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Measurement of True Calcium Absorption in Premature Infants Using Intravenous ^{46}Ca and Oral ^{44}Ca

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ABSTRACT. We have developed a method for measuring true fractional calcium absorption (α) in premature infants using two stable isotopes of calcium and tested it in seven studies in seven infants (birth weight 1543 ± 65 g, gestation 32.8 ± 7 wk). A total of 7.5 μg/kg ^{46}Ca was given as a single intravenous bolus. Immediately thereafter 1.25 mg/kg of ^{44}Ca was given in a single gavage feeding of standard infant formula (Enfamil). A metabolic isolette was used to obtain 4-h collections of urine for 24 h total. ^{46}Ca and ^{44}Ca were measured in urine by thermal ionization mass spectroscopy and expressed as the ratio to naturally occurring ^{48}Ca. The differences in the $^{46}Ca/^{48}Ca$ and $^{44}Ca/^{48}Ca$ ratios from natural levels (Δ% excess ^{46}Ca and Δ% excess ^{44}Ca) were calculated. Percent absorption (α) equals a constant times cumulative Δ% excess ^{44}Ca/Δ% excess ^{46}Ca. The calculation of α is independent of urine volume or concentration. The Δ% excess ^{46}Ca, showed the expected multiexponential decline as a function of time, and Δ% excess ^{44}Ca usually peaked during a 4- to 8-h urine collection. Calculations of α using increasingly long sampling times showed that a plateau had been reached by 12 h. α values calculated after 16–24 h in the seven infants at 2 wk of age were 41, 48, 45, 46, 25, 55, and 51%. Repeat studies at 3 wk of age were 46, 60, and 54%. These values are somewhat higher than net percent calcium absorption values reported for standard formula and thus appear very appropriate. This methodology will be very valuable in studying factors that may affect true calcium absorption in premature infants. (*Pediatr Res* 23: 589–594, 1988)

Abbreviations

IV, intravenous
α, fractional absorption

Many factors are known to affect calcium absorption in the adult where calcium absorption is a combination of primarily active and passive transport. Current evidence suggests that in the premature infant these same factors may cause different responses because of differences in both active and passive transport. Characterization of calcium absorption mechanisms in the premature is needed both to understand currently available clinical data and to design feeding regimens that will optimize calcium absorption. In adults, fecal calcium excretion is small and stable. However, in infants endogenous fecal excretion of calcium is very variable and may markedly exceed even urinary excretion of calcium (1–3). Thus traditional balance studies, which measure only net calcium absorption (true absorption − endogenous fecal excretion), are of limited usefulness in characterizing calcium absorption in infants and studying factors affecting calcium absorption. Balance studies that require total collection of stool and urine for periods of usually 72 h also are difficult to carry out in small premature infants who defecate and urinate frequently and whose skin reacts to applied adhesives. Further, cumbersome balance studies limit the ability to conduct repeated studies in the same infant.

In adults balance studies often have been supplanted by the use of radioactive tracers (4). Radioactive tracers cannot be used in infants because of the risk of their bone deposition. Further, the instability of the tracer complicates attempts to do repeated studies. These problems can be avoided by using stable rather than radioactive isotopes, but the use of stable isotopes in adults has been limited by prohibitive costs.

We have developed improved methodology to measure the calcium absorption in premature infants and report a method using two stable isotopes (one intravenous and one oral) which *1*) measures true calcium absorption; *2*) requires urine collections for less than 24 h; *3*) uses no radiation and is safe for infants; *4*) can be repeated because of the stability of the tracers and ease of study and; *5*) in infants is relatively inexpensive.

METHODS

Subjects. Seven infants were studied at 2 wk of age and three were restudied at 3 wk of age while on their standard feedings of Enfamil (460 mg Ca/liter, 313 mg P/L) plus 1 ml Poly-vi-sol containing 400 IU vitamin D. Infants were chosen between 1400–1600 g birth weight (mean ± SD birthweight 1543 ± 65) and between 30–34 wk gestation (mean ± SD gestation 32.8 ± 0.7). Studies were approved by the Human Studies Committee of Washington University and parental approval was obtained for all studies.

ISOTOPE STUDY PROTOCOL

Table 1 shows the percent natural abundance, ratio to ^{48}Ca, cost per mg, and purity of available stable isotopes. Based on dosage required to attain readily detectable enrichment and cost, ^{44}Ca was chosen as the oral isotope and ^{46}Ca was chosen as the intravenous isotope.

