

# Drug metabolism e-learning module

This e-learning resource is designed to allow you to explore drug metabolism and drug metabolising enzymes. Click on the circle in the liver to start.

Produced by University of Otago and University of Wisconsin.



# Drug Metabolism

## TheCaseSolution.com

### Overview

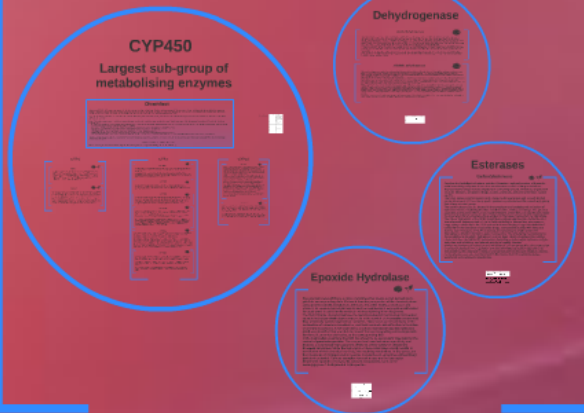
In 1947 R. T. Williams noted that enzyme-catalyzed biotransformation of drugs, dietary substances, and other synthetic and environmental agents, i.e., xenobiotics, can be by synthetic and/or synthetic reactions. Enzymes catalyzing synthetic or so-called Phase 1, reactions are thus classified broadly as all those catalyzing the oxidation, reduction, and hydrolysis of xenobiotics. Enzymes in this group include, but are not limited to, the mixed-function oxidases more commonly known as cytochrome P450 monooxygenases, monoamine and diamine oxidases, aldehyde dehydrogenases, aldehyde oxidase, alcohol dehydrogenases, quinone oxidoreductases, short-chain dehydrogenases/reductases, aldo-keto reductases, and esterases. Enzymes catalyzing synthetic, or so-called Phase 2, reactions are thus classified broadly as all those catalyzing the conjugation of xenobiotics to endogenous molecules, e.g., glucuronic acid, sulfate, glutathione, acetate, or a methyl group. Representative enzymes in this group include, but are not limited to, the UDP-glycosyltransferases, sulfotransferases, glutathione S-transferases, N-acetyltransferases, and methyltransferases.

### Phase 1

Introduce a functional group

- Increases water solubility
- Increases reactivity

Product can be excreted or metabolised further.

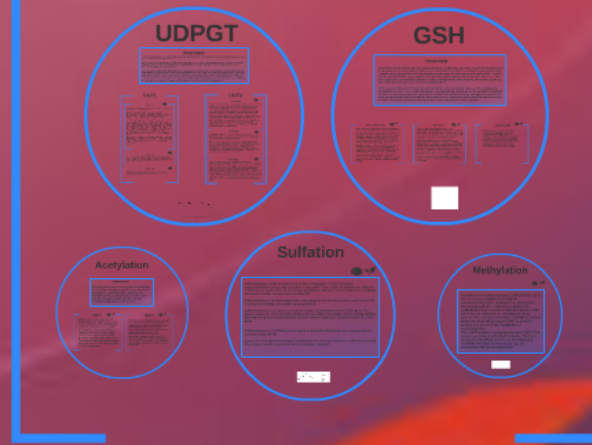


### Phase 2

Conjugates a large group



- Increases water solubility
- Increases size

Product is normally excreted.



Drug metabolising enzymes are found throughout the body but the highest concentrations are found in the liver.

These symbols indicate whether the enzyme is relevant to:

- Clinical drug metabolism 
- or
- Environmental chemical metabolism. 

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### Phase 1

Introduces a functional group  
Increases water solubility  
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Metabolised further.

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Conjugates a large group

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# Phase 1

Introduce a functional group

- Increases water solubility
- Increases reactivity

Produce can be excreted or metabolised further.

## CYP450

### Largest sub-group of metabolising enzymes

#### Overview

The cytochrome P450 (CYP) superfamily is a large and diverse group of enzymes that are involved in the metabolism of a wide range of endogenous and exogenous compounds. These enzymes are found in all eukaryotes and are particularly abundant in the liver. They are responsible for the oxidation of a wide range of substrates, including drugs, pesticides, and carcinogens. The CYP450 superfamily is divided into several families, with CYP1, CYP2, and CYP3A being the most prominent. Each family contains multiple enzymes that differ in their substrate specificity and catalytic activity. The CYP450 enzymes are heme-containing monooxygenases that use molecular oxygen and NADPH as a source of electrons to catalyze the oxidation of their substrates. The reaction typically involves the formation of a reactive iron-oxo species (Compound I) that is capable of abstracting a hydrogen atom from the substrate, followed by the insertion of an oxygen atom to form a hydroxylated product. This process is highly regulated and can be influenced by various factors, including genetic polymorphisms, environmental factors, and drug-drug interactions.

