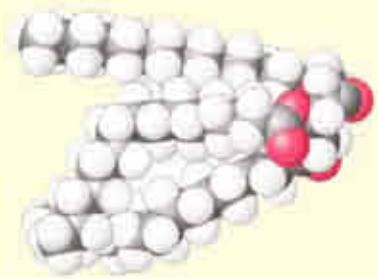
CONTENTS

CHAPTER

25

Biosynthesis of Lipids



Fats such as the triacylglycerol molecule (lower) are widely used to store excess energy for later use and to fulfill other purposes, illustrated by the insulating blubber of whales
The natural tendency of fats to exist in nearly water-free forms makes these molecules well-suited for these roles.
[*Courtesy : Upper Francois Cohier*]

Lipids play a variety of roles. "They are the principal form of stored energy in most organisms, as well as major constituents of cell membranes. Specialized lipids serve as pigments (retinal), cofactors (vitamin K), detergents (bile salts), transporters (dolichols), hormones (vitamin D derivatives, sex hormones), extracellular and intracellular messengers (eicosanoids and derivatives of phosphatidylinositol) and anchors for membrane proteins (covalently attached fatty acids, phenyl groups and phosphatidylinositol)." (Lehninger, Nelson and Cox, 1993). All organisms are, thus, able to synthesize a variety of lipids which are essential to them. It is, therefore, imperative to deal with biosynthetic pathways for some of the principal lipids present in most cells which will illustrate the strategies that are employed in assembling these water-insoluble products from simple, water-soluble precursors such as acetate. *Like other biosynthetic pathways, these reaction sequences are endergonic and reductive*. They use ATP as a source of metabolic energy and a reduced electron carrier (usually NADPH) as a reductant.

NATURE AND DISTRIBUTION OF FAT STORES

Fat is a fuel for long-term storage. Glycogen and starch are fuel meant for *long-term storage* or for the maintenance of organisms in the presence of limited amounts of oxygen. The human epitomizes this dual storage ; the ordinary adult has only enough glycogen to

maintain activity for one day or less but he can live from his fat for nearly a month. A human being is built for a daily routine in which he oxidizes glucose residues for energy immediately after meals while rebuilding glycogen reserves and converting any excess glucose to fatty acids. As the time of the last meal recedes, and glycogen supply again becomes depleted, more and more of his energy is obtained by oxidizing fatty acids previously stored as triglycerides. Even the overnight fast is sufficient to cause the amount of oxygen used for the oxidation of fatty acids to be twice that for the oxidation of glucose from glycogen at rest.

The fat is an ideal stored fuel because it is light in weight and the initial appearance on earth of organisms with large fat deposits evidently coincided with the development of the ability to move over relatively long distances without an intake of food. Salmon and ducks, for instance, are alike in building up large stores of fat before they begin their long migrations, but vertebrates of more fixed domicile, along with many insects, also can store fat for less dramatic exertion.

The importance of fat deposition began to be realized with the evolution of vertebrates and *the liver was the initial site of deposition*. Modern sharks frequently have massive livers containing cells loaded with triglycerides. With the appearance of bony fish, fat began to be deposited mainly in and around the muscle fibres, creating the oily flesh we see in salmon and sardines. Insects followed a different route and created a multipurpose organ with many of the functions of vertebrate liver, but which contains so much fat that it is known as the **fat body**. The advanced vertebrates, starting with some fish, developed a discrete **adipose tissue** by modifying the same kind of cells that produced blood cells. These adipocytes contain globules of triglyceride which constitutes 90% or more of the mass of the cell. Adipose tissue is especially abundant in subcutaneous tissues and can become the largest in the body, comprising 50% or more of the total mass of some individuals. Human can become tubs of lard. Such people are objects of humour, disdain or concern in our society. But the advantage with them is that in societies subject to famine they may be happily living on their own fat while burying the last of their formerly lean companions. In fact, the different parts of an animal cell are the sites of fatty acid metabolism : cytosol for fatty anabolism and formation of acyl-CoA and mitochondria for catabolism of acetyl-CoA by β-oxidation process (Fig. 25–1).

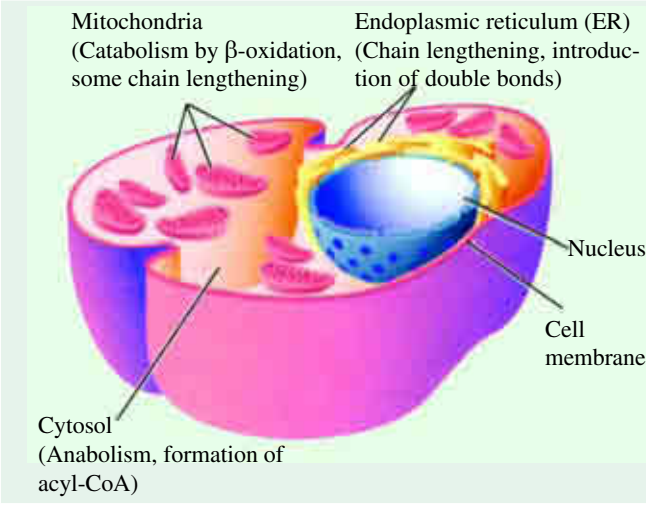


Fig. 25–1. A portion of an animal cell, showing the sites of various aspects of fatty acid metabolism

The cytosol is the site of fatty anabolism. It is also the site of formation of acyl-CoA, which is transported to the mitochondria for catabolism by the β-oxidation process. Some chain-lengthening reactions (beyond C_{16}) take place in the mitochondria. Other chain-lengthening reactions take place in the endoplasmic reticulum (ER), as do reactions that introduce double bonds.

Plants do store fat, especially in the tissues surrounding the embryos in seeds. The light weight of fuel no doubt aids in the dispersal of small seeds, but fat is also the predominant stored fuel in large seeds, where its hydrophobic nature may be of primary importance in protecting the embryo until time for development.

BIOSYNTHESIS OF FATTY ACIDS

When fatty acid oxidation was found to occur by oxidative removal of successive two-carbon

(acetyl-CoA) units, biochemists thought that the biosynthesis of fatty acid might proceed by simple reversal of the same enzymatic steps used in their oxidation. However, *fatty acid synthesis and breakdown occur by different routes, are catalyzed by different sets of enzymes and take place in different parts of the cell.*

Acetyl-CoA Transport into the Cytosol

Acetyl-CoA serves as a key intermediate between lipid and carbohydrate metabolism (Fig. 25–2). For the production of fatty acids, acetyl-CoA (which is produced in mitochondria) must first be transported across the organelle's membrane into the cytosol. Since acetyl-CoA itself cannot traverse the membrane, this transfer relies on the transport of the acetyl moiety as citrate (produced from actyl-CoA and oxaloacetate). After citrate is transferred *via* the tricarboxylate transport system from mitochondria into the cytosol, it is cleaved by **ATP-citrate lyase** to produce acetyl-CoA by the following reaction :

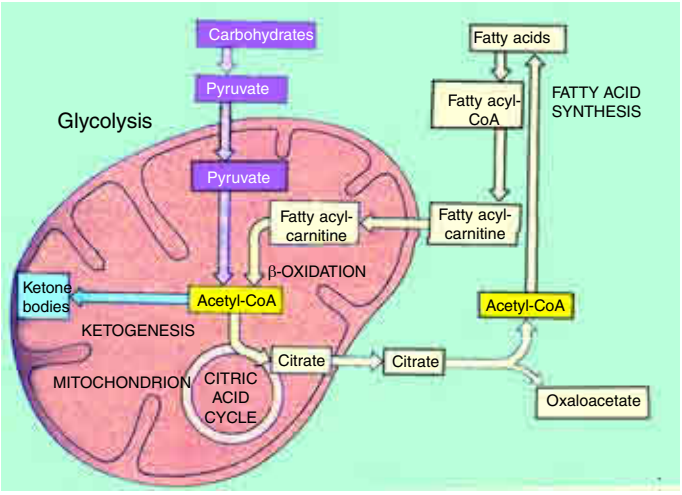


Fig. 25–2. Acetyl-CoA as a key intermediate between fat and carbohydrate metabolism

Arrows identify major routes of formation or utlization of acetyl-CoA. Citrate serves as a carrier to transport acetyl units from the mitor chondrion to the cytosol for fatty acid synthesis.

$$\text{Citrate} + \text{CoA} + \text{ATP} \xrightarrow[\Delta G' = -3{,}400 \text{ cal/mol}]{\text{ATP-citrate lyase}} \text{Acetyl-CoA} + \text{Oxaloacetate} + \text{ADP} + \text{P}i$$

Although carnitine has been assigned the role as a carrier of acetyl groups, as well as of fatty acids, current evidence supports the contention that citrate and not acetylcarnitine is the principal source of cytosolic acetyl-CoA. The acetyl-CoA is now ready to serve as a substrate with the required amounts of ATP and NADPH to form palmitate.

Production of Malonyl-CoA : The Initiation Phase

The production of malonyl-CoA is the initial committed step in the fatty acid synthesis. In 1961, Salih Vakil's observation that CO_2 greatly stimulates the incorporation of acetyl-CoA into fatty acid structures was an important finding in the elucidation of this process. In fact, his studies revealed that acetyl-CoA must be converted, rather carboxylated, into **malonyl-CoA** prior to its utilization for fatty acid synthesis. This irreversible two-step reaction is the committed step in fatty acid synthesis and, as would be expected, it is also the primary rate-limiting reaction of the process. The reaction is catalyzed by the enzyme, acetyl-CoA carboxylase.

The acetyl-CoA carboxylase from bacteria is a multienzyme complex and consists of 3 separate, functional polypeptide subunits (Fig. 25–3) :

(*a*) *biotin carboxyl carrier protein*, BCP (MW = 45,000), containing two identical subunits each of which has one mole of biotin as its prosthetic group, covalently bound in amide linkage to an ε-amino group of a lysine residue,

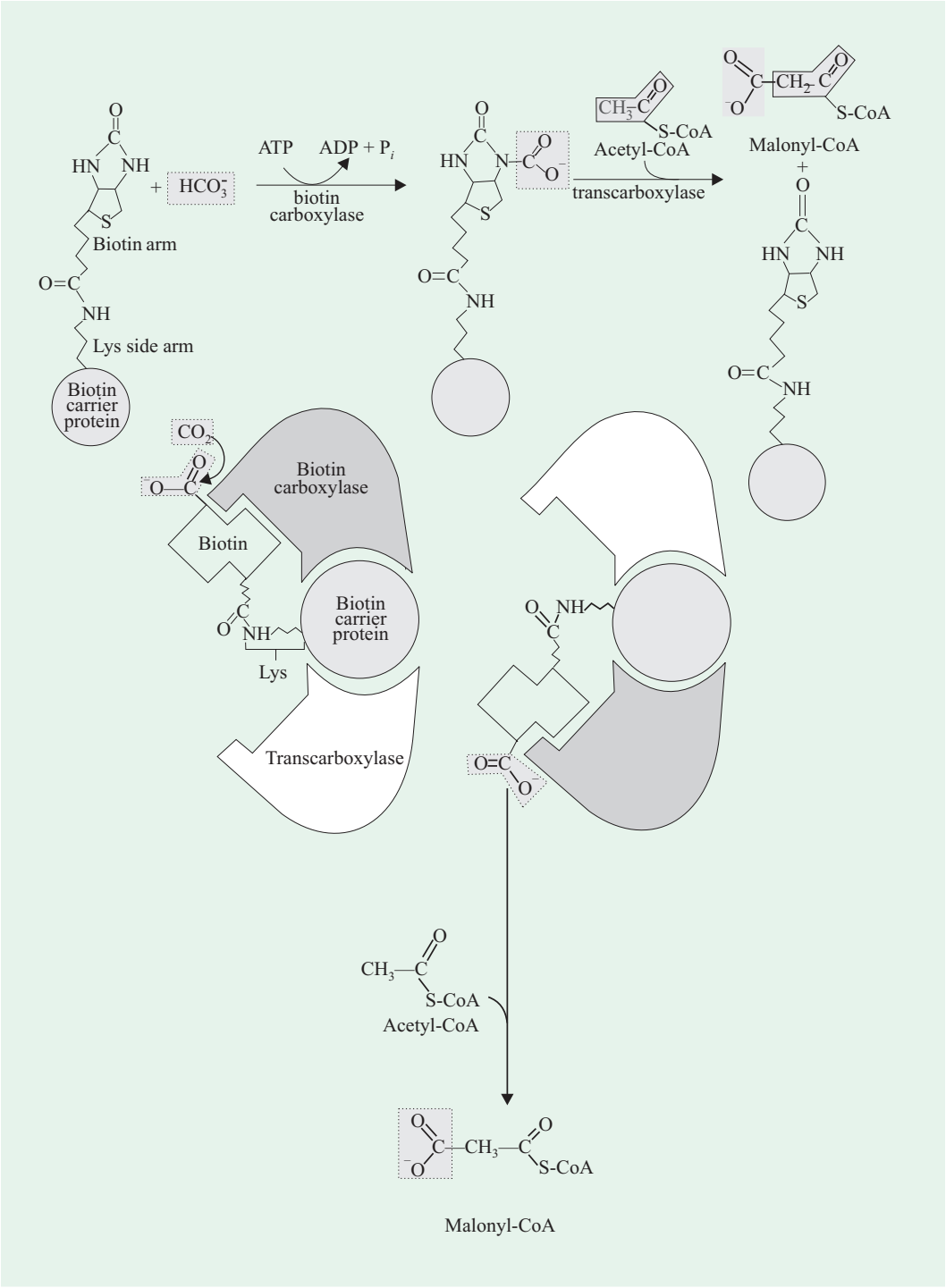


Fig. 25–3. The acetyl-CoA carboxylase reaction

Note that the long, flexible, biotin arm carries the activated CO_2 from the biotin carboxylase region to the trans-carboxylase active site, as shown in the lower diagrams. The active enzyme in each case is dark shaded.

(*Redrawn from Lehninger, Nelson and Cox, 1993*)

(*b*) *biotin carboxylase*, BC (MW = 98,0000), an enzyme with two identical subunits and which catalyzes carboxylation of the biotin unit in biotin carboxyl carrier protein in an ATP-dependent reaction, and

(*c*) *transcarboxylase*, TC (MW = 1,30,000), an enzyme with two pairs of subunits of molecular weight 35,000 and 30,000 respectively, and which catalyzes the transfer of activated CO_2 unit from carboxybiotin to acetyl-CoA, producing malonyl-CoA.

In yeast, higher plants and animals, the activities of all the three subunits are present in a single biotin-containing polypeptide chain (MW ≈ 2,20,000).

Malonyl-CoA is synthesized in two steps by the action of two enzymes, each of which employs the biotin carrier protein as one substrate. The two steps are :

First Step : The biotin carboxylase (BC) catalyzes carboxylation of biotin carboxyl carrier protein (BCP) to yield carboxybiotin carboxyl carrier protein (BCP–COO^-) ; the carboxyl group being derived from bicarbonate (HCO_3^-). This is an ATP-dependent reaction.

$$\underset{\text{(Biotinyl-enzyme)}}{BCP} + HCO_3^- + ATP \xrightarrow{\text{Biotin carboxylase}} \underset{\text{(Carboxybiotinyl enzyme)}}{BCP{-}COO^-} + ADP + Pi \qquad ...(1)$$

Second Step : The transcarboxylase transfers the "bound" CO_2 from BCP–COO^- to acetyl-CoA, forming malonyl-CoA and regenerating BCP.

$$BCP{-}COO^- + CH_3CO{-}SCoA \xrightarrow{\text{Transcarboxylase}} BCP + {}^-OOC{-}CH_2CO{-}SCoA \qquad ...(2)$$

The free energy of cleavage of carboxybiotin protein, ΔG° = 4.7 kcal/mole at pH 7.0, is sufficient to allow the compound to act as a carboxylating agent in reaction (2) as well as in other reactions with suitable acceptors. The exergonic nature of the cleavage also explains the requirement for ATP for formation of the carboxybiotin protein.

Thus, the substrates are bound to acetyl-CoA carboxylase and products are released in a specific sequence (Fig. 25–4). *Acetyl-CoA carboxylase exemplifies a ping-pong reaction mechanism* in which one or more products are released before all the substrates are bound.

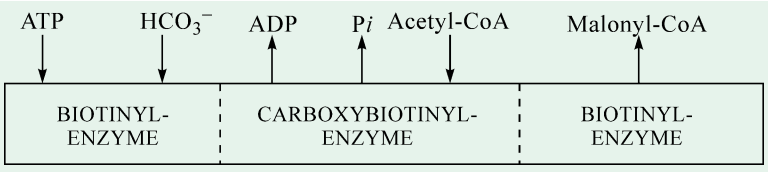


Fig. 25–4. The reaction sequence of acetyl-CoA carboxylase

Note that these reactions are "CO_2 -fixation" processes in which inorganic CO_2 is used, even by animals, to form organic compounds. The overall result of these two reactions would be the production of a mole of malonyl-CoA by the addition of a mole of CO_2 (actually as HCO_3^-) to a mole of acetyl-CoA ; the ATP mole providing energy for driving the reaction. The net equation then would be :

$$\underset{\text{(Acetyl-CoA)}}{CH_3CO - SCoA} + HCO_3^- + ATP \xrightarrow{\text{Acetyl-CoA carboxylase}} \underset{\text{(Malonyl-CoA)}}{{}^-OOC - CH_2CO - SCoA} + ADP + Pi$$

The malonyl-CoA provides 14 out of 16 carbon atoms of palmitate.

This reaction is very similar to other biotin-dependent carboxylation reactions, such as those catalyzed by pyruvate carboxylase and propionyl-CoA carboxylase.

Acetyl-CoA carboxylase is also important because it is a regulatory step ; citrate acts as an allosteric activator for the animal enzyme, but not in plant or microbial systems. The high degree of structural organization of the animal carboxylases, which are absent in their counterparts in plants, yeast and *Escherichia coli*, suggests a possible structural role, in addition to their known catalytic and regulatory functions. It could serve as an organizing matrix for a supramolecular (multienzyme) complex with other enzymes which take part in lipid biosynthesis.

Intermediates in Fatty Acid Synthesis and the ACP

Vagelos (1964) discovered that *the intermediates in fatty acid synthesis are linked to an acyl carrier protein, ACP* (*MW = # 9,000*). Specifically, the intermediates are attached to the sulfhydryl (–SH) terminus of a phosphopantetheine group (Fig. 25–5). In the degradataion of fatty acids, this

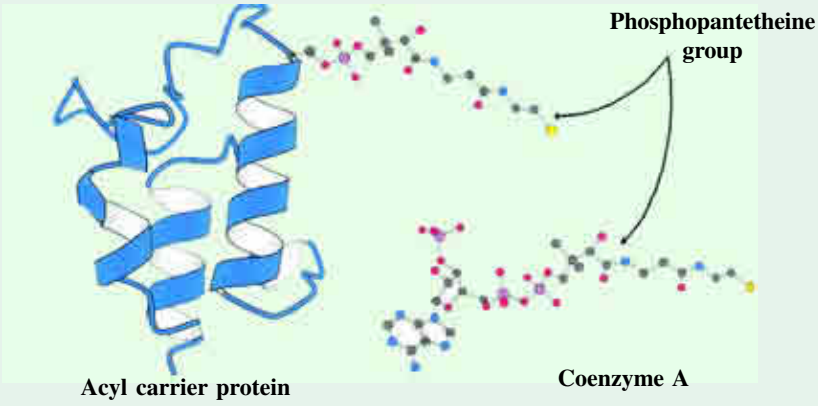


Fig. 25–5. Phosphopantetheine
Both acyl carrier protein and CoA include phosphopantetheine as their reactive units

unit is part of the CoA ; whereas in synthesis, it is attached to a serine residue of the ACP (Fig.25–6). This single polypeptide chain of 77 residues can be regarded as a giant prosthetic group, a "*macro-CoA*". The molecule apparently contains no cysteine.

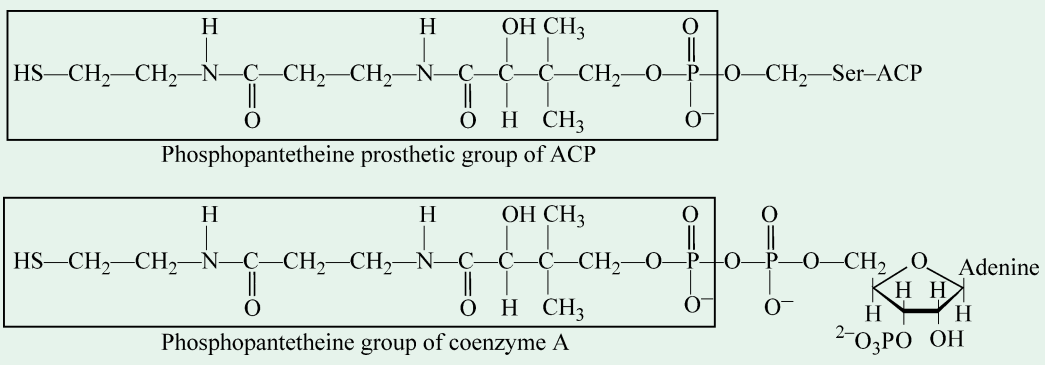


Fig. 25–6. Phosphopantetheine unit of ACP and CoA
Note that in the upper figure, the fatty acid binds to the prosthetic group by forming a thioester bond with the sulfhydryl group. In other words, the —SH group is the site of entry of malonyl groups during fatty acid synthesis.

Acyl carrier protein (ACP) of *Escherichia coli* is a small protein (Relative molecular mass, Mr = 8,860) containing the prosthetic group 4′–phosphopantetheine (Pn), an intermediate in the synthesis of coenzyme A. The thioester bond that links ACP to the fatty acyl group has a high free energy of hydrolysis. And when this bond is broken, energy is released which makes the first reaction in fatty acid synthesis (*i.e.*, condensation reaction) thermodynamically favourable. The 4′–phosphopantetheine prosthetic group of ACP serves as a flexible arm, tethering the growing fatty acyl chain to the surface of the fatty acid synthase complex and carrying the reaction intermediates from one enzyme active site to the other.

The Fatty Acid Synthase Complex

All of the reactions in the biosynthesis of fatty acids are catalyzed by a multienzyme complex, the fatty acid synthase. The detailed structure of this multienzyme complex and its location in the cell differ from one species to another, but the reaction sequence is identical in all organisms. The fatty acid-synthesizing systems from 3 sources have been investigated in some detail : that from yeast by Lynen (1952), with a particle molecular weight of 2.3×10^6 ; that from pigeon liver by Wakil (1961), with a molecular weight of 4.5×10^5 ; and that from *Escherichia coli* by Vagelos (1964). Of these systems, that from *E.coli* is perhaps the best understood at present.

Fatty acid synthase is frequently named **fatty acid synthetase**, but its action does not fit the definition of a synthetase.

