CHAPTER

6

Industrial Aspects of Vitamin D

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HISTORY OF VITAMIN D LEADING TO COMMERCIALIZATION

The late 1800s experienced the onset of the Industrial Revolution. With it, there was an increased incidence of the bone disease rickets in Europe and America. A concerted effort was made to find a way to prevent and/or cure this crippling disease of children. Scurvy and beriberi were known to be prevented by the addition of citrus fruits containing vitamin C and whole grain rice containing vitamin B_1 to the diet, and researchers began to look for foods which would cure rickets in a similar fashion. The success of these investigations led to the commercial production of vitamin D products for medicine and nutrition in humans as well as animals. An excellent summary of this history is found in "The Discovery of Vitamin D: The Contribution of Adolf Windaus" by George Wolf [1]. A brief review of this history with relation to the commercialization of the manufacture and use of vitamin D is in order.

In 1919, Sir Edward Mellanby [2], describing the results of his studies, said "The action of fats in rickets is due to a vitamin or accessory food factor which they contain, probably identical with the fat-soluble vitamin." Huldschinski [3] realized in the same year that UV light cured rickets and impacted on its causation. He additionally identified cod liver oil as an effective antirachitic agent and he and other researchers discovered the fact that sunlight exposure was also effective in preventing and curing rickets. These efforts led towards the realization that rickets was somehow related to lack of exposure to sunlight and that there was also a food substance that could be added to the diet to prevent or cure rickets. In 1922, this was followed by investigations of Harriett Chick and her coworkers [4], which showed that rickets in children could be cured by whole milk or cod liver oil.

In 1923, Goldblatt and Soames [5,6] identified a vitamin D precursor in the skin of animals which produced a substance equivalent to the fat-soluble vitamin when exposed to sunlight or UV-light.

In 1923, Hess and Weinstock [7] showed that animal skin irradiated with ultraviolet light, when fed to rachitic rats, provided absolute protection against rickets and consequently the fat-soluble vitamin was not an essential dietary trace constituent.

Merging these two cures for rickets, Steenbock and Black [8] in 1925 demonstrated that eating some foods that had been irradiated was an effective treatment for the prevention and cure of rickets. The irradiation of certain foods became standard practice in the 1920s for preventing rickets in the general population. The antirachitic factor was shown to be fat-soluble and became classified as a vitamin.

The link between irradiation and plant materials led to the conclusion that ergosterol was an antirachitic substance. The UV spectrum of provitamin D changed with UV irradiation and produced antirachitic activity; leading to the conclusion that vitamin D was derived from the provitamin.

In the 1930s, Adolph Windaus determined the formula for vitamin D_2 to be $C_{28}H_{44}O$ which was isomeric with the provitamin [9] (see also [1] for review of Windaus' work). In 1937, he also isolated 7-dehydrocholesterol from pig skin, characterized the molecule, and produced vitamin D_3 by UV irradiation of it. Thus began the commercial production of vitamin D.

Rygh [10] showed that one rat unit of cod liver oil was 100 times more effective in chicks than one rat unit of vitamin D_2. The fact that two forms of the vitamin were identified led to the commercialization of the production of both materials.

The antirachitic component of cod liver oil was shown to be identical to the newly characterized vitamin D_3 by Brockman in 1937 [11]. These results clearly established that the antirachitic substance vitamin D was a seco-steroid (a steroid in which the 9, 10 bond of the B ring has been broken, i.e. see (12)−(14) in Fig. 6.7).

The fact that exposure of chickens to direct UV irradiation resulted in protection from the effects of rickets, while ergosterol irradiated with UV light to form vitamin D_2 was not effective in chickens (although it was antirachitic in rats) led to the conclusion that the vitamin D_3 effective in chicks and made from the provitamin in cholesterol was different from ergosterol.

Although the focus on vitamin D was initially concerned with the material as an antirachitic agent in humans, it was soon realized that animals grown for commercial food were subject to the same ailment and in much greater numbers. The need to develop a material to add to animal feed for the prevention of rickets and maintenance of good health in growing livestock was recognized. As poultry and other animals began to be raised entirely or in part indoors and out of direct sunlight, it became critical to add vitamin D to the diet of these animals. Thus, vitamin D became one of the most important, if not the most important, micronutrients in the diet of livestock.

Cod liver oil was used as the major source of the antirachitic ingredient in both human and animal nutrition until synthetic methods could be developed to meet the commercial requirements. Initially, cod liver oil became the standard against which all other materials were measured in comparing their antirachitic properties. By 1936, however, Eliot and coworkers [12] and others had shown that crystalline vitamin D_3 added to milk at 400 USP units was more effective than cod liver oil or viosterol (irradiated ergosterol; vitamin D_2). Companies involved in the manufacture of vitamin D products for use in milk fortification and nutritional aids as well as animal feed ingredients became interested in the synthesis of the vitamin D for commercial purposes. Fish liver oils are naturally high in vitamin D content, but as the requirements to refine the oil developed, the vitamin content was diminished and became variable, containing 50 to 45 000 IU/gram of vitamin D_3 [13]. In the past several years, because the value has been variable and difficult to standardize for nutritional purposes, synthetic vitamin D sources are preferred.

The major vitamin D products used in industry today are vitamin D_3, vitamin D_2, 25-hydroxyvitamin D_3, and 1α-hydroxyvitamin D_3. Other derivatives such as 1α,25-dihydroxyvitamin D_3 and synthetic analogs are used primarily in pharmaceutical applications and are made in much smaller volumes. For example, Calipotriene (Dovenex), which is a derivative of vitamin D_2, is used for psoriasis as are Doxercalciferol (Hectoral) and Paricalcital (Zemplar) for secondary hyperparathyroidism associated with chronic renal failure. Large-volume products, however, are used in human and animal health and nutrition, with the largest amount of material produced for animal nutrition. This is mainly because of the large number of animals used for meat, milk, and egg production. Vitamin D is a critical component of the diet of animals, especially for those raised indoors without exposure to sunlight and no source of the vitamin. The vitamin is critical to the good health of the animal and to its efficient growth.

Vitamin D_3 has also become a critical nutritional supplement for humans as well, since large numbers of people have been found to have low vitamin D blood levels. This appears to be caused primarily by a lack of adequate exposure to sunlight (people are afraid of developing melanoma and therefore avoid sun exposure or use a sunscreen, limiting UV skin irradiation even when in sunlight). Research over the past 10 years has demonstrated that vitamin D appears to play a critical role in the maintenance of good health. The nutritional requirements of animals and the maintenance of good health in humans have placed an ever-increasing challenge to produce vitamin D and its derivatives in an efficient and economical fashion.

MANUFACTURE OF THE PROVITAMINS

The manufacture of the vitamins and their derivatives involves the synthesis of the provitamin from cholesterol (1) (Fig. 6.1) in the case of vitamin D_3 (2) or the isolation of the ergosterol (3) (Fig. 6.2) from plant sources such as yeast for the manufacture of vitamin D_2 (4). The vitamin is then generated by UV irradiation of the provitamin. It should be noted that ergosterol (3) and 7-dehydrocholesterol share similar structures with only two differences. Ergosterol possesses an additional double bond at position 22,23 and has an additional methyl group on position 24 of the steroid side chain. This fact accounts for the similar behavior of the two molecules with regard to activation. They both undergo the same photochemical transformations to form the same series of photoisomers and have very similar biological activities. The major difference is that vitamin D_2 is not active in poultry.

7-Dehydrocholesterol

The finding that vitamin D_3 derived from the irradiation of cholesterol was a more efficient form of vitamin D than that found in plant sources (particularly when used in poultry feed) led to investigations to convert cholesterol into 7-dehydrocholesterol for the commercial production of vitamin D. Isolation of cholesterol from one of its natural sources is the first step in the commercial production of vitamin D_3. Cholesterol occurs in almost all animals, and it can be extracted from the spinal cords and brains of large animals. The major commercial source is from sheep's wool. Spinal cord and brain extractions

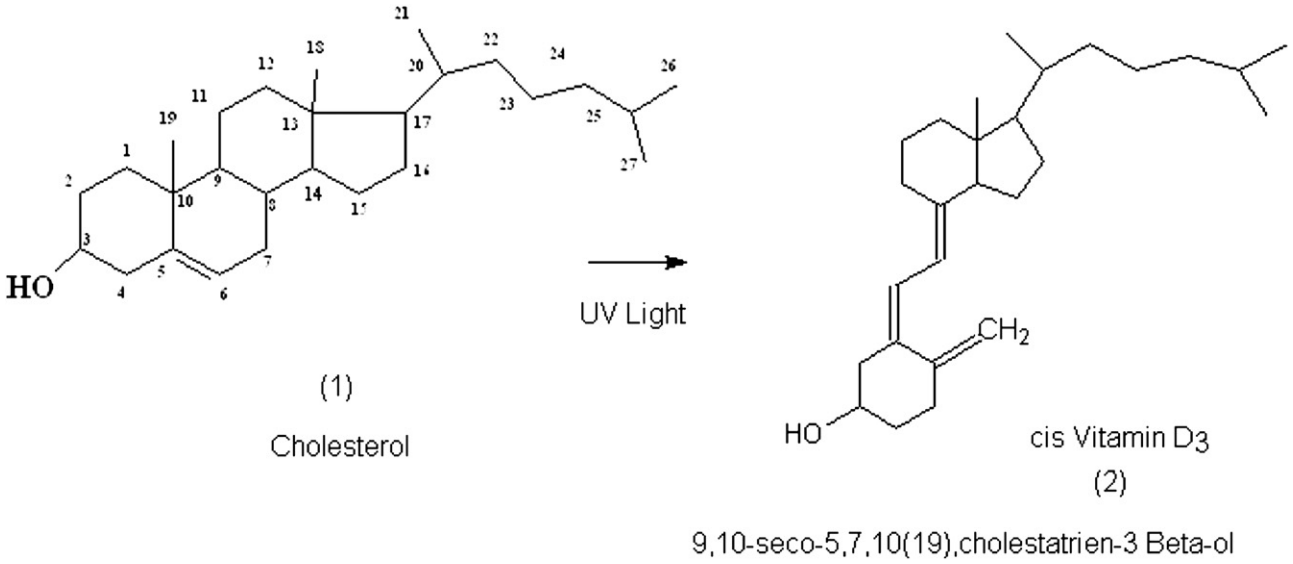

FIGURE 6.1 Cholesterol (with numbering of carbon atoms) and vitamin D_3.

