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The Kinetics of Vitamin D₃ in the Osteoblastic Cell

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Abstract

Experimental evidence is presented on the translocation of vitamin D metabolite, 1,25-(OH)₂D₃, from the membrane to the nucleus in osteoblast progenitor cells. A mathematical model permitting traversal of the cytoplasm at either a fixed velocity or by diffusion is formulated in order to determine whether transport along the cytoskeletal tracks is more consistent with the observed spatial-temporal distribution than diffusion, and it is so found. The model includes reactions in the nucleus involving D₃ to form other compounds, such as protegerin, and thus also makes predictions of the concentrations of these compounds in various regions of the cell.

1 Introduction

Bone is a composite structure of living cells. Bone contains an organic, highly mineralized matrix that is maintained partially by bone-building cells, osteoblasts and osteoclasts [17]. Other cells such as adipocytes and osteocytes also contribute to the bone's specific properties. Two types of bone make up the skeleton: flat bones and long bones. Flat bones are the skull, scapula, mandible and ileum, and long bones include, among other bones, the tibia, femur, humerus, and ribs. Flat bones develop by intramembranous ossification, whereas long bones develop by endochondral ossification.

Bone formation is tightly regulated by two key players osteoclasts (bone resorbing cells) and osteoblasts (bone forming cells) [17, 25]. The balance between active osteoblasts and osteoclasts is crucial for proper bone formation and maintenance. Osteoblasts and osteoclasts are derived from different progenitor cells. Osteoblasts arise from mesenchymal

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cells, while osteoclasts arise from the hematopoietic stem cells. 1,25 dihydroxyvitaminD₃, the active form of Vitamin D₃ (calcitriol), plays a critical role during bone formation and modulates bone growth and bone turnover [7]. Osteoblasts express the vitamin D receptor (VDR) that binds to calcitriol when it enters the cell after dissociating from the serum binding vitamin D binding protein (DBP) in the circulation [2],[15]. It is been hypothesized that calcitriol enters the cell in its free unbound hydrophobic form via diffusion through the cell membrane as stated by the free hormone hypothesis [27]. Alternative models propose a membrane receptor VDR_{mem} interact with calcitriol enhancing its uptake into the cell. Recent researchers suggested the membrane associated rapid response to steroids (MARRS) to function as VDR_{mem}. Typically, the DBP carrier proteins, exterior to the cell, are present at high concentrations and have high of rates relative to the VDR membrane receptors [13], [20], VDR_{mem}, that are present at much lower concentrations, but have higher binding affinities. These reactions are fast (seconds-minutes) and mediate catabolic effects. The nuclear receptor, VDR_{nuc}, in osteoblast cells that respond to calcitriol, is a slow (hours)¹, non-calcemic receptor mediating anabolic effects [2],[21].

After entering osteoblasts, calcitriol interacts with VDR_{nuc} and the retinoic acid receptor to initiate transcription of the gene encoding receptor activator of RANKL [28]. For this calcitriol and VDR interact with the retinoic acid receptor and translocate to the nucleus. VDR was found to interact with actin cytoskeleton and microtubules to traffic to the nuclear pores [6]. While microtubules participate in intracellular transport of D₃ [10], the details of the transport mechanism are unknown. Once in the nucleus the complex binds to target genes and activate the transcription of osteoblast specific genes such as RANKL. The RNA encoding RANKL is exported to the cytoplasm where it is translated into protein on the ribosome. The new protein is then inserted into the secretory pathway for export. The mechanism by which RANKL is converted from a membrane-bound ligand to a soluble structure is unclear. On the other hand the expression of OPG disrupts RANKL signaling. There have been a few attempts to model the dynamics of bone remodeling at the cellular level. Those known to the authors are [16], [11], [14], [23], [26].

In Section 2, evidence is presented that calcitriol molecules conjugated with quantum dots (calQDs), a fluorescent probe developed by [3], require hours to cross the cytoplasm of an osteoblast progenitor cell. Estimates in the literature on the rate of diffusion of molecules of this size indicate that transport through the cytoplasm should be faster than this, on the scale of minutes. An objective of this article is to construct a mathematical model that accommodates both transport at a fixed velocity along a specified path, as might occur along the cytoskeletal tracks, as well as transport by diffusion, in order to investigate the effects when one or the other mechanism of transport is dominant. Figure 1 shows that it takes longer than a few minutes for the calQDs to reach the nucleus; indeed, many calQDs are still in the cytoplasm at 2 hours, and do not become concentrated in the nucleus until 4 hours. That an injection of calcitriol is transported to the nucleus over the course of hours and its concentration has almost returned to its background level in the cytoplasm, while continuing to be elevated in the nucleus, will be termed the *pulse observation*.

¹It may be seen from Figure(1) the Calcitriol has clearly entered the nucleus by 2 hours.

2 Experimental results and diffusion parameters

In this section experimental results are presented on transport of vitamin D through the cytoplasm and into the nucleus. To produce the kinetics illustrated in Figure 1, C2C12 cells were treated with calQDs, as seen in the green channel, for 2 or 4 hour intervals. The 0 hour treatment has no addition of calQDs. C2C12 cells were stained for 20 minutes before imaging with 20 μ M of Hoechst 33342 (Thermo Scientific, West Palm Beach, FL) fluorescent nucleus stain. Additionally, directly before imaging, the cells were stained with 158 μ M Fm4–64 (Invitrogen, Carlsbad, CA) membrane stain. Images were taken using lasers of 405nm wavelength for the Hoechst stain, and 488nm for the QDs and Fm4–64 membrane stain. The red channel displays the membrane stain and the blue channel displays the nucleus stain. Selected z-stack images were taken using the Zeiss LSM 510 Live5 Confocal Microscope that represent the general appearance of Calcitriol bound QDs inside the cells.

A typical osteoblast is a discoid with a diameter around 30 μ m and height around 10 μ m ([9]). Estimates of the rate of diffusion of molecules in the cytoplasm and nucleoplasm may be found in the literature. According to [19] diffusivities for DNA molecules in buffer are in the range $K = 10\text{--}100 \mu\text{m}^2 \text{s}^{-1}$ for masses in the range 10–200 kDa. These are substantially more massive than calcitriol, which has a mass of around 0.4 kDa. It is estimated that the crowded nature of the cytoplasm and nucleoplasm may reduce the diffusivity by an order of magnitude or more. Similarly [12] give a range of $1\text{--}10 \mu\text{m}^2 \text{s}^{-1}$ for dextran molecules with masses in the range 30–100 kDa in the cytoplasm and nucleoplasm, and the review article of [18] suggests a reduction to 0.4–0.1 as the hydrodynamic radius increases from 10 to 100 Å. The simulations in [22] give quantitative estimates of effective diffusivity as a function of the proportion of cytoplasmic volume occupied by obstacles such as the cytoskeletal network and internal membranes. These simulations are generally in accord with the results cited above, suggesting reductions of about an order of magnitude in effective diffusivity, with near complete impermeability requiring that the volume accessible to diffusion be quite low, in the 10–50% range, depending on the nature of the obstructions. Based on such estimates, Einstein's formula for average particle dispersion due to Brownian motion, $\langle x^2 \rangle = 2Kt$, implies that molecular diffusion through a cell of the dimensions of an osteoblast would require on the order of minutes if $K \sim 1 \mu\text{m}^2 \text{s}^{-1}$, which is faster than is suggested in Figure 1. In this article diffusion rates of the magnitude cited above will be used in the membrane and nucleus, but the possibility of diffusion rates substantially lower will be considered for the cytoplasm.

