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VITAMIN D: METABOLISM AND BIOLOGICAL ACTIONS

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CONTENTS

INTRODUCTION

As a result of intensive efforts in a number of laboratories over the past two decades, a new concept of the mechanism of action of the fat-soluble vitamin D has emerged. The cornerstone of this concept is that in terms of its structure, availability, metabolism, and mode of action, vitamin D is more properly considered a steroid hormone than a vitamin in the classical sense. Thus it is now accepted that vitamin D serves as a prohormone or precursor for more

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polar metabolites, two of which, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] and 24R,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$], are the principal mediators of the activities once ascribed to the parent compound. There is also substantial evidence, at least for 1,25$(OH)_2D_3$, that its mechanism of action is similar to that of other steroid hormones such as the glucocorticoids, the mineralocorticoids, and the sex steroids, in that the steroid must interact with its receptor and this must be followed by association of the steroid-receptor complex with genetic material in the nucleus of the cells that comprise the target tissue in order to generate the full biological response.

It has been shown that 1,25$(OH)_2D_3$ has important biological actions in the three classic target organs of vitamin D: the bone, where calcium and phosphorus are mobilized or accumulated; the kidney, in which the tubular reabsorption of calcium and phosphate are increased; and the intestine, where absorption of dietary calcium is stimulated. We shall discuss several more recently recognized target tissues of the metabolites of vitamin D_3. This review focuses on the broad areas of research in the field of vitamin D that continue to be areas of current investigation: (*a*) the metabolic pathways by which various metabolites are generated and degraded, (*b*) the regulation of these pathways, and (*c*) the biological actions of metabolites of vitamin D.

METABOLISM OF VITAMIN D

Chemistry of Vitamin D Seco-steroids and Their Photochemical Production

Vitamin D and all its metabolites are seco-steroids, or compounds in which one of the rings of the cyclopentanoperhydrophenanthrene ring structure characteristic of steroids has been broken. In vitamin D, the 9,10 carbon bond of ring B is broken (see Figure 1, structure 1). Because of the 9,10 seco nature of vitamin D compounds, the A ring can become inverted from rings C and D, with rotation occurring around the bond between C-7 and C-8. Thus the α and ß designations for substituent groups on the A-ring must be drawn with reverse notation due to the inversion of the A-ring. More correctly, the 3ß-hydroxyl of vitamin D should be designated as 3R-OH while the 3ß-OH and 1α-OH of 1,25$(OH)_2D_3$ are respectively 3R-OH and 1S-OH.

Okamura, Wing, and colleagues evaluated the topology of vitamin D_3, its metabolites, and its analogs in solution by high-resolution proton magnetic resonance spectroscopy in conjunction with lathanide-induced shift studies (153, 232, 233). Their studies emphasize that in solution the seco-steroid vitamin D undergoes a dynamic equilibration between the two chair conformers (see Figure 1, structures 2 and 3). This is probably the most accurate molecular representation for all of the vitamin D seco-steroids. As discussed by Okamura

