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EDITORIAL REVIEW

The metabolism and mechanism of action of 1,25-dihydroxyvitamin D_3

In this review, I will summarize recent information concerning the metabolism and mechanism of action of 1,25-dihydroxyvitamin D_3. New concepts concerning the formation and transport of precursors will also be briefly presented.

Sources of Vitamin D[1]

Formation of vitamin D_3 in the skin

Vitamin D_3 is not a vitamin (an essential exogenous micronutrient) in a strict sense because it is formed endogenously in the skin from a precursor, 7-dehydrocholesterol [1]. Diminished sunlight exposure that has accompanied our way of life has necessitated the ingestion of the vitamin via the diet. Early work clearly established that 7-dehydrocholesterol is converted to previtamin D_3, in vitro as well as in vivo, in the presence of ultraviolet light [2–6]. Thermal isomerization of the previtamin results in the formation of the vitamin. Recently, attention has focused on the formation of vitamin D_3 in the skin of man and experimental animals, the effect of the duration of sunlight exposure, the effect of pigmentation and the role of vitamin D binding protein on the transport of the vitamin out of the skin [7–10]. The amount of previtamin D_3 formed is dependent upon the duration of sunlight exposure, the spectral properties of the incident light (light of 295 ± 5 nm being most efficient in the photolytic cleavage of the B-ring), and the amount of pigmentation present in skin. In blacks, the amount of vitamin D_3 formed after exposure to a given amount of ultraviolet light is considerably less than that in Caucasions [9]. However, if the duration and intensity of exposure is increased, then there is equivalent, or nearly equivalent, formation of vitamin D_3 in the skin of blacks when compared to Caucasians.

Holick and co-workers have hypothesized that vitamin D binding protein may help in the transport of vitamin D_3 from skin into plasma [8]. Vitamin D binding protein, a 52,000 to 58,000 molecular weight protein, binds poorly to previtamin D_3 but binds with a greater affinity to vitamin D_3. It has been suggested that vitamin D binding protein transports vitamin D_3 out of the skin into the systemic circulation and thus maintains relatively low concentrations of vitamin D_3 within skin; this allows the thermal isomerization of previtamin D_3 to vitamin D_3 to proceed at a more rapid rate by decreasing the concentrations of vitamin D_3 within the skin [8]. Whether this process is of physiological importance in vivo is not known.

Absorption of vitamin D from the diet

Vitamin D_3 (or vitamin D_2), present in food, is absorbed via the intestinal lymphatics [11]. Here the vitamin resides in the chylomicron fraction. About 50% of the vitamin in chylomicrons is transferred to vitamin D binding globulin in blood before uptake by the liver [12]. Because not all radiolabeled vitamin D_3 is transferred to vitamin D binding protein, other proteins such as albumin may play a role in the transport of vitamin D_3 [13, 14].

The formation of 25-hydroxyvitamin D_3 in the liver

Vitamin D_3, is converted to an intermediary metabolite, 25-hydroxyvitamin D_3, in the liver [15, 16]. The enzyme that catalyzes this reaction is present both in liver microsomes as well as in liver mitochondria [17, 18]. The microsomal enzyme requires NADPH and a soluble cytosolic factor for its activity. It is a cytochrome P-450-like enzyme that has recently been characterized by Yoon and DeLuca [19]. The microsomal enzyme has a lower Michaelis constant (Km) and is better regulated than the mitochondrial enzyme, which has a higher Michaelis constant (Km) and is poorly regulated [17, 18]. The administration of large doses of vitamin D_3 results in the progressive increase in circulating levels of 25-hydroxyvitamin D_3, as the process of 25-hydroxylation is poorly regulated, and at higher concentrations of vitamin D_3 the mitochondrial enzyme will form significant quantities of 25-hydroxyvitamin D_3. It is for this reason that the circulating level of 25-hydroxyvitamin D is a good index of vitamin D_3 reserves. The administration of 1,25-dihydroxyvitamin D_3 has been shown to decrease the concentration of 25-hydroxyvitamin D_3 in plasma in vivo [20]. The physiologic significance of this is not clear, as we have shown that phosphate deprivation (a maneuver that increases 25-hydroxyvitamin D_3 1α-hydroxylase activity and 1,25-dihydroxyvitamin D concentrations in plasma) does not suppress 25-hydroxyvitamin D_3 levels [21]. Conversely, phosphate ingestion (which decreases 1,25-dihydroxyvitamin D concentrations in plasma) does not alter 25-hydroxyvitamin D concentrations in blood [22].

