METABOLISM OF LIPIDS - 3

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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METABOLISM OF PHOSPHOLIPIDS

Phospholipids are a specialized group of lipids performing a variety of functions. These include the membrane structure and functions, involvement in blood clotting, and supply of arachidonic acid for the synthesis of prostaglandins (for details ***Refer Chapter 32***).

Synthesis of phospholipids

Phospholipids are synthesized from phosphatidic acid and 1,2-diacylglycerol, intermediates in the production of triacylglycerols (***Fig.14.18***). Phospholipid synthesis occurs in the smooth endoplasmic reticulum.

1. **Formation of lecithin and cephalin :** Choline and ethanolamine first get phosphorylated and then combine with CTP to form, respectively, CDP-choline and CDP-ethanolamine (***Fig.14.19***).

Phosphatidylcholine (lecithin) is synthesized when CDP-choline combines with 1,2-diacylglycerol. Phosphatidyl ethanolamine (cephalin) is produced when CDP-ethanolamine reacts with 1,2-diacylglycerol. Phosphatidyl ethanolamine can be converted to phosphatidyl choline on methylation.

Choline and ethanolamine, used for phospholipid synthesis, are mostly derived from the preexisting phospholipids. Thus, the phospholipid synthesis starting with choline or ethanolamine is regarded as ***salvage pathway***.

2. **Synthesis of phosphatidylserine :** Phosphatidyl ethanolamine can exchange its ethanolamine group with free serine to produce phosphatidylserine. The latter, on decarboxylation, gives the former.

3. **Formation of phosphatidylinositol :** CDP-diacylglycerol produced from phosphatidic acid combines with inositol to form phosphatidyl inositol (PI). This phospholipid contains ***arachidonic acid*** on carbon 2 of glycerol which serves as a substrate for ***prostaglandin synthesis***. Further, PI is important for signal transmission across membranes.

4. **Synthesis of phosphatidyl glycerol and cardiolipin :** CDP-diacylglycerol combines with glycerol 3-phosphate to form phosphatidyl glycerol 3-phosphate, which then forms phosphatidylglycerol. The latter combines with another molecule of phosphatidylglycerol to finally produce cardiolipin (***Fig.14.19***). ***Cardiolipin*** is the only phospholipid possessing ***antigenic*** properties.

5. **Formation of plasmalogens :** These are phospholipids with fatty acid at carbon 1 bound by an ether linkage instead of ester linkage. An important plasmalogen, 1-alkenyl 2-acetyl glycerol 3-phosphocholine, causes blood platelet aggregation and is referred to as ***platelet-activating factor*** (PAF). The outline of the pathway for the synthesis of plasmalogens is depicted in ***Fig.14.20***.

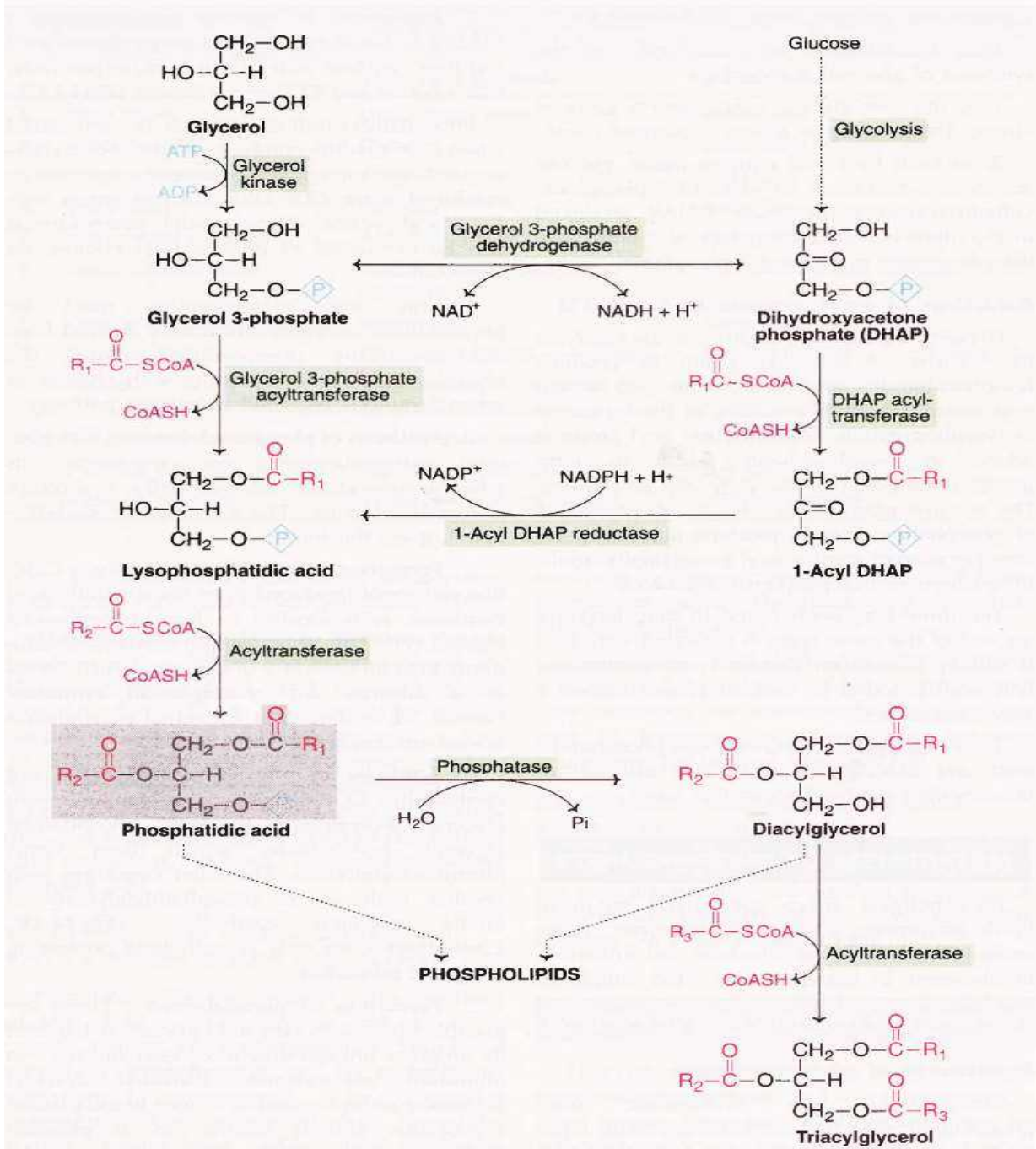


Fig. 14.18 : Synthesis of triacylglycerol.

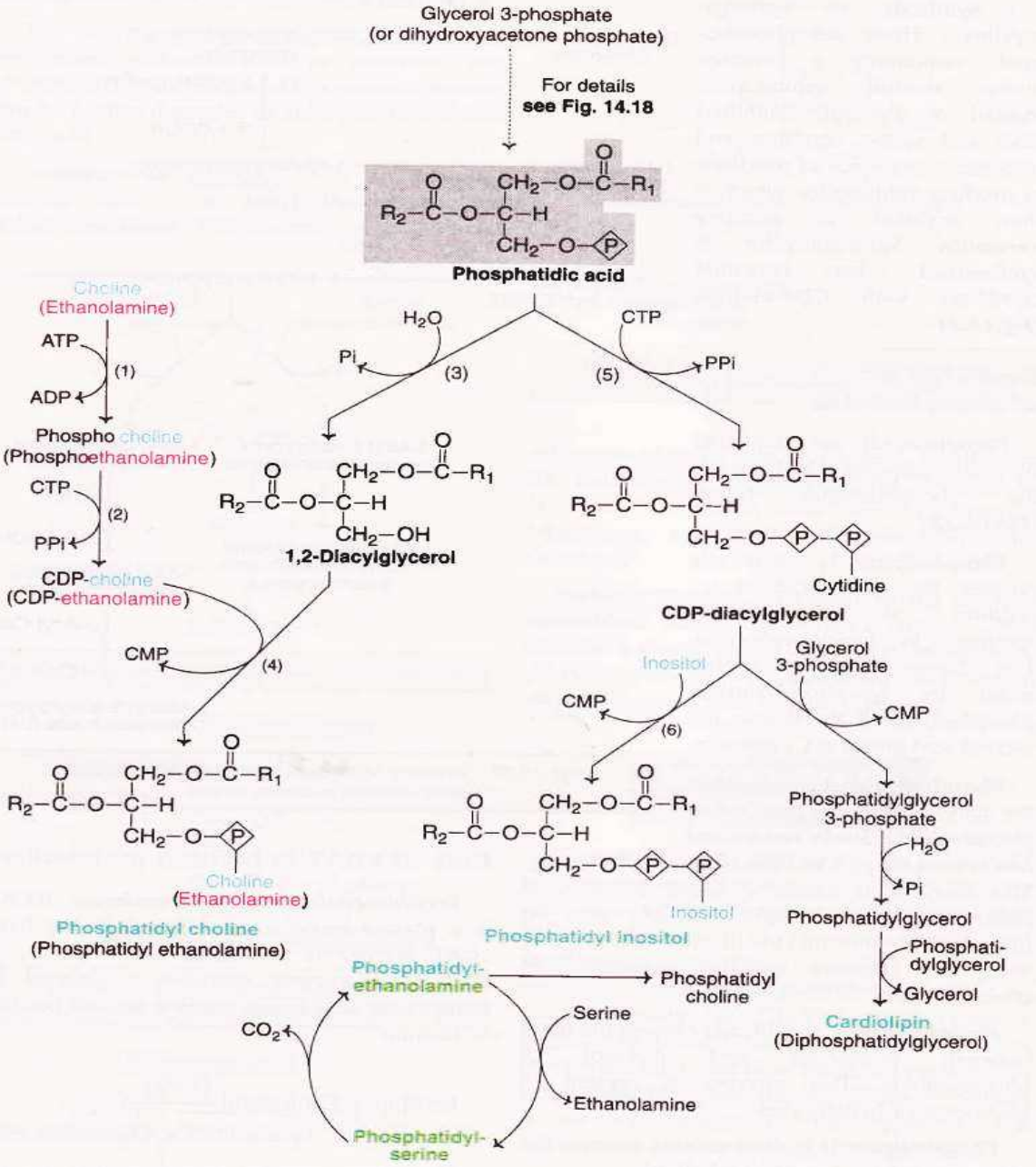


Fig. 14.19 : *Biosynthesis of phospholipids [The enzymes are numbered—(1) Choline kinase, (2) Phosphocholine cytidyltransferase, (3) Phosphatidate phosphohydrolase, (4) Phosphocholine diacylglycerol transferase, (5) CTP–Phosphatidate cytidyltransferase, (6) CDP–Diacylglycerol inositol transferase].*

CHOLESTEROL

Cholesterol, ***exclusively found in animals,*** is the most abundant animal sterol. It is widely distributed in all cells and is a major component of cell membranes and lipoproteins. Cholesterol (*Greek* : chole–bile) was first isolated from bile. Cholesterol literally means 'solid alcohol from bile.'

Cholesterol is found exclusively in animals, hence it is often called as ***animal sterol.*** The total body content of cholesterol in an adult man weighing 70 kg is about 140 g i.e., around 2 g/kg body weight. Cholesterol is ***amphipathic*** in nature, since it possesses both hydrophilic and hydrophobic regions in the structure.

Steroids are the compounds containing a cyclic steroid nucleus (or ring) namely ***cyclopentanoperhydrophenanthrene (CPPP).*** It consists of a phenanthrene nucleus (rings A, B and C) to which a cyclopentane ring (D) is attached.

There are several steroids in the biological system. These include ***cholesterol, bile acids, vitamin D, sex hormones, adrenocortical hormones,*** sitosterols, cardiac glycosides and alkaloids. If the steroid contains one or more hydroxyl groups it is commonly known as ***sterol*** (means solid alcohol).

Functions of cholesterol

Cholesterol is essential to life, as it performs a number of important functions

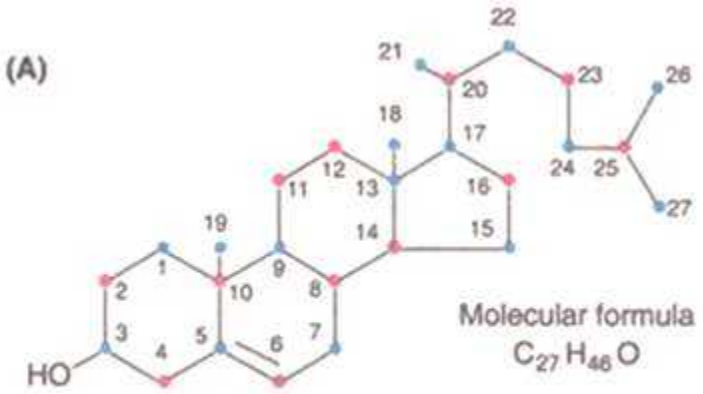
1. It is a structural component of cell membrane.
2. Cholesterol is the precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids.
3. It is an essential ingredient in the structure of lipoproteins in which form the lipids in the body are transported.
4. Fatty acids are transported to liver as cholesteryl esters for oxidation.

Structure and occurrence

The structure of cholesterol ($C_{27}H_{46}O$) is depicted in ***Fig.3.5***. It has one hydroxyl group at C_3 and a double bond between C_5 and C_6. An 8 carbon aliphatic side chain is attached to C_{17}. Cholesterol contains a total of 5 methyl groups.

Due to the presence of an –OH group, cholesterol is weakly amphiphilic. As a structural component of plasma membranes, cholesterol is an important determinant of membrane permeability properties. The occurrence of cholesterol is much higher in the membranes of sub-cellular organelles.

