◉Sheet ○Slides

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HELLO EVERYBODY :D

This is an enhanced version of Sheet #7 – Drug biotransformation. It contains **the same scientific material**, with some corrections, and a better context.

We deeply wish this would be an easy going sheet, we tried as much as possible to pick the easiest words, to put sentences literally taken from the doctor's slides, to include the most important tables, and to tell you the wonderful news whenever a thing is NOT REQUIRED RIGHT NOW *.*

We will be talking along this sheet about drug biotransformation, its main role, phases, inhibitors and inducers, many clinical examples, and exceptions for the main role.

We are exposed to many substances, through mouth, food, lungs, skin and eyes. We are bombarded by substances we call Xenobiotics (foreign to life), these substances are NOT part of the molecules that NATURALLY exist in our body, so the body will try to get rid of them, HOW? This depends on their lipid and water solubility.

Most drugs are Xenobiotics, except hormones (for instance, insulin is considered as a drug but not as a Xenobiotic).

-What is the best definition of drug biotransformation?

It is the process of transforming drug molecules to another chemical form of molecules by the biological system "Enzymes ".

* Remember that anything from the outside that affects the body's metabolic processes is called a drug, so even toxins are drugs.

-Difference in drug excretion according to the type...

Drugs differ in the way they're excreted by, according to their solubility. If drugs were water-soluble, they'll be excreted by the kidney (they'll be filtered through urine which is aqueous, and cannot be reabsorbed because they're water-soluble, same applies to lipid-soluble small molecules).

Large lipid-soluble drugs cannot be eliminated by the kidney, because even if they're filtered by the kidney they'll be reabsorbed since they're lipid-soluble and can pass through membranes. Sometimes they're excreted through bile since bile solubilizes them (the main role for bile is to absorb fat and to solubilize it to be reabsorbed by lymphatic system).

So biotransformation of drugs usually occurs on lipid-soluble not water-soluble drugs, WHY? to make them more polar and more excretable, so if the drug is water-soluble it'll be excreted from the body by urine, and if it's lipid-soluble, it'll have to be converted to a form that can be excreted by urine, which is usually more polar than the parent drug.

-Known ways for excretion at the past

At the past, maybe in the days of white and black TVs and high waist pants, it was thought that there are 3 major ways to excrete the drug :

1. Excretion of drug by urine (for polar drugs).

2. A drug that'll get metabolized to an excretable form of metabolites.

3. A drug that'll get metabolized, and then further metabolized to an excretable form.

- Ultimate role of drug metabolism

The ULTIMATE role for drug metabolism is to eliminate lipid-soluble drugs from the body and <u>to TERMINATE their action</u>.

** But sometimes there are exceptions worth to be mentioned before riding the metabolism huge roller coaster.

1 Sometimes drugs get metabolized to enhance their activity or even to produce a toxic substance. The change in the molecule makes the drug more

active as higher affinity to the receptor, to produce more effect or sometimes to produce toxins.

Ex.1 Paracetamol; an analgesic + antipyretic used mostly in cases of flu. It's metabolized by conjugation (phase 2 which we'll talk about later on). At regular doses it produces a toxic metabolite in very small amounts called "**N-acetyl-p-benzoquinoneimine.** "

At larger doses (like overdose to suicide), this toxin accumulates in the body causing damage of liver cells "necrosis of hepatic cells. " This may lead either to death or need of liver transplant (زراعة كبد).

-A Clinical example:

If someone had an overdose of paracetamol, and at the midnight his family came and told you with shivering tone that he took a whole bottle of it, you examined him very well as you learnt once in faculty of medicine, and tried to look for visible signs but you found nothing, BEWAAARE! You can't send him back home because liver toxicity appears at least after 3 days not within the first day. This means that if you sent the patient home he may get back with severe hepatic failure or may not get back at all which is definitely WORSE :,(

This patient should be sent to the hospital to be observed and treated to get rid of this toxin and get back healthy.

