A SYMBOLIC MODEL FOR THE REGULATION BY BONE METABOLISM OF THE BLOOD CALCIUM LEVEL IN RATS

JEAN-PAUL AUBERT *and* FELIX BRONNER

From the Laboratoire des Isotopes, Institut Pasteur, Paris, and the Department of Physiology and Biophysics, University of Louisville School of Medicine, Louisville, Kentucky

ABSTRACT The control by bone metabolism of the blood calcium level in young rats may be described in terms of a regulator-type system. The model presented here comprises a feedback loop involving only a proportional control in thyroparathyroidectomized, and a combination of proportional and integral controls in normal animals. It accounts for the variations observed when the system was subjected to a variety of experimental constraints. The implications, limitations, and possible extensions of the model are discussed.

INTRODUCTION

The maintenance of the blood calcium concentration at a constant level is a classic example of biological homeostasis. The mechanism whereby this is accomplished has for some time been an open question. With the discovery of the role of the parathyroid glands and the multiple effects of their hormone, it has become possible to describe qualitatively the existence and functioning of controls at various levels, *i.e.*, bone, kidney, and gut (Neuman and Neuman, 1958; McLean and Urist, 1961; Rasmussen, 1961).

However, a regulatory and particularly a homeostatic phenomenon may equally well be studied in the formal, cybernetic terms appropriate to it (Goldman, 1960). When based upon an adequate model, such a description should permit the evaluation in quantitative terms of the function and interaction of the various physiological processes making up the regulatory system. This article proposes and describes a simplified model in which the blood calcium level of the rat is regulated by the deposition of calcium in and its removal from bone.

GENERAL SYSTEMS ANALYSIS OF CALCIUM METABOLISM

Kinetic analysis of calcium metabolism, based upon the simultaneous use of tracer

calcium and mass balance techniques, has led to the definition and measurement of the following parameters (Aubert and Milhaud, 1960; Aubert *et al.*, 1963):

The pool P represents the exchangeable calcium in the organism and may be visualized as made up of several compartments, one of which is the blood. The pool is fed by calcium absorbed from the gut and resorbed from bone; the pool loses calcium *via* the urine, *via* the digestive juices, and by deposition in bone. These movements are represented by the symbol v, expressed in terms of units of mass per unit of time and measured experimentally as average values ($\bar{v}$).

Entries into the Pool

v_a = that portion of the ingested calcium absorbed in the digestive tract.

v_{o-} = calcium resorbed from bone by a variety of processes, collectively designated as "bone catabolism."

Losses from the Pool

v_u = calcium excreted in urine.

v_f = the fecal endogenous calcium, *i.e.* that fraction of the digestive juice calcium not reabsorbed in the digestive tract. For a more detailed discussion of events in the gut, see Aubert *et al.* (1963)

v_{o+} = calcium deposited in bone by a variety of processes, collectively designated as "bone anabolism."

Fig. 1 gives typical average values for 3-month old rats.

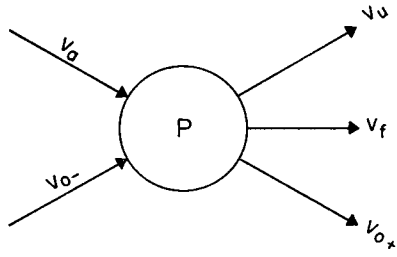

FIGURE 1. Scheme of calcium metabolism showing the pool with its inputs and outputs. In normal, 3-month old male Wistar rats, the mean values for these parameters were (Aubert *et al.*, 1964): $\bar{P}$ = 72.9 mg Ca; $\bar{v}_a$ = 35.9 mg Ca/day; $\bar{v}_{o-}$ = 32.8; $\bar{v}_u$ = 0.9; $\bar{v}_f$ = 9.4; $\bar{v}_{o+}$ = 58.4.

THEORETICAL ANALYSIS

A. Development of an Elementary Model of Regulation.

The following definitions will be used:

1. The *controlled system* is that system whose time-dependent course follows a known relationship, *e.g.* the system in which homeostasis is preserved. The *controlling system* is that system which responds to information from the controlled system by tending to overcome the disturbances that may arise in the controlled system.