If an existing IV was not in place, one was started. The infant was then placed in an isolette in which the standard bed had been replaced by a urine collection pan covered with a taut mesh material to allow passage of urine into the pan. Tubing from the

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collection pan is attached to a measured volutrol for collection of 4-h urine pools. A colostomy bag is placed over the anus to collect stool and avoid contamination of the urine samples.

A sterile solution of 3.45 μg/ml of ^{46}Ca was prepared by the NIH pharmacy and satisfied pyrogenicity and sterility standards before clinical use. A dose of 7.5 μg/kg of ^{46}Ca is given IV by slow IV push. A dose of 1.25 mg/kg of ^{44}Ca is added to a single feeding, shaken well, and the feeding given to the infant by gavage feeding. All subsequent feedings and procedures are carried out on the metabolic bed to maximize urine collection. Four-hour pools of urine are collected and stored in separate containers and frozen until analyzed.

Figure 1 shows a diagram of calcium dynamics in the infant with introduction of the oral tracer, ^{44}Ca, in the formula calcium intake with absorption of calcium into a central pool. The IV tracer, ^{46}Ca, is introduced directly into the central exchangeable calcium pool. Vu represents urinary excretion of calcium, Vf, endogenous fecal excretion of calcium, and Vo+ calcium entering bone. Calcium exiting the pool by any of these routes will contain the same ratio of the two isotopes as in the pool. For convenience, urine is used to sample the ratio of isotopes in the pool at various times.

MATERIALS AND SAMPLE PREPARATION

Acids used were Baker Ultrex HNO_3 and HCl (J. T. Baker Co., Phillipsburg, NJ). Calcium isotopes of ^{44}Ca 98.78 atom%, and ^{46}Ca 43.35 atom% were from Oak Ridge National Laboratory, Oak Ridge, TN. Deionized water was from Hydroservices, Rockville, MD. Glassware and collection vessels are acid washed in 10% HNO_3, rinsed in deionized water, and air dried undercover. All plastic ware is of polypropylene. Sample aliquots are stored in 50-ml centrifuge tubes (Falcon no. 2098) and processed in 12 ml acid washed glass centrifuge tubes (Kimax no. 45199) and stored in 1.5 ml Eppendorf microfuge tubes (Brinkmann Instruments, Westbury, NY).

MASS SPECTROMETRY

Calcium is extracted from the samples by an oxalate precipitation and purified as described previously (15). A Finnigan MAT Thermoquad (thermal ionization quadrupole mass spectrometer, Finnigan MAT, San Jose, CA) is used in the dual filament mode for all measurements. Approximately 10 μg of Ca salts, in a 5 μl drop, are loaded onto the evaporation filament and dried in two stages. The first stage uses 1.3 A through the filament for 45 s to remove the water of solution, and yields a white crystalline mass in the center of the filament. The second stage requires 1.9 A for approximately 1 s, during which time the filament glows a dull cherry red and leaves the salts as a pale yellow-green mass firmly adhered to the filament. Ions are typically produced by heating the ionization filament (uncoated with salts) with about 4 A while applying no current to the evaporation filament. Under these circumstances about 4×10^{-7} A ^{44}Ca are observed using the multiplier output. Measurements are made under computer control. The ^{44}Ca signal is maximized in several steps under software control, first by adjusting the physical position of the sample magazine and then by optimizing the lens potentials. Ion signals for all calcium isotopes except ^{40}Ca are then measured at the peak tops for fixed times. Sampling times in seconds and order of measurement are: 4,4,8,8,4:^{44}Ca, ^{42}Ca, ^{48}Ca, ^{46}Ca, ^{43}Ca. Isotope ratios are calculated for each scan relative to ^{48}Ca and corrected for fractionation by using the deviation of $^{43}Ca/^{48}Ca$ ratio from natural abundance. This correction allows one to compensate for the differential rates of isotope evaporation from the filament, and the corrected ratios of 42/48 and 44/48 are typically within 1% of natural abundance and that of 46/48 within 3%. The controlling software averages sets of 10 such ratios, and a typical filament is used to obtain two to three sets of 10 scan blocks. Interscan, interblock, and interfilament reproducibility are all within 1% of each other. Typical measurement time for three of the 10 scan blocks is about 15 min.

