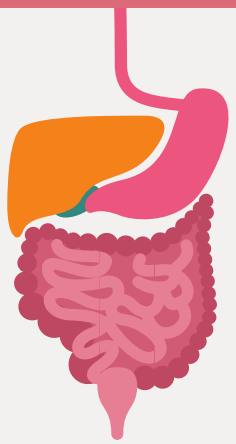
Cytochrome System & Drug Metabolism



= **Very important!**

Objectives:

- Revise the intent of drug metabolism and its different phases.
- Define the role of cytochrome system in relation to drug metabolism.
- Expand on the nature, location, nomenclature, structure, distribution & function of CYT P450.
- Focus on its regulation; directly & indirectly, its induction & inhibition in relevance to drug interactions.
- Interpret the molecular mechanism of interactions by CYT P450.
- Classify its different isoforms, their substrates, inducers & inhibitors.
- Delineate some of its genetic variations.

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Editing file

Revised by
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Drugs names | Doctors notes | Important | Extra

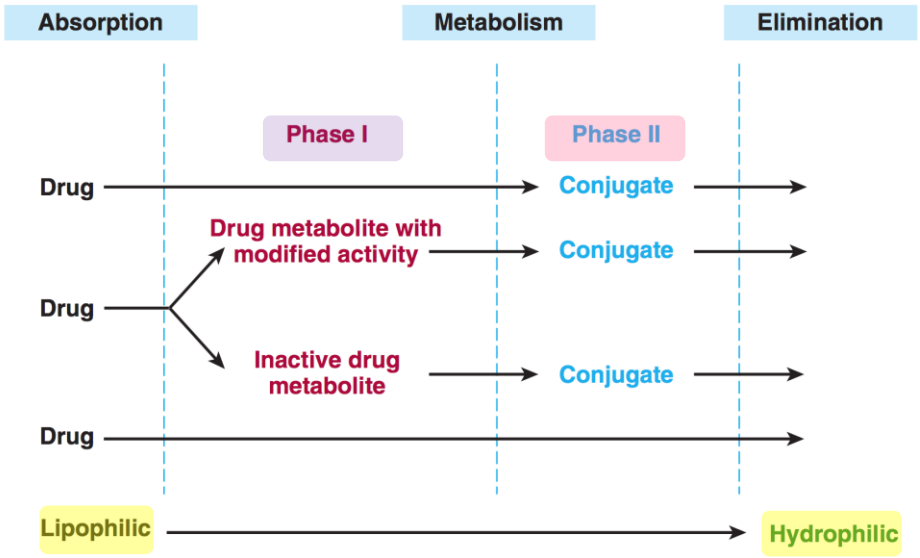
« **باذلًا وسعي** في استنقاذها من الهلاك والمرض، والألم والقلق »

Metabolism and Elimination of a substance

When a substance is identified by the body as a foreign substance (drugs, toxins, etc.) it will try to **metabolize (change)** and **eliminate** that substance out, this process occurs mainly (**NOT always**) in the **liver**.

Route of elimination

- **Liver Metabolism**
  - **Hydrophilic (Polar) product** → **Renal elimination**
  - **Lipophilic (Non-polar) product** → **Biliary elimination**



Drug metabolism occurs mainly in the "METABOLIC CLEARING HOUSE"

→ **Change form of foreign substance to become inactive and easily excreted**

Metabolism: all chemical reactions that occur in living organisms.
Metabolism = anabolism (build up molecules) **+ catabolism** (break down molecules)

The **kidneys** are one of our major excretion routes, but they **can't excrete lipophilic molecules** very well because they **cross the membranes** of kidney cells easily and they are **reabsorbed** in the distal convoluted tubules. That's why the liver is trying to metabolize (**convert**) any foreign **lipophilic** substance into **hydrophilic** form so that the **kidneys can excrete them easily**, but when the liver **produces lipophilic products** instead of hydrophilic, the **Biliary excretion** takes care of those lipophilic products.

