Drug Metabolizing Enzymes and Reaction-Phenotyping

Carl D. Davis, Ph.D.
Pharmaceutical Candidate Optimization
Metabolism and Pharmacokinetics
Bristol-Myers Squibb

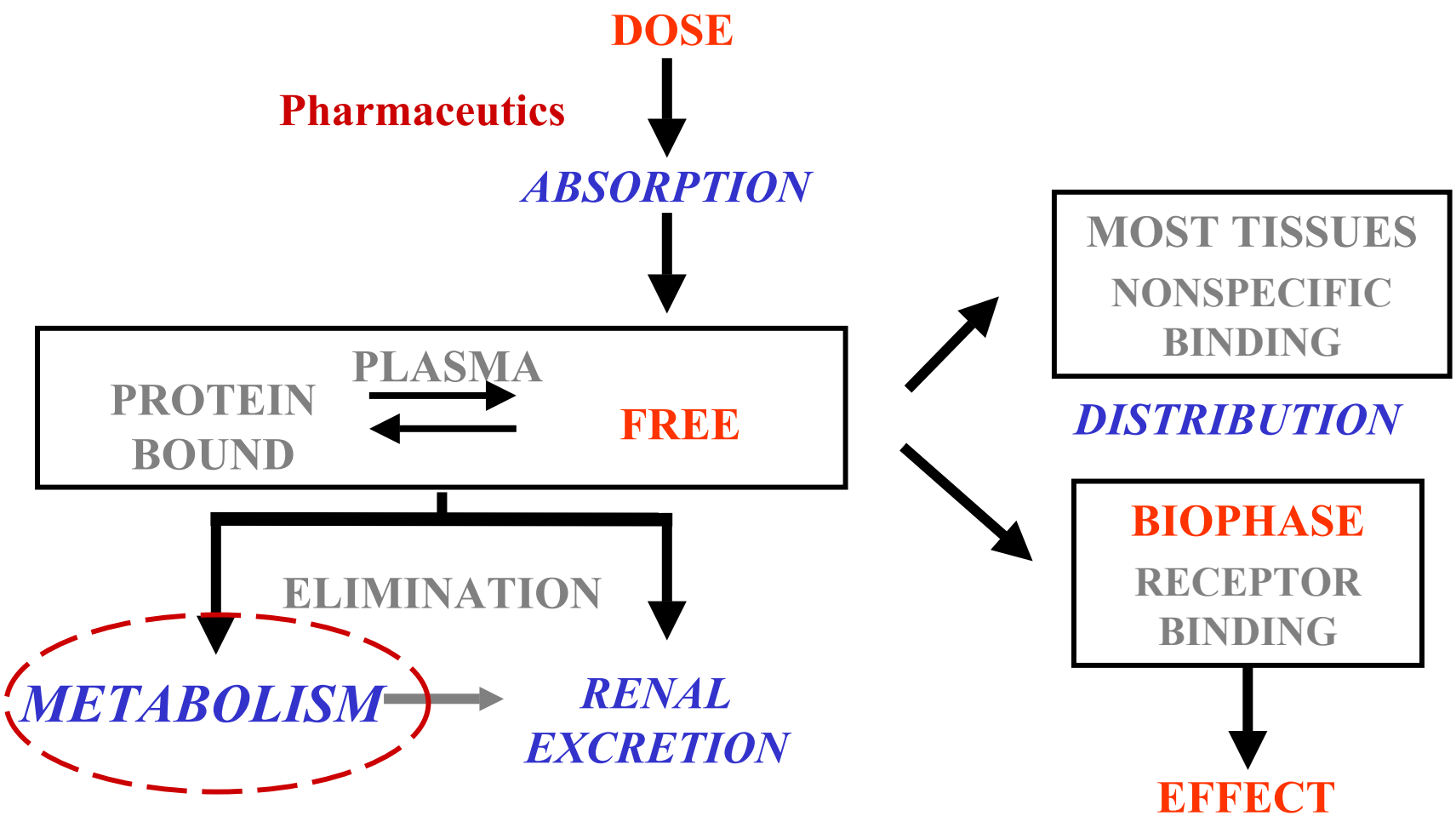
Pharmaceutical Research Institute
Wallingford, CT
carl.davis@bms.com

Presentation

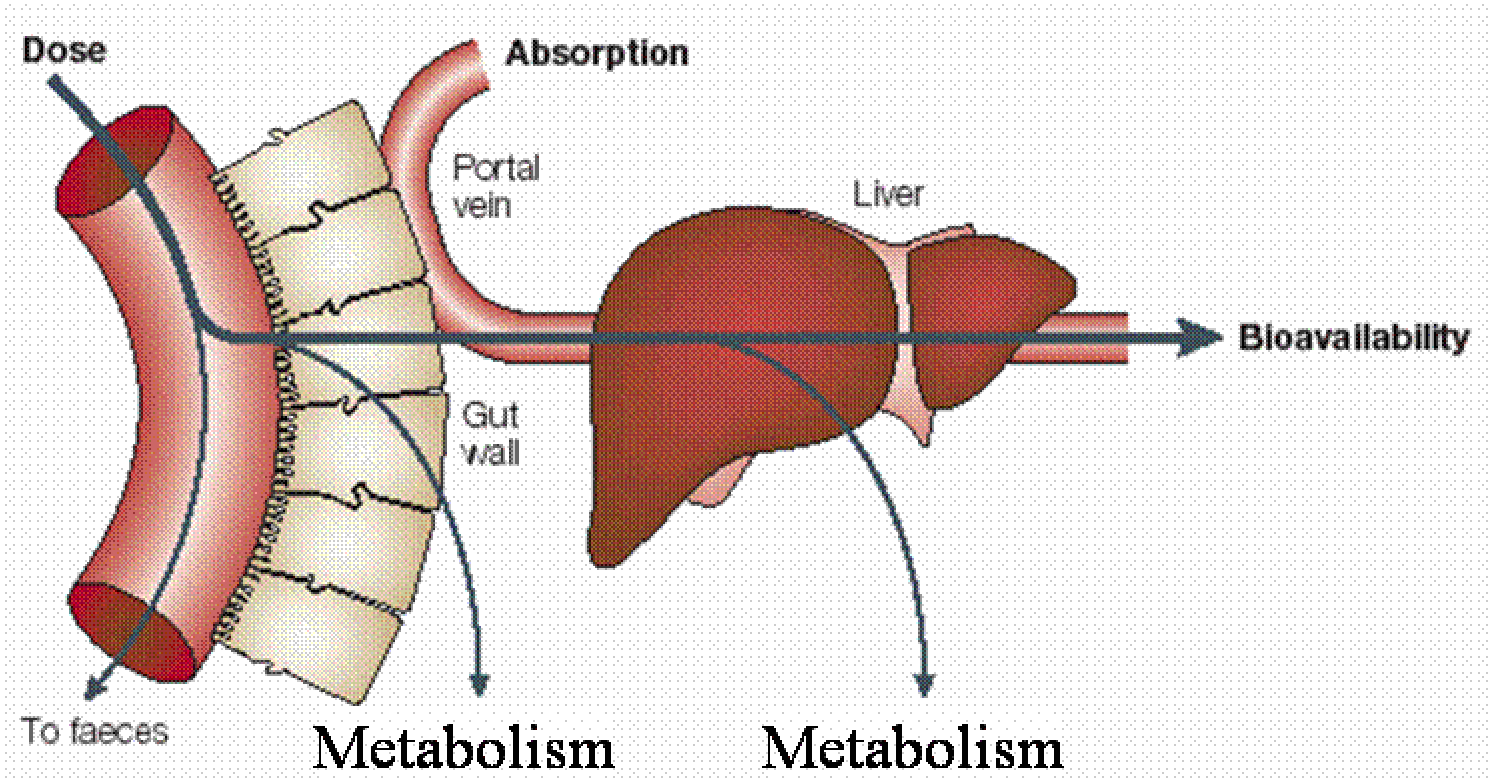
- Introduction
- Drug metabolizing enzymes
- Individual and species differences in drug metabolism
- Reaction-Phenotyping methods

The Pharmaceutical R&D Collaboration

The Fate of a Drug



Drug Metabolism



Drug Metabolism

• Drug metabolism can occur in every tissue (e.g. gut, lung and kidney). However, the major drug metabolizing enzymes (DMEs) are expressed at the highest levels in the **liver**, which thus serves as the major organ of metabolic clearance

• Drug metabolism serves to control the exposure of a potentially harmful substance. Usually via oxidation of a lipophilic xenobiotic, DMEs increase the polarity and aqueous solubility thus facilitating its elimination from the body

• DMEs also help to regulate endogenous function (e.g. cytochrome P450s are involved in steroid and fatty-acid metabolism; and the glucuronosyl-S-transferase, UGT1A1, is involved in the clearance of bilirubin)

Drug Metabolism

Factors affecting drug metabolism:

- Tissue differences
- Genetics
- Species differences
- Co-administered substrates (inhibitors or inducers)
- Auto-induction
- Diet
- Disease (especially hepatic or renal)
- Protein-binding
- Age
- Gender
- Route of administration

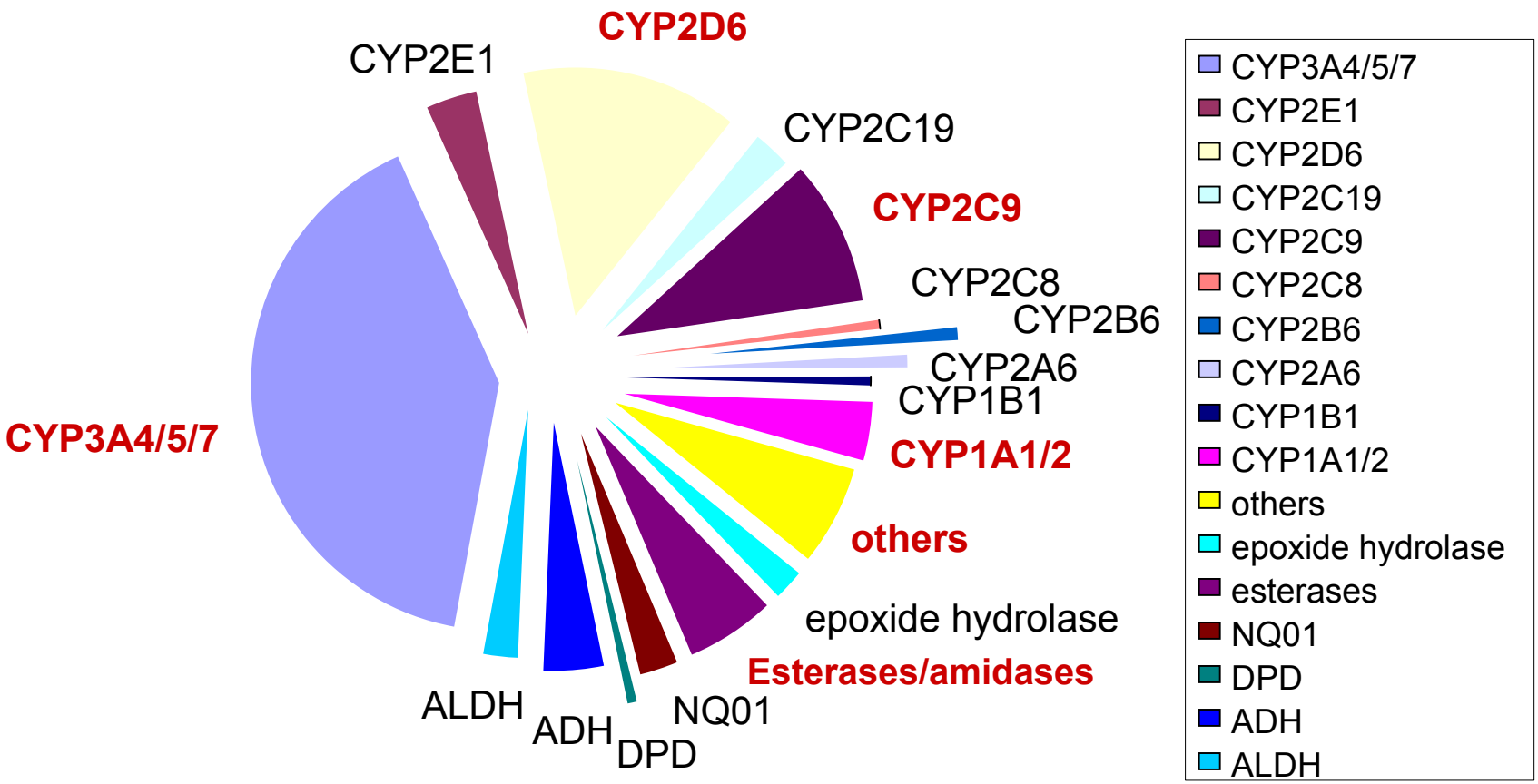
Drug Metabolism

DMEs broadly classified into two types of reactions (see Biotransformation lectures):

