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J L Omdahl, … , G Jelinek, R P Eaton

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Research Article

Kinetic data analysis was used to derive a six-compartment computer model which describes the in vivo [3H]25-hydroxyvitamin D3 ([3H]25-OHD3) metabolism in control and strontium rachitic chicks. Plasma concentrations of 25-OHD3 (13 pmol/ml) and 25, 25-dihydroxyvitamin D3 (0.9 pmol/ml) were 18 and 125% greater than controls, respectively, whereas the corresponding level for 1alpha,25-dihydroxyvitamin D3 (0.3 pmol/ml) was only 30% of control. Plasma disappearance of 25-HOD3 was fitted using a two-compartment model in which the metabolite extrapolated half-life was nearly twice as large for strontium rachitic chicks (71 compared to 41 h). Intestinal sequestration of 1alpha,25-dihydroxyvitamin D3 was assumed to be irreversible and was fitted by a single exponential term in which metabolite uptake rate and tissue concentration in strontium rickets was suppressed to 20 and 10% of control, respectively. In contrast, uptake of 25-OHD3 by the intestine was observed to occur by a reversible process in which metabolite concentration was 45% greater in the strontium rachitic compared to control group. The developed compartment model accepts time-dependent control or perturbed metabolite data for the plasma and (or) intestinal pools and provides quantitative values for metabolite pool size, flux rate, and turnover time.



# Kinetic Analysis of 25-Hydroxyvitamin $D_3$ Metabolism in Strontium-Induced Rickets in the Chick

J. L. OMDAHL, G. JELINEK, and R. P. EATON, *The University of New Mexico School of Medicine, Departments of Biochemistry and Medicine, Albuquerque, New Mexico 87131*

ABSTRACT Kinetic data analysis was used to derive a six-compartment computer model which describes the in vivo [$^3$H]25-hydroxyvitamin $D_3$ ([$^3$H]25-$OHD_3$) metabolism in control and strontium rachitic chicks. Plasma concentrations of 25-$OHD_3$ (13 pmol/ml) and 24, 25-dihydroxyvitamin $D_3$ (0.9 pmol/ml) were 18 and 125% greater than controls, respectively, whereas the corresponding level for 1$\alpha$,25-dihydroxyvitamin $D_3$ (0.3 pmol/ml) was only 30% of control. Plasma disappearance of 25-$OHD_3$ was fitted using a two-compartment model in which the metabolite extrapolated half-life was nearly twice as large for strontium rachitic chicks (71 compared to 41 h). Intestinal sequestration of 1$\alpha$,25-dihydroxyvitamin $D_3$ was assumed to be irreversible and was fitted by a single exponential term in which metabolite uptake rate and tissue concentration in strontium rickets was suppressed to 20 and 10% of control, respectively. In contrast, uptake of 25-$OHD_3$ by the intestine was observed to occur by a reversible process in which metabolite concentration was 45% greater in the strontium rachitic compared to control group. The developed compartment model accepts time-dependent control or perturbed metabolite data for the plasma and (or) intestinal pools and provides quantitative values for metabolite pool size, flux rate, and turnover time.

## INTRODUCTION

The function of vitamin D to enhance intestinal calcium absorption, bone mineral mobilization, and calcification is well appreciated (1,2). However, to effect such actions vitamin D must be metabolically activated, initially in the liver (25-hydroxylation) (3, 4) and subsequently in the kidney (1$\alpha$-hydroxylation) (5, 6), resulting in the synthesis of 1$\alpha$,25-dihydroxyvitamin D[1] (1$\alpha$,25-$(OH)_2D$)[2] (7, 8). This compound contains the greatest biological activity of any known vitamin D metabolite (9–11). Control of renal 1$\alpha$,25-$(OH)_2D$ synthesis, and consequently the physiological response of vitamin D-dependent processes, is influenced to a large extent by the action of parathyroid hormone (PTH) on the kidney 1$\alpha$-hydroxylase system (12–14). A lesion in this control system can result in the development of disease-states which affect calcium homeostasis (15–17) (e.g., renal- and vitamin D-dependent rickets). Insights into the prevention and treatment of such metabolic diseases are dependent upon understanding how perturbations in kidney 1$\alpha$-hydroxylase activity affect 1$\alpha$,25-$(OH)_2D$[1] synthesis and turnover at the whole organism level.

Experiments on this subject have succeeded in providing voluminous qualitative data on the in vivo metabolism of 25-hydroxyvitamin $D_3$ (25-$OHD_3$), yet a void exists regarding the kinetics of in vivo 25-$OHD_3$ metabolism and how the synthetic and degradative parameters are changed in disease-perturbed states. Since PTH and calcium (18, 19) are both purported to be involved in modulating 25-$OHD_3$ metabolism, we elected to study the kinetics of in vivo 25-$OHD_3$ metabolism in a strontium rachitic animal model in which there is a lesion in the PTH component of the calcium homeostatic control system. Recent studies in rats have shown that strontium's inhibition of kidney 1$\alpha$-hydroxylase and stimulation of 24-hydroxylase activities (20, 21) is due predominantly to the cation's suppression of PTH secretion.[3] This

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[1] Specific numerical subscripts for vitamin D are not used when discussing general vitamin D metabolism since the dominant forms in man ($D_2$ and $D_3$) are thought to be metabolized in an equivalent manner.

[2] *Abbreviations used in this paper*: 1$\alpha$,25-$(OH)_2D_3$, 1$\alpha$,25-dihydroxyvitamin $D_3$; 24,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin $D_3$; 25-$OHD_3$, 25-hydroxyvitamin $D_3$; PTH, parathyroid hormone.

