8. STEROIDS

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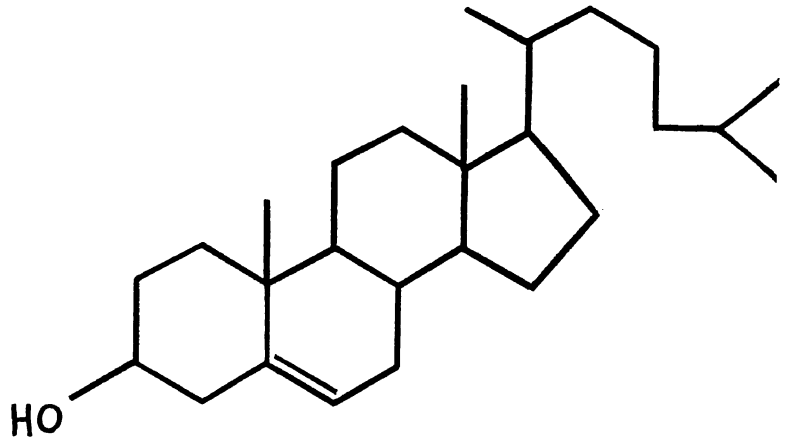
I. CHOLESTEROL: STRUCTURE AND CHEMISTRY

Carbon skeleton: four rings plus 8-carbon side-chain = 27 carbons:



Substitution: ... (dotted line) = α (alpha, away from observer).
—— (solid line) = β (beta, toward, or by observer).

The **3β-hydroxyl** group, though hydrophilic, cannot overcome the non-polarity of the rest of the molecule, hence cholesterol is **insoluble in water.**

The Δ^{5-6} double bond can be reduced two ways:

5α-H: 5α-cholestan-3β-ol; new hydrogen trans to the angular methyl group (C-19); rings A/B trans.

5β-H: 5β-cholestan-3β-ol; new hydrogen cis to C-19; rings A/B cis. This is mainly a bacterial product (coprosterol in older usage, from Gr. copros, meaning feces, where it is found).

II. OCCURRENCE AND FUNCTION

The name means **"bile-solid-alcohol."** It is found in bile, (a frequent component of gallstones, where it was first discovered), and is a crystalline solid and an alcohol.

Cholesterol occurs in **all cells** and tissues of higher organisms, but is especially abundant in nervous tissue and egg yolk. Related sterols are found in plants and higher microorganisms; these are poorly absorbed from the digestive tract. For storage, sterols are often **esterified** with unsaturated fatty acids. The total amount in a human averages about 180 grams. A typical value for blood cholesterol in developed countries is 180 mg/100 ml. Note that on a weight basis this is twice as high as the level of blood glucose.

Cholesterol has a universal function as a **component of membranes**, probably to increase their fluidity. Also it is a **precursor** of many important biological substances (see **bile acids, hormones**). In human disease, cholesterol's insolubility makes deposits troublesome, especially in **atherosclerotic plaques** and **gallstones.**

In blood, cholesterol is carried as a complex with **lipoproteins**, partly esterified with a fatty acid, and partly with the 3-OH free. (See Chapter 7, page 119). The cholesterol in blood can exchange readily with liver cholesterol, but some cholesterol pools, especially that in brain, exchange very slowly.

III. BIOSYNTHESIS OF CHOLESTEROL

A. Acetate to Squalene

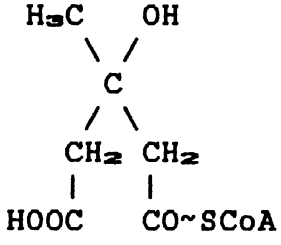
1. Formation of **ß-hydroxy-ß-methyl-glutaryl CoA** (HMG CoA)

   a. 2 acetyl CoA $\rightleftharpoons$ acetoacetyl CoA + CoA (as in ketogenesis)

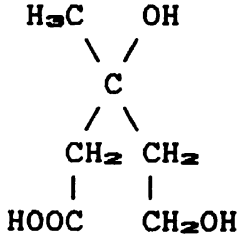
   b. acetoacetyl CoA + acetyl CoA $\rightleftharpoons$ ß-hydroxy-ß-methyl-glutaryl CoA, or HMG CoA

