Intestinal Synthesis of 24-Keto-1,25-dihydroxyvitamin D₃

A METABOLITE FORMED IN VIVO WITH HIGH AFFINITY FOR THE VITAMIN D CYTOSOLIC RECEPTOR*

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24-Keto-1,25-dihydroxyvitamin D₃ has been identified as an intestinal metabolite of 1,25-dihydroxyvitamin D₃ by ultraviolet absorbance, mass spectroscopy, and biochemical reactivity. The metabolite was produced from 25-OHD₃ by intestinal homogenates. 24-Keto-1,25-dihydroxyvitamin D₃ is present in vivo in the plasma and small intestinal mucosa of rats fed a stock diet, receiving no exogenous 25-dihydroxyvitamin D₃, and in the plasma and small intestinal mucosa of rats dosed chronically with 25-OHD₃. In contrast, in cytotoxic preparations largely free of the 5 S vitamin D-binding protein, both metabolites are about 7-fold less potent than 1,25-dihydroxyvitamin D₃. In contrast, 24-keto-25-OHD₃ was prepared in uiuo from 25S,26-(OH)₂D₃ (33, 34), a metabolite generated in detectable amounts from 1,25-dihydroxyvitamin D₃ in the intestine and thymus. In cytotoxic preparations contaminated with the 5 S vitamin D-binding protein (28, 29), 24-keto-25-OHD₃ had 2-fold lower affinity than 25-OHD₃ to the plasma vitamin D-binding protein (28, 29). It may indirectly exacerbate vitamin D toxicity by displacing biochemically active metabolites, causing their concentrations in tissues to increase. 25-OHD₃ is a primary mediator of calcium and phosphorus metabolism (8-12). Indeed, 1,25-(OH)₂D₃ is apparently the major vitamin D₃ metabolite in at least one vitamin D target tissue, intestine (13, 14). However, compounds more polar than 1,25-(OH)₂D₃, such as 24R,25-(OH)₂D₃ (15, 16) and 1,25S,26-(OH)₃D₃ (17, 18), have been identified as circulating vitamin D₃ metabolites. The functions of these metabolites are unclear, and further investigations of 1,25-(OH)₂D₃ metabolism are necessary to determine their role, relative to 1,25-(OH)₂D₃ in maintaining calcium homeostasis.

Under physiological conditions, 5,6-trans-25-OHD₃ (19) and 24R,25-(OH)₂D₃ (1, 20, 21) are the 25-OHD₃ metabolites of highest concentration in plasma. 5,6-trans-25-OHD₃ probably mimics the actions of 1,25-(OH)₂D₃ because of its pseudo-1α-hydroxyl group. 24R,25-OHD₃, or perhaps its metabolite 24-keto-25-OHD₃ (22-24) may promote bone mineralization (25). An alternative pathway of 25-OHD₃ metabolism, especially during hypervitaminosis D, provides 25-OHD₃-26,23-lactone (26, 27). (23S,25R)-25-OHD₃-26,23-lactone binds with 5-fold higher affinity than 25-OHD₃ to the plasma vitamin D-binding protein (28, 29). Thus, it may indirectly exacerbate vitamin D toxicity by displacing biochemically active metabolites, causing their concentrations in tissues to increase. The pathway proceeds through 25-OHD₃-26,23-lactone (26, 27). (23S,25R)-25-OHD₃-26,23-lactone cannot be produced in vivo from 25S,26-(OH)₂D₃ (33, 34), a kidney microsomal metabolite of 25-OHD₃ (35).

