonstrate use over a much broader period of time. Both the parent drug and metabolites can be found in hair specimens.

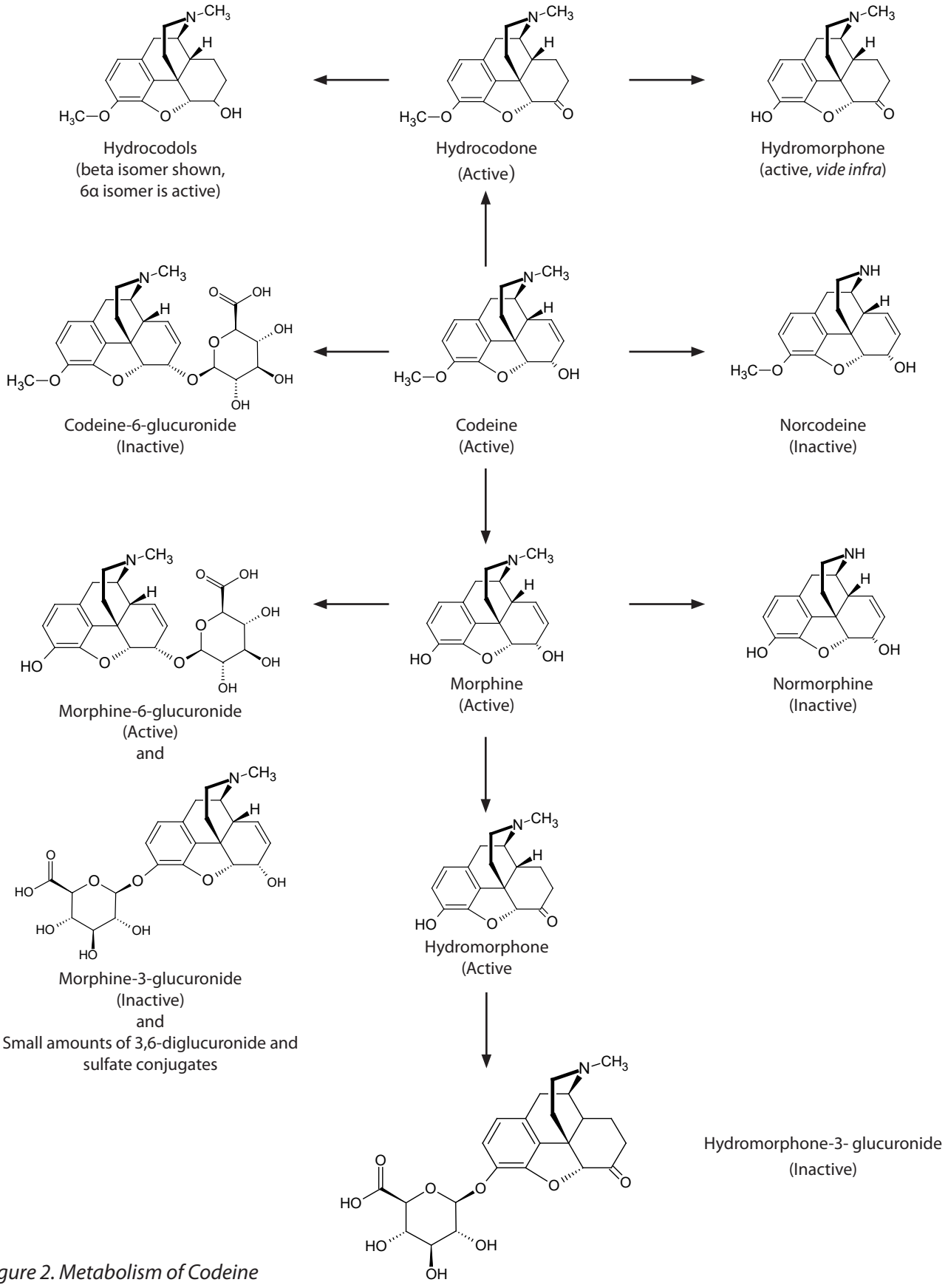


Figure 2. Metabolism of Codeine

Specialized processes, such as the formation of electrophiles, nucleophiles, and free radicals, are important to the general field of toxicology but usually play only minor roles in analytical drug toxicology and are not considered any further in this limited discussion.

