Detoxification and Biotransformation of Xenobiotics

DETOXIFICATION & BIOTRANSFORMATION

Xenobiotics = Foreign chemical substances.

Examples:

- Drugs
- Environmental toxins
- Food additives

Main site:
Liver (smooth endoplasmic reticulum)

Goal:
Convert lipid-soluble compounds ? water-soluble metabolites ? excretion.

TWO PHASES OF BIOTRANSFORMATION

Phase I ? Functionalization
Phase II ? Conjugation

This chapter focuses on Phase I.

PHASE I REACTIONS

Purpose:
Introduce or expose functional groups:

- –OH
- –NH?

- –SH
- –COOH

These reactions may:

- Inactivate drugs
- Activate prodrugs
- Produce toxic intermediates

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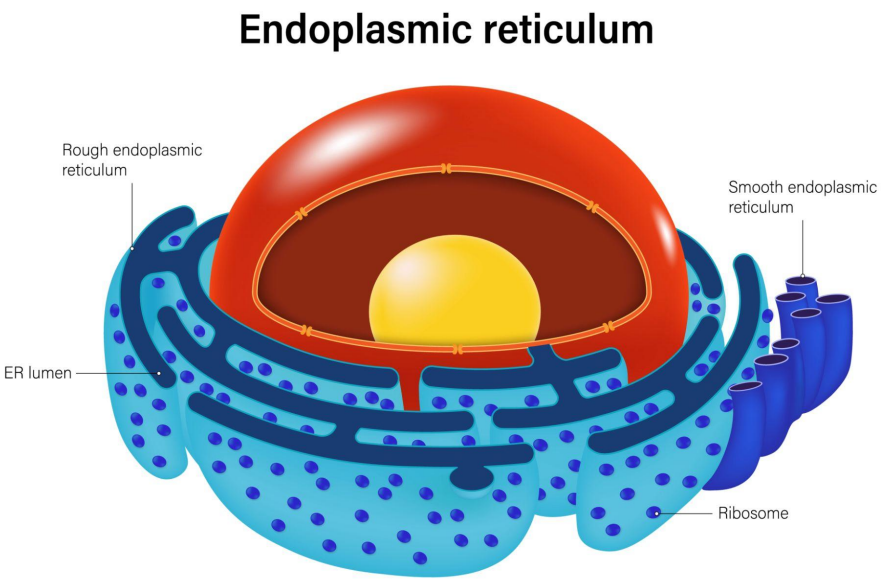

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Enzyme System

Major enzyme:
Cytochrome P450 (CYP450)

Location:
Smooth ER of hepatocytes.

Requires:

- NADPH
- O?

OXIDATIVE REACTIONS (Most Common)

Catalyzed by:
Cytochrome P450 monooxygenase system.

General reaction:

RH + O? + NADPH ? ROH + H?O

One oxygen atom incorporated into substrate.

Types of Oxidative Reactions

1. Hydroxylation
2. Dealkylation
3. Deamination
4. Sulfoxidation

Example

Barbiturate oxidation
Conversion of benzene ? phenol

Clinical Importance

- Drug interactions
- Enzyme induction
- Enzyme inhibition

Inducers:
Rifampicin, phenobarbital.

Inhibitors:
Cimetidine, erythromycin.

REDUCTIVE REACTIONS

Occur when oxygen availability is low.

Common in:

- Azo compounds
- Nitro compounds

Mechanism:
Gain of electrons (reduction).

Example:
Chloramphenicol reduction.

Often occur in:

- Liver
- Intestinal bacteria

Hydrolysis (Also Phase I)

Esterases and amidases split:

- Ester bonds
- Amide bonds

Example:
Aspirin hydrolysis.

ROLE OF CYTOCHROME P450

Hemoprotein system.

Contains:
Heme iron.

Function:
Mixed function oxidase.

Can produce reactive intermediates.

TOXIC METABOLITES

Important example:

Paracetamol (acetaminophen)

Small fraction converted to:
NAPQI (toxic metabolite)

Detoxified by:
Glutathione.

Overdose ? glutathione depletion ? liver necrosis.

