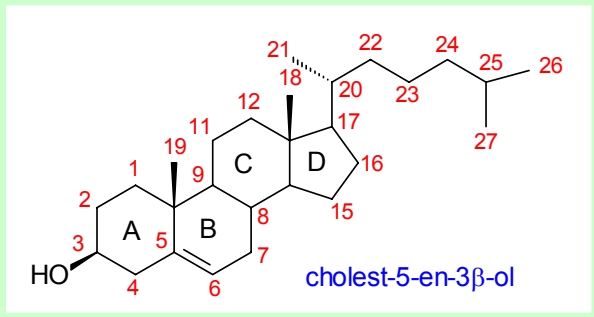
STEROLS 1. CHOLESTEROL AND CHOLESTEROL ESTERS

STRUCTURE, OCCURRENCE, BIOCHEMISTRY AND ANALYSIS

1. Cholesterol – Structure, Occurrence and Function



In animal tissues, **cholesterol** (cholest-5-en-3β-ol) is by far the most abundant member of a family of polycyclic compounds known as **sterols** (see also our web page on **plant sterols**). It can also be described as a polyisoprenoid or a triterpene. It was first recognized as a component of gallstones as long ago as 1770, while the great French lipid chemist Chevreul isolated it from animal fats in 1815. However, it was well into the 20th century before the structure was fully defined. In essence, it consists of a tetracyclic cyclopenta[*a*]phenanthrene structure with an *iso*-octyl side chain at carbon 17. The four rings (A, B, C, D) have *trans* ring junctions, and the side chain and two methyl groups (C-18 and C-19) are at an angle to the rings above the plane with β stereochemistry (as for the hydroxyl group on C-3 also). There is a double bond between carbons 5 and 6. Cholesterol has an important role in membranes and in lipid metabolism in general, so is a lipid by any definition, although I do not believe that all compounds that are soluble in organic solvents need be considered as such. The steroidal hormones, derived biosynthetically from cholesterol, and most other terpenoids are not lipids in the sense of my definition (many do not share this view), and are not discussed further here.

Cholesterol is a ubiquitous component of all animal tissues (and of fungi), where much of it is located in the membranes. However, it is not evenly distributed among cellular membranes. The highest proportion of unesterified cholesterol is in the plasma membrane (roughly 30 to 50%), while mitochondria and the endoplasmic reticulum have very low cholesterol contents, and the Golgi contains an intermediate amount. It may surprise some to learn that the brain contains more cholesterol than any other organ, and here it comprises roughly a quarter of the total free cholesterol in the human body. In plants, it tends to be a minor component only of a complex '**phytosterol**' fraction, although there are exceptions. It is nevertheless importance as a precursor of plant hormones. It occurs in the free form and esterified to long-chain fatty acids (**cholesterol esters**) in animal tissues, including the plasma lipoproteins (see below). Animals in general synthesise a high proportion of their cholesterol requirement, but they can also ingest and absorb appreciable amounts in their diets. Many invertebrates, including insects, cannot synthesise cholesterol and must receive it from the diet, although they are also able to convert plant sterols such as β-sitosterol to cholesterol. Prokaryotes lack cholesterol entirely.

It is generally believed that the main function of cholesterol is to modulate the fluidity of membranes by interacting with their complex lipid components, specifically the phospholipids such as phosphatidylcholine and sphingomyelin. In its three-dimensional structure, it is in essence a planar molecule that can interact on both sides. The tetracyclic ring structure is compact and very rigid. In addition, the location of the hydroxyl group facilitates the orientation of the molecule in a membrane bilayer, while the positions of the methyl groups appear to maximize interactions with

other lipid constituents. As an amphiphilic molecule, cholesterol is thus able to intercalate between phospholipids in lipid bilayers, spanning about half a bilayer. The interaction is mainly via van der Waals and hydrophobic forces with a contribution from hydrogen bonding of the cholesterol hydroxyl group to the polar head group and interfacial regions of the phospholipids, especially sphingomyelin. Intercalated cholesterol may also disrupt electrostatic interactions between the ionic phosphocholine head groups of nearby membrane phospholipids, leading to increased mobility of the head groups. In the absence of cholesterol, the membrane is in a fluid state that is characterized by a substantial degree of lipid chain disorder, i.e. it constitutes a *liquid-disordered* phase. The function of cholesterol is to increase the degree of order (cohesion and packing) in the membranes, leading to formation of a *liquid-ordered* phase. Thus, cholesterol is able to promote and stabilize a liquid-ordered phase over a substantial range of temperatures and sterol concentrations. In addition, high cholesterol concentrations in membranes reduce their passive permeability to solutes. These effects permit the fine-tuning of membrane lipid composition, organization and function.