Ex.2 Halothane: used in general anesthesia; metabolized then excreted by exhalation because it's a gas; part of it is metabolized into hepatotoxin. If hepatotoxicity happened, never use it on the patient again.

2 Few drugs aren't active by themselves, they need to be activated in the body to become active and produce the effect, this type of drugs is called Prodrugs, these substances are inert, if they entered the body they'll be converted into active state, so they have NO EFFECT by themselves and have to be converted.

Ex.1 Levodopa: transferred into dopamine, it's given to Parkinson disease patients. But in treatment it is not active until transforming into dopamine in the brain.

Ex.2 Codeine: a prodrug for morphine, an analgesic that works on the central nervous system.

-A thing to know:

Even though metabolism mainly occurs in the liver because it contains Cytochrome P450 system, which is an iron containing hemeprotein that metabolizes most drugs, every cell in the body can metabolize drugs (even hair follicles have drug metabolizing agents).The next biggest metabolizing machinery is intestine (small intestine to be specific). So if you remember, in first pass effect we included metabolism existence in the intestine as well as the liver because small intestine is metabolically active.

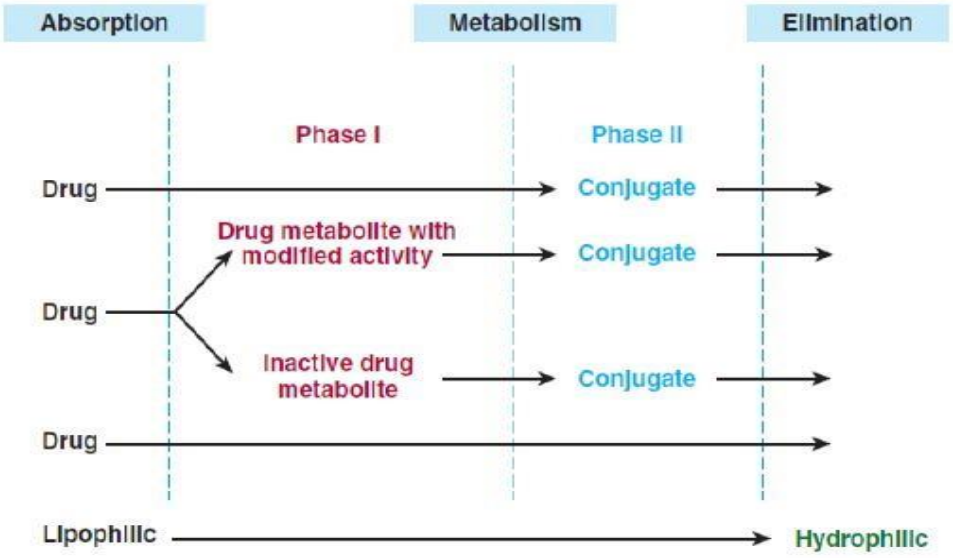


FIGURE 4–1 Phase I and phase II reactions, and direct elimination, in drug biodisposition. Phase II reactions may also precede phase I reactions.

Drug metabolizing is divided into Phase 1 (oxidation – reduction and hydrolysis) and Phase 2 (conjugation). And some "philosophy lover" doctors add Phase 3 (transporting drug molecules by active carriers) but it is not included.

- WHY it is seriously inconvenient now?

This sequence has no meaning now (it's true for certain drugs but not for all drugs), WHY? Because not all drugs need to pass both phases, some drugs will pass 1 but not 2 and they'll be excreted, some will pass 2 without passing 1, some will pass 1 then 2 and SOME WILL PASS 2 THEN 1!

- Phase 1:"Oxidation – Reduction and Hydrolysis "

Adding a bit of biochemistry to this: we all know that oxidation-reduction reactions always come together, and if any substance gets oxidized, there must be another that'll get reduced. And as we know, mostly these reactions are reversible, so the same enzyme would catalyze both reactions according to concentrations of each (substrates and products). In this phase, reactions either add a polar group or expose a polar group (like amino groups, hydroxyl groups and sulfhydryl groups), and so making the drug more excretable because of making it more water soluble.