Cytochrome System

Cytochrome system that converts lipophilic compounds into hydrophilic:

1

- **Phase 1:** introduce or unmask a **polar group** to **compound** (-OH or –NH2, etc.). Which includes:
- **Oxidation (CYPs**: the **terminal rate limiting oxidase** of the system next slide**)**
- **Reduction** and **hydrolysis**.
- The Product (metabolite) of the whole process could be: Active or more active than parent, inactive, similar to parent, toxic, or a product with different effect.
- Oxidation: loss of electrons, reduction: gain of electrons.
- Note that CYP450 action done in the 1st phase only.

Phase **I** + REDOX reactions | 6:55 min

2

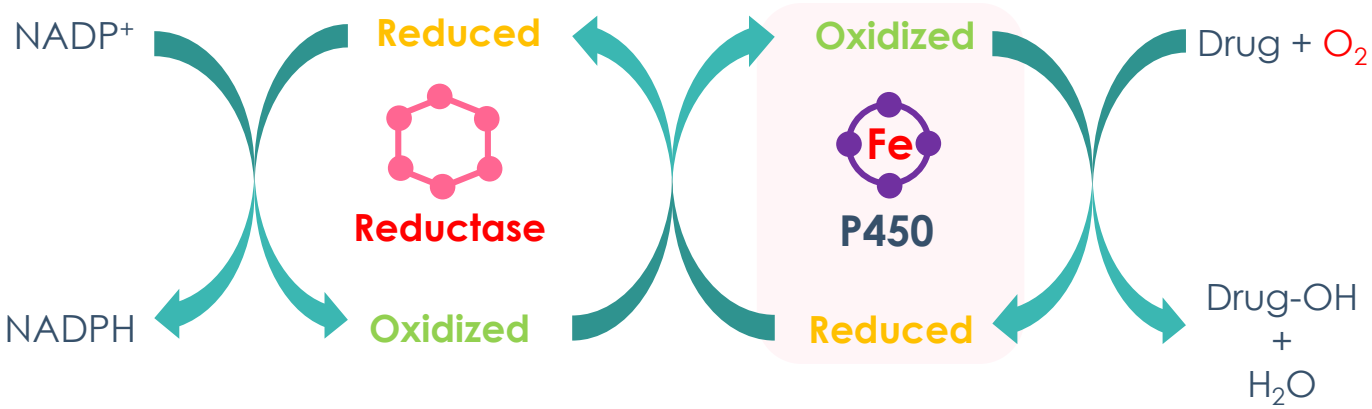
- This will cause the compound to be **slightly polar** so it can be **ready for Phase 2** (creating a **conjugation site**).
- Note that some compounds that already have **–cooh**, **-OH** or other polar groups **DON'T NEED to go through phase 1.**
- **If a compound went through phase 1 and its product is sufficiently polar it can be excreted without phase 2.**

3

- **Phase 2 (Conjugation):** it's basically taking **weak polar compound** and add it to an endogenous substrate (mostly **glucuronic acid**) so it becomes more polar. The drug is conjugated via activity of transferases,
- Usually results in inactive products, although there are exceptions.

