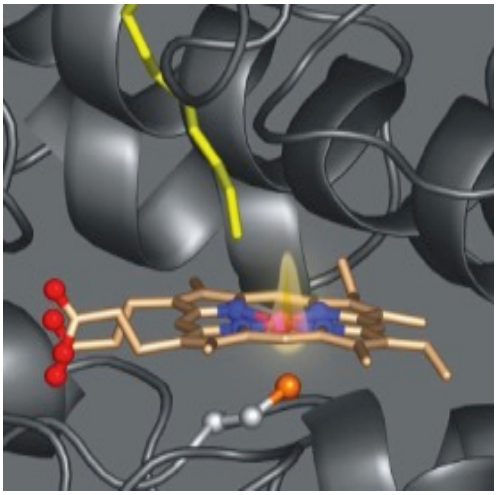
MEDCH/PCEUT 527 – ADVANCED DRUG METABOLISM 2019
Course Coordinators: Allan Rettie and Ken Thummel
When/ Where: 2.30 – 4.00 pm MWF in H074

Date	Topic	Instructor
Jan 7	Introduction	Rettie/Thummel
Jan 9	P450 I: Basics – Nomenclature, Substrate Specificity	Rettie
Jan 11	P450II: Structure-Function	Rettie
Jan 14	P450III: Reaction Mechanisms A	Totah
Jan 16	P450IV: Reaction Mechanisms B	Totah
Jan 18	Non-Heme Oxygenases	Rettie
Jan 21	*Holiday*	
Jan 23	Hydrolysis and Reduction	Rettie
Jan 25	Literature Critique I (20)	Totah
Jan 28	Acetylation/Methylation	Rettie
Jan 30	Exam 1 (80)	
Feb 1	Glucuronidation/Sulfation	Atkins
Feb 4	Glutathione Conjugation	Atkins
Feb 6	Drug Transporters I	Prasad
Feb 8	Drug Transporters II	Prasad
Feb 11	P450 Inhibition I (Reversible)	Kunze
Feb 13	P450 Inhibition II (Irreversible)	Kunze
Feb 15	Activation	Atkins
Feb 18	*Holiday*	
Feb 20	Exam 2 (70)	
Feb 22	P450 Induction (Nuclear Receptors, Stabilization)	Thummel
Feb 25	P450 Induction (Clinical , Pathophysiological Effects)	Thummel
Feb 27	Pharmacogenomics I	Thummel
Mar 1	Pharmacogenomics II	Rettie
Mar 4	Literature Critique II (20)	Rettie
Mar 6	Safety Considerations in Drug Development	Wienkers
Mar 8	Model Systems:Aristolochic Acid Case Study	Kelly
Mar 11	Cellular Toxicity	Totah
Mar 13	Chemical Toxicity	Totah
Mar 15	Toxicity:Avoidance Strategies	Totah
Mar 19	Exam 3 (80)	

MEDCH 527
AER
Jan. 7-9, 2019



CYTOCHROME P450: Structure-Function

1. **General P450 Characteristics and Taxonomy**
2. **Human P450s – Substrate and Inhibitor Selectivities**
3. **Structure-Function Aspects of Ligand Binding, P450 Reduction and Oxygen Activation**

References

P450 Homepage -http:// http://drnelson.uthsc.edu/CytochromeP450.html

Testa, B. The Biochemistry of Drug Metabolism: A 6 Part Series in *Chem. BioDivers.* (2006-2008).

Sligar, SG. Glimpsing the critical intermediate in cytochrome P450 oxidations. *Science.* 2010 Nov 12;330(006):924-5.

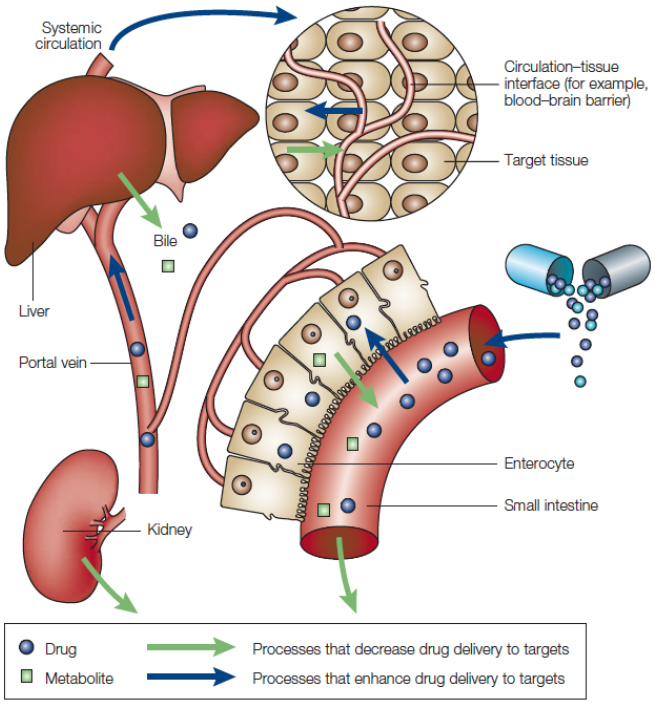
Johnson EF, et al. Correlating structure and function of drug metabolizing enzymes: Progress and ongoing challenges. *Drug Metab. Dispos.* 42:9-22 (2014).