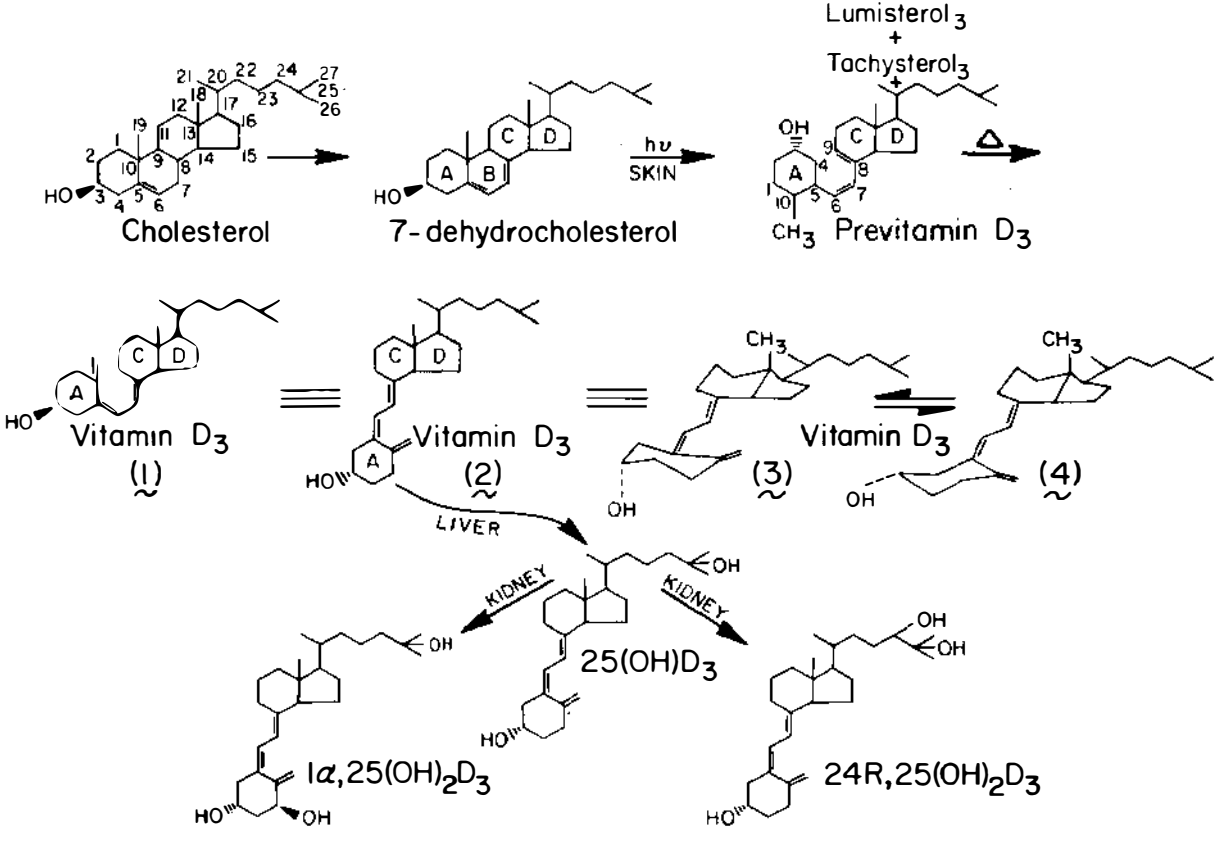


Figure 1 Key vitamin D sterols. *(Top line)* Vitamin D is produced from 7-dehydrocholesterol via previtamin D as a consequence of the uv-mediated opening of the B-ring. *(Middle line)* Summary of the evolution of the conformational representations of vitamin D. Structure 1 or 2 resulted from original chemical analysis; structures 3 and 4 represent results of recent studies (232, 233) indicating the conformational mobility of the A-ring. *(Bottom line)* Structure of the three principal metabolites; *hv* = ultraviolet light irradiation.

et al (153), any detailed understanding of the structure-function relationship of vitamin D and its metabolites demands consideration of the consequences of this dynamic interchange (which occurs several times per second) between the two chair-like conformers of the A-ring.

The precursor to the seco-steroid vitamin D and its derivatives is the provitamin D, which contains a Δ5–7 diene double-bond system in ring B (see Figure 1). This conjugated double-bond system absorbs ultraviolet (uv) light, which initiates a complex series of reactions leading ultimately to the seco-steroid previtamin D, which is in thermal equilibrium with vitamin D. The equilibrium ratio of vitamin D to previtamin D is 89:11 at 37°C.

The skin has long been recognized as the site of sunlight/photochemical–mediated synthesis of vitamin D_3. However, until the recent work of Holick and coworkers (80, 82, 84) and Kobayashi (154, 155) and coworkers, very little was known about either the sequence of events leading to the formation of vitamin D_3 in human skin or the regulation of the synthesis of this prohormone. It is now known that during exposure to sunlight, the epidermal cutaneous reservoir of 7-dehydrocholesterol, which is largely present in the Malpighii layer, is photochemically converted to previtamin D (79), which then isomerizes to vitamin D over an interval of two to three days. The blood transport protein for vitamin D and its metabolites, D-binding protein or DBP (16), preferentially removes vitamin D_3 from the skin to the general circulatory system.

Certain aspects of the photochemical production of vitamin D_3 by the skin have recently been subjected to quantitative examination. Holick et al (83) reported that a change in geographical location to more northerly latitudes or a decrease in the skin concentration of mellanin diminish the synthesis of previtamin D_3. Adams et al (2) found that there is an apparent age-correlated loss in the epidermal concentration of 7-dehydrocholesterol; this has supported the provocative suggestion that one of the factors contributing to age-related diseases of calcium metabolism may in fact be related to impaired capability of the uv-mediated production of vitamin D.

Metabolic Pathways of Vitamin D and its Derivatives

As shown in Figure 2, vitamin D_3 is hydroxylated at the C-25 position in the liver to form 25(OH)D_3, the major circulating vitamin D steroid. The kidney, functioning as an endocrine tissue, is the site of two possible further hydroxylations to form 1,25$(OH)_2D_3$ or 24,25$(OH)_2D_3$.

It is generally agreed that the kidney is the principal physiologic site of production of the dihydroxylated metabolites, although a number of other cell types have been reported to carry out these transformations in vitro. The tissues include bone (92, 215, 216), placenta (67, 201, 227, 228), intestine (151, 168), and yolk sac (39). Whether there is significant extrarenal production of

