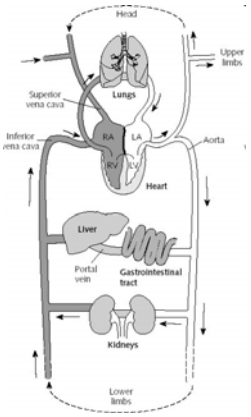
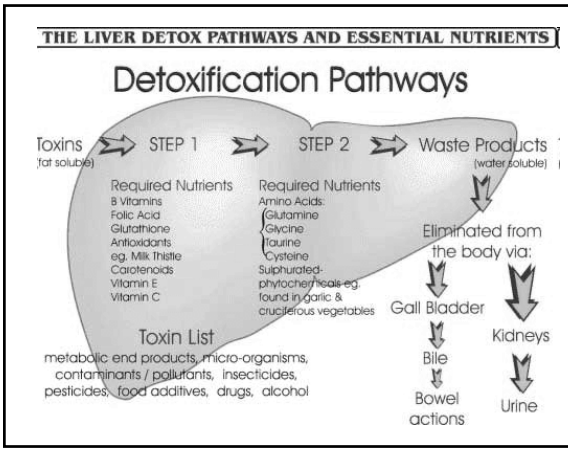
Detoxification

Chemicals entering body (mostly via food) must pass through **liver**





Detoxification

Principle of detoxification
- elimination of hydrophobic compounds from body
- formation of polar / soluble products

Two principal phases (phase I & II)
- well studied in vertebrates (mammals)
- **liver:** major organ involved in detoxification

- *plants: similar oxidating enzymes: cytochrom oxidase, phenol oxidase, peroxidase*

Phase III - elimination - both from cell & body

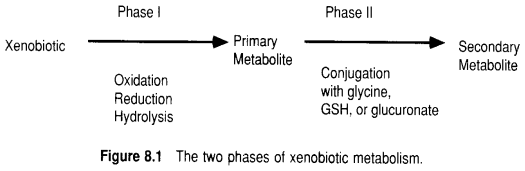


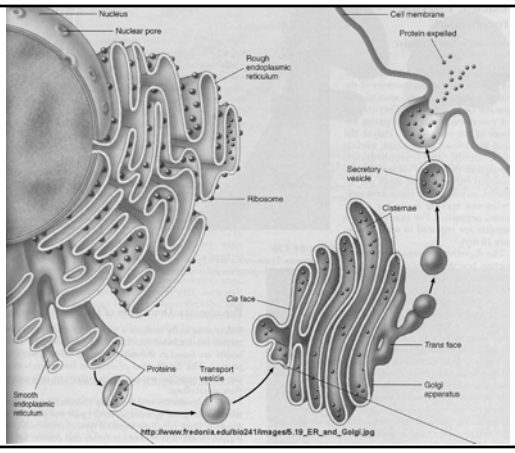
Figure 8.1 The two phases of xenobiotic metabolism.

Phase I

MFO enzymes
(mixed function oxidase, mixed function oxygenase)
- membrane enzymes bound to **Endoplasmic reticulum**
- membrane vesicles "microsomes" = S-9 fraction can be extracted from cells

MFO: principle enzymes: **cytochromes P450 (CYPs)**
- haem-containing enzymes
  (superfamily of more than 150 genes)
- several classes and subclasses
  (different substrate specificity; structure ...)

Cytochrome P450 1A (CYP1A)
– basic for detoxification of hydrophobic environmental contaminants
Cytochrome P450 19A (CYP19)
– "aromatase" involved in synthesis of estradiol (aromatization of testosterone)

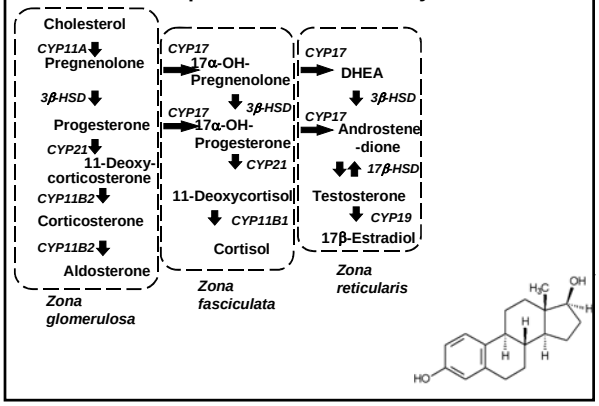


Family	Function	Members	Names
CYP1	drug and steroid (especially estrogen) metabolism	3 subfamilies, 3 genes, 1 pseudogene	CYP1A1, CYP1A2, CYP1B1
CYP2	drug and steroid metabolism	13 subfamilies, 16 genes, 16 pseudogenes	CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, 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, 11 genes, 10 pseudogenes	CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1
CYP5	thromboxane A_2 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, 2 genes, 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)

