COURSE TITLE: Enzymes and Intermediary Metabolism **COURSE CODE: BCM 218**

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Course Outline

Anabolism, catabolism & meta bolism…**meaning**

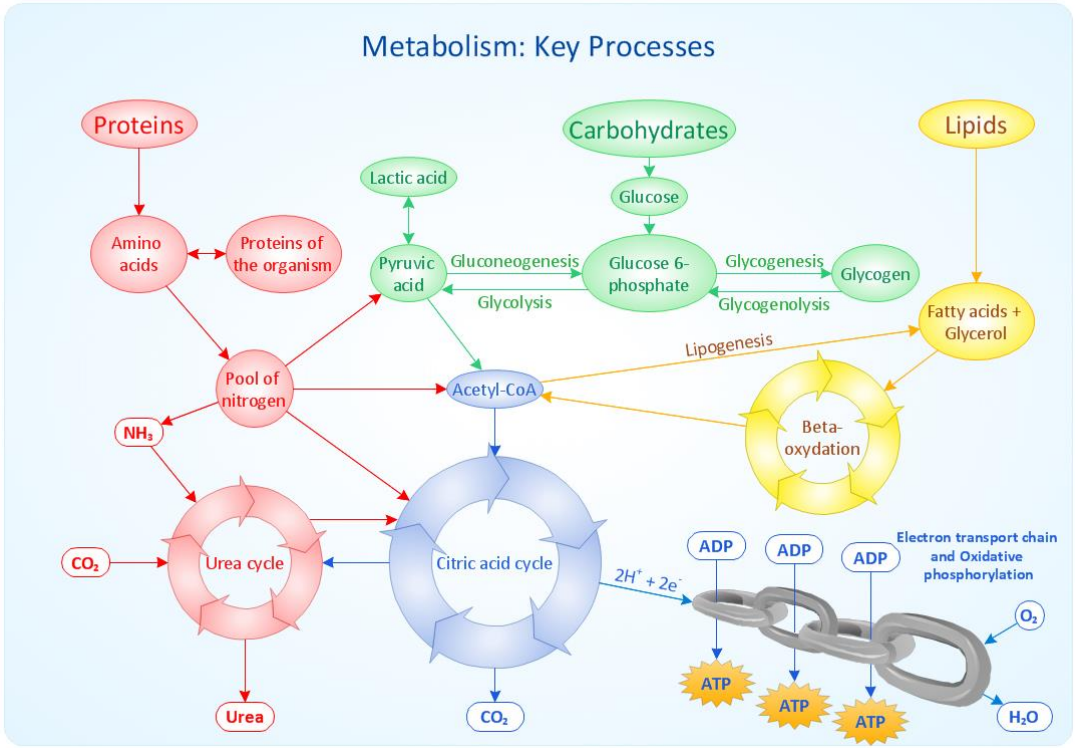
Short revision on fatty acids

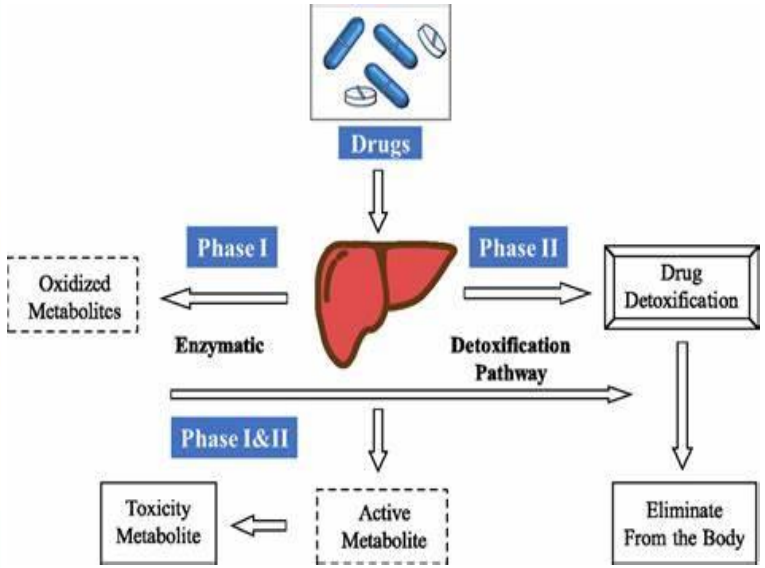
Synthesis of fatty acids

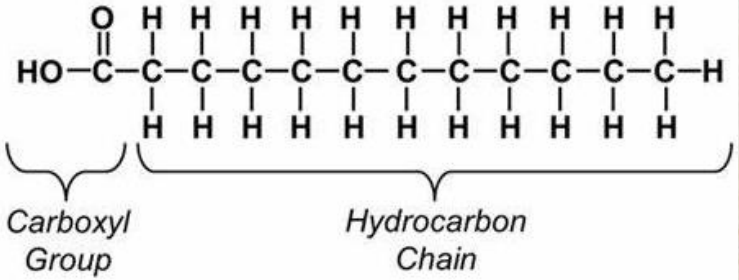
Oxidation of fatty acids (β-oxidation)

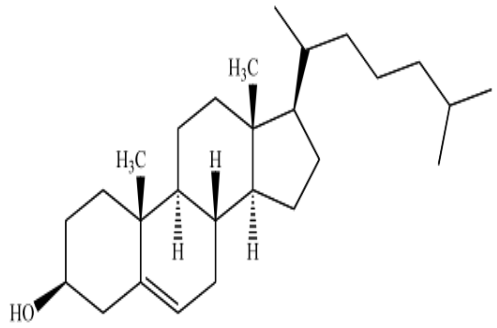
Cholesterol metabolism and regulation

Drug metabolism





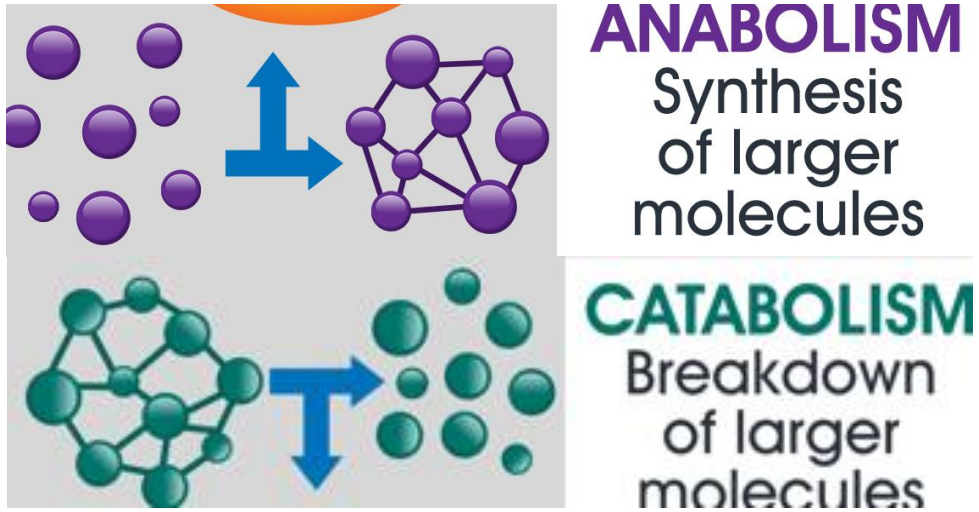




Metabolism in the simplest definition, is a sum-total of anabolism and catabolism, most of in-which the roles of enzymes cannot be over-emphasized.

Anabolism is a sequence of enzymatic-catalyzed reactions that involve the biosynthesis (production, formation or building up) of large organic molecules from simpler ones. E.g. Fatty acid synthesis, cholesterol synthesis, ketogenesis, protein synthesis, gluconeogenesis, glycogenesis, etc.

Catabolism is a sequence of enzymatic-catalyzed reactions that involves the breakdown of large organic molecules into simpler ones. E.g. β-oxidation (fatty acid oxidation), glycolysis, glycogenolysis, etc.



Fatty acids (F.As)

- F.As are components that have a carboxylic group as their only functional group, and occur in/or as lipid. They are chemically incorporated into other lipids such as TAG and phospholipids. Based on the type of bonds, F.As are classified as:
- Saturated F.As (SFAs) which are the F.As with C-C only. E.g. lauric acid, myristic acid, palmitic acid, e.t.c.

