Lecture 03/31

Tuesday, March 31, 2009
12:55 PM

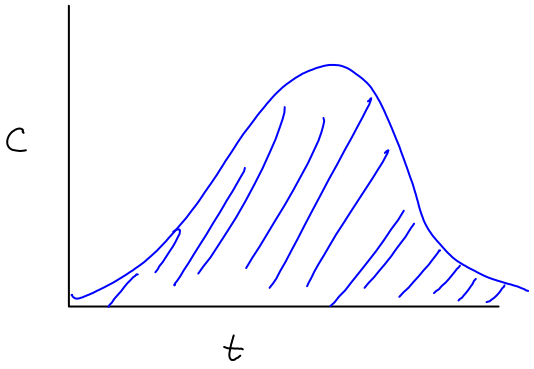
Missed. Added class too late.

Notes 04/02

Thursday, April 02, 2009
1:28 PM

Absorption (See handout

- Main paths of absorptions
  1. GI Tract
     - Goal of GI tract is meant for absorption (high surface area, lots of enzymes, etc)
  2. Pulmonary (breath)
     - System is designed to get gases into body
  3. Skin
     - Skin is designed to be a barrier to keep things out.
  - Others such as hair follicles, tear ducts... But these are minor.



AUC - area under the curve

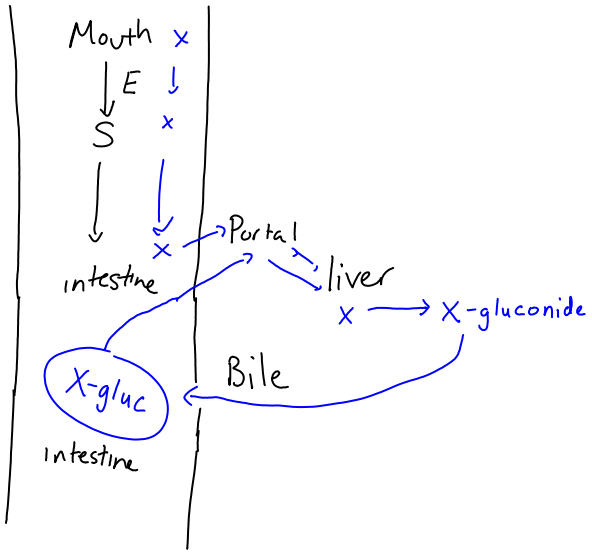
Notes 04/06

Tuesday, April 07, 2009
1:23 PM

- Absorption Handout
  - Skin: Skin is not designed to absorb
    - Layers of skin
      - Epidermis
        - varying thickness
          - Varying .15mm-1mm
          - Eyelids and scrotum very thin
        - Statum corneum: outermost keratinized layer of skin.
          - Very difficult to pass, designed to keep things out
          - Does it go through or around? Depends on chemical
      - Dermis
        - Has vascular where absorption absorbs
      - Skin has no active transport (designed to keep things out)
      - Genospecificity: skin is different in varying species
        - Most similar to human: pig or primate skin
        - Cadavers are used for testing but maintaining moisture and viability is a challenge

.	.	T1/2 (min	.
.	Partition coefficient Olive oil/water	Dermal	Oral
Carbaryl	38	870	49
Malathion	72	330	209
Dieldrin	281	210	2585
DDT	785	1560	1722

    - In general substances are more toxic when administered oral route but not always
      - Exception: nitrofen is lightly more or equal toxicity than oral
    - Curve on handout: Lag time, steady state diffusion after lag time.
  - GI Tract
    - pH has very important role as pH varies greatly through digestive tract
    - GI tract has lots of proteins that carry out transport of various ionized substances
    - Different parts
      - Esophagus
        - Not designed to absorb, very little transport
      - Stomach
        - Designed to degrade various chemicals
      - Small intestine
        - Area of 2,000ft^2 (600 times larger if it didn't have villi)
        - Fick's Equation (numerator quantity was area, higher area the higher rate of transport
      - Profusion limitation
      - Diffusion limitation
      - Active Transport
        - Normal transport that takes up pyrimidines takes up 5-bromo-uracil
        - Cobalt is absorbed through Iron transport system (divalent cation)
    - Has capability to take up relatively large compounds but there is a limit
      - molecular weights over 1,000 have great difficulty (where lung can take in, but digestive cannot)
    - Enterohepatic recirculation system

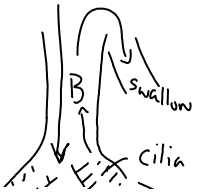


- Anatomical Considerations
- Physiological considerations
  - pH
- Absorption mechanisms
- Graph page 4
  - pH after eating a meal changes dynamically different in old vs young person.
- Table pg 4 Compare different length of GI tract of humans and rats
  - Percentage of different portions of GI tract differ in 2 organisms
- Table pg 4 Affect of fasting in LD_{50} table
  - Epoxidized soybean oil: LD50 goes down if it is fed prior to dosage
  - Polypropylene glycol: LD50 is lower when animal is fasted
- Table pg 4 table 5
  - Gavage - place directly in digestive
  - Drinking water -> sometimes animals do not drink the water (can fall in cage, be played with, etc)
- Species Extrapolation
  - Different species have different diets
    - Carnivores, herbivores, omnivores
    - Human can vomit if exposed to large amount of toxic chemical, rat cannot vomit

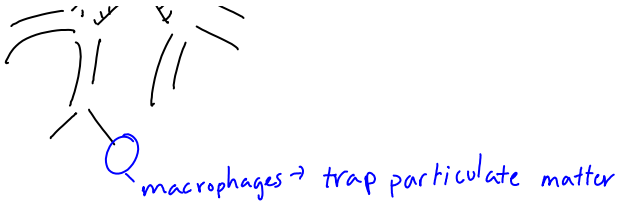
- Distance routs of administration from outside to blood

1.5um	Lung
30um	GI tract
>100um	skin

- Pulmonary absorption (pg5)
  - Pulmonary tract is a series of branching tubes
    - Number of different cell types that have cilia.
      - Muculciliary action



Potential double exposure of both pulmonary and GI when muculciliary action

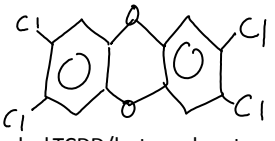


- Macrophages can travel up to region that has cilia and swallow macrophage, or macrophage can stay down in lower regions of lung
- Coal miners have black lungs due to macrophages with particulate matter deposited in lungs

1. Solid and liquid particulates:
   - Absorbed based on size
     - Large (larger 20um) filtered out before reaching lower levels of lung
     - Extremely small (<1um) come into lung and exhaled from lung
     - Deposited to greatest extent: 1um size particles
2. Gasses and vapor substances:
   - Water soluble compounds limited by diffusion rate (across cells)
   - Non water soluble compounds limited by: profusion (blood)

III. Rate of Absorption
- Animal testing is given a whole host of different ways, and the absorption is dependent on those methods of administration
- Figure 2.6 pg 5
  - 1st curve
    - Bioavailability of compound (F)
    - A,B,C all have same AUC (B and C just extended longer period of time)
      - A exceeded toxic threshold
      - B and C would not be toxic
      - Even though they have same bioavailability on A,B,C
  - 2nd curve (opposite of 1st curve)
    - Bioavailability of D,E,F varies
    - Rate is maintained

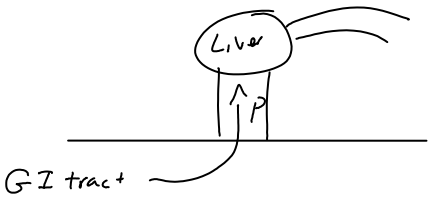
IV. Page 6: Example of TCDD absorption (dioxin), in animals most potent chemical carcinogen



Dose 1nmol labeled TCDD/kg to male rats
- Urinary excretion rate is 1-2% while fecal is 20-30%
  - Why? Nonpolar molecule, not very water soluble (urine)
- Pulmonary absorption is almost 100% (95%)
- Oral absorption high absorption (88%)
- Skin absorption (40%)

- Where was it located?
  - Liver/fat

- Page 7
  - Fig 4. pH variability between different compartments of digestive
  - Fig 1: cartoon
    - A lot of transport with respect to simple diffusion with physical characteristics of compounds will be overcome by these various types of transport



- Distribution handout (just a few minutes left)
    - Transport of blood to target tissue

Notes 04/09

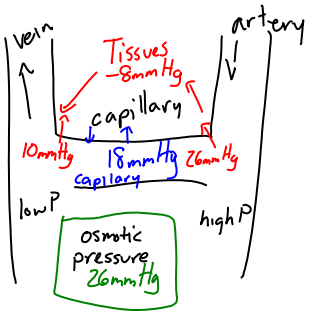
Thursday, April 09, 2009
12:53 PM

- Distribution
  - Some Examples
    - Sulfuric acid sprayed crops. Weeds died, wheat did not. # of different characteristics that weeds preferentially take up sulfuric acid. Damaging chemical component is H atom, this is what is differentially distributing.
    - Tetracycline (antibiotic) - bacteria cells concentrate tetracycline, inhibits ribosomal protein synthesis
      - Sensitivity for mammalian cells and bacteria cells is the same if tetracycline reaches appropriate site. Does not reach site in mammalian cells
  - Page 1 Perfusion varies. Cardiac output is 0.08L/heartbeat

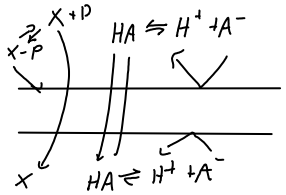
$$\frac{70\ \text{beats}}{1\ \text{min}} \times \frac{0.08\ \text{L}}{1\ \text{beat}} = 5.5\ \text{L/min}$$

total volume: 3L plasma, 2L RBC
approx every min. blood circulates all of its blood