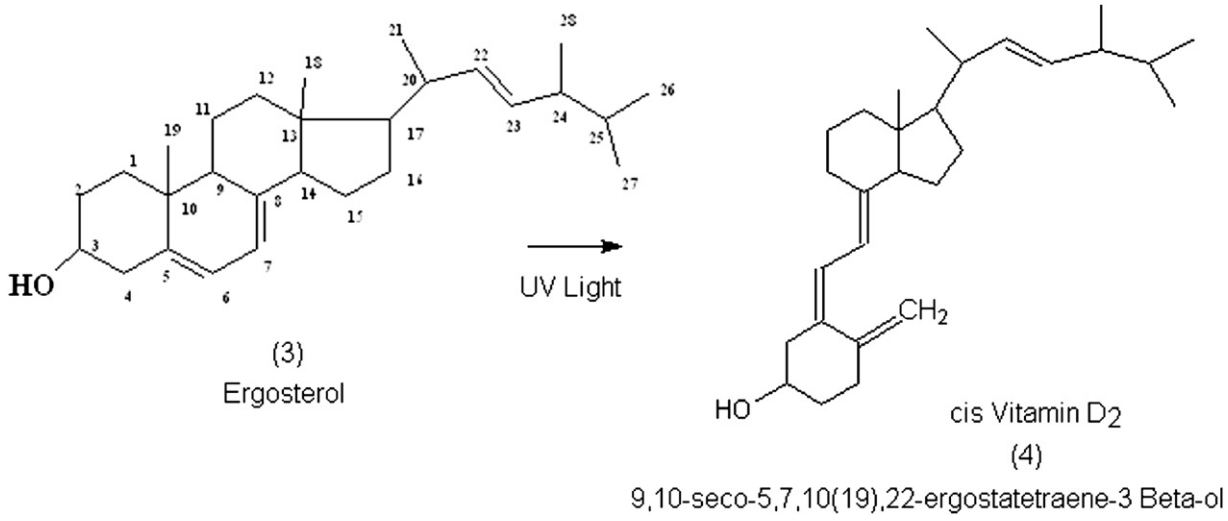

FIGURE 6.2 Ergosterol (with numbering of carbon atoms) and vitamin D_2.

were acceptable sources for many years and were used when cholesterol prices rose to levels that justified the more expensive processing costs over those for the isolation of cholesterol from wool. However, today this source of cholesterol is not acceptable because of the danger of coextracting prion protein which can give rise to transmissible spongiform encephalopathy. Cholesterol obtained from wool grease is therefore the primary acceptable source of cholesterol for the manufacture of vitamin D. Sheep's wool must be cleaned after shearing and the wool grease that is obtained as a by-product from the washing contains about 15% cholesterol. The grease is a mixture of long-chain fatty-acid esters. These are saponified to give fatty acid soaps (usually isolated as the calcium salt) and an approximately equal weight of wool grease alcohols (containing a mixture of cholesterol, lanosterol, D_2, and other sterols and fatty alcohols). The acid soaps are separated by filtration and are used as heavy greases in industry. The cholesterol is separated from the other wool wax alcohols (usually by complexation with calcium or magnesium chloride). The solid complex is broken and the product is crystallized and purified with methanol. Cholesterol of the highest quality is necessary for the preparation of 7-dehydrocholesterol since the yield and quality of the provitamin is dramatically affected by the purity of the cholesterol used as starting raw material. Companies such as the National Oil Products Company (NOPCO), and Winthrop Chemical Company, as well as others, became involved in the vitamin business because of their interest in fish oil.

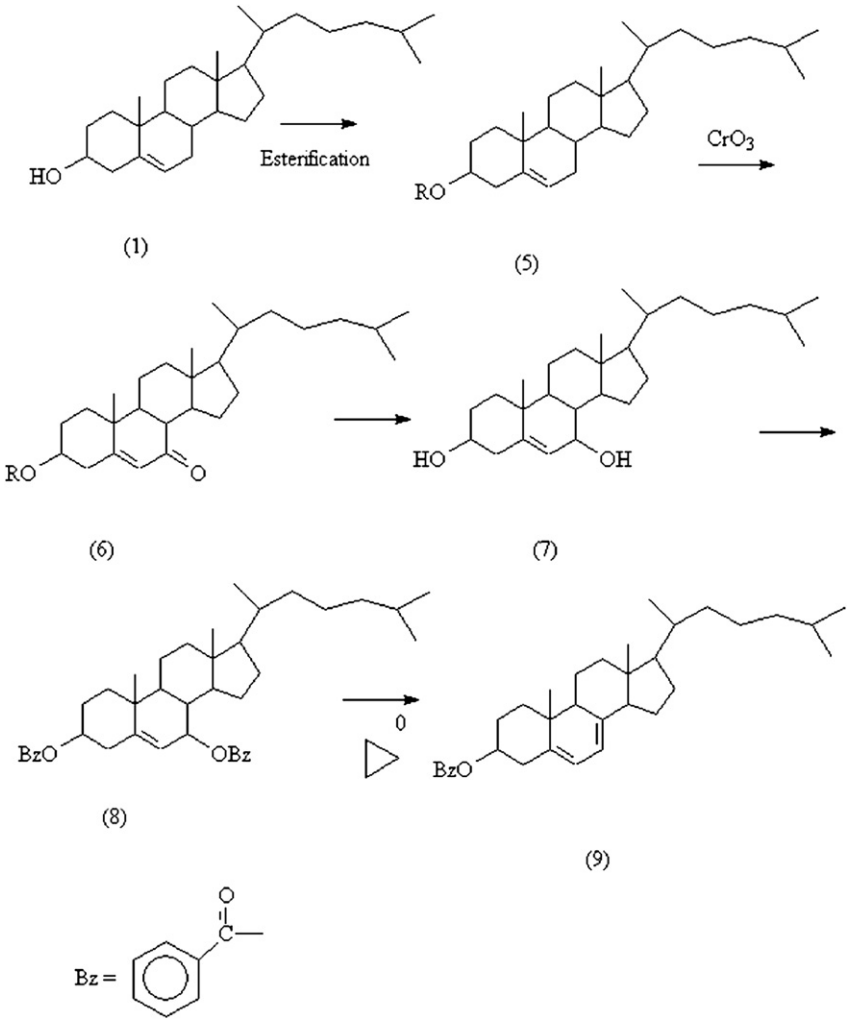

FIGURE 6.3 The Windaus procedure [10].

These were among the first companies to examine the synthetic manufacture of vitamin D_3 as an alternative to their use of cod liver oil. Several methods were investigated for the conversion of cholesterol to the provitamin. Windaus and Schenk [14,15] filed patents in 1935 assigned to the Winthrop Chemical Company for the chemical production of 7-dehydrocholesterol and its irradiation to form vitamin D_3. This procedure involved the oxidation of cholesterol (1) (with the 3-hydroxy group protected as an acetate ester (5)) to form the 7-keto cholesteryl acetate (6) which was then reduced to the 7-hydroxycholesterol (7) with aluminum isopropylate in isopropyl alcohol. The 3,7-dihydroxycholesterol is benzoylated followed by dehydration of the 3,5-dibenzoate (8) at elevated temperatures to give the 7-dehydrocholesterol benzoate (9) (see Fig. 6.3).

This procedure gave relatively low yields of the product of only about 4% and other methods were sought to improve the conversion of cholesterol into 7-dehydrocholesterol. The Windaus procedure has been improved recently to a point where the procedure is now used currently for a majority of the 7-dehydrocholesterol production.

The bromination-dehydrohalogenation process developed in the 1940s (see Fig. 6.4) was for many years the most generally used and most economical process for the production of 7-dehydrocholesterol for vitamin D_3 production. It involved the Ziegler allylic bromination [16] of the 7-position of cholesterol (1). The 3β-hydroxyl group is protected from oxidation by esterification, usually as the acetate or benzoate (5). The free radical bromine addition is accomplished by a number of agents, e.g., *N*-bromosuccinimide, *N*-bromophthalimide, or preferably 5,5-dimethyl-1,3-dibromohydantoin [17,18]. Bromine in carbon disulfide has also been employed using photo catalysis to generate the free-radical bromine [19]. 1,3-Dibromo,5,5-dimethylhydantoin (commonly called "brom 55") has been, and is still, the most efficient reagent for the introduction of bromine into the 7-position of cholesterol. This

