Drug metabolism

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Overview of the Lectures

- **Lecture 1:**
  - Phase 1 and phase 2 DM: overview on enzymes
  - The drug discovery process: position of DM
  - Oxygenases, monooxygenases: oxygen activation
  - P450 enzymes (I):
    - Introduction

- **Lecture 2:**
  - P450 enzymes (II):
    - Structure and function
    - Catalytic cycle
    - Inhibition

- **Lecture 3:**
  - P450 enzymes (III):
    - Polymorphism
    - QSAR
  - FMO:
    - Structure and function
    - Catalytic cycle

- **Lecture 4:**
  - FMO:
    - Polymorphism
  - Phase 2 drug metabolising enzymes
  - Examples of drug metabolism

Lecture 1:

Phase 1 and Phase 2 drug metabolism

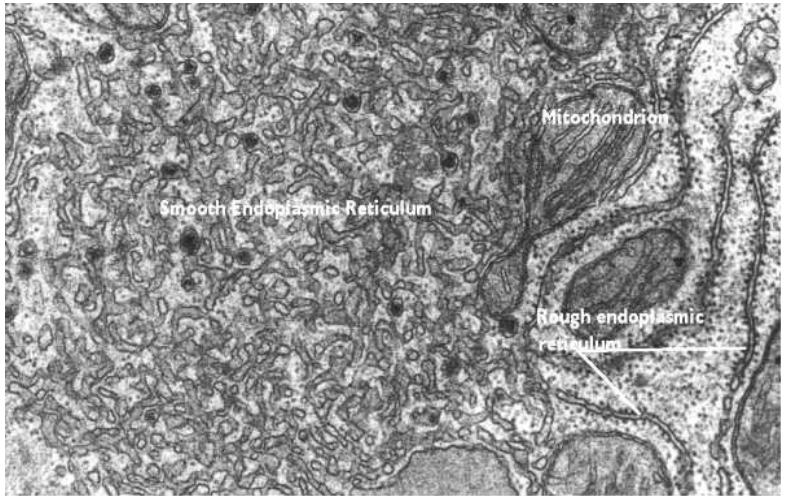
Oxygenases and monooxygenases

Introduction to cytochromes P450

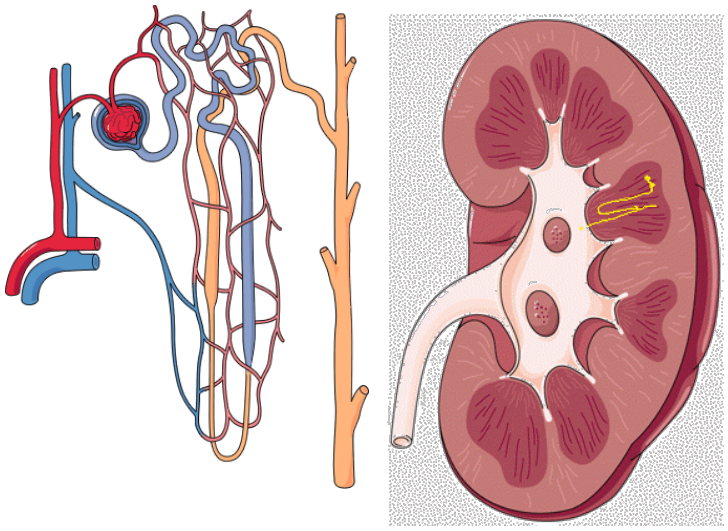
Phase 1 and Phase 2 drug metabolism

The process and organs involved

- Objective of drug metabolising enzymes:
  - To make xenobiotics more polar, more easily excreted.
- Organs mainly involved: liver and kidney



Smooth endoplasmic reticulum of hepatocytes
LIVER



Glomerular filtration
KIDNEY

Basis of drug metabolism

- Human body has a large number of enzymes to chemically modify toxins (self-defence mechanism
- Drugs are treated as toxins
- Purpose of drug metabolism is to render compounds more water soluble to allow easy excretion in urine and bile

Consequences of drug metabolism

- The metabolism of drugs can produce various pharmacological results:
  - Produce inactive metabolite (e.g. morphine-glucuronide or paracetamol-sulfate)
  - Change the pharmacological activity (e.g. acetylsalicylate (aspirin) → salicylic acid)
  - Convert an inactive pro-drug to an active compound (e.g. cyclophosphamide)
  - Convert the drug into toxic metabolites (e.g.paracetamol –imidoquinone)

Drug metabolism reactions

- Divided into two phases:
  - Phase I:
    - Polar functional group is added to or exposed on drug molecule e.g. OH, COOH, NH_2 e.t.c.
    - Reactions usually oxidations but also reductions and hydrolysis
    - After phase I compound can undergo phase II metabolism or be excreted without further biotransformation
  - Phase II:
    - May or may not be preceded by Phase I
    - Polar functional group on drug conjugated to activated endogenous substrate (e.g. glucuronic acid, sulfate, glutathione, methyl and acetly groups)
    - Results in increased water solubility

Drug metabolism: Phases

- PHASE 1: ACTIVATION
  - OXIDATIVE ENZYMES, introduction of functional groups:
    - Dehydrogenation
    - Oxidation
    - Reduction
    - Hydrolysis
    - Hydroxylation
- PHASE 2: CONJUGATION
  - CONJUGATIVE ENZYMES, linkage to highly polar carriers to facilitate excretion

Phase 1 drug metabolism

- Enzymes involved:
    - Cytochrome P450s (CYPs or P450s)
        - most important and highly abundant
        - Polymorphic
        - Inducible
        - Commonly feature in drug-drug interactions
    - Flavin containing monooxygenases (FMOs)
        - FMO3 most relevant to drug metabolism
    - Other oxygenases
        - Monoamine oxidase (MAO)
        - Xanthine oxidase (XO)
    - Dehydrogenase
        - Aldehyde oxidase (dehydrogenase)
        - Alcohol dehydrogenase
    - Esterases
        - Cholinesterases
        - Plasma and tissue esterases