During the course of investigating the pathways of 25-OHD₃-26,23-lactone biosynthesis, a unique metabolite was produced from 23S,25-(OH)₂D₃, namely 23-keto-25-OHD₃ (36, 37). 23-Keto-25-OHD₃ has 4-fold higher affinity than 25-OHD₃ for the bovine thymus cytosolic 1,25-(OH)₂D₃ receptor. 23-Ketonization is, therefore, the first modification, besides 1α-hydroxylation, that appears to enhance affinity for the 1,25-(OH)₂D₃-specific receptor. To answer the obvious question of whether a 1α-hydroxylated 23-keto-25-OHD₃ would have higher affinity than 1,25-(OH)₂D₃ for the cytosolic receptor, 23-keto-1,25-(OH)₂D₃ was prepared in vitro from 23-keto-25-OHD₃. 23-Keto-1,25-(OH)₂D₃ had 2-fold lower affinity than 1,25-(OH)₂D₃ for the bovine thymus cytosolic receptor, but appeared to be present in blood and intestine of 25-(OH)₂D₃-dosed animals, upon preliminary examination (36, 37). Because of their ability to bind with tissue 1,25-(OH)₂D₃ receptors, the in vivo presence of 23-keto derivatives of 25-OHD₃ and 1,25-(OH)₂D₃ might be significant to vitamin D toxicity.
These studies were undertaken to further examine 1,25-(OH)2D3 metabolism and to rigorously determine whether 23-keto-1,25-(OH)2D3 is a detectable metabolite of 1,25-(OH)2D3 in vivo and in vitro. This paper also reports the identification of a new 1,25-(OH)2D3 metabolite as 24-keto-1,25-(OH)2D3. 24-Keto-1,25-(OH)2D3 is produced by intestine in vitro and is present in vivo in the plasma and intestinal mucosa of rats. In contrast, 23-keto-1,25-(OH)2D3, for the cytosolic 1,25-(OH)2D3 receptor, 23-keto-1,25-(OH)2D3, in contrast, does not appear to be a quantitatively significant intestinal metabolite of 1,25-(OH)2D3 in vivo or in vitro.

**Materials and Methods**

**General—** Ultraviolet absorbance spectra were taken in 2-propanol with a Beckman Model 25 recording spectrophotometer. A molar absorptivity (α) of 18,200 liters mol−1 cm−1 was used for all vitamin D3 compounds. HPLC was performed with Waters Associates ALC/GPC 204 liquid chromatographic equipment. Calcium ions were detected at 254 nm. The normal phase HPLC columns used were DuPont Zorbax-Sil. All solvents were distilled in glass and were filtered through a 0.45-μm filter. Silica gel Sep-Paks were purchased from Waters Associates, Inc. (Milford, MA). Radioactivity was measured in a Hydroncount (J. T. Baker Chemical Co.) with a Beckman LS-300 3 channel liquid scintillation counter. Mass spectra were obtained at 70 eV from the solids probe of a Finnigan Model 4021 El/Cl/GC/MS coupled with an INCOS 2000 Data System. To obtain spectra, the probe was heated from ambient to 320 °C at an ionizer temperature of 250 °C. CI-NCI mass spectra were obtained with dichlorodifluoromethane as reagent gas. Compounds—Synthetic 1,25-(OH)2D3, 1,23S,25-(OH)2D3, 1,24R,25-(OH)2D3, 1,25S,26-(OH)2D3, and 1,25-(OH)2D3-26,23-1actone were gifts from Dr. Milan R. Uskokovic and Dr. John J. Partridge, Hoffmann-LaRoche, Nutley, NJ. The compounds were synthesized in their laboratory by the methods described (38-40). 23-Keto-1,25-(OH)2D3 was prepared from 23-keto-25-OHD3 with kidney homogenate of vitamin D-treated chickens in a standard procedure described did not produce the more polar peaks detected. Material of 1,25-(OH)2D3-26,27-3H in Intestinal Homogenates—The procedure described above for the metabolism of unlabeled 1,25-(OH)2D3 was used except for substrate, 50 μCi of 1,25-(OH)2D3 (90 Ci/mmol) in ethanol (0.1 ml) 12 h after the last dose of unlabeled 1,25-(OH)2D3. Alternatively, two male rats not receiving supplemental 1,25-(OH)2D3 were similarly dosed with 1,25-(OH)2D3 (90 Ci/mmol). HPLC was performed with a Whatman Partisil PXS 10/25 ODS-3 column (0.46 cm x 25 cm) equilibrated with 0.1% dichloromethane in methanol. The silylated metabolite eluted at 22 ml, compared to trisilyl-1,25-(OH)2D3, which eluted at 31 ml. The subject was then introduced as substrate into each of four Erlenmeyer flasks.