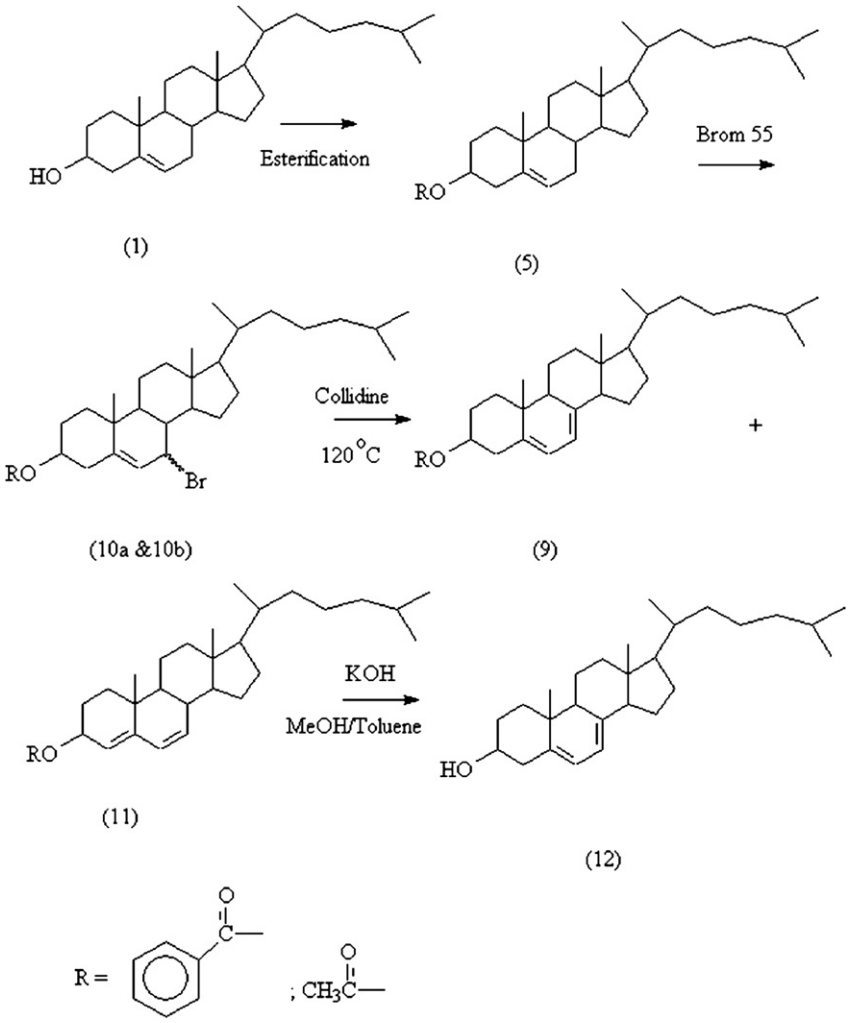

FIGURE 6.4 Preparation of 7-dehydrocholesterol from cholesterol.

bromination is not stereo-specific and conditions must be carefully controlled to optimize the production of the 7-α-bromo derivative in favor of the 7-β isomer (10a and 10b).

The elimination of the bromine atom from the 7-α position can occur by the dehydrohalogenation with the 8-beta hydrogen to generate the desired 7-dehydro conjugated diene (9). However, the allylic 4-hydrogen is also prone to elimination to produce the undesired 4,6 diene (11) probably from the 7-β-bromo confirmer. The formation of the 4,6-diene isomer lowers the amount of 5,7-isomer and also makes the purification of the 5,7-product very difficult. This can result in low yields. Dehydrohalogenation is generally accomplished through the use of various bases such as trimethyl phosphite or pyridine bases, but extreme care must be taken to avoid the generation of the unwanted 4,6-diene isomer. Holwerda [20] showed that the reaction of the 7α-bromocholesterol with 2,4,6-trimethylpyridine (collidine) followed first-order kinetics with respect to the 7-bromo substrate and zero-order kinetics with respect to the collidine. A 98% yield of the 4,6-diene was obtained in DMSO, while a 70% yield of the 5,7-diene product was observed in decalin or dioxane. This investigator then demonstrated that collidine was not acting in a normal E2 elimination. Rather, an ion-pair mechanism was proposed. The rate is dependent on the bromide ion concentration and the first-order kinetics with collidine is a result of the precipitation of collidine hydrobromide which results in a constant bromide ion concentration. Collidine is a unique base in this regard. Rappoldt and co-workers reported at the 5th Workshop on Vitamin D in 1982 [21] that the use of tetra-butylammonium fluoride was more efficient than the chloride or bromide in producing the 5,7-diene. They reported a 60—70% yield of 5,7-diene with purity of 90—100% when the 7-bromocholesterol was dissolved in THF, equilibrated with tetra butylammonium bromide to optimize the 7α-bromo isomer content and then dehydrobrominated with purified tetrabutylammonium fluoride.

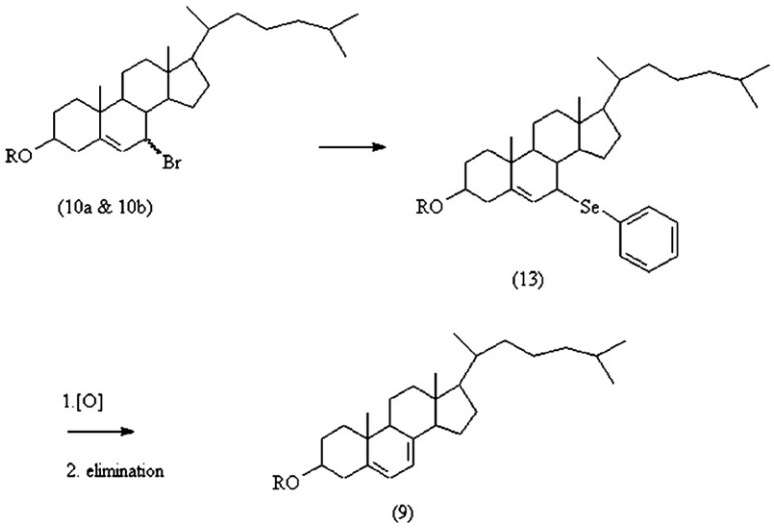

FIGURE 6.5 An alternate preparation of 7-dehydrocholesterol.

In commercial practice, the dehydrobromination is generally best accomplished using purified 2,4,6-trimethylpyridine at 120°C [22]. The yield of this process is usually on the order of 60% of the 5,7-isomer and gives rise to overall yields of only 50–60% (by weight) at best of the 7-dehydrocholesterol (12) from cholesterol. The purity of the collidine is critical, as traces of the isomeric trimethylpyridines or other pyridines results in considerably diminished yield of the desired 7-dehydrocholesterol. This is a result of the higher amounts of the 4,6-isomer that are produced together with other isomers; i.e. cholesta-5,8-diene-3β-ol [23].

The purification of the isolated de-brominated product is accomplished by recrystallization of the mixture of isomers to isolate a product with a high 5,7-diene content. This is critical to obtaining an efficient irradiation of the material to form vitamin D_3. Mixtures of solvent such as toluene and acetone can be used in various proportions to crystallize material predominantly rich in the desired 5,7-isomer.

Another method to produce 7-dehydrocholesterol involves the treatment of 7-α-bromo steroid with sodium phenyl selenolate [24] to form a 7-β-phenyl selenide (13). This compound can then be oxidized and the corresponding phenyl selenoxide eliminated to form the 7-dehydrocholesteryl ester (9) (see Fig. 6.5). This method has not replaced the direct bromination/dehydrohalogenation method because of economics but may be practical for use in the manufacture of vitamin D derivatives.

Despite early modifications of the Windaus oxidation procedure [25] to improve yield, the direct bromination/dehydrohalogenation procedure became the method of choice and continues to be used because of its economics. However, despite the many years of work to obtain better yields, it continues to give relatively low conversion yields from cholesterol (about 50%) with a concomitant buildup of the 4,6-diene by-product. Procedures to recover this material by conversion to the 5,7-diene or recycling back to cholesterol have proven inefficient and expensive.

In the last few years, modifications of the Windaus oxidation procedure have been developed and are being used for production of the majority of vitamin D_3 products in the world today. This method oxidizes a cholesterol ester at the 7-carbon atom to a carbonyl which is converted to a hydrazone with a substituted hydrazine [26]. The hydrazone is then reacted with base under mild conditions to produce 7-dehydrocholesterol in high yield and good quality at an economical cost [27]. This method has the advantage that no halogen is used in the process. This improves on the bromination/dehydrohalogenation methods which may leave bromine-containing impurities in the 7-dehydrocholesterol. This can give rise to the release of HBr during the irradiation process for the production of the vitamin. This acid generation can result in significant loss in vitamin D product. This new methodology gives fewer side reactions, leads to higher yields and produces a purer 7-dehydrocholesterol than the bromination-dehydrohalogenation methodology.

Ergosterol

Commercial ergosterol (3) of 90–100% purity is isolated exclusively from plant sources (usually yeast fermentation) and often contains up to 5 wt% of 5,6-dihydroergosterol. Many of the companies which began producing ergosterol early on were involved in yeast fermentation, such as breweries. The product

was obtained through isolation of total sterol content from the fermentation product and the subsequent separation of the provitamin from the other sterols. This isolation of the sterol fraction involves extraction of the total fat component, saponification, and extraction of the unsaponifiable (sterol containing) portion, usually with an ether. Another method is the saponification of the total fermentation material, followed by isolation of the nonsaponifiable fraction. Separation of the sterols from the unsaponifiable fraction was done by crystallization using a suitable solvent, e.g., acetone or alcohol. The ergosterol was then recrystallized from ethylene dichloride, alone or mixed with methanol. Ergosterol is particularly difficult to remove from yeast by simple extraction, usually resulting in only ca. 25% recovery. Ergosterol procedures were developed in which digestion with hot alkalies or with amines was used [28–33]. Variations of the isolation procedure have been developed; after saponification, for example, the fatty acids may be precipitated as calcium salts, which absorb the sterols. The latter are then recovered from the dried precipitate by solvent extraction. More recently new methods have been developed in China. Most of the vitamin D_2 made today comes from factories in South China where an abundant source of molasses from sugar cane or cassava is available for use in the fermentation media. In this medium, molasses represents the carbon source, yeast and urea are the nitrogen source and potassium phosphate is the phosphorus source. A variety of mineral salts including magnesium sulfate are also present. The 38-hour fed batch process controls the substrate concentration by maintaining the sugar level at 0.3–0.5%. The yield is in the range of 42 grams/liter of yeast cells containing 3–4 grams of ergosterol/100 grams of yeast cells [34]. The ergosterol is isolated by saponification of the yeast fermentate at 130°C with hot alkalis or amines, and removal of the protein and nucleic acid fraction by methanol extraction. The solvent is concentrated and the product is crystallized and isolated by filtration. Ethanol, acetone, and dichloroethylene or mixtures thereof are used to recrystallize the product. Annual production in China is over 10 metric tons by this method.

In North China where there is a preponderance of antibiotic fermentation facilities [35], ergosterol has also been produced using extraction from hyphae of penicillin production. Wet penicillin hypha contains 0.76% of ergosterol. Saponification is accomplished with 2.5 mol/L NaOH, 25% methanol solution at 100°C for 180 minutes. Extraction using ether or petroleum ether results in ergosterol with a yield of about 0.71% (4 g ergosterol/kg hyphae with a purity of 85–90%). *Aspergillus niger* for citric acid fermentation and *Rhizopus* for lactic acid fermentation are also organisms that serve as sources for ergosterol manufacture.