[3] Omdahl, J. L. *Anal. Biochem. Biophys.* In press.

observation, together with strontium's probable antagonism of the calcium-dependent mineralization process accounts for the lesions in intestinal calcium absorption and bone mineralization observed in strontium rickets. Data obtained using strontium as an ionic-agent for perturbing 25-$OHD_3$ metabolism was analyzed by using a six-compartment model which stimulates the kinetic behavior of [$^3$H]25-$OHD_3$ metabolism in chicks with normal and perturbed 25-$OHD_3$ metabolic activities. The compartmental model allows for the evaluation of plasma 25-$OHD_3$ disappearance rates and appearance rates and metabolic pool sizes for plasma 1$\alpha$,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin $D_3$ (24,25-$(OH)_2D_3$), and intestinal 25-$OHD_3$ and 1$\alpha$,25-$(OH)_2D_3$.

## METHODS

*Animals.* 1-Day-old male leghorn chicks (Dekalb-231), kindly provided by the Dekalb hatchery (Hudson, Colo.), were maintained in heated cages devoid of ultraviolet light. They were given semipurified vitamin D-deficient diet (Ca = 0.26 mmol/g and P = 0.2 mmol/g [9]) and tap water ad libitum for 16 days at which time the animals were used for experimentation.

*Dietary regimen.* The diets used during the experimental periods were similar to the previously mentioned semipurified diet except they contained purified casein, were supplemented with arginine, and differed in methionine and glycine content (casein 25.6%, arginine 1.2%, methionine 0.4%, and glycine 1.0%). Two purified diets that differed in their calcium and strontium content were used. They consisted of: (*a*) control diet (Ca = 0.13 mmol/g feed, no Sr), (*b*) strontium diet (Ca = 0.013 mmol/g feed, Sr = 0.13 mmol/g feed). At the beginning of the experimental period chicks were divided into two groups and given either control or strontium feed (pair-group fed, 15 g/chick per day), deionized water, and oral vitamin $D_3$ supplements (650 pmol bi-daily). This regimen was continued for 11 days at which time the chicks were used for the in vivo study of [$^3$H]-25-$OHD_3$ metabolism.

*In vivo [$^3$H]25-$OHD_3$ metabolism.* Radioactive 25-hydroxyvitamin $D_3$ (i.e., [26,27-$^3$H]25-$OHD_3$) was purchased from Amersham/Searle (Arlington Heights, Ill.), purified by column chromatography using Sephadex LH-20, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway N. J., (1:1, chloroform:hexane), and dissolved in 95% ethanol (ethanol/water) preparatory to intravenous (i.v.) administration. At time zero, each chick received a 0.05 ml i.v. injection (wing vein) of [26,27-$^3$H]25-$OHD_3$ ($1.2 \times 10^6$ dpm, $2.4 \times 10^4$ dpm/pmol), with a 6–8-ml blood sample obtained by cardiac puncture at a specified time post injection. Chicks were subsequently killed by cervical dislocation and the intestine (duodenum to cecum) removed, flushed with cold 0.9% NaCl, and immediately frozen. A chloroform lipid-extract was prepared from each plasma and intestinal mucosa sample by using a mixture of chloroform-methanol (22). The [26,27-$^3$H]25-$OHD_3$ (i.e., [$^3$H]25-$OHD_3$) and its metabolic products contained in the plasma lipid extracts were taken to dryness under nitrogen, dissolved in chloroform:hexane (65:35), and separated by column chromatography (1.5 × 20 cm) using Sephadex LH-20 (23) which was equilibrated and developed with the above solvent (160 ml of 65:35 mixture followed by a 50-ml strip with an 80:20 mixture). Good resolution is obtained for the separation of 25-$OHD_3$, 24,25-$(OH)_2D_3$ and 1$\alpha$,25-$(OH)_2D_3$, however, 25,26-$(OH)_2D_3$ and 1$\alpha$,25-$(OH)_2D_3$ co-migrate in the Sephadex LH-20 chromatographic system. Accordingly, an aliquot of the eluted [$^3$H]1$\alpha$,25-$(OH)_2D_3$ sample was treated with sodium metaperiodate (40 $\mu$l of $NaIO_4$, 5% wt/vol, pH 3; in 0.4 ml methanol at room temperature for 5 h), such that the presence of any [$^3$H]25,26-$(OH)_2D_3$ would be detected by a 50% loss of $^3$H from C-26 or C-27 due to periodate cleavage of the carbon-carbon bond between the vicinal hydroxyl groups.[4] Possible metabolism of [$^3$H]1$\alpha$,25-$(OH)_2D_3$ and (or) [$^3$H]24,25-$(OH)_2D_3$ to 1$\alpha$,24,25-$(OH)_3D_3$ and subsequent sequestration of the trihydroxy metabolite by intestinal tissue was evaluated by using a solvent system which resolves 1$\alpha$,24-25-$(OH)_2D_3$ (chloroform:hexane; 80:20; 170 ml) for chromatography of intestinal mucosal tissue. Plasma metabolic data for each animal was expressed as total plasma dpm with $^{131}$I-albumin used to estimate the plasma volume as 6.7% of the body weight. All radioactivity measurements were made with a Packard Liquid Scintillation Spectrophotometer model 3375 (Packard Instrument Co., Inc., Downers Grove, Ill.)