3 A mathematical model for the transport and metabolism of D_3

A system of partial differential equations is sought to model the kinematics described in Section 1 and to permit exploration of the parameter regimes which are consistent with the observations of Section 2. This entails simplifying assumptions, which are discussed below.

3.1 Spatial partition of the cell

The regions occupied by the nucleoplasm, cytoplasm and membrane are denoted by $\mathcal{R}_n$, $\mathcal{R}_c$, and $\mathcal{R}_m$ respectively. The membrane region includes the part of the cytoplasm adjacent to the membrane in which transport is purely by diffusion. The corresponding outer

boundaries of the three regions are denoted by Γ_M , $M = n, c, m$. The outward unit normal vector to a surface Γ_M is denoted by $\hat{\nu}_M$. The concentration of the D_3 hormone is indicated by D_M , $M = n, c, m$. Its nuclear receptor VDR_{nuc} is indicated by N , and the concentration of product formed by D_3 and VDR_{nuc} by $V_n = D \cdot N$. The concentration of product in the other regions is denoted by V_M , $M = c, m$. The velocity for carrying D_3 along a microtubule in the cytoplasm to the nucleus is c_D and the velocity carrying V from the nucleus through the cytoplasm is c_V .

3.2 Crossing the cell boundary

Detailed modeling of the entry of D_3 through the membrane is not attempted in the article, but will be considered in a subsequent papers, see for example, [8]. In this article to simulate the membrane receptor response, the flux, $K_{mD} \nabla \vec{D}_m$, of D_3 into the cell is taken to be the sum of a constant flux term and a term proportional to the difference between the concentration on the cell boundary and the ambient extra-cellular concentration. The latter term is incorporated because in models for molecular entry into the cell buffering and delay are found to be important [24]. By permitting backflow out of the cell the latter term affords a primitive form of buffering. The outflux of V through the cell boundary is taken to be proportional to its ambient extra-cellular concentration. Hence the boundary conditions are

$$K_{mD} \nabla \vec{D}_m \Big|_{\Gamma_m} \cdot \hat{\nu}_m = -\Phi_D(t) - H_D(D_m - \eta_D) \Big|_{\Gamma_m} \quad K_{mV} \nabla \vec{V}_m \Big|_{\Gamma_m} \cdot \hat{\nu}_m = -H_V(V_m - \eta_V) \Big|_{\Gamma_m}.$$

Taking $\Phi_D(t)$ to be non-zero for a finite duration and $H_D = 0$ approximates the MARRS hypothesis described above.

3.3 Membrane region

Transport of both D and V through the membrane region is assumed to be purely by diffusion

$$\frac{\partial J_m}{\partial t} = K_{mJ} \nabla^2 J_m, J = D, V.$$

3.4 Cytoplasm

One possible means for the translocation of vitamin D_3 to the nucleus is diffusion, but the use of a chaperon that moves along cytoskeletal tracks through the cytoplasm is another possibility. Starting from the scalar mass transport equation

$$\frac{\partial J}{\partial t} = -\nabla \cdot [\vec{\varphi}_J + J \vec{u}_J],$$

applying Fick's law $\vec{\varphi}_J = -K_{cJ} \nabla \vec{J}$, and writing the velocity field as $\vec{u}_D = -c_D \hat{u}(x)$, $\vec{u}_V = c_V \hat{u}(x)$, where $\hat{u}(x)$ is a unit vector pointing toward the cell boundary, give equations

$$\frac{\partial D_c}{\partial t} = K_{cD} \nabla^2 D_c + c_D \vec{\nabla} \cdot (D_c \hat{u}) \quad \frac{\partial V_c}{\partial t} = K_{cV} \nabla^2 V_c - c_V \vec{\nabla} \cdot (V_c \hat{u})$$

that accommodate both diffusion and fixed-velocity transport along specified paths.

3.5 Nucleus

The binding with VDR_{nuc} is described by a reaction-diffusion equation that describes the signal cross-talk with the membrane receptor to the nuclear receptor depends on a threshold effect that induces a change in the kinetics of the nuclear response, with a time lag before the signal is received. Modeling the signaling is deferred to a later article. As described above, synthesis of OPG or RANKL is a multistep process involving exporting RNA encoding to the cytoplasm where the synthesis is completed. This will be simplified to the assumption that the product is synthesized entirely in the nucleus and simply transported through the cytoplasm to the cell boundary.

The binding-unbinding process resulting in the formation of V_n is illustrated schematically by

where k_d is the rate of the destruction of D_n due to causes other than formation of V_n . The rates of conversion of D_n and V_n are given by the mass-action terms

$\mp(k_2 V_n - k_1 [R_V^N - V_n] D_n)$ where the availability of receptors R_V^N imposes a carrying capacity on the process. Incorporating diffusion terms and the possibility of D_n being synthesized within the nucleus, results in the partial differential equations

$$\frac{\partial D_n}{\partial t} = K_{nD} \nabla^2 D_n + k_2 V_n - k_1 [R_V^N - V_n] D_n + S_D - k_d D_n \quad \frac{\partial V_n}{\partial t} = K_{nV} \nabla^2 V_n - (k_2 V_n - k_1 [R_V^N - V_n] D_n). \quad (1)$$

3.6 Interface conditions

At the interfaces continuity of concentration and molar flux requires

$$J_M|_{\Gamma_M} = J_c|_{\Gamma_M}, \quad J = D, V; M = m, n \quad K_{MJ} \vec{\nabla} J_M \Big|_{\Gamma_M} \cdot \vec{\nu}_M = K_{cJ} \vec{\nabla} J_c \Big|_{\Gamma_M} \cdot \vec{\nu}_M, \quad J = D, V; M = m, n.$$

4 Non-dimensionalization

In order to study the transport of D_3 through the cell, the time scale chosen is hours. The following reference values for distance, concentration, and diffusivity are chosen

$$\bar{t}=1\text{h}, \bar{\ell}=10\mu\text{m}, \bar{C}=1\text{pM}, \bar{K}=10^4\mu\text{m}^2\text{h}^{-1}. \quad (2)$$

The reference magnitudes for length and diffusivity are based on the discussion in Section 2, with a high value for diffusivity, $K \approx 3 \mu\text{m}^2 \text{s}^{-1}$, which is consistent with the literature, being adopted. The corresponding dimensionless independent and dependent variables are denoted by

$$t'=t/\bar{t}, x'_j=x_j/\bar{\ell}, j=1, 2, 3, J'=J/\bar{C}, J=D, V, K'=K/\bar{K}.$$

