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# 1α-Hydroxylation of 24-Hydroxyvitamin $D_2$ Represents a Minor Physiological Pathway for the Activation of Vitamin $D_2$ in Mammals

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ABSTRACT: $C_{24}$-Hydroxylation was evaluated as a possible activation pathway for vitamin $D_2$ and vitamin $D_3$. Routine assays showed that 24-hydroxyvitamin $D_2$ and 1,24-dihydroxyvitamin $D_2$ could be detected in rats receiving physiological doses (100 IU/day) of vitamin $D_2$; however, 24-hydroxyvitamin $D_3$ could not be detected in rats receiving similar doses of vitamin $D_3$. In rats, 24-hydroxyvitamin $D_2$ was very similar to 25-hydroxyvitamin $D_2$ at stimulating intestinal calcium transport and bone calcium resorption. The biological activity of 24-hydroxyvitamin $D_2$ was eliminated by nephrectomy, suggesting that 24-hydroxyvitamin $D_2$ must undergo 1α-hydroxylation to be active at physiological doses. In vivo experiments suggested that when given individually to vitamin D deficient rats, 24-hydroxyvitamin $D_2$, 25-hydroxyvitamin $D_2$, and 25-hydroxyvitamin $D_3$ were 1α-hydroxylated with the same efficiency. However, when presented simultaneously, 24-hydroxyvitamin $D_2$ was less efficiently 1α-hydroxylated than either 25-hydroxyvitamin $D_3$ or 25-hydroxyvitamin $D_2$. 1,24-Dihydroxyvitamin $D_2$ was also approximately 2-fold less competitive than either 1,25-dihydroxyvitamin $D_2$ or 1,25-dihydroxyvitamin $D_3$ for binding sites on the bovine thymus 1,25-dihydroxyvitamin D receptor. These results demonstrate that 24-hydroxylation followed by 1α-hydroxylation of vitamin $D_2$ represents a minor activation pathway for vitamin $D_2$ but not vitamin $D_3$.

**V**itamin $D_3$ and vitamin $D_2$ are used for supplementation of animal and human diets in the United States. Vitamin $D_3$ is

the form of vitamin D that is synthesized by mammals, whereas, vitamin $D_2$ is the major naturally occurring form of the vitamin in plants. Vitamin $D_3$ also occurs naturally in plants and may comprise as much as 1% of the total vitamin D in alfalfa (Horst et al., 1984a,b). Whether or not vitamin $D_3$ occurs naturally in other plant species is presently unknown. Nocturnal herbivores therefore would have evolved with vi-

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tamin D$_2$ as their major (if not only) source of vitamin D. Vitamin D$_3$, on the other hand, would have served as the major vitamin D source in most diurnal species.

Shortly after their discovery, it seemed apparent that vitamin D$_2$ and vitamin D$_3$ had similar biological activity in most mammals and that birds and new world monkeys discriminated against vitamin D$_2$ in favor of vitamin D$_3$ (Bethke et al., 1946; Steenbock et al., 1932; Hunt et al., 1967). Recent research, fostered by the discovery of sensitive analytical techniques and availability of high specific activity [³H]vitamin D's, indicates that differences in the metabolism of vitamin D$_2$ and vitamin D$_3$ in mammals are perhaps widespread (Horst et al., 1982a,b). Most notable was the apparent discrimination against vitamin D$_2$ by pigs and cows and apparent preference for vitamin D$_2$ by rats. In these same experiments and in more recent studies (Hoy et al., 1988), it was shown that chicks discriminate against vitamin D$_2$ as a result of enhanced clearance of the vitamin D$_2$ metabolites 25-hydroxyvitamin D$_2$ (25-D$_2$)[1] and 1,25-D$_2$. These results corrected earlier research (Imrie et al., 1967) suggesting that discrimination against vitamin D$_2$ by chicks was a result of enhanced plasma clearance of the parent vitamin D$_2$ molecule.

