

Monitoring Drug Efficacy & Toxicity

- along with drug metabolism

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References

- Principles of Pharmacology, Golan, et al.
 - Chapter 47, *Principles of Toxicology*.
 - Chapters 2, 3, 4 on *Pharmacodynamics, Pharmacokinetics and Drug Metabolism*.

- My Wiki: <http://www.hodsdon.com/wiki>
 - Follow 'Medicine' link

Therapeutic Drug Monitoring (TDM)

or “How can you tell if a drug is working or not?”

1. Monitor clinical signs and/or laboratory measures of drug efficacy (and toxicity).
 - Blood pressure monitoring for an anti-hypertensive.
 - Blood coagulation rates (i.e. “prothrombin time” or PT) for coumadin (warfarin) anticoagulation therapy.
 - Very drug specific; not covered in detail here.
2. Measure drug levels.
 - In blood generally (either serum, plasma, whole blood, or a specific cellular component).
 - Other fluids are possible (e.g. saliva or urine).
 - Need to decide *what* and *when* to measure.

Therapeutic Drug Monitoring (TDM): *Basic Principles for Review*

- Dose-Response Curves, Therapeutic Index & Therapeutic Range (or window)
- Pharmacokinetics & Pharmacodynamics
 - Relevant because they tells us what to measure, when to measure it, and how to interpret the results.
 - Important concepts: *absorption, distribution, metabolism & elimination*.
 - A few simple equations are handy (just three).

Therapeutic Drug Monitoring (TDM):

Important differences between blood and urine.

□ Serum

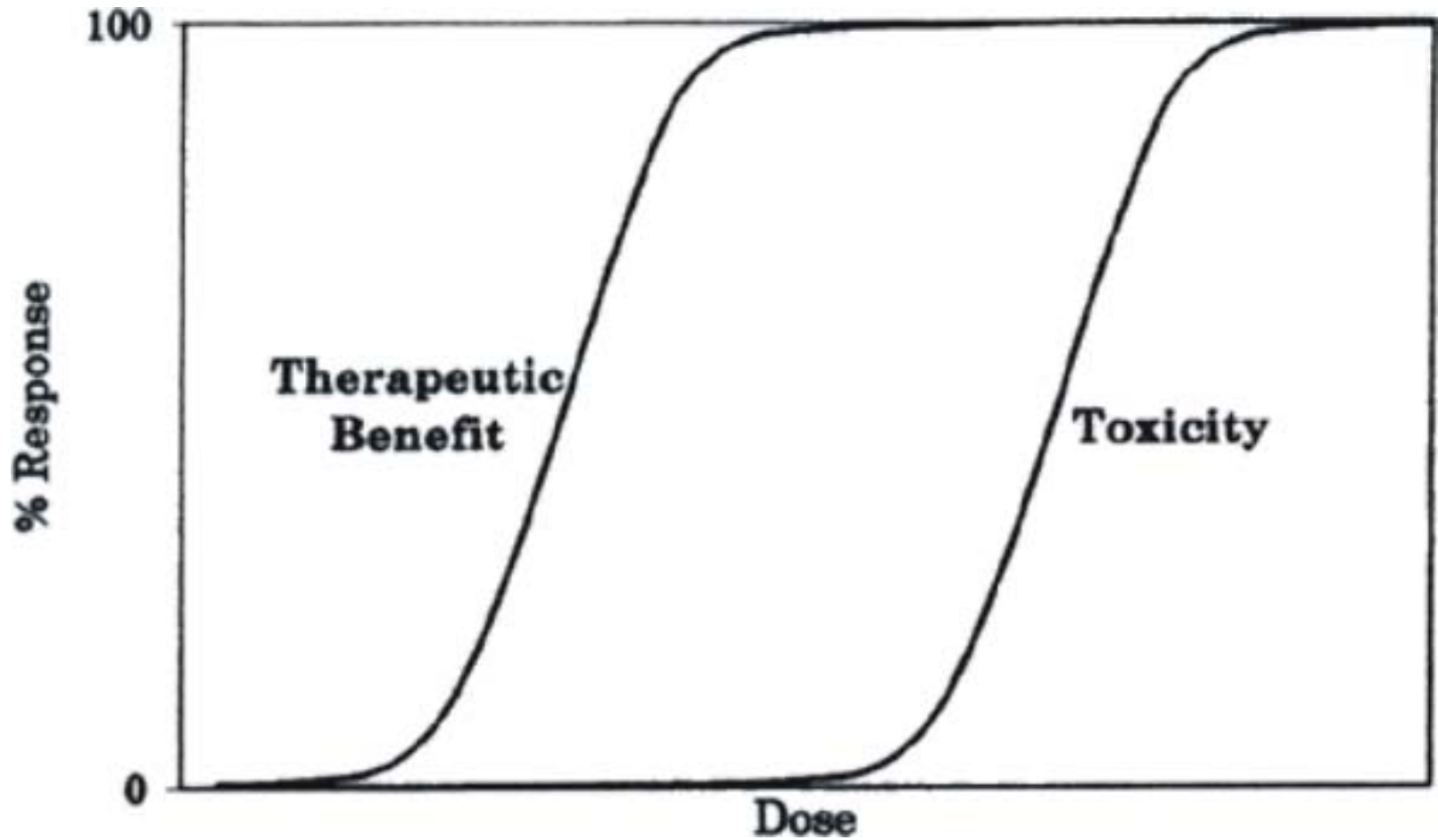
- Detection of circulating substances (i.e. drugs and toxins) generally represents a significant, **recent** exposure.
- **Quantitation** of drug/toxin levels (or their metabolites) generally correlates well with **current/ongoing** drug efficacy and toxicity.
- Slightly invasive (requires a needlestick) and also less sensitive for detection of previous exposure history.

□ Urine

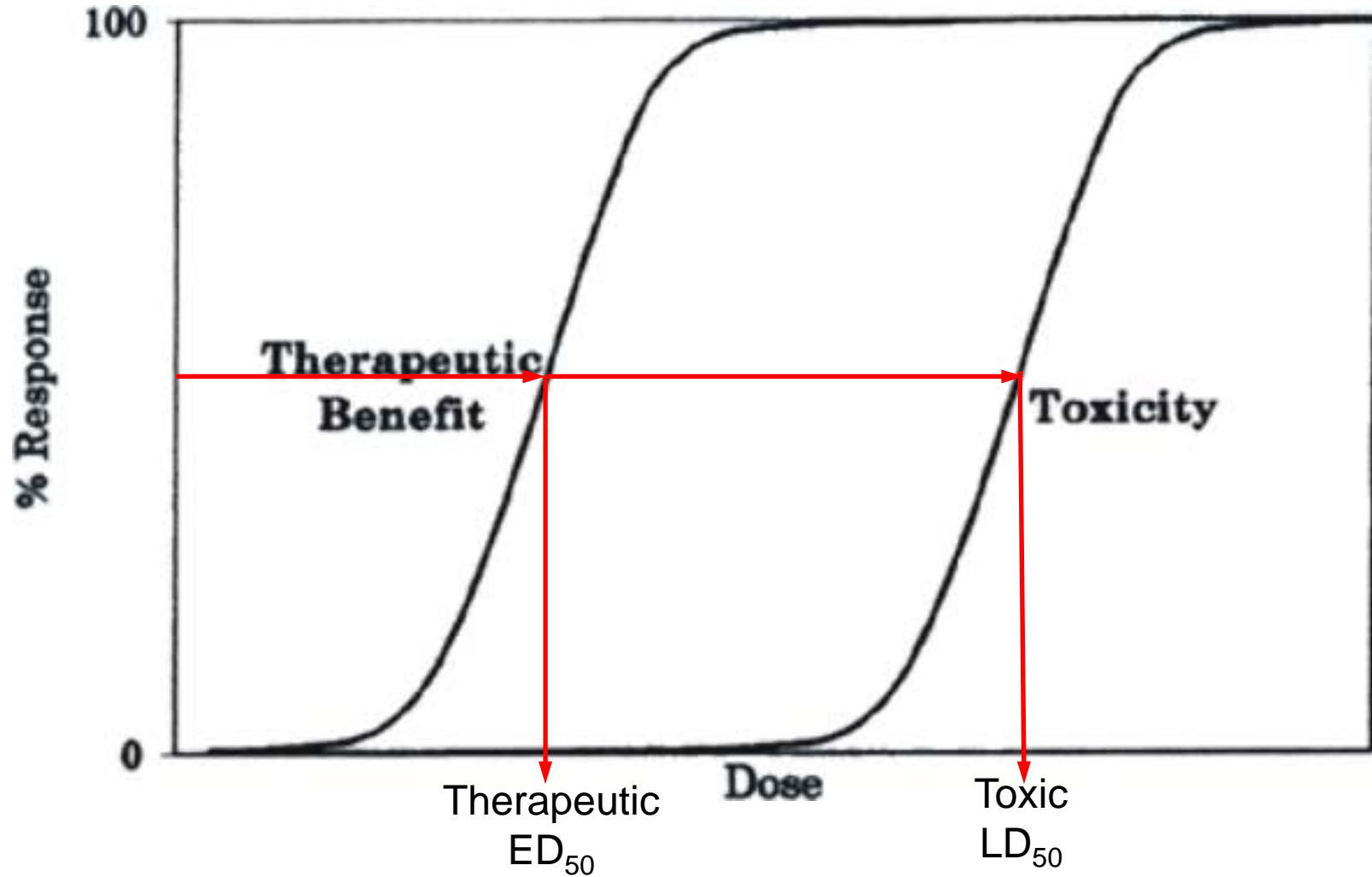
- Many drugs, toxins and their metabolites are accumulated in urine over time, thereby increasing the sensitivity of detection **for both current and past use**.
- Because urine may remain positive long after the physiological effects of a substance have disappeared, detection of a drug in urine does not always explain a patient's current clinical condition.
- Quantitation is not that useful as variability in the timing of collection compared to drug exposure and also variability in the specific gravity of urine (i.e. reflecting a patient's hydration status) results in a wide range of possible drug concentrations.

Dose-Response Curves

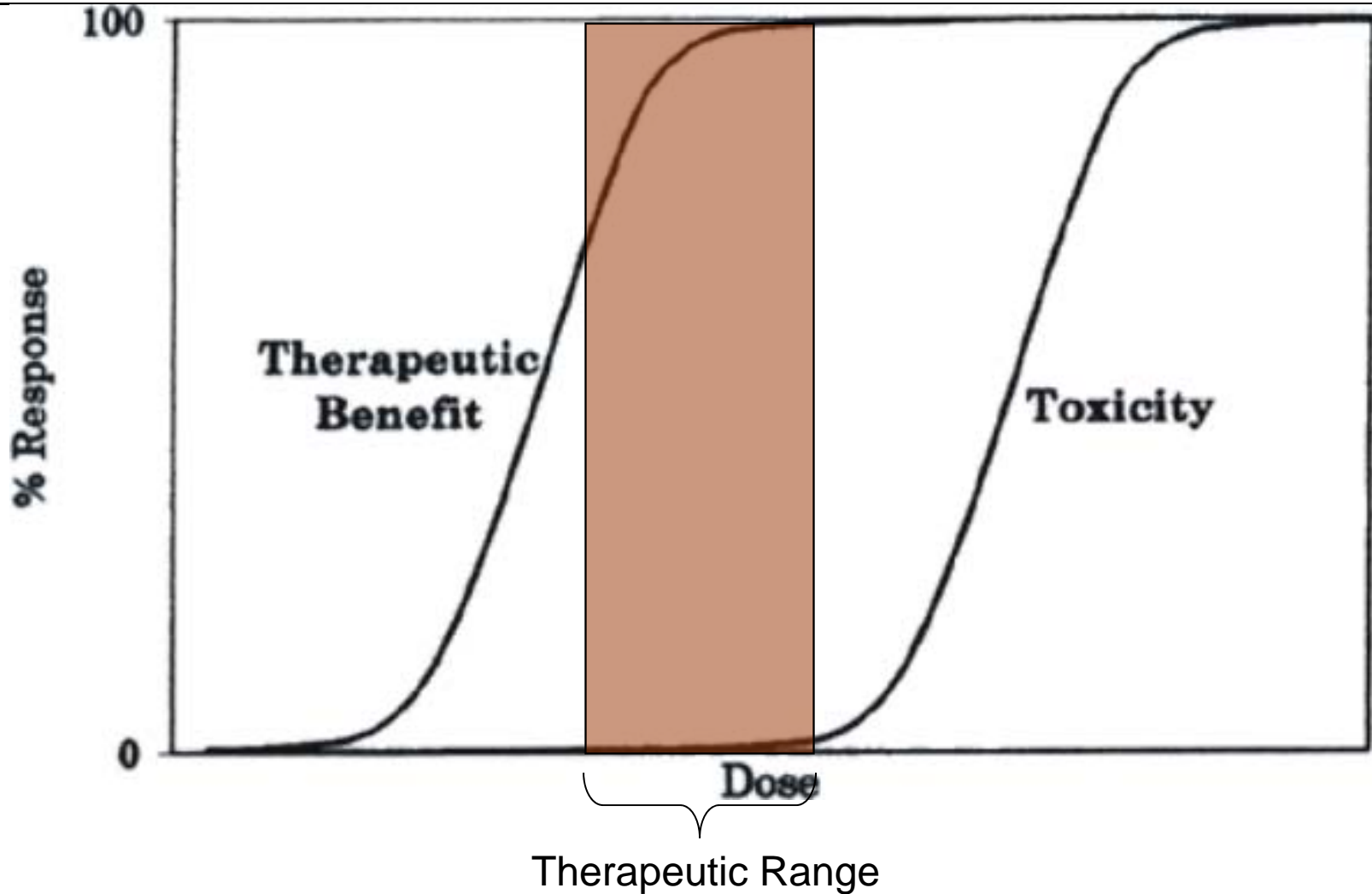
(part of “pharmacodynamics”)



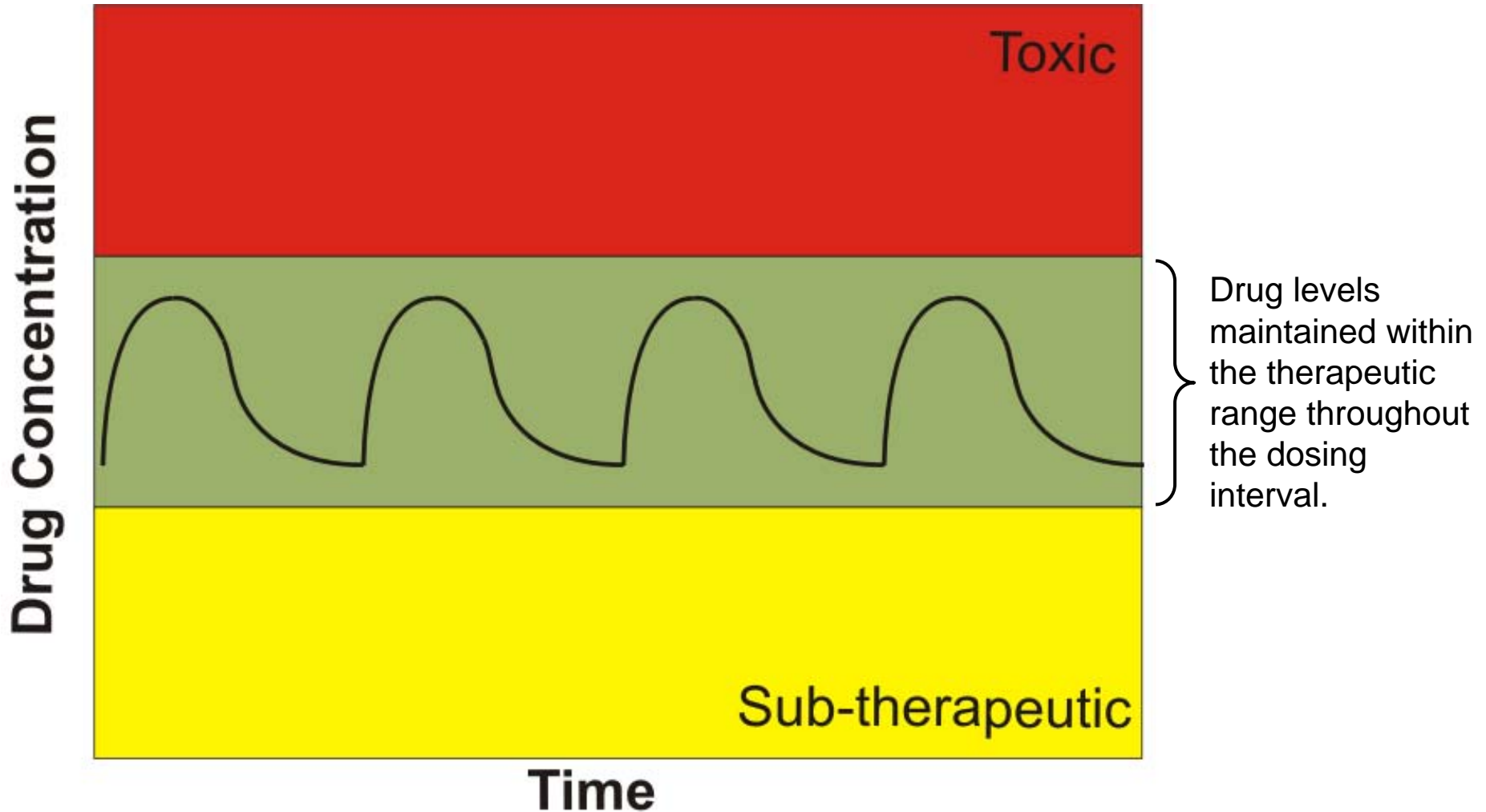
Dose-Response Curves



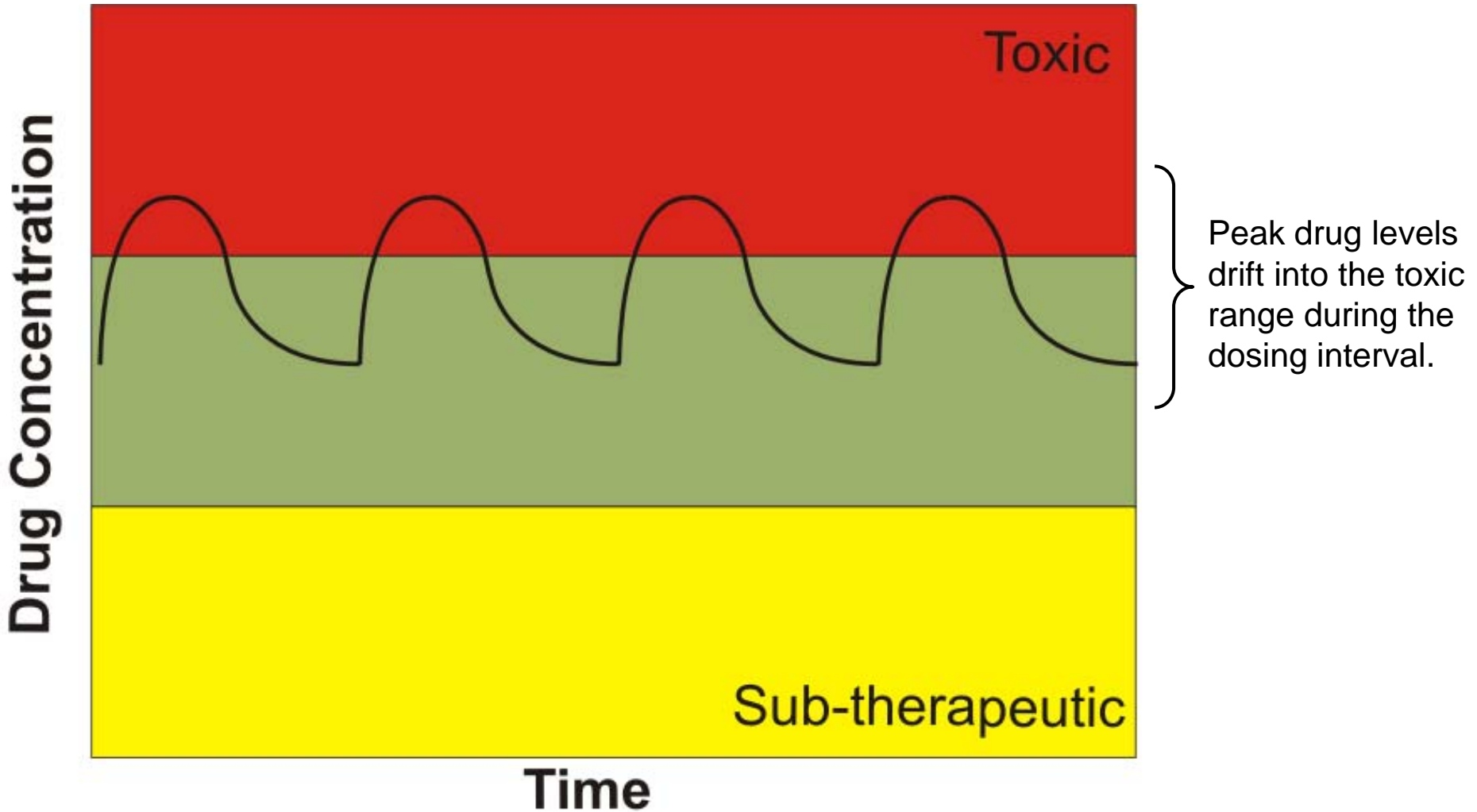
Dose-Response Curves



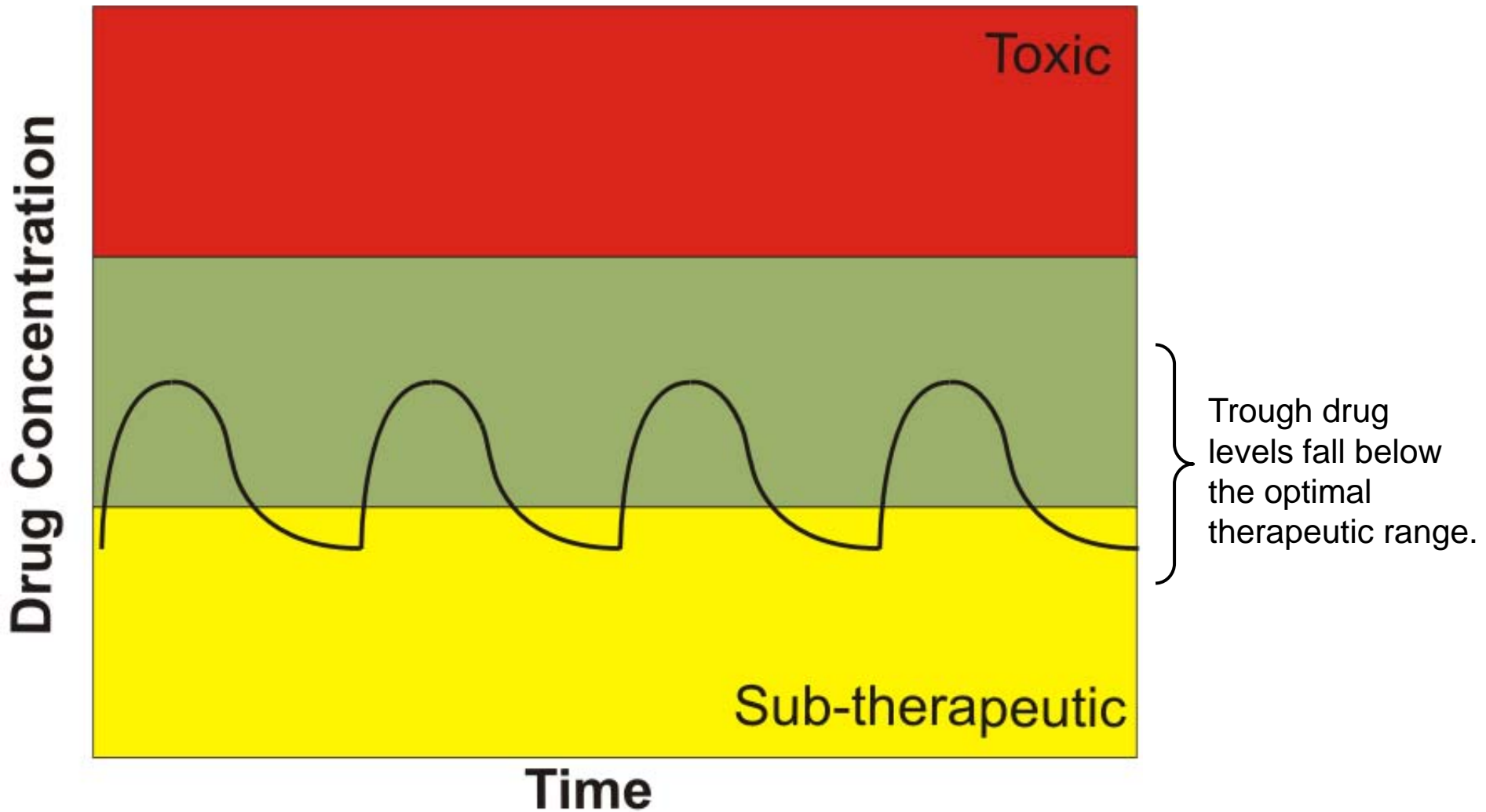
Therapeutic Range



Therapeutic Range



Therapeutic Range



Simplified Pharmacokinetics

Three Critical Equations

1) $[]_{\text{bolus}} = \text{dose} / V_d$

2) $[]_{\text{steady state}} = \text{rate}_{\text{in}} / \text{Cl}$

3) $\text{Cl} = (0.693 * V_d) / t_{1/2}$

From the above three equations, it is clear that the critical data to obtain for TDM/pharmacokinetic consultation is the **dosage, V_d** and either the **Cl or $t_{1/2}$** of the drug. Note that V_d and Cl usually given in weight-based units; hence, it is also necessary to find out the patient's weight. Of course, knowledge of the references ranges for efficacy and toxicity of the drug is critical as well.

