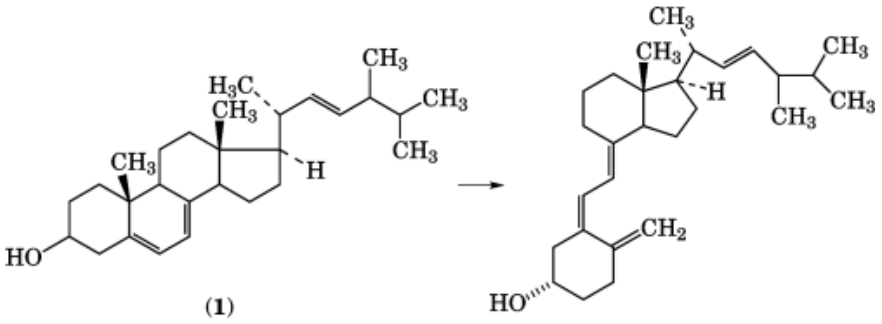
Kirk-Othmer Encyclopedia of Chemical Technology.

VITAMIN D

Vitamin D [1406-12-2] is a material that is formed in the skin of animals upon irradiation by sunlight and serves as a precursor for metabolites that control the animal's calcium homeostasis and act in other hormonal functions. A deficiency of vitamin D can cause rickets, as well as other disease states. This tendency can be a problem wherever animals, including humans, especially infants and children, receive an inadequate amount of sunshine. The latter phenomenon became prevalent with the advent of the industrial revolution, and efforts to cure rickets resulted in the development of commercial sources of vitamin D for supplementation of the diet of livestock, pets, and humans.

Research conducted during and subsequent to the 1970s revealed that vitamin D is better defined as those natural or synthetic substances that are converted by animals into metabolites that control calcium and phosphorus homeostasis and act in a variety of other hormonal-like functions.

Vitamin D_2 and vitamin D_3 are the two economically important forms of vitamin D. The other D vitamins have relatively little biological activity and are only of historical interest. Vitamin D_2 (ergocalciferol; ercalciol), (5Z,7E,22E)-(3S)-9,10-*seco*-5,7,10(19)-22-ergostatraene-3-ol **(2)**, is active in humans and other mammals, although recently (ca 1997) it has been shown to be less active than vitamin D_3 in cattle, swine, and horses. It is relatively inactive in poultry. It is prepared by the uv irradiation of ergosterol (provitamin D_2), (24-methylcholesta-5,7,22-triene-3B-ol **(1)**, a plant sterol.

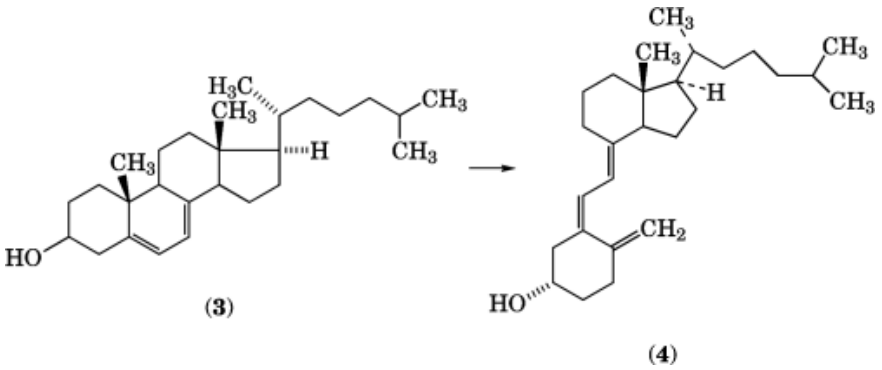


(1) **(2)**

Vitamin D_3 (cholecalciferol; calciol), (5*Z*,7*E*)-(3*S*)-9,10-seco-5,7,10(19) cholestatriene-3-ol **(4)**, is the naturally occurring active material found in all animals. It is produced in the skin by the irradiation of stored 7-dehydrocholesterol (provitamin D_3), cholesta-5,7-diene-3B-ol **(3)**.

Table 1. Vitamin D Substances

Common name	CAS Registry Number	Provitamin	Trivial name	IUPAC-IUB name
vitamin D_2	[50-14-6]	ergosterol	ergocalciferol	9,10-*seco*-5,7,10(19),22-ergostatetraen-3 β-ol
vitamin D_3	[67-97-0]	7-dehydrocholesterol	cholecalciferol	9,10-*seco*-5,7,10(19),cholestatrien-3 β-ol
vitamin D_4	[511-28-4]	22,23-dihydroergosterol		24-methyl-9,10-*seco*-5,7,10(19)-cholestatrien-3 β-ol
vitamin D_5	[71761-06-3]	7-dehydrositosterol	sitocalciferol	2,4-ethyl-9,10-*seco*-5,7,10(19)-cholestatrien-3 β-ol
vitamin D_6	[481-19-6]	7-dehydrostigmasterol		24-ethyl-9,10-*seco*-5,7,10(19)-22-ergo-statetraen-3 β-ol
vitamin D_7	[20304-51-2]	7-dehydrocampesterol		2,4-methyl-9,10-*seco*-5,7,10(19)-cholestatrien-3 β-ol

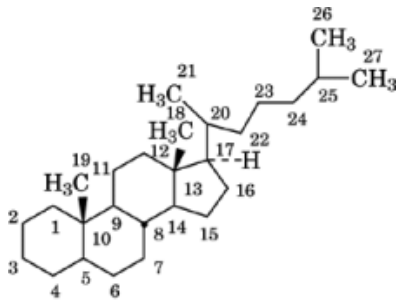


1. Nomenclature

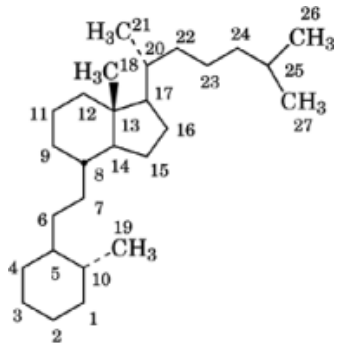
The Vitamin D compounds are steroidal materials and thus are named according to the IUPAC-IUB rules for nomenclature (1) (Table 1). Vitamin D_1 [520-91-2] is a mixture of vitamin D_2 and lumisterol.

The common name vitamin D is used throughout the pharmaceutical industry for simplicity. The trivial name calciferol has also been used extensively with the prefix ergo- and chole-, which indicate vitamin D_2 **(2)** and vitamin D_3 **(4)**, respectively (see Steroids). Vitamin D_2 was originally named calciferol in 1931 by Angus and co-workers (2). Historically, a number of substances were referred to as vitamin D and were distinguished from one another by a subscript numeral, eg, vitamin D_2, vitamin D_3, etc.

The vitamins D are 9,10-secosteroids, that is, steroid molecules with an opened 9,10 bond of the B-ring. The relationship between the provitamin steroid (perhydro-1,2-cyclopentanophenanthrene ring system) and the 9,10-secosteroid nucleus is shown in structures **(5)** and **(6)**, cholestane and 9,10-secocholestane (calcitane), respectively.



(**5**)



(**6**)

In 1981, the IUPAC-IUB Joint Commission on Biochemical Nomenclature proposed that there be a set of trivial names for the important vitamin D compounds, including calciol [67-97-0] for vitamin D, calcidiol [19356-17-3] for 25-hydroxy-vitamin D_3, and calcitriol [32222-06-3] for 1 α,25-dihydroxy-vitamin D_3. This nomenclature has met with varying degrees of acceptance, as has the proposal to use calcine [69662-75-5] (deoxy-vitamin D_2) and ercalcine [68323-40-0] (deoxy-vitamin D_3) to name the triene hydrocarbon structure for 9,10-*seco*-cholesta-5,7,10(19)-triene and 9,10-*seco*-ergosta-5,7,10(19),22-tetraene, respectively. In systematic nomenclature, calcitane can be used for the basic 27-carbon skeleton instead of 9,10-secocholestane.

1.1. Isolation and Structure Determination

The isolation and structure elucidation of vitamin D are closely related to the efforts to understand and cure rickets and related bone diseases. The advent of the use of soft coal, the migration of people to cities, and the tendency of people and animals to spend less time in sunshine caused a decline in the ability of populations to synthesize sufficient quantities of vitamin D_3. This led to the increased incidence of rickets, beginning around the mid-1600s (3).

The bone disease rickets in children, and a similar condition in adult known as osteomalacia, is characterized by the body's inability to calcify the collagen matrix of growing bone, resulting in wide epiphyseal plates and large areas of uncalcified bone called osteoid. The resultant lack of rigidity of bones leads to the ends becoming twisted and bent, particularly in long bones. The ribs develop a bumpy and uneven texture known as rosary ribs and the legs become bowed. Also, the cranium becomes soft and misshapen. In adults, no long-bone growth occurs, but new bone, which is being continually remodeled, activates cells to resorb bone, followed by osteoblast-mediated bone-growth replacement (4, 5). Metabolites of vitamin D act in the body to modulate these activities and maintain strong bone structure.

Although there is evidence that rickets was manifest in humans as early as 800 BC, it was not until 1645 that it was first described (6). The progress of finding a cure for rickets was relatively slow until the late 1800s, when a sufficient number of scientific developments began to allow workers to unravel the difficult puzzle of vitamin D metabolism. Tarret in 1889 had isolated ergosterol from ergot of rye and demonstrated that it was different from cholesterol. The similarity of the structures, however, led to difficulty in the structural elucidation of the vitamin D molecule. Mellanby (7) demonstrated the lack of a dietary component could be used to develop rickets and was able to raise D-deficient animals. This allowed research aimed at finding the antirachitic factor to be carried out.

In 1919, Huldschinski (8) realized that uv light cured rickets and impacted on its etiology. The uv light and cod liver oil were found to be useful in the treatment of the disease, and irradiation of food produced the same effect as irradiation of the animal. The link between irradiation and plant materials led to the conclusion that ergosterol was an antirachitic substance, and an extensive effort was made to characterize the chemistry of irradiated ergosterol.

Windaus in 1933 derived the skeletal structure of vitamin D_2. He found the elemental formula to be $C_{28}H_{44}O$. The side chain was unequivocally characterized by x-ray crystallography later, in 1948. Reindel and Kipphan (9) in 1932 ozonized the side chain and elucidated the C_{22} double bond. The reaction to form a Diels Alder adduct with maleic anhydride and the typical diene uv spectrum led to the conclusion there was a diene in the B ring. In 1934 Fernholz and Chakravorty (10) determined the provitamin had a 3 β-hydroxy group because the molecule could be precipitated with digitonin, a reaction characteristic of the 3 β-ol functionality in steroids.

Perbenzoic acid gave a doubly unsaturated triol monobenzoate. Only two hydroxyl groups could be acetylated, and one was tertiary. The saturated triol reacted with lead tetracetate to give an α glycol. When reacted with chromic acid, it gave a hydroxy lactone. From these observations, Windaus and Grundmann (11) described the correct structure for ergosterol **(1)**.

The observation that the uv spectrum of provitamin D changed with uv irradiation and also produced antirachitic activity led to the conclusion that vitamin D was derived from the provitamin. Windaus found the vitamin D_2 formula $C_{28}H_{44}O$ to be isomeric with the provitamins.

Bunal in 1932 used x-ray crystallography to demonstrate that many of the preparations described in the literature were mixtures and that vitamin D_2 was one crystal structure.

Calciferol, when hydrogenated catalytically, took up 4 moles of hydrogen and gave a compound with the empirical formula $C_{28}H_{52}O$. Sodium in ethanol reduction gave a dihydroproduct that reacted with 3 moles of perbenzoic acid, thus demonstrating the derivative to have three double bonds.

Ozonolysis of vitamin D_2 gave 2,3-dimethylbutanol, showing the side chain to contain the 22 double bond and a C_{24} methyl group.

Vitamin D_2 reacted with maleic anhydride to give a mono Diels-Alder adduct, which hydrolyzed to yield a dicarboxylic acid. Acetylation of the alcohols, esterification of carboxylic acids, and hydrogenation gave a compound that, when ozonized, gave a saturated ketone, $C_{19}H_{34}O$. This molecule contains the C and D ring and side chain from vitamin D_2, indicating that the B ring must have been opened by photolytic cleavage of the C_9–C_{10} bond (12). The vitamin D_2 molecule was thus shown to be a tricyclic material with four double bonds.

Windaus and Boch (13) isolated and characterized 7-dehydrocholesterol in 1937 from pig skin. They further showed that vitamin D_3 could be generated from the provitamin by uv irradiation.

Irradiated ergosterol was found not to be as antirachitic in the chick as in the rat, whereas the chick could be protected by direct irradiation. The provitamin in cholesterol was shown not to be ergosterol. Rygh (14) in 1935 found that 1 rat unit of cod liver oil was 100 times more potent in chicks than 1 rat unit of vitamin D_2. Brockmann (15) in 1936, prepared the pure crystalline 3,5-dinitrobenzoate derivative of vitamin D_3 obtained from tuna liver oil (subsequently demonstrated in other species like halibut and blue fin tuna). This material was shown to be identical to the dinitrobenzoate derivative of vitamin D_3 obtained by Windaus from the irradiation of 7-dehydrocholesterol. Thus, the chemical identity of vitamin D_2 and D_3 were well established. A

Table 2. Occurrence of the Provitamins D in Selected Plants and Animals, Parts per Thousand of Total Sterol

Source	Amount	Source	Amount
cottonseed oil	28	gallstones, man	0.25
rye grass	15	*Mytilus edulis*, sea mussel	100
wheat germ oil	10	*Modiolvs demissus*, ribbed mussel	370
carrot	1.7	*Ostrea virginica*, oyster	80
cabbage	0.5	*Ostrea edulis*, oyster	34
skin, pig	46	*Asterias rubens*, starfish	3.8
skin, chicken feet	25	common sponges	20
skin, rat	19	common coral	10
skin, mouse	9	*Aspergillus niger*, mold	1000
skin, calf	7	*Cortinellus shiitake*, mushroom	1000
skin, human infant	1.5	*Claviceps purpurea*, ergot	9000
skin, human adult	4.2	*Saccharomyces cerevisiae*, yeast	800
liver, Japanese tuna	11	*Penicillium puberculum*, mold	1000
liver, Atlantic cod	4.4	*Fucus vesiculosus*, alga, seaweed	0.8
liver, shark	1.0	*Tubifex* sp, waterworm	210
liver, halibut	0.6	*Bumbricus terrestris*, earthworm	170
liver, tuna	1.0	*Tenebrio molitor*, mealworm	120
eggs, Chinese duck	60	*Gyronomus*, sp, goat	61
eggs, cod (roe)	5.5	*Cancer pagurus*, common crab	15
eggs, hen	1.6	*Dephnia* sp, water flea	7.5
wool fat, sheep	3.9	*Musca domestica*, housefly	7.0
milk, cow	2.3	*Crangon vulgaris*, shrimp	3.8
pancreas, beef	1.8	*Homarus vulgaris*, lobster	2.5
spinal cord, beef	1.2	*Helix pomatia*, edible snail	97
blood serum, cow	0.5	*Arion empiricorum*, slug, red road snail	220
herring oil	0.5	*Littorina littorea*, periwinkle	170
heart, calf	0.32	*Sepia* sp, cuttlefish	12

more detailed description of the events leading to the structure elucidation of the vitamin Ds can be found in Reference 6.

1.2. Occurrence

The provitamins, precursors of the vitamin Ds, are distributed widely in nature, whereas the vitamins themselves are less prevalent. The amounts of provitamins D_2 and D_3 in various plants and animals are listed in Table 2.

Fish-liver oil, liver, milk, and eggs are good natural sources of the D_3 vitamin. Most milk sold in the United States is fortified with manufactured vitamin D. Fish oil is the only commercial source of natural vitamin D_3, and the content of the vitamin varies according to species as well as geographically, ie, Atlantic cod contain 100 IU/g where IU (International Unit) = 0.025 μg of vitamin D_3, whereas oriental tuna (*Percomorpli*) contain 45,000 IU/g oil.

