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Calcium Homeostasis and Parturient Hypocalcemia: An Integral Feedback Perspective

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Calcium is tightly regulated in mammals because of the critical role of calcium ion concentrations in many physiological functions. In this work, we develop a model for calcium homeostasis and identify integral feedback control as a functional module that maintains this homeostasis. We argue that maintaining calcium concentrations in a narrow range and perfect adaptation seen when the calcium homeostatic mechanism is subjected to extreme disturbances are the result of a feedback control system implementing integral control through specific interactions of the regulating hormones. Based on the constraints imposed by the suggested integral control, we arrive at a simple dynamical model for calcium homeostasis. We show that the model is biologically plausible and is consistent with known physiology. Furthermore, the utility of the integral-feedback model is revealed by examining an extreme calcium perturbation, parturient paresis in dairy cows.

1. Introduction

Calcium is an important physiological cation. Calcium salts maintain the integrity of the skeleton structure, and calcium ions in intracellular and extracellular fluids are instrumental in controlling a large number of biochemical processes. Indeed, while intracellular calcium ions are needed in the activity of a large number of enzymes and are also involved in conveying information from the surface to the interior of the cell, extracellular calcium ions are necessary for neuro-muscular excitability, blood clotting and hormonal secretion among many other functions (Griffin & Ojeda, 1996). For these important biochemical roles to be accomplished, extracellular and intracellular concentrations of calcium are maintained within a narrow range. Typically, the total serum calcium concentration is maintained between 8.5 and 10.5 mg dl^{-1} in humans (Griffin & Ojeda, 1996) and between 8 and 10 mg dl^{-1} in dairy cows (Goff *et al.*, 1996).

When calcium demand from the plasma is increased, calcium homeostasis is achieved through the influx of calcium to the blood from bone, kidney, and intestine under a tight hormonal control discussed in later sections. As a result of this hormonal control, and under normal circumstances, the blood plasma calcium concentration in humans and many animals remains constant regardless of variations in the calcium concentration of the diet and calcium demands to meet milk production and fetal growth needs.

In dairy cows, the lactational need for calcium is particularly large especially at the onset of parturition (calving). Most animals adapt to this

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large demand and plasma calcium concentrations return to normal after a transient period of reduced concentration. This remarkable adaptation takes place despite the 4–5-fold increase in the rate of calcium clearance from the plasma typically seen at parturition (Oetzel & Goff, 1998; Anderson, 1970). Yet, some cows fail to recover normal calcium levels, which disrupts nerve and muscle function and results in the clinical syndrome known as milk fever or parturient paresis (Oetzel & Goff, 1998; Anderson, 1970).

In this work, we will develop an analytical description for the dynamics of the calcium homeostatic mechanism. We will use this description to understand the normal operation of the system and to determine the causes of its failure, resulting in clinical disorders such as milk fever.

2. Dynamic Model Development

An attempt to model the calcium homeostatic mechanism in the dairy cow in terms of controlled, controlling and disturbing signals was presented in a paper by Ramberg *et al.* (1984). Controlled signals are defined to be the *plasma calcium concentration* $[\mathrm{Ca}]_p$ and *bone calcium content* M_b, while the controlling signals are taken to be *intestinal calcium absorption*, *bone calcium resorption* and *renal calcium reabsorption*. The disturbing signals are those that cause loss of calcium from the blood plasma. They take the form of *endogenous fecal calcium*, clearance via *glomerular filtration*, *placental calcium transport to the fetus during pregnancy*, *calcium deposition into the bone*, *and milk calcium secretion during lactation*. For short-term calcium regulation (hours to weeks), only control of $[\mathrm{Ca}]_p$ may be considered since it has higher priority than M_b in that time period (Ramberg *et al.*, 1984).

In order to develop a mathematical model for calcium homeostasis we start by defining $V_{bone}(t)$ to be the rate (g day^{-1}) at which calcium is transported from bone into the plasma at time t. Similarly, we define $V_{intestine}(t)$ to be the rate (g day^{-1}) at which calcium is transported into the plasma through intestinal absorption at time t. Then $V_T(t)$, the total rate of calcium introduced into the plasma, is given by

$$V_T(t) = V_{bone}(t) + V_{intestine}(t). \tag{1}$$

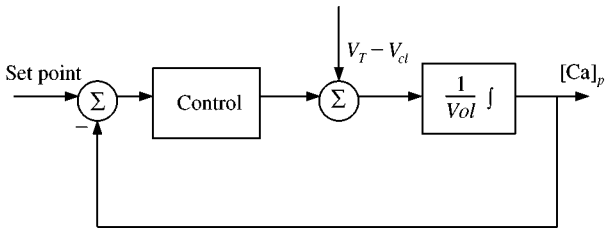

FIG. 1. Overall closed-loop system for calcium homeostasis.

Next, we denote by $V_{cl}(t)$ the calcium clearance rate from the plasma (g day^{-1}), at time t. Calcium clearance takes place through various avenues, the most important of which is milk production, but also includes feces, urine, transport to the fetus during pregnancy, and deposition into the bone. Based on the conservation of mass, we may express the rate of change of plasma calcium as follows:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{Ca}]_p = \frac{1}{vol}(V_T - V_{cl}),$$

where *vol* refers to the total plasma volume. From the above relation we can also write

$$[\mathrm{Ca}]_p = \frac{1}{vol}\int_0^t (V_T - V_{cl})\,\mathrm{d}\tau.$$

A similar model for the plasma calcium pool can be found in Hurwitz *et al.* (1983). Since the plasma calcium concentration is regulated via feedback control to accurately follow a concentration setpoint, the rate of calcium supply $V_T(t)$ must depend on the difference between that setpoint and the actual concentration $[\mathrm{Ca}]_p$. This difference is subsequently referred to as the tracking error. The nature of this dependence dictates by dynamical behavior of the regulated variables and is therefore of key significance. Based on this formulation, the overall closed-loop feedback system is shown in Fig. 1 where the *control* block signifies the dependence of V_T on the tracking error.