Phase 2 drug metabolism

- Enzymes involved:
  - UDP-glucuronosyltransferases (UGTs)
    - UDP-glucuronic acid conjugated to –OH, -COOH, -NH_2 and –SH groups
    - High capacity
  - Sulphotransferases (SULT)
  - Phosphoadenosyl phosphosulphate (PAPS) conjugated to –OH, -NH_2 and –SO_2NH_2
    - Low capacity
  - Glutathione S-transferases (GSTs)
    - Glutathione conjugated to electrophiles
    - Low capacity
  - N-acetlytransferases (NAT)
    - Acetyl-CoA conjugated to –OH, -NH_2 and –SO_2NH_2
    - Variable capacity
  - Methyltransferases
    - S-adenosyl methionine conjugated to catecholamines and phenols

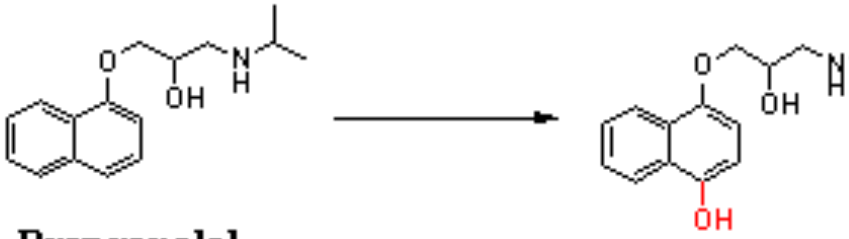
Drug Metabolising Enzymes: DME

- Phase 1 enzymes (activation):
  - Cytochrome P450s
  - Monoamino oxidase
  - Microsomal flavin monooxigenase
  - *Alcohol dehydrogenase
  - *Aldehyde dehydrogenase
  - Esterase
  - Epoxid hydrolases
- Phase 2 enzymes (conjugation):
  - UDP-Glucuronosyltransferases UGTs
  - Glutathione-S-transferase GSTs
  - *Sulphotransferases SLTs
  - *N-acetyltransferases NATs

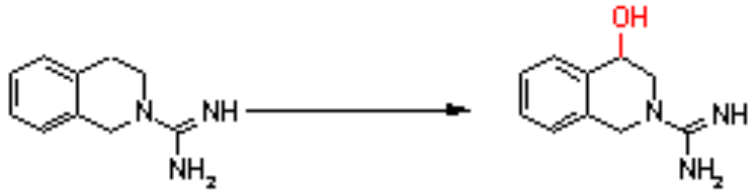
- Liver is quantitatively most important;
- DME are mainly located in on the membranes of the SER, but some are cytosolic *;
- There are multiple forms of DMEs, often with overlapping specificity (5-30 genes)

Phase I Metabolism

- **Principally oxidation, reduction or hydrolysis**

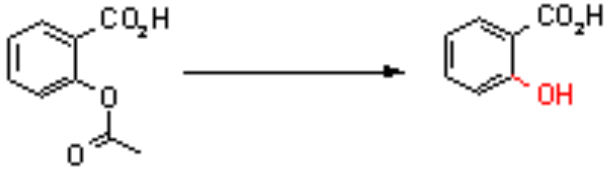


Propranolol
beta blocker
used to treat high blood pressure



Debrisoquine
Adrenergic neuron-blocking drug
used to treat high blood pressure

Nitrazepam
hypnotic drug of the benzodiazepine class
for short -term relief of severe, disabling anxiety and insomnia

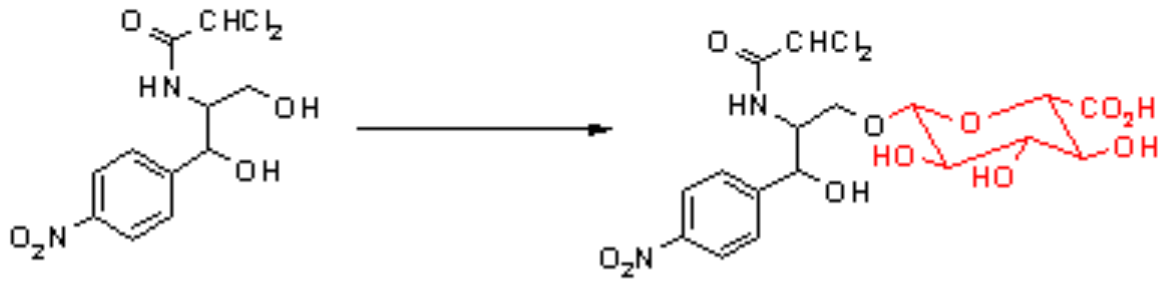


Aspirin
used for short-term treatment of pain, fever, and inflammation.
Also used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot

AstraZeneca

Phase II Metabolism

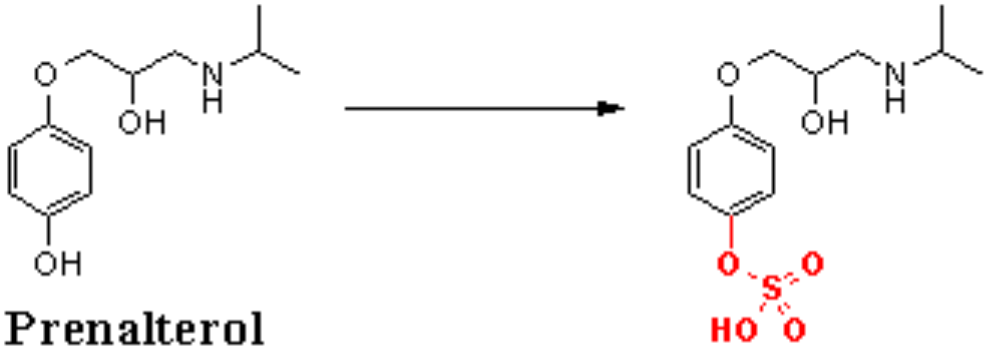
- **eg. glucuronidation**



Chloramphenicol

antibiotic useful for the treatment of a number of bacterial infections, including meningitis, plague, cholera, and typhoid fever

