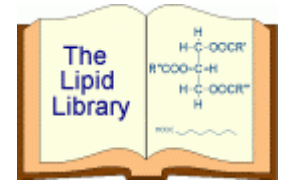


STEROLS 2. OXYSTEROLS AND OTHER CHOLESTEROL DERIVATIVES



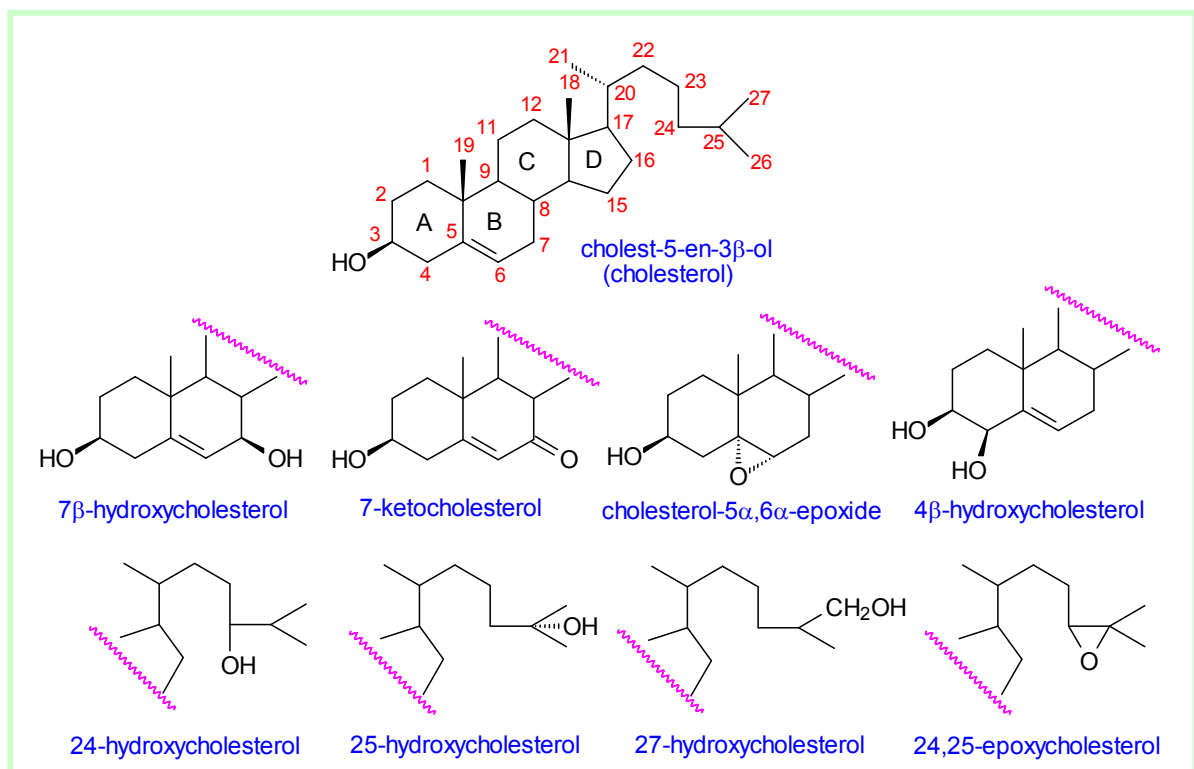
STRUCTURE, OCCURRENCE AND BIOCHEMISTRY

1. Oxysterols

Oxysterols are usually defined as oxygenated derivatives of **cholesterol**, though plant sterols can also be oxidized, and they are important as short-lived intermediates or end products in the catabolism or excretion of cholesterol. They are normally present in biological membranes and lipoproteins at trace levels only, though they can exert profound biological effects at these concentrations. They are always accompanied by a great excess (as much as 10^6 -fold) of cholesterol.

Oxysterols can be formed rapidly by non-enzymatic autoxidation of cholesterol (and cholesterol esters), when a multiplicity of different oxygenated derivatives result, but they are also synthesised by specific oxygenases in cells. Once an oxygen function is introduced into cholesterol within cells, the product can act as a biologically active mediator before it is metabolized to **bile acids** or is degraded further, processes assisted by the fact that oxysterols are able to diffuse much more rapidly through membranes than is cholesterol itself.

