Bch 303

K4 TRIACYLGLYCEROLS

Key Notes

Structure and function

Triacylglycerols (fats or triglycerides) consist of three fatty acid chains esterified to a glycerol backbone. Simple triacylglycerols have three identical fatty acids, mixed triacylglycerols have two or three different fatty acids. Triacylglycerols are the major energy store and the major dietary lipid in humans. They are insoluble in water and are stored in specialized adipose (fat) cells.

Synthesis

Triacylglycerols are synthesized from glycerol 3-phosphate, which is derived from the glycolytic intermediate dihydroxyacetone phosphate, and fatty acyl CoAs. Acyl CoA molecules are added on to glycerol 3-phosphate to form first lysophosphatidic acid and then phosphatidic acid. The phosphate group is then removed to form diacylglycerol (DAG), which is further acylated to triacylglycerol. The energy for the synthesis of triacylglycerols comes from the hydrolysis of the high-energy thioester bond in acyl CoA.

Breakdown

The fatty acids in triacylglycerols are released from the glycerol backbone by the action of lipases. The free fatty acids can then be degraded by β-oxidation to produce energy. The glycerol is converted into dihydroxyacetone phosphate which enters glycolysis.

Regulation

The concentration of free fatty acids in the blood is controlled by the rate at which hormone-sensitive triacylglycerol lipase hydrolyzes the triacylglycerols stored in adipose tissue. Glucagon, epinephrine and norepinephrine cause an increase in the intracellular level of cAMP which allosterically activates cAMP-dependent protein kinase. The kinase in turn phosphorylates hormone-sensitive lipase, activating it, and leading to the release of fatty acids into the blood. Insulin has the opposite effect; it decreases the level of cAMP which leads to the dephosphorylation and inactivation of hormone-sensitive lipase.

Related topics

Fatty acid breakdown (K2)
Fatty acid synthesis (K3)
Lipoproteins (K6)

Structure and function

Triacylglycerols (also called fats or triglycerides) consist of three fatty ac chains esterified to a glycerol backbone. Simple triacylglycerols have thr identical fatty acids esterified to the glycerol backbone, while mixed triac glycerols have two or three different fatty acid chains (*Fig. 1*). Triacylglycer constitute the major fuel store and the major dietary lipid in huma Triacylglycerols are a highly concentrated energy store. The energy yield fr the complete oxidation of fatty acids is about 39 kJ g^{-1}, compared with an ener yield of 13 kJ g^{-1} of carbohydrate or protein. The hydrophobic properties of f make them insoluble in water, and fats are stored in specialized cells call adipose cells (fat cells), which consist almost entirely of triacylglycerol. The

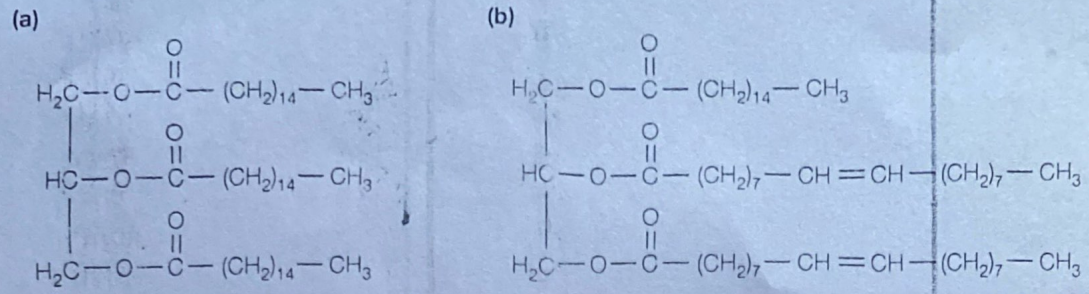


Fig. 1. Structure of (a) a simple triacylglycerol (1,2,3-tripalmitoyl-glycerol) and (b) a mixed triacylglycerol (1-palmitoyl-2,3-dioleoyl-glycerol).

cells are specialized for the synthesis and storage of triacylglycerols and for their mobilization into fuel molecules. Triacylglycerols are transported round the body in large lipid–protein particles called **lipoproteins** (see Topic K6).

Synthesis

Triacylglycerols are synthesized from **fatty acyl CoAs** and **glycerol 3-phosphate** (*Fig.* 2). The glycolytic intermediate **dihydroxyacetone phosphate** is first reduced to glycerol 3-phosphate which is, in turn, acylated by glycerol-3-phosphate acyltransferase to form **lysophosphatidic acid**. This is then reacted with a further acyl CoA molecule to form **phosphatidic acid**. Removal of the phosphate group from phosphatidic acid generates **diacylglycerol (DAG)**, which is further acylated with a third acyl CoA molecule to form **triacylglycerol** (*Fig.* 2). ATP is not involved in the biosynthesis of triacylglycerols. Instead the reactions are driven by the cleavage of the high-energy thioester bond between the acyl moiety and CoA. Both phosphatidic acid (phosphatidate) and DAG are also used in the synthesis of membrane phospholipids (see Topic E1).

Breakdown

The initial event in the utilization of both stored fat and dietary fat as energy sources is the hydrolysis of triacylglycerol by **lipases**. These enzymes release the three fatty acid chains from the glycerol backbone (*Fig.* 3). The fatty acids can then be broken down in **β-oxidation** to generate energy (see Topic K2). The glycerol backbone is also utilized, being transformed into dihydroxyacetone phosphate, an intermediate in glycolysis (*Fig.* 4). This requires two enzymes, glycerol kinase, which uses ATP to phosphorylate glycerol, producing L-glycerol 3-phosphate, and glycerol 3-phosphate dehydrogenase which produces dihydroxyacetone phosphate.

In the intestine, dietary fats are hydrolyzed by **pancreatic lipase** and the released fatty acids taken up into the **intestinal cells**. Both the digestion and uptake processes are aided by the detergent-like properties of the **bile salts** (see Topic K5).

Regulation

The breakdown of fatty acids in β-oxidation (see Topic K2) is controlled mainly by the concentration of free fatty acids in the blood, which is, in turn, controlled by the hydrolysis rate of triacylglycerols in adipose tissue by **hormone-sensitive triacylglycerol lipase**. This enzyme is regulated by **phosphorylation** and **dephosphorylation** (*Fig.* 5) in response to hormonally controlled levels of the intracellular second messenger **cAMP** (see Topic E5). The catabolic hormones **glucagon**, **epinephrine** and **norepinephrine** bind to receptor proteins on the cell surface and increase the levels of cAMP in adipose cells through activation of **adenylate cyclase** (see Topic E5). The cAMP allosterically activates

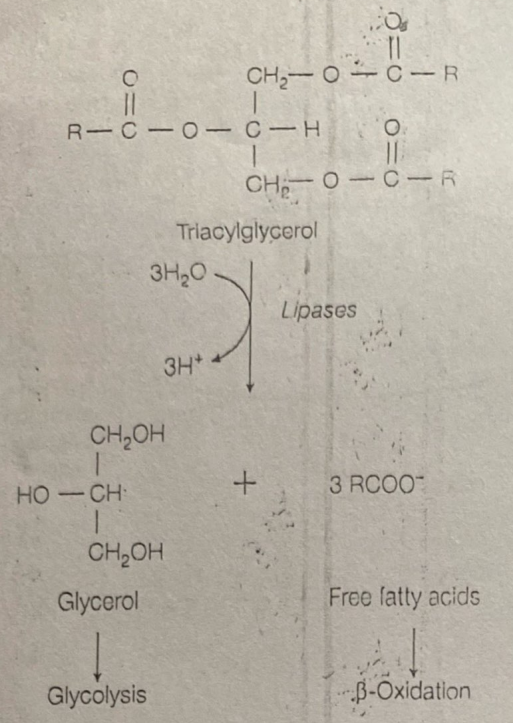


Fig. 3. Breakdown of triacylglycerols.

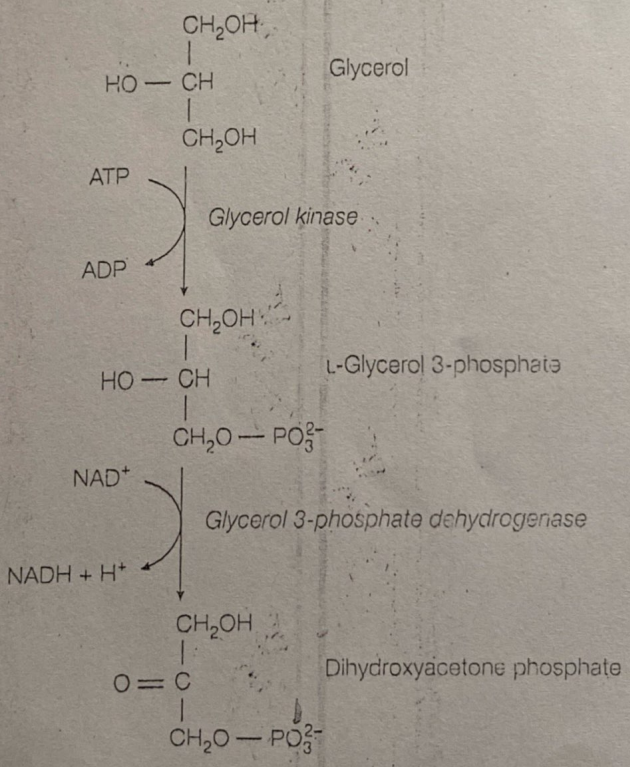


Fig. 4. Conversion of glycerol into the glycolytic intermediate dihydroxyacetone phospha

The anabolic hormone insulin has the opposite effect to glucagon and e
nephrine. It stimulates the formation of triacylglycerols through decreasing
level of cAMP, which promotes the dephosphorylation and inactivation
hormone-sensitive lipase (*Fig. 5*). Insulin also stimulates the dephosphorylat
of acetyl CoA carboxylase, thereby activating fatty acid synthesis (see To
K3). Thus fatty acid synthesis and degradation are coordinately controlled
as to prevent a futile cycle.