Much is known about the side-chain metabolism of both vitamin D$_2$ and vitamin D$_3$. For example, to become active, each must undergo 25-hydroxylation in the liver followed by 1α-hydroxylation in the kidney. In addition, the major physiologic pathway of catabolism for the vitamin D$_3$ metabolites, 25-D$_3$ and 1,25-D$_3$, is initiated by C$_{24}$-oxidation (Holick et al., 1973; Kumar et al., 1978; Reinhardt et al., 1982). C$_{24}$-Oxidized metabolites then act as substrates for further oxidation at C$_{23}$ (Napoli & Martin, 1984; Ishizuka et al., 1984; Mayer et al., 1983; Napoli & Horst, 1983; Reddy et al., 1987), leading to cleavage between C$_{23}$ and C$_{24}$ (Esvelt et al., 1979; Jones et al., 1983). Minor pathways of catabolism include C$_{26}$-oxidation and formation of lactone (Reinhardt et al., 1981; Tanaka et al., 1981; Horst et al., 1984a,b). Similarly, the 24-hydroxy derivatives of 25-D$_2$ and 1,25-D$_2$ are known (Jones et al., 1980; Horst et al., 1986; Reddy & Tserng, 1986). The metabolite 1,24,25-D$_2$ can be further hydroxylated at C$_{28}$ or C$_{26}$ (Reddy & Tserng, 1986). The C$_{22}$ alkene and C$_{24}$-(S)-methyl group in vitamin D$_2$, however, may preclude the classical side-chain oxidation reactions known to occur in the vitamin D$_3$ metabolite series such as C$_{24}$-ketonization or C$_{23}$-oxidation.

The differences in side-chain chemistry between vitamin D$_2$ and vitamin D$_3$ offer opportunities for divergence in side-chain oxidation of the two sterols; for example, the 24-position in vitamin D$_2$ is a tertiary carbon, as is the 25-position for both vitamin D$_2$ and vitamin D$_3$. In addition, the 24-position in vitamin D$_2$ is an allylic carbon, making it a far more reactive site than the corresponding position on vitamin D$_3$. On the basis of these chemical differences, 24-hydroxylation of vitamin D$_2$ may be a quantitatively significant pathway for the further metabolism of vitamin D$_2$. In support of this argument are the data of Jones et al. (1980), who demonstrated that rats receiving pharmacological doses of vitamin D$_2$ 24-hydroxylate vitamin D$_2$ to 24-D$_2$. A quantitative physiological evaluation

of the 24-hydroxylation pathway, however, was not done by this group.

The present report evaluates 24-hydroxylation of vitamin D$_2$ as a possible first step in the activation of vitamin D$_2$. We present evidence to suggest that sequential formation of 24-D$_2$ and 1,24-D$_2$ represents an activation pathway for vitamin D$_2$ in mammals.

## MATERIALS AND METHODS

*General.* High-pressure liquid chromatography and mass spectrometry were conducted as previously described (Napoli et al., 1986; Koszewski et al., 1988).

*Compounds.* Vitamin D$_2$ was purchased from Sigma Chemical Co. (St. Louis, MO). Synthetic 25(R),26-D$_2$ and 25(S),26-D$_2$ were gifts from Dr. Dudley Williams (University Chemical Laboratory, Cambridge, England). Vitamin D$_2$ metabolites used for standards were isolated from plasma of a Jersey cow given excess vitamin D$_2$ (weekly intramuscular injections of $4 \times 10^7$ units). After 4 weeks of treatment, the cow was euthanized with phenobarbital. Blood (approximately 20 L) was collected in heparinized containers. Plasma was prepared by centrifugation and stored at −20 °C. Vitamin D metabolites were extracted, purified, and identified as described (Napoli et al., 1986; Koszewski et al., 1988).

*Assays.* Assays for the vitamin D$_2$ and vitamin D$_3$ metabolites were conducted as previously described (Horst et al., 1982a,b). The extraction and chromatographic conditions used to assay 25-D$_2$, 24,25-D$_2$, and 1,25-D$_2$ were also suitable for isolation and analysis of 24-D$_2$, 24,26-D$_2$, and 1,24-D$_2$, respectively. Competitive protein binding assays and the ability of 24-D$_2$ to stimulate intestinal calcium transport (ICT) and bone calcium resorption (BCR) were determined as previously described (Horst et al., 1986). Nephrectomy was done under isoflurane anesthesia just prior to introduction of experimental metabolites. In vitro 1α-hydroxylase assays were performed as previously described (Engstrom et al., 1984).

