QUANTITATION OF CALCIUM METABOLISM. THEORY*

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There is general agreement among all who have used Ca^{45} for kinetic studies in either man or experimental animals that the decrease with time of specific activity of serum calcium can be expressed mathematically by a series of exponential terms. Beyond this, however, opinions diverge, and there is no agreement on either the number of exponential terms needed, the scheme of the body's calcium economy, or the analytical approach for solving the scheme. This paper aims at presenting a general scheme of calcium metabolism, its resolution by a combination of balance and isotope technics, and a critical review of past work.

DEFINITIONS AND NOTATION

General definitions and notation, those that apply to kinetic analysis generally, regardless of whether and how Ca metabolism is analyzed, are as follows: The pool, P, is the sum of two or more compartments, M, in units of mass, between which various exchange processes occur. Exchange is the bidirectional movement of ions between two compartments of a pool without detectable change in compartment size. Isoexchange is the movement of ions of the same element, e.g., $Ca^{45} \rightleftarrows Ca^{40}$; heteroexchange is that of different elements. This paper will consider only isoionic exchange. Unidirectional entries to and losses from a pool are detectable movements into or out of a pool that may or may not result in a change of pool size. If the amount that enters equals the amount that leaves, pool size does not change, and the pool is in dynamic equilibrium. V designates the rate in units of mass per unit time of an exchange process, and v, that of a unidirectional process.

R indicates radioactivity measurements, in counts per minute, or per cent dose; R_s indicates specific radioactivity (SA), in counts per minute per milligram Ca, or per cent dose per milligram Ca.

Definitions and notations specific to the scheme of Ca metabolism shown in Figure 1 follow. The system under analysis is a central Ca pool, the skeleton and the intestine,

and is considered to be in steady state, with v, V, and P constant dnurig the period of observation. The Ca pool obtains Ca from the skeleton and food, and loses it to the skeleton and via urine and feces. Various, analyzable exchange processes occur within P, which is made up of M, M_1, M_2, and M_3 (Figures 2 and 3). The skeleton obtains Ca from and loses it to P. The intestine obtains Ca from and loses it to the environment and P. Balance indicates the difference between input and output, either in the whole system (the body) or in a part (the pool). Traditionally, Ca balance is that in the system as a whole, or Ca intake less Ca output in excreta.

Notation for unidirectional processes and for exchanges, in milligrams per day, is as follows: Δ = Ca balance, Δ_P = Ca balance of pool, Δ_s = Ca balance of system, v_i = Ca ingested in food, v_d = Ca secreted in digestive juices, v_a = Ca absorbed from intestine, v_f = fecal endogenous Ca, v_F = total fecal Ca, v_u = urinary Ca, v_{o+} = Ca deposited
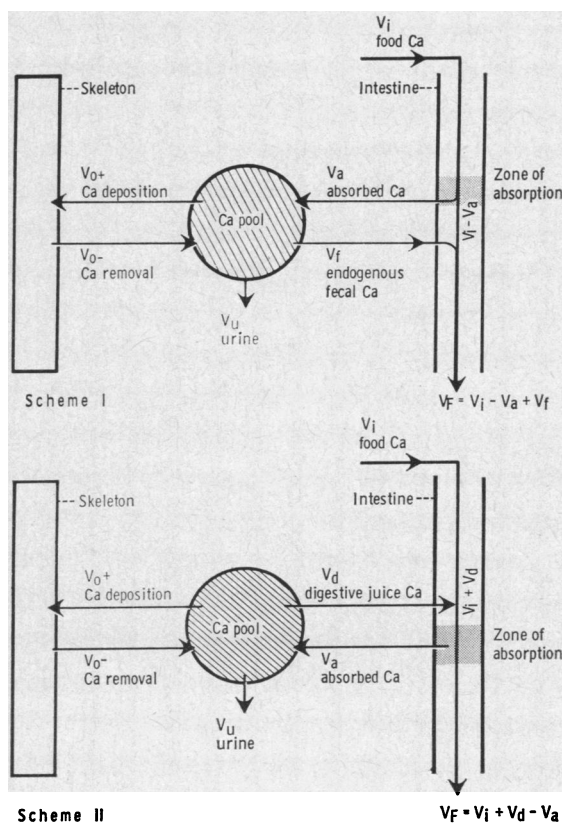
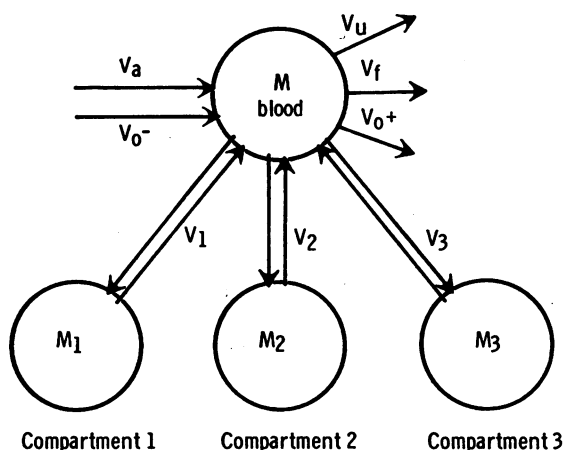FIG. 1. TWO SCHEMES OF CA METABOLISM.