• PHASE I: typically a functional group (e.g. hydroxyl) is created or exposed in a drug molecule

• PHASE II: conjugation of either the parent compound and/or its metabolite(s) involving a polar endogenous substrate that is able to react with the functional groups formed via Phase I reactions

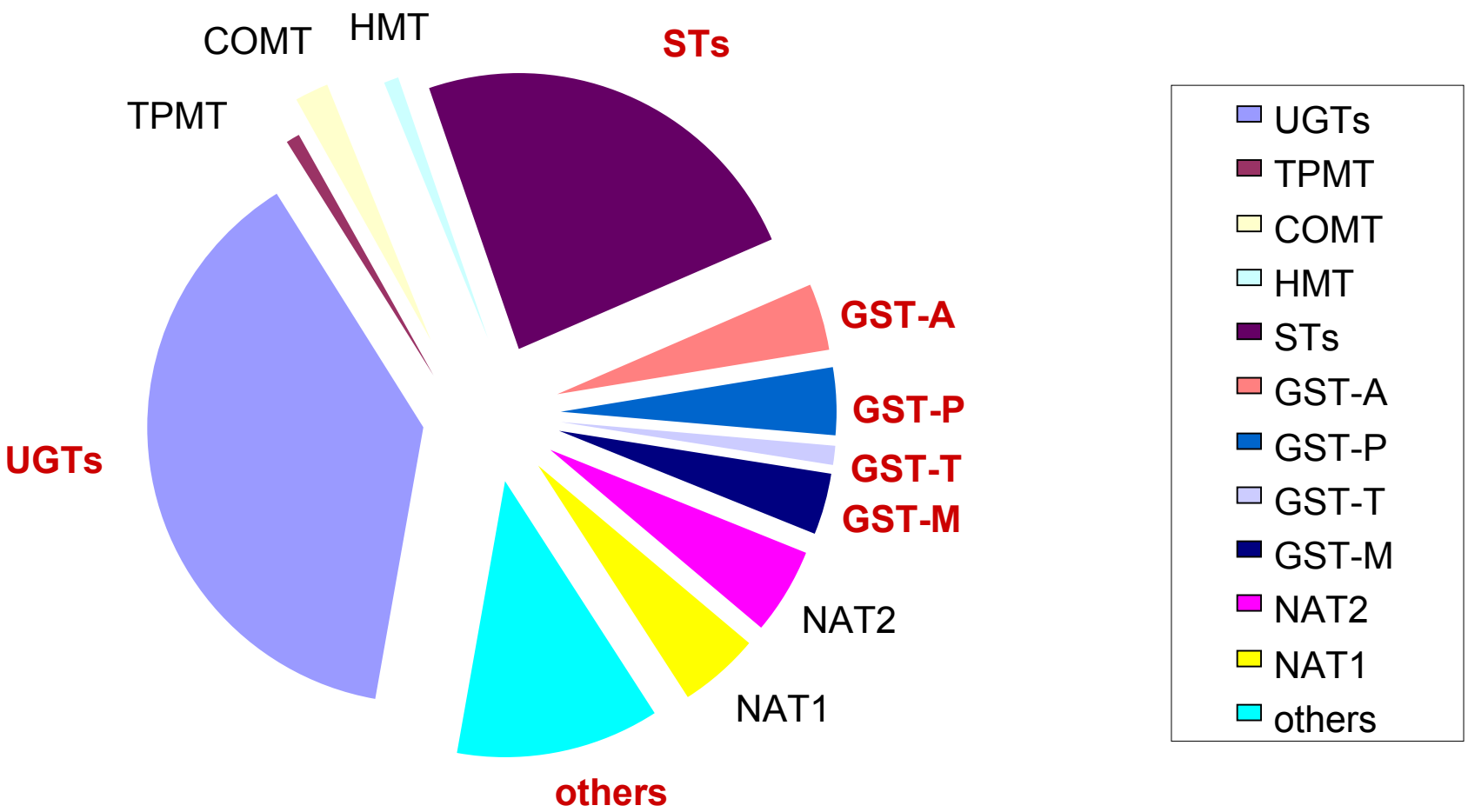
Human Phase I Enzymes of Drug Metabolism



CYP: cytochrome P450, NQ01: NADPH:quinone oxidoreductase (DT diaphorase); DPD: dihydropyrimidine dehydrogenase; ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase

Evans and Relling, Science (1999)

Human Phase II Enzymes of Drug Metabolism



HMT: histamine methyltransferase; TPMT: thiopurine methyltransferase; COMT: catechol O-methyltransferase; UGT: Uridine Glucuronosyl-S-Transferases; ST: Sulfotransferase; GST: Glutathione-S-Transferases

Evans and Relling, Science (1999)

Drug Clearance

A typical drug exhibits the following characteristics:

- Cytochrome P450-mediated clearance
  - 55 % (90% of Phase I metabolism is CYP mediated)
- Unchanged drug (i.e. non-metabolic clearance)
  - 25 % (urine, bile, expired air, faeces)
- Other metabolism
  - 20 % (UGT, ST, MAO, AO, FMO etc)

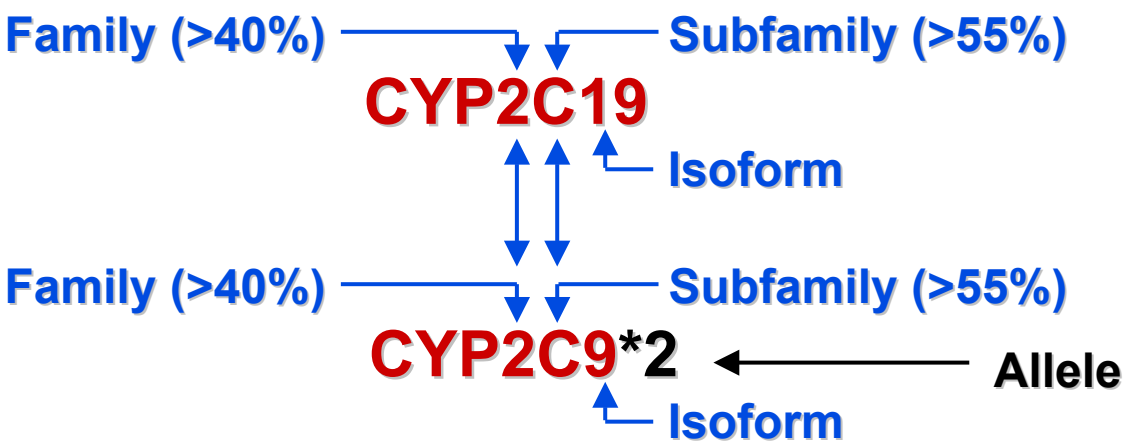
Clearance is the sum process of all in vivo elimination pathways

Any one pathway can dominate (...case-by-case analysis)

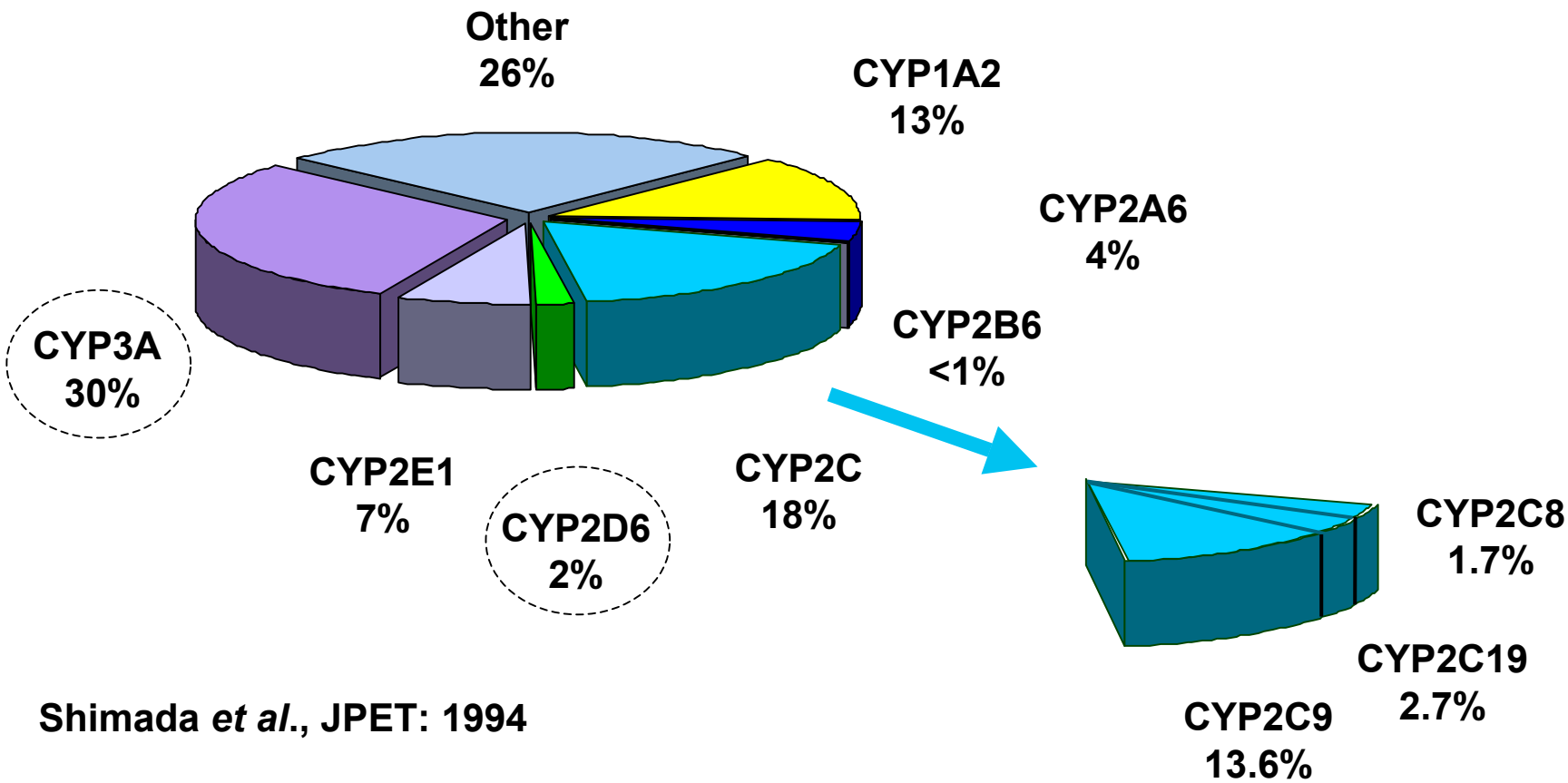
Cytochrome P450 (CYP) Enzymes

- A "super-family" of enzymes with a very broad substrate selectivity

- CYP nomenclature is based on shared homology of amino acid sequence (currently 17 families and over 50 isoforms identified in the human genome)



Relative Amounts of Individual Human Hepatic CYPs

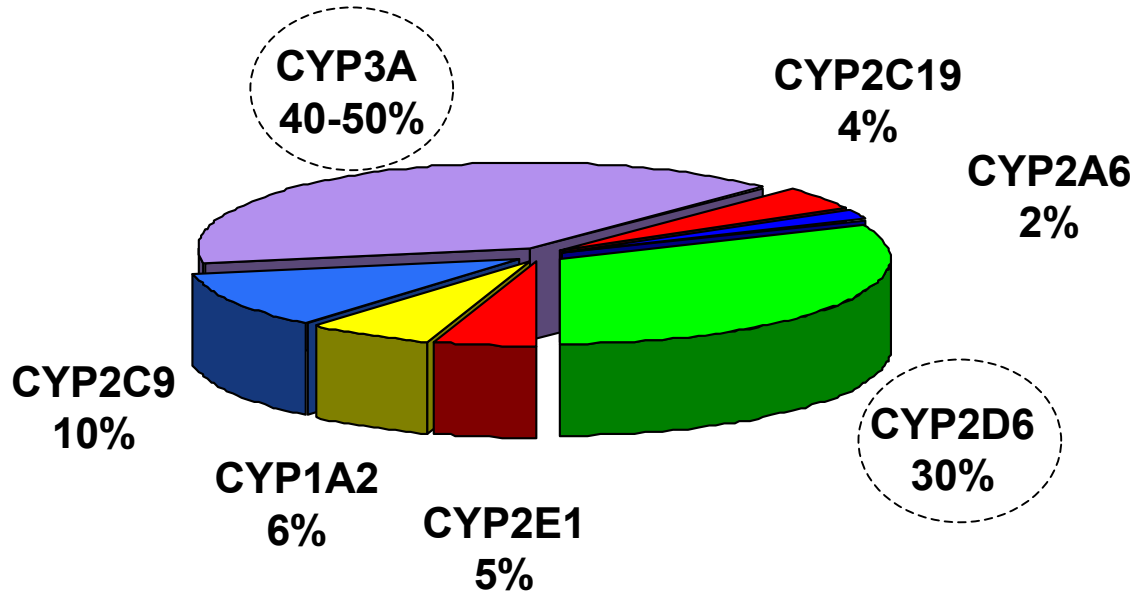


Shimada *et al.*, JPET: 1994

Lasker *et al.*, Arch. Bioch. Biophys:1998

Human Cytochromes P450 and their Relative Contribution to Hepatic Drug Metabolism