2.1. SHORTCOMINGS OF EXISTING MODEL FOR V_T

A model for V_T was provided by Ramberg *et al.* (1984). Based on experimental data, the following

expression of V_T was given:

$$V_T = 1770(0.104 - [\mathrm{Ca}]_p) \quad (\mathrm{g\,day^{-1}}),$$

where $[\mathrm{Ca}]_p$ is the plasma calcium concentration in $\mathrm{g\,l^{-1}}$. This expression for the total rate of calcium supply into the plasma represents a negative feedback model whereby the size of V_T at any given time depends on the plasma calcium concentration at that time. The type of feedback represented by this model is often referred to as *proportional feedback*. It is the simplest type of feedback possible, and is in general characterized by a feedback expression of the form $K_p e(t)$, where K_p is a real constant, and $e(t)$ is the tracking error.

Next, we analyse the proportional feedback model reported by Ramberg *et al.* in the context of the overall calcium homeostasis mechanism. We argue that while proportional feedback possesses some potential for regulation, it *cannot* be the mechanism responsible for achieving calcium homeostasis. We make our argument based on the dynamics resulting from proportional feedback. In this case, the control block in Fig. 1 is just the proportionality constant K_p. Considering that $V_T = K_p e(t) = K_P (r - [\mathrm{Ca}]_p)$, we get the following:

$$[\mathrm{Ca}]_p = \frac{1}{vol} \int_0^t [K_P (r - [\mathrm{Ca}]_p) - V_{cl}]\, \mathrm{d}\tau.$$

Therefore, the dynamics of the feeback control scheme are characterized by the differential equation

$$\frac{\mathrm{d}\,[\mathrm{Ca}]_p}{\mathrm{d}t} + \frac{K_P}{vol}[\mathrm{Ca}]_p = -\frac{V_{cl}}{vol} + \frac{K_p}{vol} r,$$

where r is that calcium concentration setpoint. From this differential equation it can be seen that if $V_{cl} = 0$, then homeostasis will indeed be achieved, as $[\mathrm{Ca}]_p$ will approach r in the steady state regardless of the initial condition on $[\mathrm{Ca}]_p$. However, further analysis of this differential equation also reveals that perfect adaptation to step changes in V_{cl} can never be achieved. Indeed, the steady-state error associated with a constant

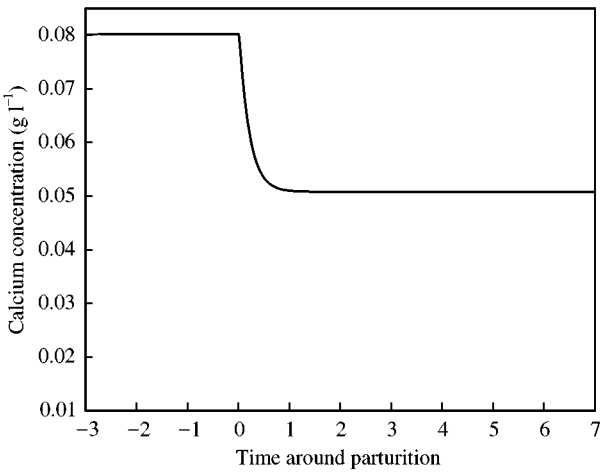

FIG. 2. Plot of simulation result for proportional model. Calcium disturbance occurs at time zero.

V_{cl} of magnitude $\overline{V_{cl}}$ will be a non-zero constant. In the case of the proportional feedback reported by Ramberg *et al.* (1984), where K_p is equal to 1770, this constant is equal to $-\overline{V_{cl}}/1770$. This implies that the plasma calcium concentration will fail to return to the setpoint value in response to a large and sudden change in V_{cl} (such as the onset of lactation around parturition), and a steady-state error will persist. This result is simulated in Fig. 2. This is contrary to the observed complete adaptation of the calcium homeostatic mechanism to an increase in calcium clearance. Another implication of the above error relation is that the steady-state error is dependent on the value of V_{cl}—indicating a lack of robustness in the regulation mechanism. Here again, this is not in agreement with the fact that the actual adaptation to the onset of lactation is robust in addition to being complete. Yet, a third piece of evidence against utility of the proportional feedback model derives from the shape of the response of the first-order differential equation to a constant change in V_{cl}. Upon applying such a constant increase in V_{cl}, $[\mathrm{Ca}]_p$ responds with a corresponding continuous *monotonic* decrease in its value until the steady-state value of $[\mathrm{Ca}]_p$ is reached. This characteristic first-order response takes place regardless of the value of any of the system parameters such as the constant of proportionality of the feedback, the plasma volume, etc. It is qualitatively different from the actual physiological time response.

3. The Necessity of Integral Feedback

We now propose that the calcium homeostatic system must employ integral feedback. This is based on a principle of feedback control theory that dictates that in order to obtain a zero steady-state error to a constant disturbance, integral feedback *must* be present. We propose a feedback model for V_T that adds to the proportional feedback another integral feedback term, resulting in what is commonly referred to by control engineers as *proportional–integral* (PI) control. The proposed expression for V_T at a given time is

$$V_T = K_p e + K_I \int e,$$

where K_p and K_I are constants and e is the calcium regulation error consisting of the difference between the setpoint r and $[\mathrm{Ca}]_p$. The schematic of the system is shown in Fig. 3. In this case, the differential equation describing the dynamics of the feedback system is second order, and is given by

$$\frac{\mathrm{d}^2[\mathrm{Ca}]_p}{\mathrm{d}t^2} + \frac{K_P}{vol}\frac{\mathrm{d}[\mathrm{Ca}]_p}{\mathrm{d}t} + \frac{K_I}{vol}[\mathrm{Ca}]_p = -\frac{1}{vol}\frac{\mathrm{d}V_{cl}}{\mathrm{d}t} + \frac{K_p}{vol}\frac{\mathrm{d}r}{\mathrm{d}t} + K_I\frac{r}{vol}.$$

Since r is a constant in the above equation, then $\mathrm{d}r/\mathrm{d}t = 0$.