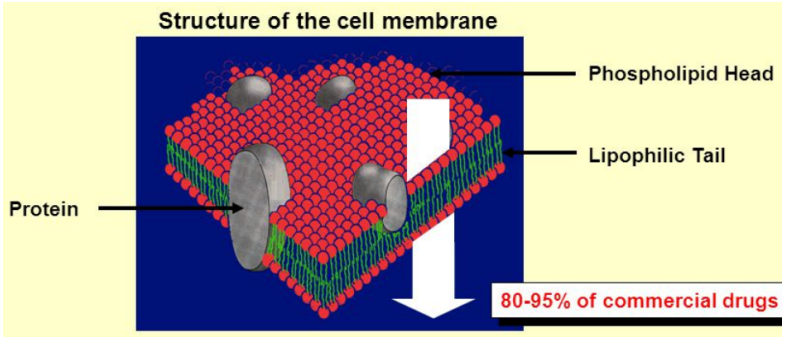
Zientek MA and Youdim K, Reaction phenotyping: Advances in experimental strategies used to characterize the contribution of drug metabolizing enzymes. *Drug Metab. Dispos.* 43:163-181 (2015).

Foti, S and Dalvie DK. Cytochrome P450 and non-cytochrome P450 oxidative metabolism: Contributions to the pharmacokinetics, safety and efficacy of xenobiotics. *Drug Metab. Dispos.* 44:1229-1245 (2016).

Manikandan P and Negini S. Cytochrome P450 structure, function and clinical significance. *Current Drug Targets* 19:38-54 (2018).

Absorption, Distribution, Metabolism and Excretion (ADME) of Orally Administered Drugs





To reach their sites of action in the body, orally administered drugs must be absorbed from the small intestine, survive first pass metabolism - typically in the liver - before eventually being excreted, usually as drug metabolites in the bile and kidney.

Therefore, clinically useful drugs must be able to cross an array of cell membranes, which are composed of a lipid bilayer. Drugs must exhibit an adequate degree of lipophilicity (logP of ~2-4) in order to able to dissolve into this lipoidal environment.

Many drug metabolism processes render lipophilic drugs more water-soluble so as to facilitate excretion via the kidneys and bile.

Most of these enzymes are found in either the microsomal or cytosolic fractions of the cell.

Cellular Location of Major Drug Metabolizing Enzymes

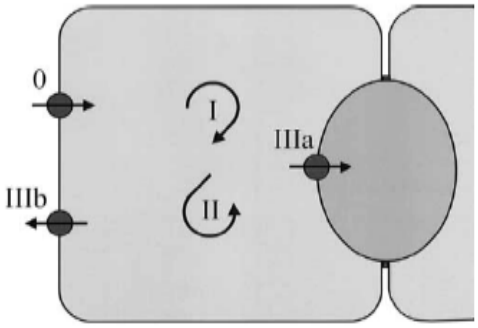
5,8 - **Endoplasmic Reticulum** ('microsomes') – P450, FMO, UGT, CES
11 - **Cytosol** – AO, SULT, NAT
9 - **Mitochondria** - MAO