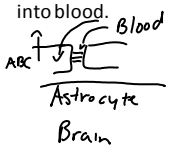
    - ◇ Once you get chemical substances into the blood, they will be mixed fairly rapidly.
  - Page 2: How much water is in the body
    - Extracellular, lymph, blood, plasma, intracellular (27L majority)
    - 57L water in average 70kg person
    - Some chemical substances are not very well distributed and will have a low volume of distribution
      - Volume of distribution of 5-10L, this means the chemical substance is predominately stuck in the vasculator
      - Volume of distribution of 7L - substance staying in blood
      - Volume of distribution of 57L, means it is uniformly distributed throughout the body
    - Figure 6-2



      - Osmotic pressure: because blood has particles in it, it has a pressure from the particles.
  - Profusion and diffusion limitation

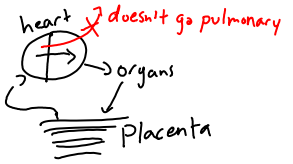


  - Table bottom pg 2: permeability vs size.
    - A small molecule like water, can do in vitro studies to determine diffusion coefficents
    - As molecular weights go up, diffusion across capillary becomes much less than in water, so diffusion coefficients can't be determined in vitro.
  - Why do various chemicals end up in 1 organ system vs another.
    - Protein binding.
    - Albumin (BSA for bovine) has numerous binding sites
    - Sometimes there is a process called cooperativity
  - Types of binding: covalent, ionic hydrogen, etc. See table
    - Suicide substrates - A+B->P and stays covalently bound (relatively rare)
  - Specific anatomical sites
    - Blood-brain barrier: why not cross well?
      1. Junction between cells and endothelium is extremely tight, so going around cells is not too easy in brain
      2. Astrocyte prevents transport of chemical cell
      3. Many of chemicals that get across one way, ABC transports in endothelium puts back into blood.

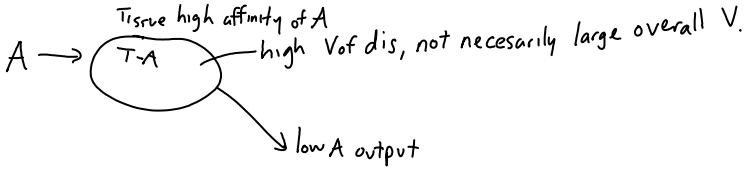


- Brain needs lots of glucose and has lots of glucose transport ability since it doesn't perform glycolysis

- Placenta
  - Number of barriers, 6 layers of tissues… some fetal some mother



- Volume of distribution
  - Parameter used to define distribution of chemical substance



  - Something that complicates volume of distribution is the fact that it is a dynamic process.
    - Pg 7, list of chemical compounds with volumes of distribution (L/Kg)

0.1L/kg → 7L/70kg (ave human) low Vof dist.

40L/kg → 2800L/70kg high Vof dist.
↳ has no physiological relivance b/c exceeds volume of blood in body. It is being sequestered to tissues throughout body.

    - Empirically it has been shown that volume of distribution is porportional to the octanol rating.
      - **Lipid soluble compounds (profusion limiated) generally have large volumes of distribution.**
    - Things that complicate:
      - Volume of distribution is dynamic (time dependent). Vascular cycles fast but other tissues etc will vary in time based on octanol rating
      - $V_D = 0.156P_{o/w} + 0.086$
  - Percent binding of compound depends on dose of compound and equilibrium of it
  - Binding proteins generally decreases concentration (pg 6)
  - Page 5: scathard plot: salicate to human serum proteins
    - 2 linear components to curve: High slope and low slope component
    - These high and low slope: high affinity low capacity, high capacity low affinity.

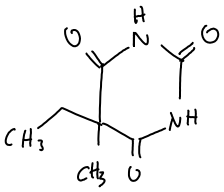
$$A + P \rightleftharpoons A\text{-}P$$

$$K_a = \frac{[A\text{-}P]}{[A][P]}$$

  -

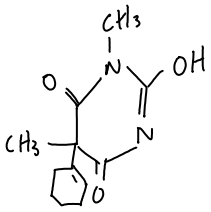
Notes 04/14

Tuesday, April 14, 2009
1:01 PM



barbitol

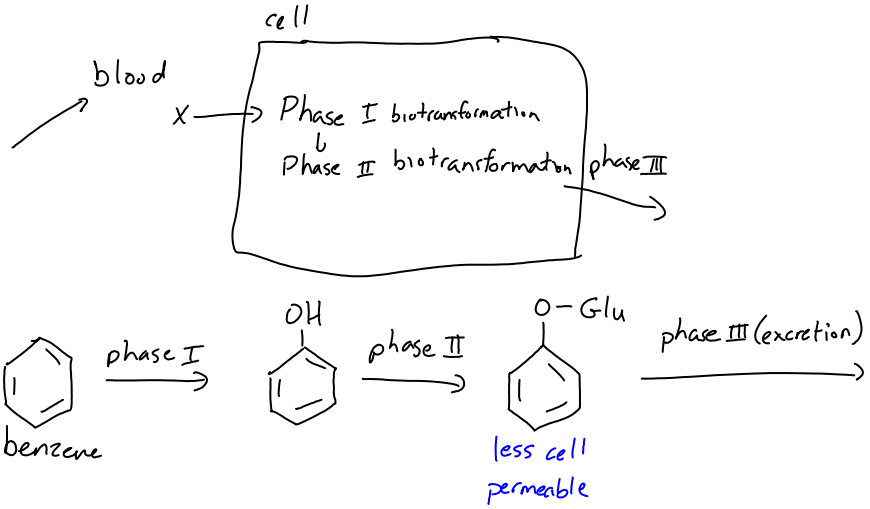
$t_{1/2}$ theoretical 55-75hrs
$t_{1/2}$ actual 55-75hrs



hexabarbitol (more lipid soluble)

~ 2-5 months
5-6 hours

More lipid soluble hexabarbitol leads to longer theoretical half life



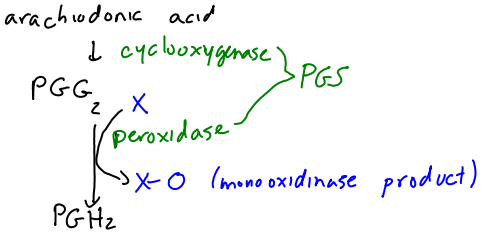
- Biotransformations:
  - Deals mainly with relatively small molecules as compared to immune system dealing with much larger molecules
  - Enzymes that carry out phase I biotransformations
    1. P450 enzymes
       - Family of proteins (humans have 57)
       - Carry out mono-oxidization reactions

$$X \xrightarrow{P450} X\text{-}O + H_2O$$

       - First 4 enzymes of p450 deal with xenobiotics
  - Biotransforamation handout
    - Molecules pictures pg 1
      - Porphin
        - Nitrogens of porphrin rings bind to xenobiotics
      - Hemochrome
        - Iron can bind to 6 positions or to 5. the 6th would bind to xenobiotic
    - Pg 2
      - Fig 2
        - Cytochrome p-450 reductase
        - Cytochrome b5
        - Substrate binds to iron moiety. Iron changes valence state as result of cytochrome p450 reductase. Then attachment of oxygen molecule
        - 1 p-450 protein can do this process for many different substrates
      - Fig 4
        - Cycle for flaven mono-oxygenase. R -> RO
      - More oxidation potential in p-450 than for flavin monooxidinases
        - Because oxygen on p-450 form is that of superoxide
        - Flavin is peroxide oxygen. Less reactive oxidation process
    - Pg 3 fig 17: example of biotransformation of benzo(a)pyrene(BP)
      - Causes cancer by inducing mutations
      - benzo(a)pyrene(BP) in it self is not carcinogenic but converts to molecule that does cause cancer
      - benzo(a)pyrene(BP) acted on by P-450 which does epoxidatoin to arene oxide (majority produced this product but many other similar compounds)
      - benzo(a)pyrene(BP) is planar molecule, each time hydroxyl group created there is capability of sterochemistry
    - Pg 4:
      - Fig 4

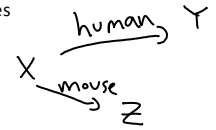
- GST (glutothione esterase)
  - Instead of epoxide hydrolase, GST can link epoxide to conjugation of other molecules
- Quinone produces superoxide (free radical oxygen) that is very chemically reactive and potential to cause damage

- Non-microsomal oxidations (not being carried out in endoplasmic reticulum)
  - Hydrocarbons --(alcohol dehydrogenase)--->alcohols -(aldehyde dehydrogenases)--->aldehydes ->acids
  - Excess of H^+ in cell, need excretion methods for hydrogen ions (Na/H antiport).
- Don't have time to talk about all the reactions: amine oxidase (later with neuro), molybdenom hydroxylase,
- prostaglandin H synthase



- Reductions
  - Not many that occur to xenobiotics in the body
  - Some microorganisms perform reductions
  - DDT (bottom pg 4) metabolized to DDE
    - Phase I biotransformation (phase I reductive halogenation)
    - GSH is cofactor

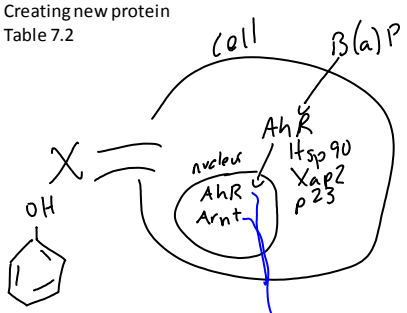
- Pg5
  - Physiological factors
    - Species



if X is toxin and $t_{1/2}$ for X→Y or X→Z is same, then mouse system OK
if Y or Z is toxin, then not a good model

Species difference in type P450
In rat: CPY2C
In human: CYP3A

    - Diet
      - Extremely important. Experiments at different places can have different diets and show variance of response in experiments
      - Human diet - large #s of different chemical substances. These substances have the capability of interacting
        - Moiety in grapefruit that can inhibit some P450 activity
    - Development and aging
    - Sex
      - Sometimes males more sensitive or females more sensitive.
    - Hormonal status
    - Pregnancy
    - Strain/interindividual differences
    - Disease
    - Diurnal and seasonal cycles
  - Chemical factors
    - Dose: certain dose metabolized to one thing, increase dose may metabolize different molecule through saturation of 1st enzyme
    - Inhibition
      - A molecule can inhibit p450 molecule
    - Activation
      - Protein is there and you are making it active by giving a chemical substance
      - Chemicals that activate p450: ethylisocyanide, acetone, & more. Make the enzyme more active than it normally is
    - Induction
      - Creating new protein
      - Table 7.2



Some Xenosensors:
- AhR
- CAR
- PXR
- PPAR

All of these form dimers with RXR and induces
Exception: ethanol: increases protein expression not through transcriptional process like all the other molecules.