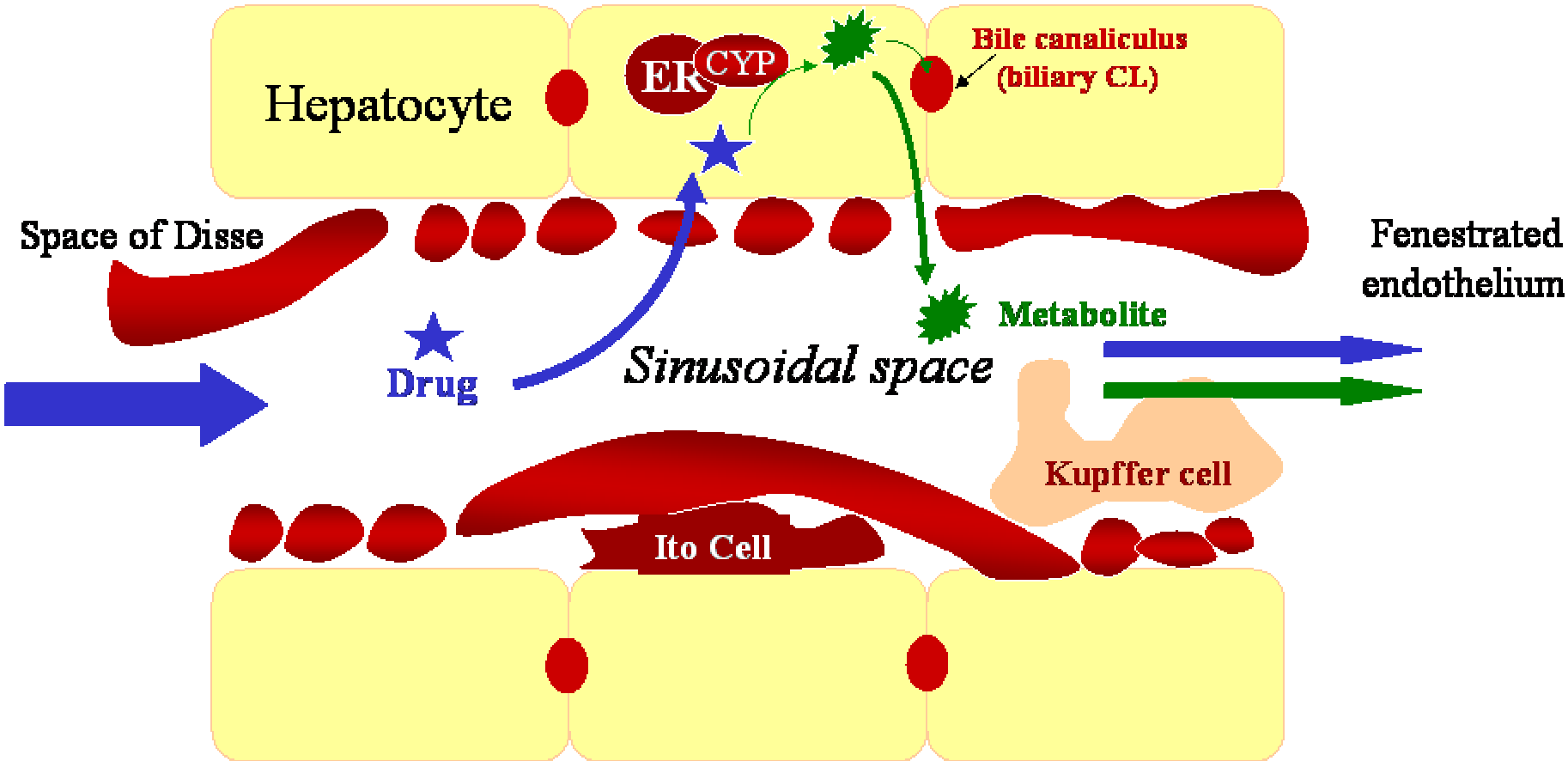
Shimada *et al*., JPET: 1994



60% of drugs are metabolized primarily by CYPs

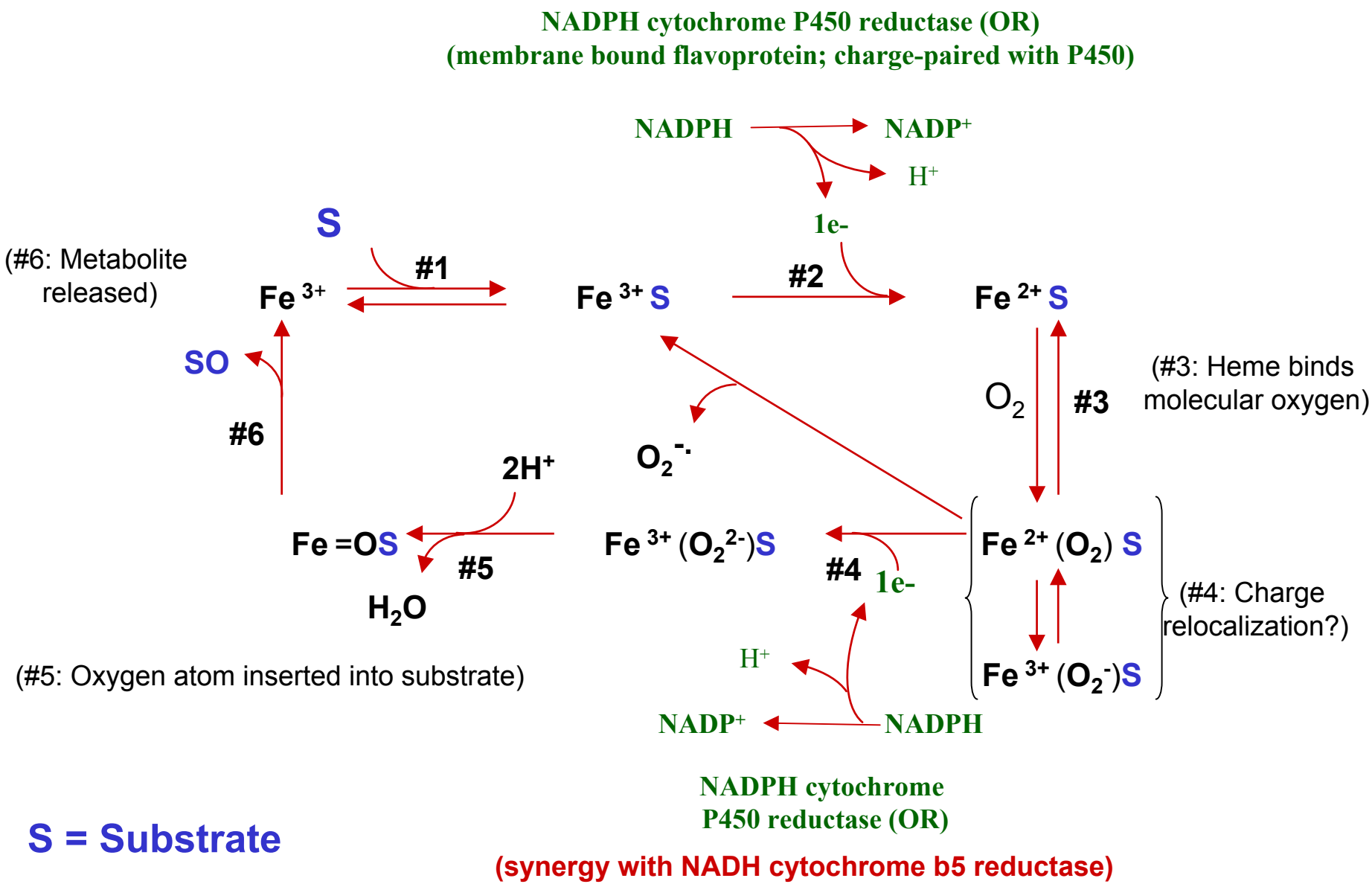
(Bertz & Granneman, Clin. PK: 1997)

Hepatic Metabolism



CYPs are found in the smooth endoplasmic reticulum (ER). Hepatocytes contain the full complement of the major DMEs including cytosolic (e.g. Sulfotransferases, Aldehyde Dehydrogenase, Xanthine Oxidase), membrane-bound (CYPs, UGTs, FMOs) and mitochondrial (e.g. MAOs)

Cytochrome P450 Mechanism



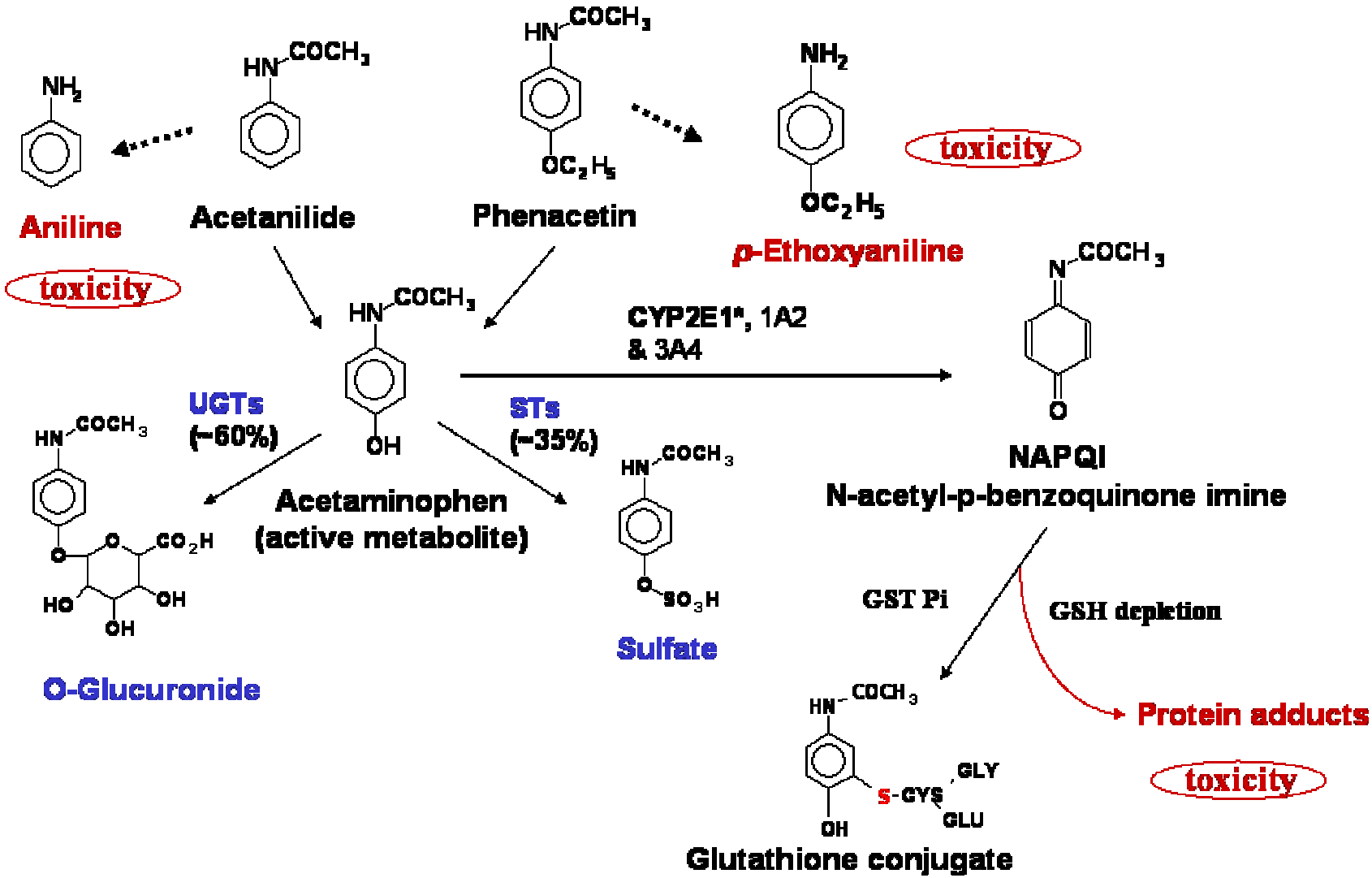
S = Substrate

Substrates, Inducers & Inhibitors of Human CYPs

	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Substrates	Caffeine Imipramine Tacrine Theophylline R-warfarin	Bupropion Midazolam Tamoxifen Verapamil Testosterone	Diclofenac Losarten Phenytoin Tolbutamide S-warfarin	Omeprazole Phenytoin Indomethacin R-warfarin	Bufuralol Codeine Desipramine Lidocaine	Acetaminophen Ethanol Chlorzoxazone Sevoflurane	Nifedipine Erythromycin Midazolam Testosterone
Inhibitors	Ciprofloxacin Furafylline Mibefradil Ticlopidine	Ketoconazole Tranylcypromine Troglitazone Orphenadrine	Fluconazole Isoniazid Sulfaphenazole Paroxetine	Cimetidine Ketoconazole Paroxetine Ticlopidine	Quinidine Methadone Cimetidine Fluoxetine	Disulfiram	Ketoconazole Erthyromycin Grapefruit juice Ritonavir
Inducers	Insulin Omeprazole (Cruciferous vegetables) (Char-grilled meat) (Tobacco)	Dexamethasone Phenobarbital Rifampin Sodium valproate	Rifampin Secobarbital	Prednisone Rifampin	None identified	Ethanol Isoniazid (Starvation)	Carbamazepine Phenobarbital Phenytoin Rifampin