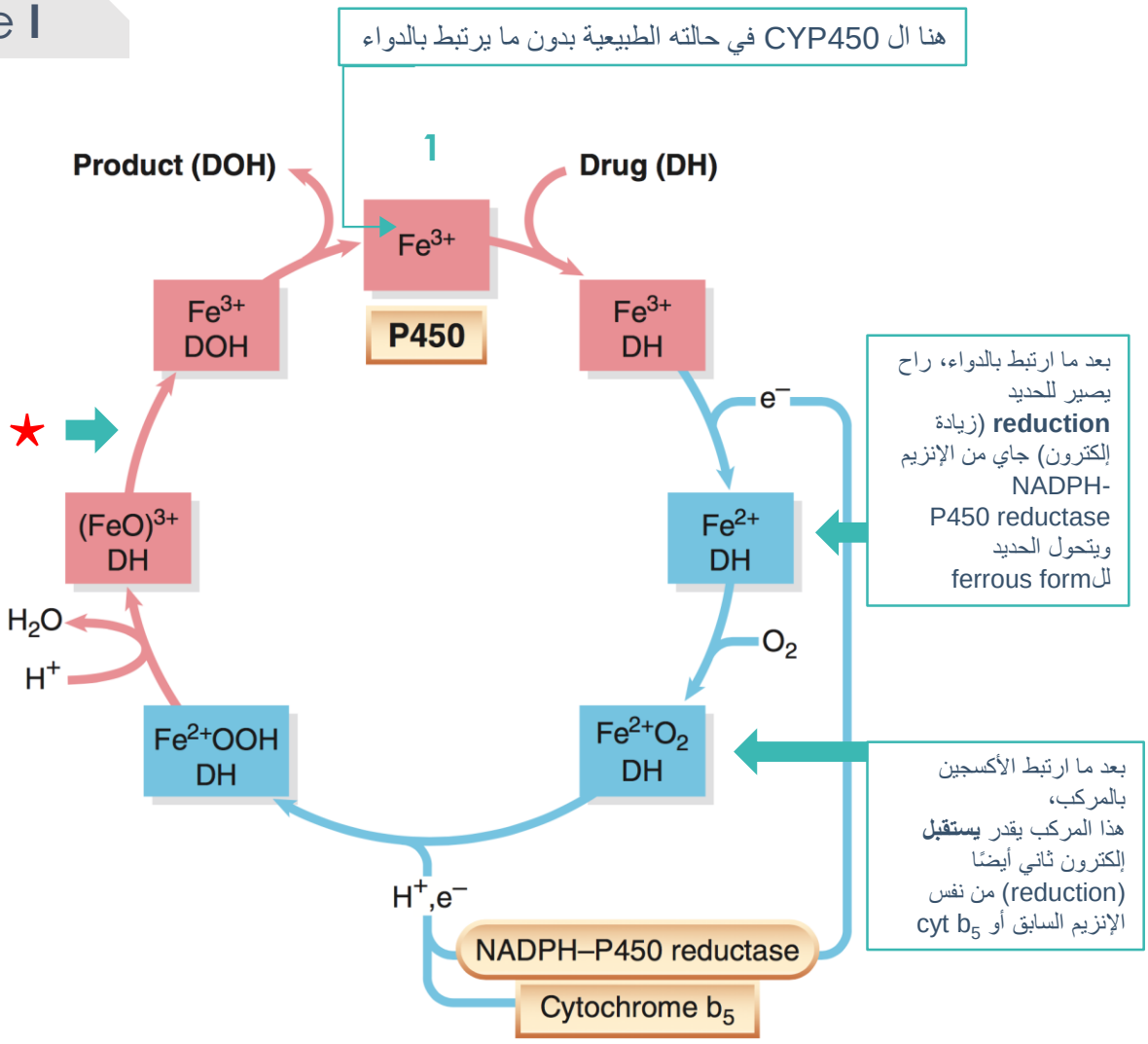
Phase **I** & **II** | 5:19 min

Not important, but if you want to understand check the next slide.



Extra explanation (phase I & II)

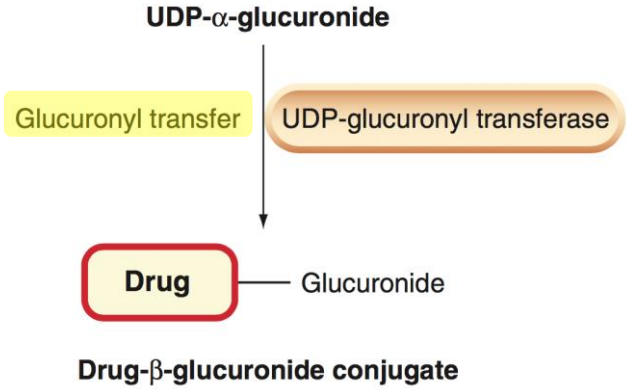
Phase I



★ قبل ما تتم هذي العملية ★

$(FeO)^{3+}$ extracts a H atom from DH (the drug) to form pair of transient free radicals: $D^{\cdot}$ & $Fe^{2+}OH^{\cdot}$. D- acquires the bound OH- radical to form hydroxylated drug (**DOH**) → which is released from the complex with **regeneration of P450** in its initial state.

Phase II



تذكروا كيف يحول الكبد البيليروبين للكونجوقيتيد فورم بنفس هالطريقة.

Cytochromes P450 (CYPs)

What are cytochromes P450?