A key consequence of integral control is that perfect adaptation is a structural property of the system. It is also a robust property that takes place regardless of the level of disturbance V_{cl} or the system parameters K_p, K_I, vol, etc. Step changes of any magnitude result in zero steady-state error after a short transient period. Furthermore, the transient response characteristics of the resulting second-order system agree quite well with the transient response characteristics seen in real data. In Fig. 4, actual calcium profiles in cows are plotted along with the computer-simulated response of the second-order system with integral feedback. The single points in the plot correspond to the average of plasma calcium concentrations for 18 calving cows over a 10-day period around the day of parturition. The solid plot corresponds to the simulated response for the second-order model when V_{cl} is increased from 20 to 70 g day^{-1} at the onset of lactation. The values of K_p and K_I used in the computer simulation were identified using data from a second set of 20 claving cows. The closeness of the fit suggests that in normal cows the homeostatic response is modeled quite well by the second-order dynamics dictated by integral feedback.

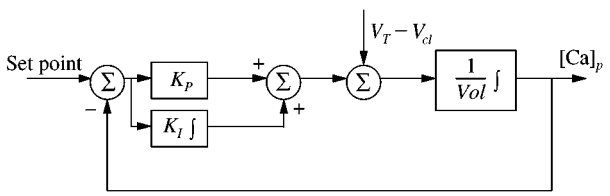

FIG. 3. Closed-loop system with PI controller.

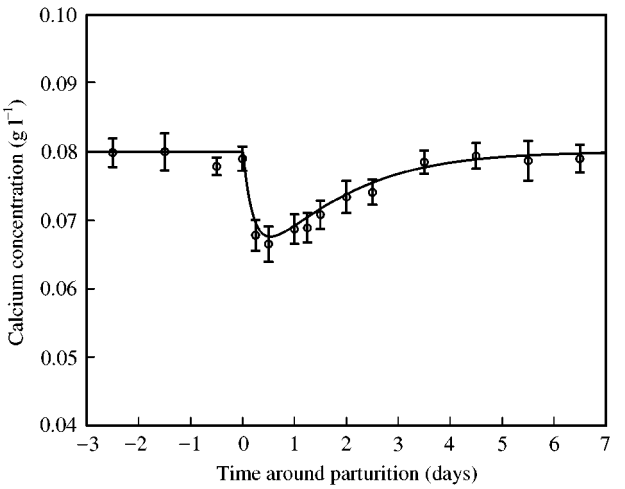

FIG. 4. Plot of actual data (⊢⊙⊣) and simulation result (▬) for second-order system. For the simulated response, V_{cl} is increased from 20 to 70 g day^{-1} at the onset of lactation. The single points in the plot correspond to the average of plasma calcium concentrations for 18 calving cows over a 10-day period around parturition. The data are taken for Jersey cows at their third or greater lactation. Heparinized blood samples were taken from the jugular vein and the plasma obtained for analysis. Plasma calcium is analysed by atomic absorption spectrophotometry. Results are presented as means ± S.E.

4. Physiological Basis

The proposed model has a number of attractive features: it has robust perfect adaptation as a structural property and it yields responses that agree with real data both during transients and in the steady state. In this section we show that it

has a physiological basis. Much of the model features are attributed directly to the presence of integral feedback. Indeed, as we have remarked earlier, integral feedback control is necessary for the robust perfect adaptation to increases in V_{cl}. So a key issue to be determined is the physiological mechanism by which integral feedback is realized. In Section 4.1 we explore two alternatives for realizing proportional integral feedback through the action of hormones.

4.1. REALIZING INTEGRATION BY MEANS OF HORMONES

We start by considering whether proportional–integral feedback can be realized with a single hormone. Suppose the total calcium input into the plasma (V_T) is proportional to the concentration of one hormone, say hormone A. If we denote this concentration by [Hormone A], then we have

$$V_T \propto [\text{Hormone A}].$$

Accordingly, PI feedback could only be explained in this case when

$$\frac{\mathrm{d}}{\mathrm{d}t}[\text{Hormone A}] \propto \left(error + K\frac{\mathrm{d}}{\mathrm{d}t}error\right).$$

However, this relation is not very likely for at least two reasons. First, it suggests that the rate of production of Hormone A must depend on the calcium error as well as the calcium error rate of change, and thus two mechanisms for the production of Hormone A must be present. Secondly, the above relation indicates that the mechanism for producing Hormone A must somehow rely on measurements of the *derivative* of the error. Direct measurement of the error is likely to be a difficult and noise-prone task.

Alternatively, if two hormones realize the PI control for calcium regulation, an elegant and quite plausible solution emerges. Suppose two hormones A and B are involved. We propose the following:

- The concentration of Hormone A is proportional to the error:

$$[\text{Hormone A}] \propto error.$$

- *The production rate of Hormone B* is proportional to the concentration of Hormone A:

$$\frac{\mathrm{d}}{\mathrm{d}t}[\text{Hormone B}] \propto [\text{Hormone A}].$$

- The rate of calcium influx into the plasma V_T is composed of two parts, one proportional to the Hormone A concentration, the other proportional to the Hormone B concentration:

$$V_T = V_A + V_B,$$

where

$$V_A \propto [\text{Hormone A}] \quad \text{and} \quad V_B \propto [\text{Hormone B}].$$

Thus, the proportional component of our PI control is given by V_A, while the integral component is giving by V_B. Furthermore, the concentration of Hormone A provides a measure of the error while the concentration of Hormone B provides a measure of the integral of the error. That the second postulate offers a plausible means for generating the integral of the error is a consequence of the underlying suggestion that only the concentration of another hormone, and not its rate of change, is needed in determining the production rate of Hormone B—much like a catalyst concentration would determine the rate of a chemical reaction.