A comprehensive list can be found at: http://medicine.iupui.edu/flockhart/table.htm

Biotransformation-Phenotyping: Phase I & II DMEs



The metabolites identified and/or specific functional groups (e.g. $-NH_2$, -OH) can help direct drug metabolism studies to look at atypical enzymes

Non-CYP Drug Metabolizing Enzymes (I)

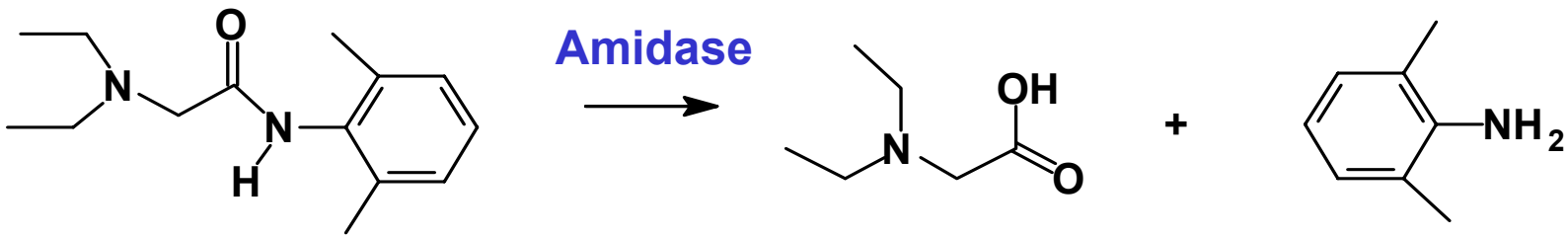
Non-CYP Oxidations

- Monoamine Oxidase (MAO; mitochondrial)
  - oxidatively deaminates endogenous substrates including neurotransmitters (dopamine, serotonin, norepinephrine, epinephrine); drugs include triptans
- Alcohol & Aldehyde Dehydrogenase (non-specific enzymes; liver cytosol)
  - ethanol metabolism
- Xanthine Oxidase (XO)
  - converts hypoxanthine to xanthine, and then to uric acid (drugs include theophylline, 6-mercaptopurine. Allopurinol is a substrate and inhibitor of xanthine oxidase
- Flavin Monooxygenases (FMOs; membrane-bound & NADPH-dependent)
  - catalyze oxygenation of nitrogen, phosphorus, sulfur; particularly facile formation of N-oxides (e.g. cimetidine)
- Many others: e.g. O-Methylation, S-Methylation, Amino Acid Conjugation: glycine, taurine, glutathione
  - metabolites or functional groups offer clues to the likely enzyme involved

Non-CYP Drug Metabolizing Enzymes (II)

Esterase Reactions: e.g. aspirin (others include procaine, clofibrate)

Amidase Reactions: e.g. lidocaine (others include peptides)



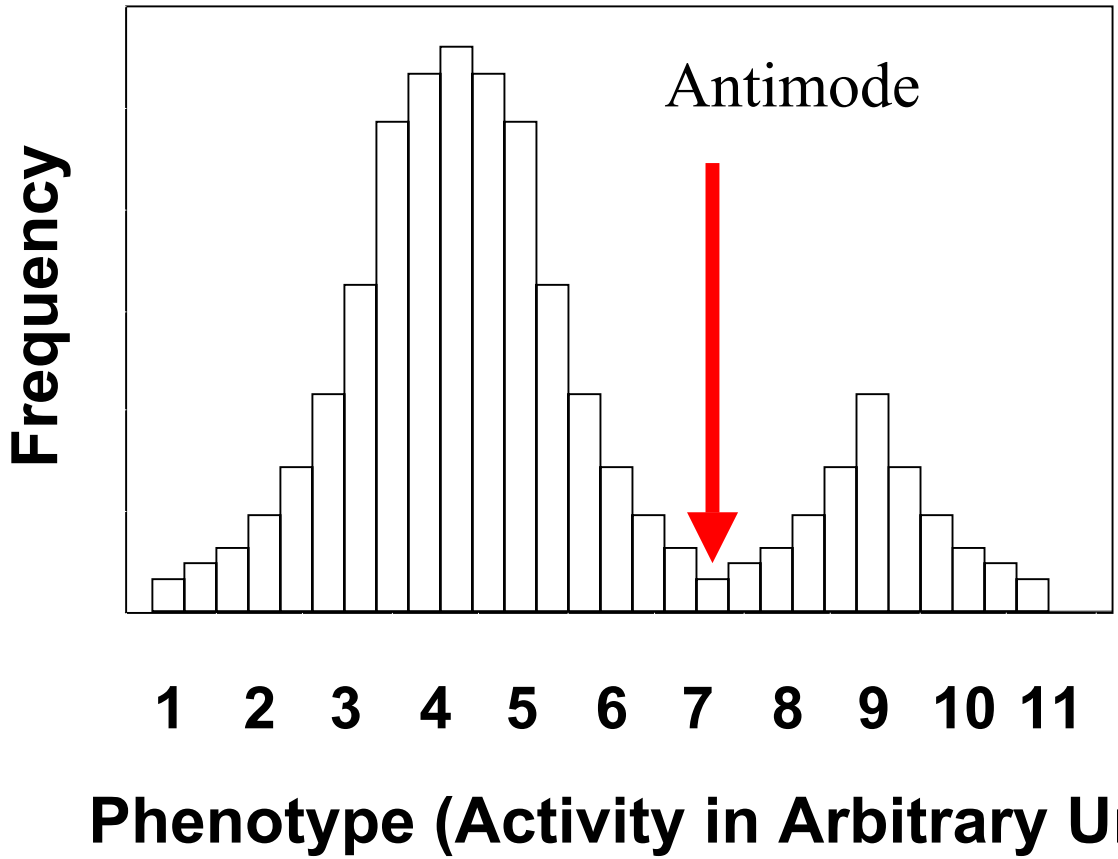
N-Acetylation: e.g. dapsone (also procainamide, isoniazid)

NAT-2 is a detoxication pathway (CYP N-hydroxyltaion pathway leads to methaemoglobinaemia)

Polymorphisms in Drug Metabolizing Enzymes

Polymorphic Distribution

Simple bimodal distribution



A trait with differential expression in >1% of the population

Frequency of CYP Polymorphic Phenotypes

Estimated Frequency Of CYP Polymorphism in Humans					
Slow Metabolizers	**2A6**	**2B6**	**2C9**	**2C19**	**2D6**
African Americans	2%		1%	4-7%	6-8%
Caucasians	20%	3-4%	1-20%	2-4%	7-10%
Asians			1%		1-2%
Japanese			1%	18-23%	
Chinese				5-17%	

Ultrafast Metabolizers	**2A6**	**2B6**	**2C9**	**2C19**	**2D6**
Africans					29%
Asians					1%
Caucasians					
- Southern Europeans					10%
- Northern Europeans					1-2%

(divers sources)

Complexities of Genetic Polymorphisms

Roche Diagnostics AmpliChip CYP450 Test - Predicted Phenotype

CYP2C19

Allele	1	3	3
1	E	E	E
2		P	P
3			P

CYP2D6

Allele	1	2	3	4	5	6	7	8	9	10	11	14A	14B	15	17	19	20	25	26	29	30	31	35	36	40	41	1XN	2XN	4XN	10XN	17XN	35XN	41XN
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
2		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
3			P	P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
4				P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
5					P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
6						P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
7							P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
8								P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
9									I	I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
10										I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
11											P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14A												P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14B													N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
15														P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
17															I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
19																P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
20																	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
25																		N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
26																			N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
29																				I	N	N	E	I	I	I	E	E	I	I	I	E	I
30																					N	N	E	N	N	N	N	N	N	N	N	N	N
31																						N	E	N	N	N	N	N	N	N	N	N	N
35																							E	E	E	E	U	U	E	E	E	U	E
36																								I	I	I	E	E	I	I	I	E	I
40																									P	I	E	E	P	I	I	E	I
41																										I	E	E	I	I	I	E	I

E	Extensive
I	Intermediate
P	Poor
U	Ultrarapid
N	Unknown

Possess at least one, and no more than two, normal functional alleles
Possess one reduced activity allele and one null allele
Carry two mutant alleles which result in complete loss of enzyme activity
Usually carry multiple copies (3-13) of functional alleles and produce excess enzymatic activity

CYP2D6 Poor Metabolizer Status Can Be Ruled Out by a Single Genotyping Assay for the -1584G Promoter Polymorphism (Gaedigk et al. Clinical Chemistry, 2003)

Examples of Human Polymorphic CYPs

Enzyme	Major Variant Alleles	Mutation	Consequence	Allele Frequency	
				Caucasians	Asian
CYP2A6	*CYP2A6*2*	L160H	inactive enzyme	1-3	0
	*CYP2A6*3*	2A6/2A7 conversions	not known	0	0
	*CYP2A6*4*	Gene deletion	no enzyme	1	15
	*CYP2A6*5*	G479L	defect enzyme	0	1
CYP2C9	***CYP2C9*2***	**R144C**	**reduced affinity for P450 reductase**	8-13	0
	CYP2C9*3	**I359L**	**altered substrate specificity**	7-9	2-3
CYP2C19	*CYP2C19*2*	Aberrant splice site	inactive enzyme	13	23-32
	*CYP2C19*3*	Premature stop codon	inactive enzyme	0	6-10
CYP2D6	***CYP2D6*2xn***	**Gene duplication/multiduplication**	**increased enzyme activity**	1-5	0-2
	*CYP2D6*4*	Defective splicing	inactive enzyme	12-21	1
	*CYP2D6*5*	Gene deletion	no enzyme	4-6	6
	*CYP2D6*10*	P34S, S486T	unstable enzyme	1-2	50
	CYP2D6*17	**T107I, R296C, S486T**	**reduced affinity for substrates**	0	n.d.
CYP2E1	*CYP2E1*2*	R76H	less enzyme expressed	0	1
	*CYP2E1*3*	V389I	no effects	<1	0
	*CYP2E1*4*	V179I	no effects	<1	n.d.
CYP3A4	***CYP3A4*2***	**S222P**	**higher Km for substrates**	3	0
	*CYP3A4*3*	M445T	unknown	0	<1

n.d.: not determined (has a very high frequency among Black Africans and African Americans)

Ingelman-Sundberg, DMD (2001)