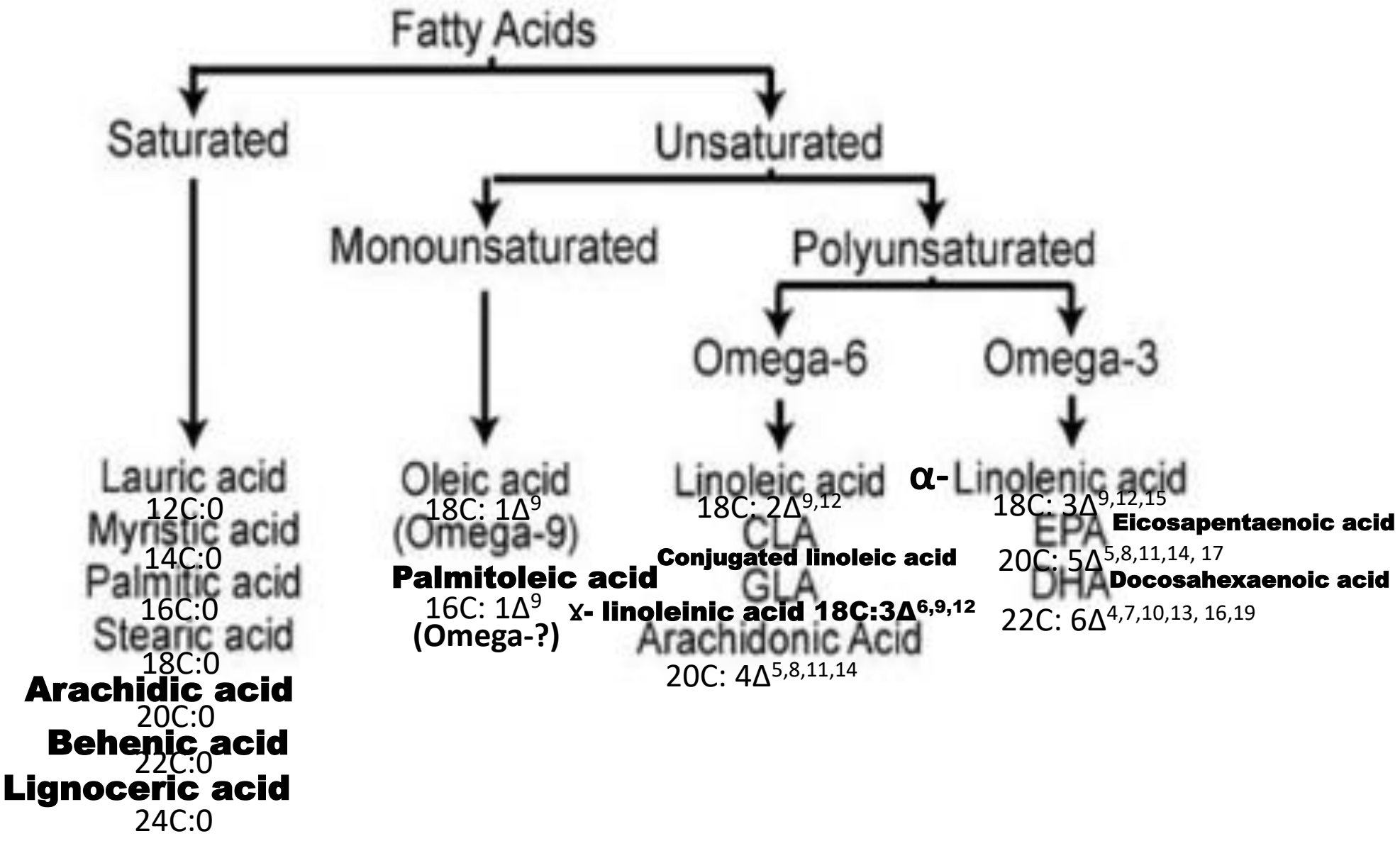
Animal fats are rich in SFAs

- Unsaturated F.As (UFAs) which are the F.As with C=C/triple bonds.

Plant oils are rich in UFAs

- UFAs are further classified into monoUFAs (MUFAs) & polyUFAs (PUFAs) based on number of the double/triple bonds.
- MUFAs contain one C=C, and may include palmitoleic acid & oleic acid.
- PUFAs contain more than one C=C, and may include linoleic acid, α-linoleinic acid, arachidonic acid, ecosapentaenoic acid, & docosahexaenoic acid.

Fatty Acids


Fatty Acids
Saturated
Unsaturated
Monounsaturated
Polyunsaturated
Omega-6
Omega-3
Lauric acid
12C:0
Myristic acid
14C:0
Palmitic acid
16C:0
Stearic acid
18C:0
Arachidic acid
20C:0
Behenic acid
22C:0
Lignoceric acid
24C:0
Oleic acid
18C: 1Δ9
(Omega-9)
Palmitoleic acid
16C: 1Δ9
(Omega-?)
Linoleic acid
18C: 2Δ9,12
CLA
Conjugated linoleic acid
GLA
ɣ- linoleinic acid 18C:3Δ6,9,12
Arachidonic Acid
20C: 4Δ5,8,11,14
α- Linolenic acid
18C: 3Δ9,12,15
EPA
Eicosapentaenoic acid
20C: 5Δ5,8,11,14, 17
DHA
Docosahexaenoic acid
22C: 6Δ4,7,10,13, 16,19

Fatty acids (F.As)

However, based on their nutritional value, F.As are classified as:

- Essential F.As which are the F.As that cannot be synthesized by the animal body, hence, should be obtained from diets (plant origin).

Examples of the essential F.As the PUFAs

- Non-essential F.As are the ones that can be synthesized by the animal body. E.g. All the SFAs including the MUFAs.

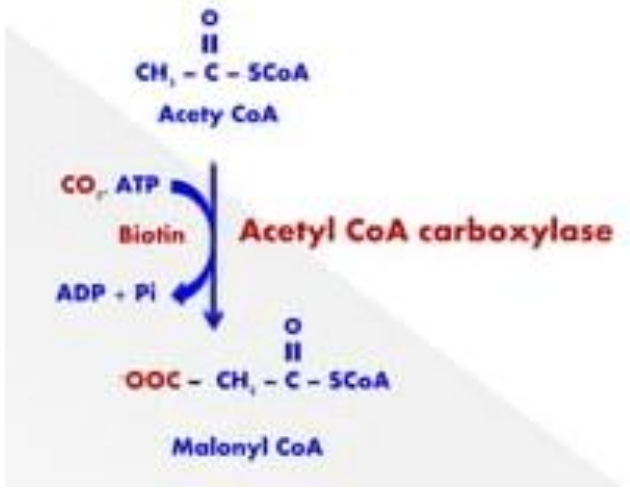
NOTE: Aside the fact that we obtain fatty acids from diets, our body can synthesize (produce) some of these fatty acids.

Now, we want to consider how fatty acids are synthesized in the body in the next slide

Biosynthesis of fatty acids

- Fatty acid synthesis is a biochemical process that involves the production of fatty acids in a living system, usually, from acetyl-CoA.
- Fatty acid synthesis primarily occurs in the cytosol of liver cells, though also occurs in cells of the mammary glands, brain, and kidneys.
- The steps involved in fatty acid synthesis include:

i. Acetyl-CoA Carboxylation: Acetyl-CoA is carboxylated to form malonyl-CoA. This reaction is an energy-driving reaction, catalyzed by an enzyme complex called, 'acetyl-CoA carboxylase', which requires biotin as a cofactor, and bicarbonate as the donor of the carboxyl group.



Fatty acids synthesis

ii. Condensation: Acetyl-CoA condenses with malonyl-CoA, giving off the CO_2, and forming acetoacetyl-CoA.

iii. Reduction: The acetoacetyl-CoA undergoes reduction by NADPH, leading to the formation of a 3-hydroxybutanoyl-CoA.

iv. Dehydration: The hydroxyl group on the newly formed chain is removed, forming a double bond between carbons, yielding crotonyl-CoA.

v. Reduction: Another NADPH-dependent reduction converts the double bond back to a single bond, yielding a fully saturated acyl chain extended by two carbons in between the chain (end of the first round of chain elongation).

vi. Repeat Cycle: This cycle of condensation, reduction, dehydration, and reduction continues repeatedly, extending the fatty acyl chain by two carbons on each round.

vii. Termination and Release: The fatty acid chain is released from the fatty acid synthase complex once it reaches a certain length, typically palmitic acid (a 16-carbon saturated fatty acid).