**RESULTS**

Unlabeled 1,25-(OH)2D3 was incubated with intestinal homogenates prepared from 1,25-(OH)2D3-treated rats. The organic extract was partially purified by Sephadex LH-20 chromatography, and a metabolite that eluted in the 1,25-(OH)2D3 area was isolated through three different HPLC steps. The last HPLC system yielded a single major component (Fig. 1A). The new metabolite migrated near 1,25-(OH)2D3 on
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**A**

In this case, however, the base peak was m/z 59, which is produced by C-24/C-25 bond cleavage. Although this peak is common in the mass spectra of 25-hydroxylated vitamin D compounds, it is usually not the base peak. The intensity of m/z 59 in this case suggests labilization of the C-24/C-25 bond. The molecular weight of 430, the side chain functionalization, and the labilization of the C-24/C-25 bond are consistent with a ketone at C-24, namely 24-keto-1,25-(OH)₂D₃.

An electron impact mass spectrum of the silylated metabolite had a molecular ion at m/z 646, and therefore indicated that only three hydroxyl groups were present (Fig. 2C). The peaks at m/z 556 and 466 represent loss of one and two (CH₃)₂SiOH groups from m/z 646, respectively. Peaks at m/z 631, 541, and 451 indicate loss of methyl groups from m/z 646, 556, and 466, respectively. The base peak at m/z 206 is the trimethylsilyl counterpart to m/z 134. The peak at m/z 73 represents (CH₃)₂Si⁺. Finally, the peaks at m/z 131 and 515 are the base peak in 1α-hydroxylated vitamin D compounds (42).

**B**

the ternary system depicted, but was distinct from other 1,25-(OH)₂D₃ derivatives. Parenthetically, this ternary system of hexane, dichloromethane, and 2-propanol resolves 1,25-(OH)₂D₃, 1,25-(OH)₂D₃-26,23-lactone, and 1,24R,25-(OH)₃D₃, a result not readily attainable with binary systems composed of either hexane or dichloromethane. The metabolite had a λₘₚₓ at 265 nm, which is consistent with a vitamin D-like cis-5,7,10(19)-triene chromophore. Based on its UV absorbance, approximately 2 μg were isolated.

Cl⁻-NCI² mass spectroscopy of the metabolite confirmed its molecular weight as 430. An electron impact mass spectrum also indicated a molecular weight of 430 (Fig. 2A). Peaks at m/z 412 and 394 indicated loss of one and two molecules of water, respectively, from the molecular ion. The peak at m/z 371 was produced by loss of 59 atomic mass units from the molecular ion. The peak at m/z 269 resulted from loss of the side chain and one water molecule. Loss of water from m/z 269 produced m/z 251. The latter two peaks indicated that the functionalization of 1,25-(OH)₂D₃ had occurred on the side chain. Peaks at m/z 152 and 134 are typical of the 5,7,10(19)-triene system of 1α-hydroxylated vitamin D compounds. They result from C-7/C-8 cleavage to give m/z 152.

Loss of water from m/z 152 yields m/z 134, which is usually

Fig. 1. Final purification of 24-keto-1,25-(OH)₂D₃. A, metabolite produced from 1,25-(OH)₂D₃; B, metabolite produced from 1,24R,25-(OH)₃D₃. A normal phase HPLC column (0.46 × 25 cm) was eluted with hexane/dichloromethane/2-propanol (8:1:1.2). The elution positions of standards are indicated: 1, 1,25-(OH)₂D₃; 4, 1,235,25-(OH)₂D₃ and 1,25-(OH)₂D₃-26,23-lactone. 1,24R,25-(OH)₃D₃ elutes in 33 ml in this system.

**C**

CI⁻-NCI² mass spectroscopy provides chemical ionisation spectra in which chloride ion addition to the molecular ion of the sample has occurred. The addition of chloride ion is readily determined by double peaks 2 mass units apart due to the two isotopes of chloride, ³⁵Cl and ³⁷Cl. Fragmentation is minimal (J. L. Napoli and B. C. Pramanik, unpublished observations).

Fig. 2. Electron impact mass spectra of 24-keto-1,25-(OH)₂D₃ produced from 1,25-(OH)₂D₃ (A), and 1,24R,25-(OH)₃D₃ (B) and electron impact mass spectrum of trisilyl-24-keto-1,25-(OH)₂D₃ (C).
The elution positions of synthetic standards are indicated: 1, 1,25-(OH)D3; 2, 24-keto-1,25-(OH)2D3; 3, 1,25-(OH)2D3; 4, 1,23S,25-(OH)3D3; 5, 24-keto-1,25-(OH)2D3; 6, 1,24R,25-(OH)3D3; 7, 1,23S,26-(OH)3D3.