Table 1. *Ca stable isotopes*

	% Natural aundance	Ratio to ^{48}Ca	Cost ($/mg)	Purity (%)
^{40}Ca	96.95	521.2		
^{42}Ca	0.646	3.519	28	95
^{43}Ca	0.135	0.7312	340	80
^{44}Ca	2.083	11.27	7	98
^{46}Ca	0.0035	0.01714	2000	40
^{48}Ca	0.186		250	98

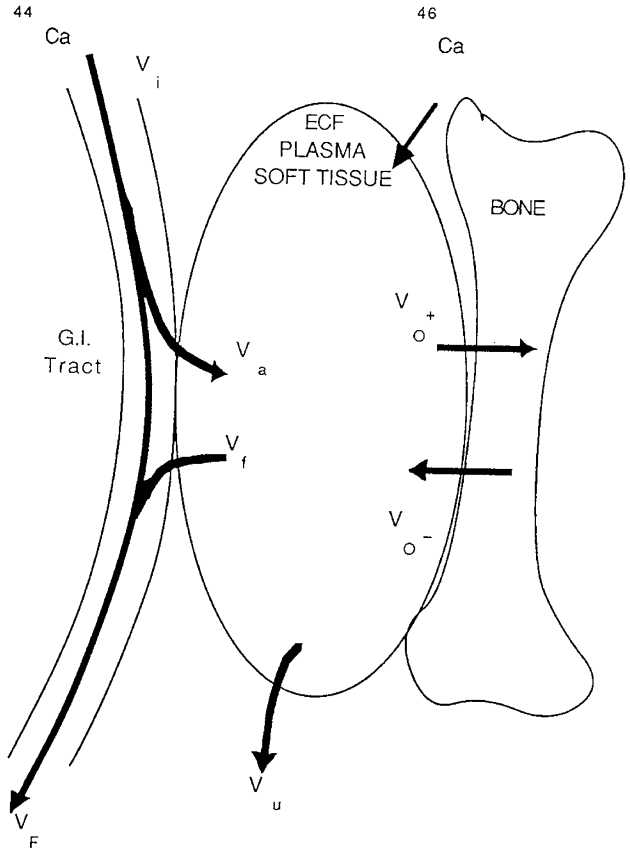

Fig. 1. Diagram of calcium dynamics in the infant where V_i is calcium intake, V_a calcium absorbed, V_f endogenous fecal excretion of calcium, V_o^+ calcium influx into bone, and V_o^- calcium efflux from bone. Oral isotope (^{44}Ca) is added to V_i and intravenous isotope (^{46}Ca) injected directly into the pool.

CALCULATIONS

Data from the analysis of the individual 4-h urine collections can be combined to give the fraction of the isotope dose excreted in urine for any chosen sampling time, (*e.g.* 12, 16, 20, 24 h). The expression used to calculate fractional absorption for any given urine sampling time is derived in the following equations in which "na" is the ratio of two Ca isotopes in nature, (*e.g.* $^{44}Ca/^{48}Ca$, $^{46}Ca/^{48}Ca$) and Δ% excess represents the degree to which a particular ratio differs from natural levels. For calcium at least, Δ% excess values are always positive since calcium isotopes are distributed uniformly in nature.

$$\text{fractional absorption} = \alpha = \frac{\text{fract. oral in urine in sample time}}{\text{fract. i.v. in urine in sample time}} \quad 1)$$

$$\alpha = \frac{[\text{Ca}]\ (\text{vol. sample})\ (\text{na}\ ^{44}\text{Ca})\ (\Delta\%\ \text{excess}\ ^{44}\text{Ca})/(^{44}\text{Ca dose})}{[\text{Ca}]\ (\text{vol. sample})\ (\text{na}\ ^{46}\text{Ca})\ (\Delta\%\ \text{excess}\ ^{46}\text{Ca})/(^{46}\text{Ca dose})} \quad 2)$$

$$\alpha = \frac{(\text{na}\ ^{44}\text{Ca})\ (^{46}\text{Ca dose})\ (\Delta\%\ \text{excess}\ ^{44}\text{Ca})}{(\text{na}\ ^{46}\text{Ca})\ (^{44}\text{Ca dose})\ (\Delta\%\ \text{excess}\ ^{46}\text{Ca})} \quad 3)$$

Equation 3 shows that the calculation of fractional absorption is independent of concentration and sample volume. Inasmuch as all isotope ratios are measured simultaneously when stable tracers are used, the determination of α is complete with one measurement, *i.e.* it is not necessary to wait for a short lived radioisotope to decay before measuring a long lived one.

RESULTS

Figure 2*A* shows the percent enrichment of the IV isotope (^{46}Ca) with time for patients 1 and 5 (the Δ% excess of each 4-h pool is plotted at the midpoint time of the pool). IV isotope enrichment curves were similarly shaped in all infants and showed the expected multiexponential decay. Figure 2*B* shows the percent enrichment of the oral isotope (^{44}Ca) in the same two patients. In these and in all patients the peak oral isotope enrichment was seen in the 0- to 4- or 4- to 8-h urine pool. Percent enrichment of the oral tracer then fell off and, during or after the 8- to 12-h pool, paralleled the IV isotope curves. α, as a function of sampling time for the two infants shown in Figure 2, is seen in Figure 3. Although α was different in these two infants, in both infants a plateau in measured α was reached after the 12 h. Similar curves for α were seen in all seven infants.