2. The *controlled signal* is that measurable physical quantity of the controlled system which carries the information function. The *controlling signal* is that measurable physical quantity of the controlling system which tends to overcome the variations that occur in the controlled system. The *disturbing signal* is the measurable physical quantity which comes from outside of the system and introduces a disturbance in the controlled system. The controlling system has as its function to maintain the controlled signal at its predetermined or *reference value.*

The preceding definitions apply to our situation as follows:

(*a*) The blood plasma is the controlled system and its calcium concentration, $[Ca_s]$, is the controlled signal. The plasma calcium concentration around which regulation occurs in normal animals constitutes the reference value, $[U]$.

(*b*) Even if the intensity of calcium absorption is controlled over the lifespan of the rat (Bronner, 1964), its rate v_a can be considered as a disturbing signal during an acute experiment, for it can be changed at will by altering the calcium intake.

Fecal endogenous calcium excretion, defined by its rate v_f, is related to the flow of calcium in the digestive juices which in turn are secreted in response to food intake. Hence v_f must also be considered a disturbing signal.

As the direction of these two processes is opposite to one another, the difference $v_a - v_f$, designated as S_i, may be considered the net disturbing signal for the system.

(*c*) The kidney would certainly appear to be a controlling system, with the rate of urinary calcium excretion, v_u, a controlling signal. It has been shown (Aubert *et al.*, 1961, 1964; Bronner *et al.*, 1965) that the intensity of bone metabolism can be related to the intensity of the disturbing signal. Bone would therefore appear to be a second controlling system, whose two controlling signals are v_{o+} and v_{o-}. Just as before, the net controlling signal of bone is the difference between these two parameters, *i.e.* $v_{o+} - v_{o-}$. This difference is the calcium balance of the system, termed Δ.

It can be seen from Fig. 1 that in young rats the ratio $\bar{v}_u/(\bar{v}_a - \bar{v}_f) = 0.04$, whereas the ratio of $\bar{\Delta}/(\bar{v}_a - \bar{v}_f) = 0.96$. In thyroparathyroidectomized rats (Aubert *et al.*, 1964), the corresponding ratios were 0.07 and 0.93. In other words, in both normal and thyroparathyroidectomized animals, bone can be considered as practically the only controlling system.

The symbolic model shown in Fig. 2 was constructed on the basis of the preceding considerations.

B. Analysis of the Model

If V_s is the volume of the controlled system, the variation in $[Ca_s]$ is given by

$$\frac{d[Ca_s]}{dt} = \frac{1}{V_s}(S_i - \Delta) \tag{1}$$

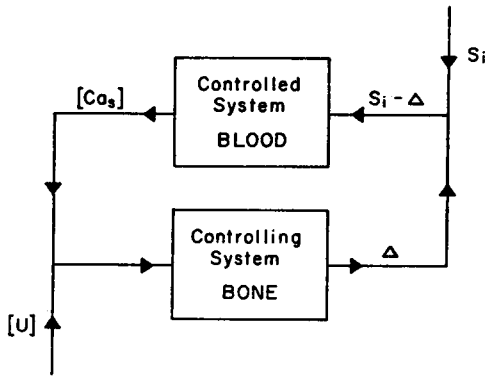

FIGURE 2. Feedback loop of regulation of the blood calcium level by bone metabolism.

1. *Thyroparathyroidectomized animals.* We shall first analyze the behavior of the model for the simplest case, *i.e.* in the absence of a hormonal regulation of $[Ca_s]$.

In a feedback loop, the controlling signal is related to the controlled signal. In the case of thyroparathyroidectomized animals this relationship between the two signals may be expressed in its simplest and most general form by

$$\Delta = K([Ca_s] - \alpha[U])^n \tag{2}$$

where K is a constant, α a coefficient less than 1 and n an integer that, for reasons of simplicity, will be considered equal to 1. In such a system, the controlling signal is proportional to the error, S_e, which equals $[Ca_s] - \alpha[U]$.