Experiments with mutant cell lines and specific inhibitors of cholesterol biosynthesis suggest that an equatorial hydroxyl group at C-3 of sterols is essential for the growth of mammalian cells. The Δ5 double bond ensures that the molecule adopts a planar conformation, and this feature also appears to be essential for cell growth, as is the flexible *iso*-octyl side-chain. The C-18 methyl group is crucial for the proper orientation of the sterol. While plant sterols appear to be able to substitute for cholesterol in supporting many of the bulk properties of membranes in mammalian cells, cholesterol is essential for other purposes.

Cholesterol also has a key role in the lateral organization of membranes and their free volume distribution, factors permitting more intimate protein-cholesterol interactions that may regulate the activities of many membrane proteins. Some membrane proteins bind strongly to cholesterol, including some that are involved in cellular cholesterol homeostasis or trafficking and contain a conserved region termed the 'sterol-sensing domain'. In addition, cholesterol forms a well-defined and essential association with the sphingolipids in the formation of the membrane sub-domains known as **rafts**, which are so important in the function of cells. It appears that the synthesis of cholesterol and of the choline-containing phospholipids is regulated co-ordinately to satisfy the requirements of membrane composition and function.

Cholesterol can flip rapidly between the leaflets in a bilayer, and the trans-bilayer distribution of cholesterol in biological membranes is uncertain. While some models propose that cholesterol is on the outer leaflet, other studies suggest that most of the sterol is in the inner leaflet of human erythrocytes, for example.

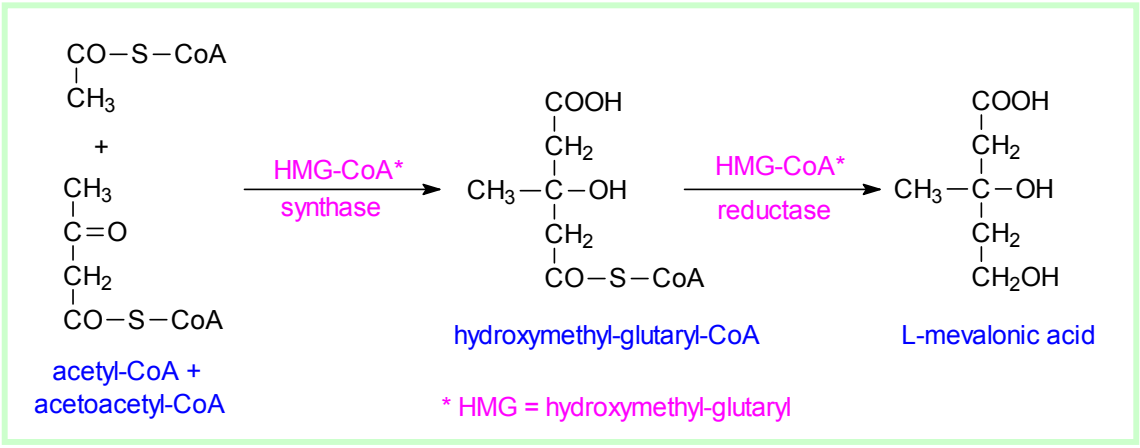
2. Cholesterol Biosynthesis

Cholesterol biosynthesis involves a highly complex series of at least 25 different enzymatic reactions, which were unravelled in large measure in the laboratories of Konrad Bloch and Fyodor Lynen, who received the Nobel Prize for their work on the topic in 1964. When the various regulatory, transport and genetic studies of more recent years are taken into account, it is obvious that this is a subject that cannot be treated in depth here. The bare bones of mechanistic aspects are therefore delineated, which with the references detailed below should serve as a guide to further study.