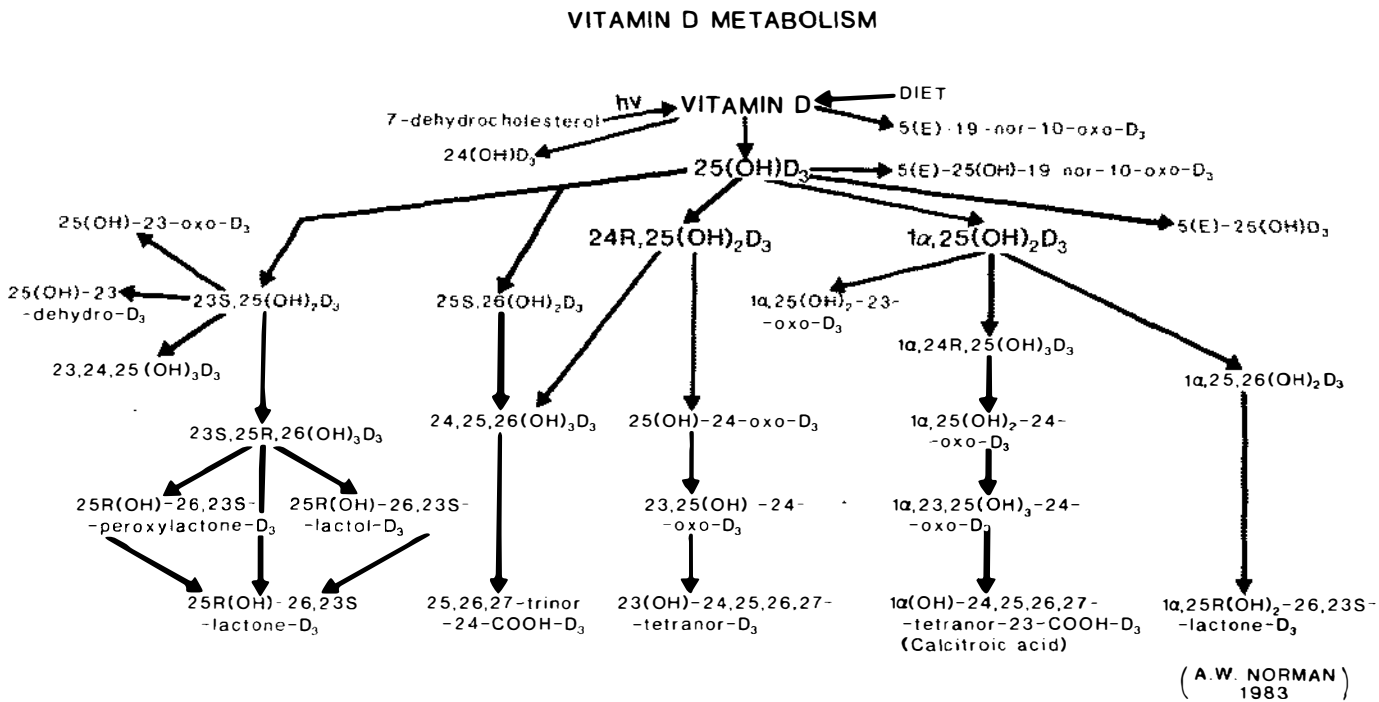


Figure 2 Summary of vitamin D metabolism. All known chemically characterized metabolites are organized into logical pathways based on steroid metabolic principles. In most instances specific product-precursor relationships have not yet been demonstrated.

$1,25(OH)_2D_3$ in the nonpregnant state remains unclear. Lambert et al (113) reported detectable $1,25(OH)_2D_3$ levels in anephric humans; anephric pigs given pharmacological doses of vitamin D_3 had plasma levels of $1,25(OH)_2D_3$ similar to those in control animals (117). On the other hand, no $[^3H]1,25(OH)_2D_3$ was observed in the blood or intestine of nephrectomized rats 20 hours after a dose of $[^3H]25\text{-}OH\text{-}D_3$ of high specific activity (171, 178a).

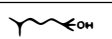
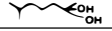
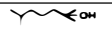
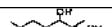
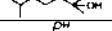
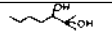
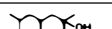
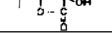
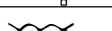
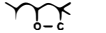
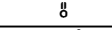
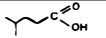
There are to date 27 chemically characterized metabolites of vitamin D. The structures of these compounds are given in Figure 3; a proposal with respect to the organization of the various metabolic pathways supporting their production is presented in Figure 2. Figure 2 is organized so that the right hand portion of the figure presents the structure of all the metabolites and analogs of $1,25(OH)_2D_3$; the left hand portion of the figure presents the structures of the non-1-hydroxylated metabolites that are derived from either $24R,25(OH)_2D_3$ or $25(OH)D_3$.

As summarized in Figure 2, the two key dihydroxylated metabolites of vitamin D, $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$, both appear to have the same metabolic pathway of catabolism. After 24-hydroxylation, this involves generation of a C-24-oxo functionality followed by C-23 hydroxylation and likely side-chain cleavage (68, 111) to yield a C-23 carboxyl metabolite (48). It is predicted that the immediate product of side-chain cleavage of the vicinal C-23-OH, C-24-oxo, is a metabolite with a C-23 aldehyde. It seems significant that both the target organ intestine (128, 138, 151) as well as kidney (128, 151) has the capability of inactivating $1,25(OH)_2D_3$. The presence of this metabolic capability in the target organ may well provide an important immediate mechanism for inactivating this biologically potent metabolite (126a). In addition to this C-24,C-23 pathway of catabolism, a second metabolic pathway has been identified for the further processing of $1,25(OH)_2D_3$ and $25(OH)D_3$. This results in the generation of a C-26,C-23-lactone derivative of either metabolite. The functional significance of the lactone pathway is not well understood.

REGULATION OF VITAMIN D METABOLISM

25-Hydroxylation of Vitamin D

The liver has long been thought to be the principal site of the 25-hydroxylation of vitamin D_3 (164), although significant activity also occurs in other tissues including lung, intestine, and kidney (95). The relative amount of activity in each tissue and the distribution of activity between microsomes and mitochondria depend on the species (95). Whether 25-hydroxylation of vitamin D is subject to feedback regulation has been a matter of some controversy, with evidence existing both for and against a role for $25(OH)D_3$ in this process (see Ref. 60 for a review). A more recent study suggests that $1,25(OH)_2D_3$ can

