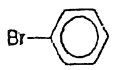
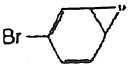
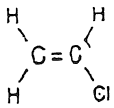
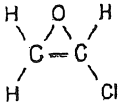
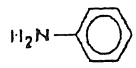
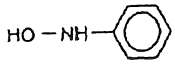
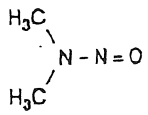
- Cytochrome P450 enzymes catalyze several types of oxidation reactions including hydroxylation of an aliphatic or aromatic carbon; epoxidation of a double bond; heteroatom oxygenation, hydroxylation or dealkylation; oxidative group transfer, cleavage of esters and dehydrogenation.

- The broad and often overlapping substrate specificity of these enzymes makes it impossible to name the enzyme for specific reactions.

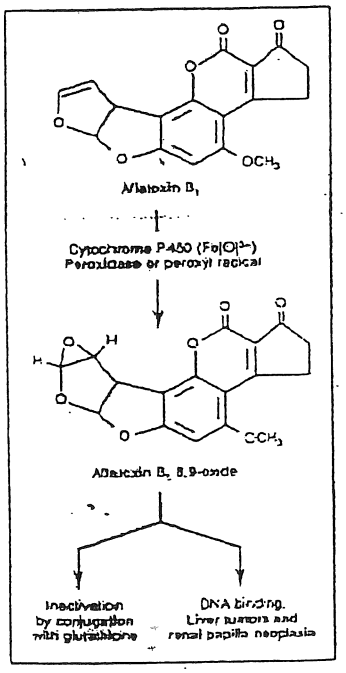
- Cytochrome P450 enzymes are "microsomal," i.e., they are concentrated in centrifugally-derived laboratory preparations enriched in SER.

- Liver tissue has the highest concentration of most biotransformation enzymes, although other organsdo have varying degrees of metabolic capacity.

Bioactivation

Compound	*Formula*	*Proposed RI*	*Type of toxicity*
bromobenzene			liver necrosis
vinyl chloride			liver cancer
aniline			methemoglobinemia
dimethylnitrosamine		H_3C^-	carcinogenesis
carbon tetrachloride	CCl_4	$^{\bullet}CCl_3$	liver necrosis
chloroform	$CHCl_3$		renal necrosis

- ✓ We mentioned that biotransformation through phase I pathways can lead toward detoxification, either directly or via continued phase II metabolism.
- ✓ This slide addresses the concept of bioactivation via phase I metabolism, i,e., toxicity occurring due to metabolism.
- ✓ Shown here are "reactive intermediates" formed due to phase I activation.
- ✓ Although these intermediates may be further metabolized and detoxified, they can also lead to pathology.



Bioactivation by Phase I enzymes

- Aflatoxin B1
- *Aspergillus flavus*
- *Penicillium puberculum*
- 1ppb carcinogenic to rats in chronic exposures

- Here we have an example of bioactivation via phase I metabolism. In

this example, aflatoxin B1 is metabolized to form a reactive epoxide (note the loss of a double bond, replaced with an epoxide, in the middle portion of the diagram, left side of the molecule).

This electrophilic epoxide can covalently bind to DNA, leading to neoplasia. Alternatively, the epoxide can be conjugated with glutathione.

Both reactions can occur in the same animal. The question of whether or not cancer develops is related to the rate of glutathione conjugation, the rate of DNA binding, and the effectiveness of DNA repair mechanisms.

Aflatoxin is a product of certain molds that can be found in improperly stored foodstuffs.