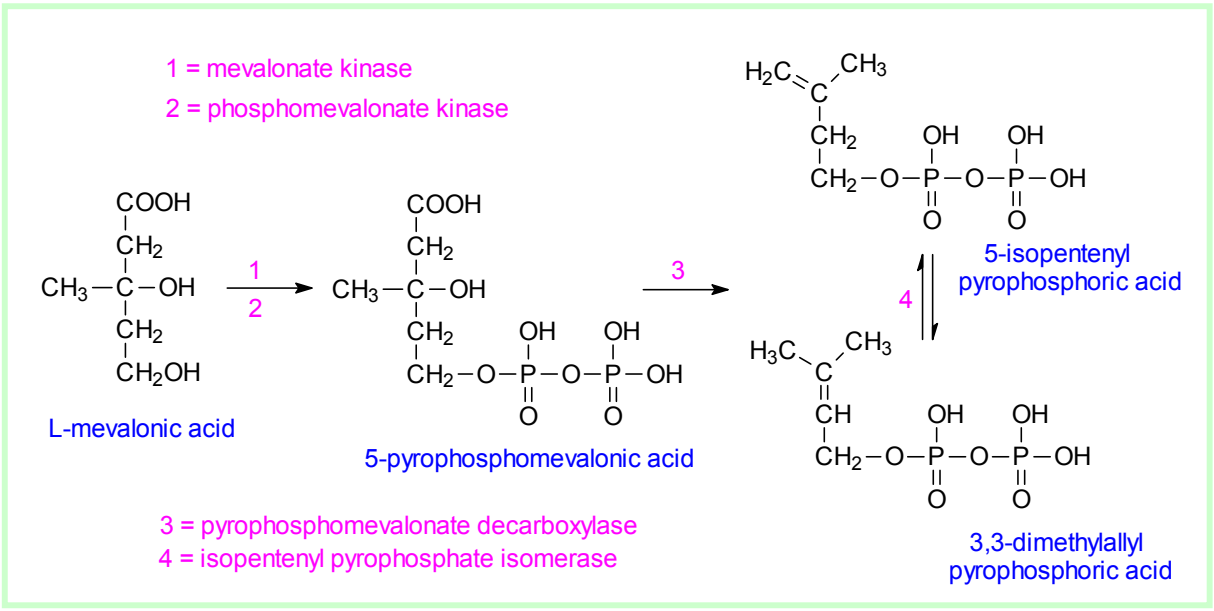
The first steps involve the synthesis of the important intermediate mevalonic acid from acetyl-CoA and acetoacetyl-CoA, both of which are in fact derived from acetate, in two enzymatic steps.

The acetyl-CoA precursor is in the cytosol as is the first enzyme, hydroxymethyl-glutaryl(HMG)-CoA synthase. The second enzyme HMG-CoA reductase is a particularly important control point,

and is widely regarded as the rate-limiting step in the overall synthesis of sterols, and its activity is regulated by several factors including a cycle of phosphorylation-dephosphorylation. This and subsequent enzymes are membrane-bound and are located in the endoplasmic reticulum. The enzyme HMG-CoA reductase is among the targets inhibited by the drugs known as 'statins', so that patients must then obtain much of their cholesterol from the diet via the circulation.



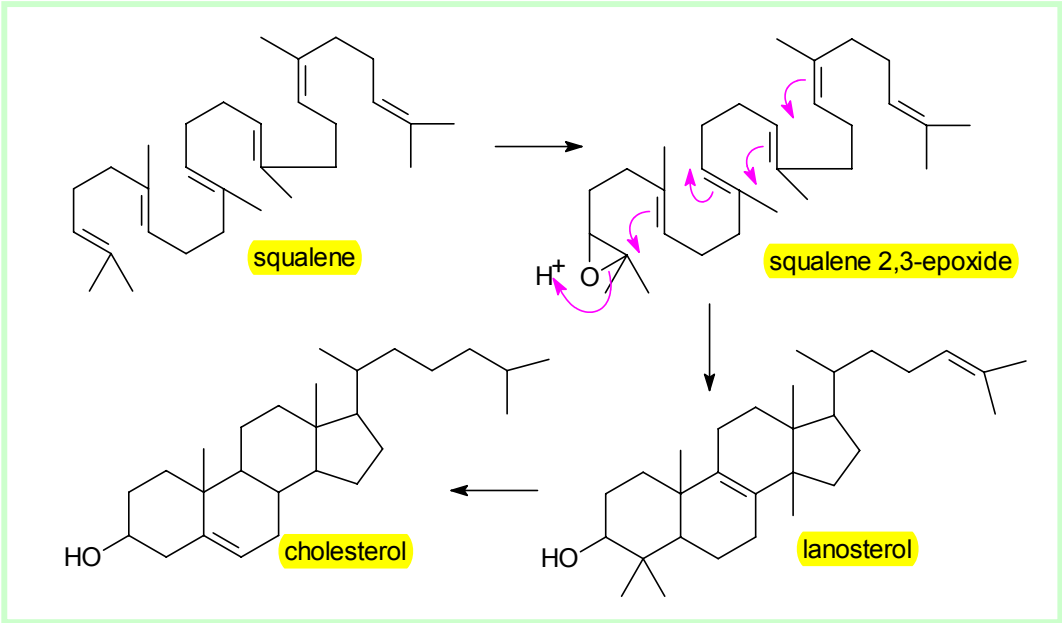
The next sequence of reactions involves first the phosphorylation of mevalonic acid by a mevalonate kinase to form the 5-monophosphate ester, followed by a further phosphorylation to yield an unstable pyrophosphate, which is rapidly decarboxylated to produce 5-isopentenyl pyrophosphoric acid. An isomerase converts part of the latter to 3,3-dimethylallylpyrophosphoric acid.