NOTE: The role of acyl-carrier protein (ACP) cannot be overemphasized in the synthesis of fatty acids

Fatty acids synthesis

- The newly synthesized fatty acids can undergo further modifications such as desaturation (introduction of double bonds) and elongation in the endoplasmic reticulum to produce a diverse array of fatty acids with varying chain lengths and degrees of unsaturation.

Regulation of fatty acid synthesis

The synthesis of fatty acid is often controlled by hormone, diets, and some cellular signals, to meet the body's energy and structural need.

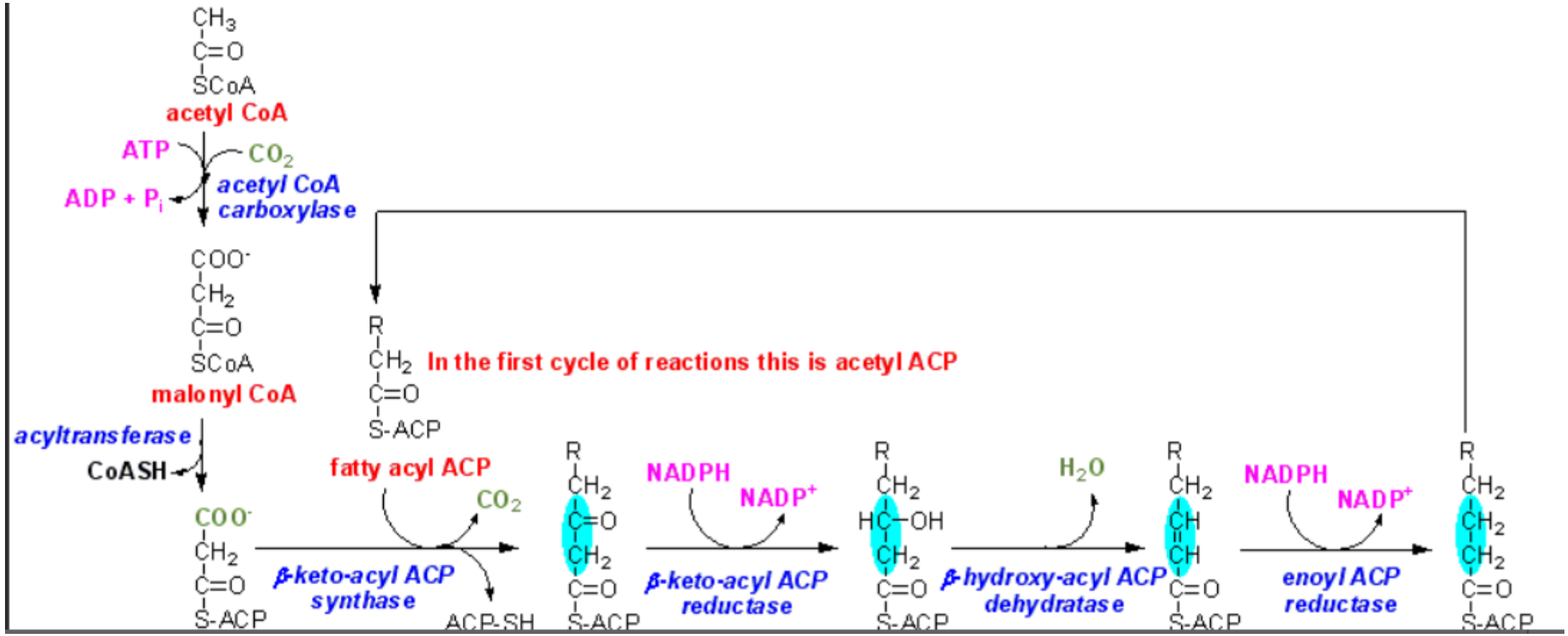
For examples:

✓ Production of insulin stimulates fatty acid synthesis

✓ Production of glucagon inhibits fatty acid synthesis

✓ Too much presence of sugar/carbohydrates in the body stimulates fatty acid synthesis

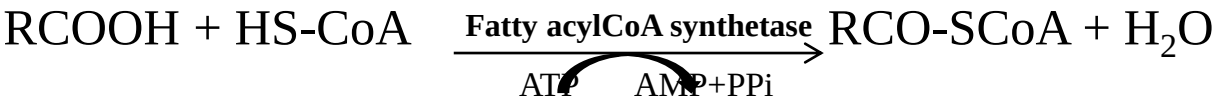
✓ The fatty acid synthase can be overexpressed by alcohol, certain drugs, and some diseases like breast cancers.


acetyl CoA
ATP
CO2
ADP + Pi
acetyl CoA carboxylase
malonyl CoA
acyltransferase
CoASH
In the first cycle of reactions this is acetyl ACP
fatty acyl ACP
CO2
ACP-SH
β-keto-acyl ACP synthase
NADPH
NADP+
β-keto-acyl ACP reductase
H2O
β-hydroxy-acyl ACP dehydratase
NADPH
NADP+
enoyl ACP reductase

Oxidation of fatty acid (β-oxidation)

- Oxidation of fatty acid (β-oxidation) is a breakdown process of fats (e.g. fatty acids) to release energy.

- Prior β-oxidation, the free fatty acids in blood are mobilized or carried by albumin, from the blood into cytosol of heart & skeletal muscle cells.
- The fatty acids in the cytosol are then activated by esterifying with co-enzyme A (HS-CoA), to form fatty acyl CoA or acylCoA, a process catalyzed by fatty acylCoA synthetase (fatty acid thiokinase).



Activation of fatty acid in the cytosol

- Acyl group from the activated fatty acids (fatty acylCoA) is transferred unto a protein called carnithine (which can cross the outer mitochondrion membrane), catalyzed by CAT-1 (translocase-1).
- After which it is conveyed into the inner motochondion membrane, catalyzed by CAT-2 (translocase-2). The carnithine now returns to the cytosol.
- β-oxidation occurs in the inner membrane of the mitochondrion (in eucaryotes) but in the cytosol of procaryotes.

β-oxidation

- **By definition**, β-oxidation is the breakdown of fatty acids (F.A) by a sequential removal of two carbon atoms from the F.A, cleaving it from the point of β-C attachment, to generate acetyl-CoA, $FADH_2$ and NADH.
- β-oxidation shortens the fatty acyl (activated F.A) chain by two C-atoms.
- β-oxidation occurs in a recurring sequence of four reactions, namely:

✓ Oxidation by FAD

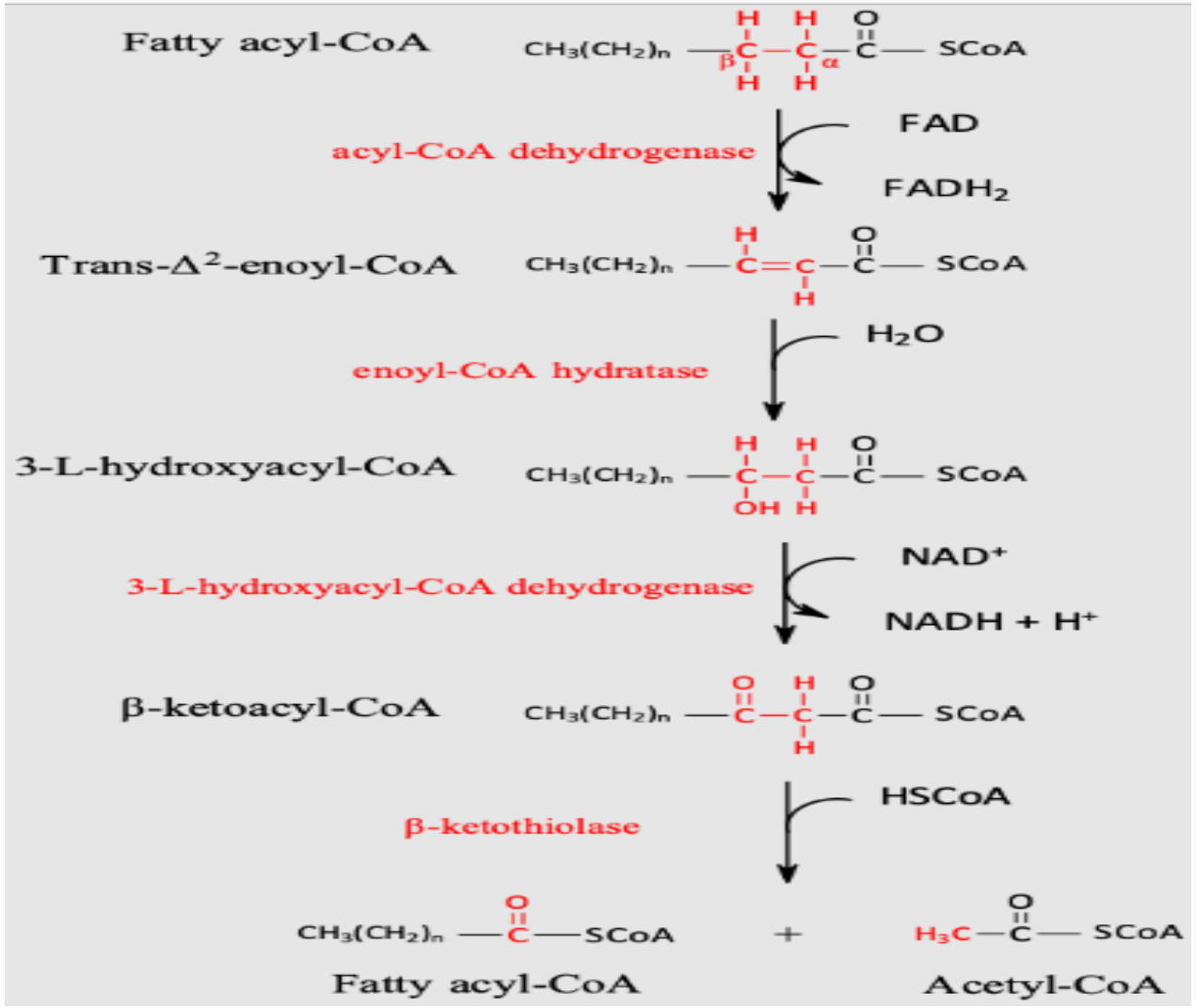
✓ Hydration

✓ Oxidation by NAD^+

✓ Thiolysis by Co-enzyme A

- The acetyl-CoA produced is further oxidized to 1$FADH_2$, 3NADH and 1GTP via the Kreb cycle (TCA or CAC).
- Via ETC & oxidative phosphorylation, 1$FADH_2$ is oxidized to 2 or 1.5ATP, 1NADH to 3 or 2.5ATP & 1GTP to 1ATP.

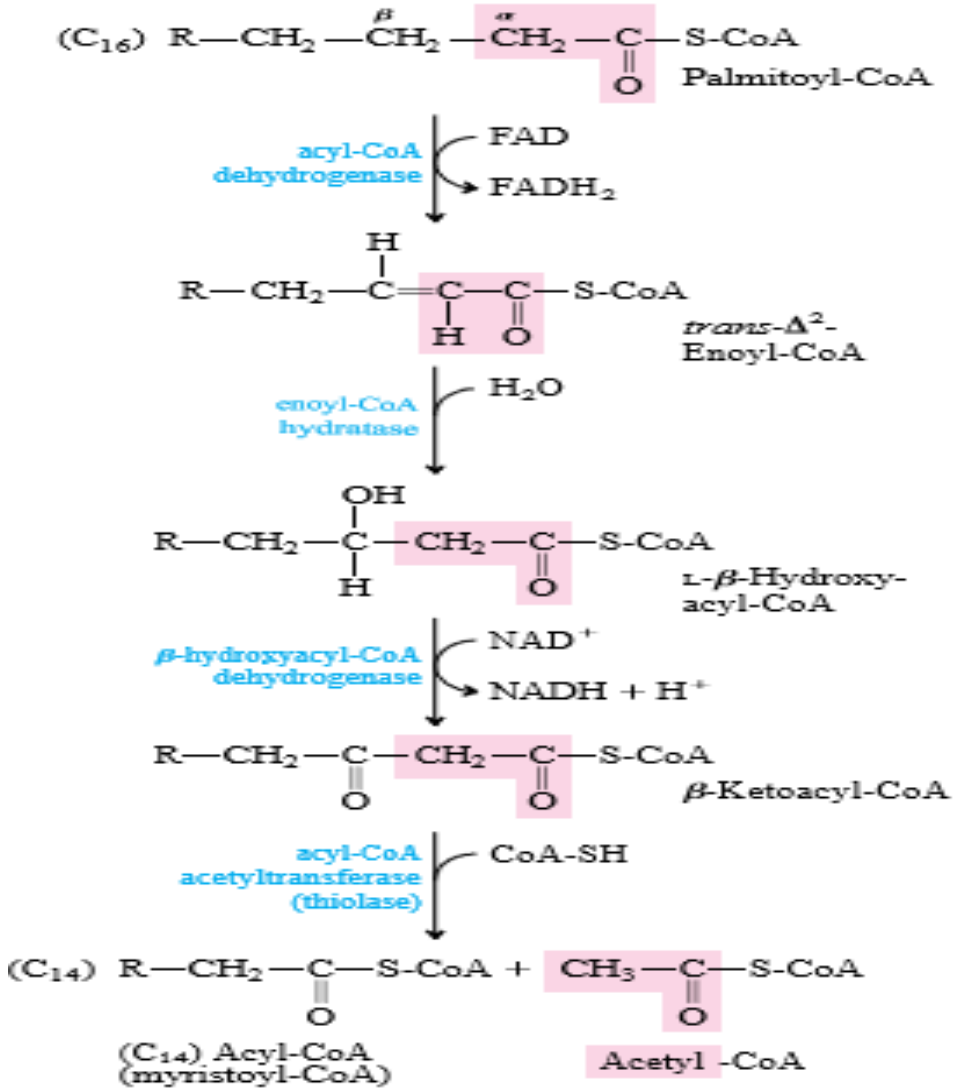
β-oxidation of F.As with even number of C



Overall Reaction:

Fatty acylCoA + xFAD + xH_2O + xNAD^+ + xHS-CoA ⟶ x$FADH_2$ + xNADH + xH^+ + x$CH_3COSCoA$

β-oxidation



Assignment 1

Show:
i. Activation of stearic acid in cytosol.
ii. β-oxidation of stearic acid.
iii. The overall β-oxidation reaction for stearic acid in the inner mitochondrial membrane.

NOTE: To be submitted on …

Overall Reaction:

$PalmitoylCoA + 7FAD + 7H_2O + 7NAD^+ + 7HS\text{-}CoA \longrightarrow 7FADH_2 + 7NADH + 7H^+ + 8CH_3COSCoA$

Recall: The acetyl-CoA produced is further oxidized to $1FADH_2$, 3NADH and 1GTP via the Kreb cycle (TCA or CAC). Via ETC & oxidative phosphorylation, $1FADH_2$ is oxidized to 2 or 1.5ATP, 1NADH to 3 or 2.5ATP; & 1GTP to 1ATP.

Energy Generated During β-oxidation

Recall: The acetyl-CoA produced is further oxidized to 1$FADH_2$, 3NADH and 1GTP via the Kreb cycle (TCA or CAC). Via ETC & oxidative phosphorylation, 1$FADH_2$ is oxidized to 2 or 1.5ATP, 1NADH to 3 or 2.5ATP; & 1GTP to 1ATP.

Thus,

3NADH × 2.5 or 3 = 7.5 or 9ATP

1$FADH_2$ × 1.5 or 2 = 1.5 or 2ATP

1GTP × 1 = 1ATP

Therefore, the total ATP generated by the complete oxidation of 1 acetylCoA = 10 or 12 ATP.