The metabolism of drugs can result in either toxication ("metabolic activation") or detoxication (sometimes referred to as "deactivation"). The enzymes for numerous drug metabolic pathways exist in multiple parts of the body. However, the liver is usually considered the primary site of drug metabolism. Although some common pathways exist between drugs, the number and types of metabolic products as a whole are typically specific to an individual drug and must be considered when reviewing and interpreting analytical results.

The two most important routes for the elimination of non-volatile drugs are renal and biliary (hepatic). Analysis of fecal material is common only when meconium is used in neonatal drug testing. In adults, even though some drugs, such as Δ^9-tetrahydrocannabinol (THC), are found in significant abundance in biliary excretion, the testing of fecal material is almost non-existent except for research purposes and will not be further addressed in this discussion. Urine is the most commonly employed matrix in clinical and forensic toxicology. Urine has historically been the only approved matrix for federally regulated workplace drug testing. A brief presentation on renal function and the application of the basic principles of renal function to drug and/or drug metabolite testing is provided below.

Generally, the kidneys are thought of as excretory organs, but they also have homeostatic, metabolic, and endocrine (hormonal) functions, as presented briefly in Table 1. Toxicologists usually only have an interest in the excretory (a combination of filtration and tubular physiologic activity) functions of the kidney both for drug and/or drug metabolite testing and specimen validity testing (SVT).

Table 1. Important Components of Kidney Function

Function	Examples
Filtration	Preparation of an ultrafiltrate from plasma primarily to remove small organic compounds and inorganic ions from plasma
Reabsorptive	Water, glucose, amino acids, electrolytes, and proteins are returned to circulating blood thereby forming a hypertonic urine and conserving these vital plasma components included in the ultrafiltrate
Secretory	Non-glomerular addition of blood substances to forming urine
Homeostatic	Maintenance of extracellular volume, acid-base balance, blood pressure, and electrolytes
Metabolic	Synthesis of glutathione, gluconeogenesis, and generation of ammonia and ammonium
Endocrine (hormonal)	Erythropoietin synthesis, activation of vitamin D, and renin release

Adapted from Reference 4.

The kidneys are normally present as a pair of organs close to each side of the vertical region between the T-11 and L-4 vertebrae of the spinal column, with the right kidney being slightly lower than the left. The primary blood supply to the kidneys derives from the inferior vena cava. The blood supply from the inferior vena cava splits into numerous afferent arterioles, each of which feeds a nephron, which is the functional unit of the kidney. Each human kidney contains approximately 0.6–1.5 million nephrons. The positioning of each nephron with respect to the renal vascularization and the macroscopic anatomy of a nephron are shown in Figure 3.