Further support for the structure as 24-keto-1,25-(OH)2D3 was provided by production of the metabolite from 1,24R,25-(OH)3D3. Incubation of 1,24R,25-(OH)3D3 with an intestinal mucosal homogenate from rats treated with 1,25-(OH)2D3 produced about 200 ng of a compound (estimated by HPLC peak height; Fig. 1B) that eluted in three HPLC systems like the metabolite produced from 1,25-(OH)2D3. It also had an essentially identical mass spectrum (Fig. 2B).

To distinguish 24-keto-1,25-(OH)2D3 and another recently characterized vitamin D3 metabolite, 23-keto-1,25-(OH)2D3 (36), their mass spectra were compared (Table I). The 23-keto compound had a molecular ion of very low intensity, most likely the result of a McLafferty rearrangement between the C-23-ketone and the C-25-hydroxyl group, which are able to align in a pseudo-six-member ring. The resultant proton transfer and loss of (CH3)2CO (m/z 58) produced a peak at m/z 372, 354 (loss of H2O from 372), and 336 (loss of H2O from 354). These were absent or of relatively low intensity in the mass spectrum of 24-keto-1,25-(OH)2D3. Instead, the spectrum of the latter compound had a more intense molecular ion and peaks resulting from dehydration of the molecular ion. It also had a relatively intense peak (compared to the molecular ion) at m/z 371. Thus, these two vitamin D3 side chains are distinguishable by mass spectroscopy.

The relationship of the newly identified metabolite to the result from C-24/C-25 bond schism. In other words, m/z 131 is the silylated counterpart of m/z 59.

The structural assignment as 24-keto-1,25-(OH)2D3 was supported by chemical reduction of the metabolite. Upon treatment with sodium borohydride, the metabolite was converted into a more polar compound which migrated with authentic 1,24,25-(OH)3D3 in an HPLC system that separates 1,25-(OH)2D3 from 1,23S,25-(OH)3D3, 1,24R,25-(OH)3D3, and 1,25S,26-(OH)3D3 by wide margins (Fig. 3).

Data obtained from Ref. 36.

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**Table I**

Mass spectral characteristics of 24-keto-1,25-(OH)2D3 and 23-keto-1,25-(OH)2D3

| m/z   | Metabolite               | Relative intensity (assignment) |
|-------|--------------------------|---------------------------------|
| 430   | 2 (M+)                   | 0.2 (M+)                        |
| 412   | 7 (M+ - 18)              |                                 |
| 394   | 14 (M+ - 2 x 18)         |                                 |
| 372   | 0.3 (M+ - 58)            | 2 (M+ - 58)                     |
| 371   | 2 (M+ - 58)              |                                 |
| 354   |                          | 10 (M+ - 58 - 18)               |
| 336   |                          | 15 (M+ - 58 - 2 x 18)           |
| 134   | 100                      | 100                             |

*a* Data obtained from Ref. 36.

*b* --, relative intensity less than 0.1%.

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**Fig. 3.** HPLC migration of the 24-keto-1,25-(OH)2D3 sodium borohydride reduction product. A normal phase HPLC column (0.46 x 25 cm) was eluted with hexane/dichloromethane/2-propanol (8:1.6). The elution positions of synthetic standards are indicated: 1, 1,25-(OH)D3; 2, 24-keto-1,25-(OH)2D3; 3, 1,25-(OH)2D3; 4, 1,23S,25-(OH)3D3; 6, 1,24R,25-(OH)3D3; 7, 1,23S,26-(OH)3D3.