Equation 1 for these animals then becomes

$$\frac{d[Ca_s]}{dt} = \frac{1}{V_s}[S_i - K([Ca_s] - \alpha[U])] \tag{3}$$

In the absence of a disturbing signal there is no variation in $[Ca_s]$ which is stabilized at $\alpha[U]$, the reference value of the operated animals. Upon application of a constant disturbing signal, $[Ca_s]$ will evolve towards a new equilibrium by an exponential path, S_e will then be at its maximum and

$$S_e(\max) = \frac{S_i}{K} \tag{4}$$

If any signal S_i is applied intermittently, then $\int \Delta\, dt = \int S_i\, dt$ between two returns of $[Ca_s]$ to the reference value. For practical purposes we can therefore agree that $\bar{\Delta} = \bar{S}_i$.

The controlling signal, Δ, may be decomposed into its two constituents, v_{e+} and v_{e-}, by means of regression equations between the intensity of bone metabolism and calcium absorption derived from studies on thyroparathyroidectomized animals

(Aubert *et al.*, 1965). In the terminology employed here, these equations can be written in the form

$$\bar{v}_{o+} = 15.1 + 0.5\bar{S}_i \quad \text{(Units : mg Ca/day)} \tag{5}$$

$$\bar{v}_{o-} = 15.9 - 0.5\bar{S}_i \tag{6}$$

Equations 5 and 6 show that the mean value of each controlling signal varies symmetrically with the mean value of the disturbing signal. If we assume that this symmetrical relationship also applies to the signals proper then, because $\Delta = v_{o+} - v_{o-}$ by definition, the simplest expression for v_{o+} and v_{o-} will be given by

$$v_{o+} = \frac{K}{2}[\mathrm{Ca}_e] \tag{7}$$

$$v_{o-} = \frac{K}{2}(2\alpha[U] - [\mathrm{Ca}_e]) \tag{8}$$

As equations 7 and 8 are linear, they can be utilized to estimate K and $\alpha[U]$ from the average experimental values. In 50 thyroparathyroidectomized animals, Aubert *et al.* (1964) found $\bar{v}_{o+} = 22.2$ mg Ca/day, $\bar{v}_{o-} = 7.0$ and $[\mathrm{Ca}_e] = 64.0$ mg/liter of serum; the resulting values for K and $\alpha[U]$ are, respectively, 0.69 liter/day and 42 mg Ca/liter. In normal animals, $[U]$ is likely to be between 100 and 110 mg Ca/liter of serum; hence α is about 0.4.

The difference in the numerical value between mean $[\mathrm{Ca}_e]$ and the estimate for $\alpha[U]$ is fairly large and may be attributed to the relative inefficiency of a proportional control system. This inference is borne out by studies on the effect of calcium intake on the serum calcium level of thyroparathyroidectomized rats. In 7 such animals maintained on a 0.6 per cent Ca diet, $[\mathrm{Ca}_e]$ was found to drop from an average of 75.7 (SE: 2.5) to 58.1 (SE: 2.6) mg Ca/liter after a fast lasting only 12 hours, and rose to 76.1 (SE: 2.3) after 12 hours of realimentation (Moukhtar, personal communication).

2. Normal animals. There is no *a priori* reason to suppose that the control system existing in ablated animals has been suppressed in normal animals. We shall assume that a hormonal factor has been superimposed and that the controlling signal may then be expressed by

$$\Delta = K'([\mathrm{Ca}_e] - \alpha[U]) - K_1[H] \tag{9}$$

where K' may or may not be equal to K of equation 2, and where $[H]$ represents the concentration of the parathyroid hormone, the effect of which is assumed to be proportional (K_1) to its concentration. As the effect of this hormone is to maintain $[\mathrm{Ca}_e]$ at the reference value $[U]$ and as α is smaller than one, the term $K_1[H]$ must be negative.

Hence, equation 1, for normal animals, becomes

$$\frac{d[\mathrm{Ca}_s]}{dt} = \frac{1}{V_s}[S_i - K'([\mathrm{Ca}_s] - \alpha[U]) + K_1[H]] \tag{10}$$

McLean and Urist (1961) have proposed that parathyroid secretion responds to a drop in the blood calcium level, which in turn is regulated by the action of parathyroid hormone on bone catabolism. The first part of this hypothesis implies that a relationship exists between hormone secretion and $[\mathrm{Ca}_s]$. In order to express this relationship in mathematical terms, it is necessary to know the nature of the repression of parathyroid hormone secretion by calcium.