1- Cytochrome Dependent Oxidations

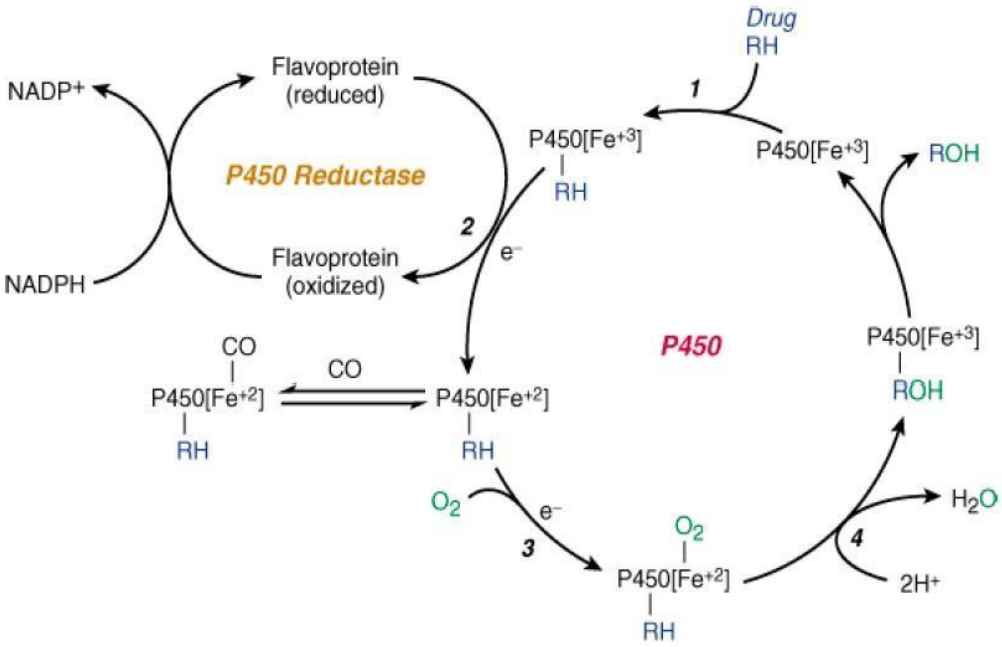
The most important family of enzymes involved in oxidation-reduction reactions of this step is Cytochrome P450 (mixed function oxidases), these enzymes for example transfer hydrocarbons into polar groups (-OH), take electrons from other molecules existing like " NADPH , P450 Reductase ", and finally give water and ROH.

- This is why P450 is called a mixed function oxidase:

1* Oxidation of hydrogen ions into water.

2* Oxidation of the drug into a hydroxyl drug.

And for a third reason that'll be explained later: Diversity of drugs metabolized by this type of enzymes.



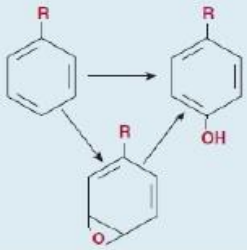
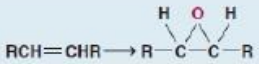
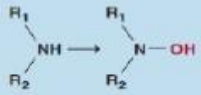
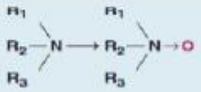
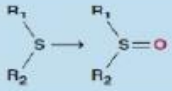
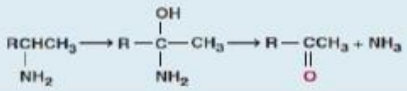
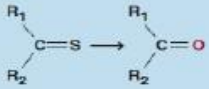
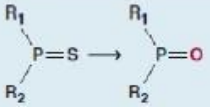
- The following table will show you the most important Phase 1 reactions of the first type.

*All reactions names are required, structural changes and drugs aren't.