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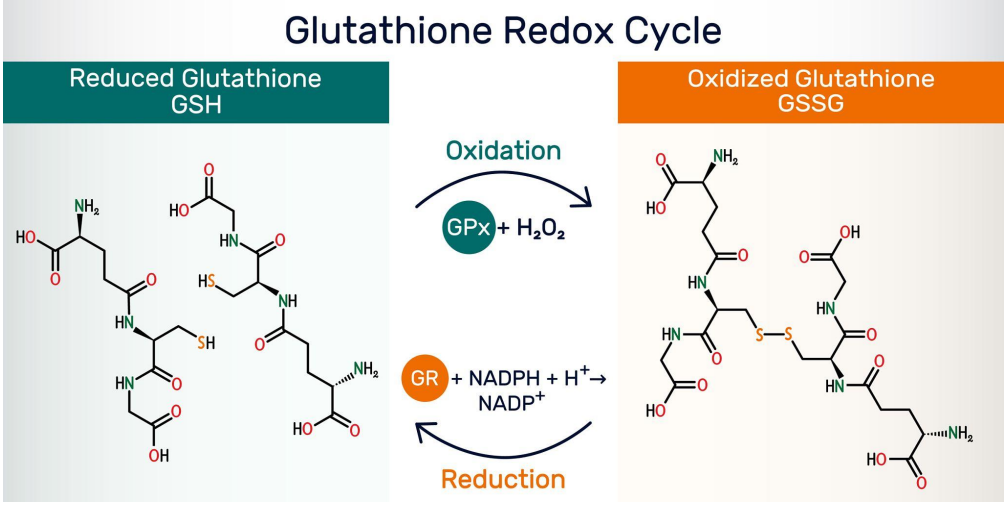
https://www.researchgate.net/publication/49701884/figure/fig5/AS%3A668545419575306%401536405057727/Acetaminophen-metabolism



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FACTORS AFFECTING PHASE I

- Age
- Genetic polymorphism
- Nutrition
- Liver disease
- Drug interactions

Example:
Slow acetylators (though acetylation is Phase II, examiner may connect).

HIGH-YIELD SUMMARY

Phase I = Functionalization
Main enzyme = CYP450
Requires NADPH + O?
Oxidation most common
Reduction under low oxygen
Toxic intermediates possible

Biotransformation is chemical modification — not always detoxification.

Sometimes the liver makes a drug safer.
Sometimes it makes it more dangerous.

HYDROLYSIS (PHASE I REACTION)

Hydrolysis breaks chemical bonds using water.

Common substrates:

- Esters
- Amides

Enzymes:

- Esterases
- Amidases

Example:
Aspirin ? Salicylic acid

Hydrolysis often occurs in:

- Liver

- Plasma
- Intestinal mucosa

Hydrolysis increases polarity but does not always make the compound fully water-soluble.

PHASE II REACTIONS

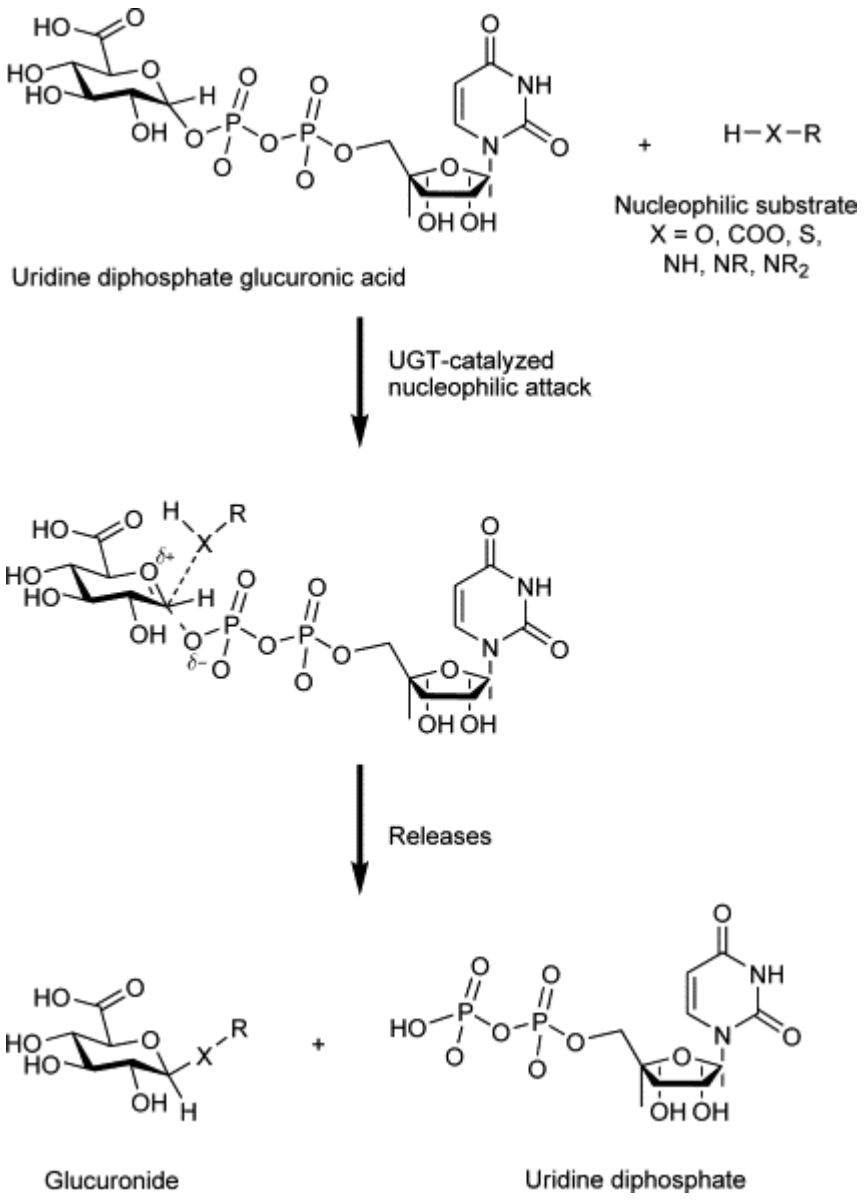
Purpose:
Conjugation.

