Nir, S., Bentz, J., Wilschut, J., & Düzgünes, N. (1983) *Prog. Surf. Sci. 13*, 1–124.
Ohki, S. (1984) *J. Membr. Biol. 77*, 265–275.
Ohnishi, S.-I., & Ito, T. (1974) *Biochemistry 13*, 881–887.
Portis, A., Newton, C., Pangborn, W., & Papahadjopoulos, D. (1979) *Biochemistry 18*, 780–789.
Rand, R. P., Fuller, N., Parsegian, V. A., & Rau, D. C. (1988) *Biochemistry 27*, 7711–7722.
Telford, J. N., & Matsumara, F. (1970) *J. Econ. Entomol. 63*, 795–800.
Tokutomi, S., Eguchi, G., & Ohnishi, S.-I. (1979) *Biochim. Biophys. Acta 552*, 78–88.
Tsien, R. Y. (1980) *Biochemistry 19*, 2396–2404.
Tsui, F. C., Ojcius, D. M., & Hubbell, W. L. (1986) *Biophys. J. 49*, 459–468.
von Zawidzki, J. (1900) *Z. Phys. Chem. 35*, 129–203.

Isotopic Labeling Affects 1,25-Dihydroxyvitamin D Metabolism†

Bernard P. Halloran,* Daniel D. Bikle, Margaret E. Castro, and Elaine Gee
Departments of Medicine and Physiology, University of California, and Division of Endocrinology, Veterans Administration Medical Center, San Francisco, California 94121

Received June 2, 1988; Revised Manuscript Received September 21, 1988

ABSTRACT: Isotope substitution can change the biochemical properties of vitamin D. To determine the effect of substituting ^3H for ^1H on the metabolism of $1,25(OH)_2D_3$, we measured the metabolic clearance rate and renal metabolism of unlabeled and ^3H-labeled $1,25(OH)_2D_3$. Substitution of ^3H for ^1H on carbons 26 and 27 [$1,25(OH)_2[26,27(n)\text{-}^3H]D_3$] or on carbons 23 and 24 [$1,25(OH)_2[23,24(n)\text{-}^3H]D_3$] reduced the in vivo metabolic clearance rate of $1,25(OH)_2D_3$ by 36% and 37%, respectively, and reduced the in vitro renal catabolism of $1,25(OH)_2D_3$ by 11% and 54%, respectively. Substitutions of ^3H for ^1H on carbons 23 and 24 as opposed to carbons 26 and 27 reduced conversion of [^3H]$1,25(OH)_2D_3$ to [^3H]$1,24,25(OH)_2D_3$ by 25% and to putative 24-oxo-1,23,25-dihydroxyvitamin D_3 by 1600%. These results indicate that substitution of ^3H for ^1H on carbons 26 and 27 or on carbons 23 and 24 can reduce the metabolic clearance rate and in vitro metabolism of $1,25(OH)_2D_3$ and quantitatively alter the pattern of metabolic products produced.

Isotopically labeled vitamin D and metabolites of vitamin D are used extensively in studies of vitamin D metabolism. These studies have proven to be extremely valuable in defining how vitamin D is metabolized and in isolating the biologically active forms of the vitamin. In most cases, it is tacitly assumed that substitution of ^3H or ^2H for ^1H or ^{14}C for ^{12}C has little, if any, effect on the physicochemical or biochemical properties of the molecules. However, isotopic labeling can alter the chromatographic properties of many molecules, including the vitamin D metabolites (Blake et al., 1975; deRidder & van Hall, 1976; Oats et al., 1978; Horning et al., 1979; Thenot et al., 1980; Halloran et al., 1984). We have shown, for example, that substitution of ^3H for ^1H on carbon 23 (C-23) and carbon 24 (C-24) can change the retention times of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$][1] and 25,26-dihydroxyvitamin D_3 [$25,26(OH)_2D_3$] on silica columns by as much as 3.7% (Halloran et al., 1984).