*Metabolism and Biological Evaluation Studies.* Standard 1,24-D$_2$ was prepared by incubating 10 μg of 24-D$_2$ in each of 10 flasks containing 5 mL of 20% kidney homogenates made from vitamin D deficient chicks (Engstrom et al., 1984). The 1,24-D$_2$ was isolated from lipid extracts of the homogenates by HPLC (Horst et al., 1982). The putative 1,24-D$_2$ was subjected to mass spectrometry for positive identification.

Experiments to study in vivo metabolism of various mono-hydroxylated vitamin D$_2$ substrates were performed in male weanling rats (Holtzman, Madison, WI). After 4 weeks on a vitamin D deficient diet (Teklad, diet no. TD81308), one group (six rats/group) of rats received daily oral supplementation (100 IU/day for the next 3 weeks) of vitamin D$_2$ (group I). Four additional groups (groups II–V) were maintained on a vitamin D deficient and calcium-deficient diet for 4 weeks. During the last 4 days of experimental diet, animals received 5 μg of either 24-D$_2$ (group II), 25-D$_2$ (group III), 25-D$_3$ (group IV), or a combination of 5 μg each of 24-D$_2$, 25-D$_2$, and 25-D$_3$ (group V). All treatments were given intraperitoneally (ip) once daily for 4 days in 100 μL of propylene glycol carrier.

Another set of experiments was designed to determine the influence of vitamin D$_2$ and vitamin D$_3$ supplementation on plasma concentrations of 25-D$_2$, 24-D$_2$, and 24-D$_3$. After 4 weeks on a vitamin D deficient diet, animals received 800 IU/day of either vitamin D$_2$ or vitamin D$_3$. After 3 weeks of treatment, blood was collected and plasma harvested for metabolite evaluation.

Table I: Bone Calcium Resorption (BCR) and Intestinal Calcium Transport (ICT) in Intact and Nephrectomized Rats Receiving Vitamin $D_2$ Metabolites

| dose | serum Ca (mg/dL) (BCR) | $^{45}Ca$(serosal)/ $^{45}Ca$(mucosal) (ICT) |
|---|---|---|
| Intact | | |
| control | $4.0 \pm 0.3$ | $2.8 \pm 0.3$ |
| 24-hydroxyvitamin $D_2$ | | |
| 25 ng | $4.5 \pm 0.3^a$ | $4.3 \pm 1.6^a$ |
| 50 ng | $4.7 \pm 0.2^a$ | $4.4 \pm 0.5^a$ |
| 100 ng | $4.6 \pm 0.4^a$ | $5.8 \pm 1.8^a$ |
| 25-hydroxyvitamin $D_2$ | | |
| 25 ng | $4.5 \pm 0.2^a$ | $4.4 \pm 4.5^a$ |
| Nephrectomized | | |
| control | $2.8 \pm 0.1$ | $2.1 \pm 0.3$ |
| 24-hydroxyvitamin $D_2$ | | |
| 50 ng | $2.7 \pm 0.1$ | $1.9 \pm 0.3$ |
| 1,25-dihydroxyvitamin $D_2$ | | |
| 12.5 ng | $3.3 \pm 0.1^a$ | $3.0 \pm 0.5^a$ |

$^a$Significantly different from controls ($P < 0.05$).

## RESULTS

*Biological Evaluation.* Plasma from a cow receiving excess vitamin $D_2$ contained a metabolite that was similar in concentration to, but slightly less polar than, 25-$D_2$ when analyzed by straight-phase HPLC chromatography (data not shown). This metabolite was identified as 24-$D_2$ by mass spectroscopy, NMR, and UV analysis (Napoli et al., 1986; Koszewski et al., 1987). The observation that this cow had similar plasma concentrations of 24-$D_2$ and 25-$D_2$ prompted us to quantitatively evaluate the biological significance of this pathway in animals receiving physiological doses of vitamin $D_2$.