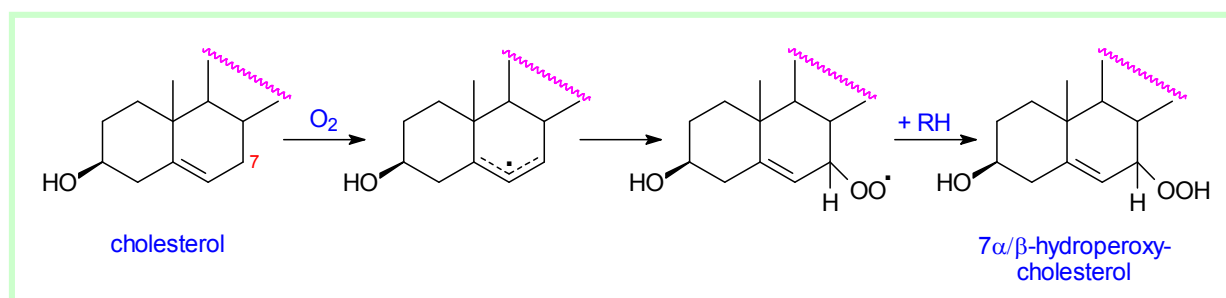
Non-Enzymatic Oxidation: There is evidence that cholesterol in a membrane environment may be attacked more readily than the polyunsaturated fatty acids by reactive oxygen species, although the opposite is true in plasma, for example. The structures of a few of the more important oxysterols are illustrated as examples of the main types of product.



Oxysterols can vary in the type (hydroperoxy, hydroxy, keto, epoxy), number and position of the oxygenated functions introduced and in the nature of their stereochemistry. Derivatives with the A and B rings and the *iso*-octyl side-chain oxidized are illustrated, but compounds with oxygen groups in position 15 (D ring) are also important biologically. Usually, they are named in relation to cholesterol, rather than by the strict systematic terminology.

Oxysterols occur in tissues both in the free state and esterified with long-chain fatty acids. For example, in human atherosclerotic lesions, 80-95% of all oxysterols are esterified. Appreciable amounts of oxysterols can be present in foods, especially those rich in such as meat, eggs and dairy products, which are most probably generated non-enzymically during cooking or processing. They can be absorbed from the intestines and transported into the circulation in chylomicrons, but the extent to which dietary sources contribute to tissue levels either of total oxysterols or of individual isomers is not known.

Mechanisms of autoxidation have been intensively studied in terms of unsaturated fatty acids, and it appears that similar mechanisms operate with sterols. As an example, the reaction mechanism leading to the production of 7-oxygenated cholesterol derivatives is illustrated. In aqueous dispersions, oxidation is initiated by a radical attack forming a delocalized three-carbon allylic radical, which reacts with oxygen to produce the epimeric products 7 α - and 7 β -hydroperoxy-cholesterol. Subsequent enzymic and non-enzymic reactions lead to the hydroxy and keto analogues, which may be accompanied by epoxy-ene and ketodienoic secondary products.



Reaction does not occur at the other allylic carbon 4, presumably because of steric hindrance. When cholesterol is in the solid state, reaction occurs primarily at the tertiary carbon-25, though some products oxygenated at C-20 may also be produced.

Epimeric 5,6-epoxy-cholesterols may be formed by a non-radical reaction involving the non-enzymatic interaction of a hydroperoxide with the double bond, a process that is believed to occur in macrophages especially and in low-density lipoproteins (LDL). In this instance, the initial peroxidation product is a polyunsaturated fatty acid; the hydroperoxide transfers an oxygen atom to cholesterol to produce the epoxide, and in so doing is reduced to a hydroxyl. Other non-radical oxidation processes include reaction with singlet oxygen, which can generate 5-hydroxy- as well as 6- and 7-hydroxy products. In addition, reaction with ozone, for example in the lung, can generate a family of distinctive oxygenated cholesterol metabolites.

Photoxidation in the retina via the action of free radicals or singlet oxygen generates unstable cholesterol hydroperoxides, which may be involved in age-related macular degeneration. For example, these compounds can quickly be converted to highly toxic 7 α - and 7 β -hydroxycholesterols and 7-ketocholesterol, depending on the status of tissue oxidases and reductases. Three separate enzymatic pathways have developed in the eye to neutralize their activity. **7-Ketocholesterol** is also a major oxysterol produced during oxidation of low-density lipoproteins, and is one of the most abundant in plasma and atherosclerotic lesions, with a high pro-apoptotic potential. It associates preferentially with membrane lipid raft domains.