Isotopic labeling can also alter metabolic properties and, in particular, the metabolism of vitamin D (Elison et al., 1961; Horning & Lertratanangkoon, 1978; Jones et al., 1983; Tanaka & DeLuca, 1982; Bell et al., 1986; Langenhove, 1986). Although there are no direct studies comparing the metabolic properties of labeled and unlabeled vitamin D, Tanaka and DeLuca (1982) showed that substitution of ^3H for ^1H in 25-hydroxyvitamin D (25-OH-D) can alter its metabolism in vitro. These investigators report that the in vitro rate of metabolic conversion of [^3H]-25-OH-D_3 to [^3H]-24,25-dihydroxyvitamin D_3 [[^3H]$24,25(OH)_2D_3$] or [^3H]$25,26(OH)_2D_3$ is dependent upon where the ^3H is incorporated into 25-OH-D_3 (Tanaka & DeLuca, 1982). Conversion of [^3H]-25-OH-D_3 to [^3H]-$24,25(OH)_2D_3$ is favored when [^3H]-25-OH-D_3 is labeled in the C-26 and -27 positions, whereas conversion to [^3H]-$25,26(OH)_2D_3$ is favored when the [^3H]-25-OH-D_3 is labeled in the C-23 and -24 positions. No study, however, has addressed the effect of ^3H-labeling on the metabolism of $1,25(OH)_2D$, the most biologically active form of vitamin D.

To determine whether substitution of ^3H for ^1H on C-23 and -24 or on C-26 and -27 changes the metabolism of $1,25(OH)_2D_3$, we examined the in vivo metabolic clearance rate (MCR) and in vitro renal metabolism of $1,25(OH)_2D_3$, $1,25(OH)_2[26,27(n)\text{-}^3H]D_3$, and $1,25(OH)_2[23,24(n)\text{-}^3H]D_3$. Our results indicate that ^3H-labeling can reduce the metabolism of $1,25(OH)_2D$ in vivo and in vitro and change the profile of metabolites produced.

Materials and Methods

Animals. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 100–125 g were fed ad libitum a semipurified, vitamin D deficient diet containing 1.2% calcium and 0.9% phosphorus (Suda et al., 1970). All rats were supplemented with vitamin D_3 (200 IU/day) and maintained on their diet for at least 10 days before experimentation.

Metabolic Clearance Rate. The metabolic clearance rate (MCR) was measured by using the constant infusion method as previously described (Halloran et al., 1986). Rats were

† This work was supported by the Veterans Administration Research Service.
* Address correspondence to this author at Veterans Administration Medical Center, m/c 11, 4150 Clement St., San Francisco, CA 94121.

[1] Abbreviations: 25-OH-D_3, 25-hydroxyvitamin D_3; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; $25,26(OH)_2D_3$, 25,26-dihydroxyvitamin D_3; 24-oxo-$1,23,25(OH)_3D_3$, 24-oxo-1,23,25-trihydroxyvitamin D_3.

Table I: Metabolic Clearance Rates of $1,25(OH)_2D_3$, $1,25(OH)_2[26,27(n)\text{-}^3H]D_3$, and $1,25(OH)_2[23,24(n)\text{-}^3H]D_3$

metabolite[a]	n	body wt[b] (g)	serum concn of $1,25(OH)_2D_3$ (pg/mL)	MCR [nL/(min·g of body wt)]
$1,25(OH)_2D_3$	12	234 ± 19	320 ± 42	374 ± 45[c]
$1,25(OH)_2[26,27(n)\text{-}^3H]D_3$ (180 Ci/mmol)	6	237 ± 23	337 ± 53	239 ± 54[d]
$1,25(OH)_2[23,24(n)\text{-}^3H]D_3$ (100 Ci/mmol)	6	231 ± 15	304 ± 20	235 ± 42[d]

[a] Form of metabolite used to calculate MCR. [b] Mean ± SD. [c] Based on the infusion rate, the MCR must be equal to or greater than 374 nL/(min·g of body weight). [d] $P < 0.001$ when compared to $1,25(OH)_2D_3$, Tukey test.