The **fatty acid synthase** system from *E.coli* consists of 7 separate polypeptides (and hence 7 different active sites) that are tightly associated in a single, organized complex (Table 25–1). The proteins act together to catalyze the formation of fatty acids from acetyl-CoA and malonyl-CoA. Throughout the process, the intermediates remain covalently attached to one of the two thiol (—SH) groups of the complex. The growing fatty acid is shifted between these two —SH groups. *One* is relatively fixed in position because it is on a cysteine residue. It acts as a parking place for acyl groups that are to be lengthened. The *other* —SH group carries the extended chain while it undergoes the reactions necessary for reduction to a saturated acyl group, and it also accepts the acetyl and malonyl groups from which the fatty acid is built. This —SH group can swing across the 7 different catalytic sites because it is located in a residue of phosphopantetheine.

Table 25–1. Seven components* of the fatty acid synthase complex from *Escherichia coli*

Component	*Abbreviation*	*E.C. No.*	*Role*
Acyl carrier protein	ACP	6.4.1.2	Carries acyl groups in thioester linkage
Acyl-CoA–ACP transacetylase	AT	2.3.1.9	Transfers acyl group from CoA to cysteine residue of KS
Malonyl-CoA–ACP transferase	MT	2.8.3.3	Transfers malonyl group from CoA to ACP
β-ketoacyl–ACP synthase	KS	2.3.1.16	Condenses acyl and malonyl groups
β-ketoacyl–ACP reductase	KR	1.1.1.36	Reduces β-keto group to β-hydroxy group
β-hydroxyacyl–ACP dehydrogenase	HD	4.2.1.17	Removes H_2O from β-hydroxyacyl-ACP, creating double bond
Enoyl–ACP reductase	ER	1.3.99.2	Reduces double bond, forming saturated acyl-ACP

* ACP has the specific task of binding the acyl intermediates during fatty acid synthesis. Of the 7 components, *ACP is not an enzyme* while the remaining are enzymatic in behaviour.

The two thiol groups are designated as 'central' and 'peripheral'. The 'central' one is the —SH group of acyl carrier protein (ACP), with the intermediates of fatty acid synthesis form a thioester and the 'peripheral' one is the —SH group of a cysteine residue in β-ketoacyl-ACP synthase, one of the 7 proteins of the multienzyme complex.

Thus, we see that the bacteria contain separate proteins to catalyze the individual reactions of fatty acid synthesis ; even the formation of malonyl-CoA occurs in 2 stages (carboxylation of biotin and transfer of the —COO^- group to acetyl-CoA). This lucky circumstance made it much easier to discover the sequence of reactions, since each reaction could be studied separately.

The Fatty Acid Synthase From Some Organisms

It may, however, be noted that the 7 active sites for fatty acid synthesis (6 enzymes + ACP) reside in 7 separate polypeptides in the fatty acid synthase of *Escherichia coli* ; the same holds good for the enzyme complex from **higher plants** (Fig. 25–7). In these complexes, each enzyme is positioned with its active site near that of the preceding and succeeding ezymes of the sequence. The flexible pantetheine arm of ACP can reach all of the active sites, and it carries the growing fatty acyl chain from one site to the next ; the intermediates are not released from the enzyme complex until the finished product is obtained.

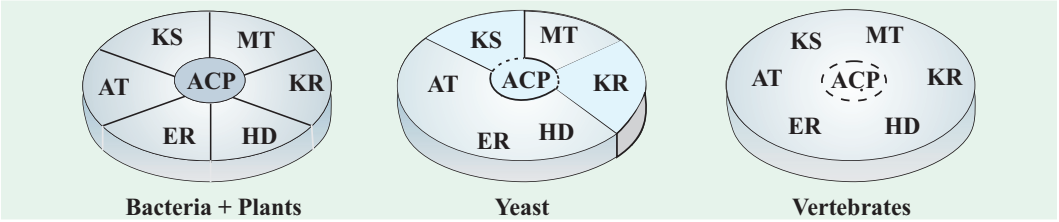


Fig. 25–7. A comparison among the fatty acid synthase complexes from different sources

Note that the fatty acid synthase from **bacteria** and **plants** is a complex where all seven activities reside in seven separate polypeptides. In **yeast**, all 7 activities reside in only 2 polypeptides. And in **vertebrates**, the 7 activities reside in a single large polypeptide.

The fatty acid synthases of yeast and of vertebrates are also multienzyme complexes, but their integration is even more complete than in *E.coli* and higher plants. In **yeast**, the seven distinct active sites reside in only two large, multifunctional polypeptides, and in **vertebrates**, a single large polypeptide (Relative molecular mass, M_r = 2,40,000) contains all seven enzymatic activities as well as a hydrolytic activity that cleaves the fatty acid from the ACP-like part of the enzyme complex. The active form of this multienzyme protein is a dimer (M_r = 4,80,000).

The organized structure of the fatty acid synthases of yeast and higher organisms enhances the efficiency of the overall process because of the following reasons :

1. The intermediates are directly transferred from one active site to the next.
2. The intermediates are not diluted in the cytosol.
3. The intermediates do not have to find each other by random diffusion.
4. The covalently-bound intermediates are secluded and protected from competing reactions.

Priming of the Fatty Acid Synthesis by Acetyl-CoA : The Priming Phase

The sequence of events that occurs during synthesis of a fatty acid is listed in Fig. 25–8. Before the condensation reactions, that build up the fatty acid chain, can begin, the two —SH groups on the enzyme complex must be charged with the correct acyl groups. The 'priming' of the system, as it is called, takes place in 2 steps :

In the *first step*, the acetyl group of acetyl-CoA is tranferred to the cysteine—SH group of the β-ketoacyl-ACP synthase. This reaction is catalyzed by **acetyl-CoA-ATP transacetylase.**

In the *second step*, the malonyl group from malonyl-CoA is transferred to the —SH group of ACP by the enzyme **malonyl-CoA-ACP transferase**, also part of the complex.

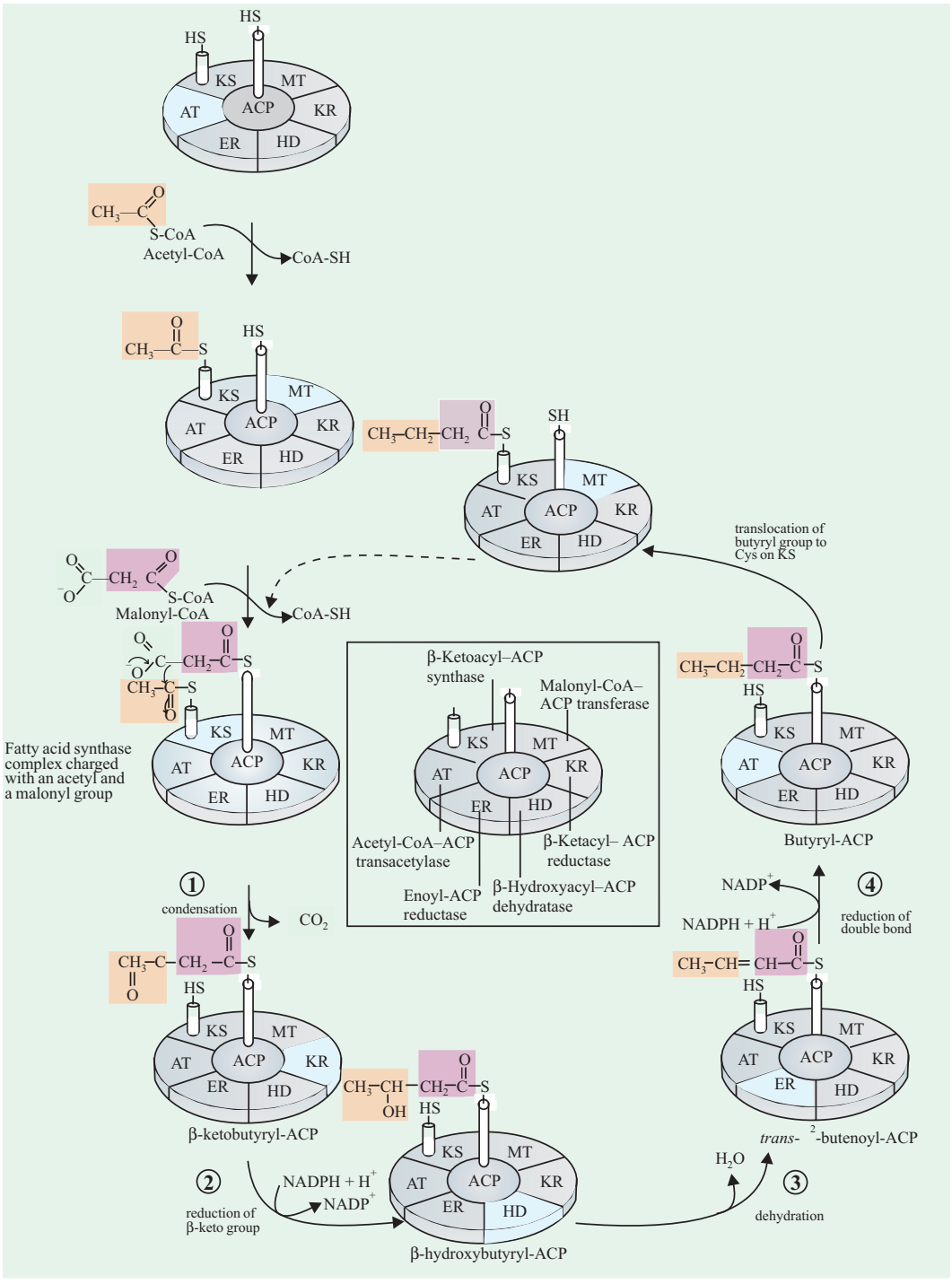


Fig. 25–8. The sequence of events occuring during fatty acid synthesis

The fatty acid synthase complex is shown schematically. Each segment of the disc represents one of the 6 enzymatic activities of the complex : acetyl-CoA–ACP transacetylase (AT); malonyl-CoA–ACP transferase (MT) ; β-ketoacyl–ACP synthase (KS), containing a critical Cys-SH residue ; β-ketoacyl–ACP reductase (KR); β-hydroxyacyl–ACP dehydratase (HD) ; and enoyl–ACP reductase (ER). At the centre is acyl carrier proteins (ACP) with its phosphopantetheine arm (Pn) ending in another —SH.

Growth of the Fatty Acyl Chain by Two Carbons : The Elongation Phase

***First Round* :** In the charged synthase complex, the acetyl and malonyl groups are very close to each other and are activated for the chain-lengthening process, which consists of the following four steps (or reactions) :

1. Condensation. The first step in the formation of a fatty acid chain is condensation of the activated acetyl and malonyl groups to form an acetoacetyl group bound to ACP through the phosphopantetheine —SH group, thus producing **acetoacetyl-ACP** ; simultaneously, a mole of CO_2 is eliminated from the malonyl group. In this reaction, catalyzed by β-*ketoacyl-ACP synthase*, the acetyl group is transferred from the cysteine —SH group of this enzyme to the malonyl group on the —SH of ACP, becoming the methyl-terminal two-carbon unit of the new acetoacetyl group. The carbon atom in the CO_2 formed in this reaction is the same carbon atom that was originally introduced into malonyl-CoA from HCO_3^- by the acetyl-CoA carboxylase reaction. Thus, CO_2 is only transiently in covalent linkage during fatty acid biosynthesis ; it is removed as such as each two-carbon unit is inserted. Thus, the net effect of condensation reaction is the extension of the acyl chain by 2 carbon atoms. Thus, the first condensation reaction in the biosynthesis of a fatty acid may be diagrammatically represented as in Fig. 25–9.

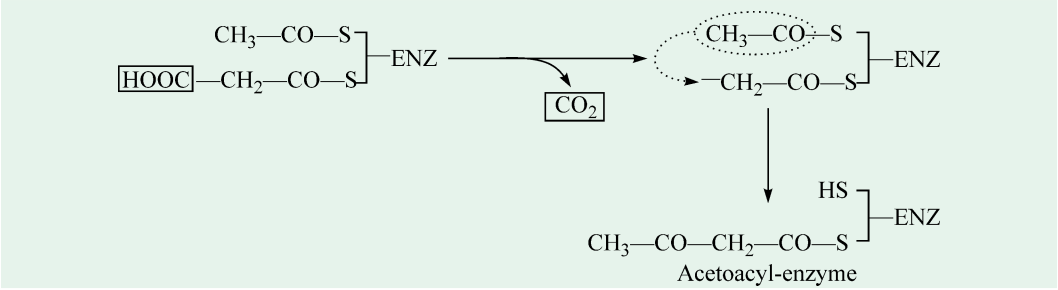


Fig. 25–9. First condensation reaction in the biosynthesis of a fatty acid

By using activated malonyl groups in the synthesis of fatty acid and activated acetate in their degradation, the cell manages to make both processes favourable, although one is effectively the reversal of the other. The extra energy, needed to make fatty acid synthesis favourable, is provided by the ATP used to synthesize malonyl-CoA from acetyl-CoA and HCO_3^- (Fig. 25–3). In effect, the condensation reaction is driven by ATP, although ATP does not directly participate in the condensation reaction. Rather, ATP is used to form an energy-rich substrate in the carboxylation of acetyl-CoA to malonyl-CoA. The free energy stored in malonyl-CoA in the carboxylation reaction is released in the decarboxylation accompanying the formation of acetoacetyl-ACP. Although HCO_3^- is required for fatty acid synthesis, its carbon does not appear in the product. Rather, all of the carbon atoms of even-chain fatty acids are derived from acetyl-CoA.

The next 3 steps in fatty acid synthesis reduce the keto (> CO) group at C-3 to a methylene (—CH_2—) group, the result being the conversion of acetoacetyl-ACP into butyryl-ACP.

2. Reduction of the Carbonyl group. The acetoacetyl group formed in the condensation step next undergoes reduction of the carbonyl group at C-3 to form **D-β-hydroxybutyryl-ACP.** This reaction is catalyzed by β-ketoacyl-ACP reductase and the electron donor is NADPH. This reaction differs from the corresponding one in fatty acid degradation in two respects :

(*a*) The D- rather than the L-epimer is formed

(*b*) NADPH is the reducing agent, whereas NAD^+ is the oxidizing agent in β oxidation. This difference exemplifies the general principle that *NADPH is consumed in biosynthetic reactions, whereas NADH is generated in energy-yielding reactions.*

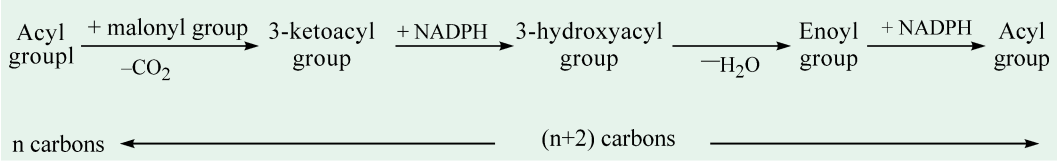
3. Dehydration. In the third step, the elements of water are removed from C-2 and C-3 of d-β-hydroxybutyryl-ACP to yield a double bond in the product, **trans-Δ^2-butenoyl-ACP** (also

called **crotonyl-ACP**). The enzyme that catalyzes this dehydration is β-*hydroxyacyl-ACP dehydratase.*

4. Reduction of the double bond. Finally, the double bond of *trans*-Δ^2-butenoyl-ACP is reduced (or saturated) to form **butyryl-ACP** by the enzymatic action of *enoyl-ACP reductase* ; again NADPH is the electron donor or the reductant. Note that FAD^+ is the oxidant in the corresponding reaction in β oxidation.

These 4 reactions, taken together, complete the first round of elongation cycle. Thus, after the first round of elongation, the C_4 (butyryl) precursor of palmitate has been synthesized from a C_2 (acetyl) and a C_3 (malonyl) unit, with the acetyl group constituting the two terminal carbons of the growing fatty acid chain (C_{15} and C_{16} in palmitate, for example).

The general sequence of condensation and reduction by fatty acid synthase may be schematically represented as :



Successive Rounds : The production of C-4 saturated fatty acyl-ACP (*i.e.*, a C_4-butyryl-ACP) completes one round through the fatty acid synthase complex in fatty acid synthesis. During the second round of elongation phase, the butyryl group is now transferred from the phosphopantetheine —SH group of ACP to cysteine —SH group of β-ketoacyl-ACP synthase (KS). To start the next cycle of 4 reactions, that lengthens the chain by 2 more carbons, another malonyl group is linked to the now vacant phosphopantetheine —SH group of ACP. Condensation occurs as the butyryl group, acting exactly as did the acetyl group in the first round, is linked to two carbons of the malonyl-ACP with simultaneous release of a mole of CO_2. The product of this condensation is a C-6 acyl group, covalently bound to the phosphopantetheine —SH group of ACP (*i.e.*, a **C_6-β-ketoacyl-ACP**). Its β-keto group is reduced in the next 3 reactions of the second round of synthesis cycle to yield the C-6 saturated fatty acyl-ACP (*i.e.*, a **C_6-fatty acyl-ATP**), exactly as in the first round of reactions.

The C_6-fatty acyl-ACP is now ready for a third round of elongation. Seven such cycles of condensation and reduction produce the **C-16 saturated palmitoyl group**, still bound to ACP. This intermediate is not a substrate for the condensing enzyme, β-ketoacyl-ACP synthase (KS) and the chain elongation generally stops at this point. Rather, it is hydrolyzed to yield **palmitate** and ACP. Small amounts of longer-chain fatty acids such as stearate (18 : 0) are also formed. In certain plants (coconut and palm, for example), chain termination occurs earlier ; a majority of the fatty acids (up to 90%) in the oils of these plants contain between 8 and 14 carbon atoms. Thus, we see that the fatty acid synthase reactions are repeated to form palmitate. The origin of carbon atoms in palmitic acid is as shown in Fig. 25–10.

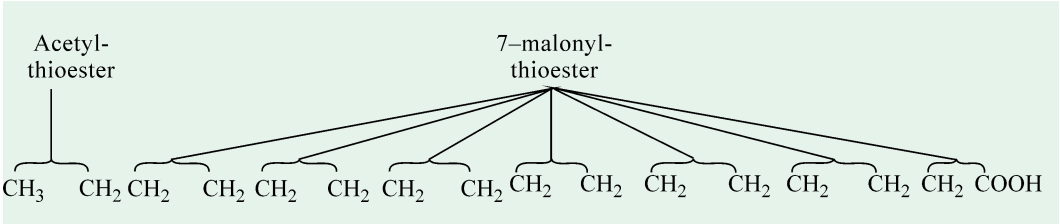


Fig. 25–10. The origin of carbon atoms in palmitic acid

Stoichiometry of Fatty Acid Synthesis

The overall reaction for the synthesis of palmitate from acetyl-CoA can be broken down into 2 parts :

(*a*) *the formation of 7 malonyl-CoA molecules*

$$7\ \text{Acetyl-CoA} + 7\ CO_2 + 7\text{ATP} \longrightarrow 7\ \text{Malonyl-CoA} + 7\ \text{ADP} + 7\ P_i \quad ...(i)$$

(*b*) *the 7 cycles of condensation and reduction*

$$\text{Acetyl-CoA} + 7\ \text{Malonyl–CoA} + 14\ \text{NADPH} + 14\ H^+ \longrightarrow \text{Palmitate} + 7\ CO_2 + 8\ \text{CoA} + 14\ NADP^+ + 6\ H_2O \quad ...(ii)$$

Hence, the overall process (the sum of Equations *i* and *ii*) for the synthesis of palmitate is :

$$8\ \text{Acetyl-CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 14\ H^+ \longrightarrow \text{Palmitate} + 8\ \text{CoA} + 7\ \text{ADP} + 7\ P_i + 14\ NADP^+ + 6\ H_2O \quad ...(iii)$$

Note that the CO_2 utilized (formation of malonyl-CoA) and the CO_2 produced (condensation reaction) cancel each other when the overall stoichiometry is tabulated.

The biosynthesis of fatty acids such as palmitate, thus, requires acetyl-CoA and the input of chemical energy in 2 forms : the group transfer potential of ATP and the reducing power of NADPH. The ATP is required to attach CO_2 to acetyl-CoA to produce malonyl-CoA ; the NADPH is required to reduce the double bonds to form the corresponding saturated fatty acyl group.

Comparison of Fatty Acid Synthesis and Degradation

Although fatty acid synthesis and degradation represent two independent cellular mechanisms, the two systems share many similarities. The one consistent feature of all synthetic and catabolic reactions is that they have a strict specificity for fatty acyl derivatives, either those of CoA or those of ACP. As illustrated in Fig. 25–11, the last 3 steps in synthesis are the biochemical reversal of the 3 key catabolic reactions of β oxidation.

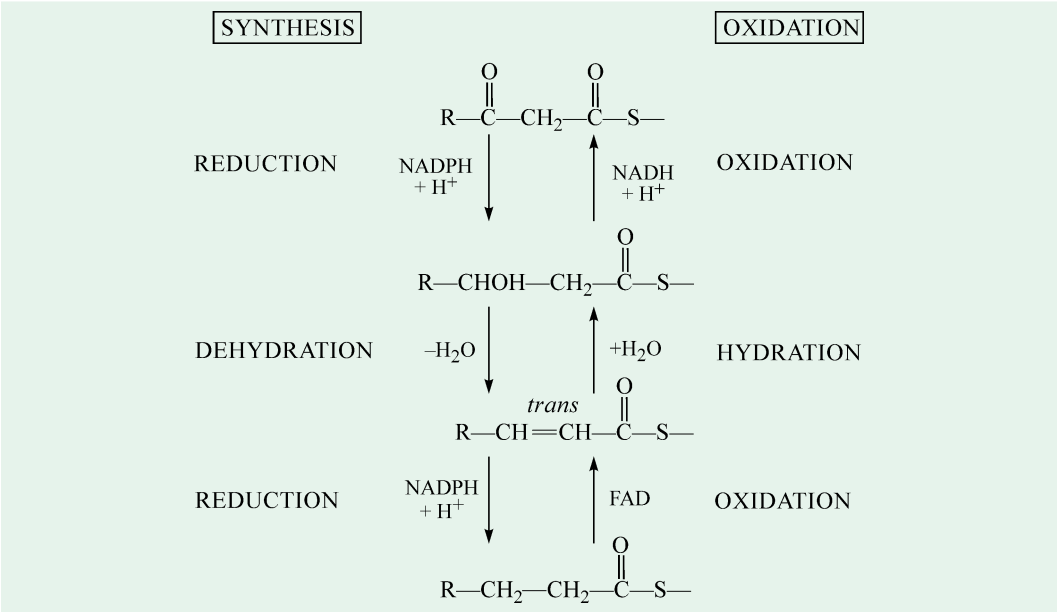


Fig. 25–11. Comparison of fatty acid synthesis and β oxidation

Since each step in the enzymatic pathway responsible for the oxidation of fatty acids is

reversible, and also that acetyl-CoA is the starting material, the proposition was advanced that fatty acid biosynthesis might occur by simple reversal of fatty acid oxidation. Rather, fatty acid oxidation consists of a new set of reactions, which once again exemplifies the principle that *synthetic and degradative pathways in biological systems are usually distinct*. The two processes (fatty acid synthesis and oxidation) are distinct from each other in following respects :

1. Fatty acid synthesis (lipogenesis) takes place in the soluble *cytosol* fraction of many tissues, including liver, kidney, brain, lung and adipose tissue, in contrast with fatty acid degradation which occurs principally or entirely in the mitochondrial matrix.
2. The intermediates in fatty acid synthesis are covalently linked to the sulfhydryl groups of an *acyl carrier protein* (ACP), whereas the intermediates in the fatty acid breakdown are bonded to coenzyme A.
3. Many of the enzymes of fatty acid synthesis in higher organisms are organized into a multienzyme complex called the *fatty acid synthase*. In contrast, the degradative enzymes do not seem to be associated.
4. The growing fatty acid chain is elongated by the sequential addition of two-carbon units, derived from acetyl-CoA. The activated donor of two-carbon units in the elongation step is *malonyl*-ACP. The elongation reaction is driven by the release of CO_2.
5. Elongation of the fatty acid synthase complex stops upon formation of *palmitate* (C_{16}). In other words, free palmitate is the main end product. Further elongation and the insertion of double bonds are carried out by other enzyme systems.
6. The source of CO_2 in fatty acid synthesis is *bicarbonate* (HCO_3^-) whereas bicarbonate has no effect upon fatty acid oxidation.
7. Also, C-3 intermediate, *malonyl-CoA* participates in the biosynthesis of fatty acids but not in their breakdown.
8. The reductant in fatty acid synthesis is *NADPH*, whereas NAD^+ and FAD serve as electron acceptors in the oxidative pathway. This difference is an excellent example of the concept that *NAD^+ and NADPH coenzymes are used in catabolic and biosynthetic reactions, respectively*.
9. The activating groups in the synthetic sequence are two different enzyme-bound thiol (–SH) groups ; whereas in the degradative process, the activating group is the –SH group of coenzyme A.
10. During fatty acid synthesis, the substrates are *ACP-S derivatives*, whereas fatty acid oxidation involves the action of the enzymes on CoA derivatives as substrates.
11. Also, during fatty acid synthesis, it is the D(–)β-hydroxyacyl–S–ACP derivative that is substrate, whereas during fatty acid oxidation, it is the CoA derivative of the L(+) β-hydroxyacyl compound, that is the substrate. Thus, the configurations of the β-hydroxyacyl intermediates differ in the two systems.