Table 2. Partial list of human placental xenobiotic- and hormone-metabolizing enzymes or isoenzymes.[6]

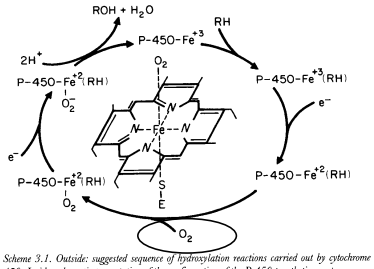
Phase	Type	Reaction (gene)	Substrate
I	MFO	*O*-Deethylase (*CYP1A1*)	7-Ethoxycoumarin
I	MFO	Aryl hydrocarbon hydroxylase (*CYP1A1*)	PAH
I	MFO	Hydroxylase (*CYP3A7*)	Cortisol
I	MFO	Aromatase (*CYP19*)	Androgens
I	MFO	Cholesterol side-chain cleavage (*CYP11A*)	Cholesterol
I	MFO	Estrogen catechol formation, 2-Hydroxylation (*CYP1A1*) 4-Hydroxylation (*CYP1B1*)	Estrogens
I	MFO	25-Hydroxycholecalciferol hydroxylase	25-Hydroxycholecalciferol
I	Oxidoreductase	17β-Hydroxydehydrogenase Type 1 Type 2	Estrone to estradiol Estradiol to estrone
I	Oxidoreductase	11β-Hydroxydehydrogenase	Cortisol/cortisone
I	Oxidation	Dehydrogenase	Alcohol/acetaldehyde
I	Oxidation	Monoamine	Norepinephrine
II	Sulfatase	Sulfate cleavage	Stercid sulfates
II	Conjugation	GST	Epoxides
II	Conjugation	Catechol-*O*-methyltransferase	Catecholamines, catechol estrogens

CYPs - example: steroid hormone synthesis

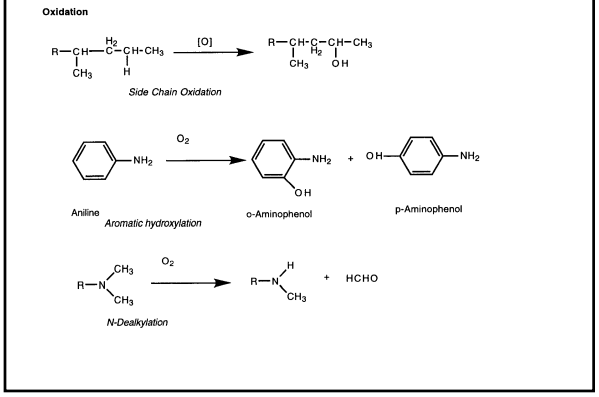


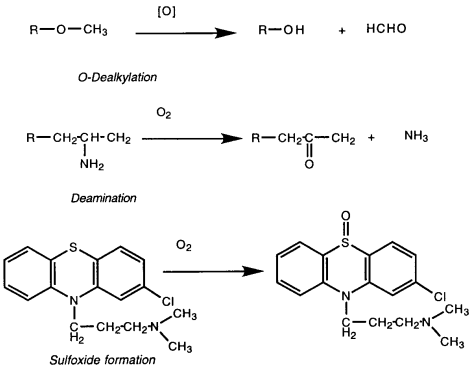
CYPs & Phase I of detoxification - major reactions

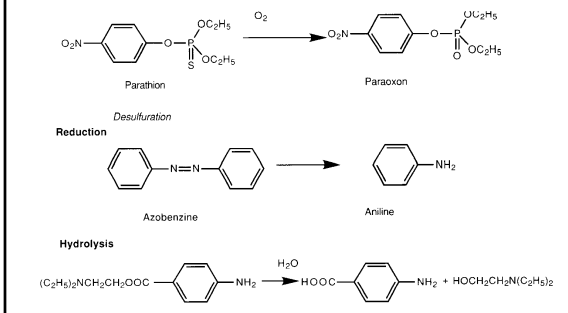
oxidation
hydrolysis
(reductions and others)



Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.