CPN	NAME	R_1	R_2	Ref	Date
1.	25(OH)D_3	H		14	1968
2.	25S,26(OH)$_2D_3$	H		194	1970
3.	1α,25(OH)$_2D_3$	OH		86,115,145	1971
4.	24R,25(OH)$_2D_3$	H		85	1972
5.	1α,24R,25(OH)$_3D_3$	OH		81	1973
6.	25R(OH)D_3-26,23S-lactone	H		229	1979
7.	1α(OH)-24,25,26,27-tetranor-23-COOH-D_3 (calcitroic acid)	OH		49	1979
8.	25,26,27-trinor-24-COOH-D_3	H		42	1979
9.	1α,25R(OH)$_2D_3$-26,23S-lactone	OH		150,204	1980
10.	25(OH)-24-oxo-D_3	H		197,230	1980
11.	24(OH)D_3	H		99,231	1981
12.	1α,25,26(OH)$_3D_3$	OH		172,203	1981
13.	25(OH)5,6-E-D_3	*		112	1981

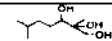
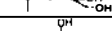
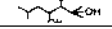
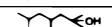
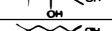
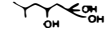
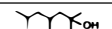
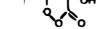
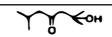
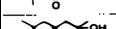
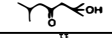
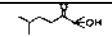
CPN	NAME	R_1	R_2	Ref	Date
14.	24,25,26(OH)$_3D_3$	H		230	1981
15.	23,24,25(OH)$_3D_3$	H		230	1981
16.	23-dehydro-25(OH)D_3	H		230	1981
17.	23S,25(OH)$_2D_3$	H		205	1981
18.	23S,25R,26(OH)$_3D_3$	H		96	1982
19.	25R(OH)D_3-26,23S-peroxylactone	H		97	1982
20.	25(OH)-23-oxo-D_3	H		90	1982
21.	1α,25(OH)$_2$-23-oxo-D_3	OH		90	1982
22.	1α,25(OH)$_2$-24-oxo-D_3	OH		127,138	1983
23.	1α,23,25(OH)$_3$-24-oxo-D_3	OH		127,137	1983
24.	23,25(OH)$_2$-24-oxo-D_3	H		128,234	1983
25.	25(OH)-19-nor-10-oxo-D_3	H**		139	1983
26.	19-nor-10-oxo-D_3	H**		139	1983
27.	25R(OH)-26,23S-lactol-D_3	H		235	1983

Figure 3 Metabolites of vitamin D_3. (*)25(OH)5,6-E-D_3 is more usually known as 25(OH)5,6-*trans*-D_3. In this compound the 5,6 double bond is *trans* (E) rather than *cis* (Z) as in vitamin D_3; this has the consequence of rotating the A-ring 180° so that the 3ß-hydroxyl group becomes a pseudo 1α-hydroxyl. (**)Both 25(OH)-19-nor-10-oxo-D_3 and 19-nor-10-oxo-D_3 have been isolated (139) as a mixture of 5,6E and 5,6Z.

reduce liver 25-hydroxylase activity (12), but whether this is a physiologically significant process remains to be established. The production of $1,25(OH)_2D_3$ by the kidney is under stringent control so that more of the hormone is available when demand for calcium is high. The nature of the regulatory factors involved in $25(OH)D_3$ metabolism and the mechanisms by which they act have been under investigation since $1,25(OH)_2D_3$ was discovered 14 years ago (53, 64, 145).

The 25-Hydroxyvitamin D_3-1- and 24-Hydroxylases

The 25-OH-D_3-1α-hydroxylase (62, 75) and probably the 25-OH-D_3-24R-hydroxylase (122, 159) belong to a class of enzymes known as mitochondrial mixed-function oxidases. The cholesterol side-chain cleavage system and 11ß-hydroxylase of the adrenal cortex are examples of similar enzyme systems. They are composed of three proteins that are internal components of the inner mitochondrial membrane; two of these proteins, a nonheme iron protein termed ferredoxin (or adrenodoxin in the case of the adrenal cortex) and ferredoxin reductase, serve to transport electrons from NADPH to the terminal component, cytochrome P-450. The cytochrome P-450 provides the substrate binding site and the catalytic site that reduces one molecule of O_2 to one molecule of water and one hydroxyl function to be incorporated into the steroid at a stereo-specific site. It is important to keep in mind the subcellular and submitochondrial location of these enzymes and the complexity of the enzymatic reaction when considering possible mechanisms of their regulation.

1,25-Dihydroxyvitamin D_3

The most powerful determinant of 1-hydroxylase and 24-hydroxylase activity in kidney cells is probably the vitamin D status, or more precisely, the $1,25(OH)_2D_3$ status of the cells or animal. In the vitamin D-deficient state, the production of $1,25(OH)_2D_3$ is maximal and $24,25(OH)_2D_3$ synthesis is low or undetectable. The situation is reversed in the presence of $1,25(OH)_2D_3$. This occurs in vivo (53, 73, 199, 202) and in primary cultures of kidney cells from chicks (69, 211), Japanese quail (91), and mice (55). For example, when chick kidney cells are grown in medium devoid of $1,25(OH)_2D_3$, the addition of the hormone to the medium causes in several hours a marked decrease in subsequently measured 1-hydroxylase activity and an increase in 24-hydroxylase activity. The dynamic nature of this regulation is suggested by the fact that the decrease in 1-hydroxylase activity and increase in 24-hydroxylase activity are reversed when $1,25(OH)_2D_3$ is removed from the medium (69, 72). Changes in both enzyme activities by $1,25(OH)_2D_3$ are also inhibited by cycloheximide and actinomycin D (6, 69), which strongly suggests a role for the expression of new genetic information and new protein synthesis in this process.