2. Reduction of HMG CoA to **Mevalonic Acid**

$$\text{HMG CoA} + 2\text{NADPH} + 2\text{H}^+ \xrightarrow[\text{Reductase}]{\text{HMG CoA}} \text{MVA} + 2\text{NADP}^+ + \text{HSCoA}$$



HMG CoA



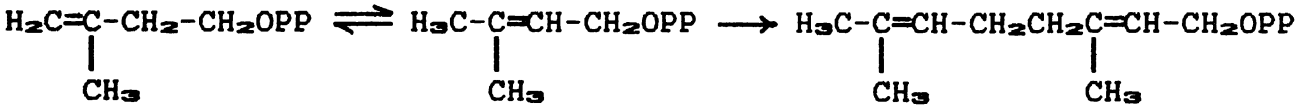
Mevalonic Acid (MVA)

This reaction is the first committed, largely irreversible step in cholesterol biosynthesis, and is an **important control point.** The enzyme is rate-limiting for the entire pathway, and is inhibited by the eventual end-product, cholesterol.

3. MVA $\xrightarrow[3 \text{ times}]{\text{ATP, Mg}^{++}}$ $H_2C{=}C(CH_3)-CH_2-CH_2OPP$ **Isopentenyl Pyrophosphate** (CO_2 is lost)

Isopentenyl pyrophosphate is the "active isoprene unit": the precursor of all isoprenoid compounds such as terpenes; vitamins A, D, E, K; sterols; rubber.

4. Isopentenyl-PP can isomerize to **dimethylallyl-PP**, which condenses with another isopentenyl-PP to form **geranyl-PP**, a monoterpene (C-10):