4.1 Cell boundary and membrane region

Changing to the dimensionless variables leads to the boundary conditions

$$\varepsilon^{-1} K'_{mD} \vec{\nabla}' D'_m \Big|_{\Gamma_m} \cdot \hat{\nu}_m = -\Phi'_D - H'_D (D'_m - \eta'_D) \Big|_{\Gamma_m} \quad \varepsilon^{-1} K'_{mV} \vec{\nabla}' V'_m \Big|_{\Gamma_m} \cdot \hat{\nu}_m = -H'_V (V'_m - \eta'_V) \Big|_{\Gamma_m}$$

where

$$\varepsilon = \frac{\bar{\ell}^2}{\bar{K}\bar{t}} = 0.01, \Phi'_J = \frac{\bar{t}}{\bar{C}\bar{\ell}} \Phi_J, H'_J = \frac{\bar{t}}{\bar{\ell}} H_J, \eta'_J = \frac{\eta_J}{\bar{C}} \quad J=D, V.$$

The diffusion equations in the membrane region become

$$\varepsilon \frac{\partial J'_m}{\partial t'} = K'_{mJ} \nabla'^2 J'_m, \quad J=D, V. \quad (3)$$

4.2 Cytoplasm

Taking the unit vectors describing the velocity field for mass transport along the cytoskeletal tracks to point outward toward the cell boundary gives the non-dimensional equations

$$\varepsilon \frac{\partial D'_c}{\partial t'} = K'_{cD} \nabla'^2 D'_c + \varepsilon c'_D \vec{\nabla}' \cdot (D'_c \hat{u}) \quad \varepsilon \frac{\partial V'_c}{\partial t'} = K'_{cV} \nabla'^2 V'_c - \varepsilon c'_V \vec{\nabla}' \cdot (V'_c \hat{u}), \quad (4)$$

where

$$c'_J = \frac{\bar{t}}{\bar{\ell}} c_J, \quad J=D, V.$$

4.3 Nucleus

Putting the independent and dependent variables in non-dimensional form gives

$$\begin{aligned}\varepsilon \frac{\partial D'_n}{\partial t'} &= K'_{nD} \nabla'^2 D'_n + \varepsilon \bar{t} k_2 V'_n - \varepsilon \bar{t} \bar{C} k_1 \left[\frac{R'_V}{\bar{C}} - V'_n \right] D'_n + \frac{\bar{t}}{\bar{C}} \varepsilon S'_D - \varepsilon \bar{t} k_d D'_n \\ \varepsilon \frac{\partial V'_n}{\partial t'} &= K'_{nV} \nabla'^2 V'_n - \left(\varepsilon \bar{t} k_2 V'_n - \varepsilon \bar{t} \bar{C} k_1 \left[\frac{R'_V}{\bar{C}} - V'_n \right] D'_n \right).\end{aligned}$$

Typical values, taken from [16], for the coefficients in (1) are given in Table 1. Thus the binding-unbinding processes are occurring over a time span of days. It is assumed that there are on the order of 1000 VDR_{nuc} or fewer per cell [1]. For a cell volume of about $5000 \mu\text{m}^3$, which is consistent with the dimensions given in Section 2, 1000 VDR_{nuc} gives a concentration of $R'_V \doteq 300 \text{pM}$, which is taken to be $O(\varepsilon^{-1})$: $R'_V = R_V^{N(1)} \varepsilon^{-1}$. Numerically a value of $k_1 = 0.01 \text{pM}^{-1} \text{d}^{-1} = 4.1667 \times 10^{-4} \text{pM}^{-1} \text{h}^{-1}$, which is typical of the values in Table 1, gives $\bar{t} \bar{C} k_1 = 4 \times 10^{-4}$, which is taken to be $O(\varepsilon)$. Thus

$$\begin{aligned}\varepsilon \frac{\partial D'_n}{\partial t'} &= K'_{nD} \nabla'^2 D'_n + \varepsilon k'_2 V'_n - \varepsilon k'_1 R_V^{N(1)} D'_n + \varepsilon^2 k'_1 V'_n D'_n + \varepsilon S'_D - \varepsilon k'_d D'_n \\ \varepsilon \frac{\partial V'_n}{\partial t'} &= K'_{nV} \nabla'^2 V'_n - \varepsilon k'_2 V'_n + \varepsilon k'_1 R_V^{N(1)} D'_n - \varepsilon^2 k'_1 V'_n D'_n.\end{aligned}\quad (5)$$

where

$$k'_2 = \bar{t} k_2 \quad k'_1 = \frac{\bar{t} \bar{C} k_1}{\varepsilon} \quad R_T^{N(1)} = \frac{R_V^N}{\bar{C}} \varepsilon \quad S'_D = \frac{\bar{t}}{\bar{C}} S_D \quad k'_d = \bar{t} k_d \quad (6)$$

4.4 Interface conditions

The non-dimensional forms of the continuity conditions are

$$J'_M|_{\Gamma_M} = J'_c|_{\Gamma_M}, \quad J = D, V; M = m, n \quad K'_{MJ} \vec{\nabla}' J'_M \Big|_{\Gamma_M} \cdot \hat{\nu}_M = K'_{cJ} \vec{\nabla}' J'_c \Big|_{\Gamma_M} \cdot \hat{\nu}_M, \quad J = D, V; M = m, n.$$

5 Three perturbation approaches

A model that makes no assumption about the magnitude of diffusivity K'_{cJ} in the cytoplasm is presented below. To frame the issues the limiting cases where $K'_{MJ} \sim 1$ for all $J = D, V; M = m, c, n$ and $K'_{MJ} \sim 1$ for all $J = D, V; M = m, n$, but $K'_{cJ} \sim 0$ are considered first.

5.1 Perturbation expansions for $K'_{MJ} \sim 1$

Inserting the expansions $J_M = \sum_{p=0}^{\infty} J_M^{(p)} \varepsilon^p$, $J = D, V, M = n, c, m$ and segregating the coefficients of like powers of ε give for ε^0 the problems

$$\begin{aligned} K'_{mJ} \vec{\nabla}' J_m^{(0)} \Big|_{\Gamma_m} \cdot \vec{\nu}_m &= 0 K'_{mJ} \nabla'^2 J_m^{(0)} = 0 J'_m|_{\Gamma_c} = J'_c|_{\Gamma_c} \\ K'_{mJ} \vec{\nabla}' J_m \Big|_{\Gamma_c} \cdot \vec{\nu}_c &= K'_{cJ} \vec{\nabla}' J'_c \Big|_{\Gamma_c} \cdot \vec{\nu}_c K'_{cJ} \nabla'^2 J'_c = 0 J'_n|_{\Gamma_n} = J'_c|_{\Gamma_n} \\ K'_{nJ} \vec{\nabla}' J'_n \Big|_{\Gamma_n} \cdot \vec{\nu}_n &= K'_{cJ} \vec{\nabla}' J'_c \Big|_{\Gamma_n} \cdot \vec{\nu}_n K'_{nJ} \nabla'^2 J_n^{(0)} = 0, \end{aligned}$$

$J = D, V$. These boundary value problems admit as solutions two arbitrary continuous functions $D_0(t')$ and $V_0(t')$ of time alone.