Vitamin D binding globulin

Vitamin D binding globulin is a protein that has a molecular weight of approximately 52,000 daltons in the rat and about 58,000 daltons in the human [23]. It binds 25-hydroxyvitamin D_3 with high affinity and binds other vitamin D metabolites, such as vitamin D_3 and 1,25-dihydroxyvitamin D_3, with lower affinity. 24,25-Dihydroxyvitamin D_3 and 25,26-dihydroxyvitamin D_3, which have both 3β-hydroxy and 25-hydroxy groups, bind to

[1] The term vitamin D refers to both vitamin D_3 (9,10-secocholesta-5,7,10(19)-triene-3β-ol) and vitamin D_2 (9,10-secocholesta-5,7,10(19)22-tetraene-3β-ol) (5Z isomers). While vitamin D_3 is formed endogenously, vitamin D_2 is not.

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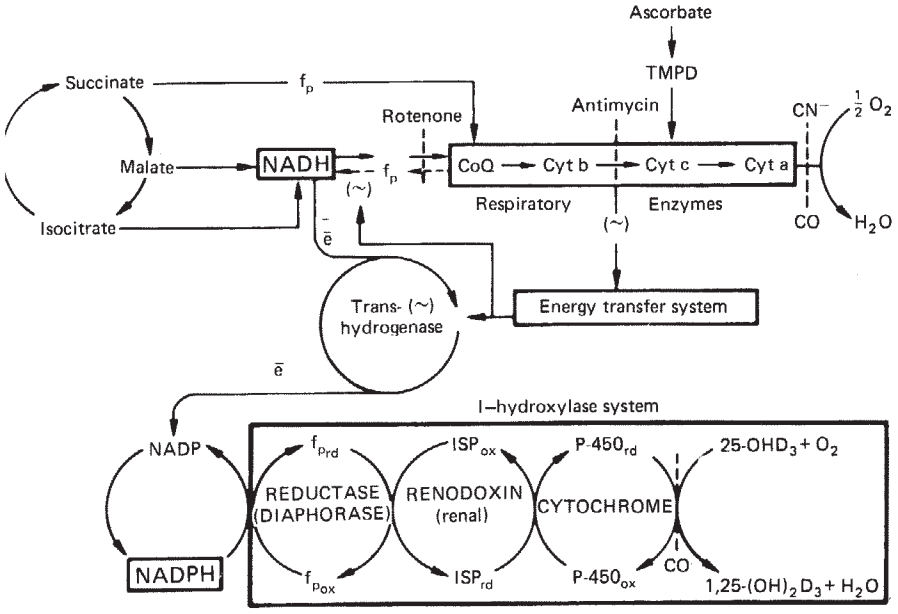

Fig. 1. *The nature of the 25-hydroxyvitamin D_3-1α-hydroxylase enzyme* (Taken from DeLuca HF, reference 1, with permission of the publisher).

vitamin D binding protein with an affinity equal to that of 25-hydroxyvitamin D_3. The distance between the 3β-hydroxy group and another hydroxyl group on the molecule is of importance as 1α-hydroxyvitamin D_3 (C-1 and C-3 hydroxyl groups in close proximity) binds poorly to vitamin D binding protein whereas 11α-hydroxyvitamin D_3 which has 3β and 11α hydroxy groups (intermediate distance between C-3 and C-25) binds with intermediate affinity [24]. Sequencing of a complementary DNA clone for vitamin D binding protein has shown that there is some homology between vitamin D binding protein and serum albumin and alpha-feto protein [25, 26]. The physiological role of this protein is uncertain. It may act as a 25-hydroxyvitamin D "buffer", and some have thought that it may help in the internalization of vitamin D sterols. The protein binds tightly to actin [26, 27]. Some investigators believe that the amounts of vitamin D binding protein influence the concentrations of "free 1,25-dihydroxyvitamin D" in plasma and that the concentrations of "free hormone" are important in determining the biologic activity of the hormone in any given condition [14, 28, 29].

The formation of 1,25-dihydroxyvitamin D_3

1,25-Dihydroxyvitamin D_3, the hormonal form of the vitamin, is formed in the mitochondria of the proximal tubules of the nephron [30–38]. The enzyme is a cytochrome P-450-like, mixed function oxidase that utilizes molecular oxygen as the source of oxygen [39–43]. The characteristics of this enzyme in the chicken have been well delineated, and a summary of the characteristics of this enzyme complex is shown in Figure 1. Warner and also Crivello suggest that in the rat and cow, the enzyme is not of the same nature as in the chicken [44, 45]. Others, however, have shown that product isolated by the former workers is not 1,25-dihydroxyvitamin D_3, and that the rat enzyme is similar to the chicken enzyme [46]. There are several factors that regulate the activity of the 25-hydroxyvitamin D_3 1α-hydroxylase enzyme [47, 48]. Many of them are functional only in certain species and under certain sets of experimental conditions. The major factors regulating the activity of the 25-hydroxyvitamin D_3 1α-hydroxylase are parathyroid hormone, the concentration of serum phosphorus, 1,25-dihydroxyvitamin D_3 itself, and serum calcium directly. A summary of the factors that are known to alter 1α-hydroxylase enzyme activity is given in Table 1 (taken from reference 47; detailed references about substances or factors altering 1,25-dihydroxyvitamin D_3 concentrations or 25-hydroxyvitamin D_3 1α-hydroxylase activity are given in this reference).