1* Epoxidation is really important. Epoxide is the O linked to 2 carbons bound to each other; it's formed by the attack of Oxygen on double bonds. By oxidation, the drug is transformed into epoxide, which is very reactive and very toxic, and if it existed it'll either kill the cells or transform them into cancerous cells.

2* Dehalogenation: drugs used for anesthesia have halogens on, metabolites of them may produce toxins and cause liver toxicity.

TABLE 4–1 Phase I reactions.

Reaction Class	Structural Change	Drug Substrates
Oxidations		
Cytochrome P450-dependent oxidations:		
Aromatic hydroxylations		Acetanilide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17α-ethinyl estradiol, naphthalene, benzpyrene
Aliphatic hydroxylations	$RCH_2CH_3 \longrightarrow RCH_2CH_2OH$ $RCH_2CH_3 \longrightarrow RCHCH_3$ (OH)	Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin
Epoxidation		Aldrin
Oxidative dealkylation		
N-Dealkylation	$RNHCH_3 \longrightarrow RNH_2 + CH_2O$	Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline
O-Dealkylation	$ROCH_3 \longrightarrow ROH + CH_2O$	Codeine, *p*-nitroanisole
S-Dealkylation	$RSCH_3 \longrightarrow RSH + CH_2O$	6-Methylthiopurine, methitural
***N*-Oxidation**		
Primary amines	$RNH_2 \longrightarrow RNHOH$	Aniline, chlorphentermine
Secondary amines		2-Acetylaminofluorene, acetaminophen
Tertiary amines		Nicotine, methaqualone
S-Oxidation		Thioridazine, cimetidine, chlorpromazine
Deamination		Amphetamine, diazepam
Desulfuration		Thiopental
		Parathion
Dechlorination	$CCl_4 \longrightarrow [CCl_3^{\bullet}] \longrightarrow CHCl_3$	Carbon tetrachloride

Names are required

Exposes:

Amine ← *N*-Dealkylation

Hydroxyl ← *O*-Dealkylation

Sulfhydryl ← *S*-Dealkylation

Understand but not required now :D

Example for dehalogenation

- Isozymes of P450 are a lot:

1. SYP3A4 = 30% of total P450 content in the liver, and it metabolizes 50% of drugs available in the markets that are eliminated by metabolism.

- WHAT is the importance of SYP3A4 ? Drug – Drug interactions (drug affecting the action of another drug), and since it does the metabolism of 50% of drugs, then we'll have a tremendous amount of drug – drug interactions possible by these drugs.

2. SYP2D6 : an enzyme that varies between people in metabolism from poor to ultra rapid. 10% of people have deficiency in this enzyme "poor metabolizers ", some others are ULTRA rapid metabolizers. Ultra rapid activity comes from having a lot of copies for CYP2D6 gene, and when transcribed, they give a lot of enzymes, so you can give a drug and it won't work because it's metabolized very fast , so we either give a higher dose or search for another drug (depending on other factors of course).

As a conclusion, you **CAN'T** give a poor metabolizer, a normal metabolizer, or an ultra rapid metabolizer the same dose (Individualization of drug therapy).

3. SYP2C9 =20% of total P450 content in the liver,This enzyme varies between people in metabolism from poor to extensive " Rapid ".

4. SYP1A2 = 15% of total P450 content in the liver (Even though the percentage isn't little but put in mind that 50% of metabolism is already taken by SYP3A4 and so "content percentage: metabolism" relation isn't parallel), this enzyme produces toxins and ACTIVATES carcinogens by epoxidation, it metabolises caffeine as well.

- Note: Substrates and their corresponding isozymes table IS NOTREQUIRED RIGHT NOW!

-Enzyme Induction

Can be defined as an increase in concentration of active enzymes, in number of enzyme molecules that are active in the liver or intestine, or in number of enzymes that can do metabolism.

- How does it happen?

ENZYME INDUCTION STARTS ON GENE LEVEL (transcription then translation).

Stimulation of the enzyme gene --> Sending mRNA to the ribosome --> Synthesis of the protein --> modification to an enzyme.