Table 25–2. lists a comparison of compounds involved in fatty acid metabolism.

Table 25–2. Comparison of compounds involved in fatty acid metabolism

Step / Component	*Degradation*	*Synthesis*
SH component	CoA	Acyl carrier protein
Intemediate SH derivative	Acetyl-CoA	Malonyl ACP + acetyl ACP
Keto ↔ hydroxy	NAD, L-β-hydroxybutyryl CoA	NADPH, D-β-hydroxybutyryl ACP
Crotonyl ↔ butyryl	FAD, electron transport system	NADPH, fatty acyl-ACP

BIOSYNTHESIS OF LONG-CHAIN FATTY ACIDS (= MITOCHONDRIAL AND MICROSOMAL FATTY ACID SYNTHESIS)

Palmitate (a C_{16} fatty acid) is the major product of the fatty acid synthase system. This system is also called as the *de novo system* in that the palmitate is constructed from acetyl-CoA (ACP) and malonyl-CoA (ACP). In plants and animals, the most important fatty acids are the C_{18} fatty acids, namely stearic, oleic, linoleic and linolenic. These C_{18} fatty acids are synthesized by *elongation systems* which differ markedly from the *de novo system*. Palmitate acts as a precursor of other long-chain fatty acids (Fig. 25–12). It may be lengthened to form stearate (18 : 0) or even larger saturated fatty acids by further addition of acetyl groups through the action of fatty acid elongation systems present in the endoplasmic reticulum (= microsomes) and mitochondria in the case of animals and in the soluble cytosol in the case of plants.

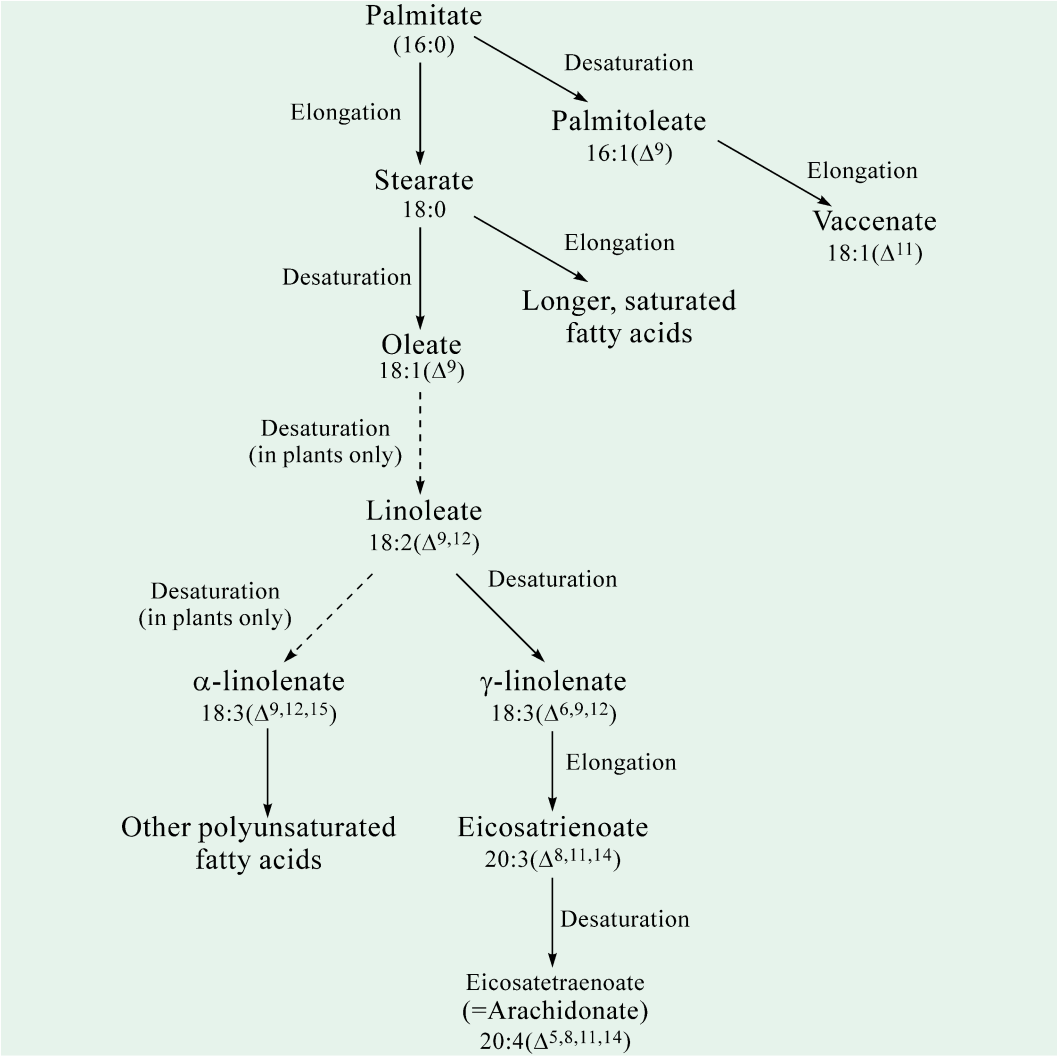


Fig. 25–12. Biosynthesis of some fatty acids in mammals

Palmitate is the precursor of stearate and longer-chain saturated fatty acids, as well as the monounsaturated fatty acids, palmitoleate and oleate. Mammals cannot convert oleate into linoleate or a-linolenate, hence required in the diet as essential fatty acids (EFAs). Conversion of linoleate into other polyunsaturated fatty acids and eicoandnoids is also outlined.

In animals :

Endoplasmic reticulum membrane

$$\text{Palmitoyl-CoA} \xrightarrow[+\ \text{NADPH}]{\text{Malonyl-CoA}} \text{Stearyl-CoA}$$

Mitochondrial outer and inner membranes

$$\text{Palmitoyl-CoA} \xrightarrow[+\ \text{NADPH}]{\text{Acetyl-CoA}} \text{Stearyl-CoA}$$

In plants :

Soluble cytosolic system

$$\text{Palmitoyl-ACP} \xrightarrow[+\ \text{NADPH}]{\text{Malonyl-ACP}} \text{Stearyl-ACP}$$

Although different enzyme systems are involved and coenzyme A, rather than ACP, is the acyl carrier directly involved in animal fatty acid synthesis, the mechanism of elongation is otherwise identical with that employed in palmitate synthesis: donation of two carbons by malonyl-ACP, followed by reduction (with NADH), dehydration and another reduction (with NADPH) to the saturated C_{18} product, stearyl-CoA. *Fasting largely abolishes chain elongation*. Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids that are present in sphingolipids.

BIOSYNTHESIS OF UNSATURATED FATTY ACIDS

Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids of animal tissues, palmitoleate and oleate. Both of them possess a single *cis* double bond between C–9 and C–10. The double bond is introduced into the fatty acid chain by an oxidative reaction catalyzed by **fatty acyl-CoA desaturase** (Fig. 25–13). The enzyme is an example of a mixed-function oxidase. Two different substrates, a fatty acyl-CoA and NADPH, simultaneously undergo two-electron oxidations.

The name **mixed-function oxidase** indicates that the enzyme oxidizes two different substrates simultaneously.

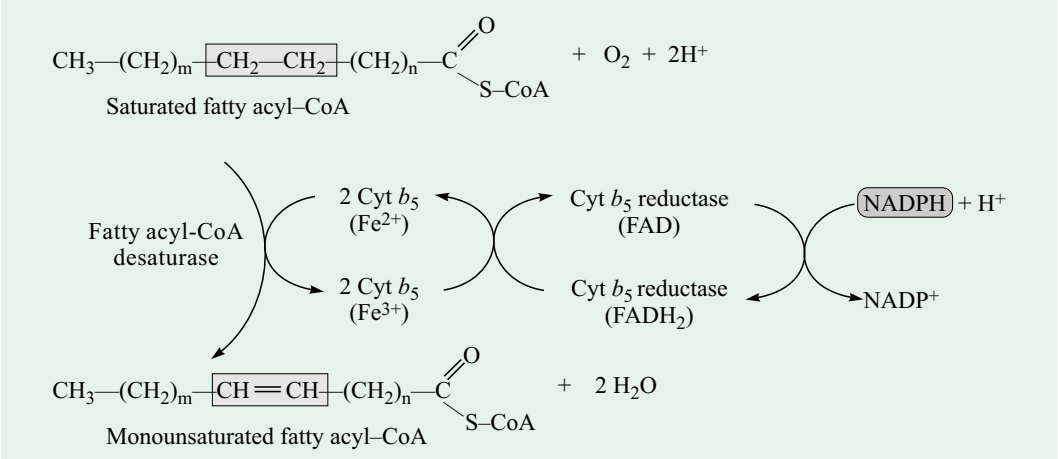


Fig. 25–13. The pathway of electron transfer in the desaturation of fatty acids by a mixed-function oxidase in vertebrates

Two different substrates– a fatty acyl–CoA and NADPH– undergo oxidation by molecular oxygen. These reactions occur on the lumenal face of the ER. A similar pathway, but with different electron carriers, exists in plants.

The path of electron flow includes a cytochrome (cytochrome b_5) and a flavoprotein (cytochrome b_5 reductase), both of which like fatty acyl-CoA desaturase itself are present in the smooth endoplasmic reticulum.

Mammalian hepatocytes can readily introduce double bonds at Δ^9 position of fatty acids but cannot introduce additional double bonds in the fatty acid chain between C–10 and the methyl-terminal end. Thus, linoleate, 18 : 2 ($\Delta^{9, 12}$) and α-linolenate, 18 : 3 ($\Delta^{9, 12, 15}$) cannot be synthesized by mammals, but plants can synthesize both. The plant desaturases that introduce double bonds at Δ^{12} and Δ^{15} positions are located in the endoplasmic reticulum. These enzymes, in fact, act not on free fatty acids but on a phospholipid called phosphatidylcholine which contains at least one oleate linked to glycerol (Fig. 25–14). Because linoleate and linolenate are necessary

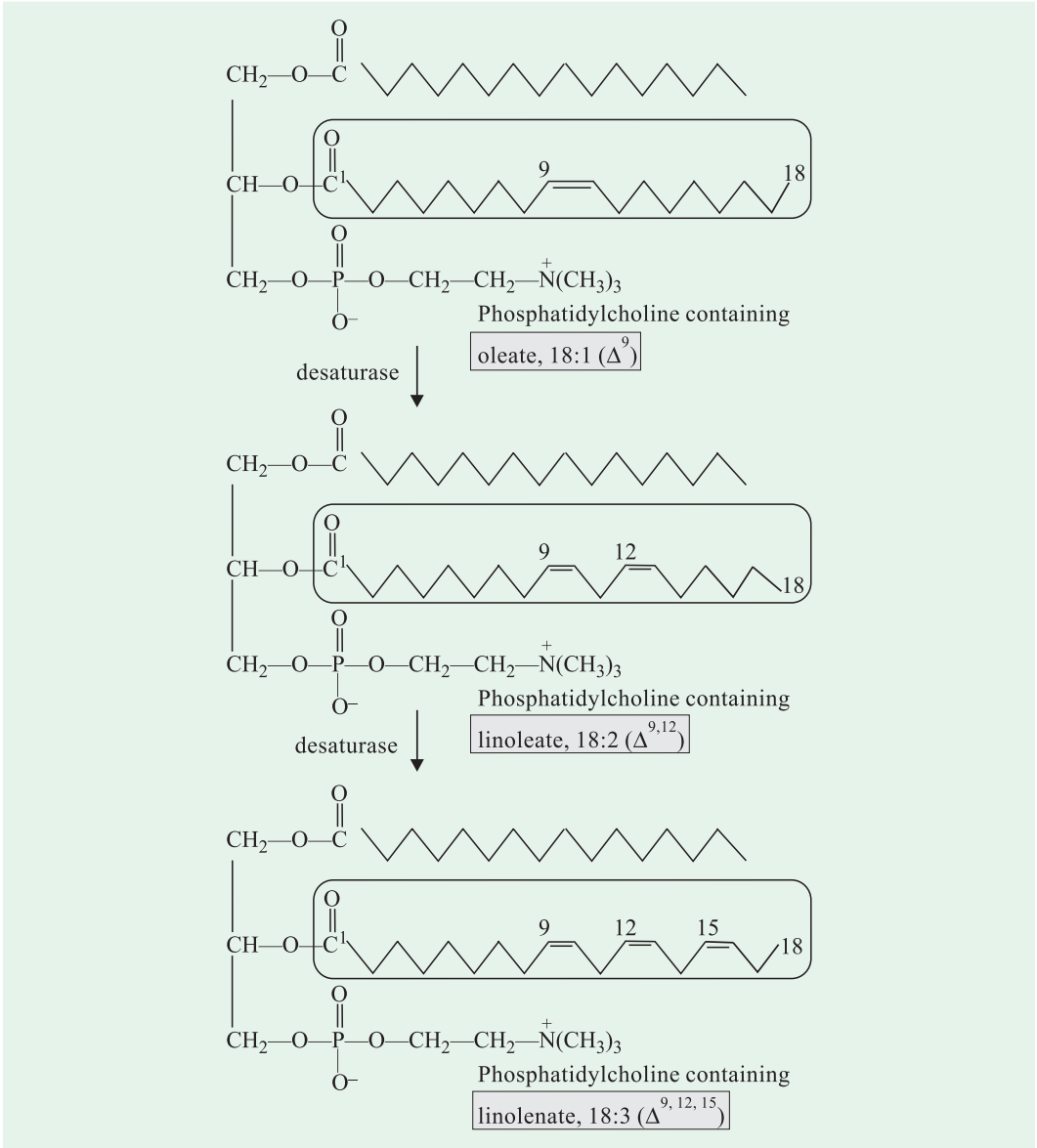


Fig. 25–14. Oxidation of phosphatidylcholine-bound oleate by desaturases, producing polyunsatureated fatty acids

precursors for the synthesis of other products, they are *essential fatty acids* (EFAs) for mammals and must be obtained from plant material in the diet. Once ingested, linoleate may be converted into certain other polyunsaturated fatty acids, particularly γ linolenate, eicosatrienoate and eicosatetraenoate (= arachidonate), which can be made only from linoleate. Arachidonate, 20 : 4 ($\Delta^{5, 8, 11, 14}$) is an essential precursor of regulatory lipids, the eicosanoids.

Families of Fatty Acids : Animal tissues contain a variety of polyunsaturated fatty acids. Of these, one series can be fabricated by the animal *de novo*. These are those fatty acids where all the double bonds lie between the 7th carbon from the terminal methyl group and the carboxyl group. Such fatty acids can be made by desaturation and chain elongation, starting with oleic acid (Fig. 25–12). Thus, oleate (which is produced from its corresponding saturated fatty acid, *i.e.*, stearate) can be elongated to a 20 : 1, *cis*- Δ^{11} fatty acid. Alternatively, a second double bond can be inserted to yield an 18 : 2 , *cis*-Δ^{6}, Δ^{9} fatty acid. However, polyunsaturated fatty acids, in which one or more double bonds are situated within the terminal 7 carbon atoms, cannot be made *de novo*. Such polyunsaturated fatty acids are essential in the diet.

There are 4 series of polyunsaturated fatty acids in the mammals (Table 25–3) : two are derived from dietary linoleate and linolenate and two are sythesized from the monounsaturated fatty acids, oleate and palmitoleate, which in their turn are formed from the corresponding saturated fatty acids. *Conjugated double bonds are not formed in animal tissues*. The four series can be recognized by the distance between the terminal methyl group (ω) and the nearest double bond. Desaturation and elongation reactions occur more extensively in liver than in extrahepatic tissues. Fasting and diabetes are marked by an inhibition of desaturation pathways.

Table 25–3. The four series of polyunsaturated fatty acids

Precursor family	*Systemic code*	*Formula*	*Series**
Linolenate	18 :3 ; 9, 12, 15	CH_3–$(CH_2)_1$— CH = CH — R	ω-3
Linoleate	18 : 2 ; 9, 12	CH_3–$(CH_2)_4$ — CH = CH — R	ω-6
Palmitoleate	16 : 1 ; 9	CH_3–$(CH_2)_5$ — CH = CH — R	ω-7
Oleate	18 : 1 ; 9	CH_3–$(CH_2)_7$ — CH = CH — R	ω-9

* Note that the family of precursor and its products generated in animals through elongation and desaturation can be identified by subtracting the number designating the last double bond from the total number of carbon atoms. The result is the same within a family. For example, with linoleate, 18 : 2 ; 9, 12 and arachidonate, 20 : 4 ; 5, 8, 11, 14 : 18 – 12 = 6 = 20 – 14.

BIOSYNTHESIS OF EICOSANOIDS

Eicosanoids is a family of very potent biological signalling molecules that act as short-range messengers affecting tissues *near* the cells that produce them. All 3 subclasses of eicosanoids (prostaglandins, thromboxanes and leukotrienes) are unstable and insoluble in water. These signalling molecules generally do not move far from the tissue that produced them and act primarily on cells very near their point of release. Eicosanoids are derivatives of the C-20 polyunsaturated fatty acid, arachidonate.

In the beginning, a specific *phospholipase*, present in most of the mammalian cells, attacks the membrane phospholipids and releases **arachidonate**. Arachidonate is then converted into **PGH_2**, the immediate precursor of many **prostaglandins** and thromboxanes (Fig. 25–15 A), by the enzymes of the smooth endoplasmic reticulum. These two reactions which lead to the formation of PGH_2 involve the addition of molecular oxygen and are both catalyzed by a bifunctional enzyme, *prostaglandin endoperoxide synthase*. Aspirin (= acetylsalicylate) irreversibly inactivates this enzyme by acetylating a serine residue essential to catalytic activity (Fig. 25–15 B). Thus, the

synthesis of prostaglandins and thromboxanes is blocked. Ibuprofen (Fig. 25–15 C), a widely-used nonsteroidal antiinflammatory drug, also inhibits this step, probably by mimicking the structure of the substrate or an intermediate in the reaction.

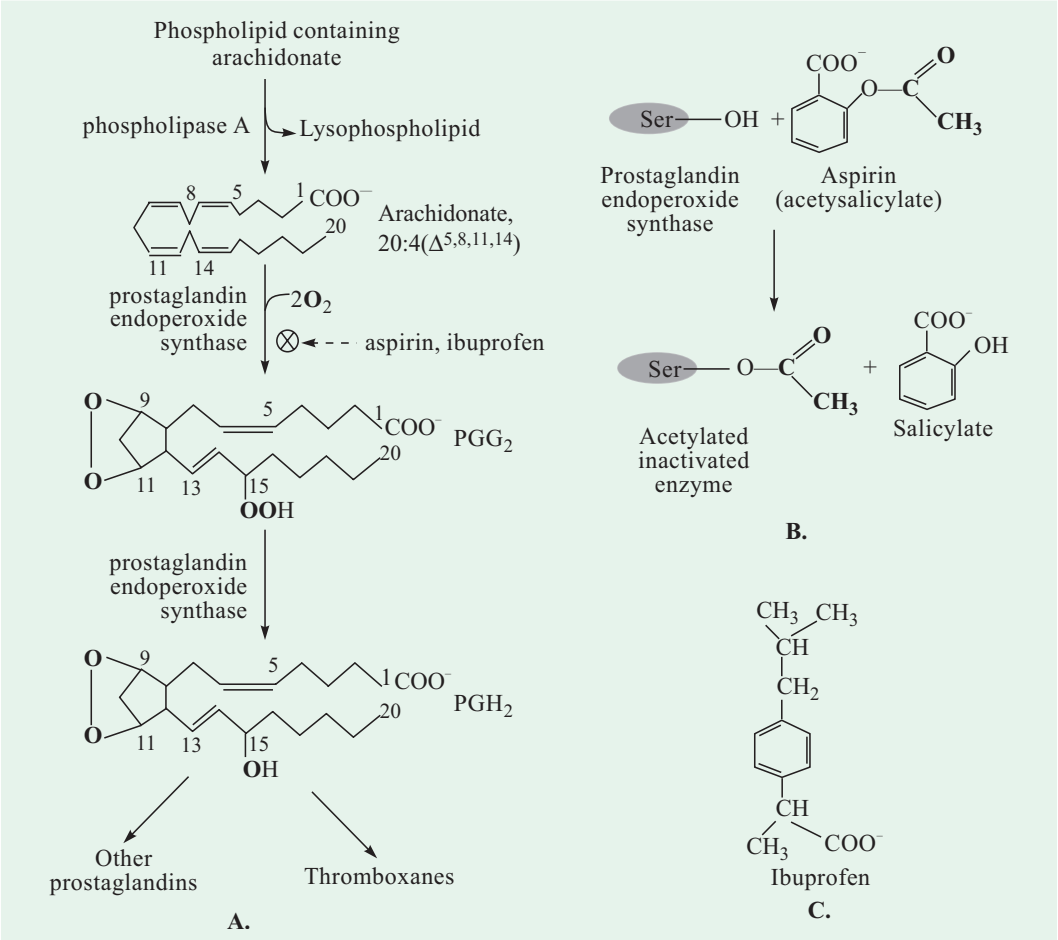


Fig. 25–15. The "cyclic" pathway from arachidonate to prostaglandins and thromboxanes

A. Production of PGH_2 from arachidonate by the action of prostaglandin endoperoxide synthase

B. Inhibitory action of aspirin

C. Chemical structure of ibuprofen

PGH_2 is enzymatically converted into **thromboxane A_2**, from which **other thromboxanes** are derived. This reaction is catalyzed by *thromboxane synthase*, an enzyme present in blood platelets

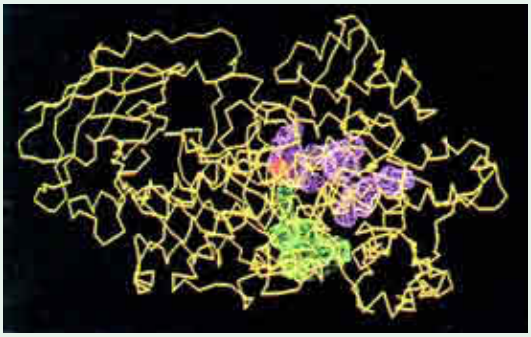


Fig. 25–16. The X-ray structure of soybean lipoxygenase-I

The protein is represented by its C_α diagram (*yellow*). Its internal cavities are outlined by dot surfaces with cavity I in *green* and cavity II in *pink*. The active site Fe atom is represented by an *orange* sphere.