Table 2 gives the individual calculations of α for the sampling times of 12, 16, 20, and 24 h for the seven infants studied as well as for repeat studies of infants 1, 4, and 7. Absorption was between 41 and 60% for all studies except one which showed an absorption of about 25%. Absorption curves for the repeated studies of infant 1 are shown in Figure 4. It is clear from inspection of Table 1 and Figures 1–4 that absorption approaches a constant value asymptotically, as expected from the discussion of the model in the Appendix. The rate at which the absorption curve approaches its asymptotic value in all studies yields a $t_{1/2}$ for the absorption process of about 2.8 h. That is, at the time of the first values of absorption listed in Table 2, the absorption process is about 88% complete and is within 2% of its asymptotic value when the sampling is continued for 24 h.

DISCUSSION

Attempts to circumvent traditional balance studies and to assess true calcium absorption in infants by using stable isotopes are not new. In 1973 and 1977 Barltrop and coworkers (1, 5, 6) used a single oral isotope ^{46}Ca and neutron activation to assess true calcium absorption in premature infants fed several experimental formulas based on standard formula. More recently Ehrenkranz *et al.* (7) used the same single isotope technique, ^{46}Ca by neutron activation, to evaluate premature infants fed either human milk or a premature formula. Although this method allows an assessment of true calcium absorption it remains dependent on complete 72-h stool collection and involves the more cumbersome processing of stool specimens. Neutron activation has far less precision than thermal ionization mass spectrometry. Finally the cost was considerably greater for isotope in these studies. Barltrop *et al.* (1) used 2 mg of ^{46}Ca and Ehrenkranz *et al.* (7) used 20–80 μg/kg of ^{46}Ca orally whereas we have used 7.5 μg/kg ^{46}Ca IV.

Barltrop and coworkers (1, 6) combined isotope studies with classical balance studies to estimate that endogenous fecal excretion is equal to true absorption-net absorption but suggest that an accurate assessment of endogenous fecal excretion requires use of an IV tracer. Use of the single oral isotope requires the assumption that the amount of tracer that is absorbed but reenters the stool as endogenous fecal excretion is very small. Ehrenkranz *et al.* (7) calculated this amount as 0.5% of the administered dose per 24-h period in three infants. Whereas this is small (1.5% in a standard 3-day study), endogenous fecal excretion appears highly variable in preterm infants, and this could represent a significant additional variable in studies (1, 2).

In 1980, Yergey *et al.* (8) published a method for measuring isotope ratios in urine using a thermal ionization probe in a