Cytochromes P450 (CYPs):

Cytochromes = colored cells
They color the liver cells dark red as they contain **iron**

P450
absorbs a very characteristic wavelength (**450** nm) of UV light when it is exposed to carbon monoxide.

- Structure: **They are a superfamily of heme-containing isozymes that are found in most cells.**
- Mainly attached to the **smooth endoplasmic reticulum** (SER) of hepatocytes.
- They are isolated in the subcellular fraction termed the MICROSOMES (therefor they're also called **Liver microsomal enzymes**).

Function

- These enzymes are part of a cascade that shuttles (transport) **electrons** from molecular oxygen (O_2) to **oxidize the drugs**, so they're responsible for most of the **OXIDATIVE METABOLISM** of:
  1. **Endogenous substances:** steroid hormones, prostaglandins, lipids, and fatty acids.
  2. **Exogenous compounds:** diet (food & beverages), **Drugs**, environmental xenobiotics.

Cytochrome P450 Isoforms:		
CYP1A2	CYP2C9	CYP2C19
CYP3A	CYP2D6	

CYPs - Distribution and Regulation

Distribution of CYPs:

- They are mainly distributed in **hepatocytes** but also in extra-hepatic sites like **enterocytes** of small intestine (most important) and very quantities in kidneys, lungs, and brain.

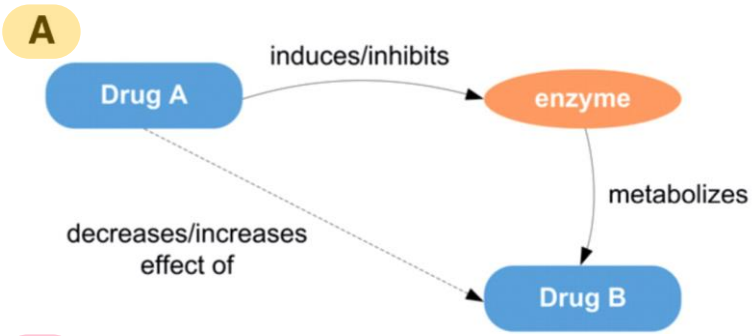
Regulation of CYPs:

- Activation or inactivation of CYPs can be processed by any food, intrinsic products or extrinsic xenobiotics as drugs (usually the lipophilic) that have to be metabolized.

Activation or inactivation can be formed:

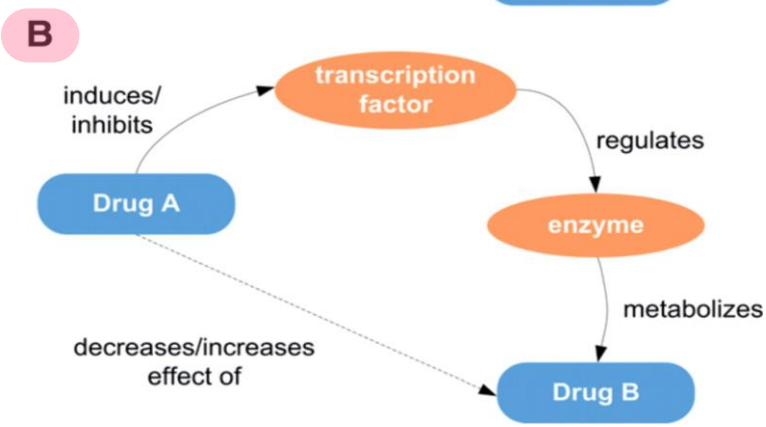
A. Directly

The drug activate the system directly



B. Indirectly:

By expression or repression of its **relevant genes** by activation or inhibition of the responsible transcription factors.