FIG. 2. MODEL OF EXCHANGEABLE CA POOL. Central compartment, with mass M, is identified with blood Ca; identity of remaining three compartments, with masses M_1, M_2, and M_3, is not specified. Total Ca pool, P, equals $M + M_1 + M_2 + M_3$. All irreversible losses from P are via M. Both pool size and rates are considered constant during an experiment. Adapted from Aubert and Milhaud (3).
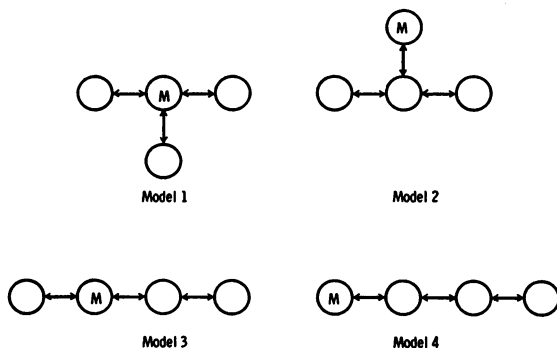

FIG. 3. DIFFERENT MODELS OF CA EXCHANGE.

in skeleton, v_{o-} = Ca removed from skeleton, and v_T = total Ca loss from P. Exchanges within P among M, M_1, M_2, and M_3, occur at rates V_1, V_2, and V_3. Further notation is: R_{sB}, R_{su}, R_{sD}, and R_{sF} = SA of serum, urinary, digestive-juice, and fecal Ca, respectively, at time t; R_u, R_D, R_F, and R_o = radioactivity present in urine, digestive juices, feces, and bone, respectively, at time t; R_f = radioactivity due to endogenous fecal Ca; R_i = radioactivity injected at $t = 0$; and R_{ing} = radioactivity ingested at $t = 0$.

DISCUSSION

I. Schemes of Ca metabolism

Figure 1 represents two possible schemes of Ca metabolism. They are alike in that the system under analysis consists of a central Ca pool, skeleton, intestine, and loss of Ca via urine.

They differ in their account of the origin of fecal endogenous Ca. Scheme I pictures fecal endogenous Ca as secreted into the bowel and not subject to intestinal absorption. This is the simplest way to account for endogenous Ca in the stool. Scheme II takes more explicit account of what is known of the formation of Ca-containing digestive juices. It derives endogenous fecal Ca from secretions into the bowel, assuming that the fraction of digestive-juice Ca absorbed is the same as that absorbed from food. Authors have used either scheme, often without specifying which. Few have studied the entire system; most have studied either absorption or bone metabolism, for example, but not both.

In human studies, Blau and his associates (1) used scheme II and calculated v_d and v_a. Heaney and Whedon (2) used scheme I and calculated v_f, v_{o+}, and P. Aubert and Milhaud (3) and Milhaud, Aubert, and Bourichon (4) also used scheme I, solving for all parameters. Bronner and Harris (5) and also Nordin (6) made no explicit choice of scheme. They calculated P, v_{o+}, and "endogenous fecal Ca," without further defining the last. Neither Krane, Brownell, Stanbury, and Corrigan (7), nor Bauer, Carlsson, and Lindquist (8), nor Dow and Stanbury (9) indicate a scheme of Ca metabolism for their calculations of P and v_{o+}.

In animal studies, Visek, Monroe, Swanson, Comar, and Hansard (10, 11) have calculated v_f and v_a in the cow, using a scheme like I. Aubert and Milhaud (3) have also used scheme I for their rat studies; as in their human studies, they solved for all parameters. Bauer and co-workers, by defining "exchangeable space" and bone "accretion," have in fact proposed a method for measuring P, v_{o+}, and v_{o-} in the rat, both in individual bones (12) and in the whole animal (13). For the latter, they had to calculate the loss of Ca in the excreta, but did not distinguish urinary from fecal endogenous loss.

Of the relationships inherent in the schemes, some are common to both. By definition,

$$\Delta_s = v_i - (v_u + v_F) \qquad [1]$$

(Δ_s here is identical with traditional Ca balance).
If P is in dynamic equilibrium, then

$$\Sigma v_{out\ P} = \Sigma v_{in\ P}; \qquad [2]$$

in other words, Δ_P must always equal 0.[1] Since the intestine does not store significant amounts of Ca, it follows, if $\Delta_P = 0$, that

$$\Delta_s = \Delta_{\text{skel}} = v_{o+} - v_{o-}. \qquad [3]$$

In scheme I, particularly, let v_T designate the total Ca loss from P per unit of time. Then,

$$v_T = v_u + v_f + v_{o+}. \qquad [4]$$

By Equation 2, losses from P must equal gains. Hence,

$$v_T = v_a + v_{o-}. \qquad [5]$$

Absorption can also be determined by

$$v_a = v_i - v_F + v_f, \qquad [6]$$

and the fraction absorbed is

$$\alpha = v_a/v_i. \qquad [7]$$

In scheme II, some Ca that leaves via v_d returns to it via v_a, but diluted with v_i. In other words, v_F contains some unabsorbed v_d and v_i. The unabsorbed v_d is equivalent to v_f in scheme I. Hence

$$v_f = v_d(1 - \alpha). \qquad [8]$$

Equation 4 again applies. Equation 5 applies as well, but absorption is also defined by

$$v_a = v_i + v_d - v_F, \qquad [9]$$

and the fraction absorbed becomes

$$\alpha = v_a/(v_i + v_d). \qquad [10]$$

Equations 8, 9, and 10 are based on the assumption that v_i and v_d are absorbed to the same extent and therefore presumably are thoroughly mixed (1).

In terms of actual values, only v_a is different in the two schemes. In particular, α is the same in both, since it follows from what was said above that the absorbed fraction of v_d is the same as that of v_i. The principal advantage of scheme II over I is that it allows calculation of what probably is a physiologically meaningful vector, v_d, whereas v_f is physiologically ill-defined.

Although both schemes account nearly equally well for what is known of Ca metabolism, the

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[1] This does not preclude changes in pool size, but only changes during the period of observation. Changes in pool size between experiments can be observed, e.g., as a result of treatment.

conceptualization each represents is meaningful only if based on experimental reality, and the system must be resolvable by available experimental procedures. The system implies the following three conditions.

First, the concept of a central Ca pool derives from experiments in which Ca^{45} is injected intravenously, and R_{sB} decreases exponentially with time. In most human studies, R_{sB} decreases monoexponentially between about 36 and 144 hours after iv injection of Ca^{45}. R_{sU} and R_{sB} are identical during that period (14, 15) and the time course of R_{sF} parallels them (15). Since the total serum Ca concentration is constant, the monoexponential decrease of R_{sB} indicates a system with constant turnover, i.e., a central pool whose v_{in} and v_{out} are equal and constant. When the linear portion of the R_{sB} curve is extrapolated to $t = 0$, the intercept is much lower than the value obtained by dividing R_i by the initial SA of the blood Ca. Hence, the blood Ca is in dynamic equilibrium with a much greater Ca mass, the Ca pool.