CYP2D6 Genotype & Nortriptyline PK {Efficacy}

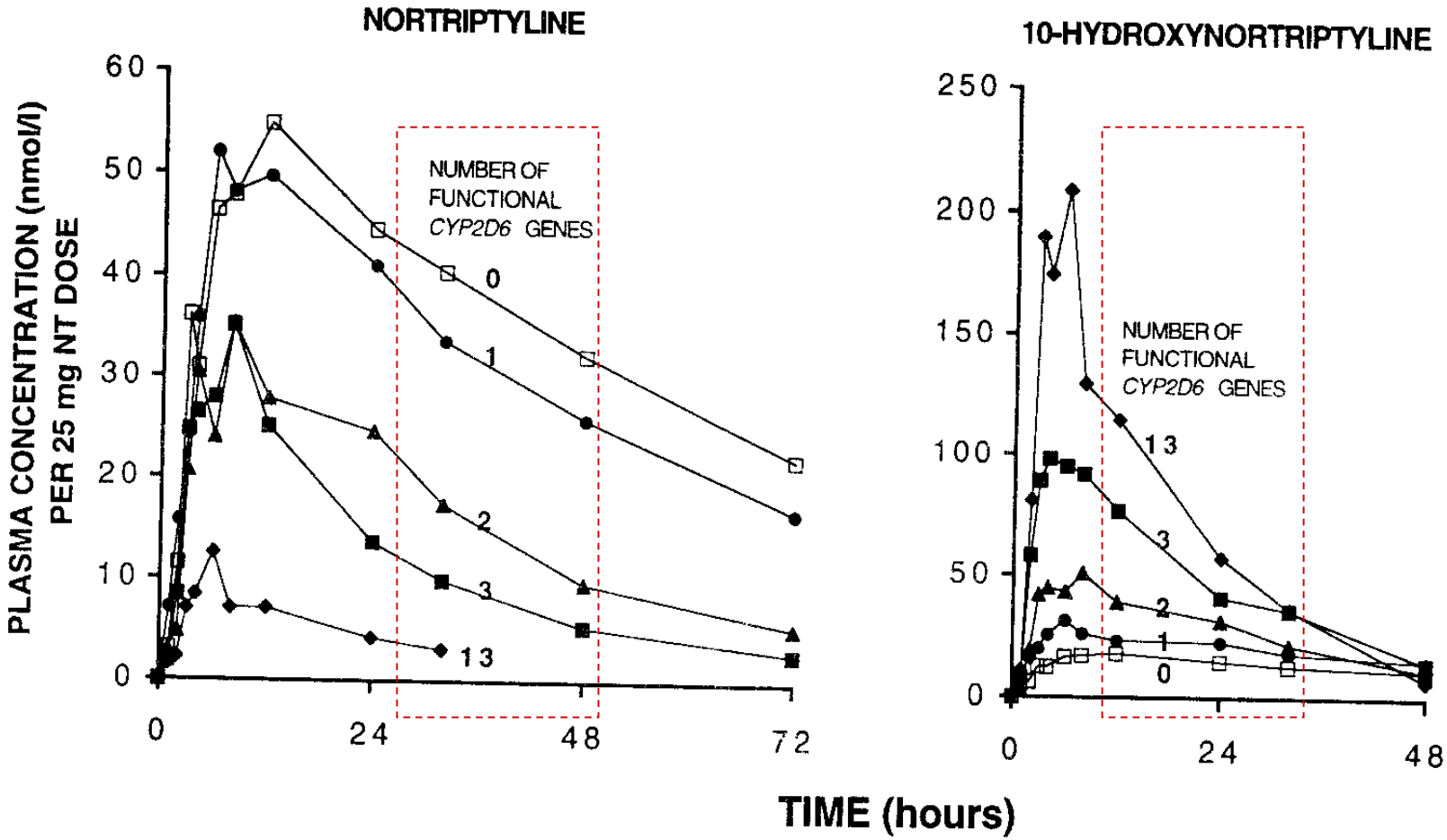
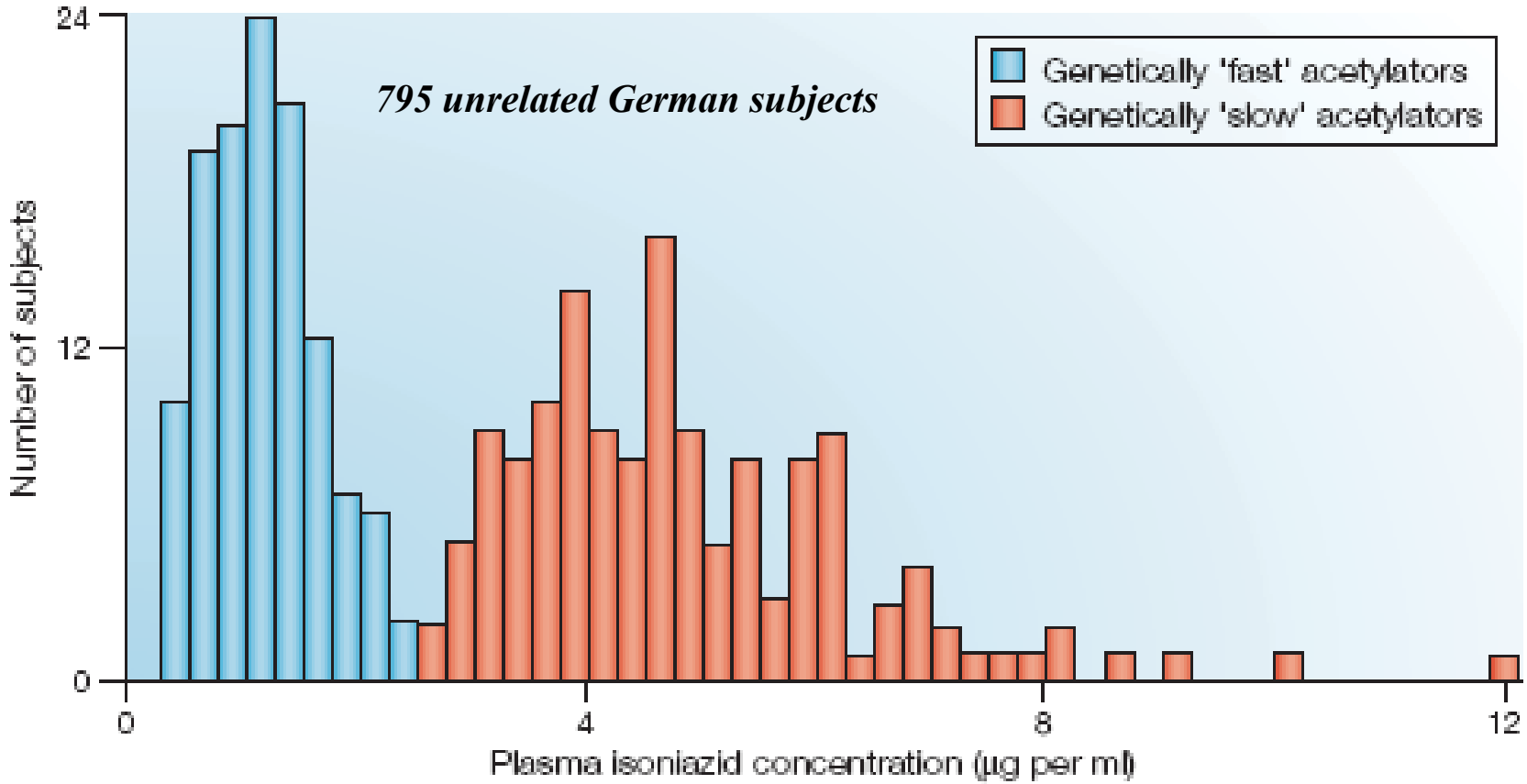


Fig. 1. Mean plasma concentrations of nortriptyline and 10-hydroxynortriptyline in different genotype groups after a single oral dose of nortriptyline. For subjects with 3 and 13 functional genes, plasma concentrations are adjusted to the 25 mg dose by means of division of the values by 2. The numerals close to the curves represent the number of functional *CYP2D6* genes in each genotype group.

Dalen P, et al. *Clin Pharmacol Ther* (1998).

NAT-2 Phenotype and Isoniazid (Phase II DME Effects)

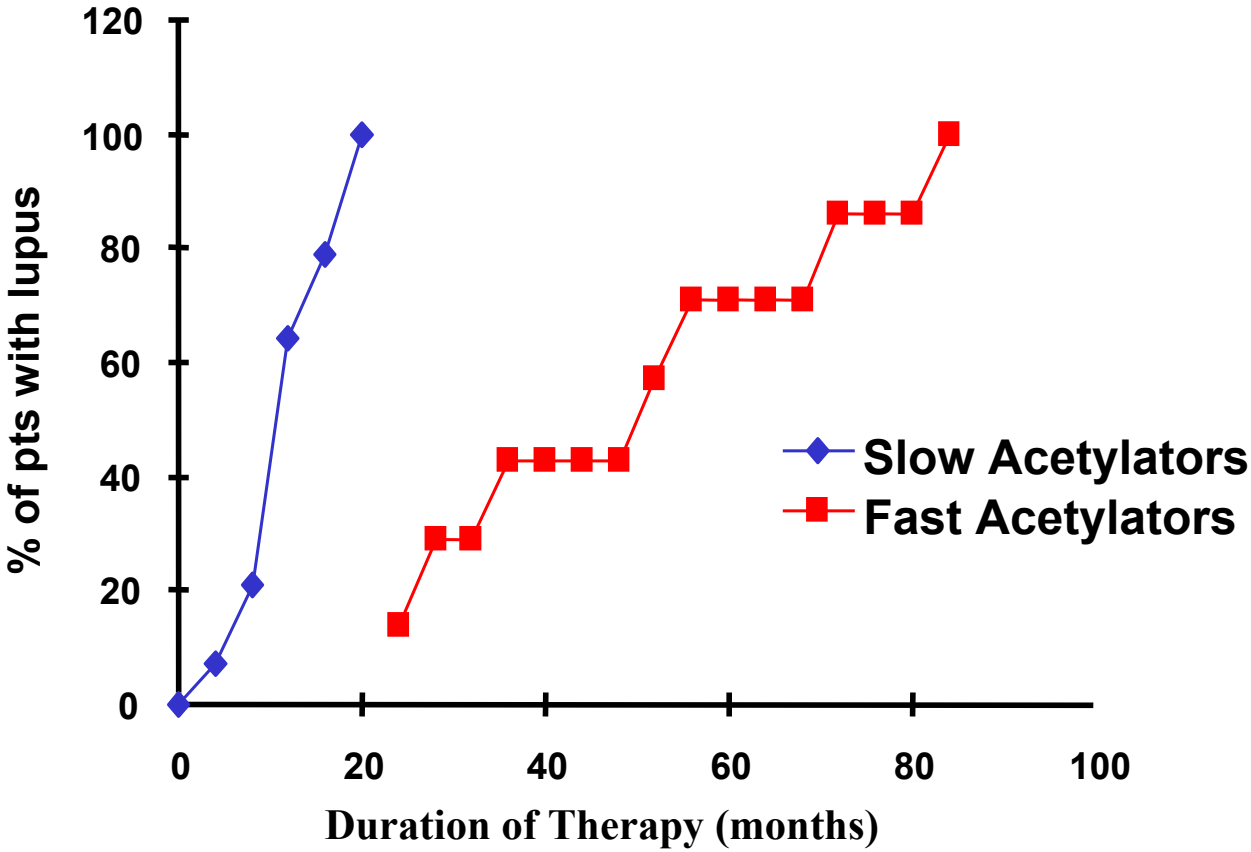


<u>Frequency of Slow Acetylator Phenotype</u>:

50% among Caucasians	**50% among Africans**
20% among Egyptians	**15% among Chinese**
10% among Japanese	

Figure adapted from Weinshilboum & Wang, Nature Reviews (2004)

Drug Induced Autoimmune Disease and NAT-2 Phenotype: Onset of Positive Antinuclear Antibody Syndrome (ANA) with Procainamide



Woosley RL, et al. *N.E.J.M.*, (1978).