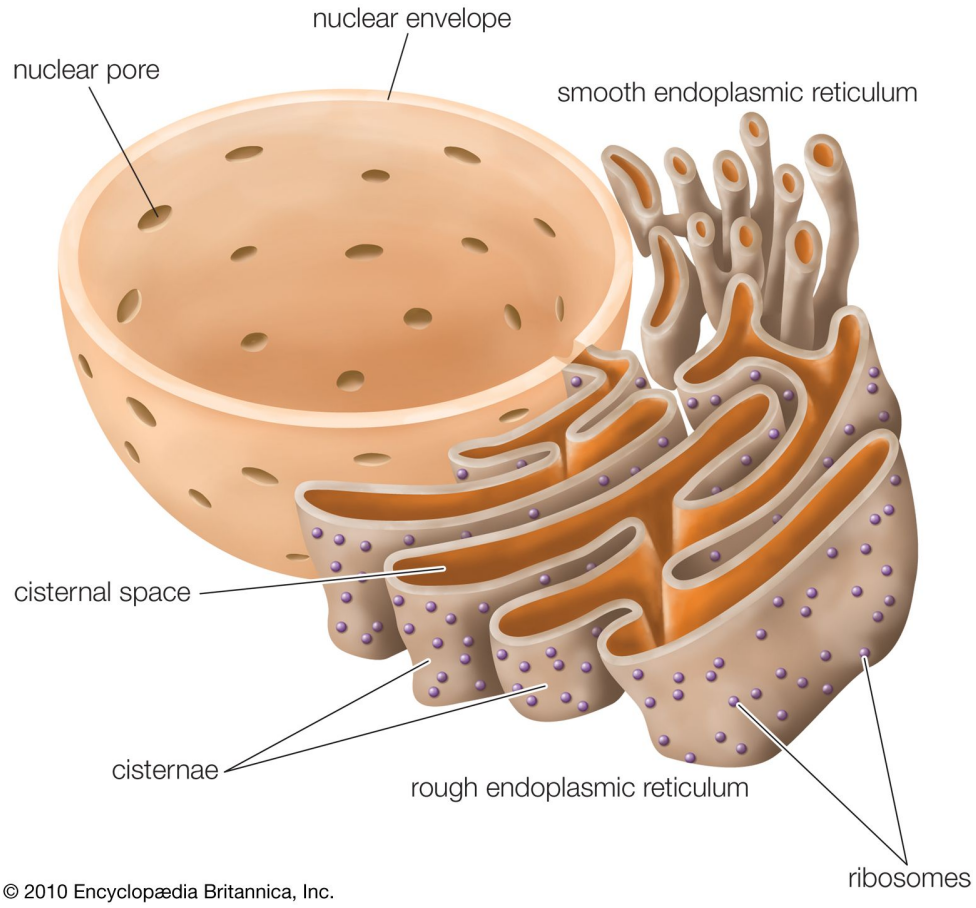
Adds large polar molecules to drug.

Result:
Highly water-soluble compound ? excreted in urine or bile.

Usually inactivates drug.