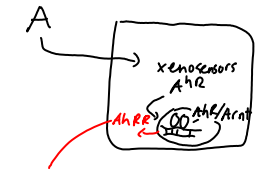
bind to DNA → induction of production of enzymes

- pg8

Notes 04/16

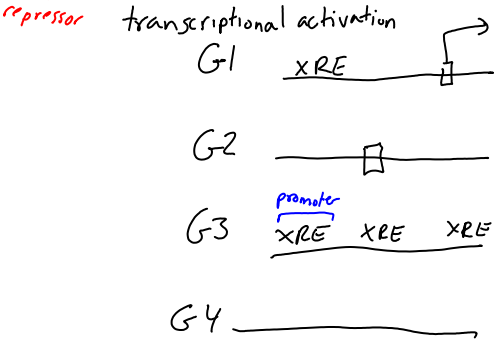
Thursday, April 16, 2009
1:05 PM

- Phase 1 biotransformations in summary



Xenosensors:
AhR
PXR
CAR

These dimerize with another molecule, go into nucleus of cell



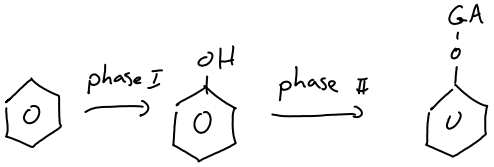
AhR receptor:

AhR → turns on Cyp1A1 which does P450
↓
BaP

TCDD is most potent inducer of AhR and this whole gene transcription processes. TCDD is th emost potent cancer causing molecule in rodents too.

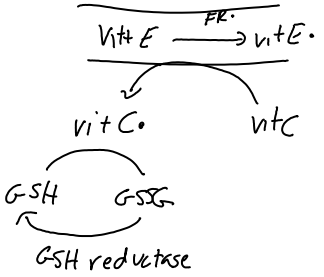
So ligands BaP or TCDD bind AhR, AhR goes in nucleus and binds/dimerizes AhR/Arnt to induce transcription. AhRR is also coded and a repressor

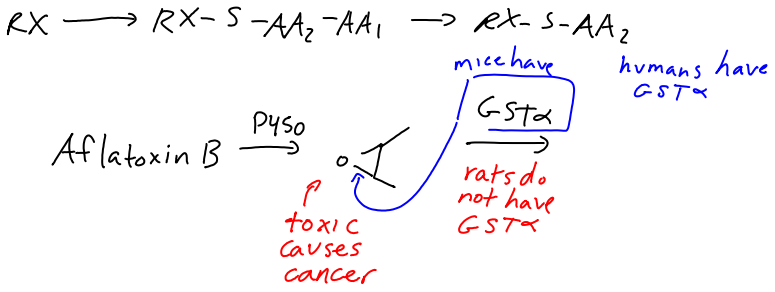
- Liver has large diversity of enzymes in addition to producing large quantity of these enzymes
- Lungs (pulmonary tract), small intestine, kidney, skin have relatively high amounts of enzyme activity
- Brain has many types of enzymes but not large quantity.



- Phase II Biotransformation
  - Glucuronidation (pg 1) - addition of sugar group (generally glucuronic acid)
    - UDPGA - activated form (also UDP-glucose but less common)
    - UDPGT - enzyme that carries out transfer
    - What does gluconidation achieve?
      1. Increases MW
      2. Decrease P_{ow} (more water soluble)
      3. Increasing ionization. (have predominately ionized forms)
    - UDPGTs are induced similarly than phase I because they have XRE promoters and are getting turned on by same xenosensors
    - XENOSENSORS signal and turn on for both phase I and phase II enzymes
    - How does adding a sugar group make more toxic at times (pg2)
      - In general the conjugated product will not be more toxic (product w/ sugar)
      - When pH changes, the gluronic acid can be a leaving group creating a reactive site that has potential for toxicity.
    - UDPGT activity across species
      - Reptiles, fish have lower UDPGT activity
      - Mammals, birds have about same UDPGT activity compared to humans
        - Cat has low UDPGT activity
        - Guinea pig has high UDPGT activity

        - Animals that have low UDPGT have high sulfation capacity and vice versa.
    - Table 4 - stability of glucuronide form has effect on toxicity
- Sulfate conjugation
    - Addition of sulfate to nucleotide forming 3' PAPS
    - 3' PAPS activated form of sulfate group - roughly equivalent to UDPGA and UDP-glucose
    - Fig 10pg 4 xenobiotic R-X-H + PAPS ---->R-X-SO_3^- + PAP
    - Sulfate biotransformations are saturated easily. Don't have capability to carry out a lot of sulfotransferase due to limited concentration of PAPS.
    - PAPS generally has highest affinity (sulfation) than glucuronidation for substrates, but has limited capability due to low [PAPS]. Glucuronidation has high capacity but not as high affinity.
    - Sulfate conjugation occurs in many sites outside of liver
    - 2 types sulfotranferase
        - Cytosolic
        - Membrane associated
    - Sulfotransferase inhibitors
- Glutathione - gly-cys-glu
    - Binds SH of cys
    - Increase MW of 200-300Da
    - In human RBC; 2mM GSH, in human hepatocyte 10mM GSH (GSH=glutathione)
    - GST - glutothione transferase
        - Glutathione will bind to xenobiotic without GST, but much better with GST enzyme
    - Glutathione (GSH) can react with reactive oxygen molecules (free radical or superoxide)
        - GSH --O_2^---> GS-SG (dimer of GSH)
        - GSH reductase: GS-SG -> GSH
        - GSH/GSSG = 100 (much more reduced form GSH than oxidized form GSSG)
    - Vitamin E in lipid membrane and can react with free radicals





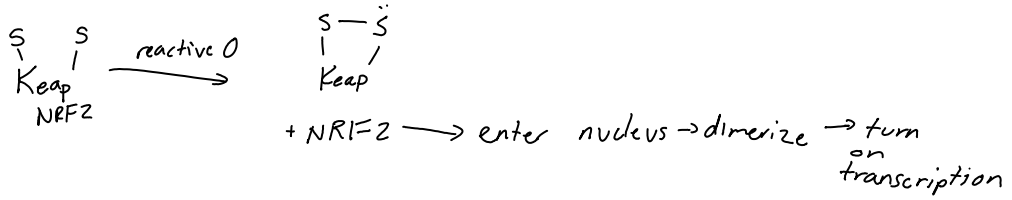
- Methylation
    - Makes more lipid soluble, in contrast to other methods to discuss above that make more water soluble.
    - Methylation more lipid soluble for metals (mercury) and can pass blood brain barrier
    - Downregulation of genes:
        - Generally methylation of gene in promotor, turns off gene
        - Methylation of kidney/liver DNA for transcription of appropriate genes for that site
    - Turning on/off transcription
        - Methylation of histones
    - Methyl donors:
        - SAM (S-adenysyl-methionine) has methyl group that can be added ad varies
        - N5THF (folate)

- Acetylation
    - Like methylation... makes more lipid soluble.
    - Acetyl donor: Acetyl-CoA

- Page 9: summary of different types of phase II transformation:
    - Glucuronidation
    - Glucosidation (didn't talk much about this.. But UDPGT adds glucose as well)
    - Sulfation
    - Acetylation
    - Methylation

- Amino acid conjugation
- Glutathione conjugation
- Lipophilic (cholesterol, fatty acids)

- Page 10:
  - Xenosensors: (AhR, Pxr, Car, GR) that turned on phase I biotransformation and phase II. These are much more preferable for phase I though
  - NRF2, phase II transformation enzyme upregulator.
    - Normally bound to actin and KEAP
      - KEAP has 2 sulfhydryl groups

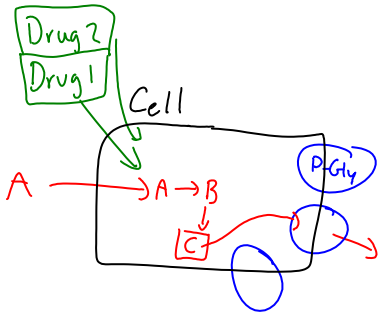


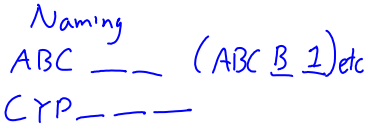
Notes 04/21

Tuesday, April 21, 2009
1:08 PM

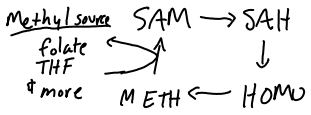
ABC transports (handout)

- A=ATP (energy requiring protein)
- ABC = ATP binding cassette protein
- Discovery
  - Giving antineoplastic drug1 gets into cancer cells
  - Up-regulation of p-glycoprotein for transport out of cell of antineoplastic drug1
  - Shift to different antineoplastic drug2 without any structural similarity to drug2 are also transferred out of cell.
  - This means there is a degree of non-specificity of chemical structure for ABC transports
  - Depends by 3-dimentional interactions that the drug has with p-glycoprotein (eflux)