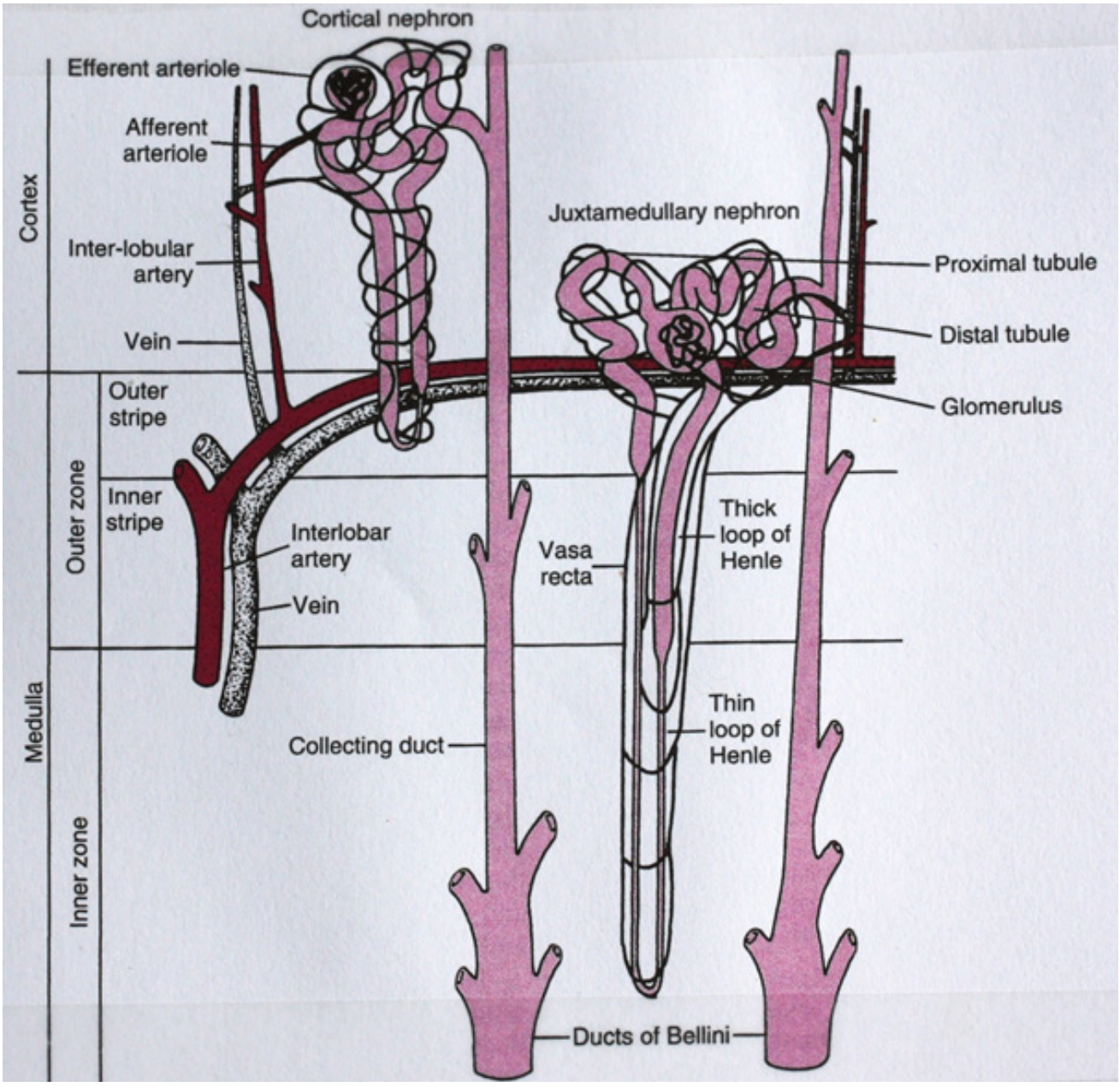


Figure 3. Vascularization and Positioning of Nephrons (p. 1258, Reference 4).

Blood filtration takes place in the glomerulus, where plasma molecules whose molecular weights are less than approximately 50,000 Da and plasma water are filtered from the blood in the capillaries that branch from the afferent arteriole in the glomerulus. Roughly 20% of the blood presented to the glomerulus is filtered.[5] Highly protein-bound (mostly albumin) molecules, such as THC, are less available for filtration than loosely bound substances, such as d-methamphetamine. Among charged species, the ultrafiltration process favors cationic species and discourages anionic species, especially polyanionic molecules such as albumin. Thus, the glomerulus acts as both a size and a charge filter, which is important for the conservation of proteins, such as albumin, and the elimination of small organic molecules, such as drugs and their metabolites, urea, creatinine, uric acid, and some steroids and their metabolites.

The reabsorption of drugs and their metabolites from the glomerular ultrafiltrate happens mostly in the tubules while the ultrafiltrate from the glomerulus is being concentrated into what will ultimately be collected in the bladder as urine. Although the glomerular filtration process is generally viewed as the source of drugs and their metabolites in formed urine, some drugs and their metabolites are both filtered and secreted by the tubules. Although numerous small organic molecules are excreted, other molecules and ionic species that need to be conserved are re-absorbed, as shown in Table 2.