And so we increase synthesis and this is **the most popular way.**

Some say that by inhibiting degradation of enzymes we'll increase the number of enzymes (enzyme accumulation), and that is true since they both lead to the same result (at the end all that we want is to increase active enzyme molecules in the body).

- Examples on inducers and their effect:

1. Polycyclic Aromatic Hydrocarbons : Environmental chemicals and pollutants present in tobacco smoke and charcoal-broiled meat, and other pyrolysis (decomposition brought by high temperatures) products.

This is an example on dangerous inducers, where induction of metabolizing enzymes in the body results in all consequences of drug metabolism, which means that the enzyme will produce toxins in higher rates, and if it works for eliminating too, it'll increase elimination rate.

This type of inducers is inert "with no action ", but it's metabolized in the body by Cytochrome P450 to an epoxide -remember what we said about them-, so yes it'll cause lung cancer and if it is excreted by urine it'll make bladder cancer. In females they're close to the breast and so will cause breast cancer.

This is all because of incomplete combustion of organic materials , for example people who always barbecue have bodies filled with polycyclic aromatic hydrocarbons that comes from meat between totally burnt and non-burnt, and so they have a lot of toxins in their bodies .Smokers -especially hookah smokers- get a lot of toxins that may result in pneumonitis and cancer. Further, WHY is it that dangerous? Water inside hookah gets rid of polar compounds, but all lipid-soluble materials (carcinogens +toxins) gather and enter the body concentrated.

2. Drugs like oral contraceptives "drugs serving to prevent pregnancy."

3. Environmental chemicals known to induce specific P450s like dioxin, dioxin is a contaminant, and small amounts of it produces carcinogens and toxins by inducing drug metabolic enzymes.

4. Cruciferous vegetables like cabbage and broccoli. Healthy food, but inducers at the same time.

5. Herbs used in medical fields.

6. Antibiotics: Rifampin , used to treat tuberculosis.

- **Auto Inducers (<u>important</u>)**: drugs that induce their own metabolism.
  - Example : Anti-epileptic drug "**Carbamazepine"**

A very important drug for treating epilepsy, it induces its metabolism and some other drugs' metabolism .

Now, what are the consequences of this on therapeutic level? What's the importance of drug being an auto inducer? What will happen?

When used in combination, Carbamazepine mostly reduces the desired effect of other drugs. For itself, auto induction will cause tolerance " NO EFFECT is observed anymore for the drug ", it eliminates itself so we need to increase the dose every time, usually it's 2 to 3 weeks and it'll complete auto induction.

Remember that half – life changes from the beginning until after 3 weeks, it becomes shorter with time, more drug is eliminated and you need to increase the dose to have the same therapeutic concentration.

- Clinical example: If an epilepsy patient returned to you after 3 weeks or more of taking the drug, you ask him if there is any improvement, he answers that Attacks keep getting back. It isn't because he didn't take the medicine as he's told, it's because you need to increase the dose; because the drug induces its own metabolism.

Other antiepliptic drugs : phenobarbital and other barbiturates , as well as phenytoin .

NOTE : Table of inducers in the slides is to know later on so CELEBRATE :D

- **Enzyme inhibition:** Inhibit the enzyme so it won't metabolize the drug.

Remember iron in P450?

P450 is a hemeprotein, so some molecules bind to it (especially to the heme) and stop its function, reducing the metabolism.

- Examples:

1- Imidazole-containing drugs

Bind tightly to the P450 heme iron and effectively reduce the metabolism of drugs through competitive inhibition.

EX. Cimetidine: used in peptic ulcer disease

2- Macrolide antibiotics

Such as erythromycin and erythromycin metabolites (CYP3A inhibitors), which complex the cytochrome P450 heme iron and inactive it.(ex: Troleandomycin; a SYP3A4 inhibitor).

3- Covalent bonding

Some drugs irreversibly inhibit P450s by covalent interaction that destroys P450 apoprotein or heme moiety (functionality).