Vitamin D_3 rarely occurs in plants. However, *Solanum glaucophyllum*, *Solanum malacoxylon*, *Cestrum diurnum*, and *Trinetum flavescens* have been shown to contain water-soluble glycosides of vitamin D analogues with 1 α,25-dihydroxy-vitamin D activity (16–22). The vitamin D content in various plant and animal materials is shown in Table 3. Vitamin D_3 occurs naturally in all animals (24).

Table 3. Distribution of Vitamin D Activity[a]

Sample	Amount[b]
phytoplankton	0
sargassum (a gulfweed)	some activity
clover hay	
sun-cured	slight
dark-cured	0
mushrooms (*Agaricus campestris*)	0.21 IU/g
milk (unfortified)	
winter (bovine)	5.3 IU/L
summer (bovine)	53 IU/L
milk (human)	63 IU/L
milk colostrum (human)	315–635 IU/L
egg yolk	150–400 IU/g
butter	4–8 IU/g
fish-liver oils	50–45,000 IU/g

[a]Ref. 23.
[b]IU (International Unit) = 0.025 μg of vitamin D_3.

2. Chemical and Physical Properties

2.1. Provitamin

The chemistry of the D vitamins is intimately involved with that of their precursors, the provitamins. The manufacture of the vitamins and their derivatives usually involves the synthesis of the provitamins, from which the vitamin is then generated by uv irradiation. The chemical and physical properties of the provitamins are discussed below, followed by the properties of the vitamins.

3 β-Hydroxy steroids which contain the 5,7-diene system and can be activated with uv light to produce vitamin D compounds are called provitamins. The two most important provitamins are ergosterol **(1)** and 7-dehydrocholesterol **(3)**. They are produced in plants and animals, respectively, and 7-dehydrocholesterol is produced synthetically on a commercial scale. Small amounts of hydroxylated derivatives of the provitamins have been synthesized in efforts to prepare the metabolites of vitamin D, but these products do not occur naturally. The provitamins do not possess physiological activities, with the exception that provitamin D_3 is found in the skin of animals and acts as a precursor to vitamin D_3, and synthetic dihydroxalated analogues of pro- and previtamins have been found to have selective activity towards nuclear and nonnuclear receptors (24–27).

2.2. Provitamin D_2

Ergosterol is isolated exclusively from plant sources. The commercial product is ca 90–100% pure and often contains up to 5 wt % of 5,6-dihydroergosterol. Usually, the isolation of provitamin D_2 from natural sources involves the isolation of the total sterol content, followed by the separation of the provitamin from the other sterols. The isolation of the sterol fraction involves extraction of the total fat component, its saponification, and then reextraction of the unsaponifiable portion with an ether. The sterols are in the unsaponifiable portion. Another method is the saponification of the total material, followed by isolation of the nonsaponifiable fraction. Separation of the sterols from the unsaponifiable fraction is done by crystallization from a suitable solvent, eg, acetone or alcohol. Ethylene dichloride, alone or mixed with methanol, has been used commercially for recrystallization. In the case of yeasts, it is particularly difficult to remove the ergosterol by simple extraction, thereby obtaining only ca 25% recovery. Industrially, therefore, the ergosterol is obtained by preliminary

digestion with hot alkalies or with amines (28–33). Variations of the isolation procedure have been developed. For example, after saponification, the fatty acids may be precipitated as calcium salts, which tend to absorb the sterols. The latter are then recovered from the dried precipitate by solvent extraction.

2.3. Provitamin D_3

Provitamin D_3 is made from cholesterol, and its commercial production begins with the isolation of cholesterol from one of its natural sources. Cholesterol occurs in many animals, and is generally extracted from wool grease obtained by washing wool after it is sheared from sheep. This grease is a mixture of fatty-acid esters, which contain ca 15 wt % cholesterol. The alcohol fraction is obtained after saponification, and the cholesterol is separated, usually by complexation with zinc chloride, followed by decomplexation and crystallization. Cholesterol can also be extracted from the spinal cords and brains of animals, especially cattle, and from fish oils.

Cholesterol 1 is converted to 7-dehydrocholesterol **(3)** (see Fig. 1). This process usually involves the Ziegler allylic bromination of the 7 position followed by dehydrobromination (34). Esterification of the cholesterol (8) is necessary to prevent oxidation of the 3 β-alcohol by the brominating agent. Allylic bromination may be accomplished with a variety of brominating agents, eg, *N*-bromosuccinimide, *N*-bromophthalimide, or preferably 5,5-dimethyl-1,3-dibromohydantoin (35). Bromine in carbon disulfide can be used if the free-radical bromination is photocatalyzed (36). A mixture of 7 α- 1 and 7 β-bromo cholesteryl esters 1 is obtained and treated with an appropriate base to dehydrohalogenate the molecule and give the 7-dehydrocholesteryl ester 1 (37, 38). Proper conditions for this reaction are necessary to generate a high yield of the desired 7-dehydro product instead of the undesired cholesta-4,6-dien-3β-ol ester **(12)**, which is formed as a by-product. Various reagents can be used to perform the dehydrohalogenation. Trimethyl phosphite or pyridine bases, particularly trimethylpyridine, have been used; the symmetrical collidine is the reagent of choice (35, 38, 39). *t*-Butylammonium fluoride has also been used to improve the yield of high quality 5,7-diene (40).

The 7 α-bromo steroid 1 can also be treated with sodium phenyl selenolate (41). The resultant 7 β-phenyl selenide 1 can be oxidized and the corresponding phenyl selenoxide eliminated to form the 7-dehydrocholesteryl ester 1.

7-Dehydrocholesterol has also been made from cholesterol by the Windaus procedure (Fig. 2); the 3,7-dibenzoate 2 is obtained (via 2 and 2 by oxidation and reduction), which undergoes thermal elimination to give the 7-dehydrocholesteryl benzoate 1 (42–44). However, the yields are substantially lower than those achieved by the bromination–dehydrobromination method.

7-Tosylhydrazone and 7-phenyl sulfoxide groups have also been introduced into cholesterol and eliminated to prepare the 5,7-diene (45, 46). The method of choice is the allylic bromination–dehydrobromination procedures, and the commercial yields in converting cholesterol to 7-dehydrocholesterol are in the range of 35–50%.

2.4. Vitamin D

The irradiation of the provitamins to produce vitamin D as well as several isomeric substances was first studied with ergosterol. The chemistry is identical for the vitamin D_3 series and yields analogous isomers. In 1932, a scheme for the irradiation of ergosterol leading to vitamin D_2 was proposed (45). Twenty years later, the mechanism of the irradiation of the provitamins to vitamin D and its photoisomers was further elucidated (46, 47). More recently, Jacobs (48) has reviewed the photochemistry. A number of products associated with the irradiation process are shown in Figure 3. The geometry and electronic characteristics of these molecules have been well established by x-ray crystallographic analysis and valence force-field calculations (50–52). The irradiation process, which converts 7-dehydrocholesterol to vitamin D also occurs in the skin of animals if sufficient sunlight is available. The photochemical and thermal isomerizations occur during the generation

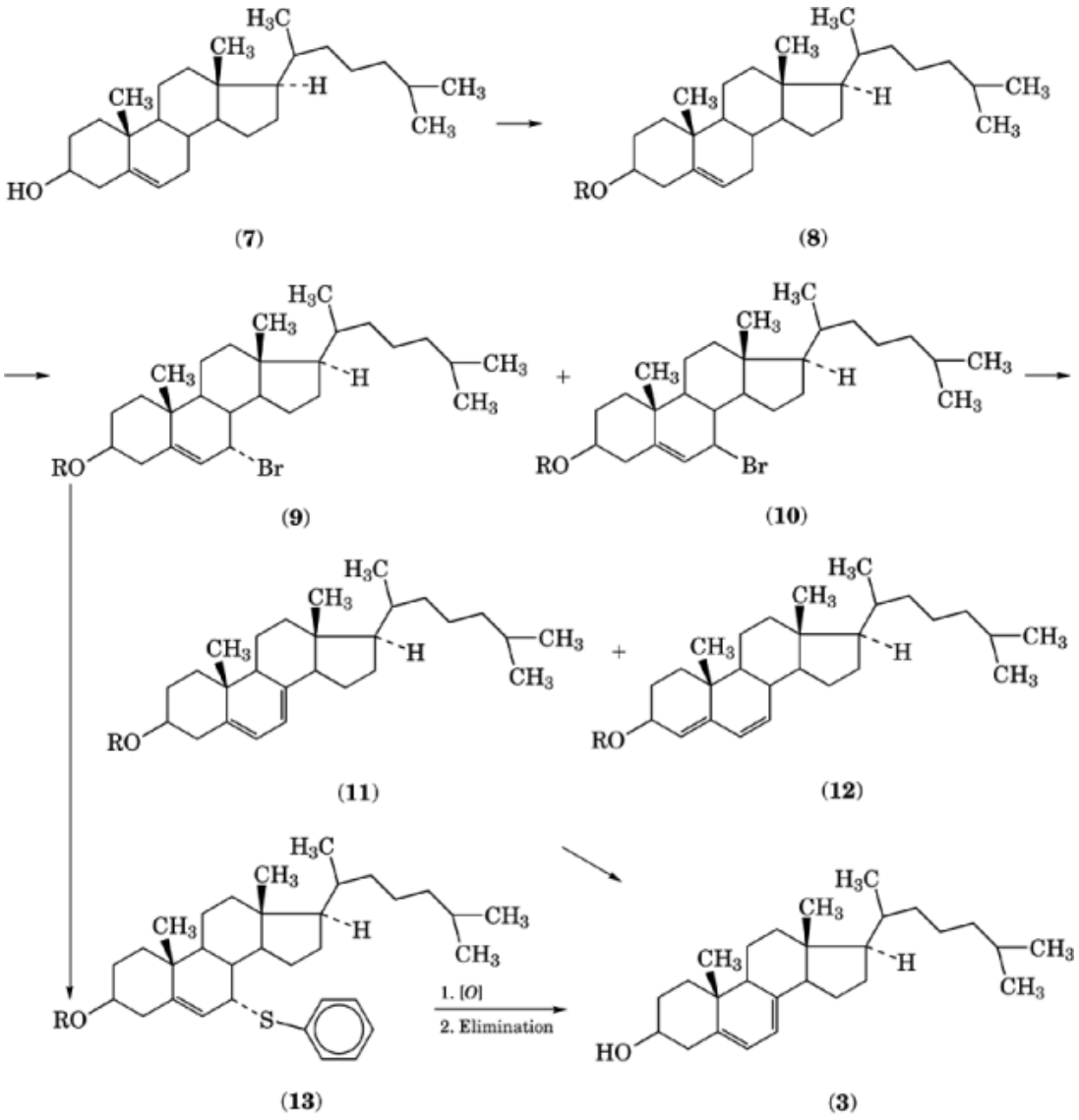


Fig. 1. Conversion of cholesterol to 7-dehydrocholesterol.

of vitamin D *in vivo* as well as during its synthetic photochemical preparation (24, 53, 54). The initial step involves ring opening of the B-ring of the sterol by ultraviolet activation of the conjugated diene. The absorbance of uv energy activates the molecule, and the $\pi \longrightarrow \pi^*$ excitation (absorption, 250–310 nm; $\lambda_{max} = 291$ nm, $\epsilon = 12,000$) results in the opening of the 9,10 bond and the formation of the (*Z*)-hexadiene, previtamin D_3 (R) [1173-13-3] 3 or previtamin D_2 (R′) [21307-015-1] 3. The uv irradiation of 7-dehydrocholesterol or ergosterol results in the steady diminution in concentration of the provitamin, initially giving rise to predominantly previtamin D. The pre- levels reach a maximum as the provitamin level drops below ca 10%. The concentration of the previtamin then falls as it is converted to tachysterol and lumisterol, which increase in concentration with continued irradiation (see Fig. 4). Temperature, frequency of light, time of irradiation, and concentration

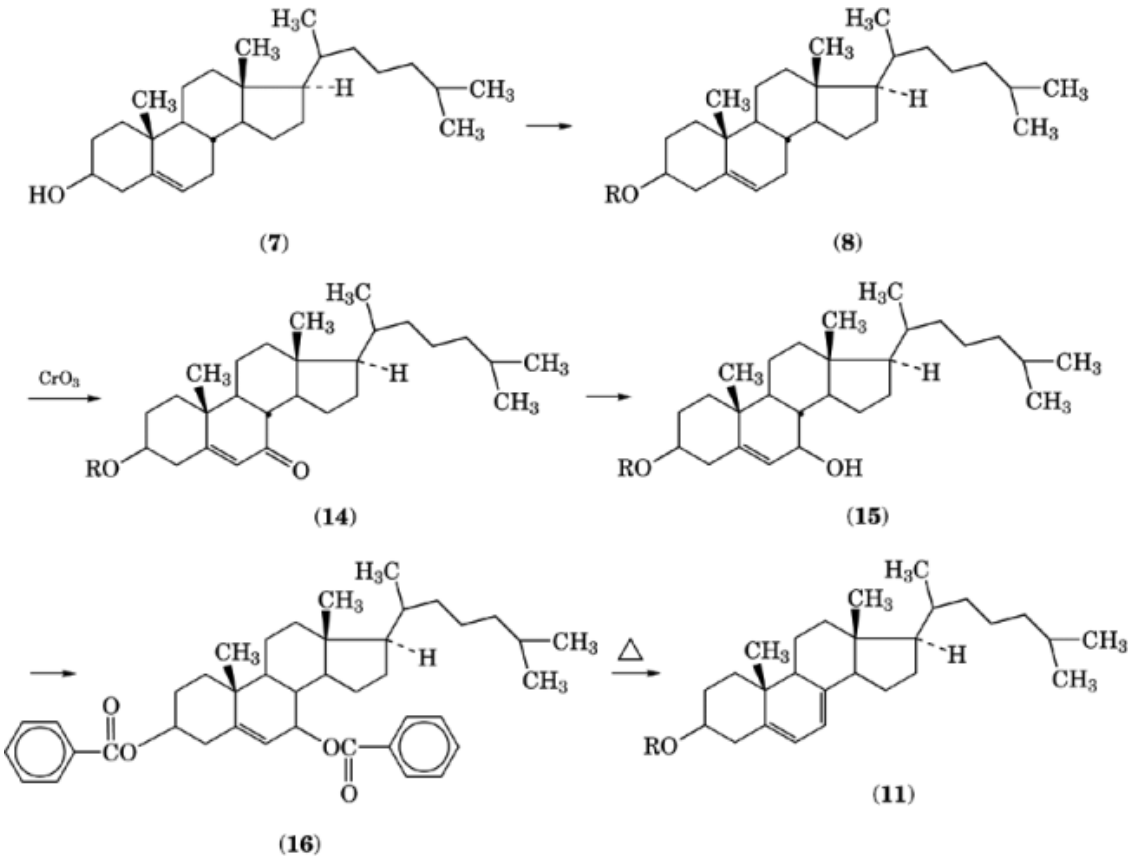


Fig. 2. The Windaus procedure (42).

of substrate all affect the ratio of products. Previtamin D undergoes thermal equilibration to vitamin D **(2)** or **(4)**, *cis*-vitamin D_2 [50-14-6] and *cis*-vitamin D_3 [67-97-0], respectively. The conversion of previtamin D 3 or 3 at temperatures of $\leq 80^{\circ}C$ by thermal isomerization to give the cis vitamin (ergocalciferol) **(2)** or cholecalciferol **(4)** involves an equilibrium, as indicated in Table 4 (55, 56).

The equilibrium composition is normally ca 80% vitamin D and 20% previtamin D. This reaction is an intramolecular {1–7} H sigmatropic shift and occurs through a rigid cyclic transition state (57).

Additionally, the $\pi \longrightarrow \pi^*$ excitation of previtamin D can result in ring closure back to the provitamin **(1)** [57-87-4] or **(3)** [434-16-2] or to lumisterol$_2$ [474-69-1] 3 or lumisterol$_3$ [5226-01-7] 3, which have the 9 β,10 α configuration. This excitation can also exhibit $(Z) \rightleftharpoons (E)$ photoisomerization to the 6,7-(E)-isomer, tachysterol$_2$ [115-61-7] 3 or tachysterol$_3$ [63902-44-3] 3 (58, 52).