**Fig. 4.** HPLC analysis of 1,25-(OH)2[3H]D3 metabolism (HPLC system 1). Metabolites were extracted from intestinal homogenates incubated with 1,25-(OH)2[3H]D3 and plasma (B) and intestinal mucosa (C) of 1,25-(OH)2[3H]D3-dosed rats. Animals in these experiments were treated with unlabeled 1,25-(OH)2D3 as described under "Materials and Methods." A normal phase HPLC column (0.46 x 25 cm) was eluted with hexane/2-propanol (9:1). The elution positions of standards are indicated: 1, 1,25-(OH)2D3; 2, 24-keto-1,25-(OH)2D3; 3, 1,25-(OH)2D3; 4, 1,23S,25-(OH)3D3; 5, 1,25-(OH)2D3-26,23-lactone; 6, 1,24R,25-(OH)2D3. Aliquots were counted for radioactivity, and the remaining material was analyzed in HPLC system 2. The data is plotted as counts/min/fraction. The broken lines represent amplification of part of the graphs. The scale (y axis) of the solid lines is placed to the left of each section. The scale of the broken line is placed to the right of each section. The two y axis legends between A and B, and B and C refer to the sections on either side. Fractions 1-11 were 2 ml each; fractions 12-20 were 1 ml each.
other intestinal homogenate-produced metabolites with 1,25-(OH)$_2$[H]D$_3$ as substrate was studied (HPLC system 1; Fig. 4A). Most of the radioactivity (90 and 87%, in the duplicates) was recovered as unreacted substrate. However, 24-keto-1,25-(OH)$_2$[H]D$_3$ represented 4.0 and 4.2% of the total recovered tritium in each analysis. A second peak, migrating closely with 1,23S,25-(OH)$_3$D$_3$ and 23-keto-1,25-(OH)$_2$D$_3$, accounted for 4.2 and 5.3% of the recovered radioactivity. Finally, a peak that co-migrated with 1,24R,25-(OH)$_3$D$_3$ accounted for 2.4 and 3.7% of the recovered radioactivity.

The occurrence of these peaks in vivo was investigated. Rats not supplemented with exogenous 1,25-(OH)$_2$D$_3$ were dosed with 1,25-(OH)$_2$[H]D$_3$. 24-Keto-1,25-(OH)$_2$[H]D$_3$ was observed (HPLC system 1) in both plasma (2.3% of recovered tritium relative to 89% for 1,25-(OH)$_2$[3H]D$_3$) and intestinal mucosa (1.4% of recovered tritium relative to 88% for 1,25-(OH)$_2$[H]D$_3$). In both plasma and intestine, radioactive tritium in each analysis. The fractions were counts/min/fraction. The fractions were collected and re-analyzed on a normal phase HPLC column eluted HPLC 1,25-(OH)$_2$D$_3$. 3, 23-keto-1,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$D$_3$-26,23-lactone; 4, 1,23S,25-(OH)$_3$D$_3$; 6, 1,24R,25-(OH)$_2$D$_3$. The data are plotted as counts/min/fraction. Fractions were 1 ml each.

The compositions of the peaks from 1,25-(OH)$_2$D$_3$-treated animals recovered from the first HPLC system (HPLC system 1; Fig. 4) were examined on a second HPLC system (HPLC system 2), which had different selectivity (2). Note that the elution order of standards in HPLC system 2 was different from HPLC system 1. Re-chromatography of the 24-keto-1,25-(OH)$_2$[H]D$_3$ peak recovered from the intestinal homogenates showed a major peak that co-chromatographed with unlabeled 24-keto-1,25-(OH)$_2$D$_3$ and some carry over of 1,25-(OH)$_2$D$_3$ obtained from the blood and intestinal mucosa of 1,25-(OH)$_2$[3H]D$_3$ dosing changes the relative abundance of 1,24R,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$D$_3$ in intestine, relative to both recovered substrate and to 1,25-(OH)$_2$[H]D$_3$-26,23-lactone and 1,24R,25-(OH)$_3$[H]D$_3$. These data show that 1,25-(OH)$_2$D$_3$ dosing changes the relative abundance of 1,24R,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$D$_3$-26,23-lactone in vivo, so that the latter becomes predominant. 1,25-(OH)$_2$D$_3$ treatment also increases the relative abundance of the peak in the 23-keto-1,25-(OH)$_2$D$_3$/1,23S,25-(OH)$_2$D$_3$ elution region.