https://en.wikipedia.org/wiki/Organelle

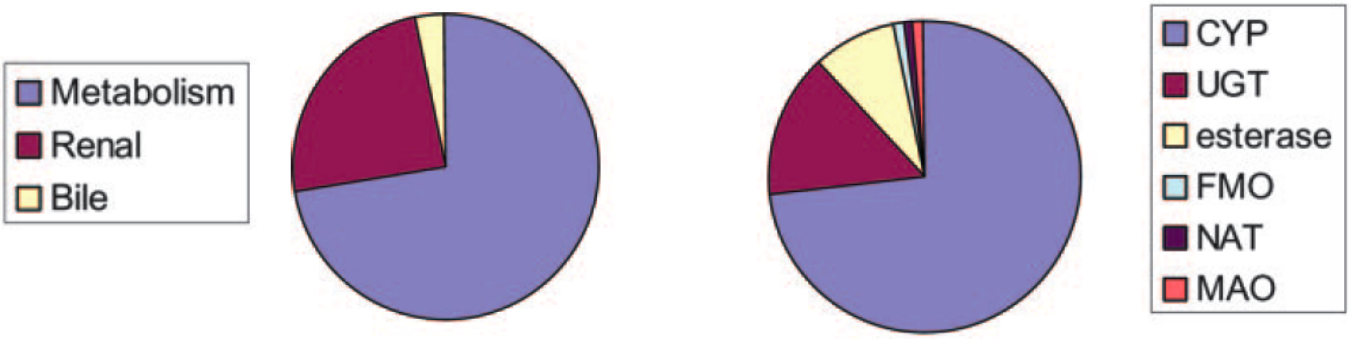
DRUG DISPOSITION PROCESSES : Role of P450s

Drug elimination is dominated by <u>metabolic processes</u>, which in turn are dominated by Phase I **cytochrome P450-mediated oxidative** metabolism.

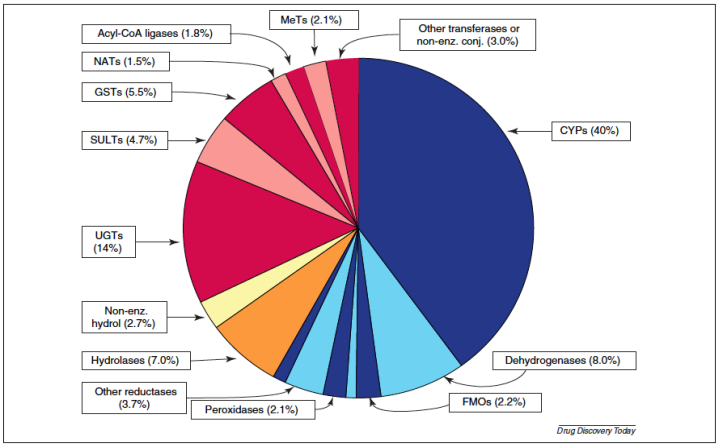
Phase 0 - Uptake
Phase I - Functionalization
Phase II - Conjugation
Phase III - Efflux