Assignment 2

Complete the table below:

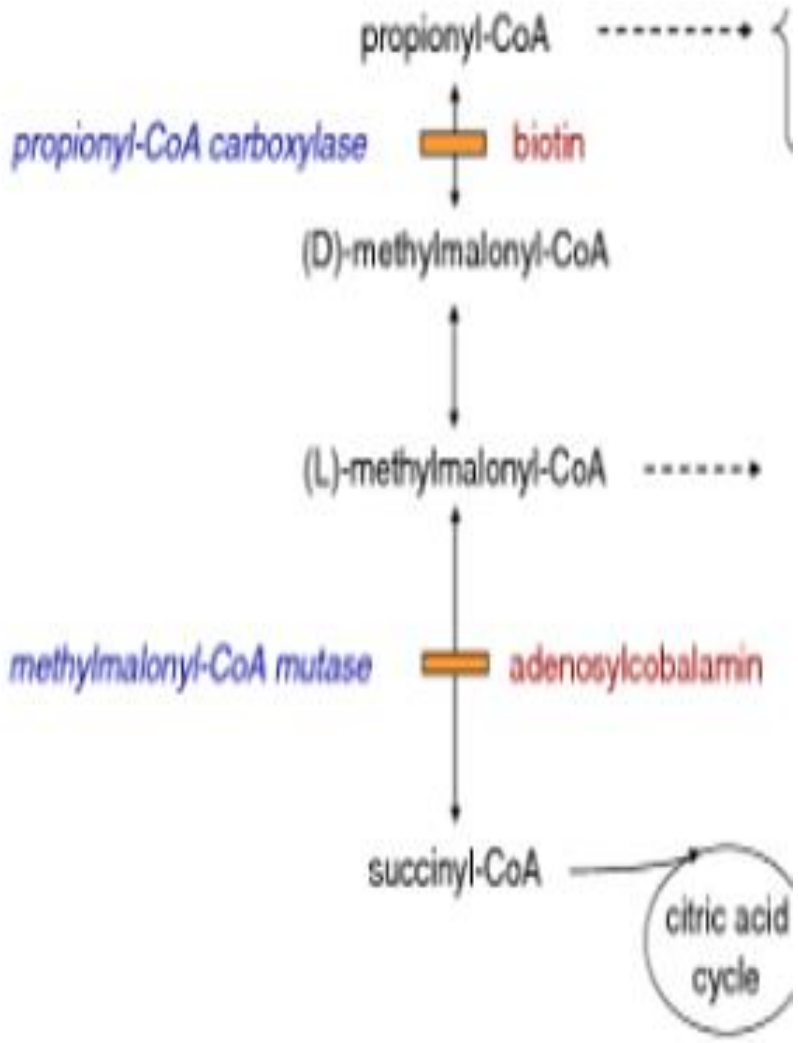
No of Carbon	Common Name	Round of β-oxidation & No of $FADH_2$ and NADH	No of AcetylCo-A	Total ATP generated at complete oxidation
12	Lauric acid		6	----------
14	Myristic acid	6		----------
16	Palmitic acid		8	----------
18	Stearic acid	8		----------
20	Arachidic acid		10	----------
22	Behenic acid	10		----------
24	Lignoceric acid		12	----------

β-oxidation (of F.As with an odd number of C)

- β-oxidation of F.As with an odd number of carbon atoms undergoes similar processes with that of even number but at its final thiolysis stage, yields a propionyl-CoA and 1 acetylCoA.
- The propionyl-CoA is thereafter carboxylated to D-methylmalonylCoA, by propionylCoA carboxylase.
- D-methylmalonylCoA is epimerized to L-methylmalonylCoA, by D-methylmalonylCoA epimerase.
- L-methylmalonylCoA is then racemerized to succinyl-CoA, by L-methylmalonylCoA mutase.
- The process above utilizes 1 molecule of ATP.
- The succinylCoA continues as an intermediate in Kreb's cycle.

NOTE: Unsaturated F.As also undergo β-oxidation but with the addition of different processes to that of the SFAs own.

Continuation of β-oxidation (of F.As with an odd number of C)



Assignment 3

Show:

i. Activation of pentadecanoic acid acid in cytosol.

ii. β-oxidation of pentadecanoic acid

iii. The overall β-oxidation reaction for pentadecanoic acid in the inner mitochondrial membrane.

iv. Amount of ATP that will be possiblly generated from pentadecanoic acid

NOTE:To be submitted on ….

Cholesterol synthesis

- All animal cells manufacture cholesterol for their use, with relative production rates varying by cell type and organ function.

- Cholesterol is a sterol lipid that is vital for various cellular functions, including the formation of cell membranes, synthesis of certain hormones, and production of bile acids.

- About 20–25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs.

Biosynthesis of Cholesterol

- The synthesis of cholesterol involves a series of enzymatic reactions, and the process can be broadly divided into several stages:

1. **Formation of acetoacetyl-CoA**

Cholesterol biosynthesis begins with the condensation of two acetyl-CoA (a two-carbon compound) into acetoacetyl-CoA, catalyzed by thiolase.

2. **Formation of HMG-CoA**

An acetyl group is transferred unto the acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). This reaction is catalyzed by the enzyme acetyl-CoA acetyltransferase or HMG-CoA synthase.

3. **Reduction of HMG-CoA to mevalonate**

HMG-CoA is reduced to mevalonate in a two-step reaction. The enzyme responsible for this conversion is HMG-CoA reductase. This is a critical regulatory step, and the activity of HMG-CoA reductase is tightly controlled, as it is the target of cholesterol-lowering drugs known as statins.

Biosynthesis of Cholesterol

4. **Conversion of mevalonate to isopentenyl pyrophosphate (IPP)**

Mevalonate undergoes a series of phosphorylation and decarboxylation reactions to produce isopentenyl pyrophosphate (IPP). This is a five-carbon compound and a building block for the synthesis of larger isoprenoid compounds.

5. **Formation of squalene**

Six IPP molecules are then combined together through a series of three reactions to form a 30-carbon linear structure known as squalene. This reaction is catalyzed by squalene synthase.

6. **Cyclization of squalene to form lanosterol**

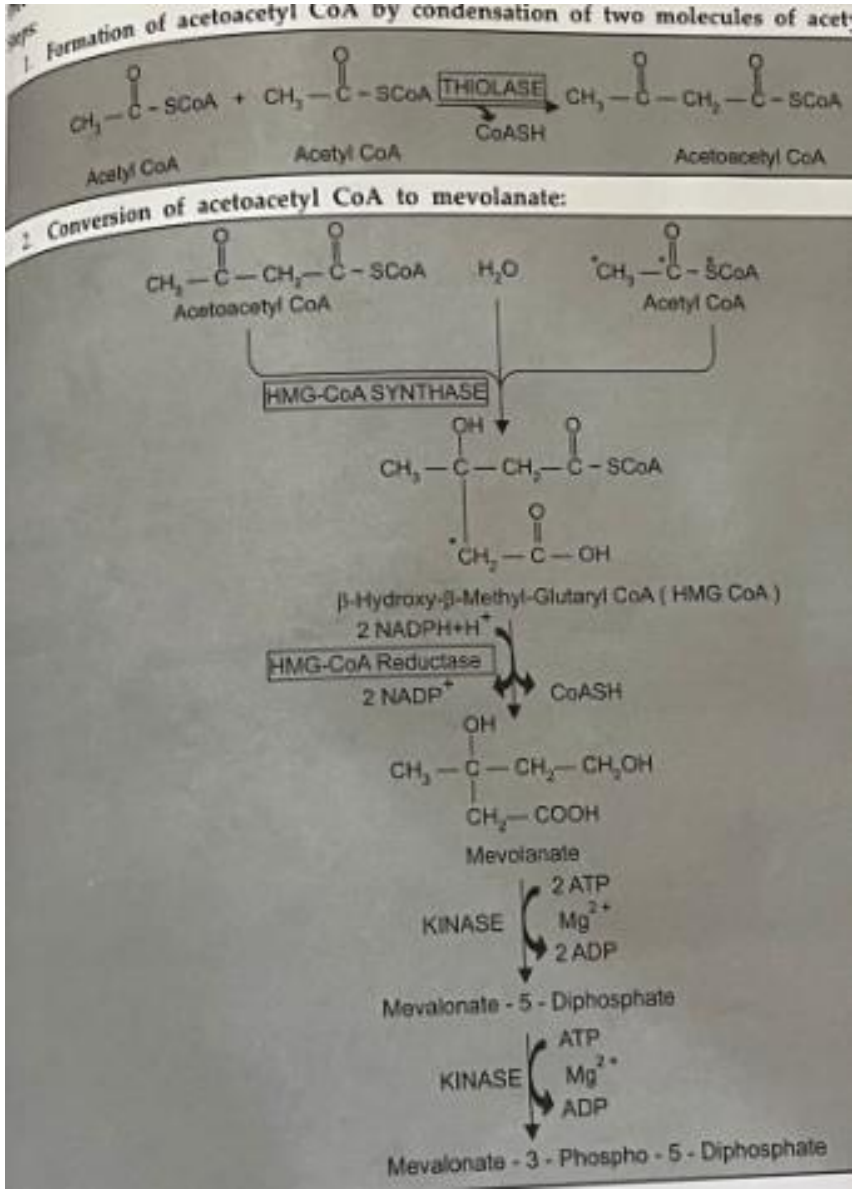
Squalene is then cyclized to form lanosterol, a tetracyclic structure. This process involves the action of several enzymes, including squalene epoxidase.