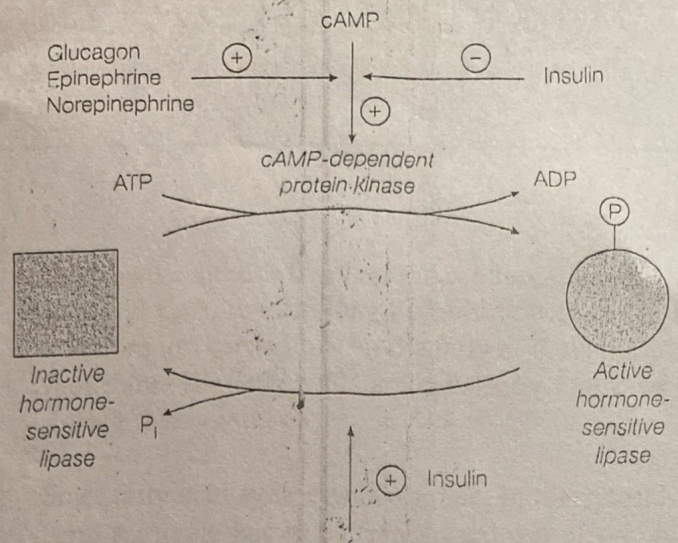


Fig. 5. Summary of the control of hormone-sensitive triacylglycerol lipase.

K3 FATTY ACID SYNTHESIS

Key Notes

Overview

Fatty acid synthesis involves the condensation of two-carbon units, in the form of acetyl CoA, to form long hydrocarbon chains in a series of reactions. These reactions are carried out on the fatty acid synthase complex using NADPH as reductant. The fatty acids are covalently linked to acyl carrier protein (ACP) during their synthesis.

Transport into the cytosol

Since fatty acid synthesis takes place in the cytosol, the acetyl CoA produced from pyruvate has to be transported out of the mitochondria. However, the inner mitochondrial membrane is not permeable to this compound, so it is first combined with oxaloacetate to form citrate which readily crosses the membrane. In the cytosol the citrate is cleaved to regenerate the acetyl CoA.

The pathway

The first committed step in fatty acid biosynthesis is the carboxylation of acetyl CoA to form malonyl CoA which is catalyzed by the biotin-containing enzyme acetyl CoA carboxylase. Acetyl CoA and malonyl CoA are then converted into their ACP derivatives. The elongation cycle in fatty acid synthesis involves four reactions: condensation of acetyl-ACP and malonyl-ACP to form acetoacetyl-ACP releasing free ACP and CO_2, then reduction by NADPH to form D-3-hydroxybutyryl-ACP, followed by dehydration to crotonyl-ACP, and finally reduction by NADPH to form butyryl-ACP. Further rounds of elongation add more two-carbon units from malonyl-ACP on to the growing hydrocarbon chain, until the C16 palmitate is formed. Further elongation of fatty acids takes place on the cytosolic surface of the smooth endoplasmic reticulum (SER).

Formation of double bonds

The enzymes for introducing double bonds into the acyl chain are also present on the cytosolic surface of the SER. The polyunsaturated fatty acids linoleate and linolenate cannot be synthesized by mammals and are therefore termed essential fatty acids as they have to be ingested in the diet.

Regulation

The key control point of fatty acid synthesis is acetyl CoA carboxylase which catalyzes the formation of malonyl CoA. Acetyl CoA carboxylase is inactivated by phosphorylation by an AMP-activated protein kinase. Thus when the energy charge of the cell is low (high AMP, low ATP) acetyl CoA carboxylase is inactive. It is reactivated by dephosphorylation by protein phosphatase 2A. Glucagon and epinephrine inhibit fatty acid synthesis by inhibiting protein phosphatase 2A, whereas insulin stimulates fatty acid synthesis by activating the phosphatase. Acetyl CoA carboxylase is also allosterically regulated: citrate activates the enzyme, whereas palmitoyl CoA inhibits it.

Related topics

Structures and roles of fatty acids (K1)
Fatty acid breakdown (K2)
Triacylglycerols (K4)

Overview

Fatty acids are synthesized by the condensation of two-carbon units. Howe in terms of the enzymic steps involved, the process is not the reverse of oxidation (see Topic K2). Fatty acid synthesis involves a separate series reactions to build up long-chain hydrocarbons from acetyl CoA units. The differences between fatty acid synthesis and breakdown are:

- fatty acid synthesis occurs in the cytosol of both prokaryotes and euka otes whereas their degradation occurs in the mitochondria of eukaryote
- fatty acid synthesis uses NADPH as the reductant whereas NADH produced in β-oxidation;
- during their synthesis, fatty acids are covalently linked to an acyl car protein (ACP) as opposed to CoA in their degradation;
- the enzyme activities of fatty acid synthesis in higher organisms are pres in a single, multifunctional polypeptide chain (as a dimer) called fatty a synthase, whereas in β-oxidation the individual activities are present separate enzymes.

Transport into the cytosol

Fatty acids are synthesized in the cytosol, but acetyl CoA is produced fr pyruvate in the mitochondria (see Topic L1). Thus the acetyl CoA must transferred from the mitochondria into the cytosol to allow fatty acid synthe to occur. However, the inner mitochondrial membrane is not readily perr able to this molecule. This problem is overcome by the condensation of ace CoA with oxaloacetate to form citrate (*Fig. 1*). This is then transported into cytosol where it is cleaved to regenerate acetyl CoA and oxaloacetate by AT citrate lyase in an energy-requiring process. The oxaloacetate, which also cani cross the inner mitochondrial membrane, is returned to the mitochondri matrix through conversion first to malate (catalyzed by malate dehydrogena and then to pyruvate (catalyzed by NADP$^+$-linked malate enzyme) (*Fig.* This latter decarboxylation reaction generates NADPH which can be used fatty acid synthesis. The remaining NADPH required for fatty acid synthesis provided by the pentose phosphate pathway (see Topic J5). Once back in t mitochondrial matrix, pyruvate is carboxylated to form oxaloacetate by py vate carboxylase with the hydrolysis of a further molecule of ATP (*Fig. 1*).

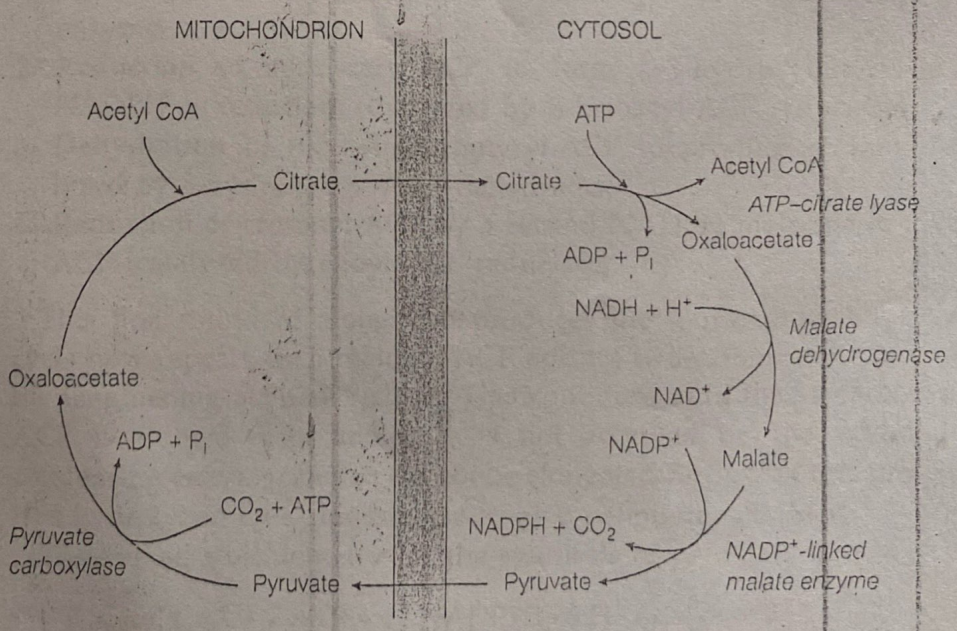


Fig. 1. Transport of acetyl CoA from the mitochondrial matrix into the cytosol.