*Plasma calcium ana strontium.* Blood was taken in heparinized syringes via cardiac puncture and plasma prepared by centrifugation (1,500 *g*, 10 min). Plasma samples were diluted with 0.5% $LaCl_3$ (1:40) and total plasma calcium and strontium were determined by atomic absorption spectrophotometry (9) (Perkin-Elmer Corp., Norwalk, Conn., model 403).

*25-$OHD_3$ plasma determination.* Vitamin D-deficient rat serum was diluted (1:6,000) with a barbital/$\beta$-lipoprotein buffer (0.05 M sodium barbital, pH 8.6, containing 200-fold diluted $\beta$-lipoprotein from vitamin D-deficient chicks) and was used in a competitive binding assay for plasma 25-$OHD_3$ as described by Belsey et al. (24). Plasma assay samples were extracted with chloroform-methanol (22). The 25-$OHD_3$ was isolated from the lipid extract through use of minisilicic acid column chromatography (25) before its quantitation by use of the binding assay.

*Bone ash determination.* Tibiae were cleaned of muscle and marrow material then extracted with ethanol- and diethyl-ether (24 h each) before ashing for 24 h at 600°C. Percent ash was determined from the bone ash weight to dry bone weight ratio.

*Kinetic analysis.* The data were analyzed and fitted to multicompartmental models using the National Institute of Health Simulation Analysis and Modeling (SAAM) program described by Berman et al., and an IBM-360 digital computer (IBM Corp., White Plains, N. Y.) (26–28). The program derives values for the parameters of an assumed model to yield a least-squares fit of the data. This is accomplished by the generation of theoretical data based on initial estimates of the kinetic parameters for the model, and the calculation of partial derivatives for each theoretical datum with respect to each adjustable parameter in the model. Equations of conditions and normal equations are generated from the partial derivatives, and corrections for the initial estimates of the parameter values are calculated. This procedure is iterated until a least-squares fit of data is obtained, which yields a set of values for the parameters along with estimates of their uncertainties.

## RESULTS

The dietary strontium treatment was designed to effect a minimal perturbation in animal weight and plasma

[4] The C-26-designated hydroxyl group is actually equally distributed between C-26 and C-27 due to the symmetry of the two carbon atoms around C-25.

calcium concentration, prompting alterations in the kidney 25-$OHD_3$ metabolism and bone mineralization. Strontium-treated chicks showed a high plasma strontium concentration and significantly lower bone ash ($P < 0.01$), indicative of rachitic bone lesions, but they did not differ statistically from the control group with regards to body weight or plasma calcium concentration (Table I). Plasma 25-$OHD_3$ concentration within the two groups remained essentially constant throughout the experimental period, with a ±10% variation. The kinetic parameters derived for 25-$OHD_3$ metabolism were therefore analyzed using steady-state theory.

### *Model nomenclature*

The following terms and figures were used in developing the kinetic model for 25-$OHD_3$ metabolism:

*Compartment.* Represented by a circle and denotes a subdivision of a model used for analysis of experimental observations.

*Compartment size (V).* The total amount of material (pmol) in a compartment (i.e., pool).

*Rate constant (λ).* The fraction of total material in a compartment that is transported to another compartment per unit time. Results are expressed in reciprocal hours ($h^{-1}$). Where number subscripts are used, the first denotes the plasma metabolite comparment into which a metabolite enters and the second, the plasma metabolite compartment from which the metabolite leaves (e.g., $\lambda_{3,1}$ indicates the fraction of metabolite which leaves compartment one, via metabolic alteration, and enters compartment three). Irreversible loss from the model system (out) is designated "O" (e.g., $\lambda_{0,1}$ indicates irreversible loss of metabolite from compartment one due to excretory, metabolic, and(or) target organ sequestration processes).

*Transport (R).* The total amount of material in a compartment that is transferred to another compartment per unit time $R = [\lambda] \times (V)]$.

TABLE I
*Physiological Parameters and Plasma Half-Life for [$^3H$]25-$OHD_3$*

| | Control | Strontium |
|---|---|---|
| Mean weight, *g* | 192.1±14.4* | 179.5±17.7 |
| Plasma calcium concentration, *mM* | 2.43±0.30 | 2.36±0.23 |
| Plasma strontium concentration, *mM* | — | 0.90±0.19 |
| Bone ash, % | 26.5±2.3‡§ | 20.4±2.0 |
| Plasma $t_{\frac{1}{2}}$ for 25-OH-$^3$H-$D_3$ | | |
| Rapid, *h* | 1.0±0.05§ | 1.8±0.12 |
| Slow-extrapolation, *h* | 41.0±2.1§ | 79.0±5.1 |

* Mean ±SD for 12 observations unless stated otherwise.
‡ Mean ±SD for five observations.
§ Significantly different from strontium group ($P < 0.01$). Unpaired Student's *t*-test.