simultaneously infused (Alza osmotic minipump, Alza Corp., Palo Alto, CA) with either $1,25(OH)_2D_3$ (18 pg/min) and $1,25(OH)_2[26,27(n)\text{-}^3H]D_3$ (21.7 dpm/min, SA = 180 Ci/mmol) or $1,25(OH)_2D_3$ (18 pg/min) and $1,25(OH)_2[23,24(n)\text{-}^3H]D_3$ (20.9 dpm/min, 100 Ci/mmol). Both $1,25(OH)_2D_3$ (a gift from Dr. Milan Uskokovic, Hoffmann-La Roche, Nutley, NJ) and $[^3H]1,25(OH)_2D_3$ (Amersham Corp., Arlington Heights, IL) were purified by using high-performance liquid chromatography before use. All infusions were for 12 days to allow the serum concentration of $1,25(OH)_2D_3$ or $[^3H]1,25(OH)_2D_3$ to reach steady state. On day 12, the rats were bled from the dorsal aorta while under light isofluorane anesthesia. The serum concentrations of $1,25(OH)_2D_3$ and $[^3H]1,25(OH)_2D_3$ were measured by using competitive protein binding assays and scintillation counting, respectively, after extraction and purification using high-performance liquid chromatography as previously described (Halloran et al., 1986; Portale et al., 1986). All samples were chromatographed before assay. Inter- and intraassay coefficients of variation for measurement of serum $1,25(OH)_2D_3$ were 16% and 10%, respectively.

The MCR, defined as the volume of blood cleared completely and irreversibly of hormone per unit time, is calculated according to the relationship MCR (mL/min) = rate of infusion of compound/serum concentration of hormone. To calculate the MCR of $1,25(OH)_2D_3$, the rate of infusion of $1,25(OH)_2D_3$ (18 pg/min) was divided by the serum concentration of $1,25(OH)_2D_3$. To calculate the MCRs of $[^3H]1,25(OH)_2D_3$, the rates of infusion of $[^3H]1,25(OH)_2D_3$ were divided by the serum concentrations of $[^3H]1,25(OH)_2D_3$.

In calculating the MCR, it is important to accurately know the rate at which the hormone is entering the blood pool. The rate of infusion of labeled hormone is calculated from the pump rate and the concentration of labeled hormone in the infusate. Although the manufacturer's reported pump rate of 8.33 nL/min was confirmed in preliminary experiments, hormone solubility (both labeled and unlabeled) decreased within 24 h of loading. This resulted in a decrease in the effective hormone concentration in the infusate. In separate experiments, the degree of solubility was observed to vary with the relative polarity of individual vitamin D metabolites but remained constant between 1 and 12 days. The percent solubilizations of $[^3H]1,25(OH)_2D_3$ and $1,25(OH)_2D_3$ were equivalent and equal to 81 ± 1%. Solubilization was taken into account in calculating all MCRs.

In calculating the MCR of unlabeled $1,25(OH)_2D_3$, it is necessary to assume that the rate of endogenous production of 1,25(OH)2D is small or insignificant compared to the rate of exogenous infusion. In previous experiments we have determined that chronic infusion of $1,25(OH)_2D_3$ at the rate of 18 pg/min will produce hypercalcemia (11.0–12.0 mg/dL) in rats weighing 200–250 g on dietary calcium and phosphorus intakes of 1.2% and 0.9%, respectively (Halloran et al., 1986). A serum calcium level of 11.0–12.0 mg/dL should suppress parathyroid hormone secretion and endogenous $1,25(OH)_2D_3$ production. Nevertheless, some endogenous production of $1,25(OH)_2D_3$ may contribute to the blood pool. The exact amount, however, cannot be determined by these methods. Consequently, our estimate of the MCR of $1,25(OH)_2D_3$ will be lower than the true MCR to the degree to which our assumption of negligible endogenous $1,25(OH)_2D_3$ production is in error.