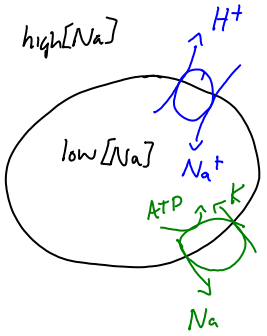
- ABC Binding motifs (pg 1, fig 1)
- Pg 1 fig 2 - MRPs have different directions on eflux capability (to/from lumen and basement membrane)
- MRPs not found uniformly on cell membrane, they are generally found at specific locations
- Some mutant ABC transporters disease causing
  - Cystic fibrosis - mutant in ABC (chloride across cell membranes)
  - drosopholia eye color mutants - ABC transportation
  - Approx 100 in E.coli are thought to be ABC transporters
- Pg 2 fig 3.
  - Parb a fig 3.
    - Various conjugates of xenobiotics to glutathione (phase II biotransformation) which is then a substrate for MRP1 for eflux
    - Other things that can happen to glutathione conjugated xenobiotic to mucaperic acid (last lecture) to a smaller molecule
  - Part b fig 3
    - Presence of glutathione allows cell to eflux etoposide with GSh without even going through phase II biotransformation
  - Part C fig 3.
    - Estrone 3-sulfate (how we metabolize estrogen) with glutathione can eflux estrogen 3-sulfate but glutathione stays in cell and is just a cofactor.
  - Part D fig 3.
    - Verapamil (calcium channel inhibitor) is a cosubstrate for eflux of GSH, though verapamil does not eflux.
    - Alter redox status of cells, makes cells more susceptible to reactive oxygen species
  - Part E fig 3.
    - H_2O_2 (hydrogen peroxide - reactive oxygen molecule) reacts with glutathione
    - Gets rid of reactive oxygen species
    - Want to maintain GSH/GSSG ratio to be high (approx 100). Glutathione reductase reduces GSSG back to GSH. If don't have enough glutathione reductase to maintain this ratio, then want to transport GSSG out of cell.
- Pg 3. fig 4.
  - Revisit next lecture methotrexate (MTX) with pharmacokinetics



  - MTX (methotrexate) gets into cell by RFC1
  - MTX is glucamylated once, and is then a substrate for MRPs1-4 for eflux
  - MTX Glucamylated 2-3 times then substrate for ABCG2 eflux
  - MTX glucamylated 4-7 times then stable inside cell and is not efluxed out of cell

- Pg 4.
  - SLC transporter family
    - 43 families on table. Now there is 55 of these gene families, and 362 genes

- 43 families on table. Now there is 55 of these gene families, and 302 genes

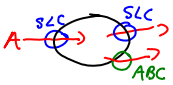


sodium hydrogen antiport

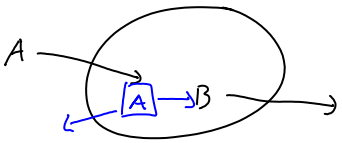
Sodium potassium antiport

Sodium hydrogen antiport does not rely directly on ATP but indirectly relies on ATP by the sodium gradient being setup by ATP depending sodium potassium antiport.

- Channels vs ABC/SLC Transporters
  - ABC transporters and SLC transporters can be saturated.
  - **ABC and SLC transporters are relatively nonspecific in terms of chemical similarities**
    - Most xenobiotics are thought to be transferred this way due to nonspecificity
  - Most channels are very specific for their substrate (ion channels etc)
  - Channels are much faster acting by orders of magnitude than ABC and SLC transporters
- In mammals
  - SLC transport only into cells
  - ABC and SLC transport of cells (eflux)



Excretion Handout



A is eliminated by cell by biotransformation to B or eflux from cell

- Clearance

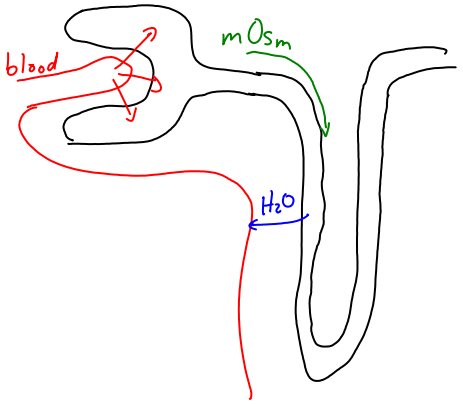
$A \rightarrow 1$ Plasma = 3L

5uM
↓ 1 hr
2.5uM (½ of chemical cleared)

$Cl = \frac{1.5L}{hr}$ $\frac{2.5uM}{5uM} = 0.5 \times 3L = 1.5L$

- Renal clearance
  - Blood flow comes into glomerius

Osmotic pressure: represented by particles in a volume



- Pg 4
  - When heart rate increases (ie exercise) the flow into glomular (RPF) increases as GFR (glomular filtration rate) increases
  - Table 12-3 If the clearance of substance over clearance of inulin (inulin only cleared by filtration)
    - If ratio is less than 1, drug is partially reabsorbed
    - =1 drug is filtered only
    - Greater than 1 drug is actively secreted
  - Table 12-5 - as you increase volume of distribution, the half life increases

30mL/mon	T1/2 69min
130mL/min	T1/2 16m
650mL/min	T1/2 3min

Notes 04/23

Thursday, April 23, 2009
1:02 PM

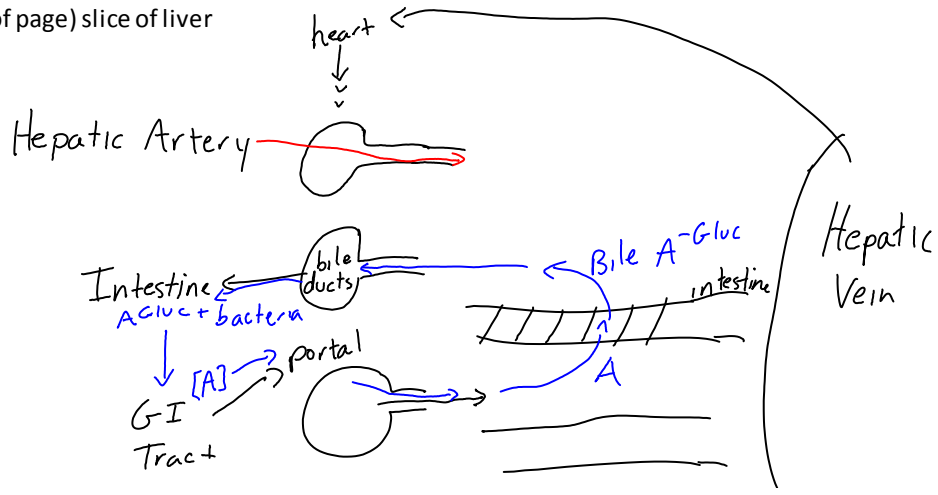
Question from last time:

As concentration increases, clearance decreases. Why?

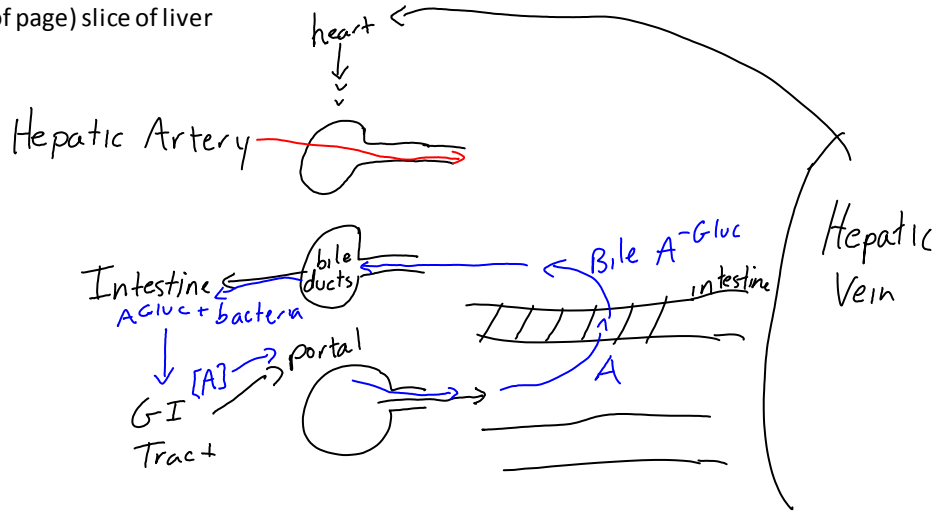
- There are a finite amount of proteins that carry out clearance that limits the rate
- The definition of clearance is based on total # of molecules.

- Excretion: Blood -> kidney
- Re-absorption: kidney -> blood
- Excretion: complete removal from body

- Excrition
  - pg 2
    - Protein bound compounds are not capable of biotransformation or excretion (some exceptions)
    - graph bound vs unbound: propanol is an exception. It is 100% E irrespective of amount of protein bound
  - Pg3
    - PAH: para-amino-hippuric acid - to measure renal plasma flow
      - Some percentage (20%) crosses glomerulus. The remainder 80% is transferred to the urine.
  - Pg 4
    - 100% inulin goes through glomerulin filtration. Used for measurment of glomerulin filtration
    - Table 12.5 : effect of volume of distribution on half life of chemical compound.
  - Pg 5
    - Table 11-6 Glomular filtration: dependent on molecular weight.
      - Insulin (small) 89% crosses from blood to nephron
      - Albumin (larger than insulin 10x) 0.1% crosses from blood to nephron
      - Small proteins generally bind to other proteins as to not be excreted by kidney.
    - Table 11-3: pH effect on clearance
      - Low pKA low excretion
  - In rats:
MW lower than 325 +/- 50	100% excreted by kidney
MW higher than 450	100% excreted by liver
Between 325-450 MW can have split	Ie 50% liver, 50% kidney or other
      - This is somewhat of a simplification as other factors besides MW come into play:
        - Lipid solubility
      - These #s change depending on the species
      - Kinetics may be completely different across species due to kidney filtration in one species and liver filtration in another
  - Pg 6
    - Fig 540 (bottom of page) slice of liver



- Fig 540 (bottom of page) slice of liver



- Low blood gas affinity are quickly excreted through exhalation
- High blood gas affinity (chloroform) slowly eliminated
- Rate of elimination of gas is the inverse of rate of absorption
- Gas with high solubility in blood

- Clearance will vary with species:
  - Circulation of blood in human takes roughly 1 minute, where the same process takes 10 seconds or so in a mouse.
  - Total clearance of chemical substance has to do with how fast it is exposed to different organ systems in the body.
  - Pg 7, fig 8
    - Kidney and liver represent about 50% of blood flow (25% liver, 20% kidney)
- Pg 7, fig 1:
  - Where different transporters are involved
  - Bile acids, xenobiotics taken into hepatocyte via transporters
  - Them being excreted by being taken across hepatocyte through canalicular lumen which leads to release to those different compounds
  - Process happens by proteins that make transport happen (not simple diffusion)

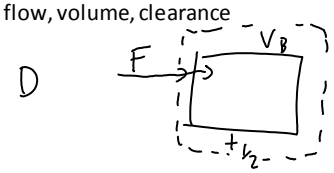
Notes 04/23 Pharmacokinetics

Thursday, April 23, 2009
2:02 PM

- Pg 1 II - plasma level time curve
  - Want to get as close to MTC as possible because it will be effective for longer period of time.
  - If Cmax exceeds MTC then will have toxicity
  - MTC and MEC varies by individual
    - Good therapeutic range = high MTC and low MEC
    - Becomes problematic when MTC and MEC are close to another

- Pg 1 bottom of pg: dosing of methotrexate
  - Methotrexate - substitutes in folate pathway as to inhibit its methylation capacity
  - Dose gets lower and lower by fracitoning over time, effect gets greater
  - Conclusion: peak concentration is not the critical parameter determining toxicity.
  - Methotrexate does not work by exceeding Cmax for toxicity.