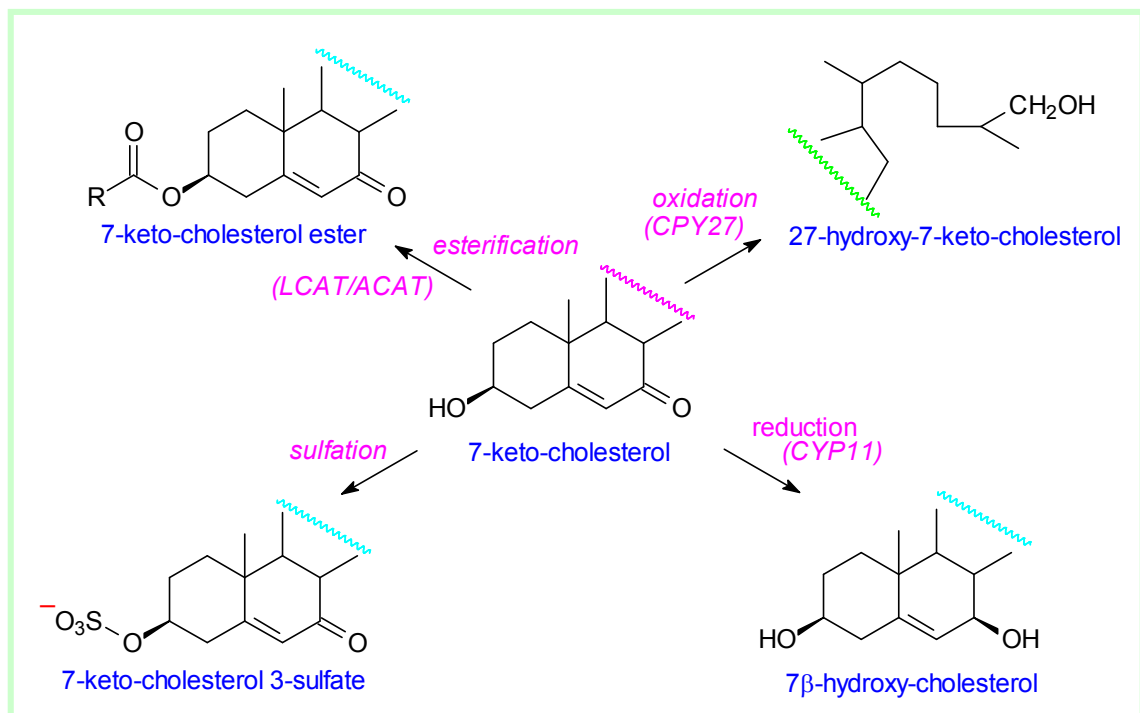
Enzymatic Oxidation: Within animal cells, oxidation of sterols is mainly an enzymic process that is carried out by several enzymes that are mainly from the cytochrome P450 family of oxygenases

(they have a characteristic absorption at 450 nm). These are a disparate group of proteins that contain a single heme group and have a similar structural fold, though the amino acid sequences can differ appreciably. They are all mono-oxygenases. For example, **7 α -hydroxycholesterol** is an important intermediate in the biosynthesis of bile acids (see below) and it is produced in the liver by the action of cholesterol 7 α -hydroxylase (CYP7A1). The reaction is under strict regulatory control, and any circulating 7 α -hydroxycholesterol represents leakage from the liver. On the other hand, **7 β -hydroxycholesterol** is produced in brain by the action of the toxic β -amyloid peptide and its precursor on cholesterol. Whether this metabolite is involved in the pathology of Alzheimer's disease has yet to be determined.

An alternative pathway to bile acids starts with **27-hydroxycholesterol**, which is produced by another cytochrome P-450 enzyme (CYP27A1) that introduces the hydroxyl group into the terminal methyl carbon (C-27). While this enzyme is present in the liver, it is found in many extra-hepatic tissues and especially the lung, which provides a steady flux of 27-oxygenated metabolites to the liver. It is involved in some of the later stages of bile acid production (see below). In addition, as a multifunctional mitochondrial P-450 enzyme in liver, it generates both 25R,26-hydroxycholesterol and 3 β -hydroxy-5-cholestenoic acid, which occur in small but significant amounts in plasma.