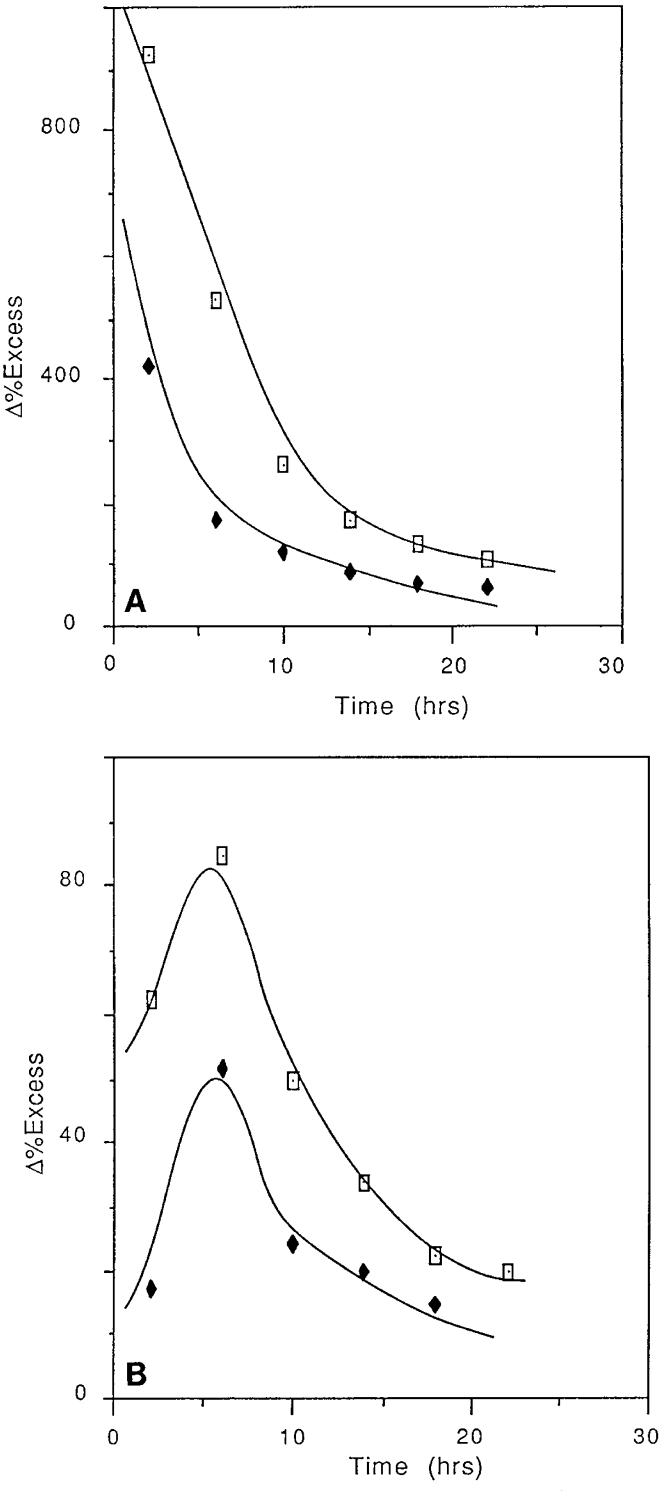

Fig. 2. *A*, Δ% excess in urine of intravenous tracer. ^{46}Ca, *versus* sample time after injection for patients 1 and 5. *B*, Δ% excess in urine of oral tracer ^{44}Ca *versus* sample time after administration in a single feeding, for patients 1 and 5.

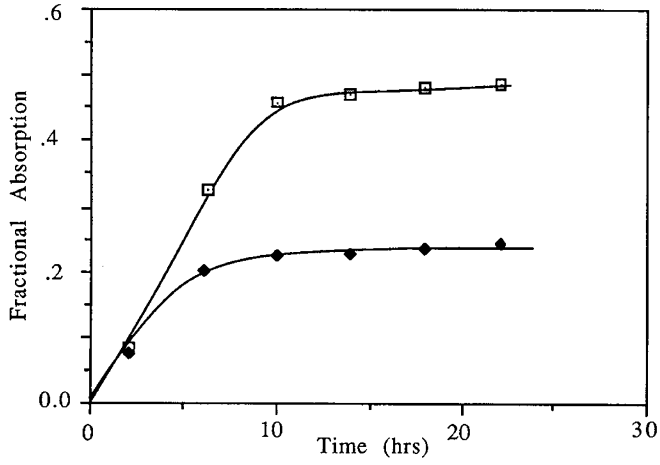

Fig. 3. Percent absorption (α) calculated from the ratio of the cumulative intravenous (^{46}Ca) and oral (^{44}Ca) isotope in urine plotted by sample time for patients 1 and 5.

Table 2. *Percent Ca absorption by urine pool for each infant*

	Infant						
Pool	1	2	3	4	5	6	7
2-Wk study							
0–12 h	38.0	45.8	39.0	42.6	22.4	52.9	46.6
0–16 h	39.1	46.9	43.2	43.6	23.0	55.5	48.2
0–20 h	40.8	48.0	44.9	44.5	23.6		50.4
0–24 h	41.4	48.4		45.9	24.6		51.1
3-Wk study							
0–12 h	41.8			59.4			45.9
0–16 h	43.9			59.6			48.3
0–20 h	45.4			59.7			52.4
0–24 h	45.9						53.6

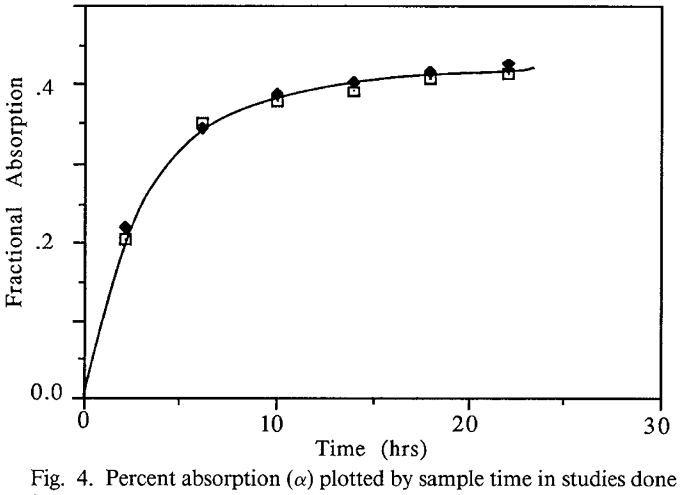

Fig. 4. Percent absorption (α) plotted by sample time in studies done at 2 wk (⊡) and at 3 wk (◆) of age in patient 1.