The catabolism of 1,25-dihydroxyvitamin D_3

1,25-Dihydroxyvitamin D_3 is metabolized by several processes in experimental animals as well as man [47, 49–53]. The precise delineation of these processes and their regulation is of importance, as circulating levels of a hormone depend not only upon its rate of formation but also upon its rate of degradation. The metabolic pathways involved in the degradation of 1,25-dihydroxyvitamin D_3 are as follows: 1) side chain oxidation to an inactive product, calcitroic acid [54–57]; 2) 24-hydroxylation to 1,24,25-trihydroxyvitamin D_3 in several tissues including the kidney, the intestine, cartilage, and perhaps other tissues as well [58–64]; 3) formation of 24-oxo-1,25-dihydroxyvitamin D_3 [65, 66]; 4) formation of 1,25-dihydroxyvitamin D_3 23,26-lactone [67]; 5) biliary excretion as polar metabolites such as 1,25-dihydroxyvitamin D_3 monoglucuronides in bile [47, 52, 68, 69]. The products that are excreted in bile are in the form of glucuronides and other polar charged materials that may be sulfates of 1,25-dihydroxyvitamin D_3. There are neutral polar steroids present in bile that could be glycosides of 1,25-dihydroxyvitamin D_3. The products of 1,25-dihydroxyvitamin D_3 undergo an enterohepatic recirculation that may be perturbed in certain pathologic states [70]. Model glucuronides and glucosides of vitamin D_3 analogs are biologically active following hydrolysis to the free sterols and thus, the aglycones of conjugates of vitamin D analogs may be liberated in the

Table 1. Factors altering serum 1,25-dihydroxyvitamin D_3 concentrations or 25-hydroxyvitamin D_3 1α-hydroxylase activity[a]

Factor	Level or activity change of substance	Effect on $1,25(OH)_2D_3$ Levels or $1,25(OH)_2D_3$ 1α-hydroxylase activity	
		Animals	Humans
Parathyroid hormone	Increase	+	+
	Decrease	−	−
Serum inorganic phosphorus	Increase	−	−
	Decrease	+	+
1,25-Dihydroxyvitamin D_3	Increase	−	?
	Decrease	+	?
Calcium (direct)	Increase	?	?
	Decrease	+	+
Calcitonin	Increase	+,−,0	+
	Decrease	?	?
Hydrogen ion	Increase	−	0
	Decrease	?	?
Sex steroids	Increase	+	+
	Decrease	?	?
Prolactin	Increase	+	0
	Decrease	?	?
Growth hormone	Increase	+	0,−,+
	Decrease	?	?
Glucocorticoids	Increase	−	−,0,+
	Decrease	?	?
Thyroid hormone	Increase	?	−[b]
	Decrease	−	+[b]
Pregnancy		+	+

[a] from Kumar, reference 47, with permission of the publisher. Minor modifications have been made.

Symbols are: +, Stimulation or increase; −, suppression or decrease; 0, no effect; ?, effect not known.

[b] Effects may be secondary to changes in calcium, phosphorus, or parathyroid hormone.

intestine following hydrolysis in that organ [71–73]. 6) Formation of 1,25,26-trihydroxyvitamin D_3 [74, 75].

In experimental animals, side chain oxidation and biliary excretion account for about 50% of the excretion of 1,25-dihydroxyvitamin D_3. The physiological role of the enterohepatic circulation has not been precisely quantitated. There are conflicting views about whether this is important in the enterohepatic physiology of 25-hydroxyvitamin D_3—a metabolite of vitamin D_3 with a much longer half-life than 1,25-dihydroxyvitamin D_3 [76]. The exact pathophysiologic role of the enterohepatic physiology of 1,25-dihydroxyvitamin D_3 in various disease states requires further examination.

Production and degradation rates of dihydroxylated vitamin D metabolites in man

1,25-Dihydroxyvitamin D_3 is rapidly cleared from the circulation of man [51–53]. Following a dose of intravenous 1,25-dihydroxyvitamin D_3 of high specific activity, <50% of the administered dose is present in plasma within a period of five minutes. A slower component of elimination has a half-life of approximately 10 hours. This corresponds well with the biologic half-life of 1,25-dihydroxyvitamin D_3 in man. The metabolic production rate and clearance rate of 1,25-dihydroxyvitamin D_3 are shown in Table 2 and are compared with the metabolic production rate of another dihydroxylated vitamin D metabolite, 24,25-dihydroxyvitamin D_3 [51, 53, 77]. The disposition of radioactivity in plasma, stool, and urine, following the administration of these substances in man, is also shown in Table 2. Bolus and continuous infusion techniques have been used to determine the metabolic clearance rate and production rate of 1,25-dihydroxyvitamin D_3. In general, they have yielded similar results [78]. 1,25-Dihydroxyvitamin D_3 concentrations in plasma decrease with age in humans, and we have shown that this is due, at least in part, to a decrease in the responsiveness of the 25-hydroxyvitamin D_3 1α-hydroxylase enzyme to parathyroid hormone in direct proportion to the decrease of glomerular filtration with age (Table 3) [79].