In humans, the specific cytochrome P-450 that produces **24S-hydroxycholesterol** (cholest-5-ene-3 β ,24-diol), cholesterol 24S-hydroxylase (CYP46A1), is located almost entirely in the smooth endoplasmic reticulum of neurons in the brain, and even the 24S-hydroxycholesterol found in plasma is derived from the brain. The enzyme is expressed in neurons, including those of the hippocampus and cortex, which are important for learning and memory, and it is responsible for most of the turnover of cholesterol in the central nervous system. **25-Hydroxycholesterol** is a relatively minor but biologically important cholesterol metabolite, and it is unusual in that it is produced in liver by an enzyme from a family of non-heme iron-containing proteins and not a cytochrome P-450, as well as by autoxidation.

24(S),25-Epoxycholesterol is not produced by the pathways described above but is synthesised in a shunt of the same mevalonate pathway that produces cholesterol. It may represent a measure of newly synthesised cholesterol.



The oxysterols formed by both autoxidation and enzymatic routes can undergo further oxidation-reduction reactions, and they can be modified by many of the enzymes involved in the metabolism

of cholesterol and steroidal hormones, such as esterification and sulfation, as illustrated for 7-keto-cholesterol.

Biological Activity: In tissues *in vivo*, the very low oxysterol:cholesterol ratio means that oxysterols have little impact on the primary role of cholesterol in cell membrane structure and function. Indeed, it is often argued that there are few reliable measurements of cellular or subcellular oxysterol concentrations, because of the technical difficulties of the very low concentrations of oxysterols in the presence of a vast excess of native cholesterol. Nonetheless, aside from their role as precursors of bile acids and some steroidal hormones, it is evident that oxysterols have a variety of roles in terms of maintaining cholesterol homeostasis and perhaps in signalling.

While cholesterol plays a key role in its own feedback regulation, there is ample evidence that oxysterols are also potent inhibitors of cholesterol biosynthesis, and **25-hydroxycholesterol** and **24(S),25-epoxycholesterol**, the latter formed as a side product in the mevalonate pathway, are especially effective. Several mechanisms appear to be involved, and it is established that they inhibit the transcription of key genes in cholesterol biosynthesis (sterol regulatory element binding protein (SREBP) transcription factors), as well as directly inhibiting or accelerating the degradation of such important enzymes in the process as HMG-CoA reductase and squalene synthase. Oxysterols may smooth out the regulation of cholesterol metabolism, preventing exaggerated responses. However, experts in the field caution that it can be difficult to extrapolate from experiments *in vitro* to the situation *in vivo*, because of the rapidity with which cholesterol can autoxidize in experimental systems and because of the difficulty of carrying out experiments with physiological levels of oxysterols.

25-Hydroxycholesterol is also reported to have a regulatory effect on the biosynthesis of sphingomyelin, which is required with cholesterol for the formation of **raft** sub-domains in membranes, and together with other oxysterols to regulate the activities of some **hedgehog proteins** involved in embryonic development.

Oxysterols are especially important for cholesterol homeostasis in the brain, which contains 25% of the total body cholesterol, virtually all of it in unesterified form, in only about 2% of the body volume. Cholesterol is a major component of the plasma membrane especially, where it serves to control the fluidity and permeability. This membrane is produced in large amounts in brain and is the basis of the compacted myelin, which is essential for conductance of electrical stimuli and contains about 70% of the cholesterol in brain. This pool is relatively stable, but the remaining 30% is present in the membranes of neurons and glial cells of gray matter and is active metabolically. Even in the foetus and the newborn infant, all the cholesterol required for growth is produced by synthesis *de novo* in the brain not by transfer from the circulation across the blood-brain barrier, which consists of tightly opposed endothelial cells lining the extensive vasculature of the tissue. The fact that this pool of cholesterol in the brain is independent of circulating levels must reflect a requirement for constancy in the content of this lipid in membranes and myelin. In adults, although there is a continuous turnover of membrane, the cholesterol is efficiently re-cycled and has a remarkably high half-life (up to 5 years). The rate of cholesterol synthesis is a little greater than the actual requirement, so that net movement of cholesterol out of the central nervous system must occur.