5.1.1 Stability condition and time constants—Using (3),(4), and (5) in the divergence theorem and employing continuity of molar flux give

$$\begin{aligned} \iiint_{\mathcal{R}_n} \left[\varepsilon \frac{\partial D'_n}{\partial t'} - \left(\varepsilon k'_2 V'_n - \varepsilon k'_1 R_V^{N(1)} D'_n + \varepsilon^2 k'_1 V'_n D'_n + \varepsilon S'_D - \varepsilon k'_d D'_n \right) \right] d\Omega + \iiint_{\mathcal{R}_c} \left[\varepsilon \frac{\partial D'_c}{\partial t'} - \varepsilon c'_D \vec{\nabla}' \cdot (D'_c \hat{u}) \right] d\Omega + \iiint_{\mathcal{R}_m} \left[\varepsilon \frac{\partial D'_m}{\partial t'} \right] d\Omega \\ + \iiint_{\mathcal{R}_n} \left[\varepsilon \frac{\partial V'_n}{\partial t'} - \left(-\varepsilon k'_2 V'_n + \varepsilon k'_1 R_V^{N(1)} D'_n - \varepsilon^2 k'_1 V'_n D'_n \right) \right] d\Omega + \iiint_{\mathcal{R}_c} \left[\varepsilon \frac{\partial V'_c}{\partial t'} + \varepsilon c'_V \vec{\nabla}' \cdot (V'_c \hat{u}) \right] d\Omega + \iiint_{\mathcal{R}_m} \left[\varepsilon \frac{\partial V'_m}{\partial t'} \right] d\Omega = \iint_{\Gamma_m} \varepsilon \end{aligned} \quad (7)$$

The coefficients of ε^1 in (7) give a system of ordinary differential equations for $D_0(t')$, $V_0(t')$

$$\begin{aligned} \Omega_{cell} \frac{dD_0}{dt'} + \left[\left(k'_1 R_V^{N(1)} + k'_d \right) \Omega_{nuc} - c'_D \Omega_{cyt} [\hat{u}] + H'_D \Sigma_{cell} \right] D_0 - k'_2 \Omega_{nuc} V_0 &= S_D \Omega_{nuc} + [-\Phi'_D + H'_D \eta'_D] \Sigma_{cell} \\ \Omega_{cell} \frac{dV_0}{dt'} - k'_1 R_V^{N(1)} \Omega_{nuc} D_0 + [k'_2 \Omega_{nuc} + c'_V \Omega_{cyt} [\hat{u}] + H'_V \Sigma_{cell}] V_0 &= H'_V \eta'_V \Sigma_{cell} \end{aligned} \quad (8)$$

where Ω_{nuc} and Ω_{cell} are the volumes of the indicated regions, Σ_{cell} is the surface area of the cell and

$$\Omega_{cyt} [\hat{u}] = \iiint_{\mathcal{R}_c} \vec{\nabla}' \cdot \hat{u} d\Omega \quad (9)$$

Thus at the ε^0 level the time evolution of the transport of D and the production of V is governed by two time constants τ_D , τ_V which are the reciprocals of the eigenvalues of the coefficient matrix

$$A = \frac{1}{\Omega_{cell}} \begin{bmatrix} \left(k'_1 R_V^{N(1)} + k'_d \right) \Omega_{nuc} - c'_D \Omega_{cyt} [\hat{u}] + H'_D \Sigma_{cell} & -k'_2 \Omega_{nuc} \\ -k'_1 R_V^{N(1)} \Omega_{nuc} & k'_2 \Omega_{nuc} + c'_V \Omega_{cyt} [\hat{u}] + H'_V \Sigma_{cell} \end{bmatrix}. \quad (10)$$

If $k'_1 k'_2 R_V^{N(1)} (\Omega_{nuc} / \Omega_{cell})^2$ is small, then

$$\tau_D^{-1} \approx \frac{1}{\Omega_{cell}} \left[(k'_1 R_V^{N(1)} + k'_d) \Omega_{nuc} - c'_D \Omega_{cyt} [\hat{u}] + H'_D \Sigma_{cell} \right] \quad \tau_V^{-1} \approx \frac{1}{\Omega_{cell}} [k'_2 \Omega_{nuc} + c'_V \Omega_{cyt} [\hat{u}] + H'_V \Sigma_{cell}]. \quad (11)$$

Thus τ_D may be negative, that is, the solutions may grow exponentially if the cytoskeletal track velocity c'_D is sufficiently large relative to the influx parameter H'_D and the reaction rate parameter $k'_1 R_V^{N(1)} + k'_d$. Thus this model predicts that the process is unstable unless the destruction/conversion process in the nucleus can process the vitamin D coming in along the cytoskeletal tracks rapidly enough, or there is a backflow mechanism ($H'_D > 0$) which permits a sufficient amount of injected vitamin D to leak out of the cell to keep from overwhelming the process.

The boundary value problems for the coefficients of the ε^1 terms are

$$\begin{aligned} K'_{mD} \vec{\nabla}' D_m^{(1)} \Big|_{\Gamma_m} \cdot \hat{\nu}_m &= -\Phi'_D - H'_D (D_0 - \eta'_D) K'_{mD} \nabla'^2 D_m^{(1)} = \frac{dD_0}{dt'} D_m^{(1)} \Big|_{\Gamma_c} = D_c^{(1)} \Big|_{\Gamma_c} \\ K'_{mD} \vec{\nabla}' D_m^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c &= K'_{cD} \vec{\nabla}' D_c^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c K'_{cD} \nabla'^2 D_c^{(1)} = \frac{dD_0}{dt'} - c'_D (\vec{\nabla} \cdot \hat{u}) D_0 D_n^{(1)} \Big|_{\Gamma_n} = D_c^{(1)} \Big|_{\Gamma_n} \\ K'_{nD} \vec{\nabla}' D_n^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n &= K'_{cD} \vec{\nabla}' D_c^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n K'_{nD} \nabla'^2 D_n^{(1)} = \frac{dD_0}{dt'} - [k'_2 V_0 - k'_1 R_V^{N(1)} D_0 + S'_D - k'_d D_0] \end{aligned}$$

and

$$\begin{aligned} K'_{mV} \vec{\nabla}' V_m^{(1)} \Big|_{\Gamma_m} \cdot \hat{\nu}_m &= -H'_V (V_0 - \eta'_V) K'_{mV} \nabla'^2 V_m^{(1)} = \frac{dV_0}{dt'} V_m^{(1)} \Big|_{\Gamma_c} = V_c^{(1)} \Big|_{\Gamma_c} \\ K'_{mV} \vec{\nabla}' V_m^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c &= K'_{cV} \vec{\nabla}' V_c^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c K'_{cV} \nabla'^2 V_c^{(1)} = \frac{dV_0}{dt'} + c'_V (\vec{\nabla} \cdot \hat{u}) V_0 V_n^{(1)} \Big|_{\Gamma_n} = V_c^{(1)} \Big|_{\Gamma_n} \\ K'_{nV} \vec{\nabla}' V_n^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n &= K'_{cV} \vec{\nabla}' V_c^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n K'_{nV} \nabla'^2 V_n^{(1)} = \frac{dV_0}{dt'} - [-k'_2 V_0 + k'_1 R_V^{N(1)} D_0] \end{aligned}$$

The solutions to these, and all higher order problems, have the form

$J_{Mq}(\vec{x}', t') = J_q(t') + J_{Mp}^{(q)}(\vec{x}', t')$, $J = D, V$, $q = 1, 2, \dots$, where $J_{Mp}^{(q)}$ are particular solutions that can be computed from the $J_r(t')$, $r = 0, \dots, q-1$. Inserting $\varepsilon^q J_q(\vec{x}', t')$ into (7) and extracting the ε^{q+1} coefficients leads to a system of ordinary differential equations of the form

$$\frac{d}{dt'} \begin{bmatrix} D_q \\ V_q \end{bmatrix} + A \begin{bmatrix} D_q \\ V_q \end{bmatrix} = \vec{F}^{(q)} \quad (12)$$

where A is given by (10). Thus all terms in the perturbation expansions are governed by the same two time constants and subject to the same stability requirement. Since with the choice of reference magnitudes (2), $\varepsilon = 0.01$, only the ε^0 term in the expansion will be of consequence if the particular solutions are of order unity. Indeed it is observed in the case of

concentric spheres, where the particular solutions can be computed analytically, that $J_{cp}^{(q)}(\vec{x}', t') \sim (K'_{cJ})^{-q}$ and thus higher order terms are negligible for $K'_{cJ} \sim 1$, but not if $K'_{cJ} \sim \varepsilon$. Convergence of the series in this latter case is unclear.