5-Isopentenyl pyrophosphate is a nucleophile, but the isomerized product is electrophylic. In the first step in the third series of reactions (not illustrated), 5-isopentenyl pyrophosphate and 3,3-dimethylallylpyrophosphate condense readily with the elimination of pyrophosphoric acid to form the monoterpenoid derivative geranylpyrophosphate. This reacts with another molecule of 5-isopentenyl pyrophosphate to produce the sesquiterpene derivative (C_{15}) farnesylpyrophosphate, two molecules of which condense to yield presqualene pyrophosphate. The last is reduced by a squalene synthase and NADPH to produce a further key intermediate **squalene**.

In the next important series of reactions, squalene is first oxidized by a squalene monooxygenase to squalene 2,3-epoxide, which undergoes cyclization catalysed by the enzyme squalene epoxide lanosterol-cyclase to form the first steroidal intermediate lanosterol (or cycloartenol in the case of

higher plants). In this remarkable reaction, there is a series of concerted 1,2-methyl group and hydride shifts along the chain of the squalene molecule to bring about the formation of the four rings. No intermediate compounds have been found. This is believed to be one of the most complex single enzymatic reactions ever to have been identified, although the enzyme involved is only 90 kDa in size. Again, the reaction takes place in the endoplasmic reticulum, but a cytosolic protein, sterol carrier protein 1, is required to bind squalene in an appropriate orientation, in the presence of the cofactors phosphatidylserine and flavin adenine dinucleotide (FAD). Finally, lanosterol is converted to cholesterol (by at least two pathways) by multiple reactions that involve the removal of three methyl groups, hydrogenation of the double bond in the side-chain, and a shift of the double bond from position 8,9 to 5,6 in ring B. A second cytosolic protein, sterol carrier protein 2, is required to bind 7-dehydrocholesterol, one of the intermediates in the process.



Synthesis occurs mainly in the liver, although the brain synthesises its own considerable supply, and cholesterol can then be exported and transported to other tissues in the form of lipoprotein complexes for uptake via low-density lipoprotein receptors, or it can be converted to bile acids secreted into the intestines. The reverse process, in which cholesterol is transferred back from peripheral tissues to the liver for catabolism (by conversion to steroids, bile acids and oxysterols) or elimination, is also important for cholesterol homeostasis (see the web-pages on **oxysterols** and on **lipoproteins**).

Elevated cholesterol and cholesterol ester levels are associated with the pathogenesis of cardiovascular disease, as is well known, but further discussion of such a complex nutritional topic is not possible here. It is less well known that a decrease in the concentration of cholesterol in the body can result in severe health problems, such as the recessive Smith-Lemli-Opitz syndrome in infants born with a decreased body concentration of the enzyme 7-dihydrocholesterol reductase. In fact, eight different inherited disorders of cholesterol biosynthesis are recognized that lead to congenital abnormalities in those afflicted. It is evident that cholesterol plays a vital part in human embryogenesis and development.