Conjugation Reaction	Conjugating Agent	Conjugating Agent Transferring Enzyme	Activated Intermediate	Functional Groups Combined with
Glucuronidation	Glucuronic acid	UDP-glucuronyl transferase	UDPGA	-OH, -COOH, -NH_2, -SH
Sulphation	Sulphate	Sulphotransferase	PAPS	-OH, -NH_2
Amino acid conjugation	Glycine	Acyl transferase	Acyl CoA	-COOH, -NH_2
Glutathione	Glutathione	Gluthaione-S-transferase	-	Alkyl halides, alkyl nitrat epoxides, lactones, etc.
Acetylation	Acetyl CoA	N-acetyl transferase	Acetyl CoA	-NH_2, -SO_2NH_2, hydrazir
Methylation	L-methionine	Methyl transferase	S-adenosyl methionine	-OH, -NH_2, -SH


Uridine diphosphate glucuronic acid
+
H−X−R
Nucleophilic substrate
X = O, COO, S,
NH, NR, NR2
UGT-catalyzed
nucleophilic attack
Releases
Glucuronide
+
Uridine diphosphate



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MAJOR PHASE II REACTIONS

1. Glucuronidation
2. Sulfation
3. Acetylation
4. Methylation
5. Glutathione conjugation

6. Glycine conjugation

GLUCURONIDATION

Most common Phase II reaction.

Enzyme:
UDP-glucuronyl transferase.

Location:
Smooth ER.

Adds:
Glucuronic acid.

Example:
Bilirubin conjugation.

Important clinically:
Newborns have low glucuronyl transferase ? risk of kernicterus.

SULFATION

Enzyme:
Sulfotransferase.

Adds:
Sulfate group.

Important for:
Steroid hormones.

ACETYLATION

Enzyme:
N-acetyl transferase (NAT).

Substrates:

- Isoniazid

- Sulfonamides
- Hydralazine

Genetic polymorphism:

- Slow acetylators
- Fast acetylators

Slow acetylators ? higher toxicity risk.

Note:
Acetylation does NOT always increase water solubility significantly.

METHYLATION

Adds methyl group.

Usually reduces activity.

Example:
Catecholamines.

GLUTATHIONE CONJUGATION

Protective mechanism.

Enzyme:
Glutathione-S-transferase.

Important in detoxifying:

- Reactive oxygen species
- Toxic intermediates (e.g., NAPQI from paracetamol)

Glutathione depletion ? toxicity.

PHASE III REACTIONS

Now the export system.

After Phase II conjugation, metabolites must be transported out of cells.

Phase III involves:

ATP-dependent transporters.

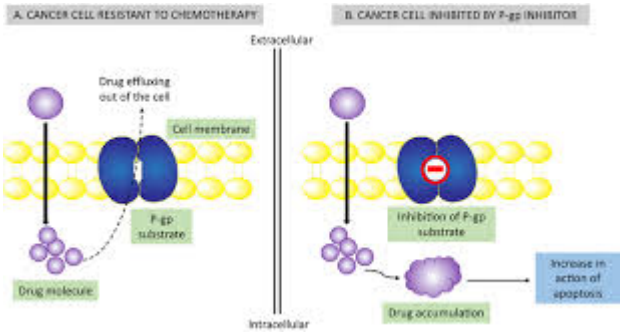
Example:

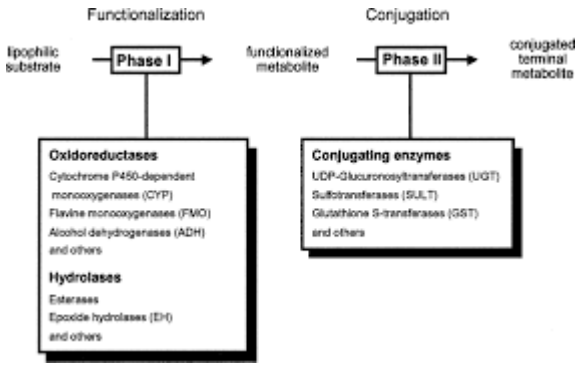
P-glycoprotein (MDR transporter)

Location:

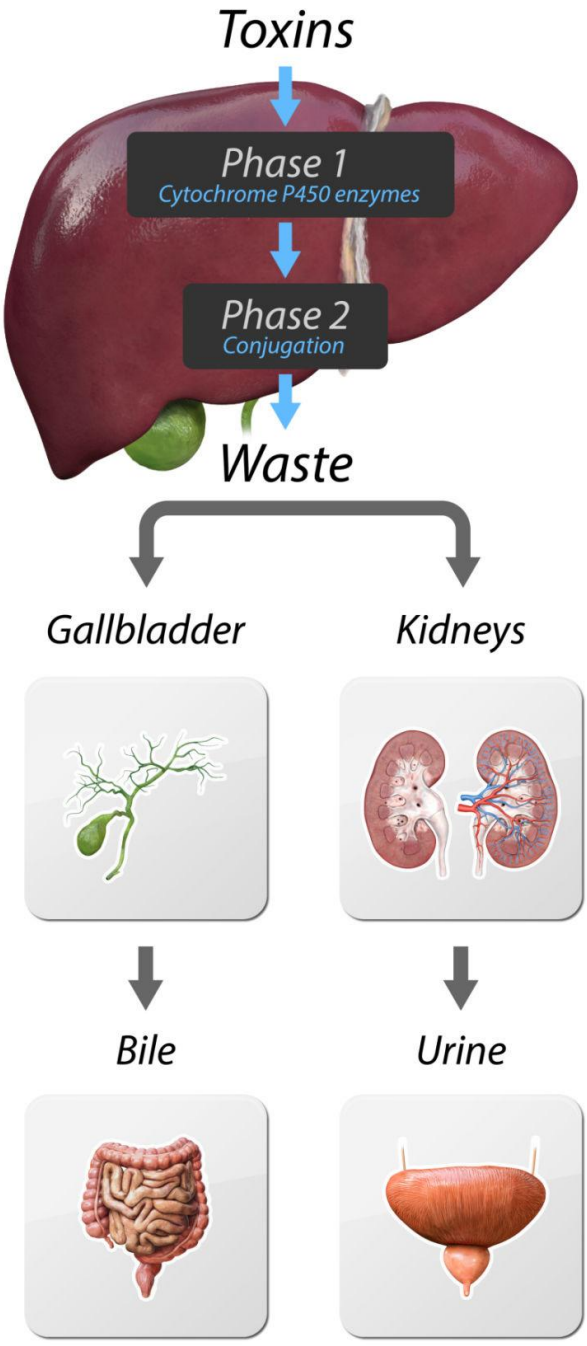
- Liver canalicular membrane
- Kidney
- Intestinal epithelium

Function:
Efflux of drugs into bile or urine.




Functionalization
Conjugation
lipophilic substrate
Phase I
functionalized metabolite
Phase II
conjugated terminal metabolite
Oxidoreductases
Cytochrome P450-dependent monooxygenases (CYP)
Flavine monooxygenases (FMO)
Alcohol dehydrogenases (ADH)
and others
Hydrolases
Esterases
Epoxide hydrolases (EH)
and others
Conjugating enzymes
UDP-Glucuronosyltransferases (UGT)
Sulfotransferases (SULT)
Glutathione S-transferases (GST)
and others

Detoxification



INTEGRATED OVERVIEW

Phase I:
Functionalization (oxidation, reduction, hydrolysis)

Phase II:
Conjugation (glucuronide, sulfate, acetyl, etc.)

Phase III:
Transport and excretion

CLINICAL CONNECTIONS

- Neonatal jaundice ? low glucuronidation
- Slow acetylators ? drug toxicity
- Paracetamol overdose ? glutathione depletion
- Multidrug resistance ? P-glycoprotein overexpression

HIGH-YIELD SUMMARY

Hydrolysis breaks esters/amides
Phase II = conjugation
Glucuronidation most common
Acetylation genetically variable
Glutathione protects against toxic metabolites
Phase III exports via ATP transporters

Biotransformation is organized chemistry.

Phase I makes the molecule reactive.
Phase II makes it soluble.
Phase III sends it away.

DETOXIFICATION & BIOTRANSFORMATION – FAQs

GENERAL CONCEPT

Q1. What are xenobiotics?
Foreign chemical substances such as drugs, toxins, and pollutants.

Q2. What is the main site of biotransformation?
Liver (smooth endoplasmic reticulum).

Q3. What is the purpose of biotransformation?
Convert lipid-soluble compounds into water-soluble metabolites for excretion.

Q4. What are the three phases of drug metabolism?
Phase I (functionalization), Phase II (conjugation), Phase III (transport/excretion).

PHASE I REACTIONS

Q5. What is the purpose of Phase I reactions?
Introduce or expose functional groups.

Q6. What is the major enzyme system in Phase I?
Cytochrome P450.

Q7. Where is CYP450 located?
Smooth ER of hepatocytes.

Q8. What cofactors are required for oxidative reactions?
NADPH and O?.

Q9. Name three types of Phase I reactions.
Oxidation, reduction, hydrolysis.

OXIDATIVE REACTIONS

Q10. What is the most common Phase I reaction?
Oxidation.

Q11. What is the general reaction of CYP450?
RH + O? + NADPH ? ROH + H?O.

Q12. What is enzyme induction?
Increased enzyme activity due to certain drugs.

Q13. Name one enzyme inducer.
Rifampicin.

Q14. Name one enzyme inhibitor.
Cimetidine.

REDUCTIVE REACTIONS

Q15. When do reductive reactions commonly occur?
Under low oxygen conditions.

Q16. Name a compound undergoing reduction.
Azo or nitro compounds.

HYDROLYSIS

Q17. What bonds are broken during hydrolysis?
Ester and amide bonds.

Q18. Name enzymes involved in hydrolysis.
Esterases and amidases.