The data in Table I summarize experiments done to determine the biological activity of 24-$D_2$ in rats. The data show that 24-$D_2$ and 25-$D_2$ are very similar in their ability to stimulate ICT and BCR. Nephrectomy abolished the activity of 24-$D_2$, which suggested that 24-$D_2$ must undergo 1$\alpha$-hydroxylation in the kidney before becoming biologically active (Table I).

*Isolation and Identification of 1,24-$D_2$.* In order to study the in vivo 1$\alpha$-hydroxylation of 24-$D_2$, standard 1,24-$D_2$ had to be synthesized. This was accomplished by incubating 24-$D_2$ with chick kidney homogenates made from vitamin D deficient chicks. Lipid extracts of the homogenates were subjected to HPLC and found to yield a peak slightly less polar than 1,25-$D_2$ on a straight-phase Zorbax (0.9 × 24 cm) HPLC column developed in 76/25/4 hexane/methylene chloride/2-propanol. In this system, 1,25-$D_2$ peaked at 35 mL, and the putative 1,24-$D_2$ peaked at 30 mL (data not shown). The 1,24-$D_2$ peak was collected and, in final preparation for mass spectral analysis, was further purified by using a Zorbax Sil column (0.45 × 25 cm) developed in 97/5/2 hexane/2-propanol/methanol (Figure 1). The final yield of product (~500 ng) was estimated by UV analysis. The mass spectrum (Figure 2) of the putative 1,24-$D_2$ showed a parent ion at $m/z$ 428 consistent with the incorporation of a single hydroxyl group into the 24-$D_2$ structure. Successive losses of water from the parent ion were readily apparent in the mass spectrum as peaks at $m/z$ 349 and 331. These data are in good agreement with the cleavage of the $C_{24}/C_{25}$ bond observed in the spectrum of 24-$D_2$ (Jones et al., 1980; Engstrom & Koszewski, 1988). The loss of 141 amu further confirmed the presence of a single hydroxyl group in the side chain. The appearance of peaks at $m/z$ 152 and 134, together with the peaks at $m/z$ 269 and 251, indicated that the additional hydroxyl group was present in the A ring. This pattern is characteristic of 1$\alpha$-hydroxylated
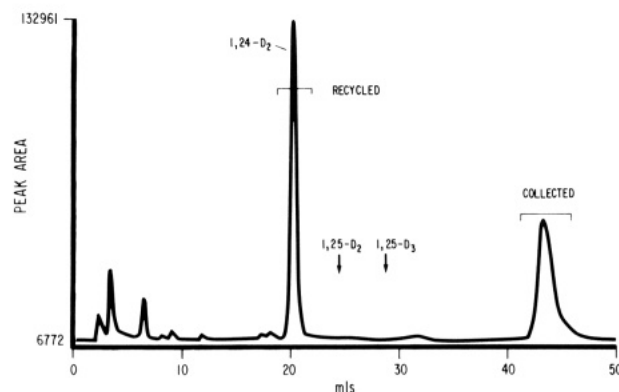


FIGURE 1: HPLC chromatogram of the semipurified 1,24-$D_2$ isolated from chick kidney homogenates outlined under Materials and Methods. The 1,24-$D_2$ was dried under $N_2$ and applied in toto to an HPLC column (Zorbax Sil 0.45 × 25 cm) developed in 97/5/2 hexane/2-propanol/methanol. The 1,24-$D_2$ was recycled one time for a total of two passes across the HPLC column. The elution volumes of 1,25-$D_2$ and 1,25-$D_3$ are also indicated.
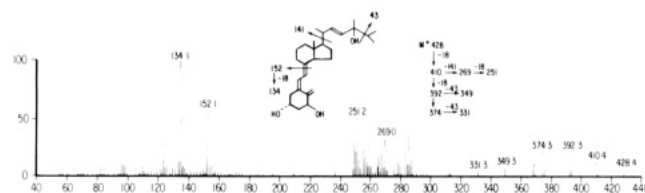


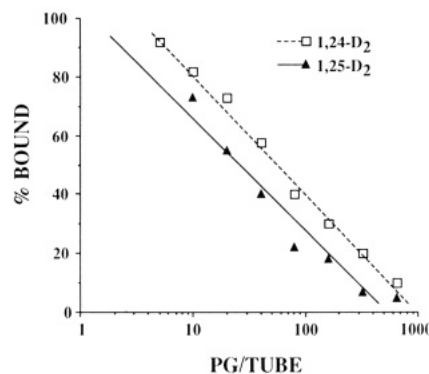FIGURE 2: Low-resolution electron impact mass spectra of 1,24-$D_2$.