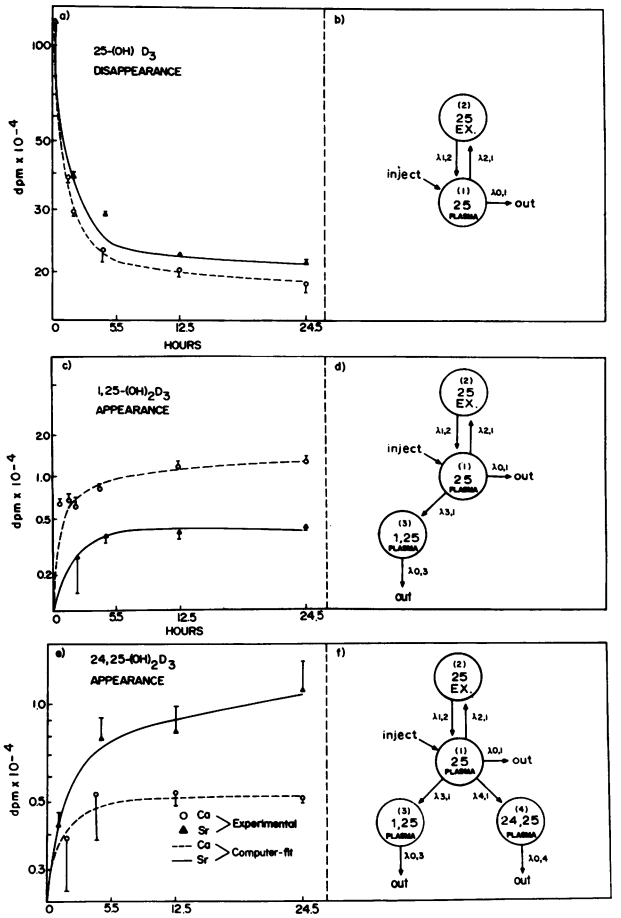


FIGURE 1 Plasma disappearance data for [$^3H$]25-$OHD_3$ and appearance data for [$^3H$]1α,25-$(OH)_2D_3$ and [$^3H$]24,25-$(OH)_2D_3$ with corresponding compartmental model for computer fit of data. Each data point is the mean ±SD for three observations. (a) [$^3H$]25-$OHD_3$ plasma disappearance curves; (b) compartment model which defines plasma [$^3H$]25-$OHD_3$ disappearance; (c) [$^3H$]1α,25-$(OH)_2D_3$ plasma appearance curves; (d) compartment model which defines plasma [$^3H$]25-$OHD_3$ disappearance and [$^3H$]1α,25-$(OH)_2D_3$ appearance; (e) [$^3H$]24,25-$(OH)_2D_3$ plasma appearance curves; (f) compartment model which defines plasma [$^3H$]25-$OHD_3$ disappearance and [$^3H$]1α,25-$(OH)_2D_3$ and [$^3H$]24,25-$(OH)_2D_3$ appearance.

### *Plasma [$^3H$]25-$OHD_3$ disappearance*

Data for [$^3H$]25-$OHD_3$ disappearance from plasma was fitted to a two-compartment model as shown in Fig. 1b. This model is the simplest compartmental system that can define the biphasic disappearance curve which consist of rapid and slow disappearance phases (Fig. 1a). To derive a calculated fit to the observed data it was found necessary to separate the plasma transfer of [$^3H$]25-$OHD_3$ into two pathways—

TABLE II

*Kinetic Parameters for [$^3H$]25-Hydroxyvitamin $D_3$ Metabolism in Control and Strontium-Treated Chicks*

| | Control | | Strontium | |
|---|---|---|---|---|
| | Rate constant ($\lambda$) | Transport ($R$) | Rate constant ($\lambda$) | Transport ($R$) |
| | ($h^{-1}$)* | *pmol/h* | ($h^{-1}$) | *pmol/h* |
| 25-$OHD_3$ Exchange with non-vascular 25-$OHD_3$ pool | 0.66±5.3% ($\lambda_{2,1}$)‡ | 93.6 | 0.38±6.5% ($\lambda_{2,1}$) | 60.3 |
| 25-$OHD_3$ Metabolically irreversible | 0.096±38% ($\lambda_{0,1}$) | 13.6 | 0.039±85% ($\lambda_{0,1}$) | 6.2 |
| 1$\alpha$,25-$(OH)_2D_3$ Appearance in plasma from 25-$OHD_3$ | 0.005±4.3% ($\lambda_{3,1}$) | 0.7 | 0.002±3.2% ($\lambda_{3,1}$) | 0.3 |
| 24,25-$(OH)_2D_3$ Appearance in plasma from 25-$OHD_3$ | 0.0027±19.5% ($\lambda_{4,1}$) | 0.4 | 0.0030±18.6% ($\lambda_{4,1}$) | 0.5 |
| 25-$OHD_3$ Exchange with gut | 0.0044±20.1% ($\lambda_{9,1}$) | 0.6 | 0.017±15.4% ($\lambda_{9,1}$) | 2.7 |
| 1$\alpha$,25-$(OH)_2D_3$ Appearance in gut from 25-$OHD_3$ | 0.0068±12.3% ($\lambda_{7,1}$) | 1.0 | 0.0012±8.2% ($\lambda_{7,1}$) | 0.2 |

* Calculated value±% SD for three observations/data point.