A majority (~60 to 70%) of administered radioactive 1,25-dihydroxyvitamin D_3 is eliminated in stool as more polar metabolites. Thus, the biliary excretion and fecal route of excretion plays a major role in the elimination of 1,25-dihydroxyvitamin D_3 in man.

In conclusion, 1,25-dihydroxyvitamin D_3 is catabolized by a variety of metabolic processes which rapidly remove it from the organism. These processes are either induced by 1,25-dihydroxyvitamin D_3 itself or are not regulated by dietary calcium and phosphorus intakes [47].

The mechanism of action of 1,25-dihydroxyvitamin D_3

The major effects of vitamin D_3 (via 1,25-dihydroxyvitamin D_3) are to increase the active absorption of calcium from the proximal intestine and to bring about the mineralization of bone [1]. In the following section, the mechanism of action of 1,25-dihydroxyvitamin D_3 in the intestine is reviewed. The intestine is a tissue in which the vitamin has its most marked effects. It has the added advantage in that biochemical events in the enterocyte can be associated with a physiological effect, namely the transport of calcium across the mucosa.

The mechanism of action of 1,25-dihydroxyvitamin D_3 in the intestine

The absorption of calcium from the intestinal lumen has both active and passive components [80]. The former (active) transport process is enhanced by 1,25-dihydroxyvitamin D_3. As shown in Figure 2, from a thermodynamic standpoint, the movement of calcium into the cell from intestinal lumen is with an electrical and a concentration gradient. On the contrary, the concentration of calcium within the cell is considerably lower than that in the extracellular fluid, and therefore, the movement of calcium out of the cell into the extracellular fluid is against a concentration gradient. The extrusion of calcium into extracellular fluid is also against an electrical gradient. Consequently, energy needs to be expended in order to move this ion out of the cell and into extracellular fluid. It is important to bear in mind that other mechanisms such as diffusion through tight junctions could exist, at least in theory, for calcium movement across the epithelial cell layer. Transcellular movement is, however, the major pathway for calcium transport across the duodenal mucosa [81].

Other considerations with respect to the movement of calcium across the intestinal cell layer include the fact that sodium is necessary for the transport of calcium across this epithelial cell, and in the absence of sodium, the active transport of calcium is greatly depressed [82]. A majority of investigators are of the view that under controlled conditions, the movement

Table 2. Metabolic clearance, production and excretion rates of 1,25-dihydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3 in normal man[a]

	MCR liter/day	PR μg/day	Biliary excretion[b] 6 hr–8 hr	Fecal excretion at 6 day–7 day	Urinary excretion
$1,25(OH)_2D_3$	44.6 ± 5.7	~1.5	15.6 ± 1%	54 ± 6%	14 ± 2% (6 day)
$24,25(OH)_2D_3$	9.2 ± 1.5	26.4 ± 7.2	15.3 ± 1.3	48.8 ± 2.7	7.4 ± 1.8% (2 day)

[a] Taken from references 51–53,77
[b] Both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ or products thereof undergo an enterohepatic circulation in man

Table 3. GFR and serum values in different groups of normal women of various ages studied before and after the infusion of bovine (1 = 34) parathyroid hormone[a]

	A	B	C
GFR *ml/min per 1.73 m²*	93 ± 3	72 ± 6	54 ± 6
Serum values			
Calcium *mg/dl*	9.2 ± 0.1	9.6 ± 0.1	9.4 ± 0.1
Phosphorus *mg/dl*			
Basal	3.4 ± 0.1	3.6 ± 0.1	3.7 ± 0.1
Incremental[b]	−0.7 ± 0.1	−0.5 ± 0.2	−0.7 ± 0.1
Alkaline phosphatase *U/liter*	21 ± 3	27 ± 2	29 ± 2
iPTH μlEq/ml	25 ± 2	25 ± 2	31 ± 3
25(OH)D *ng/ml*	45 ± 4	41 ± 2	40 ± 4
$1,25(OH)_2D$ *pg/ml*			
Basal	37 ± 7	34 ± 5	20 ± 6
Incremental[b]	64 ± 13	40 ± 11	25 ± 3
Urine cAMP *nM/100 ml GFR*			
Basal	3.1 ± 0.7	3.4 ± 0.3	3.7 ± 0.3
Incremental[b]	2.9 ± 0.4	3.5 ± 0.4	4.4 ± 0.6

[a] from Tsai et al, reference 79, with permission of the publisher
[b] Difference between levels at beginning and end of bPTH(1-34) infusion. Results are shown as mean ± SE. Group A, age, 37 ± 4 years (mean ± SD); B, age, 61 ± 6 years; C, age, 78 ± 4 years. Correlation coefficients for linear regression of variables GFR vs. age; $P < 0.001$; basal 1,25-dihydroxyvitamin D_3 vs. age, NS; incremental 1,25-dihydroxyvitamin D_3 vs. age, $P < 0.01$; incremental 1,25-dihydroxyvitamin D_3 vs. GFR, $P < 0.001$