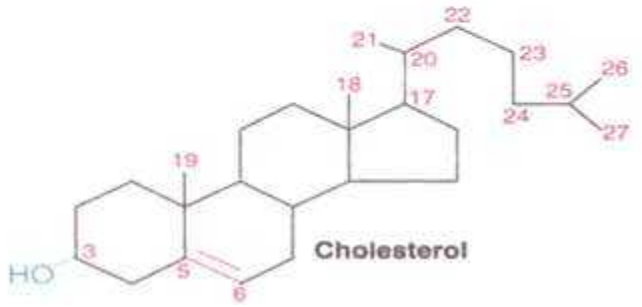
Cholesterol is found in association with fatty acids to form cholesteryl esters (esterification occurs at the OH group of C_3).



$H_3C-C-OO^-$ (acetate)

CH_3 contributes to carbons at positions 1, 3, 5, 7, 9, 13, 15, 17, 18, 19, 21, 22, 24, 26 and 27

COO^- contributes to the remaining carbon atoms



METABOLISM OF CHOLESTEROL

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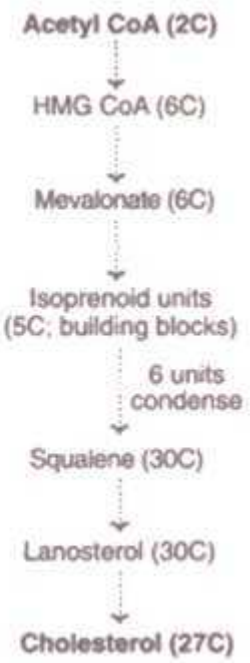
CHOLESTEROL BIOSYNTHESIS

About 1 g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by ***liver*** (50%), ***intestine*** (15%), skin, adrenal cortex, reproductive tissue etc.

The enzymes involved in cholesterol synthesis are found in the ***cytosol*** and ***microsomal fractions*** of the cell. Acetate of ***acetyl CoA*** provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by ***NADPH*** while ***ATP*** provides energy. For the production of one mole of cholesterol, 18 moles of acetyl CoA, 36 moles of ATP and 16 moles of NADPH are required.

(B)



The synthesis of cholesterol may be learnt in 5 stages

1. Synthesis of HMG CoA
2. Formation of mevalonate (6C)
3. Production of isoprenoid units (5C)
4. Synthesis of squalene (30C)
5. Conversion of squalene to cholesterol (27C).

1. **Synthesis of β-hydroxy β-methylglutaryl CoA (HMG CoA) :** Two moles of acetyl CoA condense to form acetoacetyl CoA. Another molecule of acetyl CoA is then added to produce HMG CoA. These reactions are similar to that of ketone body synthesis. However, the two pathways are distinct, since ketone bodies are produced in mitochondria while cholesterol synthesis occurs in cytosol. Thus, there exist ***two pools of HMG CoA*** in the cell. Further, two isoenzymes of HMG CoA synthase are known. The cytosomal enzyme is involved in cholesterol synthesis whereas the mitochondrial HMG CoA synthase participates in ketone body formation.

2. **Formation of mevalonate :** ***HMG CoA reductase*** is the ***rate limiting enzyme*** in cholesterol biosynthesis. This enzyme is present in endoplasmic reticulum and catalyses the reduction of HMG CoA to mevalonate. The reducing equivalents are supplied by NADPH.

3. **Production of isoprenoid units :** In a three-step reaction catalysed by kinases, mevalonate is converted to 3-phospho 5-pyrophospho-mevalonate which on decarboxylation forms isopentenyl pyrophosphate (IPP). The latter isomerizes to dimethylallyl pyrophosphate (DPP). Both IPP and DPP are 5-carbon isoprenoid units.

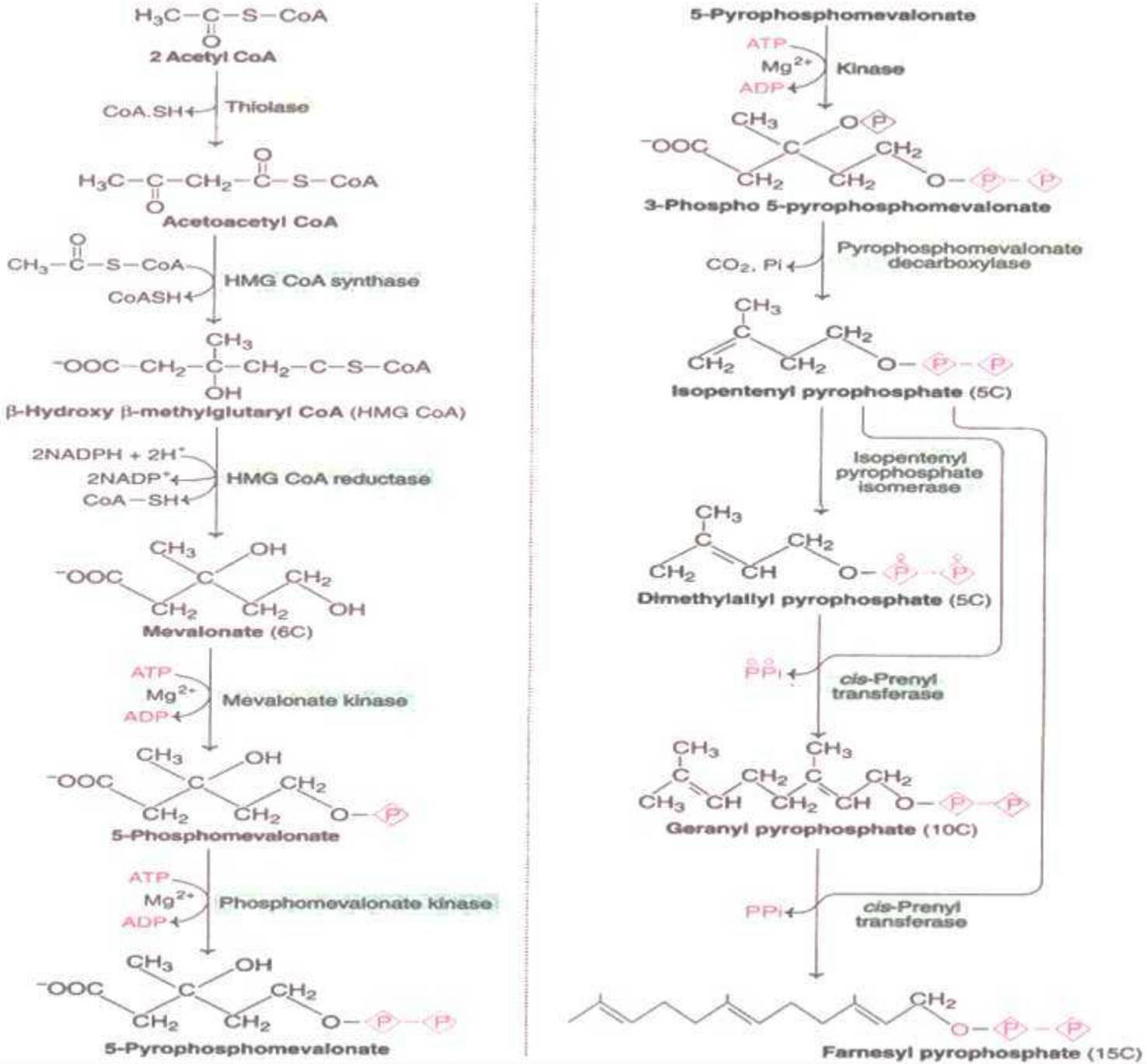
4. **Synthesis of squalene :** IPP and DPP condense to produce a 10-carbon geranyl pyrophosphate (GPP). Another molecule of IPP condenses with GPP to form a 15-carbon farnesyl pyrophosphate (FPP). Two units of farnesyl pyrophosphate unite and get reduced to produce a 30-carbon squalene.

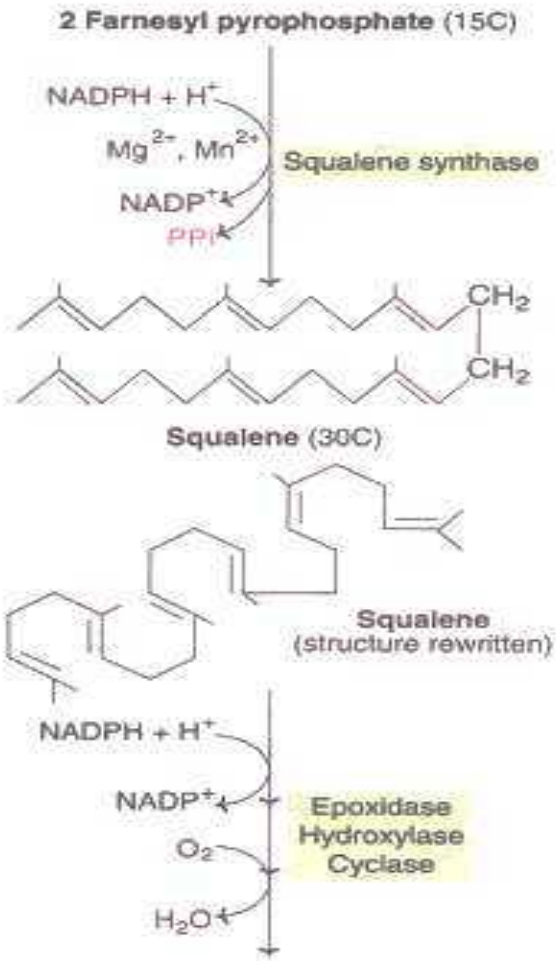
5. **Conversion of squalene to cholesterol :** Squalene undergoes hydroxylation and cyclization utilizing O_2 and NADPH and gets converted to lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions. The following are the most important reactions

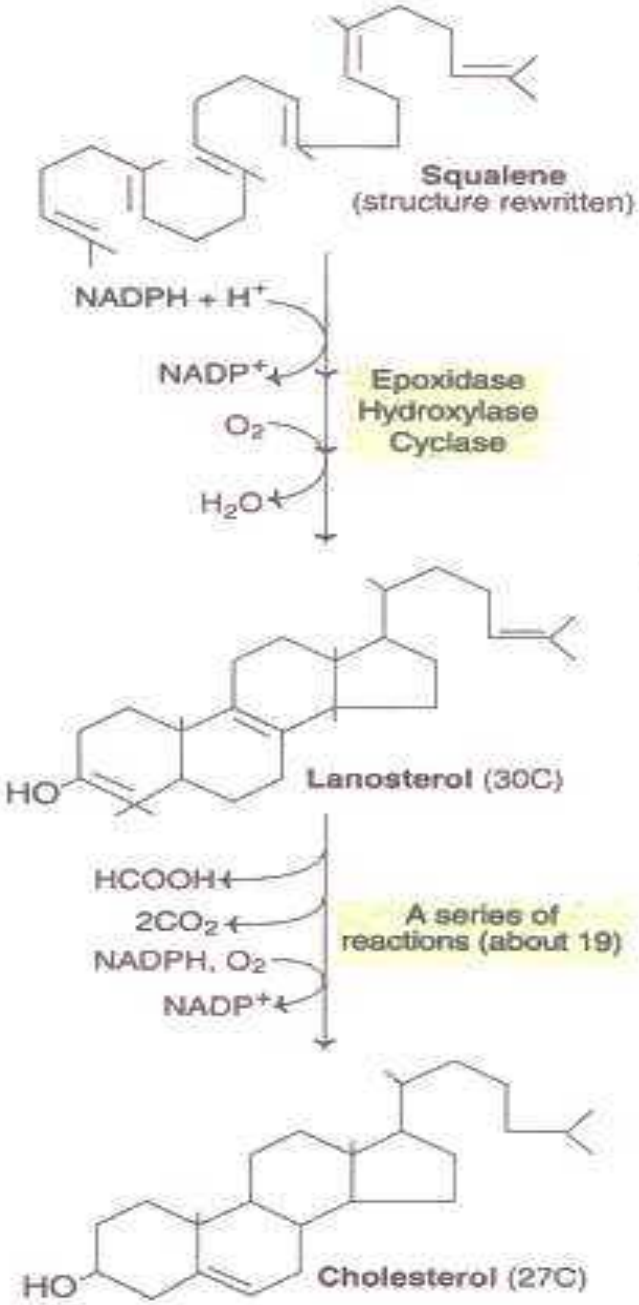
- Reducing the carbon atoms from 30 to 27.
- Removal of two methyl groups from C_4 and one methyl group from C_{14}.
- Shift of double bond from C_8 to C_5.
- Reduction in the double bond present between C_{24} and C_{25}.

The enzymes (about 19?) involved in the conversion of lanosterol to cholesterol are associated with endoplasmic reticulum. 14-Desmethyl lanosterol, zymosterol, cholestadienol and desmosterol are among the intermediates in the cholesterol biosynthesis. ***The penultimate product is 7-dehydrocholesterol which, on reduction, finally yields cholesterol.***

Cholesterol biosynthesis is now believed to be a part of a major metabolic pathway concerned with the synthesis of several other isoprenoid compounds. These include ubiquinone (coenzyme Q of electron transport chain) and dolichol (found in glycoprotein). Both of them are derived from farnesyl pyrophosphate.