In Vitro Metabolism. To determine the effect of ^3H substitution for ^1H on in vitro renal metabolism of $1,25(OH)_2D_3$, rat kidneys were perfused in vivo with 10 mL of ice-cold homogenizing buffer, excised, passed through a Krebs tissue press, and homogenized by using a Teflon pestle in 2 volumes of the following buffer: 100 mM KCl, 10 mM Tris-HCl, 10 mM NaH_2PO_4, 10 mM sodium pyruvate, 4 mM $MgSO_4$, and 10 mM EGTA with $CaCl_2$ added to a free calcium concentration of 0.01 mM at pH 6.8.

To measure metabolism of $1,25(OH)_2D_3$ and $[^3H]1,25(OH)_2D_3$, 1.0-mL aliquots of homogenized tissue were combined with either 300 pg of $1,25(OH)_2D_3$, 300 pg (279 000 dpm) of $1,25(OH)_2[26,27(n)\text{-}^3H]D_3$ (176 Ci/mmol), or 300 pg (120 000 dpm) of $1,25(OH)_2[23,24(n)\text{-}^3H]D_3$ (76 Ci/mmol) in 10 μL of ethanol, gased with oxygen, and incubated (in duplicate) in a shaking water bath at 37 °C for 0, 5, 15, or 60 min. The incubations were stopped by adding 3 mL of a 2:1 mixture of methanol/chloroform, the samples were shaken vigorously, and phase separation was accomplished by adding an additional 1 mL of chloroform and 0.8 mL of H_2O. After centrifugation at 3000 rpm for 5 min in a clinical bench-top centrifuge, the lower (chloroform) phase was removed and the upper aqueous phase reextracted twice with 1 mL of chloroform. The chloroform extracts were combined, dried under nitrogen, and resolubilized in preparation for chromatography. The extracts containing $1,25(OH)_2D_3$ were chromatographed and assayed in a manner identical with that used to measure $1,25(OH)_2D_3$ in serum (Halloran et al., 1986). The extracts containing $[^3H]1,25(OH)_2D_3$ were chromatographed by using a Zorbax-Sil column (Du Pont Instruments, Wilmington, DE) and a solvent system of 2-propanol/hexane run in a concave gradient mode (setting 9, Waters 660 programmer, Waters Associates, Milford, MA) from 3:97 to 10:90 at a flow rate of 2.0 mL/min. Tritium in the column effluent was monitored continuously by using a Flow-One radioactive flow counter (Radiomatics Instrument and Chemical Co., Tampa, FL). The amount of metabolite remaining or produced is expressed as a percent of the initial substrate. Tritium lost during hydroxylation was accounted for in calculating the amount of metabolite formed.

Statistics. Results are presented as mean ± SD. Data were analyzed by using Student's *t*-test or analysis of variance where appropriate.

Results

The MCRs of $1,25(OH)_2D_3$, $1,25(OH)_2[26,27(n)\text{-}^3H]D_3$, and $1,25(OH)_2[23,24(n)\text{-}^3H]D_3$ are shown in Table I. Assuming that endogenous production of $1,25(OH)_2D_3$ is completely suppressed by the hypercalcemia (11–12.0 mg/dL) induced by $1,25(OH)_2D_3$ administration (18 pg/min), the