Second, there is the constancy of the system during the period of observation. Either the system must be in steady state, or the time-variant parameters must be known. Suitable experimental conditions to keep some of these constant are easily attained and widely practised. Thus, on a metabolic ward, v_i can be kept constant, so v_u is relatively constant. Although v_F will vary from day to day even under these conditions, 6-day collections tend to be fairly constant, particularly if delimited by stool markers. Hence the use of average values on a daily basis for v_i, v_u, and v_F, based on experimental periods of 6 to 10 days. If v_i and v_F can reasonably be considered constant under well-defined experimental conditions, it seems as reasonable to suppose that v_a and v_f can be expressed in terms of mean daily values. If conditions during an experiment are constant, the Ca content of a subject's blood, extracellular fluid, and other body fluids can be considered constant; it therefore seems unlikely that P varies in that time. This argument is reinforced by the observation of a monoexponential decrease of pool SA during much of the experiment, and by the fact that, in successive experiments on a single patient, variations are generally within the experimental error. If

v_i, v_u, v_F, v_f, v_a, and P may reasonably be expressed as virtually constant mean daily values, so may v_{o+} and v_{o-}.

Finally, since all parameters in a resolvable system can be solved for in their appropriate units of mass, special conditions are imposed on the use of radioactive isotopes. *1)* The tracer must be an isotope of Ca, or behave like one. Isotope effects are excluded. *2)* In both schemes (Figure 1), it is assumed that during the experimental period either v_{o-} is completely unlabeled, or that a constant fraction of the radioactivity deposited via v_{o+} is returned to P. This second possibility has been considered explicitly by Heaney and Whedon (2). Most authors, however, believe that Ca returned to P by bone resorption is unlabeled. In scheme II, some of the radioactivity lost from the pool via v_d is returned to it via v_a. In order for the SA of P to decrease exponentially under these conditions, it must be assumed that v_i and v_d undergo rapid mixing and homogenization before absorption, and that the rate of absorption throughout the experiment is relatively constant. *3)* Homogenization within each compartment is assumed to be instantaneous. This is not true in fact, but the rate of homogenization is generally very much greater than the rates of exchange between two compartments.

II. *Resolution of system*

The system is resolved by a combination of isotope and balance measurements. In most studies, Ca^{r2} has been administered as a single dose either intravenously or by mouth. Although the over-all approach is similar in either case, some assumptions, methods, and the validity of certain of the experimental findings differ. Therefore, it will be useful to discuss both cases separately.

A. *Intravenous administration*. In human studies, the time-course of R_{sB} is best described by an equation of the type,

$$R_{sB} = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t} + A_4 e^{-a_4 t}, \quad [11]$$

which holds for 6 to 7 days after injection of Ca^r (3, 14, 16). Similar, but three-term equations have been proposed by Krane and associates (7) and Bauer and associates (8). The disappear-

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² Isotope measurements will be designated by Ca^r.

ance of injected Sr from the blood has also been described by a four-term equation (17). The first three terms of Equation 11 become negligible within the first 48 hours. Thereafter, the time-course of R_{sB} is described by

$$R_{sB} = A_4 e^{-a_4 t}. \quad [12]$$

If $f(t)$ is the time-course of R_{sB} and if all unidirectional losses from P are assumed to be from plasma, then at any time after injected Ca^r has become distributed homogeneously in plasma Ca, a period shown to be less than 1 minute (3), one can write for scheme I:

$$dR_u = v_u f(t) dt \quad [13a]$$

$$dR_F = v_f f(t) dt \quad [13b]$$

$$dR_{o+} = v_{o+} f(t) dt. \quad [13c]$$

Upon integration between limits, these equations become,

$$R_u]_{t_1}^{t_2} = v_u \int_{t_1}^{t_2} f(t) dt \quad [14a]$$

$$R_F]_{t_1}^{t_2} = v_f \int_{t_1}^{t_2} f(t) dt \quad [14b]$$

$$R_{o+}]_{t_1}^{t_2} = v_{o+} \int_{t_1}^{t_2} f(t) dt. \quad [14c]$$

If follows that

$$R_u]_{t_1}^{t_2}/v_u = R_F]_{t_1}^{t_2}/v_f = R_{o+}]_{t_1}^{t_2}/v_{o+}. \quad [15]$$

Moreover, at any time t,

$$R_u]_o{}^t + R_F]_o{}^t + R_{o+}]_o{}^t + M f(t) + M_1 g(t) + M_2 h(t) + M_3 i(t) = R_i, \quad [16]$$

where M = mass of Ca in serum, M_1, M_2, and M_3 are amounts of Ca in the other compartments of P, and $f(t)$, $g(t)$, $h(t)$, and $i(t)$ are the functions with time of SA of Ca in the various compartments. The same equations apply to Scheme II if v_f is replaced by $v_d(1 - \alpha)$.

Two simplified methods have been proposed on the assumption that R_{sB} is a simple monoexponential function (Equation 12), i.e., that what happens during the first 36 hours after injection can be neglected. The first, that of Bauer and collaborators (8), is based on experiments with rats (12, 13), and has been used in human studies by them and other workers (5, 6, 8, 16, 18). With it, v_f, v_{o+}, and P can be determined. The

method is based on the following relationship, derived from Equation 16:

$$R_i = Pf(t) + (v_{o+} + v_u + v_f) \int_0^t f(t)dt. \quad [17]$$

This states that at any given moment, total R_i equals the sum of radioactivity remaining in P plus R_o, R_u, and R_F. $f(t)$ is set equal to the time-course of SA. Equation 17 divided by P gives Equation 18:

$$R_i/P = f(t)$$
$$+ [(v_{o+} + v_u + v_f)/P] \int_0^t f(t)dt. \quad [18]$$

If $f(t)$ is a single-term exponential expression and if no labeled Ca has returned to the pool between times 0 and t, $(v_{o+} + v_u + v_f)/P$ equals a_4 in Equation 12, which is determined experimentally. Equation 18 can then be solved for P:

$$P = R_i/[f(t) + a_4 \int_0^t f(t)dt]). \quad [19]$$

By measuring the area under the curve described by $\int_0^t f(t)dt$ and the slope a_4 on semilog coordinates, P and a_4 can be calculated; $(v_u + v_f)$ is then calculated by applying Equation 14 to the excreta. Hence v_{o+} can be calculated.[3]