(Courtesy : Mario Amzel, The Johns Hopkins University)

(= thrombocytes). Thromboxanes induce blood vessel constriction and platelet aggregation, the early steps in blood clotting. Thromboxanes and prostaglandins both contain a ring of 5 or 6 atoms, and the pathway that leads from arachidonate to these two classes of compounds is sometimes called the **'cyclic'** pathway, to differentiate it from the **'linear'** pathway that results in the synthesis of the 'linear' molecules of leukotrienes from arachidonate (Fig. 25–17).

Leukotriene synthesis begins with the incorporation of molecular oxygen into arachidonate by the enzymatic action of the mixed-function oxidases called *lipoxygenases*. The lipoxygenases (Fig. 25-16) are found in leukocytes and in heart, brain, lung and spleen, and utilize cytochrome P-450 for their acitvity. The various leukotrienes differ in the position of the peroxide that is introduced by these lipoxygenases. Unlike the cyclic pathway, this linear pathway for leukotriene synthesis is not inhibited by aspirin or ibuprofen.

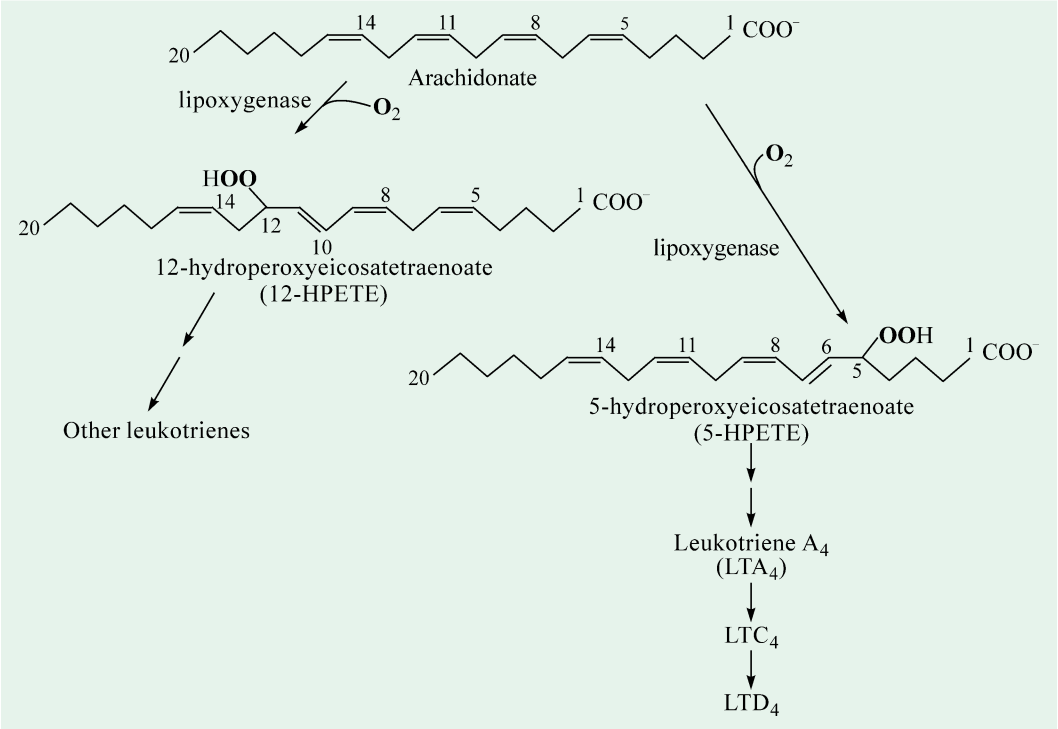


Fig. 25–17. The "linear" pathway from arachidonate to leukotrienes

BIOSYNTHESIS OF TRIACYLGLYCEROLS

Depending upon the requirements of organisms, most of the fatty acids synthesized or ingested by an organism are either incorporated into triacylglycerols for the storage of metabolic energy or incorporated into the phospholipid components of membranes. The organisms, that are not actively growing but have abundant food supply, do not convert most of their fatty acids into storage fats; but during rapid growth, the synthesis of new cell membranes requires membrane phospholipid synthesis. It is of interest to note that *both the pathways (triacylglycerol and phospholipid syntheses) begin at the same point : the formation of fatty acyl esters of glycerol.*

Humans store only about half a kilo of glycogen in their liver and muscles, which is hardly enough to supply the body's energy needs for 12 hours. In contrast, the total amount of stored triacylglycerol in a 70 kg man is about 15 kg, which is enough to supply his basic energy needs for as long as 12 weeks. When carbohydrate is ingested in excess of the capacity to store glycogen, it is converted into triacylglycerols and stored in adipose tissues. Plants also do

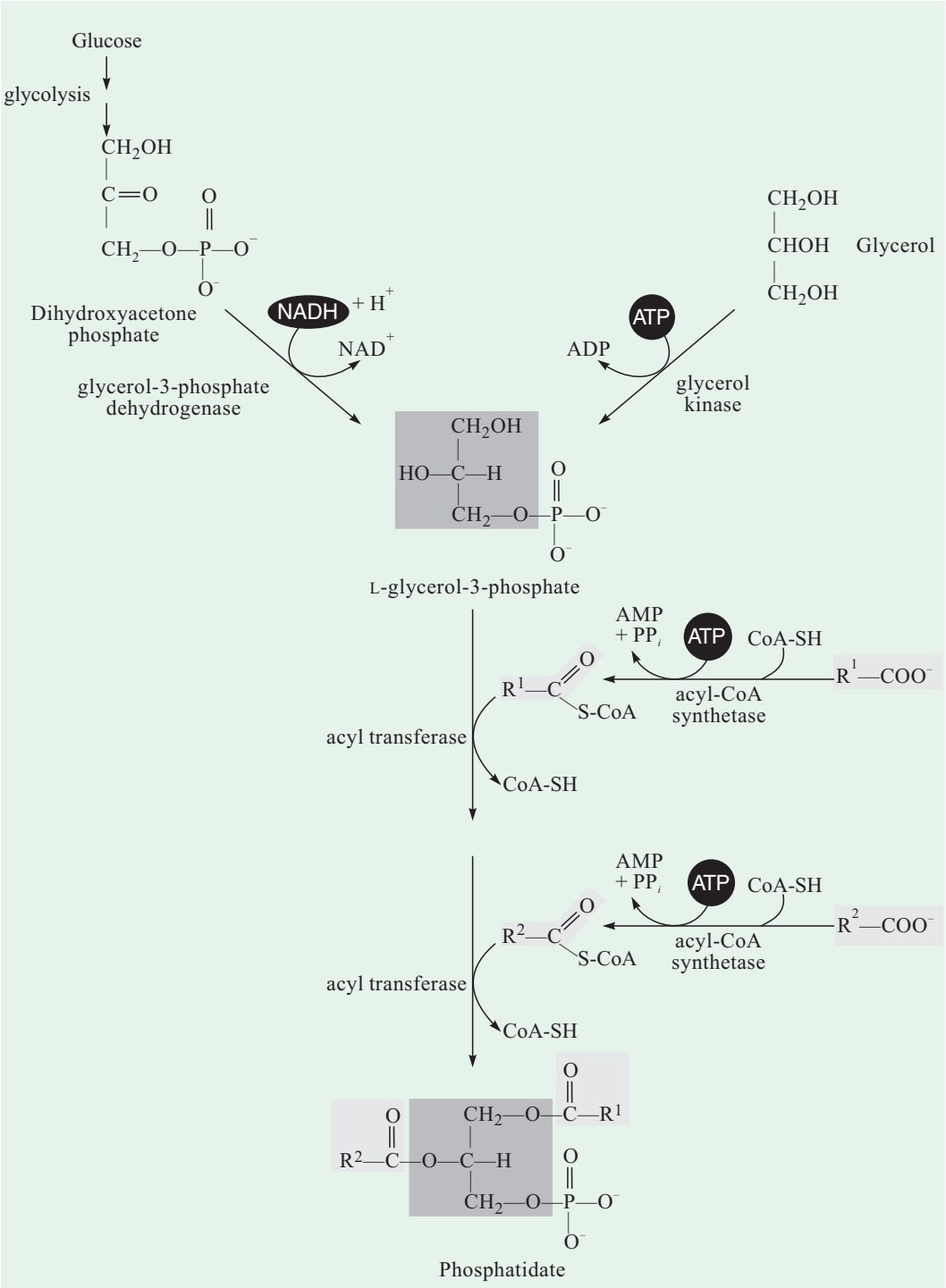


Fig. 25–18. The biosynthetic pathway to phosphatidate

Fatty acyl groups are first activated by formation of fatty acyl–CoA molecules, then they are transferred to ester linkage with L-glycerol-3-phosphate, formed in either of the 2 ways shown. Phosphatidate is shown here with the correct stereochemistry at C-2 of the glycerol molecule.

manufacture triacylglycerols as an energy-rich fuel and store them in fruits and seeds.

Triacylglycerols and glycerophospholipids (phosphatidylethanolamine, for example) share two precursors (fatty acyl-CoAs and glycerol-3-phosphate) and many enzymatic steps in their biosynthesis in animal tissues. Glycerol-3-phosphate can be formed in two ways (Fig. 25–18) :

(*a*) either from dihydroxyacetone phosphate generated during glycolysis by the action of cytosolic NAD-linked *glycerol-3-phosphate dehydrogenase*,

(*b*) or from glycerol by the action of glycerol kinase in liver and kidney.

Phosphatidate, which occurs in traces in cells, is a key intermediate in lipid biosynthesis. It can be converted either to a triacylglycerol or to a glycerophospholipid. In the pathway to triacylglycerols, phosphatidate is hydrolyzed by *phosphatidate phosphatase* to form 1, 2-diacylglycerol (Fig. 25–19). **Diacylglycerols** are then converted to **triacylglycerols** by transesterification with a third fatty acyl-CoA.

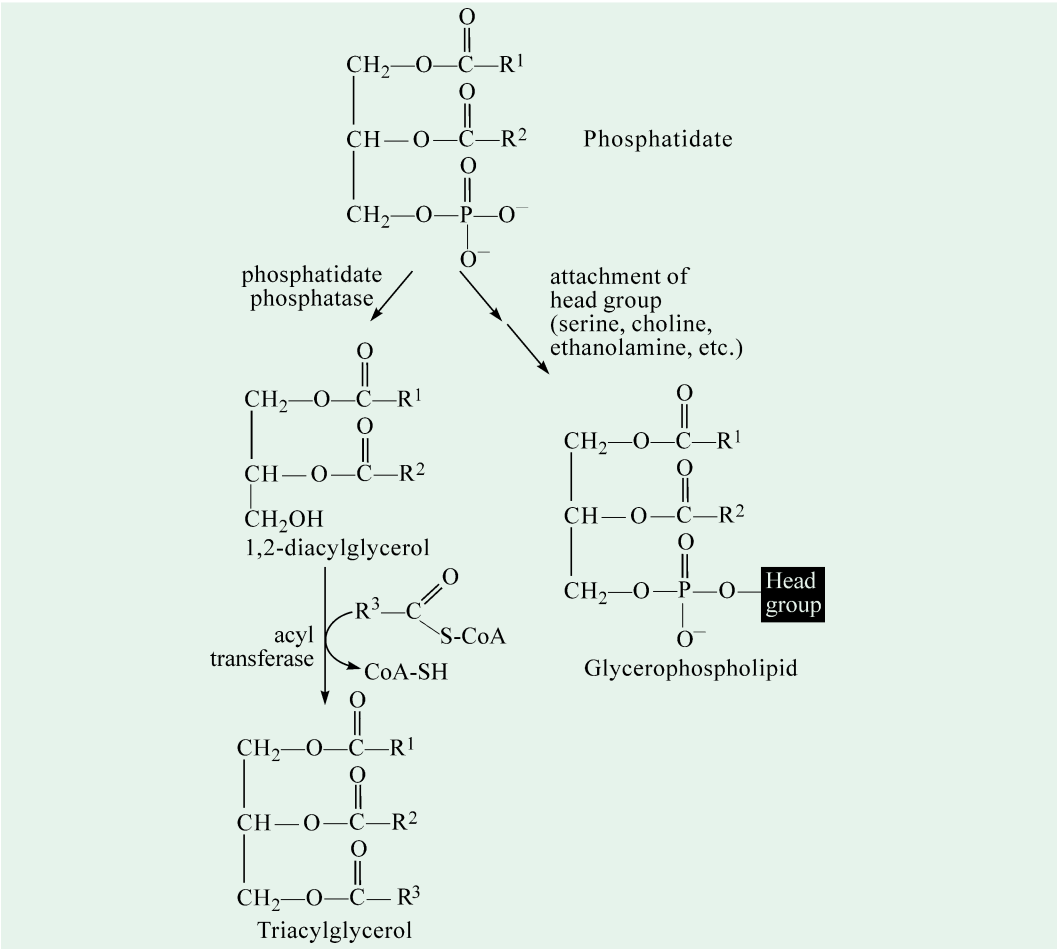


Fig. 25–19. Biosynthesis of triacylglycerols and glycerophospholipids from a common precursor phosphatidate

In men, the amount of body fat remains almost constant over long periods. However, if carbohydrate, protein or fat is consumed in excess of their energy needs, the surplus is stored in the form of triacylglycerols. This stored fat can be utilized for energy during normal body activity or during fasting. Triacylglycerol metabolism is influenced by hormones such as insulin and glucagon and also by the growth hormone and adrenalocorticoids.

BIOSYNTHESIS OF MEMBRANE PHOSPHOLIPIDS

Phospholipid biosynthesis results in the production of a large number of end products. But all of these diverse products are produced according to some basic patterns. In general, the assembly of phospholipids from simple precursors requires (Lehninger, Nelson and Cox, 1993):

1. synthesis of backbone molecule (glycerol or sphingosine),
2. attachment of fatty acid(s) to the backbone in ester or amide linkage,
3. addition of a hydrophilic head group, joined to the backbone through a phosphodiester linkage,
4. and, in some cases, alteration or exchange of the head group to yield the final phospholipid product.

In eukaryotes, the phospholipid synthesis occurs primarily at the surface of the smooth ER. Some newly-formed phospholipids remain in that membrane, but most of them move from the site of their formation to other cellular locations to act.

The first steps of glycerophospholipid synthesis are common to those of triacylglycerol synthesis : two fatty acyl groups are esterified to C_1 and C_2 of L-glycerol-3-phosphate to form phosphatidate. Usually, but not always, the fatty acid at C_1 is saturated and that at C_2 is unsaturated. A second pathway to phosphatidate is the phosphorylation of a diacylglycerol by a specific kinase. The polar head group of glycerophospholipids is attached through a phosphodiester bond, in which each of the two hydroxyls, (one on the polar head group and the other on C_3 of glycerol) forms an ester with phosphoric acid (Fig. 25–20).

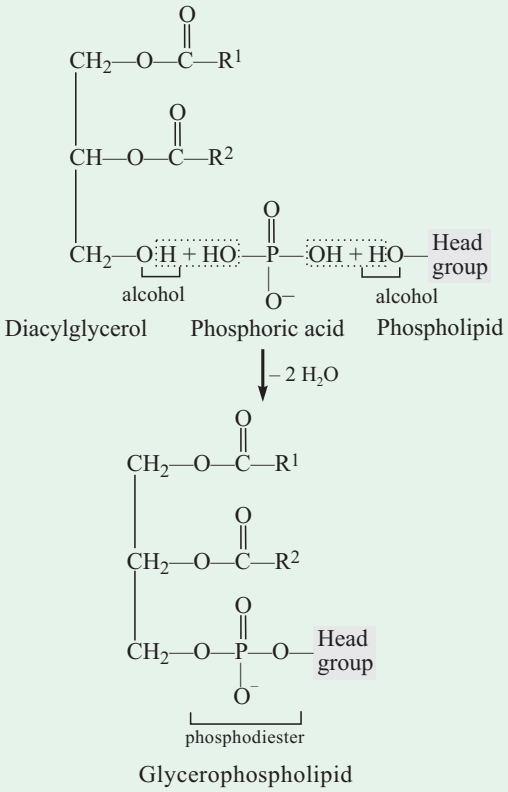


Fig. 25–20. Structure of glycerophospholipid

The phosphlipid head group is attached to a diacylglycerol by a phosphodiester bond. The bond is formed when phosphoric acid condenses with two alcohols, eliminating 2 moles of water.

Eugene P. Kennedy, in the early 1960s, discovered the importance of cytidine nucleotides in lipid biosynthesis. In the biosynthetic process, one of the hydroxyls of the phospholipid is first activated by attachment of a nucleotide, cytidine diphosphate (CDP). Cytidine monophosphate (CMP) is then displaced in a nucleophilic attack by the other hydroxyl (Fig. 25–21). The CDP is attached either to the diacylglycerol, forming an activated phosphatidate, CDP-diacylglycerol (Strategy 1), or to the hydroxyl of the head group (Strategy 2).

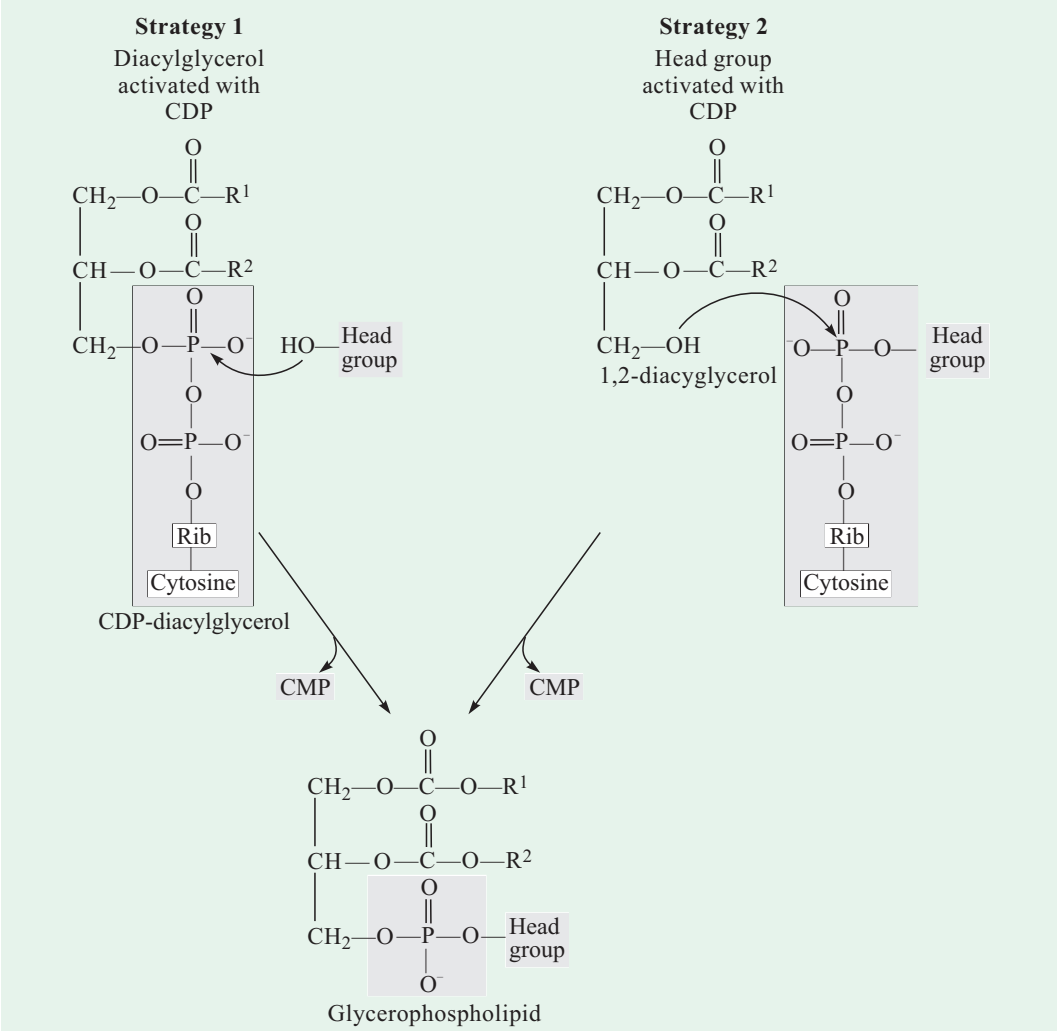


Fig. 25–21. Two general strategies for forming the phosphodiester bond of phospholipids
Note that in both cases, CDP supplied the phosphate group of the phosphodiester bond.

Phospholipid Synthesis in *Escherichia coli*

The synthesis of phosphatidylserine, phosphatidylethanolamine and phosphatidylglycerol in *E. coli* takes place through strategy 1 for head group attachment. The diacylglycerol is activated by condensation of phosphatidate with CTP to form CDP-diacylglycerol, while eliminating pyrophosphate (Fig. 25-22). CMP is displaced through the nucleophilic attack either by the hydroxyl group of serine to yield **phosphatidylserine** or by the C-1 hydroxyl of glycerol-3-phosphate to yield phosphatidylglycerol-3-phosphate. The latter is processed further by cleavage of the phosphate monoester to yield **phosphatidylglycerol**, while freeing inorganic phosphate, P*i*.