Without additional information, it is difficult to say more about the feedback control realization based on control theory alone. In Section 4.2 we will see that based on what is known of the physiology of calcium homeostasis, the above postulates are indeed very good representations of reality.

4.2. ENDOCRINOLOGY OF CALCIUM HOMEOSTASIS

It has been established in the literature that when calcium demand from the plasma is increased, calcium homeostasis is achieved through the inflow of calcium from the bone, kidney and intestine under the control of two major hormones: parathyroid hormone (PTH), and an important metabolite of vitamin D: 1-25-dihydroxycholecalciferol (1, 25-DHCC) (Griffin

& Ojeda, 1996; Conn & Melmed, 1997). Parathyroid hormone is secreted by the parathyroid glands in response to a decrease in the calcium plasma concentration from the setpoint. Experiments have shown that the production as very much a linear function of the deviation from the setpoint (Ganong, 1991; Greenspan & Baxter, 1993). PTH acts mainly on the bone and kidney. Upon the increase in PTH concentration, a process known as osteocytic osteolysis takes place, in which PTH causes the removal of bone salts from the bone matrix by lacunar osteocytes. This occurs within minutes and proceeds without actual resorption of bone matrix (Guyton, 1991). More short-term needs are met through osteocytic osteolysis. If high concentrations of PTH persist, a delayed response (hours to days), known as osteoclastic bone resorption, takes place due to the activation of the bone osteoclasts. This process involves resorbing the bone matrix itself and allows the response to PTH to continue beyond what can be handled by osteocytic osteolysis. Thus, the need for maintaining plasma calcium concentrations is deemed more important than maintaining the integrity of the bone. The effect of PTH on the kidney is to increase tubular reabsorption of calcium thus reducing calcium loss through urine. Therefore, the impact of PTH is to increase immediate calcium transfer into the blood plasma. On the other hand, the main role of 1,25-DHCC is to stimulate intestinal calcium absorption through increasing formation of a calcium-binding protein in the intestinal epithelial cell (Duke, 1993). In fact, 1,25-DHCC is considered to be the most potent stimulator of calcium absorption from the intestine. It is well known that 1,25-DHCC is produced from cholecalciferol, a biologically inactive form of vitamin D after it undergoes several hydroxylation steps in the liver and kidney (Griffin & Ojeda, 1996; Conn & Melmed, 1997; Guyton, 1991; Duke, 1993). The last hydroxylation step in the kidney takes place only under stimulation by PTH. Calcitonin, the third hormone involved in calcium homeostasis, has relatively little relevance during hypocalcemia and therefore will not be considered. In fact, calcitonin is not secreted until plasma calcium levels exceed $9.5\,\mathrm{mg\,dl^{-1}}$. Above this calcium level, plasma calcitonin is directly proportional to plasma calcium (Ganong, 1991; Greenspan & Baxter, 1993). A plot of PTH and calcitonin vs. plasma calcium shows two straight lines of negative and positive slopes, respectively, intersecting at normal calcium levels, therefore generating the calcium setpoint (Greenspan & Baxter, 1993). Hence, we anticipate that calcitonin would play the same dynamical role as PTH during periods of negative error.

In what follows, considering the case of hypocalcemia, we will put the above-mentioned relationships into mathematical terms and show that the PI controller is very efficiently implemented through hormonal interaction. Let us start by considering V_{bone}, the rate at which calcium is transported into the plasma from bone. We can express V_{bone} as a fraction of the total calcium available in bone for resorption. Hence we have

$$V_{bone} = \alpha_{bone}\, V_B, \tag{2}$$

where V_B is the quantity of calcium that is available for resorption in bone. Clearly, $0 \leqslant \alpha_{bone} \leqslant 1$. We know that PTH stimulates bone resorption, so we may model α_{bone} as a function of the PTH concentration:

$$\alpha_{bone} = f_b\,([\mathrm{PTH}]). \tag{3}$$

To a first-order approximation, and assuming that $f_b(0) = 0$, we would have $f_b([\mathrm{PTH}]) = \alpha_b[\mathrm{PTH}]$ for some constant α_b. On the other hand, we known that at any given time PTH secretion by the parathyroid gland—and hence PTH plasma concentration—is proportional to the $[\mathrm{Ca}]_p$ deficiency (Greenspan & Baxter, 1993). Thus

$$[\mathrm{PTH}] = \alpha_e e, \tag{4}$$

where e is the deviation of the calcium concentration from its setpoint and is defined as $e := setpoint - [\mathrm{Ca}]_p$. From eqns (2) and (4), we have the following equation:

$$V_{bone} = K_p e,$$

where we have defined $K_p := \alpha_b \alpha_e \mathrm{V}_B$. Therefore, PTH stimulation of bone resorption can account

for the proportional feedback component of the total calcium supply V_T. Similarly, the intestinal absorption we can express $V_{intestine}$ as a fraction of the total calcium available in the animal's diet:

$$V_{intestine} = \alpha_{intestine} V_i, \tag{5}$$

where V_i is the calcium available in the diet. As before, $0 \leqslant \alpha_{intestine} \leqslant 1$. Since intestinal absorption is stimulated by concentrations of 1,25-DHCC, we could model it as a function of the 1,25-DHCC concentration:

$$\alpha_{intestine} = f_i([1{,}25 - \text{DHCC}]). \tag{6}$$

Similarly, we could assume a first-order approximation of $f_i(\cdot)$. We can therefore write $f_i([1{,}25\text{-DHCC}]) = \alpha_i[1{,}25\text{-DHCC}]$ for some constant α_i. In this case, we have

$$V_{intestine} = \alpha_i V_i \cdot [1{,}25 - \text{DHCC}]. \tag{7}$$

We now turn to modeling the relation between PTH and the 1,25-DHCC production rate. As mentioned previously, the last hydroxylation step of cholecalciferol in the kidney takes place under PTH stimulation. Thus, assuming a large pool of cholecalciferol, and considering the considerably large half-life of 1,25-DHCC (Martin, 1985), the production of the biologically active 1,25-DHCC could be thought of as being directly proportional to the PTH concentration. Therefore, we will model the rate of production of 1,25-DHCC in the kidney to be proportional to the plasma concentration of PTH. Thus

$$\frac{\mathrm{d}}{\mathrm{d}t}[1{,}25 - \text{DHCC}] = \alpha_p[\text{PTH}],$$

which implies that

$$[1{,}25 - \text{DHCC}] = \alpha_p \int_0^t ([\text{PTH}])\,\mathrm{d}\tau. \tag{8}$$