In the second simplified method, by extrapolating the linear portion of R_{sB} to $t = 0$ (2, 19), Equation 12 can be rewritten as:

$$R_{sB} = (R_i/P)e^{-(v_T/P)t}. \quad [20]$$

Extrapolating this to $t = 0$ gives

$$R_{sB}{}^o = R_i/P, \quad [21]$$

hence P can be calculated. Since the slope of Equation 20, v_T/P, is known, v_T can be calculated; resolution into its component rates is based on Equations 14a, b, and c.

Two general methods have been proposed that solve a multitermed exponential equation for the entire system. Such methods are important, since substitution of a single-term exponential equation for a multitermed one will give erroneously high values for P and v.

In the first, Krane and co-workers (7) chose

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[3] Bauer and associates (8) have not calculated v_f as such, but have calculated $(v_u + v_f)$ by Equations 14a and b.

the following expression to describe the drop of R_{sB} with time:

$$R_{sB} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t}. \quad [22]$$

They assume that each exponential term describes the behavior of Ca in a different compartment, and express A_1, A_2, and A_3 as the percentage of the dose of Ca^{45} per gram Ca. They then define the size of each compartment, Q_1, Q_2, and Q_3, as:

$$Q_3 = 100\%/A_3 \quad [23a]$$
$$Q_2 = 100\%/(A_3 + A_2) \quad [23b]$$
$$Q_1 = 100\%/(A_3 + A_2 + A_1). \quad [23c]$$

Krane and co-workers (7) do not specify the model underlying their solution, but as they state, Equations 23a, b, and c imply a system where the radioactivity passes from one compartment to the next in successive steps, homogenization in each compartment preceding each step. This hypothesis does not seem likely. Moreover, to approximate the true situation by identifying each term of the equation with a given compartment, rather than Equations 22a, b, and c, one would use the following:

$$Q_1 = 100\%/(A_1 + A_2 + A_3) \quad [24a]$$
$$Q_1 + Q_2 = 100\%/(A_2 + A_3) \quad [24b]$$
$$Q_1 + Q_2 + Q_3 = 100\%/A_3. \quad [24c]$$

Equation 24c is merely another way of calculating P by the simplified method, e.g., with Equation 20.

In the second general method, Aubert and Milhaud (3) express the time-course of R_{sB} by Equation 11. The analysis assumes that the blood represents the central compartment whose Ca exchanges simultaneously with that of the other compartments [see also (20)]. Then, A_1, A_2, etc., and a_1, a_2, etc., represent functions of both each V and each M. These functions are complex, and a given term of the equation cannot be identified with a particular compartment. Aubert and Milhaud (3) have shown experimentally that the blood constitutes a well-defined compartment for Ca exchange. They measured the time-course of R_{sB} during the first 90 seconds after injection and showed that a maximum occurs at about 45 seconds. This maximum

amounted to 75% of the theoretical maximal value, R_i/M_{blood}. Moreover, if the nearly linear decrease of the R_{sB} curve between 45 and 90 seconds after injection was extended to $t = 0$, it intercepted the ordinate at a point within 5% of the calculated values for R_i/M_{blood}, as in their Figure 5 (3).

This type of analysis requires a well-defined model of compartments and rates, and the model shown in Figure 2 was chosen; it represents a mammillary system with the blood as the central compartment. The system is resolved as follows. Let R_i = the amount of Ca^r injected at $t = 0$, and R, R_1, R_2, and R_3 = the amount of Ca^r in the blood, and in compartments 1, 2, and 3, respectively, at time t. Also, let dR, dR_1, dR_2, and dR_3 represent the change in the amount of Ca^r per time dt. The dynamic state of the system can then be expressed by the following equations:

$$dR/dt = - [(V_1 + V_2 + V_3 + v_T)/M]R$$
$$+ (V_1/M_1)R_1 + (V_2/M_2)R_2 + (V_3/M_3)R_3 \quad [25a]$$

$$dR_1/dt = (V_1/M)R - (V_1/M_1)R_1 \quad [25b]$$

$$dR_2/dt = (V_2/M)R - (V_2/M_2)R_2 \quad [25c]$$

$$dR_3/dt = (V_3/M)R - (V_3/M_3)R_3. \quad [25d]$$

Knowing M, the total blood Ca content, one can then calculate all other unknowns and thereby resolve the system.

Besides the assumptions discussed above (see Discussion, I), this analysis also assumes that all unidirectional losses from P (v_u, v_F, and v_{o+}) occur by way of the central compartment. It seems reasonable to equate v_{o+}, the unidirectional loss of Ca from P into bone, with bone formation. As long, however, as a formal distinction is maintained between bidirectional Ca exchange and unidirectional Ca movement from pool to bone— i.e., bone formation and its component processes —the analysis described here and by schemes I and II (Figure 1) is independent of the nature of this unidirectional process (see also Discussion, III).

This type of analysis raises an important point, the kind of model into which the pool is decomposed. Equation 11 represents a function by which the plasma Ca appears to exchange at three different rates with three different Ca compartments of varying size. The equation does not,

however, indicate how the compartments of P are related. Four models are possible (Figure 3), each resolved differently and leading to different results. One or more additional exchanges may occur, so that the model includes one or several cycles. Under those conditions, the model can no longer be solved. Unidirectional entries and losses may also occur via a compartment other than the central one. Although resolution of such models is feasible, it becomes more complex.