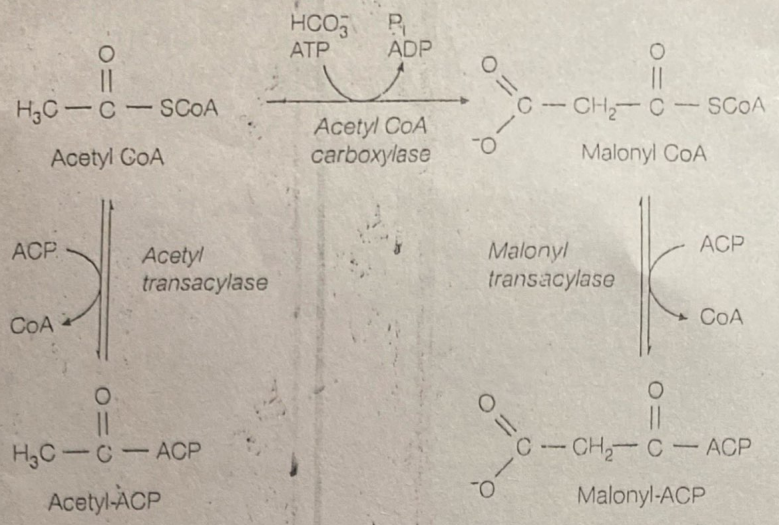


Fig. 2. Formation of acetyl- and malonyl-acyl carrier protein (ACP).

The pathway

The first committed step in fatty acid biosynthesis is the carboxylation of ac CoA to form **malonyl CoA** using CO_2 in the form of bicarbonate HCO_3^- (2). This reaction is catalyzed by the enzyme **acetyl CoA carboxylase** which **biotin** as a prosthetic group, a common feature in CO_2-binding enzymes. molecule of ATP is hydrolyzed in the reaction, which is irreversible. The el **gation steps** of fatty acid synthesis all involve intermediates linked to terminal sulfhydryl group of the **phosphopantetheine** reactive unit in A phosphopantetheine is also the reactive unit in CoA. Therefore, the next st are the formation of acetyl-ACP and malonyl-ACP by the enzymes ace **transacylase** and **malonyl transacylase**, respectively (*Fig.* 2). (For the synth of fatty acids with an odd number of carbon atoms the three-carbon propior ACP is the starting point instead of malonyl-ACP.)

The elongation cycle of fatty acid synthesis has four stages for each rou of synthesis (*Fig.* 3). For the first round of synthesis these are:

1. **Condensation** of acetyl-ACP and malonyl-ACP to form acetoacetyl-A releasing free ACP and CO_2 (catalyzed by acyl-malonyl-ACP condens enzyme).
2. **Reduction** of acetoacetyl-ACP to form D-3-hydroxybutyryl-ACP, us NADPH as reductant (catalyzed by β-ketoacyl-ACP reductase).
3. **Dehydration** of D-3-hydroxybutyryl-ACP to produce crotonyl-ACP (c lyzed by 3-hydroxyacyl-ACP dehydratase).
4. **Reduction** of crotonyl-ACP by a second NADPH molecule to give buty ACP (catalyzed by enoyl-ACP reductase).

This first round of elongation produces the four-carbon butyryl-ACP. cycle now repeats with malonyl-ACP adding two-carbon units in each cycle the lengthening acyl-ACP chain. This continues until the 16-carbon **palmitc** ACP is formed. This molecule is not accepted by the acyl-malonyl-A condensing enzyme, and so cannot be elongated further by this process. Inst it is hydrolyzed by a thioesterase to give palmitate and ACP.

The overall stoichiometry for the synthesis of palmitate is:

$$8 \text{ acetyl CoA} + 7 \text{ ATP} + 14 \text{ NADPH} + 6 \text{ H}^+ \rightarrow$$
$$\text{palmitate} + 14 \text{ NADP}^+ + 8 \text{ CoA} + 6 \text{ H}_2\text{O} + 7 \text{ ADP} + 7 \text{ P}_i$$

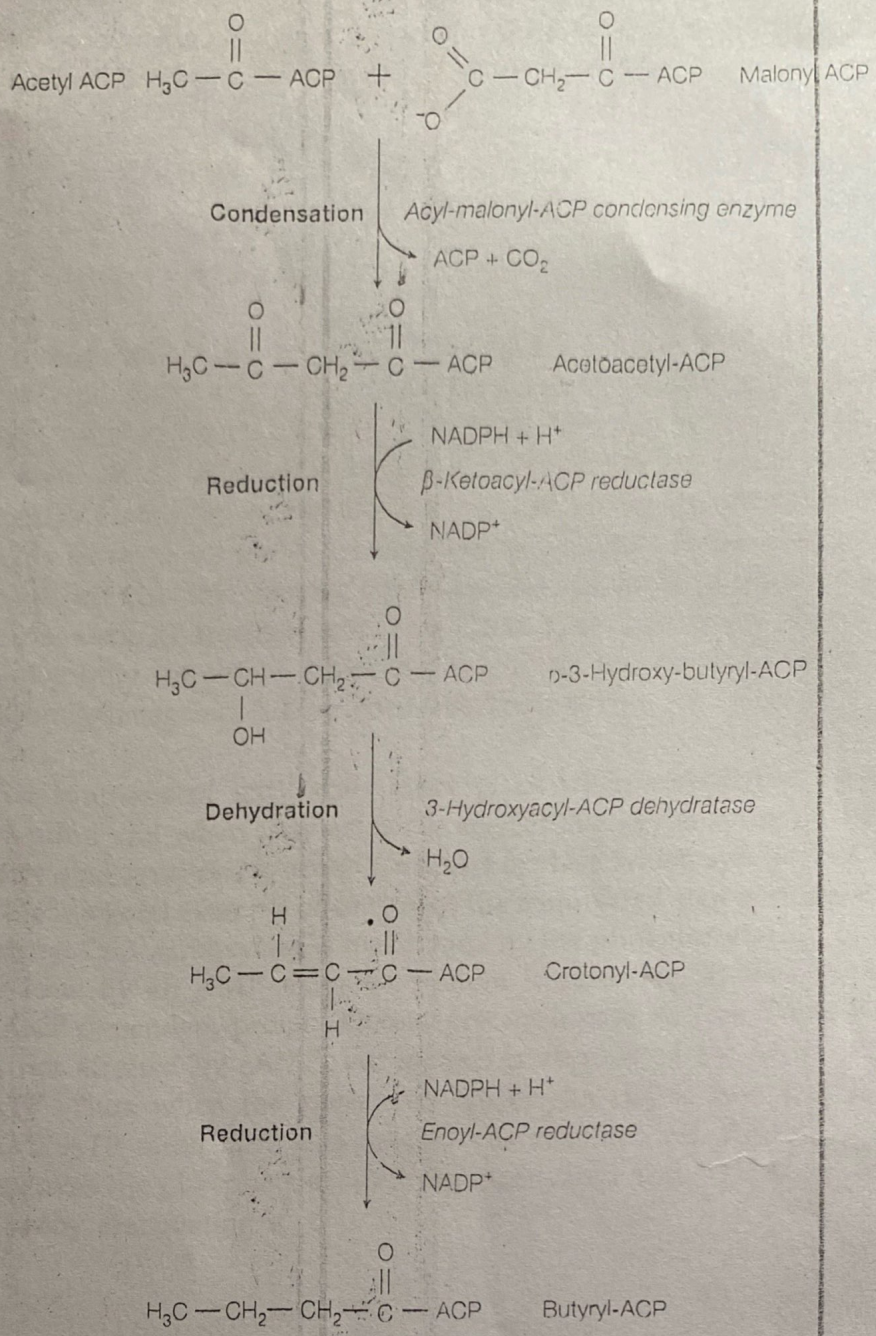


Fig. 3. The elongation cycle of fatty acid synthesis.

For each of the seven rounds of fatty acid elongation, one ATP is used in t synthesis of malonyl-CoA and two NADPH are used in the reduction reactio

In eukaryotes the elongation of fatty acids beyond C16 palmitate is carri out by enzymes located on the cytosolic surface of the smooth endoplasn reticulum (SER). Malonyl CoA is used as the two-carbon donor, and the fa acid is elongated as its CoA derivative rather than its ACP derivative.

In prokaryotes, each of the reactions of fatty acid synthesis is catalyzed by separate enzyme. However, in eukaryotes, the enzymes of the fatty acid syntl sis elongation cycle are present in a single polypeptide chain, multifunctio enzyme complex, called fatty acid synthase. The fatty acid synthase compl exists as a dimer, with the ACP moiety shuttling the fatty acyl chain betwe successive catalytic sites, and from one subunit of the dimer to the other. It is, effect, a highly efficient production line for fatty acid biosynthesis.