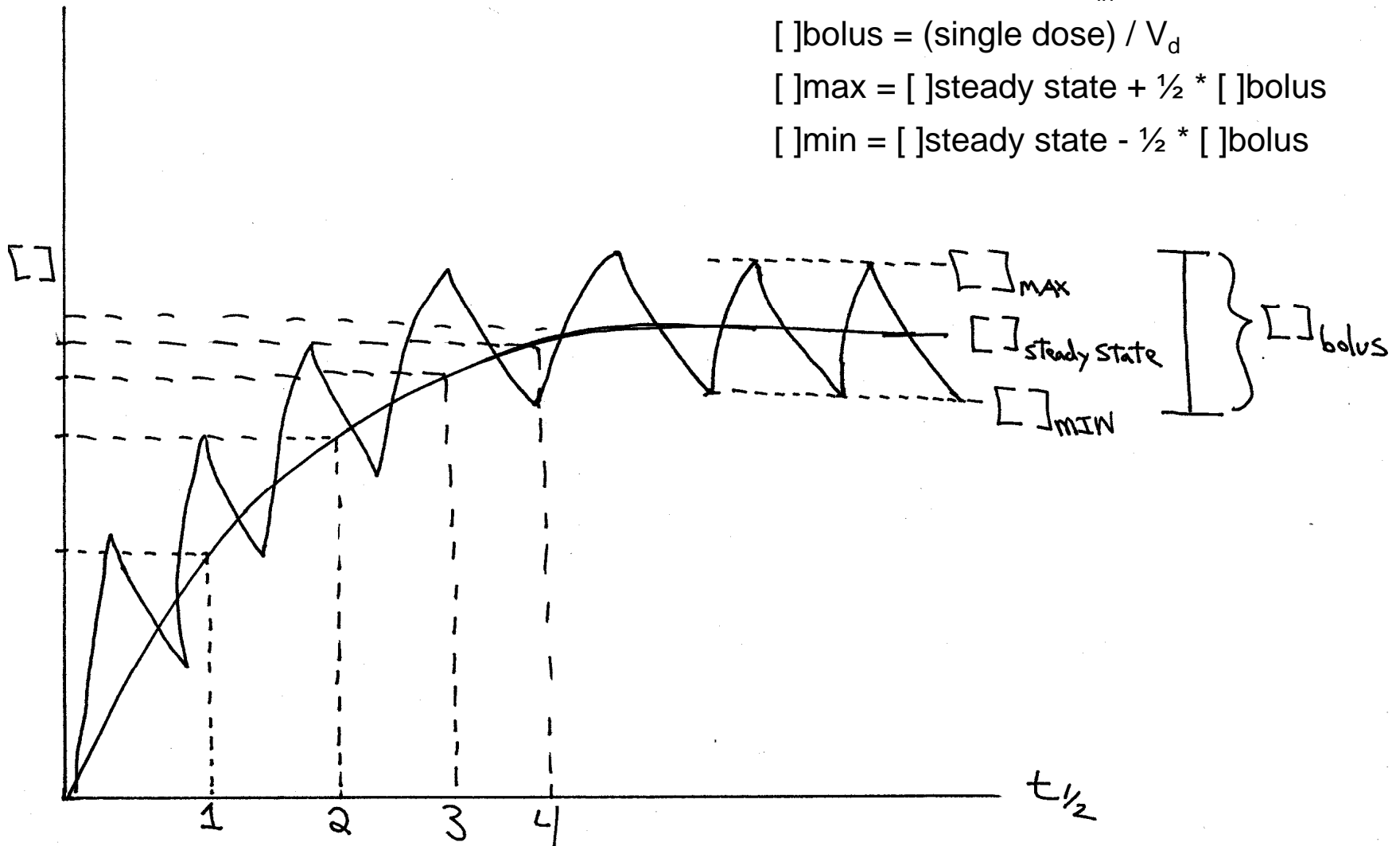
Simplified Pharmacokinetics

$$[]_{\text{steady state}} = \text{Rate}_{\text{in}} / \text{Cl}$$

$$[]_{\text{bolus}} = (\text{single dose}) / V_d$$

$$[]_{\text{max}} = []_{\text{steady state}} + \frac{1}{2} * []_{\text{bolus}}$$

$$[]_{\text{min}} = []_{\text{steady state}} - \frac{1}{2} * []_{\text{bolus}}$$

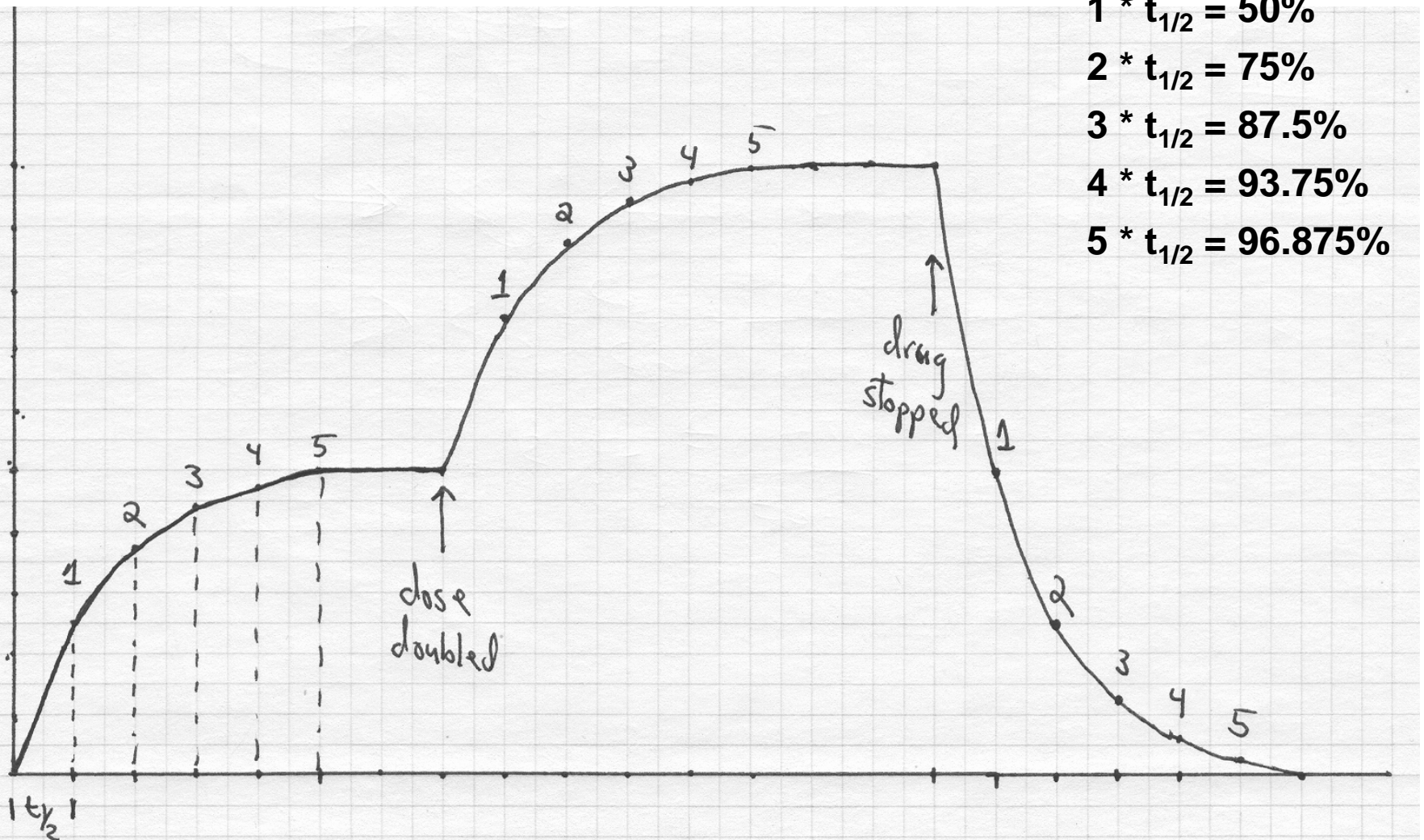




Critical Importance of the Half-life!

- Half life is the key for knowing
 - When a new medication is started, how long until the patient reaches steady state?
 - When a patient stops taking a medication, how long until it is gone?
 - When a drug dosage is changed, how long until a new steady state is achieved?

Critical Importance of the Half-life!



Simplified Pharmacodynamics

- Strictly speaking, pharmacodynamics (PD) defines the relationship between drug level and effect (think about the dose-response curves).
- In practice, it tells us *what/when* to measure drug levels and what they mean.
- In some fortunate cases, other measurements are excellent surrogates for drug levels (e.g. the “PT” and coumadin therapy).

Main PD Classifications

- 1) Maintain drug levels within the therapeutic range (e.g. phenytoin or digoxin).
- 2) Maintain drug level above some minimally effective level (e.g. vancomycin and the “MIC”), with or without avoiding toxic (high) levels.
- 3) For some antibiotics (e.g. gentamicin) efficacy is best measured using a peak level (due to “peak-dependent killing” and the “post-antibiotic effect”) and toxicity is avoided by making sure the trough levels fall below a certain threshold (toxicity due to chronic accumulation).
- 4) Most complicated situation is where only the “area under the curve” (AUC) correlates with either efficacy or toxicity and the PK are not predictable (e.g. cyclosporine).
 - a combination of trough and peak levels are most commonly used
- 5) As long as the total dose is adequate, drug monitoring is not necessary (e.g. penicillin).
 - generally applies when EITHER a drug has a very wide therapeutic range/index OR has very predictable pharmacokinetics

Classifications of Drug Toxicities

- can be overlapping

- Dose-dependent (*concentration-dependent*)
- Idiosyncratic (i.e. unpredictable, but strictly defined means *genetically determined*)
- Allergic or immunologic
- Carcinogenic
- Teratogenic
- Dependence/addiction
- etc.

Mechanisms of Drug Toxicities

- Nonspecific macromolecular damage
 - toxic metabolite of acetaminophen
- Inflammatory and immune-mediated
 - autoimmune hemolytic anemia (many drugs): methyldopa, penicillin, etc.
 - lupus-like syndrome caused by hydralazine, isoniazid and procainamide (induces antibodies against myeloperoxidase and/or DNA).
- Enzyme inhibition
 - cellular energy production: salicylate, cyanide, etc.
 - acetylcholinesterase inhibitors (pesticides)
- Receptor-mediated or “hormonal”
 - glucocorticoids (i.e. “steroids”): prednisone, cortisol, ...
 - aryl hydrocarbon receptor: dioxin

Acute versus Chronic

- Most of the acute toxicities are simply dose-dependent and predictable.
- However, many drugs cause less predictable chronic toxicities.
 - Examples include AZT (mitochondrial toxicity), phenytoin (gum hyperplasia), cyclosporine (nephrotoxicity), ...



Importance of Drug Metabolism

Just a note that it is always important to consider drug metabolism pathways when analyzing drug toxicity issues.

Drug metabolites are often responsible for toxicity.

This will be illustrated in the upcoming example cases.

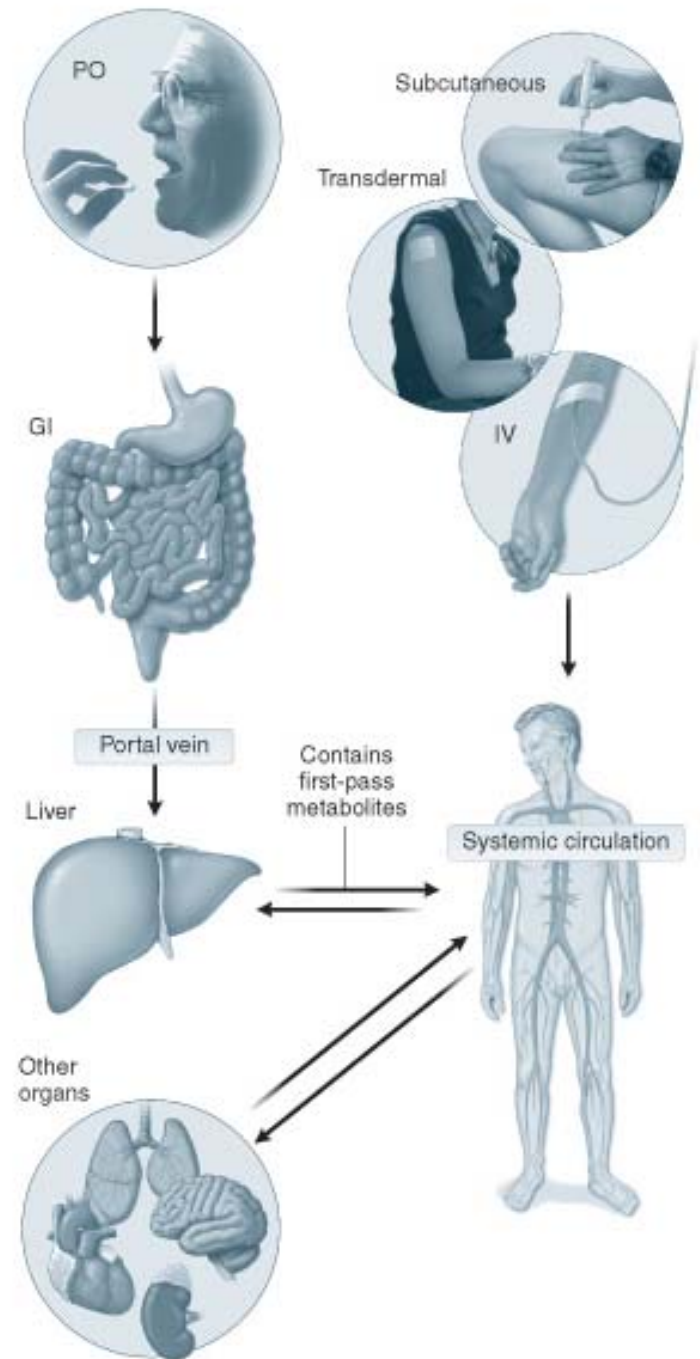


Important Effects of Drug Metabolism

- Functional inactivation
- Increased water solubility
 - Enhanced excretion
 - Redistribution away from hydrophobic tissue sites
- Occasionally, functional activation
 - The metabolites of some drugs are also active
 - “Pro-drugs” are activated by metabolic reactions

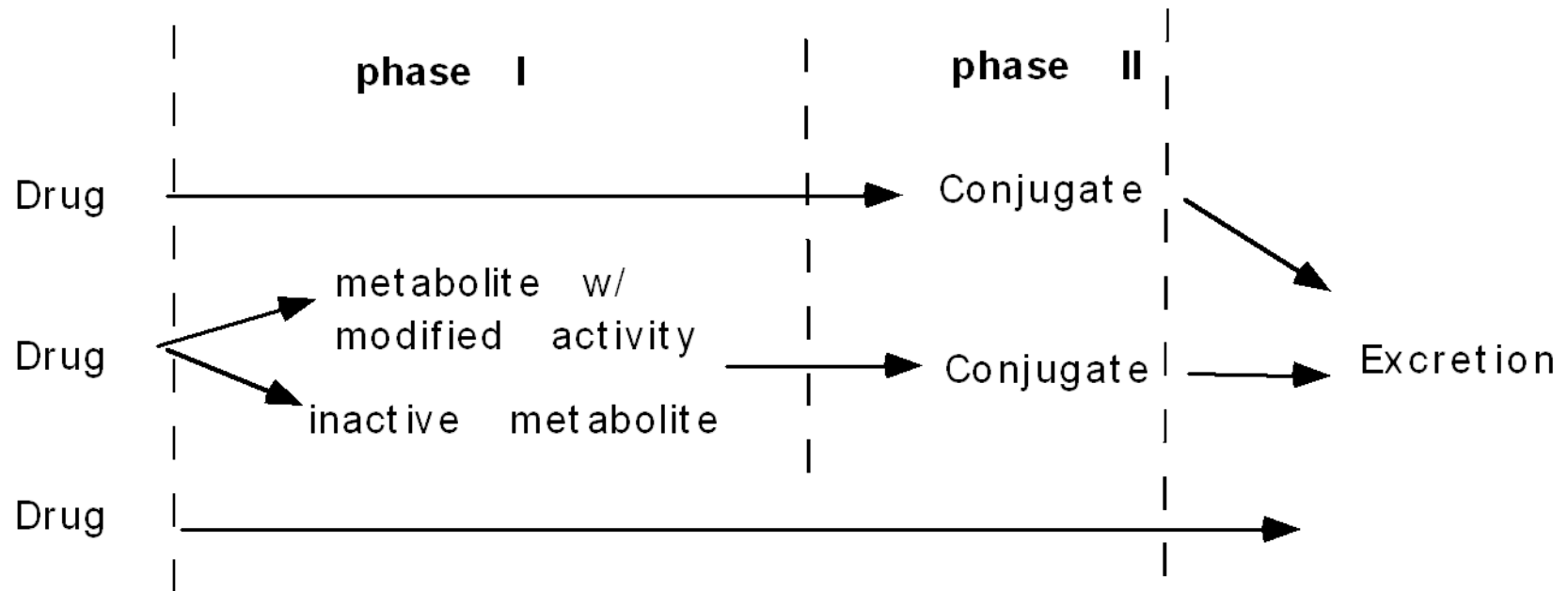
Sites of Drug Metabolism

- Liver is the most important organ.
 - Heavily perfused.
 - Highest level of drug-metabolizing enzymes.
- Others sites include skin, lungs, G.I. tract, and the kidneys.
- “First-pass metabolism” applies to orally administered drugs.



Two Major Classes of Enzymatic Reactions

METABOLISM



Phase I reactions chemically modify the drug.

Phase II reactions conjugate the drug to hydrophilic small molecules.

Phase I Reactions

Convert the drug to a more polar compound via enzymatic reactions that *add small functional groups* such as a hydroxyl, sulfhydryl or amino.

1. **Oxidation-** primarily occur via Cytochrome P450 oxidases (e.g. hydroxylation of barbiturates), but there are also a few P450-independent oxidations (e.g. dehydrogenation of alcohols).
2. **Reduction-** for a few drugs such as chloramphenicol, methadone, halothane and naloxone .
3. **Hydrolysis-** Occurs with esterases and amidases (e.g. succinylcholine and indomethicin, respectively).

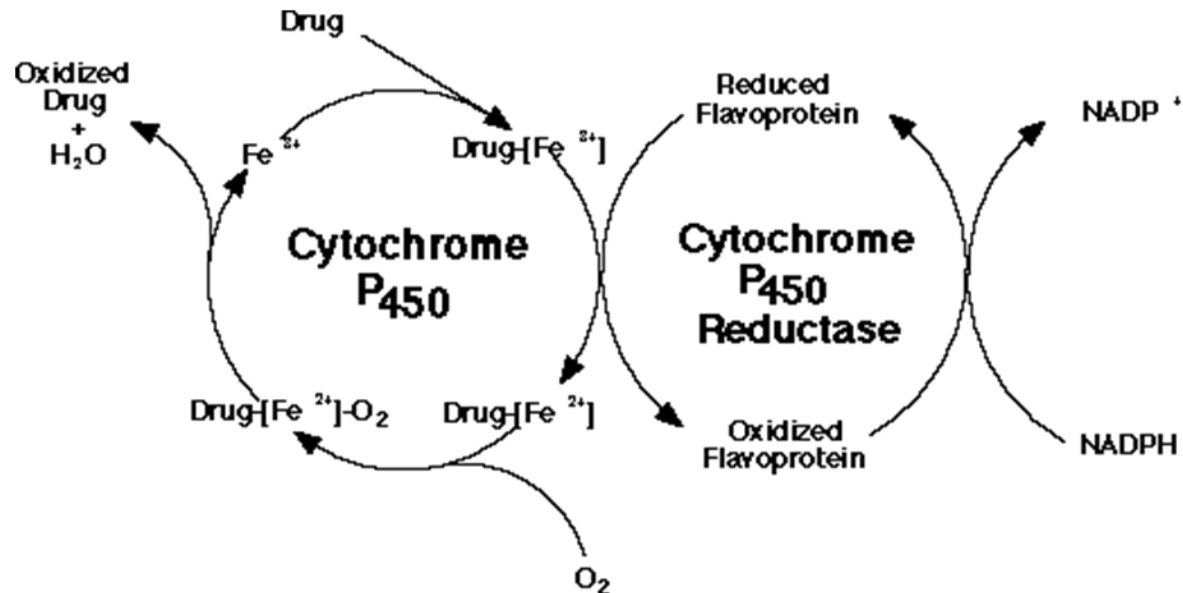
Cytochrome P450 Oxidases

Primary Phase I enzyme system and the largest metabolizer of lipid soluble drugs or xenobiotics.

Close to 50 family members of P450 enzymes.

Membrane bound enzymes in the smooth ER (“microsomal”).

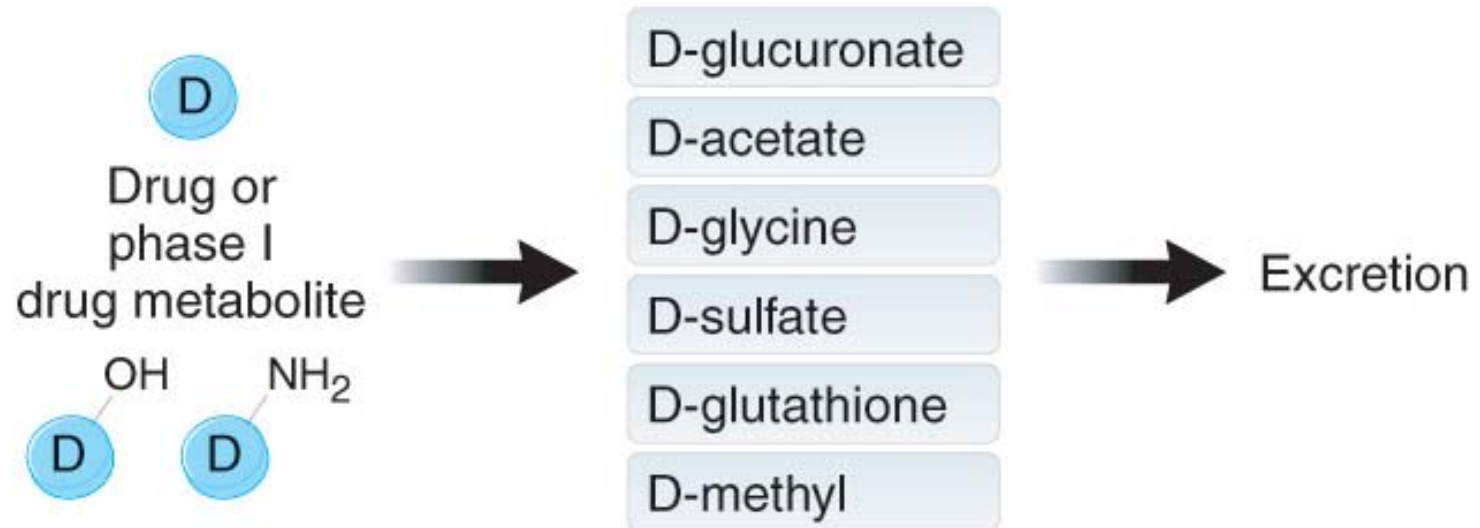
Each consists of 2 components: an oxidase and a reductase, which require molecular oxygen and NADPH as electron donor.




Phase II Reactions

Conjugation or addition of polar molecules to a drug to greatly enhance polarity and water solubility in order to facilitate excretion in feces, bile or urine. Important examples include

- (1) glucuronidation of Tylenol,
- (2) sulfation of estrogens,
- (3) acetylation of sulfonamide antibiotics, and
- (4) glutathione conjugation of Tylenol.





Inter-individual Variability in Drug Metabolism

- Age and gender (and Race?)
- Diet and drug interactions
- Co-morbidity (i.e. other diseases)
- Pharmacogenetics (separate lecture)

Age and Gender (Race?)

- **Infants:** decreased phase I and II reactions
 - mature slowly over first two weeks of life
 - bilirubin glucuronidation
 - chloramphenicol toxicity due to deficient phase II reaction (gray baby syndrome)

- **Elderly:** general decrease in hepatic capacity

- **Gender:** hormones regulate enzyme levels

- **Race:** clear differences, part of pharmacogenetics

Cytochrome P450 System

Induction and Inhibition

Inducers	Inhibitors
Barbiturates	Cimetidine
Phenytoin	Omeprazole
Carbamazepine	Acute Ethanol Toxicity
Chronic Ethanol Toxicity	Valproic Acid
Rifampin	Erythromycin
Ritonavir	Disulfram
Griseofulvin	Isoniazid
(St. John's Wort)	Ciprofloxacin

Although all enzyme systems are likely susceptible to both induction and inhibition, this has classically been defined generically for the Cytochrome P450 System given its widespread importance in drug metabolism.

Expect to see broader and more precise descriptions of these effects enter into routine clinical practice in the future.

Comorbidity (other diseases)

- Altered hepatic function
 - When hepatic function is compromised, so may drug metabolism.
 - However, this often requires extensive damage before having an effect

- Altered hepatic perfusion
 - When hepatic tissue is fully intact but not effectively perfused (e.g. from heart failure) drug metabolism may be slowed.

- Nutritional deficiency
 - Malnutrition depletes sulfation stores, glutathione, and reductive potential (NADH/NADPH levels), hindering drug metabolism.
 - Often “malnutrition” is associated with a chronic illness instead of a restricted diet, a result of catabolic syndromes (e.g. “cancer cachexia”).

Resistance to Warfarin

A 42-year-old man was admitted to hospital with chills and progressive shortness of breath on exertion. He had received an aortic valve replacement 12 years before presentation and had been taking warfarin (5.5 mg/day) since that time with an INR maintained between 2 and 3.

A diagnosis of pneumonia caused by *Pneumocystis jiroveci* was made. Serologic testing revealed a positive HIV status with a CD4 count of 150 cells/ μ L.

After successful treatment for his pneumonia, the patient was discharged from the hospital and prescribed aggressive antiretroviral therapy (zidovudine, lamivudine and lopinavir/ritonavir).



Resistance to Warfarin

At a follow-up visit one month after discharge, the patient's INR had declined to 1.1 (normal). Patient non-adherence and changes and diet were ruled out as a possible causes of the apparent warfarin resistance.

Over a period of six months his warfarin dose was slowly titrated from the initial 5.5 mg/day to a final 13 mg/day in order to maintain an INR between 2 and 3.

This most likely represented a drug interaction between the protease inhibitor combination lopinavir/ritonavir and warfarin.

Lopinavir/ritonavir both inhibit and powerfully induce the CYP3A4 enzyme complex, as well as, induce other P450 enzymes such as CYP2C9 and CYP1A2, which are both responsible for metabolism of warfarin.

Acetaminophen

– importance of drug metabolism

Illustrative Case #1

HR is a 27 y.o. man with a history of depression who took approximately 40 325 mg (standard release) acetaminophen tablets around 2 hours ago. His wife found him with the empty pill bottle and brought him into the ED. He is very emotional, describes minor stomach upset, but otherwise has no significant signs or symptoms.

How much acetaminophen is too much?

For an acute exposure, this is well established:

Toxicity is considered *possible* if maximum potential exposure is **> 7.5 g in adults or 150 mg/kg in children.**

Based on history, our patient's maximum exposure is $40 \times 325 \text{ mg} = 13,000 \text{ mg}$ or **13 g.**

Note that this is also $13,000\text{mg}/70\text{kg} = 186$ “mg/kg”.

Why is Acetaminophen Toxic?