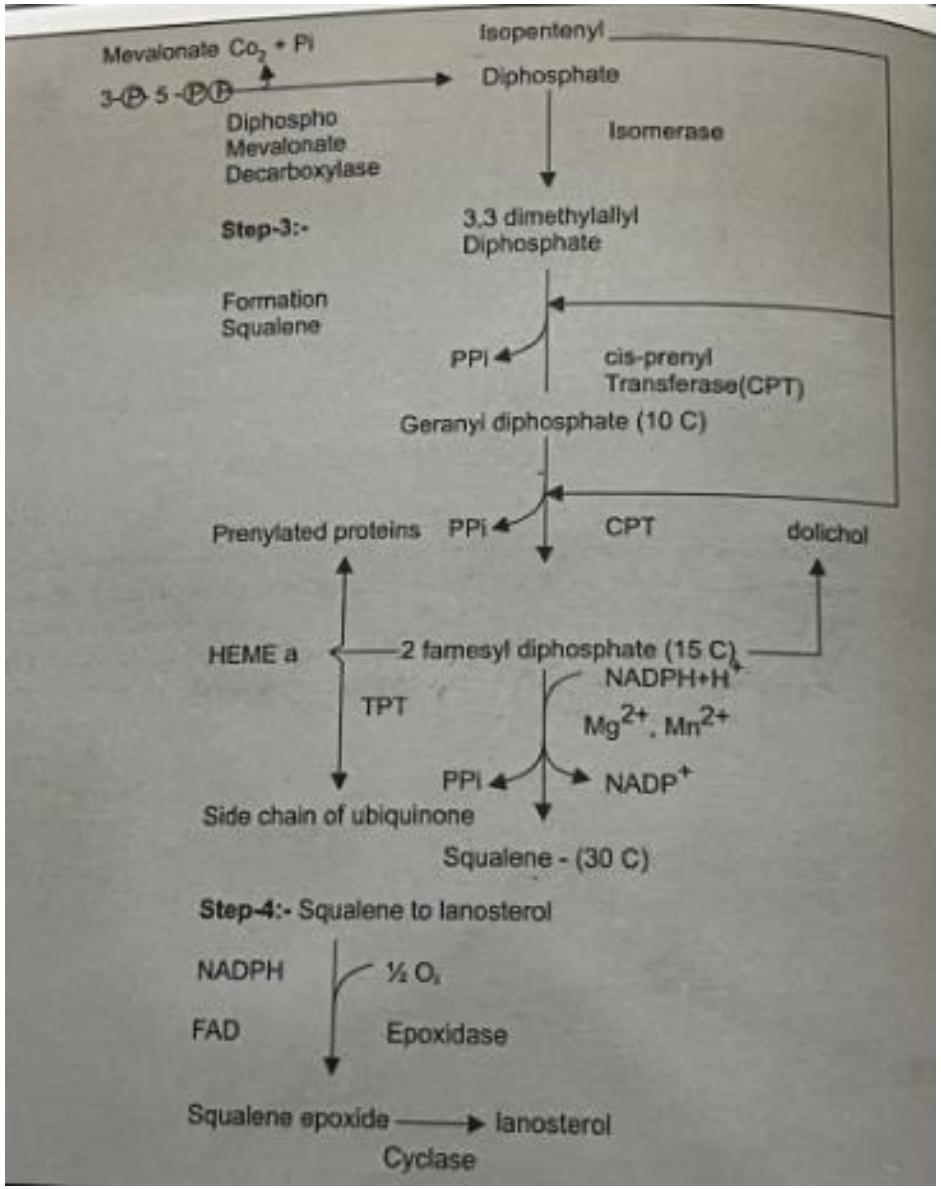
7. **Conversion of lanosterol to cholesterol**

Lanosterol is then converted into cholesterol through a series of complex reactions involving various enzymes, including lanosterol 14α-demethylase and other cytochrome P_{450} enzymes.

NOTE: Cholesterol biosynthesis is crucial because it serves as a key component of cell membranes and serves as a precursor for the synthesis of steroid hormones such as testosterone and progesterone, bile acids, and vitamin D.

Biosynthesis of Cholesterol





Pathway for cholesterol biosynthesis

Drug metabolism

- Drug metabolism, also known as drug biotransformation is the chemical modification of drugs with the overall goal of getting rid of the drug, leaving only the active ingredient to exert its effect.

- Enzymes are actively involved in the metabolism of drugs.

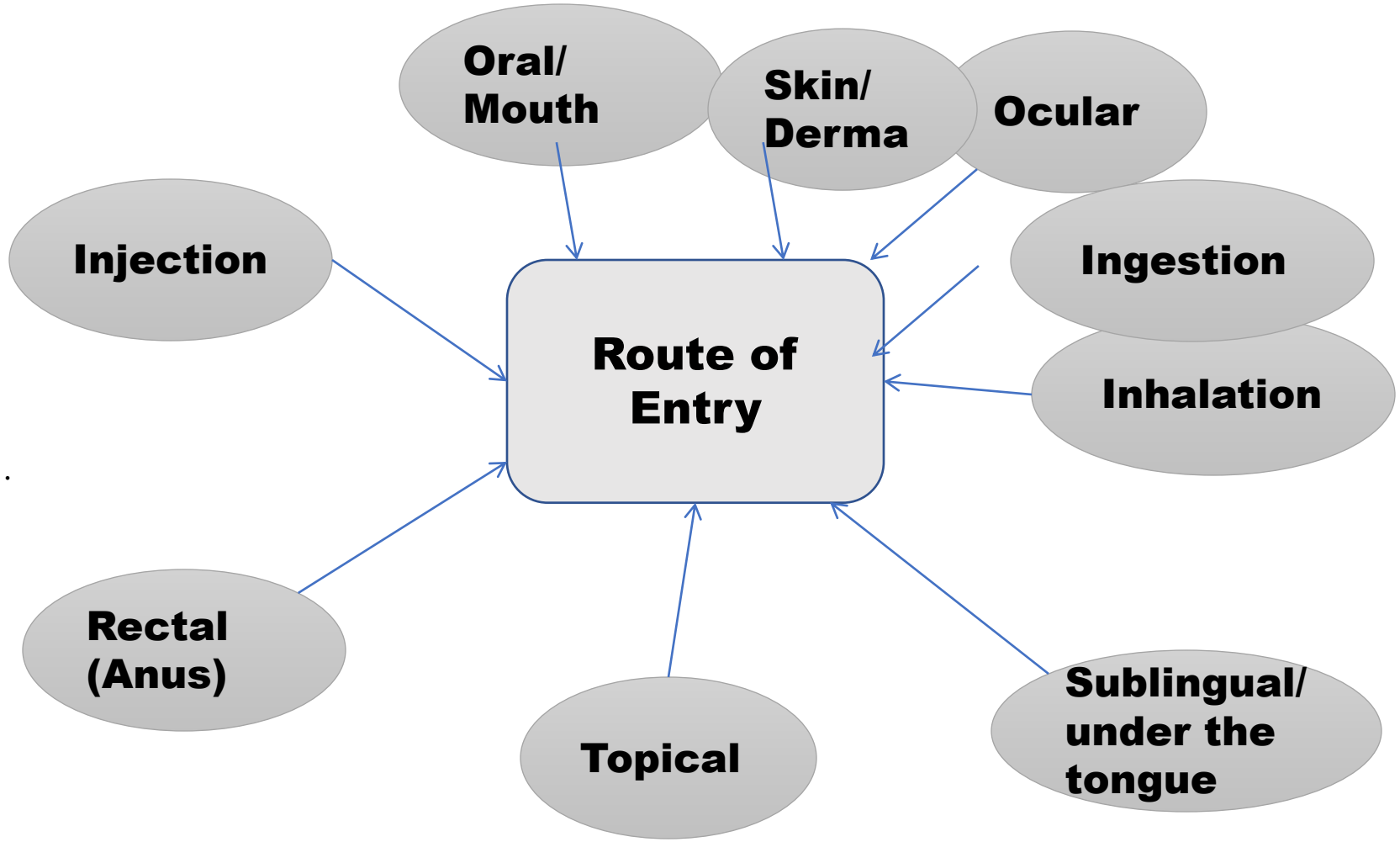
- Thus, drug metabolism is the enzyme-catalyzed chemical alterations of the drugs in the body.