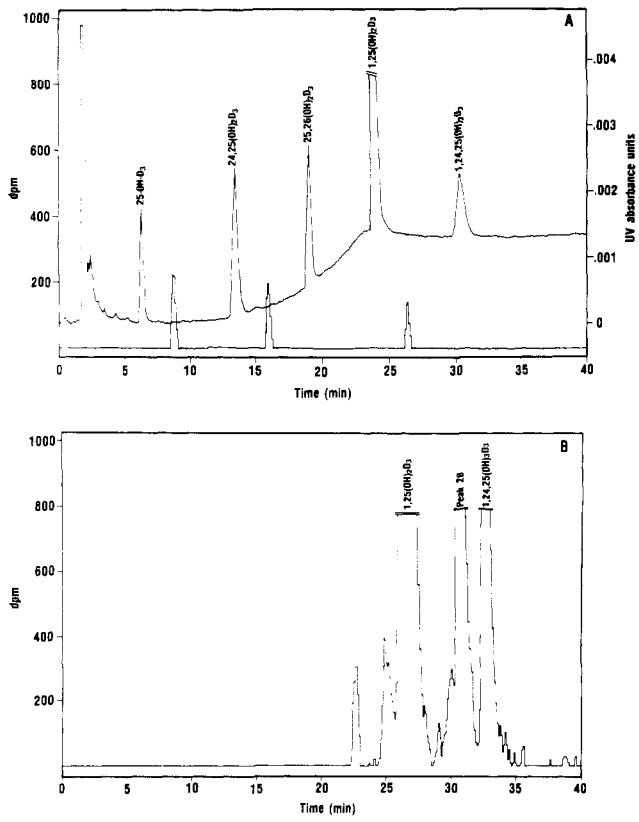

FIGURE 1: (A) Elution profile of unlabeled standards (UV absorbance) (upper trace) and ^{3}H-labeled standards (dpm) (lower trace). (B) Radioactivity trace of samples incubated for 60 min with 1,25-(OH)$_2$[26,27(n)-^{3}H]D$_3$. See text for details of chromatography system.

MCR of 1,25(OH)$_2$D$_3$ is 374 ± 45 nL/min·g of body weight). If endogenous production was not completely suppressed, the MCR would be greater. The MCRs of 1,25(OH)$_2$[26,27-(n)-^{3}H]D$_3$ and 1,25(OH)$_2$[23,24(n)-^{3}H]D$_3$ were 239 ± 54 and 235 ± 42 nL/(min · g of body weight), respectively. Both values are significantly less ($P < 0.001$, Tukey test) than the MCR of 1,25(OH)$_2$D$_3$ but are not different from each other.

The results of our in vitro metabolism studies are shown in Figures 1 and 2. Representative chromatograms of our standards and a sample are shown in Figure 1. The elution profile of nonlabeled standards (UV absorbance) for 25-OH-D$_3$, 24,25(OH)$_2$D$_3$, 25,26(OH)$_2$D$_3$, 1,25(OH)$_2$D$_3$, and 1,24,25(OH)$_3$D$_3$ is shown in Figure 1A by the upper trace. The elution profile of ^{3}H-labeled standards (dpm) for 25-OH-D$_3$, 24,25(OH)$_2$D$_3$, and 1,25(OH)$_2$D$_3$ is shown in Figure 1A by the lower trace. The radioactive trace is offset from the UV absorbance trace by the distance between pens on the chart recorder plus the dead volume between the UV and radiation detectors, which are arranged in series. The radioactivity trace of a sample incubated for 60 min with 1,25(OH)$_2$[26,27(n)-^{3}H]D$_3$ is shown in Figure 1B. The three primary metabolites remaining after 60 min of incubation are [^{3}H]1,25(OH)$_2$D$_3$, [^{3}H]1,24,25(OH)$_3$D$_3$, and an unknown metabolite (peak 28) that migrates between [^{3}H]1,25(OH)$_2$D$_3$ and [^{3}H]1,24,25(OH)$_3$D$_3$. Peak 28 has not been definitively identified, but its chromatographic properties suggest it may be 24-oxo-1,23,25-trihydroxyvitamin D$_3$ [24-oxo-1,23,25-(OH)$_3$D$_3$] (Napoli & Martin, 1984).

Figure 2 shows the amounts (unlabeled and labeled) of 1,25(OH)$_2$D$_3$ (Figure 2A), 1,24,25(OH)$_3$D$_3$ (Figure 2B), and peak 28 (Figure 2C) remaining in the assay tubes after 5, 15, and 60 min of incubation. Each point represents the mean of duplicate determinations, and each experiment was performed at least twice with virtually identical results each time. The amount of 1,25(OH)$_2$D$_3$ falls rapidly during the first 5–15 min of incubation and then falls more gradually during the next 15–60 min. As illustrated in Figure 1A, 1,25(OH)$_2$D$_3$ is more rapidly metabolized than 1,25(OH)$_2$[26,27(n)-^{3}H]D$_3$, which is more rapidly metabolized than 1,25(OH)$_2$[23,24-