- **sulphation**

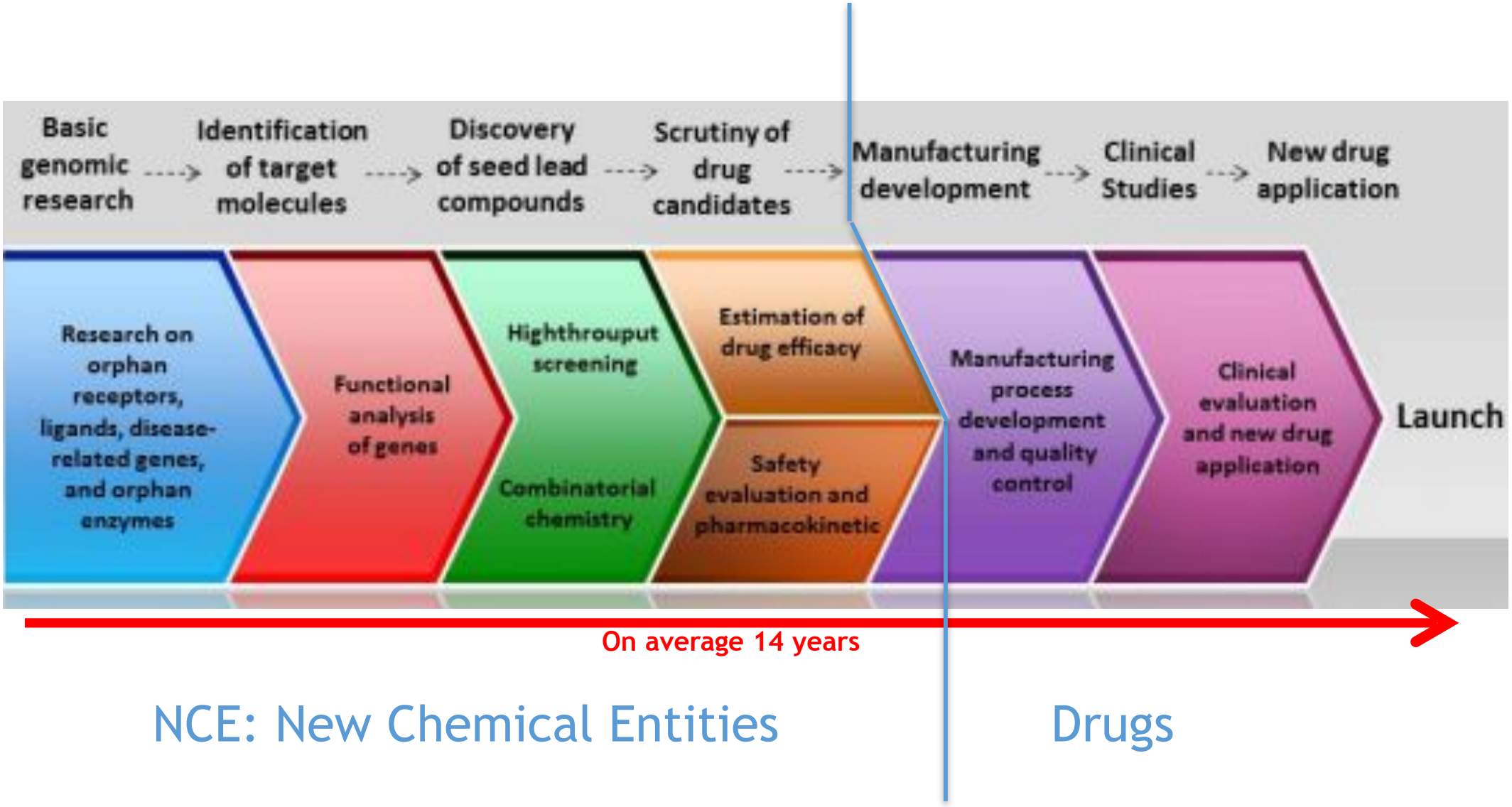


Prenalterol

cardiac stimulant which acts as a β1-adrenergic agonist

AstraZeneca

The drug discovery process



Preclinical studies of drug metabolism

- Topics dealt with at early development stages (Pharmacokinetics):
  - SOLUBILITY, RATE OF ABSORPTION
  - BIOAVAILABILITY
  - METABOLIC STABILITY
  - CLEARANCE: when too high = problems. Small chemical modification may lead to large changes in rate of metabolism.

33% NCE withdrawn for unwanted PK

- Topics dealt with at the preclinical stage (Toxicity):
  - IDENTIFICATION OF ACTIVE OR REACTIVE METABOLITES
  - SUBSTRATE METABOLIC PATHWAY
  - INTER-SPECIES COMPARISONS
  - ASSIGNMENT OF RESPONSIBLE ENZYMES
  - ASSESS THE OCCURRENCE OF POLYMORPHIC ENZYMES
  - ASSESS IF THE NCE IS INDUCER OR INHIBITOR OF DME
    - DRUG-DRUG INTERACTIONS

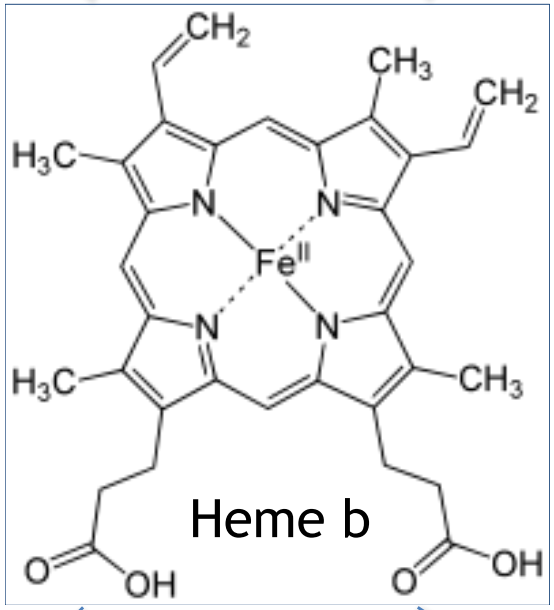
20% NCE withdrawn for toxicity

Introduction to cytochromes P450

Heme in biology

O_2 storage and transport
(Myoglobin and Hemoglobin)
Heme b

Electron transfer
(Cytochrome c)
Heme c



Heme b

O_2 reduction to H_2O
(Cytochrome c oxidase)
Heme a

O_2 catalysis
(Peroxidases, catalase, P450)
Heme b

Energy capture
Cytochrome c oxidase

Reject

New metabolites
Hormones
Peroxidases

Detoxification
Cytochrome P450

Sterol hormones
New chemicals

O_2
+
Heme catalysts

Damage
(Mutation)