‡ Indicates rate constant symbol with respect to derived compartmental models in Figs. 1 and 2, as explained in Results.

irreversible loss from the plasma (rate constant $\lambda_{0,1}$) and reversible "exchange" with an unknown extravascular compartment (rate constant, $\lambda_{2,1}$). The "exchangeable" extravascular pool allows for metabolite transport into compartment two and return to the plasma compartment one without any metabolic alteration. Although not defined, this compartment would seemingly consist of reversible metabolite exchange with low affinity lipophilic components of the general tissue mass (e.g., muscle and adipose tissue). In contrast, irreversible metabolite transport (rate constant $\lambda_{0,1}$) represents the [$^3H$]25-$OHD_3$ which is potentially available for high affinity binding, excretion, and (or) metabolic alteration. Strontium-treated chicks demonstrated a higher amount of plasma [$^3H$]25-$OHD_3$ throughout the study ($P < 0.01$) which suggested a slower metabolite disappearance rate for the experimental group (Fig. 1a). Evidence to this effect was obtained by calculating the plasma half-life values for [$^3H$]25-$OHD_3$. Animals in the strontium group had consistantly higher values in comparison to the control group for the rapid (1.8 compared to 1 h) and slow-extrapolated (41 compared to 71 h) disappearance phases (Table I). The higher half-life values could be attributed to decreases in efflux of metabolite from plasma into both the exchangeable and metabolic-irreversible 25-$OHD_3$ pools (Table II). A small but significant elevation in total plasma 25-$OHD_3$ (Table III) was also observed in the strontium group, which contributes to the longer [$^3H$]25-$OHD_3$ half-life.

TABLE III

*Metabolite Plasma Concentration and Pool Size*

| | 25-$OHD_3$ | | 1$\alpha$,25-$(OH)_2D_3$ | | 24,25-$(OH)_2D_3$ | |
|---|---|---|---|---|---|---|
| | Concentration | Total | Concentration | Total | Concentration | Total |
| | *pmol/ml** | *pmol* | *pmol/ml*‡ | *pmol* | *pmol/ml*‡ | *pmol* |
| Control | 11.0±0.5§ | 142±6 | 1.0±0.04 | 13.7±0.6 | 0.4±0.02 | 5.0±0.2 |
| Strontium | 13.0±0.6 | 158±7 | 0.3±0.01 | 3.7±0.2 | 0.9±0.04 | 11.0±0.5 |

* Mean±SD for eight observations.

‡ Calculated value±SD for three observations/data point.

§ Significantly different from strontium group ($P < 0.01$) for all data columns.

### *Plasma [$^3H$]1α,25-$(OH)_2D_3$ and [$^3H$]24,25-$(OH)_2D_3$ appearance*

Kidney 25-$OHD_3$ metabolism is essentially an irreversible process whereby synthesized 1α,25-$(OH)_2D_3$ and (or) 24,25-$(OH)_2D_3$ do not exchange with 25-$OHD_3$ (i.e., product is not converted back to substrate at a detectable rate).[5] Plasma appearance of the two metabolites was therefore fitted to respective single compartments in which metabolite transport from the kidney was irreversible (Fig. 1d, f).

Plasma appearance of [$^3$H]1α,25-$(OH)_2D_3$ was markedly suppressed in the strontium group (Fig. 1c), with transport of the metabolite being 43% of control (Table II). Calculations for metabolite plasma pool size using transport and rate constant values showed the 1α,25-$(OH)_2D_3$ pool for the strontium group (3.7 pmol) to be only 27% as large as the control pool (13.7 pmol) (Table III). This difference ($P < 0.01$) in pool size is not directly predictable on the basis of transport values for 1α,25-$(OH)_2D_3$ in the two groups but rather involves an increase in the rate of metabolite efflux from the plasma pool for the strontium treated animals ($\lambda_{0,3}$ is 0.07 $h^{-1}$ for strontium and 0.055 $h^{-1}$ for control).

Parameters describing plasma [$^3$H]24,25-$(OH)_2D_3$ appearance are in general diametric to those just described for [$^3$H]1α,25-$(OH)_2D_3$. Dietary strontium acted to increase in [$^3$H]24,25-$(OH)_2D_3$ plasma appearance (Fig. 1e), which was 25% greater than control values for transport of 25-$OHD_3$ to 24,25-$(OH)_2D_3$ (Table II). Even more illustrative of this phenomenon are the calculations for the 24,25-$(OH)_2D_3$ plasma pool size. Strontium-treated animals had a plasma pool of 11 pmol, which was over two times the estimated value for the control pool (5 pmol) (Table III). This significant difference ($P < 0.01$) appears attributable to a decrease in the plasma efflux rate for 24,25-$(OH)_2D_3$ in the strontium compared to control group (i.e., $\lambda_{0,4}$ = 0.043 $h^{-1}$ for strontium and 0.076 $h^{-1}$ for control).

### *Intestinal [$^3H$]1α,25-$(OH)_2D_3$ appearance*

Intestinal [$^3$H]1α,25-$(OH)_2D_3$ sequestration was significantly decreased ($P < 0.01$) in strontium compared to calcium-treated chicks (Fig. 2a). The intestine was treated as a metabolite sink and the data fitted to a single compartment with irreversible metabolite flow. There was a necessity for including a delay scheme (Fig. 2b, compartments five, and six) for [$^3$H]1α,25-$(OH)_2D_3$ appearance in the strontium group due to the slower rate of metabolite appearance, however, such a delay scheme was not required to fit the control data. The rate constant for intestinal [$^3$H]1α,25-$(OH)_2D_3$ appearance was extremely depressed in the strontium group (0.0012 $h^{-1}$ compared to 0.0044 $h^{-1}$ for control) as reflected by a metabolite transport rate 0.8 pmol/h which was 30% of the control value (Table II). Estimation of the steady-state 1α,25-$(OH)_2D_3$ intestinal concentration using the efflux rate constant (i.e., $\lambda_{0,7}$ = 0.039 for strontium and 0.014 for the control group) gives values of 1.5 pmol/g for the strontium group and 15.9 pmol/g for control animals (Table IV).