*Table 2. Filtration, Reabsorption, and Excretion Rates of Different Substances by the Kidneys.**

	Filtered (meq/24 h)	Reabsorbed (meq/24 h)	Excreted (meq/24 h)	Reabsorbed (%)
Glucose (g/day)	180	180	0	100
Bicarbonate (meq/day)	4,320	4,318	2	>99.9
Sodium (meq/day)	25,560	25,410	150	99.4
Chloride (meq/day)	19,440	19,260	180	99.1
Water (L/day)	169	167.5	1.5	99.1
Urea (g/day)	48	24	24	50
Creatinine (g/day)	1.8	0	1.8	0

*Glomerular filtration rate: 125 mL/min = 180 L/24 h.
Adapted from Reference 5 (p. 668).

Formed urine is used extensively by clinical and forensic analytical toxicologists to both identify substance use (e.g., the confirmed finding of benzoylecgonine in a urine specimen indicates cocaine use within the past 3–5 days) and confirm compliance with drug therapy qualitatively (e.g., finding oxycodone and/or oxymorphone and no other opiates or analgesics in a patient who has been prescribed oxycodone for chronic pain). Formed urine also contains naturally occurring small organic compounds, such as urea, creatinine, uric acid, cortisol, and 17-ketosteroids, that can be employed to great advantage in urine SVT. The advantages and disadvantages of urine as a matrix for drug and/or drug metabolite testing will be discussed in the second part of this series.

The initial and confirmatory drug test analytes and cutoffs from the 2017 Mandatory Guidelines for Federal Workplace Drug Testing Programs using Urine are listed in Table 3.[6] High-dose drugs, such as acetaminophen and meprobamate, are generally found in urine at higher levels than those in Table 3, whereas drugs such as fentanyls and lysergic acid diethylamide (LSD) are detected at lower levels.

Table 3. Urine Drug Test Analytes and Cutoffs from 2017 Mandatory Guidelines.[6]

Initial test analyte	Initial test cutoff	Confirmatory test analyte	Confirmatory test cutoff concentration
Marijuana metabolites (Δ-9-tetrahydrocannabinol-9-carboxylic acid [THCA])	50 ng/mL	THCA	15 ng/mL
Cocaine metabolite (Benzoylecgonine)	150 ng/mL	Benzoylecgonine	100 ng/mL
Codeine/Morphine	2,000 ng/mL	Codeine Morphine	2,000 ng/mL 2,000 ng/mL
Hydrocodone/Hydromorphone	300 ng/mL	Hydrocodone Hydromorphone	100 ng/mL 100 ng/mL
Oxycodone/Oxymorphone	100 ng/mL	Oxycodone Oxymorphone	100 ng/mL 100 ng/mL
6-Acetylmorphine	10 ng/mL	6-Acetylmorphine	10 ng/mL
Phencyclidine	25 ng/mL	Phencyclidine	25 ng/mL
Amphetamine/Methamphetamine	500 ng/mL	Amphetamine Methamphetamine	250 ng/mL 250 ng/mL
Methylenedioxymethamphetamine (MDMA)/ Methylenedioxyamphetamine (MDA)	500 ng/mL	MDMA MDA	250 ng/mL 250 ng/mL

Oral Fluid

Saliva is produced by three pairs of major salivary glands (parotid, sublingual, and submandibular) and numerous minor or accessory salivary glands. Oral fluid, which is commonly mis-referenced as whole saliva or mixed saliva, is saliva that is mixed with crevicular fluid, nasopharyngeal secretions, oral cavity bacteria, and oral cavity debris, such as food particles. The minor contribution from Von Ebner's glands adds a small amount of lipase activity to oral fluid. For a more complete discussion of oral fluids, including the effects of disease states and drugs on oral fluid itself, please consult Chapter 1 in Reference 7.