Side-products
(O_2^-, H_2O_2, $OH^\bullet$)

O_2
Protective processes
Catalases

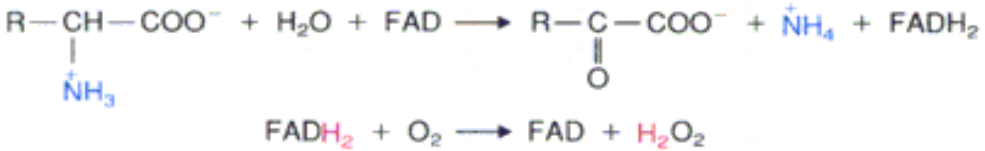
Reject

Oxygenases and monooxygenases, oxygen activation

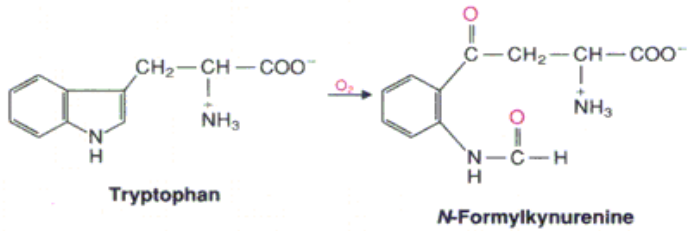
Enzymes involved in O_2 chemistry

- **Oxidases**: enzymes that catalyse the oxidation of a substrate without O_2 incorporation into the product:

  Example: D-amino acid oxidases (cofactor = FAD)



- **Oxygenases**: enzymes that catalyse the oxidation of a substrate with O_2 incorporation into the product:
  - **Di-oxygenases** incorporate both atoms of the O_2 molecule into a substrate:
    - Example, Tryptophan 2,3-dioxygenase (cofactor = haem):

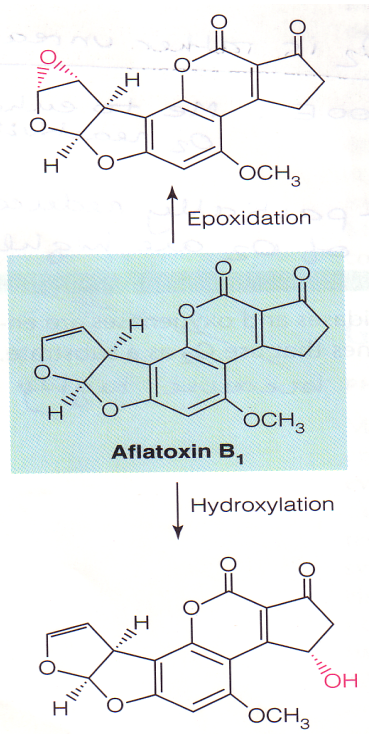


  - **Mono-oxygenase** incorporate only one atom of the O_2 molecule into a substrate, the other one produces water:

$$AH + BH_2 + O_2 \rightarrow A\text{-}OH + B + H_2O$$

Example: Cytochrome P450s:

$$RH + NAD(P)H + H^+ + O_2 \rightarrow R\text{-}OH + NAD(P)^+ + H_2O$$



Biological reactions of dioxygen, O_2

- Life originated when there was no O_2 in the atmosphere;
- The primitive cell derived its energy from glycolysis, not from respiration;
- Photosynthesis changed the whole situation: **O_2 was introduced as the first environmental "pollutant"** (Levine, 1988);
- In fact, the 21% level of atmospheric O_2 is toxic to strict anaerobic bacteria, the descendant of the primitive cell;
- By contrast, the evolved aerobic organisms learned how to use the powerful oxidising properties of O_2 , **but** developing at the same time elaborate systems to protect, repair or replace the components that may be damaged by the inevitable O_2 by-products;
- **Oxygen paradox (Koppenol 1988)**: aerobic organism need O_2 to survive, but they also must constantly defend from the toxicity of its non fully reduced by-products.

Oxygen products

- In aerobic cells, 90% of the O_2 is used for respiration:

$$O_2 + 4H^+ + 4e^- \quad \text{—>} \quad 2H_2O \qquad\qquad E° = +0.815\ V$$

- The remaining 10% is used for specialised reactions by at least 200 enzymes known to date;

- Other reactions given by O_2:

TABLE 1-1 Standard Reduction Potential for One- and Two-Electron Reduction of Dioxygen Species in Water

									E° vs. NHE, pH 7.25
		O_2	+	e^-	⟶	O_2^-			−0.33 V
O_2^-	+	e^-	+	$2H^+$	⟶	H_2O_2			+0.89 V
H_2O_2	+	e^-	+	H^+	⟶	H_2O	+	OH	+0.38 V
OH	+	e^-	+	H^+	⟶	H_2O			+2.31 V
O_2	+	$2e^-$	+	$2H^+$	⟶	H_2O_2			+0.281 V
H_2O_2	+	$2e^-$	+	$2H^+$	⟶	$2\ H_2O$			+1.349 V

Source: Sawyer (1988).

Thermodynamics

- Note that the **1e⁻ reduction to superoxide is a limiting factor to oxygen reactivity:** it has a very low E°;
- However, but once O_2^- is produced, all the other reactions are energetically favoured;
- Although the 1e⁻ reduction to is thermodynamically unfavoured, it is possible by using strong reducing agents such as activated haems, flavins, hydroquinones present in enzymes;
- These enzymes are able to
  - Either stabilise the O_2^--containing intermediate
  - Or provide pathways for 2e⁻ reactions

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Source: Sawyer (1988).

- The energetic barrier to O_2^- reactivity allows dioxygen to freely diffuse in the cell without rapidly reacting with the reducing components present in the cell (remember that NAD has E° = - 0.32 V).