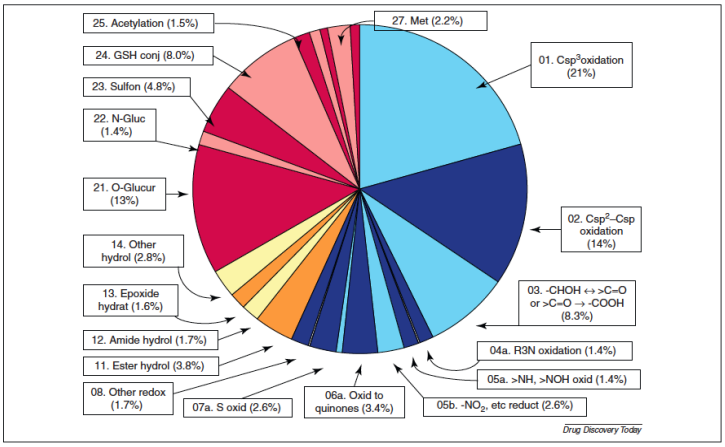


<u>Analysis of Top 200 Drugs (Williams 2004, Zanger, 2007)</u>



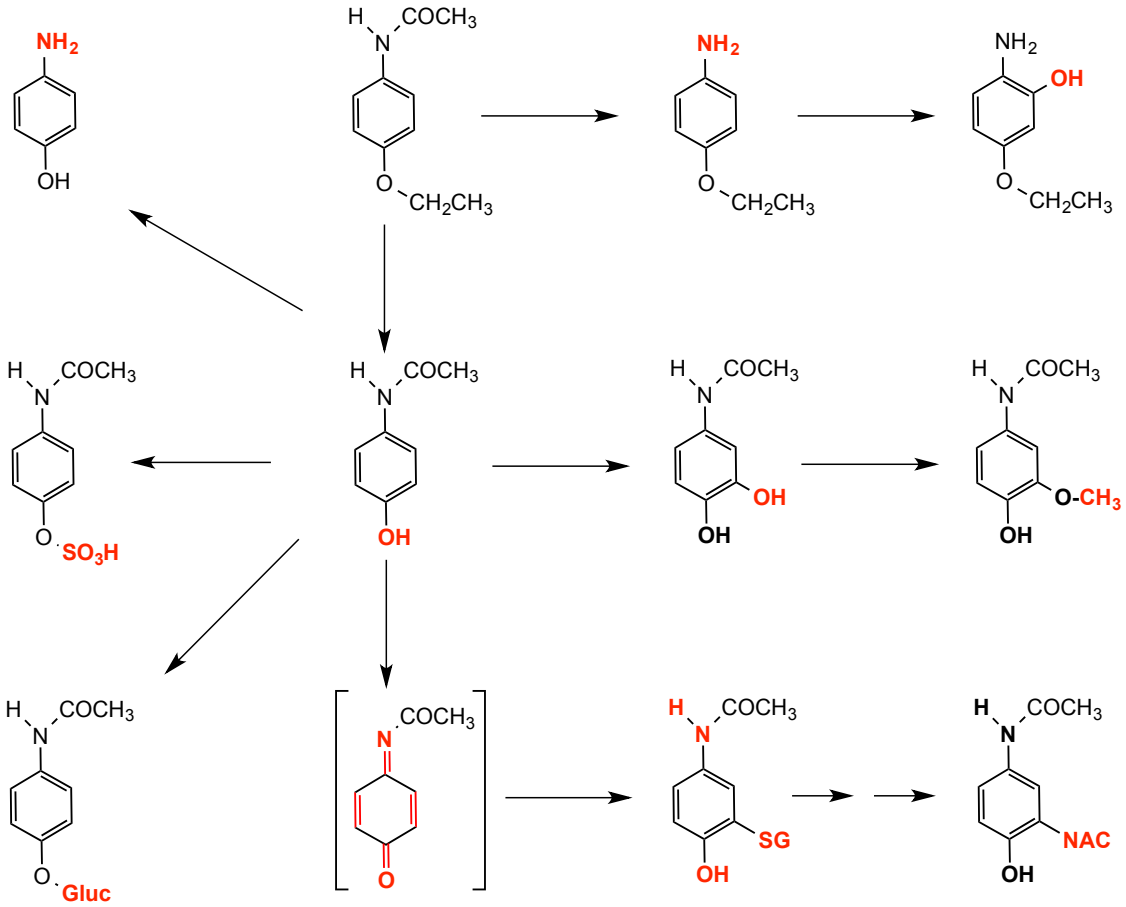
Analysis of ~7000 Metabolites (Testa, 2012)





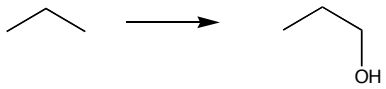
<u>Hydroxylations</u> and <u>Dealkylations</u> are the most common P450 reactions. They serve to decrease the lipophilicity of parent drug and enhance excretion.