Other photoinduced cyclization reactions can occur by conrotatory bond formation to give the 9 β,10 β-antiisomers, isopyrocalciferol$_2$ [474-70-4] 5 or isopyrocalciferol$_3$ [10346-44-8] 5 (Fig. 5), whereas thermal cyclization at $>100^{\circ}C$ leads to the two 9,10-syn isomers, (9α,10 α)-pyrocalciferol 5 [128-27-8] or 5 [10346-43-7] by a disrotatory bond formation mechanism (47). Ultraviolet over-irradiation leads to photopyro- 5 [41411-05-6] or (30) and photoisopyrocalciferols 5 [26241-65-6] or 5 [85354-28-5], respectively and to the formation of suprasterols of the type shown in structure 6 (59, 60) (Fig. 6). Prolonged irradiation of the mixture of isomers can also lead to toxisterols of the type shown in structures 6 and 6, where R = D_2 and R′ = D_3 side chains (see

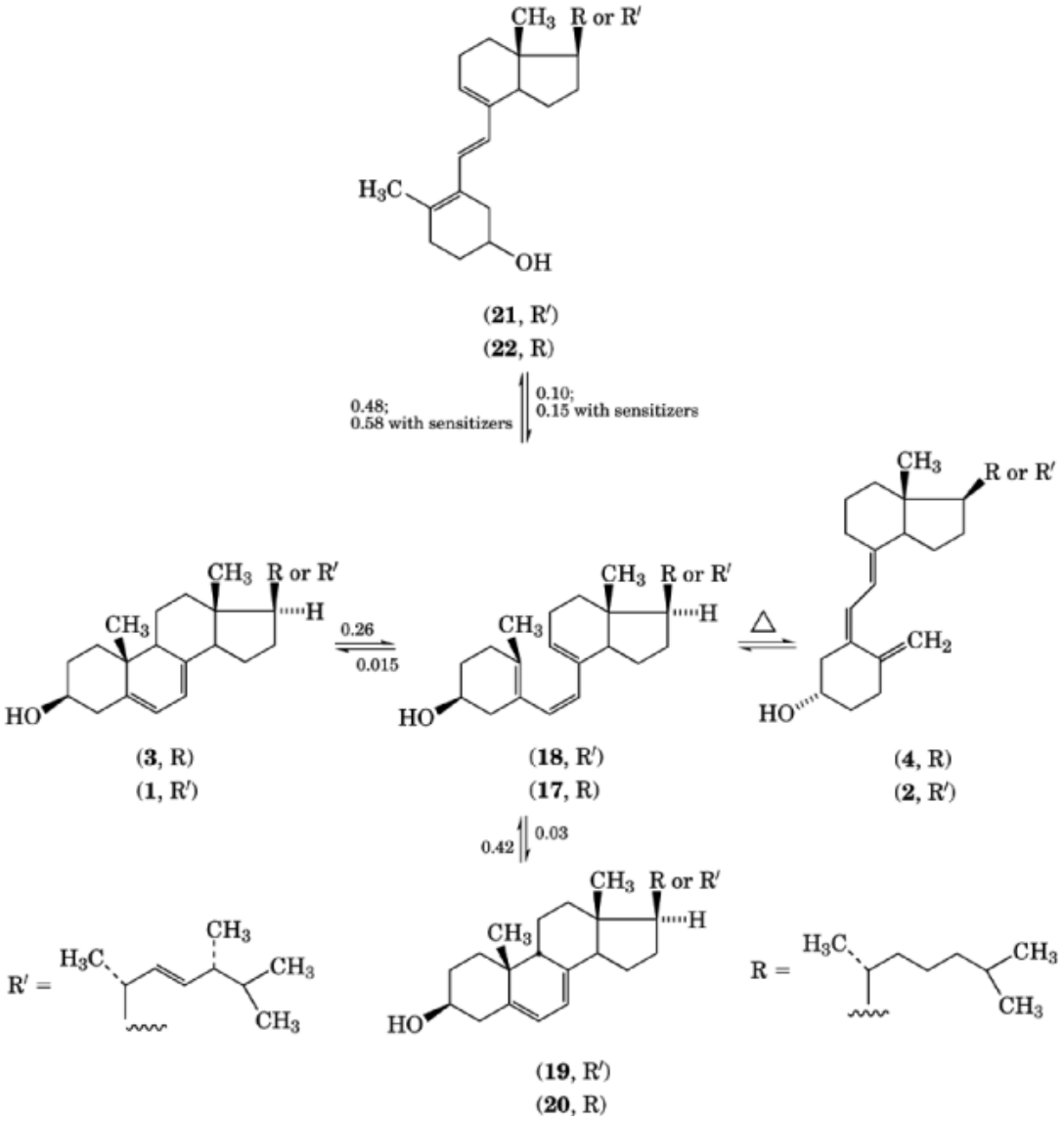


Fig. 3. Photochemical and thermal isomerization products of vitamin D manufacture (49). The quantum yields of the reactions are listed beside the arrows for the given reactions.

Fig. 3) (61–63). More than 20 members of this type of substance have been identified. There is little evidence that these materials are toxic; however, their nomenclature leads to some misunderstanding. These compounds generally show little if any biological activity and have not been found *in vivo* (15, 63–66). Normal irradiation conditions for the production of vitamin D include sufficiently low temperatures so that these products do not form, and they are not found in normal commercial samples of D_3 resins.

The irradiation of calciferol in the presence of iodine leads to the formation of 5,6-*trans*-vitamin D_2 [14449-19-5] 5 or D_3 [22350-41-0] 5 (67, 68). 5,6-*trans*-Vitamin D as well as vitamin D **(2)** or **(4)** can be converted to isovitamin D by treatment with mineral or Lewis acids. Isocalciferol 5 [469-05-6] or 5 [42607-12-5] also forms

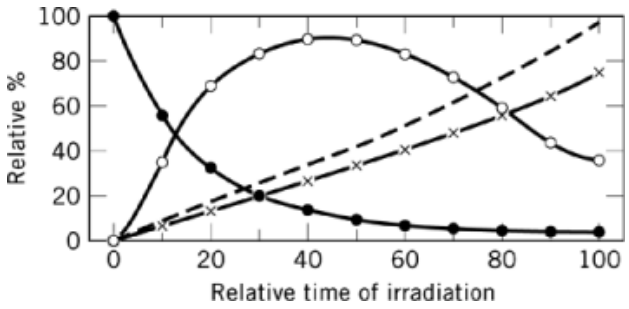


Fig. 4. Time course for uv-irradiation of 7-dehydrocholesterol (•): (○), previtamin D; (×), lumisterol; (---), tachysterol.

Table 4. Inverconversion Time of Previtamin D_3 and Vitamin D_3 at 20°C, and 40°C[a]

	Vitamin D_3 from previtamin D_3			Previtamin D_3 from vitamin D_3	
Formation, %	Days at −20°C	Days at 20°C	Hours at 40°C	Days at 20°C	Hours at 40°C
2	27	0.2	0.4	2.2	3.7
5	68	0.4	1.1	8.2	11.2
7	96	0.5	1.5		18.6
10	140	0.8	2.3		43.3
20	269	1.6	4.8		
30	474	2.6	7.8		
40	681	3.8	11.3		
50	926	5.2	15.6		
60	1230	7.0	21.2		
70	1628	9.5	29.2		
80	2204	13.3	43.4		
90	2910	23.3			

[a]Ref. 55.

upon heating of 5,6-*trans*-vitamin D. Isotachysterol 5 [469-06-7] or 5 [22350-43-2] forms from isocalciferol or vitamin D upon treatment with acid, and its production appears to be the result of sequential formation of *trans*- and isocalciferol from calciferol. These reactions are the basis of the antimony trichloride test for vitamin D (69–72).

Commercially, the irradiation of the 5,7-diene provitamin to make vitamin D must be performed under conditions that optimize the production of the previtamin while avoiding the development of the unwated isomers. The optimization is achieved by controlling the extent of irradiation, as well as the wavelength of the light source. The best frequency for the irradiation to form previtamin is 295 nm (64–66). The unwanted conversion of previtamin to tachysterol is favored when 254 nm light is used. Sensitized irradiation, eg, with fluorenone, has been used to favor the reverse, triplet-state conversion of tachysterol to previtamin D (73, 74).

The molecular extinction coefficients (at various wavelengths) of the four main components of the irradiation are shown in Table 5. The absorption of light above 300 nm is favored by tachysterol. A yield of 83% of the previtamin at 95% conversion of 7-dehydrocholesterol can be obtained by irradiation first at 254 nm, followed by reirradiation at 350 nm with a yttrium aluminum garnet (YAG) laser to convert tachysterol to previtamin D. A similar approach with laser irradiation at 248 nm (KrF) and 337 nm (N_2) has also been described (76).

The irradiation of the provitamin has been achieved using the acetate and benzoate esters, although the free alcohol form of the provitamin is usually used (77).

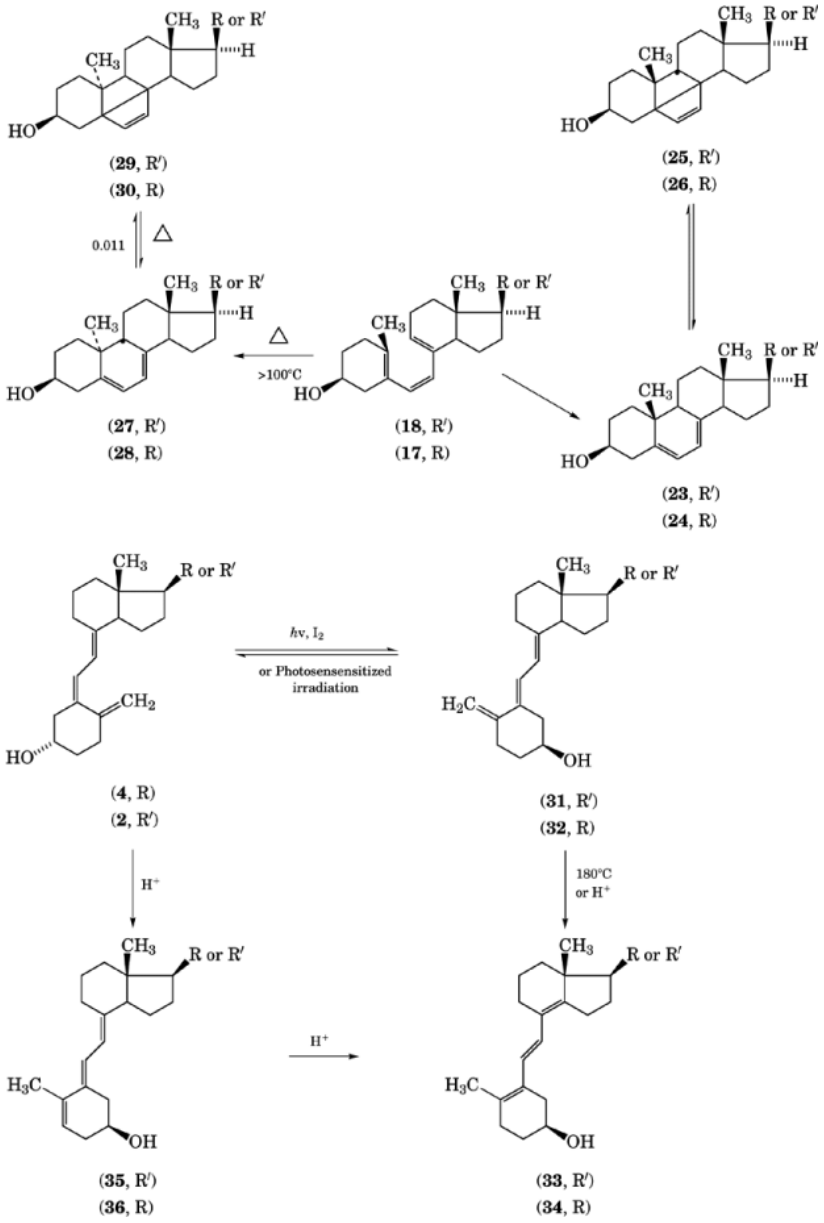
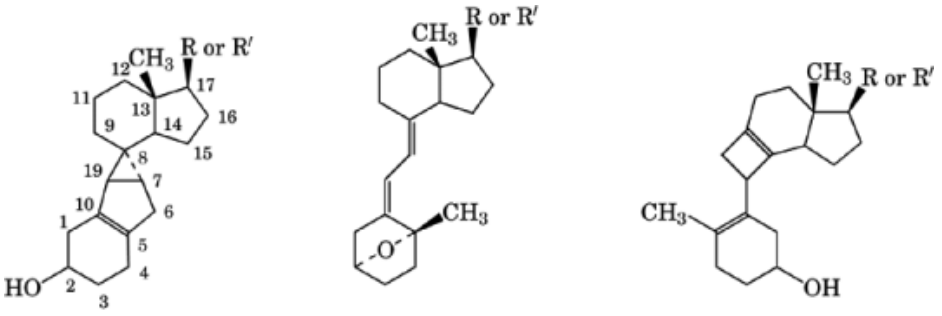


Fig. 5. Products of the isomerization of pre- and *cis*-vitamin D.

2.5. Physical Properties

The physical properties of the provitamins and vitamins D_2 and D_3 are listed in Table 6. The values are listed for the pure substances. The D vitamins are fat-soluble and, as such, are hydrophobic.



(37) (38) (39)

Fig. 6. Products of overirradition of vitamin D: suprasterol II (**37**), toxisterol-E (**38**), and toxisterol E_1 (**39**).

Table 5. Molecular Extinction Coefficients of Irradiation Products[a]

λ, nm	7-Dehydrocholesterol	Previtamin D_3	Tachysterol$_3$	Lumisterol$_3$
254	4,500	725	11,450	4,130
300	1,250	930	11,250	1,320
330	25	105	2,940	30
340	20	40	242	25
350	10	25	100	20

[a]Ref. 75.

2.6. Shipping and Handling

Vitamin D and its products are sensitive to uv light, heat, air, and mineral acids. Its sensitivity to these conditions is exaggerated by the presence of heavy-metal ions, eg, iron. Care should be taken to store and ship vitamin D and its various product forms so that exposure to these conditions is minimized. Pharmaceutical grade vitamin D_3 is supplied in the pure crystalline form. This is used in vitamin D as well as multivitamin preparations. Commercial sources of feed-grade vitamin D are usually the vitamin D_3 resin stabilized by spray- or roll drying a starch or gelatin suspension of the vitamin. These products should be stored in a cool, dry place in opaque, hermetically sealed containers under nitrogen. Vitamin D is generally recognized as safe when used in accordance with good manufacturing or feeding practices (79).

Shipping vitamin D in crystalline or resin form should be done in containers marked appropriately to indicate the material is toxic by DOT standards. Its proper DOT labeling is DOT Hazard Class 6.1, poisonous. Waste material should be burned or placed in an appropriate landfill.

The provitamins are also unstable to heat and light. They generally should be stored in a dark, cool, dry place. The provitamin is more stable if shipped with 10–15 wt % methanol rather than in a dry form.

3. Analytical and Test Methods

The development of reliable uv analysis permitted the dependable detection and assay of the provitamins and vitamins. Prior to this, the Lieberman-Bouchard chemical test was used, but the color reaction gave many false positives and was relatively inaccurate.