2 Acetyl CoA
Thiolase
CoA.SH
Acetoacetyl CoA
HMG CoA synthase
CoASH
β-Hydroxy β-methylglutaryl CoA (HMG CoA)
2NADPH + 2H+
2NADP+
HMG CoA reductase
CoA—SH
Mevalonate (6C)
ATP
Mg2+
ADP
Mevalonate kinase
5-Phosphomevalonate
Phosphomevalonate kinase
5-Pyrophosphomevalonate
Kinase
3-Phospho 5-pyrophosphomevalonate
CO2, Pi
Pyrophosphomevalonate decarboxylase
Isopentenyl pyrophosphate (5C)
Isopentenyl pyrophosphate isomerase
Dimethylallyl pyrophosphate (5C)
PPi
cis-Prenyl transferase
Geranyl pyrophosphate (10C)
Farnesyl pyrophosphate (15C)


2 Farnesyl pyrophosphate (15C)
NADPH + H+
Mg2+, Mn2+
NADP+
PPi
Squalene synthase
CH2
CH2
Squalene (30C)
Squalene
(structure rewritten)
NADPH + H+
NADP+
O2
H2O
Epoxidase
Hydroxylase
Cyclase


Squalene
(structure rewritten)
NADPH + H+
NADP+
O2
H2O
Epoxidase
Hydroxylase
Cyclase
HO
Lanosterol (30C)
HCOOH
2CO2
NADPH, O2
NADP+
A series of
reactions (about 19)
HO
Cholesterol (27C)

Regulation of cholesterol synthesis

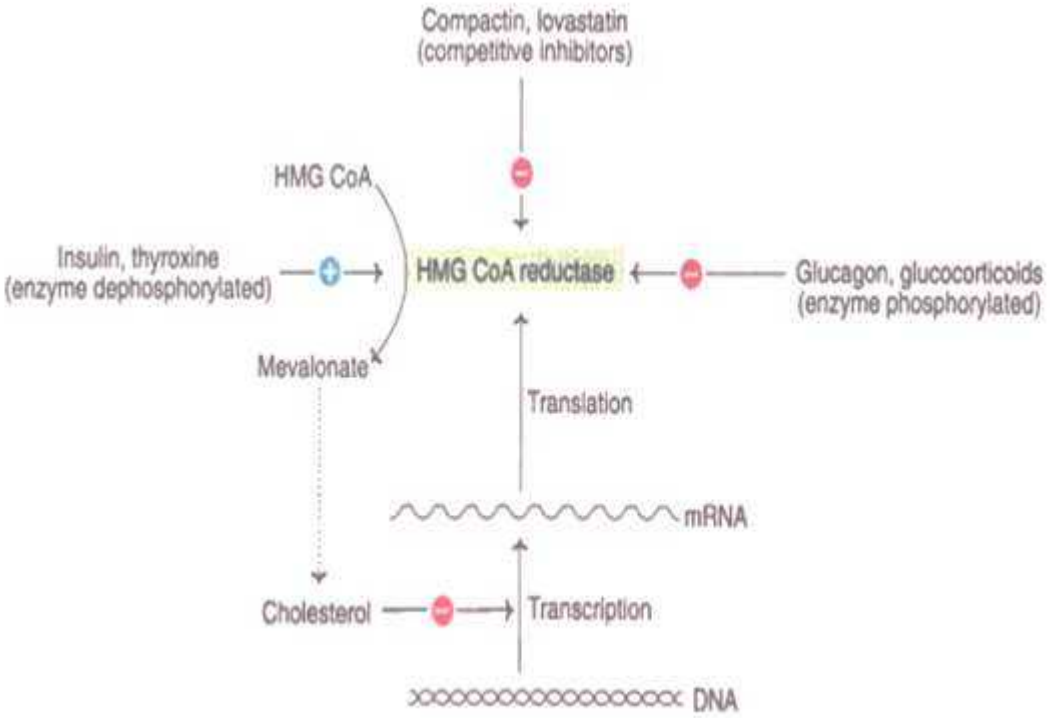
Cholesterol biosynthesis is controlled by the rate limiting enzyme ***HMG CoA reductase,*** at the beginning of the pathway (***Fig.14.28***). HMG CoA reductase is found in association with endoplasmic reticulum, and is subjected to different metabolic controls.

1. **Feedback control :** The end product cholesterol controls its own synthesis by a feedback mechanism. Increase in the cellular concentration of cholesterol reduces the synthesis of the enzyme HMG CoA reductase. This is achieved by ***decreasing the transcription*** of the gene responsible for the production of HMG CoA reductase. Feedback regulation has been investigated with regard to LDL-cholesterol taken up by the cells, and the same mechanism is believed to operate whenever cellular cholesterol level is elevated.

2. **Hormonal regulation :** The enzyme HMG CoA reductase exists in two interconvertible forms. The dephosphorylated form of HMG CoA reductase is more active while the phosphorylated form is less active. The hormones exert their influence through cAMP by a series of reactions which are comparable with the control of the enzyme glycogen synthase. The net effect is that ***glucagon*** and ***glucocorticoids*** favour the formation of inactive HMG CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, ***insulin*** and ***thyroxine*** increase cholesterol production by enhancing the formation of active HMG CoA reductase (dephosphorylated form).

3. **Inhibition by drugs :** The drugs ***compactin*** and ***lovastatin*** (mevinolin) are fungal products. They are used to decrease the serum cholesterol level in patients with hypercholesterolemia. Compactin and lovastatin are ***competitive inhibitors of*** the enzyme ***HMG CoA reductase*** and, therefore, reduce cholesterol synthesis. About 50 to 60% decrease in serum cholesterol level has been reported by a combined use of these two drugs.

4. HMG CoA reductase activity is inhibited by ***bile acids***. Fasting also reduces the activity of this enzyme.



DEGRADATION OF CHOLESTEROL

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50%) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D, coprostanol and cholestanol. The latter two are the fecal sterols, besides cholesterol.

The synthesis of primary bile acids takes place in the liver and involves a series of reactions (***Fig.14.29***). The step catalysed by ***7 α-hydroxylase*** is inhibited by bile acids and this is the ***rate limiting*** reaction. Cholic acid and chenodeoxycholic acid are the primary bile acids and the former is found in the largest amount in bile. On conjugation with glycine or taurine, ***conjugated bile acids*** (glycocholic acid, taurocholic acid etc.) are formed which are more ***efficient in their function*** as surfactants. In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as ***bile salts***.

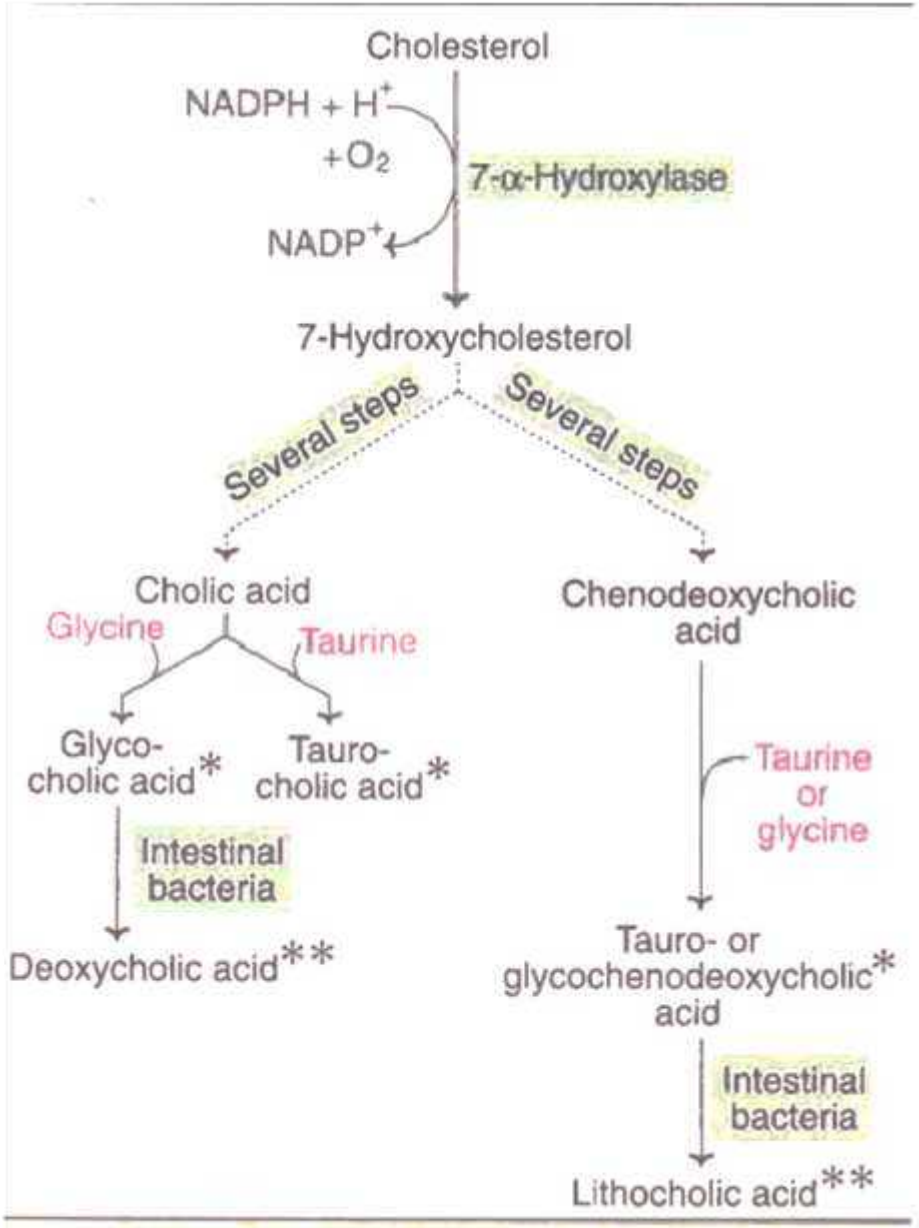


Fig. 14.29 : *Outline of bile acid synthesis (∗–Primary bile acids, ∗∗–Secondary bile acids).*

II. Synthesis of steroid hormones from cholesterol

Cholesterol is the precursor for the synthesis of all the five classes of steroid hormones

(a) Glucocorticoids (e.g. cortisol)

(b) Mineralocorticoids (e.g. aldosterone)

(c) Progestins (e.g. progesterone)

(d) Androgens (e.g. testosterone)

(e) Estrogens (e.g. estradiol).

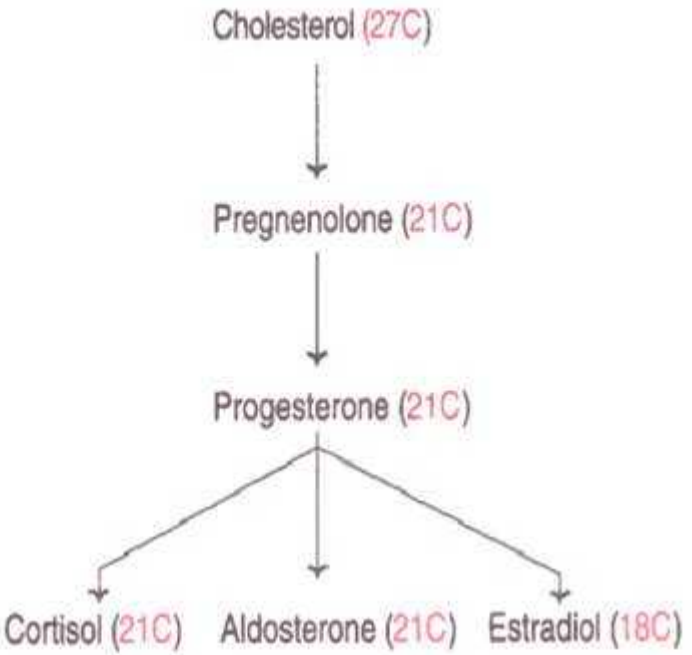


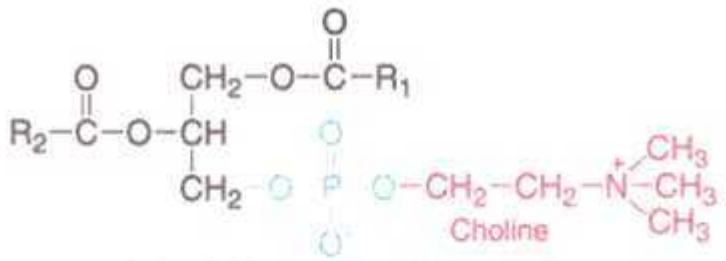
Fig. 14.30 : Outline of steroid hormone synthesis from cholesterol (Numbers in the brackets represent the number of carbon atoms).

III. Synthesis of vitamin D

7-Dehydrocholesterol, an intermediate in the synthesis of cholesterol, is converted to cholecalciferol (vitamin D_3) by ultraviolet rays in the skin.

Role of LCAT : High density lipoproteins (HDL) and the enzyme ***lecithin-cholesterol acyltransferase (LCAT)*** are responsible for the transport and elimination of cholesterol from the body. LCAT is a plasma enzyme, synthesized by the liver. It catalyses the transfer of fatty acid from the second position of phosphatidyl choline (lecithin) to the hydroxyl group of cholesterol (***Fig.14.32***). HDL-cholesterol is the real substrate for LCAT and this reaction is freely reversible. ***LCAT activity*** is associated with ***apo-A_1 of HDL***.