Phase II

Conjugation reactions:

reactive xenobiotics or metabolites formed in phase I
+
endogeneous substrates
- saccharides and their derivatives – glucuronic acid,
- aminoacides (glycine)
- peptides: glutathione (GSH)

Phase II enzymes:

glutathion S-transferase (GST)
epoxid hydrolase (EH)
UDP-glucuronosyltransferase (UDP-GTS)
sulfotransferase (ST)

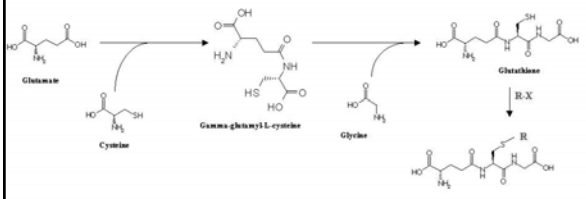
+ Excretion of conjugates in urine, sweat or bile

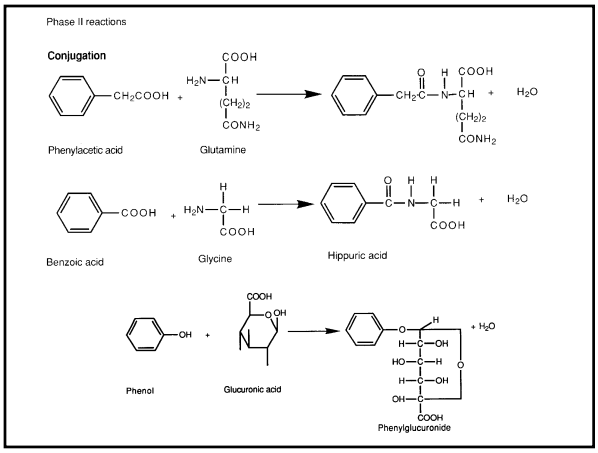
Table 3. Major phase II detoxification activities in humans

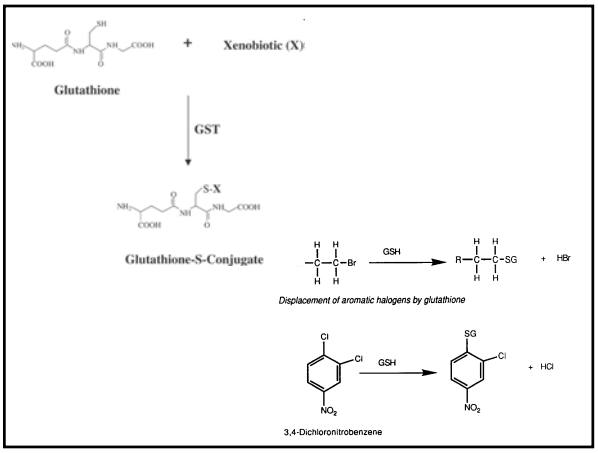
Reaction	Enzyme	Localization[a]	Substrates
H_2O	Epoxide hydrolase	Microsomes Cytosol	Epoxides
Glutathione	Glutathione transferases	Microsomes	Electrophiles
Glucuronic acid (UDPGA)[b]	Glucuronyl transferases	Microsomes	Phenols, thiols, amines, Carboxylic acids
Sulfuric acid (PAPS)[b]	Sulfotransferase	Cytosol	Phenols, thiols, amines
Methyl Group (SAM)[b]	N- and O- methyl transferases	Cytosol Microsomes	Phenols, amines
Acetic acid (Acetyl-CoA)[b]	N-acetyl transferases	Cytosol	Amines
Amino acids (Acetyl-CoA, taurine, glycine)	Amino acid transferases	Microsomes	Carboxylic acids

Glutathione:

- major donor of SH (thiol) groups in cells (MW ~ 300 g/mol)
- concentrations ~ 5 mM (1.5 g/L)



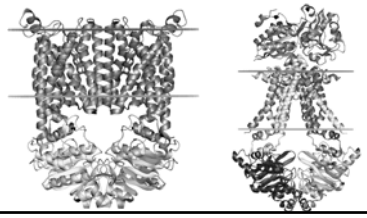




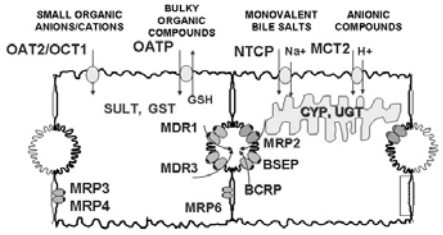
Phase III - transporters

ATP-binding cassette transporters (ABC transporters)