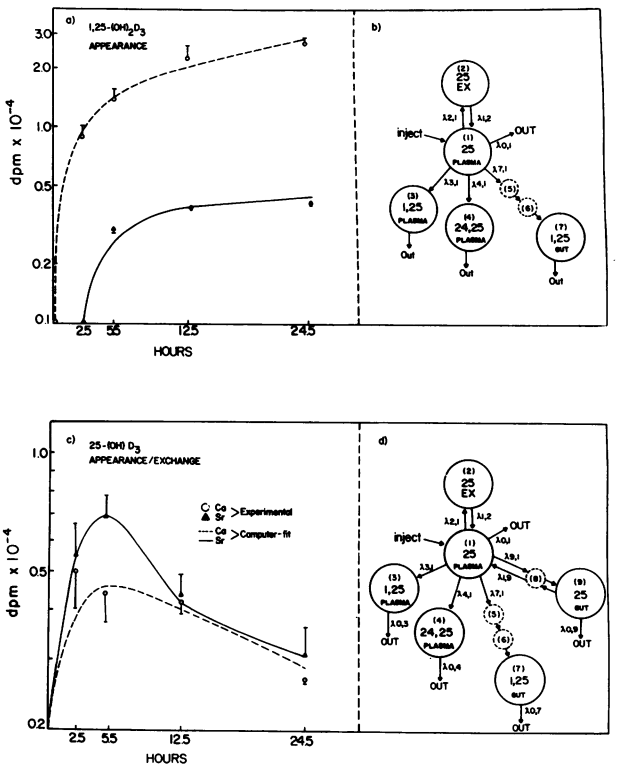


FIGURE 2 Intestinal data for [$^3$H]1α,25-$(OH)_2D_3$ appearance and [$^3$H]25-$OHD_3$ appearance (exchange). Each data point is the mean±SD for three observations. (a) [$^3$H]1α,25-$(OH)_2D_3$ intestinal appearance curves; (b) model which defines plasma data in Fig. 1 and intestinal [$^3$H]1α,25-$(OH)_2D_3$ appearance. Dotted circles are delay components which are required to fit strontium data only; (c) [$^3$H]25-$OHD_3$ appearance exchange curves for the intestine; (d) complete model which defines plasma data in Fig. 1 plus intestinal [$^3$H]1α,25-$(OH)_2D_3$ appearance and intestinal [$^3$H]25-$OHD_3$ appearance (exchange).

### *Intestinal [$^3H$]25-$OHD_3$ appearance (exchange)*

Appearance of intestinal [$^3$H]25-$OHD_3$ was linear for the first 5 h, and diminished thereafter with time (Fig. 2c). Rapid metabolite appearance and decline suggested an exchange phenomenon with plasma 25-$OHD_3$, similar to that previously discussed for the plasma metabolite and its possible extravascular ex-

[5] Personal observation.

change. Intestinal [$^3$H]25-OHD$_3$ was therefore treated as a component of the extravascular pool (i.e., compartment two) in which the data could be fitted to a single compartment with the reversible route for metabolite exchange (Fig. 2d). Similar to intestinal [$^3$H]-1$\alpha$,25-(OH)$_2$D$_3$ appearance, it was necessary to include a delay component (compartment eight) when fitting the strontium [$^3$H]25-OHD$_3$ data. As observed in Fig. 2c there was an initial higher rise in intestinal [$^3$H]25-OHD$_3$ for the strontium group, resulting in a metabolite transport which was greater than four times that observed in control chicks (Table II, $\lambda_{0.9}$ [Strontium] = 0.58 h$^{-1}$ and $\lambda_{0.9}$ [calcium] = 0.79 h$^{-1}$). Intestinal steady-state pool estimates showed the metabolite's concentration to be elevated ≅50% in the strontium group.

In contrast to the previously mentioned mucosal sequestration of [$^3$H]1$\alpha$,25-(OH)$_2$D$_3$, there was no detectable intestinal uptake of [$^3$H]24, 25-(OH)$_2$D$_3$ in this experiment. Similar to Holick et al. (29), we have never detected a significant amount of this metabolite in chick mucosal tissue after an injection of [$^3$H]25-(OH)D$_3$. It would appear, therefore, that if intestinal receptors exist for 24,25-(OH)$_2$D$_3$, they are fewer in number and (or) of lower affinity than the molecules associated with the intestinal sequestration of 1$\alpha$,25-(OH)$_2$D$_3$.

Subsequent metabolism of [$^3$H]1$\alpha$,25-(OH)$_2$D$_3$ or [$^3$H]24,25-(OH)$_2$D$_3$ to [$^3$H]1$\alpha$,24,25-(OH)$_3$D$_3$ (30) appears to have occurred to only a limited extent, as evidenced by marginally detectable radioactivity in intestinal mucosal tissue.[5] The data was not statistically significant and consequently the metabolic pathway was not included in the model for 25-OHD$_3$ metabolism.

## DISCUSSION

Dietary strontium was used as the perturbing agent to suppress 1$\alpha$,25-(OH)$_2$D$_3$ and induce 24,25-(OH)$_2$D$_3$ synthesis. Strontium appears to act by decreasing peripheral PTH concentration and possibly by directly antagonizing calcium-dependent cellular processes.[3]

TABLE IV
*Estimate of Intestinal Metabolite Concentration and Pool Size**

| | 25-OHD$_3$ | | 1$\alpha$,25-(OH)$_2$D$_3$ | |
|---|---|---|---|---|
| | Concentration | Total | Concentration | Total |
| | *pmol/g* | *pmol* | *pmol/g* | *pmol* |
| Control | 1.1±0.2 | 4.8±1.0 | 15.9±3.2 | 68.1±13.6 |
| Strontium | 1.6±0.3 | 5.4±0.8 | 1.5±0.2‡ | 5.0±0.8‡ |

* Calculated values±SD for three observations/data point.
‡ Significantly different from control group ($P < 0.01$).