FIGURE 3: Relative binding of 1,24-$D_2$ and 1,25-$D_2$ to the 1,25-D receptor from bovine thymus. Radioactive 1,25-$D_3$ was used as tracer.

vitamin D metabolites possessing an intact triene system (Napoli et al., 1986).

*Competitive Protein Binding Assays.* In vitro radioligand binding of the 1,24-$D_2$ suggested that this sterol was approximately 2-fold less competitive than 1,25-$D_2$ for [$^3$H]-1,25-$D_3$ binding sites on receptor from calf thymus (Figure 3).

*Metabolism Studies.* The plasma concentrations of the various mono- and dihydroxylated metabolites are displayed in Table II. The data show for the first time that 1,24-$D_2$ is a normal product of vitamin $D_2$ (group I) or 24-$D_2$ (groups II and V) metabolism in vivo. Also of interest in Table II are the data which show that when presented individually to vitamin D deficient animals, 24-$D_2$, 25-$D_2$, and 25-$D_3$ were 1$\alpha$-hydroxylated with the same efficiency (groups II, III, and IV). Under these conditions, plasma 1,24-$D_2$, 1,25-$D_2$, and 1,25-$D_3$ circulated at 2397 ± 412, 2130 ± 130, and 1895 ± 326 pg/mL, respectively. Coadministration of 24-$D_2$, 25-$D_2$, and 25-$D_3$ (group V) resulted in preferential 1$\alpha$-hydroxylation of 25-$D_2$ and 25-$D_3$ relative to 24-$D_2$. Under the conditions described for group V, the plasma 1,24-$D_2$, 1,25-$D_2$, and 1,25-$D_3$ were 193 ± 20, 1140 ± 75, and 772 ± 60 pg/mL,

Table II: Plasma Concentrations of Vitamin D Metabolites in Treated Rats and Cows[a]

| sterol | group/diet[b] | plasma concn (ng/mL) | | | | | | plasma concn (pg/mL) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24-D₂ | 25-D₂ | 25-D₃ | 24,25-D₂ | 24,26-D₂ | 24,25-D₃ | 1,24-D₂ | 1,25-D₂ | 1,25-D₃ |
| D₂[c] | I/A | 3.0 ±1.5 | 10.9 ±2.2 | ND[d] | 4.1 ±2.3 | 1.7 ±1.0 | ND | 8.8 ±4.1 | 151 ±44 | ND |
| 24-D₂[d] | II/B | 177 ±25 | ND | ND | 9.1 ±1 | 16.3 4.0 | ND | 2397 ±412 | ND | ND |
| 25-D₂[e] | III/B | ND | 214 ±48 | ND | 22.1 ±1.6 | ND | ND | ND | 2130 ±130 | ND |
| 25-D₃[e] | IV/B | ND | ND | 115 ±23 | ND | ND | 14.9 ±1.8 | ND | ND | 1895 ±325 |
| 24-D₂, +25-D₂ +25-D₃[f] | V/B | 48 ±12 | 97 ±22 | 84 ±12 | 19.9 ±4.7 | 14.9 ±2.1 | 12.0 ±1.4 | 193 ±20 | 1104 ±75 | 772 ±60 |
| D₂ excess (cow) | | 173 ±69 | 141 ±33 | 13.7 ±2.5 | 106 ±36 | 14.7 ±88 | 3.35 ±1.1 | ND | 41 ±7 | 4.7 ±2.4 |

[a] Means ± SEM. $N = 4$–6. [b] All diets were vitamin D deficient. A = normal calcium (0.5%); B = low calcium (0.005%). [c] Oral supplement 100 IU/day for 3 weeks. [d] Not detectable. [e] Intraperitoneal injection, 5 μg/day for 4 days. [f] 5 μg of each given simultaneously, intraperitoneally for 4 days.