In 1949 the World Health Organization adopted the biological activity of 1 mg of an oil solution containing 0.025 μg of crystalline D_3 as the analytical standard for vitamin D_3. This standard was discontinued in 1972. USP uses crystalline cholecalciferol as a standard (80). Samples of reference standard may be purchased from

Table 6. Physical Properties of Provitamins and Vitamins D_2 and D_3

Properties	Substance			
	7-Dehydro-cholesterol	Ergosterol	Vitamin D_2 (ergocalciferol)	Vitamin D_3 (cholecalciferol)
melting point, °C	150–151	165	115–118	84–85
color and form	solvated plates from ether–methanol	hydrated plates from alcohol; needles from acetone	colorless prisms from acetone	fine colorless needles from dilute acetone
CAS Registry Number	[434-16-2]	[57-87-4]	[50-14-6]	[67-97-0]
optical rotation (α^{20}_{D}), °				
acetone			82.6	83.3
ethanol			103	105–112
chloroform	−113.6	−135	52	51.9
ether			91.2	
petroleum ether			33.3	
benzene	−127.1			
coefficient of rotation per °C in alcohol			0.515	
uv max, nm	282	281.5	264.5	264.5
specific absorption, E_{max}, at 1% conc	308		458.9 ± 7.5	473.2 ± 7.8
potency[a], IU/g			40×10^6	40×10^6
biological activity			in mammals	in mammals and birds
chicken efficacy, %			8–10[b]	100
solubility, g/100 mL				
acetone at 7°C			7	
acetone at 26°C			25	
absolute ethanol	sl sol		28	
at 26°C		0.15		
at Δ°C		2.2		
ethyl acetate at 26°C			31	
water	insol	insol	insol	insol

[a]The international standard for vitamin D is an oil solution of activated 7-dehydrocholesterol (3). The IU is the biological activity of 0.025 μg of pure cholecalciferol.
[b]Studies have claimed an efficacy as high as 10% (77).

U.S. Pharmacopeial Convention, Inc., Reference Standards Order Department, 12601, Twinbrook Parkway, Rockville, Maryland 20852. One international unit of vitamin D activity is that activity demonstrated by 0.025 μ of pure crystalline *cis*-vitamin D_3. One gram of vitamin D_3 is equivalent to 40×10^6 IU or USP units. The international chick unit (ICU) is identical to the USP unit.

USP also issues vitamin D_3 capsules for AOAC determination in rats and an oil solution for the vitamin D_3 AOAC determination in chicks. Historically, the following units (shown with their approximate international unit equivalence) have been used but are currently abandoned: 1 clinical unit = $12 - 17$ IU; 1 biological unit = 0.125 IU; 1 protection unit = 0.125 IU; 1 Laquer unit = 0.14 IU; 1 Poulson unit = 0.2 IU; 1 Steenbach unit = 3 IU. The MRC, ICU, and Coward units all approximated the international unit and are also no longer in common use.

The standard chemical and biological methods of analysis are those accepted by the *United States Pharmacopeia XXIII* as well as the ones accepted by the AOAC in 1995 (81–84). The USP method involves saponification of the sample (dry concentrate, premix, powder, capsule, tablet, or aqueous suspension) with aqueous alcoholic KOH; solvent extraction; solvent removal; chromatographic separation of vitamin D from extraneous

Table 7. Methods for Chromatographic Separation of Vitamin D and Related Steroid[a]

Method	Comments
	Paper chromatography
quinoline-impregnated paper	
reversed-phase paper	
column chromatography	
alumina	
floridin	
celite	useful for multivitamin tablets
silicic acid	useful for metabolites
factice	can resolve vitamin D_2 and vitamin D_3
Sephadex LH-20	highly useful for metabolites
	High pressure liquid chromatography
Zorbax-Sil support	separates 24(*R*), 25-dihydroxy-vitamin D_3 from 24(*S*), 25-dihydroxy-vitamin D_3
	useful for metabolites
	assay of 25-hydroxy-vitamin D_3
ODS[b]-Permaphase	useful for vitamin D metabolites
silica gel	commercial vitamin D_3 assay
	Thin-layer chromatography
silicic acid	effective for irradiation mixtures
silica gel G	two-dimensional
	Gas chromatography
	separates trimethylsilyl ethers of vitamin D_3
	cyclizes vitamin D_3
	useful for 25-hydroxy-vitamin D_3
	useful for multivitamin tablets
	useful for vitamin D_2 in milk

[a] Ref. 86.
[b] ODS = octadecyl (C_{18}) silane.

ingredients; and colormetric determination with antimony trichloride and comparison with a solution of USP cholecalciferol reference standard.

The AOAC (978.42) recognizes a similar procedure, except that the unsaponifiable material is treated with maleic anhydride to remove the trans-isomer which may possibly be present (83). The antimony trichloride colorimetric assay is performed on the trans-isomer-free material. This procedure cannot be used to distinguish certain inactive isomers, eg, isotachysterol; if present, these are included in the result, giving rise to a falsely high analysis. A test must therefore be performed to check for the presence of isotachysterol.

Preferably, high pressure liquid chromatography (hplc) is used to separate the active pre- and cis-isomers of vitamin D_3 from other isomers and allows their analysis by comparison with the chromatograph of a sample of pure reference *cis*-vitamin D_3, which is equilibrated to a mixture of pre- and cis-isomers (82, 84, 85). This method is more sensitive and provides information on isomer distribution as well as the active pre- and cis-isomer content of a vitamin D sample. It is applicable to most forms of vitamin D, including the more dilute formulations, ie, multivitamin preparations containing at least 1 IU/g (AOAC Methods 979.24; 980.26; 981.17; 982.29; 985.27) (82). The practical problem of isolation of the vitamin material from interfering and extraneous components is the limiting factor in the assay of low level formulations.

A number of methods have been developed for the chromatographic separation of vitamin D and related substances and can be found in Table 7.

Table 8. Approximate uv Absorbance of 7-Dehydrocholesterol, Pre- and *cis*-Vitamin D

	(E 1% 1 cm)		
Absorbance at μg	7-Dehydrocholesterol	Previtamin D	*cis*-Vitamin D
230	35	190	250
235	40	170	280
240	50	210	325
245	60	235	360
250	85	250	400
255	120	260	430
260	150	270	460
265	200	265	470
270	270	250	450
273	282		
275	250	220	420
277	240		
280	290	180	340
282	293		
285	240	150	300
290	155	100	180
295	170	70	120
300	70	50	80

3.1. Biological Assay

The USP and AOAC recognize a biological method for the determination of vitamin D. The rat line test, however, is slow, expensive and not as accurate as the chemical or chromatographic methods. Rachitic rats are fed diets containing the vitamin D sample. This test measures bone growth on the proximal end of the tibia or distal end of the ulna, which is visualized by staining with silver nitrate. This test is not applicable to products offered for poultry feeding. The AOAC recognizes another procedure which measures the vitamin D sample activity in increasing bone ash of growing chicks compared to the activity of a USP cholecalciferol reference standard. It too is slow, expensive, and gives variable results.

3.2. Physical Methods

Vitamins D_2 and D_3 exhibit uv absorption curves that have a maximum at 264 nm and an E_{max} (absorbance) of 450–490 at 1% concentration (Table 8). The various isomers of vitamin D exhibit characteristically different uv absorption curves. Mixtures of the isomers are difficult to distinguish. However, when chromatographically separated by hplc, the peaks can be identified by stop-flow techniques based on uv absorption scanning or by photodiodearray spectroscopy. The combination of elution time and characteristic uv absorption curves can be used to identify the isomers present in a sample of vitamin D.

Infrared and nmr spectroscopy have been used to help distinguish between vitamins D_2 and D_3 (87–89). X-ray crystallographic techniques are used to determine the vitamin D structure, and gas chromatography also is a method for assaying vitamin D (49, 90–95).

3.3. Provitamin D

The molecular extinction coefficient of 7-dehydrocholesterol at 282 nm is 11,300 and is used as a measure of 7-dehydro isomer content of the provitamin (96, 97). High pressure liquid chromatography can also be used to

Table 9. Chemical Test Methods for Provitamin D

Name of reaction	Components	Results	Interpretation
revised Salkowski reaction	$CHCl_3$ + H_2SO_4 (conc)	deep red acid layer	differentiates from sterols lacking conjugated diene (red color in $CHCl_3$ layer) acid gives green fluorescence
Lieberman-Burchard reaction	$CHCl_3$; acetic acid–H_2SO_4 added dropwise	red color develops and changes to blue-violet to green	can be quantitative; acts similarly, but red color lasts longer
Tortelli-Jaffé reaction	acetic acid + 2 wt% Br_2 in $CHCl_3$	green	sterols with ditertiary double bonds; vitamin D and compounds that give similar bonds upon isomerization or reaction
Rosenheim reaction	$CHCl_3$+ trichloroacetic acid in H_2O	red color develops and changes to light blue	
Rosenheim reaction	$CHCl_3$+ lead tetraacetate in CH_3COOH is added; then trichloroacetic acid is added	green fluorescence	not given by esters of provitamin D; can be used to distinguish be-tween provitamin and provitamin ester; sensitive to 0.1 μg and is quantitative
chloral hydrate	mixture of crystalline provita-mins and chloral hydrate heated slowly; melts at 50°C	color develops and changes red to green to deep blue	other sterols, eg, cholesterol, do not react to give color
antimony trichloride reaction	$CHCl_3$+ $SbCl_3$	red color	
Chugaev reaction	glacial acetic acid plus acetyl chloride and zinc chloride heated to boiling	eosin-red greenish yellow fluorescence	1:80,000 sensitivity

analyze the provitamins. There are a variety of chemicals that show characteristic colors when reacted with the provitamins. Some of these are listed in Table 9.

The extremely low levels of vitamin D and its metabolites in biological systems make it very difficult to assay these products by traditional methods. Calcium-binding protein is not found in the intestinal mucosa of vitamin D-deficient animals. It is synthesized only in response to the presence of a material with vitamin D activity. Thus, using antiserum specific to intestinal calcium-binding protein, a radioimmunodiffusion assay (98) conducted on homogenates of intestinal mucosa of chicks fed the test material for 7–10 d allows assay of the material for vitamin D activity down to 1–200 IU. The development of this technique has allowed research to be conducted since the mid-1970s to elucidate the vitamin D metabolism and biochemistry and is now used routinely to assay vitamin D-active materials in clinical as well as research samples (6, 40, 51, 53, 55).

4. Synthesis

4.1. Manufacture

Most of the vitamin D produced in the world is made by the photochemical conversion of 7-dehydrocholesterol. Ergosterol is not used as extensively as it once was, because it offers no real price advantage and, upon irradiation, it gives vitamin D_2, which has been shown to be less active in many species. The pig, chicken, cow, and horse have been shown to discriminate against vitamin D_2 (99). Irradiation of 7-dehydrocholesterol or ergosterol is carried out by dissolving the steroid in an appropriate solvent, eg, peroxide-free diethyl ether. Solvents such as methanol, cyclohexane, and dioxane have also been used. The cooled solution is pumped through uv-transparent quartz reactors which permit the light from high pressure mercury lamps to impinge

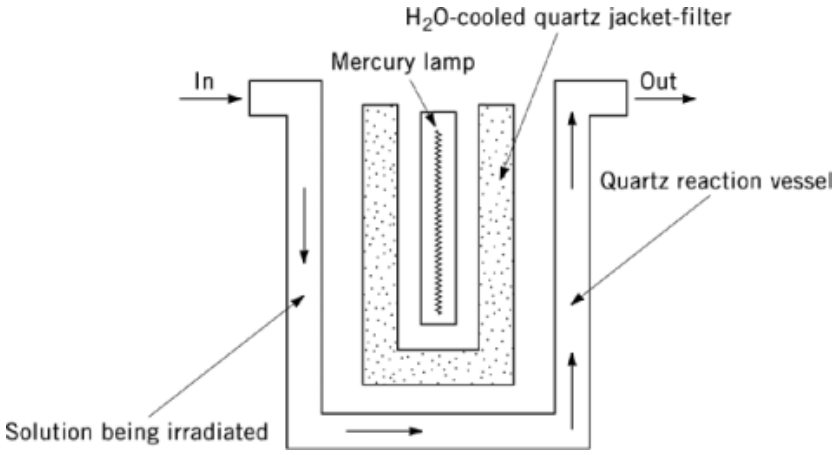


Fig. 7. Ultraviolet-light-transparent quartz reactor for sterol irradiation.

upon the solution (Fig. 7). The solution is recycled until the desired degree of irradiation has been achieved. This results in a mixture of unreacted 7-dehydrosterol, previtamin D, vitamin D, and irradiation by-products.

Among the light sources used for irradiation are carbon arcs, metal-corded carbon rod, magnesium arcs, and mercury-vapor lamps; the high pressure mercury lamp is most widely used. Higher yields and more favorable isomer distribution can be achieved if the frequency of light is kept at 275–300 nm. Several arrangements of the components of the simple reactor shown are possible and have been used. An important feature is a cooling jacket which controls the high temperature (ca 800°C) of the mercury-vapor lamps. Water solutions for cooling may contain salts for screening frequencies of light. Light below 275 nm can be filtered by aromatic compounds, as well as by a 5-wt % lead acetate or other inorganic salt solution. Glass filters can also be used as screens for frequencies which are outside the chemical filter ranges. Photosensitizers, eg, eosin, erythrosin, dibromodinitrofluorescein, and others, have been suggested to limit light frequencies and improve the isomer distribution.

The vitamin D resin is stabilized against oxidation by the addition of ≤ 1 wt% butylated hydroxyanisole or butylated hydroxytoluene.

The solvent is then evaporated, and the unconverted sterol is recovered by precipitation from an appropriate solvent, eg, alcohol. The recovered sterol is reused in subsequent irradiations. The solvent is then evaporated to yield vitamin D resin. The resin is a pale yellow-to-amber oil that flows freely when hot and becomes a brittle glass when cold; the activity of commercial resin is $20 - 30 \times 10^6$ IU/g. The resin is formulated without further purification for use in animal feeds. Vitamin D can be crystallized to give the USP product from a mixture of hydrocarbon solvent and aliphatic nitrile, eg, benzene and acetonitrile, or from methyl formate (100, 101). Chemical complexation has also been used for purification.

In 1938, it was estimated that 7.5×10^{13} quanta of light were required to convert ergosterol to 1 USP unit of vitamin D_2 (102). The value was later determined to be 9.3×10^{13} quanta.

A flow diagram for the D_3 manufacturing process is shown in Figure 8. First, ether solution containing 7-dehydrocholesterol is recirculated through a quartz uv reactor, and the ether is distilled off. Methanol is added to the 7-dehydrocholesterol–vitamin D_3 mixture, and the remaining ether is azeotroped. The resulting solution is transferred to a crystallizer, and the 7-dehydrocholesterol is crystallized and recovered by filtration. The methanol is distilled, and the vitamin D_3 resin is heated to isomerize the pre-vitamin D to the cis-vitamin D isomer. Vitamin D_3 resin is then packaged for shipment.