The cholesterol (cholesteryl) ester forms an integral part of HDL. In this manner, the cholesterol from the peripheral tissues is trapped in HDL, by a reaction catalysed by LCAT and then transported to liver for degradation and excretion. This mechanism is commonly known ***cholesterol transport.***



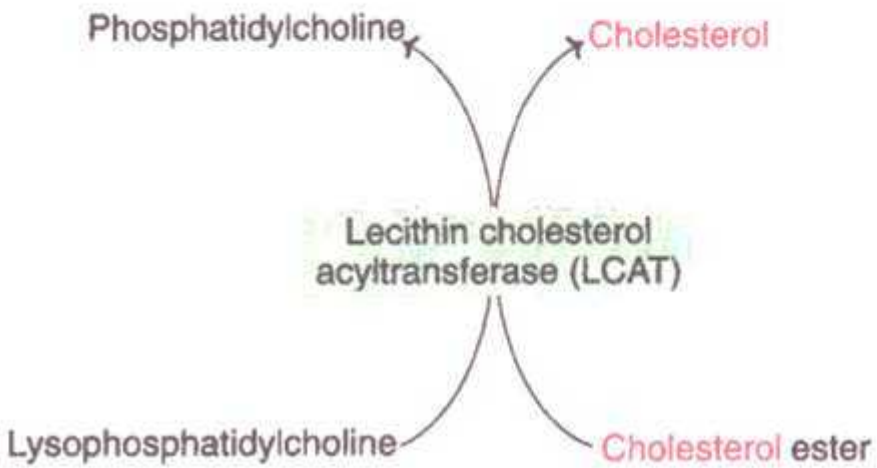
(2) **Lecithin** (phosphatidylcholine)

Transport of cholesterol

Cholesterol is present in the plasma lipoproteins in two forms

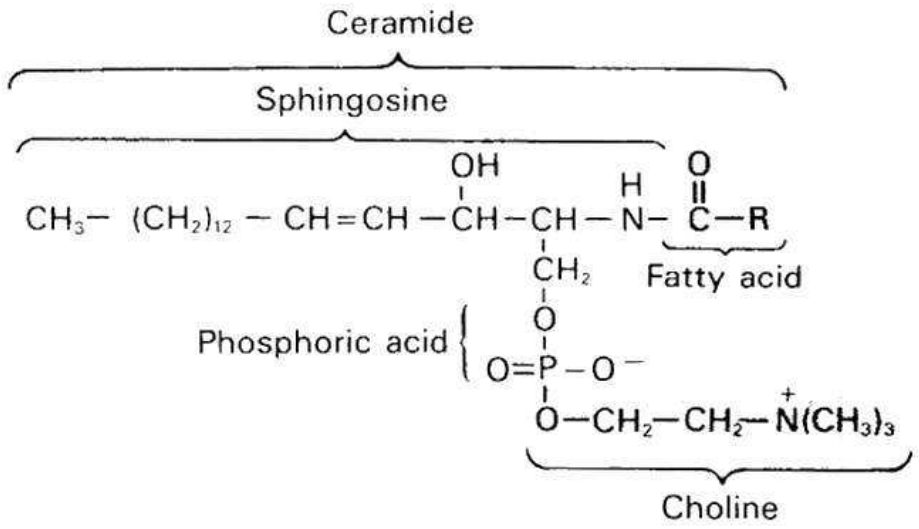
1. About 70-75% of it is in an esterified form with long chain fatty acids.

2. About 25-30% as free cholesterol. This form of cholesterol readily exchanges between different lipoproteins and also with the cell membranes.



Sphingomyelins

Sphingosine is an amino alcohol present in sphingomyelins (sphingophospholipids). They do not contain glycerol at all. Sphingosine is attached by an amide linkage to a fatty acid to produce ***ceramide***. The alcohol group of sphingosine is bound to phosphorylcholine in sphingomyelin structure (***Fig.3.3***). Sphingomyelins are important constituents of myelin and are found in good quantity in brain and nervous tissues.



6. **Synthesis of sphingomyelins :** These are phospholipids containing a complex amino alcohol, sphingosine, instead of glycerol. Palmitoyl CoA and serine combine and undergo a sequence of reactions to produce sphingosine which is then acylated to produce ***ceramide.*** Sphingomyelin is synthesized when ceramide combines with CDP-choline (***Fig.14.21***).

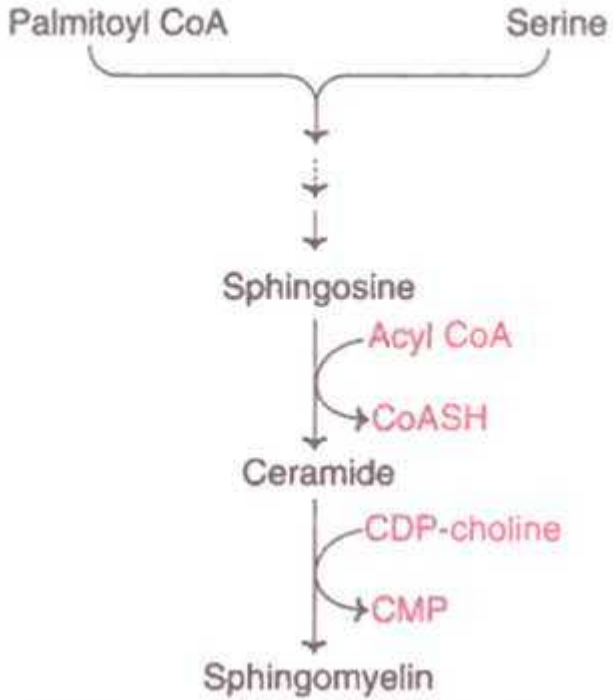
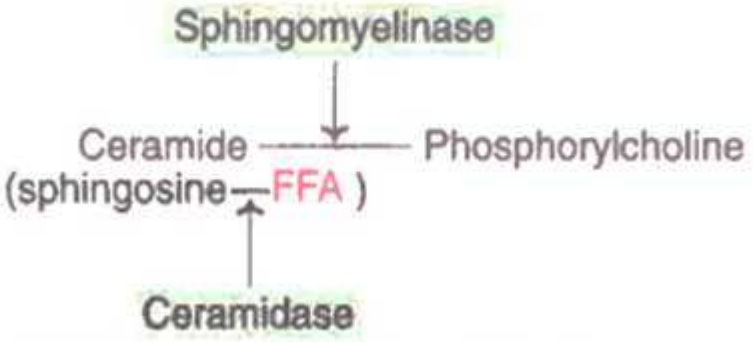


Fig. 14.21 : An outline of the synthesis of sphingomyelin.

Degradation of sphingomyelins

The enzyme ***sphingomyelinase*** of lysosomes hydrolyses sphingomyelins to ceramide and phosphorylcholine (***Fig.14.23***). Ceramide formed can be further degraded to sphingosine and free fatty acid.



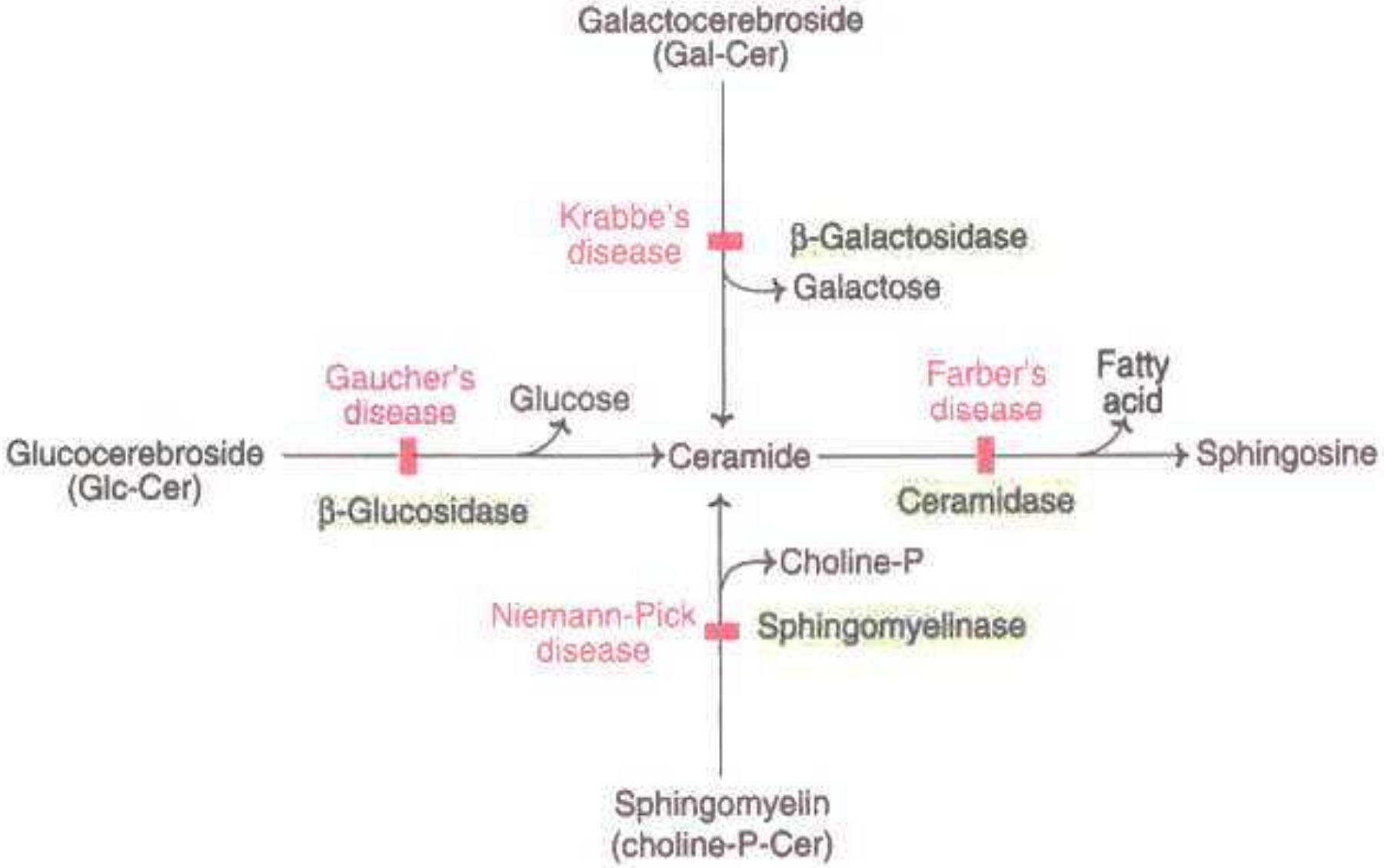


***Fig. 14.25** : Degradation of cerebrosides and sphingomyelins with metabolic disorders.*

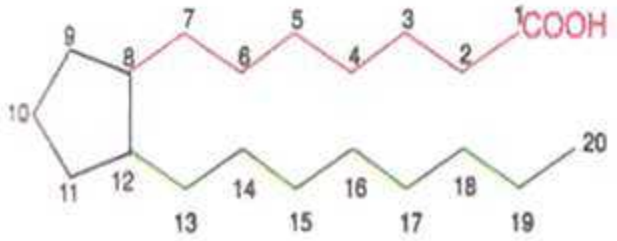
Prostaglandins

Prostaglandins and their related compounds—prostacyclins (PGI), thromboxanes (TXA), leukotrienes (LT) and lipoxins are collectively known as ***eicosaniods***, since they all contain 20 carbons (*Greek* : eikosi-twenty). Eicosanoids are considered as locally acting hormones with a wide range of biochemical functions.

History : Prostaglandins (PGs) were first discovered in human semen by Ulf von Euler (of Sweden) in 1930. These compounds were found to stimulate uterine contraction and reduce blood pressure. von Euler presumed that they were synthesized by prostate gland and hence named them as prostaglandins. It was later realized that PGs and other eicosanoids are synthesized in almost all the tissues (exception–erythrocytes). By then, however, the name prostaglandins was accepted worldwide, and hence continued.

Structure of prostaglandins

Prostaglandins are derivatives of a hypothetical 20-carbon fatty acid namely ***prostanoic acid***, hence known as ***prostanoids***. This has a cyclopentane ring (formed by carbon atoms 8 to 12) and two side chains, with carboxyl group on one side. Prostaglandins differ in their structure due to substituent group and double bond on cyclopentane ring. The different prostaglandins are given in ***Fig.32.1***.



Prostanoic acid

Synthesis of prostaglandins

Arachidonic acid (5,8,11,14-eicosatetraenoic acid) is the precursor for most of the prostaglandins in humans. The biosynthesis of PGs was described by scene Bergstrom and Bengt Samuelsson (1960). It occurs in the endoplasmic reticulum in the following stages, as depicted in ***Fig.32.3***.

1. Release of arachidonic acid from membrane bound phospholipids by phospholipase A_2—this reaction occurs due to a specific stimuli by hormones such as epinephrine or bradykinin.

2. Oxidation and cyclization of arachidonic acid to PGG_2 which is then converted to PGH_2 by a reduced glutathione dependent peroxidase.

3. PGH_2 serves as the immediate precursor for the synthesis of a number of prostaglandins, including prostacyclins and thromboxanes.

The above pathway is known as ***cyclic pathway of arachidonic acid***. In the linear pathway of arachidonic acid, leukotrienes and lipoxins are synthesized (details given later).

Cyclooxygenase–a suicide enzyme : It is interesting to note that prostaglandin synthesis can be partly controlled by suicidal activity of the enzyme cyclooxygenase. This enzyme is capable of undergoing self-catalysed destruction to switch off PG synthesis.