Aubert and Milhaud (3) have advanced arguments in favor of model 1 (Figure 3), but model 2 seems equally reasonable from what is known of Ca metabolism, particularly as in normal patients both models give similar values. Once, however, all unidirectional movements are assumed to occur by way of the blood compartment (M in Figure 3), the model chosen affects only the manner in which the Ca of P ($M + M_1 + M_2 + M_3$) is partitioned among the three other compartments (with the blood Ca constant), but has almost no effect on the values calculated for v_T and its component rates. When experimental results from a normal person, a patient with Paget's disease, and one with hypothyroidism were calculated according to scheme I (Figure 1), it was shown (3) that rates and compartment sizes varied considerably, but that the rate constants (rate/mass) varied less, indicating that the same exchange process was measured in all three cases. Aubert and Milhaud (3) postulate that M_1 is the Ca of the extracellular fluid, M_2 is the more labile portion of the tissue Ca, and M_3 is the less labile tissue Ca plus the exchangeable Ca of bone.

The relative error that arises in the simplified as compared to the general method can be estimated by neglecting the first three terms of Equation 11 [see (3) for theoretical discussion]. In all instances, values of P and v_{o+} calculated by the simplified method are larger than the true values obtained by the general method, with the error becoming relatively larger when P and v_{o+} are large. Hence, use of the simplified method is justified only when P is small, or in comparative studies.

Animal studies. Resolution of the disappearance equations obtained in animal experiments can be treated as in human studies. In the first of two methods of resolution, both developed in

work with rats, Bauer and associates (12, 13, 22) have shown that in a clearly defined portion of the diaphysis of the tibia, the Ca^{45} content, $R_o]_o{}^t$, is given by

$$R_o]_o{}^t = k_1 f(t) + k_2 \int_o^t f(t)dt, \qquad [26]$$

where $f(t)$ is a function of R_{sB}, k_1 = the exchangeable Ca of the bone, and k_2 = the rate of bone formation. Bauer and co-workers calculated k_1 and k_2 of Equation 26 by taking as reference the numerical value of Equation 26 4 hours after the rats were injected, and compared values for k_1 and k_2 obtained subsequently (8, 16, 24, and up to 120 hours). They thus obtained a series of linear equations with two unknowns and a series of values for k_1 and k_2. These were fairly constant for the bone shafts up to 120 hours, but were constant for the bone ends only during the first 48 hours [see their Table III (22)]. Resorption was measured by weighing bone and determining its Ca content at various times. Resorption, v_{o-}, must then equal the difference between v_{o+} as calculated above and the bone balance. Excretion from the body was measured by determining the total R_u and R_F and applying Equations 14a and b.

This method is theoretically sound, and is independent of the type of function that R_{sB} may have, since the Ca^{45} content of the bone is measured directly. The method cannot, however, be extended to measure all the parameters of scheme I or II in accordance with Equation 18, since in rats the time-course of R_{sB} cannot be correctly represented by a monoexponential function (3, 20).

Other authors (3, 23, 24) have applied the general method of resolution described for studies with humans to studies with rats. Hence all parameters of either scheme I or II can be calculated without killing a large number of animals. According to Milhaud, Remagen, Gomes de Matos, and Aubert (23), R_{sB} in rats between 0 and 72 hours after injection can be described adequately by a two-term exponential equation of the type,

$$R_{sB} = A_1 e^{-a_1 t} + A_2 e^{-a_2 t}, \qquad [27]$$

which is then solved with the aid of a two-compartment model. Aubert and Milhaud (3) have

shown experimentally that the calculated values for v_{o+} agree well with experimental values obtained by analysis of the whole skeleton. Bronner (20) used a model of Ca metabolism like that shown in Figure 2. He compared Ca uptake of the skeleton with that in certain tissues and organs. By calculating constants and fitting them to equations similar to Equations 25a, b, and c, he was able to show that the model adequately represented the experimental data. He did not, however, resolve the constants into absolute rates and compartment sizes.

B. *Oral administration.* Several authors have attempted to measure Ca absorption in man (1, 5, 15, 25) or animals (10, 11) by giving Ca^r orally. At first glance, this seems the simplest and most direct approach to measuring absorption, but it involves major difficulties of interpretation and technique. If α = fraction of Ca that is absorbed, then the radioactivity absorbed, R_a, is given by

$$R_a = \alpha R_{ing}. \qquad [28]$$

Obviously, the metabolic processes affecting Ca are identical for exogenous and endogenous Ca. Therefore, the physiological behavior of absorbed Ca can be analyzed as though Ca were injected intravenously. Then, if α can be determined, the system can be solved.

In one method of determining absorption, no intravenous experiment precedes the oral experiment. Analysis of this case according to scheme I follows. Equation 6 applied to radioactivity measurements[4] gives

$$R_a = R_{ing} - R_F]_o{}^t + R_f]_o{}^t. \qquad [29]$$

Equations 14a and b combined give

$$R_f]_o{}^t = (v_f/v_u)R_u]_o{}^t, \qquad [30]$$

which, when combined with Equation 29, gives

$$R_a = R_{ing} - R_F]_o{}^t + (v_f/v_u)R_u]_o{}^t. \qquad [31]$$

Since it can be assumed that the same fraction of Ca^r and Ca is absorbed,

$$\alpha = (v_i - v_F + v_f)/v_i = (R_{ing} - R_F]_o{}^t + (v_f/v_u)R_u]_o{}^t)/R_{ing}. \qquad [32]$$

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[4] When Ca^r is given intravenously, $R_f \equiv R_F$; when Ca^r is given orally, these two values must be differentiated.