5. Geranyl-PP condenses with another i-pentenyl-PP to form **farnesyl-PP**, a sesquiterpene (C-15).

6. Two farnesyl-PP condense head-to head (NADPH required) to form **squalene**, a triterpene (C-30).

B. Squalene to Cholesterol (Figure 8.1)

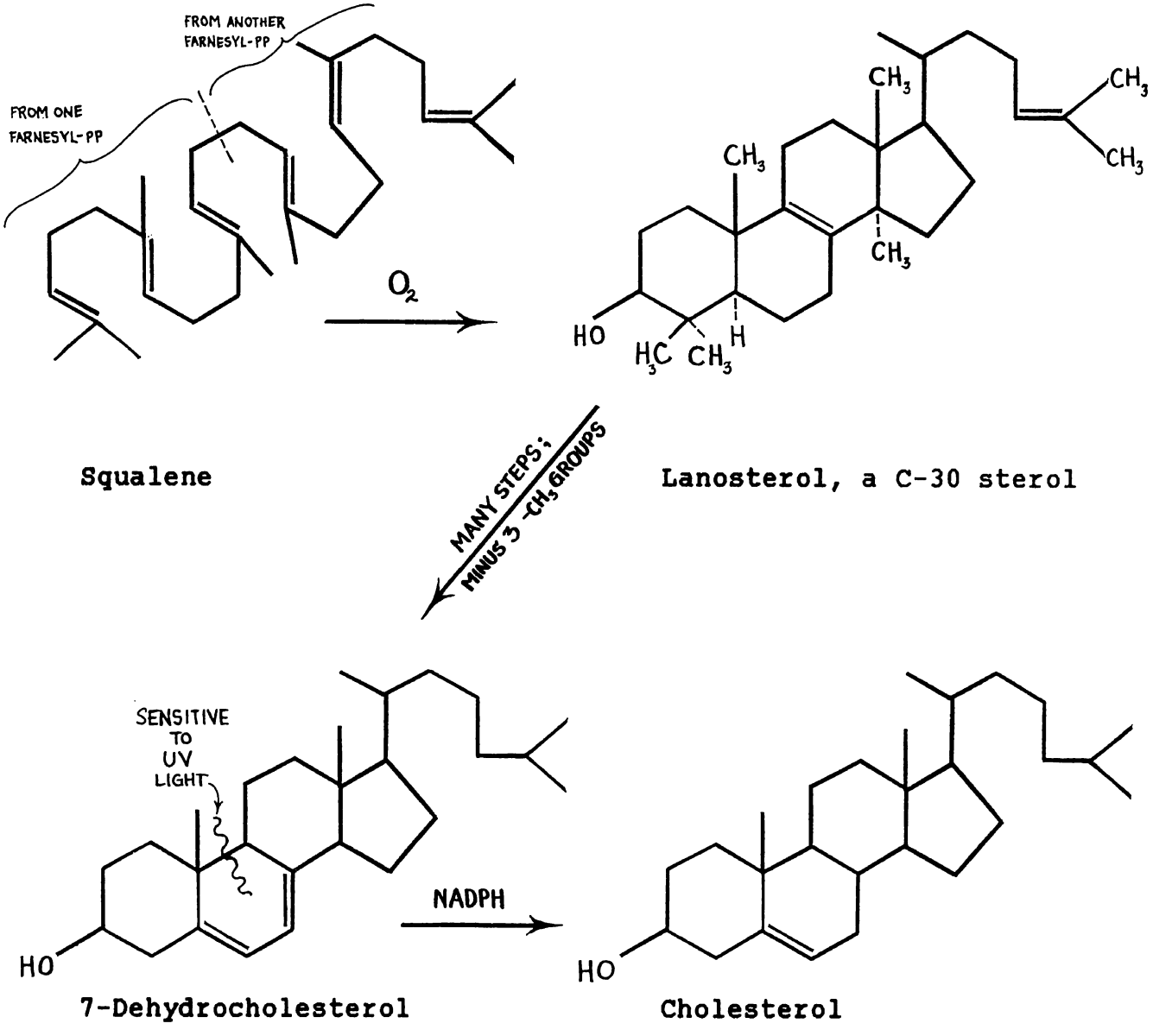


Figure 8.1. Conversion of Squalene to Cholesterol

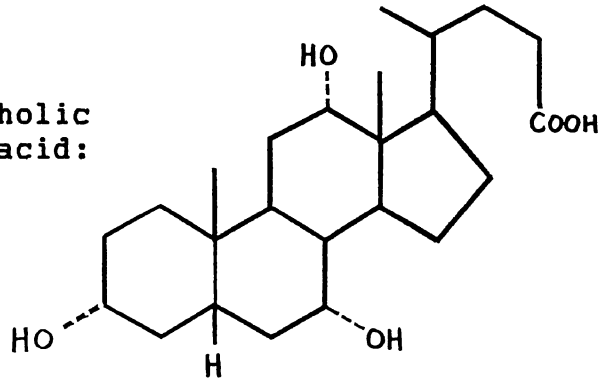
Ring B of 7-dehydrocholesterol can be split by UV light (at arrow, Figure 8.1) to form vitamin D_3. The presence of 7-dehydrocholesterol in human skin explains how sunlight leads to the formation of vitamin D. In the body, vitamin D (cholecalciferol) is transported to the liver and hydroxylated at C-25, then to the kidney and hydroxylated at 1α, to form **1α,25-dihydroxycholecalciferol**, or **calcitriol**. This is the biologically active form. Vitamin D can be regarded as a hormone because it acts on target cells by the same mechanism as the steroid hormones. The active form promotes calcium absorption in the intestine by stimulating the synthesis of a **calcium binding protein**.

IV. METABOLISM OF CHOLESTEROL

A. Secretion: Some is simply **secreted as is** into intestine.

B. Conversion to Bile Acids: This is the major route for the excretion of cholesterol in humans.

Cholic acid:



Cholanoic (C-24) acid derivatives:
Cholic acid: 3α,7α,12α-triol
Deoxycholic: 3α,12α-diol
Chenodeoxycholic: 3α,7α-diol

These are formed from cholesterol by the liver, and in human bile occur as conjugates of **glycine** or **taurine** (called bile salts) and function as detergents. Together with phospholipid, they aggregate as **micelles** and promote the emulsification, lipolysis, and absorption of fats, including the fat-soluble vitamins.

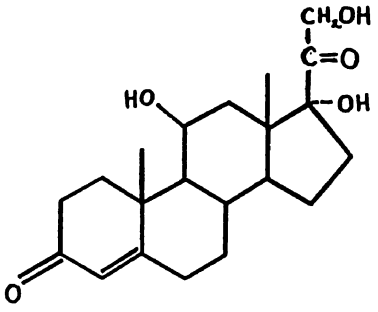
Bile salts are extensively reabsorbed (>95%) in the ileum, and *via* the portal system, undergo **enterohepatic circulation** several times per day. Bacterial action in the gut may result in some structural changes. Insufficient bile salt secretion can be a cause of gallstone formation. Further disturbance of BS metabolism can lead to malabsorption syndromes and, in extreme cases, deficiency of fat-soluble vitamins. Chenodeoxycholic acid has been useful as replacement therapy to supplement the bile acid pool to the point where cholesterol gallstones may redissolve.