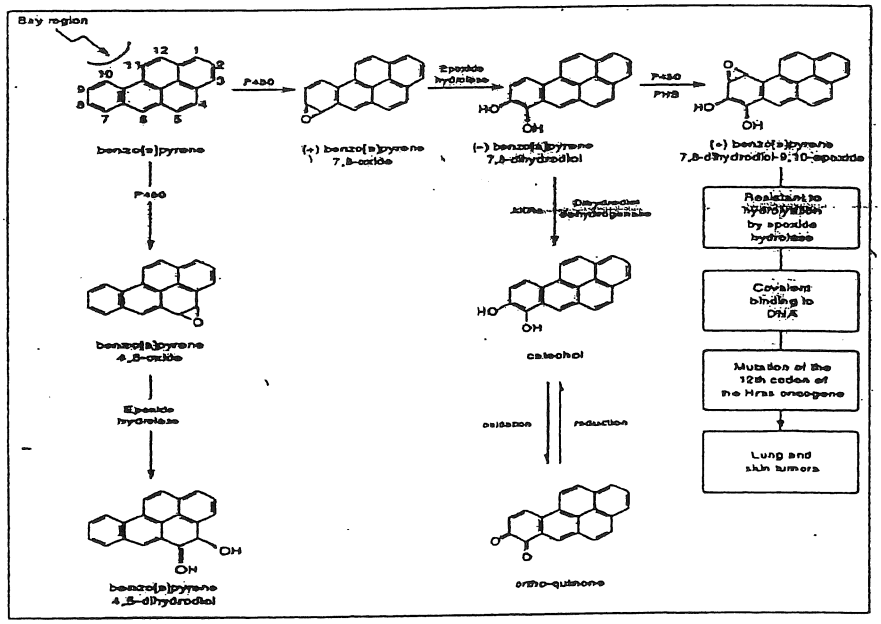


Figure 6-6. Role of epoxide hydrolase in the inactivation of benzo[a]pyrene 4,5-oxide and in the conversion of benzo[a]pyrene to its tumorigenic bay-region diolepoxide.