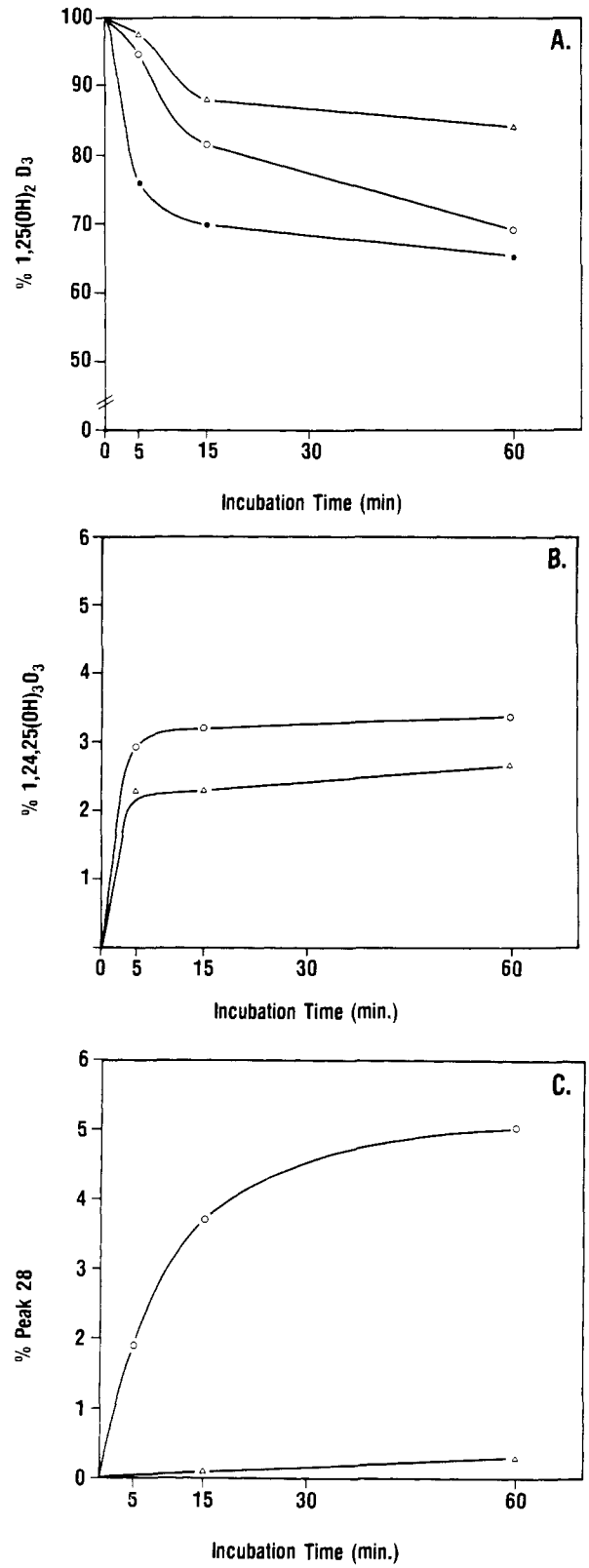

FIGURE 2: (A) Metabolism of 1,25(OH)$_2$D$_3$ (●), 1,25(OH)$_2$[26,27-(n)-^{3}H]D$_3$ (○), and 1,25(OH)$_2$[23,24(n)-^{3}H]D$_3$ (△). (B) Production of [^{3}H]1,24,25(OH)$_3$D$_3$ from 1,25(OH)$_2$[26,27(n)-^{3}H]D$_3$ (○) and 1,25(OH)$_2$[23,24(n)-^{3}H]D$_3$ (△). (C) Production of ^{3}H peak 28 from 1,25(OH)$_2$[26,27(n)-^{3}H]D$_3$ (○) and 1,25(OH)$_2$[23,24(n)-^{3}H]D$_3$ (△).