quadrupole mass spectrometer and used this methodology with Moore *et al.* (2) to examine long-term calcium kinetics in human and primate infants. They used a single IV dose of ^{46}Ca to follow decay curves and a continuous ingestion of ^{48}Ca to achieve stable serum and urine enrichments. (All measurements were made as ratios to ^{44}Ca.) Infants were studied for 9 days with total collection of stool and urine. These studies provided a great deal of information but were obviously difficult to carry out. They confirmed the high and variable fecal endogenous excretion rate using an IV tracer and defined the effects of growth on exchangeable pool size and thus deposition of calcium in bone.

Our study uses the same thermal ionization mass spectrometry methodology and again both an IV and oral tracer in an abbreviated format to look only at the question of true calcium absorption. Both the IV and oral isotopes were given as single boluses. Also, ^{44}Ca was used as the oral tracer and all measurements were made as ratios to naturally present ^{48}Ca. The data generated fit the predicted mathematical models (2, 9) (see Appendix). The fall-off in IV isotope was a multiexponential decay as seen in the previous studies. The oral tracer increased as absorption progressed, peaked at the 6-h midpoint, and then fell off parallel to the IV curve. The curves for α as a function of time became maximal at the 10-h midpoint. Similar studies in an adult showed the curve became maximal at 20 h (10). This difference probably represents the more rapid transport time of the infant and the more rapid completion of absorption. From the practical point it means that any measurement of α made as soon as 12 h after giving the isotope can be used as a valid measure of true calcium absorption. We have elected to continue to carry the 4-h urine collections to 24 h and thus have the possibility of more accurate calculations of α.

Inasmuch as we hope to use this methodology to look at the effects of interventions, reproducibility of studies over a short time interval is important. The stability of stable isotopes thus becomes another advantage. Baseline isotope levels, residual from the initial study, can be subtracted from future samples without worry of interval decay. In three infants studied there was little difference in percent calcium absorption during the short period between 2 and 3 wk of age. Classical balance studies have suggested an increase in percent net calcium absorption with increasing age but primarily after 4 wk of age (11). Ehrenkranz *et al.* (7) found no change in percent calcium absorption with increasing age in a limited number of studies. A large number of infants studied over longer periods of time using this method will be required to address the question of developmental changes in α. However, it would appear that at 2 to 3 wk of age, repeated study immediately before and after intervention will be a valid method.

Although the cost of isotope must be placed in perspective relative to the labor costs of such studies (the labor savings of doing a 24-h rather than a 72-h study alone may compensate for isotope costs), limiting isotope costs is an important consideration. Thus the smaller dose of isotopes is an advantage in small infants. Table 1 lists all of the naturally occurring isotopes of calcium and their abundance expressed both as a percentage of the normal naturally occurring calcium (percent natural abundance) and as a ratio to the abundance of ^{48}Ca which has arbitrarily been chosen as the reference isotope. The cost and purity of available isotopes has been added to Table 1. ^{44}Ca, which is the least expensive, would be unsuitable as an IV tracer because the dose required to create a readily detectable enrichment would be too large for IV administration to infants; however, it is ideal for oral administration in formula with a very high total calcium content. ^{46}Ca has been bypassed in studies of adults or children because of its extremely high cost; ^{42}Ca is preferred. In infants, the tracer dose of ^{46}Ca used is so small as to be cost effective. Total isotope cost per study runs between $50 and $75.

The important question, however, is do our results make sense? Infants of 1400–1600 g birth weight were purposely chosen so that feeding a standard, lower mineral-containing, formula would be acceptable to all concerned with the infants care. This group has been the best studied using standard balance studies where percent absorption ranged between 30–50% (12). Using ^{46}Ca orally only and neutron activation, Barltrop *et al.* (1) described true percentages of absorption as somewhat higher than net absorption but still between 30–40%. Our measures of true absorption seem appropriately higher. Classical balance studies have always shown better absorption of calcium from breast milk

although the exact combination of factors responsible for this still remain unclear (12, 13). Attempts to mimic some of the nutritional factors, such as calcium:phosphorus ratios, fat composition, and protein quality, have resulted in premature formulae from which net calcium absorptions have been reported to be 65–75% (15). Using neutron activation of ^{46}Ca given orally, Ehrenkranz *et al.* (7) reported percentages of true calcium absorption of more than 80% in infants fed human milk or premature formula. However, they acknowledge a concern that because isotope was given as a clear solution in the middle of a feeding (and not mixed with the formula) that "some of the enriched calcium may have been absorbed as if from a clear solution rather than as part of a common dietary pool, yielding artificially high values." In our studies the isotope was added to a single feeding, usually about 1 oz of formula, vigorously shaken, and then allowed to stand for 10–15 min. In more recent studies we have used overnight equilibration and recommend that this be done to assure more complete equilibration between formula and tracer. Our preliminary studies have shown a higher α in breast milk-fed infants but have not shown values to be higher in infants fed premature formula; however, more study is needed to evaluate the multiple effects of diet.