C. Steroid Hormones: Quantitatively minor, but of major importance physiologically. The examples shown below are the principal members of each type secreted in the human.

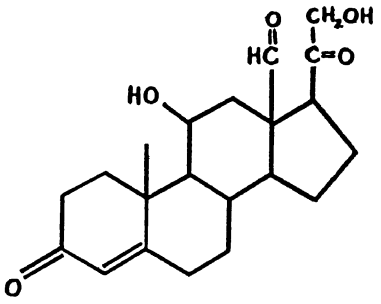
1. Major types

   a. adrenal steroids (corticosteroids)

      i. **glucocorticoids** ii. **mineralocorticoids**



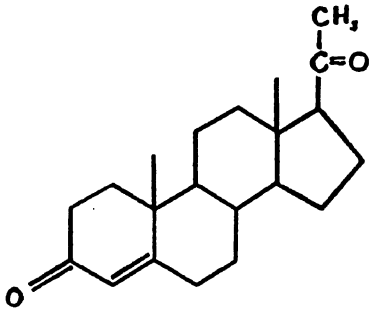
Cortisol



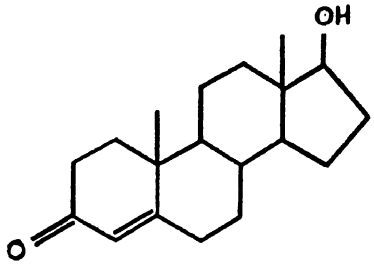
Aldosterone

   b. Gonadal steroids

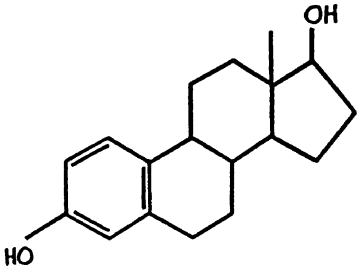
      i. **progestational** ii. **androgens** iii. **estrogens**



progesterone



testosterone



estradiol

2. Formation from Cholesterol

a. All steroid-producing tissues cleave the side-chain of cholesterol between carbons 20 and 22 to form **pregnenolone**. This is the rate-limiting step. In most cases, pregnenolone is then converted to **progesterone**.

b. Adrenal cortex: hydroxylations occur in sequence at positions **17, 21, 11, 18**. Except: the *zona fasciculata*, which makes cortisol, has no 18-hydroxylase, and the *zona glomerulosa*, which makes aldosterone, has no 17-hydroxylase. Some conversion to androgens (especially **dehydroepiandrosterone**, or **DHEA**) and estrogens may also occur. In a patient with Cushing's syndrome, these androgens may cause signs of virilization such as hirsutism.

c. Gonads: The *corpus luteum* stops at progesterone. The testis, after 17-hydroxylation, cleaves the remaining side-chain to form **19-carbon** steroids (androstenedione, then testosterone). The ovary also does this, in fact makes testosterone, but then aromatizes ring A (resulting in the loss of carbon 19) to form **18-carbon** steroids (estradiol, estrone).

3. Metabolism: reductions occur involving ring A, and the products are excreted in bile and/or urine as conjugates of **sulfate** or **glucuronic acid**. Androgens are oxidized at C-17 to ketones of the class known as **17-ketosteroids**. Urinary 17-KS are indicative of androgen metabolism of both testicular and adrenal origin.

V. ACTION OF STEROID HORMONES

A. Mechanism

In contrast to most peptide hormones, which interact with a receptor on the plasma membrane without penetrating the cell, steroid hormones **enter target cells** and are bound by a **receptor** in the **cytoplasm**. The hormone-receptor complex then **diffuses to the nucleus** and binds to chromatin of selected genes, inducing the **synthesis** (or repression) of particular **proteins**.