What is a Drug?

A drug is a substance, be it natural, synthetic, exogenous or endogenous, that is used or intended to be used to modify a physiological process or a pathological state, for the benefits of the recipient.

...before considering the metabolism of drugs, lets consider the different routes by which drugs get through into our body

ROUTES OF ENTRY OF DRUGS



Routes of drug administration

For any drug to exert its pharmacological effects, it must reach its site of action, and this is by passing through the appropriate route of administration.

1- Topical/local route: drugs are applied and produce their actions at the site of application.

E.g. as eye/ear drops, powder, cream, shea butter, face mask lotion (on skin), medicinal eye pencil (tiro on conjuntiva).

Assignment: Are there any advantages and disadvantages associated with the topical/local route of administering drugs?

2-oral route: drugs are placed in the mouth and swallowed using water

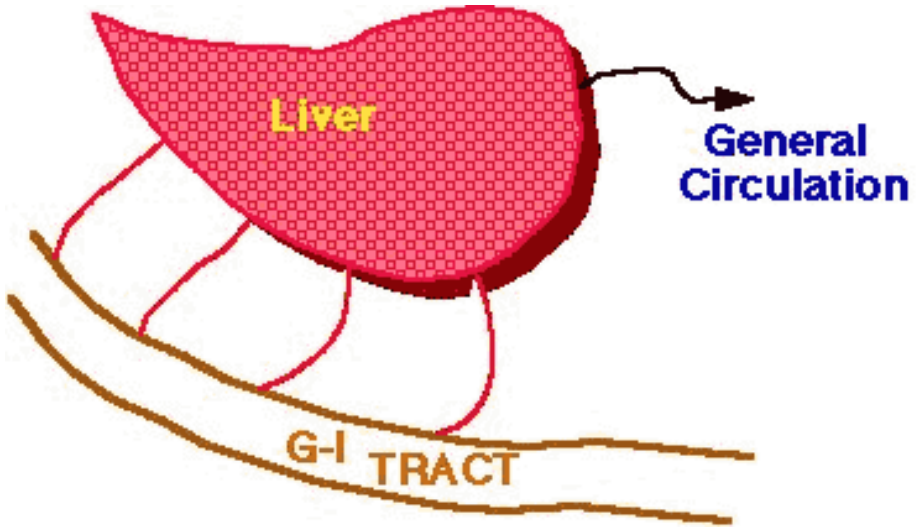
Advantages

- Easy to use (self-administered)
- Available in different dosage forms, e.g paracetamol (tablet, syrup)
- Easily spit out if not tolerated.

Disadvantages

- First-pass effect
- Cannot be used in an emergency or for unconscious patients
- The bitter, unpleasant & irritating ones cause nausea and vomiting
- Food-drug interaction, e.g. malaria drugs with vit.C, milk, fruit
- Staining of teeth, e.g. tetracyclin

First pass effect:



- The first pass effect is the term used for the liver metabolism of drug when it is absorbed from the gut and delivered to the liver via the portal circulation.

- The greater the first pass effect, the lower the bioavailability of the drug (the rate and extent of the drug reaching systemic circulation).

3- Buccal/Sublingual route:

- Some drugs are taken as smaller tablets which are held in the mouth **(buccal tablet)** or under the tongue **(sublingual tablet).**

- Buccal tablets are often harder tablets (about 4 hours disintegration time), designed to dissolve slowly. Though, there are softer sublingual tablets (about 2 min disintegration time), may be used for the rapid relief.

3- Buccal/Sublingual route (Cont.)

Advantages

1- Avoid hepatic first pass - The liver is by-passed thus there is no loss of drug by first pass effect.

2- Rapid absorption - Because of the good blood supply to the area, absorption is usually quite rapid.

3- Drug stability - pH in mouth relatively neutral (stomach - acidic). Thus a drug may be more stable.

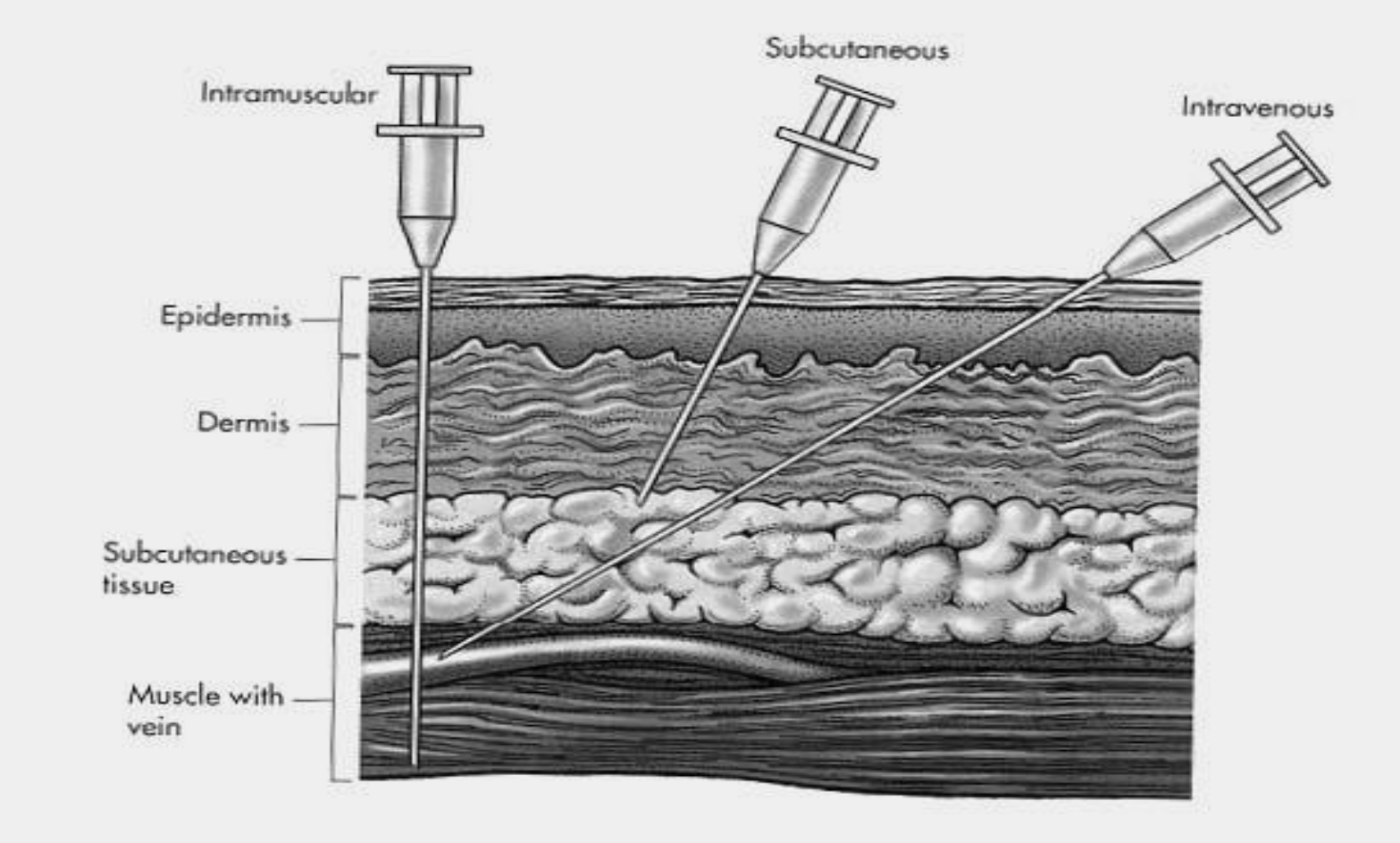
3- Buccal/Sublingual route (Cont.)