From this, v_f and α can be calculated as follows:

$$v_f = v_u(v_F R_{\text{ing}} - v_i R_F]_o{}^t)/$$
$$(v_u R_{\text{ing}} - v_i R_u]_o{}^t) \quad [33]$$

$$\alpha = 1 - [(v_u R_F]_o{}^t - v_F R_u]_o{}^t)/$$
$$(v_u R_{\text{ing}} - v_i R_u]_o{}^t)]. \quad [34]$$

In the analysis according to scheme II, Equations 9 and 10 are combined and applied to the radioactivity measurements, as in Equation 29. The value for $R_D]_o{}^t$ is substituted, by analogy with Equation 30, to give:

$$R_F]_o{}^t = (1 - \alpha)R_{\text{ing}}$$
$$+ (1 - \alpha)R_u]_o{}^t(v_d/v_u). \quad [35]$$

This can also be derived from Equation 31 by substituting $v_d(1 - \alpha)$ for v_f. Since it is independent of the scheme used, Equation 34 defines α. Equation 36, derived from Equations 34 and 35, gives v_d:

$$v_d = v_u(v_i R_F]_o{}^t - v_F R_{\text{ing}})/$$
$$(v_F R_u]_o{}^t - v_u R_F]_o{}^t). \quad [36]$$

If R_F of Ca^r is recorded for more than 5 to 7 days, unabsorbed Ca^r will have been excreted in the stool; Ca^r then excreted in the stool is endogenous, and analysis of this latter part of the curve can proceed as for iv injection.

In a second method for determining absorption, the oral experiment can be compared with an intravenous one. If an iv experiment is performed shortly before or after the absorption study, data of both studies can be combined to calculate α, on the assumption that no significant metabolic change has occurred between the two studies. Two methods of calculating absorption in this way have been proposed. Both are based on Equation 28 and are independent of the scheme (I or II) used. First, the urine method [method B in (26)] is based on the fact that in a stable system, R_u of Ca^r is proportional to the amount introduced into the system, or:

$$\alpha = [(R_u]_o{}^t/v_u)_{\text{ingested}}/(R_u]_o{}^t/v_u)_{\text{injected}}], \quad [37]$$

where $t \cong 6$ days. Second, in the pool method (4), P is calculated by the simplified method, by extrapolating the linear portion of the disappearance curve to the origin as if absorption were complete:

$$\alpha = P_{\text{injection}}/P_{\text{ingestion}}. \quad [38]$$

In either method, α can be calculated without the need for a Ca balance, in either the intravenous or the oral part of the experiment. The two methods are not fully equivalent, however, since they rest on somewhat different assumptions. Let α_r be the real and α_m the measured value of α; and let P and v_T be values in an injection experiment, and P' and v_T' be values in an oral experiment. If R_{s_B} is considered a monoexponential function, Equations 37 and 38 then become, respectively, in the urine and pool methods:

$$\alpha_m = \alpha_r(v_T/v_T')[1 - e^{-(v_T'/P')t}/$$
$$1 - e^{-(v_T/P)t}] \quad [39]$$

$$\alpha_m = \alpha_r(P/P'). \quad [40]$$

In the urine method, $\alpha_m = \alpha_r$ if $P = P'$ and $v_T = v_T'$. The value of $e^{-(v_T/P)t}$ in Equation 39 is small, however, when $t = 6$ days. Hence the relationship between α_m and α_r is determined essentially by v_T/v_T'. In the pool method, on the other hand, the relationship between α_m and α_r is determined only by P/P' and is completely independent of the rates of turnover of the pool. Nevertheless, if v_T/P remains constant in the two experiments, as measurement of R_{s_u} can easily verify, the urine method is better than the pool method, since it is independent of the type of function (monoexponential or not) that may represent the time-course of R_{s_u}. Moreover, when P is determined by the simplified method, the error can be fairly large (see above).

General resolution of the system is possible and all parameters (by either scheme I or II) can theoretically be solved for in an oral experiment. If a tracer dose of Ca^r is added to a test meal, general resolution of the system is possible by the radioactivity balance method, even if comparison with an iv experiment is not feasible. It is necessary to subject the patient to the usual balance study to determine v_u, v_F, v_i, and Δ. In scheme I, v_f and α are calculated from Equations 33 and 34. By using the simplified method and extrapolating the curve of the logarithm of R_{s_u} to $t = 0$, one can calculate a $P_{\max}$ and $v_{T\max}$ as if absorption were complete. The true P and v_T can then be calculated by setting

$$P = \alpha P_{\max} \quad [41]$$

and

$$v_T = \alpha v_{T\max}. \quad [42]$$

Since v_u is determined experimentally and v_f is calculated by Equation 33, one can solve for v_{o+} by Equation 4 and for v_{o-} by Equation 3 once Δ is determined. Finally, v_a is given by Equation 7. In scheme II, α and v_d are given by Equations 34 and 36, respectively, and P and v_T are then calculated as above, by Equations 41 and 42. Values of v_f, v_{o+}, v_{o-}, and v_a are given by Equations 8, 4, 3, and 9, respectively.

When comparison with an iv experiment is possible, combination of either the pool or the urine method with a Ca balance study gives α, P, v_T, v_u, v_i, and Δ. In scheme I, v_a, v_{o-}, v_{o+}, and v_f are given by Equations 7, 5, 3, and 4, respectively. In scheme II, combination of Equations 9 and 10 gives v_d and v_a; v_f, v_{o+}, and v_{o-} are given by Equations 8, 4, and 3, respectively.

In the case of Ca absorption of a Ca^r-labeled compound, a Ca balance has no meaning unless all dietary Ca is in the form of the labeled compound. This can be done in animal, but not easily in human studies. For resolution of the system, data from an iv study must be used. Since, however, α calculated for the labeled compound need bear no relation to α of the food in general, resolution of the system may not be meaningful.

On the other hand, if absorption of a specific Ca compound is to be compared with that of dietary Ca in general, experiments (by both injection and ingestion can be performed simultaneously with two different isotopes of Ca, e.g., Ca^{45} and Ca^{47} (26). The α of the Ca compound can then be calculated by either urine or pool method.