Therefore, eqns (7) and (8) together yield the following relationship:

$$V_{intestine} = \alpha_p \alpha_i V_i \int_0^t ([\text{PTH}])\,\mathrm{d}\tau. \tag{9}$$

Replacing [PTH] in the above equation by its expression in eqn (4), we get

$$V_{intestine} = K_I \int_0^t (e)\,\mathrm{d}\tau, \tag{10}$$

where $K_I := \alpha_i \alpha_p \alpha_e V_i$. This, we now propose, is the means by which integration is realized. V_{bone} and $V_{intestine}$ contribution to the total calcium inflow V_T implements the proposed PI controller.

5. Studying Calcium Homeostatic Disorders using Dynamic Models: A Parturient Paresis Case Study

Occasionally, the calcium homeostatic mechanism in dairy cows experiences some failures. On the day of calving, dairy cows typically produce 10 l of colostrum containing 23 g or more of calcium, approximately six times as much calcium as the extracellular calcium pool contains. Most animals adapt to the onset of lactation by rapidly increasing intestinal calcium absorption and bone calcium resorption. However, in some cows, the calcium regulatory mechanism breaks down. These animals become severely hypocalcemic, which disrupts neuromuscular signaling, resulting in recumbency and the clinical syndrome referred to as parturient paresis, or simply *milk fever* (Anderson, 1970; Oetzel & Goff, 1998). Milk fever affects about 6% of the dairy cows in the US each year according to the 1996 National Animal Health Monitoring Survey. The economic impact of the disease is exacerbated by the fact that cows that experience milk fever are more susceptible to other disorders such as mastitis, displaced abomasum, and ketosis. Usually, milk-fever cows are treated with intravenous calcium injections that keep them alive until intestinal and bone resorption adapt to the large calcium clearance. Figure 5 shows the plasma calcium concentration plot vs. time for a milk-fever cow treated with IV calcium infusion. We now propose to use the integral feedback model to study milk fever. While the linear model proposed (with f_i and f_b approximated by linear relations) describes quite well the calcium homeostasis function in healthy animals, it must be modified to account for nonlinear effects inherent in the

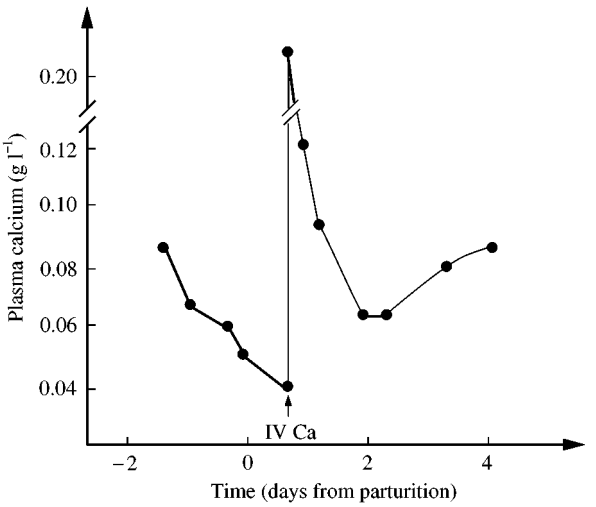

FIG. 5. Plasma calcium concentration in a cow with clinical milk fever treated with 10.5 g calcium intravenously over a 12-min period at day 0.5 after parturition.

calcium homeostatic mechanism that become significant in milk fever. The first of the nonlinear effects that are introduced in our linear model takes the form of a saturation in the proportional control term. The physical justification of this saturation follows from the observation that calcium reserves from bone are limited by the osteocytic osteolysis process and cannot be increased indefinitely in proportion to [PTH]. Beyond a certain limit, an increase in [PTH] does not lead to a corresponding increase in bone resorption of calcium. Therefore, using the same terminology as before, we could express V_{bone}, the rate of calcium provided by the bone as

$$V_{bone} = Sat_L(K_p e),$$

where Sat_L is a saturation function whereas $Sat_L(x)$ is equal to x if x is less than or equal to L and assumes the constant value L when x exceeds L. The second key nonlinear effect introduced into the model reflects the impact of reduced $[Ca]_p$ on the intestinal absorption processes. Experiments by Daniel (1983), demonstrated a highly significant correlation between plasma calcium levels and the amplitude and rate of both gut and abomasal motility in cows. This observation is explained in terms of the general effects of a depression in the levels of ionized calcium on smooth muscle contractility and

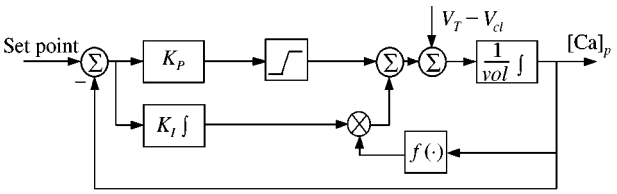

FIG. 6. Nonlinear model for calcium homeostasis. f denotes the intestinal nonlinear reduction factor.

neuromuscular transmission. The effect of low plasma calcium on the supply rate of calcium through intestinal absorption has been modeled as a non-linear, monotonically increasing factor multiplying the absorption coefficient. This factor assumes a value of unity at the setpoint indicating that gut motility does not play a role around normal levels of calcium, and becomes progressively smaller than one for lower levels for $[Ca]_p$. This can be mathematically modeled by the following equation:

$$V_{intestine} = \left[K_I \int e d\tau \right] f([Ca]_p)$$

with f being the nonlinear multiplication factor or function. This multiplication factor is difficult to measure experimentally. We adopted a multiplication factor that is a quadratic function of $[Ca]_p$, which has been obtained by considering the product of the rate and amplitude linear regression equations for rumen motility given by Daniel (1983) which is then normalized to give unity value at normal $[Ca]_p$ levels. It should be pointed out here that obtaining an accurate shape of such a multiplication factor is neither practically feasible nor important. The main point here is to study the qualitative effects that such a multiplicative factor may have on the regulatory system dynamics, and in particular whether it can lead, in computer simulations, to a breakdown in the calcium regulatory mechanism similar to what is observed in milk-fever animals. The rationale behind this argument is more elaborated upon through a theorem provided in a subsequent section.