Formation of double bonds

In eukaryotes the SER has enzymes able to introduce double bonds i fatty acyl CoA molecules in an oxidation reaction that uses molecular oxyg This reaction is catalyzed by a membrane-bound complex of three enzyn NADH-cytochrome b_5 reductase, cytochrome b_5 and a desaturase. The ove reaction is:

$$\text{saturated fatty acyl CoA} + \text{NADH} + \text{H}^+ + \text{O}_2 \rightarrow \text{mono-unsaturated acyl CoA} + \text{NAD}^+ + 2\,\text{H}_2\text{O}$$

The reaction may be repeated to introduce more than one double bond int fatty acid.

Mammals lack the enzymes to insert double bonds at carbon atoms beyo C-9 in the fatty acid chain. Thus they cannot synthesize linoleate and lino nate, both of which have double bonds later in the chain than C-9 (linole has *cis*, *cis* Δ^9, Δ^{12} double bonds, and linolenate has all-*cis* Δ^9, Δ^{12}, Δ^{15} dou bonds). Hence, in mammals linoleate and linolenate are called essential fa acids since they have to be supplied in the diet. These two unsaturated fa acids are also the starting points for the synthesis of other unsaturated fa acids, such as **arachidonate**. This C20:4 fatty acid is the precursor of seve biologically important molecules, including the prostaglandins, prostacycli thromboxanes and leukotrienes (see Topic K1).

Regulation

The synthesis of fatty acids takes place when carbohydrate and energy a plentiful and when fatty acids are scarce. The key enzyme in the regulation fatty acid synthesis is **acetyl CoA carboxylase** which synthesizes malonyl Co This is a good example of **control at the committed step** of a metabolic pathwa Acetyl CoA carboxylase is inactivated by the **phosphorylation** of a single seri residue by an **AMP-activated protein kinase** (*Fig.* 4) (see Topic C5). Unl cAMP-dependent protein kinase (protein kinase A) (see Topic K4), this kin is not affected by cAMP, but instead is stimulated by AMP and inhibited ATP. Thus when the energy charge of the cell is low (i.e. there is a h AMP:ATP ratio) fatty acid synthesis is switched off. **Protein phosphatase** removes the phosphate group from inactivated acetyl CoA carboxylase (*Fig.* thereby reactivating it.

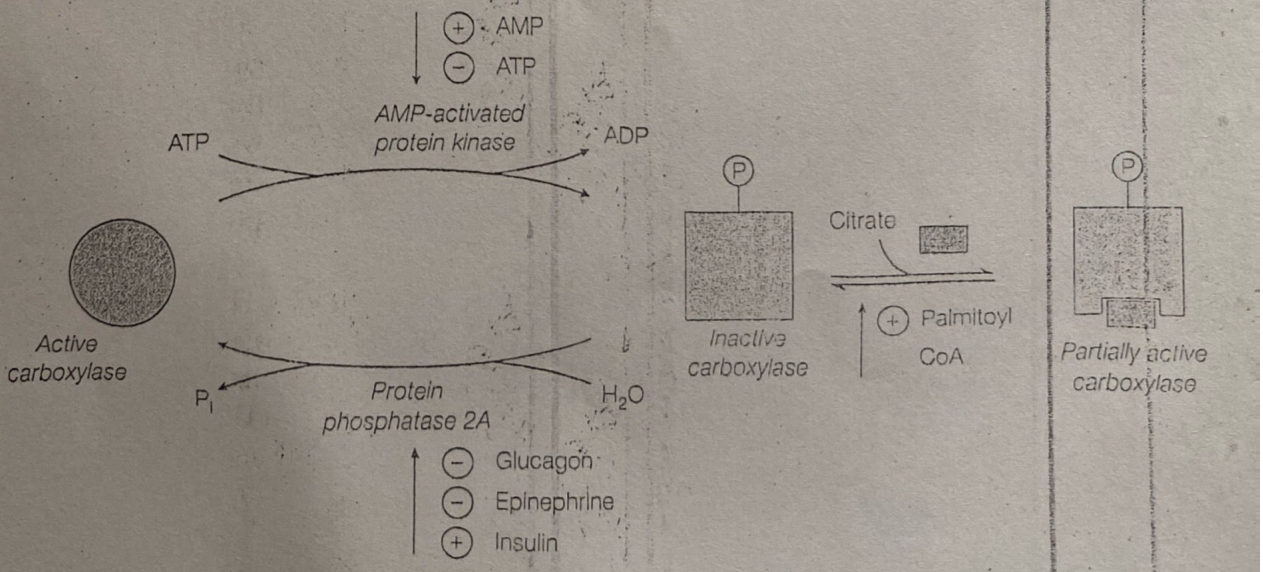


Fig. 4. Summary of the control of acetyl CoA carboxylase by phosphorylation and allosteric regulation.

Acetyl CoA carboxylase is also subject to hormonal regulation. When ene is required, **glucagon** and **epinephrine** inhibit protein phosphatase 2A, t keeping acetyl CoA carboxylase in the inactive form (*Fig. 4*) and blocking f acid synthesis. In the well-fed state, when blood glucose levels are high, insu stimulates acetyl CoA carboxylase, possibly by activating protein phospha 2A (*Fig. 4*), thereby leading to an increase in fatty acid synthesis.

As well as its control by phosphorylation/dephosphorylation, acetyl C carboxylase is also **allosterically regulated**. The citric acid cycle intermed **citrate**, the level of which is high when both acetyl CoA and ATP are ab dant, allosterically stimulates acetyl CoA carboxylase. This results in conversion of the inactive phosphorylated form into a partially active form t is still phosphorylated (*Fig. 4*), thereby activating fatty acid synthesis so t the excess acetyl CoA is 'stored' as fatty acid residues within triacylglycero adipose tissue. In contrast, high levels of **palmitoyl CoA**, which is abund when there is an excess of fatty acids, antagonize the effect of citrate on ace CoA carboxylase, reducing its activity (*Fig. 4*) and switching off further fa acid synthesis.

K4 TRIACYLGLYCEROLS

Key Notes

Structure and function

Triacylglycerols (fats or triglycerides) consist of three fatty acid chains esterified to a glycerol backbone. Simple triacylglycerols have three identical fatty acids, mixed triacylglycerols have two or three different fatty acids. Triacylglycerols are the major energy store and the major dietary lipid in humans. They are insoluble in water and are stored in specialized adipose (fat) cells.

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Related topics

Fatty acid breakdown (K2)
Fatty acid synthesis (K3)
Lipoproteins (K6)

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Ketone bodies

When in excess, acetyl CoA produced from the β-oxidation of fatty acids i converted into acetoacetate and D-3-hydroxybutyrate. Together with acetone these compounds are collectively termed ketone bodies. Acetoacetate and D-3 hydroxybutyrate are produced in the liver and provide an alternative supply of fuel for the brain under starvation conditions or in diabetes.

Related topics

Structures and roles of fatty acids (K1)
Fatty acid synthesis (K3)
Triacylglycerols (K4)
Citric acid cycle (L1)

Overview

Fatty acid breakdown brings about the oxidation of long-chain fatty acids. fatty acids are first converted to their **acyl coenzyme A (CoA)** derivatives then degraded by the successive removal of two-carbon units from the enc the fatty acid as **acetyl CoA**. The pathway produces $FADH_2$ and NADH direc The acetyl CoA produced can also enter the citric acid cycle and produce furt $FADH_2$ and NADH (see Topic L1). The $FADH_2$ and NADH are then oxidi by the respiratory electron transport chain to yield energy in the form of A (see Topic L2).

Activation

Fatty acid breakdown occurs in the cytosol of prokaryotes and in the m chondrial matrix of eukaryotes. Before entering the mitochondrial matrix, fatty acid is **activated** by forming a **thioester link** with CoA (*Fig. 1*). This re tion is catalyzed by **acyl CoA synthase** (also called **fatty acid thiokinase**) wh is present on the outer mitochondrial membrane, and uses a molecule of A The overall reaction is irreversible due to the subsequent hydrolysis of PP two molecules of P_i.

Transport into mitochondria

Small- and medium-chain acyl CoA molecules (up to 10 carbon atoms) are reac able to cross the **inner mitochondrial membrane** by diffusion. However, lon chain acyl CoAs do not readily cross the inner mitochondrial membrane, a require a specific transport mechanism. To achieve this, the longer chain a CoAs are conjugated to the polar **carnitine** molecule, that is found in b plants and animals. This reaction, catalyzed by an enzyme on the outer f of the inner mitochondrial membrane (**carnitine acyltransferase I**), removes CoA group and substitutes it with a carnitine molecule (*Fig. 2*). The acylca tine is then transported across the inner mitochondrial membrane by **carnitine/acylcarnitine translocase**. This integral membrane transport prot (see Topic E3) transports acylcarnitine molecules into the mitochondrial ma and free carnitine molecules out. Once inside the mitochondrial matrix the a group is transferred back on to CoA, releasing free carnitine, by the enzy **carnitine acyltransferase II** which is located on the matrix side of the in mitochondrial membrane (*Fig. 2*).