IRRADIATION OF 7-DEHYDROCHOLESTEROL AND ERGOSTEROL

The photochemical conversion of 7-dehydrocholesterol (12) is used to manufacture most of the vitamin D produced in the world today as vitamin D_3. Ergosterol (3) is irradiated to form vitamin D_2 (4), although this form of the vitamin is not used as extensively as it once was. It offers no real price advantage and has been shown to be less active in the pig, chicken, cow, and horse [36].

These molecules contain a 5,7-diene moiety in the B ring which can be excited by UV light of 250–350 nm wavelength leading to a π to π^* excitation. This results in the cleavage of the 9,10 bond of the B ring to form a secosteroid (2,4) (secosteroids are steroids in which two of the B-ring carbon atoms, C9 and C10, are not joined).

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 [37]. The value was later determined to be 9.3×10^{13} quanta.

The product of this reaction is pre vitamin D (14), commonly referred to as "pre." Pre undergoes thermal equilibrium to form cis vitamin D (2), commonly called "cis." Because these two substances form equilibrium mixtures with concentrations dependent on temperature and time, both are considered the active form of vitamin D.

The equilibrium composition is normally around 80% vitamin D and 20% pre vitamin D in commercial production in which the resin is heated at 60–80°C for several hours.

The reaction is an antarafacial hydride {1–7} sigmatopic shift which involves a rigid cyclic transition state [40].

At −20°C, the pre isomer equilibrates to less than 5% cis in a month and it takes 2204 days to form 80% cis. At 20°C, 80% cis is formed in 13 days while at 40°C, 80% cis is formed in hours. Conversely, 100% cis at 40°C forms 10% pre in 43 hours (see Fig. 6.6).

Pre vitamin D can also undergo photoisomerization to undesired by-products. Irradiation with UV light, leads to (*Z*)–(*E*) photoisomerization to the 6,7-(*E*)-isomer, tachysterol (15). The B-ring 9,10 bond can also reform to regenerate the starting provitamin (7-dehydrocholesterol), as well as its isomer, lumisterol (16) which results from the free rotation about the 6,7 bond giving rise to the 10α-methyl isomer. The quantum yields of these reversible reactions are shown in Figure 6.7. They form equilibrium mixtures which are

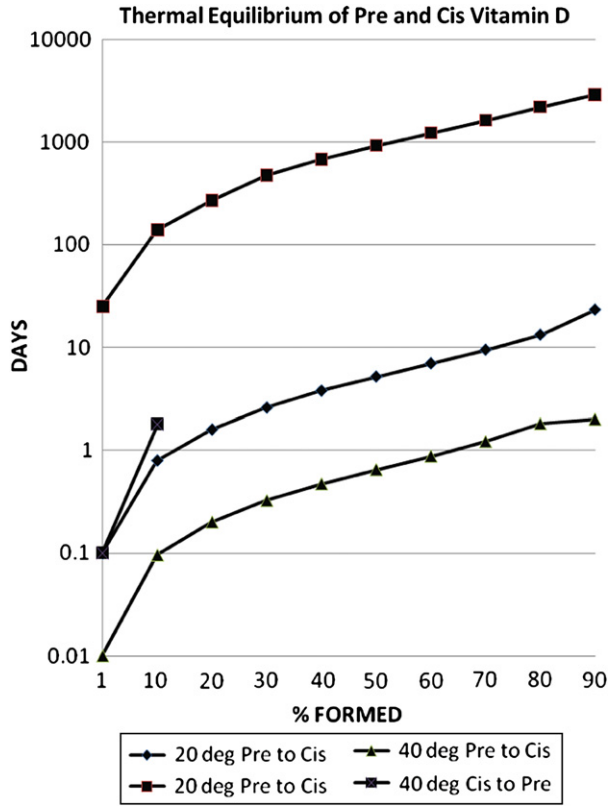

FIGURE 6.6 Thermal equilibrium of pre and *cis* vitamin D.

dependent on the wavelength of light used for the reaction as well as time and temperature.

It has been shown by theoretical calculations and confirmed experimentally that the optimum wavelength of light for the conversion of the provitamin into pre vitamin D is 296 nm [41]. A combination of photocyclization and photoisomerization pathways results in the formation of a variety of isomers. For a review of the extensive work done on the photochemistry of vitamin D see Jacobs and Laarhoven [42] as well as Dauben, McInnis and Michno [43].

It is assumed that only cZc conformers undergo photocyclization and that the rest undergo only photoisomerization resulting in a complex mixture of isomers.

Care must be exercised in the irradiation process to assure product with a normal composition of isomers. These isomers can give rise to many other by-products in the event of overirradiation or exposure to air, heat, and light (see Table 6.1 for a summary of normal products in D_2 and D_3 irradiation and Table 6.2 for a description of abnormal products which might form under various conditions which could result from improperly controlled irradiation and heating procedures).

The irradiation of 7-dehydrocholesterol (7-DHC) or ergosterol with UV light from Hg lamps gives rise to linear formation of the corresponding pre vitamin D with the concomitant reduction in provitamin concentration. As the pre levels build, irradiation of pre begins to generate tachysterol and lumisterol and these isomers build in concentration with continued irradiation. Pre also is in equilibrium with the 7-dehydrocholesterol. In addition to time of irradiation and frequency of the light, temperature, solvent, and concentration of substrate affect the ratio of isomers in the product (see Fig. 6.8).

Toxisterols, suprasterols, and other unusual forms of the type shown below (Fig. 6.9) can also form upon prolonged heating and irradiation [45–49]. There is little evidence that these materials are toxic despite their nomenclature.

These materials show little if any biological activity and are not found in vivo [11,49–52].

It is important to note that normal irradiation procedures for the production of vitamin D utilize sufficiently low temperatures and controlled irradiation so as to limit these products, and that the by-products of overirradiation and heating as shown in Table 6.3 do not form; they are not found in normal commercial samples of D_3 products.

The most common commercial process involves the use of mercury lamps as the light source; however, several papers and patents have also been published in which light sources generating UV light near the optimum for conversion of provitamin to pre vitamin D (about 292 nm) are described.

Bromine eximer lamps [53] that emit 292 nm light have been proposed for use in vitamin D_2 and D_3 manufacture. Laser light formed by an eximer or exciplex emitter that emits quasi-monochromatically according to the corona discharge mechanism [54] can give very specific monochromatic light at 282 nm. 7-Dehydrocholesterol (7-DHC) conversion is limited and the unused 7-DHC is recovered and recycled. While pure pre vitamin is obtained, it is produced at a low rate of conversion and thus the cost/photon is not economical for large-scale commercial production.

The maximum molecular extinction coefficients (at various wavelengths) of the four main components of the irradiation are 4500 at 254 nm and 1250 at 300 nm for 7-dehydrocholesterol, 725 at 254 nm, 930 at 300 nm and 105 at 330 nm for pre vitamin D_3; 11 450 at 254 nm and 11 250 at 300 nm and 2940 at 330 nm for tachysterol; 4130 at 254 nm and 1320 at 300 nm for lumisterol [53]. It is clear that the absorption of light above 300 nm is favored by tachysterol with a quantum yield of 0.48 compared to 0.26 for the conversion of 7-DHC to pre. Based on this, another scheme to produce vitamin D involves the continued irradiation of provitamin to high conversion with light of low frequency (250–300 nm) that results in a mixture of pre and tachysterol. The product containing a high percentage of tachysterol is then subjected to a secondary

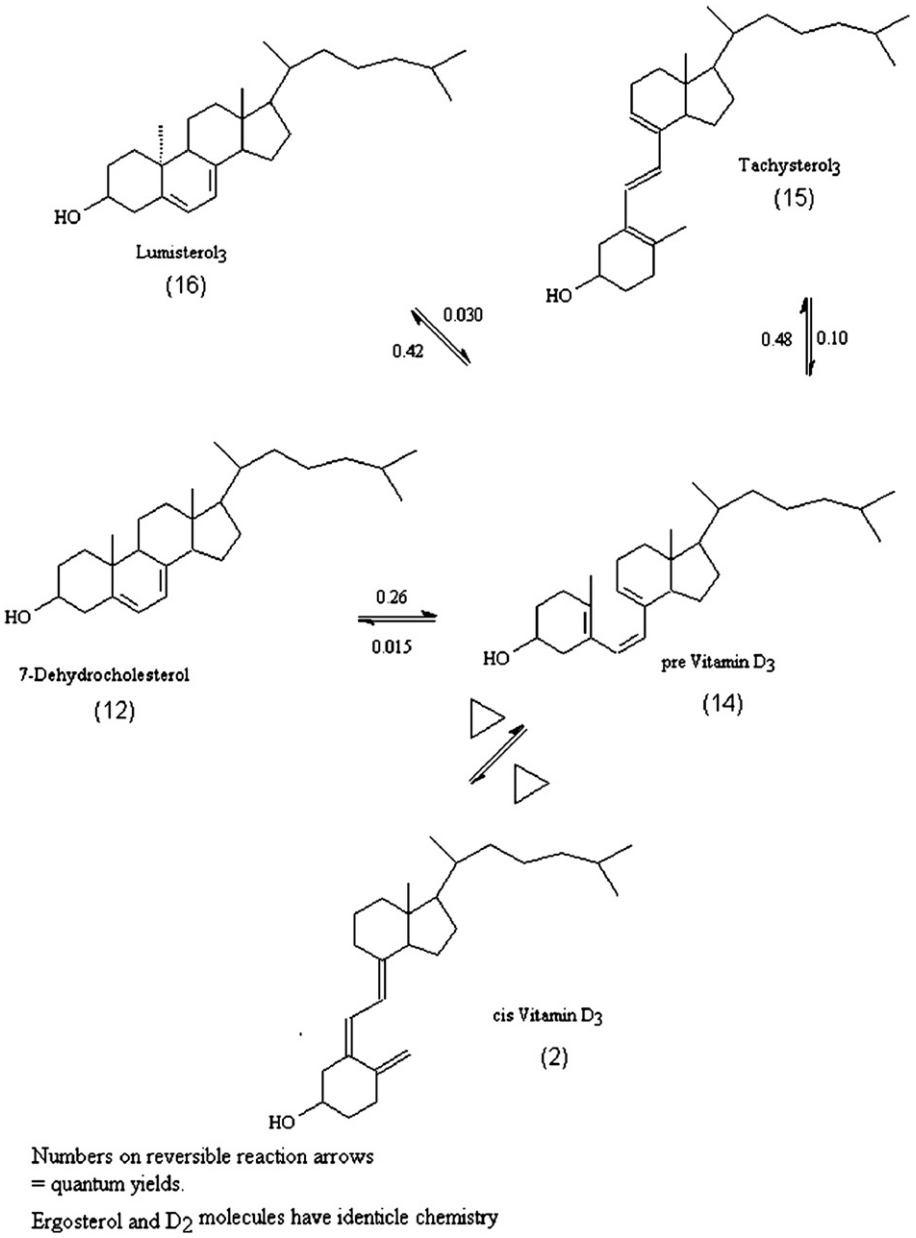

FIGURE 6.7 UV irradiation of 7-dehydro steroids to make vitamin D.

irradiation with light using a laser at about 330−360 nm. For example, 350 nm light, which is generated with an yttrium aluminum garnet (YAG) laser, has been used. N_2 laser at 337 nm and XeF at 350 nm have also been used [55−58].