- Pg 2:
  - III Pharmacokinetic models
    - Dose, flow, volume, clearance



    - Linear/catenary model:

    - Physiological pharmacological model (blood flow or perfusion model)
      - Benefits
        - Can model without taking into account route of administration of a compound
        - Can model across species
    - Fig 5-1
      - Two phases
        1. Distribution phase: rapid process due to volume of blood flow. High slope
        2. Elimination phase: lower slope
    - Fig 5-4:
      - See equation of combined line.
- Pg 3
  - IV nonlinear pharmocokinetics (not in 1st order elimination process)
    - Riboflavin nonlinear due to saturation of protein in gut wall
    - Etc more examples table 16-1
    - Figure 16.1 graph plasma concentration vs time
      - Linear: compound B - first order kinetics
      - As concentration goes up, has two options
        1. Continue to get rid of compound linearly (line C)
        2. Get rid of compound as in line A at zero order kinetics.
           - Saturation (absorption protein, biotransformation) occurs which is zero order
    - Figure 16.2: AUC vs Dose
    - Summary: As you give chemical compound at low doses, in general you will get rid of it by 1st order. As dose is increased saturation may occur to go by zero order kinetics in which the rate depends on the concentration of the component that is saturated.
- Pg 4
  - Figure 16-12
    - 2 drugs are given at exact same dose
      - B is linearly excreted because it is not protein bound
      - A is eliminated much slower because it is 90% protein bound
      - Protein binding slows down elimination process.
  - Figure 16-14
    - Opposite effect by mention on previous page (saturation slows down elimination)
    - Here larger the amount of chemical, the faster the elimination.
      - Concentration enhances excretion rate.

- Continue 4/28

- Pg 5 Clearance graphs
  - Methotrexate
    - Extrapolates volume of distributio nbetween difference of species
  - Caffiene
    - Monkey does not fit well because it is a longer lifespan animal compared to mouse, rat, & rabbit
    - Next graph

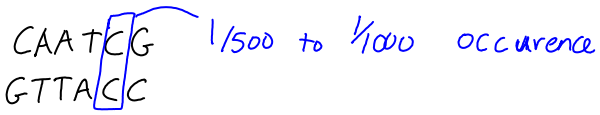
- Pg 7
  - Chemical 2,3,7,8-TCDD
    - Guin

Notes 04/28 Toxicogenetics

Tuesday, April 28, 2009
1:45 PM

- Toxicogenetics - determines why each individual shows variability to response
  - For prescribed drugs: potential of 600 fold difference (2-3 orders of magnitude different)
  - Drug doses works in about 60% of population, 20% not high enough, 20% too high of dose

- Toxicogenetics (& pharmakogenetics/ecogenetics)
  - Phenylketonuria (PKU) genetic disorder
    - Unable to convert phenylalanine -> tyrosine
    - Excess phenylalanine leads to mental retardation
  - SNP - single nucleotide polymorphism



    - SNP outcomes:
      1. No change, silent mutation
      2. Mutation can happen in splice sites (introns) which does not code for protein

- Continued pg 9 on 4/30
  - Fig 1:
    - Drug metabolizing enzyme SNP: Gene altered by SNP cannot metabolize compound as quick as WT. Metabolic deficiency
    - Drug receptor SNP:
    - Both drug met enzyme and drug receptor SNP: highest toxicity
  - Genetics becomes extremely complex because there is potential for many SNP to effect toxicity (this example is only 2 SNPs)

- Pg 10:
  - Fig 3
    - Cartoon of gene for AH receptor
    - Mice brother sister mated for 20 generations so that all genes are exact same. Utilizing these types of mice, takes genetic factor out of toxicity
    - Not mimicing human population with SNP so flipside is inbred mice do not fit human population
  - Fig 1
    - Model of ABC protein mRNA given that there is a SNP between these two RNAs
    - Large number of nucleotides in structure of RNA. Out of all NT, position 3435 difference C vs U leads to completely different structure of RNA
    - What does this lead to? If this RNA codes for protein, the difference between proteins is only at that single locus.
      - Can cause alteration of 1/2life or RNA could be affected depending of structure of RNA

Notes 04/30 Toxicodynamics Part 1

Tuesday, June 02, 2009
2:45 AM

- Toxicodynamics: assuming chemical has gotten into body and gone to appropriate cell in body… what does it do to cause toxicit y
  - Talks about mechanism in which toxicity occurs

- Pg 1
  - Adaptive Response: When exposed to chemical in **low dose**, adaptive response that leads to protection of various toxic events
    - HSP (heat shock protein)
      - AH receptor cells bound to HSP in cells.
      - Expose mammalian cells to 43^0C the cells die (heat shock)
      - Expose to 42.5^0C for a few hours they don't die when exposed to 43^0C
      - Example of adaptive response
      - HSP protective against heat as well as many other toxic phenomenon
        - Expose to reactive oxygen species = death
        - Or 42.5^0C and then reactive oxygen species = no die
      - HSP proteins are chaperone proteins that shuttle proteins around cell
    - Hormesis - relatively low doses of any toxic substances can have beneficial effects
      - Example: background radiation allows better growth.
      - Dilute it far enough, may potentially have beneficial effects
  - Death of cells
    - Cell death thresholds
    - Sometimes can kill 10-15% of organ cells can have little impact on organ

    B. How to determine cell death (difficult to determine but some methods):
      1) Boundary function: Give dye and if cell is healthy, will repulse the dye. Blue if cell is dead (membrane integrity)
      2) Energy metabolism: If cell no longer creating ATP may be indication of cell death
      3) Protein synthesis: not making proteins, usually cell dead
      4) Transcription: not transcribing, usually cell dead

    C. Differentiation of apoptosis and necrosis (oncosis): these are 2 ways of describing cell death
      1) Apoptosis
        - Embryo develops limb, cell death in between each digits that leads to the hand.
        - In brain, large number of neurons that originally develop and large number of them die

- Pg2
  - Top pg 2 events of aptoposis
- Bottom pg 4 events of necrosis

- Pg2
  - Apoptosis:
    - Signs on handout (surface contact, shrinkage, organelles intact, nuclear change, phagocytosis)
    - Sometimes DNA laddering due to DNA fragmentation (fig 3.7)
    - Low amounts of apoptosis aren't detectible due to phagocytosis
    - Fig 3.8: cartoon of what happens in apoptosis
      - Priming (reversible)
      - Once triggered (Ca relase), not reversible. Lead to cell death.
      - Ca low internal concentration inside cells (13,000x more concentrated outside of cell)

- Pg3
  - 2 types apoptotic events
    1) Extrinsic (fig 1 shows 2 paths to get effector capases)
      - Series of events which leads to activation of several caspase that leads to cell death
      - Intracellular induction that induces at mitochondria
      - Leads to release of cytochrome C from mitochromdria and

    2) Intrinsic
      - Cleave of DNA molecules

  - Necrosis was once believed tot a metabolically controlled process (physical: breaking of membrane, burning)
  - Now we know there is programmed necrosis (always knew of programmed apoptosis)

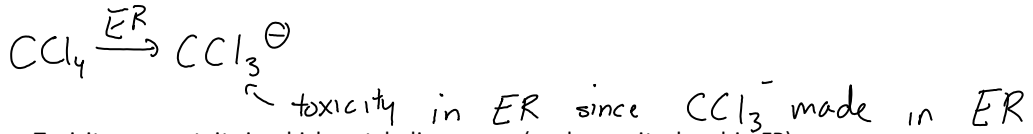
- Pg4
  - Necrosis:
    - Reverse of apoptosis (expands or swells rather than shrinks)
    - Mechanisms which it occurs

1) Ubiquitiniation of cellular proteins
    - Ubiquitin is small protein (76aa) that gets attached to lysine residues in cells in order to target specific cell proteosome.
    - In order to carry out degredation, must first be targeted by proteosome. This is facilitated by ubiquitin
    - Ubiquitin also has other functions
2) Depletion of ATP
    - (pg 6 fig 3.13) umol ATP/g dry weight tissue.
    - Permeability of cells. Low ATP levels, will initiate changing cell membrane
3) Reactive Oxygen Intermediates (ROS)
    - Generally exposure of toxic increases amount of ROS
    - Talked about this ROS pathway with respect to glutathione.
        - Vitamin E took up extra electron, passed vit C, oxidized glutathione, enzyme that rejuvenates reduced form of glutathione.
        - Overall redox state in cell that is determined by lots of different molecular couples (NADP, NADPH)
        - Top pg 8 fig 1: redox couple of GSH/GSSG in plasma, cytoplasm, mitochondria
4) Loss of calcium homeostasis
    - Extracellular concentration: 1.3mM, cytoplasmic: 100nm
    - Depletion of ATP no longer able to maintain membrane asymmetry

- Pg5
    - Different kinds of cell death
        - Not just apoptosis/necrosis. Sometimes there is characteristics of both (listed fig 1 top pg 5)
        - Normal cells that become unattached undergo onocis (type of cell death)
        - Cancer cells can overcome onocis and remain alive after unattachment
    - Mitochondrial pathway suggested leading also to necrosis (diagram pg 3 showed apoptosis only)

- Pg6
    - Table 3.1
        - Idea that different kinds of cell death are completely different processes
    - Bottom diagram. Theory that autophagy and apoptosis are completely different processes (not overlapping)
    - Others believe that this is all part of single process that leads to cell death.