Q19. Give one example of hydrolysis reaction.
Aspirin ? Salicylic acid.

PHASE II REACTIONS (CONJUGATION)

Q20. What is the purpose of Phase II reactions?
Increase water solubility by conjugation.

Q21. What is the most common Phase II reaction?
Glucuronidation.

Q22. Which enzyme performs glucuronidation?
UDP-glucuronyl transferase.

Q23. Why are newborns prone to kernicterus?
Low glucuronyl transferase activity.

TYPES OF CONJUGATION

Q24. What is acetylation?
Addition of acetyl group via N-acetyl transferase.

Q25. What is special about acetylation?
Shows genetic polymorphism (slow and fast acetylators).

Q26. Name one drug that undergoes acetylation.
Isoniazid.

Q27. What is glutathione conjugation?
Detoxification of reactive intermediates.

Q28. Which enzyme catalyzes glutathione conjugation?
Glutathione-S-transferase.

Q29. What happens in paracetamol overdose?
Glutathione depletion ? liver toxicity.

PHASE III REACTIONS

Q30. What is Phase III reaction?
Transport of conjugated metabolites out of cells.

Q31. Which transporter is involved in Phase III?
P-glycoprotein.

Q32. Phase III transport requires what?
ATP.

Q33. Where are Phase III transporters found?
Liver, kidney, intestine.

CLINICAL INTEGRATION

Q34. Can Phase I reactions sometimes produce toxic metabolites?
Yes.

Q35. Which metabolite of paracetamol is toxic?
NAPQI.

Q36. Which antioxidant neutralizes NAPQI?
Glutathione.

Q37. What is enzyme induction effect on drug levels?
Decreases drug levels by increasing metabolism.

Q38. What is enzyme inhibition effect on drug levels?
Increases drug levels.

Q39. Which phase is usually responsible for drug inactivation?
Phase II.

Q40. Are Phase II reactions always inactivating?
Mostly, but not always.

INTEGRATED QUESTIONS

Q41. Which phase introduces functional groups?
Phase I.

Q42. Which phase increases polarity the most?
Phase II.

Q43. Which phase involves ATP-dependent transport?
Phase III.

Q44. Which phase shows genetic polymorphism commonly?
Acetylation in Phase II.

Q45. What is the overall goal of detoxification?
Enhance elimination of xenobiotics.

DETOXIFICATION & BIOTRANSFORMATION – MCQs

1. Xenobiotics are:

A. Endogenous hormones
B. Foreign chemical substances
C. Vitamins
D. Enzymes

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2. The major site of drug biotransformation is:

A. Kidney
B. Brain
C. Liver
D. Spleen

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3. Phase I reactions primarily:

A. Increase molecular size
B. Introduce functional groups
C. Conjugate glucuronic acid
D. Excrete drug

---

4. The most important Phase I enzyme system is:

A. Catalase
B. Cytochrome P450
C. Transferrin
D. Pepsin

---

5. Cytochrome P450 is located in:

A. Mitochondria only
B. Nucleus
C. Smooth ER
D. Ribosomes

---

6. Phase I oxidation requires:

A. NADH only
B. NADPH and O?
C. ATP only
D. FAD only

---

7. General CYP450 reaction:

A. RH + H?O ? ROH
B. RH + O? + NADPH ? ROH + H?O
C. RH + CO? ? RCOOH
D. RH + ATP ? ROH

---

8. The most common Phase I reaction:

A. Reduction
B. Oxidation
C. Conjugation
D. Acetylation

---

9. Reduction reactions commonly occur:

A. In high oxygen state
B. In low oxygen state
C. Only in kidney
D. Only in plasma

---

10. Hydrolysis breaks:

A. Double bonds
B. Ester and amide bonds
C. Peptide bonds only
D. Disulfide bonds

---

11. Esterases catalyze:

A. Oxidation
B. Hydrolysis
C. Conjugation
D. Methylation

---

12. Phase II reactions are also called:

A. Functionalization
B. Conjugation
C. Reduction
D. Oxidation

---

13. The most common Phase II reaction:

A. Sulfation
B. Acetylation
C. Glucuronidation
D. Methylation

---

14. Enzyme responsible for glucuronidation:

A. Sulfotransferase
B. UDP-glucuronyl transferase
C. Acetyl transferase
D. Catalase

---

15. Low glucuronyl transferase in newborns predisposes to:

A. Hemolysis
B. Kernicterus
C. Rickets
D. Pellagra

---

16. Acetylation is catalyzed by:

A. NAT
B. CYP450
C. GST
D. UGT

---

17. Genetic polymorphism is seen in:

A. Glucuronidation
B. Acetylation
C. Sulfation
D. Reduction

---

18. Slow acetylators are at risk of:

A. Drug toxicity
B. Rapid elimination
C. Hypoglycemia
D. Hypocalcemia

---

19. Glutathione conjugation protects against:

A. Hyperglycemia
B. Reactive toxic intermediates
C. Iron overload
D. Ketosis

---

20. Toxic metabolite of paracetamol:

A. NAPQI
B. Bilirubin
C. Acetyl-CoA
D. Lactate

---

21. Glutathione depletion results in:

A. Renal failure
B. Liver necrosis
C. Hypothyroidism
D. Anemia

---

22. Phase III reactions involve:

A. Conjugation
B. Oxidation
C. ATP-dependent transport
D. Hydrolysis

---

23. P-glycoprotein is:

A. Oxidase enzyme
B. Conjugating enzyme
C. Efflux transporter
D. Hormone

---

24. Phase III transport requires:

A. NADPH
B. Oxygen
C. ATP
D. FAD

---

25. Drug enzyme induction causes:

A. Increased drug levels
B. Decreased drug metabolism
C. Increased drug metabolism
D. No effect

---

26. Enzyme inhibition leads to:

A. Faster drug clearance
B. Increased drug levels
C. Decreased toxicity
D. No change

---

27. Which phase may generate toxic metabolites?

A. Phase I
B. Phase II
C. Phase III
D. All phases

---

28. Sulfation involves addition of:

A. Acetyl group
B. Methyl group
C. Sulfate group
D. Glucose

---

29. Acetylation does NOT always:

A. Inactivate drug
B. Increase water solubility
C. Modify drug
D. Affect activity

---

30. Hydrolysis often occurs in:

A. Bone
B. Plasma
C. Brain
D. Thyroid

---

31. Conjugated metabolites are usually excreted via:

A. Lungs
B. Sweat only
C. Urine or bile
D. Skin

---

32. Which reaction usually increases polarity the most?

A. Oxidation
B. Conjugation
C. Reduction
D. Hydrolysis

---

33. CYP450 contains:

A. Copper
B. Iron
C. Zinc
D. Cobalt

---

34. Mixed-function oxidase refers to:

A. Hydrolysis enzyme
B. CYP450 system
C. Transferase
D. Peptidase

---

35. Rifampicin is a:

A. CYP inhibitor
B. CYP inducer
C. Transferase inhibitor
D. Sulfotransferase inhibitor

---

36. Cimetidine is a:

A. CYP inducer
B. CYP inhibitor
C. Conjugating enzyme
D. Transporter

---

37. Bilirubin conjugation is an example of:

A. Oxidation
B. Reduction
C. Glucuronidation
D. Acetylation

---

38. Which phase is primarily responsible for water solubility?

A. Phase I
B. Phase II
C. Phase III
D. None

---

39. Reactive oxygen species are detoxified by:

A. Glucuronidation
B. Glutathione conjugation
C. Acetylation
D. Methylation

---

40. Which compound undergoes hydrolysis?

A. Aspirin
B. Glucose
C. Cholesterol
D. Thyroxine

---

41. Phase I reactions may:

A. Inactivate drug
B. Activate prodrug
C. Produce toxic intermediates
D. All of the above

---

42. Which enzyme system uses NADPH?

A. Transferrin
B. CYP450
C. Pepsin
D. Renin

---

43. Which phase occurs after conjugation?

A. Phase I
B. Phase II
C. Phase III
D. Hydrolysis

---

44. Drug resistance in cancer is associated with:

A. Increased CYP450
B. P-glycoprotein overexpression
C. Increased acetylation
D. Decreased glucuronidation

---

45. Acetylation occurs mainly in:

A. Kidney
B. Liver
C. Heart
D. Muscle

---

46. Which reaction type is most common overall?

A. Reduction
B. Hydrolysis
C. Oxidation
D. Methylation

---

47. Phase I increases drug:

A. Lipid solubility
B. Hydrophobicity
C. Reactivity
D. Molecular weight drastically

---

48. Glutathione-S-transferase participates in:

A. Phase I
B. Phase II
C. Phase III
D. Digestion

---

49. Which phase is directly linked to ATP-binding cassette transporters?

A. Phase I
B. Phase II
C. Phase III
D. Hydrolysis

---

50. The ultimate goal of detoxification is:

A. Drug activation
B. Increase lipid solubility
C. Facilitate excretion
D. Produce ROS

ANSWER KEY

1. B
2. C
3. B
4. B
5. C
6. B
7. B
8. B
9. B
10. B
11. B
12. B

13. C

14. B

15. B

16. A

17. B

18. A

19. B

20. A

21. B

22. C

23. C

24. C

25. C

26. B

27. A

28. C

29. B

30. B

31. C

32. B

33. B

34. B

35. B

36. B

37. C

38. B

39. B

40. A

41. D

42. B

43. C

44. B

45. B

46. C

47. C

48. B

49. C

50. C

GENERAL CONCEPT

Q1. What are xenobiotics?
Foreign chemical substances not normally produced in the body.