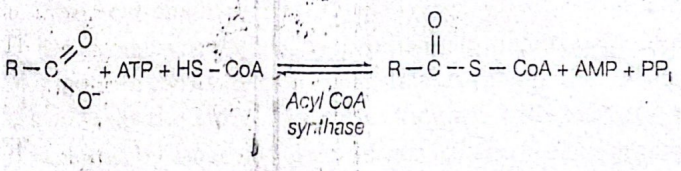


Fig. 1. Activation of a fatty acid.

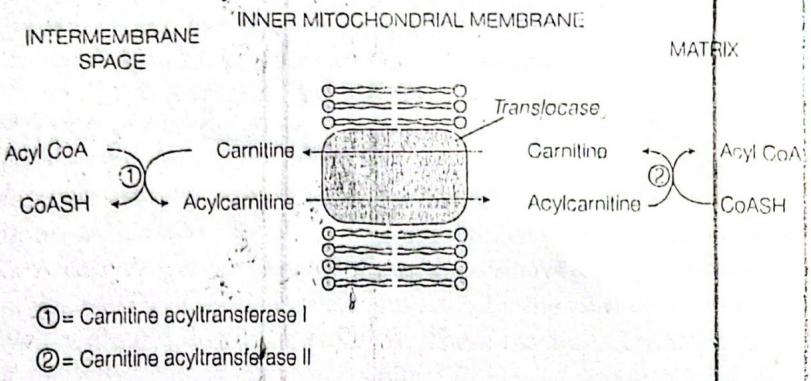


Fig. 2. Transport of fatty acids across the inner mitochondrial membrane.

β-Oxidation pathway

The individual reactions involved in the degradation of fatty acids by oxidation are as follows (*see Fig. 3*):

1. **Oxidation** of the fatty acyl CoA to enoyl CoA forming a *trans* Δ^2-dou bond on the fatty acyl chain and producing $FADH_2$ (catalyzed by acyl C **dehydrogenase**).
2. **Hydration** of the *trans* Δ^2-enoyl CoA to form 3-hydroxyacyl CoA (cataly by **enoyl CoA hydratase**).
3. **Oxidation** of 3-hydroxyacyl CoA to 3-ketoacyl CoA, producing NA (catalyzed by **hydroxyacyl CoA dehydrogenase**).
4. Cleavage, or **thiolysis**, of 3-ketoacyl CoA by a second CoA molecule, giv acetyl CoA and an acyl CoA shortened by two carbon atoms (catalyzed **β-ketothiolase**).

Thus, the breakdown of individual fatty acids occurs as a repeating seque of four reactions: **oxidation** (by FAD), **hydration**, **oxidation** (by NAD^+) **thiolysis**. These four reactions form one 'round' of fatty acid degradation (*3*) and their overall effect is to remove two-carbon units sequentially in form of acetyl CoA from the fatty acid chain. The cleavage of the Δ^2 (o bond of the fatty acyl chain (see *Fig. 3*, top structure, for nomenclature) g fatty acid breakdown its alternative name, **β-oxidation**. The shortened acyl C then undergoes further cycles of β-oxidation until the last cycle, when the CoA with four carbon atoms is split into two molecules of acetyl CoA. Th C16 saturated acyl CoA, such as palmitoyl CoA, would be completely degra into eight molecules of acetyl CoA by seven rounds of degradation, leadin the overall equation:

$$\text{palmitoyl CoA} + 7\,\text{FAD} + 7\,\text{NAD}^+ + 7\,\text{CoA} + 7\,\text{H}_2\text{O} \rightarrow$$
$$8\,\text{acetyl CoA} + 7\,\text{FADH}_2 + 7\,\text{NADH} + 7\,\text{H}^+$$

Mitochondria contain three acyl CoA dehydrogenases which act on sh medium- and long-chain acyl CoAs, respectively. In contrast, there is just each of the enzymes enoyl CoA hydratase, hydroxyacyl CoA dehydroge and β-ketothiolase which all have a broad specificity with respect to the le of the acyl chain.

In animals the acetyl CoA produced from fatty acid degradation cannot be verted into pyruvate or oxaloacetate. Although the two carbon atoms from a CoA enter the citric acid cycle, they are both oxidized to CO_2 in the react catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase

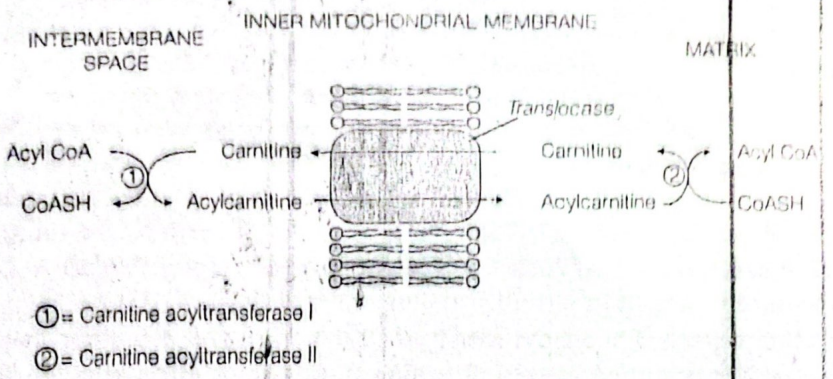


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Thus, the breakdown of individual fatty acids occurs as a repeating seque of four reactions: **oxidation** (by FAD), **hydration**, **oxidation** (by NAD^+) **thiolysis**. These four reactions form one 'round' of fatty acid degradation (*3*) and their overall effect is to remove two-carbon units sequentially in form of acetyl CoA from the fatty acid chain. The cleavage of the Δ^2 (o bond of the fatty acyl chain (see *Fig. 3*, top structure, for nomenclature) g fatty acid breakdown its alternative name, **β-oxidation**. The shortened acyl C then undergoes further cycles of β-oxidation until the last cycle, when the CoA with four carbon atoms is split into two molecules of acetyl CoA. Th C16 saturated acyl CoA, such as palmitoyl CoA, would be completely degra into eight molecules of acetyl CoA by seven rounds of degradation, leadin the overall equation:

$$\text{palmitoyl CoA} + 7\,\text{FAD} + 7\,\text{NAD}^+ + 7\,\text{CoA} + 7\,\text{H}_2\text{O} \rightarrow$$
$$8\,\text{acetyl CoA} + 7\,\text{FADH}_2 + 7\,\text{NADH} + 7\,\text{H}^+$$

Mitochondria contain three acyl CoA dehydrogenases which act on sh medium- and long-chain acyl CoAs, respectively. In contrast, there is just each of the enzymes enoyl CoA hydratase, hydroxyacyl CoA dehydroge and β-ketothiolase which all have a broad specificity with respect to the le of the acyl chain.

In animals the acetyl CoA produced from fatty acid degradation cannot be verted into pyruvate or oxaloacetate. Although the two carbon atoms from a CoA enter the citric acid cycle, they are both oxidized to CO_2 in the react catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase

double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3. To overcome this problem an **isomerase** converts the *cis*-Δ^3 bond into a *trans*-Δ^2 double bond, and the resulting *trans*-Δ^2-enoyl CoA can then continue down the β-oxidation pathway (see *Fig. 4*).

Another enzyme, in addition to the isomerase, is required for the oxidation of **polyunsaturated fatty acids** which have a double bond at an even-numbered carbon atom. In this case the 2,4-dienoyl intermediate resulting from the action of acyl CoA dehydrogenase is acted on by **2,4-dienoyl CoA reductase** to form *cis*-Δ^3-enoyl CoA (*Fig. 4*). This is then converted by the isomerase into the *trans* form which continues down the pathway. These reactions are important since over half the fatty acids of plant and animal lipids are unsaturated (and often polyunsaturated).

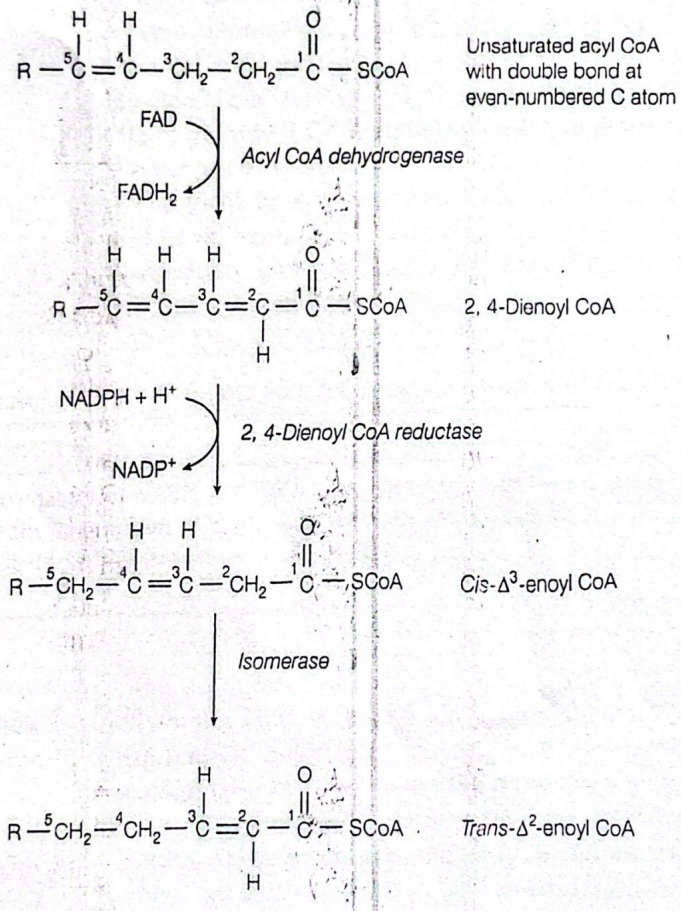


Fig. 4. Accessory enzymes required for the metabolism of unsaturated fatty acids.