The secondary irradiation is also accomplished by using a photosensitizer to give light in the area of 350 nm. This provides a high yield of vitamin D product low in provitamin, lumisterol, and tachysterol. Photosensitizers, such as eosin, erythrosin, dibromodinitrofluorescein, and others, can also be used to promote this photoconversion of tachysterol back to pre vitamin D by absorbing light of lower wavelengths from lamps and emitting very specific wavelength light useful in this conversion. The photosensitizer can also be bound to a solid matrix to facilitate the removal of the potentially toxic material from the final product [59,60].

To date, the economics of these light sources have not proven to be practical in the large-scale manufacture of vitamin D_3. The fact that they give high yields may justify their use primarily in the manufacture of more expensive vitamin D derivatives such as 25-hydroxyvitamin D, 1α-hydroxyvitamin D and 1α,25-dihydroxyvitamin D.

In commercial practice, the irradiation is carried out in a facility having vessels and equipment adequate in

TABLE 6.1 Normal Photochemical Vitamin D Products and Their CAS Registry Numbers [44–46]

Photopyrocalciferol$_2$ [41411-05-6]	Photopyrocalciferol$_3$ [85320-70-3]	Ultraviolet over-irradiation
Photoisopyrocalciferol$_2$ [26241-65-6]	Photoisopyrocalciferol$_3$ [85354-28-5]	Ultraviolet over-irradiation
5,6-*trans*-vitamin D$_2$ [14449-19-5]	5,6-*trans*-vitamin D$_3$ [22350-41-0]	irradiation of calciferol in the presence of iodine [58,59]
Isocalciferol$_2$ [469-05-6]	Isocalciferol$_3$ [42607-12-5]	treatment of trans D with mineral or Lewis acids; also forms from trans D with heat
Isotachysterol$_2$ [469-06-7]	Isotachysterol$_3$ [22350-43-2]	from isocalciferol or vitamin D (via trans) upon treatment with acid

size to manufacture many kilograms per day of vitamin D. A flow diagram for a typical commercial production facility is show in Figure 6.10.

Irradiation of 7-dehydrocholesterol or ergosterol is carried out by dissolving the steroid in an appropriate solvent, e.g., peroxide-free diethyl ether. Solvents such as ethanol, methanol, cyclohexane, and dioxane can also be used but should be free of dissolved oxygen. The cooled solution is pumped through UV-transparent quartz reactors which permit the light from a high-pressure mercury lamp to impinge upon the solution. A commercial-type lamp assembly is shown in Figure 6.11. Nitrogen gas is passed through the inner lamp well (section 9) to minimize ozone formation. Cooled water is passed through the outer well (section 7). The important feature here is the cooling jacket which controls the high temperature (ca. 800°C) of the mercury-vapor lamps and can be used as a light filter (see below).

The irradiation of the 5,7-diene provitamin to make vitamin D must be performed under conditions that optimize the production of the pre vitamin while avoiding the development of unwanted isomers. 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 the most widely used, however.

The solution is recycled until the desired degree of irradiation has been achieved. The resulting solution contains a mixture of unreacted 7-dehydrosterol, pre vitamin D, vitamin D, and irradiation by-products. Higher yields, with a more favorable isomer distribution, can be achieved if the frequency of light is kept at 275–300 nm. The optimum frequency for the irradiation is 295 nm.

Water solutions for cooling may contain salts for screening frequencies of light to ensure more optimum frequencies of light. Light below 275 nm can be filtered by aromatic compounds. Inorganic salt solutions such as 5-wt% lead acetate can also be used to filter the unwanted low frequencies; glass filters can also be used as screens for frequencies which are outside the chemical filter ranges. Photosensitizer solutions can be used similarly to emit specific frequencies of UV light.

When the desired amount of conversion of the provitamin to pre has been achieved, usually between 20–30%, the solution containing the vitamin D resin is stabilized against oxidation by the addition of $\leq$1 wt% butylated hydroxyanisole or butylated hydroxytoluene. The solution is then transferred to the isolation unit and the solvent is evaporated and recovered for reuse. The unconverted provitamin is recovered from an appropriate solvent, e.g., alcohol or methanol, by precipitation in a crystallization unit. The recovered sterol is reused in subsequent irradiations.

The solution containing the vitamin D is then evaporated to recover the solvent and the residual oil is heated to isomerize the pre vitamin D to the cis isomer. The resulting vitamin D 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.

TABLE 6.2 Abnormal Photochemical Vitamin D Products and their CAS Registry Numbers [44–46]

Isopyrocalciferol$_2$ [474-70-4]	Isopyrocalciferol$_3$ [10346-44-8]	Photo induced cyclization of pre; conrotatory bond formation to give the 9β,10β-antiisomers
(9α,10α)-pyrocalciferol$_2$ [128-27-8]	(9α,10α)-pyrocalciferol$_3$ [10346-43-7]	Thermal cyclization at >100°C leads to 9,10-syn isomers by disrotatory bond formation mechanism [57]
Photopyrocalciferol$_2$ [41411-05-6]	Photopyrocalciferol$_3$ [85320-70-3]	Ultraviolet over-irradiation
Photoisopyrocalciferol$_2$ [26241-65-6]	Photoisopyrocalciferol$_3$ [85354-28-5]	Ultraviolet over-irradiation
5,6-*trans*-vitamin D$_2$ [14449-19-5]	5,6-*trans*-vitamin D$_3$ [22350-41-0]	irradiation of calciferol in the presence of iodine [58,59]
Isocalciferol$_2$ [469-05-6]	Isocalciferol$_3$ [42607-12-5]	treatment of trans D with mineral or Lewis acids; also forms from trans D with heat
Isotachysterol$_2$ [469-06-7]	Isotachysterol$_3$ [22350-43-2]	from isocalciferol or vitamin D (via trans) upon treatment with acid

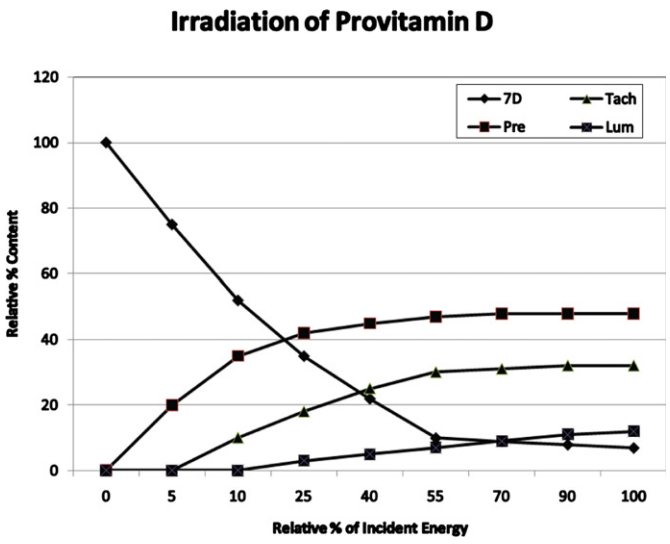

FIGURE 6.8 Approximate course of irradiation of provitamin D [41].

Vitamin D can be crystallized from a mixture of hydrocarbon solvent and aliphatic nitrile, e.g., benzene and acetonitrile, or from methyl formate to give the USP product [63,64].

Chemical complexation as well as column chromatography is also used for purification of the resin to obtain crystalline vitamin D for food and pharmaceutical usage.

Vitamin D products are formulated in a variety of matrices to protect the vitamin from exposure to air, heat, light, and minerals which cause it to degrade. These formulations also allow for the dilution of the high-potency pure product into its final dosage form with adequate distribution so as to assure uniform dosage of the food, feed, or pharmaceutical preparation.

Vitamin D_2 is made from ergosterol using the same type of technology. The same isomer distribution occurs and the irradiation must be carried out with similar care as described above for vitamin D_3.

METABOLITE MANUFACTURE

1α-Hydroxyvitamin D_3

1α-Hydroxyvitamin D_3 is becoming an important supplement in poultry diets. It has the ability to reduce tibial dyscondroplasia and is additive with phytase in promoting phosphorus utilization [65,66]. The product was originally prepared by Barton in 1973 and many preparations have been reported in the literature since. The one used most frequently is a modification of the Barton method [67,68]. The commercial process [69] starts by treating vitamin D_3 with SO_2 to produce two cyclic adducts. The 3-OH group is protected with a silicon protecting group. The SO_2 is removed with the formation of a derivative of a single isomer (5,6-transvitamin D_3) followed by allylic oxidation to introduce the 1α-hydroxy function. After de-protection and crystallization, the 1α-hydroxytrans vitamin D_3 is photochemically isomerized to 1α-hydroxyvitamin D_3.