If cholesterol itself cannot cross the blood-brain barrier, its metabolite **24(S)-hydroxycholesterol** is able to do so with relative ease. When the hydroxyl group is introduced into the side chain, this oxysterol effects a local re-ordering of membrane phospholipids such that it is more favourable energetically to expel it, and this can occur at a rate that is orders of magnitude greater than that of cholesterol *per se*, though still only 6-7 mg per day. There is a continuous flow of this metabolite from the brain into the circulation, where it is transported by lipoprotein particles to the liver for further catabolism, i.e. it is hydroxylated in position 7 and then converted to bile acids. Especially high levels of 24(S)-hydroxycholesterol are observed in the plasma of human infants, while

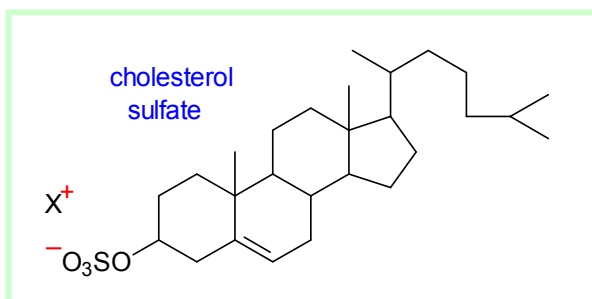
reduced levels are found in patients with neurodegenerative diseases, including multiple sclerosis and Alzheimer's disease (it may be protective against β -amyloid peptide, the amyloidogenic peptide found in brain in this condition). In contrast, **7 β -hydroxycholesterol**, produced by this protein, is pro-apoptotic, but any links with the disease are unproven. **27-Hydroxycholesterol** diffuses across the blood-brain barrier from the blood stream into the brain, where it is further oxidized and then exported. This flux may regulate certain key enzymes within the brain, and there are suggestions that the balance between the levels of 24- and 27-hydroxy-cholesterol may also be relevant to the generation of β -amyloid peptides.

Oxysterols do appear to be important for many aspects of cholesterol turnover and transport, and there have been many reports of involvement in disease processes, especially atherosclerosis and the formation of human atherosclerotic plaques, but also cytotoxicity, necrosis, inflammation, immuno-suppression, phospholipidosis and gallstone formation. For example, they are enriched in pathologic cells and tissues, such as macrophage foam cells, atherosclerotic lesions, and cataracts. They may regulate some of the metabolic effects of cholesterol. However, as cautioned above, effects observed *in vitro* may not necessarily be of physiological importance *in vivo*. Similarly, it has been argued that plasma oxysterols could serve as markers of oxidative stress, but the experimental difficulties in analysis have been such that their value has been limited. Sample handling remains a problem, but the newer methods of mass spectrometry with electrospray ionization now enable direct analysis of even the reactive hydroxy-, hydroperoxy- and ozonide-containing oxysterols.

Catabolism. Because of their increased polarity relative to cholesterol, oxysterols can exit cells relatively easily. Some are converted to inert sterol esters and stored in this form, a proportion is further oxidized and converted to bile acids (see below) and some are converted to sulfate esters (especially at the 3-hydroxyl group) or glucuronides for elimination.

2. Cholesterol 3-sulfate

The strongly acidic sulfate ester of cholesterol occurs in all mammalian cells, but it is especially abundant in keratinized tissue, such as skin and hooves. Although present at low levels, it can be the main sulfolipid in many cell types, but especially kidney, and reproductive and nervous tissues. In many organs, it appears to be concentrated in epithelial cell walls or in plasma membranes. Cholesterol sulfate is the main circulating sterol sulfate in plasma, although it is there accompanied by dehydroepiandrosterone sulfate, the function of which is unknown. In addition, 7-ketocholesterol sulfate has been found in primate retina, while 24-hydroxycholesterol occurs in bovine brain as its sulfate ester.



Cholesterol sulfate may have a role in ensuring the integrity and adhesion of the various skin layers, while also regulating some enzyme activities. For example, it functions in keratinocyte differentiation, inducing genes that encode for key components involved in development of the barrier. However, the function of this lipid is still only partly understood. It may play a part in cell adhesion, differentiation and signal transduction. In addition, it has a stabilizing role, for example in protecting erythrocytes from osmotic lysis and regulating sperm capacitation.