The strontium prompted changes in reaction and exchange rates and steady-state metabolite pool sizes for 1$\alpha$,25-(OH)$_2$D$_3$ and 24,25-(OH)$_2$D$_3$ were quantitated by performing an analysis of in vivo [$^3$H]25-OHD$_3$ metabolism. Kinetic metabolite data from the plasma and intestinal pools were coupled with current concepts regarding 25-OHD$_3$ metabolism to generate a computer-derived model for 25-OHD$_3$ metabolism and intestinal metabolite sequestration, as shown in Fig. 3. No attempt was made to incorporate a nonvascular exchange pathway for plasma [$^3$H]24,25-(OH)$_2$D$_3$ and [$^3$H]1$\alpha$,25-(OH)$_2$D$_3$ since data was not available to address this particular subject. Also, during model development we attempted to fit the experimental data using a three-compartment model for plasma [$^3$H]25-OHD$_3$ disappearance as well as delay compartments for plasma [$^3$H]24,25-(OH)$_2$D$_3$ and [$^3$H]-1$\alpha$,25-(OH)$_2$D$_3$ appearance. None of these modifications improved the fit given by the basic six-compart-

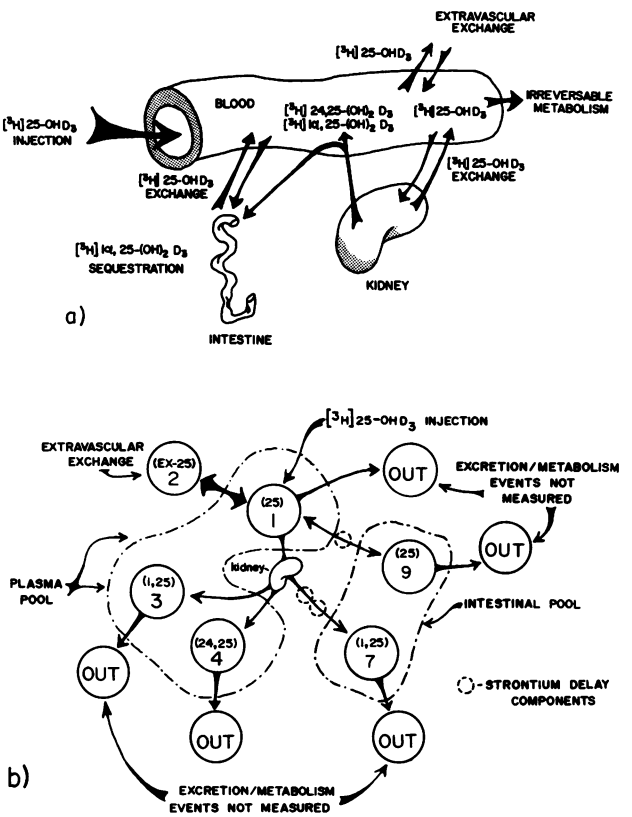


FIGURE 3 Schematic diagrams for organ involvement in [$^3$H]25-OHD$_3$ metabolism and the developed compartmental model. (a) A summary of the organ involvement and metabolic sequence which was used in building the model for [$^3$H]25-OHD$_3$ metabolism in the chick; (b) compartment model for [$^3$H]25-OHD$_3$ metabolism-sequestration showing the separation of plasma and intestinal pools and the function of the "out" components. This figure is a schematic of Fig. 2d.

ment model. Closeness of fit between experimental and computer-generated data was good, however, which suggest that the six-compartment model is appropriate for the major pathways of 25-$OHD_3$ metabolism.

An enlarged 25-$OHD_3$ plasma pool in strontium-treated chicks was indicated by the slower disappearance curve for [$^3$H]25-$OHD_3$ (Fig. 1a; Table I) and subsequently verified by measurement of the plasma 25-$OHD_3$ concentration. The biphasic plasma [$^3$H]25-$OHD_3$ disappearance curve is similar to that shown for cholesterol, 25-$OHD_3$ and 1$\alpha$,25-$(OH)_2D_3$ in patient studies (31–33), although the metabolite's disappearance in the chick occurs much faster (6- to 10-fold increase) than that observed in humans.

The necessity for a 25-$OHD_3$ nonvascular exchange pool in the model for plasma [$^3$H]25-$OHD_3$ disappearance (Fig. 1b) suggest a general tissue distribution and exchange for the metabolite which is similar to vitamin $D_3$ (34). Specific binding of 25-$OHD_3$ has been observed in a variety of tissues for both the rat and chick (35, 36). What fraction of the total tissue sequestered 25-$OHD_3$ is attributable to such high affinity binding is currently unknown, however, data from this study showed that ≅80% of the total 25-$OHD_3$ was contained in an exchangeable nonvascular pool (i.e., $\lambda_{1,2}$ [control] = 0.16 $h^{-1}$ and $\lambda_{1,2}$ [strontium] = 0.087 $h^{-1}$). Possible tissue uptake and exchange of 25-$OHD_3$ is exemplified in the present study by the kinetics of intestinal [$^3$H]25-$OHD_3$ binding and turnover (Fig. 2d). The egress of [$^3$H]25-$OHD_3$ from the intestine nearly coincided with the decrease in rate of plasma [$^3$H]25-$OHD_3$ disappearance (i.e., ≅5.5 h after pulse [$^3$H]25-$OHD_3$ injection.) Since 25-$OHD_3$ is not known to be metabolized in the intestine it seems that the decrease in intestinal content was mainly reflective of the metabolite's retrograde movement into the vascular system. An additional input of [$^3$H]25-$OHD_3$ into the plasma pool could be attributed to enterohepatic circulation of the metabolite (37).