B. Function

1. Adrenal hormones

a. **Glucocorticoids** promote gluconeogenesis by inducing key enzymes such as pyruvate carboxylase. Proteins, especially those of muscle, are broken down to amino acids, which the liver converts into glucose which is partly released to the circulation and partly stored as liver glycogen.

Cortisol (also known as hydrocortisone) and synthetic steroids such as prednisone and prednisolone also have anti-inflammatory and anti-immune effects. When used in high doses over a long time they cause, besides muscle wasting, lipolysis on the extremities but accumulation of fat on the face and trunk ("Cushingoid" features).

b. **Mineralocorticoids** promote retention of Na^+, along with H_2O, by the kidney, and excretion of H^+ and K^+. A high level of aldosterone causes hypertension; deficiency, excessive loss of salt.

2. Gonadal Hormones

a. **testosterone**: promotes development of male secondary sex characteristics, and also has anabolic activities. With FSH, it promotes spermatogenesis by seminiferous tubules.

b. **estradiol**: promotes development of female secondary sex characteristics, proliferative phase of endometrium, and, with FSH, development of ovarian follicle and finally, ovulation.

c. **progesterone**: supports secretory phase of endometrium, luteal phase of ovary, and inhibits further ovulation.

C. Regulation

1. Adrenal hormones

a. The hypothalamus puts out **Corticotropin Releasing Hormone (CRH)** which stimulates the pituitary to release **Adrenocorticotrophic Hormone (ACTH)** , which then signals the adrenal cortex to secrete

cortisol. Negative feedback by cortisol occurs on both pituitary and hypothalamus. In extreme cases, such as when steroid drugs are used in high doses for a long time, adrenal **atrophy** may occur due to prolonged inhibition of ACTH production. Conversely, lack of cortisol production, as in an enzymatic defect in the adrenal, may lead to adrenal **hyperplasia** due to excessive ACTH production in an attempt by the pituitary to compensate for the lack of steroid hormone.

b. Production of **aldosterone** is regulated largely by the renin-angiotensin system. In response to a perceived drop in perfusion pressure, the kidney produces **renin**, which converts **angiotensinogen**, a peptide made by the liver, to angiotensin I. Then this is converted by an enzyme in lung tissue to **angiotensin II**, which induces production of aldosterone in the *zona glomerulosa* of the adrenal. Potassium also induces formation of aldosterone. Thus an excess of renin or aldosterone causes hypertension; deficiency results in salt loss.

2. Gonadal hormones: Trophic hormones are the same in both sexes. The hypothalamic **Gonadotropin Releasing Hormone (GnRH)**, which is the same as **Luteinizing Hormone Releasing Hormone (LHRH)**, stimulates the pituitary to release both **Follicle Stimulating Hormone (FSH)**, (formerly known as ICSH) and **Luteinizing Hormone (LH)**.

In the male, FSH induces spermatogenesis in seminiferous tubules; feedback is by a glycoprotein, **inhibin**. LH stimulates production of **testosterone** by Leydig cells. Feedback is on both pituitary and hypothalamus.

In the female, FSH induces follicular development and production of **estradiol**. At mid-cycle, there is a positive feedback effect by estrogen, causing a surge of both FSH and LH production by the pituitary. Luteinization ensues, with production of **progesterone** by the *corpus luteum*.

VI. CHOLESTEROL LEVELS

Since a high level of cholesterol in blood, especially that contained in LDL, is a risk factor in atherosclerotic heart disease, much attention is being given to factors that lower cholesterol levels.

A. Cholesterol-Lowering Agents

1. **Diet**: Cholesterol itself can be avoided by substituting vegetable products for meat and dairy products. In addition, consuming more polyunsaturated fats and fewer saturated fats has a cholesterol-lowering effect.

2. **Exercise**: This activity seems to increase the HDL/LDL ratio, which correlates negatively with atherosclerosis.