Example of inter-connected metabolic pathways for the drug, phenacetin

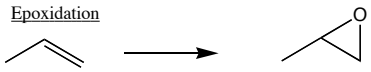


Common P450 reactions

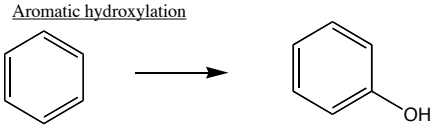
Aliphatic hydroxylation



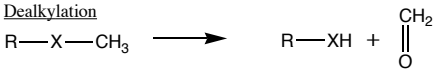
Epoxidation



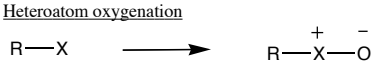
Aromatic hydroxylation



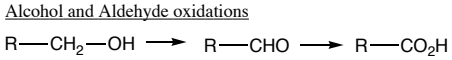
Dealkylation



Heteroatom oxygenation

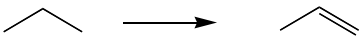


Alcohol and Aldehyde oxidations

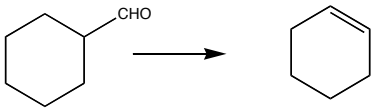


Uncommon P450 reactions

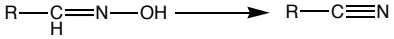
Dehydrogenation



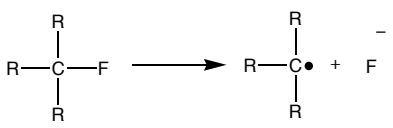
Deformylation



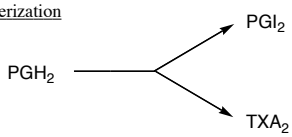
Dehydration



Reductive dehalogenation



Isomerization



P450s are the major oxidoreductase drug-metabolizing enzymes

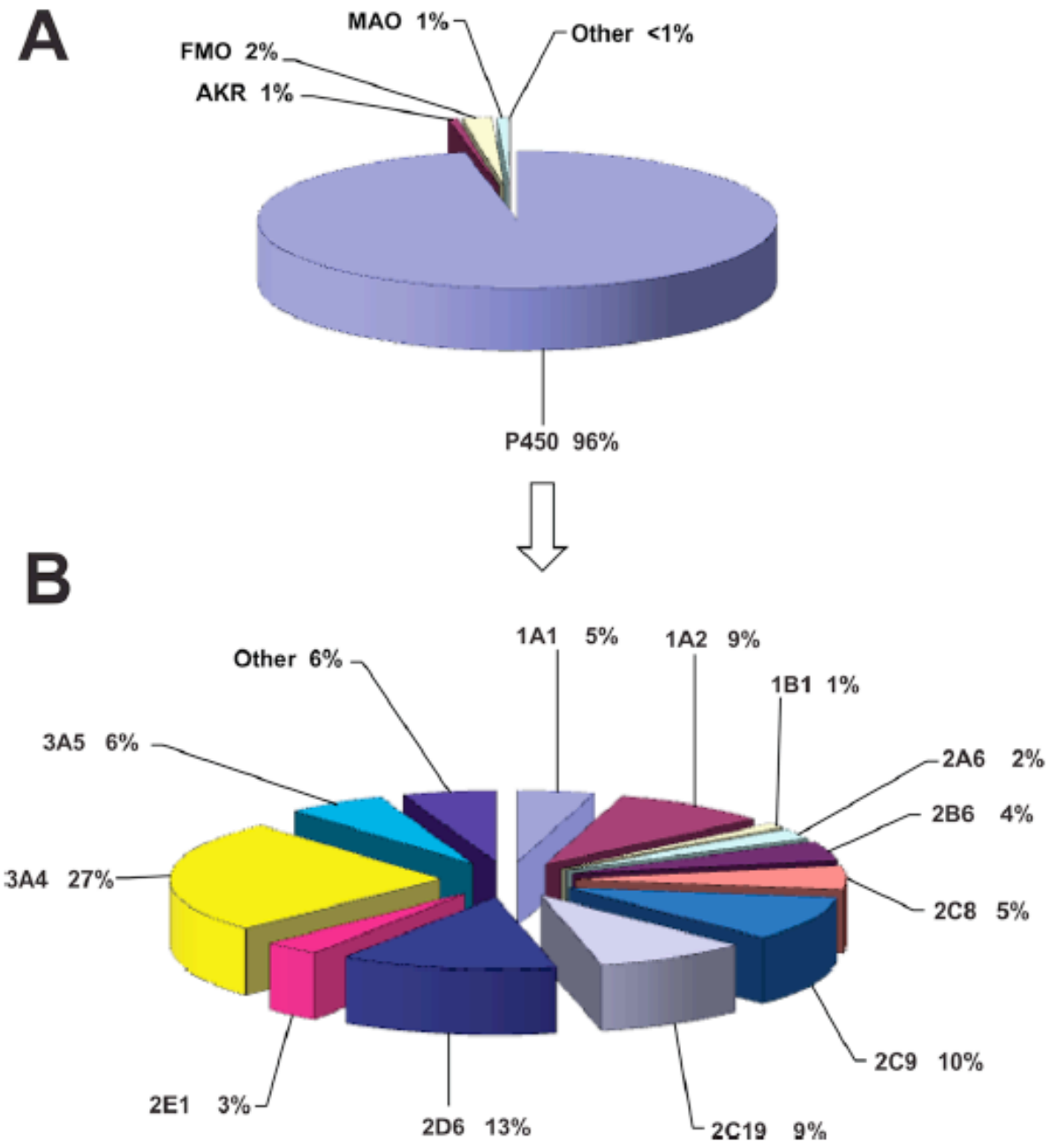


Figure 3. (A) Human oxidoreductases participating in the metabolism of drugs (calculation for drugs under development and marketed drugs). n = 4192 reactions; 860 drugs used in calculations. (B) Human P450 enzymes in the metabolism of drugs (data calculated for minor and major reactions, drugs under development, and marketed drugs). n = 4058 reactions; 860 drugs used in calculations.