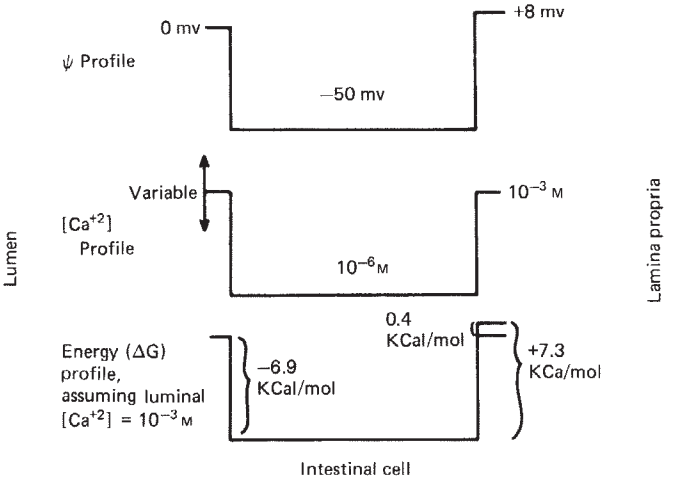

Fig. 2. *Thermodynamic considerations in the movement of calcium across the intestinal cell.* (Taken from Wasserman et al, reference 80, with permission of the publisher).

of calcium across the intestinal cell layer is dependent upon protein synthesis [83, 84]. Some investigators, however, have suggested that calcium transport can occur in the absence of protein synthesis (and specifically calcium binding protein synthesis) when protein synthesis inhibitors are given [85, 86]. Others have shown that while total protein synthesis is severely diminished following the administration of protein synthesis inhibitors, the synthesis of calcium binding protein is not completely abolished [87]. Most investigators currently agree that protein transport is central to the movement of calcium across the cell and is dependent upon receptor mediated mechanisms. Thus, events that occur in the nucleus of the cell are vital in facilitating transcellular calcium movement. Events at other sites may also play a role, though to what extent remains controversial. Following the administration of 1,25-dihydroxyvitamin D_3 in vivo, the transport of calcium in the intestine is biphasic, involving cells located at the tip of villus and those located at the crypt [88]. Halloran and DeLuca have demonstrated that after the administration of 1,25-dihydroxyvitamin D_3 to the rat, there is an initial increase in calcium transport that reaches a maximum at about six hours, followed by a decline and a nadir at about 18 hours. Following this is another increase in calcium transport that reaches a maximum at 24 to 48 hours. When a second injection of 1,25-dihydroxyvitamin D_3 is given at 48 hours, only the first rapid component of an increase in calcium transport is noted. The suggestion is that there are two populations of cells responding differently—one at the tip of the villus that responds quickly and another population at the crypt that migrates up the villus (in 24 to 48 hours) and then plays a part in calcium transport. The latter population is then still able to respond to the initial phase of 1,25-dihydroxyvitamin D_3 stimulated calcium transport.

The effect of 1,25-dihydroxyvitamin D_3 on intracellular and nuclear events in the intestinal cell

Vitamin D_3 via its active metabolite induces the synthesis of calcium binding and other proteins in different animal species. Wasserman first reported the induction of chick intestinal calcium binding protein following the administration of vitamin D_3 to vitamin D-deficient chicks [89, 90]. This protein has a molecular weight of approximately 28,000 in chick intestine; its synthesis is closely related to the vitamin D induced uptake of calcium by the intestinal cell. The precise manner in which it helps in the movement of calcium across the intestinal cell is uncertain; however, it does not act as a "buffer" for calcium [80, 91]. It is vitamin D-dependent in the intestine and an increased flux of Ca^{++} across intestinal cells by diffusional processes in the absence of vitamin D, such as occurs with an

increase in dietary calcium does not increase the synthesis of the protein [80, 91]. Calcium binding proteins are also present in mammalian intestinal cells. In man, intestinal calcium binding protein has a molecular weight of approximately 28,000 [92, 93]; in the rat, the protein has a molecular weight of approximately 10,000 [94]; and in the cow, the molecular weight is approximately 8,500 daltons [95]. The amino acid sequence of bovine and porcine calcium binding protein have been determined, and the presumed sequence of rat intestinal calcium binding protein has been deduced from the sequence of a cDNA clone [96–98].