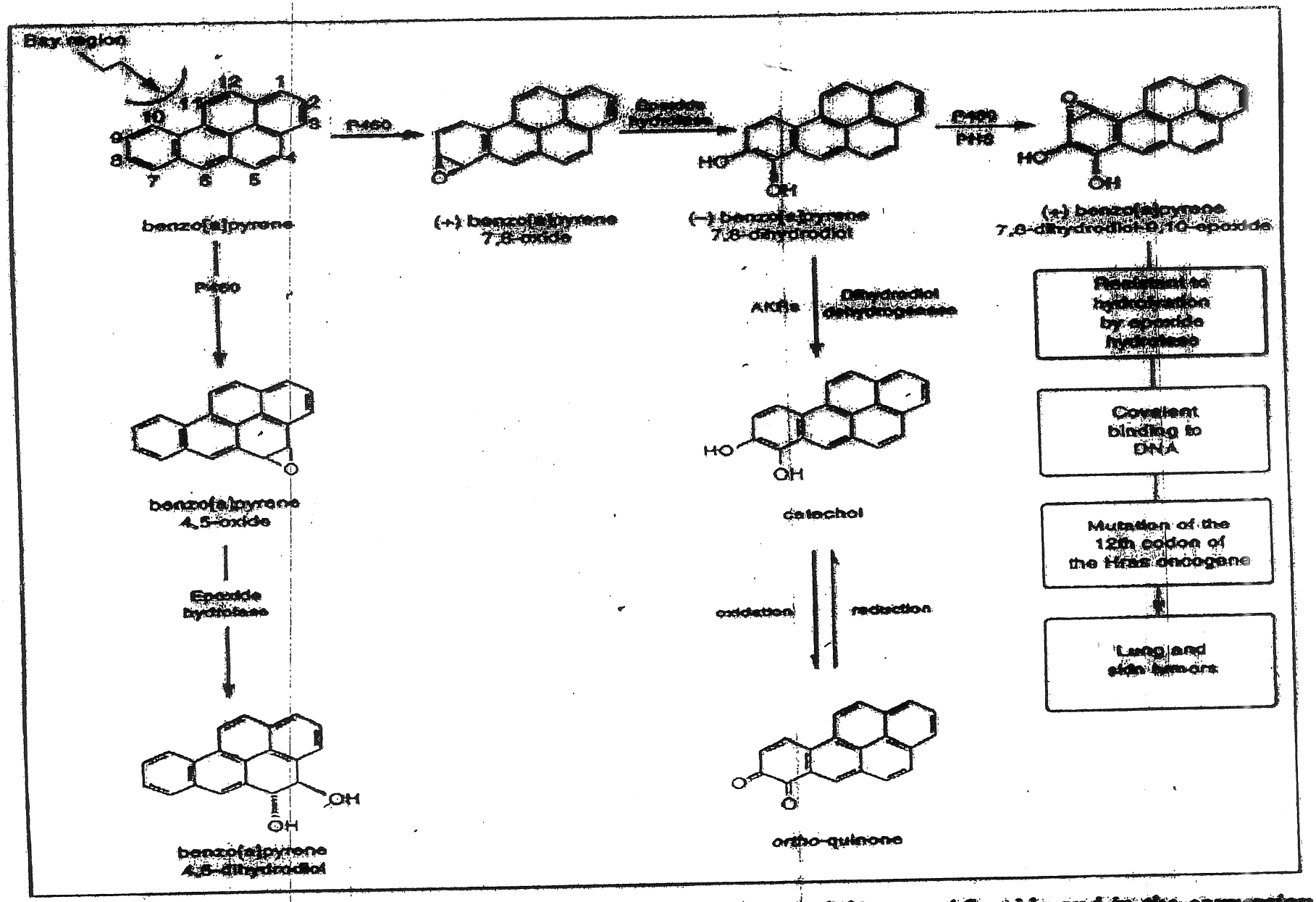


Figure 6-6. Role of epoxide hydrolase in the inactivation of benzo[a]pyrene 4,5-oxide and in the conversion of benzo[a]pyrene to its tumorigenic bay-region diolepoxide.

- Benzo[a]pyrene is a good example of a carcinogen requiring phase I activation to exert carcinogenic potential.

Although you will not be required to reconstruct this flow chart, note that there are multiple pathways that can occur.

The dynamics of these pathways and subsequent repair mechanisms determine the likelihood of tumorogenesis.

There are two cytochrome P450 pathways that can form reactive intermediates (epoxides).

The 4,5 epoxide does not have nearly the carcinogenic potential as the 7,8 epoxide. Note the the epoxides may form diols through epoxide hydrolases, and that the 7,8 dihydrodiol can be further metabolized by cytochrome P450 to yield a 7,8 dihydrodiol, 9,10 epoxide.

Due to the configuration of this molecule, it can readily intercalate into DNA, leading to altered cell growth and cancer.

Types of conjugation reactions for a number of specific functional groups

Conjugation reaction	*Functional group*
glucuronic acid conjugation	–OH; –COOH; $–NH_2$; NH; –SH; –CH
sulfate conjugation	aromatic –OH; aromatic $–NH_2$; alcohols
glycine conjugation	aromatic $–NH_2$; –COOH
acetylation	aromatic $–NH_2$; aliphatic $–NH_2$; hydrazines; $–SO_2NH_2$
methylation	aromatic –OH; $–NH_2$; NH; –SH
glutathione conjugation	epoxide, organic halides

- **Now, let's focus on phase II reactions, most of which involve** conjugating a substrate to another molecule (or part of a molecule).
- This conjugation generally renders the product to be more polar.
- An increase in the polarity facilitates excretion.

- In order for phase II metabolism to occur, the substrate must have a "functional handle" on it's molecule to support the conjugation reaction (a place that facilitates conjugation to a "donor molecule" such as glucuronic acid moiety or a sulfate moiety.
- For example, glucuronide or sulfate conjugation reactions may occur at the site of an hydroxyl or carboxyl group on the substrate molecule; for glutathion conjugations, epoxides or organic halides are common "joining" moieties.
- Note that phase II substrates may be parent compounds or metabolites of phase I reactions.