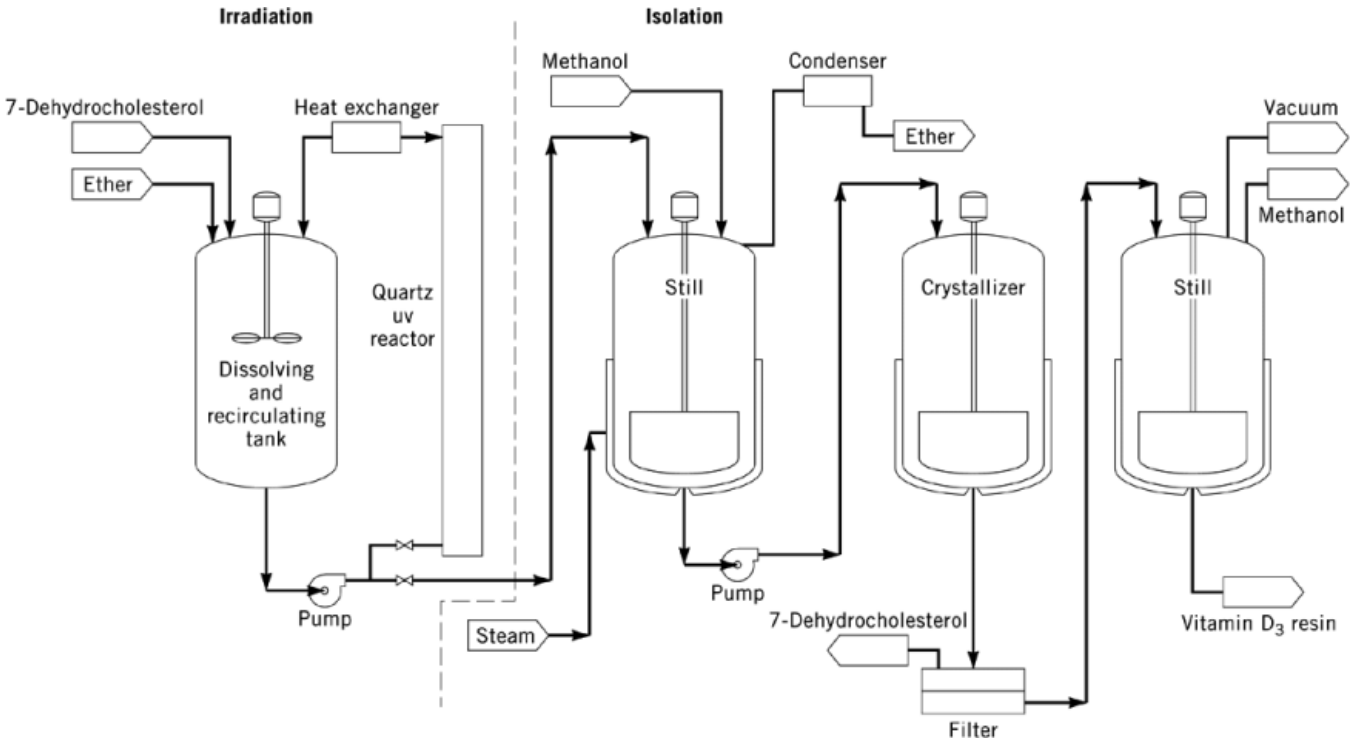
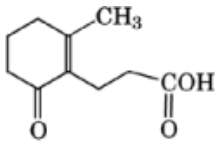


Fig. 8. Vitamin D irradiation process.

4.2. Total Synthesis

Poor yields encountered during the manufacture of vitamin D stimulated early attempts to synthesize vitamin D. In 1959 Inhoffen synthesized vitamin D_3 from 3-methyl-2-(2-carboxyethyl)-2-cyclohexenone **(40)**, using the Wittig reaction extensively (103).



(40)

5,6-*trans*-Vitamin D_3 was prepared, followed by photochemical isomerization into vitamin D_3. Direct preparation of the previtamin and vitamins D is described in References 104 and 105.

The discovery that vitamin D_3 was metabolized to biologically active derivatives led to a significant effort to prepare 25-hydroxy vitamin D_3 and, subsequently, the 1 α-hydroxy and 1,25 dihydroxy derivatives. Initial attempts centered around modification of steroidal precursors, which were then converted to the D derivatives by conventional means.

Chemical syntheses of 25-hydroxy-vitamin D_3 were achieved by several groups of researchers (106–112). Many of these syntheses depended on the availability of the precursor 25-oxo-27-norcholesterol. Grignard reaction followed by introduction of the 7-dehydro function and irradiation allows 25-hydroxy-vitamin D_3 to be

isolated (109). Fucosterol (stigmasta-5,24(28)-dien-3 β-ol) as well as bile acids, pregnenolone, and desmosterol have also been used as starting materials for the synthesis of 25-hydroxy intermediates. 24,25-Dihydroxy-vitamin D_3 [40013-87-4] was isolated and chemically characterized in 1972 (113). The first synthesis of this important D_3 catabolite was of a racemic mixture, and it was followed by the stereospecific syntheses of the two epimers (114–123). The biosynthesized material migrates exclusively with the synthetic 24(*R*) epimer.

The yield of the first chemical synthesis of 1,25-dihydroxy-vitamin D_3 was <0.005% (124). A key intermediate compound was 1,25-dihydroxy-cholesterol (109, 125–130).

Subsequent synthesis of Vitamin D metabolites involved oxidative degradation of the vitamin D molecule to obtain the C- and D-ring portion with the intact side chain. Recombination of this molecule with an appropriate structure containing the A-ring was then carried out by a Wittig-type condensation.

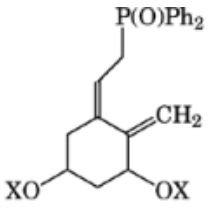
The chemical syntheses of 1,24(*R*),25-trihydroxy-vitamin D_3 [56142-94-0] and 1,24(*S*),25-trihydroxy-vitamin D [56142-95-1] were reported (131, 132) in 1975. The chemical synthesis of 25,26-dihydroxy-vitamin D_3 [29261-12-9] has also been described, and it has been determined that the biologically occurring epimer is 25(*R*),26-dihydroxy-vitamin D_3 (117, 133–135). The 23,25-dihydroxy-24-oxo metabolite has been isolated (136) as well. 1 α-Hydroxycalcitroic acid (1-hydroxy-24-nor-9,10-secochola-5,7-10(19)-trien-23-oic acid) [71204-89-2], 25-hydroxy-26,23-lactone vitamin D_3 [71203-34-6], and homocalcitroic acid (9,10-secohola-5,7,10(19)-trien-24-oic acid) have also been reported, and the biological activity of these metabolites has been studied (137). The synthetic chemistry associated with these and other metabolites of vitamin D_2 and vitamin D_3 is described in References 6, 40, and 138, and more recently in References 16, 51, 55, (139–141)

The discovery that vitamin D metabolites play a much larger biochemical role than just maintaining calcium homeostasis has stimulated a number of groups around the world to develop more economical chemical syntheses for the vitamin D metabolites and analogues, which might be useful in studying and treating D_3-related diseases and conditions. Many of these methods are reviewed in References 139 and 140.

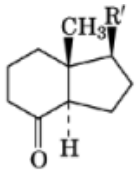
The most useful synthetic routes include the following pathways to the vitamin D structures and their derivatives:

Photochemical ring opening of 7-dehydrocholesterol derivatives which have ring A or the side chain modified (142, 143).

A phosphine oxide of type **(41)** can be coupled with Grundman's ketone **(42)** to produce the D_3 skeleton (105, 144–151).



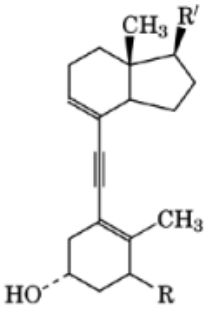
(**41**, X = a protective group)



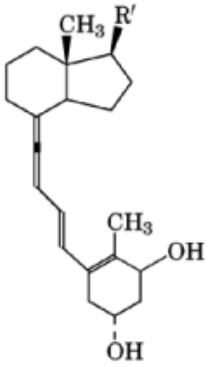
(**42**, R′ = cholesterol side chain or derivative)

Synthetic dienynes like **(43)** are semihydrogenated to form previtamin D and are then rearranged to the D structure (152–155).

Vinyl allenes **(44)** are rearranged with heat or metal catalysis and photosensitized isomerization to produce the vitamin D triene (156–160).

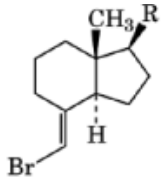


(**43**, R = H, OH)
R′ = cholesterol side chain or derivative

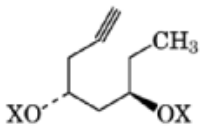


(**44**, R′ = cholesterol side chain or derivative)

Intermolecular cross-coupling of **(45)** with a synthetic alkyne structure like **(46)** leads to the D_3 skeleton (161, 162).



(**45**, R = cholesterol side chain or derivative)



(**46**, X = protective group)

Direct modification of vitamin D_2 or D_3 or its metabolites to give derivatives by a variety of synthetic methods has also been used extensively (163–171).

Whereas these preparations are extremely useful for obtaining sufficient quantities of the D_3 metabolites and analogues for study and as possible therapeutic treatments, their cost is high compared to the cost of manufacture of vitamin D_3. For this reason, the metabolites are unlikely to be successful in replacing vitamin D_3 as an ingredient in animal or human nutrition.

5. Biochemistry

5.1. Biochemistry

Vitamin D is introduced into the bloodstream either from the skin after natural synthesis by the irradiation of 7-dehydrocholesterol stored in the epidermis (172) or by ingestion and absorption of vitamin D_2 or vitamin D_3 through the gut wall (40). Between 60 and 80% of the vitamin introduced in the blood is taken up by the liver, where cholecalciferol is transferred from chylomicrons to a vitamin D-binding protein (DBP), an α-globulin specific for vitamin D and its metabolites but one which does not bind with previtamin D in the skin (173). Cholecalciferol is hydroxylated in the liver at the C-25 position (51, 141, 174). This hydroxylation occurs in the endoplasmic reticulum and requires NADPH, a flavoprotein, cytochrome P-450, Mg^{2+}, and O_2 (175). 25-Hydroxylation also occurs in intestinal homogenates of chicks (176), but does not appear to occur outside the liver in mammals (177). 25-Hydroxy-cholecalciferol is the main circulating form of vitamin D_3, and its normal concentration level is 15–47 ng/mL (178).

25-Hydroxy vitamin D pools in the blood and is transported on DBP to the kidney, where further hydroxylation takes place at C-1 or C-24 in response to calcium levels. 1-Hydroxylation occurs primarily in the kidney mitochondria and is catalyzed by a mixed-function monooxygenase with a specific cytochrome P-450 (52, 179, 180). 1 α- and 24-Hydroxylation of 25-hydroxycholecalciferol has also been shown to take place in the placenta of pregnant mammals and in bone cells, as well as in the epidermis. Low phosphate levels also stimulate 1,25-dihydroxycholecalciferol production, which in turn stimulates intestinal calcium as well as phosphorus absorption. It also mobilizes these minerals from bone and decreases their kidney excretion. Together with PTH, calcitriol also stimulates renal reabsorption of the calcium and phosphorus by the proximal tubules (51, 141, 181–183).

Vitamin D receptors have been identified in intestine, bone, and kidney. Receptors have also been shown, to a lesser degree, to be present in the pituitary, brain, skin, reproductive organs, and cells of the immune system, suggesting vitamin D may play a broader role in controlling cellular proliferation and differentiation (184). The nuclear receptor (genomic) for 1,25-dihydroxy vitamin D_3 has been shown to be a member of the super-family of transactivating regulators of gene transcription similar to the receptors of other steroid hormones (185). Nongenomic activation by 1,25-dihydroxy vitamin D_3 on the cellular and subcellular level has been observed and is believed to be responsible for calcium transport in tissue known as transcaltachia (186, 187).

Further side-chain oxidation of vitamin D_3 metabolites may be necessary for phosphate transport (188, 189). 24,25-Dihydroxycholecalciferol is produced by kidney mitochondria, but can also be produced elsewhere (190, 191). 1 α,24,25-Trihydroxy-vitamin D_3 stimulates intestinal calcium mobilization to the extent of ca 60%

of the 1 α,25 activity and has 10% of the 1 α,25 activity in causing bone resorption (192). It is less active in chicks and is excreted.

More than 20 other, naturally occurring metabolites of vitamin D have been isolated and characterized, and many derivatives have been synthesized. Their function is the subject of continuing research (16, 51, 141, 162).

Although it is being found that vitamin D metabolites play a role in many different biological functions, metabolism primarily occurs to maintain the calcium homeostasis of the body. When calcium serum levels fall below the normal range, 1 α,25-dihydroxy-vitamin D_3 is made; when calcium levels are at or above this level, 24,25-dihydroxycholecalciferol is made, and 1 α-hydroxylase activity is discontinued. The calcium homeostasis mechanism involves a hypocalcemic stimulus, which induces the secretion of parathyroid hormone. This causes phosphate diuresis in the kidney, which stimulates the 1 α-hydroxylase activity and causes the hydroxylation of 25-hydroxy-vitamin D to 1 α,25-dihydroxycholecalciferol. Parathyroid hormone and 1,25-dihydroxycholecalciferol act at the bone site cooperatively to stimulate calcium mobilization from the bone (see Hormones). Calcium blood levels are also influenced by the effects of the metabolite on intestinal absorption and renal resorption.

Interaction of vitamin D and its metabolites with sex hormones has been demonstrated, particularly in birds in which the egg-laying functions combine calcium needs and reproductive activity. The metabolites of vitamin D behave as hormones. As such, they play an active role in the endocrine system, along with other hormones, to maintain the various body functions. Several biological influences of metabolites of vitamin D have been studied, including effects related to cancer (193–197), skin diseases (198–201), immunomodulatory effects (202, 203), and Alzheimer's disease (204–206) (Fig. 9).

The metabolism of vitamin D_2 follows a pathway similar to that described for Vitamin D_3.

5.2. Vitamin D_3 Deficiency

Vitamin D_3 deficiency is uncommon in normal adults. However, when it does occur, it can be serious, particularly in pregnant women. Some vitamin D_3 deficiency can occur because of a large reduction of fat intake, which decreases D_3 absorption. Strict vegetarians also risk reduced vitamin D_3 intake. Premature infants and elderly people who are exposed to minimal sunlight and consume little vitamin D_3 also have a reduced capacity to metabolize D_3 and can develop vitamin D_3 deficiency.

Clinical stresses which interfere with vitamin D_3 metabolism, can result in calcium deficiency leading to osteomalacia and osteoporosis (secondary vitamin D deficiency). These stresses include intestinal malabsorption (lack of bile salts); stomach bypass surgery; obstructive jaundice; alcoholism; liver or kidney failure decreasing hydroxylation of vitamin D_3 to active forms; inborn error of metabolism; and use of anticonverdiants that may lead to increased D_3 requirement.

People who experience the exclusion of sunlight (living in northern climates; cultures where apparel limits sunlight), as well as infants and children that are confined to bed or limited outdoor activity because of weather or illness, also can show a tendency towards vitamin D_3 deficiency.

Vitamin D deficiency in animals may be caused by the fact that the vitamin is not available to the livestock. Modern animal husbandry subjects animals to total confinement with little or no exposure to sunlight. This mandates that they be given vitamin D-fortified diets. The vitamin is sensitive to oxidation, heat, light, and minerals, and significant losses may occur in the fortified feed unless the product is adequately protected. Mycotoxins in feeds also interfere with utilization of vitamin D in feeds (207–209).

Symptoms of D_3 deficiency in animals include poor appetite, stunted growth, and weight loss; increased incidence of irritability and convulsions (tetany); some growth abnormalities; decreased egg production in poultry with reduced hatchability and thin eggshell quality; and birth of weak, dead, or deformed offspring in other animals.

For a more detailed description of symptoms, see References 4, 5, 210, and 211.

