PART

V

Nutrition Biochemistry

Fruits and vegetables are nutritious. Besides containing carbohydrates proteins, lipids, minerals and some fibres, they also possess appreciable amounts of vitamins which are *sine quo non* for life.

"What drives life... is a little electric current, kept up by sunshine."
— *Albert Szent-Györgyi : Chemistry of Muscular Contraction, 1960.*

Pictured above is a 2 1/2-year-old boy with severe rickets, which is due to the deficiency of vitamin D in the body. The disease was once common in cold climates where heavy clothing blocks the ultraviolet (UV) component of sunlight necessary for the production of vitamin D_3 in skin. Cholecalciferol, (vitamin D_3) is produced in the skin by UV irradiation of 7-dehydrocholesterol, the provitamin form. In the liver, a hydroxyl group is added at C-25; in the kidney, a second hydroxylation at C-1 produces the active hormone, 1,25-dihydroxycholecalciferol. This hormone regulates the metabolism of Ca^{2+} in kidney, intestine, and bone. Dietary vitamin D prevents rickets.

C H A P T E R

33

Fat-Soluble Vitamins

HISTORICAL RESUME

Vitamins have a back-dated history. The dreaded disease **scurvy** was one of the prevalent diseases in Europe during 15th and 16th centuries. The disease is said to have afflicted the crusadors. Scurvy was reported by Vasco de Gama during his sea voyages and Jacques Cartier in 1535 had reported death of about 25% of his sailing crew due to scurvy. On the recommendation of Sir James Lancaster, an English privateer, the ships of East India Company in 1601 carried oranges and lemons to prevent scurvy. In 1757, James Lind, a British naval surgeon, stated that fresh fruits and vegetables alone are effective to protect the body from various maladies and urged the inclusion of lemon juice in the diet of sailors to prevent scurvy. And some 40 years later, the Admiralty took his advice. After limes were substituted for lemons in 1865, British sailors began to be known as "limeys." Similarly, **rickets** was also attributed to faulty diets and Guérin (1838) produced it experimentally in puppies to prove the dietary connection.

In 1887, Admiral Takaki, Director-General of the Medical Services in Japan, demonstrated that another scourge **beriberi** could be prevented by enriching the diet with meat, vegetables and milk and at the same time decreasing the amount of milled rice in the case of Japanese

CHRISTIAAN EIJKMAN (LT, 1858-1930)

Eijkman, a Dutch physician, was a member of a medical team that was sent to the East Indies to study beriberi in 1886. At that time, all diseases were thought to be caused by microorganisms. When he microoganism that caused beriberi could not be found, the team left the East Indies. Eijkman stayed behind to become the director of a new bacteriological laboratory. In 1896, he accidentally discovered the cause of beriberi when he noticed that chickens being used in the laboratory had developed symptoms characteristic of the disease. He found that the symptoms had developed when a cook had started feeding the chickens rice meant for hospital patients. The symptoms disappeared when a new cook resumed feeding them chicken feed. Later, it was recognized that thiamine (vitamin B_1) is present in rice hulls but not in polished rice. For this work, Eijkman shared the **1929 Nobel Prize in Medicine or Physiology,** with Frederick G. Hopkins.
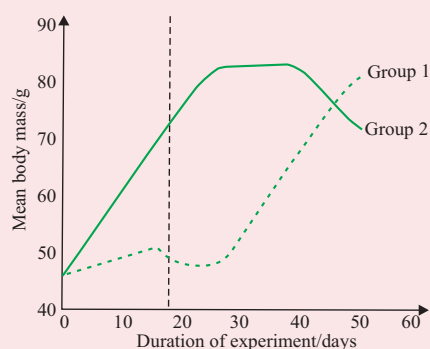
sailors. Later, Eijkman (1897), a Dutch physician, found that experimental beriberi could be induced in hens when fed with polished rice without bran. Such hens could be cured by giving them the rice polishings. Eijkmann, for a time, believed that the rice polishings contained something that neutralized the beriberi toxin in the polished rice. In 1906, however, Frederick Gowland Hopkins ascribed the diseases such as scurvy and rickets to the lack of some '*dietary factors*'. Six years later (*i.e.*, in 1912), Hopkins in collaboration with Casimer Funk of Poland, who was

FREDERICK GOWLAND HOPKINS (LT, 1861-1947)

Hopkins, a British biochemist, was born in Eastbourne, East Sussex, England on June 20, 1861. He was one of the founders of the science of Biochemistry, which he developed at Cambridge. His greatest work was in the identification and isolation of vitamins. He shared the **1929 Nobel Prize in Medicine or Physiology** for his discovery of essential nutrient factors now known as vitamins–needed in animal diets to maintain health, along with Christiaan Eijkman of The Netherlands for his discovery of vitamin B_1 (thiamine).

Frederick Gowland Hopkins worked out the importance of vitamins in the diet in the early 1900s. His classic experiment, described in the adjoining graph, helped people understand the need for vitamins as additional food factors in a healthy diet.

Basically, he showed that the milk-deficient rats did not grow properly. He suggested that the milk contained an 'accessory food factor', or 'vital amine'– hence the name *vitamin,* whcih the rats need to stay healthy. Later work by American researchers showed this compound to be fat-soluble. We now know this compound to be vitamin A.

Hopkins died on May 16, 1947 at Cambridge.

Between 1906 and 1912, Hopkins and his team at Cambridge University studied accessory food factors. In this experiment two groups of rats are used. Group 1 rats were fed on a diet of purified casein (milk protein), starch, cane sugar, lard and salts. Group 2 rats were fed the same diet but with a milk supplement.

Those on the artificial diet (1) stopped growing and lost body mass; those with the milk supplement (2) grew normally, even though the milk contributed only 4 per cent of the total food eaten.

working at the Lister Institute of London, suggested the **vitamin theory** which postulates that *'specific diseases such as beriberi, scurvy and rickets are each caused by the absence from the diet of a particular nutritional factor.'*

Funk, for the first time, also isolated the dietary factor from rice polishings which acted as antiberiberi substance. Since this factor was an amine and necessary to life, Funk at the suggestion of Dr. Max Nierenstein introduced the term 'vitamine' ($vita^L$ = life) to denote it.

Since subsequent studies showed that not all these substances are amines, the terminal letter 'e' was dropped from its spelling at the suggestion of Sir. J.C. Drummond (1919), who also proposed their **alphabetical nomenclature**. In fact, the various vitamins have no structural resemblance to each other, but because of a similar general function in metabolism, they are studied together. Although these molecules serve nearly the same roles in all forms of life, but higher animals have lost the capacity to synthesize them.

DEFINITION

The vitamin concept has undergone extensive revisions during the history of biochemistry. However, Franz Holfmeister's (LT, 1850-1922) definition— 'vitamins are substances which are indispensable for the growth and maintenance of the animal organism, which occur both in animals and plants and are present in only small amounts in food'— still holds good, although it has been interpreted in various ways.

Table 33–1. **Differences among enzymes, hormones and vitamins**

Enzymes	Hormones	Vitamins
1. These are specific catalytic substances.	These are regulatory sub-stances. Hormones are steroids (estradiol, testosterone) or peptides (insulin, ocytocin) or amino acid derivatives (thyroxine, adrenalin).	These are accessory nutrients.
2. All enzymes are proteinaceous in nature.		Vitamins belong to diverse groups chemically.
3. These are synthesized by the animal cells.	These are synthesized within some part of the body.	These are not synthesized by most animal cells but supplied to the body from outside source (mainly as food). Plants can, however, synthesize them.
4. These can initiate and continue reactions.	These cannot initiate reactions but can influence the rate at which they proceed.	—
5. These are not consumed during growth.	They are consumed during growth.	—
6. These are involved in coenzyme syntems.	These are not involved in coenzyme systems.	These are involved in coenzyme systems.
7. These operate in biological systems.	Only some hormones are involved in enzyme systems.	Most vitamins are involved in enzyme systems.
Examples : Pepsin, Trypsin, Urease, Diastase, Lipase, Catalase, Maltase, Alcohol dehydrogenase, Cytochrome oxidase.	*Examples:* Estradiol, Testosterone, Relaxin, Progesterone, Insulin, Thyrotropin, Ocyto- cin, Secretin, Intermedin, Thyroxine, Adrenalin.	*Examples :* Fat-soluble vitamins A, D, E, K ; Water-soluble vitamins B, C.

The term '**vitamin**', in its modern sense, usually refers to *the substances distinct from major components of food, required in minute quantities (i.e., oligodynamic in nature) and whose absence causes specific deficiency diseases*. As the living organisms cannot synthesize most of these compounds, a steady supply of them is *sine quo non* for life. Their ultimate source is the plant or bacterial world.

> **Sine quo non** is a Latin phrase, which means an indispensable condition.

Differences with hormones— Indeed, for some time, it was believed that the distinction between vitamins and hormones was no longer tenable. But there exists a fundamental difference between these two classes of active substances : *the hormones are regulatory substances whereas the vitamins are accessory nutrients*. The vitamins also differ from the hormones in that they are supplied to the body from some external source (*i.e.,* chiefly from the food ingested) whereas the hormones are synthesized within some part of the body of an organism. Moreover, most vitamins and some hormones are involved either directly or indirectly in enzyme systems in order to carry out biochemical functions. Some vitamins are also known to be coenzymes.

A comparison of enzymes, hormones and vitamins is presented in Table 33–1.

GENERAL CHARACTERISTICS

The vitamins are characterized for some general facts, which are listed below :
1. Vitamins are of widespread occurrence in nature, both in plant and animal worlds.
2. All common foodstuffs contain more than one vitamin.
3. The plants can synthesize all the vitamins whereas only a few vitamins are synthesized in the animals.
4. Human body can synthesize some vitamins, *e.g.*, vitamin A is synthesized from its precursor carotene and vitamin D from ultraviolet irradiation of ergosterol and 7-dehydrocholesterol. Some members of the vitamin B complex are synthesized by microorganisms present in the intestinal tract. Vitamin C is also synthesized in some animals such as rat.
5. Most of vitamins have been artificially synthesized.
6. All the cells of the body store vitamins to some extent.
7. Vitamins are partly destroyed and are partly excreted.
8. Vitamins are nonantigenic.
9. Vitamins carry out functions in very low concentrations. Hence, the total daily requirement is very small.
10. Vitamins are effective when taken orally.
11. Synthetically-made vitamins are just as nutritionally good as natural vitamins.
12. Old people need about the same amounts of vitamins as young people.

CLASSIFICATION

In 1913, McCollum and Davis described a lipid-soluble essential food factor in butter fat and egg yolk. In 1915, a water-soluble factor in wheat germ necessary for the growth of young rats was also described. Since then, two categories of vitamins are usually recognized : fat-soluble and water-soluble. These two groups discharge rather different functions.

A. Fat-soluble vitamins. These are oily substances, not readily soluble in water and their biochemical functions are not well understood. They contain only carbon, hydrogen and oxygen. Their examples are vitamins A, D, E and K. They, however, play more specialized roles in certain group of animals and in particular type of activities. For instance, they function in the formation of a visual pigment (vitamin A), in the absorption of calcium and phosphorus from the vertebrate intestine (vitamin D), in protecting mitochondrial system from inactivation (vitamin E) or in the formation of a blood clotting factor in vertebrates (vitamin K). The individual fat-soluble vitamins bear a closer resemblance to each other chemically. In fact, the 4 fat-soluble vitamins can be regarded as lipids. Vitamins A, E and K are terpenoids, and vitamin D is a steroid. All four are

isoprenoid compounds, since they are synthesized biologically from units of isoprene (for structure, refer page), a building block of many naturally-occurring oily, greasy or rubbery substances of plant origin. Unlike the water- soluble vitamins (B and C), fat-soluble vitamins can be strored in the body, *e.g.*, an adult's liver can store enough vitamin A to last several months or longer. However, because *fat-soluble vitamins are storable*, their excessive intakes can result in toxic conditions (hypervitaminoses).