The 1α-hydroxyvitamin D_3 in addition to its many applications for pharmaceutical uses by several companies is formulated in a starch matrix for use in animal feed products at a concentration of 0.04%. The major use of the product is in animal feeds and volume currently approximates 100 kg of crystalline product per year which will produce 20 000 000 metric tons of feed (5 μg of 1α-hydroxyvitamin D_3/kg of finished feed).

The fact that the process starts with crystalline D_3 causes the product to be substantially more expensive than the vitamin D itself. The benefits of using the product to replace vitamin D must overcome this cost differential. It is, therefore, used as a supplement to vitamin D_3 to achieve its additive benefits.

25-Hydroxyvitamin D_3

25-Hydroxyvitamin D_3 is also of commercial use, primarily in animal nutrition but also pharmaceutically for osteoporosis and other bone disease treatments. Its

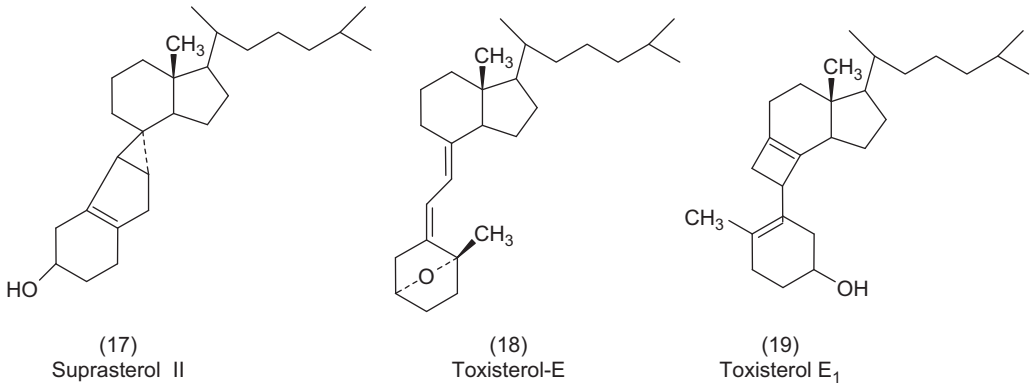

FIGURE 6.9 Unusual by-products of heat and prolonged irradiation of vitamin D_3.

TABLE 6.3 UV Absorbance Maxima of 7-Dehydrocholesterol, Pre- and *Cis*-Vitamin D [61]

Absorbance	7-Dehydrocholesterol	Pre vitamin D	*cis*-Vitamin D
@ (nm)	(E 1% 1 cm)		
260		270	
265			484
273	282		
282	293		
293	170		

use in animal feeds is claimed to promote bone development, weight gain, and feed efficiency. It should be noted that when administered to an animal, vitamin D is hydroxylated in the liver soon after absorption to 25-hydroxyvitamin D which then circulates in the blood. Feeding the metabolite bypasses this initial biological process. Therefore, one must evaluate the toxicity, cost, and benefits of this type of administration.

The 1α-hydroxyvitamin D is not a naturally occurring material but upon absorption into the body undergoes 25-hydroxylation in a manner similar to (and at an equivalent rate to) the vitamin D. This process by-passes the kidney 1α-hydroxylation of the 25-hydroxyl metabolite, making the 1α,25-dihydroxyvitamin D_3 hormone available at an accelerated rate.

25-Hydroxyvitamin D is made commercially primarily by a process which involves the fermentation of a double mutant yeast to form 5,7,24-cholestatrienol [70–72].

The 25-hydroxyvitamin D_3 is used at 62.5 μg/kg finished feed and current usage is estimated to be approximately 15 000 000 metric tons of feed per year. Thus, approximately 937.5 kg of 25-hydroxyvitamin D_3 are used.

The active hormonal form of vitamin D_3, 1,25-dihydroxyvitamin D_3 or calcitriol, can be made from the 25-hydroxyvitamin D [73].

1,25-Dihydroxyvitamin D_3 and its derivatives are used primarily in pharmaceutical preparations and are made by a variety of processes.

ANALYTICAL

Vitamin D

The early history of vitamin D led to the use of biological testing to determine the effectiveness of vitamin D products to reduce the effects of rickets. This ultimately led to standardized rat and chick tests in which laboratory animals are fed special diets devoid of vitamin D to produce rachitic conditions in the animal. The test animals are then dosed with the test substance and the bone growth at the proximal end of the tibia or distil end of the ulna are compared after staining with silver nitrate. Rats can be used to test vitamin D_2 or D_3, but the results can lead to false positives for use of the product in poultry, since chickens do not respond to

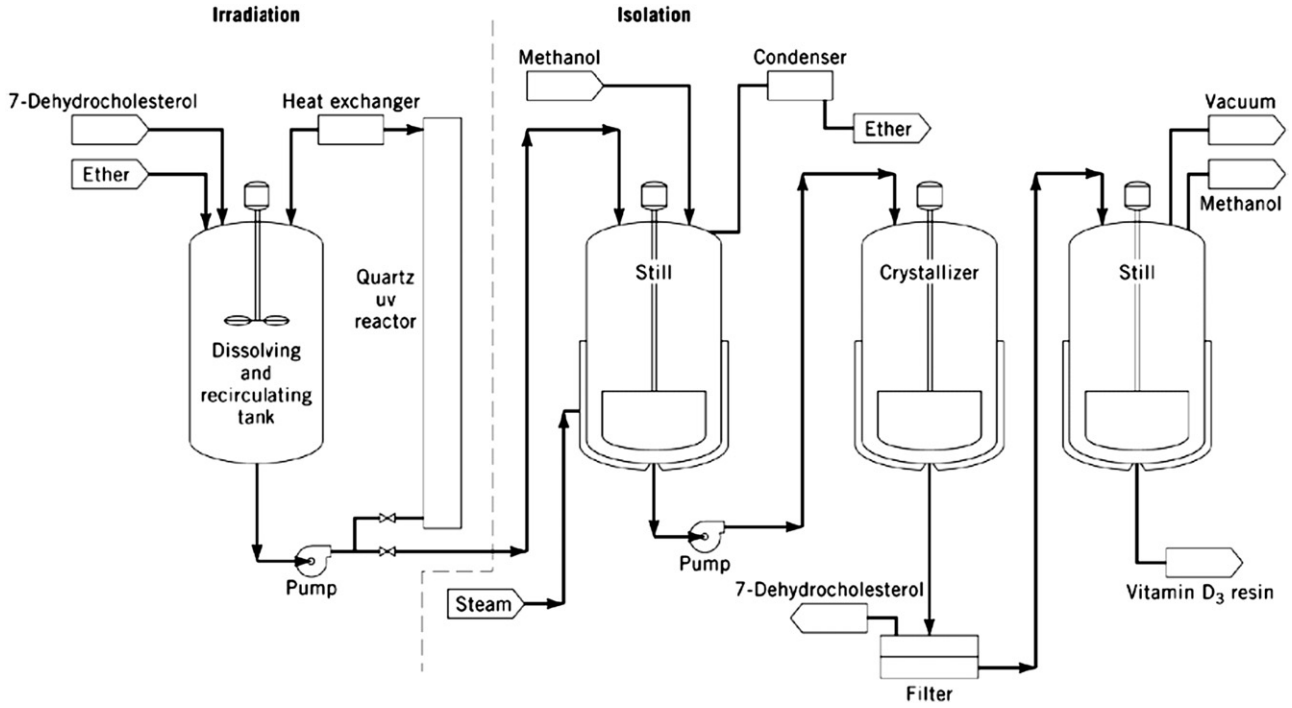

FIGURE 6.10 Vitamin D_3 manufacturing flow diagram [61, p. 239].

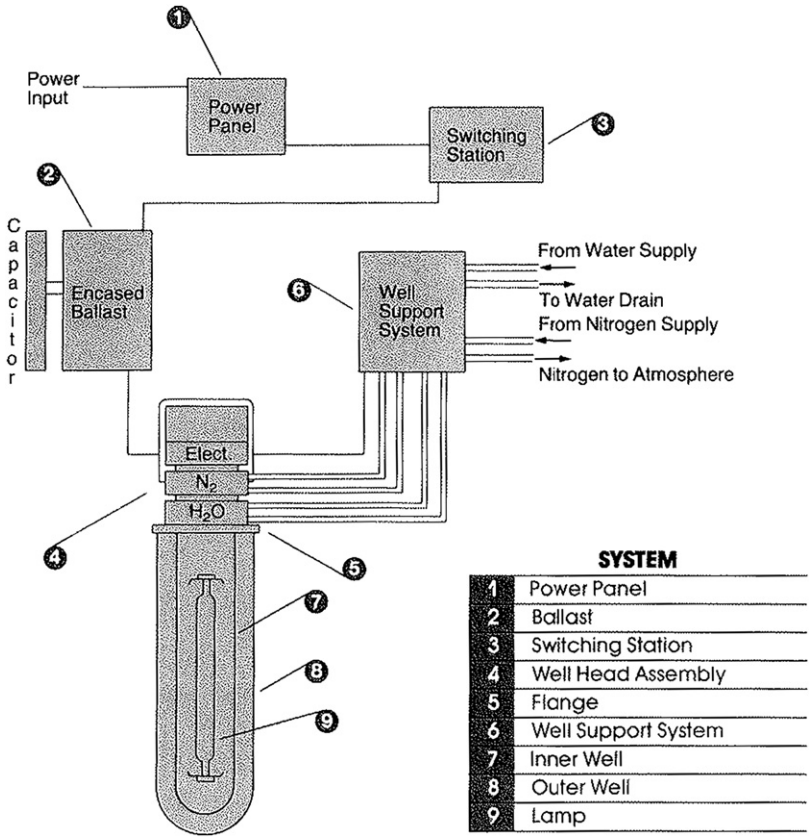

FIGURE 6.11 Commercial photochemical UV lamp system [62].

vitamin D_2. Therefore, chickens must be used to evaluate product that is to be used in poultry. This method is still approved by the AOAC (see AOAC 45.3.03 Chick Bioassay for Poultry Feed Supplement.932.16 [74], while material used for all other animals can use the AOAC Rat Bioassay 45.3.02 Rat Bioassay 936.14 [75]). These methods are slow, expensive, and lead to variable results.