Herein we present only the development of the methodology to determine true calcium absorption. Methodology is currently being developed to measure endogenous fecal excretion using the IV tracer appearance in the stool. The IV tracer decay curve has recently been used to calculate exchangeable calcium pool size in these infants (16). Inasmuch as careful 4-h urine collections are done over a 24-h period for the current methodology, a 24-h urine calcium is automatically available. It should thus be possible to expand the current methodology not only to give calcium retention (calcium balance) but also to calculate nonexchangeable calcium retention as a measure of bone calcium accretion.

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APPENDIX

Calcium from dietary sources is either absorbed into the body or passed through the bowel and excreted. Calcium not initially excreted in feces distributes throughout the body and will eventually be excreted through the kidneys or the endogenous fecal path. The latter is subject to resorption/recirculation lower in the bowel. In this analysis, we focus only on the process absorption of dietary calcium from the bowel. A subsequent analysis will deal with processes related to total body distribution of calcium.

Three aspects of calcium metabolism are shown in Appendix Figure 1. Compartments 1 and 2 represent the gut and the rapidly turning over internal component of calcium metabolism, respectively. Although it is difficult to relate model compartments to physiological entities, compartment 2 is thought to correspond closely with plasma, extracellular fluid, and perhaps a small portion of rapidly turning over bone surface and soft tissue. Compartment 2 exchanges its calcium with other body pools as shown. The transfer rate constants L_{ji} indicate the direction of calcium transfers with the L_{01} defining irreversible losses to feces and urine. Dietary calcium enters compartment 1, a portion is transferred to compartment 2 at rate L_{21}, the balance being excreted at rate L_{01}. Calcium in compartment 2 is either distributed internally, excreted into the urine at a rate L_{02} or returned to the bowel via the endogenous fecal route L_{12}.

Two different tracers are administered simultaneously, one each into compartment 1 and 2. All measurements are effectively made in compartment 2. We have constructed our model so that the rate of appearance of any isotope is proportional to the isotope abundance in compartment 2. Accordingly, because plasma isotope levels are reflected in urine, one can determine, from urinary measurements alone, the isotope dilution in compartment 2.

The differential equations describing the time course of the tracers in Figure A-1 are:

$$\text{Oral } dX_1/dt = -(L_{21} + L_{01})X_1 + L_{12}X_2 + D_1\partial(0) \quad 1)$$

$$\text{IV } dX_2/dt = L_{21}X_1 - (L_{21} + L_{02})X_2 + D_2\partial(0) \quad 2)$$

where X_i are tracer masses and $D_i\partial(0)$ are time zero tracer doses in the respective compartment. This system of equations can be conveniently expressed in Laplace transform notation and solved by normal linear algebra methods. There are two solutions of this system that are of interest for the present work. The first involves administration of the tracer to compartment 1 at $t = 0$, X_{10}, with the time course of this tracer being followed in the second compartment. This solution is noted as X_{12}. The second solution of interest, X_{22}, is for administering a tracer to the second compartment at time zero, X_{20}, then following its time course in the second compartment. The two solutions are:

$$X_{12} = \frac{X_{10}\,L_{21}}{(w_1^2 - 4w_2)^{1/2}} * [e^{-bt} - e^{-at}] \quad 3)$$

$$X_{22} = \frac{X_{20}}{(w_1^2 - 4w_2)^{1/2}} * [(\beta - b)e^{-bt} - (\beta - a)e^{at}] \quad 4)$$

where a, b, β, θ, w_1, and w_2 are all terms involving the four rate constants of the system:

$$a,b = (1/2)\,[w_1 \pm (w_1^2 - 4w_2)^{1/2}],$$

$$\beta = L_{01} + L_{21},\ \theta = L_{02} + L_{12},$$

$$w_1 = \beta + \theta,\ w_2 = \beta\theta - (L_{02}L_{21}).$$

α of an orally administered agent is defined as the ratio of the area under the curve of the time-dependent dilutions of the oral