Figure 6 shows the resulting control system, while Fig. 7 shows the behavior of the system for low and high values of K_p and K_I.

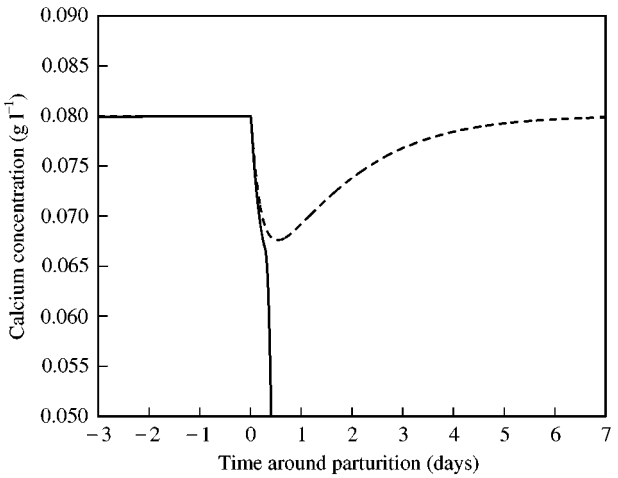

FIG. 7. (▬▬) Result of the simulation for low values of K_p and K_I. The model includes the reduction function $f(\cdot)$ and the saturation. (▬ ▬ ▬) Result of the simulation for high values of K_p and K_I.

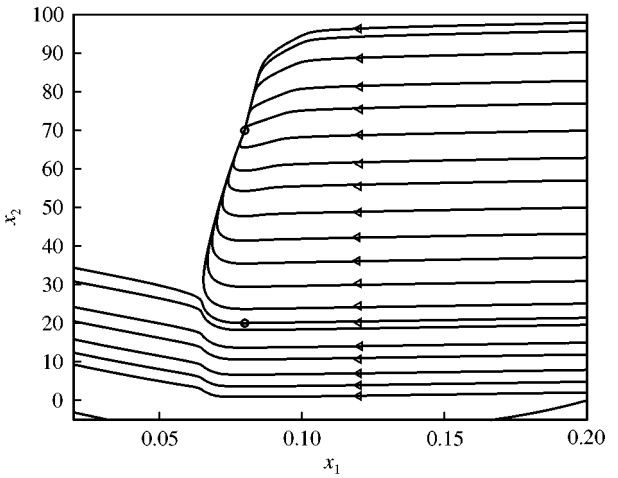

FIG. 8. Phase portrait for $K_p = 3000$ and $K_I = 12\,000$. The circles on the plot represent equilibrium points before and after the calcium clearance disturbance.

6. Mathematical Analysis

For the model depicted in Fig. 6, we have shown using numerical simulation that for a set of values of the model parameters, a breakdown in the calcium concentration level characteristic of that seen in milk-fever animals does indeed take place. In what follows, we prove analytically that this breakdown will occur for a continuum of values of the model parameters, and is not limited to a carefully chosen set of parameters. Let us consider the state space equations of the system shown in Fig. 6, given by

$$\dot{x}_1 = \frac{1}{vol}\left(Sat_L(K_p(r - x_1)) + f(x_1)\, x_2 - V_{cl}\right),$$

$$\dot{x}_2 = K_I\,(r - x_1),$$

where x_1 is the output of the calcium pool integrator, x_2 is the output of the PI block integrator, r is the calcium setpoint (i.e. the normal calcium concentration that should be tracked at all times), L is the saturation limit in the proportional branch and $f(\cdot)$ is the nonlinear function corresponding to the effect of excessive decrease of $[\mathrm{Ca}]_p$ on intestinal absorption. Obviously, the equilibrium point of this system can be computed to be

$$\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} r \\ V_{cl} \end{pmatrix}.$$

We could study this system numerically through phase portrait analysis. In this analysis, the differential equations governing the system are solved for different sets of initial conditions for x_1 and x_2. The resulting solutions are plotted in the x_1–x_2 plane. The results are shown in Figs 8 and 9. The trajectory of interest to us is the one passing through the (0.08, 20) point, since this is the pre-disturbance equilibrium point. It is interesting to see that for $K_p = 4300$ and $K_I = 1800$, the post-disturbance trajectory goes to the new equilibrium point (0.08, 70), which corresponds to the $[\mathrm{Ca}]_p$ setpoint and the new clearance rate, while for decreased values of these parameters, the regulatory system breaks down. This breakdown is characterized by the post-disturbance x_2 state increasing without bound. We could reach a similar result analytically. In fact, we provide a theorem which proves that for a range of relationships between the model parameters, instability (corresponding to milk fever) will occur in this model after a step increase in the calcium clearance V_{cl}.