Oxidation of odd-chain fatty acids

Fatty acids having an **odd number of carbon atoms** (which are relatively rare in nature) are also degraded by the β-oxidation pathway in the same way as those with an even number of carbon atoms. The only difference is that in the final round the five carbon acyl CoA intermediate is cleaved into one molecule of the C3 **propionyl CoA** and one molecule of the C2 acetyl CoA. The propionyl CoA is then converted into succinyl CoA which enters the citric acid cycle (see Topic L1).

Regulation

The major point of control of β-oxidation is the availability of fatty aci The major source of free fatty acids in the blood is from the breakdown **triacylglycerol** stores in adipose tissue which is regulated by the action hormone-sensitive triacylglycerol lipase (see Topic K4). Fatty acid breakdo and fatty acid synthesis are coordinately controlled so as to prevent a fut cycle (see Topic K3).

Energy yield

For each round of degradation, one $FADH_2$, one NADH and one acetyl C molecule are produced. Each NADH generates three ATP molecules, and ea $FADH_2$ generates two ATPs during oxidative phosphorylation (see Topic L In addition, each acetyl CoA yields 12 ATPs on oxidation by the citric a cycle (see Topic L1). The total yield for each round of fatty acid degradati is therefore 17 ATP molecules.

The complete degradation of palmitoyl CoA (C16:0) requires seven roun of degradation and hence produces $7 \times 5 = 35$ ATP molecules. A total of eig acetyl CoA molecules are produced and hence another $8 \times 12 = 96$ ATP. Th the total ATP yield per molecule of palmitate degraded is $35 + 96 = 131$ AT However, one ATP is hydrolyzed to AMP and PP_i in the activation of palm tate to palmitoyl CoA, resulting in two high-energy bonds being cleaved. Th the net yield is **129 ATPs** (*Table 1*).

The yield of ATP is reduced slightly for unsaturated fatty acids, since t additional metabolic reactions which enable them to be degraded by t β-oxidation pathway either involve using NADPH or bypass an FADI producing reaction (see *Fig. 4*).

Table 1. Calculation of the ATP yield from the complete oxidation of palmitate

Degradative step	ATP yi
7 x 5 ATP for oxidation of NADH and $FADH_2$ produced by each round of degradation	35
8 x 12 ATP for the breakdown of acetyl CoA by the citric acid cycle	96
–2 ATP equivalents for the activation of palmitate	–2
	Total = 129

Ketone bodies

When the level of acetyl CoA from β-oxidation increases in excess of tl required for entry into the citric acid cycle, the acetyl CoA is converted i **acetoacetate** and D-3-**hydroxybutyrate** by a process known as **ketogenes** D-3-hydroxybutyrate, acetoacetate and its nonenzymic breakdown prod **acetone** are referred to collectively as **ketone bodies** (*Fig. 5*).

Two molecules of acetyl CoA initially condense to form acetoacetyl CoA a reaction which is essentially the reverse of the thiolysis step in β-oxidati The acetoacetyl CoA reacts with another molecule of acetyl CoA to fo **3-hydroxy-3-methylglutaryl CoA (HMG CoA)** (*Fig. 5*). This molecule is th cleaved to form acetoacetate and acetyl CoA. (HMG CoA is also the start point for cholesterol biosynthesis; see Topic K5.) The acetoacetate is then eit reduced to D-3-hydroxybutyrate in the mitochondrial matrix or undergoe slow, spontaneous decarboxylation to acetone (*Fig. 5*). In diabetes, acetoacet is produced faster than it can be metabolized. Hence untreated diabetics h high levels of ketone bodies in their blood, and the smell of acetone can of be detected on their breath.

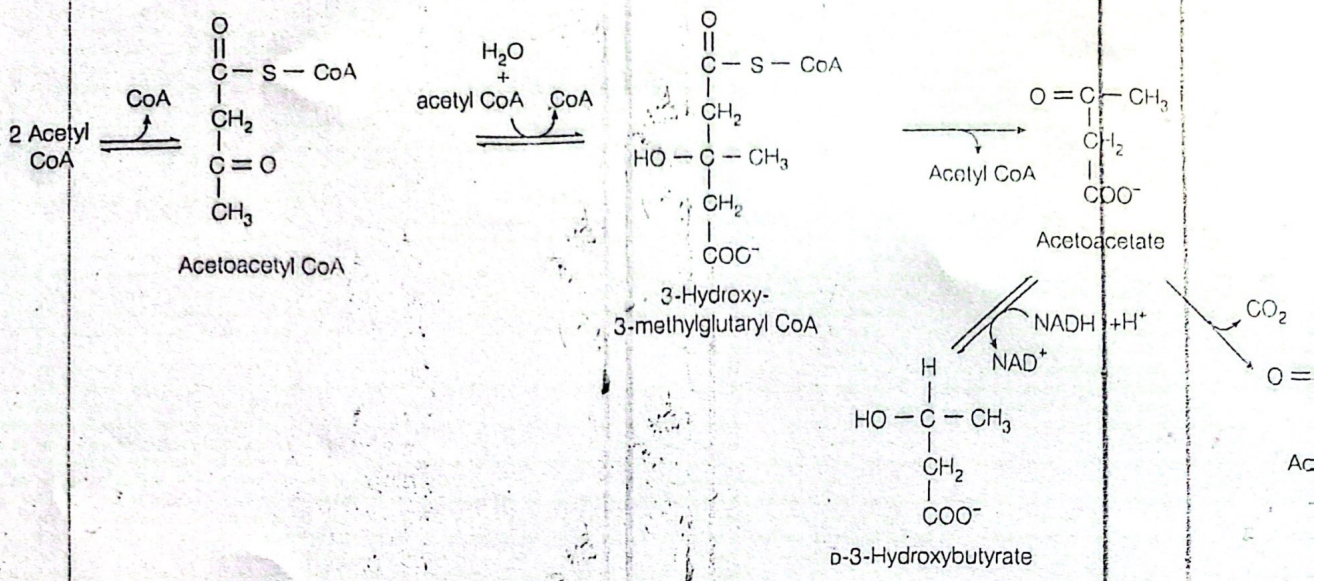


Fig. 5: Conversion of acetyl CoA to the ketone bodies acetoacetate, acetone and D-3-hydroxybutyrate.

Acetoacetate and D-3-hydroxybutyrate are produced mainly in the liver a are not just degradation products of little physiological value. They are us in preference to glucose as an energy source by certain tissues such as the he muscle and kidney cortex. Although glucose is normally the major fuel for t brain, under conditions of starvation or diabetes this organ can switch to usi predominantly acetoacetate.

BCH 303

K5 CHOLESTEROL

Key Notes

Functions of cholesterol

Cholesterol is a component of cell membranes and is the precursor of steroid hormones and the bile salts.

Biosynthesis of cholesterol

All 27 carbon atoms of cholesterol are derived from acetyl CoA. First acetyl CoA and acetoacetyl CoA combine to form HMG CoA which, in turn, is reduced to mevalonate by HMG CoA reductase. Mevalonate is converted into the five-carbon isoprene compounds 3-isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate. These two compounds condense to form the C10 geranyl pyrophosphate, which is elongated to the C15 farnesyl pyrophosphate by the addition of another molecule of isopentenyl pyrophosphate. Two molecules of farnesyl pyrophosphate condense to form the C30 squalene, which is then converted via squalene epoxide and lanosterol to cholesterol.

Regulation of cholesterol biosynthesis

Cholesterol can either be obtained in the diet or synthesized in the liver. High levels of cholesterol and its metabolites decrease the amount and inhibit the activity of HMG CoA reductase, the enzyme that catalyzes the committed step in cholesterol biosynthesis. This enzyme can also be inhibited therapeutically by the compound lovastatin.

Bile salts

Bile salts (bile acids) are the major excretory form of cholesterol. These polar compounds are formed in the liver by converting cholesterol into the activated intermediate cholyl CoA, and then combining this compound with either glycine, to form glycocholate, or taurine, to form taurocholate. The detergent-like bile salts are secreted into the intestine where they aid the digestion and uptake of dietary lipids.