The elucidation of the chemical nature of the vitamins led to the use of chemical methods of analysis which were primarily dependent upon colorimetric procedures. These were approved by the major official governing organizations and used for many years as the official methods of analysis.

The AOAC chemical method (Colorimetric method 975.42 45.1.17) [76] involves "saponification" of the sample (dry concentrate, premix, powder, capsule, tablet, or aqueous suspension) to release the vitamin from its matrix, with aqueous alcoholic KOH. The vitamin is then extracted using an appropriate solvent and the solvent containing the vitamin D is removed. Vitamin D is separated from extraneous ingredients by a chromatographic separation and the potency of the vitamin D is determined by a colorimetric determination with antimony trichloride in comparison with a solution of USP cholecalciferol reference standard. The procedure includes a step to treat unsaponifiable material with maleic anhydride to remove any *trans*-isomer which may be present and lead to a falsely high result. The antimony trichloride colorimetric assay is performed on the *trans*-isomer-free material. This procedure cannot be used to distinguish isotachysterol and, if present, also gives rise to a falsely high result. A test must therefore be performed to check for the presence of isotachysterol.

The USP XXXII [77] and AOAC 2010 (HPLC 979.24 [78]) both now recognize high-pressure liquid chromatography (HPLC) as the preferred method of analysis. HPLC allows the separation of the active pre- and *cis*-isomers of vitamin D_3 from other isomers and provides a means to analyze the active content by comparison with the chromatograph of a sample of pure reference *cis*-vitamin D_3. Equilibration of a solution of the standard to a mixture *of* pre- and cis-isomers [12,13] is included in the procedure in order to evaluate the total active isomer content of the sample. The sensitivity of this method provides information on isomer distribution and allows for the accurate evaluation of 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, i.e., oils containing 100 000 IU cholecalciferol/g; resins 20 000 000 IU cholecalciferol/g; and powders and aqueous dispersions at

25 000 IU cholecalciferol/g (AOAC Methods 979.24; 980.26; 981.17; 982.29; 985.27) [79–81]. The limiting factor in the assay of low-level formulations is the isolation of the vitamin material from interfering and extraneous components which may obscure the vitamin D HPLC peak.

Vitamin D products formulated in a variety of matrices are then usually mixed with carriers to form premixes and final dosage compositions. The assay of the vitamin requires the matrix to be broken in such a way as to assure complete availability of the vitamin. Additionally, the ability to separate it from the formulated mixture must be efficient. The above methods all address this issue. A particularly useful extraction procedure involves the use of dimethyl sulfoxide [82]. The usual HPLC procedures utilize UV detection for quantification of the elution peaks.

Recently HPLC has been coupled with mass spectrographic detectors to enable the assay of vitamin D to much more significant detection limits [83,84].

A number of methods have been developed for the paper, thin-layer, and column chromatographic separation of vitamin D and related substances but these are more tedious and difficult to perform on low-level samples. Gas chromatography requires derivatization and has been applied to metabolite analysis as well as assay of multivitamin tablets and vitamin D_2 in milk and other formulations [85–90].

The USP [91] requires the following tests for the pure crystalline vitamin D:

Identity by:

(a) IR;

A typical infrared spectrum of cholecalciferol will have the following parameters; the IR spectrum of the sample should be identical to the IR spectrum of a USP reference standard of cholecalciferol.

Wavelength	Peak Ht.	Characteristic
3305	Strong	-OH stretch
2934	Strong	-CH stretch & bend
1642	Weak	-C=C- stretch
1458, 1438, 1375	Medium	-CH stretch & bend
1053	Strong	-C-OH stretch

(b) UV;

A typical UV curve for a 10 μg/ml solution of cholecalciferol has a molar extinction coefficient of 18 692 at λ_{max} 264.8 ($E^{1\%}_{max} = 484$).

(c) Chemical color (with acetic acid and sulfuric acid turns bright red changing to violet and then blue green).

Thin Layer Chromatography: USP <621> [77] (developed with $SbCl_3$ in Acetyl Chloride which gives a yellow/orange color) with retention time compared to a USP reference standard.

Specific Rotation; USP <781s> [92] α_D = between +105° and +112°.

Assay: by High Pressure Liquid Chromatography (HPLC) USP <621> [77] against a USP reference standard.

The international standard for vitamin D is an oil solution of activated 7-dehydrocholesterol. The International Unit (IU) is the biological activity of 0.025 μg of pure cholecalciferol. One gram of vitamin D_3 is equivalent to 40×10^6 IU or USP units.

Samples of reference standard may be purchased from US Pharmacopeial Convention [93]. Reference standards are also available from the World Health Organization (WHO) as well as the European (EP) and British Pharmacopeia (BP). 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.

The various isomers of vitamin D exhibit characteristically different UV absorption curves. Cis vitamins D_2 and D_3 exhibit UV absorption maximum at 265 nm with an E_{max} (absorbance) of 450–490 at 1% concentration (Table 6.3).

Mixtures of the isomers are difficult to distinguish but the pure substances and their concentrates can be assayed using their UV absorption. When chromatographically separated by HPLC, the vitamin D peaks can be identified by stop-flow techniques based on UV absorption scanning or by photodiode-array spectroscopy as well as mass 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 [94–96].

Provitamin Assay

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 [97,98].

High pressure liquid chromatography can also be used to analyze the provitamins.

There are a variety of chemicals that show characteristic colors when reacted with the provitamins. Some of these are listed below.

- The **Salkowski reaction (revised)** treats the provitamin with $CHCl_3$ and H_2SO_4 (conc.) to give a deep red color in $CHCl_3$ layer and green fluorescence in the acid layer which differentiates from sterols lacking a conjugated diene.

- The **Lieberman-Burchard reaction** is run in $CHCl_3$ with acetic acid−H_2SO_4 added dropwise. A red color develops and changes to blue-violet to green. The test can be quantitative and acts similarly to the Salkowski reaction, but the red color lasts longer.
- The **Tortelli-Jaffe reaction** is run in acetic acid with 2 wt% Br_2 in $CHCl_3$ which turns green with sterols having ditertiary double bonds including vitamin D and compounds that give similar bonds upon isomerization or reaction.
- The **Rosenheim reaction** is run in $CHCl_3$ with trichloroacetic acid in H_2O. A red color develops and changes to light blue. When run with $CHCl_3$ and lead tetraacetate in CH_3COOH followed by the addition of trichloroacetic acid, the reaction gives a green fluorescence which is not given by esters of provitamin D and can be used to distinguish between provitamin and provitamin ester. The test is quantitative to 0.1 μg.
- A mixture of crystalline provitamins and **chloral hydrate** heated slowly melts at 50°C and color develops and changes from red to green to deep blue while other sterols, e.g., cholesterol, do not react to give color.
- The **antimony trichloride reaction** with $CHCl_3$ and $SbCl_3$ gives a red color.
- The **Chugaev reaction** adds glacial acetic acid plus acetyl chloride and zinc chloride to the provitamin which is heated to boiling. An eosin-red greenish yellow fluorescence develops with a sensitivity of 1:80 000 [99].

Assay of 25(OH)D

The extremely low levels of vitamin D and its metabolites in biological systems make it very difficult to assay the vitamin D products in these environs by traditional methods.

The ability to assay these materials was initially developed by Haddad [100] with the use of a competitive protein-binding assay (CPBA) for 25-hydroxyvitamin D (25(OH)D).

25(OH)D is especially vulnerable to matrix effects in any protein-binding assay because of its lipophilic properties. These were overcome by the utilization of chromatographic sample purification prior to the complex formation with the calcium.

A nonchromatographic radio immunoassay for circulating 25(OH)D was developed by Napoli and Hollis using an antigen that would generate an antibody that was cospecific for $25(OH)D_2$ and $25(OH)D_3$ [101]. The study of vitamin D and its metabolites and their effects in clinical disease over the past 30 years was made possible by the ability to assay these materials. The need to assay large numbers of samples to evaluate the vitamin D blood levels of large populations requires an ability to perform these assays with a rapid, accurate, and valid method.

In 2001, Nichols Diagnostics introduced the fully automated chemiluminescence CPBA ADVANTAGE 25(OH)D assay system [102] in which nonextracted serum or plasma is introduced directly into a mixture containing human D-binding protein (DBP), acridinium-ester-labeled anti-DBP, and $25(OH)D_3$-coated magnetic particles. Another chemiluminescence assay was developed in 2004 by the DiaSorin Corporation [103]. The assay, LIAISON 25(OH)D, is very similar to the ADVANTAGE assay but uses an antibody as a primary binding agent as opposed to the human DBP and is a radio immune assay (RIA) method. See Hollis [104] for a review of these methods and their application to the important assay of blood levels of 25(OH)D.

DIETARY REQUIREMENTS

Humans

Dietary Reference Intakes of vitamin D_2 and vitamin D_3 (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies (formerly National Academy of Sciences) were established in 1997 [105].

They recommended 200 IU/day from 1 month to 50 years of age and 400 IU/day from 51 years to 70 years and 600 IU/day after reaching the age of 71. In 2008, the American Academy of Pediatrics (AAP) issued recommendations for intakes for vitamin D that exceed those of FNB to 400 IU/day and the Surgeon General of the United States indicated that all citizens should make sure they took a minimum of 400 IU/g day to ensure good health. Many studies over the past 15 years have led to the suggestion that 1000−2000 units and as high as 5000 IU/day of vitamin D_3 per day are necessary to provide enough vitamin D to maintain 25-hydroxyvitamin D blood levels (37.5−50 nmole/L; 15−20 ng/ml) sufficiently high to provide all of the functions the vitamin and its metabolites serve to influence.

The Food Nutrition Board established an expert committee in 2008 to review the DRIs for vitamin D (and calcium). The FNB issued its report, updating as appropriate the DRIs for vitamin D and calcium, in November 2010. The new recommendations are 600 IU/day from age 1 year to 70 years and 800 IU/day from age 70 and older. Upper level intake limits were set at 4000 IU/day for 9 year olds and older. As noted above, there is much evidence which suggests higher levels than this are needed and this problem will be one of ongoing concern [105a].