Kinetics

- An additional barrier to O_2 reactivity is the slow kinetics;
- Reaction of O_2 with various organic molecules is very thermodynamically favoured, but in reality the occur extremely slowly at room temperature without initiators or catalysts:

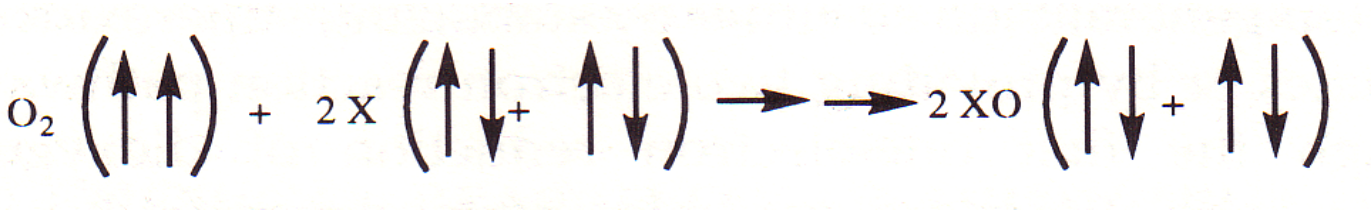
TABLE 1-2 Heats of Formation of the Oxygenation of Simple Organic Compounds with O_2

					ΔH, kcal/mol
$CH_4(g)$	+	$1/2O_2(g)$	$\longrightarrow$	$CH_3OH(g)$	−30
$C_6H_6(g)$	+	$1/2O_2(g)$	$\longrightarrow$	$C_6H_5OH(g)$	−43
$C_6H_5OH(g)$	+	$1/2O_2(g)$	$\longrightarrow$	$C_6H_4(OH)_2(g)$	−42
$C_2H_4(g)$	+	$1/2O_2(g)$	$\longrightarrow$	$C_2H_4O(g)$	−25

Source: Holm (1987).

Spin restrictions of O_2

- The problem is the spin restriction: O_2 has a triplet ground state (2 unpaired e^- with parallel spins), while the majority of organic molecules are in a singlet ground state (no unpaired electrons):



- It follows that for O_2 to react with organic molecules must violate the *spin conservation law: "the overall spin state must be the same before and after each elementary steps of a reaction"*;

- Catalysts containing metals can break down this kinetic spin restriction, through the formation of activated high spin species, we will see later how P450 can do these steps.

Reactive oxygen species (ROS) and oxidative stress

- These are:
  - Superoxide ($O_2^{\bullet -}$), 1e$^-$ reduction;
  - Hydrogen peroxide (H_2O_2), 2e$^-$ reduction;
  - Hydroxyl radicals ($OH^{\bullet}$), 3e$^-$ reduction;

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Source: Sawyer (1988).

- They are produced in incomplete reductions (errors) but also as ordinary products of some enzymes (xantine oxidase, amino acid oxidases produce H_2O_2);

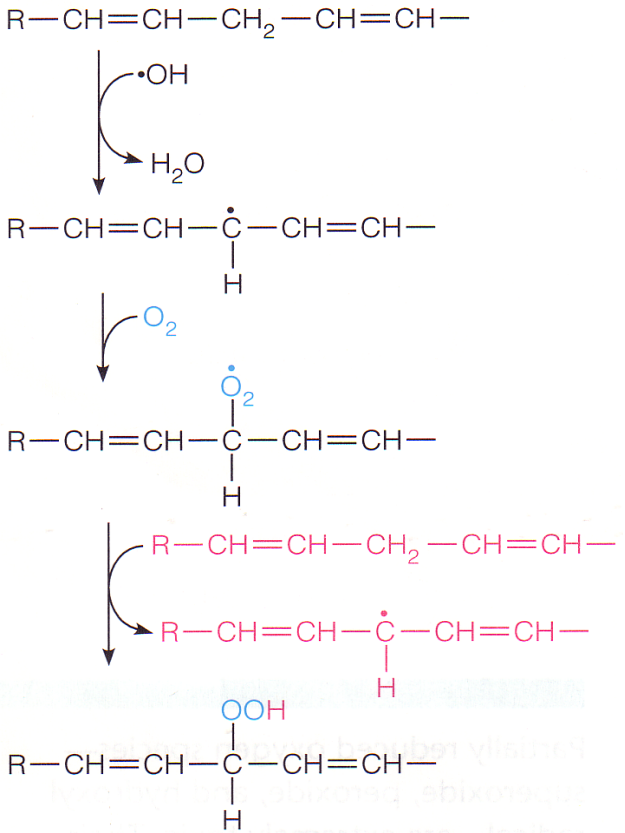
- ROS can be produced in large amounts: 0.02 pmoles per cell or 0.15 moles per whole body;

- Respiration can lead to 1-2% of the electrons to give ROS;

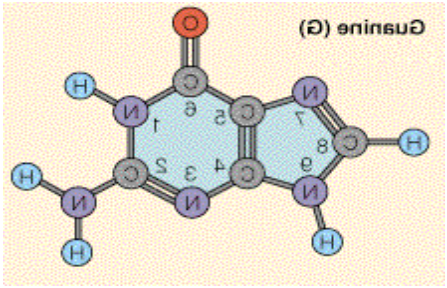
- Whatever the source, they are toxic and cause what is called **oxidative stress.**

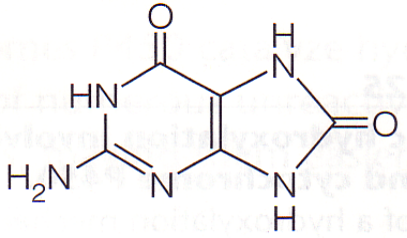
Hydroxyl radicals ($OH^{\bullet}$)

- It is a very toxic radical;
- It damages proteins, membranes, lipids, nucleic acids:
  - It inititates the oxidation of fatty acids in membranes with a chain reaction called lipid peroxidation;
  - It can alter bases to give for example 8-oxoguanine and thymine glycol; these are mutagenic because cause non-Watson-Crick base pairs and/or block replication;
  - $OH^{\bullet}$ are the most active mutagen resulting from ionizing radiation.