Vitamin D

Vitamin D is derived via cholesterol in a series of reactions, one of which requires the action of UV light to break the bond between two carbon atoms. Deficiency of vitamin D causes rickets in children and osteomalacia in adults.

Steroid hormones

The steroid hormones are derived from cholesterol by a series of reactions that involve the heme-containing cytochrome P450 enzymes. These mono-oxygenases require both O_2 and NADPH to function. There are five classes of steroid hormones: (1) the progestagens (e.g. progesterone); (2) the androgens (e.g. testosterone); (3) the estrogens (e.g estrone); (4) the glucocorticoids (e.g cortisol); and (5) the mineralocorticoids (e.g. aldosterone).

Related topics Membrane lipids (E1) Lipoproteins (K6)

Functions of cholesterol

Cholesterol is a steroid. It is an important constituent of cell membrar where, in mammals, it modulates their fluidity (see Topic E1). Cholesterc also the precursor of steroid hormones such as progesterone, testosterone cortisol, and the bile salts (see below).

Biosynthesis of cholesterol

Animals are able to synthesize cholesterol *de novo* by an elegant series reactions in which all 27 carbon atoms of cholesterol are derived from ace CoA. The acetate units are first converted into C5 isoprene units, that are th condensed to form a linear precursor to the cyclic cholesterol.

The first stage in the synthesis of cholesterol is the formation of isopente **pyrophosphate** (*Fig. 1*). Acetyl CoA and acetoacetyl CoA combine to fo **3-hydroxy-3-methylglutaryl CoA (HMG CoA)**. This process takes place in liver, where the HMG CoA in the mitochondria is used to form ketone bod during starvation (see Topic K2), whereas that in the cytosol is used to synthes cholesterol in the fed state (under the influence of cholesterol). HMG CoA is th reduced to **mevalonate** by **HMG CoA reductase** (*Fig. 1*). This is the commit step in cholesterol biosynthesis and is a key control point. Mevalonate is co verted into **3-isopentenyl pyrophosphate** by three consecutive reactions ea involving ATP, with CO_2 being released in the last reaction (*Fig. 1*).

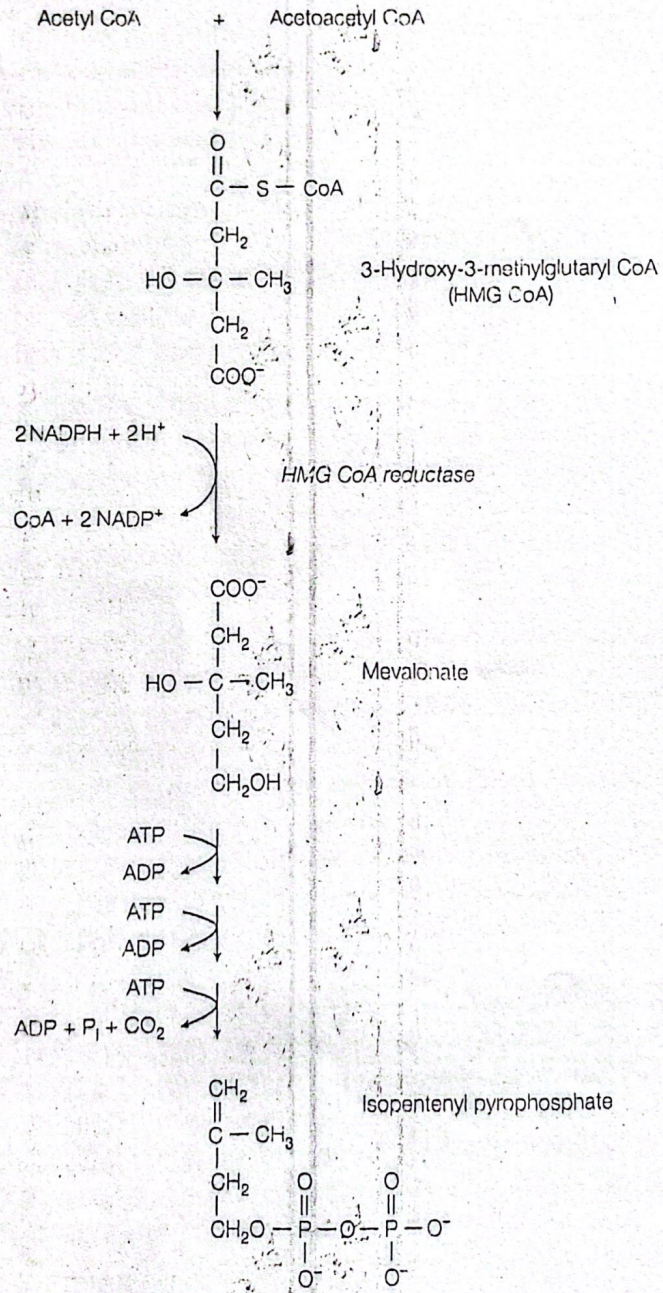


Fig. 1. Synthesis of isopentenyl pyrophosphate.

The C5 isoprene units in isopentenyl pyrophosphate are then condensed form the C30 compound squalene (*Fig. 2*). First, isopentenyl pyrophospl isomerizes to **dimethylallyl pyrophosphate** (*Fig. 2a*), which reacts with anot molecule of isopentenyl pyrophosphate to form the C10 compound gera **pyrophosphate** (*Fig. 2b*). Another molecule of isopentenyl pyrophosphate t reacts with geranyl pyrophosphate to form the C15 compound farne **pyrophosphate**. Next, two molecules of farnesyl pyrophosphate condense form **squalene** (*Fig. 2b*).

Squalene is then converted into **squalene epoxide** in a reaction that uses and NADPH (*Fig. 2b*). The squalene epoxide cyclizes to form lanosterol, a finally cholesterol is formed from lanosterol by the removal of three met groups, the reduction of one double bond by NADPH, and the migration the other double bond (*Fig. 2b*).

Farnesyl pyrophosphate and the C20 compound geranylgeranyl pyroph **phate** (which is formed by the condensation of another isopente pyrophosphate with farnesyl pyrophosphate) are covalently linked to cyste residues in a number of proteins, promoting their association with membra ~~(see Topic E2)~~. **Dolichol**, which contains some 20 isoprene units is used to ca the biosynthetic precursor of the N-linked oligosaccharides that are sub quently attached to proteins ~~(see Topic I5)~~.

Regulation of cholesterol biosynthesis

Cholesterol can be obtained either from the diet or it can be synthesized *novo*, mainly in the liver. Cholesterol is transported round the body in lipop tein particles ~~(see Topic K6)~~. The rate of synthesis of cholesterol is depend on the cellular level of cholesterol. High levels of cholesterol and its metal lites control cholesterol biosynthesis by:

- feedback-inhibiting the activity of **HMG CoA reductase**, the enzyme wh catalyzes the committed step in cholesterol biosynthesis ~~(see Topic G5)~~;
- decreasing the amount of HMG CoA reductase by reducing the synthe and translation of its mRNA;
- decreasing the amount of HMG CoA reductase by increasing its rate degradation.

In addition, HMG CoA reductase, like acetyl CoA carboxylase in fatty a synthesis ~~(see Topic K3)~~, is inactivated by phosphorylation by an AM activated protein kinase, retained in this form under the influence of glucag during starvation.

HMG CoA reductase can be inhibited therapeutically by administering drug **lovastatin**, based on the fungal products mevinolin and **compactin**, wh competitively inhibit the enzyme and hence decrease the rate of choleste biosynthesis. Therefore, these compounds are routinely used for the treatm of **hypercholesterolemia** (high levels of blood cholesterol) ~~(see Topic K6)~~.

Bile salts

Bile salts (or bile acids) are polar derivatives of cholesterol and constitute major pathway for the excretion of cholesterol in mammals. In the liver, chol terol is converted into the activated intermediate **cholyl CoA** which then rea either with the amino group of glycine to form **glycocholate** (*Fig. 3a*), or w the amino group of taurine ($H_2N\text{-}CH_2\text{-}CH_2\text{-}SO_3^-$, a derivative of cysteine) form **taurocholate** (*Fig. 3b*). After synthesis in the liver, the bile salts gly cholate and taurocholate are stored and concentrated in the gall bladder, bef release into the small intestine. Since they contain both polar and nonpo