- protein superfamily (one of the largest, and most ancient in all extant phyla from prokaryotes to humans)
- transmembrane proteins - transport across extra- and intracellular membranes (metabolic products, lipids, sterols, drugs)

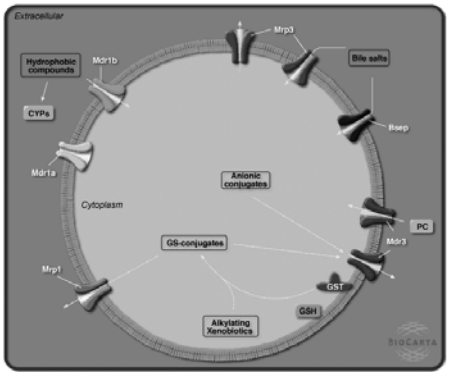


Phase III - transporters



- MRP (MDR) - multidrug resistance-associated protein family
- OATP: Organic anion transporting polypeptide
- P-glycoprotein
- … many others

Phase III - transporters



Detoxification enzymes may be induced by substrates

- **CYP1A – induction via AhR**
  - -Substrate: hydrophobic organochlorine compounds (PCDDs/Fs, PAHs PCBs ...)
  - *[see also: lectures on nuclear receptors]*

- **Other CYPs** - substrate-induced
- **Phase II enzymes** - by reactive toxicants
- **ABC transporters** - by respective chemicals

AhR dependent CYP1 induction

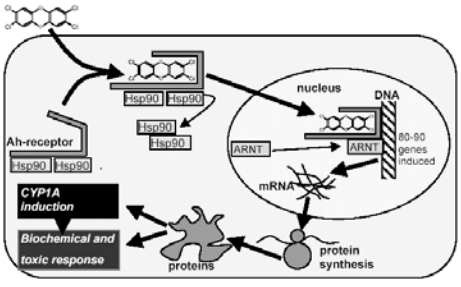


Figure 5. The mechanism of CYP1A induction mediated through the aryl hydrocarbon receptor (AhR). (Figure by M. Engwall).

Induction of detoxication enzymes

-> increased **energetic demand** (ATP, metabolism)
-> may lead to **resistance** to toxic compounds

-> **activation of pro-mutagens**/pro-carcinogens

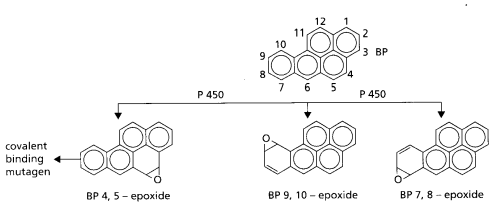
-> increase of **oxidative reactions**
production of Reactive Oxygen Species (ROS)
[see oxidative damage and stress lectures]

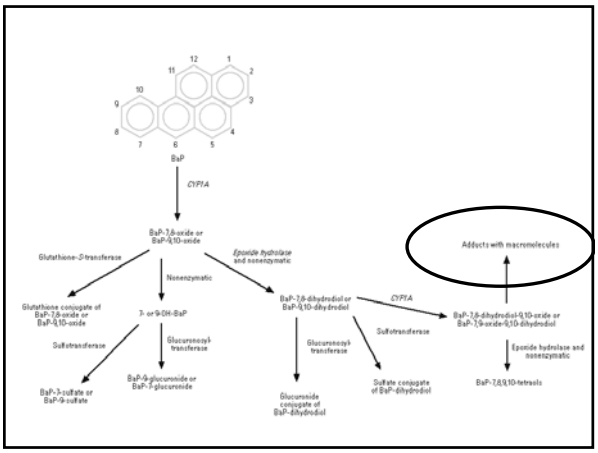
-> **side toxic effects** *[see nuclear receptor lectures]*
- increased degradation of endogeneous compounds (retinoids – regulatory molecules degraded by CYP1A

- crosstalk with other mechanisms & receptors

Activation of promutagens by CYPs

Benzo[a]pyrene





Aflatoxin B1

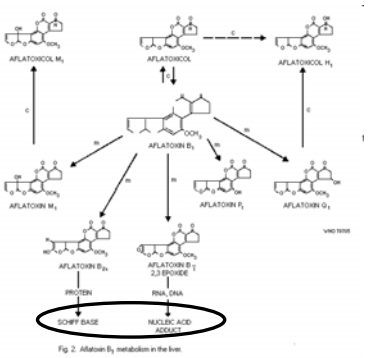


Fig. 2. Aflatoxin B_1 metabolism in the liver.