(n)-^{3}H]D_3. After 60 min of incubation, 35% of the initial 1,25$(OH)_2D_3$, but only 18% of the 1,25$(OH)_2$[23,24(n)-^{3}H]D_3, had been converted to other metabolites. Using analysis of variance, there were significant differences between the rates of metabolism of 1,25$(OH)_2D_3$ and 1,25$(OH)_2$[23,24(n)-^{3}H]D_3 ($P < 0.003$) and between the rates of metabolism of 1,25$(OH)_2D_3$ and 1,25$(OH)_2$[26,27(n)-^{3}H]D_3 ($P < 0.04$). Substitution of ^{3}H for ^{1}H on C-23, and -24, as opposed to C-26 and -27, decreased conversion of [^{3}H]1,25$(OH)_2D_3$ to [^{3}H]-1,24,25$(OH)_3D_3$ by 25% ($P < 0.004$) (Figure 2B) and to peak 28 by 1600% ($P < 0.001$) (Figure 2C). Because of the small amount produced, we were unable to measure unlabeled 1,24,25$(OH)_3D_3$.

In calculating the amount of 1,24,25$(OH)_3D_3$ produced from 1,25$(OH)_2$[23,24(n)-^{3}H]D_3, it was assumed that one ^{3}H atom was lost with the addition of the OH group at C-24. In calculating the amount of peak 28 produced, no corrections for loss of ^{3}H were made because of the uncertainty of the identity of peak 28. If peak 28 is 24-oxo-1,23,25$(OH)_2D_3$, it is possible that the amount of peak 28 produced from 1,25-$(OH)_2$[23,24(n)-^{3}H]D_3 is 3 times greater than reported due to the loss of three ^{3}H atoms.

Discussion

Our results indicate that substitution of ^{3}H for ^{1}H can alter the metabolism of 1,25$(OH)_2D_3$. Substitution of ^{3}H for ^{1}H on C-26 and -27 or on C-23 and -24 can reduce the in vivo MCR of 1,25$(OH)_2D_3$ by as much as 37%. Substitution of ^{3}H for ^{1}H can also reduce the in vitro metabolism of 1,25-$(OH)_2D_3$ by as much as 54%, with the largest effect occurring when ^{3}H is substituted on C-23 and -24. Furthermore, the products formed from metabolism of 1,25$(OH)_2D_3$ vary dramatically depending upon the specific site of ^{3}H substitution.

There is a well-established theoretical basis for the isotope effects observed with 1,25$(OH)_2D_3$ (Bigeleisen, 1949; Melander, 1980). Substitution of a heavy isotope for a light isotope increases the strength of the molecular bond. If this bond is involved in a rate-limiting reaction during metabolism of the molecule, metbolism will be inhibited (primary isotope effect). Since the isotope effect is largest for hydrogen and since the products formed during 1,25$(OH)_2D_3$ metabolism (at least in vitro) either include [viz., 1,24,25$(OH)_3D_3$] or likely include [viz., peak 28 = 24-oxo-1,23,25$(OH)_3D_3$] cleavage of the carbon–hydrogen bonds at C-24 or C-23, it is likely that there will be large isotope effects for 1,25$(OH)_2D_3$. Furthermore, it is predictable, on the basis of the observation that much of the metabolism of 1,25$(OH)_2D_3$ involves 24-hydroxylation, that metabolism of 1,25$(OH)_2$[23,24(n)-^{3}H]D_3 will be inhibited to a greater degree than that of 1,25-$(OH)_2$[26,27(n)-^{3}H]D_3. This prediction is borne out by our results in vitro (Figure 2A). Furthermore, the relative proportions of products formed should vary depending on the specific site of substitution. Substitution of ^{3}H for ^{1}H at C-26 and -27 should reduce hydroxylation at these carbons, whereas ^{3}H substitution for ^{1}H at C-23 and -24 should reduce hydroxylation at these carbons. Again, these predictions are borne out by our results (Figure 2B, C). That the in vivo MCRs of 1,25$(OH)_2$[26,27(n)-^{3}H]D_3 and 1,25$(OH)_2$[23,24-(n)-^{3}H]D_3 did not differ suggests that clearance in vivo involves more than one pathway.