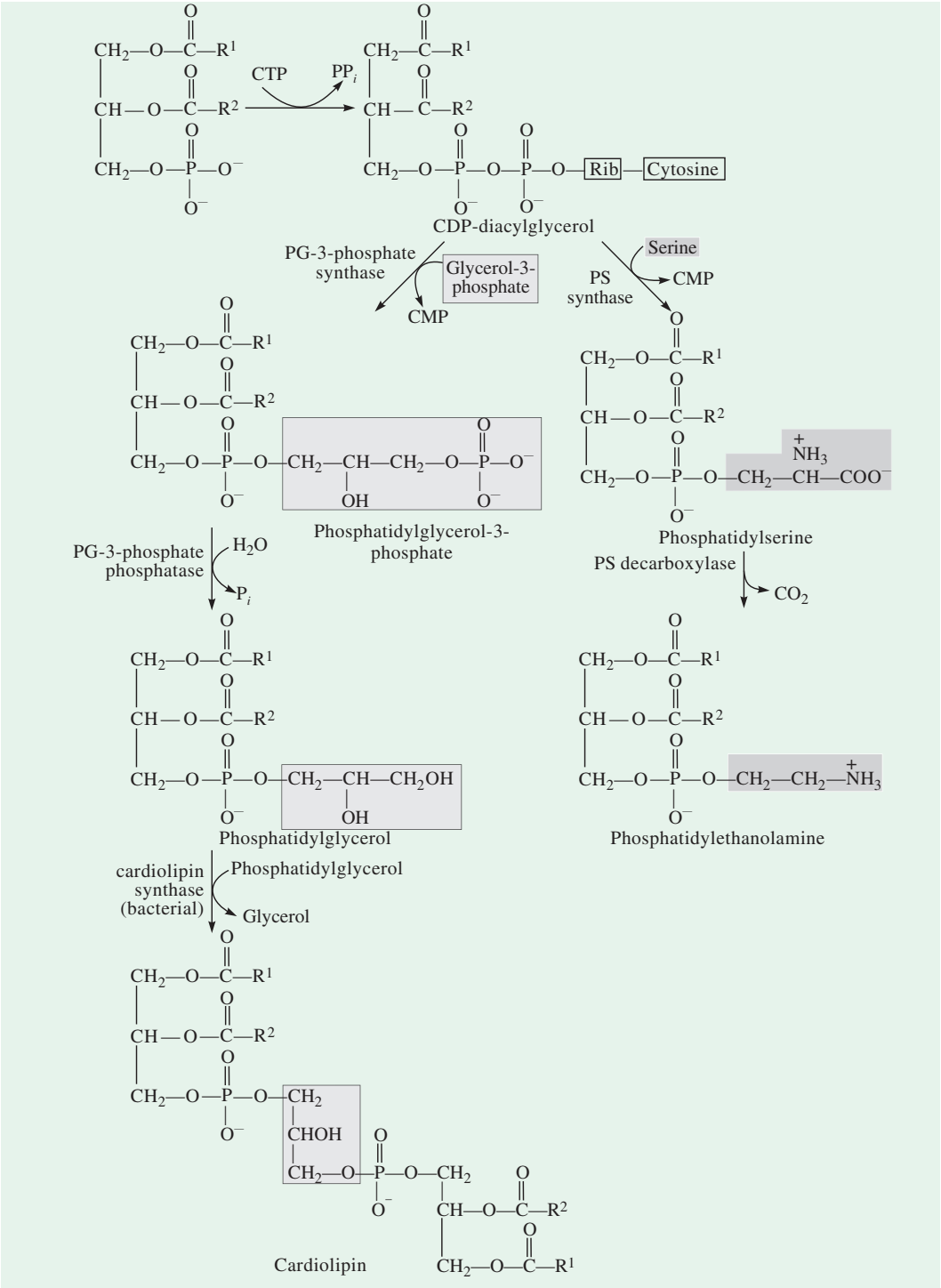


Fig. 25–22. Phospholipid biosynthesis in *Escherichia coli*

Initially, a head group (either serine or glycerol-3-phosphate) is attached *via* a CDP-diacylglycerol intermediate, *i.e.*, through Strategy 1. For phospholipids other than phosphatidylserine, the head group is further modified, as shown in the diagram.

PG = Phosphatidylglycerol; PS = Phosphatidylserine

The two products (phosphatidylserine and phosphatidylglycerol) can serve as precursors of other membrane lipids in bacteria (Fig. 25–22). Decarboxylation of the serine moiety in phosphatidylserine by the enzyme phosphatidylserine decarboxylase yields **phosphatidylethanolamine.** Condensation of two moles of phosphatidylglycerol produces cardiolipin, and a mole of glycerol is eliminated in the process. In cardiolipin, the two diacylglycerol moles are joined through a common head group.

Phospholipid Synthesis in Eukaryotes

In eukaryotes, the synthesis of acidic phospholipids (phosphatidylglycerol, cardiolipin and the phosphatidylinositols) takes place by the same strategy as employed for phospholipid synthesis in bacteria. **Phosphatidylglycerol** is made exactly like that in bacteria. However, **cardiolipin** synthesis differs slightly as phosphatidylglycerol condenses with CDP-diacylglycerol (Fig. 25–23) and not with another mole of phosphatidylglycerol as in *E. coli* (Fig. 25–22).

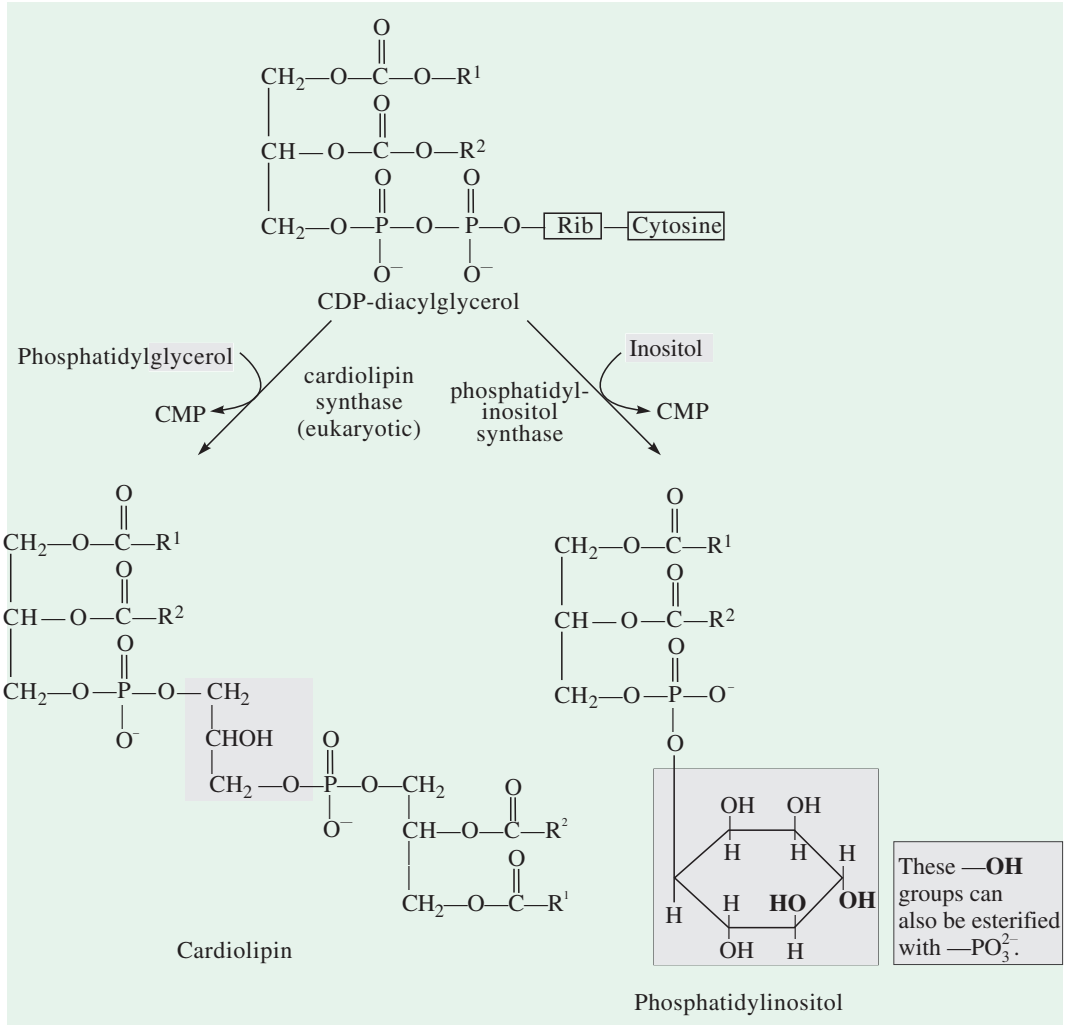


Fig. 25–23. Biosynthesis of cardiolipin and phoshatidylinositol in eukaryotes through Strategy 1

The synthesis of **phosphatidylinositol** takes place by the condensation of CDP-diacylglycerol with inositol. Phosphatidylinositol and its phosphorylated derivatives play a central role in signal transduction in eukaryotic individuals.

Interrelationship among the Eukaryotic Pathways to Phosphatidylserine, Phosphatidylethanolamine and Phosphatidylcholine

In yeast, as also in bacteria, phosphatidylserine may be formed by condensation of CDP-diacylglycerol and serine. And phosphatidylethanolamine can be synthesized from phosphatidylserine in the reaction catalyzed by phosphatidylserine decarboxylase (Fig. 25–24).

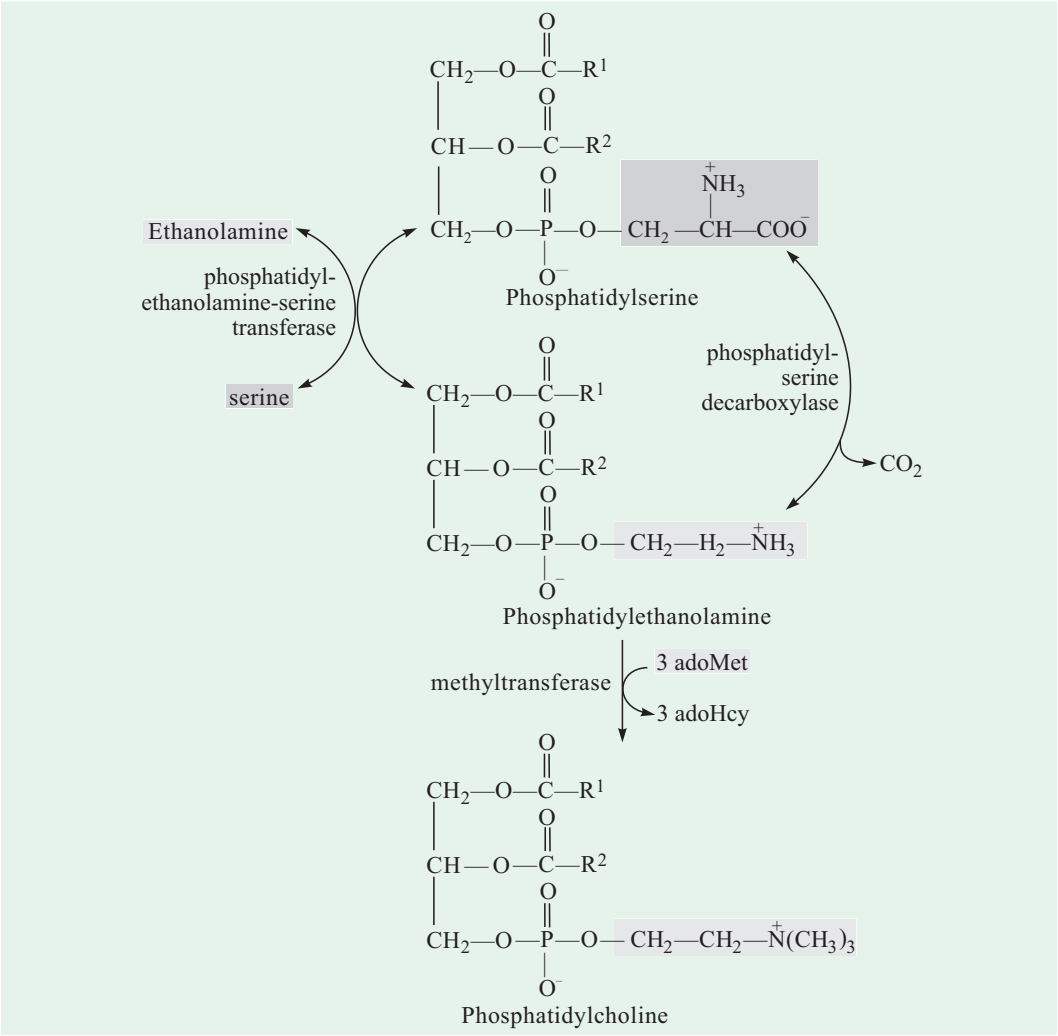


Fig. 25–24. The "salvage" pathway of the synthesis of phosphatidylethanolamine and phosphatidylcholine in yeast

Note that phosphatidylserine and phosphatidylethanolamine are interconverted by a reversible head group exchange reaction.

adoHcy = S-adenosylhomocysteine

An alternative pathway to phosphatidylserine is a head group exchange reaction, in which free serine displaces ethanolamine. Phosphatidylethanolamine may also be converted into **phosphatidylcholine** (lecithin) by the addition of 3 methyl groups to its amino group.

Mammals, however, do not synthesize phosphatidylserine from CDP-diacylglycerol. Instead, they derive it from phosphatidylethanolamine *via* the head group exchange reaction, as depicted in Fig. 25–24. *In mammals, the synthesis of all nitrogen-containing phospholipids occurs by*

Strategy 2: phosphorylation and activation of the head group followed by condensation with diacylglycerol. As an instance, choline is resused (or "salvaged") by first being phosphorylated and then converted into CDP-choline by condensation with CTP. A diacylglycerol displaces CMP from CDP-choline, forming phosphatidylcholine (Fig. 25–25).

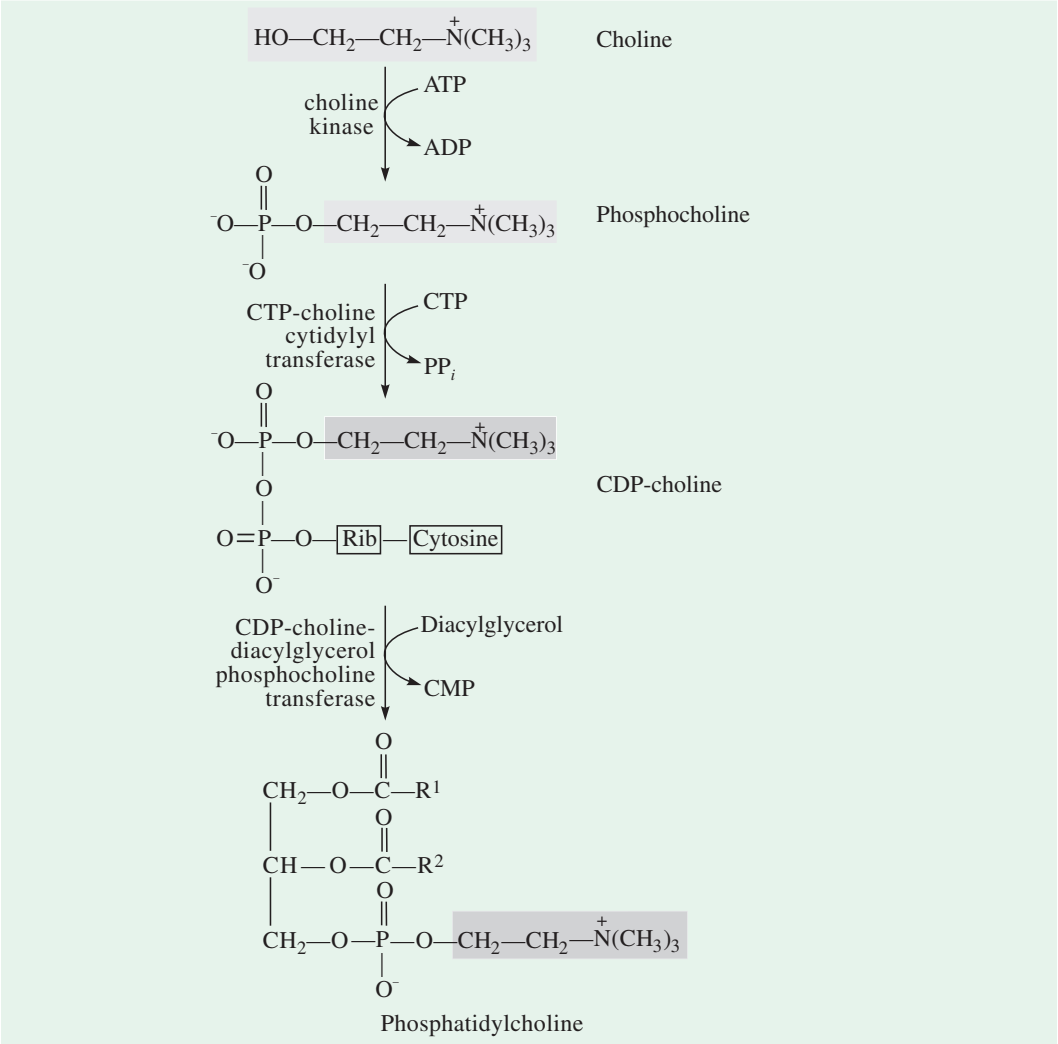


Fig. 25–25. Biosynthesis of phosphatidylcholine from choline in mammals according to Strategy 2
The same strategy is employed for salvaging ethanolamine in phosphatidylethanolamine synthesis.

Ethanolamine, obtained in the diet, is converted into phosphatidylethanolamine by an analogous salvage pathway. In the liver, phosphatidylcholine is also produced by methylation of phosphatidylethanolamine, using s-adenosylmethionine. In all other tissues, however, phosphatidylcholine is produced only by condensation of diacylglycerol and CDP-choline. Fig. 25–26 summarizes the pathways leading to the formation of phosphatidylcholine and phosphatidylethanolamine in various organisms.

Biosynthesis of Sphingolipids

The biosynthesis of sphingolipids occurs in 4 stages (Fig. 25–27) :

1. synthesis of a C_{18} amine, **sphinganine** from palmitoyl-CoA and serine,

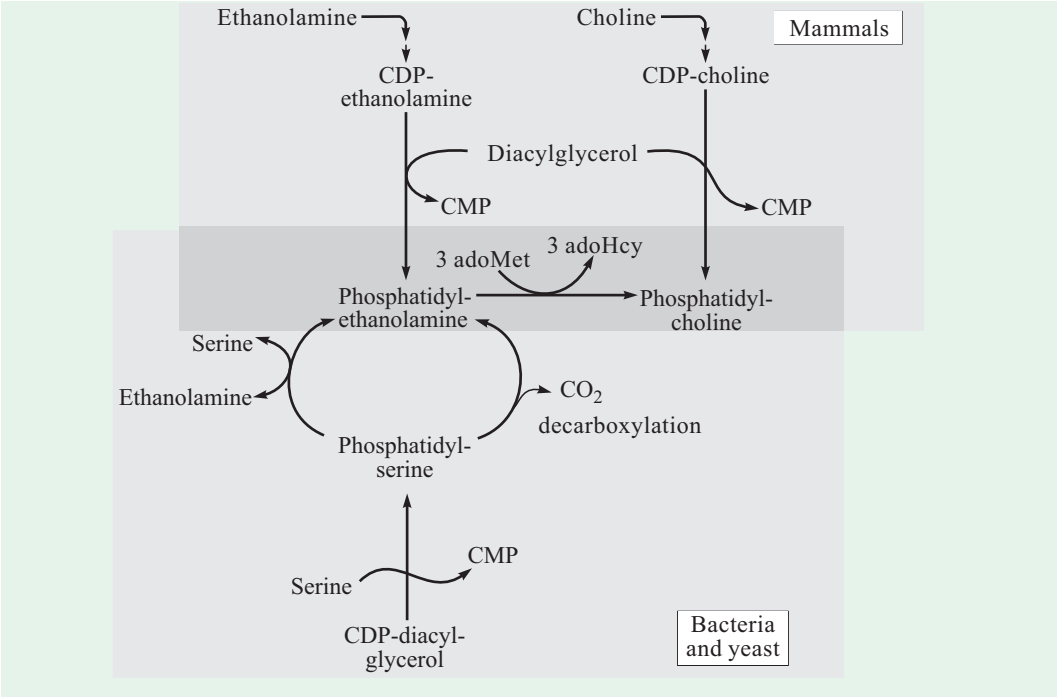


Fig. 25–26. Summarized pathways leading to phosphatidylcholine and phosphatidylethanolamine

Note that the conversion of phosphatidylethanolamine to phosphatidylcholine in mammals occurs exclusively in the liver.

2. attachment of a fatty acid in amide linkage to form **ceramide**,
3. desaturation of the sphinganine moiety to form **sphingosine**, and
4. attachment of a head group to produce a sphingolipid such as a **sphingomyelin** or **cerebroside**.

The pathways leading to the formation of sphingolipids share some features in common :

1. NADPH provides reducing power and fatty acids enter as their activated CoA derivatives.
2. In cerebroside formation, sugars enter as their activated nucleotide derivatives. Head group attachment in sphingolipid synthesis has several novel features. Phosphatidylcholine, rather than CDP-choline, serves as the donor of phosphocholine in the synthesis of sphingomyelin from the ceramide. In glycolipids, the cerebrosides, and gangliosides, the head group is a sugar, attached directly to C_1 hydroxyl of sphingosine in glycosidic linkage, rather than through a phosphodiester bond ; the sugar donor is a UDP-sugar, either UDP-glucose or UDP-galactose.