Drugs and their metabolites can enter saliva as it is being formed (as shown in Figure 4) or can enter the final product (oral fluid) by a number of pathways. These include

- transfer from whole blood that is in contact with forming saliva (primarily basic drugs, except when direct transport is possible; Figure 4);
- as a nasopharyngeal contaminant from the insufflation of drugs (e.g., cocaine);
- when smoke passes through the oral cavity on its way to the lungs (e.g., from a marijuana cigarette);

- when drugs leach from uncoated tablets, pills, or powders while the formulation is in the oral cavity;
- from gastric reflux when the gastric contents contain drug and/or metabolite (e.g., benzoylecgonine after oral cocaine ingestion);
- from pulmonary efflux (e.g., smoked methamphetamine); and
- from the intake of dust from a solid drug form or an aerosol from a liquid form of a drug.

For a more complete discussion, please see Chapters 1, 2, and 9 in Reference 7.

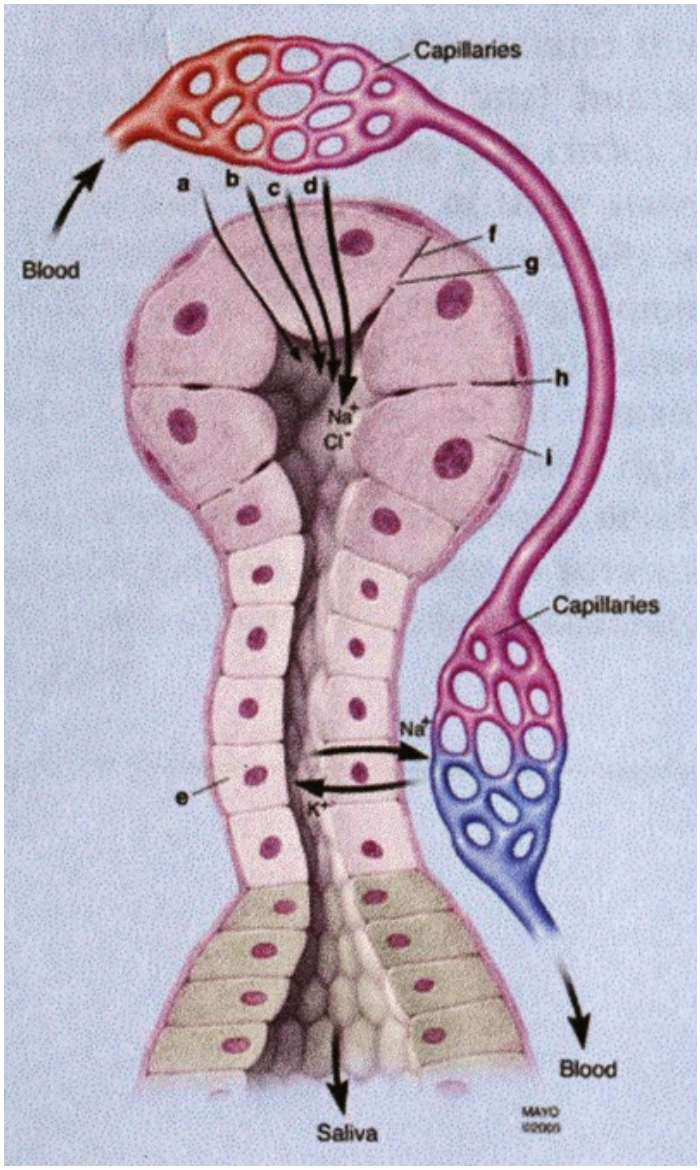


Figure 4. Mechanisms of Transport of Proteins and Ions to and from Plasma into the Salivary Ducts (Reference 8).

Legend:

a = ultrafiltration
b = active transport or passive diffusion across the cell membrane
c = simple filtration through cell membrane pores
d = transepithelial movement of water along NaCl gradient via channel proteins
e = creation of hypotonic salivary solution via ductal Na+ reabsorption
f = acinar cell membrane
g = cell membrane pore
h = intercellular space
i = acinar cell

In the transfer of normal plasma constituents from capillary arterial blood to saliva and the reabsorption of some constituents back into capillary venous blood, formed saliva at the ductus or exit from the salivary gland is vastly different in composition from the blood plasma from which it was created. Similarities between the formation of urine and the generation of saliva are obvious, although the products differ in their function. The so-called "ion trapping" of drugs (i.e., the preferential transfer of basic drugs from the almost neutral environment of blood plasma to the more acidic environment typical of saliva) is highly dependent on a number of factors discussed in Reference 7, unless active transport of a drug is possible.