5.1.2 Example: Ellipsoidal osteoblast—The discussion of Section 2 suggests modeling the cell as a 1.5 : 1.5 : 0.5 ellipsoid. For illustrative purposes the nucleus is taken to be a 0.6 : 0.6 : 0.2 ellipsoid and the membrane region is assumed of negligible width. Both nucleus and cell are centered in the xy -plane. Pictures of osteoblasts indicate that the nucleus is not generally concentric (Figure 2). If the nucleus is centered at the origin and the velocity field runs radially outward from the origin, then $\nabla \cdot \hat{u} = 2/r$. A spherical cell with a spherical nucleus with the same volumes as the ellipsoidal cell has radii $r'_m \doteq 1.04$ and $r'_n \doteq 0.42$. Table 2 gives the geometric parameters occurring in the coefficient matrix (10) for the case concentric ellipsoids and spheres and for the case of eccentric nuclei abutting the membrane.

For purposes of illustration the PTH binding-unbinding numbers in Table 1 will be used, since the system of differential equations used in [16] for this process is closest to the assumed form (1) used in this article. The dimensionless values for OPG/RANKL and PTH corresponding to those of Table 1 are given in Table 3. As indicated above $R_v^{N(1)} = 3$ follows from a value of about 1000 VDR_{nuc} . The speed of transport along the cytoskeletal tracks will be taken to be $c'_D = c'_V = 0.2$, which is consistent with transport times across the cytoplasm of several hours. Figure 3 shows that, in the absence of the possibility of outflux of D from the cell, $H_D = 0$, for PTH the stability of the system (12) requires a value of the exogenous destruction parameter k'_d that is slightly larger than the value in Table 3. The stability thresholds for OPG/RANKL are similar, but in this case the thresholds are orders of magnitude larger than the value given in Table 3. Alternatively letting the flux parameter H'_D be sufficiently positive produces stability for the value of k'_d given in Table 3, as is shown in Figure 4. Over the ranges of k'_d and H'_D shown in Figures 3 and 4 the other time constant τ_V is virtually invariant, having a value in the 0.25–0.3 h range for all four geometric configurations.

Figure 5 shows the concentrations of D_0 and V_0 predicted by (8) for the cases of an ellipsoid with nucleus abutting the cell membrane and a sphere with concentric nucleus. A constant injection rate $\Phi'_D = -1$ for thirty minutes with no backflow ($H'_D = 0$) was assumed. At the ε^0 level the solution has no spatial dependence, higher order terms are negligible, and thus for the given magnitudes of the diffusivities the diffusion is "instantaneous" relative to the time scale (hours) of the destruction/conversion process in the nucleus. This is not in accord with the pulse observation. Note also that V_0 is about a factor of $\varepsilon^{-1} = 100$ smaller than D_0 .

5.2 Perturbation expansion for $K'_{cD} \sim 0$

In the limiting case $K'_{nD} = K'_{mD} = 1$, $K'_{cD} = 0$ the problem for ε^0

$$K'_{mD} \nabla'^2 D_m^{(0)} = 0 \quad K'_{mD} \vec{\nabla}' D_m^{(0)} \Big|_{\Gamma_m} \cdot \hat{\nu}_m = 0, \quad K'_{mD} \vec{\nabla}' D_m^{(0)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c = 0$$

admits a solution $D_m^{(0)}(t)$ of time alone, which an application of the divergence theorem implies must satisfy the differential equation

$$\Omega_m \frac{d}{dt'} D_m^{(0)}(t) + H'_D \Sigma_{cell} D_m^{(0)}(t) = (-\Phi'_D + H'_D \eta'_D) \Sigma_{cell}. \quad (13)$$

Thus the membrane and cell boundary equations completely determine $D_m^{(0)}(t)$. In the cytoplasm the solution to the first order hyperbolic partial differential equation (c.f 4)

$$\frac{\partial D_c^{(0)}}{\partial t'} = c'_D \vec{\nabla}' \cdot (D_c^{(0)} \hat{u}) \quad D_c^{(0)} \Big|_{\Gamma_c} = D_m^{(0)}(t) \quad (14)$$

has a unique solution for given boundary datum $D_m^{(0)}(t)$. In the nucleus

$$K'_{nD} \nabla'^2 J_n^{(0)} = 0 \quad K'_{nD} \vec{\nabla}' J_n^{(0)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n = 0$$

for $J = D, V$ admits a solution $D_n^{(0)}(t), V_n^{(0)}(t)$, which an application of the divergence theorem implies must satisfy the system

$$\frac{d}{dt} D_n^{(0)}(t) - k'_2 V_n^{(0)}(t) + (k'_1 R_V^{N(1)} + k'_d) D_n^{(0)}(t) = \Omega_n^{-1} \iiint_{\mathcal{R}_n} S'_D(\vec{x}, t) d\Omega \quad \frac{d}{dt} V_n^{(0)}(t) + k'_2 V_n^{(0)}(t) - k'_1 R_V^{N(1)} D_n^{(0)}(t) = 0. \quad (15)$$

Transportation of D_3 from the cytoplasm can be introduced into the system through the

source term S'_D provided that $D_c^{(0)} \Big|_{\Gamma_c}$ can be continued into the nucleus as an integrable function.

5.2.1 Example: Spherical osteoblast—For a spherical cell with concentric spherical cytoplasm and nucleus the cytoplasm equation (14) can be solved explicitly. Let

$r = r'_n, r'_c, r'_m$ denote the outer boundaries of the three regions. The solution to the membrane equation (13) has time constant

$$\tau_D = H'_D \Sigma_{cell} / \Omega_m = 3H'_D \frac{r_m^2}{r_m^3 - r_c^3}$$

If the parameters are chosen to make this equal to a few hours, then the solution $D_m^{(0)}(t)$ will be a pulse of the observed width. The assumption $H'_D=0$ does not lead to a pulse-like solution, however. The solution in the cytoplasm is

$$D_c^{(0)}(r', t') = \frac{D_m^{(0)} \left(\frac{r' + t' c'_D - r'_c}{c'_D} \right) r'^2_c}{r'^2}.$$

Thus the shape of the pulse that is formed in the membrane is simply translated through the cytoplasm at speed c'_D . The above formula for $D_c^{(0)}(r', t')$ continues naturally into $\mathcal{R}_n$ with an integrable singularity at the origin, however

$$D_c^{(0)}(r'_n, t') = \frac{D_m^{(0)} \left(\frac{r'_n + t' c'_D - r'_c}{c'_D} \right) r'^2_c}{r'^2_n}$$

also could be used. Thus the utility of this model depends upon alternative reasonable extensions yielding similar predictions. It is easily shown that the eigenvalues of the coefficient matrix of (15) are both positive and thus there is not a stability problem. This model will not be pursued further in this article. The two features of interest in the context of the main model presented below are that the model is stable for all choices of parameters for the equations in the nucleus, but consistency with the pulse observation requires that the flux parameter $H'_D > 0$.