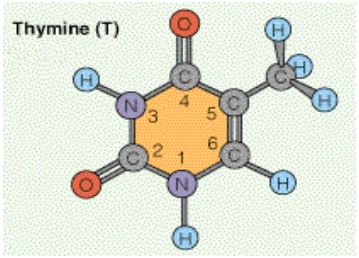


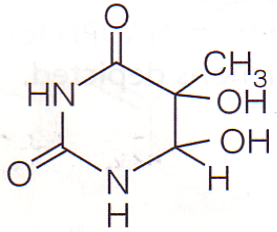
Lipid peroxidation





8-Oxoguanine





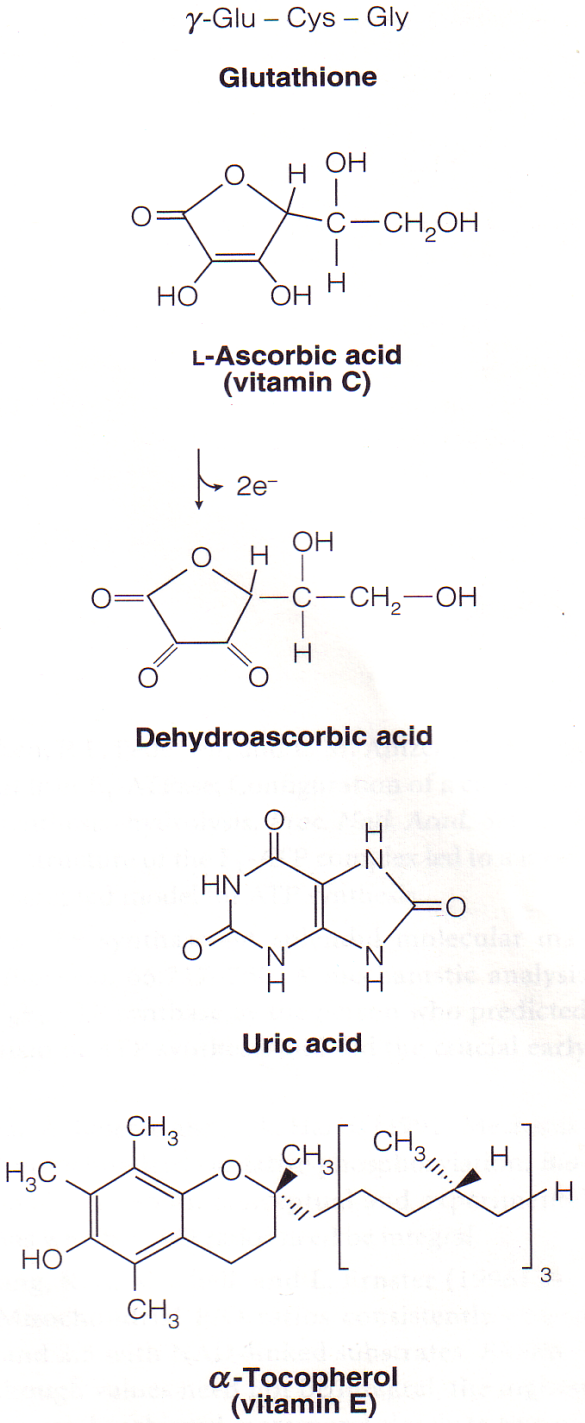
Thymine glycol

Superoxide radicals ($O_2^{\bullet -}$)

- It is a free radical that can combine with another free radical, nitric oxide ($NO^{\bullet}$), used by the cell as signalling agent, to give peroxynitrite, $OONO^-$
- Peroxynitrite is very toxic and causes:
  - lipid peroxidation
  - Nitration of tyrosyl hydroxyl groups in proteins that damages particulary membrane proteins

Defenses from oxidative stress

- Antioxidants:
  - They act trapping the radicals before they cause too much damage:
    - Glutathione
    - L-ascorbic acid (vit. C)
    - Uric acid
    - α-Tocopherol (vit. E)
- Endogenous enzymes:
  - They engage the ROS in reactions that ultimately give water:
    - SOD
    - Catalase
    - Peroxidase



Enzymes clearing ROS

- Superoxide dismutase:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

  - Cu/Zn SOD is present in cytosol of eukaryotic cells;
  - Mn SOD is present in mitochondria and bacteria;
  - Fe SOD is present in cyanobacteria and some plants;
  - Recently a Ni SOD has been described.

- Catalase, one of the highest enzymes turnover (> 40000 molecules/sec):

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

- Peroxidase, widely distributed, in erythrocytes we find the glutathione peroxidase:

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

  - Glutathione peroxidase contains an unusual amino acid, the selenocysteine, where the S of cys has been replaced by Se; this is related to the current interest in dietary supplement of Se to prevent cancer.

Respiratory burst

- Respiratory burst occurs for example following phagocytosis;
- It causes a high O_2 intake, but the mechanism is not yet known;
- The O_2 intake is used here to deliberately produce O_2^- and H_2O_2 to kill the material/bacterium engulfed;
- In this case toxic ROS are produced for a specific purpose under “controlled” conditions.