As stated above, urine is a fairly stable matrix. However, collected neat oral fluid generally behaves differently from urine when stored at room or refrigerated temperature. Neat oral fluid may remain completely liquid with no macroscopic evidence of inclusions or precipitates, form a gel that may or may not revert to the liquid state on repeated inversion or vortexing, remain liquid but form precipitates, or remain liquid but form string-like inclusions. What neat oral fluid does upon storage differs from donor to donor and may even change day-to-day for a given donor.

Although neat oral fluid may be employed as a drug and/or drug metabolite testing matrix, at the time of this writing, the use of a so-called pad-type oral fluid collector (e.g., Intercept, Quantisal, NeoSal, Oral-Eze), which employs a fiber pad for collection and a buffer-preservative to avoid matrix changes such as those previously described and to preserve drugs and their metabolites, appears to be a desirable approach. For a pad-type collector, an indicator that shows when an oral fluid collection is complete is a necessity. Unlike urine collection, all collections of oral fluid should be witnessed collections. A same-sex collector/witness is not required for oral fluid collection.

Hair

As seen in Figure 5, human hair grows from the matrix cells (melanocytes and keratinocytes) in the germination center, which is the papilla of the anagen follicle. Mitotic cell division forces the layers above the germination center toward the outside. As the forming hair is pushed upward, keratin expression occurs, followed by pigment incorporation, if any pigment will be incorporated. Subsequently, hardening and dehydration of the hair take place, followed by removal of the inner root sheath before the mature hair protrudes from the skin. As the germination center in the papilla is well vascularized (Figure 6), there exists a distinct opportunity for drug and/or drug metabolite to be incorporated into growing hair. In addition to the deposition of drugs and their metabolites into forming hair in the papilla, the germination center possesses all of the enzymes required for metabolism, such as phase II glucuronidation, which may explain the presence of highly polar glucuronides in the formed hair shaft.

Formed or even partially formed hair shafts are usually exposed to eccrine and apocrine sweat, where an apocrine gland is present, and sebum (produced by sebaceous glands), as can be visualized by looking at Figure 6. Such contact with sweat and sebum also provides an opportunity for drug deposition into hair shafts. For a more complete discussion, please see Reference 10.