B. Water-soluble vitamins. Most of these are universally vitamins since they perform the same general functions wherever they occur. Besides C, H and O, they also contain nitrogen. They are catalytic factors and as such form vital links in the chains of biochemical reactions characteristic of all living objects. For instance, thiamine is required whenever sugars are oxidized aerobically to release energy. The individual water-soluble vitamins bear no closer resemblance to each other chemically. The biochemical or coenzyme function of nearly all of these is known. The common water-soluble vitamins are vitamins of B complex such as B_1 through B_{12} (vitamins B_4, B_8, B_{10} and B_{11}, however, do not exist) and the vitamin C. Choline, inositol, *p*-aminobenzoic acid, bioflavonoids and α-lipoic acid are frequently included in this category. Many nutritionists, however, do not consider them as true vitamins, although their dietary deficiencies in animals lead to the development of characteristic symptoms. Moreover, none of them except α-lipoic acid is a part of the coenzyme system. The B-series of vitamins, being water-soluble and excretable, are *required daily in meagre amounts* (in milligrams or even less) for the normal growth and good health of humans and many other organisms. It is virtually impossible to 'overdose' on them.

STORAGE OF VITAMINS IN THE BODY

The vitamins can be stored in the body to a slight extent. The liver cells are, however, rich in certain **fat-soluble vitamins**. For instance, the amount of *vitamin A* contained in the liver is sufficient enough to meet its requirement without any additional intake for about 6 months. Similarly, the quantity of *vitamin D* stored ordinarily in the liver is sufficient to maintain a person without any additional intake of vitamin D for about 2 months. The storage of *vitamin K* is, however, relatively slight.

The **water-soluble vitamins** are stored even in lesser amounts in the cells. Evidently, in cases of deficiency of *vitamin B compounds*, clinical symptoms appear rather early, that is within a few days. Similarly, absence of *vitamin C* can induce deficiency symptoms within a few weeks. Vitamin C is stored in the adrenal cortex.

DAILY HUMAN REQUIREMENTS OF VITAMINS

The requirement of vitamins varies considerably depending on the nature of the individual consuming them. Some general facts regarding vitamin requirement may be listed below :

1. Greater the size of the individual, higher are his vitamin needs.
2. Younger ones require higher quantities of vitamins than do the elders.
3. The vitamin requirements increase when a person performs exercise.
4. During ailments, the vitamin requirements are ordinarily enhanced.
5. Under certain specific conditions of metabolic disorders when the vitamins cannot be properly utilized, the requirement for one or more specific vitamins is at extreme.
6. Growing children require comparatively high quantities of vitamin D.
7. During pregnancy and lactation, the vitamin D requirement by the mother is greatly enhanced.
8. The requirements of vitamin B complex (*esp.*, that of vitamin B_1) are increased under conditions of greater utilization of carbohydrates.

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Table 33–2. **Recommended daily dietary allowances***

	Age[†]	Weight	Height	Energy	Protein	Fat-soluble vitamins			Water-soluble vitamins						
						A[‡]	D_3	E	B_1	B_2	B_5	B_6	B_9	B_{12}	C
	years	kg	cm	kcal	g	µg	µg	mg	mg	mg	mg	mg	µg	µg	mg
INFANTS	0.0–0.5	6	60	650	kg × 2.2	420	10	3	0.3	0.4	6	0.3	30	0.5	35
	0.5–1.0	9	71	970	kg × 2.0	400	10	4	0.5	0.6	8	0.6	45	1.5	35
CHILDREN	1–3	13	90	1300	23	400	10	5	0.7	0.8	9	0.9	100	2.0	45
	4–6	20	112	1700	30	500	10	6	0.9	1.0	11	1.3	200	2.5	45
	7–10	28	132	2400	34	700	10	7	1.2	1.4	16	1.6	300	3.0	45
MEN	11–14	45	157	2700	45	1000	10	8	1.4	1.6	18	1.8	400	3.0	45
	15–18	66	176	2800	56	1000	10	10	1.4	1.7	18	2.0	400	3.0	60
	19–22	70	177	2900	56	1000	7.5	10	1.5	1.7	19	2.2	400	3.0	60
	23–50	70	178	2700	56	1000	5	10	1.4	1.6	18	2.2	400	3.0	60
	51+	70	178	2400	56	1000	5	10	1.2	1.4	16	2.2	400	3.0	60
WOMEN	11–14	46	157	2200	46	800	10	8	1.1	1.3	15	1.8	400	3.0	50
	15–18	55	153	2100	46	800	10	8	1.1	1.3	14	2.0	400	3.0	60
	19–22	55	163	2100	44	800	7.5	8	1.1	1.3	14	2.0	400	3.0	60
	23–50	55	163	2000	44	800	5	8	1.0	1.2	13	2.0	400	3.0	60
	51+	55	163	1800	44	800	5	8	1.0	1.2	13	2.0	400	3.0	60
Pregnant				+300	+30	+200	+5	+2	+0.4	+0.3	+2	+0.6	+400	+1.0	+20
Lactating				+500	+20	+400	+5	+3	+0.5	+0.5	+5	+0.5	+100	+1.0	+40

* The recommended allowances can be attained with a variety of common foods, including those nutrients also for which human requirements have been less well defined.

† All the entries for the age range represent allowances for the midpoint of the specified age range. However, in the case of age range 23–50 years, the reference is to the man and woman at age 25 years.

‡ Retinol equivalents : 1 retinol equivalent = 1 µg retinol or 6 µg β-carotene.

Note that there is no recommended dietary allowances (RDAs) for vitamin K, vitamin B_3 (pantothenic acid), and vitamin B_7 (biotin) as intestinal bacteria synthesize the body's requirement.

(Adapted from Food and Nutrition Board, National Academy of Sciences— National Research Council, Revised 1980)

The daily requirement of any vitamin (refer Table 33–2) for any individual is not a fixed quantity and varies according to the rate of metabolism. *In general, in all cases of high metabolic activity (such as heavy muscular work, during pregnancy and lactation and in growing children), the vitamin requirement is proportionately high.* Normally, a man doing ordinary work can obtain enough vitamin from his balanced diet.

The intestinal organisms may synthesize vitamins in significant amounts and play a vital role in regulating the quantity of vitamin available to the organism. Most of the vitamins of B complex group (such as thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, folic acid) and vitamain K are some of the vitamins synthesized by the intestinal organisms. These may be absorbed to varying extents and utilized. This fact renders rather 'inaccurate' the figures for daily requirement of the various vitamins. Certain organisms, however, destroy vitamins. Supplementing the diet with certain antibiotics and sulfa drugs enhances the growth of these organisms.

In measuring human requirements of vitamins, certain units have been used. In the beginning, these were arbitrarily fixed and were mainly based on the amount necessary to check avitaminosis in animals under standard conditions. With the passage of time, the various vitamins were synthesized and so it became possible to base the unit on the weight of purified preparations.

AVITAMINOSES
(Deficiencies of Vitamins)

A lack of one or more vitamins leads to characteristic deficiency symptoms in man. Multiple deficiencies caused by the lack of more than one vitamin are, however, more common in human beings. Vitamin deficiencies occur rather frequently in certain parts of the world for socioeconomic reasons. Avitaminosis may be of following 2 types :

   A. *primary or direct*— This arises due to inadequate intake of vitamins resulting from chronic alcoholism, dietary fads etc.

   B. *secondary* or *'conditioned deficiency'*— This arises due to other factors such as malabsorption, increased excretion, allergies, anorexia, gastrointestinal disorders etc.

Vitamin deficiency, whether primary or secondary, leads to:

(*a*) a gradual decrease in tissue levels of the vitamin(s) deficient,

(*b*) a biochemical lesion,

(*c*) an anatomic lesion, and

(*d*) finally cellular pathology and disease.

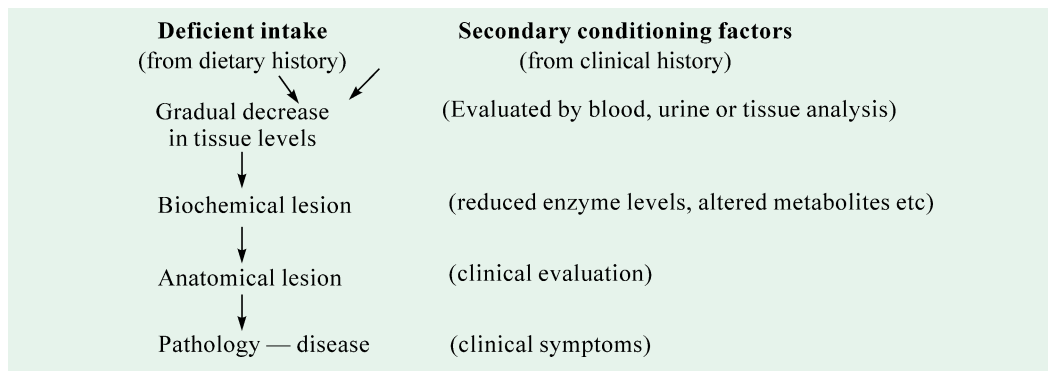This sequence is schematically represented in Fig. 33–1.

Deficient intake
(from dietary history)

Secondary conditioning factors
(from clinical history)

Gradual decrease
in tissue levels

(Evaluated by blood, urine or tissue analysis)

Biochemical lesion

(reduced enzyme levels, altered metabolites etc)

Anatomical lesion

(clinical evaluation)

Pathology — disease

(clinical symptoms)

Fig. 33–1. Sequence of events occurring in a tyical avitaminosis

An account of **fat-soluble vitamins** is given below.

VITAMIN A

A. History. It was first recognized as an essential nutritional factor by Elmer McCollum in 1915 and then isolated from fish-liver oil by Holmes in 1917. On account of its established role in the visual process, it is often called as **antixerophthalmic factor** or the **"bright eyes" vitamin**. It was first synthesized in 1946 by Milas.

B. Occurrence. *Liver oils of various fishes are the richest natural sources of vitamin A.* Shark and halibut contain maximum amount whereas the cod-liver has lowest amount. Depending upon the species of fish and the time of year of catch, the fish livers contain 2,000 to 100,000 I.U. per gram of vitamin A. The amount present in human liver is much less (500 to 1,000 I.U.per gram). However, polar bear liver is an extremely concentrated source of vitamin A. Other noteworthy sources are butter, milk and eggs and, to a lesser extent, kidney. In its provitamin form (*i.e.*, as carotenes) it is supplied by all pigmented (particularly yellow) vegetables and fruits such as carrots, pumpkins, cantaloupes, turnips, peppers, peas, sweet potatoes, papayas, tomatoes, apricots, peaches, plums, cherries, mangoes etc. Yellow corn is the only cereal containing significant amounts of carotene. New-born infants have low quantities of vitamin A content that is rapidly augmented because colostrum and breast milk furnish large amounts of the vitamin. The milk of well-fed mothers, however, contains sufficient amounts of vitamin A (in ester form) for the infant's need. However, vitamin A is absent from vegetable fats and oils (olive oil, linseed oil, groundnut oil). It is added to margarine during its manufacture from these oils.

The β-carotene content of some items are presented in Table 33–3.