Oxygen metabolism and Human diseases

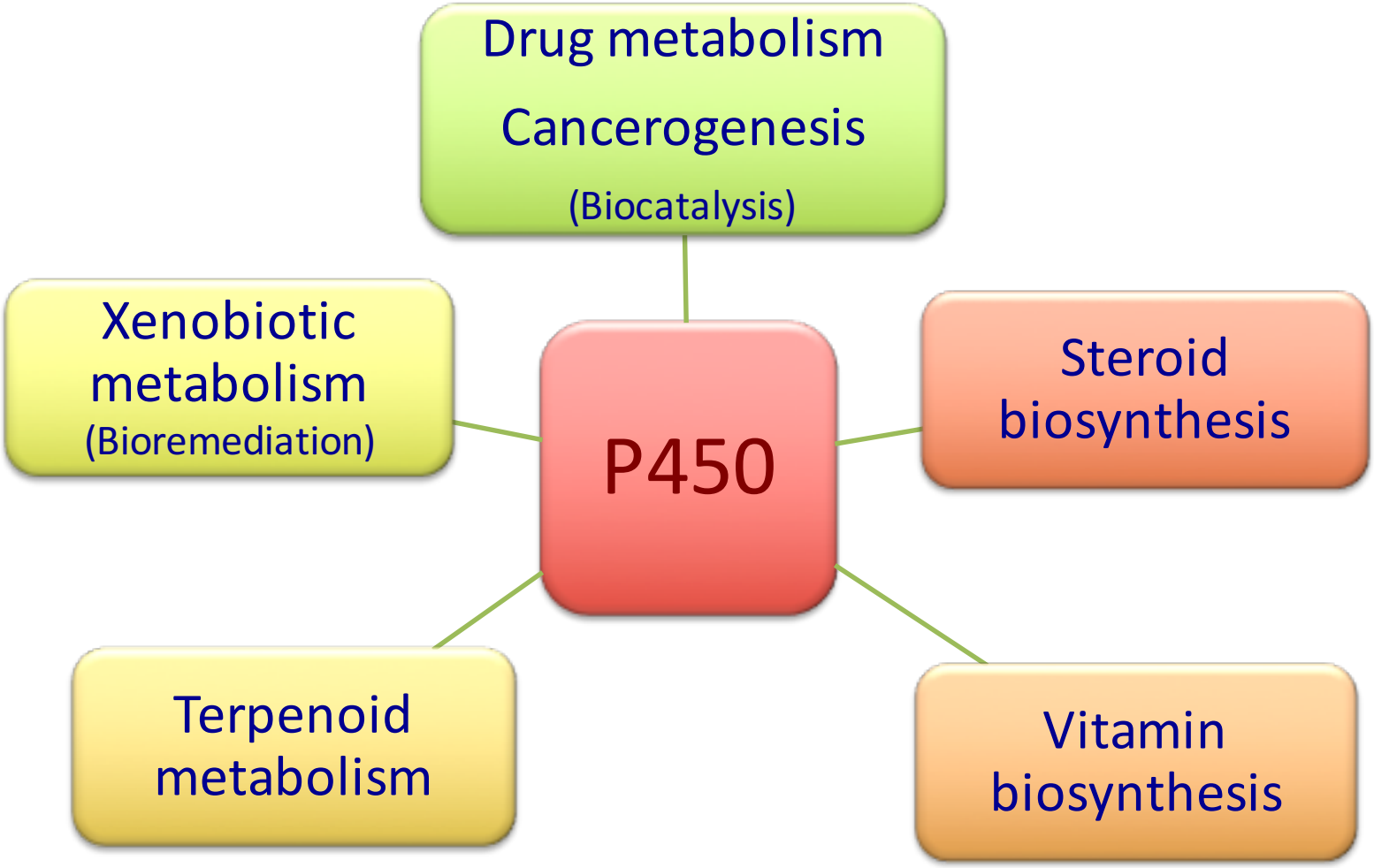
- Oxidative damage has been linked to:
  - Cardiovascular diseases; cancer; stroke; neurodegenerative diseases; chronic inflammatory diseases;

- Dietary supplements of vit C and E can help prevent these diseases;

- Correlations:
  - Cancer can derive from the alterations caused by OH• (8-oxoguanine, 5-hydroxycytosine); DNA lesions derived from oxidative stress increases with age;
  - Human mutations on the gene that codifies for the human Cu/Zn SOD has been found to correlate with the neurodegenerative disorder amyotrophic lateral sclerosis (Lou Gehrig's disease);
  - Peroxynitrite ($OONO^-$) has been found to play a role in causing multiple sclerosis (MS). Interestingly, people with gout who have high levels of uric acid, hardly ever develop MS.
  - Mutations in the mitochondrial genes that encode for the respiratory complexes have been shown to cause optic nerve degeneration and muscle disease;
  - Mutations on the mitochondrially encoded cyt c oxidase has been associated with Alzheimer's disease;

Cytochromes P450: Introduction

Relevance of P450 in biology:



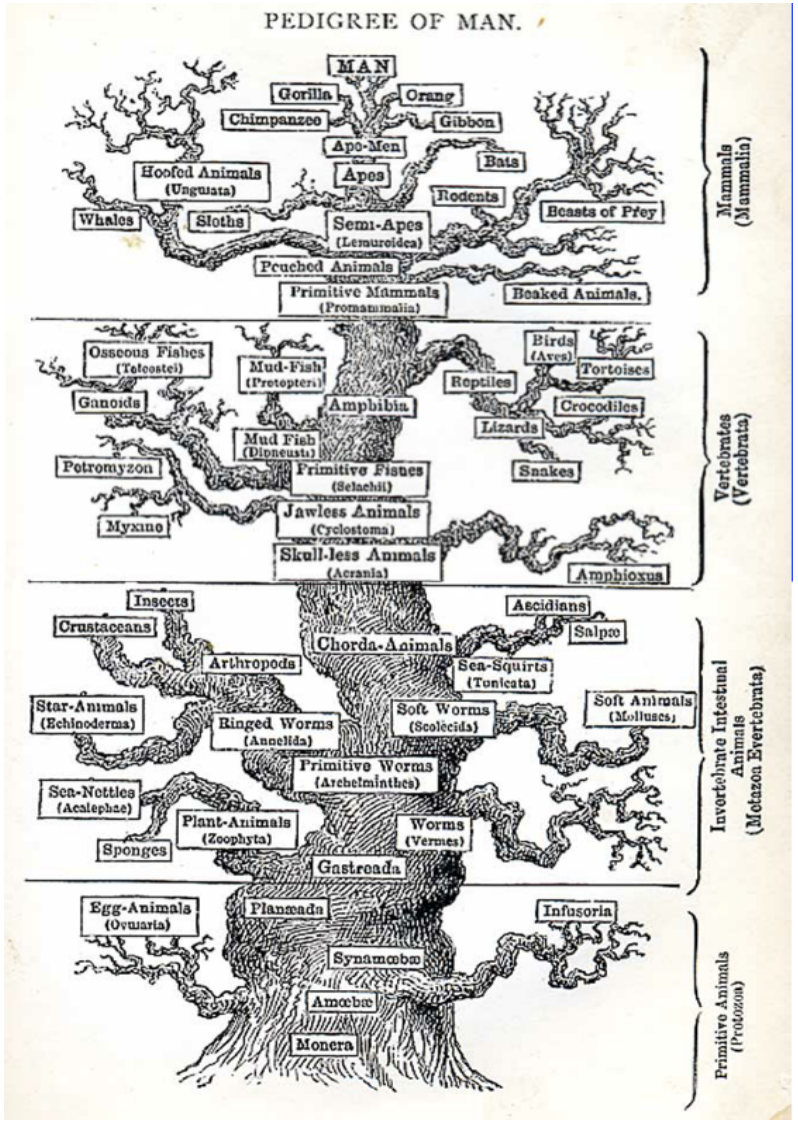
General reaction:

$$R\text{-}H + O_2 + 2e^- + 2H^+ \rightarrow R\text{-}OH + H_2O$$

Number of named P450 Sequences

(yr 2014)