On the basis of *in vitro* studies with explanted parathyroid glands Raisz *et al.* (1965) have discussed the possibility of the control of parathyroid secretion by the action of calcium at the cell membrane. This suggestion can certainly be formulated in mathematical terms. On the other hand, it is also possible that this kind of feedback control is described by the more general theory of control of protein biosynthesis at the genetic level and to apply to our situation the analytical principles formulated by Goodwin (1963). As either possibility at the present would be speculative, we shall instead use what is mathematically the simplest expression, a linear relationship between $[\mathrm{Ca}_s]$ and the rate of hormone appearance in the circulation. This relationship can be assumed to approximate the true function, at least over the narrow range within which $[\mathrm{Ca}_s]$ varies in normal animals.

It may also be assumed that the rate of hormone degradation is proportional to the hormone concentration. Then the equation describing the variation of hormone concentration during the time dt may be written

$$\frac{d[H]}{dt} = K_2([L] - [\mathrm{Ca}_s]) - K_3[H] \tag{11}$$

In equation 11, the rate of parathyroid hormone appearance is proportional to the feedback signal ($[L] - (\mathrm{Ca}_s]$) that will be assumed to equal 0 when $[\mathrm{Ca}_s] \geq [L]$. The term $[L]$ then corresponds to the value of $[\mathrm{Ca}_s]$ when hormone appearance ceases. Moreover, it follows from equation 11 that the term $K_1[H]$ in equation 10 expresses the functioning of an integral control added to the already existing proportional control.

When S_i is constant, it follows from equations 10 and 11 that if the determinant $(K_3 - K_1/V_s)^2 - 4K_1K_2/V_s$ is positive, the system will proceed without oscillation and the evolution of $[\mathrm{Ca}_s]$ with time will be given by an equation of the type

$$[\mathrm{Ca}_s] = A + Be^{-bt} + Ce^{-ct} \tag{12}$$

If the determinant is negative, the system will proceed with damped oscillations and the time-dependent function of $[\mathrm{Ca}_s]$ will be given by an equation of the type

$$[\mathrm{Ca}_s] = P\int_0^t e^{-\beta t}\sin \omega t\, dt + Qe^{-\beta t}\sin(\omega t + \ ) \tag{13}$$

The functions given by equations 12 and 13 are bounded when $t \geq 0$ and the limit for $[Ca_s]$, when t increases, is

$$[Ca_s]_{\lim (S_i)} = \frac{K_1 K_2[L] + K_3(K'\alpha[U] + S_i)}{K' K_3 + K_1 K_2} \tag{14}$$

When $S_i = 0$, the system becomes stabilized at the reference value $[U]$ and we can rewrite equation 14 in the form of the maximum error

$$S_s(\max) = [Ca_s]_{\lim (S_i)} - [U] = \frac{S_i}{K'}\left(\frac{[L] - [U]}{[L] - \alpha[U]}\right) \tag{15}$$

Comparison of equation 15 with equation 4 shows that in normal animals the error resulting from the functioning of the proportional control alone (S_i/K') is further reduced by a factor resulting from the functioning of the integral control.

When 7-week old male Sprague-Dawley rats ($n = 55$, mean body wt: 230g), that had been kept on a 1.6 per cent Ca intake from weaning, were allowed access to 0.05, 0.5, and 1.5 per cent Ca intake *ad libitum* during a 2-week experimental period, S_i varying from 3.6 to 69.3 mg Ca/day, the variation in $[Ca_s]$ about a mean value of 106.7 mg Ca/liter of serum was found to be very small (Bronner *et al.* 1965). These results imply that in equation 15 the numerical value of $[L]$ must be very close to that of $[U]$.

As was true for the ablated animals, one may also in the case of the normal animals decompose the controlling signal, Δ, into its two constituents v_{o+} and v_{o-}. From studies with 90 3-month old male Wistar rats (mean body wt: 150g) that had been kept on a 0.4 per cent Ca intake from weaning and allowed access to food *ad libitum* during the experiment, Aubert *et al.* (1961) were able to derive regression equations between the intensity of bone metabolism and that of the disturbing signal, when the latter ranged between 3.6 and 36 mg Ca/day. The equations were

$$\bar{v}_{o+} = 45 + 0.5\overline{S_i} \quad \text{(units : mg Ca/day)} \tag{16}$$