Oral studies have both theoretical and physiological limitations. To resolve all parameters of either scheme I or II after oral administration of Ca^r, one must assume that αR_{ing} behaves like an equivalent amount of R_i. Absorption is not an instantaneous process; rather, it takes 2 to 3 hours before R_{sB} reaches its peak after an oral dose (27). The error becomes even larger with the simplified method, which presupposes instantaneous homogenization of the entire pool. Sufficiently frequent blood sampling after ingestion of tracer, however, could establish an equation of the type:

$$R_{sB} = - (A_1 + A_2 + A_3 + A_4)e^{-at} + A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + A_3 e^{-a_3 t} + A_4 e^{-a_4 t}. \quad [43]$$

From this, the system could be resolved, since Equation 43 takes into account the time of absorption.

There are also physiological considerations. To use an oral study for the resolution of the system, one must assume all dietary Ca to be labeled homogeneously before the study. Since this is impractical, Ca^r is usually added to milk (27) or to a particular Ca compound, like a Ca salt (28). Absorption of milk Ca must be assumed not to differ from that of the general dietary Ca, an assumption that remains unproved. In the second case, one can measure Ca absorption of the salt in question, but cannot, as shown above, resolve the entire system. Oral experiments, therefore, do not appear to be suitable for study of Ca metabolism in general. The absorbability of Ca in specific foods or compounds can be readily determined in conjunction with an iv study.

III. Long-term behavior of R_{sB}.

All the human studies discussed so far involve periods of 6 to 8 days after isotope administration. Then the monoexponential decrease of R_{sB} observed between 2 and 6 to 7 days ceases, and the curve assumes a new slope. Two interpretations of this phenomenon have been offered.

Heaney and Whedon (2) believe the new and flatter slope of the R_{sB} curve, seen about 1 week after injection of radioactivity, is due to the return of label into P, resulting from resorption of labeled bone after a period θ. Dansky and Heaney [quoted in (2)] have derived a mathematical expression that accounts for recycling in an open single-compartment system:

$$x_n(t) = \sum_{n=0}^{N} \frac{x_0(0)e^{K(t-n\theta)}[k_b'(t - n\theta)]^n}{n!}, \quad [44]$$

where x_0 = the specific radioactivity at $t = 0$, K = the rate constant of loss from the pool, k_b' = the rate constant of loss from bone, and θ = time interval required for recycling the tracer. Heaney and Whedon (2) have applied Equation 44 at the end of the second cycle—i.e., for 2θ and when $n = 1$—in order to calculate the fraction of v_{o+} that returns to P under these conditions. This fraction was found to vary between 20 and 40%, with a mean of 32%.

Aubert and Milhaud (3) attribute the same break observed in the R_{sB} curve to a slow, physicochemical phenomenon. They propose to solve for it by adding a fifth compartment, M_4, to the four-compartment mammillary exchange system shown in Figure 2. According to their calculations, the true v_{o+} is about 25% smaller than that calculated by the general methods discussed above.

Although both Heaney and Whedon, and Aubert and Milhaud arrive at similar estimates of the magnitude of this slow phenomenon, they differ profoundly in interpretation. Heaney and Whedon consider it to be due to bone resorption; Aubert and Milhaud [also Krane and associates (7)] attribute it to a slow exchange. Only further studies can resolve this point, especially in the light of the "slow exchange process" described by Marshall, Rowland, and Jowsey (29) *in vivo* and by Neuman and Neuman (30) *in vitro*. The process described by Marshall and associates, however, is more akin to a translocation of Ca in the skeleton without measurable effect on P and is in no way comparable to exchange as defined here.

IV. *Special methods of system analysis*

In Ca^r infusion studies, Rich, Ensinck, and Fellows (31, 32) have attempted to correlate estimated changes in pool size with clinical states. Rich (31) administered a mixture of Ca gluconate and Ca glycerophosphate labeled with Ca^r at a constant rate (110 mg Ca per hour) for 4 hours, and measured radioactivity in serum and excreta. P and v_{o+} were calculated by the method of Bauer and associates (8). The size of the rapidly exchanging Ca P was calculated by use of the curve representing increase in R_{sB} during infusion. Rich defined this P at time t by the relationship: E_R = *total radioactivity injected by time t/serum SA at time t* (Equation 45). In later studies (32), high-specific-activity Ca^{45} in isotonic saline was infused by a constant-rate pump, and the "miscible calcium" was defined as the rate at which Ca^{45} was administered divided by the slope of the regression curve fitted to the rising specific-activity values measured during the experiment. In these studies, only "miscible Ca" was determined.

To use the method of Bauer and associates (8) for calculating P and v_{o+}, it is essential to define a zero time. This cannot be done precisely in Rich's method, and his later studies (32) abandon this approach. E_R, as defined by Equation 45, is not only a function of the size of exchangeable P, but equally a function of all the rates. Whatever scheme is chosen to resolve the system, if two subjects have the same size pool, but different rates of exchange, the serum Ca will undergo exchange with different quantities of Ca during the infusion. Consequently, E_R will differ in the two cases, even though the pools are in fact identical. This argument applies also to the rates of irreversible loss and gain of the central compartment, but over a 4-hour period, these are small in comparison to the rates of exchange.

There is also no way of predicting at present the effect of the Ca infusion (110 mg Ca per hour) on the parameters of the system, even if serum Ca concentration is unchanged. In the later studies, however, the total Ca infused was negligible ($\cong < 30$ µg Ca).

Despite these criticisms of the theoretical significance of the E_R, it is interesting to note that the rate constants V_1/M_1, V_2/M_2, V_3/M_3 are relatively invariant (3). Hence, a major change in M is likely to be accompanied by a comparable change in V. This may explain why Rich was able to distinguish by this test major alterations in E_R associated with certain diseases.

In another analysis, Krane and associates (7) define the Ca pool of man at time t as: P_t = *body retention of radioactivity at time t/serum SA at time t* (Equation 46). As defined, this "pool" must necessarily be a function of time. Krane and associates, however, claim that P_t approaches a limit at $t = 9$ days, thus permitting a comparison of P_t (9 days) in different patients. They state: "The concept of a calcium 'pool' makes no assumption based on the shape of the curves, but reflects the fact that a lower specific activity at any time means a larger mass of dilution." (7).