respectively. Both the plasma 1,24-D₂ and 1,25-D₃ were significantly lower ($P < 0.01$) than the 1,25-D₂. Similarly, the plasma 1,24-D₂ was significantly lower ($P < 0.01$) than the 1,25-D₃. Similar results were observed in in vitro assays using rat kidney homogenates. The results from these assays showed that when these substrates were presented simultaneously at saturating concentrations (5 μM), the renal 1α-hydroxylase enzyme produced similar amounts of 1,25-D₂ and 1,25-D₃ [1.9 and 1.95 pmol/(min·mg of tissue), respectively]. Production of 1,24-D₂, on the other hand, was <0.2 pmol/(min·mg of tissue). Due to the lack of adequate amounts of 24-D₂ substrate, the kidney hydroxylases could not be studied with each metabolite individually.

The plasma concentrations of 24,25-D₂, 24,25-D₃, and 24,26-D₂ are also presented in Table II. The data show that 24,26-D₂ (a pathway unique to vitamin D₂) is produced by rats receiving physiological doses of vitamin D₂. The data also indicate that 25-D₂ was preferentially metabolized to 24,25-D₂, whereas, 24-D₂ was preferentially metabolized to 24,26-D₂.

To determine if the 24-hydroxylation pathway may exist for vitamin D₃, studies were conducted in rats receiving 800 IU of either vitamin D₂ or vitamin D₃. The results (not shown) demonstrate that, in vitamin D₂ dosed rats, 25-D₂ and 24-D₂ circulated at 14.1 ± 3.2 and 15.9 ng/mL, respectively. Vitamin D₃ dosed rats, on the other hand, had no detectable 24-D₃, and 25-D₃ circulated at 26.3 ng/mL.

## DISCUSSION

This report identifies a new activation pathway for vitamin D₂ in mammals. The process involves initial C₂₄-hydroxylation of vitamin D₂ followed by 1α-hydroxylation. The presence of circulating 1,24-D₂ in vitamin D₂ and 24-D₂ dosed rats, the high affinity of 1,24-D₂ for receptor binding sites, and the similarities in biological activity of 25-D₂ and 24-D₂ support the argument that the pathway leads to vitamin D₂ activation. At the doses tested (800 IU/day for 3 weeks), the equivalent pathway could not be demonstrated for vitamin D₃. In that regard, Wichmann et al. (1981) have isolated small quantities of 24-D₃ from chick plasma. However, in their experiments, the chicks were dosed with pharmacological amounts (10 million IU) of vitamin D₃. Whether 24-D₃ was produced by chicks receiving physiological doses of vitamin D₃ was not addressed by these researchers. Although not yet demonstrated in biological systems, the compound 1,24-D₃ has been chemically synthesized and found to stimulate BCR and ICT (Smith et al., 1982). Additionally, 1,24-D₃ has been evaluated and found to be effective in the treatment of osteoporosis (Shiraki et al., 1985). Therefore, like 1,24-D₃, the metabolite
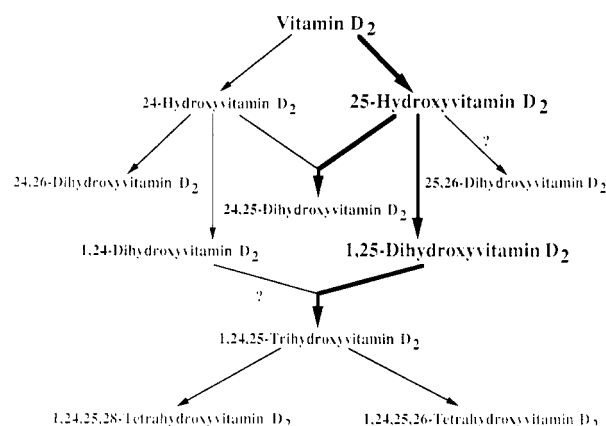


FIGURE 4: Known pathways of vitamin D₂ metabolism in mammals. The (?) denotes anticipated pathways not yet confirmed, and the bold arrows indicate the major physiological pathway for vitamin D₂ metabolism.