Parathyroid Hormone

Another regulator of $25(OH)D_3$ metabolism for which substantial evidence exists is parathyroid hormone. Parathyroid hormone (PTH) stimulates production of $1,25(OH)_2D_3$ in kidney homogenates in vitro and decreases that of $24,25(OH)_2D_3$ in chicks (69) and Japanese quail (10); it has the same effects on serum levels of the metabolites in rats (60) and man (120, 183). Similar direct effects of PTH on $25(OH)D_3$ metabolism in whole cell studies in vitro have been demonstrated in primary cultures of kidney cells from chick (69, 211), monkey (100), and mouse (55) and of kidney slices from chick (175), guinea pig (106), and rat (4). In the latter study, it was reported that while PTH decreased 24-hydroxylase in both young and adult rats, it stimulated production of $1,25(OH)_2D_3$ only in young rats. The reason for this change in responsiveness with age is unclear, but it did not appear to be related to an alteration in cyclic AMP response due to age.

The mechanism by which PTH acts on $25(OH)D_3$ metabolism has not been completely elucidated. In chick kidney cell cultures, the addition of insulin to the medium enhanced the response of $25(OH)D_3$ metabolism to PTH, but this did not occur via an effect of total cyclic AMP production (71). Cyclic AMP has been reported to alter $25(OH)D_3$ metabolism in much the same way as PTH does (175); a similar effect has been noted for forskolin (71a), which elevates intracellular cyclic AMP levels by direct interaction with adenyl cyclase. PTH activates cyclic AMP–dependent protein kinase and stimulates the phosphorylation of specific intracellular proteins in chick kidney cells (141), but the role, if any, of these proteins in $25(OH)D_3$ metabolism has not yet been identified.

Calcitonin

There are several reports (58, 88) that calcitonin has a stimulative effect on $1,25(OH)_2D_3$ accumulation in vivo as well as on 1-hydroxylase activity measured in defined segments of rat kidney tubules in vitro following administration of the hormone in vivo (103). It is unclear whether this is a direct effect of calcitonin on renal cells. Lorenc et al (119) reported that calcitonin stimulated $1,25(OH)_2D_3$ accumulation in intact but not thyroparathyroidectomized rats, which led the authors to suggest that the effect is an indirect one, perhaps mediated through the parathyroid gland. Assessment of the effect of calcitonin on kidney cells in vitro has yielded conflicting results. There are reports of both stimulation (114) and inhibition (170) of $1,25(OH)_2D_3$ production by calcitonin in freshly isolated chick renal tubules, but the hormone has been observed to have no effect in primary cultures of chick cells (74). The latter result would be consistent with the interpretation above that the effect of calcitonin observed by several investigators in vivo is not exerted directly on the kidney cell.

Calcium and Phosphorus

There is much evidence (reviewed in 54, 212) to suggest that the prevailing in vivo levels of serum calcium and to a lesser extent, phosphate, affect $25(OH)D_3$ metabolism, possibly independently of the parathyroid gland (212). Although no effects of variations in these ions have been observed in primary cultures of chick kidney cells (72, 211), Armbrecht (5) recently reported that rat renal slices prepared from vitamin D–replete rats on a high calcium diet show decreased $1,25(OH)_2D_3$ when high concentrations of calcium were present in the media. Kidney slices from vitamin D–deficient rats required concomitant treatment with $1,25(OH)_2D_3$ in order to respond to high calcium concentrations in the media with slightly decreased $1,25(OH)_2D_3$. Further examination and documentation are needed to determine whether calcium and phosphate have direct, independent roles in regulating $25(OH)D_3$ metabolism. It should be pointed out that in the above studies with rat kidney slices, no effect of the calcium concentration of the media on the production of $24,25(OH)_2D_3$ was observed, although a positive relationship between serum calcium and $24,25(OH)_2D_3$ levels is usually found in vivo (54).

Estrogens

There are abundant reports of avian models in which in vivo administration of estradiol, alone or in a combination with testosterone or progesterone, enhanced 1-hydroxylase activity measured subsequently in vitro (8, 9, 25, 163). There is equally convincing evidence that this effect is not exerted directly on the renal cell; under a number of experimental conditions, estradiol has not been observed to stimulate 1-hydroxylase activity in cultured chick kidney cells (70, 184, 211). The precise mechanism of the effect observed in vivo is unknown, but the interesting proposition has been put forth that it is secondary to a stimulative effect of estrogen on D-binding protein (DBP) levels (16). According to this theory, as DBP increases in response to estrogen, the free concentration of $1,25(OH)_2D_3$ falls, bringing about increased 1-hydroxylase levels. Alternatively or in conjunction with this process, estrogen may modulate the response of the kidney to PTH. A recent report (52) indicates that estradiol causes a threefold increase in the number of PTH receptors in the kidney. This effect could lead to elevated 1-hydroxylase activity in the absence of changes in circulating PTH concentrations.