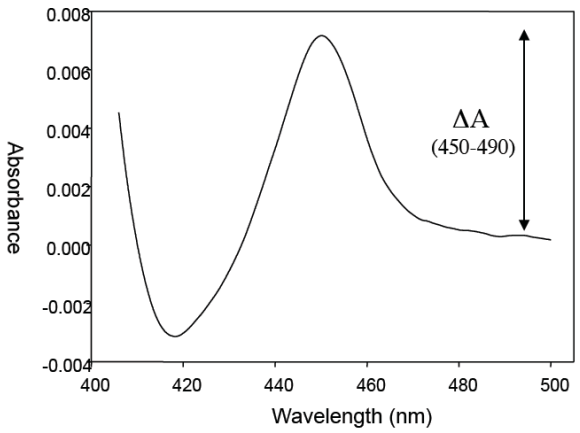
Cytochrome P450s - Basic characteristics

- Named for wavelength of maximal absorption of the Fe^{2+}-CO complex

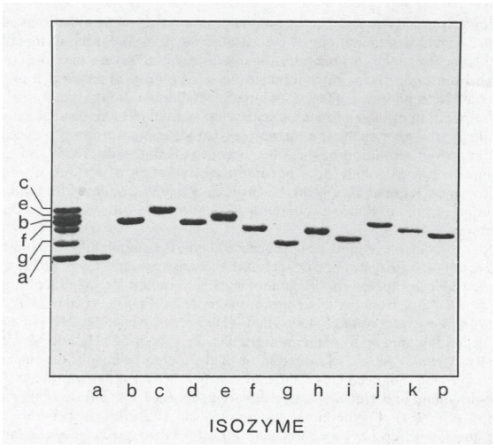
- Occurs at 450 ± 4 nm; ε ~ 100 mM cm^{-1}

- Superfamily of heme-containing oxygenases with MWt of 55 ± 5 kDa

- ~ 500 amino acids



Fe^{2+}-CO vs Fe^{2+} difference spectrum of P450



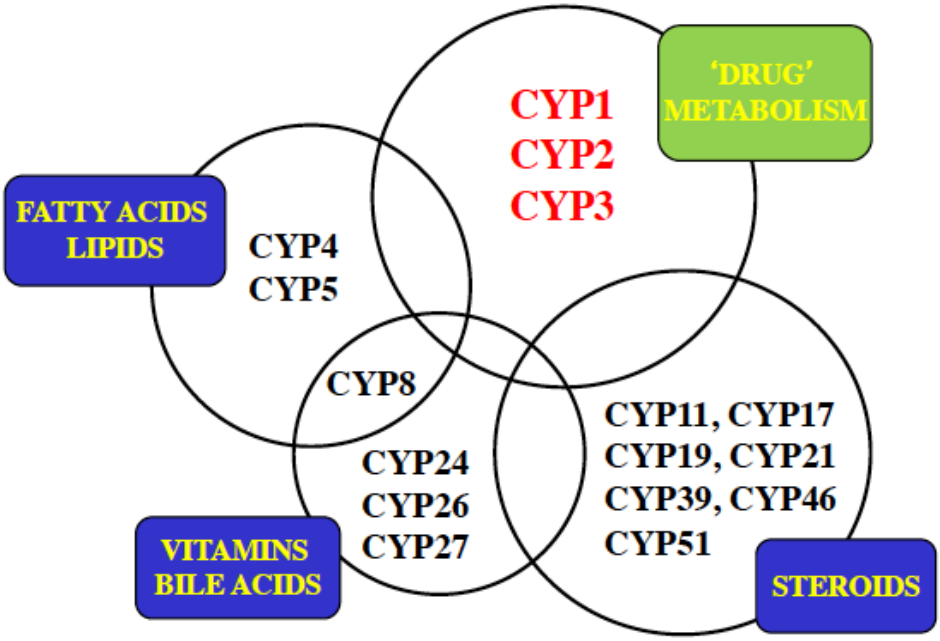
Purified P450s from Rat
(Levin et al., 1980)

Difference spectrum can be used to quantitate P450 by applying ε = 91 mM^{-1} cm^{-1} according to the relationship:

$$[P450]\ (\mu M) = \frac{\Delta A\ (450\text{-}490) \times 1000}{91 \times 1} = 0.007 \times 11 = 0.08\ \mu M$$

- P450s are ubiquitous in nature, >10000 named genes
- There are >200 P450 genes in rice, and in plants P450s often represent up to ~1% of the total genome.
- Humans have 57 full-length P450 genes, coding for 58 human P450 enzymes.
- The 58 functional human P450s are arranged into 18 families that are conserved in all mammals.
- Only 3 P450 families are important to hepatic drug clearance.

P450 SUBSTRATE CLASSES



- The CYP1, CYP2 and CYP3 P450 families are important to drug clearance.