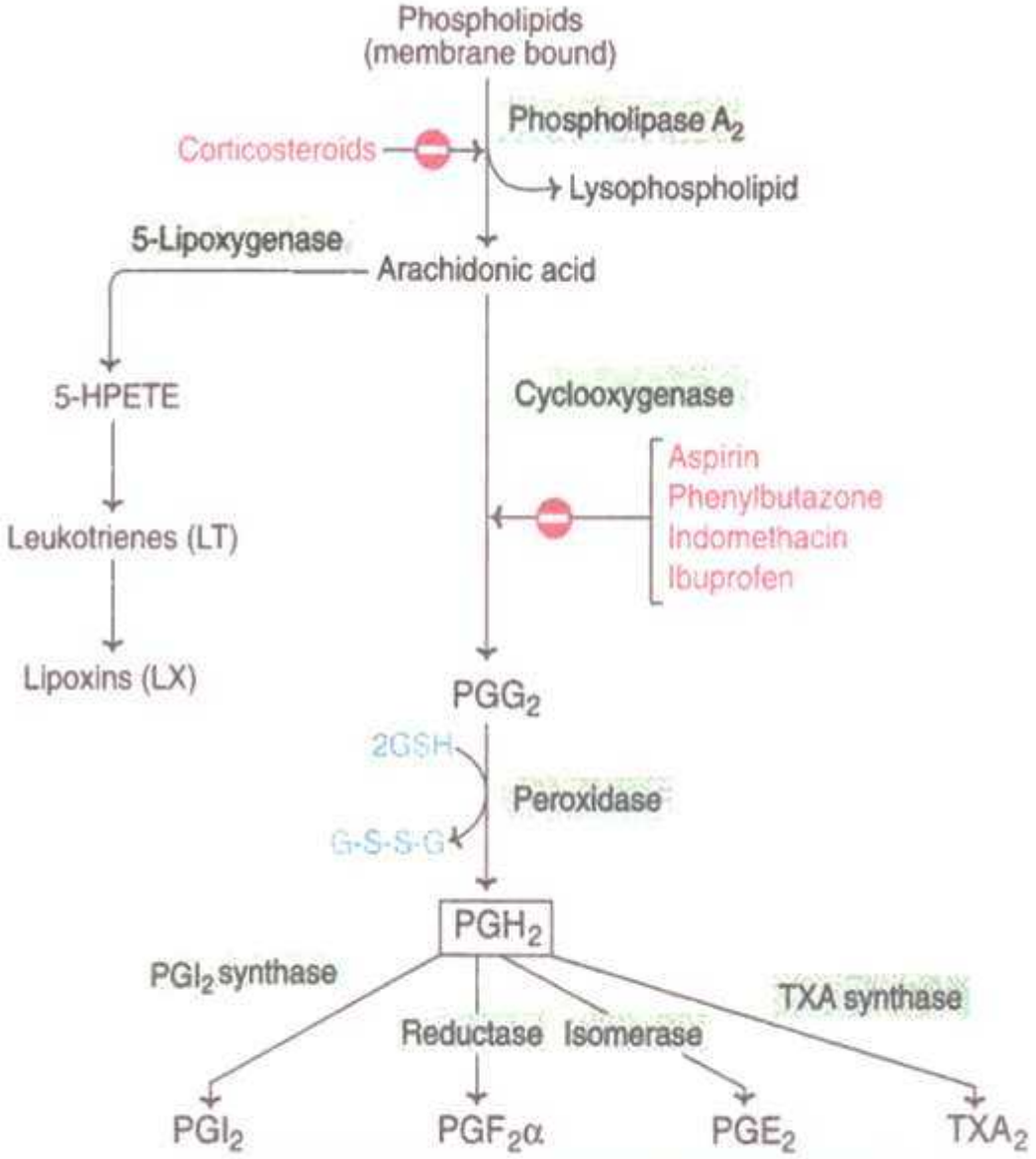


Fig. 32.3 : Overview of biosynthesis of prostaglandins and related compounds (5-HPETE–5-Hydroxyperoxycicosatetraenoic acid; PG–Prostaglandins; PGI_2–Prostacyclin I_2; TXA_2–Thromboxane A_2).

Inhibition of PG synthesis : A number of structurally unrelated compounds can inhibit prostaglandin synthesis. Corticosteroids (e.g. cortisol) prevent the formation of arachidonic acid by inhibiting the enzyme phospholipase A_2.

Many non-steroidal ***anti-inflammatory drugs*** inhibit the synthesis of prostaglandins, prostacyclins and thromboxanes. They do so by ***blocking*** the action of the enzyme ***cyclo-oxygenase.***

Aspirin inhibits PG synthesis : Aspirin (acetyl salicylic acid) has been used since nineteenth century as an antipyretic (fever-reducing) and analgesic (pain relieving). The mechanism of action of aspirin however, was not known for a long period. It was only in 1971, John Vane discovered that aspirin inhibits the synthesis of PG from arachidonic acid. Aspirin irreversibly ***inhibits*** the enzyme ***cyclooxygenase.*** Other antiinflammatory drugs, such as indomethacin and phenylbutazone act as reversible inhibitors of the enzyme cyclooxygenase.

Degradation of prostaglandins : Almost all the eicosanoids are metabolized rapidly. The lung and liver are the major sites of PG degradation. Two enzymes, namely 15-α-hydroxy PG dehydrogenase and 13-PG reductase, convert hydroxyl group at C_{15} to keto group and then to C_{13} and C_{14} dihydro-derivative.

Biochemical actions of prostaglandins

Prostaglandins act as ***local hormones*** in their function. They, however, differ from the true hormones in many ways. Prostaglandins are produced in almost all the tissues in contrast to hormonal synthesis which occurs in specialized glands. PGs are not stored and they are degraded to inactive products at the site of their production. Further, PGs are produced in very small amounts and have low half-lives.

Prostaglandins are involved in a variety of biological functions. The actions of PGs differ in different tissues. Sometimes, PGs bring about opposing actions in the same tissue.

Overproduction of PGs results in many symptoms which include pain, fever, nausea, vomiting and inflammation.

Prostaglandins mediate the regulation of blood pressure, inflammatory response, blood clotting, reproductive functions, response to pain, fever etc.

1. **Regulation of blood pressure :** The prostaglandins (PGE, PGA and PGI_2) are vasodilator in function. This results in increased blood flow and decreased peripheral resistance to ***lower the blood pressure***. PGs serve as agents in the treatment of hypertension.

2. **Inflammation :** The prostaglandins PGE_1 and PGE_2 ***induce*** the symptoms of ***inflammation*** (redness, swelling, edema etc.) due to arteriolar vasodilation. This led to the belief that PGs are natural mediators of inflammatory · reactions of rheumatoid arthritis (involving joints), psoriasis (skin), conjunctivitis (eyes) etc.

3. **Reproduction :** Prostaglandins have widespread applications in the field of reproduction. PGE_2 and PGF_2 are ***used for*** the medical ***termination of pregnancy*** and ***induction of labor.*** Prostaglandins are administered to cattle to induce estrus and achieve better rate of fertilization.

4. **Pain and fever :** It is believed that pyrogens (fever producing agents) promote prostaglandin biosynthesis leading to the formation of PGE_2 in the hypothalamus, the site of regulation of body temperature. ***PGE_2*** along with histamine and bradykinin ***cause pain.*** Migraine is also due to PGE_2. Aspirin and other non-steroidal drugs inhibit PG synthesis and thus control fever and relieve pain.

5. **Regulation of gastric secretion :** In general, prostaglandins (PGE) inhibit gastric secretion. ***PGs*** are used for the ***treatment of gastric ulcers.*** However, PGs stimulate pancreatic secretion and increase the motility of intestine which often causes diarrhea.

6. **Influence on immune system :** Macrophages secrete PGE which decreases the immunological functions of B-and T-lymphocytes.

7. **Effects on respiratory function :** PGE is a bronchodilator whereas PGF acts as a constrictor of bronchial smooth muscles. Thus, PGE and PGF oppose the actions of each other in the lungs. PGE_1 and PGE_2 are used in the treatment of asthma.

8. **Influence on renal functions :** PGE increases glomerular filtration rate (GFR) and promotes urine output. Excretion of Na^+ and K^+ is also increased by PGE.

9. **Effects on metabolism :** Prostaglandins influence certain metabolic reactions, probably through the mediation of cAMP. PGE decreases lipolysis, increases glycogen formation and promotes calcium mobilization from the bone.

10. **Platelet aggregation and thrombosis :** The prostaglandins, namely prostacyclins (***PGI_2***), ***inhibit platelet aggregation***. On the other hand, thromboxanes (TXA_2) and prostaglandin E_2 promote platelet aggregation and blood clotting that might lead to thrombosis. PGI_2, produced by endothelial cells lining the blood vessels, prevents the adherence of platelets to the blood vessels. ***TXA_2*** is released by the ***platelets*** and is responsible for their spontaneous ***aggregation*** when the platelets come in contact with foreign surface, collagen or thrombin. Thus, prostacyclins and thromboxanes are antagonists in their action. In the overall effect PGI_2 acts as a vasodilator, while TXA_2 is a vasoconstrictor. The balance between PGI_2 and TXA_2 is important in the regulation of hemostasis and thrombosis.

Biomedical applications of PGs

As described above, prostaglandins perform diversified functions. And for this reason, PGs (or other derivatives) are the most exploited in therapeutic applications. They are used in the ***treatment of gastric ulcers, hypertension, thrombosis, asthma*** etc. Prostaglandins are also employed in the medical termination of pregnancy, prevention of conception, induction of labor etc.

Inhibitors of prostaglandin synthesis (e.g. aspirin, ibuprofen) are utilized in controlling fever, pain, migraine, inflammation etc.

Mechanism of action of PGs

The mechanism of action of prostaglandins is not known for certain. They bind to the specific cellular receptors and bring about their action at the molecular level. It is believed that PGs may act through the mediation of cyclic nucleotides. PGE increases cAMP levels whereas PGF elevates cGMP.

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All the original contributors of the concept and findings published are gratefully acknowledged while preparing the e-content for the students of Biotechnology and allied sciences

METABOLISM OF LIPIDS -2

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E –content

Course: M.Sc.
Subject: Biochemistry; Biotechnology
Topic: Metabolism of Lipids
Subtopic: **Synthesis of Fatty Acids and Ketone Bodies**
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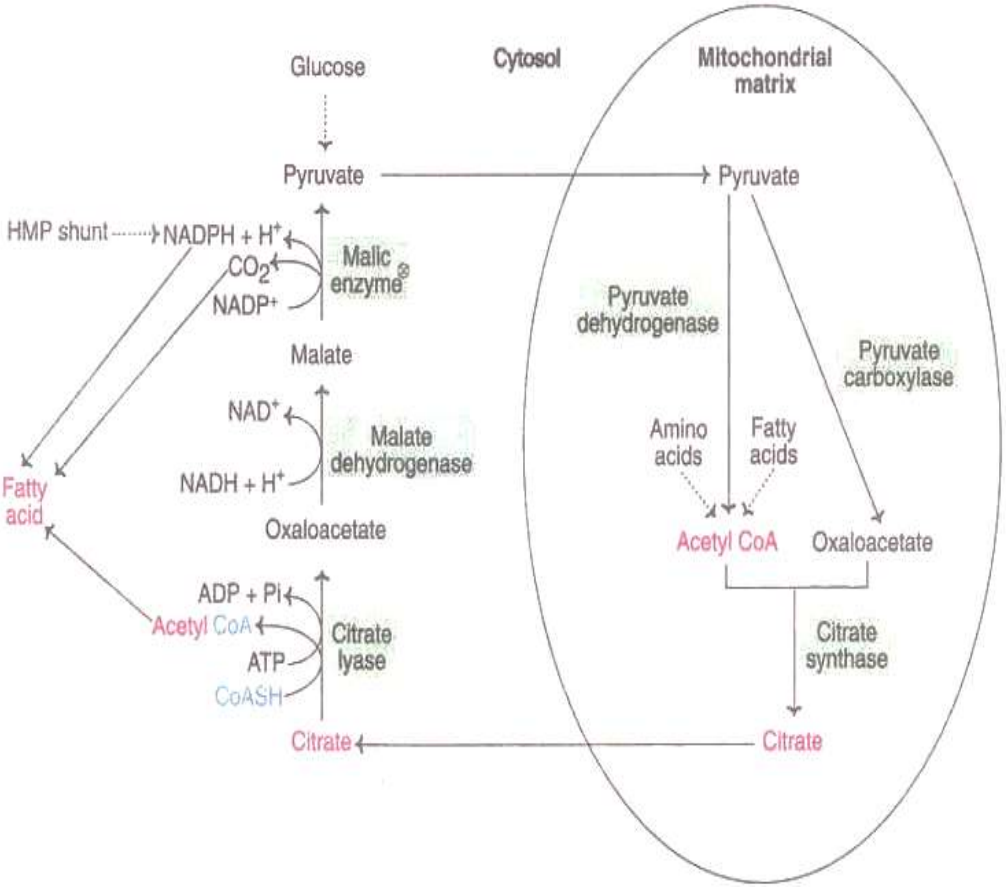
BIOSYNTHESIS OF FATTY ACIDS

I. Production of acetyl CoA and NADPH
II. Conversion of acetyl CoA to malonyl CoA
III. Reactions of fatty acid synthase complex

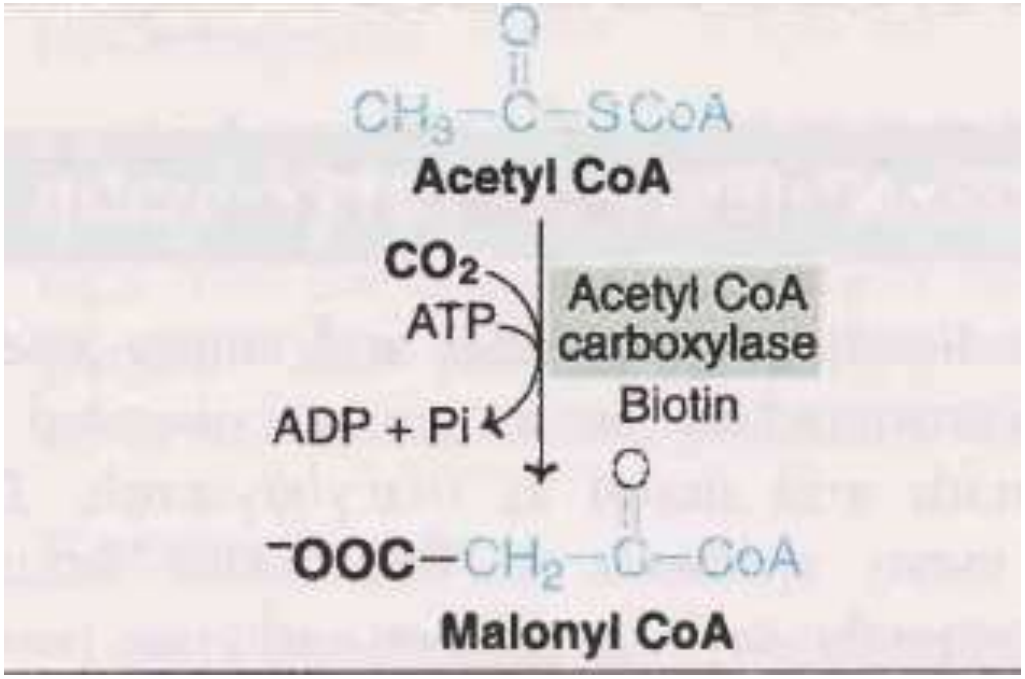
I. Production of acetyl CoA and NADPH

Acetyl CoA and NADPH are the prerequisites for fatty acid synthesis. Acetyl CoA is produced in the mitochondria by the oxidation of pyruvate and fatty acids, degradation of carbon skeleton of certain amino acids, and from ketone bodies. Mitochondria, however, are not permeable to acetyl CoA. An alternate or a bypass arrangement is made for the transfer of acetyl CoA to cytosol. Acetyl CoA condenses with oxaloacetate in mitochondria to form citrate. Citrate is freely transported to cytosol where it is cleaved by ***citrate lyase*** to liberate acetyl CoA and oxaloacetate. Oxaloacetate in the cytosol is converted to malate (***Fig.14.14***).