Pituitary Hormones

There is evidence that the pituitary gland plays a role in regulation of $25(OH)D_3$ metabolism. Although some evidence suggests that prolactin has a role in stimulating $1,25(OH)_2D_3$ production in chicks (13, 187, 189), no evidence that

prolactin affects $25(OH)D_3$ metabolism in rats (126, 185, 190) or humans (110) has been found. Hypophysectomy in the rat is reported to result in lowered circulating $1,25(OH)_2D_3$ levels and increased $24,25(OH)_2D_3$ levels (50, 158, 190). These effects were reversed by the administration of growth hormone for several days to two weeks (158, 190). Additionally, Gray (63) has reported that hypophesectomized rats not only have decreased $1,25(OH)_2D_3$ levels, but they do not respond to phosphate deprivation with increased levels of this steroid as do intact animals. More recently, it has been reported that administration of growth hormone and thyroid hormone overcomes this refractoriness to dietary phosphate deprivation (65). It was postulated that these hormones might play a permissive role in the response of $25(OH)D_3$ metabolism to hypophosphatemia. It should be noted that in studies of growth hormone–deficient children, hormone replacement therapy was observed to have no effect on plasma $1,25(OH)_2D_3$ levels or on other parameters of calcium metabolism observed (61).

The mechanism of the effect of growth hormone on $25(OH)D_3$ metabolism has not been investigated. Possibilites include direct effects on the 1- and 24-hydroxylases, changes in serum DBP levels that could alter total and free $1,25(OH)_2D_3$ concentrations in a manner analogous to that proposed for estrogens above, or an alteration in the renal responsiveness to PTH.

The current status of putative regulatory factors of $25(OH)D_3$ metabolism is summarized in Table 1. It is not possible to accommodate the many variations in experimental protocol represented by the citations in Table 1 in such an abbreviated format; however, the major thrust and conclusions of each report are indicated. In the table, observations in the literature regarding the regulation of $25(OH)D_3$ metabolism are classified according to the type of experimental protocol that was employed. Studies in which intact animals were used for both treatment and assessment of $25(OH)D_3$ metabolism are cited under the heading *In vivo;* studies in which intact animals were treated but $25(OH)D_3$ metabolism was measured in isolated kidney cells, kidney homogenates, or mitochondria are cited under the heading *In vivo* → *In vitro;* studies in which intact cell preparations were used for treatment with the indicated factor and for measurement of $25(OH)D_3$ metabolism are cited under the heading *In vitro*. The arrows indicate whether the authors concluded that the regulatory factor increased (↑), decreased (↓), or did not alter (→) the net production of either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$.

At present, the best candidates for direct modulation of the renal metabolism of $25(OH)D_3$ are $1,25(OH)_2D_3$ and PTH. It is clear however, that this metabolic pathway is not isolated from other endocrine systems in the body but can respond indirectly or directly to changes in pituitary and gonadotrophic hormones and possibly to changes in other hormones.

Table 1 Summary of factors proposed to regulate the metabolism of 25-hydroxyvitamin D_3[a]

Regulatory factor	Reference					
	In vivo		In vivo → In vitro		In vitro	
	$1,25(OH)_2D_3$	$24,25(OH)_2D_3$	$1,25(OH)_2D_3$	$24,25(OH)_2D_3$	$1,25(OH)_2D_3$	$24,25(OH)_2D_3$
$1,25(OH)_2D_3$	↓ 24 148 199	↑ 24 148 199	↓ 53 73 202	↑ 105 159	↓ 55 69 91 149 211	↑ 55 69 91 100 149 211
PTH	↑ 24 57 60 89 120 183	↓ 24 57 60 89	↑ 4 10 69 202	↓ 4 10 202	↑ 11 55 71 91 106 170	↓ 11 55 71 91 100
Calcitonin	↑ 58 88 → 119		↑ 103		↑ 114 ↓ 170 → 74	
Increased extracellular calcium	↓ 18 174 212	↑ 18 174	↓ 6 9 73 → 6	↑ 9 202	↓ 5 → 72 211	→ 5 72 188 211 ↑ 100
Decreased extracellular phosphate	↑ 63 169 174 212	↓ 174	↑ 66 → 15 73	→ 6	→ 72 211	→ 72 188 211
Estrogens			↑ 8 9 25 163	↓ 8 9 25 163	→ 70 184 211	→ 70 184 211
Prolactin	↑ 189 → 110 126 185 190		↑ 187		↑ 186 13	→ 186
Growth hormone	↑ 63 65 158 185 190 → 61				↑ 186 → 13	→ 186

[a]Observations in the literature regarding the regulation of $25(OH)D_3$ metabolism have been classified according to the type of experimental protocol employed. On the left are citations to papers in which intact animals were used for both treatment and assessment of $25(OH)D_3$ metabolism. In the references in the center of the table, intact animals were treated but $25(OH)D_3$ metabolism was measured in isolated kidney cells, kidney homogenates or mitochondria. At the right of the table are references to papers in which intact cell preparations were used for treatment with the indicated factor and for measurement of $25(OH)D_3$ metabolism. The arrows indicate whether the authors concluded that the regulatory factor increased (↑), decreased (↓) or did not alter (→) the net production of either $1,25(OH)_2D_3$ or $24,25(OH)_2D_3$.

BIOLOGICAL ACTION OF VITAMIN D

General Model of Action

It is now generally accepted that most of the biological responses produced in target tissues by vitamin D, particularly by $1,25(OH)_2D_3$ in intestine and bone, are mediated via pathways analogous to those used by other steroid hormones. According to this model, target tissues are defined by the presence of specific, high-affinity receptors/binding proteins for the hormone in question. After association of the steroid hormone with its receptor, the steroid hormone-receptor complex is tightly associated with the nuclei or chromatin of the cell. Here, a selective stimulation of DNA transcription occurs that results in the biosynthesis of new messenger RNA molecules that are then translated to produce the proteins necessary to generate the biological response of the hormone in question. In the case of $1,25(OH)_2D_3$, there is abundant evidence that the intracellular localization of the steroid in target tissues is indeed nuclear (see 146 for review). In fact, the nuclear localization of the steroid initially led to the concept that this active metabolite of vitamin D is actually a hormone and not a vitamin (142).