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Fig. 7. Competitive binding protein assays of 1,25-(OH)₂D₃ and derivatives. Either crude chick intestinal cytosol (A) or calf thymus cytosol substantially free of the 5 S vitamin D-binding protein (B) was used as the source of receptor. Points are the mean of triplicates. Individual values deviated ±10% from the mean. The compounds tested were 1,25-(OH)₂D₃ (●); 24-keto-1,25-(OH)₂D₃ (○); 1,24R,25-(OH)₃D₃ (▲); 23-keto-1,25-(OH)₂D₃ (△); and 1,23S,25-(OH)₃D₃ (■).

Table II

| Compound          | Chick cytosol | Thymus cytosol |
|-------------------|---------------|----------------|
|                   | 50% Displacement | Relative affinity | 50% Displacement | Relative affinity |
| 1,25-(OH)₂D₃      | 0.1           | 100            | 0.1              | 100              |
| 24-Keto-1,25-(OH)₂D₃ | 0.63          | 15             | 0.1              | 100              |
| 1,24R,25-(OH)₃D₃  | 0.87          | 15             | 0.1              | 100              |
| 23-Keto-1,25-(OH)₂D₃ | 3.63          | 2.8            | 0.2              | 50               |
| 1,23S,25-(OH)₃D₃  | 5.34          | 1.9            | 0.63             | 16               |

* Amount/tube to produce 50% displacement of 1,25-(OH)₂D₃ [26,27-³H]D₃.

* Binding potency relative to 1,25-(OH)₂D₃.

A new metabolite of 1,25-(OH)₂D₃ has been unequivocally identified as 24-keto-1,25-(OH)₂D₃. The structural assignment is supported by diverse evidence. The ultraviolet absorbance and mass spectra of the metabolite and its trisilyl derivative showed that it is a side chain ketone derivative of 1,25-(OH)₂D₃. The mass spectra also suggest that the ketone function is at C-24 and exclude a C-23-ketone from consideration. Chemical reduction of the metabolite produced a compound that coeluted with 1,24,25-(OH)₃D₃ on an HPLC system that readily separates 1,25-(OH)₂D₃, 1,23,25-(OH)₃D₃, 1,24,25-(OH)₃D₃, and 1,25,26-(OH)₃D₃. Furthermore, CI-NCI mass spectroscopy of the reduction product indicated a molecular weight consistent with 1,24,25-(OH)₃D₃. Synthesis of a metabolite in vitro from 1,24,25-(OH)₃D₃, with chromatographic and mass spectral characteristics identical with the metabolite produced from 1,25-(OH)₂D₃, provided the final demonstration that the structural assignment as 24-keto-1,25-(OH)₂D₃ is correct.

24-Keto-1,25-(OH)₂D₃ is produced in small intestine in vitro from 1,25-(OH)₂D₃ at low substrate concentrations and is present in the plasma and small intestinal mucosa of rats maintained on a stock diet. It is also present in the plasma and small intestinal mucosa of rats dosed chronically with 1,25-(OH)₂D₃. These data indicate that 24-keto-1,25-(OH)₂D₃ is on the pathway of 1,25-(OH)₂D₃ metabolism under physiological circumstances and, during states of 1,25-(OH)₂D₃ excess, at least one vitamin D target tissue (intestine). Under the experimental conditions reported here, however, there was about 60-fold less 24-keto-1,25-(OH)₂D₃ than 1,25-(OH)₂D₃ in intestinal mucosa under physiological conditions.

The affinity of 24-keto-1,25-(OH)₂D₃ was measured for the 1,25-(OH)₂D₃ cytosolic receptor in chick intestinal mucosa and in calf thymus (Fig. 7). In each case, 1,24R,25-(OH)₂D₃ and 24-keto-1,25-(OH)₂D₃ were indistinguishable and were more active than the C-23-oxidized derivatives. In the bovine thymus cytosol preparation, which lacks substantial 5 S vitamin D-binding protein contamination, 1,25-(OH)₂D₃, 24-keto-1,25-(OH)₂D₃, and 1,24R,25-(OH)₂D₃ were equipotent; in the chick cytosol preparation, which contains the 5 S vitamin-D binding protein, the two metabolites had 15% of the affinity of 1,25-(OH)₂D₃ (Table II).
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taminant are likely to reflect affinity of ligand for receptor more accurately than those obtained with crude preparations. On the other hand, differences in receptor structure have not been clearly ruled out.