CYP Polymorphisms & Adverse Drug Reactions (ADRs)

P450 Enzyme	Variant Alleles and frequencies in Caucasians	Examples of ADRs associated with the varaiant ADR alleles
CYP1A2	*CYP!A2*1F* (68%)	Antipsychotics: tardive dyskinesia
CYP2C9	*CYP2C9*2* (8 – 13%), *CYP2C9*3* (7-9%)	Warfarin: haemorrhage Phenytoin: phenytoin toxicity Tolbutamide: hypoglycaemia
CYP2C19	*CYP2C19*2* (13%), *CYP2C19*3* (0%)	Mephenytoin: toxicity Diazepam: prolonged sedation
CYP2D6	*CYP2D6*4* (12-21%), *CYP2D6*5* (4-6%) *CYP2D6*10* (1-2%), *CYP2D6*17* (0%)	Propafenone: arrhythmias Metoprolol: bradycardia Nortriptyline: confusion Opioids: dependence Phenformin: lactic acidosis Perhexilene: hepatotoxicity
CYP3A4	*CYP3A4*1B* (5.5%)	Epidophyllotoxins: treatment-related leukaemias

Pirmohamed and Park, Toxicology (2003): Adapted from Ingelman-Sundberg et al. (1999), Ingelman-Sundberg (2001) and Pirmohamed and Park (2001)

Clinical Consequences of CYP2D6 Polymorphisms

CYP2D6 Poor metabolizers
Increased Risk of Toxicity

Debrisoquine	Postural hypotension and physical collapse
Sparteine	Oxytocic effects
Flecainide	Ventricular tachyarrhythmias
Perhexiline	Neuropathy and hepatotoxicity
Phenformin	Lactic acidosis
Propafenone	CNS toxicity and bronchoconstriction
Metoprolol	Loss of cardioselectivity
Nortriptyline	Hypotension and confusion
Terikalant	Excessive prolongation in QT interval
Dexfenfluramine	Nausea, vomiting and headache
L-tryptophan	Eosinophilia-myalgia syndrome
Indoramin	Sedation
Thioridazine	Excessive prolongation in QT interval

Failure to Respond

Codeine	Poor analgesic efficacy
Tramadol	Poor analgesic efficacy
Opioids	Protection from oral opiod dependence

CYP2D6 Ultra-Rapid Metabolizers
Increased Risk of Toxicity

Encainide	Proarrhythmic effects
Codeine	Morphine toxicity

Failure to Respond

Nortriptyline	Poor efficacy at normal dosages
Propafenone	Poor efficacy at normal dosages

(Shah: Drug Safety, 2004)

Prodrug Effects

- codeine metabolized to morphine: abdominal pain in CYP2D6 ultra-rapid metabolizers; no analgesia in CYP2D6 PMs

Dosage

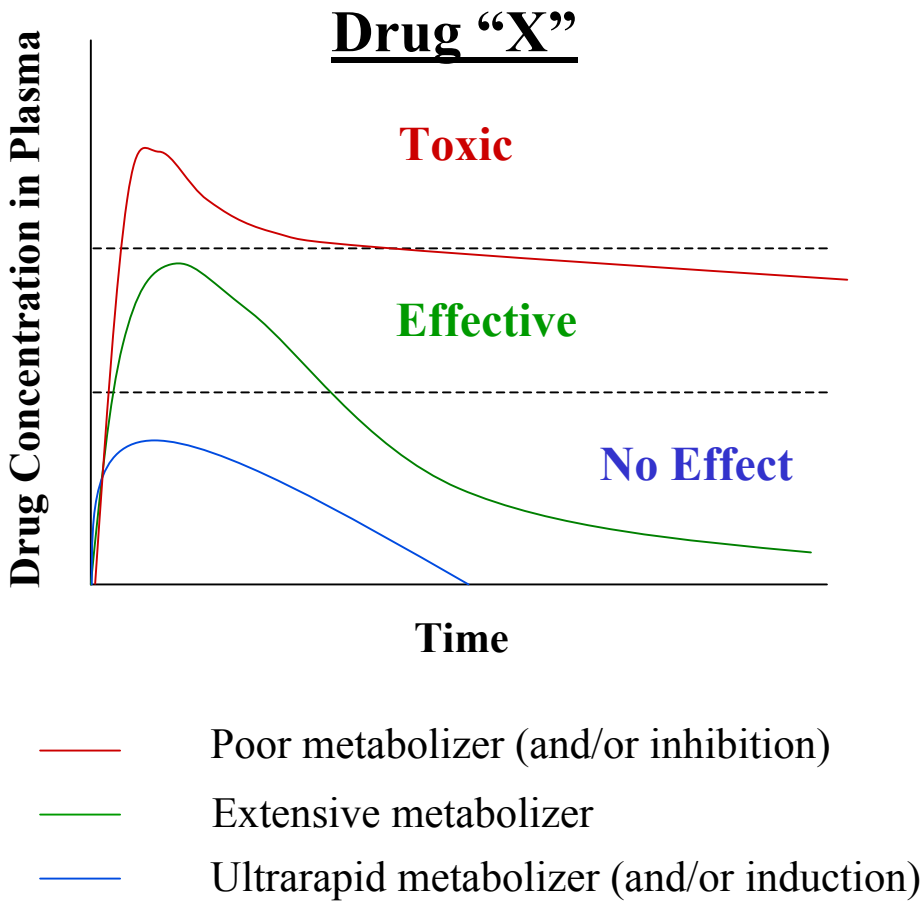
- clearance of S-warfarin by CYP2C9*3 reduced by 90% vs. CYP2C9 wt.
  - give 0.5 mg/day instead of normal 5-8 mg/day
- omeprazole: CYP2C19 PM AUC = 12 x CYP2C19 EM AUC
  - give 1-2 mg instead of normal 20 mg

A Perspective on Drug Therapy

- **Adverse Drug Reactions (ADRs) accounted for 5% of all hospital admissions in 1993**
- **ADRs reported in 6.7% of hospitalized patients (1998)**
- **ADRs accounted for 106,000 deaths in the US in 1994 (the same year there were 743,460 deaths from heart disease)**
- **4% of drugs introduced into the UK between 1974 and 1994 were withdrawn because of ADRs**

Pirmohamed and Park, Toxicology (2003)

Metabolic Clearance and Systemic Exposure



Metabolic clearance in the gut or liver (i.e. first-pass effect) can govern total absorption, systemic exposure and the clinical outcome

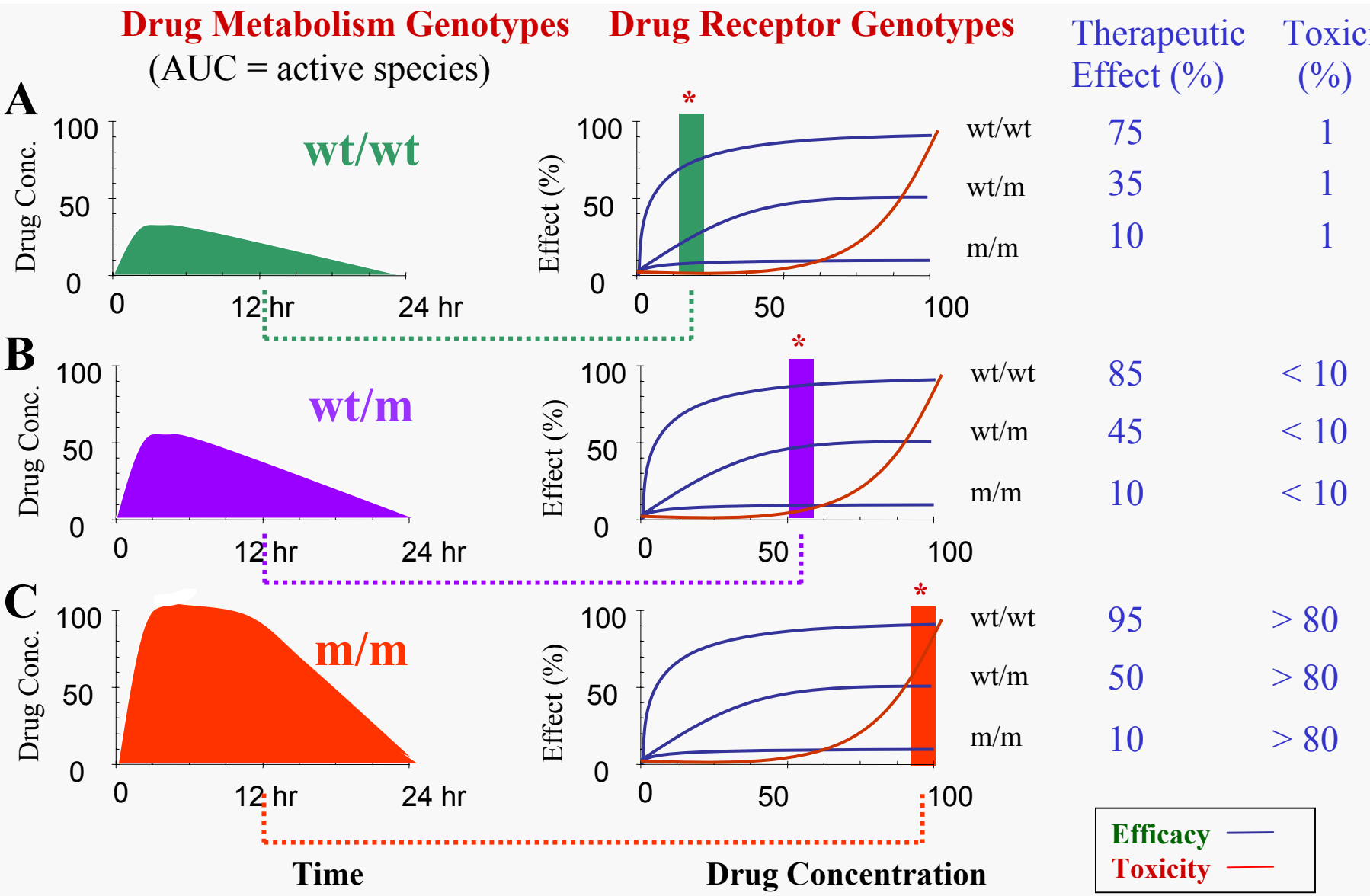
Genetic polymorphisms of DMEs and Drug targets that Increase the Risk of Adverse Drug Reactions

Enzyme/target/gene	Drug	Adverse drug reaction
Metabolic enzymes		
Pseudocholinesterase (butyrylcholinesterase)	Suxamethonium chloride (succinylcholine)	Prolonged apnoea
N-acetyltransferase 2 (NAT2)	Sulphonamides	Hypersensitivity
	Hydralazine	Lupus erythematosus
	Isoniazid	Neuropathy
Dihydropyrmidine dehydrogenase (DPD)	Fluorouracil	Myelotoxicity
Thiopurine methyltransferase (TPMT)	Azathioprine	Myelotoxicity
	Mercaptopurine	Myelotoxicity
UDP-glucuronosyltransferase 1A1 (UGT1A1)	Irinotecan	Diarrhoea, myelotoxicity
Cytochrome P450 (CYP) 2C9	Warfarin	Haemorrhage
	Glipizide	Hypoglycaemia
	Phenytoin	Increased toxicity
CYP2C19	Diazepam	Prolonged sedation
CYP2D6	Some antiarrhythmics	Proarrhythmic effects
	Some antipsychotics	Extrapyramidal symptoms
	Metoprolol	Bradycardia
Drug targets and related structures		
Ryanodine receptor (RYR1)	Suxamethonium chloride	Malignant hyperthermia
	Inhalational anaesthetics	Malignant hyperthermia
Cardiac ion channels	See section 1.2	Torsade de pointes
Coagulation factor V	Oral contraceptive pills	Thromboembolic disease
Prothrombin	Oral contraceptive pills	Thromboembolic disease
Dopamine D_3 receptor (DRD3)	Antipsychotics	Tardive dyskinesias
Various (see section 1.5)	Opioids	Addiction

(Güzey & Spigset: Drug Safety, 2002)

Genetically Regulated Heterogeneity in Drug Effects

Exposure (PK) Sensitivity (PD) (Evans and Relling, Science, 1999)



DRUG-INDUCED-ARRHYTHMIAS and ION CHANNEL POLYMORPHISMS

Prolonged QT syndrome arrhythmias:

- **Characterized by an abnormal cardiac repolarization and possibly syncope, seizures, and sudden death (torsade de pointes)**

- **Associated with both cadiovascular and non-cardiovascular drugs**
  - quinidine, procainamide, *N*-acetylprocainamide, sotalol, amiodarone, disopyramide, phenothiazines, tricyclic antidepressants, cisapride, and nonsedating antihistamines such as astemizole and terfenedine

    Braunwald: Heart Disease: A Textbook of Cardiovascular Medicine, 6th ed., Copyright © 2001 W. B. Saunders Company

- **Linked to cardiac ion channel subclinical mutations**

  L. Baumbach *et al*. Am. J. Human Genetics (2001); N. Makita *et al.* Circulation, (2002).

(Adapted from Pohl: NIH, 2002)

Reasons for Drug Withdrawal (post 1990)

Drug	Year of withdrawal	Reason(s) for withdrawal
Dilevalol	1990	Hepatotoxicity
Triazolam	1991	Neuropsychiatric reactions
Terodiline[a]	1991	QTI prolongation and TdP
Encainide	1991	Proarrhythmic effects
Fipexide	1991	Hepatotoxicity
Temafloxacin	1992	Hypoglycaemia, haemolytic anaemia and renal failure
Benzarone	1992	Hepatotoxicity
Remoxipride	1993	Aplastic anaemia
Alpidem	1993	Hepatotoxicity
Flosequinan	1993	Excess mortality, possibly due to arrhythmias
Bendazac	1993	Hepatotoxicity
Soruvidine	1993	Myelotoxicity following DI
Chlormezanone	1996	Hepatotoxicity and severe skin reactions
Tolrestat	1996	Hepatotoxicity
Minaprine	1996	Convulsions
Pemoline	1997	Hepatotoxicity
Dexfenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Fenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Terfenadine[a]	1998	DI, QTI prolongation and TdP
Bromfenac	1998	Hepatotoxicity following prolonged administration
Ebrotidine	1998	Hepatotoxicity
Sertindole[a]	1998	QTI prolongation and potential for TdP
Mibefradil	1998	Rhabdomyolysis following DI Concerns on potential DI including risk of TdP
Tolcapone	1998	Hepatotoxicity
Astemizole[a]	1999	DI, QTI prolongation and TdP
Trovafloxacin	1999	Hepatotoxicity
Grepafloxacin[a]	1999	QTI prolongation and TdP
Troglitazone	2000	Hepatotoxicity
Alosetron	2000	Ischaemic colitis
Cisapride[a]	2000	DI, QTI prolongation and TdP
Droperidol[a]	2001	QTI prolongation and TdP
Levacetylmethadol[a]	2001	DI, QTI prolongation and TdP
Cerivastatin	2001	Rhabdomyolysis following DI

a Drugs withdrawn specifically due to the risk of TdP.