II. Formation of malonyl CoA

Acetyl CoA is carboxylated to malonyl CoA by the enzyme ***acetyl CoA carboxylase*** (***Fig.14.15***). This is an ATP-dependent reaction and requires ***biotin*** for CO_2 fixation. The mechanism of action of acetyl CoA carboxylase is similar to that of pyruvate carboxylase (***Refer Chapter 7, Fig.7.29***). Acetyl CoA carboxylase is a regulatory enzyme in fatty acid synthesis (details given later).



III. Reactions of fatty acid synthase complex

The remaining reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as ***fatty acid synthase (FAS) complex.*** In eukaryotic cells, including man, the fatty acid synthase exists as a dimer with two identical units. Each monomer possesses the activities of seven different enzymes and an ***acyl carrier protein (ACP)*** bound to 4'-phosphopantetheine. Fatty acid synthase functions as a single unit catalysing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. In the lower organisms (prokaryotes), the fatty acid synthesis is carried out by a multienzyme complex in association with a separate acyl carrier protein. This is in contrast to eukaryotes where ACP is a part of fatty acid synthase.

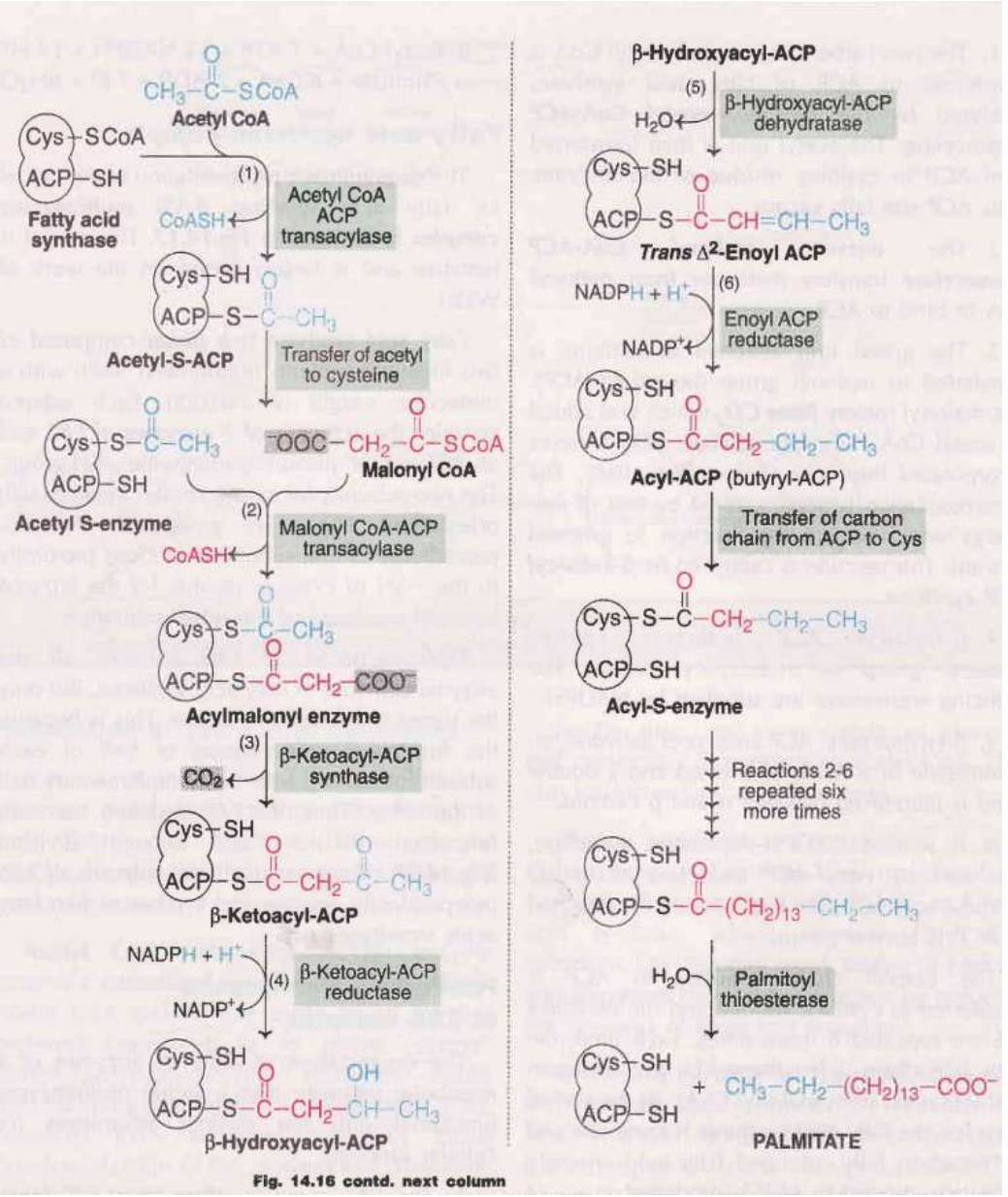


Fig. 14.16 contd. next column

Fig. 14.16 : *Biosynthesis of long chain fatty acid-palmitate. (Cys–Cysteine; ACP–Acyl carrier protein; The pathway repeats 7 times to produce palmitate; the first two carbons at the methyl end are directly from acetyl CoA, the rest of the carbons come from malonyl CoA).*

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, ***acetyl CoA-ACP transacylase***. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme ***malonyl CoA-ACP transacylase*** transfers malonate from malonyl CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety ***loses CO_2*** which was added by acetyl CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain. The decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward. This reaction is catalyzed by ***β-ketoacyl ACP synthase.***

4. β-Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH.

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catalysed by ***enoyl-ACP reductase*** occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group.

The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid—namely palmitate—bound to ACP is produced.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

Summary of palmitate synthesis

Of the 16 carbons present in palmitate, only two come from acetyl CoA directly. The remaining 14 are from malonyl CoA which, in turn, is produced by acetyl CoA. The overall reaction of palmitate synthesis is summarized

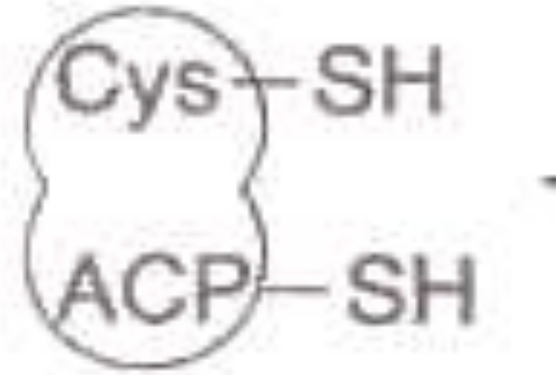
$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 14\ H^+ \longrightarrow \text{Palmitate} + 8\ \text{CoA} + 7\ \text{ADP} + 7\ \text{Pi} + 6H_2O$$

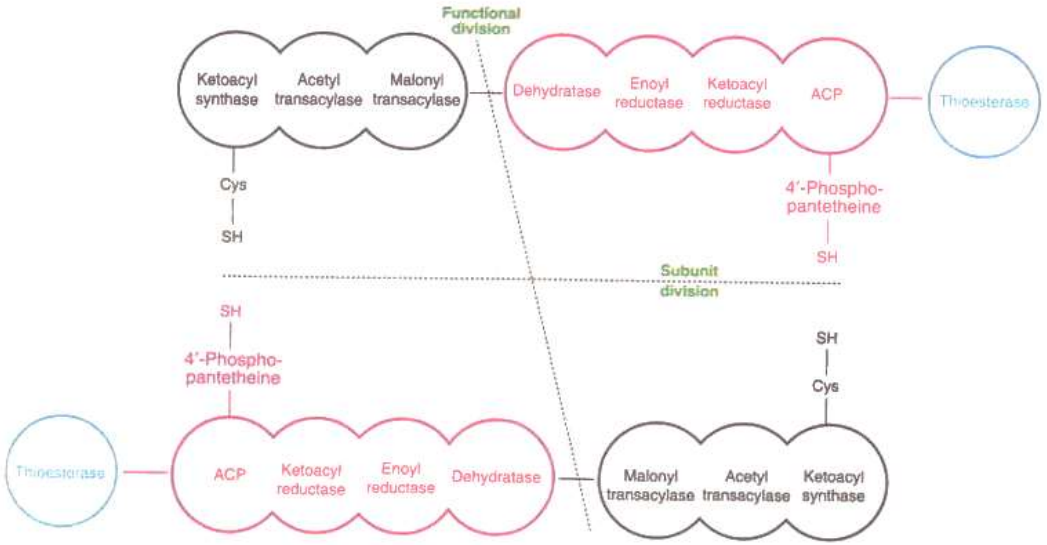
Fatty acid synthase complex

The diagrammatic representation of the model for fatty acid synthase (FAS) ***multienzyme complex*** is depicted in ***Fig.14.17***. This model is tentative and is largely based on the work of Wakil.

Fatty acid synthase is a ***dimer*** composed of two identical subunits (monomers), each with a molecular weight of 240,000. Each subunit contains the activities of 7 enzymes of FAS and an ACP with 4'-phosphopantetheine –SH group. The two subunits lie in antiparallel (head-to-tail) orientation. The –SH group of phosphopantetheine of one subunit is in close proximity to the –SH of cysteine residue (of the enzyme ketoacyl synthase) of the other subunit.

Each monomer of FAS contains all the enzyme activities of fatty acid synthesis. But only the dimer is functionally active. This is because the functional unit consists of half of each subunit interacting with the complementary half of the other. Thus, the FAS structure has both functional division and subunit division (***Fig.14.17***). The two functional subunits of FAS independently operate and synthesize two fatty acids simultaneously.





FAS

Functional significance of FAS complex

The organization of different enzymes of a metabolic pathway into a single multienzyme functional unit has distinct advantages for cellular function

1. The FAS complex offers ***great efficiency*** that is free from interference of other cellular reactions for the synthesis of fatty acids.

2. Since the entire process of the metabolic pathway is confined to the complex, there are no permeability barriers for the various intermediates.

3. The multienzyme polypeptide complex is coded by a single gene. Thus, there is a ***good coordination*** in the synthesis of all enzymes of the FAS complex.

Regulation of fatty acid synthesis

Fatty acid production is controlled by enzymes, metabolites, end products, hormones and dietary manipulations. Some of the important regulatory mechanisms are discussed hereunder.

Acetyl CoA carboxylase : This enzyme controls a ***committed step in fatty acid synthesis.*** Acetyl CoA carboxylase exists as an inactive protomer (monomer) or an active polymer. Citrate promotes polymer formation, hence increases fatty acid synthesis. On the other hand, palmitoyl CoA and malonyl CoA cause depolymerization of the enzyme and, therefore, inhibit fatty acid synthesis.

Availability of NADPH : The reducing equivalents for fatty acid synthesis are provided by NADPH which come either from citrate (acetyl CoA) transport or hexose monophosphate shunt. About 50-60% of required NADPH is obtained from ***HMP shunt,*** which significantly influences fatty acid synthesis.

Hormonal influence : Hormones regulate acetyl CoA carboxylase by a separate mechanism—phosphorylation (inactive form) and dephosphorylation (active form) of the enzyme. Glucagon, epinephrine and norepinephrine inactivate the enzyme by cAMP-dependent phosphorylation. Insulin, on the other hand, dephosphorylates and activates the enzyme. Thus, ***insulin promotes fatty acid synthesis*** while ***glucagon inhibits.***

Dietary regulation : Consumption of high carbohydrate or fat-free diet increases the synthesis of acetyl CoA carboxylase and fatty acid synthase, which promote fatty acid formation. On the other hand, ***fasting*** or ***high fat diet decreases*** fatty acid production by reducing the synthesis of these two enzymes.

Oxidation of Unsaturated fatty acids

β-Oxidation of unsaturated fatty acids poses a problem since the location of a cis bond can prevent the formation of a trans-Δ^2 bond. These situations are handled by an additional two enzymes, Enoyl CoA isomerase or 2,4 Dienoyl CoA reductase.