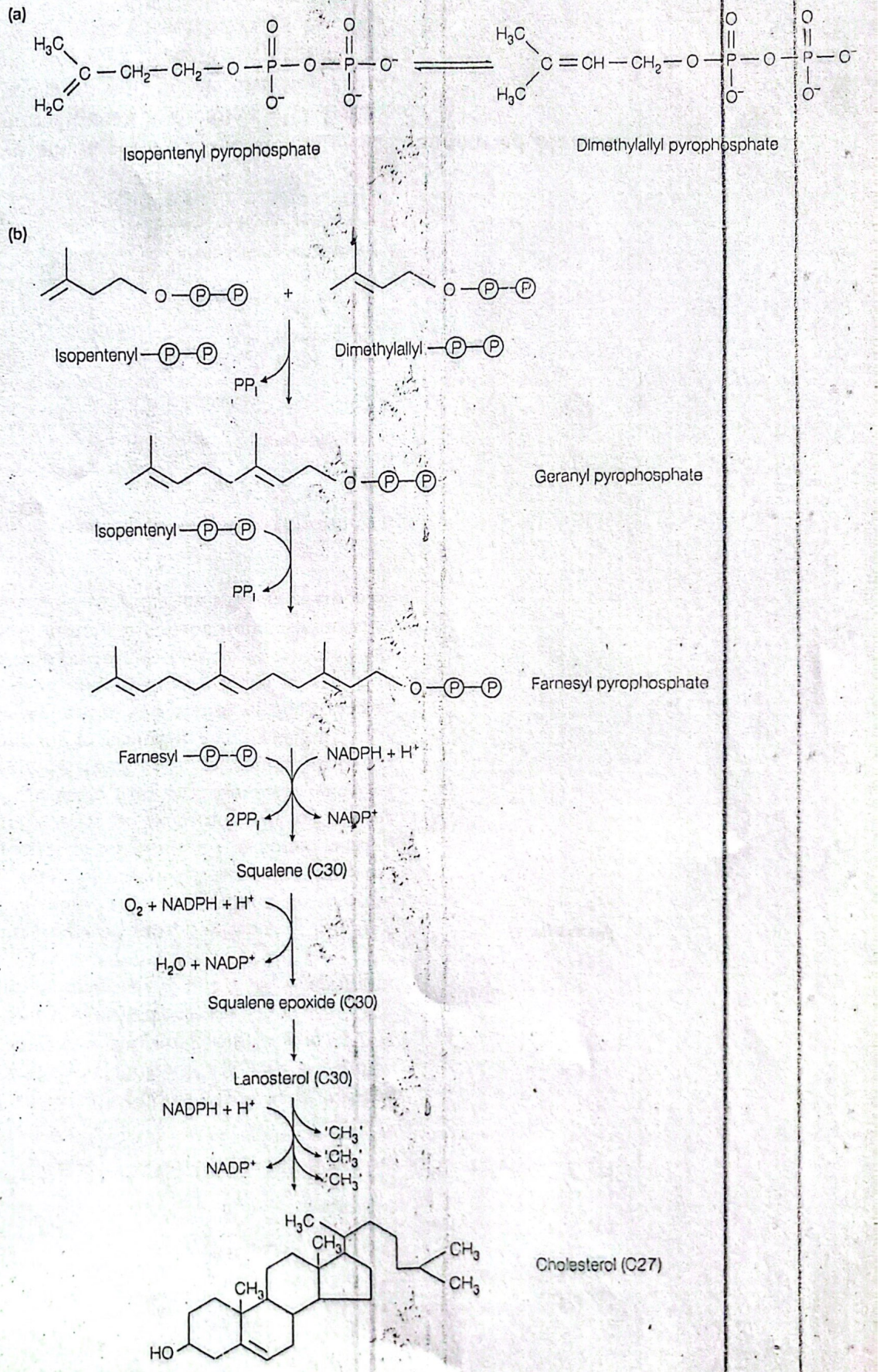


Fig. 2. Synthesis of squalene and cholesterol from isopentenyl pyrophosphate. (a) Isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate; (b) synthesis of cholesterol.

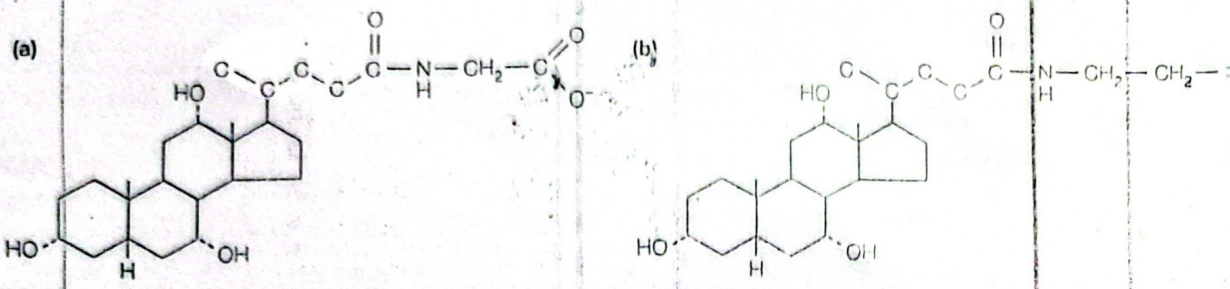


Fig. 3. Structures of the bile salts (a) glycocholate and (b) taurocholate.

regions (that is are amphipathic molecules), the bile salts are very effe detergents and act to solubilize dietary lipids. The resulting increase in surface area of the lipids aids their hydrolysis by lipases and their uptake intestinal cells (see Topic K4). The intestinal absorption of the lipid-sol vitamins A, D, E and K also requires the action of the bile salts.

Vitamin D

Vitamin D is derived from 7-dehydrocholesterol by the action of the UV ponent of sunlight on the skin. UV light brings about photolysi 7-dehydrocholesterol between C-9 and -10, leading to a rearrangement c double bonds of the molecule to form previtamin D_3 (*Fig. 4*). This mol spontaneously isomerizes to form vitamin D_3 (cholecalciferol). Subsec hydroxylation reactions take place in the liver and kidneys to produce dihydroxycholecalcerol (1,25(OH)D_3), the active hormone (*Fig. 4*). Ric which is caused by a deficiency of vitamin D, was historically a common di of childhood in Britain due to the low vitamin D content of the national and lack of exposure to sunlight. Even today, people whose cultures re the body to be clothed so that no skin is exposed to sunlight have pro in maintaining an adequate vitamin D intake. In adults this takes the fo osteomalacia – the softening or weakening of the bones.

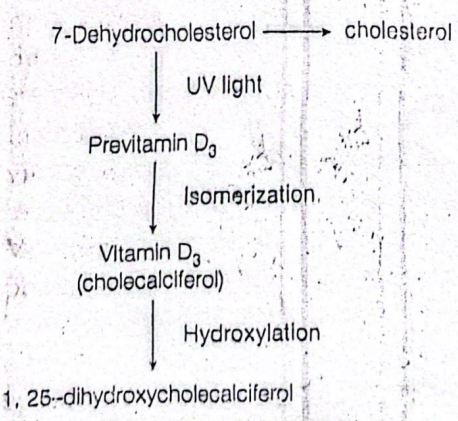


Fig. 4. Formation of vitamin D.

Steroid hormones

Cholesterol is the precursor of the five major classes of steroid hor (*Table 1*). The synthesis of steroid hormones is initiated by the removal o carbon unit from carbon 20 of the cholesterol side chain to form pregne the common precursor of all steroid hormones (*Fig. 5*). A series of re catalyzed by cytochrome P450 modify pregnenolone to give rise to th vidual hormones (*Fig. 5*).

Table 1. Classes of steroid hormone

Class	Site of synthesis	Hormone	Action
Progestagens	Corpus luteum	Progesterone	Prepares uterine lining for egg implantation; maintenance of pregnancy
Androgens	Testis	Testosterone	Development of male secondary sex characteristic
Estrogens	Ovary	Estrone	Development of female secondary sex characterist
Glucocorticoids	Adrenal cortex	Cortisol	Promotes gluconeogenesis and glycogen formation enhances fat and protein degradation
Mineralocorticoids	Adrenal cortex	Aldosterone	Increases reabsorption of Na^+ and excretion of K^+ H^+ by kidney tubules

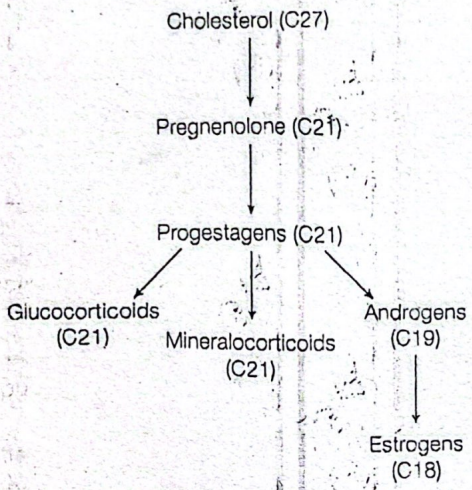


Fig. 5. Biosynthetic pathway for the synthesis of the steroid hormones.

The cytochrome P450s are a group of **heme-containing** enzymes (see I M4) that get their name from the wavelength maximum of their absorb spectra when bound to carbon monoxide. They are present in both the m chondria and the SER of many cells, and consist of a family of structu related enzymes with different substrate specificities. The enzymes all cat so-called **mono-oxygenase** reactions, in which one oxygen atom from m ular oxygen is inserted into the substrate molecule, and the other oxygen forms water. The electrons required to bring about the reduction of oxyg form water are supplied by specialized electron transport chains which are tionally linked to the P450 enzymes. These electron transport chains us have NADPH as the ultimate electron donor, so a cytochrome P450-cata reaction is often characterized by the involvement of both O_2 and NADP