1,25-(OH)₂D₃ was reported to undergo side chain metabolism in vivo by Harnden et al. (44) and Kumar et al. (45). They demonstrated that the intestine was one site of conversion (46) and rigorously identified 1,24,25-(OH)₃D₃ as a rat intestinal 1,25-(OH)₂D₃ metabolite in vitro (47). In contrast, Ohnuma and Norman (48) were unable to detect 1,24,25-(OH)₃D₃ as an in vitro product of 1,25-(OH)₂D₃ metabolism with rat intestinal mucosal homogenates. Instead, they found a product which co-migrated with a radioactive peak extracted from the plasma of rats dosed with 1,25-(OH)₂[26,27-H]D₃. This peak had been tentatively identified as 1,25-(OH)₂D₃-22-oxo-D₃ (49). The present report is consistent with the results of Kumar et al. (47). However, we also detected a product that co-migrated with authentic synthetic 1,25-(OH)₂D₃-26,23-lactone in two HPLC systems with different selectivities. About 10-fold less 1,25-(OH)₂D₃-26,23-lactone was observed than 1,24,25-(OH)₃D₃ in the homogenates at the incubation time studied. Clearly, rat intestine can produce both metabolites but, in brief incubation times, seems to accumulate more 1,24,25-(OH)₂D₃.

Larger amounts of 1,24,25-(OH)₃[3H]D₃ than 1,25-(OH)₂[3H]D₃ were detected in the plasma and intestine of rats not treated with exogenous 1,25-(OH)₂D₃. This is consistent with the results of Ramberg et al. (50) that showed about 4-fold more 1,25-(OH)₂D₃ was converted into 1,24,25-(OH)₃D₃ than into 1,25-(OH)₂D₃-26,23-lactone in normal bovine. On the other hand, the present work also shows that chronic 1,25-(OH)₂D₃ dosing produces a significant change in the relative amounts of 1,24,25-(OH)₃[3H]D₃ and 1,25-(OH)₂[3H]D₃-26,23-lactone in vivo, such that the latter metabolite predominates. By analogy to 25-OHD₃-26,23-lactone biosynthesis, which increases during chronic vitamin D₃ treatment (26-28), it is reasonable to expect that one cause of the metabolism warrant further study.

Evidence of significant production of 23-keto-1,25-(OH)₂D₃ by intestinal homogenates was obtained. Nor was a substantial presence of this putative metabolite observed in vivo relative to 24-keto-1,25-(OH)₂D₃. Our conclusions are based on HPLC comparisons of radiolabeled 1,25-(OH)₂[3H]D₃ metabolic products in vitro and in vivo with standards. Notably, our standard 23-keto-1,25-(OH)₂D₃ was produced from two different substrates with known side chains, namely 1,23S,25-(OH)₂D₃ and 23-keto-25-OH-D₃ (36, 37). Thus, the key functionality was already in place in our starting materials. Moreover, the mass spectral data obtained from 23-keto-1,25-(OH)₂D₃ produced in our laboratory, were distinct from those of 24-keto-1,25-(OH)₂D₃ and the metabolite isolated by Ohnuma et al. (52), but showed a fragmentation pattern similar to that of 23-keto-25-OH-D₃. Furthermore, it was reported that the putative 23-keto-1,25-(OH)₂D₃ is the sole precursor of the putative 1,25,26-(OH)₃-23-oxo-D₃ (53). Consequently, both of these structural assignments and conclusions based on them concerning C-23 ketonization as a major route of intestinal 1,25-(OH)₂D₃ metabolism warrant further study.

In summary, this report describes the identification of a physiological 1,25-(OH)₂D₃ metabolite produced by intestine as 24-keto-1,25-(OH)₂D₃. 24-Keto-1,25-(OH)₂D₃ is rapidly generated from 1,25-(OH)₂D₃ and is present in a vitamin D₃ target tissue. It has high affinity for the cytosolic 1,25-(OH)₂D₃ receptor. Its physiological function, if any, is not certain, but it is intriguing to consider that the metabolite acts in situ as a mediator of calcium homeostasis. Alternatively, it may be part of an inactivation pathway. Further research will determine which of these is the more viable hypothesis.

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