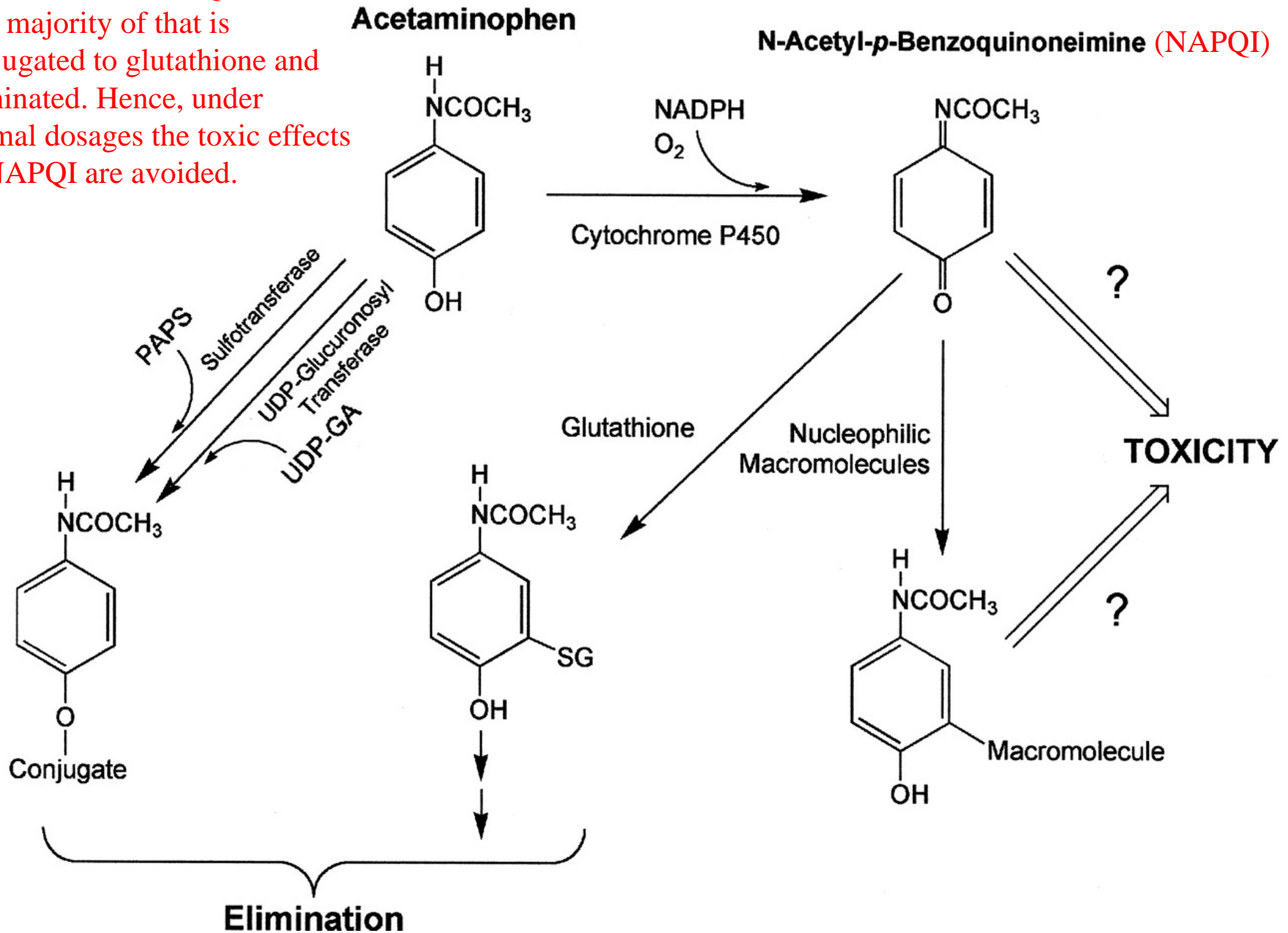
Acetaminophen is a strong analgesic and antipyretic with weak anti-inflammatory properties.

Its therapeutic effects are attributed to central inhibition of prostaglandin synthetase.

However, its toxic effects are unrelated to its therapeutic effects (i.e. for many drugs toxicity is simply “too much therapy”).

The toxic effects of acetaminophen are the consequence of an undesired *metabolite*. This is another common mechanism of drug toxicity.

Normally, only 5 – 15% is converted into NAPQI and a vast majority of that is conjugated to glutathione and eliminated. Hence, under normal dosages the toxic effects of NAPQI are avoided.



Four Phases of Acetaminophen Poisoning

Phase I (0.5 – 24 h): Consequences of G.I. distress – anorexia, nausea, malaise, pallor, vomiting and diaphoresis. Little or NO signs of hepatotoxicity. The patient may appear normal.

Phase II (24 – 72 h): Initial phase of hepatotoxicity. RUQ pain may be evident. G.I. distress lessens. Hepatitis – elevated liver enzymes. Decreased hepatic function – elevated PT/INR, elevated unconjugated bilirubin. Possible decreased renal function.

Phase III (72 – 96 h): Sequelae of hepatic necrosis – massively elevated liver enzymes, coagulation defects, encephalopathy, jaundice. Renal failure and myocardial dysfunction may be present (multi-system organ failure).

Phase IV (4 d – 2 wk): Either death (result of multi-system organ failure) or if hepatic damage is reversible, complete resolution may occur. Liver transplant is another possibility.

Back to our patient...

Potential toxic exposure (13 g) and symptoms consistent with phase I of acetaminophen toxicity (i.e. he complains of G.I. distress).

No physical signs of other drugs (but cannot safely rule them out).

What to do next?

- 1) Laboratory Studies
- 2) Treatment

What lab tests should we get?

- 1) **Serum Overdose Panel** (designed to rule out many common and treatable overdoses where laboratory monitoring is particularly valuable):
 - Acetaminophen – **120 $\mu\text{g/ml}$**
 - Salicylate – not detected.
 - Alcohol panel (ethanol, methanol, isopropanol and acetone) – not detected.
 - Barbiturates – not detected.
 - Tricyclic Antidepressants – not detected.

What lab tests should we get?

2) Hepatic and Renal Damage/Function

- Electrolytes (mM):
 - Na – 141, K – 4.1, Cl – 103, Bicarbonate – 28.
- Basic Renal Function:
 - Cr – 0.8, BUN – 12.
- Liver Enzymes:
 - AST – 26, ALT – 21, AP – 84, LDH – 120.
- Evaluation of Liver *Function*:
 - PT/INR – 11.5s/1.1, Glucose – 95,
 - Total Billirubin – 0.93, Ammonia – 15



What about the acetaminophen level?
(120 $\mu\text{g/ml}$)

Therapeutic Range is 10 – 20 $\mu\text{g/ml}$.

Toxicity is expected for “acute” *peak* levels greater than 150 – 200 $\mu\text{g/ml}$.

But, you can't expect to catch the *peak* drug level for overdoses. How do you deal with this?

Predicting a maximum peak level

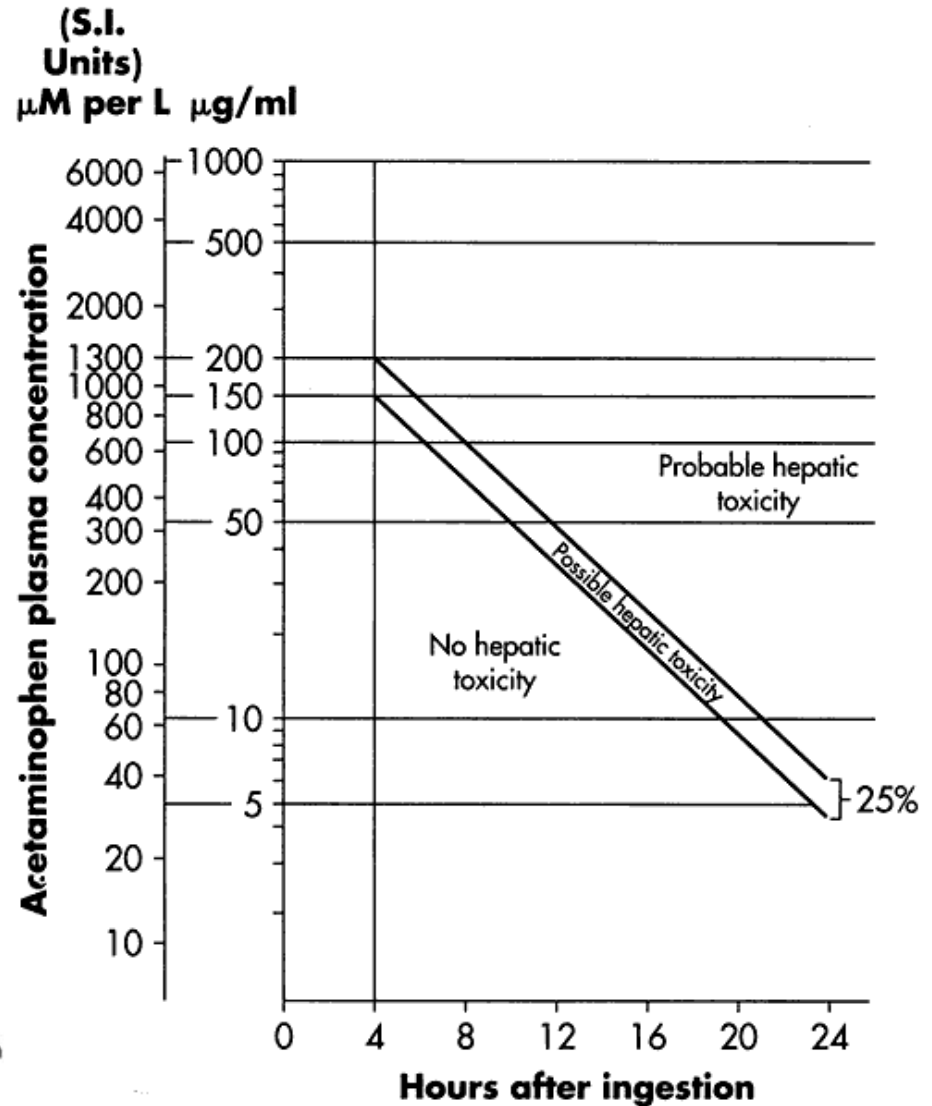
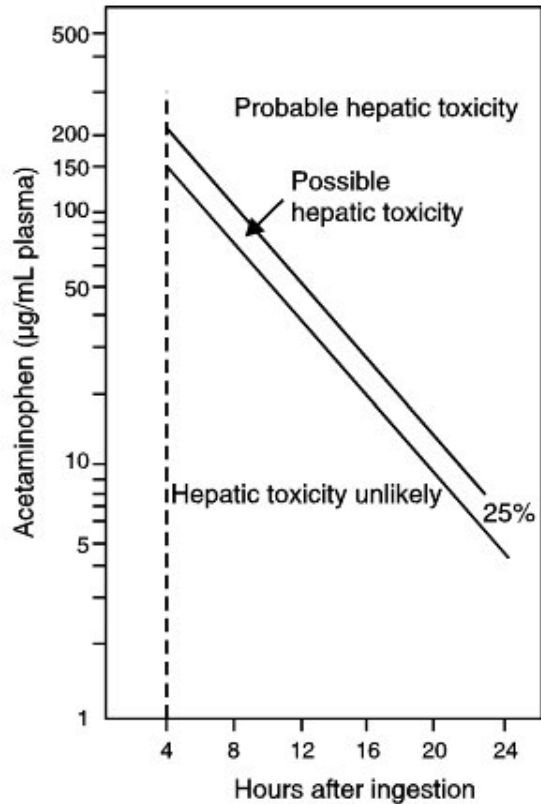
- 1) What information do you need?
 - Dose (13g), Patient Weight (70kg), Volume of distribution (~1 L/kg), Bioavailability (~60 – 98%, but in overdose tends to be on the higher side as metabolism reaches saturation).

- 2) How do you calculate a maximum peak level?
 - 95% of 13 grams is ~ 12 grams.
 - Volume of distribution = 1 L/kg * 70 kg = 70 Liters.
 - Peak Level = dose/Vd = 12g/70L = **171 µg/ml** (multiplied by 1000 to convert g/L into µg/ml).

- 3) What factors might prevent the patient from actually achieving this theoretical maximum?
 - Delayed absorption due to delayed gastric emptying, formation of “concretions” and intestinal irritation.
 - Ongoing elimination during delayed absorption (peak later and lower).
 - Actual dose less than reported/anticipated.
 - Unfortunate inter-individual variability (often as much as 50 – 100%) in Volume of Distribution, Bioavailability, Elimination Half-life, etc.
 - Hence, although rough PK calculations useful, monitoring of serum drug levels remains essential.

Rumack-Matthew Nomogram

Acetaminophen Toxicity Nomogram



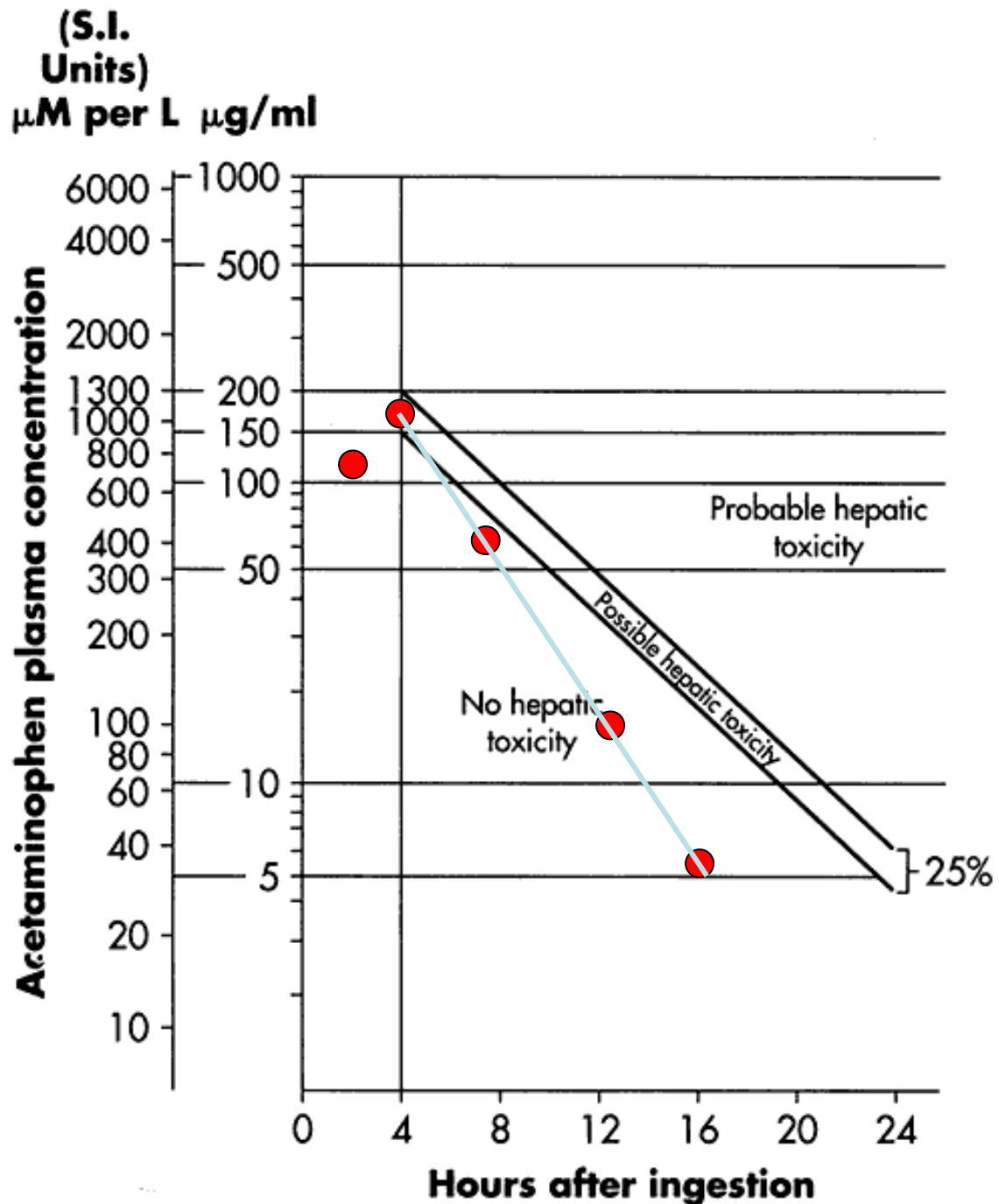
The Rumack-Matthew nomogram, relating expected severity of liver toxicity to serum acetaminophen concentrations.

From Smilkstein MJ, Bronstein AC, Linden C, et al, "Acetaminophen Overdose: A 48-Hour Intravenous N-Acetylcysteine Treatment Protocol," *Ann Emerg Med*, 1991, 20(10):1058, with permission.

What happens with our patient?

Treatment is initiated as a follow-up level at ~ 4 hours post-ingestion was > 150 mg/ml.

A series of levels measured every 4 hours is consistent with successful treatment as the half-life is less than 4 hours (blue line represents ~ 2.5 – 3 hr half-life).



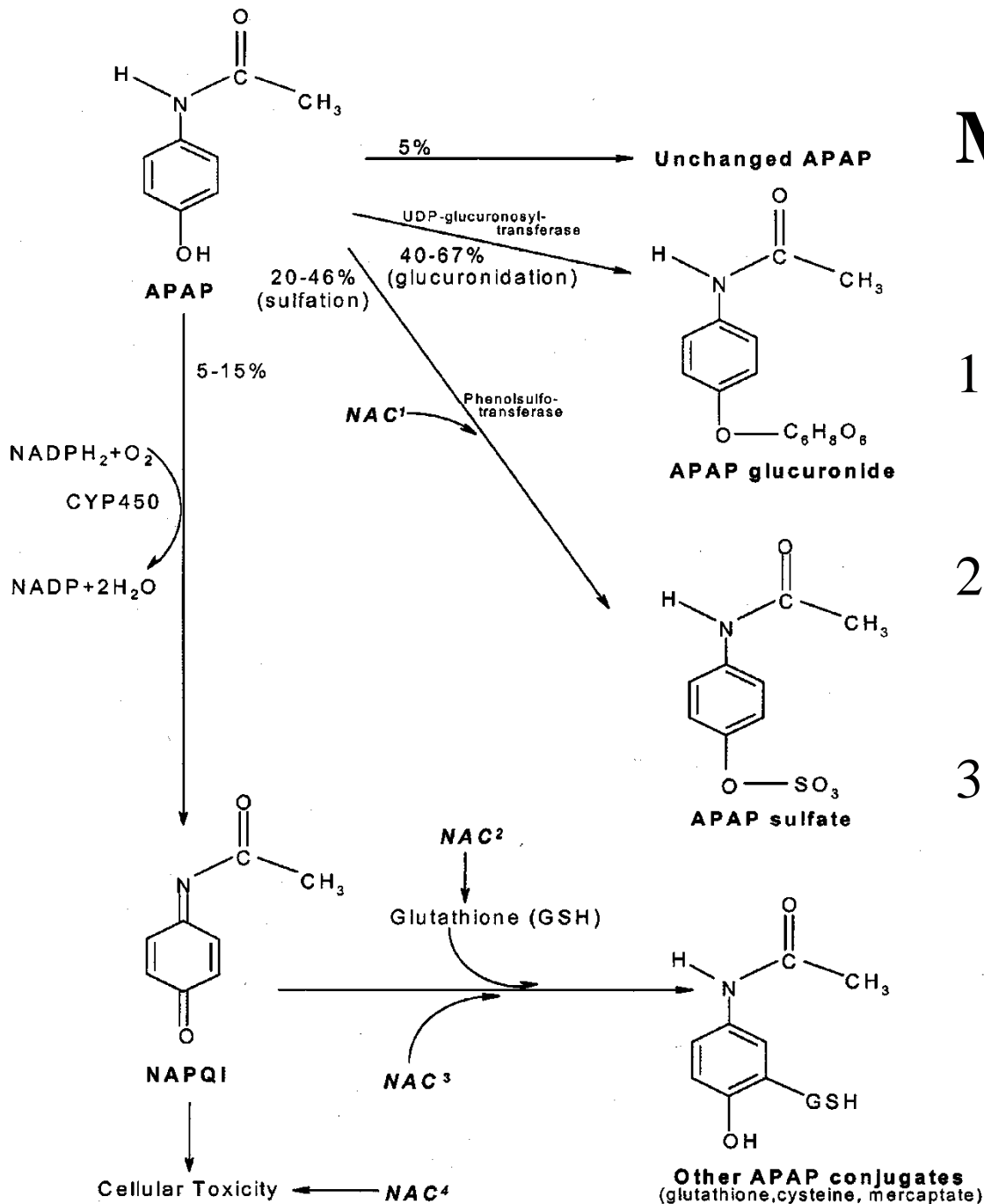
Treatment of Acetaminophen Toxicity

1) Generic

- Decontamination: gastric lavage (if early) and activated charcoal (PO or via NG tube)
- Supportive: not indicated during Phase I, but relevant for other phases.

2) Antidote: N-acetylcysteine (NAC)

- Maximal effectiveness if given in first 8 hours.
- Given P.O. in the US (see handout); may require an anti-emetic as reputedly very noxious.
- However, also demonstrates effectiveness is given after hepatotoxicity begins (i.e. Phase II).
- Two mechanisms likely. First, replenishes reductive sulfation stores for detoxification of APAP and NAPQI. Second, may have a direct protective and/or regenerative effect on tissue, sometimes used in multi-system organ failure due to other causes.



Mechanism for N-acetylcysteine:

1. Provides a substrate for APAP sulfation.
2. Replenishes glutathione stores.
3. Can substitute for glutathione for detoxification of NAPQI.

Acetaminophen Case #2

R.W. is a 31 y.o. woman with a PMH of major depression and alcohol abuse. She is currently in the process of divorcing an abusive husband and is separated from her 8 y.o. son.

Approximately 24 hours prior to presenting to the YNHH ED, the patient took approximately 48 tablets containing 500 mg Acetaminophen for a total dose of 24 grams. These were taken after approximately 8-10 hours of heavy “binge” drinking by the patient.

She subsequently lost consciousness and upon awakening complained of nausea, vomiting and right upper quadrant abdominal pain.

Acetaminophen Case #2

Past Medical History: Significant only for major depression and alcohol abuse. She has had two previous suicide attempts and has been under treatment of a psychiatrist. However, she last visited her psychiatrist about one year ago and stopped taking her psychiatric medications at that time.

Medications: none

Allergies: none

Social History: Drinks ½ pint of hard alcohol per day and smokes tobacco occasionally. She has been a Jehovah's Witness since 1986 and refused all blood products during the admission.

Physical Examination

- ❑ Vital Signs: Afebrile, BP 156/93, HR 90, RR 20, SaO₂ 98% (on room air)
- ❑ She is alert and oriented x 4 and in no acute distress.
- ❑ HEENT: PERRL, EOMI, *no scleral icterus*, oropharynx clear
- ❑ Neck: Supple, no lymphadenopathy, no carotid bruits, no JVD
- ❑ Heart: sinus tachycardia, regular rhythm, no G/M/R
- ❑ Lungs: clear to auscultation bilaterally
- ❑ Abdomen: soft, nondistended, *mild RUQ tenderness*
- ❑ Extremities: *no asterexis*, no C/C/E, no petechiae/ecchymosis
- ❑ Neuro: non-focal
- ❑ Rectal: normal tone, heme negative

Initial Laboratory Values

Electrolytes and Renal Function: Na 137, K3.3, Cl 106, HCO₃-19.8, BUN 9, Cr 1.0

Liver Enzymes: *ALT 654, AST 884*, Alk Phos 86, Amylase 89 (for the pancreas)

Liver Function: Glucose 255, Total Protein 6.4, Albumin 3.6, Bilirubin T/D 1.44/0.37, PT 13.7 (12.2), PTT 26.1 (note that four hours later: PT 16.4 (12.1), PTT 31.9)

Serum Overdose Panel: Acetaminophen 19 µg/ml, Salicylates 3 µg/ml, Alcohols/TCAs/Barbiturates - all negative.

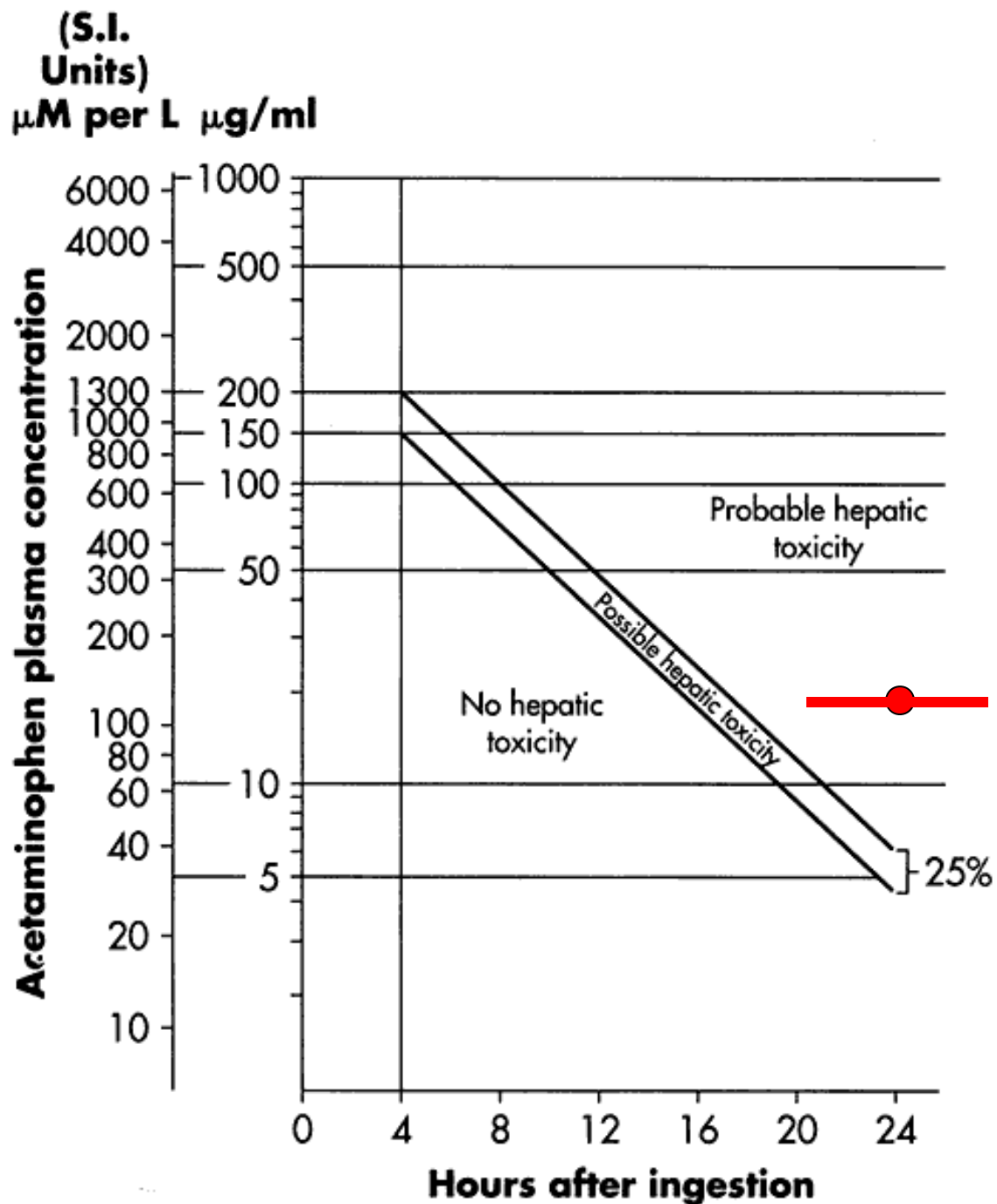
CBC: WBC 5.0, Hgb 12.8, Hct 40.7, MCV 89, Platelet 60

One more example of using the Rumack-Matthew Nomogram...