BIOSYNTHESIS OF CHOLESTEROL

This extremely difficult pathway was elucidated by Konrad Bloch, Feodor Lynen, John Kornforth and George Popják in the late 1950s. Cholesterol, an essential molecule in many animals including man, is not required in the mammalian diet because the liver can synthesize it from simple precursors. Like long-chain fatty acids, cholesterol is made from acetyl-CoA but the assembly plan is quite unlike each other. The synthesis of cholesterol takes place in 4 stages (Fig. 25–28) :

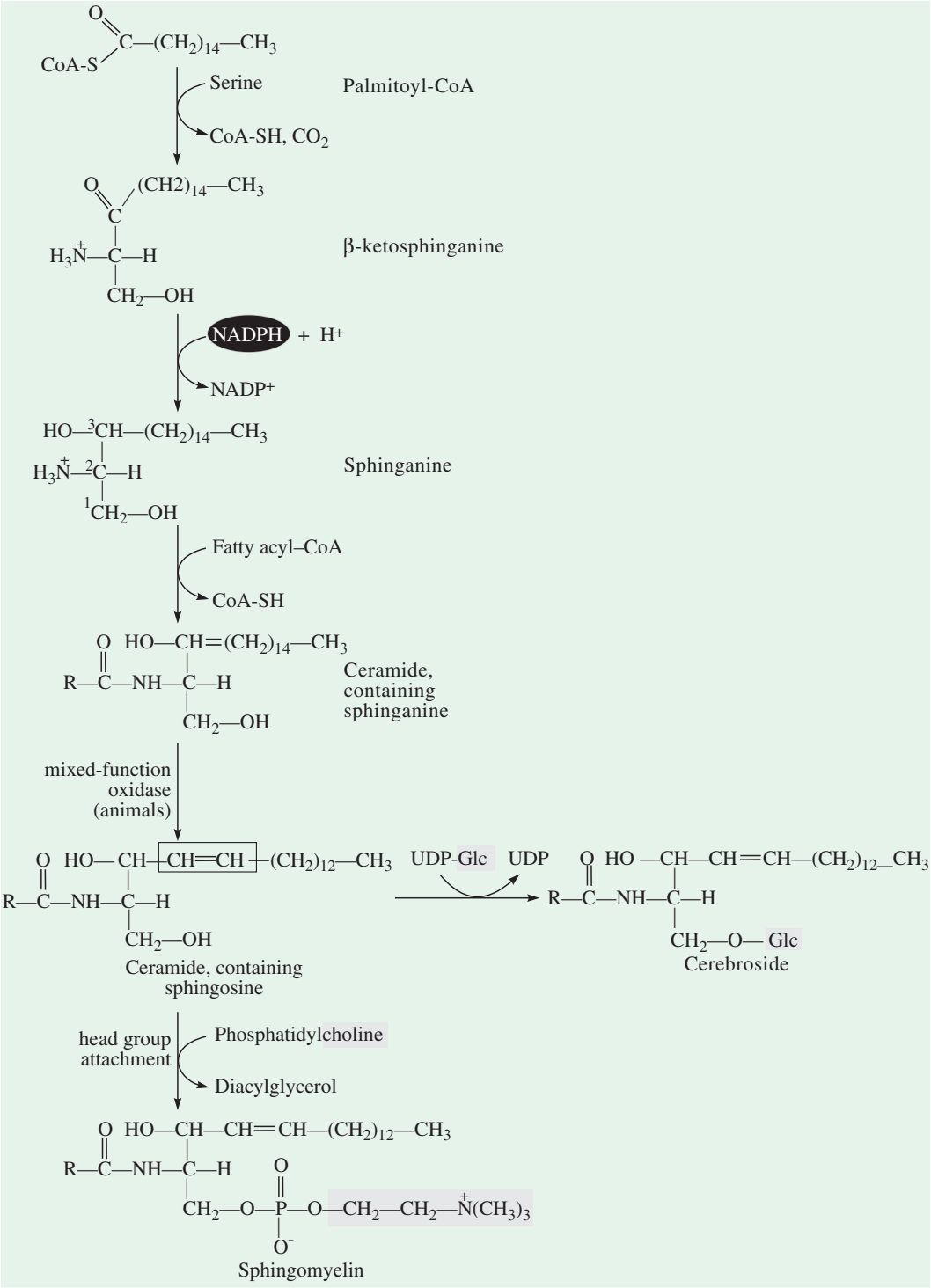


Fig. 25–27. Biosynthesis of sphingolipids

The synthesis involves first the condensation of palmitate and serine to produce sphinganine, followed by acylation of sphinganine to produce a ceramide. In animals, a double bond (shown in rectangle) is then created by a mixed-function oxidase. At last, a head group is added : phosphatidyl choline, to form sphingomyelin; or glucose, to form a cerebroside.

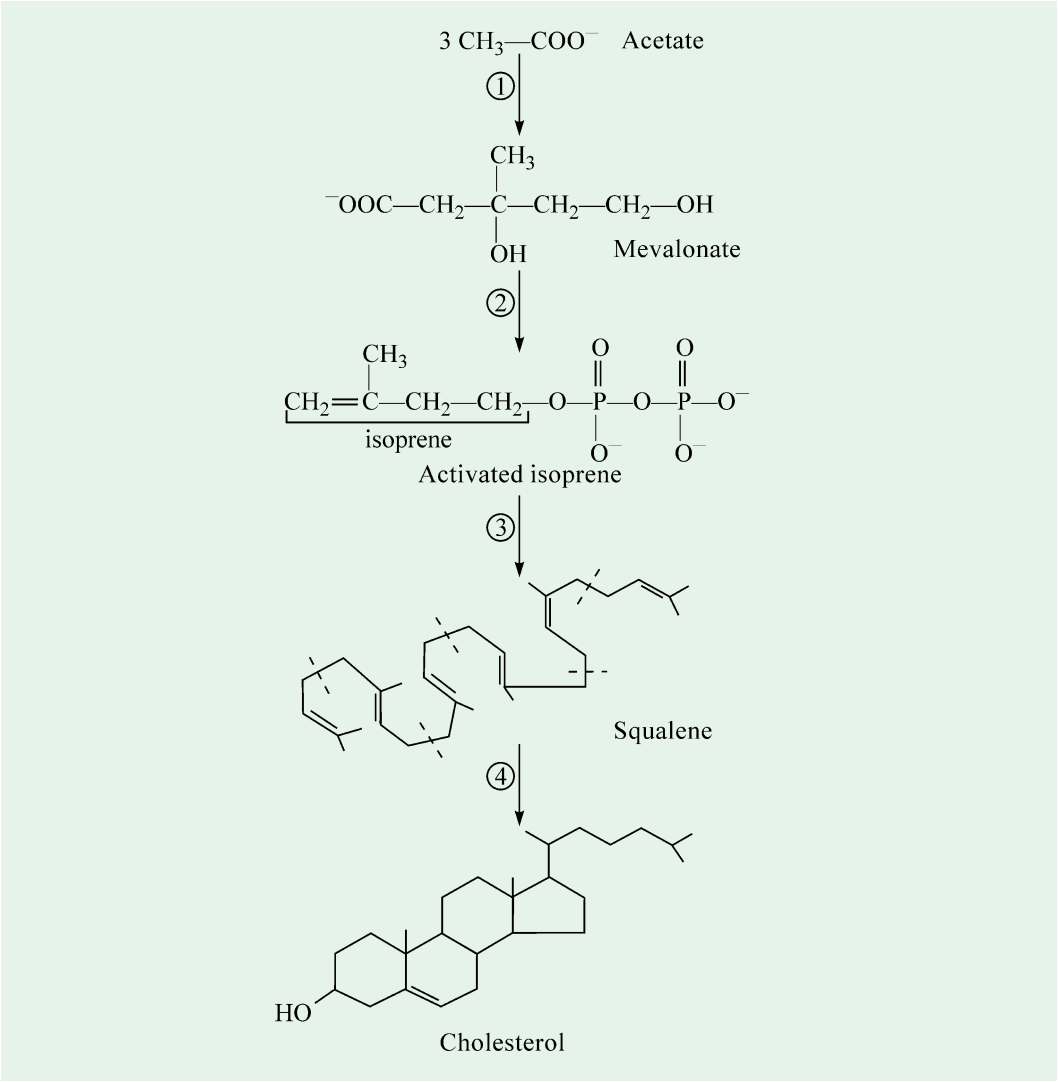


Fig. 25–28. Summarized scheme of cholesterol biosynthesis

The isoprene units in squalene are shown separated by a dashed line.

Stage 1 : *Conversion of 3 acetate units to mevalonate.*

In this stage, 3 acetate units condense to form mevalonate, an intermediate of cholesterol synthesis (Fig. 25-31) In effect, utilizing thiolase, to form acetoacetyl-CoA which then condenses with a third mole of acetyl-CoA to yield a C-6 compound, β-hydroxy-β-methylglutaryl-CoA (HMG-CoA), using HMG-CoA synthase as an enzyme. These first two reactions are reversible and do not commit the cell to synthesize cholesterol or other isoprenoid compounds.

The third reaction, *i.e.*, the reduction of HMG-CoA to mevalonate, is a committed step, wherein 2 moles of NADPH each donate two electrons. This reaction is catalyzed by HMG-CoA reductase (Fig. 25–29), an integral membrane protein of the smooth endoplasmic reticulum. It is the major point of regulation for cholesterol synthesis. It may, however, be pointed out that HMG-CoA is converted into mevalonate in the cytosol, but it is converted into acetyl-CoA and acetoacetate in the mitochondria (Fig. 25–30).

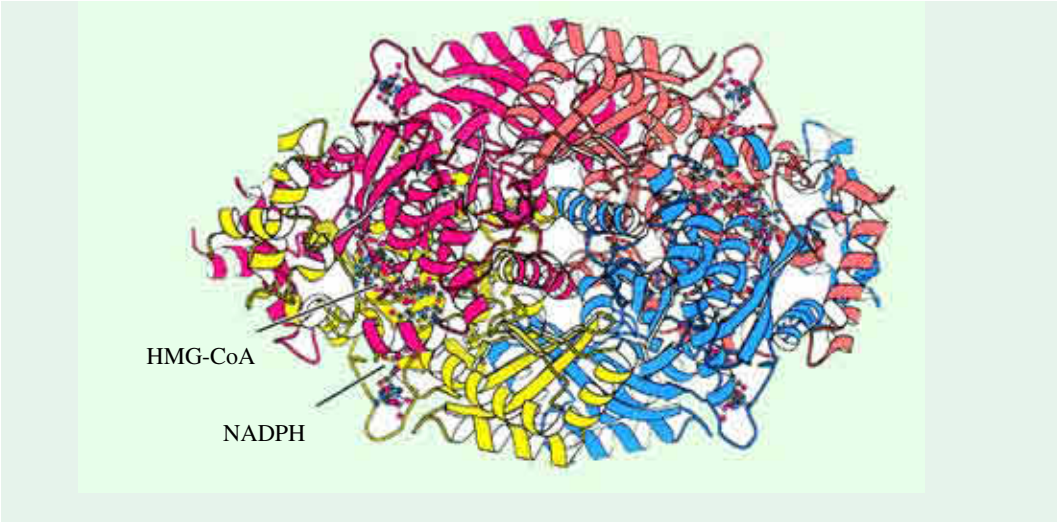


Fig. 25–29 . HMG-CoA reductase
The structure of a portion of the tetrameric enzyme is shown.

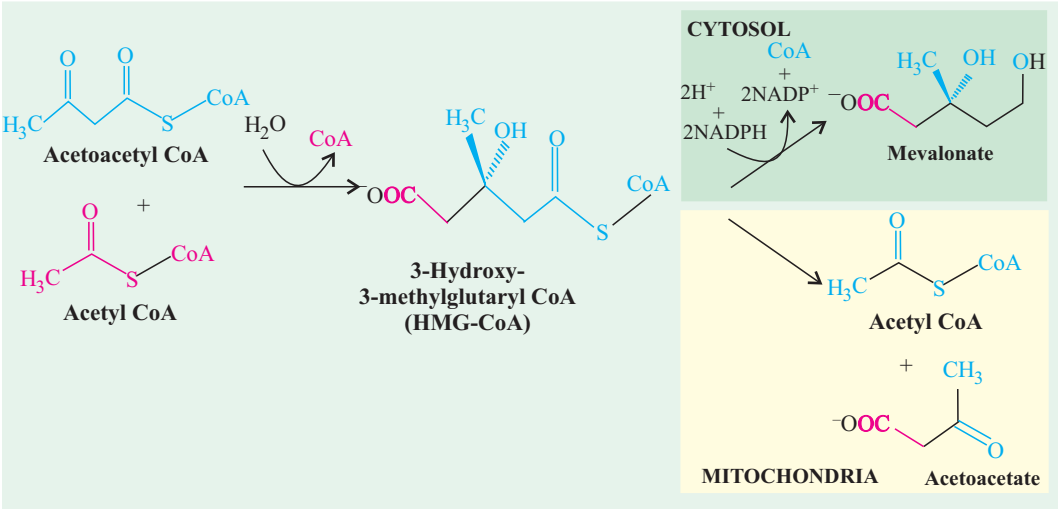


Fig. 25–30. Fates of 3-hydroxy-3-methylglutaryl CoA
In the cytosol, HMG-CoA is converted into mevalonate. In mitochondria, it is converted into acetyl CoA and acetoacetate

Stage 2 : *Conversion of mevalonate to 2 activated isoprenes*

Here, the three phosphate groups are tranferred from 3 ATP molecules to mevalonate (Fig. 25-32). The phosphate group attached to C-3 hydroxyl group of mevalonate in the intermediate compound, 3-phospho-5-pyrophosphomevalonate is a good leaving group. In the next reaction, this phosphate and the adjacent carboxyl group both leave, thus producing a double bond in the C-5 product, Δ^3-isopentenyl pyrophosphate. This is the first of the two activated isoprenes essential to cholesterol formation. Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, dimethylallyl pyrophosphate.

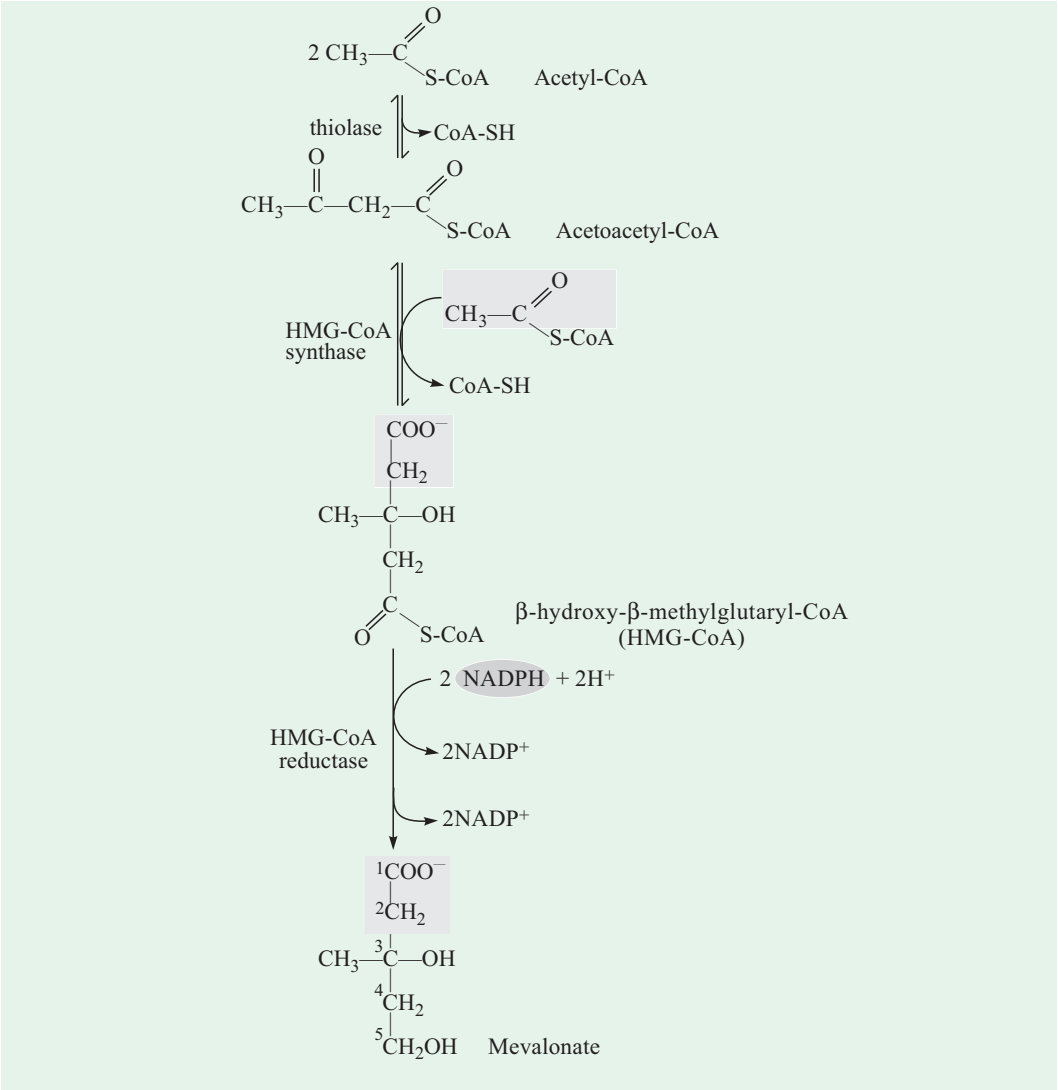


Fig. 25–31. Formation of mevalonate from acetyl-CoA

The origin of C-1 and C-2 in mevalonate from acetyl-CoA is shown anclosed in shaded rectangles.

Stage 3 : *Condensation of 6 activated isoprenes to squalene*

Isopentenyl pyrophosphate and dimethylallyl pyrophosphate now undergo a 'head-to-tail' condensation wherein one pyrophosphate group is displaced and a C-10 chain, **geranyl pyrophosphate** is formed (Fig. 25-33). The 'head' is the end to which pyrophosphate is joined. Geranyl pyrophosphate then undergoes another 'head-to-tail' condensation with **farnesyl pyrophosphate**. Finally, two moles of farnesyl pyrophosphate undergo yet another 'head-to tail' condensation, forming **squalene** and eliminating both pyrophosphate groups. Squalene has 30 carbon atoms, 24 in the main chain and 6 in the form of methyl group branches.

Geranyl and farnesyl pyrophosphates derive their common names from the sources from which they were first isolated. **Geraniol**, a component of rose oil, has the smell of geraniums. **Farnesol** is a scent present in the flowers of a tree, *Farnese acacia*. Many natural scents of plant origin are synthesized from isoprene units. **Squalene** was first isolated from the liver of sharks (*Squalus*).

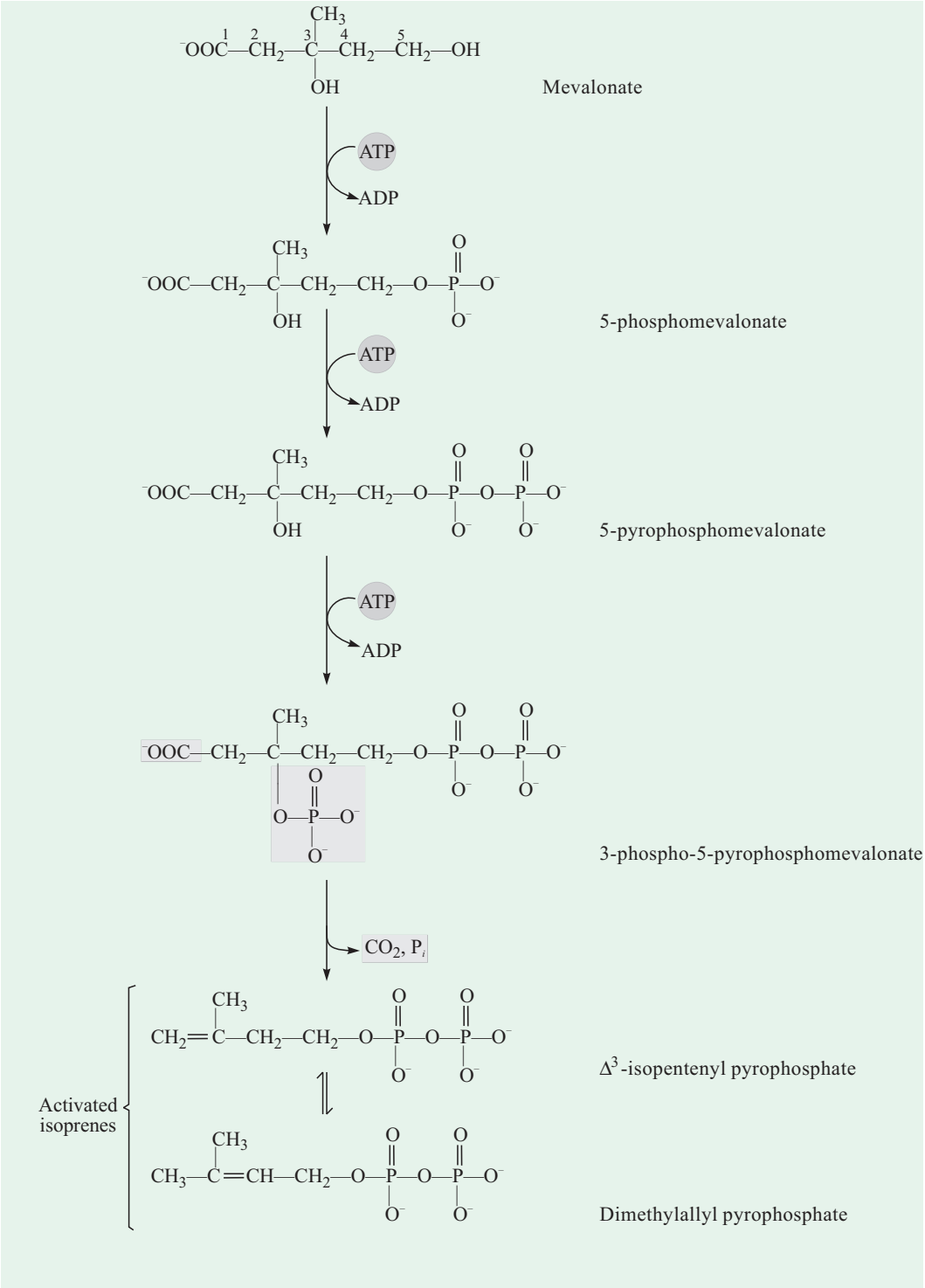


Fig. 25–32. Conversion of mevalonate into activated isoprene units

Note that 6 isoprene units combine to form squalene. The leaving groups of 3-phosho-5-pyrophosphomevalonate are shown in light-screened areas.