Whatever the conformation of the hydrocarbon chain, β-oxidation occurs normally until the acyl CoA (because of the presence of a double bond) is not an appropriate substrate for acyl CoA dehydrogenase, or enoyl CoA hydratase:

•If the acyl CoA contains a *cis-Δ^3 bond*, then *cis-Δ^3*-Enoyl CoA isomerase will convert the bond to a *trans-Δ^2* bond, which is a regular substrate.
•**If the acyl CoA contains a *cis-Δ^4 double bond*, then its dehydrogenation yields a 2,4-dienoyl intermediate,** which is not a substrate for enoyl CoA hydratase. However, the enzyme 2,4 Dienoyl CoA reductase reduces the intermediate, using NADPH, into ***trans-Δ^3*-enoyl CoA. As in the above case, this compound is converted into a suitable intermediate by 3,2-Enoyl CoA isomerase**.
To summarize:
•*Odd-numbered* double bonds are handled by the isomerase.
•*Even-numbered* double bonds by the reductase (which creates an odd-numbered double bond)

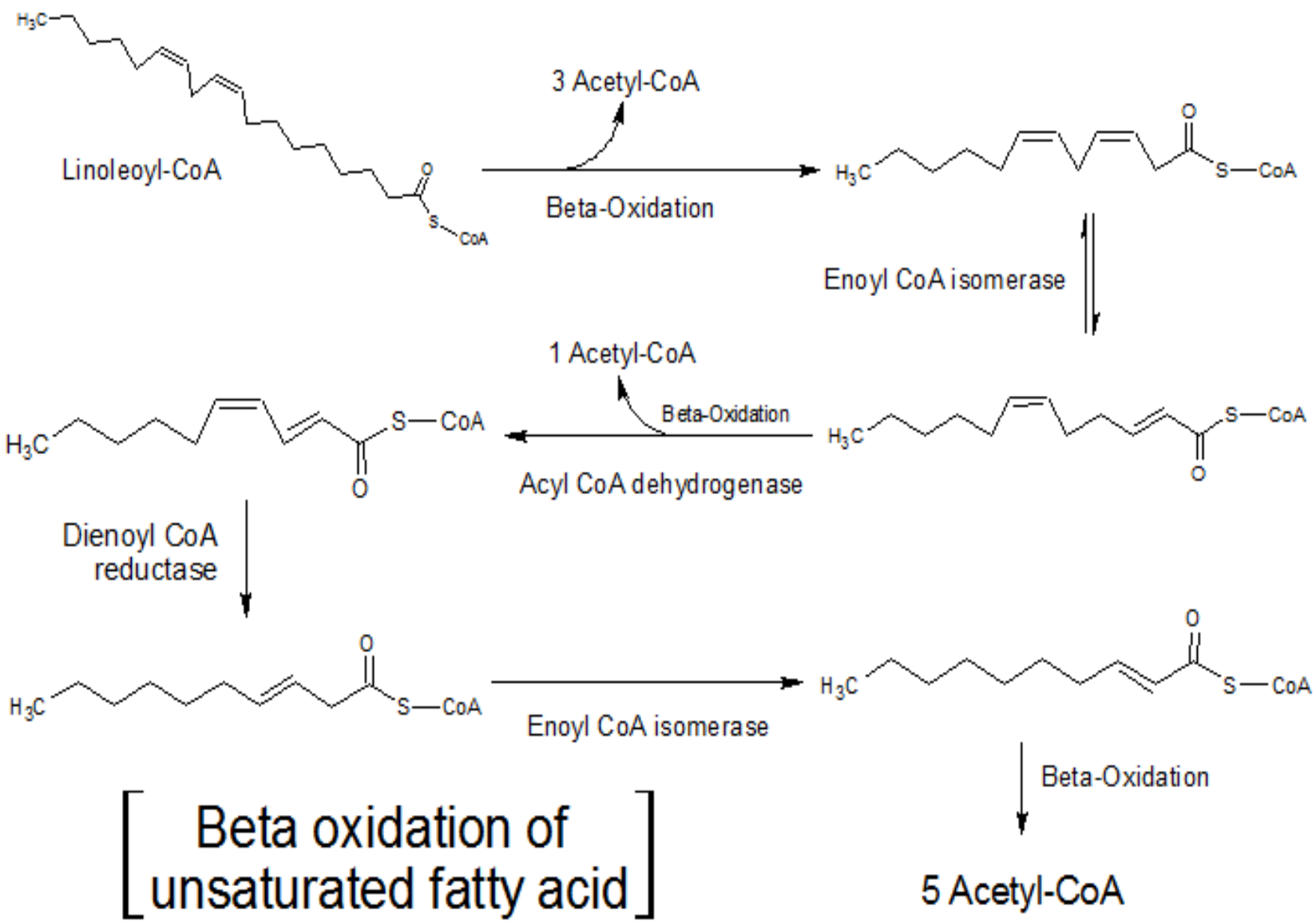

Linoleoyl-CoA
3 Acetyl-CoA
Beta-Oxidation
Enoyl CoA isomerase
1 Acetyl-CoA
Beta-Oxidation
Acyl CoA dehydrogenase
Dienoyl CoA reductase
Enoyl CoA isomerase
Beta-Oxidation
5 Acetyl-CoA
Beta oxidation of unsaturated fatty acid

Table 14.3 Comparison of fatty acid synthesis and oxidation

		Fatty acid synthesis	*β-Oxidation*
1.	Major tissues	Liver, adipose tissue	Muscle, liver
2.	Subcellular site	Cytosol	Mitochondria
3.	Precursor/substrate	Acetyl CoA	Acyl CoA
4.	End product	Palmitate	Acetyl CoA
5.	Intermediates are bound to	Acyl carrier protein	Coenzyme A
6.	Coenzyme requirement	NADPH (supplying reducing equivalents)	FAD and NAD^+ (get reduced)
7.	Carbon units added/degraded	Malonyl CoA	Acetyl CoA
8.	Transport system	Citrate (mitochondria ⟶ cytosol)	Carnitine (cytosol ⟶ mitochondria)
9.	Inhibitor	Long chain acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits carnitine acyltransferase I)
10.	The pathway increased	After rich carbohydrate diet	In starvation
11.	Hormonal status that promotes	High ratio of insulin/glucagon	Low ratio of insulin/glucagon
12.	Status of enzyme(s)	Multifunctional enzyme complex	Individual enzymes

Desaturation of fatty acid chains

A microsomal enzyme system called ***fatty acyl CoA desaturase*** is responsible for the formation of unsaturated fatty acids. This reaction also involves flavin-dependent cytochrome b_5 reductase, NADH and molecular O_2. The monounsaturated fatty acids—namely oleic acid and palmitoleic acid—are, respectively, synthesized from stearate and palmitate.

Mammals lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10. Hence, linoleic acid (18 : 2; 9, 12) and linolenic acid (18 : 3; 9, 12, 15) are essential for man in the diet. However, arachidonic acid (20 : 4; 5, 8, 11, 14) can be synthesized from linoleic acid by desaturation and chain elongation. Arachidonic acid is the precursor for eicosanoids (prostaglandins and thromboxanes), a group of compounds with diversified functions, discussed elsewhere (***Chapter 32***).

SYNTHESIS OF LONG CHAIN FATTY ACIDS FROM PALMITATE

Palmitate is the end product of the reactions of fatty acid synthase system that occurs in cytosol. Further, ***chain elongation*** can take place either in ***mitochondria*** or in ***endoplasmic reticulum*** (microsomes), by separate mechanisms. The microsomal chain elongation is more predominant and involves successive additions of malonyl CoA with the participation of NADPH. These reactions are similar to that catalysed by fatty acid synthase. A specific group of enzymes, namely ***elongases***, bring about fatty acid chain elongation.

The mitochondrial chain elongation is almost a reversal of β-oxidation of fatty acids. Acetyl CoA molecules are successively added to fatty acid to lengthen the chain. The reducing equivalents are derived from NADPH.

SYNTHESIS OF TRIACYLGLYCEROLS

Triacylglycerol (TG) synthesis mostly occurs in ***liver*** and ***adipose tissue,*** and to a lesser extent in other tissues. Fatty acids and glycerol must be activated prior to the synthesis of triacylglycerols. Conversion of fatty acids to acyl CoA by thiokinase is already described (***See Fig. 14.5***).

Synthesis of glycerol 3-phosphate

Two mechanisms are involved for the synthesis of glycerol 3-phosphate

1. In the liver, glycerol is activated by glycerol kinase. This enzyme is absent in adipose tissue.

2. In both liver and adipose tissue, glucose serves as a precursor for glycerol 3-phosphate. Dihydroxyacetone phosphate (DHAP) produced in glycolysis is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate.

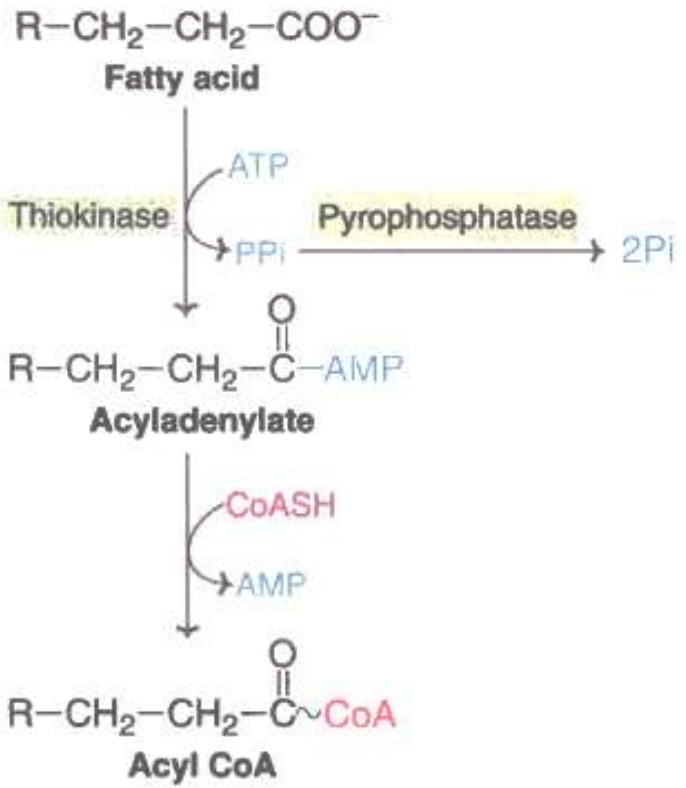


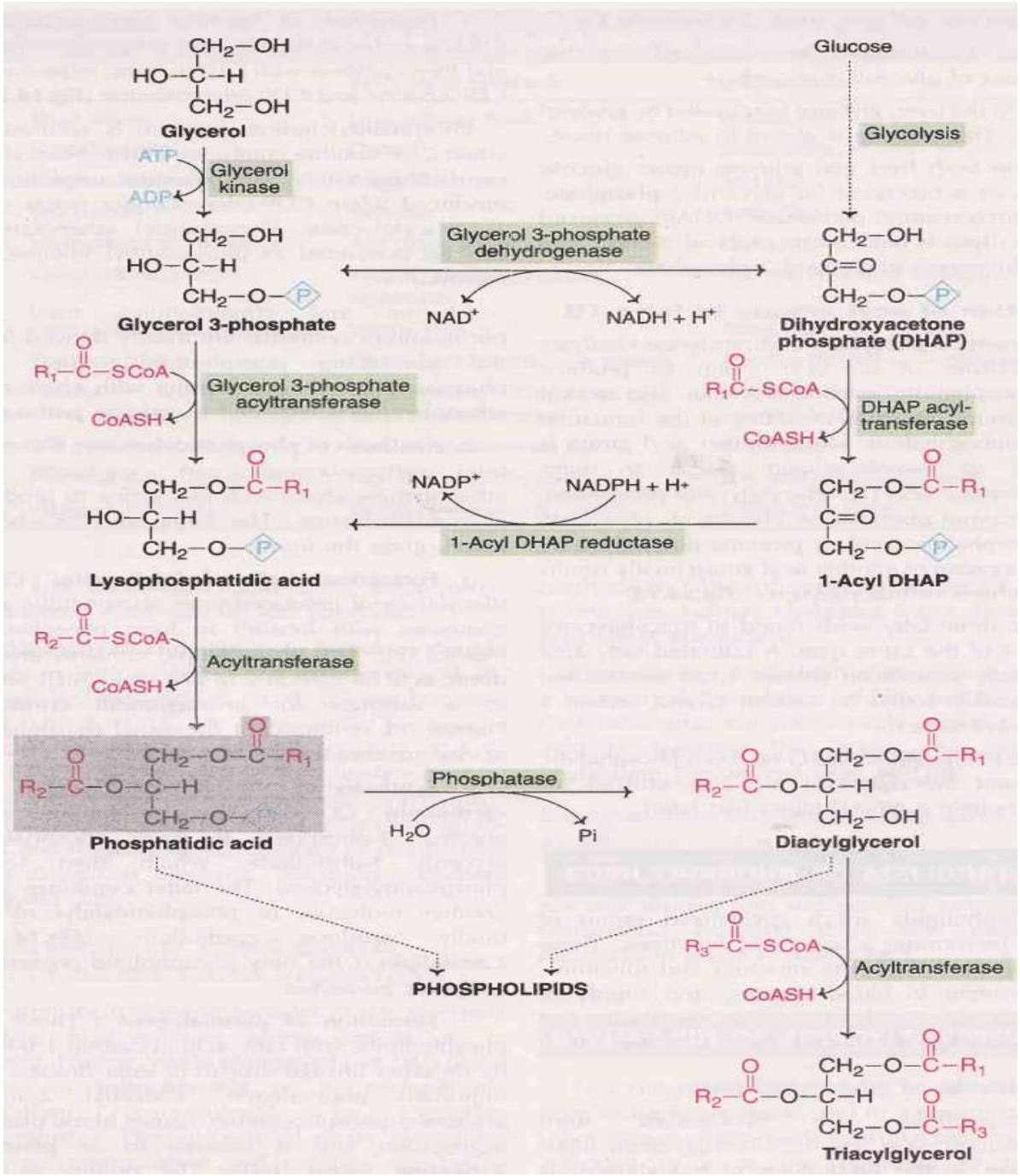
Fig. 14.5 : Activation of fatty acid to acyl CoA by the enzyme thiokinase.

Addition of acyl groups to form TG

Glycerol 3-phosphate acyltransferase catalyses the transfer of an acyl group to produce lysophosphatidic acid. DHAP can also accept acyl group, ultimately resulting in the formation of lysophosphatidic acid. Another acyl group is added to lysophosphatidic acid to form phosphatidic acid (1,2-diacylglycerol phosphate). The enzyme phosphatase cleaves off phosphate of phosphatidic acid to produce diacylglycerol. Incorporation of another acyl group finally results in synthesis of triacylglycerol (***Fig.14.18***).