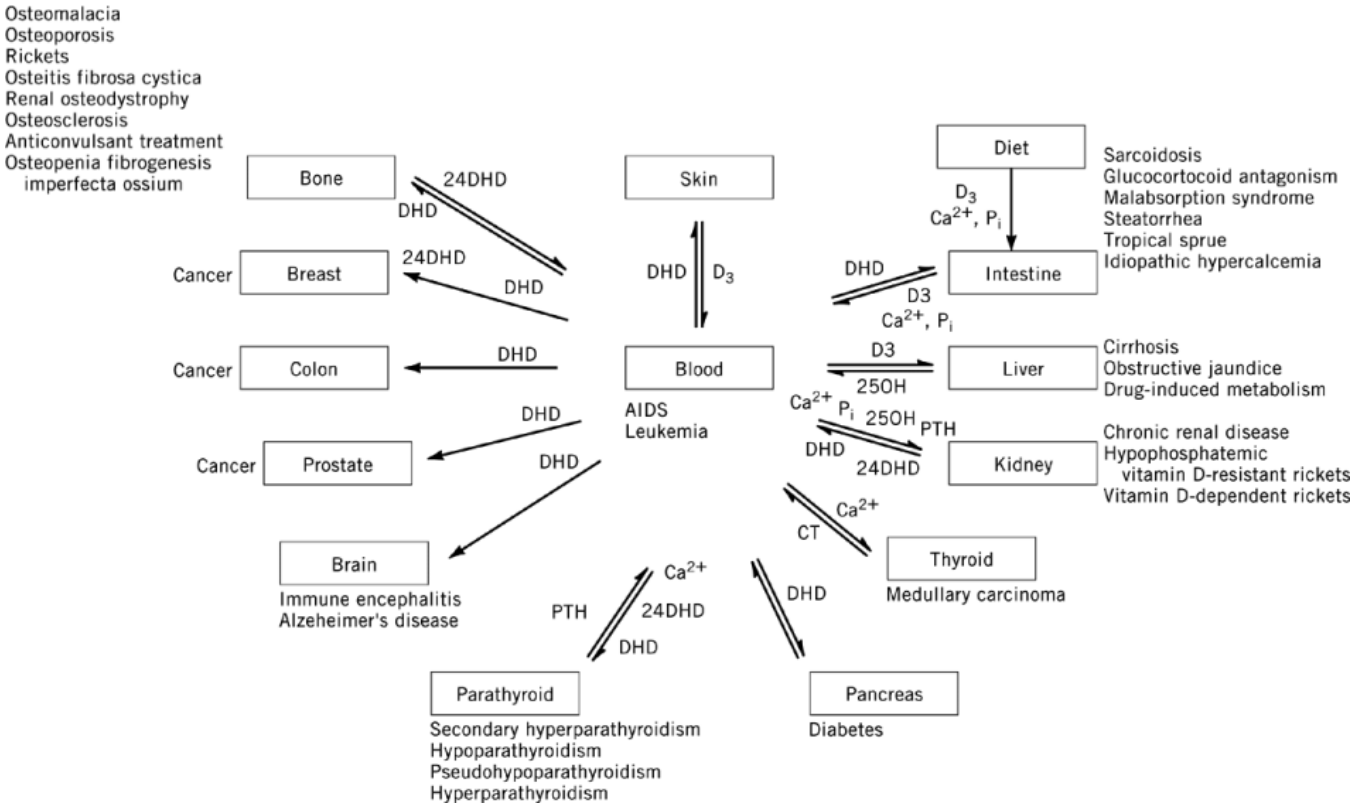


Fig. 9. Human disease states in which vitamin D has been implicated. D_3=vitamin D_3; DHD=1a,25−dihydroxyvitamin D_3; 24DHD=24(*R*),25-dihydroxyvitamin D_3; 25OH=25-hydroxyvitamin D_3; PTH=parathyroid hormone; CT=calcitonin; P_i=inorganic phosphorus.

5.3. Dietary Requirements

Vitamin D is essential for growth and maintenance of good health. According to the National Research Council (NRC), the vitamin D requirement for optimum health is ca 400 IU/d in humans, regardless of age. This amount of vitamin D gives ample protection from rickets, provided a sufficient amount of the other essential nutrients, including calcium and phosphorus, is supplied (212). Recommended NRC amounts of vitamin D per kilogram of feed for various species are as follows: starting and growing chicks, 200 IU; laying and breeding hens, 500 IU; turkeys, 1100 IU; ducks, 200 IU; quail, 480–900 IU; geese, 200 IU; and swine 125–220 IU. Calves require 600 IU per 100 kg of body weight (213–215). Practical feeding levels are somewhat higher, in order to assure delivery of the vitamin to the animals.

The present (ca 1997) maximum safe level of vitamin D_3 for long-term feeding in most species is four to ten times the NRC dietary requirements. Short-term (<60 d), most species can tolerate 100 times their apparent dietary requirements (210).

Animals exposed to sunlight for extended lengths of time do not require substantial dietary vitamin D. Current livestock management practices place an emphasis on high productivity, and most feed manufacturers recommend vitamin D supplementation of diets. Recommendations for practical levels of vitamin D in feeds for various animals, as recommended by feed manufacturers, are listed in Table 10.

Table 10. Practical Feeding Levels of Vitamin D, 10^6 IU/t

Animal	Amount	Animal	Amount
Poultry		*Dairy cattle*	
chickens		calf starter	1–2
broilers	2–4	calf milk replacer	2–4
replacement birds	1–3	replacement heifers	1–2
layers	1–3	dry cows	1–2
breeding hens	2–4	lactating cows	2–4
turkeys		bulls	2–4
starting	3–5		
growing	2–4	*Beef cattle*	
breeding	3–5	calf starter	1–2
ducks		replacement heifers	1–2
market	1–3	feedlot	2–3
breeding	2–4	dry pregnant cows	1–2
		lactating cows	1–2
Swine		bulls	1–2
prestarter (to 10 kg)	2–3		
starter (10–35 kg)	1–2	*Sheep*	
growing-finishing (35 kg to market)	1–2	fattening lambs	2–3
gestation	1–2	breeding	1–2
lactation	2–3		
boars	2–3	*Other*	
		dogs	5–1
Fish		cats	1–2
trout	1–2	horses	1–2
catfish	1–2		

Historically, rickets prevention or cure was used to evaluate adequate vitamin D_3 nutrient levels. More recently, in the absence of uv light, Edwards (216) found different vitamin D_3 levels were required for the optimization of the various effects of vitamin D_3 in poultry, ie, 275 IU/kg for growth, 503 IU/kg for bone ash, 552 IU/kg for blood plasma calcium, and 904 IU/kg for rickets prevention.

5.4. Toxicity

Vitamin D toxicity was known as early as the year 1429 (217). Accidental toxicity has been reported in monkeys, dogs, horses, pigs, chinchillas, and humans, and particularly in cattle when extremely high doses of vitamin D have been used to treat milk fever.

Vitamin D intoxication causes 25-hydroxy vitamin D_3 blood levels to go from a normal of 30–50 ng/mL to 200–400 ng/mL. At this high level, the metabolite can compete with 1 α-25-dihydroxy vitamin D_3 for receptors in the intestine and bone and induce effects usually attributed to the dihydroxy vitamin D_3. Thus, 25-hydroxy vitamin D_3 is believed to be the critical factor in vitamin D intoxication. Vitamin D_2 is metabolized slower than vitamin D_3 and thus appears to be less toxic (218).

The overall effect in most animals is to stimulate intestinal absorption of calcium with a concomitant increase in serum calcium and a reduction in parathyroid hormone (PTH). Modest hypercalcemia allows the glomerular filtration rate to remain stable and hypercalciuria to occur because of increased filtered load of calcium and reduction of tubular resorption of calcium with reduced PTH. However, with further increases in serum calcium, the glomerular filtration rate decreases, resulting in an even more rapid increase in serum calcium and the subsequent fall in urinary calcium.

Table 11. Estimated Safe Upper Dietary Levels[a]

Species	NRC recommended dietary daily requirement, IU/kg	Exposure, multiple of daily requirement	
		Long-term (<60 d)	Short-term (>60 d)
chicken	200	200	14
quail	1200	100	4
turkey	900	100	3.9
cow	300	83	3.9
catfish	1000		20
trout	1800		555
horse	400		5.5
sheep	275	91	8
pig	220	150	10

[a]Ref. 210.

Polyuria, along with vomiting can cause extracellular fluid to be reduced, contributing to further renal function disruption. Renal dysfunction then, becomes the major contributor to loss of calcium homeostasis, and the resulting hypercalcemia during vitamin D intoxication. Loss of appetite, gastrointestinal disturbances, head and joint pain, and muscle weakness are typical clinical symptoms. Death from hypervitaminosis D is usually caused by renal failure. Cardiovascular and kidney mineralization results. Respiratory tract mineralization has also been observed, as well as calcification in the salivary gland. The severity of effects of toxic levels of vitamin D depend on the dose, functional state of the kidneys, composition of the diet, as well as differences in toxicology of the various species (210).

Vitamin D withdrawal is an obvious treatment for D toxicity (219). However, because of the 5–7 d half-life of plasma vitamin D and 20–30 d half-life of 25-hydroxy vitamin D, it may not be immediately successful. A prompt reduction in dietary calcium is also indicated to reduce hypercalcemia. Sodium phytate can aid in reducing intestinal calcium transport. Calcitonin glucagon and glucocorticoid therapy have also been reported to reduce serum calcium resulting from D intoxication (210).

Vitamin D_3 is nonirritating to rabbit eye and dermal tests. It has an oral LD_{50} of 35–42 mg/kg in rats; 42 mg/kg in mice (136 mg/kg IP), and 4–80 mg/kg in dogs (10 mg/kg IP and 5 mg/kg IM and IV). It is nontoxic on inhalation.

The metabolites of vitamin D are usually more toxic than the vitamin because the feedback mechanisms that regulate vitamin D concentrations are circumvented. 25-Hydroxycholecalciferol has a one-hundredfold increase in toxicity over vitamin D_3 when fed to chicks (220) and 1 α,25-dihydroxy vitamin D_3 is several times more toxic than the 25-hydroxy analogue. Vitamin D_2 seems to have less toxicity than vitamin D_3, a circumstance which is believed to be caused by the more efficient elimination of 25-hydroxy and the 1 α,25-dihydroxy vitamin D_2 from the animals. Estimated safe upper dietary levels are given in Table 11.

5.5. Disease States

Rickets is the most common disease associated with vitamin D deficiency. Many other disease states have been shown to be related to vitamin D. These can involve a lack of the vitamin, deficient synthesis of the metabolites from the vitamin, deficient control mechanisms, or defective organ receptors. The control of calcium and phosphorus is essential in the maintenance of normal cellular biochemistry, eg, muscle contraction, nerve conduction, and enzyme function. The vitamin D metabolites also have a function in cell proliferation. They interact with other factors and receptors to regulate gene transcription.

In the treatment of diseases where the metabolites are not being delivered to the system, synthetic metabolites or active analogues have been successfully administered. Vitamin D_3 metabolites have been successfully used for treatment of milk fever in cattle, turkey leg weakness, plaque psoriasis, and osteoporosis and

Table 12. Price History of Vitamin D Materials[a]

Year	Source	Vitamin D	
	Cod-liver oil, $/L	Ergocalciferol (crystalline vitamin D_2), $/g	Cholecalciferol D_3 $/g[b]
1955	1.51	0.60	0.045
1960	1.40	0.54	0.045
1965	1.71	0.48	0.045
1970	1.70	0.48	0.045
1975	5.50	0.63	0.045
1980	8.00	0.63	0.045
1985	7.25	[c]	24[d]
1990	13.00	[c]	24[d]
1995	13.00	[c]	24[d]

[a] Adapted from Ref. 221.
[b] 850,000 IU/g unless otherwise indicated.
[c] D_2 costs were not quoted after 1983.
[d] 40×10^6 IU/g.

renal osteodystrophy in humans. Many of these clinical studies are outlined in References 6, 16, 40, 51, and 141. The vitamin D receptor complex is a member of the gene superfamily of transcriptional activators, and 1,25 dihydroxy vitamin D is thus supportive of selective cell differentiation. In addition to mineral homeostasis mediated in the intestine, kidney, and bone, the metabolite acts on the immune system, β-cells of the pancreas (insulin secretion), cerebellum, and hypothalamus.

Vitamin D metabolites may therefore play an active role in diseases related to these functions, ie, leukemia, cancer (breast, colon, prostate), and autoimmune diseases (AIDS, immune encephalitis, and diabetes) (51, 141, 193–197, 202, 203).

6. Economic Aspects

Vitamin D is available in a variety of forms. Cod liver oil and percomorph liver oil historically were good sources of vitamin D. Recent cost increases of these materials have caused a decline in their market position. Cod liver oil sold for $0.40–0.45/L in 1970 and as high as $1.45/L in 1979 and $3.43/L in 1996. The prices of the cod liver oils and of vitamin D_2 and vitamin D_3 from 1955 to 1995 are shown in Table 12.

Vitamin D_3 is produced as a resin ($20 - 30 \times 10^6$ IU/g) which sold in 1980 for ca $\$1.02/10^6$ IU. The 1979 prices of vitamin D were $\$24.25/10^9$ IU ($9.70/kg of product at 400,000 IU/g) (222). In the United States in 1979, $\$3.6 \times 10^6$ worth of vitamin D was sold. The 1996 price for vitamin D_3 products was $13.50–14.00/g for resin and $24.00/g (223) for crystalline material. Vitamin D_3 feed-grade formulated products sell for approximately $\$0.03–0.04/10^6$ units of activity.

Estimates of world demand in 1979 were as high as 1300×10^{12} IU of vitamin D_3. This was divided into thirds for Europe, the United States, and the rest of the world, respectively. Of this demand, 90% was estimated for animal-feed fortification and 10% for food and pharmaceutical uses. It is estimated that the demand will be $1500–1600 \times 10^{12}$ IU in 1997 for animal usage and 100×10^{12} IU for human use. The United States will require approximately 500 TU (1 trillion units = 25 kg *cis*-vitamin D_3 or 17 t of resin) for animal use and 30 TU (approximately 1 t of crystalline *cis*-vitamin D_3) for human use. This represents approximately 50 t of vitamin D_3 resin/yr for animal use worldwide and about 2.5 t of crystalline vitamin D_3 for human use. A substantial proportion of the vitamin D_3 is imported, and with all uses included, it is estimated that 80–90% of the sales are of vitamin D_3.

The vitamin D_2 volume is estimated at only 1200 kg/yr, exclusive of Chinese sources.

7. Uses

Most of the vitamin D sold is synthetic. Vitamin D_2 as a concentrate or in microcrystalline forms is used in many pharmaceutical preparations, although vitamin D_3 is preferred by many manufacturers and consumers. Vitamin D_2 in the form of irradiated yeast has been used as a feed supplement for cattle, swine, and dogs, but its use has declined in favor of Vitamin D_3. In the United States, swine usage accounts for 13% and poultry for 41% of vitamin D consumption in animal agriculture. The beef and dairy industries account for 44% of vitamin D consumption, of which 22% is by dairy calves. The remaining 1% of total consumption of D vitamins is largely in prepared pet foods. European usage in 1994 was estimated at: 58% cattle, 19% swine, 20% poultry, and 3% other. Crystalline vitamin D_3 is used for medicinal preparations and formulations in the pharmaceutical industry, as well as for the fortification of fresh and evaporated milk and nonfat dry milks. Preparations based on the use of the resin are less expensive than those using crystalline vitamin D. Essentially all milk produced in the United States is fortified with vitamin D_3. Cereals and margarine are also fortified with vitamin D_3. The vitamin must be diluted to a proper dosage form, and many supplement preparations are marketed, eg, tonics, drops, capsules, and tablets; oil-based injectables are also available. Combination formulations are used widely, particularly with vitamin A for humans and with vitamins A and E for animals. Preemulsified products are used for increased bioavailability. Water-miscible formulations have also been developed (224). Solutions of vitamin D in oil or in oil-on-dry carriers, eg, corn or flour, are used in animal feeds. These diets contain high levels of minerals, and the vitamin D formulation is not stable unless it is protected. Several stable forms are patented and sold commercially; they include beadlets or powders of dry suspensions in gelatin, carbohydrates, wax, and cellulose derivatives (89, 225–228).

Animal uses employ resin in various stabilized forms at levels of 200,000; 400,000; 500,000; and 1×10^6 units of D_3 per gram. Combination products containing A and D are also available, with 650,000 units of vitamin A and 325,000 units vitamin D per gram of product being the most common dosage form.

Approximately 200 kg/yr of Vitamin D_3 formulations are also marketed as rat poisons. The metabolites of vitamin D_3 and synthetic derivatives are being used or developed for treatment of osteoporosis, skin psoriasis, and other diseases in humans. 1 α-Hydroxy vitamin D_3 is being used for milk fever in cows, and 25-hydroxy vitamin D_3 has been proposed for eggshell thickness in poultry and is being marketed as an animal dietary nutritional supplement.

BIBLIOGRAPHY

"Vitamins (Vitamin D)" in *ECT* 1st ed., Vol. 14, pp. 828–849, by H. R. Rosenberg, E. I. du Pont de Nemours & Co., Inc.; in *ECT* 2nd ed., Vol. 21, pp. 549–573, by S. B. Greenbaum, Diamond Shamrock Chemical Co.; "Vitamin D" under "Vitamins" in *ECT* 3rd ed., Vol. 24, pp. 186–213, by A. L. Hirsch, A. L. Laboratories, Inc.