In addition to the increase in synthesis of calcium binding protein in vivo and in vitro, Bishop et al have shown that 1,25-dihydroxyvitamin D_3 increases the synthesis of another protein (protein 6) of pI ~5.1 and molecular weight >18,000 within 30 minutes of the addition of 1,25-dihydroxyvitamin D_3 to chicken embryonic duodena [99]. Thirty minutes after the addition of 1,25-dihydroxyvitamin D_3 to duodena, the radioactive leucine incorporated in the protein is about one and a half times that seen in control duodena; approximately three times more radioactive leucine is incorporated into experimental duodena at one hour, and about 1.5 times more at 20 hours. The incorporation of radioactivity into another protein (protein 4), is inhibited by about 40% within 30 minutes of the addition of 1,25-dihydroxyvitamin D_3. The inhibition is sustained for at least 20 hours. Whether the decrease in incorporation of radioactive leucine into protein 4 is due to an acceleration in degradation or due to a decrease in actual synthesis or both is not certain.

Recently, Shinki et al showed that 1,25-dihydroxyvitamin D_3 increased ornithine decarboxylase activity in chick intestinal cells following the administration of 1,25-dihydroxyvitamin D_3 to vitamin D-deficient chicks [100]. This response occurred within two hours of the administration of 1,25-dihydroxyvitamin D_3 [100]. The change in ornithine decarboxylase activity is temporally related to the increase in calcium transport. The induction of ornithine decarboxylase activity occurred within crypt and villus cells, but the rate of increase of putrescine synthesis was much higher in the villus cells [101]. Spermidine N1-acetyltransferase activity also increases following the administration of 1,25-dihydroxyvitamin D_3 [102]. Initial reports by Shinki et al reported no increase in 1,25-dihydroxyvitamin D_3 induced activity of S-adenosylmethionine decarboxylase activity [100]. Recently, however, Steeves and Lawson noted an increase in the activity of this enzyme in chick intestine after the administration of 1,25-dihydroxyvitamin D_3 [103]. Mezzetti, Moruzzi and Barbiroli reported an increase in spermine binding protein following the administration of 1,25-dihydroxyvitamin D_3 [104]. It is likely that polyamine synthesis is involved in the response of the intestinal cell to 1,25-dihydroxyvitamin D_3. The exact relationship of the observed responses to calcium transport are not certain, as Steeves has shown that inhibitors of ornithine decarboxylase such as DL-α-difluoromethylornithine HCl (DFMO) and S-adenosylmethionine decarboxylase such as methylglyoxal bis(guanylhydrazone) (MGBG) inhibit the respective enzymes but do not alter 1,25-dihydroxyvitamin D_3–induced calcium transport [103]. It is possible, however, that the decarboxylase inhibitors did not completely deplete the intestinal cells of polyamines. The relationship between polyamines and 1,25-dihydroxyvitamin D_3 requires further study.

The effects of 1,25-dihydroxyvitamin D_3 on the induction of calcium binding protein and other proteins are mediated by a receptor–dependent mechanism. Brumbaugh and Haussler have demonstrated the presence of high molecular weight (~64,000), high affinity, low capacity receptors specific for 1,25-dihydroxyvitamin D_3 in intestinal tissues [105, 106]. Brumbaugh and Haussler have reported on a temperature–dependent translocation of receptors from cytoplasm into the nucleus [106]. Free receptors occur in the cytoplasm whereas occupied receptors are predominantly nuclear in localization [107, 108]. Following the administration of 1,25-dihydroxyvitamin D_3, there is an increase in the chromatin template activity, as well as an induction of the activity of RNA polymerase II that results in the synthesis of messenger RNA [109, 110]. Messenger RNA levels for calcium binding protein increase following the administration of 1,25-dihydroxyvitamin D_3 [111]. Recently, Pike and co-workers have purified the intestinal 1,25-dihydroxyvitamin D_3 receptor and have raised monoclonal antibodies to it [112, 113]. Similar work has been performed by Simpson and DeLuca using different purification methods [114]. A radioimmunoassay for the receptor has been developed and has been used to quantify receptors in fibroblasts of humans with 1,25-dihydroxyvitamin D resistance [115, 116]. Trypsin cleavage of the receptor produces fragments that bind hormone but not DNA or a specific monoclonal antibody, showing that there are distinct domains for binding to hormone or other ligands [117]. The receptor also contains reactive sulfhydryl groups in its DNA binding dormain [118]. Receptors are necessary for 1,25-dihydroxyvitamin D action. The strongest evidence supporting the contention that receptors are necessary for 1,25-dihydroxyvitamin D action comes from reports of 1,25-dihydroxyvitamin D_3 resistance in patients with vitamin D-dependency rickets type II. In these patients, clinical rickets is accompanied by excessively high levels of 1,25-dihydroxyvitamin D_3 in the plasma and a resistance to the action of the hormone [119]. There is decreased localization of 1,25-dihydroxyvitamin D_3 in the nuclei of fibroblasts obtained from these patients, and a direct assay of receptors in the fibroblasts of such patients has revealed that there is both a decreased number and an altered affinity of receptors for the hormone [116]. 1,25-Dihydroxyvitamin D_3 does not induce the activity of 25-hydroxyvitamin D_3-24-hydroxylase as it does in tissue from normal subjects [119]. Wecksler, Okamura and Norman have defined the requirements for optimal receptor binding to vitamin D molecules [120]. The C-1 and C-25 hydroxyl groups are most important for receptor binding followed by the C3 hydroxyl. Similar results were obtained by Eisman and DeLuca [121]. Shortening of the side chain also reduces binding substantially [120].