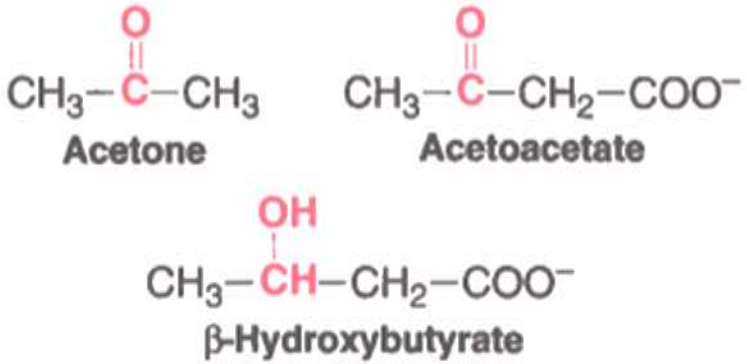
The three fatty acids found in triacylglycerol are not of the same type. A saturated fatty acid is usually present on carbon 1, an unsaturated fatty acid is found on carbon 2, and carbon 3 may have either.

The intermediates of TG synthesis phosphatidic acid and diacylglycerol are also utilized for phospholipid synthesis (described later).


Glycerol
ATP
ADP
Glycerol kinase
Glycerol 3-phosphate
Glycerol 3-phosphate dehydrogenase
NAD+
NADH + H+
Glucose
Glycolysis
Dihydroxyacetone phosphate (DHAP)
CoASH
Glycerol 3-phosphate acyltransferase
DHAP acyl-transferase
NADP+
NADPH + H+
1-Acyl DHAP reductase
Lysophosphatidic acid
1-Acyl DHAP
Acyltransferase
Phosphatase
H2O
Pi
Phosphatidic acid
Diacylglycerol
PHOSPHOLIPIDS
Triacylglycerol

Ketone Bodies

The compounds namely ***acetone, aceto-acetate*** and ***β-hydroxybutyrate*** (or 3-hydroxy-butyrate) are known as ketone bodies (***Fig.14.10***). Only the first two are true ketones while β-hydroxybutyrate does not possess a keto (C=O) group. ***Ketone bodies are water-soluble*** and ***energy yielding***. Acetone, however, is an exception, since it cannot be metabolized.



Utilization of ketone bodies

The ketone bodies, being water-soluble, are easily transported from the liver to various tissues. The two ketone bodies—acetoacetate and β-hydroxybutyrate serve as important sources of energy for the ***peripheral tissues*** such as skeletal muscle, cardiac muscle, renal cortex etc. The tissues which lack mitochondria (e.g. erythrocytes) however, cannot utilize ketone bodies. The production of ketone bodies and their utilization become more significant when glucose is in short supply to the tissues, as observed in ***starvation***, and ***diabetes mellitus***.

Ketoacidosis

Both acetoacetate and β-hydroxybutyrate are strong acids. Increase in their concentration in blood would cause acidosis. The carboxyl group

Ketogenesis

The synthesis of ketone bodies occurs in the ***liver***. The enzymes for ketone body synthesis are located in the ***mitochondrial matrix***. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies. Ketogenesis occurs through the following reactions (***Fig.14.11***).

1. Two moles of acetyl CoA condense to form acetoacetyl CoA. This reaction is catalysed by thiolase, an enzyme involved in the final step of β-oxidation. Hence, acetoacetate synthesis is appropriately regarded as the reversal of thiolase reaction of fatty acid oxidation.

2. Acetoacetyl CoA combines with another molecule of acetyl CoA to produce β-hydroxy β-methyl glutaryl CoA (HMG CoA). ***HMG CoA synthase***, catalysing this reaction, ***regulates the synthesis of ketone bodies.***

3. HMG CoA lyase cleaves HMG CoA to produce acetoacetate and acetyl CoA.

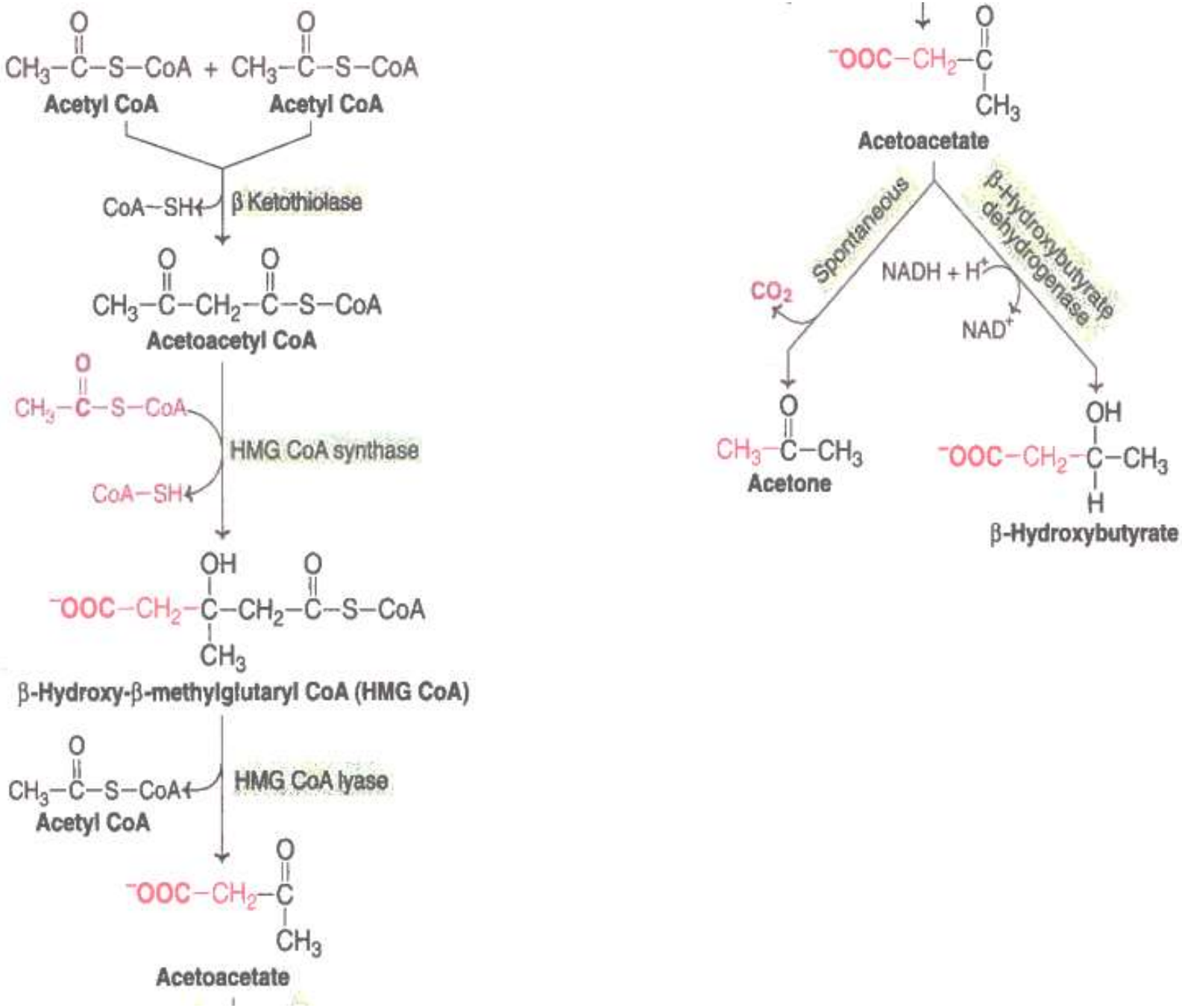
4. Acetoacetate can undergo spontaneous decarboxylation to form acetone.

5. Acetoacetate can be reduced by a dehydrogenease to β-hydroxybutyrate.

The carbon skeleton of some amino acids (ketogenic) is degraded to acetoacetate or acetyl CoA and, therefore, to ketone bodies, e.g. leucine, lysine, phenylalanine etc.

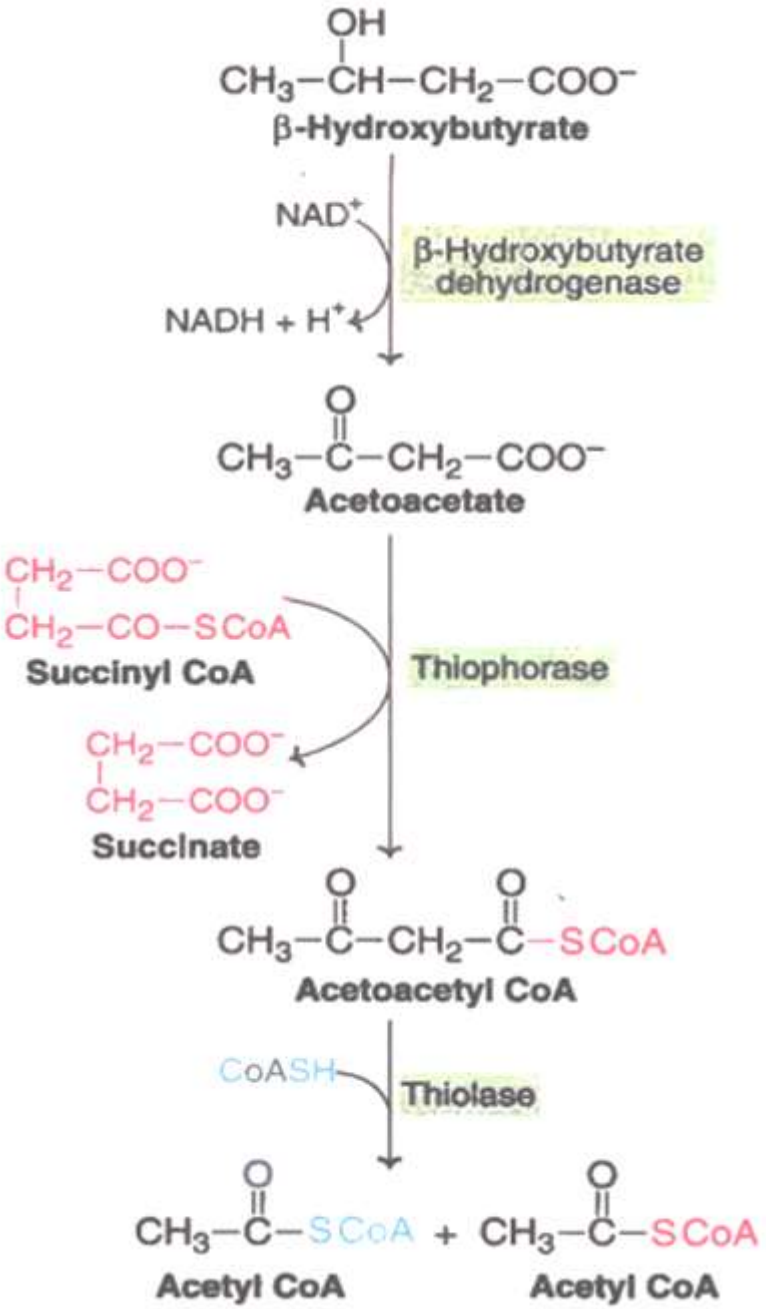
Regulation of ketogenesis

The ketone body formation (particularly overproduction) occurs primarily due to non-availability of carbohydrates to the tissues. This is an outcome of excessive utilization of fatty acids to meet the energy requirements of the cells. The hormone ***glucagon stimulates ketogenesis*** whereas ***insulin inhibits.*** The increased ratio of glucagon/insulin in diabetes mellitus promotes ketone body formation. This is due to disturbances caused in carbohydrate and lipid metabolisms in diabetes, as discussed elsewhere (***Chapter 36***).


Acetyl CoA
Acetyl CoA
CoA–SH
β Ketothiolase
Acetoacetyl CoA
HMG CoA synthase
CoA–SH
β-Hydroxy-β-methylglutaryl CoA (HMG CoA)
HMG CoA lyase
Acetyl CoA
Acetoacetate
Spontaneous
β-Hydroxybutyrate dehydrogenase
NADH + H+
NAD+
CO2
Acetone
β-Hydroxybutyrate

Reactions of ketone bodies : β-Hydroxybutyrate is first converted to acetoacetate (reversal of synthesis) and metabolized. Acetoacetate is activated to acetoacetyl CoA by a mitochondrial enzyme ***thiophorase*** (succinyl CoA acetoacetate CoA transferase). The coenzyme A is donated by succinyl CoA, an intermediate in citric acid cycle. ***Thiophorase is absent in liver,*** hence ketone bodies are not utilized by the liver. Thiolase cleaves acetoacetyl CoA to two moles of acetyl CoA (***Fig.14.12***).

The summary of ketone body synthesis, utilization and excretion is depicted in ***Fig.14.13***.



Overproduction of ketone bodies

In normal individuals, there is a constant production of ketone bodies by liver and their utilization by extrahepatic tissues. The concentration of ketone bodies in blood is maintained around 1 mg/dl. Their excretion in ***urine*** is very low and undetectable by routine tests (***Rothera's test***).

When the rate of synthesis of ketone bodies exceeds the rate of utilization, their concentration in blood increases, this is known as ***ketonemia.*** Ketonemia is predominantly due to incresed production of ketone bodies rather than the deficiency in their utilization. The term ***ketonuria*** represents the excretion of ketone bodies in urine. The overall picture of ketonemia and ketonuria is commonly referred to as ***ketosis.*** Smell of acetone in breath is a common feature in ketosis. Ketosis is most commonly associated with starvation and severe uncontrolled diabetes mellitus.

Starvation : Starvation is accompanied by ***increased degradation of fatty acids*** (from the fuel reserve triacylglycerol) to meet the energy needs of the body. This causes an over-production of acetyl CoA which cannot be fully handled by citric acid cycle. Furthermore, TCA cycle is impaired due to deficiency of oxaloacetate, since most of it is diverted for glucose synthesis to meet the essential requirements (often unsuccessful) for tissues like brain. The result is an accumulation of acetyl CoA and its diversion for ***overproduction of ketone bodies.***

Diabetes mellitus : Diabetes mellitus is associated with insulin deficiency. This results in impaired carbohydrate metabolism and increased lipolysis, both of them ultimately leading to the accumulation of acetyl CoA and its conversion to ketone bodies. In severe diabetes, the ketone body concentration in blood plasma may reach 100 mg/dl and the urinary excretion may be as high as 500 mg/day.

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