TABLE 6.4 Practical Feeding Levels of Vitamin D, 10^6 IU/ton [111]

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	Feedlots	2–3
Breeding	2–4	Dry pregnant cows	1–2
		Lactating cows	1–2
Swine		Bulls	1–2
Prestart (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		**Fish**	
Dogs	5–1	Trout	1–2
Cats	1–2		
Horses	1–2		

* *Poultry cannot absorb vitamin D_2.*

Animals

Rickets prevention or cure initially was the purpose for feed fortification with vitamin D_3. Adequate levels were determined to be those sufficient to prevent rickets. In 1995, Edwards [106] found that, in the absence of UV light, different vitamin D_3 levels result in the optimization of various effects of vitamin D_3 in poultry. For example, 275 IU/kg is required for growth, 503 IU/kg for optimum bone ash, 552 IU/kg for proper blood plasma calcium, and 904 IU/kg for rickets prevention.

The National Research Council recommends the following amounts of vitamin D per kilogram of feed for various species: 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 [107–109].

Higher levels are usually used in common practice in order to make sure the animals receive adequate dosage of the vitamin. Most species can safely tolerate four to ten times the NRC requirements during long-term feeding and short-term (<60 d) most species can tolerate 100 times their apparent dietary requirements [110].

Animals produce vitamin D_3 when exposed to sunlight and do not require substantial dietary vitamin D. However, many animals are raised indoors with little exposure to sunlight and modern livestock management practices place an emphasis on high productivity. As a result, most feed manufacturers recommend vitamin D_3 supplementation of diets. Recommendations for practical levels of vitamin D_3 in feeds for various animals, as recommended by feed manufacturers, are listed in Table 6.4.

ECONOMIC ASPECTS

Vitamin D_3 is available in a variety of forms. Cod liver oil and percomorph liver oil were good sources of vitamin D_3 historically but crude cod liver oil processing involves alkali refining, bleaching, winterization, and deodorization. This vigorous treatment of the vitamin-containing oil substantially depletes the vitamin activity. Fully cleaned and deodorized cod liver oil is sold with synthetic vitamins added back. Most of the cod liver oils on the market fall into this category. This is the so-called high-vitamin cod liver oil, standardized at a maximum of 2500 IU vitamin A per gram (12 500 IU per teaspoon) and 250 IU vitamin D_3 (1250 IU per teaspoon).

Lower-potency oils are sold with the ratio of vitamin A to D_3 of 10:1. In 2010, cod liver oil with a potency of 1700 IU vitamin A and 170 IU vitamin D_3 sold for approximately $5.50 to $7.75 per kilogram.

Most of the vitamin D sold is synthetic as opposed to natural. The term “natural” is often used to describe an “organic” product. It should be noted that vitamin D_3 is the vitamin found in nature; it is made by all animals.

Vitamin D_2 and vitamin D_3 (crystalline and resin materials) are Generally Recommended As Safe (GRAS) by the United States Food, Drug and Cosmetic Act for both human nutrition [112] and animal feeds [113].

Pharmaceutical formulations are made with USP crystalline vitamin D_2 and vitamin D_3. The pure crystalline vitamin at 40 MIU/gram or resin (at 25–30 MIU/

gram) must be diluted in a carrier to a concentration that is practical for use in uniform dosing of the final product. Many supplement preparations such as tonics, drops, capsules, tablets, and oil-based injectables are also marketed.

The primary regulations governing vitamin D fortification are 21 CFR 184.1950 and 184.1(b) (2). These regulations state that vitamin D may be added to a limited number of foods for the functional use of nutrient supplementation. The food categories included on the approved list include breakfast cereals, milk, milk products, grain products and pastas, infant formula, and margarine. Essentially all milk produced in the United States is fortified with vitamin D_3. Preparations based on the use of vitamin D_3 resin are less expensive than those using crystalline vitamin D_3 and have been used for many years.

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 because it is the form occurring naturally in animals.

Vitamin D_2 has been used as a feed supplement for cattle, swine, and dogs, but its use has declined in favor of vitamin D_3. Vitamin D_2 cannot be used in poultry and many feed producers would prefer not to use both D_2 and D_3 to avoid the possibility of a mix-up that would allow D_2 to get into poultry feed which could result in catastrophic losses. Table 6.5 shows the metric tons of feed grade 500 000 IU/gram used by species in 2010. The usage levels shown for dairy and beef in 2010 are approximately 30% and 50% lower, respectively, than they have been recently due to current market fluctuations.

TABLE 6.5 Animal Use of Vitamin D by Species in the United States and Europe. In Metric Tons of 500 000 IU/gram Equivalent

US	1994%	2010%	2010 MT D_3 500
Poultry	41	49	
Layers/breeders			101
Broilers			306
Turkeys			73
Swine	13	11	109
Beef and dairy	44	32	
Dairy			280
Beef			35
Pet foods	2	8	76
Total tons of 500 000 IU/gm			980
Europe	**1994**		
Cattle	58		
Swine	19		
Poultry	20		
Other	3		

Formulations combining vitamin A and D or A, D, and E (the fat-soluble vitamins) are used widely. These may be pre-emulsified to insure increased bioavailability.

Vitamin D is not water-soluble. In order to make it compatible with aqueous systems, it is formulated by dissolving the vitamin in oil and using surfactants to enable the emulsification of the oil in an aqueous media [114].

In animal feeds, oil solutions of vitamin D_3 or solutions of the vitamin in oil-on-dry carriers, e.g., corn or flour, can be used. Animal diets usually contain high levels of minerals, and the vitamin D_3 in the feed is thus exposed to the minerals as well as to air, heat, light, and moisture when the feed is in use. Because of this, when used in most feeds, vitamin D_3 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. They can be spray dried or drum dried [115–119].

Animal feed formulations usually employ resin in these various stabilized forms and are sold predominantly at levels of 500 000 or 1×10^6 units of vitamin D_3 per gram. Combination products containing vitamins A and D are also available, with 1 000 000 units of vitamin A and 200 000 units of vitamin D per gram of product being the most common dosage form.

During the past year we have seen vitamin D_3 in a price range of $10.00 to $100.00 USD per Million International Units (MIU). Raw material plays a large part in market pricing and with today's cholesterol price, the intrinsic value of vitamin D_3 is approximately $50.00/MIU. The estimate of world usage of vitamin D_3 as 500 000 IU/gram material in animal feed is estimated in Table 6.6. The total tonnage is approximately 77.5 metric tons of D_3 as crystals equivalent in animal feed, 15.3 metric tons of crystals in pharmaceutical use and about 4.5 metric tons in human food, or 97.3 metric tons of D_3 crystals worldwide.

Eighty percent of the world market is made in China by four main producers. About 1000 MT is produced in Crenzach, Germany, which is mainly consumed by its manufacturer for internal use. This is supplemented in the market with 25-hydroxyvitamin D_3 product with sales revenue in 2008 reported to be 35 million euros at an average price of €350/kg or approximately 100 000 kg of the metabolite. Another major supplier is planning production of vitamin D_3 in India and is planning to be in operation by 2011.

TABLE 6.6 2010 World Usage of Vitamin D_3 [111]

	Feed Grade			Food & Pharma	Food Grade		
	MT D3 500 000 IU/GM						
Country	-	-	MT OF 40 MIU/GR	MT OF 40 MIU/GR	MT OF 100,000 IU/GR	MT OF 40 MIU/GR	MT OF 40 MIU/GR
Europe		1300	16.25	6.5	600	1.5	
USA		1000	12.5	5.5	800	2	
China		800	10	1.5	200	0.5	-
Brazil		300	3.75				
Asia		1200	15				
South America		500	6.25				
Turkey				0.5			
Iran				0.3			
Japan				0.3			
India				0.7			
Australia					200	0.5	
Other		1100	13.75				
TOTAL		6200	77.5	15.3	1800	4.5	97.3

Approximately 200 kg/year of vitamin D_3 formulations have been sold 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. 1α-Hydroxyvitamin D_3 is being used for milk fever in cows, and more importantly for the treatment of tibial dyschondroplasia and to facilitate phosphorus utilization. 25-Hydroxyvitamin D_3 has been proposed for eggshell thickness in poultry and is being marketed as an animal dietary nutritional supplement. Additionally it is used for human pharmaceutical purposes. As mentioned earlier, vitamin D_2 derivatives such as Calipotriene (Dovenex) a 24-hydroxy,25-cyclopropyl derivative is used for psoriasis, and 1-α-hydroxyvitamin D_2 (Doxercalciferol; Hectoral) and 19-nor-1α,25 dihydroxyvitamin D_2 (Paricalcital; Zemplar) are used for secondary hyperparathyroidism associated with chronic renal failure.

STORAGE AND SHIPPING

Vitamin D is sensitive to air, heat, UV light, and mineral acids. These sensitivities are exaggerated by the presence of heavy-metal ions, e.g., iron. Therefore, care should be taken to store and ship vitamin D and its various product forms by methods that minimize exposure to these conditions.

Pharmaceutical grade vitamin D_3 is packaged in the pure crystalline form. It usually is packaged in 100, 500, or 1000 g hermetically sealed pouches. The high potency of the product allows for smaller amounts of the material to be used in formulations and the smaller package size minimizes the need to store open packages of vitamin D. The crystalline vitamin D is used in vitamin D food and pharmaceutical applications as well as multivitamin preparations.

Commercial sources of feed-grade vitamin D are usually a vitamin D_3 resin stabilized by spray or by roll drying a starch or gelatin suspension of the vitamin. These products should be stored in a cool, dry area, preferably in opaque, hermetically sealed containers under an inert atmosphere such as nitrogen, CO_2, or an inert gas.

Shipping vitamin D in crystalline or resin form should be done in containers marked appropriately to indicate that the material is toxic by DOT standards. Its proper DOT labeling is DOT Hazard Class 6.1, poisonous (this is because of its high degree of potency per gram).

Waste material should be placed in an appropriate landfill or preferably burned.

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

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