Stage 4 : *Conversion of squalene to the steroid nucleus*

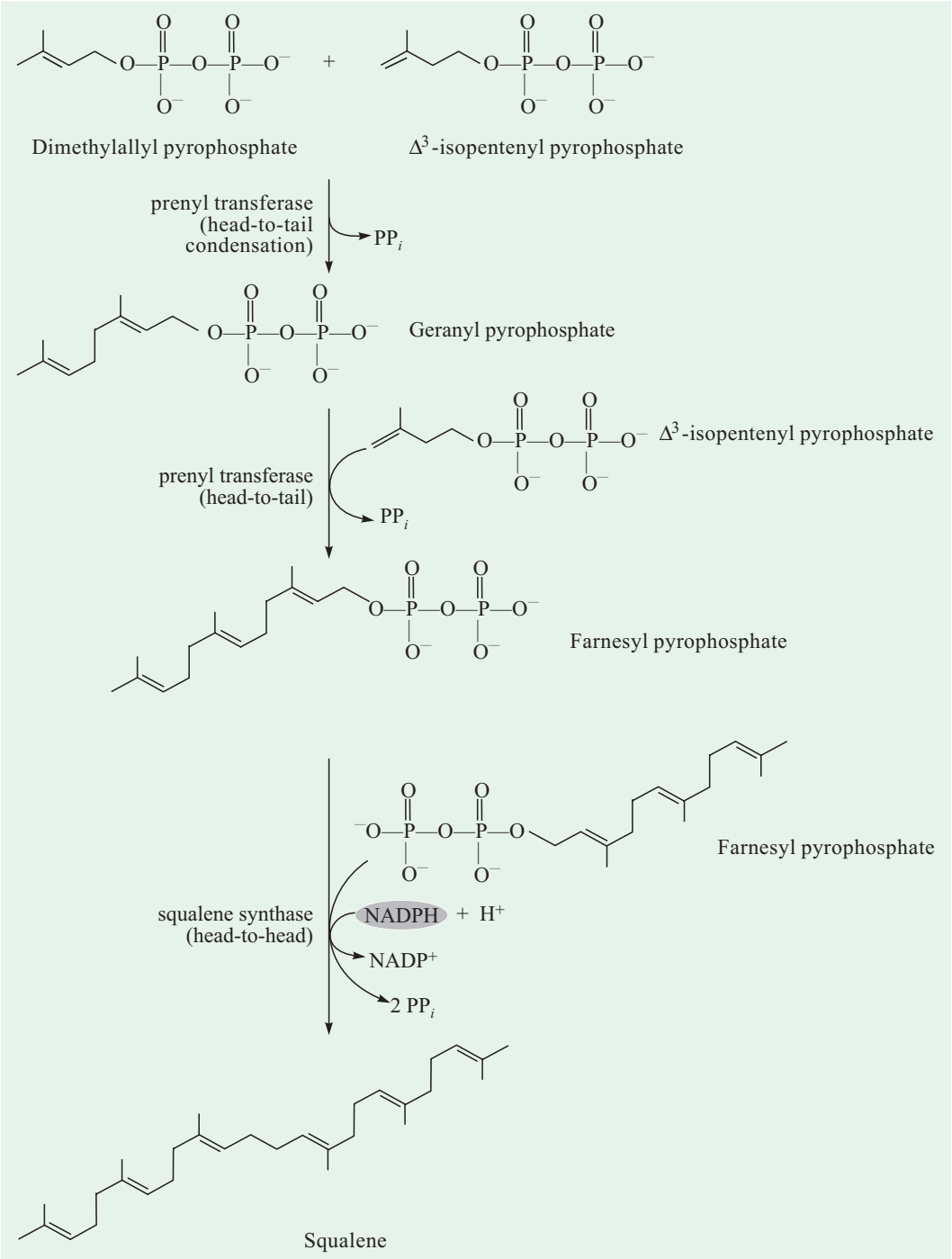


Fig. 25–33. Formation of squalene (C-30) by successive condensations of activated isoprene (C-5) units

All of the sterols are alcohols with a OH group at C-3 and have a steroid nucleus containing 4 fused rings (hence, so named). One oxygen atom from oxygen is added to the end of squalene

chain forming an epoxide, **squalene-2,3-epoxide** (Fig. 25–34). The reaction is catalyzed by squalene monooxygenase, another mixed-function oxidase enzyme. NADPH reduces the other oxygen atom of oxygen to water. The double bonds of the product, squalene-2,3-epoxide are positioned so that a concerted reaction can convert the linear squalene epoxide into a cyclic structure. In animals, this cyclization results in the formation of **lanosterol**, which contains the 4 rings characteristic of the steroid nucleus. Lanosterol is finally converted into **cholesterol** in a series of about 20 reactions, including the migration of some methyl groups and the removal of others.

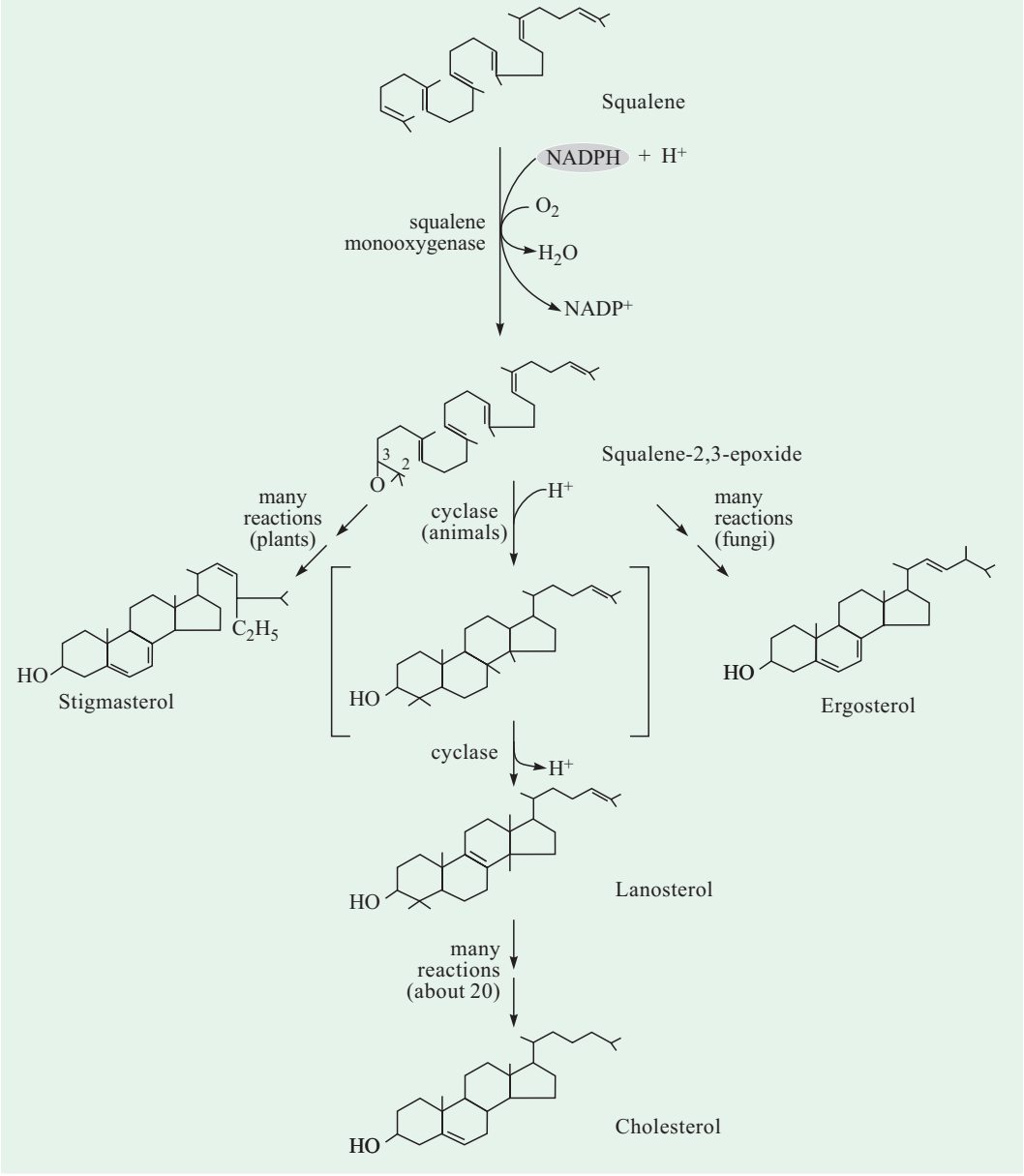


Fig. 25–34. Conversion of linear squalene into cyclic steroid nucleus
The first step in this sequence is catalyzed by a mixed-function oxidase (a monooxygenase), for which the cosubstrate is NADPH. The product is an epoxide, which in the next step is cyclized to the steroid nucleus. Note that the final product of these reactions in animal cells is cholesterol, but in other organisms slightly different sterols are produced.

In other organisms apart from animals, **closely-related sterols**, instead of cholesterol, are formed. In such cases, after the formation of squalene-2,3-epoxide, the synthetic pathways diverge slightly, yielding other sterols. As an instance, stigmasterol is formed in many plants and ergosterol in fungi (Fig. 25–34).

Most of the cholesterol synthesis takes place in the vertebrate liver cells (Fig. 25–35). While a small fraction of this is incorporated into the membranes of hepatocytes, most of it is exported in the form of either bile acids or cholesteryl esters. **Bile acids** and their salts are relatively hydrophilic cholesterol derivatives and help in lipid digestion. **Cholesteryl esters** are relatively hydrophobic and are formed in the liver through the action of acetyl-CoA—cholesteroyl acyl transferase (ACAT) on cholesterol. This enzyme catalyzes the transfer of a fatty acid from coenzyme A to the hydroxyl group of cholesterol (Fig. 25–36).

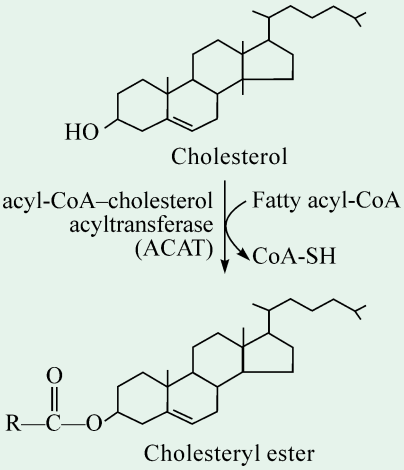


Fig. 25–36. Synthesis of cholesteryl ester

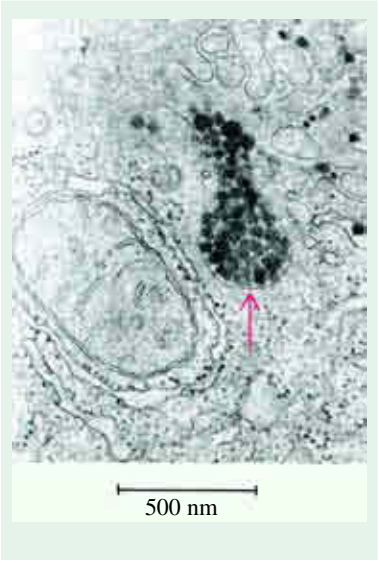


Fig. 25–35. Site of cholesterol *synthesis*

Electron micrograph of a part of a liver cell actively engaged in the synthesis and secretion of very low density lipoprotein (VLDL). The arrow points to a vesicle that is releasing its content of VLDL particles.

(Courtesy of Dr. George Palade)

Plasma Lipoproteins

Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are insoluble in water. These lipids must, however, be moved from liver and intestine, the tissues of their origin to the tissues where they will be stored or consumed. They are transported in the blood plasma in the form of **plasma lipoproteins**. These are macromolecular aggregates of specific carrier proteins (called apolipoproteins) with various combinations of phospholipids, cholesterol, cholesteryl esters and triacylglycerols.

Apolipoproteins or **apoproteins** ('apo' designates the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles. Each lipoprotein particle is a spherical aggregate, consisting of a core of hydrophobic lipids surrounded by a shell of polar lipids and apoproteins (Fig. 25–37). Lipoproteins are classified according to increasing density into very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), which may be separated by ultracentrifugation (Table 25–4) and visualized by electron microscopy.

Table 25–4. Major classes of human plasma lipoproteins

Lipoprotein	*Density (g/mL)*	*Composition (wt %)*				
		Protein	*Free cholesterol*	*Cholesteryl esters*	*Phospho-lipids*	*Triacyl-glycerols*
Chylomicrons	< 0.95	2	1	3	9	85
VLDL	0.95 — 1.006	10	7	12	18	50
LDL	1.007 — 1.063	23	8	37	20	10
HDL	1.064 — 1.210	55	2	15	24	4

(*Modified from D. Kritchevsky, 1986*)

Each class of lipoprotein performs a specific function which is determined by its point of synthesis, lipid composition and apoprotein content. Lipoproteins of human plasma contain at least 9 types of apolipoproteins (Table 25–5), based on their size, their reaction with specific antibodies and their distribution in the lipoprotein classes. These protein components act as signals, targeting lipoproteins to specific tissues or activating enzymes that act on the lipoproteins. They also solubilize hydrophobic lipids.

Table 25–5. Apolipoproteins of human plasma lipoproteins

Apolipoprotein	*Mol. Wt.*	*Lipoprotein association*	*Functions (if known)*
ApoA–I	28,331	HDL	Activates LCAT
ApoA–II	17,380	HDL	—
ApoB–48	2,40,000	Chylomicrons	—
ApoB–100	5,13,000	VLDL, LDL	Binds to LDL receptor
ApoC–I	7,000	VLDL, HDL	—
ApoC–II	8,837	Chylomicrons,VLDL, HDL	Activates lipoprotein lipase
ApoC–III	8,751	Chylomicrons, VLDL, HDL	Inhibits lipoprotein lipase
ApoD	32,500	HDL	—
ApoE	34,145	Chylomicrons,VLDL, HDL	Triggers clearance of VLDL and chylomicron remnants

(*Modified from Vane DE and Vane JE, 1985*)

Chylomicrons

The chylomicrons, Fig. 25–37 the first major lipoprotein type, are the largest of the lipoproteins and are between 180 and 500 nm in diameter. They have very low density (d < 0.95 g/cm^3) because of a high triacylglycerol contents (about 85%) in them (Table 25–4). Chylomicrons are synthesized in the smooth endoplasmic reticulum of epithelial cells lining the small intestine. They then move through the lymphatic system to enter the bloodstream. The apoproteins of chylomicrons consist of apoB-48, apoI, apoC-II. ApoC-II activates lipoprotein lipase in the capillaries of adipose, heart, skeletal muscle and lactating mammary tissues, allowing the release of free fatty acids to these tissues. Chylomicrons, thus, carry fatty acids obtained in the diet to the tissue in which they will be consumed or stored as fuel.

Very low-density lipoproteins (VLDLs) : Triacylglycerols and cholesterol in excess of the liver's own requirements are exported into the blood in the form of very low-density lipoproteins ($0.95< d <1.006$ g/cm^3). Excess carbohydrate in the diet can also be converted into triacylglycerols in the liver and exported as VLDLs. Besides triacylglycerols, VLDLs contain some cholesterol, cholesteryl esters, as well as apoB–100, apoC–I, apoC–II, apoC–III and apoE. These lipoproteins are transported in the blood from the liver to adipose tissue, where activation of lipoprotein lipase by apoC–II causes the release of free fatty acids from the triacylglycerols of the VLDL. Thus, triacylglycerols in VLDL, as in chylomicrons, are hydrolyzed by lipases on capillary surfaces.

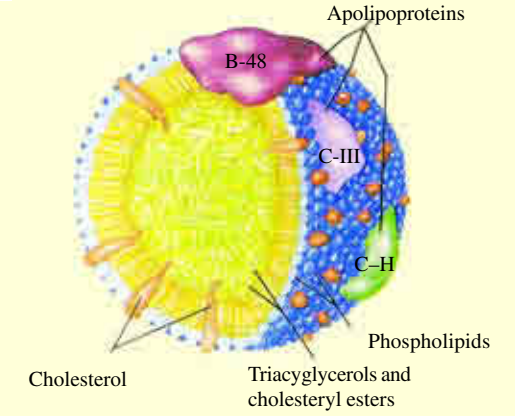


Fig. 25–37. Molecular structure of a chylomicron The surface is a layer of phospholipids with head groups facing the aqueous phase. Triacylglycerols sequestered. in the interior (*yellow*) make more than 80% of the mass. Several apolipoproteins that protrude from the surface (B-48, C-II, C-III) act as signals in the uptake and metabolism of chylomicron contents. The diameter of chylomicrons ranges between 100 and 500 nm.

Low-density lipoproteins (LDLs) : The loss of triacylglycerols converts VLDL to low-density liprotein. LDL (Fig. 25–38) is the major carrier of cholesterol in blood. It has a diameter of 22 nm and a mass of 3,000 kd. It contains a core of about 1,500 esterified cholesterol molecules; linoleate is the most common fatty acyl chain in these esters. This highly hydrophobic core is surrounded by a shell of phospholipids and unesterified cholesterols. The shell also contains a single copy of apoB–100, which is recognized by target cells. *The role of LDL is to transport cholesterol to peripheral tissues and regulate do novo cholesterol synthesis at these sites.*

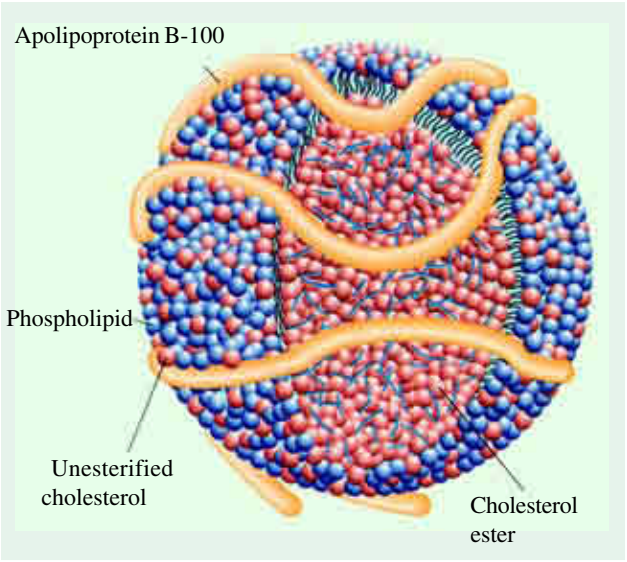


Fig. 25–38. LDL cholesterol Each particle is approximately 22 nm (220 Å) in diameter and consists of approximately 1500 cholesterol ester molcules, surrounded by a mixed monomolecular layer of phospholipids and cholesterol, and a single molcule of the protein apolipoprotein B, which interacts specifically with the LDL receptor projecting from the plasma membrane. Apolipoprotein B-100 (apoB-100) is one of the largest single polypeptide chains known with 4,636 amino acid residues and has a molecular weight of 5,13, 000 daltons.

High-density lipoproteins (HDLs) : The fourth major lipoprotein type, high-density lipoproteins, has a high density ($1.063 < d <1.210$ g/cm^3) and is synthesized in the liver as small, protein-rich particles containing relatively little cholesterol and cholesteryl esters. HDLs contain apoC–I and apoC–II, among other apolipoproteins, as well as the enzyme, lecithin-cholesterol acyl transferase (LCAT). The enzyme catalyzes the formation of cholesteryl esters from lecithin (phosphatidylcholine) and

cholesterol (Fig. 25–39). After release into the bloodstream, the nascent (or newly synthesized) HDL collects cholesteryl esters from other circulating lipoprotiens. Chylomicrons and VLDLs, after the removal of their triacylglycerols by lipoprotein lipase, are rich in cholesterol and phosphatidylcholine. LACT on the surface of nascent HDL converts this phosphatidylcholine and cholesterol to cholesteryl esters, which enter the interior of the nascent HDL, converting it from a flat disc to a sphere– a mature HDL. This cholesterol-rich lipoprotein now returns to the liver, where the cholesterol is unloaded. Some of this cholesterol is converted into bile salts.

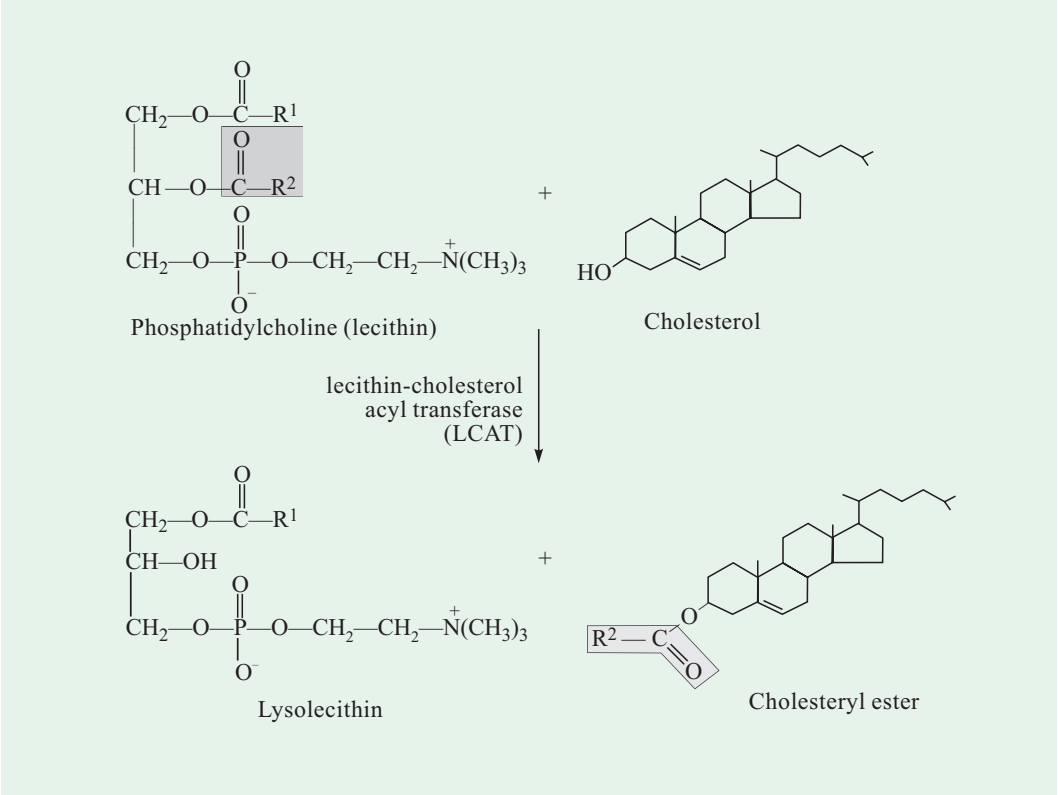


Fig. 25–39. The reaction catalyzed by lecithin-cholesterol acyl transferase (LCAT)

LCAT is present on the surface of HDL and is stimulated by the HDL component, apoA-I. The cholesteryl esters accumulate within nascent HDLs, converting them to mature HDLs.

Regulation of Cholesterol Biosynthesis

Cholesterol synthesis is a complex and energy-expensive process. In mammals, cholesterol production is regulated by intracellular cholesterol concentration and by the hormones, glucagon and insulin. The rate-limiting step in the pathway to cholesterol is the conversion of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) into mevalonate (Fig. 25–40). This reaction is catalyzed by a complex regulatory enzyme called HMG-CoA reductase. It is allosterically inhibited by unidentified derivatives of cholesterol and of the key intermediate mevalonate (Fig. 25–40). HMG-CoA reductase is also hormonally regulated. The enzyme exists in phosphorylated (inactive) and dephosphorylated (active) forms. Glucagon stimulates phosphorylation (inactivation) and insulin promotes dephosphorylation, activating the enzyme and favouring cholesterol synthesis.