Sterol sulfates have been detected occasionally in lower life forms, such as the sea star, *Asterius rubrius*, and the marine diatom, *Nitzschia alba*.

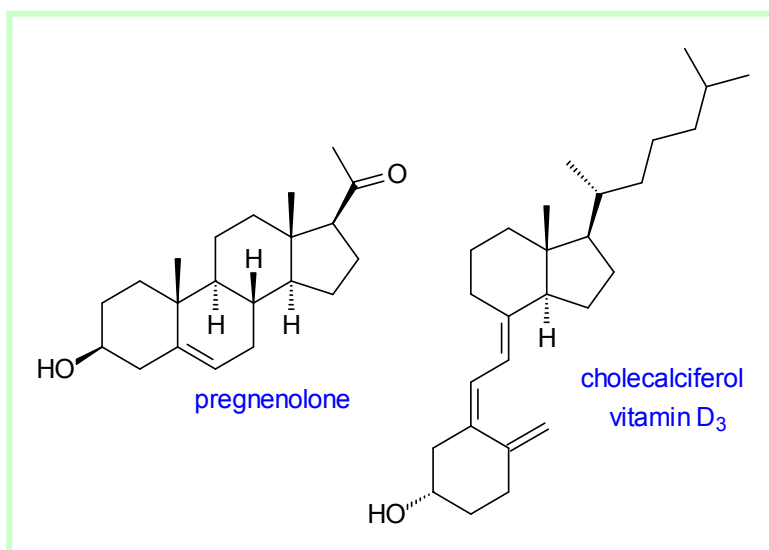
3. Cholesterol Glycosides and Other Cholesterol Derivatives

Cholesterol is found linked covalently to specific proteins where it functions to anchor the protein in a membrane, but this is discussed in our web page on [proteolipids](#). Cholesteryl glucoside and acyl cholesteryl glucoside have been found in the skin of snakes. Cholesteryl glucoside occurs also in human fibroblasts, and some rat tissues, where it may act as a mediator of signal transduction in the early stages of stress. As with plant and fungal steryl glycosides, these have a sugar β -glucosidic linkage. In addition, a cholesterol-conjugate with glucuronic acid has been isolated from human liver (33 nmol/g wet tissue) and plasma, but its origin, function and metabolic fate are unknown.

Some bacterial species contain cholesterol glycosides, and four unusual glycolipids, i.e. cholesteryl- α -glucoside, cholesteryl-6'-O-acyl- α -glucoside, cholesteryl-6'-O-phosphatidyl- α -glucoside, and cholesteryl-6'-O-lysophosphatidyl- α -glucoside, occur in the pathogenic bacterium *Helicobacter pylori*, for example. The key enzyme involved in their biosynthesis is a membrane-bound, UDP-glucose-dependent cholesterol- α -glucosyltransferase. These lipids appear to support the pathogenicity of the organism. Cholesterol 6-O-acyl- β -D-galactopyranoside and its non-acylated form are significant components of membranes of the spirochete *Borrelia burgdorferi*, which is the causative agent of Lyme disease. Sterol glycosides are more common constituents of plants (see our web page on [plant sterols](#)).

4. Steroidal Hormones and Vitamin D

These subjects are too big to be discussed in depth here. In brief, in addition to the bulk sterols, animal tissues produce small amounts of vital steroidal hormones, including oestrogens and progesterone, which are made primarily in the ovary and placenta during pregnancy, and testosterone mainly in the testes. Pregnane neurosteroids are produced in the central nervous system. Conversion of cholesterol to pregnenolone in mitochondria is the rate-limiting step and involves first hydroxylation and then cleavage of the side-chain.



Vitamin D encompasses two main sterol metabolites that are essential for the regulation of calcium and phosphorus levels and thence for bone formation in animals. However, these have many other functions, especially in relation to the immune system. Ultraviolet light mediates cleavage of 7-dehydrocholesterol in the skin to produce pre-vitamin D, which rearranges spontaneously to form the secosteroid vitamin D₃ or cholecalciferol. The newly generated vitamin D₃ is transported to the liver where it is subject to 25-hydroxylation and thence to the kidney for 1 α -hydroxylation to generate a high affinity ligand for the vitamin D receptor. Vitamin D₂ or ergocalciferol is derived from ergosterol and is obtained from plant and fungal sources in the diet.

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