Animals	6,502
Insects	3,571
Mammals	1,056
Other vertebrates	922
Non-insect invertebrates	953
Plants	7,533
Fungi	6,418
Protozoa	247
Bacteria	1,306
Archaea	48
Viruses	2



Courtesy: David Nelson

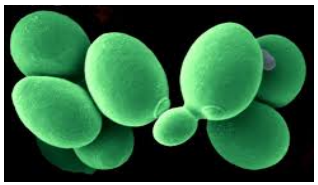
Number of P450s per genome

- *Arabidopsis thaliana*: 275
- *Drosophila melanogaster*: 90
- *Caenorhabditis elegans*: 80
- *Homo sapiens*: 57
- *Mycobacterium tuberculosis:* 20
- *Bacillus subtilis:* 7
- *Saccharomyces cerevisiae*: 3
- *Escherichia coli*: 0

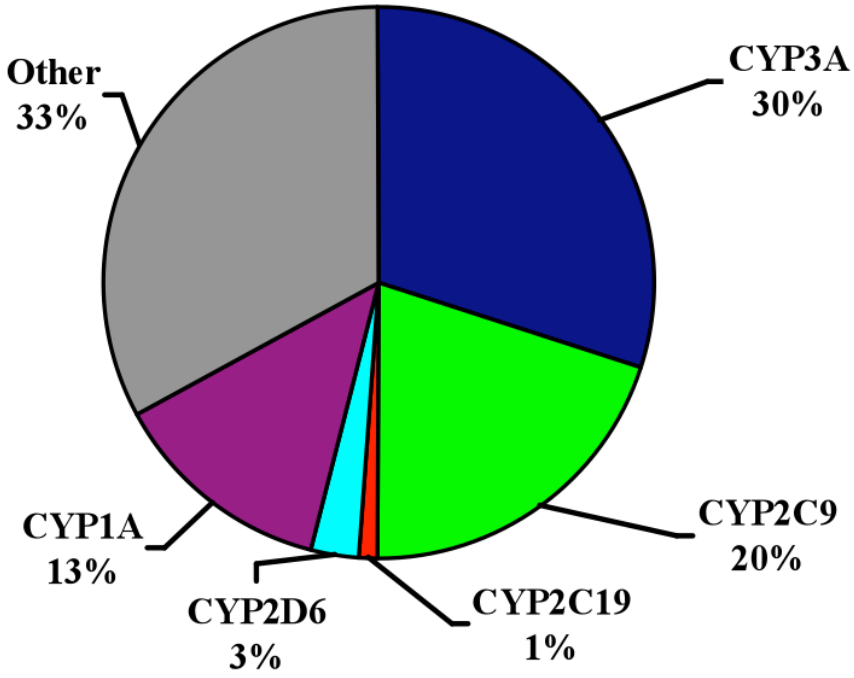




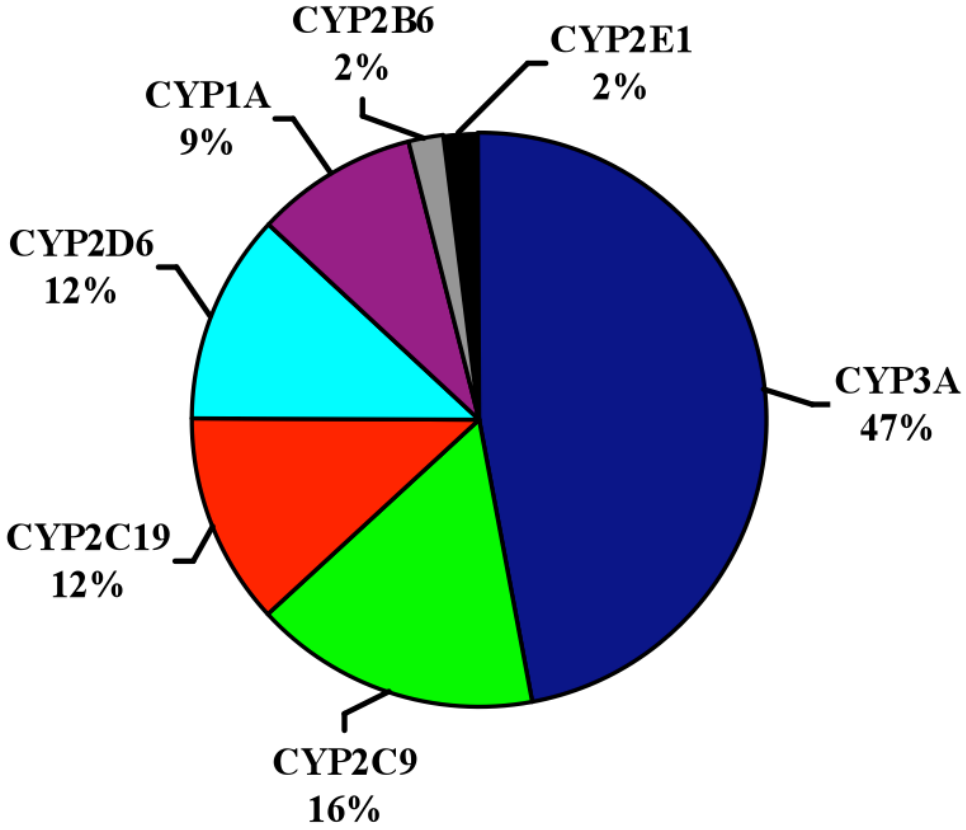





Conc. *versus* relevance to metabolism



Conc. in liver



Relevance to metab.

Example: 2D6 is only 3% of hepatic P450 but it metabolises 12% of drugs!


KEEP
CALM!
TO BE
CONTINUED...