Table 32–3. β-**carotene content of some fruits and vegetables**

Fruit/vegetable	β–carotene (μg/100 g)
Mango (ripe)	300
Orange	210
Tomato	190
Drumstick leaves	7,000 – 8000
Coriander leaves	7,000
Carrot	1,500 – 3,000
Radish leaves	3,600
Mint leaves	1,800
Cabbage	1,300

Table 33–1. presents vitamin A content of some animals including those of polar regions and fishes.

Table 33-1. **Vitamin A content of some animal foods**

Source	Biological Name	Vitamin A (I.U./g of food)
Weddel seal	*Leptonychotes weddelli*	444
Ox liver*		550
Cod liver*		600
Southern elephant seal	*Mirounga leonina*	1,160
Antarctic husky	*Canis familiaris*	10,570
Halibut liver oil*		30,000
Arctic bearded seal	*Erignathus barbatus*	12,000 – 14,000
Polar bear	*Thalarctos maritimus*	24,000 – 35,000

*   Commonly recommended as source of vitamin A

Vitamin A originates in marine algae, and then passes up the food chain to reach the large carnivorous animals. Toxic levels of vitamin A may accumulate in the livers of a wide range of creatures such as Polar bears, seals, porpoises, dolphins, sharks, whales, Arctic foxes and huskies. Even a small meal of southern Australian seal liver, say 80g, may produce illness in man. Most of the foods recommended as source of vitamin A contain well below the toxic levels of vitamin A, but one– Halibut liver oil– contains dangerously high amounts of vitamin A.

As regards the vitamin A contents of **polar animals**, one can see that, in reality, very little quantities of livers of these animals are required to kill a human being : 30–90 g of polar bear liver or halibut liver, 80–240 g of bearded seal liver and 100-300 g of Antarctic husky liver is enough to kill a human being.

C. Structure. Vitamin A is found in two forms A_1 and A_2. The carotenoids that give rise to vitamin A in animal body is named as provitamin A. These inculde α-, β- and γ-carotenes and cryptoxanthin. β-*carotene is most potent of all these forms*. A molecule of β-carotene is made of eight 5-carbon isoprenoid units, linked to form a long chain of 40 carbon atoms with an ionone ring at each end. It is an orange-red hydrocarbon and upon hydrolysis yields 2 moles of vitamin A_1 (Fig. 33–2). During hydrolysis, a cleavage occurs at the mid point of the carotene in the polyene chain connecting the 2

> **α- and γ-carotenes** as well as cryptoxanthin, however, yield only 1 molecule of vitamin A since in them one of the two rings present in their molecule differs in structure from that of vitamin A.
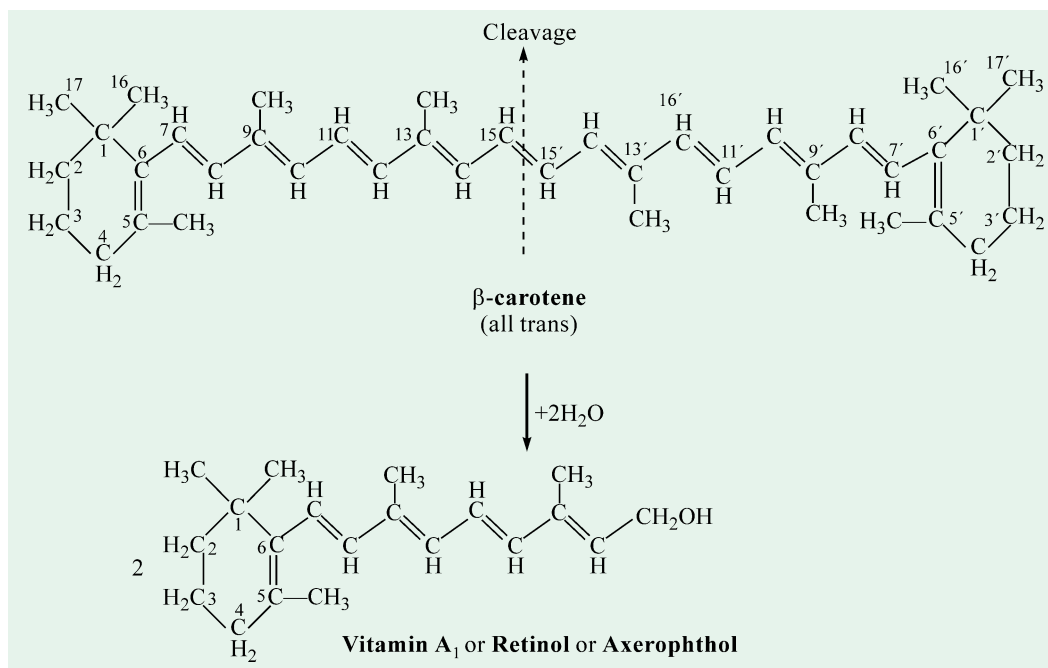
β-ionone rings. This conversion usually takes place in livers of fishes and mammals. Vitamin A_1 is a complex primary alcohol called retinol, having the empirical formula, $C_{20}H_{29}OH$. The terminal hydroxyl group is ordinarily esterified. It contains β-ionone ring.

Fig. 33–2. Hydrolysis of β-carotene

Another form of vitamin A, present in fresh-water fishes, is known as vitamin A_2 (Fig. 33–3). It differs from vitamin A_1, which is found in salt-water fishes, in possessing an additional conjugate double bond between carbon atoms 3 and 4 of the β-ionone ring. Its potency is 40% that of vitamin A_1.
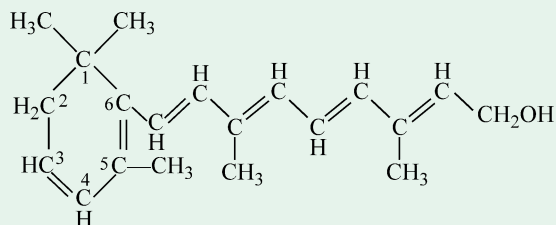
Fig. 33–3. Vitamin A₂ or 3-dehydroretinol

(all *trans*)

D. Properties. Ordinarily retinol is a viscid, colourless oil but by careful fractionation it has also been isolated as pale yellowish needles. It gives a characteristic absorption band in ultraviolet (UV) spectrum at 328 *m*µ. It is soluble in fat and fat solvents but insoluble in water. Loss of vitamin A in cooking, canning and freezing of foodstuffs is small; oxidizing agents, however, destroy it. It is destroyed on exposure to UV light. Vitamin A is relatively unstable in air unless protected by antioxidants including vitamin E.

E. Metabolism. In the tissues, the metabolic transformation of retinol is carried out by enzymes. The dietary β-carotene is split into retinal (Fig. 33–4) by an enzyme of the intestine. Retinal is
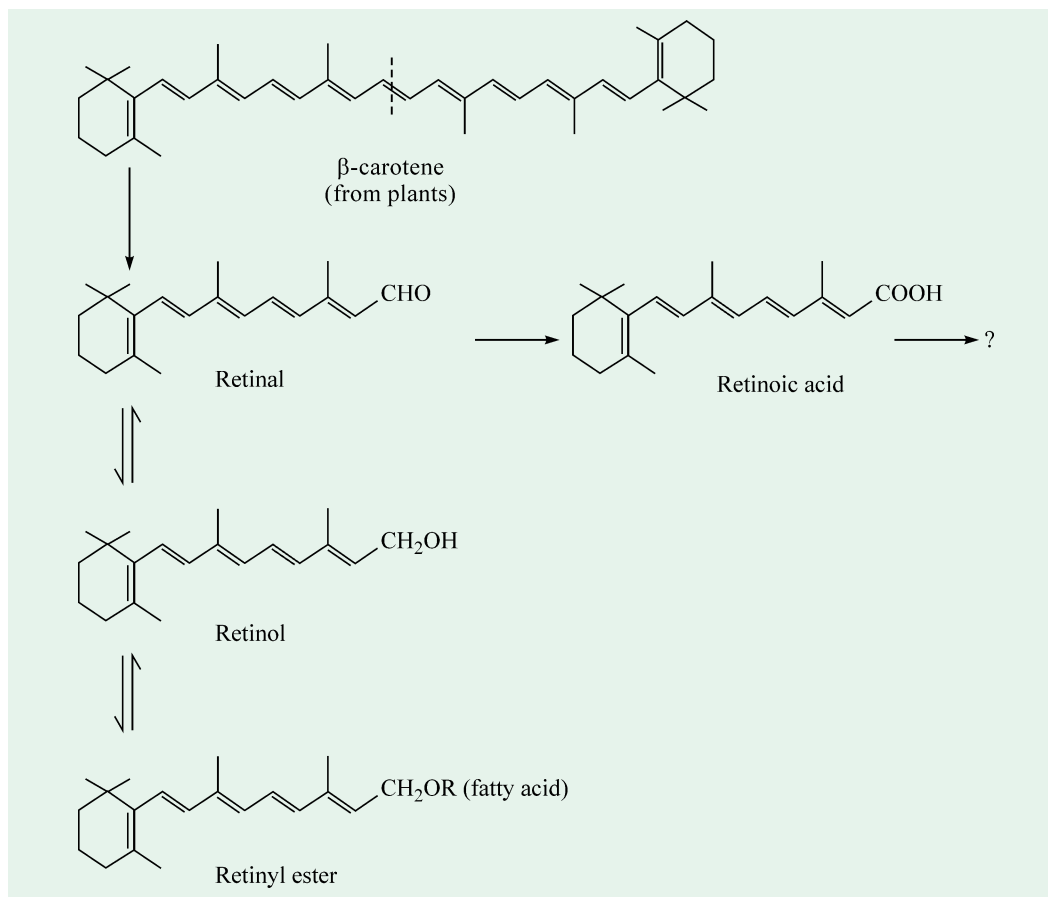

Fig. 33–4. Formation of retinoic acid

(Adapted from Ganguli J, 1973)

then reduced by another enzyme to retinol which, in turn, is converted to retinyl ester by reacting with a fatty acid like palmitic. The retinyl ester, on the contrary, is enzymatically hydrolyzed to produce retinol which is re-esterified with palmitic acid to produce retinyl ester. This is absorbed through the lymphatic system and is stored in the liver.

Liver stores vitamin A in large quantities mainly in the form of retinyl ester. But the vitamin A that circulates in the blood is in the form of retinol and is bound to a specific carrier protein called retinol binding protein (RBP). The retinyl ester present in the liver, therefore, has to be converted to retinol before it mixes with the blood. Liver can also successively convert retinol to retinal and retinal to retinoic acid. Retinoic acid is quickly absorbed from the intestine through the portal system and is rapidly excreted back into the intestine through the bile.

Vitamin A helps maintain the epithelial cells of the skin and the linings of the digestive, respiratory and genito-urinary systems. These linings play a protective role against cancer-causing agents (or carcinogens), viruses and bacteria and are rendered vulnerable by a deficiency of vitamin A. Vitamin A guards against cancer by protecting cell walls from undesirable oxidation, and scavenging the products of oxidation— free radicals, which are linked to the development of cancer.

F. Deficiency. *Vitamin A is perhaps the most important as it affects the various metabolic processes in the body. It has profound effect on epithelial structures, in general.* Vitamin A deficiency leads to the onset of many diseases like nyctalopia or night blindness (inability to see in night), xerophthalmia (scaly condition of the delicate membrane covering the eyes), keratomalacia (softening of the cornea), phrynoderma or "toad skin" (hard and horny skin) and stunted growth.