Following localization of the $1,25(OH)_2D_3$ receptor complex in the nucleus, increased RNA synthesis occurs (214). It has been clearly demonstrated that mRNA coding for vitamin D–dependent calcium-binding protein (CaBP) is specifically stimulated by $1,25(OH)_2D_3$ in chick intestine (30, 46, 191, 192). Synthesis of other proteins has also been reported to depend on vitamin D. In the intestine, these include a 39,000–42,000-dalton protein that is part of the outer mitochondrial membrane (78), a spermine-binding protein (132), ornithine decarboxylase (179), and a 76,000-dalton cytosolic protein (104). In bone, $1,25(OH)_2D_3$ stimulates the production of the bone gamma carboxyglutamic acid (gla) protein (165), a 6,000-dalton CaBP containing a γ-carboxyglutamic acid residue, the carboxylation of which depends on vitamin K. It may well be that with increasingly sophisticated and sensitive techniques, it will be discovered that the synthesis of other proteins is responsive to $1,25(OH)_2D_3$.

Receptors for 1,25-Dihydroxyvitamin D_3 and Vitamin D–Dependent Calcium-Binding Protein

The biochemical properties of the chick intestinal receptor for vitamin D_3 have been the subject of intense study and were recently reviewed in detail (146). This binding protein has a high affinity for $1,25(OH)_2D_3$, with a K_d of $1–50 \times 10^{-11}$ M, and is highly specific, as shown by competition studies with chemically synthesized analogs of $1,25(OH)_2D_3$ (45, 107, 166, 223).

Although it was originally believed that the unoccupied receptor existed primarily in the cytoplasmic compartment of the cell, it now appears that this observation was the result of the standard procedure of extracting receptor with buffers of high ionic strength; when a buffer of physiologic ionic strength (e.g. 0.15 M KCl) is used, approximately 70% of the unoccupied receptor appears in the nuclear fraction (218, 219). The occupied receptor is less salt-extractable than the occupied receptor (217), which suggests that a physical-chemical change resulting from the binding of the steroid increases its affinity for the chromatin.

As summarized in Table 2, the receptor for $1,25(OH)_2D_3$ is by no means limited to the classical target tissues of intestine, bone, and kidney but has a wide distribution in a number of tissues. In addition, a number of human and nonhuman cell lines have been shown to contain $1,25(OH)_2D_3$ receptor-like proteins. Norman et al (146) reviewed these and the biochemical basis upon which the presence of each of these receptors have been reported. If it is assumed that a target tissue of $1,25(OH)_2D_3$ is defined by the presence of a receptor, a biological response to the hormone should be apparent in a tissue with a receptor. One response that has been examined in a number of tissues is the presence of vitamin D–dependent CaBP. The results of such investigations are also summarized in Table 2. For a more detailed discussion of the chemical and physical properties of vitamin D–dependent CaBP, see Norman et al (146) and Wasserman (220).

Although there is a strong association between the presence of receptors for $1,25(OH)_2D_3$ and vitamin D–dependent CaBP in a variety of tissues from a number of animal species, the function of this protein in these tissues is uncertain. Recent advances in the study of the molecular biology of CaBP, its messenger RNA, and its gene (43, 94) will undoubtedly contribute to an understanding of its biological function. The overall effect of $1,25(OH)_2D_3$ on most of the tissues or cells listed in Table 2 is also not well understood. In addition to advances in the study of the biological effects of $1,25(OH)_2D_3$ in the classical target tissues for vitamin D, the intestine, bone, and kidney, advances have recently been made in two areas that may represent previously unrecognized actions of $1,25(OH)_2D_3$. These are the effect of vitamin D status on insulin secretion and a possible role for $1,25(OH)_2D_3$ in cellular growth and differentiation.

1,25-Dihydroxyvitamin D_3 in the Pancreas

The presence of vitamin D–dependent CaBP in the pancreas initially was reported by Christakos et al (27). Roth et al (176) have since elegantly demonstrated that CaBP is localized exclusively in the insulin-secreting beta cells; Narbaitz et al (140) have shown that $[^3H]1,25(OH)_2D_3$ is also localized

Table 2 Tissue and species distribution of receptor proteins for $1,25(OH)_2D_3$ and vitamin D–dependent CaBP

Tissue	Species	Reference[a]	
		$1,25(OH)_2D_3$ Receptor	CaBP
Intestine	Chick	20, 213	27, 206, 208, 210, 221
	Rat	109, 226	44, 101, 209
	Mouse	33	23
	Hamster	—	102
	Guinea pig	—	56
	Cow	—	56, 195
	Pig	—	7, 51, 56
	Monkey	—	222
	Man	224	3, 40, 130, 160
Kidney	Chick	31	27, 178, 208
	Rat	26	173, 177, 207
	Mouse	33	—
	Man	—	160
Bone	Chick	108, 129	27, 28
	Rat	123	—
	Mouse	33	—
Parathyroid gland	Chick	21, 93, 225	27
	Rat	193	—
	Cow	93	—
	Pig	—	7, 147
	Man	93, 225	—
Pancreas	Chick	31, 161, 162	27, 136, 176
Pituitary	Chick	162	—
	Rat	121, 162	—
	Cow	60a	—
Placenta	Rat	29, 162	22, 41
	Mouse	—	23
	Man	29	—
Chorioallantoic membrane	Chick	36	214a
Egg shell gland	Chick	35	34, 98
	J. Quail	196	—
Skin	Rat	(a)	(a)
Yolk sac	Rat	(a)	ND
Parotid gland	Rat	(a)	ND
Uterus	Rat	(a)	(a)
Monocytes	Human	(a)	ND
Macrophages	Human	(a)	ND
T-Lymphocytes (activated)	Human	(a)	—
Leukocytes	Man	167	ND
Thymus	Bovine	—	—
Cerebellum	Bovine, human	ND	(a)
Mammary tissue	Human, bovine	(a)	ND