#### CYP1

CYP1 enzymes are primarily involved in the metabolism of aromatic hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines. They are found in various tissues, including the liver, lung, and skin. CYP1 enzymes are characterized by their ability to catalyze the oxidation of these substrates to hydroxylated products, which are more water-soluble and easier to excrete. CYP1B1 is the most well-studied member of this family and is known to be involved in the metabolism of several carcinogenic PAHs.

#### CYP2

CYP2 enzymes are a large and diverse group of enzymes that are involved in the metabolism of a wide range of substrates, including drugs, pesticides, and carcinogens. They are found in various tissues, including the liver, lung, and skin. CYP2 enzymes are characterized by their ability to catalyze the oxidation of these substrates to hydroxylated products, which are more water-soluble and easier to excrete. CYP2D6 is the most well-studied member of this family and is known to be involved in the metabolism of several important drugs, including antidepressants and antipsychotics. CYP2C9 and CYP2C19 are also important members of this family and are involved in the metabolism of many other drugs.

#### CYP3A

CYP3A enzymes are a large and diverse group of enzymes that are involved in the metabolism of a wide range of substrates, including drugs, pesticides, and carcinogens. They are found in various tissues, including the liver, lung, and skin. CYP3A enzymes are characterized by their ability to catalyze the oxidation of these substrates to hydroxylated products, which are more water-soluble and easier to excrete. CYP3A4 is the most well-studied member of this family and is known to be involved in the metabolism of many important drugs, including statins, immunosuppressants, and anticancer drugs. CYP3A5 is another important member of this family and is also involved in the metabolism of many drugs.

## Dehydrogenase

#### Alcohol Dehydrogenase

Alcohol dehydrogenase (ADH) is a heme-containing enzyme that is primarily found in the liver and is responsible for the oxidation of ethanol to acetaldehyde. It is a member of the alcohol dehydrogenase family, which also includes enzymes that oxidize other alcohols, such as propanol and butanol. ADH is a dimeric enzyme with two active sites per monomer. The active site contains a zinc atom coordinated to a cysteine residue and a histidine residue. The reaction catalyzed by ADH is reversible and is coupled to the reduction of NAD+ to NADH. ADH is highly specific for ethanol and is inhibited by disulfiram, a drug used to treat alcoholism.

#### Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) is a heme-containing enzyme that is primarily found in the liver and is responsible for the oxidation of aldehydes to carboxylic acids. It is a member of the aldehyde dehydrogenase family, which also includes enzymes that oxidize other aldehydes, such as formaldehyde and acetaldehyde. ALDH is a dimeric enzyme with two active sites per monomer. The active site contains a zinc atom coordinated to a cysteine residue and a histidine residue. The reaction catalyzed by ALDH is reversible and is coupled to the reduction of NAD+ to NADH. ALDH is highly specific for acetaldehyde and is inhibited by disulfiram, a drug used to treat alcoholism.



## Esterases

#### Carboxylesterases

Carboxylesterases are a class of enzymes that catalyze the hydrolysis of esters, amides, and other carbonyl compounds. They are found in various tissues, including the liver, lung, and skin. Carboxylesterases are characterized by their ability to catalyze the hydrolysis of these substrates to carboxylic acids and alcohols. They are highly specific for their substrates and are inhibited by various inhibitors, including organophosphates and carbamate pesticides. Carboxylesterases are also involved in the metabolism of many drugs, including antipsychotics, antidepressants, and anticancer drugs. The reaction catalyzed by carboxylesterases is reversible and is coupled to the hydrolysis of water. The reaction is highly regulated and can be influenced by various factors, including genetic polymorphisms, environmental factors, and drug-drug interactions.



## Epoxide Hydrolase

Epoxide hydrolase (EH) is a class of enzymes that catalyze the hydrolysis of epoxides to dihydroxy compounds. They are found in various tissues, including the liver, lung, and skin. EH is a dimeric enzyme with two active sites per monomer. The active site contains a zinc atom coordinated to a cysteine residue and a histidine residue. The reaction catalyzed by EH is reversible and is coupled to the hydrolysis of water. EH is highly specific for epoxides and is inhibited by various inhibitors, including organophosphates and carbamate pesticides. EH is also involved in the metabolism of many drugs, including antipsychotics, antidepressants, and anticancer drugs. The reaction catalyzed by EH is reversible and is coupled to the hydrolysis of water. The reaction is highly regulated and can be influenced by various factors, including genetic polymorphisms, environmental factors, and drug-drug interactions.



# CYP450

## Largest sub-group of metabolising enzymes

### Overview

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme co-factor and, therefore, are haemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, usually integrated as P450-containing systems. The heme P450 is derived from the cytochrome peak at the N-terminus of the absorption maximum of the enzyme (450 nm) which is the redox active and coordinated heme chromophore.

CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses. However, the enzymes have not been found in E. coli. More than 2,500 distinct CYP proteins are known.

Most CYPs require a protein partner to deliver one or more electrons to reduce the heme (and eventually molecular oxygen). Based on the nature of the electron transfer proteins, CYPs can be classified into several groups:

- Microsomal P450 systems in which electrons are transferred from NADPH via cytochrome P450 reductase (usually CYP<sub>11B</sub>, POR, or CYPOR). Cytochrome b<sub>5</sub> (CYP3A4) can also contribute reducing power to this system, either directly or indirectly by cytochrome b<sub>5</sub> reductase (CYP3A5).
- Mitochondrial P450 systems, that employ adrenodoxin-reductase and adrenodoxin from NADPH as P450.
- Mitochondrial P450 systems, that employ a ferredoxin reductase and ferredoxin by ferredoxin-NADP reductase.
- CYP450-S450-like systems in which both electrons required for the CYP come from cytochromes b<sub>5</sub>.
- P450-NADPH systems employed by yeast. A P450 domain and binding reductase is bound to the CYP.
- P450-like systems, which do not require external reducing power. Notable ones include CYP19 (aromatase synthase), CYP11A (steroid 17 $\alpha$ -hydroxylase), and CYP19A (alkene oxidase synthase).

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (R) while the other oxygen atom is reduced to water:



Humans have 57 genes and more than 58 paralogues divided among 18 families of cytochrome P450 genes and 43 subfamilies.

**CYP1**

**CYP1A1**

CYP1A1 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs), for example, and polycyclic aromatic hydrocarbons (PAHs). The induction of CYP1A1 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP1A1 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP1A2**

CYP1A2 metabolizes the endogenous substrate and its expression is induced by heterocyclic aromatic hydrocarbons (HAHs) and is induced by cigarette smoke. The induction of CYP1A2 is a marker for exposure to HAHs and is used to assess the risk of cancer. CYP1A2 is induced by cigarette smoke, and is used to assess the risk of cancer. CYP1A2 is induced by cigarette smoke, and is used to assess the risk of cancer. CYP1A2 is induced by cigarette smoke, and is used to assess the risk of cancer.

**CYP2**

**CYP2B6**

CYP2B6 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP2B6 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP2B6 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP2C8**

CYP2C8 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP2C8 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP2C8 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP2C9**

CYP2C9 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP2C9 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP2C9 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP2C19**

CYP2C19 acts on 50% of drugs in phase I metabolism. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP2C19 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP2C19 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP2D6**

CYP2D6 is responsible for the metabolism and elimination of approximately 25% of clinically used drugs. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP2D6 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP2D6 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP2E1**

Most phase I drug metabolism by CYP2E1, either directly or via facilitated oxidation from the heme P450, is mediated by cytochrome b<sub>5</sub> and adrenodoxin reductase. CYP2E1 is induced by cigarette smoke and is used to assess the risk of cancer. CYP2E1 is induced by cigarette smoke, and is used to assess the risk of cancer. CYP2E1 is induced by cigarette smoke, and is used to assess the risk of cancer.

**CYP3A**

**CYP3A4**

CYP3A4 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP3A4 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP3A4 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP3A5**

CYP3A5 is a phase I liver drug metabolizer. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP3A5 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP3A5 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

**CYP3A7**

CYP3A7 is an enzyme belonging to the cytochrome P450 family. It is involved in the metabolic activation of several hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs). The induction of CYP3A7 by the tobacco smoke carcinogen benzo(a)pyrene is a good example of a phase I enzyme induction. Many CYP3A7 inducers are hydrocarbons, such as the tobacco carcinogen benzo(a)pyrene.

## Epoxide Hy

Epoxide hydrolases (EH) are a class of enzymes that catalyze the hydrolysis of epoxides to yield the corresponding diols. If viewed from the concept of xenobiotic metabolism, EH lead a life in phase II, as epoxide hydrolysis may as well be regarded as a phase II reaction because water is added to the molecule without the loss of any atoms. The first EH to be characterized was the membrane-bound EH from sheep vesicular stomatitis virus (VSV-EH).

Alcohol dehydrogenase (ADH) is a class of enzymes that catalyze the oxidation of alcohols to aldehydes or ketones. ADH is found in all eukaryotes and is involved in the metabolism of a wide range of alcohols. The most common form of ADH is ADH1A, which is involved in the metabolism of ethanol. ADH1A is induced by alcohol and is used to assess the risk of liver disease. ADH1A is induced by alcohol, and is used to assess the risk of liver disease. ADH1A is induced by alcohol, and is used to assess the risk of liver disease.

# Largest sub-group of metabolising enzymes

## Overview

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a haem co-factor and, therefore, are haemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorised as P450-containing systems. The term P450 is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with carbon monoxide.

CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses. However, the enzymes have not been found in *E. coli*. More than 21,000 distinct CYP proteins are known.

Most CYPs require a protein partner to deliver one or more electrons to reduce the iron (and eventually molecular oxygen). Based on the nature of the electron transfer proteins, CYPs can be classified into several groups:

- Microsomal P450 systems in which electrons are transferred from NADPH via cytochrome P450 reductase (variously CPR, POR, or CYPOR). Cytochrome b5 (cyb5) can also contribute reducing power to this system after being reduced by cytochrome b5 reductase (CYB5R).
- Mitochondrial P450 systems, that employ adrenodoxin reductase and adrenodoxin to transfer electrons from NADPH to P450.
- Bacterial P450 systems, that employ a ferredoxin reductase and a ferredoxin to transfer electrons to P450.
- CYB5R/cyb5/P450 systems in which both electrons required by the CYP come from cytochrome b5.
- FMN/Fd/P450 systems originally found in *Rhodococcus* sp. in which a FMN-domain-containing reductase is fused to the CYP.
- P450 only systems, which do not require external reducing power. Notable ones include CYP5 (thromboxane synthase), CYP8 (prostacyclin synthase), and CYP74A (allene oxide synthase).

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (RH) while the other oxygen atom is reduced to water:



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## CYP2

### CYP2B6

CYP2B6 enzymes synthesise cholesterol, steroids and other lipids. Its expression is induced by phenobarbital. The enzyme is known to metabolize some xenobiotics, such as the anti-cancer drugs cyclophosphamide and ifosfamide.

Transcript variants for this gene have been described; however, it has not been resolved whether these transcripts are in fact produced by this gene or by a closely related pseudogene, CYP2B7. Both the gene and the pseudogene are located in the middle of a CYP2A pseudogene found in a large cluster of cytochrome P450 genes from the CYP2A, CYP2B and CYP2F subfamilies on chromosome 19q.

CYP3A4 is induced by glucocorticoids. It is involved in the metabolism of many drugs, including acetaminophen, erythromycin. The enzyme undergoes deactivation by CYP3A4. Also, many substances are many protoxins being toxic.

CYP3A4 is the most common member of this family, it is an iron atom. In humans, it is part of a cluster of cytochromes.



...xylase). It is involved in the metabolism of many drugs, including acetaminophen, erythromycin. The enzyme undergoes deactivation by CYP3A4. Also, many substances are many protoxins being toxic.

# Table of CYP450 isoforms

Family	Function	Members	Names
CYP1	drug and steroid (especially estrogen) metabolism, benzo(a)pyrene toxification	3 subfamilies, 3 genes, 1 pseudogene	CYP1A1, CYP1A2, CYP1B1
CYP2	drug and steroid metabolism	13 subfamilies, 16 genes, 16 pseudogenes	CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1
CYP3	drug and steroid (including testosterone) metabolism	1 subfamily, 4 genes, 2 pseudogenes	CYP3A4, CYP3A5, CYP3A7, CYP3A43
CYP4	arachidonic acid or fatty acid metabolism	6 subfamilies, 12 genes, 10 pseudogenes	CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1
CYP5	thromboxane A2 synthase	1 subfamily, 1 gene	CYP5A1
CYP7	bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus	2 subfamilies, 2 genes	CYP7A1, CYP7B1
CYP8	varied	2 subfamilies, 2 genes	CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)
CYP11	steroid biosynthesis	2 subfamilies, 3 genes	CYP11A1, CYP11B1, CYP11B2
CYP17	steroid biosynthesis, 17-alpha hydroxylase	1 subfamily, 1 gene	CYP17A1
CYP19	steroid biosynthesis: aromatase synthesizes estrogen	1 subfamily, 1 gene	CYP19A1
CYP20	unknown function	1 subfamily, 1 gene	CYP20A1
CYP21	steroid biosynthesis	2 subfamilies, 1 gene, 1 pseudogene	CYP21A2
CYP24	vitamin D degradation	1 subfamily, 1 gene	CYP24A1
CYP26	retinoic acid hydroxylase	3 subfamilies, 3 genes	CYP26A1, CYP26B1, CYP26C1
CYP27	varied	3 subfamilies, 3 genes	CYP27A1 (bile acid biosynthesis), CYP27B1 (vitamin D3 1-alpha hydroxylase, activates vitamin D3), CYP27C1 (unknown function)
CYP39	7-alpha hydroxylation of 24-hydroxycholesterol	1 subfamily, 1 gene	CYP39A1
CYP46	cholesterol 24-hydroxylase	1 subfamily, 1 gene	CYP46A1
CYP51	cholesterol biosynthesis	1 subfamily, 1 gene, 3 pseudogenes	CYP51A1 (lanosterol 14-alpha demethylase)