Xerophthalmia or **xerosis** is a major cause of blindness in childhood and is still a major health problem in Far East such as Hong Kong, Jakarta, Manila and Dhaka. The disease affects large number of children but few adults. Xerophthalmia is characterized by drying of the eyes and hence so named (*xeros*^{G} = dry ; *ophthalmos*^{G} = eyes). The lacrymal glands become stratified and keratinized and cease to produce tears. This makes the external surface dry and dull. The ulcers develop. The bacteria are not washed away. The eyelids swell and become sticky. This results in frequent exudation of blood, causing severe infection to the eye. If left untreated, blindness results. Incredible, yet true, about 1400 cases of xerophthalmia were reported in 1904 among Japanese children. Besides, during World War I, many xerophthalmic cases were reported in Denmark because of the fact that butterfat was shipped out of that country in huge quantities and people had to live on substitutes with no retinol.

Keratomalacia (*kerato*^{G} = hair ; *malakia*^{G} = softness) is a corneal disease, occurring maximally in pre-school children of 3–4 years. This usually happens suddenly in young children with kwashiorkor *esp*., after an episode of diarrhea or infection. At first the cornea loses its lustre, undergoes a necrosis and develops a few pin-point ulcers, which later coalesce to form a large, white ulcerative area. The perforation of the cornea may follow sometime, causing the release of aqueous humour and prolapse of the iris. Occasionally, instead of extensive ulceration and perforation of the cornea, the whole eyeball may shrink. Keratomalacia is unfortunately still prevalent on a wide scale in many parts of India (South and Eastern) and Indonesia and some other countries of Asia and Africa.

Phrynoderma is a skin lesion and is characterized by follicular hyperkeratosis. In it, the skin *esp*., on the outer aspects of forearms in the regions of the elbows and of the thighs and buttocks becomes rough and spiky. In severe cases, the trunk is also affected.

Also, the damaged epithelial structures in diverse organs such as the eyes, the kidneys or the

respiratory tract often become infected. It is for this reason that vitamin A has been called an '*anti-infection*' *vitamin*. The vaginal epithelium may become cornified, and epithelial metaplasia of the urinary tract may contribute to pyuria and hematuria. Increased intracranial pressure with wide separation of cranial bones at the sutures may occur.

In the alimentary tract, the deficiency of vitamin A causes damage to the intestinal mucosa, resulting in diarrhea.

Vitamin A is an important factor in tooth formation. In its deficiency, there is a defective formation of enamel so that the dentin is exposed. Evidently, sound tooth formation does not occur. Vitamin A deficiency may result in retardation of mental and physical growth and in apathy. Anemia with or without hepatosplenomegally is usually present.

Other deficiency diseases that have been attributed to vitamin A are atrophy of the testes and disturbances of the female genital organs.

According to a report, presented at a meeting of the National Seminar on Corneal Disasters held in New Delhi (1981), India accounts for about one-third of the total blind population of the world, that is about 9 million blind people and that every year about 25,000 children go blind in India due to malnutrition.

Researches conducted by the National Institute of Nutrition (NIN), Hyderabad (1978), show that about 30% of the blind in India lost their sight before they were 21. NIN studies have also revealed that about 10% of the school children belonging to the poorer socioeconomic groups show signs of vitamin A deficiency. It is however, worth-mentioning that the vitamin A deficiency is mainly a result of ignorance rather than the non-availability of food or of the resources to acquire the food as is the case with protein calorie malnutrition. According to NIN researches, if 40 g of green leafy vegetables are included in the existing diet without any other changes, the child will get the necessary vitamin A.

G. Hypervitaminosis A. Vitamin A is less toxic to man and other animals when it is taken in large doses over a long period of time. The children receiving overdosages of 500,000 units of vitamin A per day exhibit tender swellings over the bones, limited motion and definite hyperosteoses.

Human adults consuming 500,000 units or more show nosebleed, weakness, headache, anorexia and nausea. Continuous excessive intake of the vitamin is dangerous because it results in fragile bones and abnormal fetal development. In the case of plant carotenoids, a dietary oversupply (for example, eating too many carrots on a daily basis) results in **carotenosis**, a condition readily diagnosed by yellowing of the skin. These toxic effects develop presumably due to the fact that viatmin A, like vitamin D, is not readily excreted and consequently tissue levels may build up dangerous concentrations.

H. Human requirements. The recommended dietary allowance (RDA) of vitamin A is about 5,000 International Unit (I.U.). Growing children, adults and pregnant and lactating mothers require high doses of up to 8,000 I.U. It is also possible that some individuals require more than the minimal requirement due to either faulty absorption or some other reason. For vitamin A, the World Health Organization (WHO) has chosen as one International Unit the biologic activity of 0.000344 mg (0.344 μg) of synthetic vitamin A-acetate, which is equivalent to 0.30 μg of retinol. In fact, one μg of β-carotene, the provitamin form, gets converted to about 0.167 μg of retinol, the true vitamin form. Another way of putting it is that the retinol equivalent of 1 μg of β-carotene is 0.167. A diet consisting of 1/2 pint of milk, 1 ounce of butter and an adequate amount of

1 pint	=	0.568 litre (British Units)
	=	0.473 litre (U.S. Units)
1 ounce	=	27.09 gm

green vegetables or carrots daily is sufficient enough to meet minimal requirement. The optimal requirement of this vitamin is met with by taking 1 pint of milk and cod liver oil daily.

Fatality Due to Exessive Intake of Vitamin A

A three-man team of explorers from the Australasian Antarctic Expedition started their expedition to explore Antarctica in January, 1912. The team was led by Douglas Mawson and the other two members were Lt. BES Ninnis and Xavier Mertz, a Swiss scientist. Disaster struck on December 14, 1912, when Ninnis fell into a very deep pit and died. With him also went precious food supplies. With most of their food gone, Mawson and Mertz decided to return to their base at Commonwealth Bay, which is at the shores of Antarctica. From here, they could take the ship back to their country. But Commonwealth Bay was about 315 miles from where they were stationed. Covering that distance in the inhospitable surroundings of Antarctica would have taken them weeks, and they had only 10 days' food left with them. They had 6 huskies with them (Huskies are Eskimo dogs, used as ponies in Antarctic region). Both knew that sooner or later they would have to eat those dogs to remain alive.

So they did kill the huskies and ate their flesh, but the flesh was stringy (*i.e.*, fibrous) and they could not eat it. But they found the liver softer and easier to eat, so they took generous quantities of liver. Mertz was a near vegetarian; he could not eat the stringy flesh, so he took more liver than Mawson. Little did he realize that he was taking fatal amounts of vitamin A in this form. On New Year's Eve, Mertz began to feel ill. Next day, he complained of stomachache. Few days later, both men began displaying typical symptoms of vitamin A poisoning, although Mawson was affected less. Their skin was falling off their bodies in strips and their hair was dropping out in handfuls. A week later, Mertz fell into a delirious sleep and never woke. His was the first known case of death due to overdose of vitamin A. Mawson, however, survived, and ultimately did return to Commonwealth Bay.

I. Treatment. For xerophthalmia, the treatment consists in giving 1,500 μg/kg/24 hr of vitamin A orally for 5 days and then continued with intramuscular injection of 7,500 μg of vitamin A in oil daily until recovery occurs.

J. Vitamin A and the vision. George Wald (Nobel Laureate, 1966) of Harvard University has made major contributions to our understanding of the role of vitamin A in visual process. The retina of the human eye contains 2 types of receptor cells, *rods* and *cones*. Animals which have vision only in bright light ("*day vision*") like pigeons have only cones while animals which can see in night or dim light ("*night vision*") like owls have only rods. Thus, the rods are concerned with seeing at low illumination and the cones are responsible for colour vision.

I. Rod vision. The rods contain a photosensitive visual pigment known as **rhodopsin**. It is a conjugated protein and upon illumination splits into a protein called opsin and a carotenoid called retinene$_1$ (Fig. 33–5). It is actually this reaction which may initiate an enzymic reaction responsible for visual mechanism. The retinene$_1$, released by bleaching of rhodopsin, is reduced to vitamin A$_1$ by NADH in the presence of retinene1 reductase, an enzyme present in the retina. Vitamin A$_1$ of mammals is in all-*trans* form like the retinene liberated by bleaching rhodopsin. The isomerization of all-trans retinene1 and vitamin A$_1$ to Δ"-*cis* forms is catalyzed by the enzyme *retinene isomerase*. The major site of this reaction is the liver. Opsin in dark reacts with Δ"-cis retinene1 to regenerate rhodopsin. The series of reactions leading from vitamin A1 to rhodopsin constitutes the major events in '*dark adaptation*'. And the reactions leading to bleaching of rhodopsin constitute what is known as '*light adaptation*'.
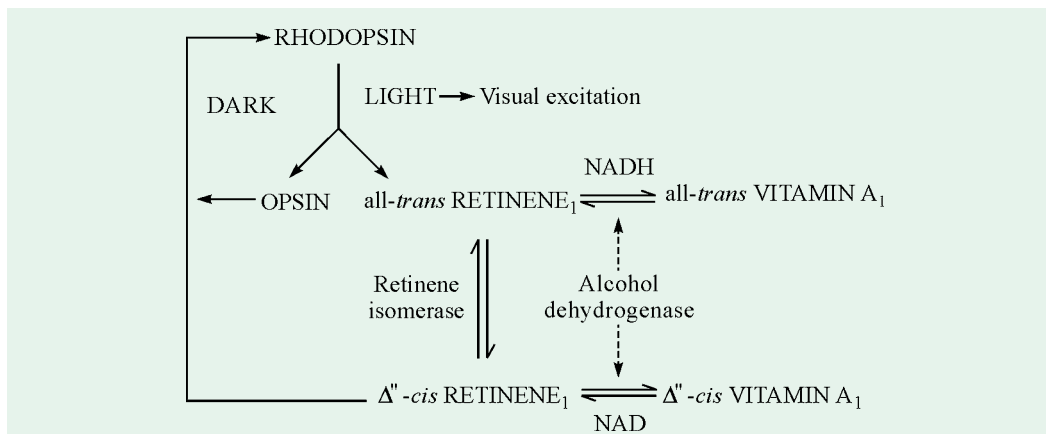
Fig. 33–5. Visual cycle in vertebrates.

Whereas the retina of mammals, birds, frogs and marine fishes contain rhodopsin, all fresh-water fishes have another visual pigment **porphyropsin** in their retina. Wald has shown that porphyropsin undergoes the same cyclic changes on bleaching and regeneration as in the case of rhodopsin except that here retinene A_2 and vitamin A_2 come in picture.

II. Cone vision. According to Young-Helmholtz trichromatic theory, there are present at least 3 closely related pigments in cones. These are **iodopsin, chlorolabe** and **erythrolabe**. The last two pigments absorb actively in the green and the red portions of the spectrum respectively. When light strikes the retina, it bleaches one or more of these pigments based upon the quality of light. The pigments are converted to all-trans retinene and the protein, opsin. This reaction produces the nerve impulse which is read as blue, green or red based on the pigment affected.
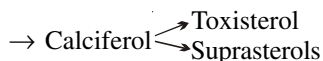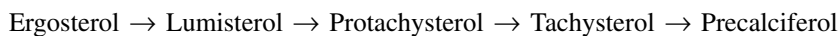
VITAMIN D

A. History. The first demonstration of the existence of vitamin D was shown by Elmer McCollum in 1922 who found that cod liver oil was effective in preventing rickets, a disease induced in rats by providing low calcium diet. On account of its preventive action on rickets, vitamin D is often called as **antirachitic factor**. It is also known as '**sunshine vitamin**' as its provitamin form present in human skin is easily converted to the active form by irradiation with ultraviolet light. At least 10 different compounds are known to have antirachitic properties and are designated as D_2, D_3 etc, but the two, namely, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are more important. Vitamin D_3 was, however, first isolated by Brockmann and others.