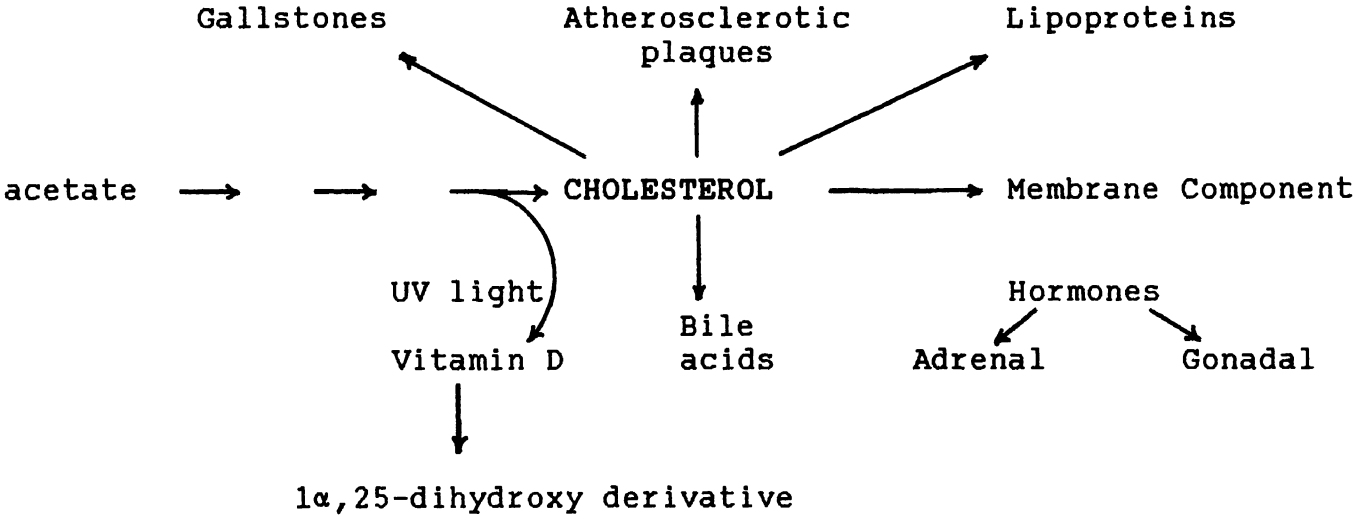
3. **Drugs**: Some that have been helpful are: **clofibrate** and **nicotinic acid**, which lower cholesterol levels by poorly-understood mechanisms; **compactin**, which inhibits HMG CoA reductase, the controlling step in cholesterol synthesis; and **cholestyramine**, a resin which, taken orally, binds bile acids and promotes their excretion rather than en-

terohepatic circulation, causing the liver to replace them by new synthesis from cholesterol, some of which comes from the blood.

B. Familial Hypercholesterolemia

This condition results from a heritable lack of functional receptors for LDL. Since endocytosis of LDL cannot occur, intracellular HMG CoA reductase becomes more active, while at the same time blood levels of LDL reach very high levels (See Chapter 11). Diet has little effect on the homozygous condition; drugs can help somewhat.

VII. CHOLESTEROL IN PERSPECTIVE



VIII. REVIEW QUESTIONS ON STEROIDS

DIRECTIONS: Each of the questions or incomplete statements below is followed by five suggested answers or completions. Select the one that is BEST in each case and fill in the corresponding space on the answer sheet.

1. In man,

A. all plasma cholesterol is carried as cholesterol esters.
B. cholesterol is made only by liver cells.
C. cholesterol in brain exchanges readily with cholesterol in plasma.
D. cholesterol is entirely metabolized to CO_2 and H_2O.
E. conversion of cholesterol to bile acids involves shortening the side chain.

2. Calcitriol is the most active form of

A. vitamin K
B. vitamin E
C. vitamin D
D. vitamin B_{12}
E. vitamin A

3. Estriol is excreted in the urine as the conjugate with

A. glucuronic acid
B. cysteine
C. glycine
D. glutamine
E. protein

4. Which of the following stimulates the synthesis of calcium binding protein in intestinal mucosa?

A. calcium
B. 1,25-dihydroxycholecalciferol
C. alpha-tocopherol
D. calcitonin
E. parathyroid hormone

5. In mammals, which of the following can NOT take place?

A. estrone $\longrightarrow$ estradiol
B. estrone $\longrightarrow$ dihydrotestosterone
C. 17-hydroxypregnenolone $\longrightarrow$ testosterone
D. progesterone $\longrightarrow$ estrogen
E. cholesterol $\longrightarrow$ estrogen