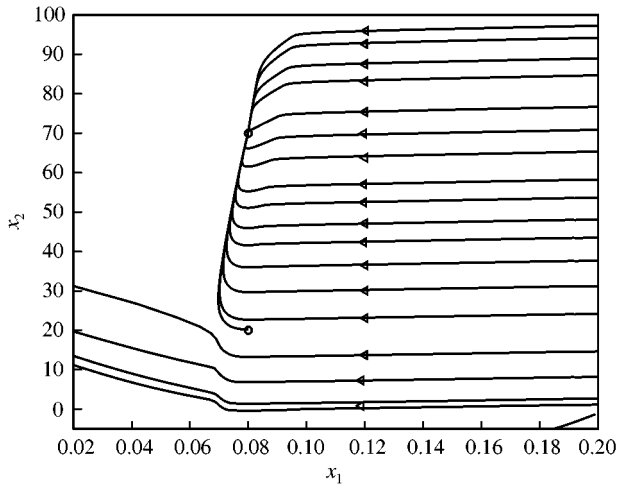

FIG. 9. Phase portrait for $K_p = 4300$ and $K_I = 1800$. The circles on the plot represent equilibrium points before and after the calcium clearance disturbance.

Theorem. *Consider the dynamical system described above with the initial conditions* $x_1(0) = x_{10} < r$ *and* $x_2(0) = x_{20} < \bar{x}$. *If*

- $L < V_{cl}$,
- $f(x_{10}) \leqslant vol\,\beta/(\bar{x} + K_I r^2/(\alpha - \beta))$ *where* $0 < \beta < \alpha := V_{cl} - L/vol$,

then $x_1(t) (t \geqslant 0)$ *will be monotonically decreasing and for some* $T \leqslant x_{10}/(\alpha - \beta)$, $x_1(T) = 0$.

Proof. Let $T = \min\{t > 0 : x_1(t) = 0\}$. Define $T' = \min(T, x_{10}/(\alpha - \beta))$. We know that

$$x_2(t) = x_2(0) + \int_0^t \dot{x}_2(\tau)\,\mathrm{d}\tau = x_2(0) + \int_0^t K_I(r - x_1(\tau))\,\mathrm{d}\tau, \tag{11}$$

where $0 < t < T'$. (11) implies that

$$x_2(t) \leqslant \bar{x} + K_I T' r \leqslant \bar{x} + K_I r \frac{x_{10}}{\alpha - \beta} \leqslant \bar{x} + K_I \frac{r^2}{\alpha - \beta}. \tag{12}$$

Also for $t \geqslant 0$, we have

$$\dot{x}_1 \leqslant \frac{f(x_1(t))\,x_2(t)}{vol} - \alpha. \tag{13}$$

So for $0 \leqslant t \leqslant T'$,

$$\dot{x}_1(t) \leqslant \frac{f(x_1(t))}{vol}\left(\bar{x} + \frac{K_I r^2}{\alpha - \beta}\right) - \alpha. \tag{14}$$

Therefore for $t = 0$

$$\dot{x}_1(0) \leqslant \frac{\beta}{\bar{x} + K_I r^2/(\alpha - \beta)}\left(\bar{x} + \frac{K_I r^2}{\alpha - \beta}\right) - \alpha = \beta - \alpha < 0. \tag{15}$$

Since f is monotonically increasing, it follows from eqn (14) that $x_1(t)$ is decreasing and

$$\dot{x}_1(t) \leqslant \dot{x}_1(0) \leqslant \beta - \alpha < 0, \quad 0 \leqslant t \leqslant T'. \tag{16}$$

Thus,

$$x_1(T') = x_1(0) + \int_0^{T'} \dot{x}_1(\tau)\,\mathrm{d}\tau \leqslant x_1(0) + (\beta - \alpha)T' = 0. \tag{17}$$

This implies that $T \leqslant T'$, and therefore $T = T' \leqslant x_{10}/(\alpha - \beta)$, which completes the proof. □

7. Discussion

In this paper, we considered the application of control theory concepts in the modeling and analysis of blood plasma calcium homeostasis in dairy cows. A dynamic model based on integral feedback control was proposed. This model captures the observed perfect adaptation of the homeostatic mechanism in response to increased calcium demand. A physiological basis for integral feedback in which PTH and 1,25-DHCC interact with each other to produce an integral effect was proposed. The dynamic model proposed for healthy animals is a linear model. However, when nonlinearities modeling the saturations in the bone response to PTH and the reduction in gut motility after an excessive decrease in calcium concentration were introduced, our proposed dynamic model produced the homeostatic breakdown characteristic of milk fever.

One of the points advocated through this model is that homeostasis may be quite naturally described in the context of dynamical systems using the language and perspectives of feedback control theory. This point of view has many advantages, which we illustrate using the example of calcium homeostasis. As mentioned in the previous sections, it is possible to reach important conclusions about the nature of the underlying calcium feedback mechanism in dairy cows using only qualitative features of the calcium concentration profiles. One key feature that is reflected in the data is the consistent and robust adaptation of the calcium homeostasis mechanism to the onset of lactation. The profound implications of this remarkable adaptation on the structure of the underlying homeostatic mechanism seem to have gone unrecognized in the literature, as evidenced by the proportional feedback homeostasis model used by Ramberg *et al.* When one invokes feedback control theory, however, the necessity of integral feedback is inescapable. With integral feedback in place, a simple dynamic model of the calcium homeostatic mechanism was obtained. This model not only possesses the property of perfect adaptation, but also exhibits transient characteristics prior to adaptation that are similar to those seen in actual data. Yet, the most important implication of integral feedback does not lie in producing a simple dynamical model that agrees well with the actual data. Rather, it lies in the severe structural constraints that it imposes on the underlying homeostatic mechanism. Such constraints explain the role of PTH and 1,25-DHCC in homeostasis and the nature of the interaction between these two hormones. Indeed PTH measures the error and leads to bone resorption in proportion to that error, all the while leading to the production of 1,25-DHCC at a rate proportional to the error. As a result, the 1,25-DHCC hormone concentrations will be proportional to the integral of the error. In turn 1,25-DHCC results in intestinal absorption, thus implementing integral feedback. Therefore, the function of PTH and 1,25-DHCC hormones and the nature of their interaction is a direct result of the requirements of integral control—so much so that the mere existence of two hormones responsible for calcium homeostasis, along with their function and the nature of their mutual interaction, may be hypothesized based on the requirements of integral feedback control alone, without prior knowledge of the endocrinology of calcium homeostasis. The significance of this may be better appreciated when one considers that 1,25-DHCC and its role in calcium homeostasis was discovered as late as the 1970s. Even when one takes for granted the presence of two hormones responsible for calcium homeostasis during hypocalcemia, in the absence of explanations that rely on integral feedback the only explanation for the need for two hormones (as opposed to a single hormone) is redundancy. Based on the arguments put forth in this article, that explanation must be abandoned.