In animal tissues, cells can obtain the cholesterol they require either from the diet, via the circulating low-density **lipoproteins** (LDL)) or they can synthesise it themselves as outlined above. A protein within cells that is able to sense the existing concentration of cholesterol controls the levels. Thus, a sterol regulatory element-binding protein (specifically 'SREBP-2'), which is synthesised as an inactive precursor and inserted into the endoplasmic reticulum, encounters an escort protein termed SREBP cleavage-activating protein (SCAP). It is this second protein that is the cellular cholesterol sensor. When the latter recognizes that cellular cholesterol levels are

inadequate, it activates a transcription factor, which stimulates the expression of the genes coding for the LDL receptor and for the key enzyme in cholesterol biosynthesis, HMG-CoA reductase. This in turn stimulates the rate of cholesterol uptake and synthesis. Conversely, when cellular cholesterol levels are higher, the SCAP fails to activate the transcription factor and uptake and synthesis of cholesterol are not enhanced.

Within cells, there are large differences in cholesterol concentrations among the organelles. These differences are maintained by mechanisms that can involve either vesicle formation or non-vesicular pathways that utilize specific transport proteins.

When increased levels of plasma sterols other than cholesterol are found in plasma, they usually serve as markers for abnormalities in lipid metabolism associated with disease states. For example, premature atherosclerosis and xanthomatosis occur in two rare lipid storage diseases, Cerebrotendinous xanthomatosis and sitosterolemia. In the former, cholestanol is present in all tissues, while in sitosterolemia, dietary campesterol and sitosterol accumulate in plasma and red blood cells. Inhibition of cholesterol biosynthesis may be associated with the appearance of some of the precursor sterols in the plasma.

Cholesterol is the biosynthetic precursor of steroidal hormones (glucocorticoids, oestrogens, progesterones, androgens and aldosterone), vitamin D, bile acids and cholesterol esters. It is found bound covalently to specific membrane proteins or **proteolipids** (see web page), which have vital functions in embryonic development. It must be supplied from exogenous sources to the primitive nematode *Caenorhabditis elegans*, where it does not appear to have a major role in membrane structure, but rather some ill-defined signalling functions controlling development.

3. Cholesterol Esters

Cholesterol esters, *i.e*. with long-chain fatty acids linked to the hydroxyl group, are much less polar than free cholesterol and appear to be the preferred form for transport in plasma and for storage. They do not contribute to membranes but are packed into intracellular lipid particles. Because of the mechanism of synthesis (see below), plasma cholesterol esters tend to contain relatively high proportions of the polyunsaturated components typical of phosphatidylcholine (Table 1). Arachidonic and “adrenic” (20:4(n-6)) acids can be especially abundant in cholesterol esters from the adrenal gland.

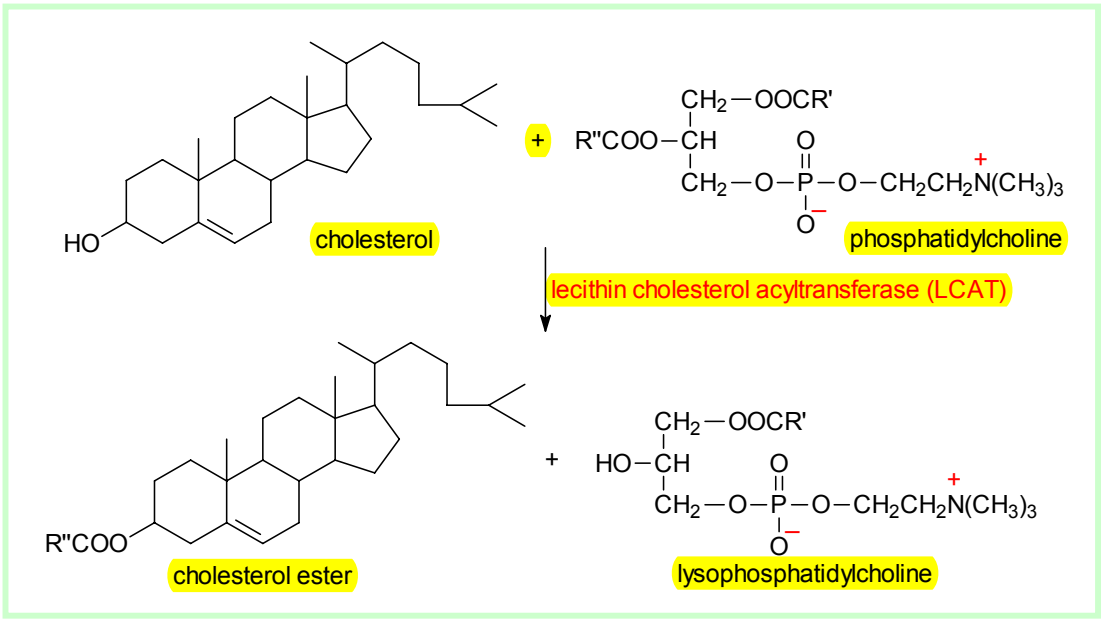
Table 1. Fatty acid composition of cholesterol esters (wt % of the total) from various tissues.