4- Suicide drugs: Inactivators

Drugs that aren't even substrates for P450 but they destroy it (ex: grapefruit).

5- Competitive inhibition

Substrates compete with each other for the same active site of the enzyme, affecting each other's metabolism.

6- Deficiency of cofactors weakening drug metabolism

7- Inhibitors of nucleic acid and protein synthesis weaken enzyme synthesis and so, drug metabolism.

8- Malnutrition (سوء التغذية).

9-Impairment "weakness " of hepatic function.

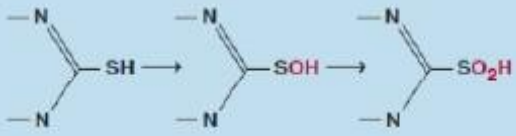
NOTE: Table of inhibitors in the slides **isn't required now *.***

2- Cytochrome Independent System

The table below shows the second type of phase 1 reactions.

* ALL reactions' names are required , structural changes are to understand , and drugs aren't required :D

The Cytochrome independent system reactions are either Oxidations , reductions , or hydrolysis.

Cytochrome P450-independent oxidations:		
Flavin monooxygenase (Ziegler's enzyme)	$R_3N \longrightarrow R_3N^+ \rightarrow O^- \xrightarrow{H^+} R_3N^+OH$	Chlorpromazine, amitriptyline, benzphetamine
	$RCH_2N(H)-CH_2R \longrightarrow RCH_2-N(OH)-CH_2R \longrightarrow RCH=N(O^-)-CH_2R$	Desipramine, nortriptyline
		Methimazole, propylthiouracil
Amine oxidases	$RCH_2NH_2 \longrightarrow RCHO + NH_3$	Phenylethylamine, epinephrine
Dehydrogenations	$RCH_2OH \longrightarrow RCHO$	Ethanol
Reductions		
Azo reductions	$RN=NR_1 \longrightarrow RNH-NHR_1 \longrightarrow RNH_2 + R_1NH_2$	Prontosil, tartrazine
Nitro reductions	$RNO_2 \longrightarrow RNO \longrightarrow RNHOH \longrightarrow RNH_2$	Nitrobenzene, chloramphenicol, clonazepam, dantrolene
Carbonyl reductions	$RCR' \longrightarrow RCHR'$ (O; OH)	Metyrapone, methadone, naloxone
Hydrolyses		
Esters	$R_1COOR_2 \longrightarrow R_1COOH + R_2OH$	Procaine, succinylcholine, aspirin, clofibrate, methylphenidate
Amides	$RCONHR_1 \longrightarrow RCOOH + R_1NH_2$	Procainamide, lidocaine, indomethacin

Info related to some reactions:

1- Alcohol oxidation (Dehydrogenations) : Ethanol metabolism is well known , alcohol dehydrogenase then aldehyde dehydrogenase , but some of it gets metabolized by CYP2E1 and it induces it .

2- Hydrolysis : Ex .Esters , their hydrolysis produces 2 compounds that are more polar than them.

- Phase 2: Conjugation

Sometimes it isn't enough to increase polarity by reactions of phase 1, so we reach to the second step where we conjugate the drug with polar groups by activating these polar or very polar molecules to bind to the drug which is again (lipid- soluble), to become water soluble.

TABLE 4–3 Phase II reactions.