As a result, several themes readily emerge from the application of control theory to the study of biological systems. One major theme relates to the advantages of using the ideas of control theory in the derivation of necessary conditions for the structure of biological system and the type of control used to achieve homeostasis. These conditions can be used to eliminate some hypotheses and favor others. At this stage, known biology could be used to gain more confidence in the favored hypotheses.

Another advantage of the dynamical point of view of homeostasis in the context of feedback control is achieved during disease. When homeostatic mechanisms break down, a dynamic description of homeostasis could provide suggestions for identifying the causes of the breakdown, while eliminating those possibilities that are not consistent with the model dynamics. In the case of calcium homeostasis, as a result of the nonlinear effects introduced to model saturation in the bone response to PTH and reduction in gut motility during severe hypocalcemia, a breakdown in calcium concentration levels has been seen in simulation and can be proven to take place for a continuum of values of the model parameters. As a result, the dynamic model would suggest that a reduction in bone responsiveness to PTH may be an important factor leading to milk fever. At the same time, it can be shown that for breakdown to take place in this case, the reduced bone responsiveness of PTH must be accompanied by a reduction in gut motility and that neither effect alone can be responsible for milk fever. We have only proposed

one scenario for milk fever, but other possibilities may exist. Even in this case, the dynamical model based on integral feedback control can serve as a key test for the viability of candidate mechanisms for milk fever and to suggest further experiments or courses for treatment.

The successful understanding of calcium regulation mechanism through verifiable models can have two other important implications. First, active regulation of calcium through artificial means, e.g. using implanted devices, becomes possible. In engineering terms, this corresponds to designing the controller for a given system model. Many effective techniques exist in the control systems literature for this purpose. Secondly, the added understanding of calcium regulation gained by studying the dynamics of the calcium feedback loop may shed new light on other calcium diseases that affect humans, most notably osteoporosis. This is achieved by incorporating the dynamical model for calcium homeostasis, which includes the coupling dynamics of PTH and 1,25-DHCC with existing models in the literature that capture the dynamics of osteoblast and osteoclast cell populations (Suda *et al*., 1992a, b; Kroll, 2000). Since osteoblasts are responsible for bone formation while osteoclasts lead to bone resorption, it is the ratio and activity pattern of these populations that determine the net bone loss during osteoporosis. Therefore by studying these cell population dynamics we hope to obtain a better understanding of the dynamics of bone resorption.

Finally, we would like to compare the results reported in this work to those published recently by Saunders *et al.* (2000) where the authors attribute calcium homeostasis to a control scheme they refer to as *Integral Rein Control* (IRC). The basic idea behind IRC is that the equilibrium value for calcium is determined by functions dependent on the level of a chromogranin-derived peptide CgA, which is hypothesized to inhibit both calcitonin and PTH. This equilibrium is therefore independent of the external variables (being the input of calcium from the gut). Once this setpoint is fixed, the balance between calcitonin and PTH adjusts dynamically to compensate for the disturbance, thus maintaining calcium at its equilibrium. This scheme works locally, yielding a zero steady-state error to disturbances, provided the equilibrium obtained this way is stable. Here, we argue that IRC does not adequately describe calcium homeostasis for several reasons. We will only elaborate on the most salient of these reasons.

First, the model developed by Saunders *et al.* ignores some of the known physiology of calcium regulation. For example, the model considers gut input as a disturbance. This is in contradiction with the known literature where intestinal absorption is itself a controlling variable whose level is determined by the concentration of 1,25-DHCC. The production of 1,25-DHCC is inturn controlled by the PTH concentration. In fact, as mentioned in previous sections, it has been irrefutably established that PTH and 1,25-DHCC in concert are the major players responsible for calcium homeostasis. Calcitonin plays a role, but only during hypercalcemia. Without calcitonin, calcium clearance will still take place and eventually brings the calcium concentration to its setpoint. In fact, an excess or deficiency in PTH or 1,25-DHCC produces dramatic clinical disorders while an excess or deficiency in calcitonin (e.g. after thyroidectomy) produces few discernible abnormalities (Greespan & Baxter, 1993). The IRC model hinges critically on both calcitonin and PTH and ignores completely 1,25-DHCC. In fact, without calcitonin, the IRC control model breaks down completely.

Secondly, the model indicates that when a large disturbance is present, e.g. calcium clearance from the plasma during milk production, the concentration of PTH should be above normal even after perfect adaptation has taken place and plasma calcium concentration levels are back to normal. This is not what is observed in animal experiments (J. P. Goff, pers. comm.). Instead, when the plasma calcium concentration adapts to the constant disturbance and assumes its normal level, so does the PTH concentration.

Thirdly, the IRC model has a limited range of operation before a bifurcation destroys the equilibrium. Moreover, the behavior of the model is wildly oscillatory and lightly damped even to small step disturbances (computer simulation of the IRC model, results not shown in this paper). This is contrary to what is known about the remarkable stability of the calcium homeostatic system.

However, we would like to emphasize that the model and analysis developed in this work and the work of Saunders *et al.* converge in that they both provide another piece of evidence in support of an emerging theme that integral control is a functional building block in a wide range of physiological mechanisms, ranging from the cellular level to systemic physiology. This perspective was recently discussed in the work of Yi *et al.* (2000) where the necessity of integral control for robust perfect adaptation in bacterial chemotaxis was studied. These results as well as those reported in this article seem to point to the prevalence of integral control in mechanisms where physiological quantities must be maintained within a narrow range despite internal and external disturbances. Further work is needed to catalog and uncover the architecture of these systems where integral control is at work.

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