The **designation D_1** is not used, however, because D_1 was the designation for the first crystalline material, which later turned out to be a mixture.

B. Occurrence. The best natural sources of vitamin D are the liver oils of many fishes such as cod and halibut. The flesh of oily fishes (*e.g.*, sardine, salmon, herring) is also excellent source. Egg yolks are fairly good but milk, butter and mushrooms are poor. The diets of infant may contain only small amounts of vitamin D; cow's milk contains only 0.1 to 1 µg/quart (1 µg = 40 IU). Cereals, vegetables and fruits contain only negligible amounts. Eggs and yolks contain 3 to 10 µg/g. Most marketed cow's milk is fortified with 10 µg of vitamin D per quart and most commercially–prepared milks for infant formulae are also fortified. Vitamin D_2 is of plant origin and is produced commercially by irradiation with ultraviolet light of a provitamin known as ergosterol which is found in plants, especially in ergot (hence so named) and yeast. Vitamin D_3,

on the contrary, is of animal origin and can be produced from 7-dehydrocholesterol also by irradiating with ultraviolet light. The 7-dehydrocholesterol is also a provitamin found naturally occurring in animals. Vitamins D_2 and D_3 both have about the same degree of activity in the human beings. In nature, these vitamins occur as esters. *Like vitamin A, vitamin D is absent from vegetable fats and oils* and is added to margarine during its manufacture.

C. Structure. The transformation of ergosterol ($C_{28}H_{44}O$) to the active form D_2 takes place through a series of intermediate steps illustrated as below :

Ergosterol → Lumisterol → Protachysterol → Tachysterol → Precalciferol

→ Calciferol ⟨→Toxisterol / →Suprasterols

Similarly, cholecalciferol ($C_{27}H_{44}O$) is produced from 7-dehydrocholesterol through a series of intermediaries as follows :

7-dehydrocholesterol→ Lumisterol$_3$ → Tachysterol$_3$ → Precalciferol$_3$

 → Cholecalciferol
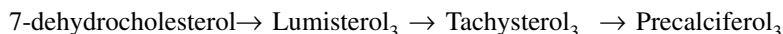
During the activation of the provitamins, the ring B is cleaved between carbon atom 9 and 10 to produce vitamins D_2 and D_3 (Fig. 32–6).
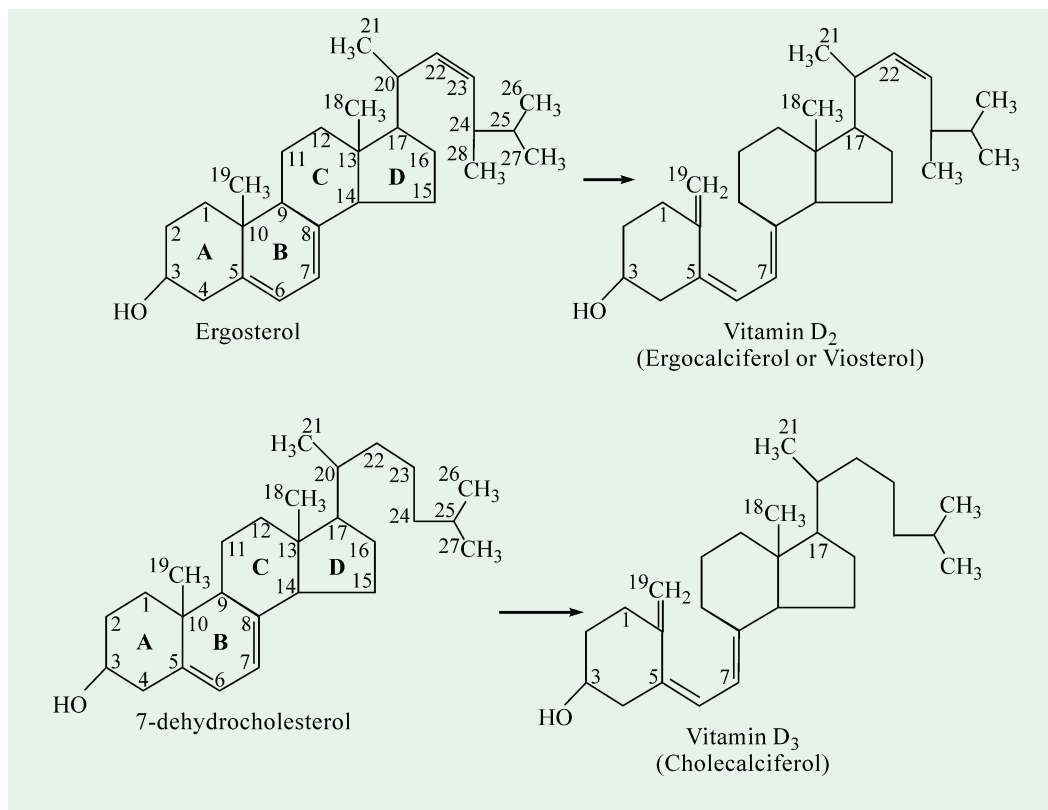

Fig. 33–6. Activation of provitamins of the vitamin D group

Note that in both the cases, the effect of irradiation is the opening of ring B.

It may, however, be noted that the two vitamins, D_2 and D_3 and also D_4 and D_5 (Fig. 33–7), differ only in their side chains attached to C_{17}.
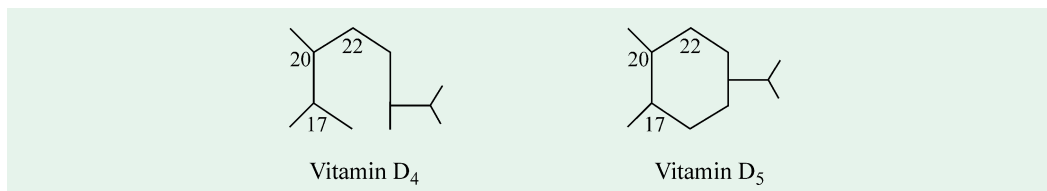
Fig. 33–7. Side chains of vitamins D4 and D5

Other forms of vitamin D may be obtained by irradiation of other sterols. Vitamin D_4, for example, is produced by irradiation of 22-dihydroergosterol. Its potency is 50—70% that of vitamin D_2.

However, researches conducted by DeLuca *et al* (1968) indicate that the biologically active form of vitamin D_3, present in animals such as rat, has a slightly different structure. It is identified as 25-hydrocholesterol (Fig. 33–8). It is a more polar compound and has an additional OH group at C_{25}. It is one and a half times more potent than the vitamin in curing ricket. Also, it stimulates bone metabolism and intestinal calcium transport more rapidly than the vitamin. It is synthesized
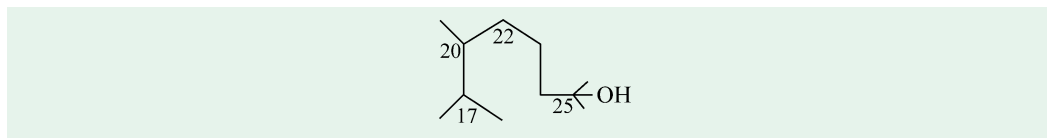
Fig. 33–8. Side chain of 25-hydrocholesterol
or 25–hydroxycholecalciferol

in the liver and is then converted into *1,25-dihydroxycholecalciferol* [1,25(OH)$_2$D$_3$] in the kidneys. Cholecalciferol and its derivatives are **seco-steroids**, *i.e.,* ring A is not rigidly fused to ring B. Because of its conformational mobility, ring A of a seco-steroid exists in two equilibrated conformers. Now as the in vitro mode of action of vitamin D is understood, it has been proposed that *1,25(OH)2D$_3$ is actually a hormone and not a vitamin* as it fits the traditional description of a hormone. 1,25(OH)$_2$D$_3$ is produced in the kidneys (organ) and transported by the blood to intestinal mucosa and bone (target tissues), where it functions in the processes for the absorption, reabsorption and mobilization of calcium and phosphate ions. In conjunction with parathormone and calcitonin, it has a major role in homeostasis of Ca and P in the body's fluids and tissues. Now *because a hormone-receptor protein has been identified for 1,25(OH)$_2$D$_3$, the status of vitamin D as a hormone is well established.*

D. Properties. Vitamin D is a white and almost odourless crystalline substance, soluble in fat and fat solvents. It is fairly heat resistant and also relatively resistant to oxidation. It is not affected by acids and alkalies.

E. Metabolism. The provitamin D_3 can be synthesized within the human body so that it may, in fact, not be required in the diet. This may, henceforth, not be treated as a vitamin. In the past when man lived mainly outdoors and with minimum clothing, there was no hindrance for the penetration of ultraviolet light from the sun to convert it into the active form. In the far northern areas, however, the amount of light is not adequate for conversion and as such fish liver oils serve as excellent source of vitamin D in these areas. The increased need of this vitamin is usually felt in growth and in pregnancy to provide for the needs of the foetus.

Vitamin D plays an important role in calcification of bones and teeth. It encourages the absorption, into the blood, of calcium salts and phosphates. Calcium passage across duodenum occurs mainly by diffusion and active transport of Ca^{2+} occurs across the ileal mucosa. Both these processes are related in dificiency of vitamin D. The subsequent release of bound calcium is also

markedly stimulated by vitamin D but only in the presence of parathyroid hormone. *On the whole, the function of vitamin D is to cause increased absorption, longer retention and better utilization of calcium and phosphorus in the body.*

There is considerable difference in the potency of these 2 common forms of vitamin D. For example, vitamin D_2 is a powerful antirachitic agent for man and for the rat but not for the chicken. Vitamin D_3, on the contrary, is much more potent for the chicken than either for man or the rat.

F. Deficiency. The most characteristic symptom of vitamin D deficiency is the childhood disease known as rickets. Deficiency of it in human adults leads to osteomalacia, a condition that might also be termed "adult rickets".

Rickets (derived from an old English word, *wrickken* = to twist) is primarily a disease of growing bones. In it, the deposition of inorganic materials on the matrix of bones (*i.e.*, calcification) fails to occur, although matrix formation continues. Rickets is unusual below the age of 3 months (mo). It may occur in older children with malabsorption. Clinical manifestations of rickets in children usually manifest in the first year or in the second year. One of the early signs of rickets is **craniotabes**, which is due to thickening of the outer table of the skull and is detected by pressing firmly over the occiput or posterior parietal bones. A ping-pong ball like sensation will be felt. Craniotabes near the suture line may, sometimes, be present in normal premature infants.

> **CLINICAL IMPLICATIONS**
> **RICKETS**
>
> As described by Daniel Webster in 1645, in rickets, " .... the whole bony structure is as flexible as softened wax, so that the flaccid and enervated legs can hardly support the superposed weight of the body ; hence the tibia, giving way beneath the overpowering weight of the frame, bend inwards ; and for the same reason the legs are drawn together at their tops ; and the back, by reason of the bending of the spine, sticks out in a hump in the lumbar region .... the patients in their weakness cannot (in the most severe stages of the disease) bear to sit upright, much less stand .....".

Costochondral junctions become prominent to give appearance of a beaded ribs, the **rachitic rosary**. Thickening of the wrists and ankles are other early evidences of osseous changes. Increased sweating, especially around the head, may also be present.