Phase II Conjugation Enzyme Cofactors

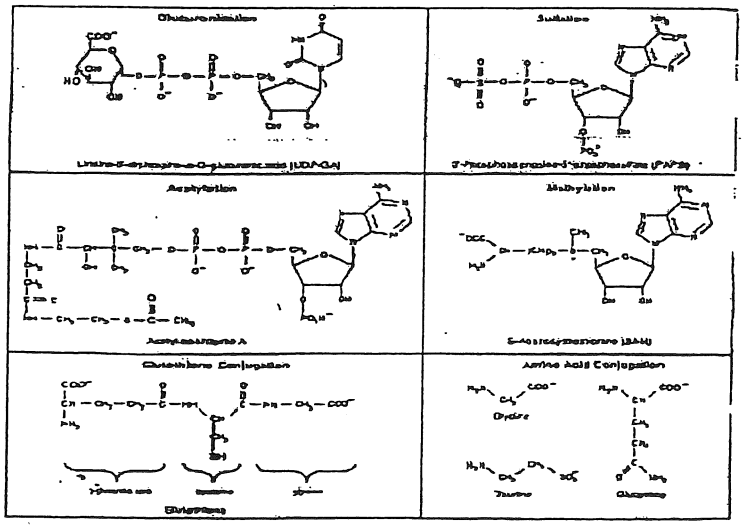


Figure 6–11. Structures of cofactors for phase II biotransformation.
The functional group that reacts with or is transferred in the metabolism is shown in bold.