When drugs play a role in regulation of the CYT P450, they are termed:

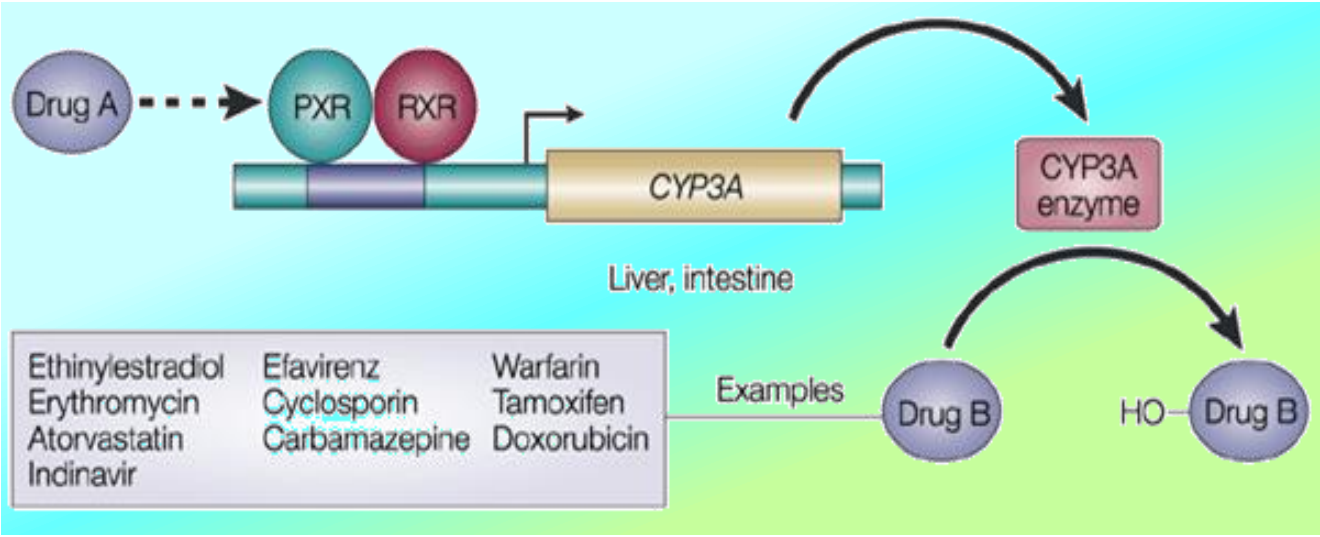
1) **Enzyme inducer if they activate the enzyme.**
2) **Enzyme Inhibitors if they inactivate the enzyme.**

→ **This causes drug-drug interactions** (pharmacokinetics)

CYPs - Drug-Drug interaction

Molecular Basis Of Drug–drug Interaction and their outcome:

The orphan nuclear **receptor PXR** (**pregnane** X receptor) is a transcription factor that **regulates the expression of the CYP P450 genes**.



Drug A-Inducer	Drug A-Inhibitor
An enzyme **inducer** that: 1. Binds and **activates PXR** 2. PXR translocate in nucleus dimerize (joins up) with **RXR** (**retinoid** X receptor**).** 3. The heterodimer **PXR/RXR** will induce **expression** of CYT P450 isoenzymes to **increase metabolism of drug B.**	An enzyme **inhibitor** that**:** 1. Binds and **prevents PXR** activation. **2. Repression** of CYPs. **3. Decrease** in Drug B metabolism.
Outcome	**Outcome**
Increase metabolism of the inducer itself which will **decrease** its pharmacological actions leading to **tolerance or even complete nullification** and also it will increase co-administrated drugs metabolism. **(Decreased EFFICACY)**	**Retard** (decreased) **metabolism and excretion** of inhibitor and co-administrated drugs leading to **prolong** action of those drugs. **(Increased TOXICITY)**

Simple diagram explains the enzyme inducers & inhibitors

CYP-450

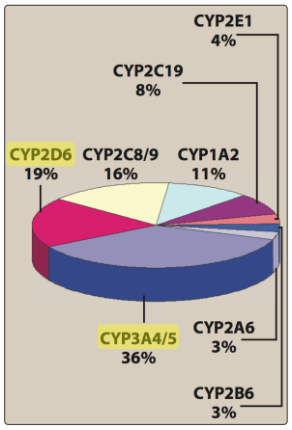
Classification:

The family name is indicated by the **Arabic number** that follows CYP, and the capital letter after number designates the subfamily, for example, CYP3A. A second number indicates the specific isozyme, as in CYP3A4. **So →**

- Families designated by Numbers → CYP3A4

- Sub families designated by Letters → CYP3A4

الدكتور ركز بشكل كبير نعرف كل المعلومات المتعلقة ب CYP**3A4** & **2D6** لأن أغلب الأدوية تشتغل عليهم



CYT P450 3A4\5 - (most common, 30% of CYP)

Found in the liver & GIT. Responsible for the metabolism of:
- Most calcium channel blockers, Most benzodiazepines, Most HIV protease inhibitors, Most HMG-CoA-reductase inhibitors, Cyclosporine, Most non-sedating antihistamines, Cisapride.