In addition to the effects noted on calcium binding protein, there are significant effects of 1,25-dihydroxyvitamin D_3 on the uptake of calcium by endoplasmic reticulum and Golgi apparatus [122, 123]. 1,25-Dihydroxyvitamin D_3 also induces cyclic AMP and cyclic GMP production in embryonal duodena [124, 125]. In summary, there is both induction and suppression of several proteins in the cytoplasm of the intestinal cell. The effects appear to be mediated by intracellular receptors for the hormone.

Effects of 1,25-dihydroxyvitamin D_3 on the luminal cell membrane

Vitamin D_3 or 1,25-dihydroxyvitamin D_3 alter the biochemical and morphological characteristics of the intestinal cell membranes. Following the administration of vitamin D_3 to vitamin D-deficient animals, there is an increase in the size of the villus as well as an increase in the size of microvilli; an alteration in the numbers of intracellular organelles also occurs [126–130]. We have observed rapid changes in the morphology of intestinal cells following the administration of 1,25-dihydroxyvitamin D_3 [131]. The villi become larger and more regular following the administration of 1,25-dihydroxyvitamin D_3. The microvilli also become more uniform in appearance. Brush border membrane vesicles from vitamin D-deficient animals have depressed calcium uptake when compared with those from vitamin D-replete animals [86, 132]. This process is not dependent upon the synthesis of new protein [86]. There is an increase in the synthesis of phosphatidyl choline (by an increase in the activity of CDP-choline:sn-1,2-diacylglycerolcholine phosphotransferase), that increases the ratio of phosphatidyl choline to phosphatidyl ethanolamine [133]. The increase in phosphatidylcholine synthesis occurs at the same time as the increase in calcium transport. Transmethylation pathways involved in the synthesis of phosphatidylcholine from phosphatidyl ethanolamine are not altered. The acylation of lysophosphatidyl choline is also enhanced by 1,25-dihydroxyvitamin D_3 as is the deacylation of phosphatidyl choline [134]. At early times after the administration of 1,25-dihydroxyvitamin D_3 to vitamin D-deficient chicks, the phosphatidyl choline to phosphatidyl ethanolamine ratio is not changed [135]. Rasmussen and co-workers have suggested that alteration in the ratio of phosphatidyl choline to phosphatidyl ethanolamine alters the fluidity of the membrane, and thereby allows calcium to diffuse into the cell [136]. Direct measurements of membrane fluidity in intestinal cells, however, have not confirmed this hypothesis [137].

Wasserman and his colleagues have noted alterations in the rate of ^{32}P incorporation into high molecular weight proteins upon the administration of vitamin D_3 to vitamin D-deficient animals [80, 138]. Incorporation of radiolabeled phosphorus into high molecular weight proteins has also been found by Lawson and Wilson, and de Jong, Ghijsen and Van Os following the administration of vitamin D analogs to vitamin D-deficient animals [139, 140]. Kowarsky and Schachter have noted the induction of a protein that they have termed "intestinal membrane calcium binding protein" upon the administration of vitamin D to vitamin D-deficient animals [141]. There have been other reports of the synthesis of other membrane proteins following the administration of vitamin D_3 analogs to vitamin D-deficient animals [142, 143]. These proteins appear to be induced in a temporal sequence that parallels the increase in calcium transport following the administration of vitamin D, and it is possible that they may play a role in the translocation of calcium across the brush border membrane. The specific activity of cell surface enzymes such as maltase and sucrase is diminished [144]. The sensitivity of cell membranes to proteolytic enzymes is also altered following the administration of vitamin D_3 to vitamin D-deficient animals [145]. Wilson and Lawson have reported the increased synthesis of a membrane protein that resembles actin and have suggested that this may be important in the translocation of calcium across brush border membranes [146]. Alkaline phosphatase and calcium–dependent ATPase activities are also enhanced in luminal cell membranes [147–149]. Thus, vitamin D analogs noticeably alter the structure and composition of cell surface proteins and lipids in a time frame that is similar to that of the increase in calcium transport brought about by the sterols. The precise association of any single event with calcium transport is not clear. Certainly, a calcium specific, transmembrane translocating protein has not been identified.

Events at the contraluminal border of the intestinal cell

Based on some of the thermodynamic considerations given above, it is likely that 1,25-dihydroxyvitamin D_3 is involved in increasing the active extrusion of calcium from the intestinal cell at the basolateral membrane. Recently, Ghijsen and Van Os have noted that there is an increase in the activity of a calcium–dependent ATPase isolated from basolateral membrane following the administration of 1,25-dihydroxyvitamin D_3 [150]. Meyer and Wasserman have also observed vitamin D_3 induced ATP-dependent increases in calcium transport in basolateral membranes [151]. This event may be important in the movement of calcium out of the cell and into the extracellular fluid.