To examine this statement more closely, let us consider that R_{sB} decreases monoexponentially. Equation 46 then becomes:

$$P_t = [R_o]_o{}^t + P(R_{sB})_t]/(R_{sB})_t$$
$$= P + R_o]_o{}^t/(R_{sB})_t. \quad [47]$$

Using Equations 14c and 20, one can write

$$R_o]_o{}^t/(R_{sB})t = [R_i(v_{o+}/v_T)(1 - e^{-(v_T/P)t})]/$$
$$(R_i/P)e^{-(v_T/P)t} = P(v_{o+}/v_T)(e^{(v_T/P)t} - 1), \quad [48]$$

so that Equation 47 becomes

$$P_t = P[1 + (v_{o+}/v_T)(e^{(v_T/P)t} - 1)]. \quad [49]$$

In other words, the "pool at time t" becomes a rather complex function of P and of v_{o+} and v_T. Moreover, P_t increases indefinitely with time. The notion of a "pool at time t" does not add to what can be learned by solving the system.

The excretion index defined by Bronner and associates (33, 34) consists of three parameters after iv injection of Ca^r where $t =$ days:

$$A = R_u]_o{}^{6t}$$
$$B = v_u]_o{}^{6t} \quad [50]$$
$$C = A/B.$$

Rewritten in terms of Equation 14a:

$$A = v_u \int_o^6 f(t)dt \cong R_i(v_u/v_T)$$
$$B = 6v_u \quad [51]$$
$$C = (1/6)\int_o^6 f(t)dt \cong (R_i/v_T).$$

The integral in Equation 51 is independent of the nature (mono- or multiexponential) of the SA curve. Values of A, B, C can be obtained simply by measuring SA, Ca concentration, and the volume of 6 successive 24-hour urine samples after iv injection of tracer. The way these parameters vary indicates variations of v_u and v_T. Since v_{o+} is the major component of v_T, variation in the excretion index suggests how v_u and also v_{o+} are affected. Although the excretion index does not give a complete description of Ca metabolism, it can be useful in clinical screening studies, e.g., to evaluate the effect of drugs.

In a recent preliminary publication, Bhandarkar, Bluhm, MacGregor, and Nordin (35) propose to measure Ca absorption by giving patients labeled $CaCl_2$ and measuring R_{sB} after 2 hours. To support the claim that R_{sB} is a measure of absorption, these workers plotted R_{sB} against "net calcium absorption," $(v_i - v_F)$, and observed a linear relation with an error of

approximately $\pm 42\%$. Clearly, such a test provides only an indication, not a true measure, of absorption; it may be useful in certain screening studies.

CONCLUSION

The preceding discussion has shown that a number of conditions must be met to obtain meaningful answers in a kinetic analysis of Ca metabolism. *a*) There are experimental conditions for isotope administration. To study a system in a steady state, the tracer introduced must perturb it as little as possible. Since the blood represents a well-defined compartment, high-specific-activity Ca^r is best administered by rapid, iv injection. In other words, the mass of Ca^r introduced must be negligibly small in relation to that of the blood.

b) The disappearance curve must be established. The precision of measurement of this fundamental parameter is a function of the number of measurements made, particularly during the early part of the curve, when it falls off rapidly. When the general method of resolution is used in human studies, blood samples should be obtained at 2, 4, 8, 15, 30, 45, 60, 75, 120, 180, 360, 540, and 720 minutes, and 24 and 48 hours after injection. The rest of the curve is more easily obtained by measuring R_{s_u}. The size of the blood Ca compartment is determined from plasma volume. The simplified method may be used when the patient is a small child, or when many patients are being studied for comparative purposes and their pools are known to be small. Then, all determinations may be of R_{s_u}.

c) To measure Ca intake and output, the diet must be weighed and its Ca content determined. Measurement of urinary Ca and Ca^r output presents no difficulties. The principal problem with fecal Ca output is to establish the correct time correspondence between intake and output, in terms of both balance and kinetic measurements. Since P becomes homogenized between 36 and 48 hours after injection, the simplest method is to give a suitable marker, e.g., 1 g carmine, at 36 hours, e.g., at the evening meal of day 2, and to collect all stools separately thereafter. Six days later, another marker is given. Total Ca and Ca^r is then measured in all stools for that period, i.e., from the stool with

the first trace of the first marker through, but excluding, the stool with the first trace of the second marker.

For analysis of results, the general method of resolution best minimizes all sources of error (see Discussion, II). Choice of model (Figure 3) will affect primarily the determination of M and V within P, and only slightly that of uni-directional processes. Consequently, an investigator may treat data according to more than one model; if this is done systematically, it may indicate an ultimate choice of model. At present, experimental evidence does not permit a clearcut choice between schemes I and II (Figure 1), but as data accumulate, one scheme or the other will be preferred for consistent interpretation. Again, data may be treated according to both schemes.

Although the simplified method leads to erroneously high values for P and v_{o+}, it can be used in comparative series where the true P is small, and where greater simplicity of analysis and calculation becomes important. The simplified method also has a place as a diagnostic tool in a metabolic ward, provided its limitations are taken into account.

In absorption studies, oral administration of Ca^r is theoretically valid only to measure Ca absorption for a given Ca compound, or a single, (uniformly labeled) Ca-containing food. For this, data from an iv study must be available. Under these conditions, α is best calculated by the urine method (Equation 37). In screening studies on the effect of a drug on Ca metabolism, evaluation of the excretion index is the most economical method (see Discussion, IV).

SUMMARY

A detailed, formal analysis of Ca metabolism has been presented that permits measurement of sizes and exchange rates of compartments constituting the Ca pool, as well as the following unidirectional rates: bone formation and resorption, absorption, urinary Ca output, and endogenous fecal Ca loss. The last can also be expressed as a fraction of the rate of formation of digestive juice Ca.

Two possible schemes of Ca metabolism have been presented and analyzed; these differ in their account of the origin of endogenous fecal Ca.

Calculations according to either scheme are detailed.

Both general and special methods of analysis of Ca metabolism have been presented, their advantages and drawbacks discussed, and their applicability evaluated.

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