- Pg7
    - 3 curves fig 2
        - Some people thing this is all part of continuum for cell death. Autophagy will be caused at very low doses. Dose increases leads to apoptosis. Dose increases goes to necrosis.
    - Fig 1 (top pg 7):
        - number of molecules that are induced if you have ER stress or unfolded protein response (UPR)
            - UPR indication that proteins have become unfolded in some way
            - 3 pathways diagrammed
                - Ability to get to cell death as a result of having alteration of alterations of ER



        - Toxicity occurs at site in which metabolism occurs (nucleus, mitochondria, ER).

- Pg 8
    - All sites of cell are potential for toxicity (fig 3.78)
        - Distribution determines
            - Protein binding and transport to specific areas
        - Biotransformation determines
            - Where biotransformation occurs… if byproduct is toxic it will affect area of where biotrans occurs
            - IE CCl_4 in ER
            - OH radical has very short half life so anywhere it is created is where toxicity occurs

- ROS
    - Vitamin A, E, glutathione
    - Enzymes that transform ROS
        - H_2O_2 enzymes
            - Glutathione peroxidase
            - Peroxyredoxin
            - Catalase
        - Any enzymes that facilitate making of OH radical?

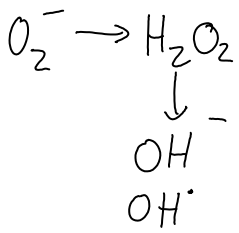
- Divalent cations, divalent ions.
- Many of divalent cations have to be sequestered to proteins otherwise they make toxic molecules
- Cu, Fe, not found freely floating (protein bound)

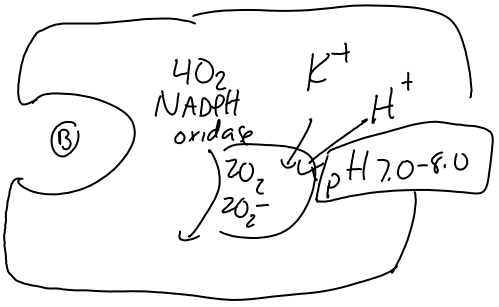
Notes 05/12 Toxicodymanics Part 2

Tuesday, May 12, 2009
1:04 PM

Toxicodynamics

Pg 14

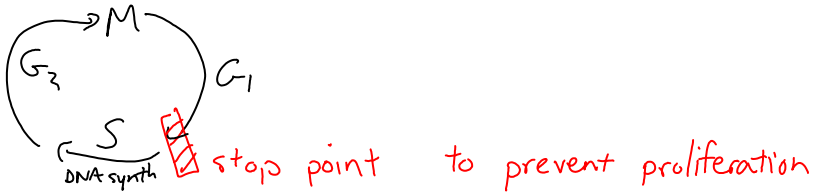




- negative charges going into vesicle, compensated by positive charges going in. Which would make electroneutral, except superoxide contribution is molar, K/H doesn't compensate enough with only mmolar amounts
- 90% of negative charge is countered by negative charge coming out of strychnine dependent transporter for choride ions.
- Reactive oxygen species that initiates this process, is the 1st step in destruction in an organism

- Frequent events with toxicity (can we put these in sequence?)
  - Decrease intracellular pH
  - Increase in intracelluar calcium
  - Depletion of glutathione
  - Depletion of ATP
  - Alteration in mitochondrial membrane transport

- Pg 15
  - Fig 1
    - Stress response: Oversimplified -> pathways target more things
  - Table 1
    - What genes are turned on or off in this process?
      - Cell cycle



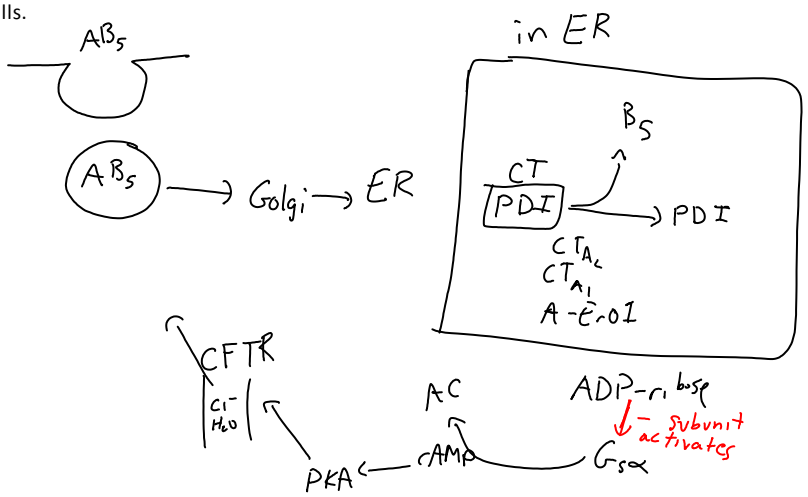
        - If mutation occurs, cell cycle may get signal to arrest as to not transcribe mutated DNA
  - Fig 2
    - Permanent (enduring): adaptive response: Phase I, Phase II
    - Transient: stress response
    - GSH is both transient and enduring

Notes 05/12 Toxinology

Tuesday, May 12, 2009
1:21 PM

- Lots of organisms produce toxins.
  - Natural organisms make more toxic/complicated toxins than ones we can produce
    - Many of the toxins are proteins or peptide
      - anthrax
      - Snake toxins are a number of different proteins
    - Organisms that don't produce toxins
      - Fungal toxins that are very complex chemical moieties and often for antibiotics
        - Aflotoxin B1
        - Xeralinone - 14 member ring mimic of estrogen
        - Trycothacines

  - Example: cholera toxin
    - Has 2 chromosomes
    - TCP and cholera toxin gene
      - TCP gene for attachment to intestine
      - Quorum sensing - 1 microorganism can send signal to another organism
      - If density in intestine is high then they will release into stool and go and infect someone else
      - Colera toxin has 5 subunits
        - A - 28kDa
        - B - 5 aprox 11,000Da.
          - Cholera toxin only attacks very specific cells in very specific proliferation process by beta subunit
        - Overall MW of 60-70kDa (5 subunits), so difficulty crossing cell membrane.
          - But it causes toxicity? How does it do that?
          - Proteins follow pathway to get out of cells
            - When make protein at ribosome, and do intracellular manipulations, protein at ribosome goes to golgi (further manipulation/folding), protein goes to cell surface and released by exocytosis
            - Colera toxin goes inside cell by endocytosis and follow reverse process that proteins get out of cells.



Cholera toxin: leads to increase in cell signaling molecule cAMP
cAMP activates PKA, (activate CFTR by PKA and not ATP)
Huge release of H_2O and Cl^- out of cells as a result of cholera toxin

Function of beta subunit, target specific site on specific cells that toxin want sto get into. Beta unit, is target for GM1 gangliosie.
Alpha subunit, alpha activates ADP-ribose to Gsalpha

Notes 05/12 DNA Structure and Mutagenesis

Tuesday, May 12, 2009
1:44 PM

Ave grade:
Grad 60/125
Ugrad 40/100

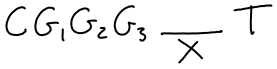
- Page 1:
  - I. Components of DNa
    - Sugars in RNA/DNA (ribose/deoxyribose)
    - BP 2 pyrines, 2 pyrimidines in DNA and RNA
    - DNA structure: bases are all parallel to each other, planar molecules

- Pg 2:
  - I. Structure of DNA
    - A, B, Z DNA forms
  - II. DNA replication is semiconservative
  - III. Genetic code

- Pg 3:
  - Replication fork - large # of proteins involved in this process (16 about)
  - V. Mutations
    - Figure 4.17: frame shift mutations
    - When nucleotides can be exposed to nitrous acid - takes off amine group and oxidizes
      - Cytosine to uracil - non complimentary BP. Becomes a problem with replication in which 2 different pieces of DNA with different sequences
- Pg 4
    - Figure 4.19
      - Replication in presence of something that imitates nucleotide
        - BrdU (bromodeoxyuridine) looks like Thiamine, polymerase uses BrdU instead
    - Keto enol shift, critical for way that polymerase reads base.
      - Polymerase can read keto form different than enol form

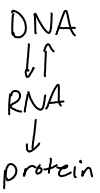
- Pg 5
  - VI. Mutations are concentrated at hotspots
    - Figure 4.20, particularly sensitive at 200 place. Mutations occur in specific sites and not uniformly across all DNA (like ta rgeting all G)
    - Table 2
      - Compounds that cause methylation an ethylating can be mutagenic
      - Methylation and ethylation can be added to xenobiotics to facilitate elimination or reduce toxicity, etc. Does not make compo und more water soluble (makes more lipid soluble)
      - Methylation/ethylation generally does not enhance excretability due to lower water solubility
      - Methylation at specific NT can cause polymerase to read NT as wrong NT
    - Figure 4.21
      - Methylated cytosine leads to mutation after replication

- Pg 6
  - Mutation rate of the Gs are dependent on nucleotides right next to G.