XENOBIOTICS	***STEROLS/BILE ACIDS***	***FATTY ACIDS***	***'ORPHANS'***
CYP1A1	CYP1B1	CYP2U1	CYP2A7
CYP1A2	CYP11A1	CYP2J2	CYP2S1
CYP2A6	CYP11B1	CYP4A11	CYP2W1
CYP2A13	CYP11B2	CYP4F2	CYP3A43
CYP2B6	CYP17A1	CYP4F3	CYP4B1
CYP2C8	CYP19A1	CYP4F8	CYP4A22
CYP2C9	CYP21A2	CYP4F22	CYP4F11
CYP2C18	CYP51A1	CYP4Z1	CYP4V2
CYP2C19	CYP7A1	CYP5A1	CYP4X1
CYP2D6	CYP7B1	CYP8A1	CYP20A1
CYP2E1	CYP8B1		
CYP2F1	CYP27A1	***VITAMINS A/D***	
CYP3A4	CYP39A1	CYP2R1	
CYP3A5	CYP46A1	CYP24A1	
CYP3A7		CYP26A1	
CYP4F12		CYP26B1	
		CYP26C1	
		CYP27B1	
		CYP27C1	

P450 TAXONOMY - Basic Nomenclature Rules:

- When describing a P450 gene, *CYP1A2* for example, *CYP* is italicized and designates the gene as a segment coding for **cy**tochrome **P**450. The first arabic numeral designates the P450 family. This is followed by a capital letter designating the subfamily, and another arabic numeral to distinguish members within a subfamily.

- When describing the gene product, either CYP or P450 can be used in front of the family designation; for example, CYP1A2.

- P450 'isoforms' are assigned to specific families on the basis of amino acid sequence homology. **The P450 protein sequences within a given family are >40% identical** (some exceptions exist).

- **P450 sequences within the same sub-family are > 55% identical.** The degree of homology for distinct gene products from the same sub-family varies between 55 and >98% - e.g. human CYP2C9 and CYP2C19 sequences are 92% identical, but the two enzymes have distinct substrate selectivities.

- When considering genetic variants of a P450 gene, an asterisk is placed after the arabic numeral for sub-family designation, and each allelic form is assigned an arabic number, e.g. *CYP2C9*2* represents the first allelic form of this gene discovered (relative to the reference sequence which usually has the *1 designation).

- Homologous P450s are related genes that can be identified on the basis of sequence similarity alone, e.g. human CYP2C9, rat CYP2C11 and monkey CYP2C43. They likely evolved from a common ancestor before species divergence.

- Orthologous P450s are related gene products that maintain functional similarities. Examples of P450 species orthologs are the CYP2E1 enzymes found in the rat, rabbit, monkey, and human, - all of which have very similar catalytic properties. In contrast, it is often difficult to identify species orthologs to the human CYP2C isoforms.

How much of each of the major P450s is present in human liver and intestine microsomes? [Data from Sim-CYP]

LIVER

Sim-Healthy Volunteers — Sim-CYP

Enzyme Abundances (pmol/mg-protein) and Turnover Rate Constants (1/h)

	EM		PM		IM		UM		Turnover	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
CYP1A2	52	67	0	0	0	0	0	0	0.0183	56
CYP2A6	20	173	0	0	0	0	0	0	0.0267	56
CYP2B6	17	122	6	200	0	0	0	0	0.0217	56
CYP2C8	24	81	0	0	0	0	0	0	0.0301	56
CYP2C9	73	54	29	73	0	0	0	0	0.0067	56
CYP2C18	1	106	0	0	0	0	0	0	0.0267	56
CYP2C19	14	106	0	0	0	0	0	0	0.0267	56
CYP2D6	8	61	0	0	0	0	16	61	0.0099	56
CYP2E1	61	61	0	0	0	0	0	0	0.0176	63
CYP2J2	1.2	175	0	0	0	0	0	0	0.0194	56
CYP3A4	137	41	0	0	0	0	0	0	0.0193	68
CYP3A5	103	65	0	0	0	0	0	0	0.0193	68
CYP3A7	35.4	61	0	0	0	0	0	0	0.019	68

INTESTINE

Sim-Healthy Volunteers

Intestine | Colon

Enzyme Abundances (nmol/small intestine) and Turnover Rate Constants (1/h)

	EM		PM		IM		UM		Turnover	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
CYP2C9	12.9	60	3.8	60	0	0	0	0	0.03	20
CYP2C19	1.5	60	0	0	0	0	0	0	0.03	20
CYP2D6	0.8	60	0	0	0	0	1.6	60	0.03	20
CYP2J2	1.4	60	0	0	0	0	0	0	0.03	20
CYP3A4	66.2	60	0	0	0	0	0	0	0.03	20
CYP3A5	24.6	60	0	0	0	0	0	0	0.03	20

- A P450 enzyme can be present at relatively low amounts in human liver microsomes, yet still provide a large contribution to overall drug metabolism e.g. CYP2D6.
- CYP2E1 (the ‘solvent P450’) is an example of the converse - high levels in human liver, but metabolizes few drugs.