5.3 Perturbation expansions for general $K'_{c,j}$

It was observed above that for solutions to (8) $V_0 \sim \varepsilon D_0$. Adopting this ansatz yields perturbation expansions $D_M = D_M^{(0)} + \varepsilon^2 D_M^{(2)} + \dots$, $V_M = \varepsilon V_M^{(1)} + \varepsilon^3 V_M^{(3)} + \dots$, $M = m, c, n$. The lowest order problems now incorporate terms of order ε^q , $q = 0, 1$, thus obviating the assumption $K'_{M,j} \sim 1$. The resulting lowest order problem for $D^{(0)}$ is independent of $V^{(1)}$

$$\begin{aligned} K'_{mD} \vec{\nabla}' D_m^{(0)} \Big|_{\Gamma_m} \cdot \hat{\nu}_m &= \varepsilon \left(-\Phi'_D - H'_D (D_m^{(0)} - \eta'_D) \Big|_{\Gamma_m} \right) \varepsilon \frac{\partial D_m^{(0)}}{\partial t'} = K'_{mD} \nabla'^2 D_m^{(0)} D_m^{(0)} \Big|_{\Gamma_c} = D_c^{(0)} \Big|_{\Gamma_c} \\ K'_{mD} \vec{\nabla}' D_m^{(0)} \Big|_{\Gamma_c} \cdot \vec{\nu}_c &= K'_{cD} \vec{\nabla}' D_c^{(0)} \Big|_{\Gamma_c} \cdot \vec{\nu}_c \varepsilon \frac{\partial D_c^{(0)}}{\partial t'} = K'_{cD} \nabla'^2 D_c^{(0)} + \varepsilon c'_D \vec{\nabla}' \cdot (D_c^{(0)} \hat{u}) D_n^{(0)} \Big|_{\Gamma_n} = D_c^{(0)} \Big|_{\Gamma_n} \\ K'_{nD} \vec{\nabla}' D_n^{(0)} \Big|_{\Gamma_n} \cdot \vec{\nu}_n &= K'_{cD} \vec{\nabla}' D_c^{(0)} \Big|_{\Gamma_n} \cdot \vec{\nu}_n \varepsilon \frac{\partial D_n^{(0)}}{\partial t'} = K'_{nD} \nabla'^2 D_n^{(0)} - \varepsilon k'_1 R_V^{N(1)} D_n^{(0)} + \varepsilon S'_D - \varepsilon k'_D D_n^{(0)}. \end{aligned} \quad (16)$$

and thus more tractable analytically. The corresponding problem for $V^{(1)}$

$$\begin{aligned}
K'_{mV} \vec{\nabla}' V_m^{(1)} \Big|_{\Gamma_m} \cdot \hat{\nu}_m &= \left(-H'_V \left(\varepsilon V_m^{(1)} - \eta'_V \right) \Big|_{\Gamma_m} \right) \varepsilon \frac{\partial V_m^{(1)}}{\partial t'} = K'_{mV} \nabla'^2 V_m^{(1)} V_m^{(1)} \Big|_{\Gamma_c} = V_c^{(1)} \Big|_{\Gamma_c} \\
K'_{mV} \vec{\nabla}' V_m^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c &= K'_{cV} \vec{\nabla}' V_c^{(1)} \Big|_{\Gamma_c} \cdot \hat{\nu}_c \varepsilon \frac{\partial V_c^{(1)}}{\partial t'} = K'_{cV} \nabla'^2 V_c^{(1)} - \varepsilon c'_V \vec{\nabla}' \cdot \left(V_c^{(1)} \hat{u} \right) V_n^{(1)} \Big|_{\Gamma_n} = V_c^{(1)} \Big|_{\Gamma_n} \quad (17) \\
K'_{nV} \vec{\nabla}' V_n^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n &= K'_{cV} \vec{\nabla}' V_c^{(1)} \Big|_{\Gamma_n} \cdot \hat{\nu}_n \varepsilon \frac{\partial V_n^{(1)}}{\partial t'} = K'_{nV} \nabla'^2 V_n^{(1)} - \varepsilon k'_2 V_n^{(1)} + k'_1 R_V^{N(1)} D_n^{(0)}
\end{aligned}$$

then can be solved with $D_n^{(0)}$ in the last equation a known quantity.

5.3.1 Example: Spherical osteoblast—For a spherical cell with concentric spherical cytoplasm and nucleus solution by integral transforms is feasible. Let $r=r'_n, r'_c, r'_m$ denote the outer boundaries of the three regions. The prime notation is dispensed with for the independent and dependent variables, but retained for parameters. When the field describing the cytoskeletal tracks is taken to be $\hat{u} = r, \hat{u}$ the outward radial unit vector, the solution depends only on the radial variable r . Thus

$$\begin{aligned}
\nabla^2 J &= \frac{2}{r} \frac{\partial J}{\partial r} + \frac{\partial^2 J}{\partial r^2}, \vec{\nabla} \cdot (J \hat{u}) = \frac{2}{r} J + \frac{\partial J}{\partial r}, \vec{\nabla} J = \frac{\partial J}{\partial r} \hat{r} \text{ and the problem for } D^{(0)} \text{ is} \\
K'_{mD} \frac{\partial D_m^{(0)}}{\partial r}(r'_m, t) &= \varepsilon \left[-\Phi'_D - H'_D \left(D_m^{(0)}(r'_m, t) - \eta'_D \right) \right] \varepsilon \frac{\partial D_m^{(0)}}{\partial t} = K'_{mD} \left(\frac{2}{r} \frac{\partial}{\partial r} + \frac{\partial^2}{\partial r^2} \right) D_m^{(0)} D_m^{(0)}(r'_c, t) = D_c^{(0)}(r'_c, t) \\
K'_{mD} \frac{\partial D_m^{(0)}}{\partial r}(r'_c, t) &= K'_{cD} \frac{\partial D_c^{(0)}}{\partial r}(r'_c, t) \varepsilon \frac{\partial D_c^{(0)}}{\partial t} = K'_{cD} \left(\frac{2}{r} \frac{\partial}{\partial r} + \frac{\partial^2}{\partial r^2} \right) D_c^{(0)} + \varepsilon c'_D \left(\frac{2}{r} + \frac{\partial}{\partial r} \right) D_c^{(0)} D_n^{(0)}(r'_n, t) = D_c^{(0)}(r'_n, t) \quad (18) \\
K'_{nD} \frac{\partial D_n^{(0)}}{\partial r}(r'_n, t) &= K'_{cD} \frac{\partial D_c^{(0)}}{\partial r}(r'_n, t) \varepsilon \frac{\partial D_n^{(0)}}{\partial t'} = K'_{nD} \left(\frac{2}{r} \frac{\partial}{\partial r} + \frac{\partial^2}{\partial r^2} \right) D_n^{(0)} - \varepsilon k'_1 R_V^{N(1)} D_n^{(0)} + \varepsilon S'_D - \varepsilon k'_d D_n^{(0)}.
\end{aligned}$$