	Fatty acids						
	16:0	18:0	18:1	18:2	18:3	20:4	22:4
Human							
plasma	12	2	27	45		8	
liver	23	10	28	22		6	
Sheep							
plasma	10	2	27	35	7		
liver	17	9	29	7	4	3	
adrenals	13	7	35	18	2	4	2

Data from - Christie, W.W. *et al*. *Lipids*, **10**, 649-651 (1975); Nelson, G.J. *Comp. Biochem. Physiol*., **30**, 715-725 (1969); Horgan, D.J. and Masters, C.J. *Aust. J. Biol. Sci*., **16**, 905-915 (1963); Nestel, P.J. and Couzens, E.A. *J. Clin. Invest*., **45**, 1234-1240 (1966).

Cholesterol esters are major constituents of the adrenal glands, and they accumulate in the fatty lesions of atherosclerotic plaques. Esters of steroidal hormones are also present in the adrenal glands, where they are concentrated in cytosolic lipid droplets adjacent to the endoplasmic reticulum. 17β-Estradiol, the principal oestrogen in fertile women, is transported in lipoproteins in the form of a fatty acid ester. Such esters may be biologically inert storage or transport forms.

In plasma, and the high-density lipoproteins (HDL) in particular, cholesterol esters are synthesised largely by transfer of fatty acids to cholesterol from position *sn*-2 of phosphatidylcholine by the enzyme lecithin cholesterol acyl transferase (LCAT). The enzyme also promotes the transfer of cholesterol from cells to HDL. It has been established that human LCAT is a relatively small glycoprotein with a polypeptide mass of 49 kDa, increased to about 60 kDa by a complex carbohydrate moiety. Most of the enzyme is produced in the liver and circulates in the blood stream bound reversibly to HDL, where it is activated by the main protein component of HDL, apolipoprotein A-I. As cholesterol esters accumulate in the core of the lipoprotein, cholesterol is removed from its surface thus promoting the flow of cholesterol from cell membranes into HDL. This in turn leads to morphological changes in HDL, which grow and become spherical. Subsequently, cholesterol esters are transferred to the other lipoprotein fractions LDL and VLDL, a reaction catalysed by cholesteryl ester transfer protein. LCAT is of great importance for cholesterol homeostasis and it is a suggested target for therapeutic intervention against atherosclerosis.



In other animal tissues, a further enzyme acyl-CoA:cholesterol acyltransferase (ACAT) synthesises cholesterol esters from CoA esters of fatty acids and cholesterol. ACAT exists in two forms, both of which are intracellular enzymes found in the endoplasmic reticulum, and possess multiple hydrophobic regions predicted to function as trans-membrane domains. ACAT1 is present in many tissues, but especially in macrophages and adrenal and sebaceous glands, which store cholesterol esters in the form of cytoplasmic droplets. It is also responsible for the synthesis of cholesterol esters in arterial foam cells in human atherosclerotic lesions. ACAT2 is found mainly in the liver and small intestine, and it is believed to be involved in the supply of cholesterol esters to the nascent lipoproteins.

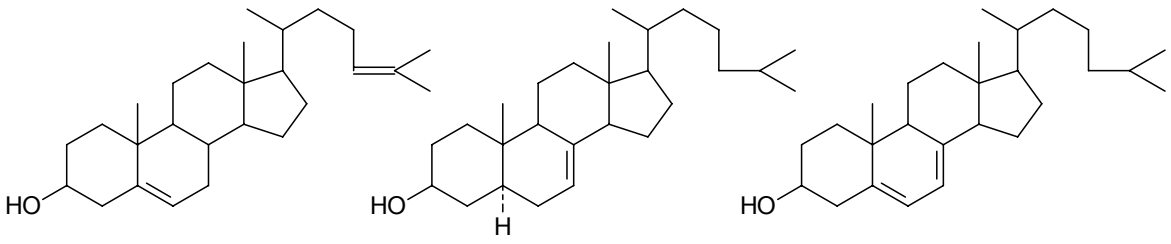
Cholesterol ester hydrolases in animals liberate cholesterol and free fatty acids when required for membrane and lipoprotein formation, and they also provide cholesterol for hormone synthesis in adrenal cells. Many cholesterol ester hydrolases have been identified, including a carboxyl ester hydrolase, a lysosomal acid cholesterol ester lipase, hormone-sensitive lipase and hepatic cytosolic cholesterol ester hydrolase. These are located in many different tissues and organelles and have multiple functions. A neutral cholesterol ester hydrolase has received special study, as it