Isotope effects on in vivo and in vitro metabolism are not without precedent. Tanabe et al. (1969) observed a 250% increase in the half-life of butethal when ^{2}H was substituted for ^{1}H in the 3′-position to form 3′-dideuteriobutethal. Substitution of ^{2}H for the *N*-methyl hydrogens of morphine reduces its potency in mice (Elison et al., 1961), and glucose turnover rates determined with [6-^{14}C]glucose during insulin infusion are lower than those determined with [2-^{3}H]glucose but higher than those determined with [3-^{3}H]glucose (Bell et al., 1986).

In summary, our results clearly demonstrate that isotope effects occur for 1,25$(OH)_2D_3$ and must be taken into account in interpreting the outcome of studies that rely on the use of radioactively labeled 1,25$(OH)_2D_3$. Measurement of metabolic clearance in vivo and metabolism in vitro may depend on whether or not 1,25$(OH)_2D_3$ is labeled and what labeling scheme is used. Clearly, radiolabeling in vitamin D research should not be abandoned, but caution should be exercised in interpreting results.

Registry No. 1,25$(OH)_2D_3$, 32222-06-3; 1,25$(OH)_2$[26,27(n)-^{3}H]D_3, 117897-60-6; 1,25$(OH)_2$[23,24(n)-^{3}H]D_3, 73890-65-0; 1,24,25$(OH)_3D_3$, 50648-94-7; 24-oxo-1,23,25-trihydroxyvitamin D_3, 87678-01-1.

References

Bell, P. M., Firth, R. G., & Rizza, R. A. (1986) *J. Clin. Invest. 78*, 1479.

Bigeleisen, J. (1949) *Science 110*, 14.

Blake, M. I., Crespi, H. L., & Katz, A. A. (1975) *J. Pharm. Sci. 64*, 367.

deRidder, J. J., & van Hall, H. J. M. (1976) *J. Chromatogr. 121*, 96.

Elison, C., Rapoport, H., & Laursen, R. (1961) *Science 134*, 1078.

Halloran, B. P., Bikle, D. D., & Whitney, J. O. (1984) *J. Chromatogr. 303*, 229.

Halloran, B. P., Bikle, D. D., Levens, M. J., Castro, M. E., Globus, R. K., & Holton, E. M. (1986) *J. Clin. Invest. 78*, 622.

Horning, M. G., & Lertratanangkoon, K. (1978) in *Stable Isotopes: Applications in Pharmacology, Toxicology and Clinical Research* (Baillie, T. A., Ed.) p 55, University Park Press, London.

Horning, M. G., Thenot, J. P., Bouwsma, O., Nowlin, J., & Lertratanangkoon, K. (1979) *Adv. Pharm. Ther. 7*, 245.

Jones, G., Kung, M., & Kano, K. (1983) *J. Biol. Chem. 258*, 12920.

Langenhove, A. (1986) *J. Clin. Pharm. 26*, 383.

Malander, L. (1980) *Reaction rates of isotopic molecules*, Wiley-Interscience, New York.

Napoli, J. L., & Martin, C. A. (1984) *Biochem. J. 219*, 713.

Oats, J. A., Seyberth, H. W., Frolich, J. C., Sweetman, B. J., & Watson, J. T. (1978) in *Stable Isotopes*, p 281, Macmillan Press, London.

Portale, A. A., Halloran, B. P., Murphy, M. N., & Morris, R. C. (1986) *J. Clin. Invest. 77*, 1.

Suda, T., DeLuca, H. F., & Tanaka, Y. (1970) *J. Nutr. 100*, 1049.

Tanabe, M., Yasuda, D., & LeValley, S. (1969) *Life Sci. 8*, 1123.

Tanaka, Y., & DeLuca, H. F. (1982) *Arch. Biochem. Biophys. 213*, 517.

Thenot, J. P., Ruo, T. I., Stec, G. P., & Atkinson, A. J. (1980) in *Recent Developments in Mass Spectrometry in Biochemistry and Medicine* (Frigerio, A., & McCamish, M., Eds.) p 373, Elsevier, Amsterdam.