Substrates	Inhibitors = (Toxicity)	Inducers = (no response)
Immunosuppressant; Cyclosporine	○ Immunosuppressant; • Cyclosporine	○ Rifampicin
Azole Antifungals; Fluconazole	○ Azole Antifungals; • Fluconazole, Ketoconazole, Itraconazole	○ Phenytoin
Antibiotics; Erythromycin, Clarithromycin	○ Antibiotics; • Erythromycin, Clarithromycin, Troleandomycin	○ Carbamazepine
○ Ca^{2+} channel blockers • Amlodepine, Verapamil ○ Statins; Atorvastatin ○ Antiarrhythmic; Amidarone ○ Cancer Chemotherapy: • Cyclophosphamide, Tamoxifen ○ Non-Sedating Antihistaminics • Astamizole ○ Benzodiazipines • Midazolam, Clonazepam	○ Protease Inhibitors • **Ritonavir** ○ Cimetidine ○ Chloramphenicol ○ Nefazadone ○ Grape Fruits	○ Barbiturates ○ Dexamethasone ○ Progestins

CYPs- Genetic variations

Genetic polymorphisms in CYT P450 isoenzymes have been observed and are reasons behind the **ALTERED RESPONSE** to drug therapy.

CYP2D6

This isoenzyme has the **most frequent** polymorphisms in all CYT P450 and When polymorphism occurs → ↓ metabolizing capacity of CYP2D6 i.e those who exhibit the polymorphism become poor metabolizers:

1- Metabolism of some drugs neuroleptics, tricyclic antidepressants, antianginals agent (perihexiline), antiarrhythmics (propafenone & metoprolol) is **suppressed** → so side effects & toxicity **develop**. i.e.:

- Neuropathy after therapeutic doses of perihexiline
- Severe Bradyarrhythmias →heart block on therapeutic dose of propafenone or metoprolol.

2- The pro-drugs cannot be converted to their therapeutically active metabolite; e.g. poor analgesia with codeine & tramadole because they are not transformed into active forms.

It Absent in 7% of Caucasians, 1-2% non-Caucasians Hyperactive in up to 30% of East Africans.

Catalyzes primary metabolism of:	Inhibited by:
○ Codeine ○ Many **B-blockers** ○ Many **tricyclic antidepressants**	○ Fluoxetine ○ Paroxetine ○ Haloperidol ○ Quinidine

CYP2C9

Warfarin, phenytoin, & tolbutamide are examples of drugs with **narrow therapeutic index** that are metabolized by **CYP2C9**. Clearance of these drugs is impaired in genetic variation of the enzyme this **will ↑ toxicity**

Primary metabolism of:	Inhibited by:
○ Most NSAIDs (including COX-2) ○ S-warfarin (the active form) ○ Phenytoin	○ Fluconazole

CYP2C19

Polymorphism in CYP2C19 shows increased & prolonged action of its substrates as omeprazole. This has been an advantage as in those variants → ↑ cure rates in peptic ulcer patient with *Helicobacter pylori* Benefit.