In strontium-induced rachitic chicks the rate of renal 1$\alpha$,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ synthesis and subsequent transport into the plasma compartment occurred at 43 and 125% of control values, respectively. Such transport-rate values are reflective of the two metabolites differing plasma-pool sizes (i.e., strontium had a smaller 1$\alpha$,25-$(OH)_2D_3$ and a larger 24,25-$(OH)_2D_3$ plasma pool). Relative to the control group, strontium acted to decrease plasma 1$\alpha$,25-$(OH)_2D_3$ (3.7 compared to 13.7 pmol) and increase plasma 24,25-$(OH)_2D_3$ (11 compared to 5 pmol). Such a decrease in the 1$\alpha$,25-$(OH)_2D_3$ pool is demonstrable in hypoparathyroid patients (14). Whether a concomitant increase in the plasma 24,25-$(OH)_2D_3$ pool also occurs, as demonstrated in this study, has not been established (13). To date, an increase in serum 24,25-$(OH)_2D^1$ concentration has been established only in patients with elevated vitamin D intakes (38).

The plasma concentration ratio of 25-$OHD_3$ to 1$\alpha$,25-$(OH)_2D_3$ was 10 for control and 43 for strontium animals, which is lower than that reported for humans (range 40–1,000) (13, 23). Such low precursor-product ratios in the chick appear due to the high values for 1$\alpha$,25-$(OH)_2D_3$ relative to 25-$OHD_3$, which were augmented by use of a control diet that contains one-half the normal amount of calcium (i.e., 0.13 mmol/g compared to 0.26 mmol/g). The relatively high rate of 25-$OHD_3$ metabolism may also be contributory as exemplified kinetically by the control chick's catalytic-potential to replace its 1$\alpha$,25-$(OH)_2D_3$ plasma pool every 20 h (i.e., plasma pool divided by transport rate, Tables II, III).

The model for the intestinal appearance of 1$\alpha$,25-$(OH)_2D_3$ and 25-$OHD_3$ was developed assuming an irreversible process for [$^3$H]1$\alpha$,25-$(OH)_2D_3$, and a reversible process for [$^3$H]25-$OHD_3$ due to the lack of definitive information on possible exchange processes. These assumptions were based on data for the metabolites appearance and (or) exchange in mucosal tissue (Fig. 2) and *in vitro* binding constant for 1$\alpha$,25-$(OH)_2D_3$ with the intestinal cytosol receptor (39). Intestinal handling of the two metabolites was strikingly different. A rapid influx and subsequent efflux was observed for [$^3$H]25-$OHD_3$, whereas [$^3$H]1$\alpha$,25-$(OH)_2D_3$ was continuing to increase in concentration at 24 h after the pulse [$^3$H]-25-$OHD_3$ injection. Such data is characteristic of specific and nonspecific hormone sequestration by a target organ, in which high- and low-cellular binding affinities are observed for biologically active (e.g., 1$\alpha$,25-$(OH)_2D_3$) and nonactive forms (e.g., 25-$OHD_3$) of the hormone (40). The presence of an intestinal cytosol receptor which has a greater affinity for 1$\alpha$,25-$(OH)_2D_3$ than for 25-$OHD_3$ has been described (39) and shown to have a $K_d$ of 2.2 nM; indicating that the binding process is approaching irreversibility. Saturation of such high affinity-binding sites occurs in vitro at ≅10 nM 1$\alpha$,25-$(OH)_2D_3$ (39), which indicates that such intestinal binding sites are nearly saturated with 1$\alpha$,25-$(OH)_2D_3$ in the present study (intestinal concentration range, 1.6–16 nM; Table IV). It would appear, therefore, that *de novo* synthesized metabolite would most effectively compete for binding sites in newly synthesized epithelial cells. Accordingly, we assumed that 1$\alpha$,25-$(OH)_2D_3$ intestinal binding and turnover was strongly dependent upon the epithelial cell life and therefore constituted prodominantly an irreversible process. Such a high tissue specificity for 1$\alpha$,25-$(OH)_2D_3$ became especially evident when the intestinal concentration of metabolite was 5 to 15 times greater than that of plasma. In contrast, 25-$OHD_3$ was present in intestinal tissue at only ≅10% of the concentration observed in plasma.

The genesis of suppressed intestinal-calcium absorption in strontium rickets became evident when 25-$OHD_3$ metabolism was analyzed kinetically during strontium feeding. Intestinal 1$\alpha$,25-$(OH)_2D_3$ content was suppressed to 10% of control due to a fivefold decrease in rate of renal synthesis and over a twofold increase in metabolite turnover rate (i.e., $\lambda_{0,7}$ [control] = 0.014 $h^{-1}$ and $\lambda_{0,7}$ [strontium] = 0.039 $h^{-1}$). It is possible from such results to quantitate both the turnover and synthetic components of 25-$OHD_3$ metabolism and to determine how metabolic perturbations affect both parameters. Such a comprehensive insight is not obtainable from either ambient metabolite concentrations or kidney hydroxylase activities alone.

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