Let $\hat{D}_M(r, s)$ denote the Laplace transform of $D_M(r, t)$. Taking transforms of the partial differential equations in (18) and solving the resulting ordinary differential equations give in the case of zero initial conditions

$$\begin{aligned}
\hat{D}_m^{(0)}(r, s) &= c_1(s) \sinh \left(\sqrt{\frac{\varepsilon s}{K'_{mD}}} r \right) r^{-1} + c_2(s) \cosh \left(\sqrt{\frac{\varepsilon s}{K'_{mD}}} r \right) r^{-1} \\
\hat{D}_c^{(0)}(r, s) &= c_3(s) M \left(-\frac{\sqrt{4\varepsilon s K'_{cD} + c'^2_D + c'_D}}{\sqrt{4\varepsilon s K'_{cD} + c'^2_D}}, 2, \frac{\sqrt{4\varepsilon s K'_{cD} + c'^2_D} r}{K'_{cD}} \right) e^{-1/2 \frac{r \left(\sqrt{4\varepsilon s K'_{cD} + c'^2_D + c'_D} \right)}{K'_{cD}}} + c_4(s) U \left(-\frac{\sqrt{4\varepsilon s K'_{cD} + c'^2_D}}{\sqrt{4\varepsilon s K'_{cD} + c'^2_D}}, \frac{\sqrt{4\varepsilon s K'_{cD} + c'^2_D} r}{K'_{cD}} \right) \quad (19) \\
\hat{D}_n^{(0)}(r, s) &= \sinh \left(\sqrt{\frac{\varepsilon \left(s + R_V^{N(1)} k'_1 + k'_d \right)}{k'_{nD}}} r \right) c_5(s) r^{-1} + \frac{S'_D}{s \left(s + R_V^{N(1)} k'_1 + k'_d \right)}.
\end{aligned}$$

where M and U are Kummer functions. The transforms $\hat{V}_M, M = m, c$ for the corresponding problem for $V^{(0)}$ have similar forms to the corresponding $\hat{D}_M$

$$\begin{aligned}\hat{V}_n^{(1)}(r, s) &= \Delta(s)^{-1} (V_{n1}(s)c_5(s) + V_{n2}(s)c_6(s)) \\ V_{n1}(s) &= k_1 R_V^{N(1)} \left(K_{nD}'^2 s(k_2 + s) \sqrt{\frac{\varepsilon(k_1' R_V^{N(1)} + k_d' + s)}{K_{nD}'}} \sinh \left(r \sqrt{\frac{\varepsilon(k_1' R_V^{N(1)} + k_d' + s)}{K_{nD}'}} \right) + r S_D' \varepsilon(-k_1' K_{nV}' R_V^{N(1)} + k_2' K_{nD}' - \right. \\ V_{n2}(s) &= -2s \varepsilon(k_2' + s) (k_1' R_V^{N(1)} + k_d' + s) \sinh \left(r \sqrt{\frac{\varepsilon(k_2' + s)}{K_{nV}'}} \right) (-k_1 K_{nV}' R_V^{N(1)} + k_2' K_{nD}' - k_d' K_{nV}' + K_{nD}' s - K_{nV}' s) \\ \Delta(s) &= K_{nD}' K_{nV}' r s(k_2' + s) (k_1' R_V^{N(1)} + k_d' + s) \left(\sqrt{\frac{\varepsilon(k_2' + s)}{K_{nV}'}} - \sqrt{\frac{\varepsilon(k_1' R_V^{N(1)} + k_d' + s)}{K_{nD}'}} \right) \left(\sqrt{\frac{\varepsilon(k_1' R_V^{N(1)} + k_d' + s)}{K_{nD}'}} + \sqrt{\frac{\varepsilon(k_2' + s)}{K_{nV}'}} \right)\end{aligned}$$

Substituting into the transforms of the five boundary and interface conditions into (18) results in a linear system of equations for the unknown coefficients $c_q(s)$. Substituting these solutions into the relevant equation in (19) and inverting numerically using the fast Fourier transform ([5], [4]) gives the solution as a function of time.

Figure 6 shows that for $K_{cD}' = 10^{-2}$ the predicted migration of vitamin D and the reaction product V is similar in magnitude to that for $K_{cD}' \sim 1$ in Figure ?? (Lower pane) and shows only slight spatial dependence. It is inconsistent with the pulse observation in not predicting the accumulation of D in the nucleus over the course of hours. Figures 7, 8 and 9 show the corresponding results for $K_{cD}' = 10^{-3}$, $K_{cD}' = 10^{-4}$ and $K_{cD}' = 10^{-5}$ respectively. The latter two figures are consistent with the pulse observation, however the lower panes show that the ansatz $V_0 \sim \varepsilon D_0$ becomes less accurate with decreasing K_{cD}' , thus calling into question how quantitatively correct these graphs are. The graphs seem plausible otherwise, and thus may remain qualitatively correct.

Although the complexity of the formulas for the solutions of this section make finding explicit stability criteria difficult, empirically instabilities similar to the type present the system (8) were observed in the sense that decay times became very large as some critical value was approached. Table 4 shows minimum values of either k_d' or H_D' , with the other fixed, that permitted decay of the concentration to zero over a four day period. Typically values only slightly below these produced nonsensical solutions, and so these are good approximations to the stability thresholds. For $K_{cD}' = 10^{-1}$ the results of columns 1–4 are similar to those of Figures 3 and 4. For $K_{cD}' = 10^{-4}$ and 10^{-5} the value of k_d' seemed to have little effect on the minimum value of H_D' required for stability and only to affect the concentration of D in the nucleus, as is predicted by the model of Section 5.2.

6 Summary and discussion

This article represents an initial attempt to produce a spatial-temporal model of transport and metabolism of vitamin D₃ in an osteoblast using partial differential equations. A model was sought that is consistent with the observations, summarized in Figure 1, that molecules of

calcitriol migrate from the cell boundary through the cytoplasm and accumulate in the nucleus over the course of a few hours. The use of the scalar mass transport equations in the cytoplasm permitted both diffusion and fixed-velocity transport, as might be the case if transport along the cytoskeletal tracks is an important mechanism. The resulting model, presented in Section 3, admits approximation by a pair of linear boundary value problems (16,17) for D_3 and the output product that are analytically tractable for a simple geometry such as concentric spherical cell components.

Estimates in the literature for the diffusivity for molecules as large or larger than D_3 are summarized in Section 2. The estimated diffusivities suggest that transport by diffusion should proceed sufficiently rapidly that, viewed over the course of hours, little spatial inhomogeneity would be observable. This is an implication of Einstein's formula for average particle dispersion for Brownian motion which is confirmed by the model presented here (see Figure 6). The diminution of the diffusivity due to the crowded nature of the cytoplasm is estimated in the articles cited in Section 2 to be about an order of magnitude, however the model required a diffusivity about three or four orders of magnitudes smaller than indicated in the literature to produce the sort of spatial inhomogeneity observed in Figure 1 (see Figures 6–8). The simulations presented in [22] suggest that such impermeability would require that a majority of the cytoplasmic volume be inaccessible to diffusion.