Cited Publications

1. P.Karlson (chairman) and co-workers, IUPAC-IUB Joint Commission on Biochemical Nomenclature. "Nomenclature of Vitamin D recommendations 1981," *Eur. J. Biochem.* **124**, 223 (1982).
2. T. C. Angus and co-workers, *Proc. R. Soc. London, Ser. B* **105**, 340 (1931).
3. W. F. Loomis, *Sci. Am.* **223**(6), 77 (1970).
4. H. M. Frost, *Bone Dynamics in Osteoporosis and Osteomalacia*, Surgery Monograph Series, Charles C. Thomas, Publisher, Springfield, Ill., 1966.

5. H. F. DeLuca in R. B. Alfin-Slater and D. Kratchevsky, eds., *Nutrition and the Adult: Micronutrients*, Plenum Press, New York, 1980, p. 207.
6. A. W. Norman, *Vitamin D—The Calcium Homeostatic Steroid Hormone*, Academic Press, Inc., New York, 1979, p. 3.
7. E. Mellanby, *J. Physiol.* (*London*) **52**, L 111 (1919); *Lancet* **196**, 407 (1919).
8. K. Huldschinski, *Dtsch. Med. Wochenschr.* **45**, 712 (1919).
9. F. Reindel and H. J. Kipphan, *Justus Liebigs Ann. Chem.* **493**, 181 (1932).
10. E. Fernholz and P. N. Chakravorty, *Ber. Deutsch. Chem. Ger. A.* **67**, 2021 (1934).
11. A. Windaus and H. Grundmann, *Justus Liebigs Ann. Chem.* **524**, 295 (1936).
12. Ref. 6, 39–48.
13. A. Windaus and F. Bock, *Z. Physiol. Chem. Hoppe-Seglers* **245**, 168 (1937).
14. O. Rygh, *Nature* (*London*) **136**, 396 (1935).
15. H. Brockmann and co-workers, *Z. Physiol. Chem. Hoppe-Seglers* **241**, 104 (1936); **245**, 96 (1937).
16. A. W. Norman, K. Schaefer, H.-G. Grigoleit, D. v. Herath, eds., *Vitamin D. Chemical, Biochemical and Clinical Update, Proceedings of the Sixth Workshop on Vitamin D*, Merano, Italy, March, 1985, Walter de Gruyter, Berlin, 1985, p. 55.
17. R. H. Wasserman, J. D. Henion, M. R. Haussler, and T. A. McCain, *Science* **194**, 853 (1976).
18. M. Peterlik and co-workers, *Biochem. Biophys. Res. Commun.* **70**, 797 (1976).
19. J. P. Simonit, K. M. L. Morris, and J. C. Collins, *J. Endocrinol.* **68**, 18 (1976).
20. H. Zucker and W. A. Rambeck, *Centralbl. Veterinaermed.* **28**, 436 (1981).
21. W. A. Rambeck and co-workers, *Z. Pflanzenphysiol.* **104**, 9 (1981).
22. R. H. Wasserman and co-workers, *Nutr. Rev.* **33**, 1 (1975); *J. Nutr.* **106**, 457 (1976); *Biochem. Biophys. Res. Commun.* **62**, 85 (1975).
23. Ref. 6, p. 49.
24. M. F. Hollick in Ref. 16, p. 219.
25. A. W. Norman and co-workers, *J. Biol. Chem.*, 13811–13819 (1994).
26. M. C. Dormanen and co-workers, *Biochem. Biophys. Res. Comm.* **201**, 394–401 (1994).
27. I. Nemere and co-workers, *J. Biol. Chem.* **269**, 23750–23756 (1994).
28. U.S. Pat. 2,395,115 (Feb. 19, 1946), K. J. Goering (to Anheuser-Busch, Inc.).
29. U.S. Pat. 2,874,171 (Feb. 17, 1959), H. A. Nelson (to Upjohn Co.).
30. U.S. Pat. 3,006,932 (Oct. 31, 1961), J. Green, S. A. Price, and E. E. Edwin (to Vitamins, Ltd.).
31. U.S. Pat. 2,794,035 (May 28, 1957), O. Hummel (to Zellstaff-Fabrik Waldhof).
32. U.S. Pat. 2,865,934 (Dec. 23, 1958), R. A. Fisher (to Biofermentation Corp.).
33. Ger. Pat. 1,252,674 (Oct. 26, 1967), K. Petzoldt, K. Klieslich, and H. J. Koch (to Schering A.G.).
34. K. Ziegler, *Ann.* **551**, 80 (1942).
35. S. Bernstein, L. J. Benovi, L. Dorfman, K. S. Sax, and Y. Subbarow, *J. Org. Chem.* **14**, 433 (1949); U.S. Pat. 2,498,390 (Feb. 21, 1950), S. Bernstein and K. J. Sax (to American Cyanamid Co.).
36. U.S. Pat. 2,446,091 (May 4, 1968), J. Van Der Vliet and W. Stevens (to Hartford National Bank and Trust Co.).
37. H. Schaltagger, *Helv. Chim. Acta* **33**, 2101 (1950).
38. D. N. A. Holwerda, Doctoral Dissertation, Imperial University Leydon, 1970.
39. F. Hunziker and F. X. Mullner, *Helm. Chim. Acta* **41**, 70 (1958).
40. A. W. Norman, K. Schaefer, D. V. Herrath, and H. G. Grigoleit, eds., *Vitamin D: Chemical, Biochemical, and Clinical Endocrinology of Calcium Metabolism, Proceedings of the Fifth Workshop on Vitamin D*, Williamsburg, Va., Feb. 1982, Walter de Gruyter, Berlin, 1982, p. 1133.
41. W. G. Salmond, M. A. Serta, A. M. Cain, and M. C. Sobala, *Tetrahedron Lett.* **20**, 1683 (1977).
42. U.S. Pat. 2,098,984 (Nov. 16, 1937), A. Windaus and F. Schenck (to Winthrop Chem. Co., Inc.).
43. L. E. Fieser, *J. Am. Chem. Soc.* **75**, 4394 (1953).
44. U.S. Pat. 2,505,646 (Apr. 25, 1950), W. E. Meuly (to E. I. du Pont de Nemours & Co., Inc.).
45. A. Windaus, F. VonWerder, A. Luttringhaus, and E. Fernholz, *Ann.* **499**, 188 (1932).
46. L. Velluz, G. Amiard, and B. Goffinet, *Bull. Soc. Chim. France* **22**, 1341 (1955).
47. E. Havinga, R. J. DeKoch, and M. Rappoldt, *Tetrahedron* **11**, 276 (1960).
48. H. J. C. Jacobs, *Pure & Appl. Chem.* **67**(1), 63 (1995).
49. J. G. Bell and A. A. Christie, *Analyst* **99**, 385 (1974).
50. P. B. Braun, J. Hornstra, C. Knobles, E. W. M. Rutten, and C. Romer, *Acta Crystallogr.* **B29**, 463 (1973).

51. E. Havinga, *Experimentia* **29**, 1181 (1973).
52. A. W. Norman, R. Bouillon, M. Thomasset, eds., *Vitamin D. A Pluripotent Steroid Hormone: Structural Studies, Molecular Endocrinology and Clinical Applications. Proceedings of the Ninth Workshop on Vitamin D*, Orlando, Florida, May 1994, Walter de Gruyter, Berlin, 1994, p. 89.
53. J. I. Pedersen, J. G. Ghazarian, N. R. Orme-Johnson, and H. F. DeLuca, *J. Biol. Chem.* **251**, 3933 (1976).
54. A. W. Norman and co-eds., *Vitamin D; Basic Research and its Clinical Application, Proceedings of the Fourth Workshop on Vitamin D*, Berlin, Feb. 1979, Walter de Gruyter, Berlin, 1979, p. 173.
55. K. H. Hanewald, M. P. Rappoldt, and J. R. Roborgh, *Rec. Trav. Chim. Pays-Bas* **80**, 1003 (1961).
56. A. W. Norman, K. Schefer, H.-G. Grigoleit, D. Herrath, eds., *Vitamin D. Molecular, Cellular and Clinical Endocrinology. Proceedings of the Seventh Workshop on Vitamin D*, April, 1988, Walter de Gruyter, Berlin, 1988, p. 83.
57. A. Verloop, A. L. Koevoet, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **76**, 689 (1957).
58. A. L. Hoevoet, A. Verloop, and E. Havinga, *Rec. Trav. Chim.* **74**, 788 (1955).
59. W. G. Dauben and co-workers, *J. Am. Chem. Soc.* **80**, 4117 (1958).
60. W. H. Okamura, M. L. Hammond, A. J. C. Jacobs, and J. Von Thiegil, *Tetrahedron Lett.* **52**, 4807 (1976).
61. E. Havinga, *Chimia* **30**, 27 (1976); D. H. R. Barton and co-workers, *Chem. Comm.*, 65 (1976).
62. F. Boosman, H. J. C. Jacobs, E. Havinga, and A. Van den Gen, *Tetrahedron Lett.* **7**, 427 (1975).
63. A. W. Norman and co-eds., *Vitamin D Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism; Proceedings of the Third Workshop on Vitamin D*, Asilomar, Pacific Grove, Calif., Jan. 1977, Walter de Gruyter, Berlin, 1977, p. 15.
64. S. Kobayshi and M. Yasumura, *J. Nutr. Sci. Vitaminol.* **19**, 123 (1973); S. Kobayshi and co-workers, *J. Nutr. Sci. Vitaminol.* **26**, 545 (1980).
65. D. H. R. Barton, R. H. Heese, M. M. Pecket, and E. Rizzardo, *J. Am. Chem. Soc.* **95**, 2748 (1973).
66. K. Pfoertner and J. D. Weber, *Helv. Chim. Acta* **55**, 921, 937 (1972).
67. A. Verloop, A. L. Koevoet, R. Van Moorselase, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **78**, 1004 (1959).
68. A. Verloop, A. L. Koevoet, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **74**, 1125 (1955).
69. T. Kobagashi, *J. Vitaminol.* (*Jpn*). **13**, 255 (1967).
70. H. H. Inhoffen, G. Quinkert, J. H. Hess, and H. M. Erdman, *Chem. Ber.* **89**, 2273 (1956).
71. C. H. Nields, W. C. Russell, and A. Zimmerli, *J. Biol. Chem.* **136**, 73 (1940).
72. J. B. Wilkie, S. W. Jones, and O. L. Kline, *J. Amer. Pharm. Assoc. Sci. Ed.* **47**, 395 (1958).
73. S. C. Eyley and D. H. Williams, *J. Chem. Soc. Chem. Comm.*, 858 (1975).
74. E. C. Snoeren, M. R. Daha, J. Lugtenburg, and E. Havinga, *Rec. Trav. Chim. Pays-Bas* **89**, 261 (1970).
75. W. G. Dauben and R. B. Phillips, *J. Am. Chem. Soc.* **104**, 355 (1982).
76. V. Malatesta, C. Willis, and P. A. Hackett, *J. Am. Chem. Soc.* **103**, 6781 (1981).
77. U.S. Pat. 3,661,939 (May 9, 1972), (to Nisshin Flour Milling Co., Ltd.).
78. P. S. Chen and H. B. Bosman, *J. Nutr.* **83**, 133 (1964).
79. Code of Federal Regulations, Title 21-Food and Drugs, Part 582.5923, p. 541, April 1, 1995.
80. *Chron. WHO* **3**, 747 (1965).
81. *The United States Pharmacopeia 23 USP 23-NF 18*) The United States Pharmacopeial Convention, Inc., Rockville, Md., 1995.
82. P. Cunniff, ed., *Official Methods of Analysis of the International Association of Official Analytical Chemists*, 16th ed., Washington, D.C., 1995.
83. F. Mulder, *J. Assoc. Off. Anal. Chem.* **60**, 989 (1977).
84. H. Hofsass, N. J. Alicino, A. L. Hirsch, L. Amieka, and L. D. Smith, *J. Assoc. Off. Anal. Chem.* **61**, 774 (1978).
85. F. Mulder and co-workers, *J. Assoc. Off. Anal. Chem.* **64**, 61 (1981); **65**, 1228 (1982); **68**, 822 (1985).
86. Ref. 6, p. 64.
87. J. Carol, *J. Pharm. Sci.* **50**, 451 (1961).
88. W. W. Morris, Jr., J. B. Wilkie, S. W. Jones, and L. Friedman, *Anal. Chem.* **34**, 381 (1962).
89. R. Strohecker and H. M. Henning, *Vitamin Assay-Tested Methods*, CRC Press, Cleveland, Ohio, 1966, p. 281.
90. U.S. Pats. 2,777,797 and 2,777,798 (Jan. 15, 1977), M. Hochberg and M. L. MacMillan (to Nopco Chemical Co.).
91. J. R. Evans, *Clin. Chim. Acta* **42**, 167 (1972).
92. T. K. Murray, K. C. Day, and E. Kodicek, *Biochem. J.* **98**, 29P (1966).
93. L. V. Aviolo and S. W. Lee, *Anal. Biochem.* **16**, 193 (1966).

94. D. Sklan, P. Budowski, and M. Katz, *Anal. Biochem.* **56**, 606 (1973).
95. D. O. Edlund and F. A. Filippini, *J. Assoc. Anal. Chem.* **56**, 1374 (1973).
96. T. G. Hogness, A. E. Sidwell, Jr., and F. P. Zscheile, Jr., *J. Biol. Chem.* **120**, 239 (1937).
97. W. Huber, G. W. Ewing, and J. Kriger, *J. Am. Chem. Soc.* **67**, 609 (1945).
98. R. H. Wasserman and co-workers, *Vitam. Horm.* (*N.Y.*) **32**, 299 (1974).
99. R. L. Horst and co-workers, Ref. 55, p. 93.
100. U.S. Pat. 3,334,118 (Aug. 1, 1967), K. Schaff, S. Schmuhler, and H. C. Klein (to Nopco Chemical Co.).
101. U.S. Pat. 3,665,020 (May 23, 1972), R. Marbet (to Hoffmann-LaRoche, Inc.).
102. R. S. Harris, J. W. M. Bumker, and L. M. Moser, *J. Am. Chem. Soc.* **60**, 2579 (1938).
103. H. H. Inhoffen and co-workers, *Chem. Ber.* **91**, 2309 (1958); H. H. Inhoffen and co-workers, *Chem. Ber.* **92**, 1564 (1959).
104. T. M. Dawson, J. Dixon, P. S. Littlewood, B. Lythgoe, and A. K. Saksena, *J. Chem. Soc. C*, 2960 (1971).
105. B. Lythgoe, M. E. N. Nambudiry, and J. Tideswell, *Tetrahedron Lett.* **31**, 3685 (1977).
106. J. A. Campbell, D. M. Squires, and J. C. Babcock, *Steroids* **13**, 567 (1969).
107. S. J. Halkes and N. P. VanVliet, *Rec. Trav. Chim. Pays-Bas* **88**, (1969).
108. J. W. Blunt and H. F. DeLuca, *Biochemistry* **8**, 671 (1969).
109. M. Morisaki, J. Rubio-Lightbourn, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 457 (1973).
110. J. J. Partridge, S. Faber, and M. R. Uskokovic, *Helv. Chim. Acta* **57**, 764 (1974).
111. U.S. Pat. 4,172,076 (Apr. 4, 1977), A. Hirsch and J. Pikl (to Diamond Shamrock, subsequently assigned to A. L. Labs, Jan. 1, 1982).
112. U.S. Pat. 4,226,770 (Oct. 7, 1980), E. Kaiser.
113. M. H. Holick and co-workers, *Biochemistry* **11**, 4251 (1972).
114. Y. Tanaka, H. Frank, H. F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry* **14**, 3293 (1975).
115. Y. Tanaka, H. F. DeLuca, N. Ikekawa, M. Morisaki, and N. Koizumi, *Arch. Biochem. Biophys.* **170**, 620 (1975).
116. H.-Y. Lam. H. K. Schnoes, H. F. DeLuca, and T. C. Chen, *Biochemistry* **12**, 4851 (1973).
117. M. Seki, J. Rubio-Lightbourn, J. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 2783 (1973).
118. J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *C. R. Acad. Sci.* **278**, 529 (1974).
119. J. Redel and co-workers, *J. Steroid Biochem.* **6**, 117 (1975).
120. N. Ikekawa, M. Morisaki, N. Koizumi, Y. Kato, and T. Takeshita, *Chem. Pharm. Bull.* **23**, 695 (1975).
121. G. Milhaud, M.-L. Labat, and J. Redel, *C. R. Acad. Sci.* **279**, 827 (1974).
122. M. Seki, N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, 15 (1975).
123. J. J. Partridge, V. Toome, and M. R. Uskokovic, *J. Am. Chem. Soc.* **98**, 3739 (1976).
124. E. J. Semmler, M. F. Holick, H. K. Schnoes, and H. F. DeLuca, *Tetrahedron Lett.*, 4147 (1972).
125. K. Ochi and co-workers, *J. Chem. Soc. Perkin I*, 165 (1979).
126. T. A. Narwid, J. F. Blunt, J. A. Iacobelli, and M. R. Uskokovic, *Helv. Chim. Acta* **57**, 781 (1974).
127. J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 1854 (1973).
128. M. Morisaki, K. Bannai, and N. Ikekawa, *Chem. Pharm. Bull.* **21**, 1853 (1973).
129. M. Morisaki, J. Rubio-Lightbourn, N. Ikekawa, and T. Takeshita, *Chem. Pharm. Bull.* **21**, 2568 (1973).
130. H. E. Paaren, D. E. Hamer, H. K. Schnoes, and H. F. DeLuca, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2080 (1978).
131. N. Ikekawa and co-workers, *Biochem. Biophys. Res. Commun.* **62**, 485 (1975).
132. J. J. Partridge, S. J. Shivey, E. G. Baggiolini, B. Hennessy, and M. Uskokovic, in A. W. Norman and co-eds., *Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism*, Walter de Gruyter, Berlin, 1977, p. 47.
133. H.-Y. Lam, H. K. Schnoes, and H. F. DeLuca, *Steroids* **25**, 247 (1975).
134. J. Redel, P. Bell, F. Delbarre, and E. Kodicek, *C. R. Acad. Sci.* **276**, 2907 (1973).
135. J. Redel and co-workers, *Steroids* **24**, 463 (1974); J. Redel, N. Bazely, Y. Tanaka, and H. F. DeLuca, *FEBS Lett.* **94**, 228 (1978).
136. A. W. Norman and co-workers, *Biochemistry* **22**, 1798 (1983).
137. H. F. DeLuca and H. K. Schnoes, in A. W. Norman and co-eds., *Vitamin D: Recent Basic Advances and Their Clinical Application*, Walter de Gruyter, Elmsford, N.Y., 1979.
138. D. E. M. Lawson, *Vitamin D*, Academic Press, Inc., New York, 1978.
139. H. Dai and G. H. Posner, *Synthesis*, 1383 (1994).