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	UDP glucuronic acid	UDP glucuronosyltransferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides	Nitrophenol, morphine, acetaminophen, diazepam, *N*-hydroxydapsone, sulfathiazole, meprobamate, digitoxin, digoxin
Acetylation	Acetyl-CoA	*N*–Acetyltransferase (cytosol)	Amines	Sulfonamides, isoniazid, clonazepam, dapsone, mescaline
Glutathione conjugation	Glutathione (GSH)	GSH-*S*-transferase (cytosol, microsomes)	Epoxides, arene oxides, nitro groups, hydroxylamines	Acetaminophen, ethacrynic acid, bromobenzene
Glycine conjugation	Glycine	Acyl-CoA glycinetransferase (mitochondria)	Acyl-CoA derivatives of carboxylic acids	Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid
Sulfation	Phosphoadenosyl phosphosulfate	Sulfotransferase (cytosol)	Phenols, alcohols, aromatic amines	Estrone, aniline, phenol, 3-hydroxycoumarin, acetaminophen, methyldopa
Methylation	S-Adenosylmethionine	Transmethylases (cytosol)	Catecholamines, phenols, amines	Dopamine, epinephrine, pyridine, histamine, thiouracil
Water conjugation	Water	Epoxide hydrolase (microsomes)	Arene oxides, *cis*-disubstituted and monosubstituted oxiranes	Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbamazepine epoxide
		(cytosol)	Alkene oxides, fatty acid epoxides	Leukotriene A_4

* * All graphs and tables were taken from Katzung's Basic and Clinical Pharmacology 11th Edition book.

For this table, you JUST need to know the following and some info related to some reactions that'll be written below:

- Types of Conjugations I put in boxes (rectangles), their active donor "endogenous reactant", and name of the transferase.

These are synthetic reactions because you are synthesizing a new molecule, and this requires energy more than oxidation - reduction reactions in the body (more expensive, more demanding), the energy we need is to activate the donor, wait … WHAT ????

YES! For example, in acetylation which is a type of conjugation reactions, we need to activate acetate group by converting to acetyl CoA, in this form it can acetylate the drug.

- Another example: Glucuronidation, which is conjugating glucuronic acid (a very polar molecule that contains 4 hydroxyls, 1 carboxyl, and 1 carbonyl) to UDP " Uridine Diphosphate " to become activated .

- AND another example: phosphoadenosine phosphosulfate or PAPS is the active form which sulfate is converted into because it cannot attach by its own.

- Information related to reactions:

1* Glutathione (GSH) is awesome, and its role in your body is crucial so you don't accumulate toxins inside. If you remember in biochemistry 1, it's formed by joining 3 amino acids together (glutamic acid, cysteine, glycine), the sulfhydryl group (-SH) of cysteine attaches to molecules, allowing GSH to walk between cells and attach to any toxins existing, so if you don't have glutathione you'll get diseases, and if it's depleted you'll have diseases and toxicity of drugs .

Think with me a bit, WHY is it easily depleted SINCE IT'S THAT IMPORTANT :O ?

Because there is no natural exogenous source for it, its sources are cysteine and other amino acids, so what is the solution ???

We can give N- acetyl cysteine, NOT glutathione,WHY ?? Because if glutathione is given orally it'll be digested, and if it's given intravenous it'll be very irritable and it'll cause thrombosis to veins, so N- acetyl cysteine will transform inside into cysteine and will be used in GSH synthesis.

2* In sulfation, you want PAPS to give sulfate, sulfate is also easily depleted in the body so what's the source of it? Either diet by cruciferous vegetables or from sulfur containing amino acids.

SO sulfate and glutathione conjugations are easily depleted in the body.

-Newborn Glucuronidation Deficiency

Glucuronidation and sulfation occur together mostly, some drugs that get glucuronidated get sulfated.

- Like paracetamol :

60 %– 65 % glucurodinated

30% sulfated

5% or less toxicated

New born sulfate but don't glucuronidate, so they only depend on sulfation, and if they don't have sulfur they'll develop toxicity, that's why YOU SHOULD NEVER EVER GIVE PARACETAMOL TO A NEWBORN TO REDUCE FEVER !

*** NOTE** : Highlighted words and numbers are edited oradded info.

FINALLY O.O

It was a really long journey which we talked through about a lot of things we trust their importance for future you :D

PLEEEEASE refer to the doctor's slides .

"We are your colleagues, students just like you and we're still learning, if you found anything we missed that'll make this sheet better, generously tell us and we'll gladly add it" :D!

BEST OF LUCK AMAZING FUTURE DOCTORS *.*