A level measured ~ 24 hours after the overdose was 19 $\mu\text{g}/\text{ml}$.

Again, the red bar represents a degree of uncertainty in the timing (arbitrary choice of ± 4 hours).

The placement of the level in the region of “probable hepatic toxicity” is clear and also unnecessary as the patient has already elevated liver enzymes and early signs of hepatic failure.



Hospital Course

The patient did well and never became encephalopathic.

Of course, she received a complete treatment of N-acetylcysteine (beginning ~24 hours after the overdose).

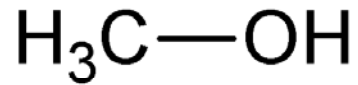
Her peak liver enzymes occurred about 36 hours after admission: AST 5870, ALT 5440.

She never developed signs of multi-organ failure and maintained good ABGs, cardiac function, Cr, urine output, etc.

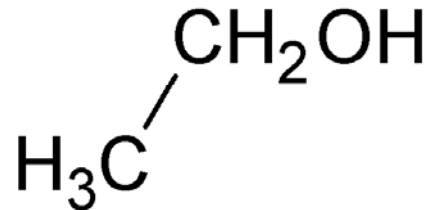
She was discharged on hospital day #4 with normal PT/PTT, AST 219, ALT 1860 and Bilirubin T/D of 0.64/0.26.

Toxic Alcohols

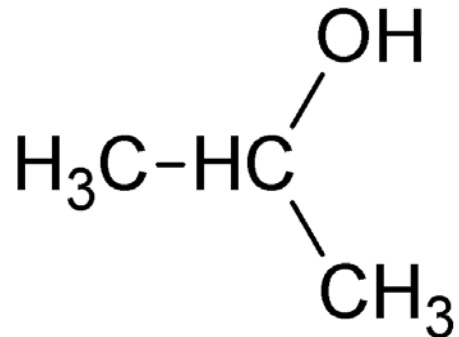
Methanol



Ethanol

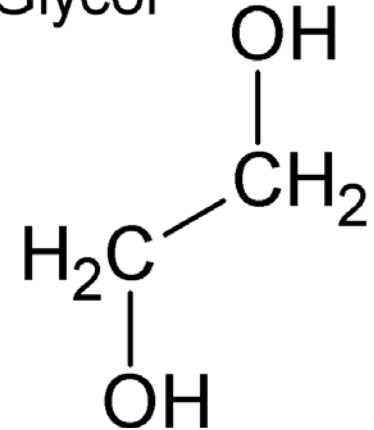


Isopropanol

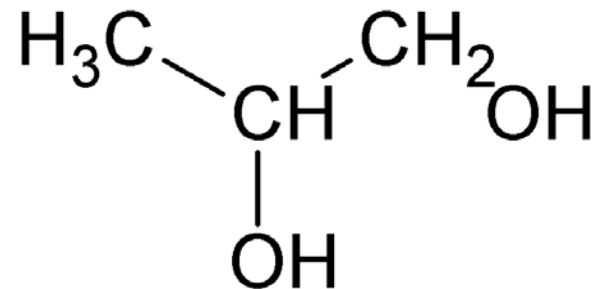


Toxic Glycols

Ethylene Glycol



Propylene Glycol



– more drug metabolism

All alcohols and glycols act on the CNS as a “sedative-hypnotic”

- Respiratory depression (bradypnea)
- CNS depression (inebriation)
- Hypothermia
- Tachycardia and Hypotension
- Isopropanol is a much stronger CNS depressant than the others and induces coma at around 100 mg/dl (i.e. 0.1 % w/v).

**Serum Ethanol
Level (mg/dL)****Clinical effects on the non-
tolerant individual**

< 50 mg/dl

Mild muscular incoordination

50-100

Incoordination; driving increasingly dangerous

100-150

Mood, personality, behavioral changes; driving is dangerous

150-200

Prolonged reaction time; driving is very dangerous

200-300

Nausea, vomiting, diplopia, marked ataxia

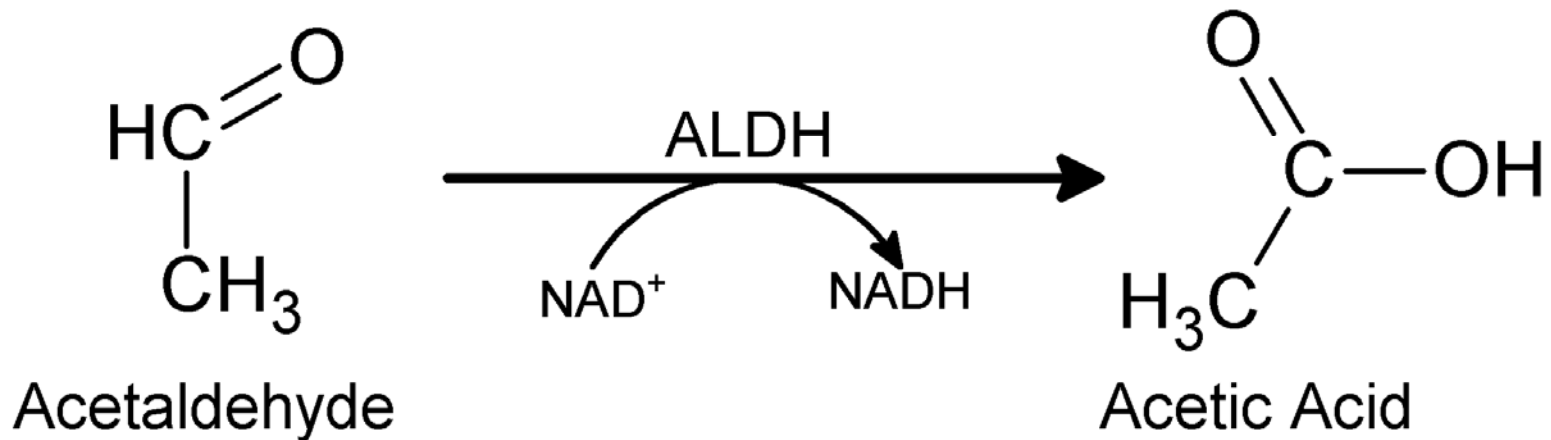
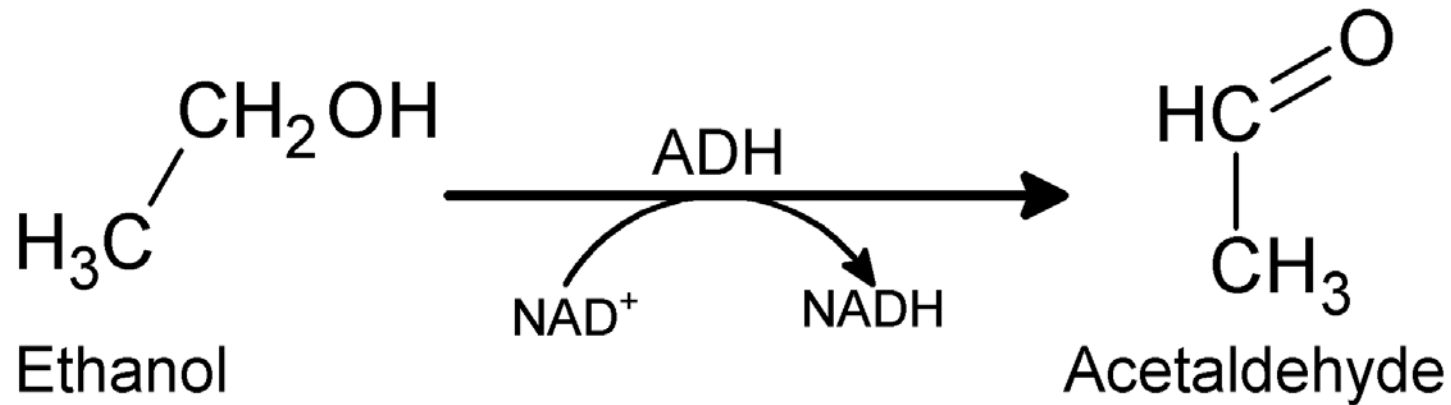
300-400

Hypothermia, dysarthria, amnesia

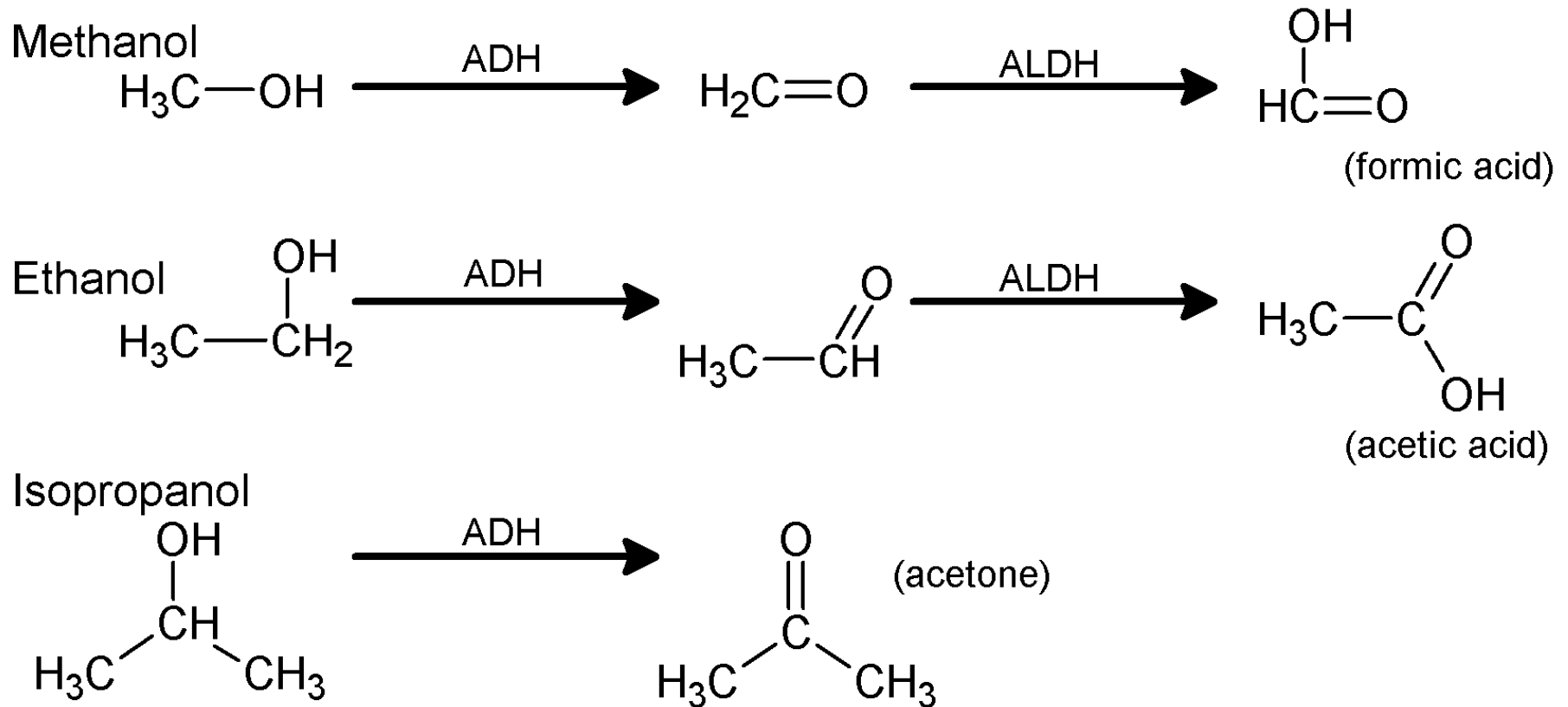
400-700

Coma, respiratory failure, death

“Metabolic” toxicity is a result of metabolism by *alcohol dehydrogenase (ADH)* and *aldehyde dehydrogenase (ALDH)*



Metabolism by ADH and ALDH



Methanol Poisoning

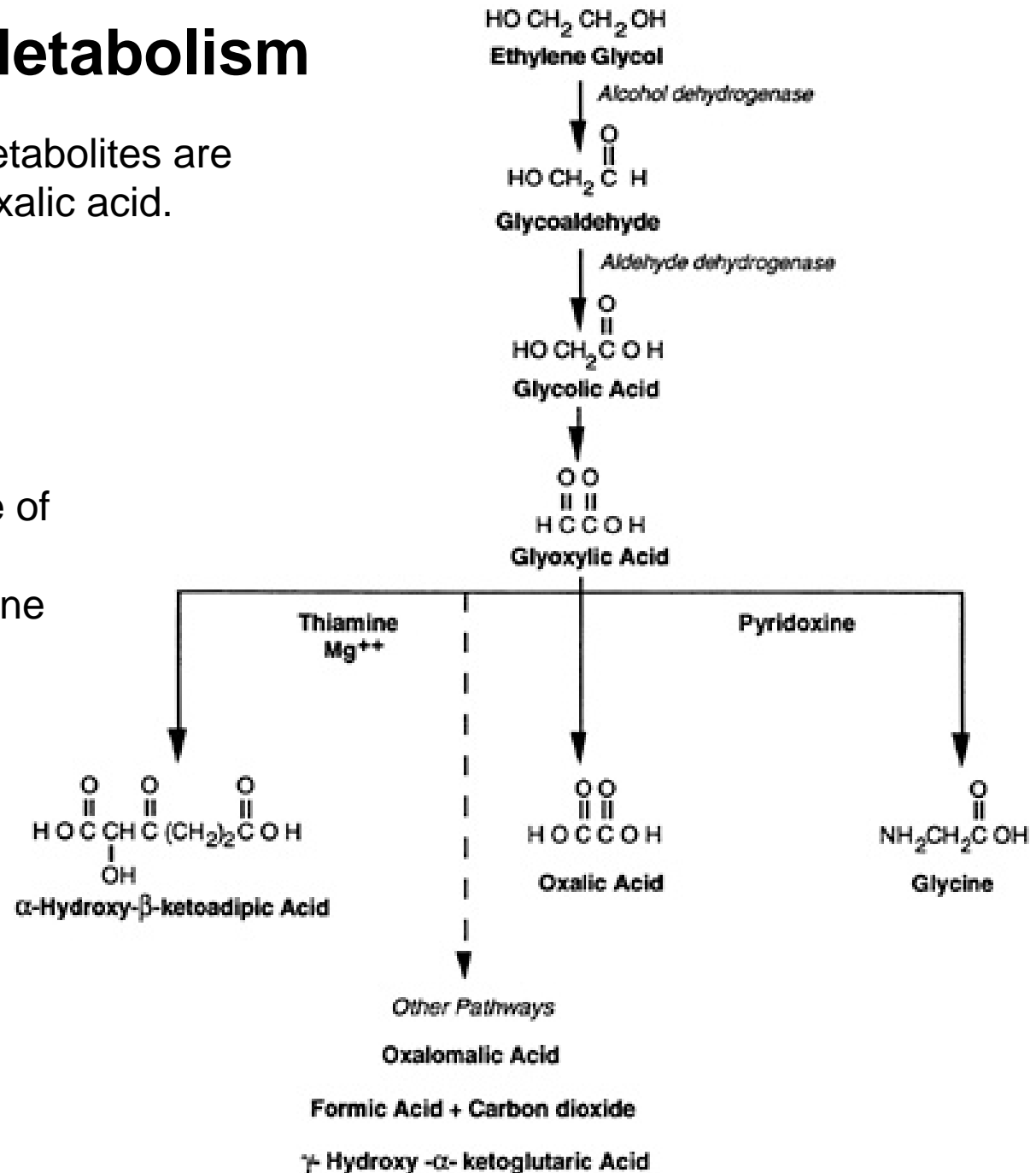
- Formaldehyde and formic acid accumulate because there is no endogenous metabolic pathway for detoxification.
- A minor pathway for elimination can be aided by folate administration.
- Methanol causes a CNS/respiratory depression like other alcohols and its metabolites poison tissue (oxidative phosphorylation) resulting in
 - an elevated anion gap metabolic acidosis
 - noncardiogenic pulmonary edema
 - Gastritis with N/V, anorexia and abdominal pain
 - Occasional pancreatitis
 - **Most common is damage to the retina and optic nerve resulting in “snow fields”, blurred vision, hyperemic optic discs, mydriasis, papilledema and eventually blindness.**

Ethylene Glycol Metabolism

Note that the primary toxic metabolites are glycolic acid, glyoxylic acid, oxalic acid.

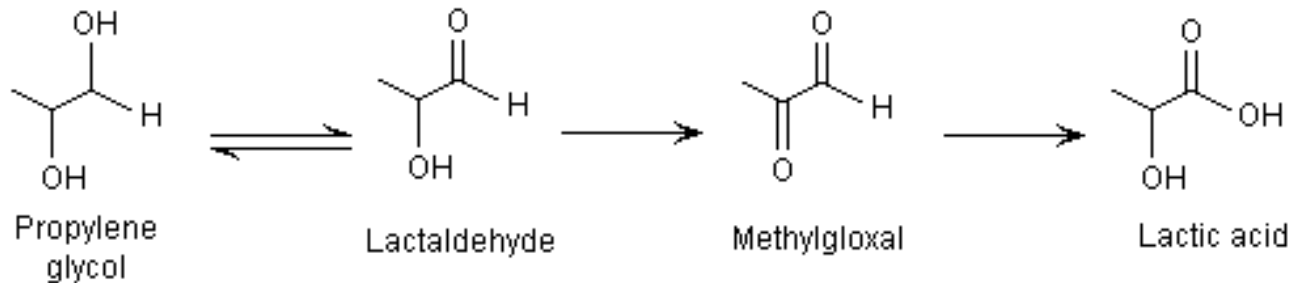
Precipitation of oxalic acid in tissues causes multisystem organ failure in untreated ingestions

Primary treatment is blockade of alcohol dehydrogenase with ethanol or fomepizole. Thiamine and pyridoxine are given therapeutically to detoxify glyoxylic acid and prevent its conversion into oxalic acid.

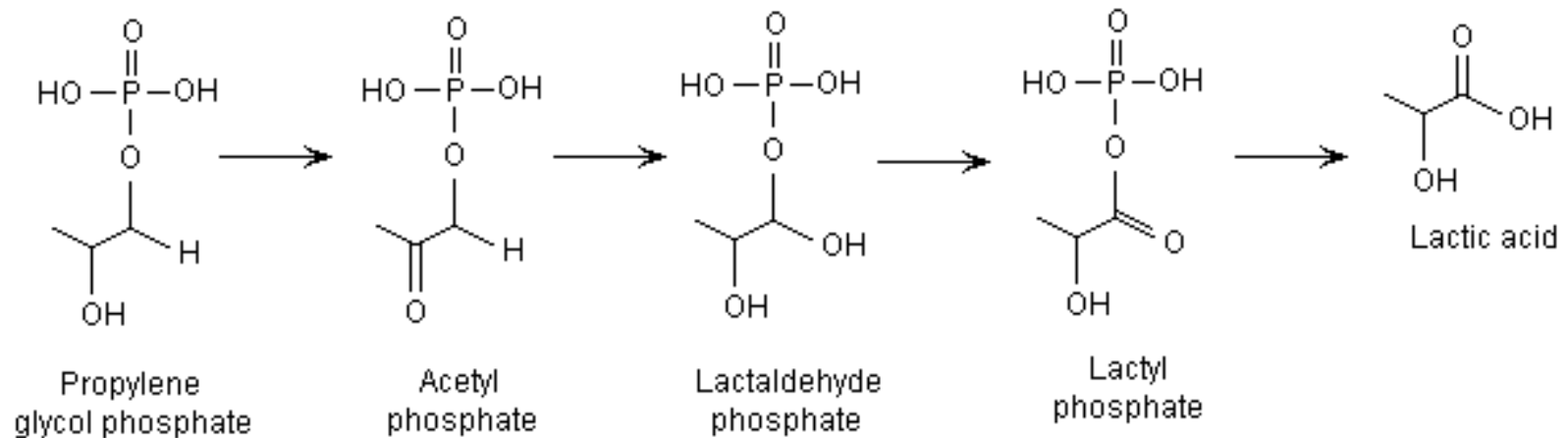


Propylene Glycol Metabolism

Pathway 1



Pathway 2



Stuporous with blurred vision...

- An 84-year-old woman weighing 121 lb (55 kg) with no previous history of alcoholism was stuporous on presentation at the emergency department. Her family had found her obtunded and reported that she had complained earlier of blurred vision and had had one episode of emesis.
- On physical examination, her blood pressure was 107/54 mm Hg, pulse rate 60 beats per minute, and respirations 16 per minute. Her lungs were clear and heart rate was regular, with occasional premature beats. Funduscopic examination was unremarkable, and neurologic examination showed no evidence of focal deficits. The remainder of the examination was unremarkable.

Laboratory Values

- Na 146, K 4.2, Cl 107, HCO_3^- 14 (low)
- Anion Gap = $146 - (107 + 14) = 25$ (high)
- BUN 10, Cr 1.4, Glucose 148, Lactate 2.1
- Liver Enzymes normal
- Serum Ethanol < 10 mg/dl (enzymatic method)
- ABG: pH 7.12 (dangerously acidic), PaO₂ 71, PCO₂ 30
- Measured serum osmolality = 354
- Calculated Osmolality = 307.7 ($2 * \text{Na} + \text{BUN}/2.8 + \text{Glc}/18$)
- Osmolal Gap = 46.2 (measured – calculated)
- Urinalysis revealed calcium oxalate crystals.

Summary and Differential Diagnosis

- 84 y.o. woman with severe CNS depression, blurred vision, a metabolic acidosis with an elevated anion gap (possible respiratory contribution), an osmolal gap of 46.2 and a finding of calcium oxalate crystals in the urine.
- A toxic alcohol or glycol ingestion is highly suspected. Although blurred vision could suggest methanol poisoning, oxalate crystals in the urine is most consistent with ethylene glycol.
- Note that with an osmolal gap of 46.2, expect a high ethylene glycol level ($46.2 * 5.8 = 268$).

An explanation...

- ❑ On further questioning, the family reports keeping a 2 L soda bottle containing antifreeze diluted with water in the kitchen.
- ❑ Suspect that patient's poor baseline eyesight may have led to mistaken ingestion.
- ❑ Confirmed when a sample from the patient's bedside drinking glass contained antifreeze and an ethylene glycol level was confirmed in the patient's blood (217 mg/dl).

Treatment

- Gastric lavage and oral activated charcoal.
- 5% dextrose and sodium bicarbonate administered IV.
- An intravenous IV loading dose of 10% ethanol given for a target of 100 mg/dl EtOH:
 - $V_d = 0.54 \text{ L/kg} * 55 \text{ kg} = 29.7 \text{ L (297 dl)}$
 - $(100 \text{ mg/dl} * 297 \text{ dl}) / (1000 \text{ mg/g} * 0.7939 \text{ g/ml})$
 - 37.4 ml of 100% EtOH or 374 ml of 10% EtOH
- A maintenance dose of 75 ml/hr was required to maintain a constant 100 mg/dl (frequently monitoring of EtOH level necessary).
 - $(20 \text{ mg/dl/hr} * 297 \text{ dl}) / (1000 \text{ mg/g} * 0.7939 \text{ g/ml})$
 - Requires 7.5 ml/hr of 100% EtOH or 75 ml/hr of 10% EtOH
- Patient also given IV thiamine and pyridoxine to encourage non-toxic metabolism of ethylene glycol.
- Because of extremely high ethylene glycol level, hemodialysis also performed to speed removal.

Chronic Toxicity of Anti-Retroviral Therapy

- L.R., a 31 y.o. HIV+ female, presents to the ED with 3 day h/o fever, tachycardia, and general malaise. Fever not associated with chills, cough or dysuria. She reports intermittent watery diarrhea and abdominal cramping over the past two months.
- Her diarrhea is associated with early satiety and bloating after PO intake; these abdominal symptoms are often accompanied by chest pressure and sweats.
- She also reports episodes of aching leg pain progressing to involve her lower back, generally lasting 1/2 to 1 day, over the last two months.
- L.R. has been on an anti-retroviral regimen of d4T, ddI, and nelfinavir for the past six months.

Physical Exam

- ❑ Vitals: T 100.7; HR 115; RR 20; BP 130/90; O2 98 % on room air
- ❑ HEENT: within normal limits
- ❑ Lungs: clear to auscultation bilaterally
- ❑ Heart: regular but tachycardic; III/VI systolic ejection murmur radiating to LUSB.
- ❑ Abdomen: mildly distended but soft and non-tender, no palpable masses, no hepatosplenomegaly, normal active bowel sounds, guaiac negative stool.
- ❑ Pelvic: WNL except single non-tender, movable inguinal lymph node.

Lab Tests

- Na⁺ 136, K⁺ 3.4, Cl⁻ 98, HCO₃⁻ **11.8**
- BUN 11, Cr 0.8, Glucose 138
- WBC **17.9** (66^N 14^B 3^{Meta} 11^L 5^M)
- Hb 14.8, Hct 42.5, Plt 236,000
- ABG: pH 7.31, pCO₂ 32, pO₂ 111
- Lactic Acid **10.3** (nml = 0.5 - 1.3)
- LDH **441** (90 -190), ALT **63** (0-40), AST **78** (7-40), Alk Phos 86 (70-230)

What's Going On?

- The initial leading diagnosis was infection and dehydration, most likely G.I. in origin.
- This conclusion was supported by the patient's malaise and G.I. symptoms, fever and elevated WBC. The negative chest x-ray, negative urinalysis and a lack of cough or dysuria ruled out other potential sites of infection.
- However, this diagnosis failed to account for several of her other findings including *mild lactic acidosis and elevated LFTs (evidence of hepatic injury)*.



Could LR have liver damage?