HUMAN LIVER P450 CHEATSHEET

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm

Typical amount of total spectral P450 in human liver microsomes is 300-500 pmol/mg.

CYP3A4

- A major constitutive isoform in human liver and intestine, responsible for the metabolism of up to 50% of all drugs that are cleared by oxidative processes
- Highly inducible form of P450 (e.g. by rifampin, phenytoin, phenobarbital)
- Key drug substrates include midazolam, lovastatin, alfentanil, nifedipine, R-warfarin, lidocaine, quinidine, carbamazepine, ethynyl estradiol, erythromycin
- **Marker reactions**: midazolam 1'-hydroxylation, testosterone 6 alpha-hydroxylation,
- **Inhibitors**: CYP3cide, SR-9186, azamulin (ketoconazole, itraconazole)
- **Activator**: alpha-naphthoflavone

CYP3A5

- Present at significant levels in humans in only ~15% of the adult Caucasian population due to genetic polymorphism.
- Similar, albeit slightly distinct substrate specificity to CYP3A4.
- **Marker reaction:** midazolam 1'-hydroxylation
- **Inhibitor:** ketoconazole (all azoles typically weaker inhibitors than for CYP3A4)

CYP2D6

- Relatively uninducible form that prefers to metabolize basic drugs.
- Highly polymorphic, > 60 alleles known.
- Key substrate classes, beta-blockers, many CNS drugs.
- **Marker reaction:** dextromethorphan O-demethylation
- **Inhibitor:** quinidine

CYP2C9

- Major form, prefers to metabolize mildly acidic drugs
- Key substrates: phenytoin, tolbutamide, S-warfarin.
- **Marker reaction:** S-warfarin 7-hydroxylation, diclofenac 4'-hydroxylation
- **Inhibitor**: sulfaphenazole, benzbromarone **Activator**: dapsone

CYP2C19

- Important polymorphic isoform, prefers basic or neutral substrates
- Key substrates: omeprazole, citalopram, proguanil.
- **Marker reaction:** (S)-mephenytoin 4'-hydroxylation
- **Inhibitor:** (S)-benzylnirvanol

CYP2C8

- Key substrates: taxol, carbamazepine, some overlap with CYP3A4
- **Marker reaction:** paclitaxel 6-alpha-hydroxylation, amodiaquine de-ethylation.
- **Inhibitor:** montelukast

CYP2B6

- Highly inducible
- Key substrates: bupropion, efavirenz, propofol, cyclophosphamide
- **Marker reaction :** bupropion hydroxylation
- **Inhibitor:** thiotepa, (clopidogrel, 2-phenyl-2-(1-piperdinyl)propane)

CYP2A6

- Key substrates: coumarin, nicotine and several tobacco smoke carcinogens. Some overlap with CYP2E1 substrates
- **Marker reaction:** coumarin 7-hydroxylation
- **Inhibitor:** 8-methoxypsoralen, tranylcypromine

CYP1A2

- Inducible by cigarette smoke and polycyclic aromatic hydrocarbons
- Key substrates: caffeine, theophylline, phenacetin, several some pro-mutagens (2-acetylaminofluorine and by-products of charcoal broiled meats)
- **Marker reaction:** caffeine N-3 demethylation, phenacetin O-deethylation
- **Inhibitor:** furafylline

CYP2E1 .

- Inducible by ethanol
- Key substrates: ethanol, acetaminophen, volatile anesthetics (enflurane and sevoflurane), and a myriad of organic solvents.
- **Marker reaction:** chlorzoxazone 6-hydroxylation
- **Inhibitor:** diethyl dithiocarbamate (disulfiram metabolite)

Summary - Diagnostic Substrates and Inhibitors (in vitro primarily)

[1]nM Ki [2]Mechanism-based

Isoform	Typical substrate	Inhibitor
1A2	Caffeine	Furafylline[2]
2A6	Coumarin	8-Methoxypsoralen[2]
2B6	Bupropion	2-Phenyl-2-(1-piperdinyl)propane[2]
2C8	Amodiaquine	Montelukast[1]
2C9	Flurbiprofen, (S)-Warfarin	Sulfaphenazole[1]
2C19	(S)-Mephenytoin	(S)-Benzylnirvanol[1]
2D6	Dextromethorphan	Quinidine[1]
2E1	Chlorzoxazone	Disulfiram[2]
3A4/5	Midazolam	Ketoconazole[1], TAO[2]
		CYP3cide[2], SR-9186 (both 3A4)