6. Serum cholesterol levels may be significantly changed by a diet with a low ratio of:

A. diglycerides to monoglycerides
B. saturated to polyunsaturated fatty acids
C. lactose to glucose
D. vitamin K to vitamin D
E. cephalin to lecithin

7. All of the following depend on micellar activity for their absorption EXCEPT

A. glycine
B. vitamin E
C. cholesterol
D. vitamin A
E. stearic acid

8. Some virilization may be seen in a patient with Cushing's syndrome, but not in a patient on high doses of prednisone because

A. prednisone has weak glucocorticoid activity and prolonged use does not cause adrenal atrophy
B. prednisone has weak mineralocorticoid activity and is a strong glucocorticoid
C. prednisone cannot produce a Cushingoid toxicity syndrome
D. virilization in the patient with Cushing's syndrome is caused by adrenal androgens
E. Cushing's syndrome is an autosomal dominant trait

DIRECTIONS: For each of the questions or incomplete statements below, ONE or MORE of the answers or completions is correct. On the answer sheet fill in space

A if only 1, 2, and 3 are correct
B if only 1 and 3 are correct
C if only 2 and 4 are correct
D if only 4 is correct
E if all are correct

FILL IN ONLY ONE SPACE ON YOUR ANSWER SHEET FOR EACH QUESTION

Directions Summarized				
(A) 1,2,3 only	(B) 1,3 only	(C) 2,4 only	(D) 4 only	(E) All are correct

9. Biosynthesis of cholesterol involves:

1. lanosterol
2. squalene
3. dimethylallyl pyrophosphate
4. succinyl CoA

10. In fasting there is lowered activity of liver HMG CoA reductase and squalene oxidocyclase, and therefore less synthesis of

1. HMG CoA
2. ubiquinone
3. acetoacetate
4. cholesterol

11. Cholesterol can be converted to

1. androgens
2. corticosteroids
3. bile acids
4. polyunsaturated fatty acids

12. In man, cholesterol

1. is converted to glycocholic acid by intestinal bacteria.
2. is a part of cell membranes
3. can be catabolized mainly to acetyl CoA.
4. is a precursor of cortisol.

13. Which of the following promotes mineralization of bone?

1. decreased levels of ascorbic acid
2. increased levels of plasma calcium
3. parathyroid hormone
4. 1,25-dihydroxycholecalciferol

14. Vitamin D

1. is not itself active in stimulating calcium transport by intestine and calcium mobilization from bone *in vitro*.
2. is produced by ultraviolet light acting on delta-5,7 sterols.
3. is converted to the 1,25-dihydroxy-derivative which stimulates the intestinal mucosa to transport calcium.
4. induces the kidney to excrete phosphate.

15. High doses of hydrocortisone over 2 months would probably cause

1. a rise in liver glycogen
2. larger skeletal muscles
3. atrophy of the adrenal cortex
4. a rise in blood ACTH

16. Agents that lower blood cholesterol include

1. cholestyramine
2. compactin
3. clofibrate
4. nicotinic acid

17. The mechanism of action of progesterone involves

1. interaction with a membrane-bound receptor
2. penetration of the hormone into the cell
3. activation of adenylate cyclase
4. synthesis of new protein

FILL IN ONLY ONE SPACE ON YOUR ANSWER SHEET FOR EACH QUESTION

Directions Summarized				
(A) 1,2,3 only	(B) 1,3 only	(C) 2,4 only	(D) 4 only	(E) All are correct

18. The biosynthesis of testosterone involves

1. isopentenyl pyrophosphate
2. pregnenolone
3. lanosterol
4. cortisol

19. A lack of receptors for LDL will probably induce

1. a low level of blood LDL
2. high activity of HMG CoA reductase
3. decreased plasma cholesterol
4. decreased endocytosis of LDL

20. The surge of gonadotropins around the time of ovulation

1. is caused by the effect of GnRH on the ovary
2. is caused by a positive feedback of estradiol secreted during the follicular phase
3. starts the development of follicles that release estradiol during the luteal phase
4. starts the development of the *corpus luteum* that releases progesterone during the luteal phase

IX. ANSWERS TO QUESTIONS ON STEROIDS

1. E	11. A
2. C	12. C
3. A	13. C
4. B	14. A
5. B	15. B
6. B	16. E
7. A	17. C
8. D	18. A
9. A	19. C
10. D	20. C