140. W. H. Okamura and C. D. Zhu, *Chemical Reviews* **95**(c), 1877 (1995).
141. A. W. Norman, R. Bouillon, and M. Thomasset, eds., *Vitamin D, Structure, Function Analysis and Clinical Application, Proceedings of the Eighth Workshop on Vitamin D*, Paris, July 5, 1991, Walter de Gruyter, Berlin, 1991.
142. D. H. R. Barton, R. H. Hesse, M. M. Pechet, and E. Rizzardo, *J. Chem. Soc., Chem. Commun.*, 203–204 (1974).
143. D. H. R. Barton, R. H. Hesse, M. Pechet, and E. Rizzardo, *J. Am. Chem. Soc.* **95**, 2748 (1973).
144. B. Lythgoe and co-workers, *Tetrahedron Lett.*, 3863–3866 (1975).
145. B. Lythgoe and co-workers, *J. Chem. Soc., Perkin Trans.*, **1** 590–595 (1978).
146. B. Lythgoe, T. A. Moran, M. E. N. Nambudiry, and S. Ruston, *J. Chem. Soc., Perkin Trans.*, **1**, 2386–2390 (1976).
147. B. Lythgoe and co-workers, *J. Chem. Soc., Perkin Trans.*, **1**, 387–395 (1978).
148. J. V. Frosch, I. T. Harrison, B. Lythgoe, and A. K. Saksena, *J. Chem. Soc., Perkin Trans.*, **1**, 2005–2009 (1974).
149. E. G. Baggiolini and co-workers, *J. Org. Chem.* **51**, 3098–3108 (1986).
150. J. DeSchrijver and R. J. De Clercq, *Tetrahedron Lett.* **34**, 4369 (1993).
151. G. H. Posner and C. M. Kinter, *J. Org. Chem.* **55**, 3967–3969 (1990).
152. L. Castedo, J. L. Mascarenas, A. Mourino, and L. A. Sarandeses, *Tetrahedron Lett.* **29**, 1203–1206 (1988).
153. L. Castedo, A. Mourino, and L. A. Sarandeses, *Tetrahedron Lett.* **29**, 1203–1206 (1988).
154. S. A. Barrack, R. A. Gibbbs, and W. J. Okamura, *J. Org. Chem.* **53**, 1790–1796 (1988).
155. J. M. Aurrecoechea and W. H. Okamura, *Tetrahedron Lett.* **28**, 4947–4950 (1987).
156. W. H. Okamura, J. M. Aurrecoechea, R. A. Gibbs, and A. W. Norman, *J. Org. Chem.* **54**, 4072–4083 (1989).
157. E. M. VanAlstyne, A. W. Norman, and W. H. Okamura, *J. Am. Chem. Soc.* **116**, 6207–6216 (1994).
158. M. L. Hammond and co-workers, *J. Am. Chem. Soc.* **100**, 4907 (1978).
159. P. Condran and co-workers, *J. Am. Chem. Soc.* **102**, 6259 (1980).
160. W. H. Okamura and co-workers, in Ref. 53, 5–12.
161. B. M. Trost, J. Dumas, and M. Villa, *J. Am. Chem. Soc.* **114**, 9836–9845 (1992).
162. K. Nagasawa, Y. Zako, H. Ishihara, and I. Shimizu, *Tetrahedron Lett.* **32**, 4937–4940 (1991).
163. M. Sheves and Y. Mazur, *J. Am. Chem. Soc.* **97**, 6249–6250 (1975).
164. H. E. Paaren, H. F. DeLuca, and H. K. Schnoes, *J. Org. Chem.* **45**, 3253–3258 (1980).
165. M. Kabat and co-workers, *Tetrahedron Lett.* **32**, 2343–2346 (1991).
166. S. R. Wilson, A. M. Venkatesan, C. E. Augelli-Szafran, and A. Yasmin, *Tetrahedron Lett.* **32**, 2339–2342 (1991).
167. D. R. Andrews and co-workers, *J. Org. Chem.* **51**, 1635–1637 (1986).
168. H. H. Inhoffen, G. Quinkert, and S. Schutz, *Chem. Ber.* **90**, 1283–1286 (1957).
169. H. H. Inhoffen, G. Quinkert, H.-J. Hess, and H. Hirschfeld, *Chem. Ber.* **90**, 2544–2553 (1957).
170. D. R. Andrews, D. H. R. Barton, R. H. Hesse, and M. M. Pechet, *J. Org. Chem.*, **51**, 4819–4828 (1986).
171. L. Vanmaele, P. J. De Clercq, and M. Vandewalle, *Tetrahedron Lett.* **41**, 141–144 (1985).
172. M. F. Holick, *Am. J. Clin. Nutr.* **60**, 619–630 (1994).
173. M. F. Holick and co-workers, *Endocrinology* **135**, 655 (1994).
174. G. Ponchon, A. L. Kennan, and H. F. DeLuca, *J. Clin. Invest.* **48**, 1273 (1969).
175. T. C. Madhok and H. F. DeLuca, *Biochem. J.* **184**, 491 (1979); E. Axen, T. Bergman, and K. Wikvall, *J. Steroid Biochem. Mol. Biol.* **51**, 97 (1994).
176. S. A. Holick, M. F. Holick, T. E. Tavela, H. K. Schnoes, and H. F. DeLuca, *J. Biol. Chem.* **251**, 397 (1976).
177. E. B. Olson, Jr., J. C. Knutson, M. H. Bhattacharyya, and H. F. DeLuca, *J. Clin. Invest.* **57**, 1213 (1976).
178. J. A. Eisman, R. M. Shepard, and H. F. DeLuca, *Anal. Biochem.* **80**, 198 (1977).
179. D. R. Fraser and E. Kodicek, *Nature* (*London*) **228**, 764 (1970).
180. J. G. Ghazarian, C. R. Jefcoate, J. C. Knutson, W. H. Orme-Johnson, and H. F. DeLuca, *J. Biol. Chem.* **249**, 3026 (1974).
181. L. T. Boyle, L. Miravet, R. W. Gray, M. F. Holick, and H. F. DeLuca, *Endocrinology* **90**, 605 (1972).
182. T. C. Chen, L. Castillo, M. Korycka-Dahl, and H. F. DeLuca, *J. Nutr.* **104**, 1056 (1974).
183. H. F. DeLuca and co-workers, in C. Hansch, P. G. Sammes, and J. B. Taylor, eds., *Comprehensive Medicinal Chemistry*, Vol. **3**, Pergamon Press, Oxford, U.K., 1991, p. 1129.
184. A. W. Norman and co-workers, *J. Steroid Biochem. Mol. Biol.* **41**, 231 (1992).
185. K. E. Lowe, A. C. Maiyor, and B. W. Norman, *Crit. Rev. Eukar. Gene. Exp.* **2**, 65–109 (1992).
186. D. T. Baran, *J. Cell. Biochem.* **270**, 303 (1994).
187. D. W. Beno and co-workers, *J. Biol. Chem.* **270**, 3542 (1995).
188. R. Kumar, D. Harnden, and H. F. DeLuca, *Biochemistry* **15**, 2420 (1976).

189. D. Harnden, R. Kumar, M. F. Holick, and H. F. DeLuca, *Science* **193**, 493 (1976).
190. M. F. Holick and co-workers, *Biochemistry* **11**, 4251 (1972).
191. Y. Tanaka, L. Castillo, H. F. DeLuca, and N. Ikekawa, *J. Biol. Chem.* **252**, 1421 (1977).
192. M. F. Holick and co-workers, *J. Biol. Chem.* **248**, 6691 (1973).
193. J. N. M. Heersche and J. A. Kanis, eds., *Bone and Mineral Research*, Vol. **8**, Elsevier Science B.V., Amsterdam, the Netherlands, 1994, p. 45.
194. M. Gross and co-workers, *J. Bone Miner. Res.* **1**, 457 (1986).
195. T. V. Wijngaarden and co-workers, *Cancer Res.* **54**, 5711 (1994).
196. R. J. Skowronski, D. M. Pechl, and D. Feldman, *Endocrinology* **136**, 20 (1995).
197. M. Inaba and co-workers, *Blood* **82**, 53 (1993).
198. J. A. MacLaughlin and co-workers, *Proc. Natl. Acad. Sci.* **82**, 5409 (1985).
199. S. Morimoto and co-workers, *Calcif. Tissue Int.* **39**, 209 (1986).
200. S. Morimoto and co-workers, *Calcif. Tissue Int.* **38**, 119 (1986).
201. K. J. Kragballe, *J. Cell. Biochem.* **49**, 46 (1992).
202. J. M. Lemire, *J. Cell. Biochem.* **49**, 26 (1992).
203. E. P. Amento, *Steroids* **49**, 55 (1987).
204. M. S. Saporito and co-workers, *Brain Res.* **633**, 189 (1994).
205. M. S. Saporito and co-workers, *Exp. Neurol.* **123**, 295 (1993).
206. S. Carswell, *Exp. Neurol.* **124**, 36 (1993).
207. H. Kohler and co-workers, *Zentralbl. Veterinaermed. Reihe B.* **25**, 89 (1978).
208. F. H. Bird, *Poult. Sci.* **57**, 1293 (1978).
209. B. Jedek and coworkers, *Zentralbl. Veterinaermed. Reihe B.* **25**, 29 (1978).
210. *Vitamin Tolerances of Animals*, National Research Council, National Academy Press, Washington, D.C., 1987, p. 10.
211. Y. H. Hull, *Encyclopedia of Food Science and Technology*, Vol. **4**, John Wiley & Sons, Inc., New York, 1991, Pts. 1–8, p. 2713.
212. *Recommended Dietary Allowances*, 7th ed., National Academy of Science, National Research Council, Washington, D.C., 1968, p. 1964.
213. *Nutritient Requirements of Poultry*, 9th ed., National Academy of Science, National Research Council, Washington, D.C., 1994, p. 15.
214. *Nutritient Requirements of Swine*, 9th ed., National Academy of Science, National Research Council, Washington, D.C., 1988, p. 35.
215. *Nutritional Requirements of Dairy Cattle*, 3rd ed., National Academy of Science, National Research Council, Washington, D.C., 1966, p. 1349.
216. H. Edwards, "Factors Influencing Leg Disorders in Broilers," p. 21 in (Proceedings of the Maryland Nutrition Conference, March 23, 1995, University of Maryland, College Park, Maryland).
217. W. Putscher, *Z. Kinderheilkd.* **48**, 269 (1929).
218. D. O. Harrington and E. H. Page, *J. Am. Vet. Med. Assoc.* **182**, 1358 (1983).
219. K. Diem and C. Lentner, eds., *Scientific Tables*, Ciba Geigy, Ltd., Basel, Switzerland, 1971, p. 464.
220. R. L. Morrissey and co-workers, *J. Nutr.* **107**, 1027 (1977).
221. *Oil, Paint and Drug Reporter*, **167**, No. 2, p. 23 (1955) and subsequent issues.
222. *Chemical Economics Handbook*, Stanford Research Institute, Menlo Park, Calif., 1980.
223. *Chem. Mktg. Rep.* **251**, No. 5, p. 39 (1997).
224. U.S. Pat. 2,417,299 (Mar. 11, 1947), L. Freedman and E. Green (to U.S. Vitamin Corp.).
225. U.S. Pat. 2,702,262 (Feb. 15, 1955), Burley and A. E. Timrech (to Charles Pfizer and Co., Inc.).
226. U.S. Pat. 2,827,452 (Mar. 18, 1978), H. Schlenk, D. M. Sand, and J. A. Tillotson (to University of Minnesota).
227. U.S. Pat. 3,067,104 (Dec. 4, 1962), M. Hochberg and C. Ely (to Nopco Chemical Co.).
228. U.S. Pat. 3,143,475 (Aug. 4, 1964), A. Koff and R. F. Widmer (to Hoffmann-LaRoche, Inc.).

General References

229. Refs. 5, 6, 16, 51, 62, and 141, are also general references.
230. C. E. Bells, in W. H. Sebrell and R. S. Harris, eds., *The Vitamins*, 1st ed., Vol. **2**, Academic Press, New York, 1954, p. 132.
231. G. F. Combs and H. F. DeLuca, *The Vitamins: Fundamental Aspects in Nutrition and Health*, Academic Press, Inc., 1992, p. 151.
232. E. D. Collins and A. W. Norman, in L. J. Macklin, ed., *Handbook of Vitamins*, 2nd ed., Marcel Dekker, New York, 1991, Chapt. 2, p. 59.
233. H. F. DeLuca in R. B. Olfin-Slater and D. Kritchevsky, eds., *Nutrition and the Adult: Micronutrients*, Plenum Press, New York, 1986, p. 205.
234. W. Friedrich, *Vitamins*, Walter de Gruyter, New York, 1988, p. 141.
235. A. W. Norman, in M. Brown, ed., *Present Knowledge in Nutrition*, 6th ed., International Life Sciences Institute–Nutrition Foundation, Washington, D.C., 1990, Chapt. 12, p. 108.

ARNOLD L. HIRSCH
Alpharma Inc.

Related Articles

Vitamins, survey; Pharmaceuticals; Feed and feed additives