$$\bar{v}_{o-} = 45 - 0.5\overline{S_i} \tag{17}$$

In these, as in the ablated animals, $\bar{v}_{o+}$ and $\bar{v}_{o-}$ can be seen to have varied symmetrically in response to the mean controlling signal and therefore Equations 7 and 8 may be modified to read

$$v_{o+} = \frac{K'}{2}[Ca_s] - \frac{K_1}{2}[H] \tag{18}$$

$$v_{o-} = \frac{K'}{2}(2\alpha[U] - [Ca_s]) + \frac{K_1}{2}[H] \tag{19}$$

In the study referred to above, Bronner *et al.* (1965) showed that $\bar{v}_{o+}$ did not vary much from the mean value of 71.4 mg Ca/day, whereas $\bar{v}_{o-}$ decreased nearly linearly as $\bar{S}_i$ increased. In these animals, equations 18 and 19 must be replaced by

$$v_{o+} = \frac{K'}{2}[\mathrm{Ca}_s] \tag{20}$$

$$v_{o-} = \frac{K'}{2}(2\alpha[U] - [\mathrm{Ca}_s]) + K_1[H] \tag{21}$$

The significance, in physiological terms, of these two different sets of equations has been discussed elsewhere (Bronner and Aubert, in preparation). Here we merely wish to discuss their significance in terms of the maximum regulating capacity of the system.

Regulation by the parathyroids is efficient whether S_i is negative, zero, or positive. However, there exists an upper limit, S_i (max), for positive values of S_i beyond which the capacity of the system is overcome.

We can estimate S_i (max) as follows: The lower limit of v_{o-} is zero and at that limit the only remaining controlling signal is v_{o+} which must then equal S_i. As S_i (max) corresponds to the value of S_i at which the system is still regulated at $[U]$, S_i (max) equals the value of v_{o+} when $v_{o-} = 0$. In the case of equations 18 and 19 this is given by $K'\alpha[U]$ and in the case of equations 20 and 21 by $K'[U]/2$.

Although the numerical value of S_i has been found to be about 70 mg Ca/day in rats where the regulation of $[\mathrm{Ca}_s]$ is described by equations 20 and 21 (Bronner *et al.*, 1965), one can imagine a situation where this value is exceeded. In such a case, $[\mathrm{Ca}_s]$ could be maintained at $[U]$ only with the aid of a hormone acting in a direction opposite to that of parathyroid hormone, such as calcitonin (Copp *et al.*, 1962) or thyrocalcitonin (Foster *et al.*, 1964; Hirsch *et al.*, 1964). It should be possible to take account in the above analysis of such a hormone when its mode of action becomes known.

A final remark needs to be made concerning a possible regulatory role of the pool. In theory, a pool in which only processes of exchange take place cannot by itself play such a role. Experimentally the pool did not increase with increasing S_i (Bronner *et al.*, 1965). Hence the only role the pool can play is to increase the apparent volume of the controlled system.

CONCLUSION

The model developed here represents an attempt at a unifying expression for the regulation of the blood calcium level by bone metabolism in normal and thyroparathyroidectomized rats.

In either group bone metabolism responds *via* a feedback loop to variations in the blood calcium level, but the type of response differs in the two groups. In ablated animals, the control is of the proportional type, whereas in normal animals an integral control, due to the parathyroid hormone, is added to the proportional control.

The model accounts for the principal experimental findings, (*a*) for the pro-

nounced stability of the blood calcium level in normal animals, (*b*) for a decrease in this level in ablated animals and its marked variation in them with calcium input, and (*c*) for the variations in the intensity of bone metabolism in either group with variations in calcium input.

The model also includes a number of uncertainties. Some of these appear as predictions, *e.g.* the non-oscillatory behavior of the system in thyroparathyroidectomized animals. Others appear as alternatives, *e.g.* the possibility of oscillatory or non-oscillatory behavior of the system in normal animals.

Finally, the model does not take into account a hormone acting in a direction opposite to that of the parathyroid hormone. The need for such a hormone becomes apparent when the maximum regulating capacity of the system is analyzed. This point is of theoretical importance, as the expression chosen for the rate of appearance of parathyroid hormone may in fact express the resultant of the rates of appearance of the two hormones. In that case, the regulatory capacity of the parathyroid system has been overestimated.

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