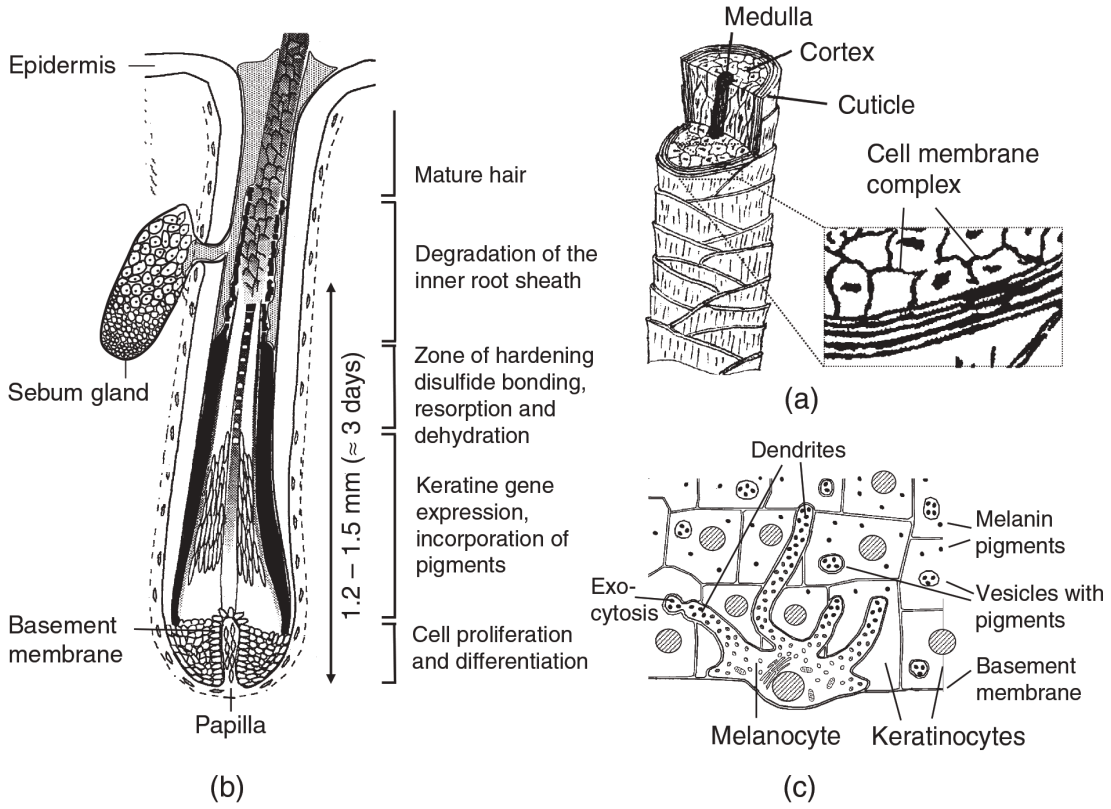


Figure 5. Hair Growth (p. 18, Reference 9).

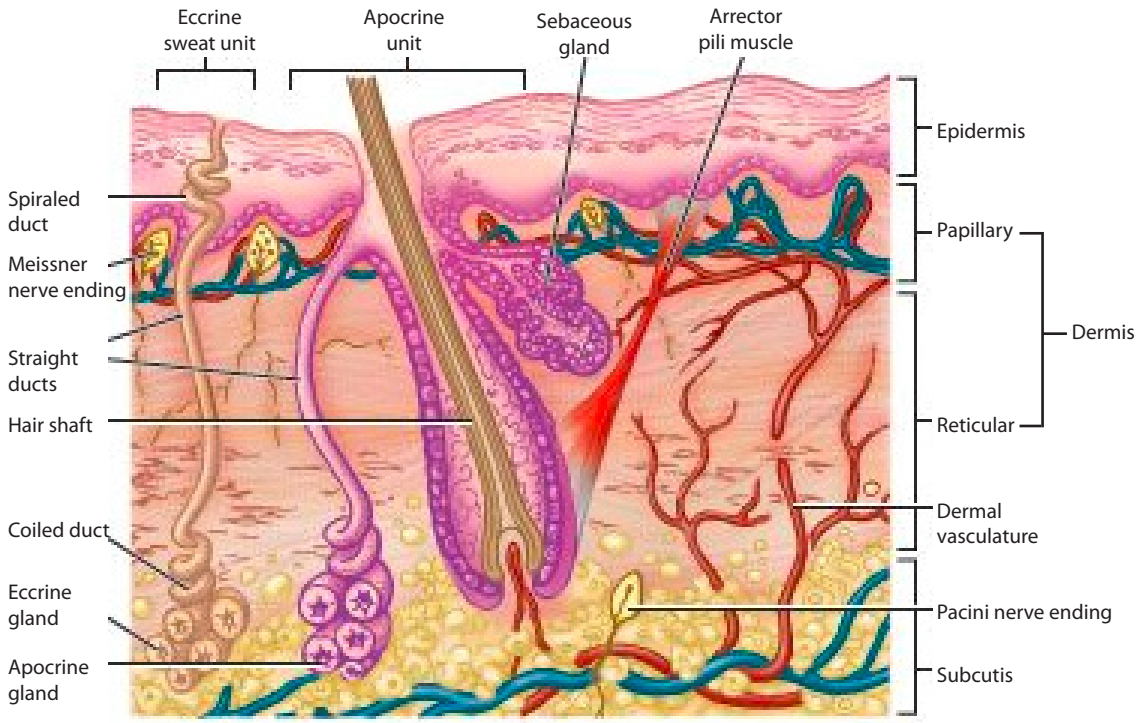


Figure 6. General Human Hair Follicle and Accompanying Glands (p. 1725, Reference 1).

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