    - If X=A, G3 most mutated
    - Conclusion: Nontarget NT can be important in determining mutagenic events
  - VII. Spontaneous rates of mutation
    - 10^{-5}-10^{-6} chance locus has mutation
    - 10^{-9}-10^{-10} single NT mutation
    - Silent mutation - mutation occurs but doesn't change amino acid
    - Forward versus reverse mutations
      - Assays used to detect mutagenesis traditionally look at reverse mutaions
        - AMES test took bacteria, altered bacteria to need His (doesn't normally need His), mutate back to normal phenotype in WT
        - Forward mutation from WT to mutation
  - VIII. Alterations in classical genetics

Simplistic model:



    - But also siRNA, miRNA, piwiRNA.
    - NT repeat diseases

- Pg 7
  - Fig 2. DNA repeat diseases.

CGG

- Fragile X (Excess repeats in **5' regulatory region** in gene, causes mental retardation.)
  - WT: 6-50 CGG repeats
  - Heterozygous 50-200 CGG repeats
- SMB, Huninton's disease, etc dominant in **protein coding region**
  - Dominant gene
- Brings variability of length of DNA depending on # of sequence repeats

IX. Aspects of mechanisms
- Table 1-2. things that cause mutations
  - UV light
  - Etc
  - 3/4 of mutagens are carcinogens
- Table 1: enzymes involved in biotransformation of mutagenic chemicals
  - Many mutagenic chemicals become mutagenic as a result of process of biotransformations we do on these compounds
  - Benz[a]pyrine talked about before
  - P-450 create epoxides, arene oxides, etc which can enhance ability to attack DNA and make mutagenic

- Page 8
  - Table 1: continued
    - Phase II reactions that lead to mutagenesis
  - Fig 12: benzo[a]pyrene
    - Epoxidized at 7.8 position, broken down to doil, subsequently epoxide again, to create 9,10 epoxide which can attack bases of DNA
      - This creates bulky adduct

- Continued 5/14
- Pg 10
  - Table 1:
    - Error rates of DNA polymerase types (alpha, beta, etc)
- Pg 13 fig 1, there is proof reading, and mismatch repair to further refine to bring error rate to 10^{-9} for A and B family
  - Y family pols, highest error
    - Y family can see through adducts such as below. A and B can't do polymerase below

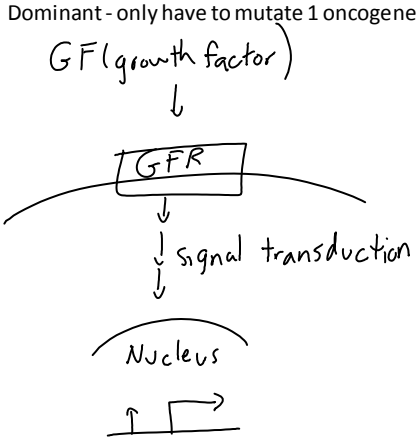
- Mutations
  - BP substitution
  - Frameshift
- Clastogenesis (ring chromosome, break chromosome, dicentric centromeres)
- Aneuploidization (trisomy 21, 18, 13) - 3 copies

- How do we detect mutations
  - AMES test
    - Mutate bacteria at hisdidine locus to create auxotroph bacteria (need histidine to survive)
    - Measuring reverse mutation back to prototrophy
    - Used bacteria - Salmonella typhimurium
    - Steps to modify AIMS to detect better
      - Take out DNA repair in microorganism
      - Get rid of LPS gene, gets rid of transporters that get rid of chemical from bacteria.
      - Mimic biotransformation that occurs in humans: Many chemicals in human become mutagens by biotransformation. S-9 mix takes homoginized liver from a rat that has been induced to produce mutagen biotransformation.
    - Ames test is cheap
  - TK (thymidine kinase) mouse lymphoma cell assay
    - Measures forward mutation (aims is reverse mutation) forward mutation is more realistic
    - Mammalian cell DNA
    - More expensive but more similar to human
  - Chromosomal chinease hampster ovary cells
    - Takes into account clastogenesis
  - Sister chromatid exchange (bottom pg 14)
    - Label 1 chromatid with florescent dye.
    - Measures recombinant events for single chromosome
    - Chemical agents can induce recombinant events and measured by florescence
    -

Notes 05/14 Carcinogenesis

Thursday, May 14, 2009
2:24 PM

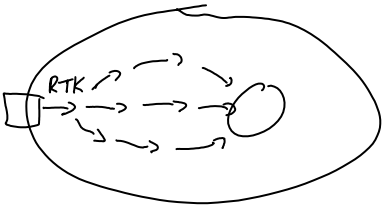
- Pg 1
  - Fig 9.66 compare types of cancer that are due to genetics versus environment
- Pg 2
  - Fig 6.1 Chemical agents put on skin of mice to determine if agents could cause cancer.
- Pg 3
  - 3 sets of genes in cancer process
    1) Oncogenes
    2) Tumor suppressor genes
    3) Stability genes
  - Oncogenes
    - Dominant - only have to mutate 1 oncogene to get effect



  - Tumor supressor genes
    - Recessive - must alter both copies for effect

Missed 5/19 lecture

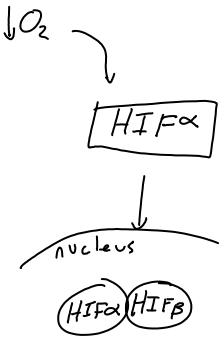
Continue pg 11. 5/21



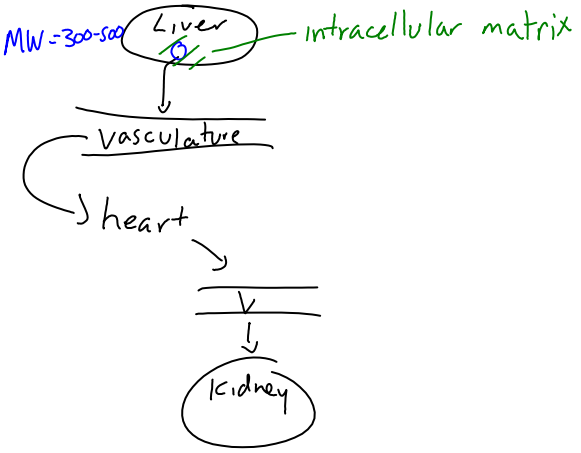
- Pg 12
  - Figure 2: single tumor cell proliferate
    - As tumor gets larger and larger, there is a metabolic stress associated with not having enough nutrients
    - Tumor growth reaches maximum, series of events for angiogenesis (additional vasculator) to overcome this metabolic stress.
  - Figure 3:
    - c-jun normally ….. Leads to degradation, short half life
    - v-gun, has longer half life due to loss of ubiquitiniation signal
    - Viruses can cause carcinogenesis, because they in some ways alter DNA
  - Figure 3: not talk much about. Just p53 affects many things
- Pg13
  - Ends of chromosomes have 6NT repeats. Each time chromosome is duplicated, some of those NT are lost

- When telomeres are very short it will stop proliferating and go into process called senescence
- If you have up-regulation of telomerase, it can reestablish telemeres at the end of chromosomes
- Table 3
  - Different species have different responses to carcinogens
  - Selecting species for assays is important

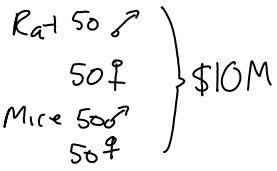
- Pg 14
  - VEGF - vascular growth factor. Growth of blood vessels from existing blood vessels (angiogenesis)
  - Vasculargenesis - new vessels
  - Angiogenesis - branching of existing vessels
  - Inducing of VEGF - HIF∝
    - HIFalpha normally created in cell but also destroyed by proline hydroxylase
    - Build up of HIFalpha, goes into nucleus, dimerize with another HIFbeta, induce response that alters hypoxia
      - A response to hypoxia is creating blood vessels so VEGF would be one that is induced
      - HIFbeta is the same as Arnt

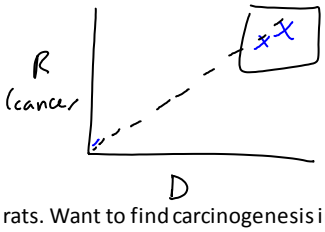


  - Figure 1: intravastation and other steps in metastatic cascade



- Pg 15
  - Maximum tolerated dose



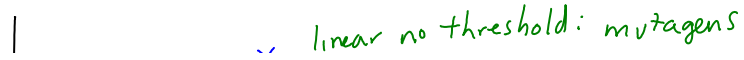


Linear non theshold

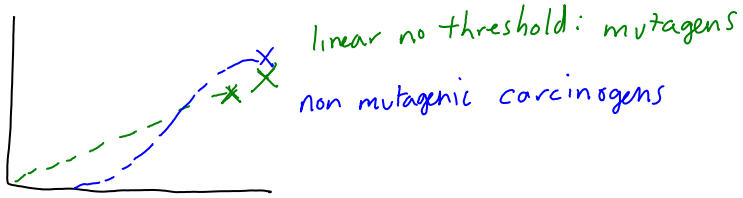
Assay is 10 million so cannot do more than about 200 rats. Want to find carcinogenesis in larger numbers so use high dose and extrapolate to low dose.

At high doses, cell death occurs. Loss of cells causes cellular proliferation. Cellular proliferation and mutations occur.

  - TCDD - not mutagenic but carcinogenic. Induces process of Arnt/Ahr for upregulation of CYP enzymes. The CYP enzymes do not impact TCDD though, so how does it cause cancer? We don't know.
    - Benzoate pyrene (B(a)P) also binds Ahr, upgreg of CYP, and CYP breaks down B(a)P
  - Dose response curve:



- Dose response curve:



  - PXR, CAR, PPAR all dimerize to RXR
  - Liver tumors in the male B6C3F1 mouse
  -
  - Alpha2u-globulin: high concentration found in rat blood. In male rates it causes kidney tumors. The carcinogen can be reason for kidney tumors or the native alpha2u-globulin tumor.
  - TCDD is planar: 4angstrom by 9 angstrom. Similar size molecules will bind to Arnt just as TCDD does.