Signs of advanced rickets are easily identified. These are listed below :

1. Head : Craniotabes may obliterate before the end of the 1st year, although the rachitic process continues. The softness of the skull may result in flattening and, at times, permanent asymmetry of the head. The anterior fontenel is larger than normal; its closure may be delayed until after the 2nd year of life. The central parts of the parietal and frontal bones are usually thickened, forming prominences or bosses, which give the head a box-like appearance (**catput quadratum**).

2. Thorax : The sides of the thorax become flattened, and the longitudinal grooves develop posterior to the rosary. The sternum with its adjacent cartilage projects forward leading to protruding chest (*pigeonbreast*). Along the lower border of the chest develops a horizontal depression (*Harrison groove*), which corresponds with the costal insertions of the diaphragm.

3. Spinal cloumn : Moderate degree of lateral curvature (*scoliosis*) is common and a *kyphosis* (=increased convexity in the region of thoracic spine) may appear in the dorsolumbar region when sitting. *Lordosis* (=forward curvature of the lumbar spine) may be seen in the erect position.

4. Pelvis : The pelvic entrance is narrowed by a forward projection of the promontory; the exit, by a forward displacment of the caudal part of the sacrum and coccyx. In the female, these changes, if they become permanent, add to the hazards of childbirth and necessitate caesarean section.

5.   Extremities :   The epiphyseal enlargement at the wrists and ankles becomes more noticeable. Bending of the softened shafts of the femur, tibia and fibula results in bowlegs (*knock-knees*) ; the femur and the tibia may also acquire an anterior convexity. *Coxa vara* is sometimes the result of rickets.

Deformities of the spine, pelvis and legs results in reduced stature, **rachitic dwarfism**.

6.   Ligaments :   Relaxation of ligaments helps to produce *deformities* and partly accounts for knock-knees, weak ankles, kyphosis and scoliosis.

7.   Muscles :   The muscles are poorly developed and lack tone. As a result, the rachitic children are late in standing and walking. Abdomen becomes protuberant (*potbelly*) because of marked hypotonia of abdominal wall muscles, visceroptosis and lumbar lordosis.

8.   Sense organ :   Avitaminosis D in early infancy results in *bilateral lamellar cataracts*.

9.   Dentition :   Eruption of temporary teeth may be delayed in rachitic children. The first tooth in such babies appears between 6th and 9th month, at which time it has appeared in half of the normal babies. In deficiency of vitamin D, the formation of teeth becomes defective and leads to the development of *dental caries*.

Chemical analysis of the bones of rachitic children reveals the presence of low inorganic and high organic and water contents in them. The ratio of calcium to phosphorus (C/P), however, remains constant. In the blood serum, there is usually a normal content of calcium but the phosphate content is reduced (1.5–3.5 mg/dL), against a normal value of 4.5–6.5 mg/dL in healthy infants. Vitamin D deficiency is also accompanied by generalized aminoaciduria, a decrease of citrate in bone and its increased urinary excretion, decreased ability of the kidneys to make an acid urine, phosphaturia, and, occasionally, mellituria. The parathyroid glands hypertophy in rickets.

Rickets in itself is not a fatal disease but complications and intercurrent infections such as pneumonia, tuberculosis, and enteritis are more likely to cause death in rachitic children than in normal children.

Rickets is most prevalent where climate or custom prevents individuals from exposure to sun, whereby checking vitamin D production by irradiation of the skin. In the seventeenth century. this disease was so common in England that it used to be known as "*English disease*". A study conducted by the Indian Social Institute, New Delhi (1981) shows that about 168 children per 1,000 of live births die of rickets in India.

In **osteomalacia** (*osteon*^{G} = bone ; *malakia*^{G} = softness), the action of bones is essentially like that in rickets. However, the bones become softer than the rachitic bones and the C/P ratio does not remain constant. The loss of calcium is greater than that of phosphorus and there is a relative gain in magnesium content. The disease is prevalent in India, China and Arab, particularly among women because of the custom that keeps them indoor and also prevents them from exposure to sun. This is particularly true of Bedouin Arab women who are clothed so that only their eyes are exposed to sunlight. The serum calcium is reduced, sometimes to such an extent that tetany develops.

Vitamin D$_3$ deficiency also leads to a disease called **idiopathic steatorrhea** or **celiac disease**. Like osteomalacia, the disease is characterized by demineralization of the bones which may result in deformities or dwarfism. In fact, celiac disease is *indirectly* a vitamin D deficiency because the primary abnormality appears to be, in part, a fatty diarrhea. The fat is not absorbed in the intestine and is passed out in stool along with calcium salts and vitamin D.

G. Hypervitaminosis D. Overdosing of calciferol to the children and adults as well produces demineralization of bone. Serum concentrations of both calcium and phosphate are greatly raised, resulting in metabolic calcification of many soft tissues and the formation of renal calculi. The latter disorder may block the renal tubules causing hydronephrosis.

The use of very high doses of vitamin D is not danger-free, however. The toxic effects caused by excess dosage include anorexia, nausea, polyuria, weakness, headache etc. The toxicity is due to the diminished excretion of this vitamin, rather than its storage in the liver. *The water-soluble vitamins, on the contrary, if given in excess pass out immediately in the urine and are henceforth nontoxic.* Much of the whole milk available in urban areas and evaporated milk are fortified with vitamin D concentrate so that 1 quart of fresh, whole milk or a cane of evaporated milk contains the required amount (*i.e.*, 10 μg).

H. Human requirements. Vitamin D requirement is greatly influenced by the amount of ultraviolet light to which the individual is exposed. Half an hour of direct sunlight on the cheeks of a baby each day is sufficient to generate the minimal daily requirement of vitamin D. For adults also, exposure to sunlight for 30 minutes a day is believed to satisfy the daily requirement (about 10 μg or 400 IU) of vitamin D. As effective UV rays do not penetrate glass windows, exposure to sun through window glass is of little importance. Smoke also hinders the progress of these rays and as such city sunshine is not much beneficial. It is for these and some other reasons that vitamin D should be included in the diet. This is particularly true for older people. The recommended daily allowance of vitamin D is 400 I.U. for infants, pregnant women and lactating mothers. For adults, 400 units are adequate. One International Unit is defined as the biologic activity of 0.025 μg of pure crystalline vitamin D_3.

VITAMIN E

A. History. The presence of this active principle was first demonstrated in vegetable oils by Evans and Mattill independently in 1920. This was designated as vitamin E or **antisterility factor** on account of the development of sterility in animals in its absence. In 1936, two compounds with vitamin E activity were isolated from wheat germ oil by Evans and his associates and given the name, α- and β-tocopherol (*tokos*[G] = childbirth ; *pheros*[G] = to bear; *ol* = an alcohol). Subsequently, five other tocopherols were obtained from various cereal grains like wheat germ, corn oil, rice etc.

B. Occurrence. The tocopherols are of widespread occurrence in many plant oils such as wheat germ, rice, corn, cottonseed, soybean and peanut but not olive oil. The are also present in small amounts in meat, milk, eggs, leafy plant and some fruits. *Fish liver oils, so abundant in vitamin A and D, are devoid of vitamin E*. Of all the tocopherols discovered so far, the α-form has the widest distribution and greatest biologic activity. The relative *biologic potencies* of various tocopherols are :

<div align="center">

α-tocopherol—100

β-tocopherol—25

γ-tocopherol—19

</div>

The vitamin E content of some oils are presented in Table 33–4.

Table 33–4. **Vitamin E content of some oils**

Oil	Vitamin E (mg/100 g)
Groundnut	261
Wheatgerm	225
Soybean	166
Linseed	110
Palm	56
Mustard	32

C. Structure. Vitamin E is the collective name for a group of closely related lipids called tocopherols. The tocopherols are derivatives of 6-hydroxychroman (also known as tocol) bearing an isoprenoid side chain at carbon 2. The structure of a-tocopherol ($C_{29}H_{50}O_2$) is given in Fig. 33–9.
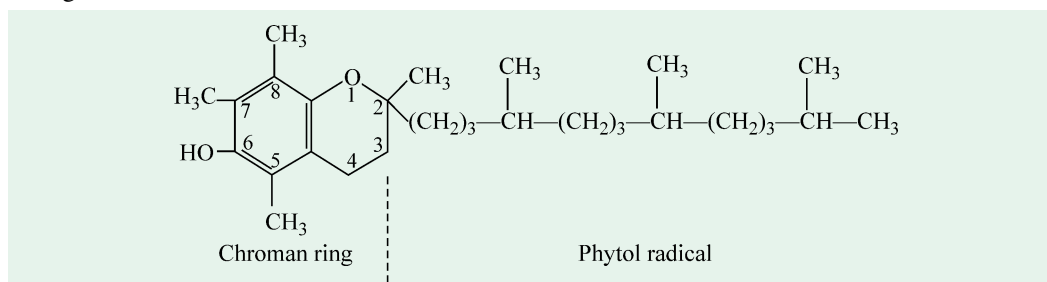

Chroman ring   Phytol radical

Fig. 33–9. α-tocopherol

(5,7,8-trimethyl tocol)

The various tocopherols differ from each other in substituents on carbons 5, 7 and 8. These substituents are methyl groups and hydrogen atoms. a-tocopherol contains 3 methyl groups whereas other tocopherols are short one or two methyl groups on the aromatic ring (refer Table 33–3).

Table 33–3. **Composition of the variable segment of different tocopherols**

Types of Tocopherol		Substituents at Carbon Atoms		
		5	7	8
Alfa	α	CH_3	CH_3	CH_3
Beta	β	CH_3	H	CH_3
Gamma	γ	H	CH_3	CH_3
Delta	δ	H	H	CH_3
Epsilon	∈	CH_3	H	H
Zeta	ζ	CH_3	CH_3	H
Eta	η	H	CH_3	H

It is noteworthy that the presence of all 3 methyl groups attached to the benzene ring is necessary for full activity. d-tocopherol has but one methyl group and is almost without activity. A slight change in the structure of the tocopherols, for example shortening the side chain, may greatly diminish their physiologic activity.
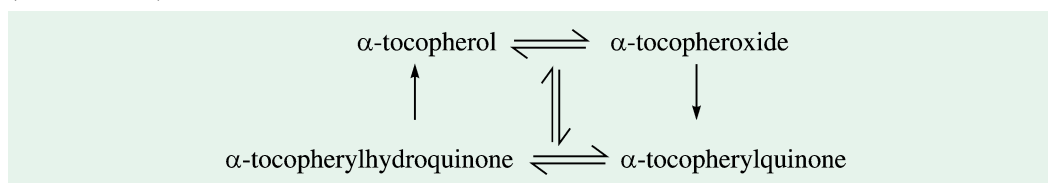
D. Properties. Vitamin E is a light yellow oil. It is resistant to heat (up to 200°C) and acids but acted upon by alkalies. It is easily but slowly oxidized and is destroyed by UV rays. The tocopherols are excellent antioxidants. They prevent other vitamins presents in food (*e.g.,* vitamin A) from oxidative destruction. It is found in the nonsaponifiable fraction of the vegetable oils.

E. Metabolism. *Tocopherols act as antioxidants, i.e.,* they can prevent the oxidation of various other easily oxidized substances such as fats and vitamin A. It is for this reason that they are commercially added to foods to retard their spoilage. It may be recalled that vitamin A is essential for reproduction. *Whereas the beneficial action of vitamin A is mainly on the ectoderm and endoderm, that of vitamin E is on the mesodermal tissue.* But, very likely, vitamin E influences all the 3 germinal layers of the embryo by preventing the too rapid destruction of vitamin A. Certain substances such as phenols and vitamin C (ascorbic acid) stimulate the antioxidant property of vitamin E.