DI = drug interactions; **QTI** = QT interval; **TdP** = torsade de pointes.

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(Shah: Drug Safety, 2004)

Reaction-Phenotyping

- Predict the in vivo metabolic clearance and the contribution of individual Drug Metabolizing Enzymes to the total *in vivo* clearance
  - A drug with a metabolic clearance (e.g. >40% of the total clearance) and metabolized by a polymorphic enzyme and/or a primary enzyme (e.g. >30-50% of the total metabolic clearance) has an increased relative risk of drug-drug interactions and/or individual variation
  - Reaction-phenotyping data can refine the human dose projection

Species Differences in Drug Metabolizing Enzymes

❖ Orthologs of the major DMEs are found in most species; however, within a species even a single amino-acid change can alter the substrate affinity of an enzyme and, potentially, the metabolic clearance of a compound

- e.g. succinylcholine: a prolonged apnea in patients is associated with an aspartic acid→glycine substitution at amino acid 70 of butyrylcholinesterase

❖ Notable species differences include:

- ➢ Dogs: deficient in NAT (cannot acetylate aromatic amines)
- ➢ Guinea-pigs: deficient in ST activity; no N-hydroxylation
- ➢ Cats: poor UGT activity (unable to glucuronidate phenols)
- ➢ Rats: often very rapid metabolizers; CYP2C is the major family in the liver with significant gender differences
- ➢ Cynomolgus monkeys: reported to have low CYP1A2 activity

Cannot rely entirely on animal pharmacokinetics (PK) data to predict human PK

In Vitro Metabolism Studies

- **Isolated hepatocytes**
  - "Gold Standard" for in vitro metabolism studies (contain a full complement of hepatic DMEs)
    - Human hepatocytes are easy to use
      - fresh cells are not readily available
    - Can be cryopreserved

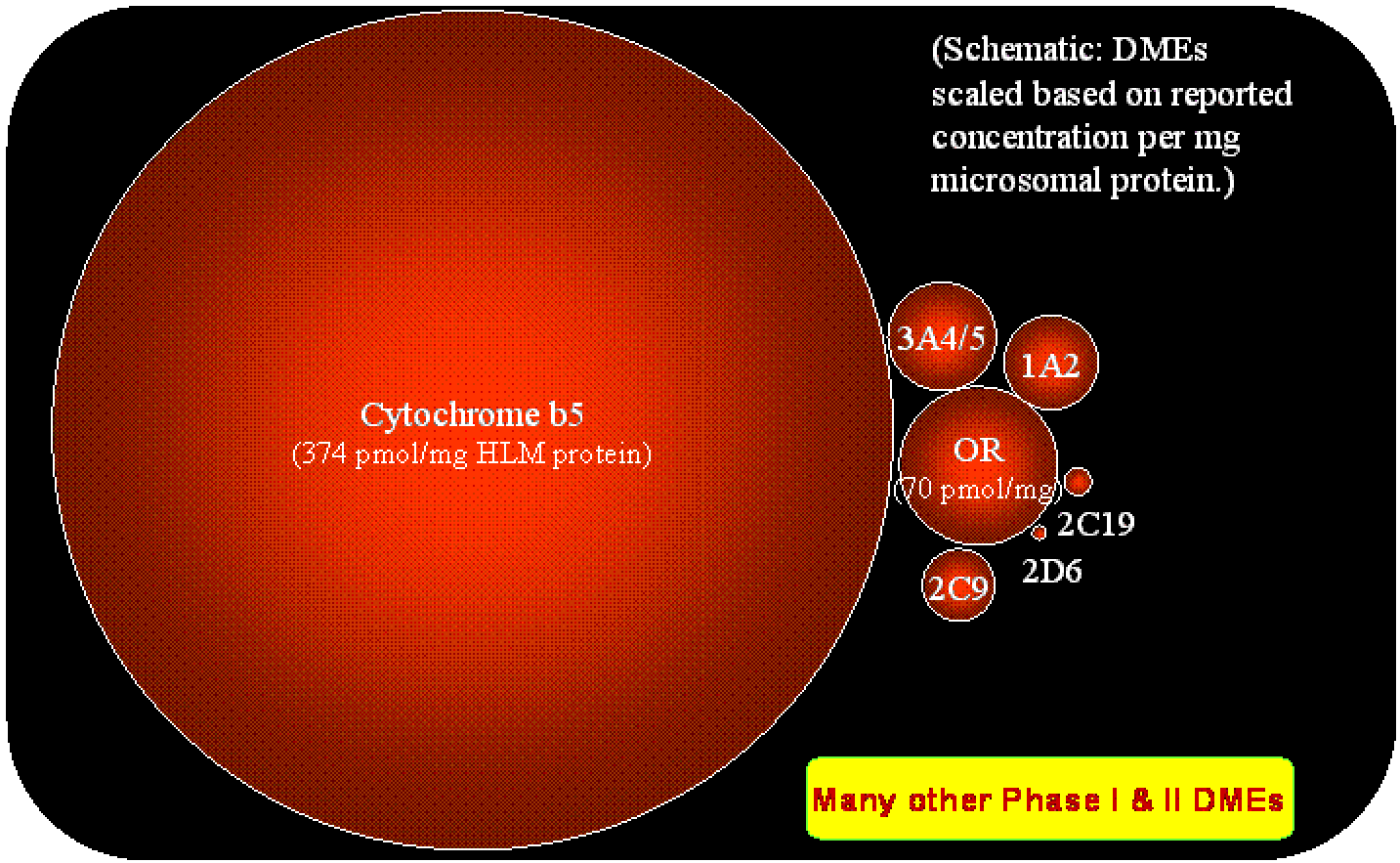
- **Liver Microsomes** (endoplasmic reticulum)
  - Contain the membrane-bound enzymes (CYPs, FMOs and UGTs)
  - Human Liver Microsomes (HLM) are relatively easy to prepare in bulk amounts and can be stored frozen for long periods with enzyme activity maintained

- **Liver S9** (cytosolic fraction)
  - Contains cytosolic enzymes (e.g. STs, XO, ADHs, NATs)
  - Otherwise similar to HLM in terms of advantages and limitations

- **Recombinant/reconstituted enzyme systems** (single functional enzyme systems)
  - Allow mechanistic studies of isolated metabolic pathways
    - More artificial than other in vitro DME systems

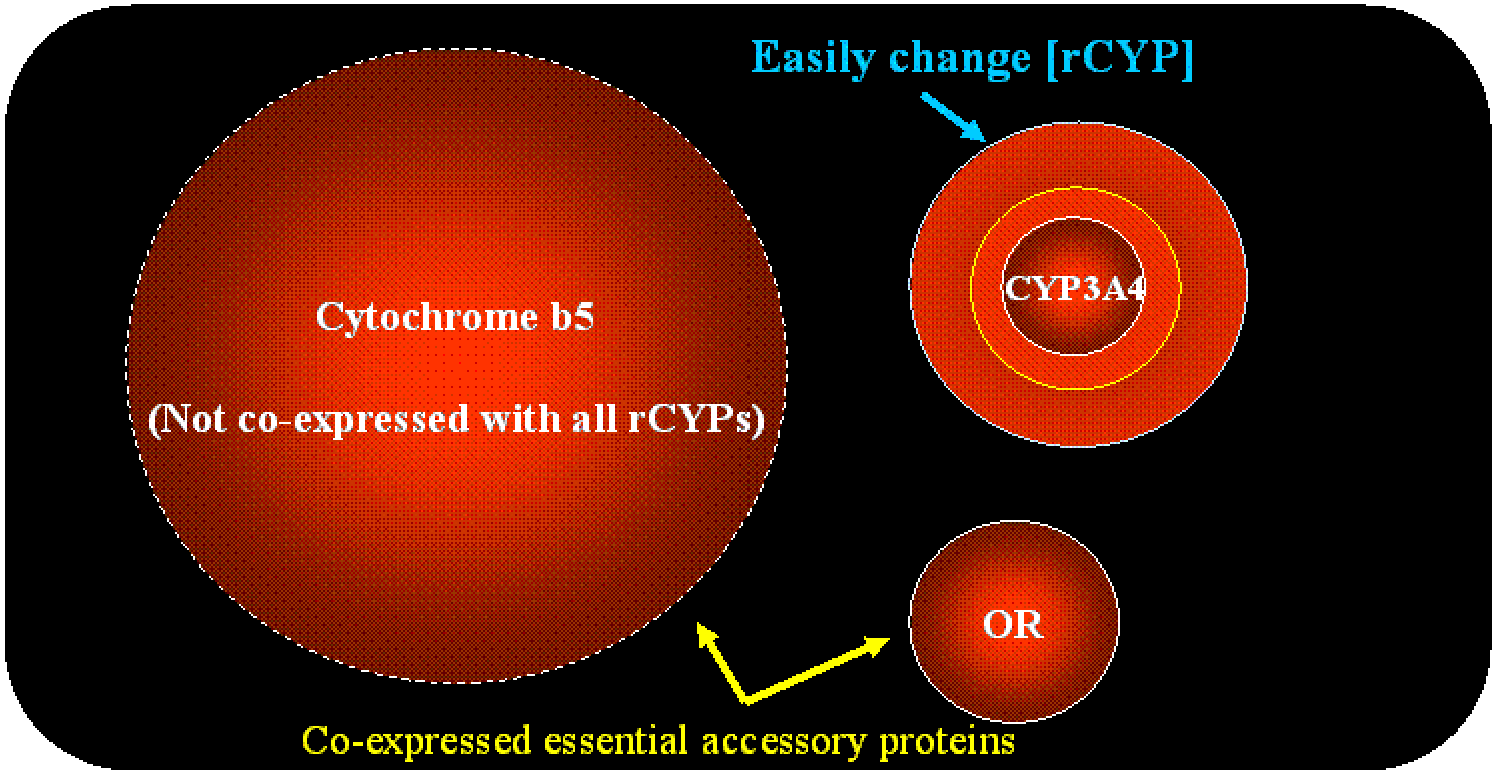
- **Liver Slices**
  - Similar to hepatocytes in that they contain the full complement of hepatic DMEs
    - Harder to prepare than other systems and not used as often

Relative Expression of Membrane-Bound Major CYPs and Electron Transfer Accessory Proteins in Human Liver Microsomes (HLM)



- HLMs contain a multitude of native DMEs and endogenous accessory proteins

Recombinant CYPs (rCYPs): Simplified DME Systems



Microsomes prepared from human CYP modified cDNA recombinant expression systems:

- E.Coli bacteriosomes (University of Dundee/Cypex)
- B Lymphoblast cells (BD/Gentest)
- Baculovirus infected insect cells (BD/Gentest - SUPERSOMES™)

Reaction-Phenotyping Methods

- **Intrinsic clearance can be measured in HLM and scaled to predict the hepatic in vivo clearance in humans**

- **The effect of co-incubated CYP-selective chemical or monoclonal antibody inhibitors on rates of metabolism in HLM can be used to identify primary DMEs**

- **Incubations with recombinant CYPs can be scaled to predict hepatic in vivo clearance using Relative Activity Factors (RAFs) and/or relative hepatic abundance of the enzymes**

- **A correlation of rate of metabolism can be made with a panel of HLM donors ($n \geq 10$) that have been phenotyped for the major DMEs**

Each method has its own limitations

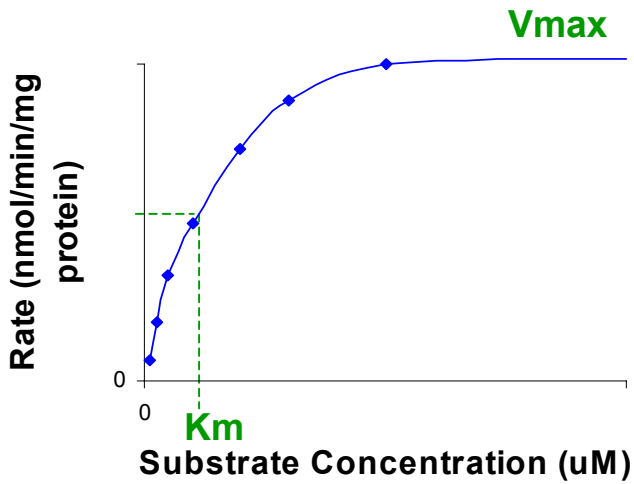
Reaction-Phenotyping Methods: Calculating Intrinsic Clearance