  - Table 9
    - Potency of carcinogens varies

- Pg16:
  - Fig 9.58
    - Change 12th codon, G-T change. Activate ras oncogene leading to overall cancer process
  - Fig 2:
    - Radiation exposure (linear nonthreshold response)
  - Fig 9.64
    - Estrogen dependent carcinogen. Conditions of removal of ovaries and injected estrogen

- Pg17
  - Fig 1: roles of different genes in cancer
    - Certain genes responsible for initiation process, genes responsible for metastasis. Some fall between both categories
  - Fig 1: some types of mutagenesis are preferable for DNA in the B-form.
  - Long period of time from exposure to development of cancer
    - Leukemia is 10-15yrs as opposed to 20-25yrs. Leukemia cells are not attached to an organ, so it is quicker to develop.

Notes 05/21 Immunotoxicology

Thursday, May 21, 2009
2:32 PM

- Immune system is very complex as to deal with a multitude of insults
- Two major types of immunity
  1) Innate immunity - consist of a number of cells that you have
  2) Acquired immunity - consists of making antibodies
- Different species have different innate vs. acquired immunity
- Immune system has several types of cells (pg1 Table 12-2)
- Two things can happen for immunotoxicology
  1) Decrease effect of immune system: Many mutagens are also immunosuppressant agents
     - May be preventing ability to rid the body of tumor cells after cancer develops
  2) Increase effect of immune system:
     - Hypersensitivity - immune response to drugs. 7-8% of hospital are due to drug interactions
     - Autoimmune

- Pg2
  - Fig 1: all cells derived from stem cells
    - Cells created in bone marrow (lymphocytes), some go on to create T cells at thymus while some go on to create B cells
      - B cells responsible for creating antibodies
- Pg4
  - Fig 2: key fig in handout: Antigen is taken in
    - Shows 10 different aspects of overall process. Many chemical substances perturb different points in this process

Notes 05/26

Thursday, May 28, 2009
1:09 PM

Missed Lecture
Inhalation Toxicology

Notes 05/28 Reproductive Toxicology

Thursday, May 28, 2009
1:08 PM

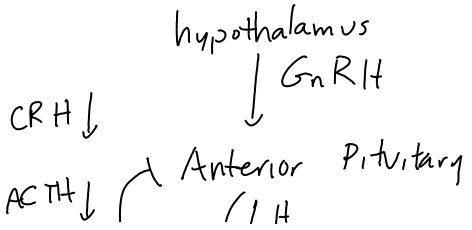
Review Mon june 8th at 12 noon

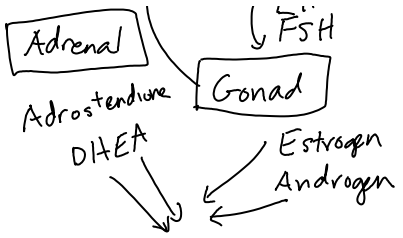
- Beryllium toxicity
  1. Acute toxicity
  2. Cancer

  - Chronic beryllium disease (CBD)
    - Formation of vesicles in lung as a result of mediating a process through the immune system
    - HLA-DPB1^{69} human lymphocyte polymorphism (glutamic acid at 69 position).
      - 1/3 of people have this polymorphism
      - Risk factor for chronic beryllium disease
      - Complicated: US government has a lot of beryllium chemistry labs, govt discovered this polymorphism. Tested those people with beryllium disease and many had this polymorphism, govt tried to remove these people form beryllium research

- Reproductive Toxicology
  - Examples:
    - Hypothesized fall roman empire due to lead toxicity (wine from certain crucibles). Increase in infertility
    - DBCP (dibromochloropropane) - industrial exposure in mid 1970s in California. Decreased levels of sperm. UC agency founded for center of occupational health
  - Endocrine disruption: chemicals that interfere with steroid hormone activity
    - Bioaccumulation of chemicals in the body - result from increase in industrialization since WW2
    - Fish exposure to chemical upregulation in female hormones… feminization of fish population
  - Male and female germ cells develop at different times.
    - female
      - 8 weeks gestation: female 600,000 eggs
      - 20 weeks gestation: female 6x10^{6} eggs
      - >20 weeks: # of eggs decrease during lifetime

- Pg2
  - Initiation of reproduction starts in CNS, then hypothalamus, etc tec (fig 16.7)
    - Some chemicals affect CNS or not reproductive organs directly but have reproductive toxicity
  - Table 1: experiments in animals are relatively appropriate for predicting effect in human
- Pg3
  - Development of follicles
    - development of single egg cell takes approx 3 months
    - Sperm take approx 10-12weeks (approx same as eggs) from initial stem cell into sperm
    - Both eggs and sperm go through a number of sequential steps to maturate so that fertilization can occur
    - At each stage for egg maturation, loss of some follicles (large number of follicles go through initial stages)
      - Chemical substances have capability for being specific for specific stages in this overall process
    - Overall process (fig 2):
      1. hypothalamus release GnRH to pituitary
      2. Pituitary release FSH or LH
      3. Ovaries develop follicles (follicogenesis)
      4. Release of single oocyte
      5. If fertilized, implants in uterus
- Pg 4
  - Fig 16-6
    - Release of GnRH release FSH or LH. Concentration of FSH and LH change over time. Surge of LH and FSH during release of oocyte
    - Steroid hormones are going through changes as well… surge of estradiol just prior to release. Surge progeseterol just after release.
    - Endometrium sloughing and increasing based on cycle
  - Table 16-23
    - Environmental chemicals that interfere with female reproductive capability
    - Complexity of this system keeps us using animal models since cell culture and human oocyte tests are not ideal (hard to get oocytes,





- Table 16-20
  - Endpoints people measure to determine if reproductive toxicity has ocured

- Pg5
  - Graph right hand side
    - Early in gestation, 600,000 eggs
    - Peak egg amount in gestation
    - Overtime loss off eggs in lifetime
  - Left hand side.
    - Curve of loss of eggs overtime roughly equivalent between human and mouse (though mouse has significantly lower amount of eggs)
  - Table 6
    - Simple compound (lead) can affect every aspect of reproductive toxicity. (both male and female)

- Pg6
  - Table 2.5

    A **granulosa cell** is a somatic cell of the sex cord that is closely associated with the developing female gamete (called an oocyte or egg) in the ovary of mammals.

    Pasted from <http://en.wikipedia.org/wiki/Granulosa>
  - Table 2.6

    The **theca folliculi** comprise a layer of the ovarian follicles. They appear as the follicles become tertiary follicles.

    Pasted from <http://en.wikipedia.org/wiki/Thecal_cell>
  - Table 2.7

    An **oocyte**, **ovocyte**, or rarely **ocyte**, is a female gametocyte or germ cell involved in reproduction. In other words, it is an immature ovum, or egg cell. An oocyte is part of the ovary development. The germ cells produce a primordial germ cell (PGC) which becomes an oogonium which marks the start of mitosis. After mitosis stops (due to actions of retinoic acid and the mesenephros) meiosis starts. This stage the oogonia is now an Oocyte (pronounced *oh'a-site*).

    Pasted from <http://en.wikipedia.org/wiki/Oocyte>

- Pg 7
  - Fig 1: endrocrine disrupting chemicals. All produce same endpoint though have different mechanisms of producing endpoint.
    - Diethylstilbestrol - cross placenta carcinogen
    - 17B-estradiol - bind to estrogen receptor
    - Genistein - works at one of 2 different kinds of estrogen receptor.
  - Table 1: VCH
    - VCH: 2 sites for epoxidation in biotransformation. Turns into VCD
    - Changes in FSH until later time.
      - VCH works directly at ovary and does not effect upstream processes because changes in upstream processes come subsequently.
  - Fig 4: VCD reduces ovarian weights by about 1/2
  - Fig 1 various endocrine disrupting chemicals:
    - Chemicals that mimic estrogen are endocrine disruptors.
    - Chemicals that block estrogen are also endocrine disruptors.
- Pg8
  - Table 1: indicator of developmental toxicity. Giving DES to animals. Take mike that are WT and get a phenotype from DES treat ment
    - Take a mouse with estrogen receptor removed, don't see these same DES endpoints. So DES toxicity is mediated through estrogen receptor.
    - DES binds to both alpha and beta estrogen receptor
  - Cartoon on left hand side (fig 3). Interaction of estrogen receptor with AhR receptor
    - Xenosensor Ah receptor has interactions with estrogen receptor
    - Better diagramed on pg 9. fig 1
  - Table 2:
    - Effect of genistein which works specifically at 1 of the estrogen receptors (DES binds to both)
    - Genostein works not only at beta, but also has tyrosine kinase activity. It is not the tyrosine kinase activity that is responsible for toxicity
    - Knockout mouse B estrogen receptor shows no toxicity, so beta receptor is target toxicity for genistein
    - As exceed dose affinity for chemical can hit level in which in affects other lower affinity xenosensors (AhR, CAR, PXR, ER)

- Pg9
  - Fig 1 (rt hand side) TCDD reacting at estrogen receptor.
    - Ah receptor can go 2 directions
      1. Turns on estrogenicity without estrogen present (mimic of estrogen)

2. Leads to the degradation of the estrogen receptor (prevent estrogenicity of other compounds)

- Picture left side pg9
  - FSH and LH have opposite effects on granulosa cells (proliferation vs differentiation)
  - Luteinizaing hormone and epidermal growth factor both go on single pathway ERK1/2 that will effect gene expression
  - LH effect on a cell will not be independent on all other effects on the cell.

- Pg10
  - Male reproduction
    - Sperm produced in seminiferous tubule. Stems cells in outer region of seminiferous tubule. Undergo gradual maturation as they go through center. Seminiferous tubule filled with sertoli cell (comparable to female granulola)
    - Leydig cells - produce hormones and other sorts of things
    - DNA is wrapped around octamones of proteins. 140,150nt of DNA in octamere bunched together. 2 types of chromatin
      - Heterochromatin (very dense, for areas that are not transcribed actively)
      - Eukromatin (actively transcribed genes)
    - In sperm protamine replaces all the histones to pack even denser. This prevents from damage that could occur (oxidative damage). Protamine is sort of super heterochromatin form.