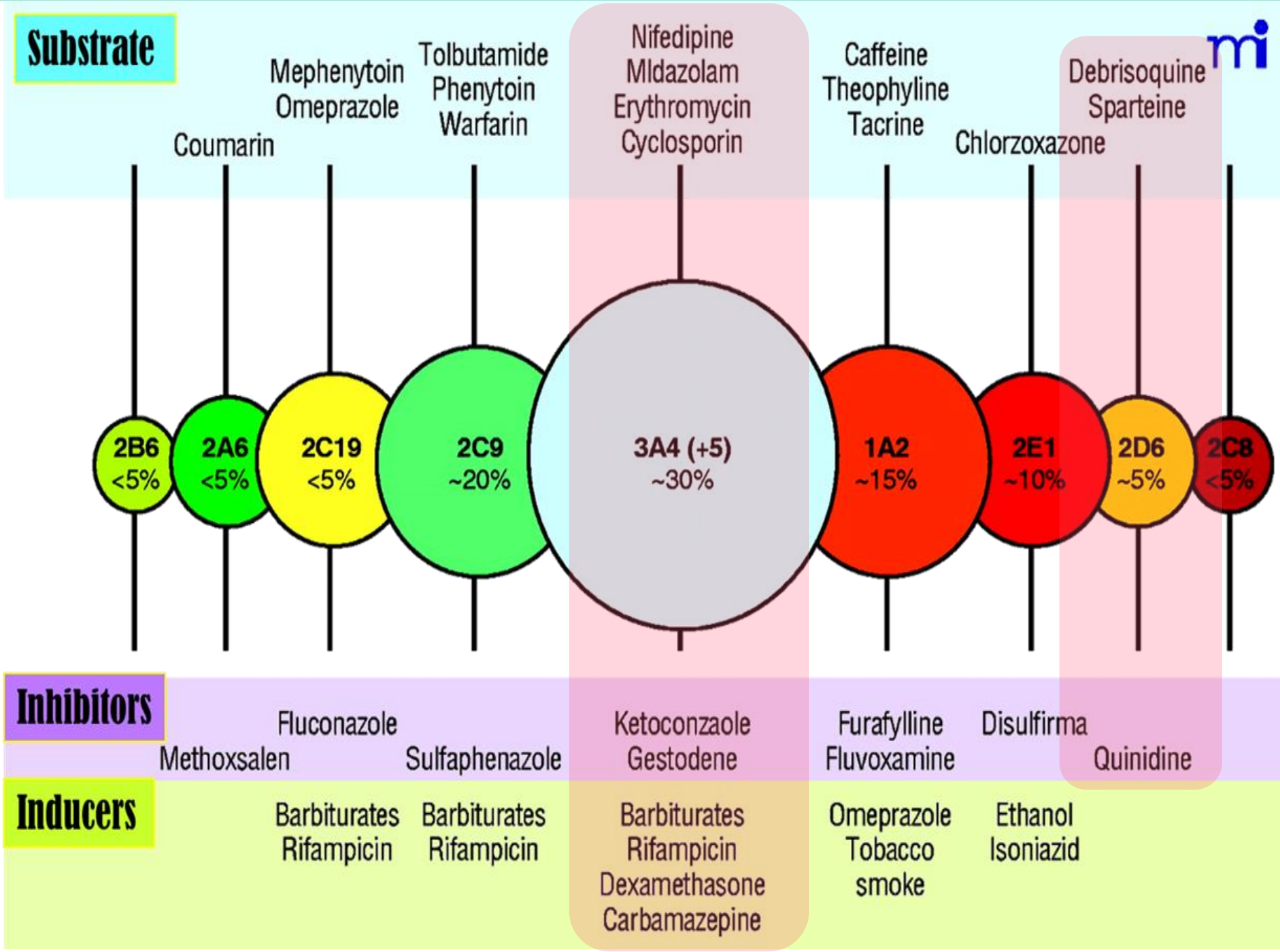
Primary metabolism of:	Inhibited by:
○ Diazepam ○ Omeprazole ○ Phenytoin	Omeprazole, Isoniazid, Ketoconazole

CYP1A2

Induced by smoking tobacco

primary metabolism of:	Inhibited by:
Theophylline, Imipramine, Propranolol, Clozapine	Many fluoroquinolone antibiotics, Fluvoxamine, Cimetidine

The doctor focused on **3A4** & **2D6**, He said it is imp to know the drugs included under these enzymes