Q2. What is biotransformation?
Chemical modification of xenobiotics to increase water solubility for excretion.

Q3. Where does most biotransformation occur?
Liver.

Q4. Which cellular organelle is mainly involved?
Smooth endoplasmic reticulum.

Q5. What are the three phases of drug metabolism?
Phase I – Functionalization
Phase II – Conjugation
Phase III – Transport and excretion

PHASE I REACTIONS

Q6. What is the purpose of Phase I reactions?
To introduce or expose functional groups.

Q7. Name the major enzyme system in Phase I.
Cytochrome P450.

Q8. Where is Cytochrome P450 located?
Smooth ER of hepatocytes.

Q9. What cofactors are required for CYP450 reactions?
NADPH and oxygen.

Q10. Write the general oxidation reaction.
RH + O? + NADPH ? ROH + H?O.

Q11. What types of reactions occur in Phase I?
Oxidation, reduction, hydrolysis.

Q12. Which is the most common Phase I reaction?
Oxidation.

Q13. Can Phase I reactions produce toxic metabolites?
Yes.

OXIDATIVE REACTIONS

Q14. What is mixed-function oxidase?
Another name for the CYP450 system.

Q15. What metal is present in Cytochrome P450?
Iron (heme).

Q16. What is enzyme induction?
Increased synthesis of CYP450 enzymes.

Q17. Name one CYP450 inducer.
Rifampicin.

Q18. Name one CYP450 inhibitor.
Cimetidine.

Q19. What is the clinical effect of enzyme inhibition?
Increased plasma drug concentration.

REDUCTIVE REACTIONS

Q20. When do reductive reactions occur commonly?
Under low oxygen conditions.

Q21. Give one example of a compound undergoing reduction.
Azo compounds.

HYDROLYSIS

Q22. What bonds are broken during hydrolysis?
Ester and amide bonds.

Q23. Name enzymes involved in hydrolysis.
Esterases and amidases.

Q24. Give one example of hydrolysis reaction.
Aspirin ? Salicylic acid.

PHASE II REACTIONS

Q25. What is the purpose of Phase II reactions?
Conjugation to increase water solubility.

Q26. Which Phase II reaction is most common?
Glucuronidation.

Q27. Which enzyme catalyzes glucuronidation?
UDP-glucuronyl transferase.

Q28. Why are neonates prone to kernicterus?
Low glucuronyl transferase activity.

Q29. What is acetylation?
Addition of an acetyl group via N-acetyl transferase.

Q30. What is unique about acetylation?
Genetic polymorphism (slow and fast acetylators).

Q31. Name one drug metabolized by acetylation.
Isoniazid.

Q32. What is glutathione conjugation?
Detoxification of reactive intermediates by glutathione.

Q33. Which enzyme catalyzes glutathione conjugation?
Glutathione-S-transferase.

CLINICAL CORRELATION

Q34. What is the toxic metabolite of paracetamol?
NAPQI.

Q35. How is NAPQI detoxified?
By glutathione conjugation.

Q36. What happens in paracetamol overdose?
Glutathione depletion ? liver necrosis.

PHASE III REACTIONS

Q37. What is Phase III reaction?
ATP-dependent transport of metabolites out of cells.

Q38. Name one Phase III transporter.
P-glycoprotein.

Q39. Where is P-glycoprotein found?
Liver, intestine, kidney.

Q40. What is its function?
Efflux of drugs into bile or urine.

Q41. What clinical condition is associated with P-glycoprotein overexpression?
Multidrug resistance in cancer.

INTEGRATED THINKING QUESTIONS

Q42. Which phase increases drug polarity slightly?
Phase I.

Q43. Which phase increases water solubility markedly?
Phase II.

Q44. Which phase requires ATP?
Phase III.

Q45. Can biotransformation ever activate a drug?
Yes, in case of prodrugs.

Q46. What happens to lipid-soluble drugs without biotransformation?
They accumulate in tissues.

Q47. Which phase shows genetic variability commonly tested in exams?
Acetylation in Phase II.

Q48. Why is liver disease clinically important in drug metabolism?
Reduced biotransformation ? drug toxicity.

Q49. Which antioxidant is crucial in detoxification?
Glutathione.

Q50. What is the ultimate aim of detoxification?
To facilitate elimination of xenobiotics.