Intrinsic clearance (CLint) is the enzyme-mediated clearance that would occur without physiological limitations (e.g. hepatic blood flow)

Michaelis-Menten Kinetics (Simple form)

$$\text{Rate of Metabolism, } v = \frac{Vmax * C_E}{Km + C_E}$$

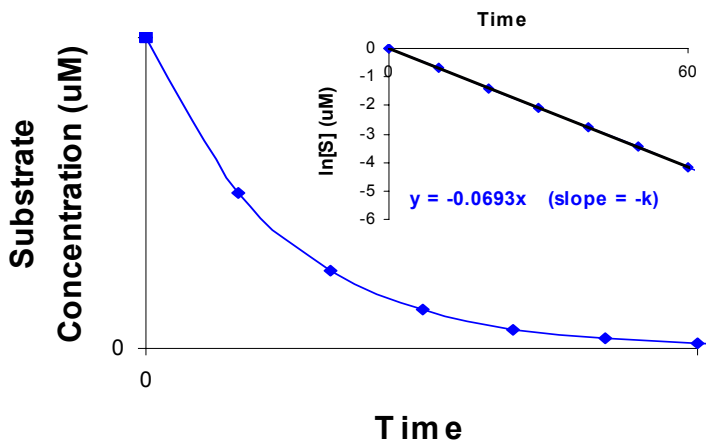
$$CLint = Vmax/Km$$



When $C_E << Km$

$$C = C_0 * e^{-kt} \qquad t1/2 = \ln 2/k$$

$$CLint \text{ (ml/min/mg)} = \frac{\ln 2}{t1/2 * [HLM]}$$



Reaction-Phenotyping Methods: Scaling Intrinsic Clearance to In Vivo Hepatic Clearance

Initial rate / Half-Life/ k
(hepatocyte/tissue/microsomes/S9)

↓

$CLint_{in\ vitro}$

↓ Scaling factors

$CLint'_{in\ vivo}$

↓ Models of hepatic clearance

CLh as %QH

In Vivo Clearance

Human Scaling Factors
45 mg microsomal protein/g liver; 26 g liver/kg bodyweight

Well-Stirred Model:

$$CLh = \frac{fub * CLint'_{in\ vivo} * QH}{fub * CLint'_{in\ vivo} + QH}$$

Parallel Tube Model:

$$CLh = QH - QH * \exp(-fub * CLint'_{in\ vivo}/QH)$$

Dispersion Model:

$$CLh = QH * \frac{1-4a}{(1+a)^2 * \exp[(a-1)/2Dn] - (1-a)^2 * \exp[-(a+1)/2Dn]}$$

$a = (1 + 4Rn * Dn)^{1/2}$; $Dn = 0.17$ (dispersion no.);
$Rn = fub * CLint'_{in\ vivo}/QH$ (efficiency no.)

(Fub = fraction unbound; QH = hepatic blood-flow)

Reaction-Phenotyping Methods

Enzyme	Inhibitor	Index Substrate
CYP1A2	Furafylline	Phenacetin
CYP2C9	Sulfaphenazole	Tolbutamide
CYP2C19	Tranylcypromine; (+)-N-3-benzyl-nirvanol	(S)-mephenytoin
CYP2D6	Quinidine	Bufuralol
CYP3A4	Ketoconazole	Testosterone
		Midazolam
		Nifedipine

(CYP-selective inhibitory MAbs are also available)

Incubation conditions are chosen to optimize the selectivity of the inhibitor

Significant inhibition (e.g. >80% decrease in HLM turnover/rate/intrinsic clearance) clearly signifies a primary metabolic clearance pathway

Reaction-Phenotyping Methods: Scaling rCYP to HLM Activity

Example:

$$V(s) = \sum_{i=1}^{n} A_i * V_i(s)$$

$$A_i = \frac{K_{cat}\ HLM\ CYP_i}{K_{cat}\ rCYP_i} * [CYP_i]_{HLM}$$

$V_i(s)$ = individual Concentration-Velocity function of ECN compound for each CYP
A_i = $rCYP_i$ Scaling Factor; K_{cat} is the Capacity/Turnover Number (pmol product/pmol CYP/min)

If K_{cat} rCYP = K_{cat} HLM (for Index Reaction)

$A_i = [CYP_i]_{HLM}$ (i.e. relative abundance in the liver)

$$f_i\ (\%) = \frac{A_i * CL_i}{\sum_{i=1}^{n} A_i * CL_i} * 100$$

(f_i (%) = % fraction of total metabolized by CYP)

(other methods can be used)

Example of Reaction-Phenotyping: Mirtazapine

- **Mirtazepine is metabolized to three major metabolites in vitro**

Mirtazapine 8-Hydroxylation

	% Predicted Contribution of CYP [Mirtazapine]		
CYP	2.5µM	25µM	250µM
1A2	30%	50%	55%
2C8	-	-	-
2C9	-	-	10%
2D6	65%	40%	15%
3A4	5%	10%	20%

Reaction Mediated by CYP1A2 and CYP2D6
CYP2D6 is important at therapeutic levels
[Confirmed by Chemical Inhibitors]

Mirtazapine N-Demethylation

	% Predicted Contribution of CYP [Mirtazapine]		
CYP	2.5µM	25µM	250µM
1A2	45%	20%	10%
2C8	<5%	5%	15%
2C9	-	-	-
2D6	-	-	-
3A4	50%	65%	75%

Reaction Mediated by CYP1A2 and CYP3A4

Mirtazapine N-Oxidation

	% Predicted Contribution of CYP [Mirtazapine]		
CYP	2.5µM	25µM	250µM
1A2	85%	45%	15%
2C8	-	-	-
2C9	-	-	-
2D6	-	-	-
3A4	15%	55%	85%

Reaction Mediated by CYP1A2 and CYP3A4
CYP1A2 most important at Therapeutic Levels

Total Biotransformation of Mirtazapine

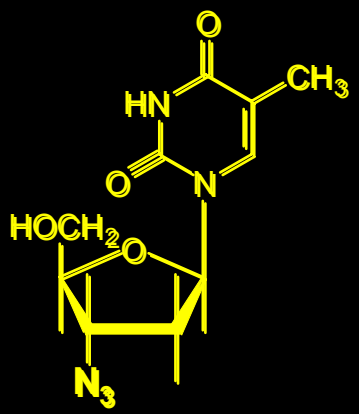
	% of Total Biotransformation [Mirtazapine]		
CYP	2.5µM	25µM	250µM
8-OH	55%	45%	30%
N-demethyl	45%	50%	55%
N-Oxide	<5%	5%	15%

Störmer et al. JPET (2000)

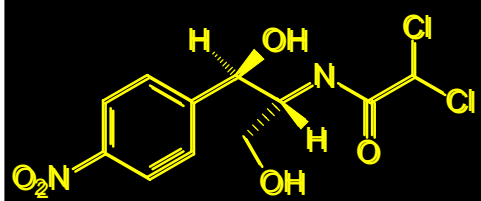
Other Developments

Glucuronidation & UGT Phenotyping

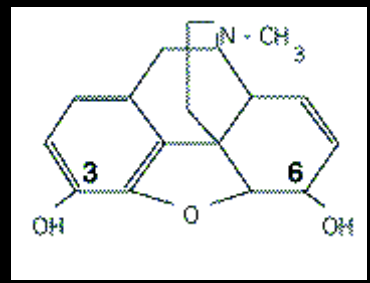
Zidovudine



Chloramphenicol



Morphine



Zidovudine Elimination:

- gluc. conjugate (67 %)
- renal excretion (90 % DRM)

DRM = Drug Related Material

Chloramphenicol Elimination:

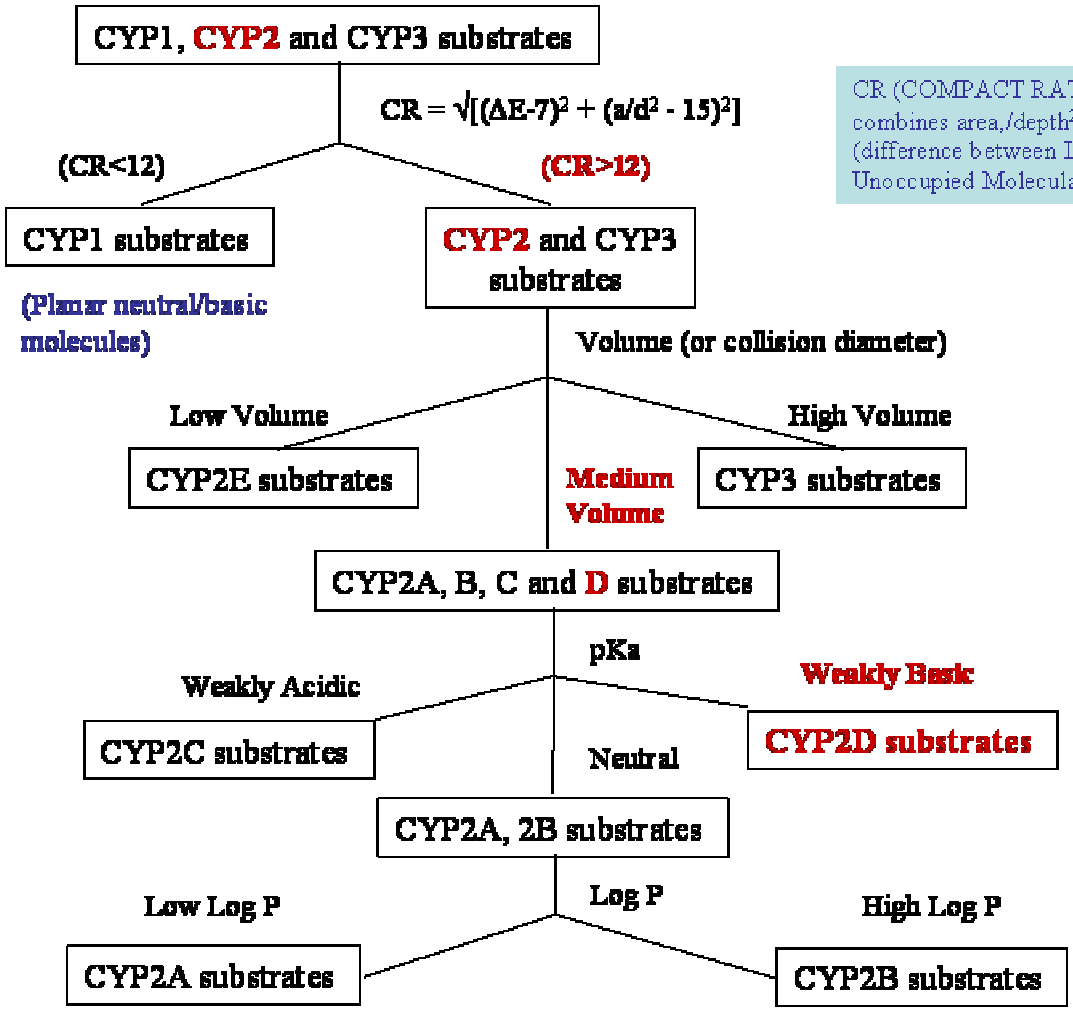
- gluc. conjugate (90 %)
- renal excretion (90 % DRM)

Morphine Elimination:

- gluc. conjugate (70 %)
- renal excretion (< 90 % DRM)

- Direct glucuronidation can serve as the major metabolic clearance pathway
- UGT1A1 polymorphism (e.g. Gilbert syndrome and hyperbilirubinemia)
- UGT-DDIs, and thus implications of UGT reaction-phenotype, are being explored (irinotecan a more recent example)

In Silico Screening: Substrate Specificity of CYPs



(Lewis and Dickins: DDT, 2002)

Summary

• Metabolism is the major contributor to the systemic exposure and total in vivo clearance of many drugs and thus an important consideration in Drug Discovery and Development

• The liver is the major organ of metabolic clearance (however, drug metabolism can occur elsewhere)

• The cytochromes P450 are the major enzymes of drug metabolism, but there are many others to consider on a case-by-case basis

• Inter- and intra-individual differences in drug metabolizing enzymes, including known polymorphisms of the enzyme and/or the drug-target, can have a significant effect on systemic exposure and thus the clinical outcome

• In vitro reaction-phenotyping methods: (i) enable a prediction of human pharmacokinetics and dosages, (ii) allow the significance of individual human-specific drug metabolizing enzymes to be determined