In fact, the biochemical activity of tocopherol lies in its capacity to protect mitochondrial system from inactivation by fat peroxides. Thus, in mitochondria obtained from vitamin E-deficient animals, a marked deterioration in activity is found due to peroxidation of unsaturated fatty acids which are usually present in these particles. Addition of vitamin E prevents this deterioration by acting as antioxidant for peroxidation.

It has been observed that tocopherol-deficient muscles (*esp*., cardiac and skeletal) show a high oxygen uptake. Administration of tocopherol brings down the oxygen consumption to normal.

The catabolism of α-tocopherol involves both the oxidative cleavage of the chroman ring to yield quinone or hydroquinone-like compounds and the degradation of the isoprenoids side chain (Simon, 1956)

$$\alpha\text{-tocopherol} \rightleftharpoons \alpha\text{-tocopheroxide}$$

$$\alpha\text{-tocopherylhydroquinone} \rightleftharpoons \alpha\text{-tocopherylquinone}$$

F.   Deficiency. The characteristic symptoms of experimentally-induced vitamin E deficiency vary from animal to animal. In mature female rats, **sterility** develops because of reabsorption of fetus after conception while in males, the germinal epithelium of the testes degenerates and the spermatozoa become nonmotile.

Avitaminosis E in herbivorous animals like rabbits and guinea pigs leads to acute **muscular dystrophy** (atrophy of muscle fibres), which ultimately results in creatinuria ; young chicks exhibit capillary damage and **encephalomalacia** ; hen eggs show **low hatchability** and monkeys reveal **hemolytic anemia**. There is, however, little evidence that man is ever short of vitamin E.

Finally, *as is true for almost all the vitamins, avitaminosis E prevents normal growth*. It also sometimes causes degenatation of the renal tubular cells.

G.   Human requirements. Vitamin E is not a problem in human nutrition because it is ubiquitous in foods. However, the minimum daily requirement of vitamin E for adults is 30 I.U. for men and 25 I.U. for women. The pregnant and lactating mothers, however, require 30 I.U. daily. For infants and children,. the vitamin E requirement is at the rate of 1 to 1.25 I.U. per kilogram of body weight. One International Unit of dl-α-tocopherol is equivalent to the biologic activity of 1.1 mg of pure compound or 0.67 mg of d-α-tocopherol.

VITAMIN K

A.   History. Henrick Dam (1929), a Danish investigator, found that newly-hatched chicks, fed on artificial diets, develop hemorrhage, a fatal disease characterized by prolonged blood-clotting period. The term vitamin K (K for Danish koagulations) was then proposed by Dam (1934) himself to designate the active factor which cured or prevented this disease. On account of its blood-clotting power, it is also called as **antihemorrhagic factor** or coagulation **vitamin**.

Henrick Dam (LT, 1895-1976) was on a lecture tour of the United States and Canada when the Nazis occupied his homeland, Denmark, in 1940. He spent the war years at the University of Rochester, and it was during this period that he received his Nobel Prize (1943).
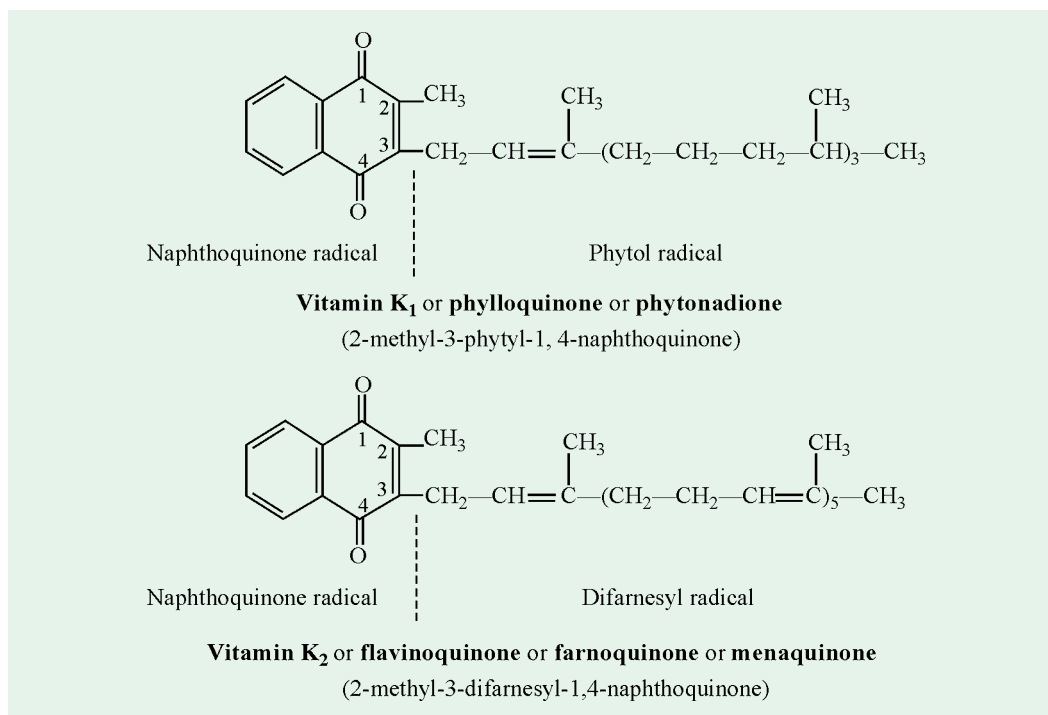
Vitamin K₁ or phylloquinone or phytonadione
(2-methyl-3-phytyl-1, 4-naphthoquinone)

Vitamin K₂ or flavinoquinone or farnoquinone or menaquinone
(2-methyl-3-difarnesyl-1,4-naphthoquinone)

Fig. 33–10. **Two vitamins of K group**

Of the 2 naturally-occurring forms of this vitamin, vitamin K_1 was first isolated by Dam *et al* from alfalfa in 1939 and the other form, vitamin K_2 from fish meal by Doisy *et al*, also in 1939.

B. Occurrence. Vitamin K_1 occurs in green vegetables like spinach, alfalfa, cabbage etc. Fruits and cereals are poor sources. Vitamin K_2 is found in some intestinal bacteria. A rich source of K_2 is putrefied fish meal. Their relative *biologic potencies* are :

<div align="center">

vitamin K_1—100

vitamin K_2—80

</div>

C. Structure. Chemically, the two forms of vitamin K (Fig. 33–10) are derivatives of quinones and differ from each other in the composition of their side chain present at carbon 3 of the naphthoquinone ring. It is a phytol radical in vitamin K_1 ($C_{31}H_{46}O_2$) and a difarnesyl radical in vitamin K_2 ($C_{41}H_{56}O_2$). Vitamin K_1, found in plants, has 4 isoprene units in its side chain whereas vitamin K_2, found in animals, contains in its side 6 isoprene units, each with a double bond.

Analogues— The various analogues of naphthoquinone, however, have also been shown to possess vitamin K activity for animals. This is due to their structural resemblance. The common examples are menadione and phthiocol (Fig. 33–11).
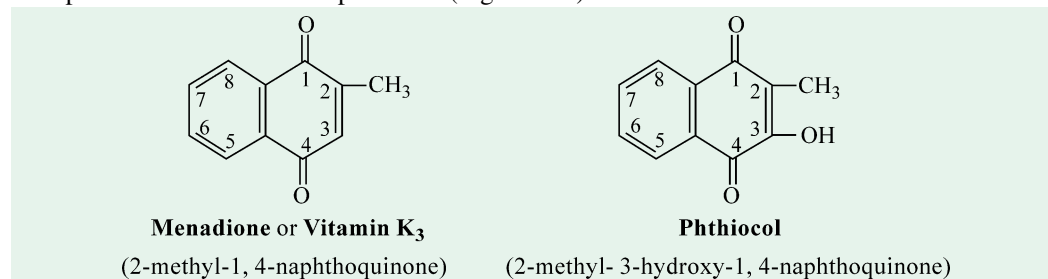

Menadione or **Vitamin K₃**
(2-methyl-1, 4-naphthoquinone)

Phthiocol
(2-methyl- 3-hydroxy-1, 4-naphthoquinone)

Fig. 33–11. **Two analogues of vitamin K**

Menadione, which is sometimes referred to as vitamin K_3, is twice as potent as vitamin K_1. It is soluble in oil, sparingly so in water and is not oxidized in air when protected from light. Its diphosphate ester is water-soluble and is widely used clinically.

D.   Properties. Vitamin K_1 is a yellow viscid oil but vitamin K_2 is a yellowish crystalline solid. It is sensitive to light and is, therefore, kept in dark bottles. It is destroyed by irradiation, strong acids, alkalies and oxidizing agents.

E.   Metabolism. Vitamin K plays an essential role in the biosynthesis of prothrombin— a blood plasma protein needed in the process of blood clotting and produced in liver. The process of blood coagulation may be summarized as below.
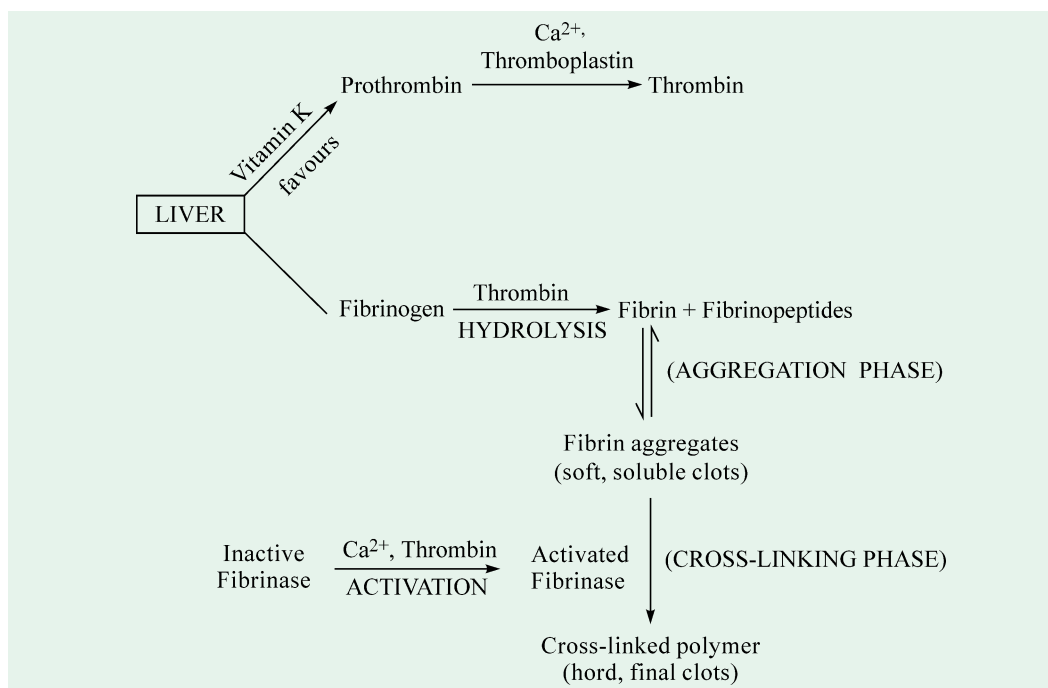

Fig. 32–12. **Probable mechanism of blood coagulation**

[Note the role of vitamin K in the synthesis of prothrombin which is a precursor of thrombin, the latter has dual function of (*a*) hydrolysing fibrinogen to fibrin and (*b*) activating fibrinase of FSF, which brings about clotting.]