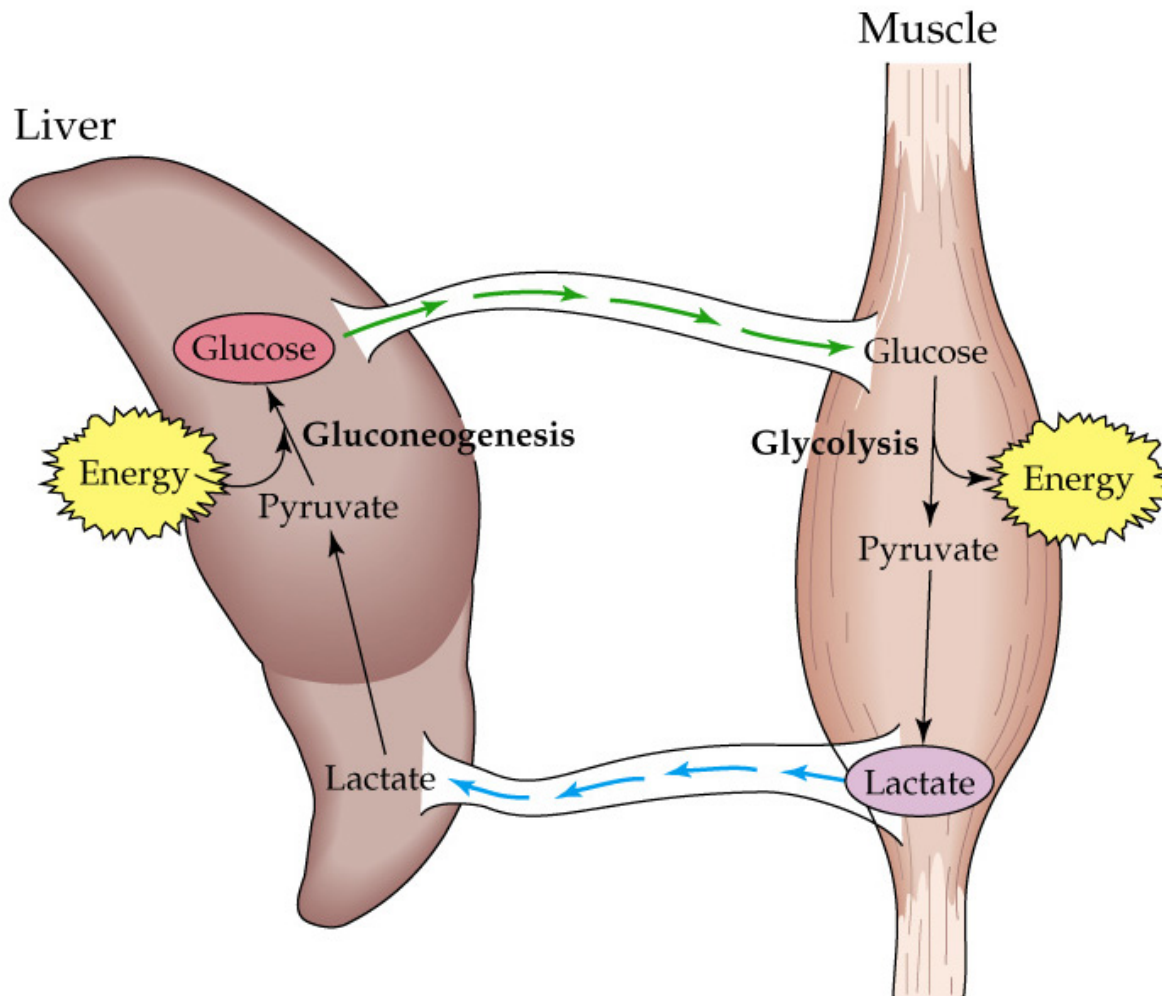
- Since the introduction of antiretroviral therapy, there have been numerous reports of hepatotoxicity and lactic acidosis related to Nucleoside Analogue Reverse Transcriptase Inhibitors (nRTIs), e.g. AZT, d4T, ddI, etc..
- Early papers documented several severe cases of rapidly progressing liver disease and acidosis that has in some cases proven fatal.

How Might this Happen?

- nRTIs are designed to inhibit HIV reverse transcriptase by terminating growing DNA transcripts.
- However, nRTIs also inhibit human γ -polymerase, found in mitochondria.
- Chronic inhibition of γ -pol leads to massive mitochondrial dysfunction and a severe energy crisis throughout the body.
 - we can't survive without our mitochondria!
- Famously “predicted” by Tommy Cheng (Pharm., YMS) from *in vitro* studies
 - provided a relative “risk” ranking of nRTIs based on activity against γ -pol that correlated nearly perfectly with clinical experience



The Always Important Cori Cycle



When the muscle switches to anaerobic metabolism, lactate is secreted into the bloodstream.

The excess circulating lactate is taken up by the liver and converted back into reduced sugar chains by gluconeogenesis, which requires a lot of NADH.

Physiologic Consequences of Chronic Mitochondrial Dysfunction

- 1) Energy starvation in tissue due to poor aerobic oxidation.
 - lactic acid produced due to anaerobic metabolism of glucose.
 - results in weakness and muscle pain (myopathy).
 - can also result in a peripheral neuropathy.
- 2) The liver has to metabolize all this lactic acid and attempt to keep up with glucose demands of the body.
 - for a variety of reasons, triglyceride production by the stressed liver dramatically increases resulting in
 - severely elevated blood triglyceride levels,
 - hepatic steatosis (fat deposits) which
 - ultimately damages the liver (hepatitis) and
 - eventually the liver begins to fail (lactic acidosis, hypoglycemia, etc.).
- 3) Dyslipidemia (fat deposits) are often seen in other tissues.

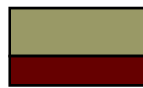


Many of the following slides were “borrowed” from Greg Howe, PhD

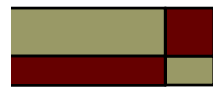
PHARMACOGENETICS

Pharmacogenetics

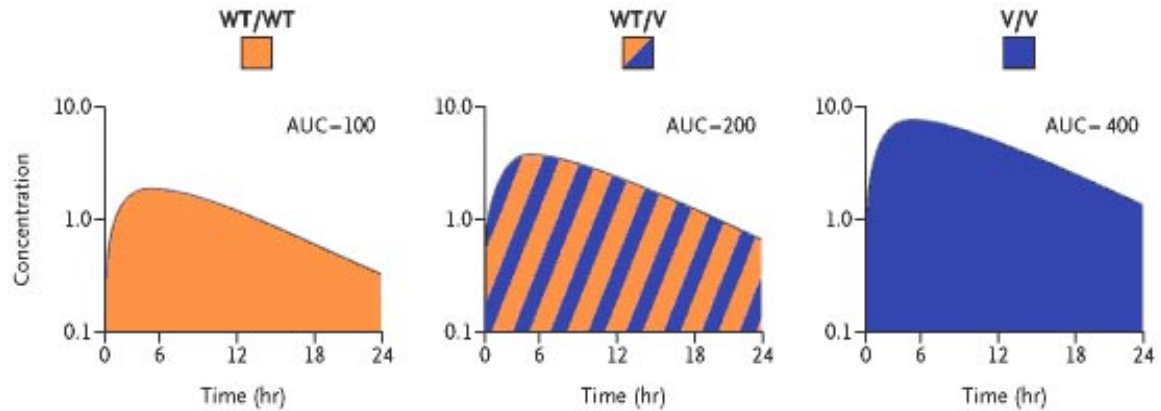
- Genetic variation that *personalizes* an individual's response to a drug.
- Two flavors
 - Variation in drug response (pharmacodynamics)
 - e.g. drug receptor polymorphisms
 - Variation in drug disposition (pharmacokinetics)
 - Drug metabolizing enzymes (vast majority to date)
 - Drug transporters



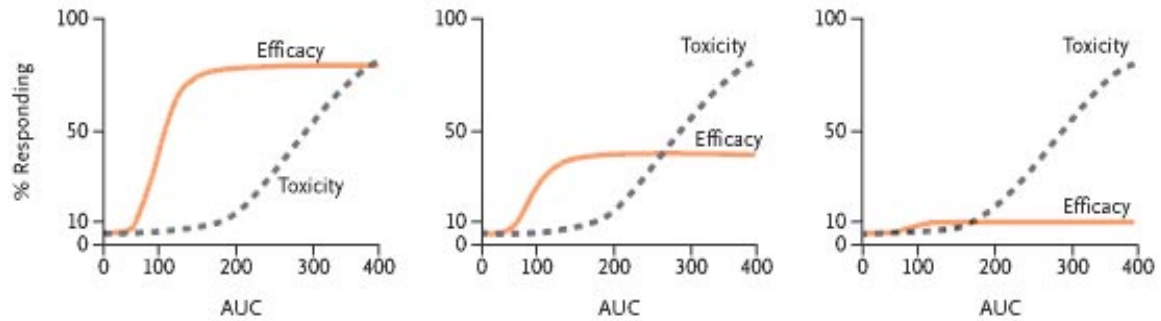
Genotype



Drug Metabolism (Degradation)



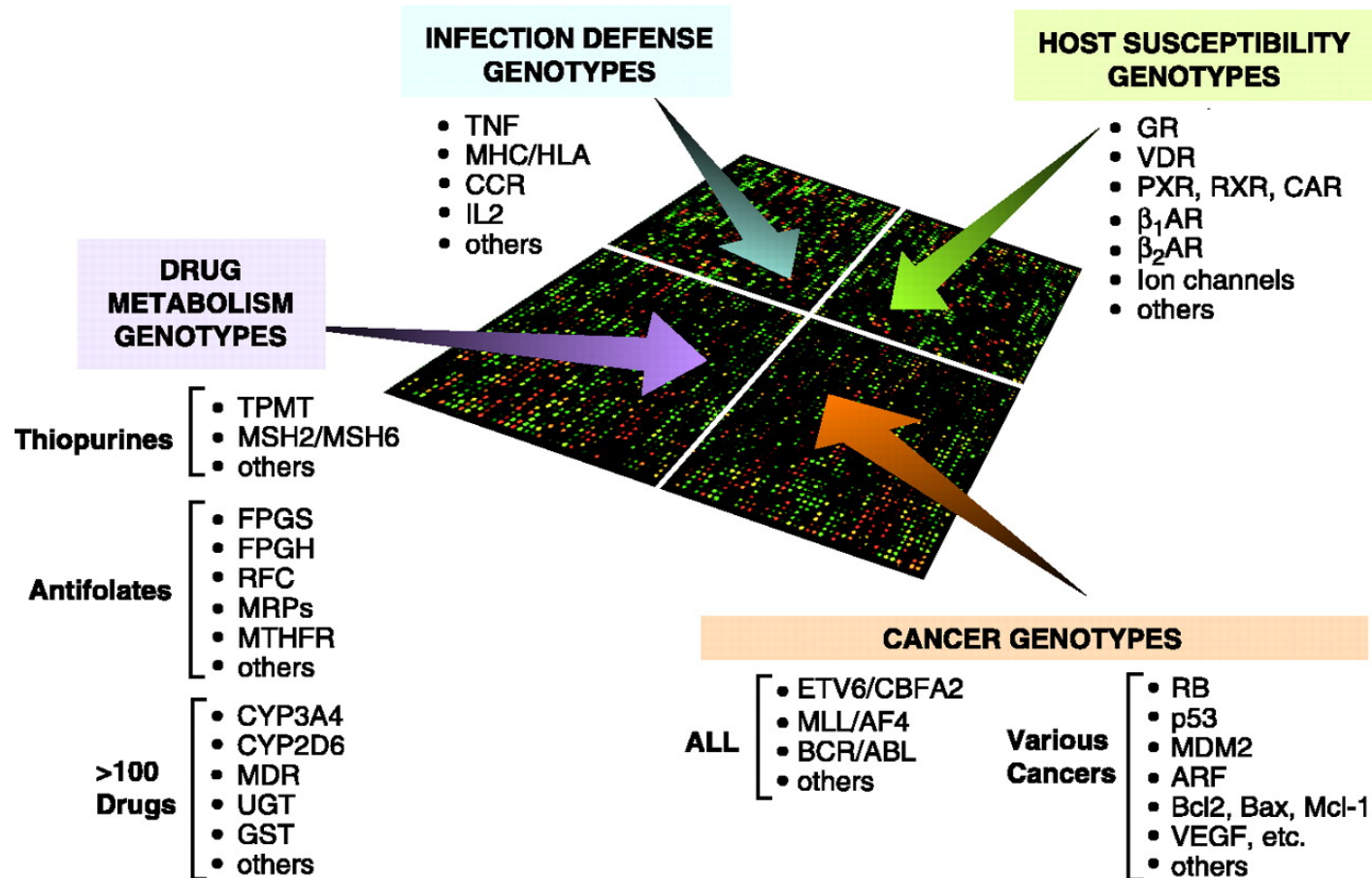
Drug Receptor (Efficacy)



Polygenic Drug Response

Metabolism genotype	+	Receptor genotype	Response	
			Efficacy	Toxicity
	+		65%	Low (5%)
	+		32%	Low
	+		9%	Low
	+		79%	Moderate (15%)
	+		40%	Moderate
	+		10%	Moderate
	+		80%	High (80%)
	+		40%	High
	+		10%	High

Molecular Diagnostics



Drug Development

- General public supports medical research. They want cures.
- Cures are generally in the form of drugs.
- Pharmaceutical companies discover 90% of these drugs.
- It takes 12 years on average for an experimental drug to travel from lab to medicine chest.
- Only five in 5,000 compounds that enter preclinical testing make it to human testing.
- Only one of the five tested in people is approved.
- The cost of this development has been estimated to be in the hundreds of millions (>800 million in 2003). Only half is out of pocket- the rest is lost investment.
- Only 3 out 10 drugs generate enough profits to cover R&D costs.



Adverse Drug Reactions

- The overall incidence of serious and fatal adverse drug reactions (ADR) in the hospitalized patient populations was 6.7% and 0.32% or 2,200,000 serious and 106,000 fatal ADRs in 1994.
- Deaths from ADRs ranks somewhere between the 4th and 6th most common cause of death in the US.

Vioxx – Cox-2 Painkiller from Merck

- ❑ Inhibits the activity of the enzyme cyclooxygenase which mediates the synthesis of endogenous prostaglandins which causes the joint pain of arthritis.
- ❑ Dollars spent to develop the drug (\$800 million)
- ❑ Annual sales of Vioxx before taken off market (\$2.5 billion)
- ❑ Market of Cox-2 drugs (\$9 billion by 2010)
- ❑ Useful painkillers on market to replace Cox-2 painkiller (aspirin- problems of GI bleeding).
- ❑ 1-2% of people are susceptible to cardiovascular problems when taking Cox-2 painkillers.
- ❑ A state jury found Merck liable on April 28, 2006 for the death of a 71-year-old man who had a fatal heart attack within a month of taking Vioxx and ordered the company to pay \$7 million in non-economic compensatory damages and \$25 million in punitive damages.
- ❑ In a prior loss, Merck was ordered to pay one plaintiff 253.4 million dollars -- reduced to 26 million dollars under Texas caps on punitive damages.
- ❑ In Nov. 2007, Merck announced an agreement to pay \$4.85 billion to settle about 27,000 lawsuits. Total amount earned by plaintiffs' firms will be nearly \$2 billion in fees at their standard rates of 33 to 40%.



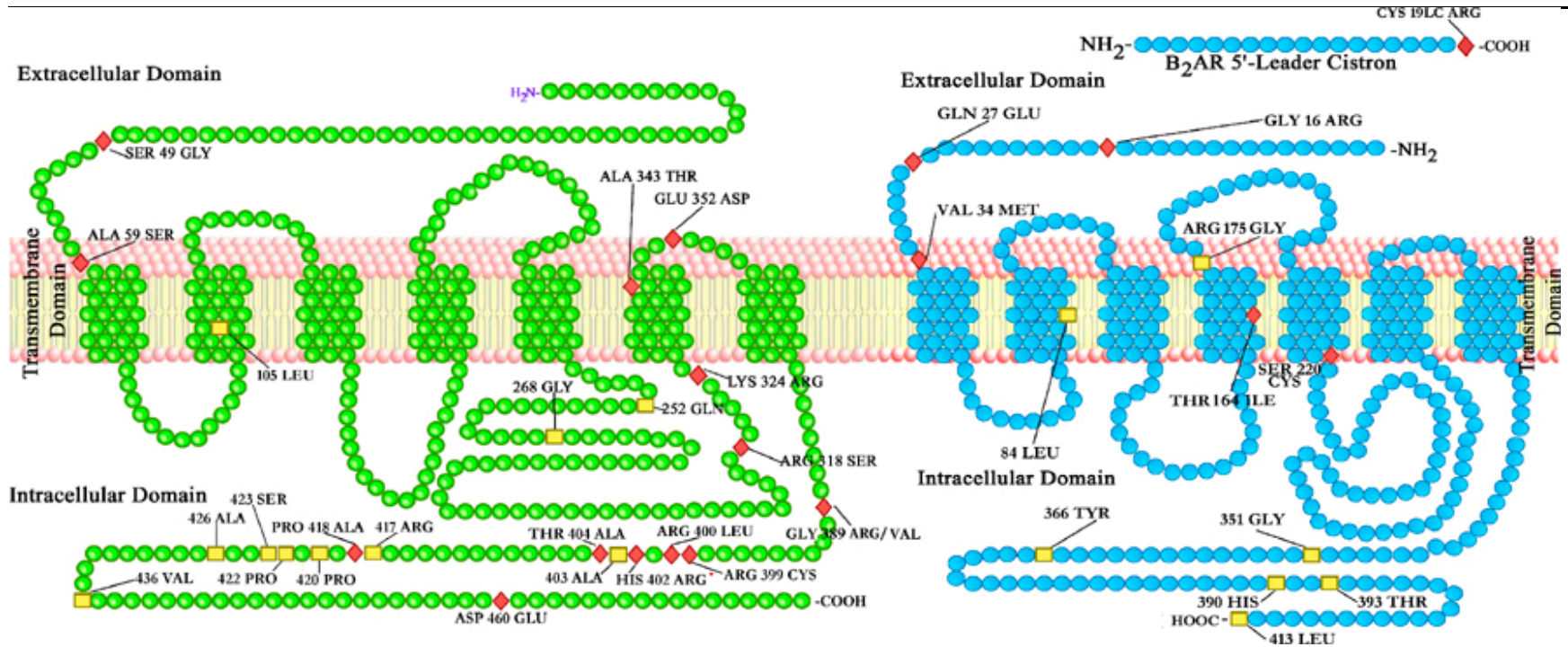
Pharmacogenetics – Drug Response

- ❑ Angiotensin converting enzyme (ACE inhibitors, e.g. enalapril)
- ❑ Adrenergic receptors (e.g. albuterol)
- ❑ Dopamine receptors (e.g. haloperidol)
- ❑ Serotonin Transporter (antidepressants)
- ❑ Glycoprotein IIb/IIIa (e.g. aspirin)

Pharmacogenetics of the human beta-adrenergic receptors

The Pharmacogenomics Journal (2007) 7, 29–37.

M R G Taylor

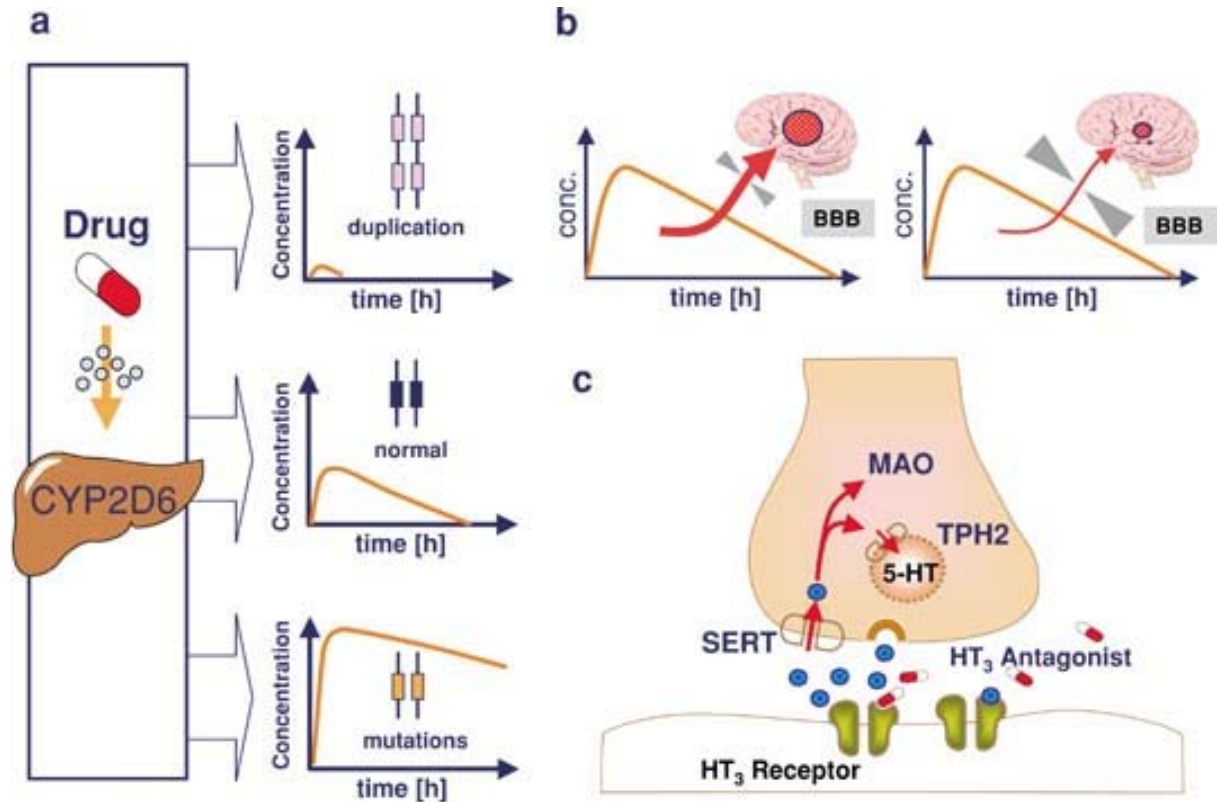


Beta receptor agonists (e.g. albuterol – asthma) and beta receptor antagonists (e.g. propranolol for hypertension) are a widely prescribed and critical set of medication.

Location of reported ADRB1 and ADRB2 polymorphisms. The location of the reported (literature and in National Center of Biotechnology Information database) polymorphisms is shown for the ADRB1 and ADRB2 genes and the ADRB2 leader peptide sequence. Amino-acid positions are numbered and the wild-type and polymorphic variant are indicated. Red diamonds indicate sites of missense polymorphisms that alter the amino-acid sequence of the protein. Yellow squares indicate polymorphisms of the DNA sequence that do not translate into an alteration of the amino-acid residue (silent polymorphisms).

Polygenic Drug Response

Drug-response phenotype is a complex trait. (a) The *HT3* antagonist tropisetron is a CYP2D6 substrate. After receiving the same dose, patients with high enzyme activity due to gene duplication will not achieve effective drug concentrations. (b) Because the drug is a Pgp substrate, transfer from blood to central nervous system will be influenced by the level of Pgp expression, an additional source of variability, at the blood-brain barrier (BBB). (c) The magnitude of response at the *HT3* receptor is influenced not only by drug concentration but also by genetic polymorphisms in the receptor and concentration of neurotransmitter in the synaptic cleft. Serotonin concentration is influenced by proteins involved in biosynthesis (TPH2, tryptophane hydroxylase 2), transport (SERT, high-affinity serotonin reuptake transporter), and catabolism (MAO, monoamine oxidase). Genetic polymorphisms that affect function have been described for all of the genes encoding these proteins. A pharmacogenetic analysis of nonresponse or poor response (observed in 30% of patients) should include all of these candidate genes.



Eichelbaum M, et al. 2006.
Annu. Rev. Med. 57:119–37

Cancer Pharmacogenomics

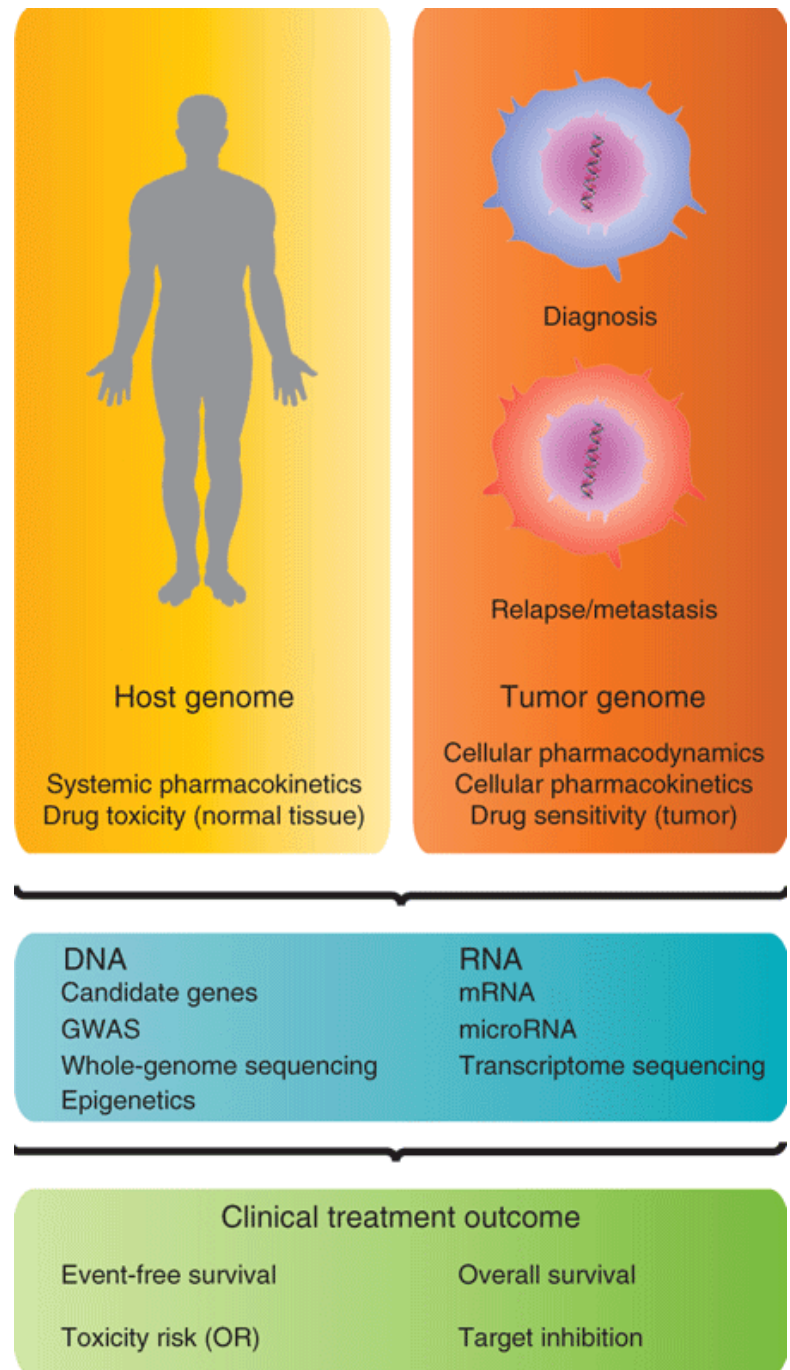
Clinical Pharmacology & Therapeutics (2011) **90** 3, 461–466.

doi:10.1038/clpt.2011.126

S W Paugh, G Stocco, J R McCorkle, B Diouf, K R Crews and W E Evans

“In cancer pharmacogenomics, there are at least two genomes of importance: the patient's germline genome (inherited genome variation) and the tumor genome (inherited genome variation plus acquired genome variation). Moreover, there may be additional acquired genome variations in metastatic or recurrent tumor cells that influence drug response and treatment outcome. A comprehensive pharmacogenomic strategy interrogates multiple mechanisms of genome variation in both germline and tumor genetic material (blue box), assessing their influence on multiple drug-response phenotypes (green box). GWAS, genome-wide association studies.”