Phase II Conjugation Enzyme Cofactors

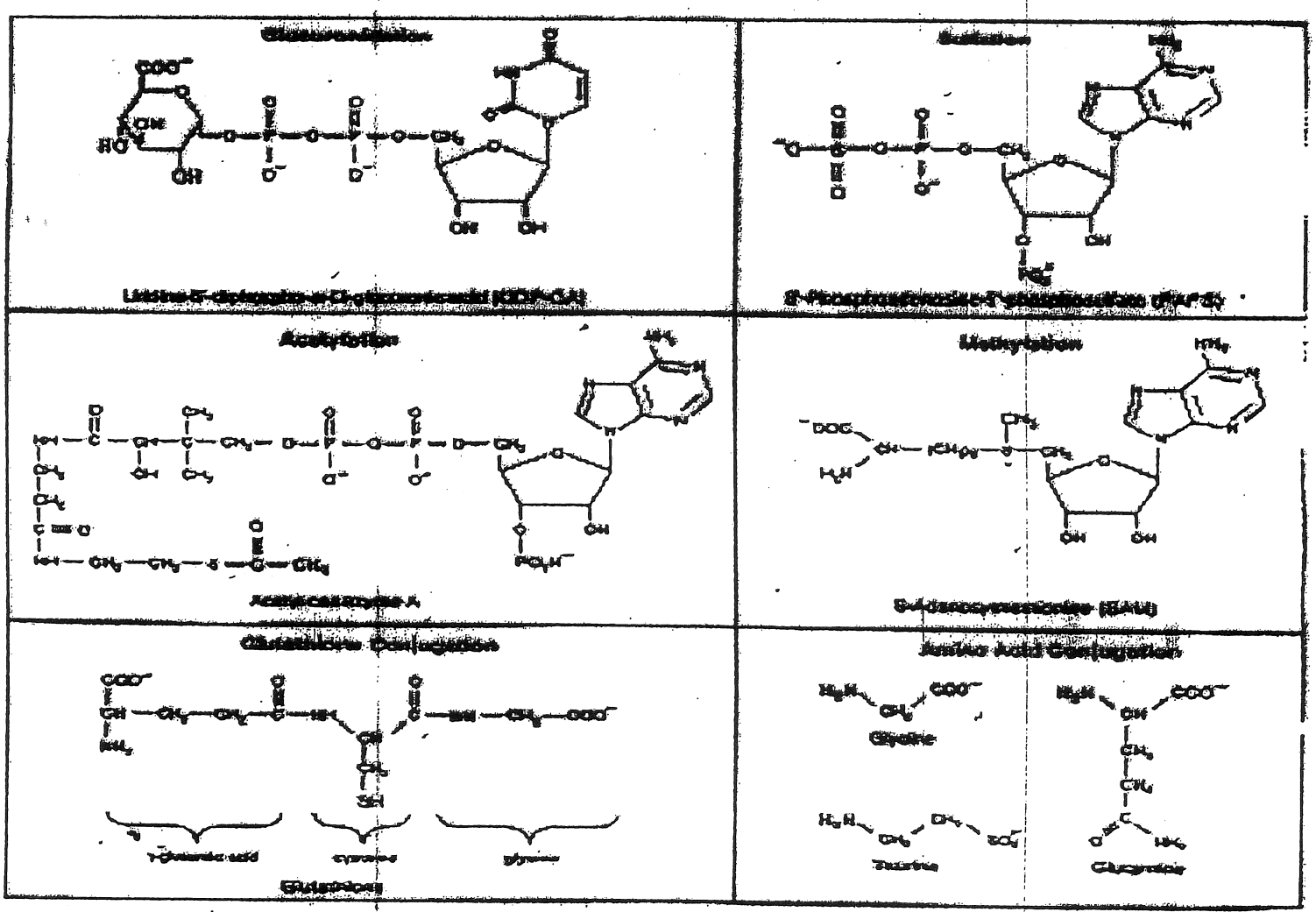


Figure 6–M. Structures of cofactors for phase II biotransformation.

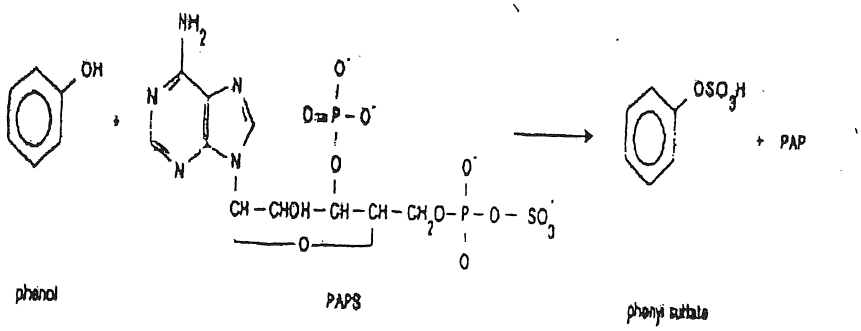
The functional group that reacts with or is transferred to the xenobiotic is shown in bold.

- We already learned that cofactors are necessary for most phase I reactions.
- Cofactors are also essential for phase II reactions (to provide the "donor" polar molecule).

Examples are given above.

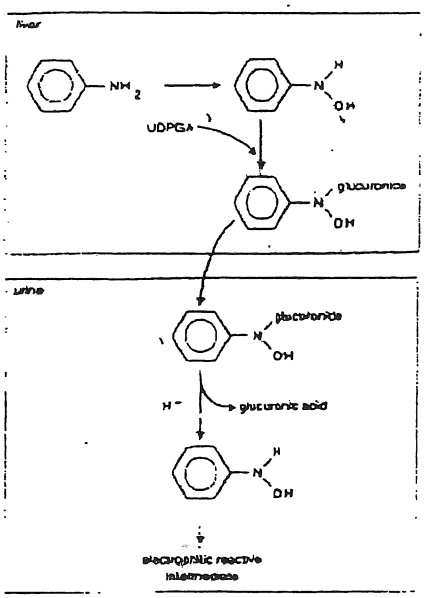
- In the case of glucuronidation, (uridine diphosphate glucuronic acid (UDPGA) is the necessary cofactor, and serves as the donor of a glucuronic acid moiety.
- What are the respective cofactors necessary for ST and GST reactions?

Sulfotransferase-mediated conjugation



- Here is a detailed diagram illustrating the ST-mediated conjugation of phenol.
- Note that the phenyl sulfate product contains only the sulfate portion of the donor molecule, not the PAP portion; this gets biochemically "recycled" for subsequent reactions in the body.

Example of (indirect) bioactivation via phase II reactions:



Glucuronides may be unstable under certain circumstances (eg some aromatic amines). Compounds may be 'bio-activated' in the liver to form N-hydoxyl-derivatives, after which they can be 'bio-inactivated' by forming N-glucuronides. However, in an acid environment (i.e., urine), the glucuride is a good leaving group, allowing the substance to exert a possible carcinogenic effect on the bladder epithelium.