Disadvantages

1- Holding the dose in the mouth is inconvenient.

2- Small doses only can be accommodated easily.

4- Parenteral route:



4- Parenteral route (Cont.)

A- Intravascular (IV, IA):

- placing a drug directly into blood stream.

-May be - Intravenous (into a vein) or - intraarterial (into an artery).

B-Intramuscular:(into the skeletal muscle).

C- Subcutaneous: (under the skin), e.g. insulin.

D- Intradermal: (into the skin itself) is used for skin testing some allergens.

E- Intrathecal: (into the spinal canal) is most commonly used for spinal anesthesia .

F- Intraperitoneal: (infusion or injection into the peritoneum) e.g. peritoneal dialysis in case of renal insuffeciency.

Advantages of parenteral routes

- precise, accurate, and immediate onset of action
- 100% bioavailability.
- suitable for injection of drug in aqueous solution (rapid action) and drug in suspension or emulsion (sustained release).

Disadvantages of parenteral routes

1- risk of embolism/embolus lodges in the artery to obstruct the blood flow.

2- high concentrations attained rapidly leading to a greater risk of adverse effects.

3- Pains at the injection site for certain drugs

3- Expertise is needed.

5-Rectal route:

Most commonly by suppository/purgative/laxative or enema.

Advantages

1- By-pass liver - Some of the veins draining the rectum lead directly to the general circulation, thus by-passing the liver. Reduced first-pass effect.

2- Useful - This route may be most useful for patients unable to take drugs orally (unconscious patients) or with younger children, or if the patient is nauseous or vomiting.

5- Rectal route (Cont.)

Disadvantages

1- Erratic absorption - Absorption is often incomplete and erratic.

2- Not well accepted.

6- Inhalation route:

- Used for gaseous and volatile agents and aerosols.

- solids and liquids are excluded if larger than 20 micron. the particles impact in the mouth and throat.

Advantages

A- Large surface area

B- thin membranes separate alveoli from circulation

C- high blood flow

- **As result of that a rapid onset of action** due to rapid access to circulation.

6- Inhalation route (Cont.)

Disadvantages

1- Most addictive route of administration because it hits the brain so quickly.

2- Difficulties in regulating the exact amount of dosage.

3- Sometimes patient having difficulties in giving themselves a drug by inhaler.

Factors that determine the choice of route for administering drugs

1. Physical properties of the drug, e.g. is the drug solid, liquid or gas.
2. Condition of the patients, e.g. unconscious, vomiting, etc.
3. Rapidity of the desired response.
4. Site of desired action, e.g. systemic or contact action.
5. Effect of digestive juices and first-pass effect.
6. Accuracy of dosage.

Drug Metabolism

Metabolism of drugs makes drugs more polar, and enhances excretion of the drugs.

Three (3) possibilities occur when drug enters the body

(a) Some are excreted away unchanged: It depends on the volatility or polarity of the drug.

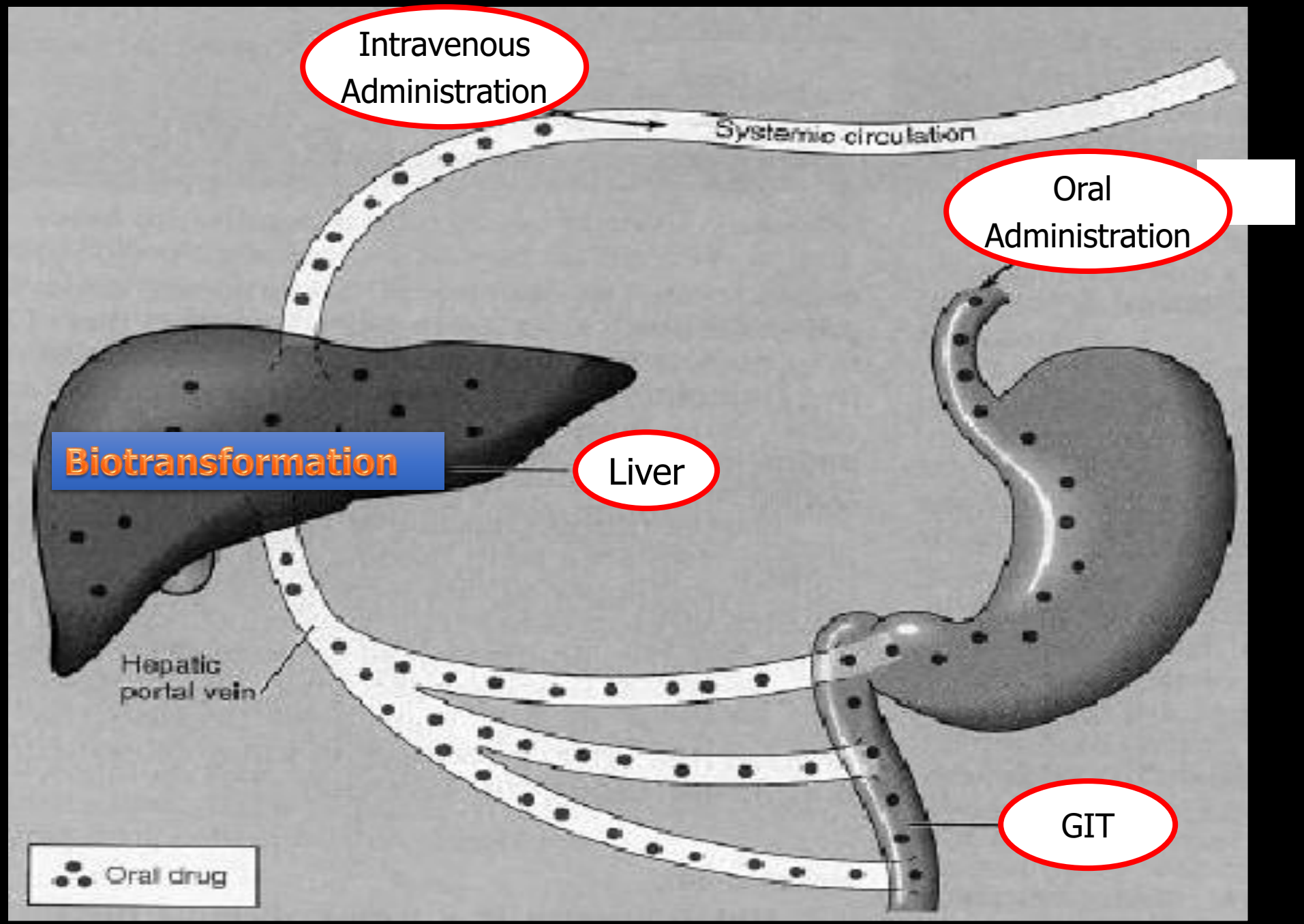
(b) Some undergo spontaneous reaction by their catalyst/enzymes e.g. activated or deactivated in the gut.

(c)Some are metabolized and excreted: 98% of drugs fall under this category.

Sites of Drug Metabolism

- Metabolism of drugs occurs in many tissues
- E.g. gastrointestinal tract (GIT), lungs, kidney, brain, liver, brain, and plasma.

…but mostly in the liver because all of the blood in the body passes through the liver.


Intravenous Administration
Systemic circulation
Oral Administration
Biotransformation
Liver
Hepatic portal vein
GIT
Oral drug

Phases of drug metabolism (Phase I & Phase II)

Drugs are metabolized via two phases

Enzyme	Phase I	Phase II
Types of reactions	Oxidation e.g. +/- O_2, H_2, H_2O Reduction Hydrolysis	Conjugations e.g. Glutathione conjugation
Increase in hydrophilicity	Small	Large
General mechanism	Exposes functional group	Polar compound added to functional group
Consquences	May result in metabolic activation	Facilitates excretion

Factors affecting drug metabolism

The factors affecting drug metabolism are generally classified into two:

1. Host factors
2. Intrinsic (chemical) factors

The hos factors include:

– Gender
– Age
– Diet and nutrition
– Enzyme induction and inhibition
– Hepatic injury
– Stress
– Plasma protein binding/carrier proteins

The intrinsic (chemical) factors include:

• Route of administration
• Dose or concentration of the drug
• First-pass effect
• Drug-to-drug interaction
• Solubility of the drug