Also, check out what Jeffrey Sklar is doing...



Pharmacogenetics – Drug Metabolism

- Most drug metabolizing enzymes are genetically polymorphic in humans (gene frequencies generally range from 1 – 10%).
- Probably confers an evolutionary advantage
 - Diversity promotes adequate response to a new environmental toxin (xenobiotic).
 - Good correlation of P450 genetics with diet
- Complicates drug therapy.
- Pharmacogenetic Testing (Molecular Diagnostics Labs)
 - Take a look at “The Pharmacogenetics and Pharmacogenomics Database”: <http://www.pharmgkb.org>

Important Pharmacogenetic Examples

Enzyme	Medication
Thiopurine S-methyltransferase (TPMT)	6-thioguanine, mercaptopurine, azathioprine
Plasma (pseudo)cholinesterase	Succinylcholine
N-acetyl transferase (NAT1)	Isoniazid
UDP glucuronosyltransferase 1A (UGT1A1)	Irinotecan
Dihydropyrimidine Dehydrogenase	5-Fluorouracil
CYP2D6 (cytochrome P450 isoenzyme)	Codeine

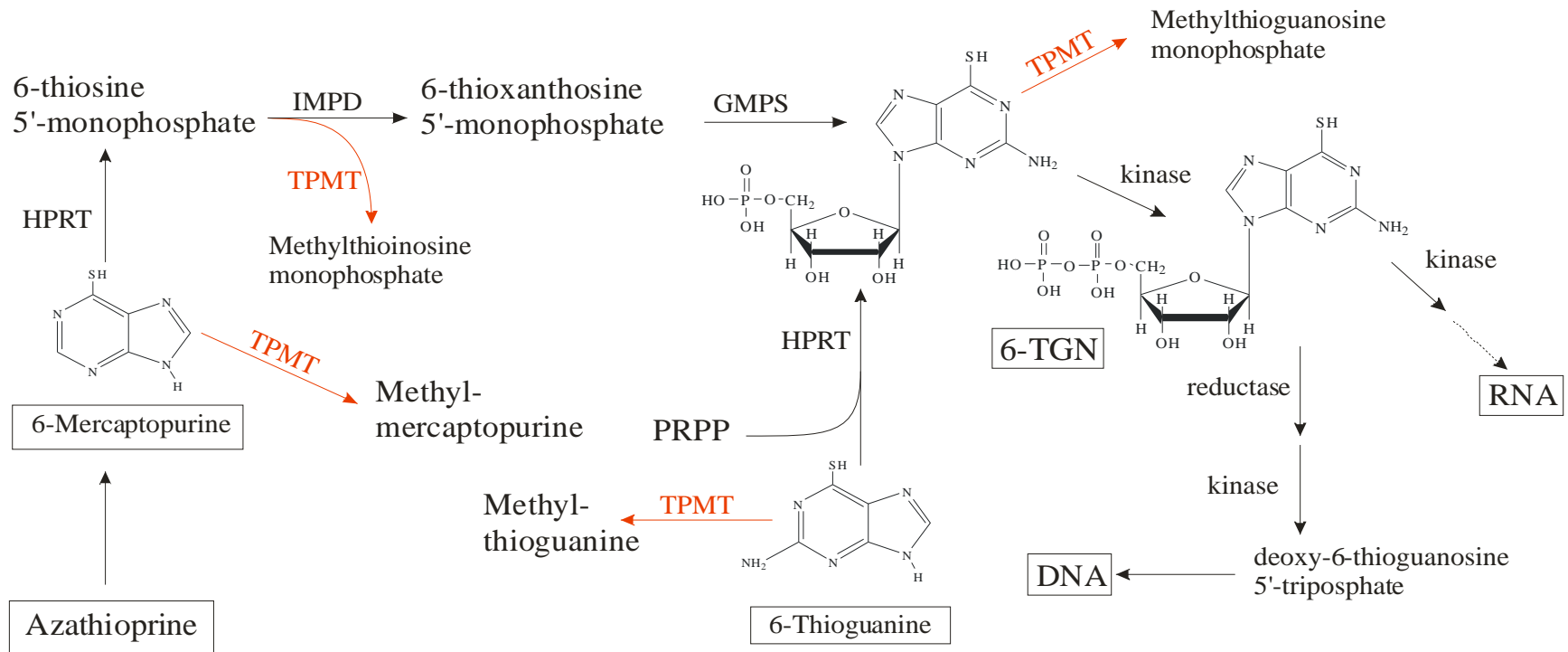
Pharmacogenetic Case

HPI: 56 y.o. female, 140 lbs., presents with bleeding gums, epistaxis, and bloody stools. Also describes excessive fatigue and mild chest pain on exertion.

PMH: Previously diagnosed with psoriasis and put on azathioprine (100 mg/day PO) about one month ago.

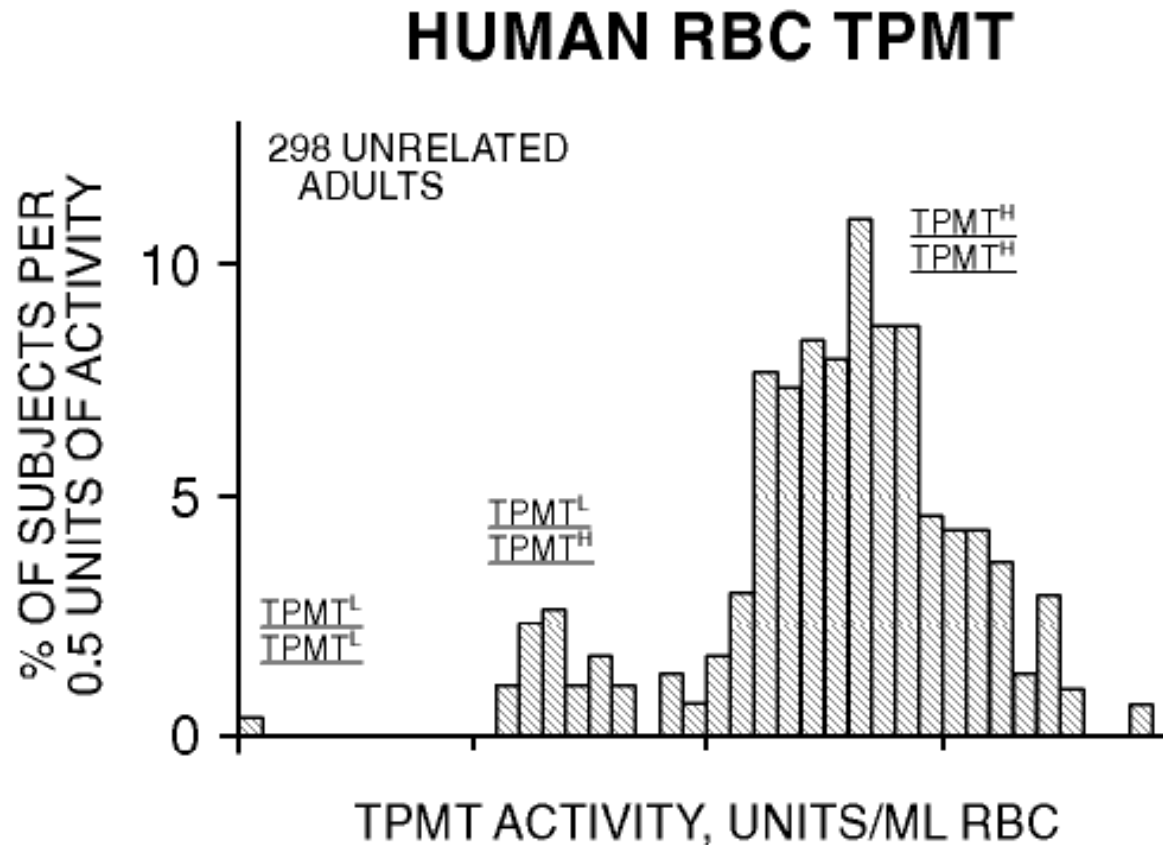
Lab: Hct 18%, Hb 6, WBC 800, Platelets 1,000:
“Pancytopenia” – all blood cell levels strongly suppressed.

Role of TPMT in Metabolism of 6-thiopurine (6-TP) Medications



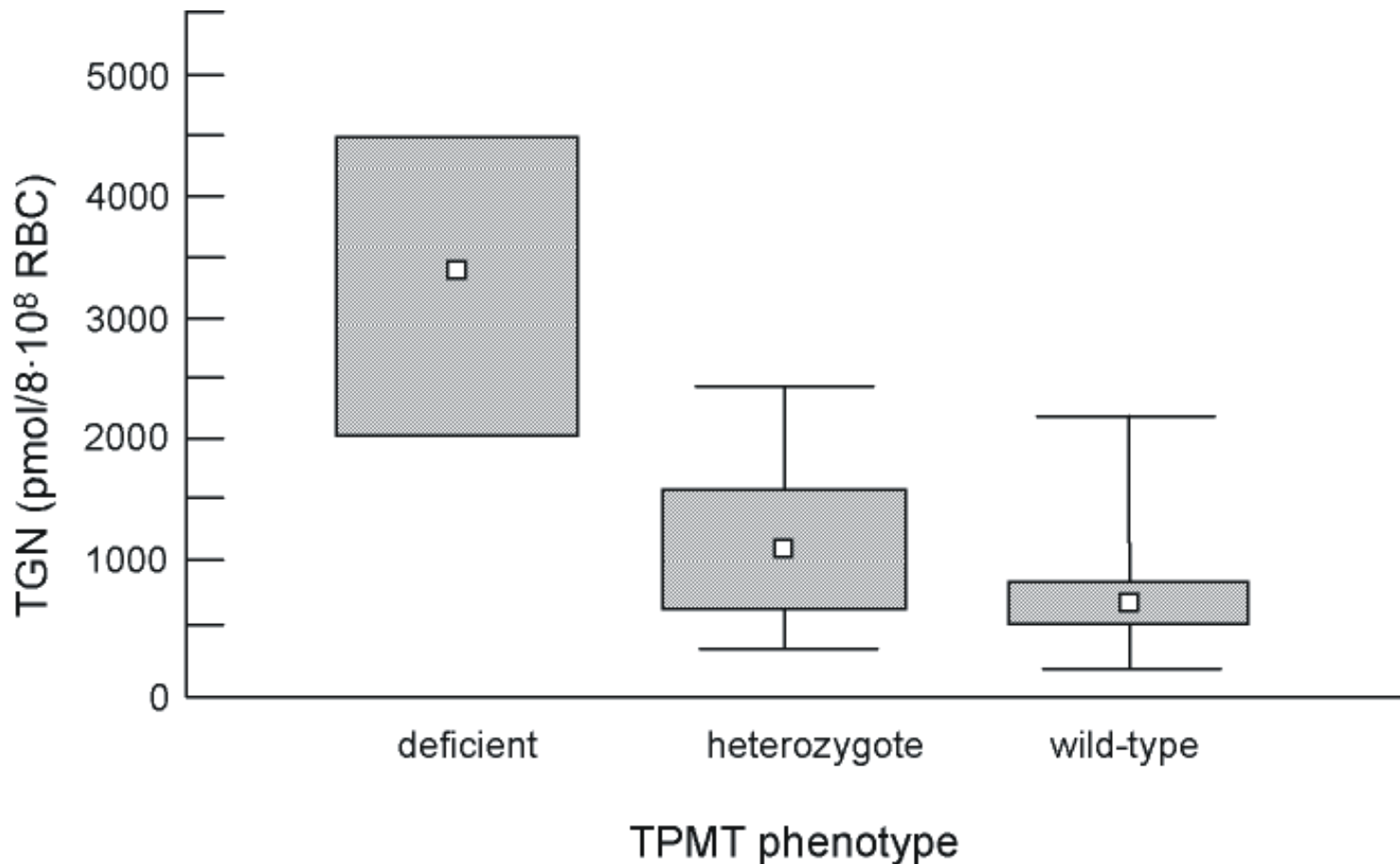
Both **efficacy** and **toxicity** are dependent on the level of 6-TGNs.

Inter-individual Heterogeneity in TPMT Activity Recognized by Weinshilboum over 20 Years Ago.



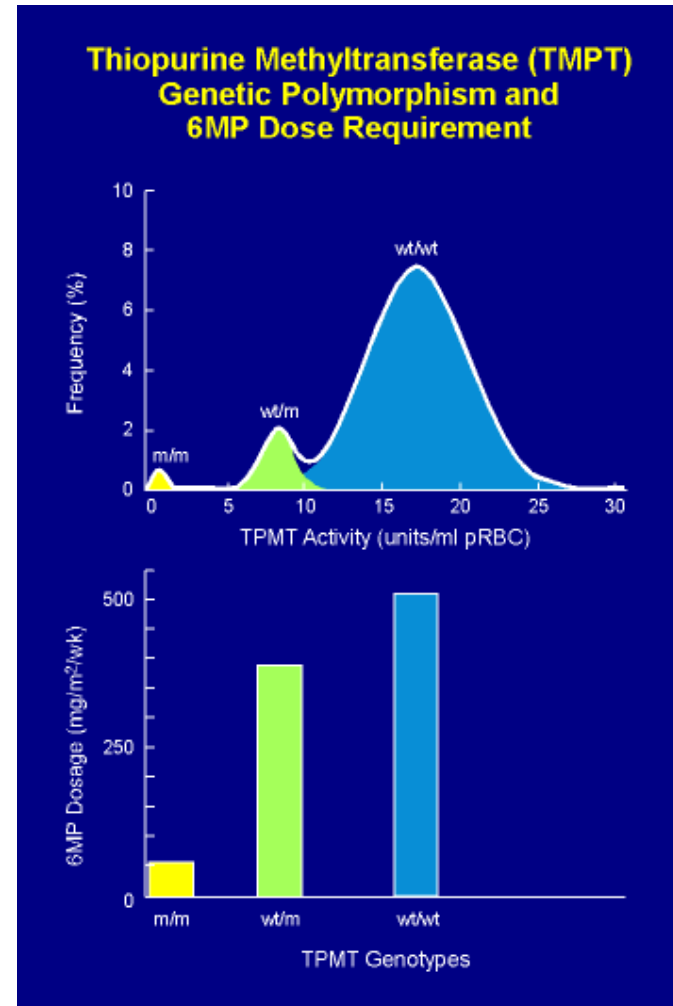
Weinshilboum and Sladek
Am J Hum Gen 32(5):651-62, 1980

Relationship between Inherited Variations in TPMT Activity and Serum Levels of (active) 6-TGNs

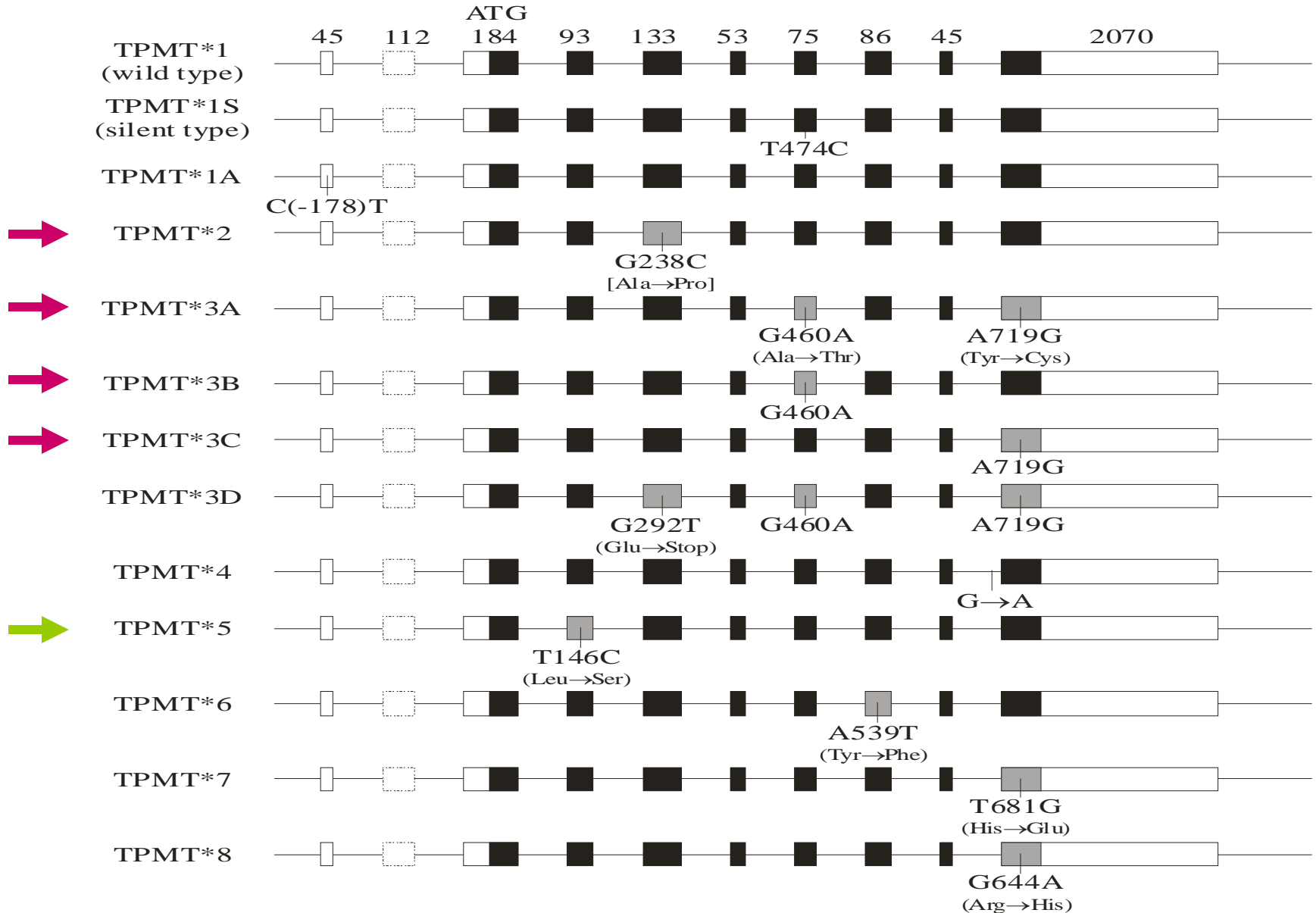


Pharmacogenetics of TPMT

- Heterogeneity in tissue TPMT activities associated with a limited number of genetic polymorphisms.
- “Zygoty” for these polymorphisms correlates very well with
 1. 6-TGN levels in tissue
 2. Risk for toxicity
 3. Efficacy, indirectly
- TPMT genotyping is becoming routine clinically and is recommended before starting any patient on any 6-TP medication.
- Genotype-based dosing regimens have been established



Polymorphisms in the Human TPMT Gene





Clinical Consequences of Inherited Polymorphisms in the TPMT Protein Sequence

- Multiple studies have demonstrated the increased risk of toxicity from 6-TP medications due to TPMT deficiency:
 - increased risk of life-threatening myelosuppression
 - risk for a secondary brain tumor after irradiation
 - greater risk for chemotherapy-associated acute myelogenous leukemia

- On the other hand, unusually high TPMT activities and consequent low 6-TGN levels have been associated with a higher rate of leukemic relapse (i.e. decreased efficacy).

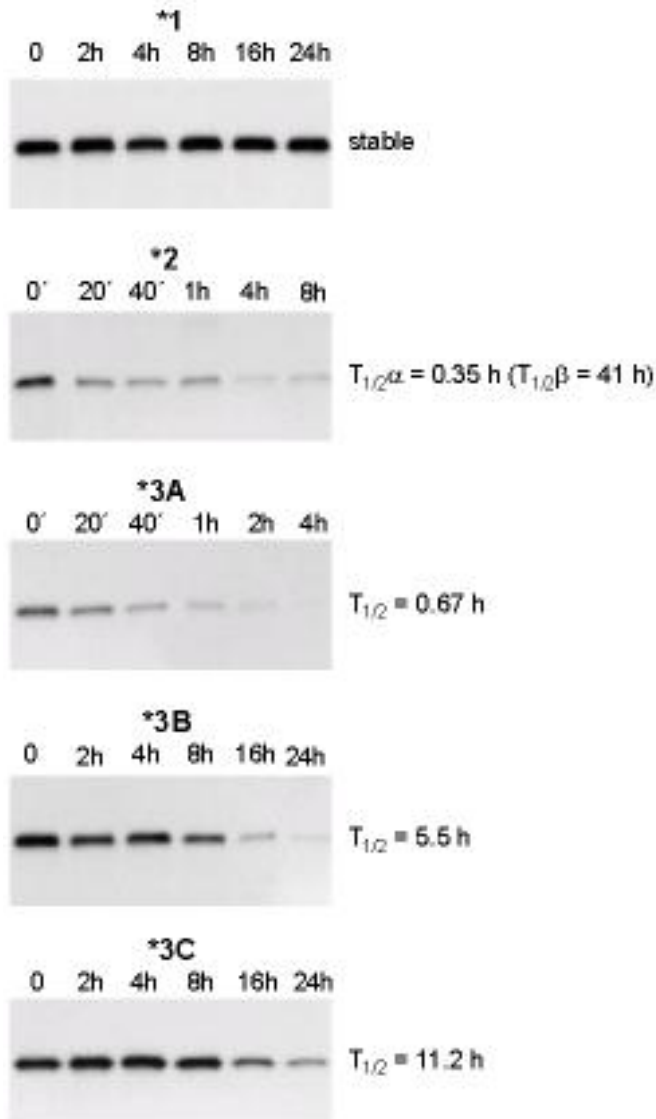
- Recent studies have demonstrated a relationship between clinical resistance to 6-MP therapy for inflammatory bowel disease (IBD) and decreased concentrations of 6-TGNs



6-TP TDM and TPMT Testing

- Before 6-TP drugs are started, TPMT genotype and/or RBC TPMT activity can be assessed and used to guide 6-TP dosage.
- During treatment, RBC 6-MP, 6-MMP and 6-TGN levels can be measured to guide therapy.

Pulse-chase analysis of rh TPMT in COS-1 cells



Biochemical mechanism of decreased tissue TPMT activity

(W. Evans at St. Jude's and R. Weinshilboum at Mayo):

- 1) Decreased tissue TPMT activities are a result of decreased steady-state enzyme levels and not an inherent catalytic defect of the enzyme.
- 2) Experiments performed in yeast, COS-1 cells and reticulocyte lysates have demonstrated that **deficiency is a consequence of increased protein degradation** (synthesis is unchanged).
- 3) Increased TPMT protein degradation requires
 - association with protein folding chaperones (hsp70, hsp90 and hop),
 - polyubiquitylation of TPMT,
 - an intact proteasome,
 - and is stabilized by addition of excess SAM, suggesting the importance of native-state stabilization in targeting of the polymorphs for intracellular degradation.



A General Phenomenon?

Intermediate frequency (~ 1 – 10%) genetic polymorphisms appear to exist in the protein sequences of *many* “small molecule” enzymes.

A large number of these polymorphisms reduce tissue enzyme levels by similarly destabilizing the protein and targeting it for proteasomal degradation.

A General Phenomenon?

Aromatase (cytochrome P450 19) [CYP19]

- involved in estrogen biosynthesis
- potential importance in breast cancer pathogenesis and treatment

Reduced (NADPH)/quinone oxidoreductase I [NQO1]

- reduces oxidized metabolites of xenobiotic quinones
- involved in metabolism of various endogenous quinones including vitamin E
- generates “antioxidants”
- seen as a “chemoprotective” agent

Phenylethanolamine N-methyltransferase [PNMT]

- involved in epinephrine synthesis
- implicated in numerous psychiatric and neurological disorders.

Catechol O-methyltransferase [COMT]

- degradation of catecholamines and pharmaceutical L-dopa,
- cSNPs associated with development of estrogen-based cancers and a wide spectrum of mental disorders

Histamine N-methyltransferase [HNMT]

- involved in degradation of histamine (a major neurotransmitter)
- cSNP has been associated with schizophrenia and asthma

Nicotinamide N-methyltransferase [NNMT]

- suggested involvement in idiopathic Parkinson's and hepatic cirrhosis

Multiple Sulfotransferases [SULT]: 1A1, 1A2, 1A3, 1E1

- sulfation of steroids, catecholamines,
- Implicated in breast cancer

Uridine Diphosphate Glucuronyl Transferase

UGT1A

- Uridine diphosphate glucuronyl transferase (UGT) catalyzes a phase II conjugation step that increases the solubility of drug metabolites. Its normal activity is the glucuronidation of bilirubin.
- At least 15 transcripts exist from two loci, UGT1A and UGT2A.
- UGT1 gene locus in humans is located on chromosome 2. There are 5 exons, of which exons 2-5 are at the 3' end of all isoforms of UGT. Exon 1 can be encoded by multiple first exons (at least 13 exist).