involved in the removal of cholesterol esters from macrophages, so reducing the formation of foam cells and thence the development of fatty streaks within the arterial wall, a key event in the progression of atherosclerosis.

4. Other Animal Sterols

Cholesterol will oxidize slowly in tissues or foods to form a range of different products with additional hydroperoxy, epoxy, hydroxy or keto groups, and these can enter tissues via the diet. There is increasing interest in these from the standpoint of human health and nutrition, since accumulation of oxo-sterols in plasma is associated with inhibition of the biosynthesis of cholesterol and bile acids and with other abnormalities in plasma lipid metabolism. Those similar **cholesterol oxides or oxysterols** produced in tissues by specific microsomal or mitochondrial oxidations are discussed in a further document on this web site.

A number of other sterols occur in small amounts in tissues, most of which are intermediates in the pathway from lanosterol to cholesterol, though some of them have distinct functions in their own right. **Lanosterol**, the first sterol intermediate in the biosynthesis of cholesterol, was first found in wool wax, both in free and esterified form, and this is still the main commercial source. It is found at low levels only in most other animal tissues. There have been interesting speculations on the evolutionary significance of lanosterol biosynthesis. As oxygen is required, it cannot be produced by primitive organisms, hence its absence from prokaryotes. When sterols became available to eukaryotes, much greater possibilities opened for their continuing evolution. The production of cholesterol from lanosterol is then seen as 'molecular streamlining' by evolution, removing protruding methyl groups that hinder the interaction between sterols and phospholipids in membranes.



desmosterol (5,24-cholestadien-3β-ol) | lathosterol (5α-cholest-7-en-3β-ol) | 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol)

Further examples of animal sterols include **7-dehydrocholesterol** (cholesta-5,7-dien-3β-ol) in the skin, which on irradiation with UV light is converted to vitamin D_3 (cholecalciferol). Desmosterol (5,24-cholestadien-3β-ol) may be involved in the process of myelination, as it is found in relative abundance in the brains of young animals but not in those of adults. It is also found in appreciable amounts in spermatozoa and astrocytes. There is a rare genetic disorder in which there is an impairment in the conversion of desmosterol to cholesterol, desmosterolosis, with serious consequences in terms of mental capacity. In human serum, the levels of **lathosterol** (5α-cholest-7-en-3β-ol) were found to be inversely related to the size of the bile acid pool, and in general the concentration of serum lathosterol is strongly correlated with the cholesterol balance under most dietary conditions. The isomeric saturated sterols, **cholestanol** and **coprastanol**, which differ in the stereochemistry of the hydrogen atom on carbon 5, are formed by microbial biohydrogenation of cholesterol in the intestines, and together with cholesterol are the main sterols in faeces. In the rare lipid storage disease, Cerebrotendinous xanthomatosis, cholestanol is present in all tissues.

5. Analysis

With animal tissues, especially those of clinical importance such as plasma, the cholesterol content is often determined by using enzymatic methods from commercially available kits that are suited to routine analysis of large numbers of samples. For the total cholesterol content, it is necessary to hydrolyse the cholesterol ester fraction first, and this usually requires more vigorous conditions than with glycerolipids. For more accurate or detailed analysis of animal and plant sterols, a sterol fraction is first isolated from lipid extracts by thin-layer or column chromatography, following hydrolysis if necessary. Individual components can then be determined by gas chromatography in the presence of an internal standard (*e.g*. epicoprostanol or betulin), often after conversion to trimethylsilyl ether derivatives to give sharper peaks. Mass spectrometry may be required for identification of individual components.

Sterol esters are transmethylated for GC analysis of the fatty acid components, although the reaction may again be much slower than with glycerolipids. Intact sterol esters are best analysed by reversed-phase HPLC.

Recommended Reading

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