[a]ND = not detected. (a) = Reference citation is available in Tables 3 and 4 of Ref. 146.

exclusively in the beta cells. Combined with the earlier observation that vitamin D depletion inhibits insulin secretion (143), these results represent another case in which there is good correlation between a biological effect of $1,25(OH)_2D_3$, a receptor for the steroid, and a vitamin D–dependent CaBP in the same group of cells.

1,25-Dihydroxyvitamin D_3 in Stem Cell Differentiation and Growth

A recent development that may be of great significance with respect to understanding the vitamin D endocrine system was the discovery by Suda and coworkers that $1,25(OH)_2D_3$ suppresses the growth and proliferation of both murine (1) and human (134) myeloid leukemia cells, both of which are tumor cell lines. At the same time, Colston et al (32) reported the presence of $1,25(OH)_2D_3$ receptor in a malignant melanoma cell line and the observation that $1,25(OH)_2D_3$ significantly increased the cell doubling time of these cells. This was followed by the report that $1,25(OH)_2D_3$ suppresses the growth of a murine granulocyte-macrophage progenator cell line (CFU-C) (135), the report that $1,25(OH)_2D_3$ mediates the differentiation of human promyelocytic leukemia cells (HL-60) into mature granulocytes (198), and the report that $1,25(OH)_2D_3$ prolongs the survival time of mice inoculated with myeloid leukemia cells (87). Provvedini et al recently detected $1,25(OH)_2D_3$ receptors in human leukocytes (167). Collectively, these reports support the concept that $1,25(OH)_2D_3$ has a fundamental role in stem cell differentiation and growth.

Biological Actions of 24,25-Dihydroxyvitamin D_3

The evidence for and against a possibly unique, physiologically significant action of $24,25(OH)_2D_3$ was recently reviewed in detail (146). DeLuca and coworkers published a number of studies with 24,24-difluoro-25-hydroxyvitamin D_3 in which 24-hydroxylation would presumably be blocked by the presence of the fluorine atoms (19, 152, 200). The activity of this compound in several classic measurements of vitamin D activity was identical to that of $25(OH)D_3$. Assuming that the fluorine atoms do effectively block hydroxylation and that both fluorine atoms behave chemically more like hydrogen atoms than hydroxyl functions, these authors concluded that hydroxylation at the 24-position is not required for any actions of vitamin D in calcium metabolism. They suggest that the $24,25(OH)_2D_3$ leads to a catabolic pathway and has no intrinsic physiological significance.

Other evidence is more supportive of a biologically significant role for $24,25(OH)_2D_3$. Receptor-like binding proteins for $24,25(OH)_2D_3$ have been

identified in parathyroid gland (131), chondrocytes (38), endochondrial bone (181), and limb bud mesenchymal cells (181). It has been shown that $24,25(OH)_2D_3$ is necessary in combination with $1,25(OH)_2D_3$ for normal hatching of chick embryos (76) and Japanese quail embryos (144) and for regression of hyperplastic parathyroid glands in vitamin D–deficient chicks (77). A number of reports from several laboratories present results suggesting that $24,25(OH)_2D_3$ in combination with $1,25(OH)_2D_3$ is necessary for normal bone metabolism (15, 47, 59, 116, 125, 156, 236). In addition, $24,25(OH)_2D_3$ alone has been reported to be active in stimulating DNA and proteoglycan synthesis in cultured rat and rabbit chondrocytes (37), in stimulating DNA synthesis and ornithine decarboxylase activity in epiphyses and diaphyses of rat bone (182), in supressing bone resorption in uremic humans (118) and rats (157), and in interactions with somatostatin in rat calvaria (180). However, a receptor-like protein for $24,25(OH)_2D_3$ in postnatal osteoblasts or osteoclasts has not been identified (124).

Clearly the possibly physiologically significant action of $24,25(OH)_2D_3$ acting alone or in combination with other vitamin D metabolites remains a complex issue. In addition to the need for further documentation of specific actions of $24,25(OH)_2D_3$, other questions remain. For example, is a further metabolite of $24,25\text{-}(OH)_2D_3$ responsible for those actions attributed to the steroid? Does the apparent requirement for the combined presence of the two metabolites actually represent a sequence of steps requiring each of the metabolites alone? It is hoped that the next several years will see the design of careful experiments to address these questions.

SUMMARY

In the last few years, it has become clear that the vitamin D endocrine system is comprised of many more target cells and tissues than were imagined a decade ago; in addition to the intestine, kidney, and bone, the vitamin D endocrine system now includes the beta cells of the pancreas, breast tissue, placenta, the pituitary gland, cells of the reticuloendothelial system, and several other cells and tissues. The complexity of the metabolic pathway by which the active metabolite(s) of vitamin D are produced and further metabolized has emerged, as has the complexity of its regulation.

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