The presence of transport of D_3 at a fixed velocity requiring only hours to traverse the cytoplasm and reach the nucleus, coupled with a conversion/destruction process proceeding over the course of days raises the possibility of instability. It was found that, for plausible parameter values, the model predicted that such instability could arise. For the limiting case of high diffusivity in the cytoplasm, this instability could be avoided, for a fixed cytoskeletal velocity, by assuming some combination of sufficiently high conversion/destruction in the nucleus and backflow of injected D_3 out of the cell. For the case of low diffusivity in the cytoplasm, which caused the model to agree better with the experimental observations presented, the rate of conversion/destruction of D_3 in the nucleus has no apparent effect on stability, and the rate of backflow from the cell required for stability is less. Thus the model does not support the extreme case of the cell boundary being completely impermeable to outflow of injected D_3 , but the amount of outflow needed decreases with the diffusivity of the cytoplasm.

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Figure 1. Transport of calcitriol through the cytoplasm to the nucleus. The background concentration in the cell at 0 hrs is normalized to 1.

Figure 2.

Ellipsoidal geometry of an osteoblast assumed in Section 5.1.2. Left: Cross-section in xy plane. Right: Three-dimensional view.

Figure 3.

The time constant τ_D as a function of the exogenous destruction parameter k_d' . The membrane flux parameters are $H_D'=0$, $H_V'=1$ and cytoskeletal track speeds are $c_D'=c_V'=0.2$. The other reaction parameters are the PTH values given in Table 3 and the geometric parameters are given in Table 2. The two cases labeled "Eccentric" are the extreme cases in which the nucleus abutts the membrane.

Figure 4.

The time constant τ_D as a function of the membrane flux parameter H'_D . The other membrane flux parameter is $H'_V=1$ and cytoskeletal track speeds are $c'_D=c'_V=0.2$. The reaction/destruction parameters are the PTH values given in Table 3 and the geometric parameters are given in Table 2. The two cases labeled "Eccentric" are the extreme cases in which the nucleus abutts the membrane.

Figure 5.

Solutions D_0 (blue) and $V_0(\times 100)$ (magenta) of the system (8). The PTH values of k'_1 and k'_2 of Table 3 and exogenous destruction parameter $k'_d=9$ were used. See the text for other parameters. Upper pane: Ellipsoidal cell with ellipsoidal nucleus abutting the membrane. Lower pane: Spherical cell with concentric spherical nucleus.

Figure 6.

Migration of D, as approximated by $D^{(0)}$, [Upper pane] and V, as approximated by $\varepsilon V^{(0)}$, [Lower pane] to and from the nucleus for a spherical osteoblast with concentric interfaces. The parameters used were those of Table 3 (PTH) and

$K'_{cD} = 10^{-2}$, $K'_{nD} = K'_{mD} = 1$, $c'_D = c'_V = 0.2$, $H'_D = H'_V = 0.1$. The positions of the interfaces are $r'_n = 0.42$, $r'_c = 1$, $r'_m = 1.04$. The coloring of the graph indicates position: Blue: $r' = 1.02$; Purple: $r' = 0.8$; Brown: $r' = 0.5$; Green: $r' = 0.4$.

Figure 7.

Migration of D [Upper pane] and V [Lower pane] to and from the nucleus for a spherical osteoblast with concentric interfaces. The parameters used were those of Table 3 (PTH) and $K'_{cD}=10^{-3}$, $K'_{nD}=K'_{mD}=1$, $c'_D=c'_V=0.2$, $H'_D=H'_V=0.1$. The positions of the interfaces are $r'_n=0.42$, $r'_c=1$, $r'_m=1.04$. The coloring of the graph indicates position: Blue: $r' = 1.02$; Purple: $r' = 0.8$; Brown: $r' = 0.5$; Green: $r' = 0.4$.

Figure 8.

Migration of D [Upper pane] and V [Lower pane] to and from the nucleus for a spherical osteoblast with concentric interfaces. The parameters used were those of Table 3 (PTH) and $K'_{cD}=10^{-4}$, $K'_{nD}=K'_{mD}=1$, $c'_D=c'_V=0.2$, $H'_D=H'_V=0.1$. The positions of the interfaces are $r'_n=0.42$, $r'_c=1$, $r'_m=1.04$. The coloring of the graph indicates position: Blue: $r' = 1.02$; Purple: $r' = 0.8$; Brown: $r' = 0.5$; Green: $r' = 0.4$.

Figure 9.

Migration of D [Upper pane] and V [Lower pane] to and from the nucleus for a spherical osteoblast with concentric interfaces. The parameters used were those of Table 3 (PTH) and $K'_{cD}=10^{-5}$, $K'_{nD}=K'_{mD}=1$, $c'_D=c'_V=0.2$, $H'_D=H'_V=0.1$. The positions of the interfaces are $r'_n=0.42$, $r'_c=1$, $r'_m=1.04$. The coloring of the graph indicates position: Blue: $r' = 1.02$; Purple: $r' = 0.8$; Brown: $r' = 0.5$; Green: $r' = 0.4$.

Table 1

Typical binding-unbinding rates.

	binding (k_1)	unbinding (k_2)	elimination (k_d)
OPG-RANKL	$0.01 \text{ pM}^{-1} \text{ d}^{-1}$	10 d^{-1}	0.35 d^{-1}
RANK-RANKL	$6 \times 10^{-4} \text{ pM}^{-1} \text{ d}^{-1}$	$2 \times 10^{-2} \text{ d}^{-1}$	
PTH	$0.02 \text{ pM}^{-1} \text{ d}^{-1}$	3 d^{-1}	86 d^{-1}

Table 2

Geometric parameters occurring in coefficient matrix. The maximum of $\Omega_{\text{cyt}}[u]$ occurs when the nucleus is concentric, the minimum when it abutts the cell membrane.

Parameter	Ellipsoid	Sphere
Ω_{cell}	4.71	4.71
Ω_{nuc}	0.31	0.31
Σ_{cell}	17.07	13.59
$\Omega_{\text{cyt}}[\hat{u}](\text{max})$	10.34	11.38
$\Omega_{\text{cyt}}[\hat{u}](\text{min})$	8.48	9.79

Table 3

Dimensionless values of reaction parameters.

	k_1'	k_2'	k_d'	$R_V^{N(1)}$
OPG/RANKL	0.042	0.42	0.015	3
PTH	0.083	0.125	3.58	3

Table 4

Approximate minimum values for stability as a function of the diffusion rate K_{cD} .

K_c	H'_D	k'_d	H'_D	k'_d	H'_D	k'_d
10^{-1}	0	8	0.08	3.58	0.16	0
10^{-2}	0	9	0.09	3.58	0.16	0
10^{-3}	0	*	0.10	3.58	0.14	0
10^{-4}	0	*	0.03	3.58	0.03	0
10^{-5}	0	*	0.005	3.58	0.005	0

* indicates that $k_d = 100$ did not suffice.