and IV tracers. α can be calculated from the ratio of the integrals over time of the two tracers, *i.e.*:

$$\alpha = \frac{\int_0^t X_{10}dt}{\int_0^t X_{22}dt}$$

Obtaining accurate estimates of α will depend on the interval of data collection. Our model can be used to define the relationship between the accuracy of estimation and the collection interval. If the time intervals of this expression are set between zero and ∞, then the result is:

$$\alpha = \frac{X_{10}L_{21}}{X_{20}(L_{01} + L_{21})} \quad 5)$$

It can be seen from equation 5 that the α becomes a constant determined by the tracer doses and a ratio of transfer rate constants. At times before the final value is reached, α is at some intermediate value, but approaching the final value asymptotically. The data presented herein show that the α in infants approaches a constant value after about 12 h.

Inasmuch as all of the parameters in our model cannot be determined *a priori* and must be determined by fitting a model to the data, it is important to establish whether this is possible. A commonly used approach is to perform an identifiability analysis. This technique assumes infinite data of pure quality and can be used to determine the number of unknown parameters that can be estimated from data. The identifiability analysis documented below will show that all parameters of our model are identifiable.

The form of the Laplace transform of the solution of equation 3, the time-dependent behavior of the oral tracer in plasma, is:

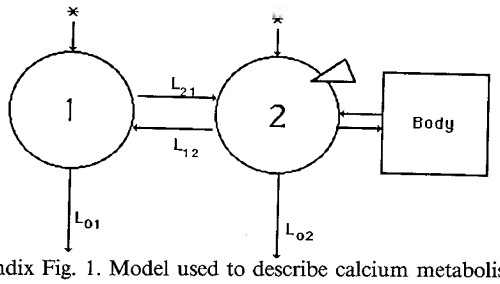
$$L(X_{12}) = \frac{X_{10}L_{21}}{s^2 + w_1 s + w^2} \quad 6)$$

which can be rearranged into a form that groups the similar powers of "s," the Laplace variable, in both numerator and denominator:

$$L(X_{12}) = \frac{Os_1 + (X_{10}L_{21})s^0}{s^2 + w_1 s^1 + w_2 s^0} \quad 7)$$

The equation for the time-dependent behavior of oral tracer in plasma can also be expressed as the sum of exponentials. Only two exponential terms are involved as there are only two compartments being considered from the tracer distribution as seen in Appendix Figure 1. The sum of exponentials is:

$$Y2 = Ae^{-at} + Be^{-bt}$$



Appendix Fig. 1. Model used to describe calcium metabolism where rates of transfer from compartment i to compartment j are delineated by L_{ji}.

The Laplace transform of the solution of this equation is:

$$L(y2) = A/(s+a) + B/(s+b)$$

$$L(y2) = \frac{(A + B)^1 + (Ab + Ba)s^0}{s^2 + (a + b)s^1 + abs^0} \quad 8)$$

Equating the coefficients of like powers of "s" from the numerators and denominators of equations 7 and 8, according to the method of Laplace transform identifiability analysis we have:

$$A + B = 0 \quad a)$$

$$Ab + Ba = L_{21} \quad b)$$

9)

$$(a + b) = w_1 = \beta + \theta \quad c)$$

$$ab = w_2 = \beta\theta - L_{21}L_{12} \quad d)$$

where β and θ are defined above.

Solving equations 9 for the values of the transfer rate constants, L_{ji}, in terms of the fitted parameters of the sum of exponentials leads to the conclusion that the L parameters are identifiable in this system. That is, L_{21} is equal to the sum of the exponents and coefficients given in equation 9b. From equation 9c, because L_{02}, the rate of urinary excretion, and L_{01}, the rate of total fecal excretion, can both be determined independently from measurements involving the tracers we have the expression;

$$(a + b) - (L_{21} + L_{12}L_{02}) = L_{01}$$

Thus L_{12} can be determined independently and all of the rate constants are uniquely identified. Because a related expression could be generated by the use of equation 9d, the system is overdetermined. The analysis is in fact a specific case of the general system analyzed previously (1). Godfrey *et al.* (2) concluded that the system shown in Appendix Figure 1 cannot be uniquely identified unless at least one of the rate constants can be determined independently as we do with L_{02} and L_{12}.

REFERENCES

1. Jacquez JA 1985 Compartmental Analysis in Biology and Medicine, 2nd ed. University of Michigan Press, Ann Arbor, pp. 501–503
2. Godfrey KR, Jones RP, Brown RF 1980 Identifiable pharmacokinetic models: the role of extra inputs and measurements. J Pharm Biochem 8:633–648