Multiple effects of 1,25-dihydroxyvitamin D_3 on the enterocyte

There is considerable evidence now that 1,25-dihydroxyvitamin D_3 has multiple effects both at the luminal membrane, within the cell and at the basolateral membrane. Shultz, Bollman and Kumar have performed experiments to show that the uptake of calcium by brush border membrane vesicles alone is not sufficient to cause the movement of calcium through the intestinal epithelial cell layer [152]. The control point resides within the cell, such as at the level of protein synthesis or at the contraluminal membrane. Wasserman et al have suggested that the luminal events (uptake of calcium into the cell) are more rapid in the onset than the cellular events (such as calcium binding protein synthesis) [135]. The former (luminal) events are associated with the synthesis of phospholipids and are not protein–dependent, whereas the intracellular events are protein–dependent [135].

In conclusion, 1,25-dihydroxyvitamin D has diverse effects on the enterocyte. While luminal membrane calcium uptake may not be protein–dependent, the movement of this divalent cation across the cell is dependent on the synthesis of proteins induced by receptor mediated mechanisms in a manner analogous with that of other steroids such as estrogens. Contraluminal enzymes such as calcium ATPase are most probably involved in the extrusion of calcium out of the cell into the extracellular fluid.

Effects of vitamin D and its metabolites on the kidney

The nature of the 25-hydroxyvitamin D_3 1α-hydroxylase, its localization and regulatory factors have been covered in a previous section. The effect of vitamin D_3 and its various metabolites on ion transport are much less marked on the kidney than on the intestine and conflicting data have been

reported. Vitamin D_3 increases the tubular reabsorption of phosphate and calcium in the intact rat and dog [153, 154]. Similarly, 25-hydroxyvitamin D_3 increases the reabsorption of sodium, calcium, phosphate, and bicarbonate in the intact rat and dog [154–157]. In the thyroparathyroidectomized dog and rat, the antiphosphaturic effect of 25-hydroxyvitamin D_3 is absent; infusion of parathyroid hormone restores the antiphosphaturic effect [154–157]. The effect of 25-hydroxyvitamin D_3 on phosphate reabsorption can occur in the thyroparathyroidectomized rat in the presence of vasopressin [158]. The effect of 25-hydroxyvitamin D_3 on calcium reabsorption may be a distal tubular effect [159].

1,25-Dihydroxyvitamin D_3 also increases the reabsorption of phosphate in the intact dog and hamster [158, 160, 161]. The effect is noted only in the presence of parathyroid hormone [158, 160, 161]. Vasopressin is also able to mimic the action of parathyroid hormone in this respect [158]. In the parathyroidectomized rat, 1,25-dihydroxyvitamin D_3 has either no effect or a phosphaturic effect [158, 160–163]. The effect of 1,25-dihydroxyvitamin D_3 on calcium transport is not clearly established. Some have found a decrease in the amount of urinary calcium excreted following the administration of 1,25-dihydroxyvitamin D_3; others have not been able to find a decrease (in fact an increase, in some instances) in urinary calcium excretion following the administration of 1,25-dihydroxyvitamin D_3 [160, 161, 164, 165]. Kurnik and Hruska have suggested that the effect of 1,25-dihydroxyvitamin D_3 on phosphate transport is mediated by changes in lipid composition of membrane [166].

The localization of the 25-hydroxyvitamin D_3-1α-hydroxylase enzyme has been alluded to above [34, 35]. The enzyme is a mitochondrial enzyme located exclusively in the proximal tubules [36–38]. Receptors for 1,25-dihydroxyvitamin D_3 are located in the distal tubule of the kidney [167]. One report suggests that there are receptors in the proximal tubule also [168]. Examination of the vitamin-D–dependent calcium binding protein in the tubule reveals that it is located in the distal tubule [168–170]. Based on available information, it is clear that 1,25-dihydroxyvitamin D_3 exerts a significant effect on the distal tubule by inducing the synthesis of calcium binding protein. As receptors for the hormone are also present in this segment of the tubule, it is very likely that receptors are involved in the induction of the calcium binding protein.

Summary

Much has been learned about the formation of the active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3. Information concerning its formation and catabolism has allowed a clear understanding of factors involved in the maintenance of plasma concentrations of the hormone. The effects of 1,25-dihydroxyvitamin D_3 on calcium transporting cells in the intestine are marked and well defined. The tissue (intestinal tissue) is easily isolated and manipulated and hence, this is an ideal tissue in which to examine the mechanism of divalent cation transport. The mechanism by which 1,25-dihydroxyvitamin D_3 brings about this effect should help in understanding sterol hormone action.

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Note added in proof

There is a high degree of homology between NH_2-termini of rat and human vitamin D binding protein [25, 172, 173].

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