1,24-D₂ may have potential as a therapeutic agent in the prevention or treatment of some calcium-related diseases.

The presence of the C₂₄-activation pathway for vitamin D₂ therefore represents a deviation in the metabolic pathways for vitamin D₂ and vitamin D₃. The presence of an alternate activation pathway may aid in explaining the differences in efficacy between vitamin D₂ and vitamin D₃ for treatment of some bone diseases. For example, vitamin D₂ is superior to vitamin D₃ or 25-D₃ at curing bone lesions occurring in patients taking phenobarbitone/phenytoin (Christiansen et al., 1975; Tjellesen et al., 1985). These anticonvulsant treatments may target 25-D₃ or 1,25-D₃ to deactivation by stimulating target tissue catabolic enzymes; whereas, vitamin D₂ metabolites (24-D₂, 25-D₂, and 1,25-D₂) may be spared this process due to their different molecular configuration.

The major known pathways of vitamin D₂ metabolism in animals receiving normal dietary vitamin D₂ are depicted in Figure 4. As described, vitamin D₂ is preferentially hydroxylated at C₂₅ with C₂₄-oxidation present as a minor pathway. At present, we are unable to pinpoint the major in vivo source of the vitamin D₂ C₂₄-hydroxylase; however, in vitro evidence from our laboratory suggests that 24-D₂ production in liver homogenates far exceeds production of 25-D₂ (Engstrom & Koszewski, 1988). Nevertheless, a major route of 25-D₂ metabolism subsequently results in the formation of 24,25-D₂. On the other hand, only a small amount of 24-D₂ is metabolized to 24,25-D₂, with hydroxylation at C₂₆ to yield 24,26-D₂ being a major pathway (Koszewski et al., 1988). Activation of either 24-D₂ or 25-D₂ then results from C₁-hydroxylation with the major physiological pathway for ac-

tivation proceeding through $C_1$-hydroxylation of 25-$D_2$ to form 1,25-$D_2$. Relative to the vitamin $D_2$ activation process is our observation that 24-$D_2$ is less efficiently $1\alpha$-hydroxylated in the presence of 25-$D_2$ and 25-$D_3$ (Table II, group V). This phenomenon suggests that, in the normal vitamin D replete state, 24-$D_2$ is relatively ineffective at competing for the $1\alpha$-hydroxylase enzyme; however, in the absence of competitors, 24-$D_2$ is $1\alpha$-hydroxylated in vivo at an apparent rate equal to that of 25-$D_2$ and 25-$D_3$ (Table II, group II). Also proposed in Figure 4 is the converging of the two activation pathways to the formation of 1,24,25-$D_2$, a metabolite with little or no inherent BCR and ICT activity (Horst et al., 1986). Further metabolism of the 1,24,25-$D_2$ metabolite to tetrahydroxy metabolites has been demonstrated and reported by Reddy et al. (1986).

The $C_{24}$-activation pathway for vitamin $D_2$ therefore represents the most notable deviation to date from the well-understood pathways for vitamin $D_3$ metabolism. Although this information was collected largely from rat experiments, recent findings of 24-$D_2$ in humans undergoing vitamin $D_2$ therapy (Hollis et al., 1986) argues this pathway may also be active in other mammalian species. This information could be of potential significance in providing a more complete understanding of vitamin D activation in mammals, as well as providing possible explanations for described differences in biological activity between vitamin $D_2$ and vitamin $D_3$.

## ACKNOWLEDGMENTS

**Registry No.** $D_2$, 50-14-6; $D_3$, 67-97-0; 24-$D_2$, 58050-56-9; 25-$D_2$, 21343-40-8; 25-$D_3$, 19356-17-3; 1,24-$D_2$, 124043-51-2; 1,25-$D_2$, 60133-18-8; 1,25-$D_3$, 32222-06-3; 24,26-$D_2$, 109028-06-0; 24,25-$D_2$, 58050-55-8; 24,25-$D_3$, 40013-87-4; Ca, 7440-70-2.

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