The formation of the blood clot (refer Fig. 33–12) is caused by the enzymic hydrolysis of the soluble plasma protein, fibrinogen to the insoluble protein, fibrin and fibrinopeptides. This transformation is catalyzed by an enzyme, *thrombin*. Thrombin itself is not present in the blood but is produced from its precursor, prothrombin in the presence of Ca^{2+} ions and another protein called *thromboplastin* (= thrombokinase). In the next step, fibrin forms soft, fibrous networks (or soft clots). Then in the presence of Ca^{2+}, thrombin activates fibrinase, an enzyme precursor found in blood plasma. Fibrinase is also known as fibrin-stabilizing factor (FSF). Finally, fibrous networks of fibrin link with each other under the influence of activated FSF to produce cross-linked fibrin (or hard clots).

The vitamins K are fat-soluble and are absorbed only in the presence of bile. As a result, the absorption occurs in the upper portion of the small intestine where bile salts are present. The avitaminosis K may occur where bile is prevented from entering the intestinal tract. This is true for most of the fat-soluble vitamins but is, in particular, important in the case of vitamin K because of its blood clotting action.

Like vitamin E and coenzyme Q, the vitamin K has also been ascribed a role in electron transport system (ETS) and oxidative phosphorylation in mitochondria. The vitamin K_1 and K_2 both activate electron transport in the succinate oxidase of cardiac muscle preparations that have been made inactive by treating with isooctane. The bacterial extracts or liver mitochondria, when Antagonists irradiated, require vitamin K for oxidative phosphorylation. This suggests a possible role of vitamin K in oxidative phosphorylation. The specific site of action is believed to occur between NADH and cytochrome *b*. It has been suggested that a phosphate ester of vitamin K, upon oxidation, transfers phosphate to ADP to form ATP.

Antagonists— Two antagonists of vitamin K are dicumarol and warfarin (Fig. 33–13) ; both antagonists prevent blood clotting. Dicumarol was first isolated from mouldy clover hay. It is often given to patients, who have suffered heart attacks caused by blood clots, as as preventive measure against

> **Dicumarol** is also spelt as dicoumarol.

further clotting in the blood vessels. Warfarin (name derived from the initials of the Wisconsin Alumni Research Foundation, which sponsored the research on the compound) is a synthetic analogue of vitamin K. It is extremely poisonous to rats, causing death by internal bleeding. Ironically, this potent rodenticide is also a valuable anticoagulant drug for the treatment of human patients in whom excessive bleeding is dangerous— surgical patients and victims of coronary thrombosis.

Dicumarol
[3,3′–methylenebis (4-hydroxy-1,2-benzopyrone)]
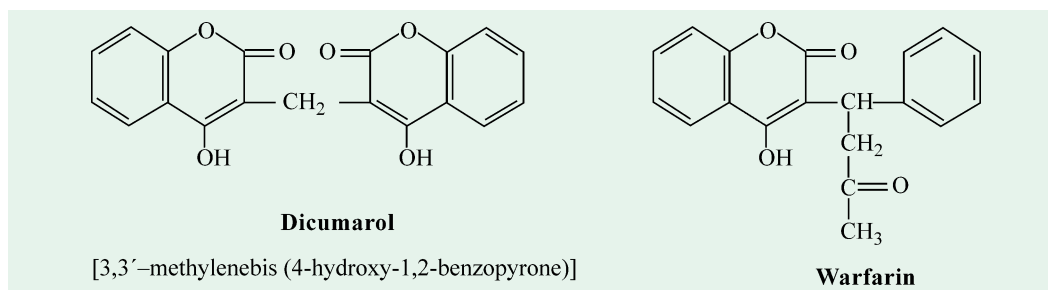
Warfarin

Fig. 33–13. **Two vitamin K antagonists**

Note that both dicumarol and warfarin lack an isoprenoid side chain.

In 1988, by introducing the antioxidant vitamin E, David Gershon and his colleagues at the Technion–Israel Institute of Technology, have succeeded in reducing cell damage and increasing the life span of nematode worms. The damage body cells sustain is due to oxidation, an underlying mechanism of ageing, which also damages the disposal system. Paradoxically, the oxygen we depend on for life is a source of our age-associated decline in function. Similar researches conducted on humans might shed light on how to intervene and retard ageing in them.

F.   Deficiency. Deficiency of vitamin K causes loss of blood-clotting power. The infants may also show signs of vitamin K deficiency by developing **hemorrhage**. This disease persists by the time the bacteria develop in the intestine. Administration of this vitamin to pregnant mothers before parturition decreases the onset of this disease. In man, however, avitaminosis K results in **steatorrhea** with diminished intestinal absorption of lipids.

In general, vitamin K deficiency is rarely found in higher animals as this is provided by food and also synthesized by intestinal bacteria.

G.   Human requirements. *There is seldom a lack of sufficient vitamin K in human beings.* As such, no standard requirement has been set.

COENZYME Q

Crane *et al* (1959) have demonstrated that coenzyme Q and certain other ubiquinones are components of mitochondrial lipids. These substances serve as electron transport agents and are

involved in the formation of ATP at a cytochrome *a*. Roles (1967) has classified the coenzyme Q group as vitamins because of their ability to cure (or protect against) vitamin E deficiency in several animal species. Some of the coenzymes also participate in electron transport and/or oxidative phosphorylation.
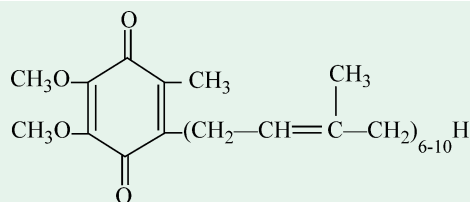

Fig. 33–14. **Coenzyme Q**

Various homologues of coenzyme Q, containing 6 to 10 isoprene units, have been isolated from various microbes, chloroplasts of green plants and mitochondria of animals. These have the same quinonoid nucleus (Fig. 32–14) but differ in the number of isoprenoid units in the side chain. For example, coenzyme Q from animal source has 10 isoprene units and is, henceforth, called coenzyme Q_{10} or *ubiquinone*$_{50}$ (50 carbon atoms, *i.e.,* 10 isoprene units, in the side chain), whereas the one from bacteria has less than 10 isoprene units. *Mycobacteria, however, contain no coenzyme Q.*

STIGMASTEROL

Another alleged fat-soluble vitamin is stigmasterol. It is a plant sterol and has been isolated from soybean and wheat germ oils. It in present in alfalfa and fresh cream. Chemically, it resembles ergosterol closely and contains only 2 double bonds at carbon postitons 5—6 and 22—23 (Fig. 33–15). The absence of stigmasterol causes stiffness of the wrists and elbows of the guinea pigs. For this reason, it is commonly called as '**antistiffness factor**'. The muscles atrophy and become streaked.
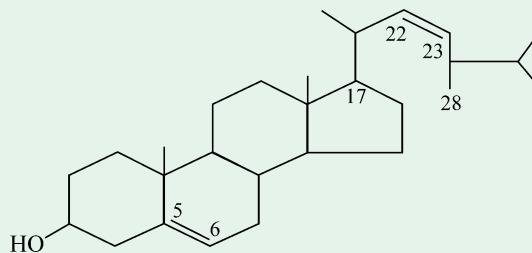

Fig. 33–15. **Stigmasterol**

Contents

Table 33–4. **Fat-soluble vitamins**

Vitamin	Common name(s)	Chemical name	Sources	Metabolic functions	Deficiency diseases*	Daily requirement of man
A	Antixerophthalmic factor or 'Bright eyes' vitamin	A_1— Retinol, $C_{20}H_{30}O$ A_2— 3-dehydroretinol $C_{20}H_{28}O$	Fish liver oils, butter, milk, eggs and kidneys; Pigmented (esp., yellow) vegetables and fruits	Visual cycle; Membrane integrity	Demyelinization; Nyctalopia; Xerophthalmia; Keratomalacia; Phrynoderma	5,000 I.U.
D	Antirachitic factor or 'Sunshine' vitamin	D_2— Ergocalciferol $C_{28}H_{44}O$ D_3— Cholecalciferol $C_{27}H_{44}O$	Fish liver oils, egg yolks, milk, butter and mushrooms	Calcification of bones and teeth	Rickets (in children); Osteomalacia (in adults); Celiac disease	400 I.U.
E	Antisterility factor	α-tocopherol, $C_{29}H_{50}O_2$ β-tocopherol, $C_{28}H_{48}O_2$ γ-tocopherol, $C_{28}H_{48}O_2$	Plant oils like wheat germ, rice cottonseed, corn, soyabean etc; Also meat, milk and eggs	Act as antioxidants; Control O_2 consumption; Participate in nucleic acid metabolism	Sterility in rats; Muscular dystrophy in rabbits and guinea pigs; Encephalomalacia in young chicks	30 I.U.
K	Antihemorrhagic factor or Coagulation vitamin	K_1— Phylloquinone $C_{31}H_{46}O_2$ K_2— Farnoquinone $C_{41}H_{56}O_2$	Green vegetables like spinach, alfalfa, cabbage etc.; Also fruits and cereals	Biosynthesis of prothrombin; Oxidative phosphorylation; Electon transport system	Hemorrhage (in infants); Steatorrhea (in adults)	0.001 mg

* Deficiency diseases have been mentioned in relation to man, if not stated otherwise.

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PROBLEMS

1. In contrast to water-soluble vitamins, which must be a part of our daily diet, fat-soluble vitamins can be stored in the body in amounts sufficient for many months. Suggest an explanation for this difference based on solubilities.

2. In the presence of warfarin, an analogue of vitamin K, several proteins of the blood coagulation pathway are ineffective because they cannot bind Ca^{2+} efficiently. Why ?

3. What is the chemical reaction in which vitamin K participates ? How is this reaction involved in blood coagulation and bone formation ?

4. If one takes vitamin E to protect his heart, how much should he take ?

5. Will taking vitamins give one extra energy ?

6. When taking vitamin E, is it okay to take other medicine ?

7. Should one take multivitamin pills containing vitamin D every day as one gets older ?

8. How much exposure to sunlight a person needs to supply his body with enough vitamin D ?

9. Vegetables oils are fortified with :
   (*a*) vitamin A
   (*b*) vitamin D
   (*c*) vitamin E
   (*d*) vitamin K

10. What needs to be stored in dark bottles ?
   (*a*) biotin
   (*b*) nicotinic acid
   (*c*) riboflavin
   (*d*) retinol

11. One claim put forth by purveyors of health foods is that vitamins obtained from natural sources are more healthful than those obtained by chemical synthesis. For example, it is claimed that pure L-ascorbic acid (vitamin C) obtained from rose hips is better for you than pure L-ascorbic acid manufactured in a chemical plant. Are the vitamins from the two sources different ? Can the body distinguish a vitamin's source ?

12. How much vitamin D a person needs daily to help prevent osteoporosis ?

13. Is it safe to take very high doses of vitamin D ?

14. Vitamin A is the essential dietary factor for the formation of :
   (*a*) rhodopsin
   (*b*) biliverdin
   (*c*) hemoglobin
   (*d*) biotin

15. Which of the following is both a vitamin and a hormone ?
   (*a*) ascorbic acid
   (*b*) calciferol
   (*c*) thiamine
   (*d*) riboflavin

16. Does your skin ever lose its ability to make vitamin D from sunlight ?

17. When is a good time to take your multivitamin/mineral tablet ?

18. If a person takes a daily multivitamin and supplements, can he get away with less fruits, vegetables and grains ?

19. Can one take vitamins with thyroid pills ?

20. The most nutritive substance on Earth is :
   (*a*) Spinach
   (*b*) *Spirulina*
   (*c*) *Laminaria*
   (*d*) Soybean