UGT1A Gene

CLINICAL PHARMACOLOGY & THERAPEUTICS
2004;75(6):495-500

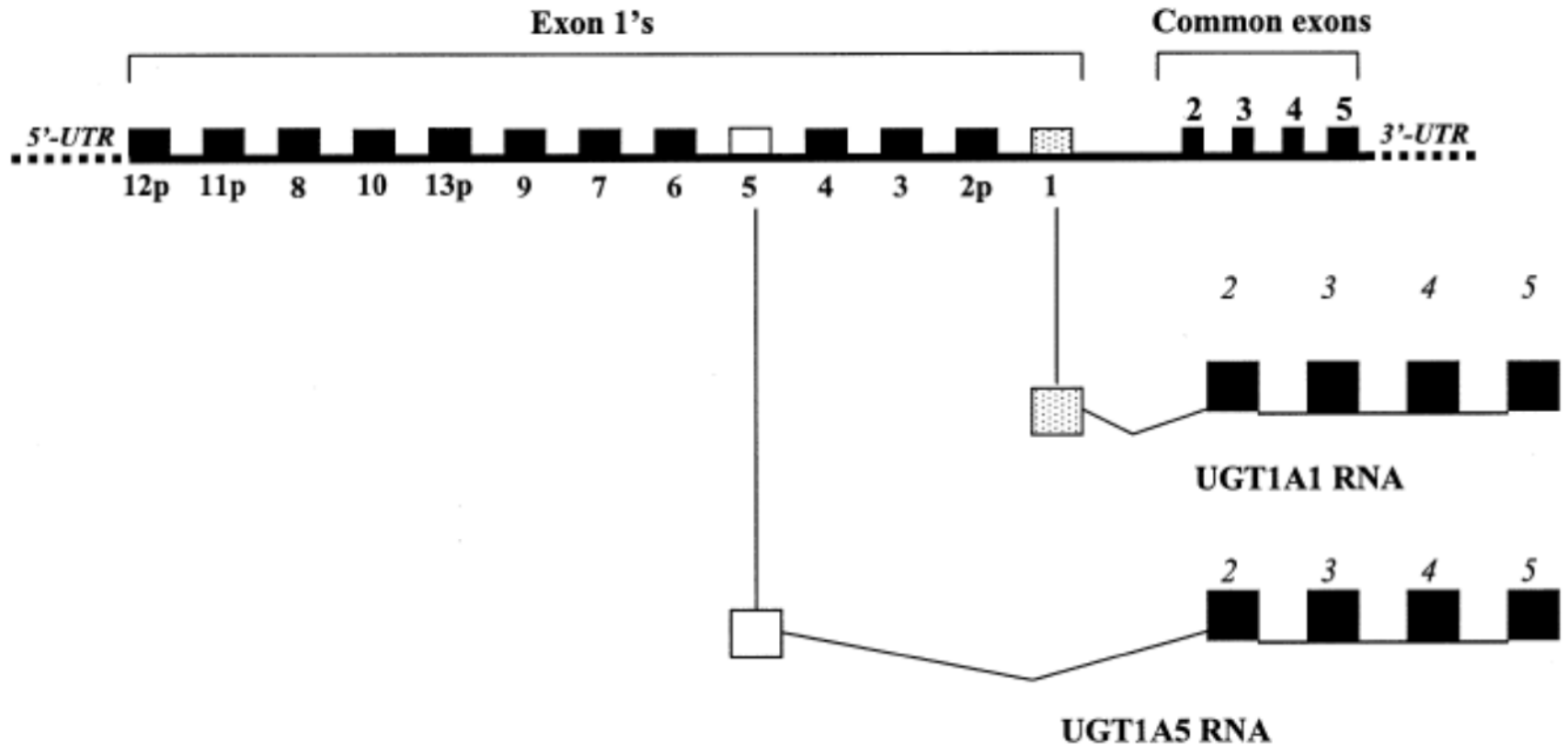


Fig 1. Structure of human uridine diphosphate–glucuronosyltransferase (UGT) 1A gene, containing 13 uridine diphosphate–glucuronosyltransferase genes, each consisting of 1 unique exon (12p to 1) and 4 common exons (2-5). First exons are alternatively spliced to ribonucleic acid (RNA) (as shown for the 1A1 and 1A5 isoforms in this example). With the exception of the pseudogenes 12p and 13p, all exons have their own promoters. The proximal part of these promoters contains a TATA box that is important for transcription.

Clinical Significance- UGT1A1*28

- Pharmacokinetic studies of irinotecan, an anticancer drug derived from camptothecin, and used with ovarian and colon cancer patients, have shown large inter-individual variability to its exposure.
- Adverse events for patients receiving irinotecan-based therapy are diarrhea, neutropenia, nausea, vomiting and alopecia (hair loss)- found in 20% to 35% of patients.
- SN-38 is an active metabolite of irinotecan and is responsible for the pharmacological and toxic effect of irinotecan.
- SN-38 is glucuronidated by UGT1A1 isoenzyme.
- The addition of 2 extra TA bases to the TATAA box, that is found in the variant allele UGT1A1*28, is the cause of this variability.
- The TA addition interferes with binding of the transcription factor IID and results in 30% reduction in expression of UGT1A.
- Patients who are homozygous for the UGT1A1*28 polymorphism should be considered for a reduced initial dose of irinotecan.

CYP2D6

- The human cytochrome P450 2D6 (CYP2D6) gene produces a debrisoquine 4-hydroxylase involved in the metabolism of endogenous compounds and drugs.
- This enzyme is classified as polypeptide 6, subfamily D, family 2, superfamily cytochrome P450.
- The CYP2D6 gene is located on chromosome 22q13 and is transcribed into a mRNA containing nine exons. A 9432 base pair genomic sequence containing the entire gene and several kilobases of intergenic sequence serves as a full-length gene.
- CYP2D6 has at least 70 allelic variants

Cytochrome P-450 2D6 Mutations Detected

CYP2D6 allele	Nucleotide change	Effect on Enzyme Metabolism
*1	None (wildtype)	Normal
*2	2850C>T	Normal
*3	2549A>del	Inactive
*4	1846G>A	Inactive
*5	Gene Deletion	Inactive
*6	1707T>del	Inactive
*7	2935A>C	Inactive
*8	1758G>T	Inactive
*9	2613-2615 delAGA	Partially active
*10	100C>T	Partially active
*11	883G>C	Inactive
*12	124G>A	Inactive
*17	1023C>T	Partially active
Gene Duplication	Gene Duplication	Increased/decreased (depends on gene)

CYP2C19

- The human cytochrome P450 2C19 (CYP2C19) gene encodes the enzyme mephenytoin 4-hydroxylase), which is involved in the metabolism of compounds from classes of anticonvulsants and others.
- This enzyme is classified as polypeptide 19, subfamily C family 2, of superfamily cytochrome P450.
- The CYP2C19 gene is located on chromosome 10q24 and is transcribed into mRNA containing eight exons and a protein of 490 amino acids.
- CYP2C19 has two major variant alleles but also other minor variants that result in enzyme deficiency.

Cytochrome P-240 2C19 Mutations Detected

CYP2C19 allele	Nucleotide change	Effect on Enzyme Metabolism	Caucasian
*1	None (wildtype)	Normal	85%
*2	681G>A	Inactive	15%
*3	636G>A	Inactive	minor
*4	1A>G	Inactive	
*5	1297C>T	Inactive	
*6	395G>A	Inactive	
*7	IVS5+2T>A	Inactive	
*8	358T>C	Inactive	

Clinical Significance- CYP2D6 and CYP2C19

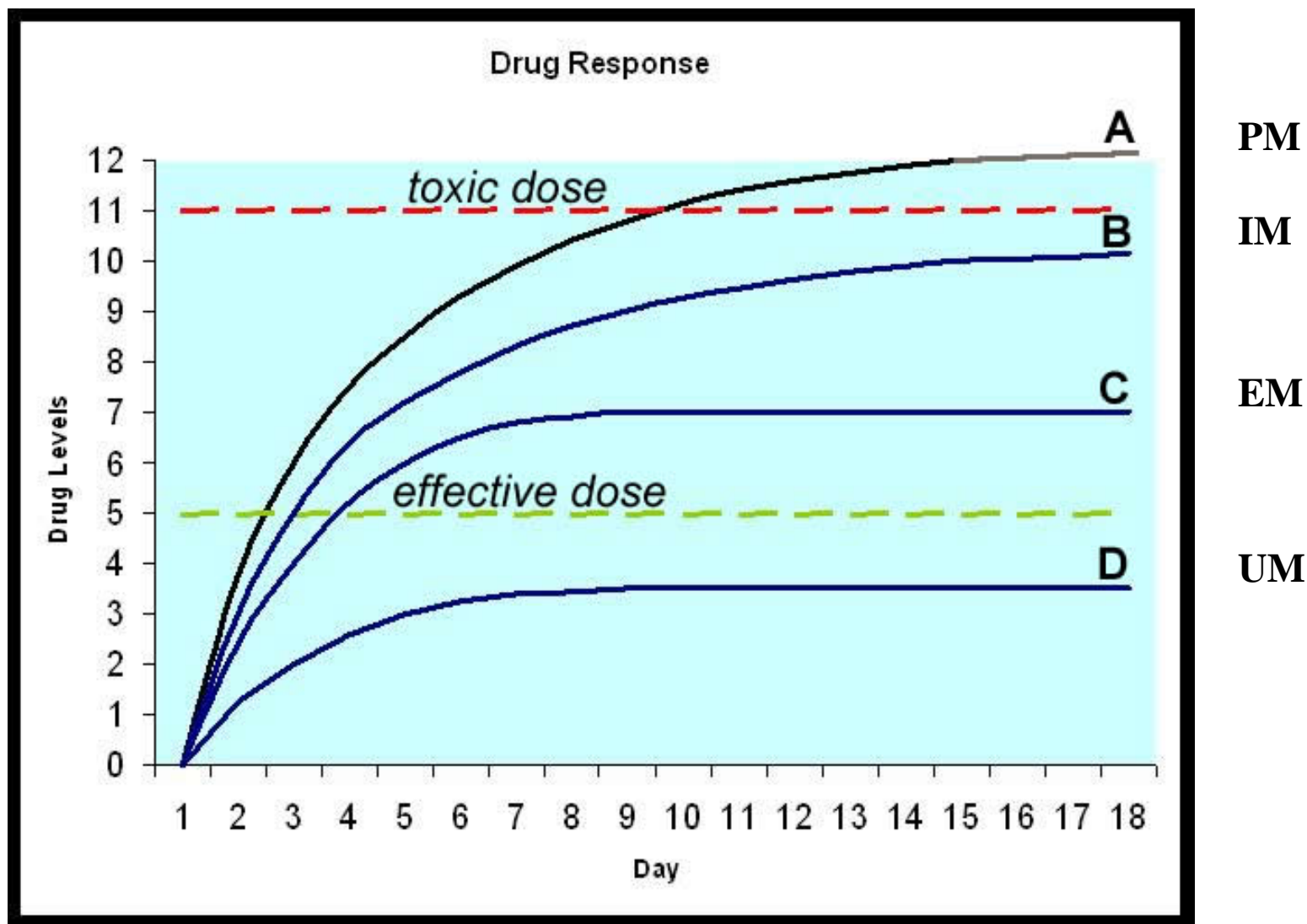
- CYP2D6 (cytochrome P450 2D6) is the best studied of the CYP genes and approximately 10% of the population has a slow acting form of this enzyme and 7% a super-fast acting form. Thirty-five percent are carriers of a non-functional 2D6 allele, especially elevating the risk of ADRs when these individuals are taking multiple drugs.
- Drugs that CYP2D6 which metabolizes 25% of all prescription drugs includes Prozac, Zoloft, Paxil, Effexor, hydrocodone , amitriptyline, Claritin, cyclobenzaprine, Haldol, metoprolol, Rythmol, Tagamet, tamoxifen, and the over-the-counter diphenylhydramine drugs, Allegra, Dytuss, and Tusstat.
- CYP2C19 (cytochrome P450 2C19) is associated with the metabolism of carisoprodol, diazepam (Valium), Dilantin, and Prevacid (Ulcers).



Four Metabolizing Groups

- People are divided into four groups:
 - Poor metabolizer (PM)- lack functional enzymes
 - Intermediate metabolizer (IM)- heterogeneous for one deficient allele or carry 2 alleles with reduced activity
 - Extensive metabolizer (EM)- two normal alleles
 - Ultraextensive metabolizer (UEM)- multiple gene copies of functional alleles.
- Among Caucasian populations most people are EM with 5-10% being PM and a similar amount are UEM.
- Among African and Asian populations PMs are infrequent

Pharmacogenetic Effect of Cytochrome p450 Genotypes



- A. **PM** poor metabolizer, absent or greatly reduced ability to clear or activate drugs.
- B. **IM** intermediate metabolizer. Heterozygotes for normal and reduced activity genes.
- C. **EM** extensive metabolizer. The norm.
- D. **UM** Ultra Metabolizer. Greatly increased activity accelerating clearance or activation

Roche AmpliChip CYP450 First FDA

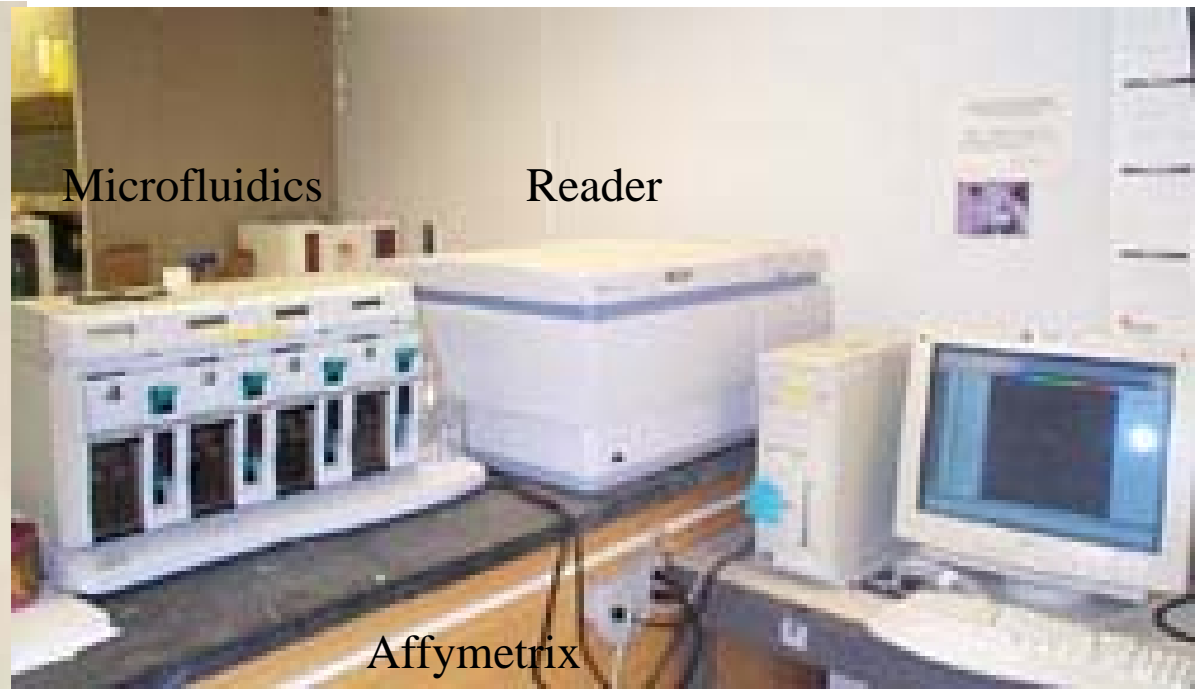
Approved Microarray

Dec. 2004

- Tests for a patient's CYP2D6 and CYP2C19 genotype from genomic DNA extracted from whole blood.
- Tests for 76 CYP2D6 variants
- Tests for 2 CYP2C19 variants



GeneChip® System 3000Dx



Microfluidics

Reader

Affymetrix

Preliminary Average Dose Recommendations for Antidepressant Drugs

Drug	Usual Dose	UM%	EM%	IM%	PM%
CYP2D6-dependent Desipramine (<i>des-IP-ra-meen</i>)	50 (10-100) mg	260	130	30	30
Mianserin	60 (30-70) mg	300	110	90	70
CYP2C19- dependent Clomipramine	50 (50-200) mg		100	90	50

CYP2C9

- ❑ The human cytochrome P450 2C9 (CYP2C9) gene produces a mephenytoin hydroxylase involved in the metabolism of endogenous compounds and toxic chemicals.
- ❑ This enzyme is classified as polypeptide 9, subfamily IIC, family 2.
- ❑ The CYP2C9 gene is located on chromosome 10q24 and is transcribed into mRNA containing ten exons. A 50707 base pair genomic sequence containing the entire gene and several kilobases of intergenic sequence serves as a full-length gene.
- ❑ There are 5 major allelic variants.

CYP2C9 Variants

Cytochrome P-450 2C9 Mutations Detected		
CYP2C9 allele	Nucleotide change	Effect on Enzyme Metabolism
*1	None (wildtype)	Normal
*2	430C>T	Inactive
*3	1075A>C	Inactive
*4	1076T>C	Inactive
*5	1080C>G	Inactive
*6	818delA	Inactive

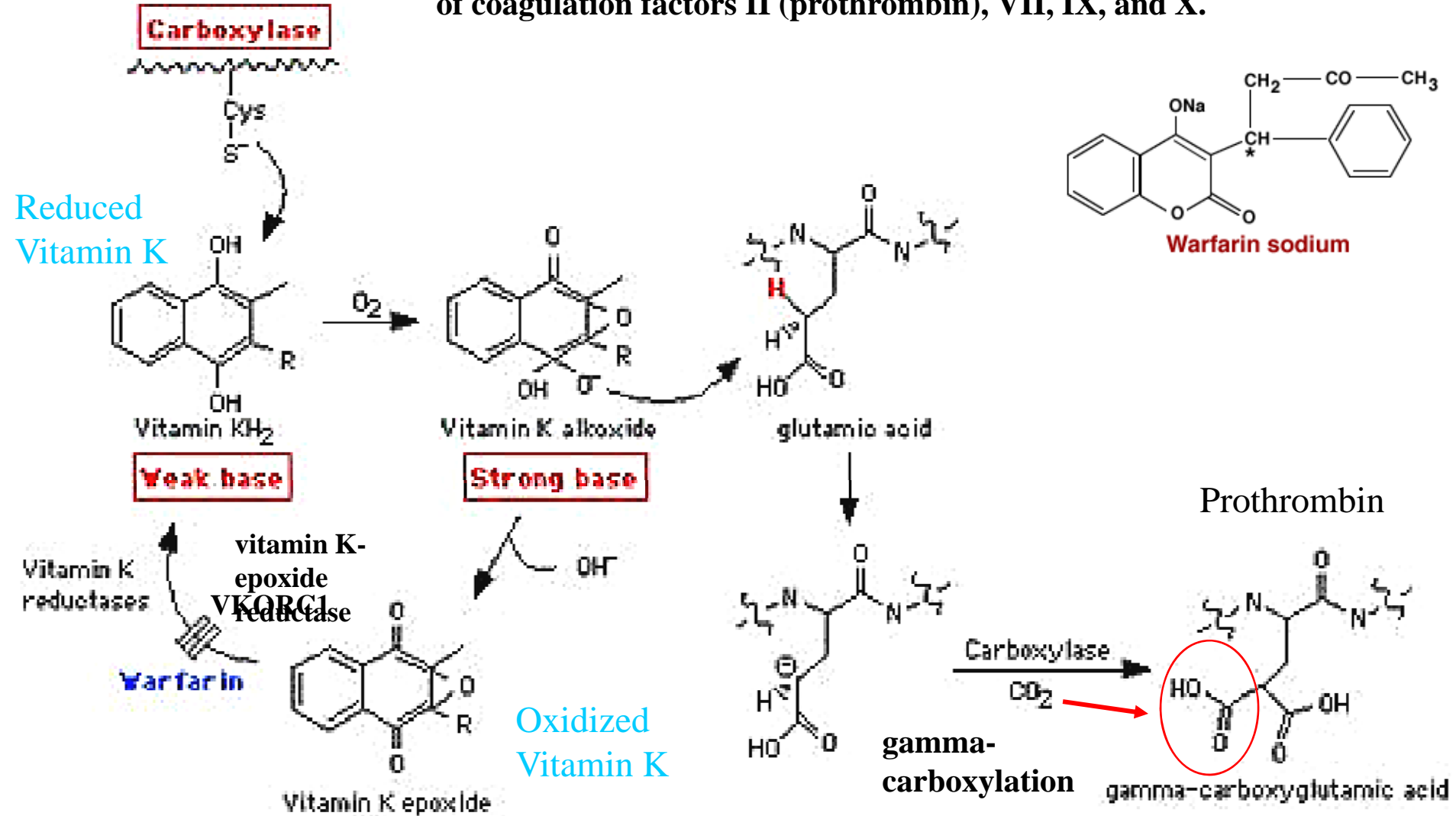


Clinical Significance CYP2C9

- CYP2C9 (cytochrome P450 2C9) is the primary route of metabolism for Coumadin (warfarin) and Dilantin (phenytoin-antiepileptic).
- Approximately 10% of the population are carriers of at least one allele for the slow-metabolizing form of CYP2C9 and may be treatable with 50% of the dose at which normal metabolizers are treated.
- Other drugs metabolized by CYP2C9 include Amaryl, isoniazid, sulfa, ibuprofen, amitriptyline, Hyzaar, THC (tetrahydrocannabinol), naproxen, and Viagra.
- Approximately 5-10% of all drugs are metabolized by this enzyme

Warfarin

- Warfarin impairs the hepatic enzymes vitamin K-epoxide reductase (VKOR1) and vitamin K reductase, which are required for the "recycling" of oxidized vitamin K into reduced vitamin K.
- Reduced vitamin K is required for the normal posttranslational gamma-carboxylation of select glutamic acid residues in the N-terminal domain of coagulation factors II (prothrombin), VII, IX, and X.





Thrombosis and Warfarin

- ❑ Warfarin is the most widely prescribed oral anticoagulant drug in the United States, with approximately 30 million prescriptions per year.
- ❑ Warfarin has a very narrow therapeutic range, outside of which the patient can suffer from clotting or bleeding events. The drug is responsible for 29,000 emergency room visits for bleeding events every year and responsible for the largest number of drug-related fatalities.
- ❑ Warfarin is given to people with an increased tendency for thrombosis or to people that have already formed a blood clot (thrombus).
- ❑ Common clinical indications for warfarin use are artificial heart valves, deep venous thrombosis, and pulmonary embolism.
- ❑ Dosing of warfarin is complicated because it interacts with many commonly used medications and chemicals present in food.
- ❑ Therapeutic effect monitoring of the degree of anticoagulation is required by blood testing and determining the internal normalized ratio (INR).
- ❑ During the initial stage, the INR is checked as often as every day; the intervals can be lengthened if the patient manages stable therapeutic INR levels on an unchanged warfarin dose. The target (INR) level tends to be 2-3.

CYP2C9 and *VKORC1* Genotypes

CYP2C9 polymorphisms

Frequency

Caucasians

CYP2C9*1- wild type allele	cys144/leu359	
CYP2C9*2-	arg144/leu359	0.14
CYP2C9*3-	cys144/Ile359	0.086

VKORC1 polymorphisms

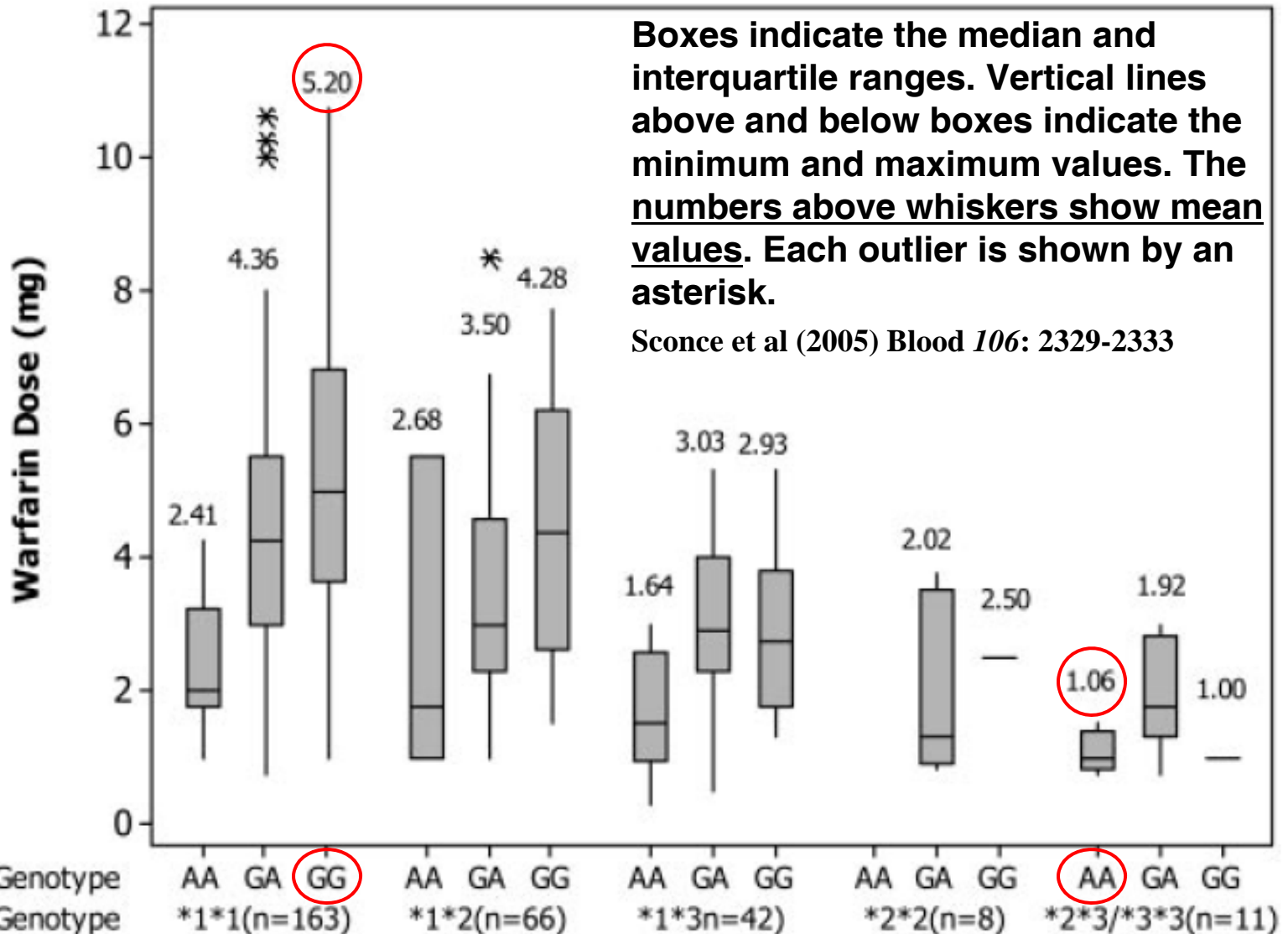
Frequency

Relative to A*	Relative to first nucleotide†	NCBI	Our study	Region
-4931T>C	381	0.47	0.42	5' flanking
-4451C>A	861	0.26	0.33	5' flanking
-2659G>C	2653	0.17	0.21	5' flanking
-1877A>G	3435	0.14	0.02	5' flanking
-1639G>A	3673	0.29	0.42	5' flanking
497T>G	5808	0.18	0.30	Intron 1
1173C>T	6484	0.31	0.42	Intron 1

*The position of the SNP is relative to the A of the ATG initiation codon (denoted nucleotide +1).