Summary

- Major organ for drug metabolism: Liver
- Major organ for drug excretion: Kidneys
- Drug metabolism is important for turning lipid soluble drugs → water soluble (thus easily excreted)
- **Phases of Drug Metabolism:**
  - Phase I: is responsible for changing main drug → **metabolite**
  - Phase II: is responsible for **conjugation**
- Main role of CYTP450 is to control Phase I.
- CYTP450 has exogenous and endogenous substrates.
- Drugs are exogenous substrates, while hormones and fatty acids are endogenous.
- **Activation or Inactivation of CYTP450 can be achieved:**
  - Directly: by inducing or inhibiting the metabolism
  - Indirectly: by repression of its relevant genes
- **Outcome of Drug-Drug interaction:**
  - Inducers → ↑ metabolism → ↓ efficacy
  - Inhibitors → ↓ metabolism → ↑ toxicity
- Most important Isoforms of CYTP450:
  - **CYTP3A4** (36%)
  - **CYTP2D6** (19%)

MCQs

1- "A 50 years old, patient was treated for the last 3 years by the hypocholestrolemic agent; atorvastatin. Yesterday he began to complain of severe muscle pains, weakness and reddish discoloration of urine. He receives daily multivitamins and his lab results last week, proved that he has become diabetic, for which he was prescribed metformin. He was also started on a course of fluconazole for a concomitant fungal infection. From drug history, the diagnosis of his current state was likely rhabdomyositis (severe muscloskeletal toxicity) and was verified by the lab finding of severe elevation in creatinine phosphokinase. "
Which one of the following drug-drug interaction on CYT 3A4 is the likely cause of his current state?
A- Metformin + Atrovastatin
B- Atrovastatin + Fluconazole
C- Fluconazole+ Multivitamins
D- Fluconazole+ Multivitamins

2- An enzyme inducer will bind to:
A- O_2
B- CYP-450
C- PXR
D- RXR

3- CYPs in phase 1 are mainly responsible for:
A- Conjugation
B- Oxidation
C- Reduction
D- Hydrolysis

4- A 46 years old, patient 2 months ago start TB treatment, after repetitive check of BP she has a high BP and physician prescribe to her a drug. which one of flowing drug its therapeutic dose has no effect on her case :
A- Verapamil
B- Propanolol
C- Furosemide
D- Captopril

5- A 22 women who has unprovoked seizures 1 month ago neurologist prescribe phenytoin to her after first dose (she was taken a Diclofenac (metablized by CYP2C9) to arthritis before phenytoin dose) she come to ER on ambulance she develop dysrhythmia, severe hypotension, ataxia then coma most likely she had:
A- Increase CYP2C9
B- Decrease CYP2C9
C- Moderate CYP2C9
D- Diclofenac interaction CYP2C9

Answers: 1-B 2-C 3-B 4-A 5-B

MCQs

6- Which of the following cytochrome P450 isoenzymes is involved in the metabolism of largest number of drugs in human beings and has been implicated in some dangerous drug interactions:
A- CYP3A4
B- CYP2C9
C- CYP2E1
D- CYP 1A2

7- Which of the following types of drug metabolizing enzymes are inducible:
A- Microsomal enzymes
B- Non-microsomal enzymes
C- Both microsomal and nonmicrosomal enzymes
D- Mitochondrial enzymes

8- Select the antibiotic which inhibits drug metabolizing isoenzyme CYP3A4 resulting in potentially fatal drug interaction with terfenadine:
A- Erythromycin
B- Clindamycin
C- Gentamicin
D- Vancomycin

9- The majority of drug biotransformation occurs by which cytochrome family?
A- CYP_1
B- CYP_2
C- CYP_3
D- None of the above

10- A prodrug is:
A- The prototype member of a class of drugs
B- The oldest member of a class of drugs
C- A drug that is stored in body tissues and is then gradually released in the circulation
D- An inactive drug that is transformed in the body to an active metabolite

11- Induction of drug metabolizing enzymes involves:
A- A conformational change in the enzyme protein to favor binding of substrate molecules
B- Expression of enzyme molecules on the surface of hepatocytes
C- Enhanced transport of substrate molecules into hepatocytes
D- Increased synthesis of enzyme protein

Answers: 6-A 7-A 8-A 9-c 10-D 11-D

Thank you for checking our team!

Pharmacology 435

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Sources:

1. 435's slides.
2. Pharmacology (Lippincotts Illustrated Reviews Series), chapter 1, 5th & 6th edition.
3. Wikipedia.
4. Basic & Clinical Pharmacology by Katzung, chapter 4,12th edition.
5. Rang & Dale's pharmacology, chapter 9, 7th edition.