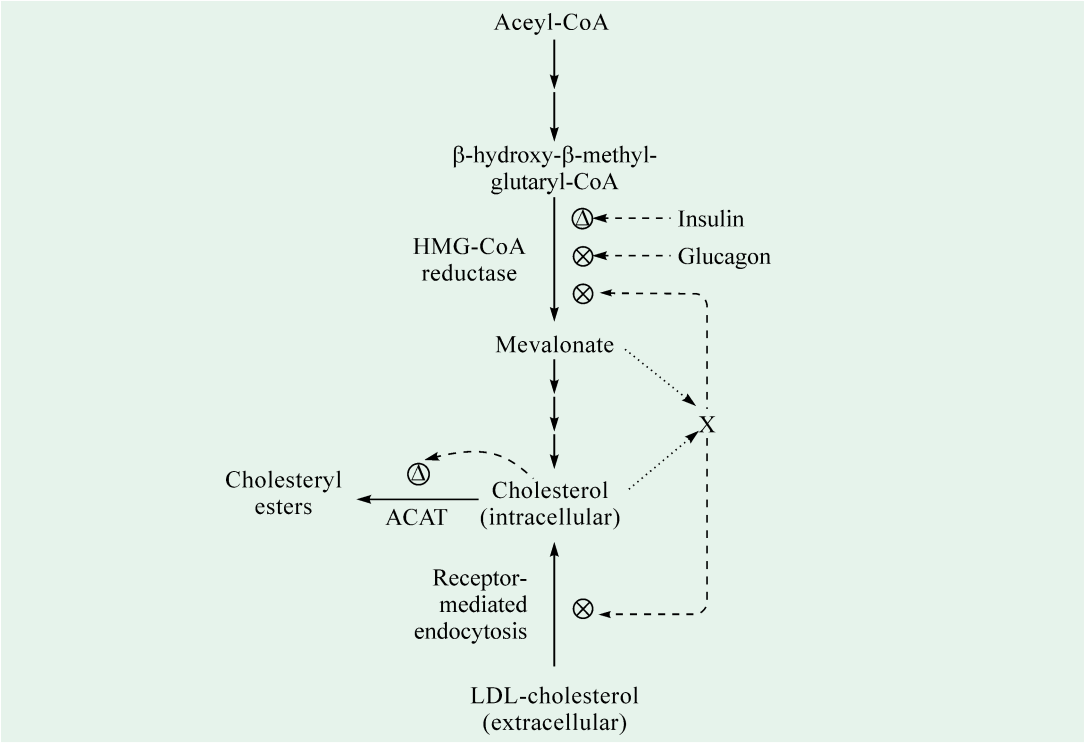


Fig. 25–40. Regulation of cholesterol biosynthesis

Glucagon acts by promoting phosphorylation of HMG-CoA reductase, insulin by promoting dephosphorylation. X represents unidentified metabolites of cholesterol and mevalonate, or other unidentified second messengers.

(*Adapted from Lehninger, Nelson and Cox, 1993*)

High intracellular cholesterol inhibits the acitivity of HMG-CoA reductase. It also slows the synthesis of new molecules of the enzyme. Furthermore, high intracellular concentrations of cholesterol also activate ACAT, increasing esterification of cholesterol for storage. Finally, high intracellular cholesterol causes reduced production of the LDL receptor, slowing the uptake of cholesterol from the blood.

Overproduction of cholesterol can lead to serious disease. When the amount of cholesterol synthesized and obtained in the diet exceeds the amount required for the synthesis of membranes, bile salts and steroids, the pathological accumulation of cholesterol in blood vessels *i.e.*, atherosclerotic plaques (Fig. 25-41) can develop into a heart disease in humans called **atherosclerosis**, characterized by the obstruction of blood vessels (Fig, 25–42). Heart failure from occluded coronary arteries is a major cause of death in industrialized societies.

Studies have revealed that atherosclerosis results from an inborn error called **familiar hypercholesterolemia** (FH) which affects both homozygous and heterozygous individuals. The cholesterol level in the plasma of

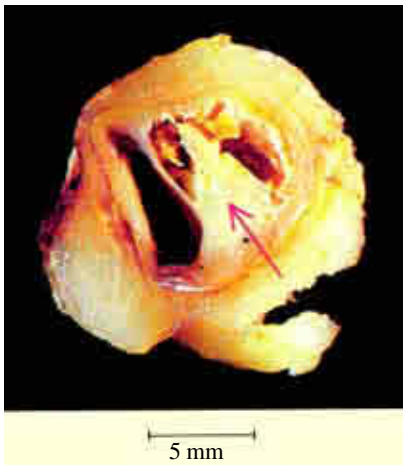


Fig. 25–41. An atherosclerotic plaque

A plaque (marked by an arrow) blocks most of the lumen of this blood vessel. The plaque is rich in cholesterol.

[Courtesy of Dr. Jeffrey Sklar.]

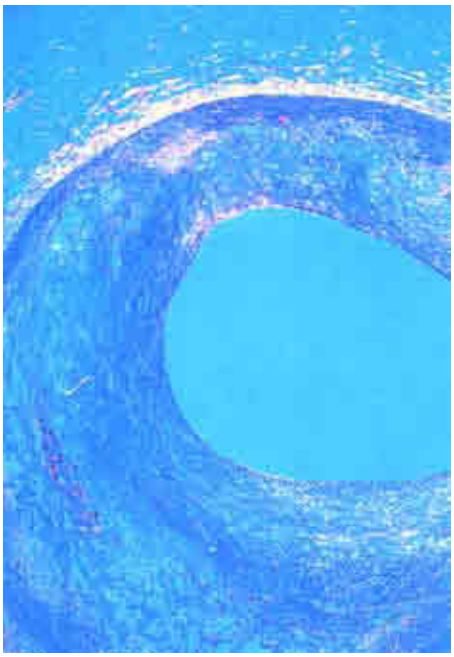
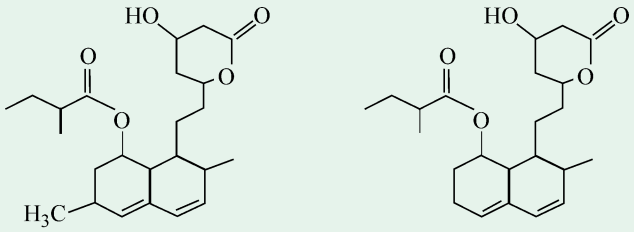


Fig. 25–42. Atherosclerosis
A human heart artery considerably blocked by cholesterol as veiwed under polarized light

homozygotes is typically 680 mg/dl, compared with 300 mg/dl in heterozygotes. *In FH, cholesterol is deposited in various tissues because of high concentration of LDL-cholesterol in the plasma*. Nodules of cholesterol called *xanthomas* are prominent in skin and tendons. More harmful is the deposition of cholesterol in atherosclerotic plaques, causing arterial narrowing and leading to heart attacks. In reality, most homozygotes die of coronary artery disease in childhood. The disease in heterozygotes has a milder and more variable clinical manifestation. *The molecular defect in most cases of familial hypercholesterolemia is an absence or deficiency of functional receptors for LDL.* Homozygotes have almost no receptors for LDL, whereas heterozygotes have about half the normal number. As a result, the entry of LDL into liver and other cells is impaired, leading to an increased plasma level of LDL. Thus, cholesterol obtained in the diet is not cleared from the blood ; it accumulates and contributes to the formation of atherosclerotic plaques. This genetic disorder results from a mutation at a single autosomal locus.

Clinical assay results are often expressed in mg/dl, which is equal to mg per 100 ml.

Two natural products derived from fungi, **lovastatin** (also called **mevinolin**) and **compactin** (Fig. 25–43) are being widely used to lower the plasma cholesterol level. Both are potent competitive inhibitors of HMG-CoA reductase, the key control point in the biosynthetic pathway. The consequent increase in the number of LDL receptors on liver cells leads to a decrease in the LDL level in blood.



Lovastatin **Compactin**

Fig. 25–43. The two competitive inhibitors of HMG-CoA reductase
Note that their structure resembles 3-hydroxy-3-methylglutaryl-CoA, the substrate of the reaction catalyzed by HMG-CoA reductase.

BIOSYNTHESIS OF STEROID HORMONES

There are five major classes of steroid hormones : glucocorticoids, mineralocorticoids, androgens, estrogens and progestagens. All these steroid hormones in humans are derived from cholesterol. Glucocorticoids and mineralocorticoids are synthesized in the cortex of the adrenal gland ; androgens in the testis ; estrogens in the ovary ; and progestagens in the corpus luteum. **Glucocorticoids** (such as corticosterone) promote gluconeogenesis and the formation of glycogen and enhance the degradation of fat and protein ; they also enable animals to respond to stress–indeed, the absence of glucocorticoids can be fatal. **Mineralocorticoids** (primarily aldosterone)

act on the distal tubules of the kidney to increase the reabsorption of Na^+ and the excretion of K^+ and H^+, which lead to an increase in blood volume and blood pressure. **Androgens** (such as testosterone) influence the development of secondary sex characteristics in males. **Estrogens** (such as estradiol) are responsible for the development of female secondary sex characteristics. Progesterone, a **progestagen**, prepares the lining of the uterus for implantation of an ovum ; it is also essential for the maintenance of pregnancy ; it also participates, along with estrogens, in the ovarian cycle. The steroid hormones are effective at very low concentrations and are hence synthesized in relatively small quantities. Little cholesterol is consumed in their production, in contrast to bile salts.

The synthesis of steroid hormones requires removal of some or all of the carbons in the "side chain" that projects from C-17 of the D ring of cholesterol. Side chain removal takes place in the mitochondria of tissues that make steroid hormones. It involves first the hydroxylation of two adjacent carbons in the side chain (C-20 and C-22) and then cleavage of the bond between them (Fig. 25–44). Formation of individual hormones also involves the introduction of oxygen atoms.

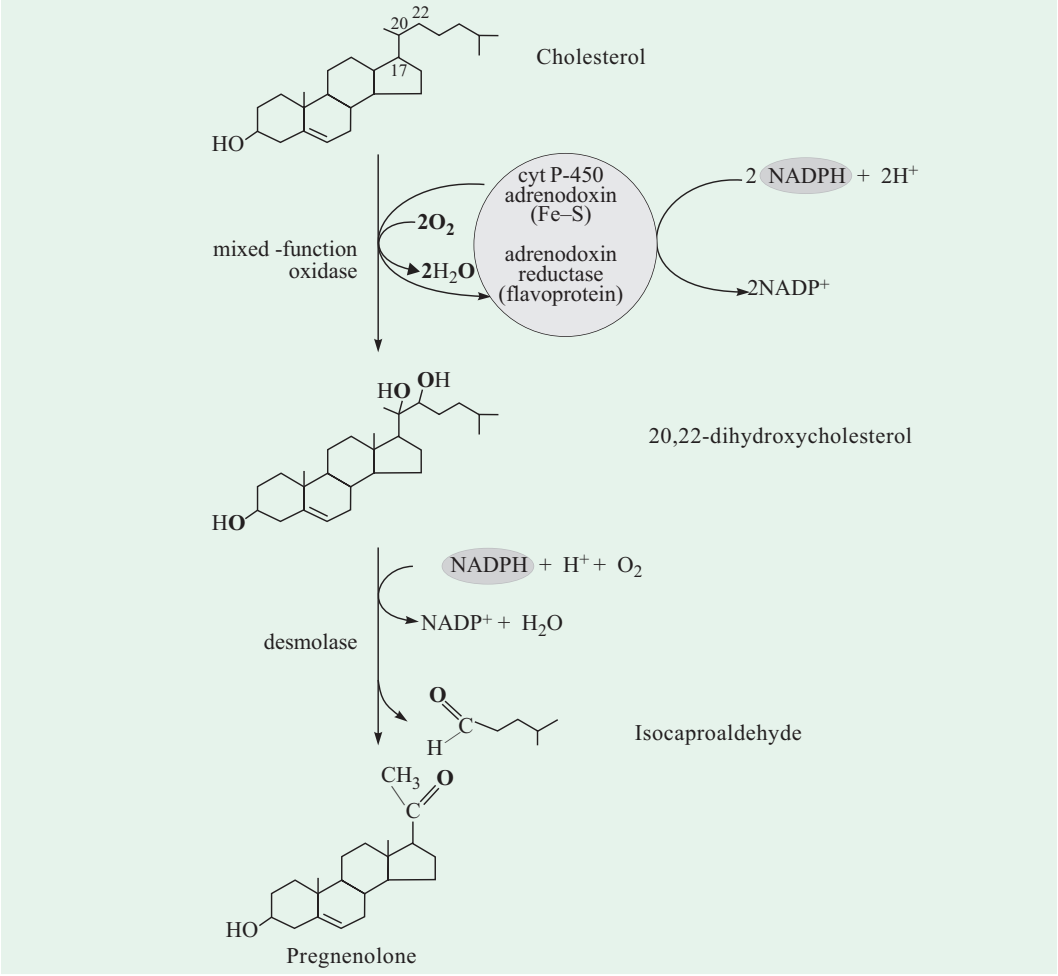


Fig. 25–44. Side chain cleavage in the synthesis of steroid hormones
This involves oxidation of adjacent carbons. Cytochrome P-450 acts as electron carrier in this mixed-function oxidase system, which also requires the electron-transferring proteins, adrenodoxin and adrenodoxin reductase. This side chain-cleaving system is found in mitochondria of the adrenal cortex, where active steroid production occurs. Note that pregnenolone is produced by the cleavage of 6 carbon atoms from the side chain of cholesterol. Pregnenolone is the precursor of all other steroid hormones.

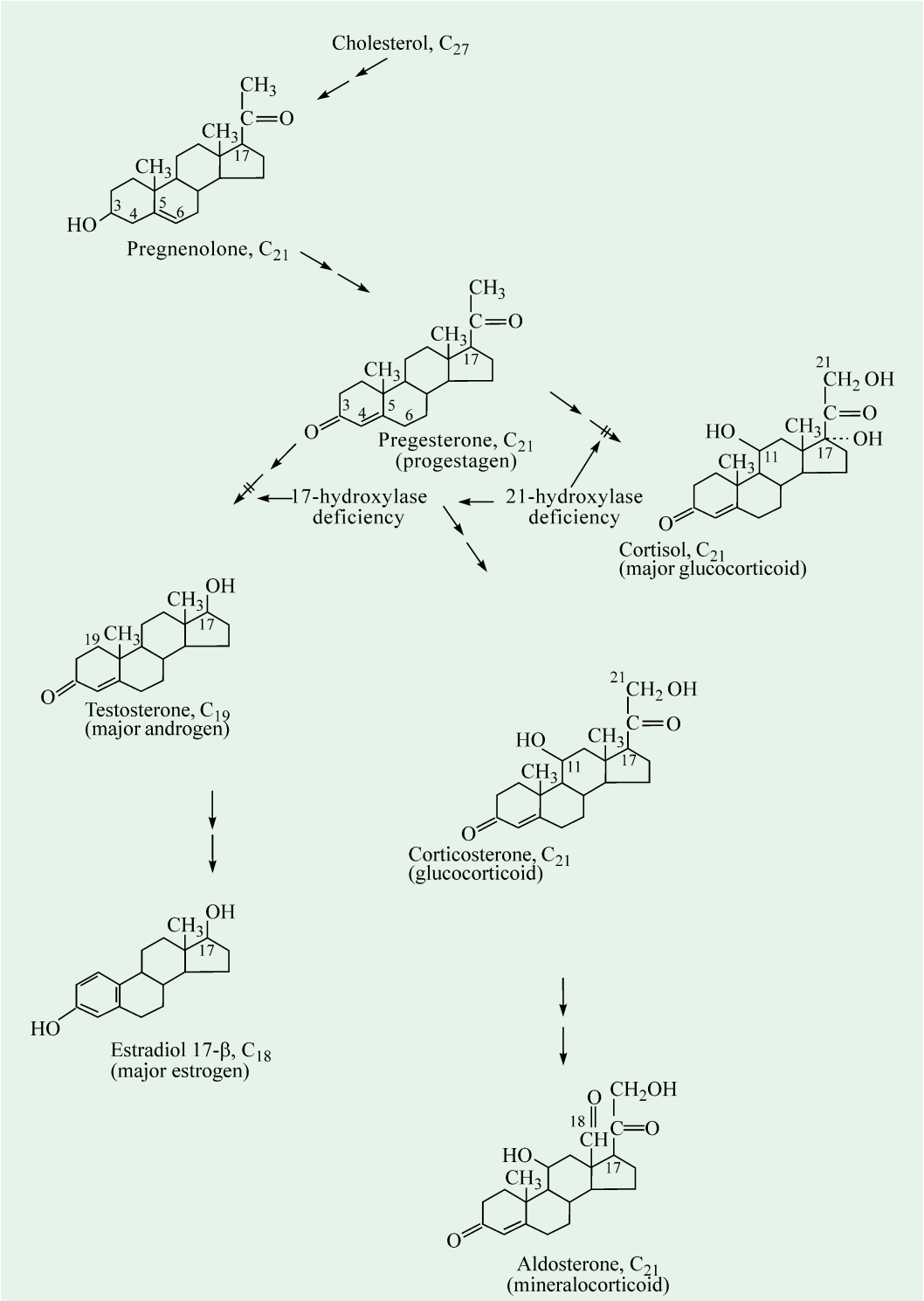


Fig. 25–45. **Steroid hormone production from pregnenolone**

All of the hydroxylation and oxygenation reactions in steroid biosynthesis are catalyzed by monooxygenases (mixed-function oxidases of mitochondria) that require molecular oxygen and NADPH. Mitochondrial cytochrome P-450 acts as an electron carrier in this monooxygenase system, which also requires the electron-transferring proteins adrenodoxin and adrenodoxin reductase.

The removal of 6 carbons from C_{27} **cholesterol** yields the C_{21} structure, **pregnenolone**, which is common to many steroid hormones. Pregnenolone is then converted into C_{21} **progesterone** (a progestagen) in a two-step process that includes oxidation of the OH group of the C-3 to a keto group and isomerization of the double bond from C-5,6 to C-4,5 (Fig. 25–45).

The 3 principal steroid hormones are cortisol, corticosterone (both glucocorticoids) and aldosterone (a mineralocorticoid). Hydroxylation of C-11, C-17 and C-21 converts progesterone into cortisol (Fig. 25–45) and, in this reaction series, hydroxylation of C-17 must occur prior to that of C-21. The C-11 hydroxylation can take place at any stage of the conversion. For aldosterone synthesis, progesterone is first hydroxylated at C-21 and then at C-11 to produce **corticosterone**. The C-18 methyl group of corticosterone is then oxidized to an aldehyde in the last step, which yields **aldosterone**.

Progesterone is also the precursor of androgens, which in turn are precursors of estrogens (Fig. 25–45). Synthesis of testosterone (C_{19}) involves loss of C-20 and C-21 from progesterone (C_{21}) ; the steroid product of these reactions is another androgen, **androstenedione** (C_{19}) which has a keto group at C-17. This keto group is then reduced to a hydroxyl group to produce **testosterone. Estrogen** (C_{18}) is synthesized from testosterone by the loss of its C-19 methyl group and conversion of A ring into an aromatic structure. **Estrone** (C_{18}), another estrogen, is produced in an analogous manner from androstenedione.

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PROBLEMS

1. Compare and contrast fatty acid oxidation and synthesis with respect to
   (*a*) site of the process.
   (*b*) acyl carrier.
   (*c*) reductants and oxidants.
   (*d*) stereochemistry of the intermediates.

(*e*) direction of synthesis or degradation.

(*f*) organization of the enzyme system.

2. For each of the following unsaturated fatty acids, indicate whether the biosynthetic precursor in animals is palmitoleate, oleate, linoleate, or linolenate.

(*a*) 18 : 1 cis-Δ^{11}

(*b*) 18 : 3 cis-Δ^{6}, Δ^{9}, Δ^{12}

(*c*) 20 : 2 cis-Δ^{11}, Δ^{14}

(d) 20 : 3 cis-Δ^{5}, Δ^{8}, Δ^{11}

(e) 22 : 1 cis-Δ^{13}

(f) 22 : 6 cis-Δ^{4}, Δ^{7}, Δ^{10}, Δ^{13}, Δ^{16}, Δ^{19}

3. What is the role of decarboxylation in fatty acid synthesis ? Name another key reaction in a metabolic pathway that employs this mechanistic motif.

4. The serine residue in acetyl-CoA carboxylase that is the target of the AMP-dependent protein kinase is mutated to alanine. What is a likely consequence of this mutation ?

5. What is a potential disadvantage of having many catalytic sites together on one very long polypeptide chain ?

6. Write a balanced equation for the synthesis of a triacylglycerol, starting from glycerol and fatty acids.

7. Write a balanced equation for the synthesis of phosphatidyl serine by the *de novo* pathway, starting from serine, glycerol, and fatty acids.

8. What is the activated reactant in each of the following biosyntheses ?

(a) Phosphatidyl serine from serine

(*b*) Phosphatidyl ethanolamine from ethanolamine

(*c*) Ceramide from sphingosine

(*d*) Sphingomyelin from ceramide

(*e*) Cerebroside from ceramide

(*f*) Ganglioside G_{M1} from ganglioside G_{M2}

(*g*) Farnesyl pyrophosphate from geranyl pyrophosphate

9. Would you expect the reaction catalyzed by cardiolipin synthase to be strongly exergonic or strongly endergonic ? Explain your reasoning.

10. Identify a pathway for utilization of the four carbons of acetoacetate in cholesterol biosynthesis. Carry your pathway as far as the rate-determining reaction in cholesterol biosynthesis.

11. Explain why a deficiency of steroid 21-hydroxylase leads to excessive production of sex steroids (androgens and estrogens).

12. After a person has consumed large amounts of sucrose, the glucose and fructose that exceed caloric requirements are transformed to fatty acids for triacylglycerol synthesis. This fatty acid synthesis consumes acetyl-CoA, ATP, and NADPH. How are these substances produced from glucose ?

13. Write the net equation for the biosynthesis of palmitate in rat liver, starting from mitochondrial acetyl-CoA and cytosolic NADPH, ATP, and CO_2.

14. In the condensation reaction catalyzed by β-ketoacyl-ACP synthase (Fig. 25–), a four-carbon unit is synthesized by the combination of a two-carbon unit and a three-carbon unit, with the release of CO_2. What is the thermodynamic advantage of this process over one that simply combines two two-carbon units ?

15. The biosynthesis of palmitoleate (Fig. 25–), a common unsaturated fatty acid with a *cis* double bond in the Δ^9 position, uses palmitate as a precursor. Can this be carried out under strictly anaerobic conditions ? Explain.

16. Use a net equation for the biosynthesis of tripalmitoylglycerol (tripalmitin) from glycerol and palmitate to show how many ATPs are required per molecule of tripalmitin formed.

17. Write the sequence of steps and the net reaction for the biosynthesis of phosphatidylcholine by the salvage pathway from oleate, palmitate, dihydroxyacetone phosphate, and choline. Starting from these precursors, what is the cost in number of ATPs of the synthesis of phosphatidylcholine by the salvage pathway ?

18. The rate-limiting step in the early stages of cholesterol biosynthesis is the conversion of β-hydroxy-β-methylglutaryl-CoA to mevalonate, catalyzed by HMG-CoA reductase (Fig. 25–). The liver of a fasting animal has decreased reductase activity. When the flow through this reaction is reduced, what is the effect on the formation of ketone bodies from acetyl-CoA ? How does this explain increased ketosis during fasting ?

19. Cells from a patient with familial hypercholesterolemia (FH) and cells from an individual without that disease were incubated with LDL particles containing radioactively-labeled cholesterol. After incubation, the incubation medium was removed and the radioactivity of the cells measured. The cells were treated to remove any bound material, lysed, and internal cholesterol content measured. Results are given below. What mutation of the gene for the LDL receptor protein could account for the results ?

Cell Type	*Radioactivity of Cell*	*Cholesterol Content*
Normal	3,000 cpm/mg cells	Low
FH	3,000 cpm/mg cells	High

20. The combination of bile salt-binding resin and an HMG CoA reductase inhibitor is very effective in reducing serum cholesterol for most patients with high cholesterol. Why is this treatment much less effective for patients with familial hypercholesterolemia ?