Bodin et al
(2005)
Blood 106:
135-140

Distribution of Warfarin Dose by *CYP2C9* and *VKORC1* genotype





Contribution to Warfarin Dose Variability

Regression Analysis

	<u>A</u>	<u>B</u>
□ Age	17%	
□ Height	16%	
□ CYP2C9 polymorphisms	18%	10%
□ VKORC gene mutations	15%	25%

A= Sconce et al (2005). Blood 106, 2329

B= Rieder et al (2005). NEJM 352, 2285

Warfarin Drug Labeling Revision

Medicare reimbursement

- The warfarin drug labeling was revised on August 16, 2007 by the FDA to include genomic information.
 - It states that lower initial doses should be considered for patients with genetic variations in CYP2C9 and VKORC1.
- Physicians are not required to perform genetic testing before initiating warfarin therapy, nor delay the initiation of warfarin therapy.
- Labeling is intended to inform physicians that up to 30% of their patients (i.e. those who carry the CYP2C9 and/or VKORC1 genetic variations) may be at risk for an adverse response to warfarin.
- Centers for Medicare & Medicaid Services reviewed the evidence and determined that the test is insufficient to guide health decisions and will not be reimbursed .



Camptothecin



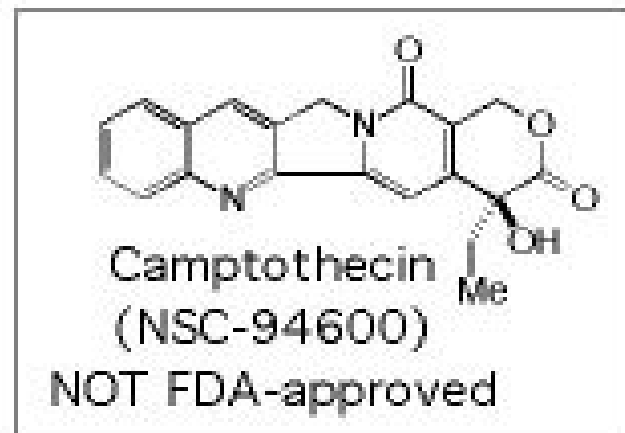
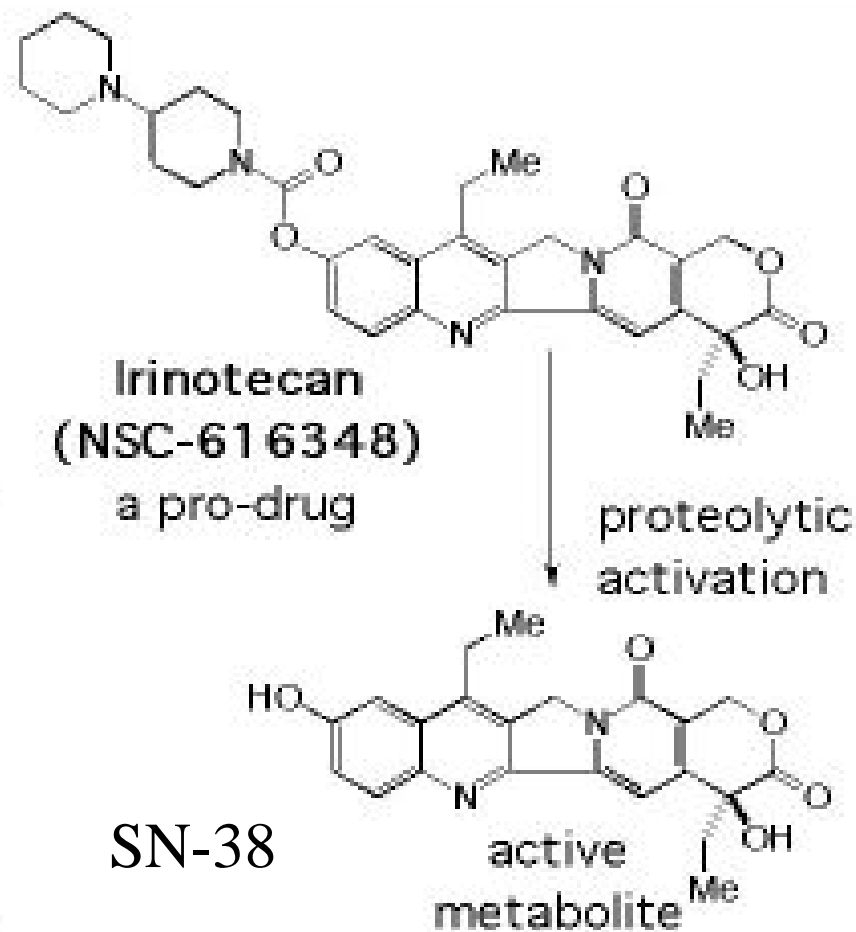
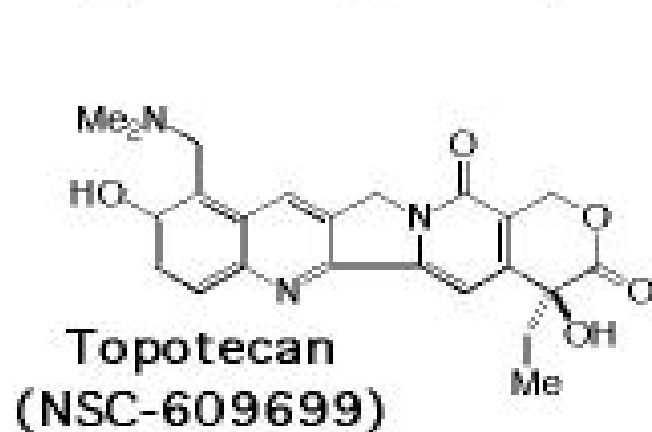
Wani and Wall

- ❑ Derived from Chinese tree *Camptotheca acuminata*.
- ❑ Mansukh Wani and Monroe Wall reported the structure in 1966 at Research Triangle Institute in North Carolina.
- ❑ Inhibits DNA topoisomerase I which prevents DNA replication.
- ❑ Same group reported the structure of Taxol (Pacific yew tree) in 1971.

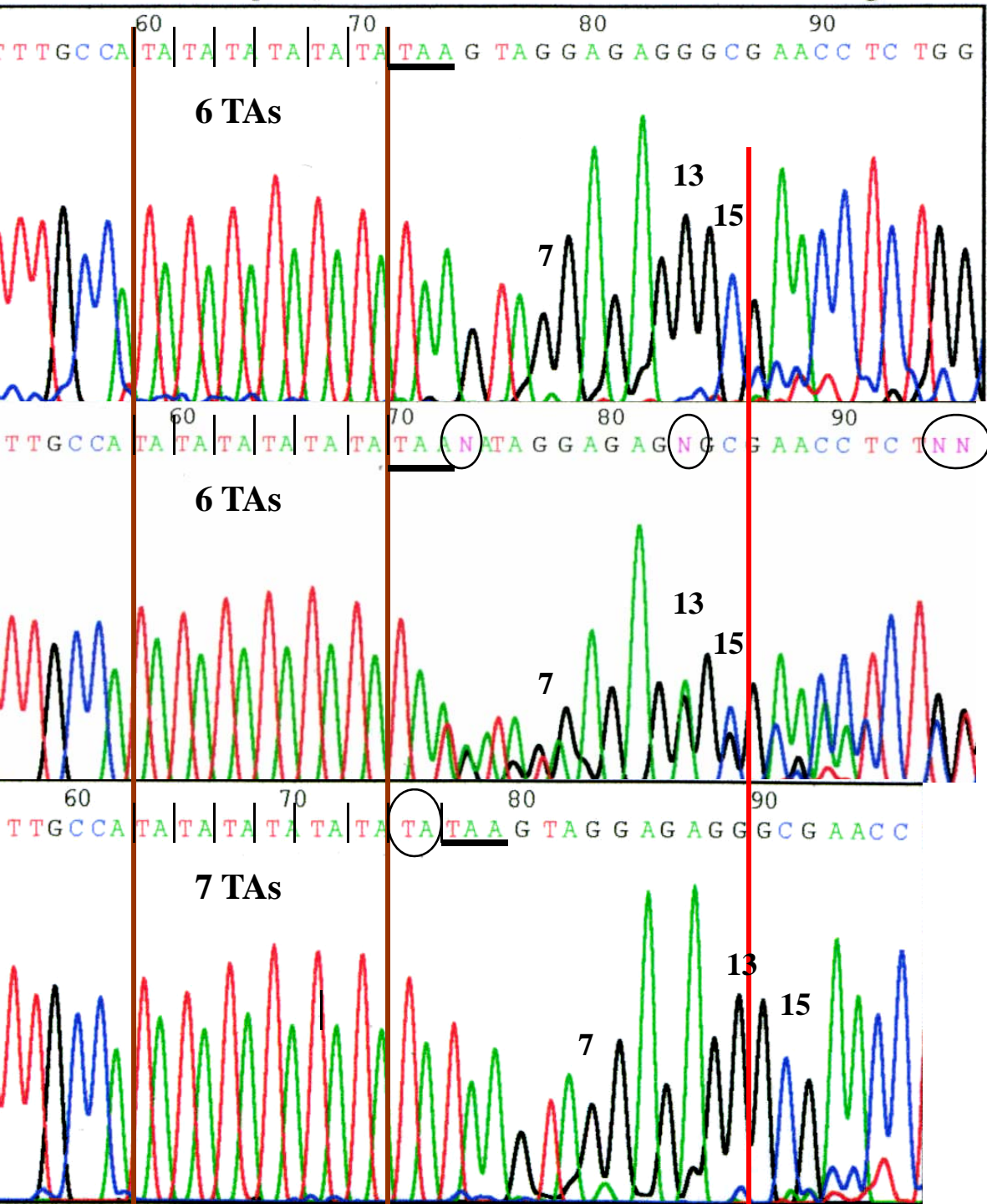


HAPPY TREE
Camptotheca acuminata

FDA-approved drugs - Topoisomerase-I Poisons.



Topoisomerase I poisons stabilize a covalent complex between the enzyme and DNA in which one DNA strand has been broken effectively blocking the "religation" reaction carried out by Topoisomerase I. Both FDA-approved Topoisomerase I poisons are derivatives of the natural product Camptothecin.



DNA Sequencing of UGT1A1

Normal

Allele frequency
16%-40% depending
on ethnic background

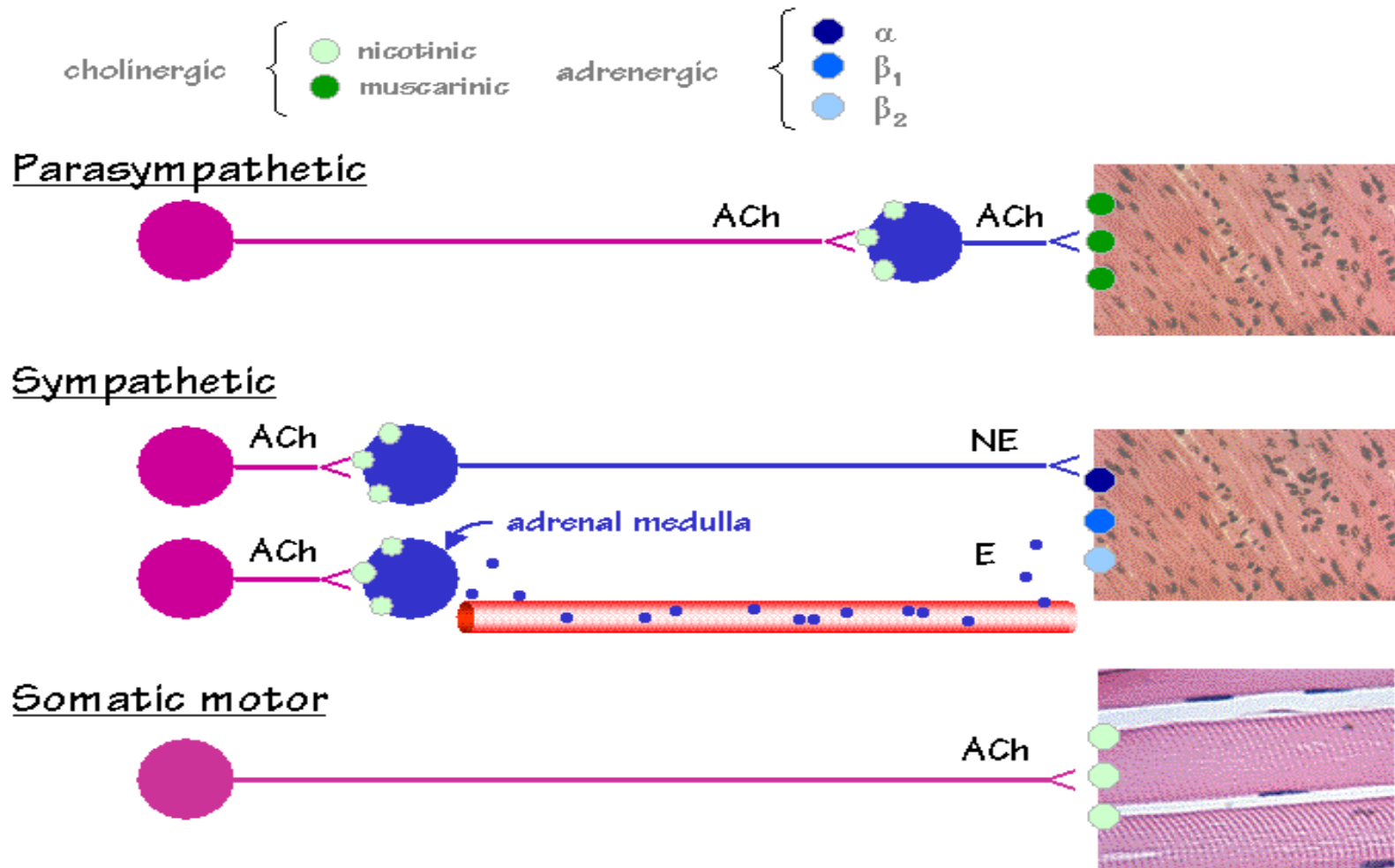
**Heterozygous
Mutant**

**Homozygous
Mutant**



EXTRA CASES (FOR FUN)

Toxicities of Autonomic Drugs



Autonomics Case #1

- 16 m.o. girl brought to ED by parents after becoming unresponsive.
- On way to hospital, had brief episode of eye rolling, produced large mucous stool, then became limp.
- On presentation to ER, suffers seizure lasting 30 minutes, which is eventually controlled with diazepam and phenytoin.

Autonomics Case #1 cont'd

- VS: RR 35, P 135; pupils 3 mm, sluggish response to light.
- Child passed a large bloody stool, then resumed seizure activity. Severe respiratory distress required intubation.
- IV phenobarbital and midazolam given to control seizures; stabilized with Glasgow Coma Score 3.



Symptoms--Summary

- Unresponsive
- Constricted pupils
- Episodes of bowel incontinence
- ...what could this be?

ACh Overdose!

- ❑ Excessive ACh stimulation at central and peripheral receptors can lead to a diversely presenting cholinergic toxidrome.
- ❑ Classic presentation is unresponsive patient, pinpoint pupils, muscle fasciculations, diaphoresis, emesis, diarrhea, salivation, lacrimation, urinary incontinence (**S.L.U.D.G.E.**).

Causes of ACh Overdose

- ❑ Exogenous ACh ingestion is almost never a cause of overdose because the compound is unstable outside neurons.
- ❑ Most cases of ACh toxicity are due to acetylcholinesterase inhibitors (AChEIs).
- ❑ Common non-pharmaceutical sources of AChEIs include organophosphates and carbamates (common pesticide ingredients).

Insecticide Poisoning?

- ❑ Child's pseudocholinesterase level was 0 (3 - 9 u/ml normal).
- ❑ Parents reported garden had recently been sprayed with pesticide that was found to contain organophosphates.
- ❑ Mother had used pesticide to treat girl's head lice on day of admission.
- ❑ Moral: don't use poison as shampoo!

Autonomics Case #2

- 65 y.o. man presents to ED after an episode of syncope and emesis at his birthday party.
- On presentation, he is anxious and diaphoretic and complains of non-pleuritic chest pain and headache.
- PMH: HTN, CAD (CABG x 2, 5 years ago), Type II DM, depression.
- Meds: Cozaar, Aspirin, Paxil.

Physical Exam

- ❑ VS: T 98.5, P 115, RR 22, BP 210/150
- ❑ General: Anxious, diaphoretic, slightly overweight man
- ❑ HEENT: Pupils dilated to 9 mm.
- ❑ Skin: warm, moist.
- ❑ Chest: Lungs CTA. Tachycardic, bounding pulse.
- ❑ Abdomen: absent bowel sounds.



Fight or Flight...

- What can cause unrestrained sympathetic activation?
- Cocaine, Methamphetamine, PCP...
- Dopamine? Norepinephrine?
- What else?



Monoamine Oxidase Inhibitors

- ❑ MAOIs were introduced in 1960s as therapy for depression.
- ❑ Classic interaction between old MAOIs and tyramine (ingredient in fermented foods like wine and cheese).
- ❑ Tyramine displaces NE, and in absence of degradation leads to sympathetic activation.

Other Causes

- The newer MAOIs are more selective and avoid this interaction.
- In addition, SSRIs (like Paxil) are not associated with irreversible sympathetic interaction by themselves.
- SSRI + MAOI can lead to “Serotonin Syndrome,” characterized by muscle rigidity, hyperthermia, changes in mental status.

Course of Action

- Patient was treated with IV Fenoldopam (D_1/α_2 agonist), which relieved hypertension and gradually slowed heart rate.
- Once stabilized, he admitted that he had run out of Paxil the day before and had taken “some of my old pills” that he found in his medicine cabinet.
- These proved to be iproniazid, an old non-selective MAOI.

Autonomics Case #3

- 39 y.o. man presents to ED with orthopnea and altered mental status (confusion).
- PMH notable for CHF, PAF, panic disorder, GERD, and EtOH abuse.
- In last 3 days had become fatigued and lost appetite; stopped alcohol.
- Meds: digoxin, ticlopidine hydrochloride, disopyramide phosphate, paroxetine.

Case #3 - Physical Exam

- ❑ VS: P 140, BP 115/65, RR 35, T 37.3 C
- ❑ HEENT: WNL
- ❑ Chest: Lungs CTA, heart sounds distant
- ❑ Abdomen: Liver tender to palpitation
- ❑ G/U: small amount of tea-colored urine
- ❑ Extremities: cold; pretibial edema
- ❑ Neuro: somnolent, agitated; unable to follow commands; hyperreflexic.

Selected Labs

- Hct 43.6, HbG 14.5, WBC 13.1, Platelets 4.3
- BUN 54; Creatinine 4.1
- Troponin T neg
- CK 10,776 (43-239); CK-MB 25.9 (0-3.6)
- ALT 4375 (4-41); AST 12,283 (10-29)
- PTT 24 (11-15; INR 11.9); FSP 108 (<5)
- Myoglobin 181 (<60)
- Drugs: Paroxetine 70 (<23); Ticlopidine 160 (<50)

Lab indications

- CK, Myoglobin levels w/ no Troponin elevation ==> Rhabdomyolysis
- BUN, Creatinine ==> Renal Failure
- ALT, AST ==> Hepatic Failure
- FSP ==> DIC
- Drug levels ==> overdose or ???

Cytochrome Testing

- Patient was tested for abnormalities of CYP2D6 and CYP2C19, isoforms of cytochrome P450 that metabolize paroxetine and ticlopidine, respectively.
- Patient was found to have severely reduced amount of functional CYP2D6.
- Slow metabolism of paroxetine proposed to have caused symptoms of serotonin syndrome.

Immunosuppressants

□ Cytotoxic Agents

- Methotrexate, Azathioprine, Cyclophosphamide

□ Corticosteroids

- Prednisone, Dexamethasone

□ Signal Transduction Inhibitors

- Calcineurin Inhibitors (Cyclosporin A), FK506 (Tacrolimus)

□ “Biologic Modifiers” (monoclonal antibodies)

- Anti- CD25, Anti-TNF IgG1

Calcineurin Inhibitors

- Many immunosuppressive regimens are based on calcineurin inhibitors (e.g. cyclosporin A and tacrolimus).
- Side effects include nephrotoxicity, neurotoxicity, diabetogenicity, increased susceptibility to opportunistic infections, and certain *de novo* malignancies.

Immuno Case #1

- A year after having a renal transplant, a 76 year-old woman on cyclosporin A develops nausea and mild diarrhea.
- Her daughter stops by her home to check on her and finds her her confused and extremely lethargic.
- She is immediately taken to the ER.
- Physical Exam:
 - Temp = 98.7
 - BP = 120/80
 - Edema in lower extremities

Lab Tests

- Blood and urine cultures negative
- WBC = 6,000 (no sign of infection)
- Cr = 5 (normal 0.5-1.7)
- BUN = 45 (normal 8-25)
- BUN/Cr < 10 (indicates “intrinsic” renal failure)
- Na⁺ = 130 (normal = 135-145), Cl⁻ = 88 (normal 97-110), HCO₃⁻ = 18, K⁺ = 5.5 (normal 3.7-4.5)
- Summary: Acute renal failure (intrinsic) with mild acidosis, elevated anion gap and abnormal electrolytes. No overt signs of infection.



Differential Diagnosis

- ❑ Infection due to excess immunosuppression (not likely).
- ❑ Transplant Rejection due to subtherapeutic cyclosporine therapy.
- ❑ Nephrotoxicity due to chronically excessive cyclosporine therapy (toxicity).

Cyclosporine PK and PD Overview

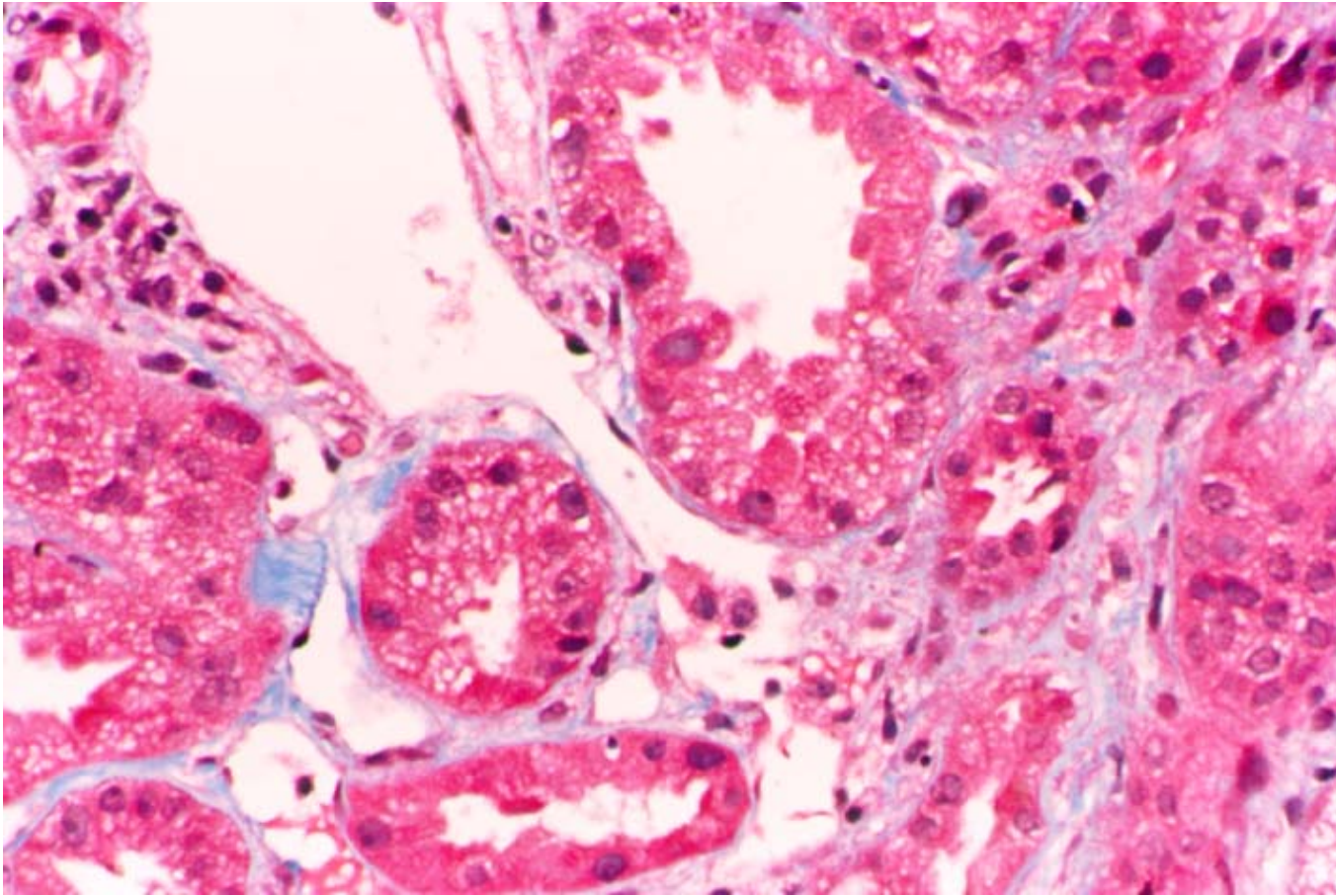
- Cyclosporine is usually given once a day PO.
- G.I. absorption is incomplete and variable.
 - Peak levels seen between 2 – 4 hours.
- Extensively metabolized by many pathways.
 - Approximately 10 – 15 major metabolites with varying functional activity.
 - Excreted primarily through the biliary system.
 - Terminal half-life between 10 – 24 hours.
- Both toxicity and efficacy seem to be a consequence of long term “overall exposure” and does not correlate well with acute levels.



Monthly Cyclosporine Levels

- Trough levels taken just before morning dose.
- Therapeutic range (trough): 100 – 500 ng/ml.
- This patient's levels were between 150 and 350 ng/ml for the past few months, though a slight upwards trends is apparent.
- Today's level is 375 ng/ml.
- Next step is usually to do a renal (kidney) biopsy.

Renal Biopsy Shows Classic Cyclosporine-Induced Toxicity



Serum [Cyclosporine]

- How did she become toxic?
- Were her drug levels too high?
- Trough levels, measured just before a regular dose, were monitored monthly and always within the therapeutic range.
- So, then why toxic?

Efficacy/Toxicity vs. the AUC, Trough (C0) and Peak (C2) Levels

- The efficacy and toxicity of cyclosporine are best estimated by the total “area under the curve” (AUC) of drug concentration during the recurring period of administration.
- However, because cyclosporine has variable absorption, distribution and metabolism, trough levels do NOT correlate very well with the AUC (reflected in the typical wide reference ranges for trough levels).
- Next best approach is to combine trough levels (just before next dose) with the serum concentration approximately two hours after each dose, known as a “C2” level.
 - However, this has really only been demonstrated for the immediate post-transplant period (first two weeks), when the patient is still hospitalized and under more controlled conditions.
- For this patient we can only speculate that over the past few months she has had a higher than usual “overall exposure” or AUC.
 - Possibly, this could have been detected by “C2” levels, though we can’t know that for certain.
 - A common scenario is that a patient is started on another medication (or over the counter “supplement”) that alters cyclosporine metabolism (St. Johns Wort!!!)

Use of AUC and C2 Levels for Cyclosporine TDM

