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J Clin Invest. 1969;48(9):1705-1713. <https://doi.org/10.1172/JCI106136>.

Research Article

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Study of Calcium Absorption in Man: A Kinetic Analysis and Physiologic Model

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ABSTRACT A physical model of calcium absorption was developed from analysis of data obtained on 23 subjects, including 13 patients having a variety of abnormalities of calcium metabolism. The model was tested and found consistent in all subjects studied. This technique provides a quantitative description of the rate of entry of oral dose of ^{45}Ca into the circulation as a function of time by analysis of serum or forearm radioactivity in response to intravenous and oral administration of ^{45}Ca . The kinetics of the absorption process as proposed by the model are characterized by an initial delay phase of 15–20 min, by a maximal rate of absorption at 40–60 min after ingestion, and by 95% completion of the absorption within $2\frac{1}{2}$ hr. Partial identification of the physiological counterparts of the model was possible by introduction of the isotope at various levels of the gut. Although the region of the duodenum was found to have the greatest rate of absorption per unit length in normal subjects, it was least responsive to stimulation by parathyroid hormone and suppression by calcium loading. Furthermore, the response of the gut to parathyroid hormone was delayed, whereas the suppression of absorption by intravenous or oral calcium loading was rapid and dramatic. The implications of these observations are discussed.

This work was presented in part at the 1966 annual meeting of The American Federation for Clinical Research.

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Received for publication 15 October 1968 and in revised form 1 April 1969.

INTRODUCTION

The use of tracer techniques has greatly facilitated the study of intestinal absorption of calcium. The fraction of radioactive calcium (^{45}Ca or ^{47}Ca) recovered in the stool after an oral dose of the isotope has provided reasonable estimates of calcium absorption in patients (1–4). True net calcium absorption has been obtained by correcting for endogenous fecal excretion determined by the fractional dose recovered in the stool after intravenous administration of isotope. More recently this time-consuming and cumbersome approach has been avoided by analysis of the fraction of radioactivity excreted in urine (5, 6) or appearing in the forearm as determined by external monitoring (7) after oral and intravenous administrations of the isotope. This technique is based on the assumption (8) that fractional loss of the absorbed portion of the oral dose is identical with that of the intravenous dose and also permits quantification of the fraction of dose absorbed. The relative rate of appearance of the isotope in the circulation by serial blood samples (4, 9–11) or by external monitoring of the forearm (12) after oral administration alone has been used as a rough and rapid means to estimate relative calcium absorption in various states of altered calcium metabolism. Data obtained by these techniques are inadequate for kinetic analysis of the absorption process. As a result of the limitations of existing techniques, little progress has been made in understanding this fundamental process despite the importance of calcium absorption in numerous disease states.

This report presents a technique for the study of calcium absorption through the construction of a physical model that accounts quantitatively for the rate of cal-

cium absorption as a function of time and provides a framework for physiological interpretation of the process. This technique involves the analysis of serum or forearm radioactivity after oral and intravenous administration of ^{45}Ca .

METHODS

The population studied consisted of 10 normal volunteers between the ages of 21 and 55 yr and 13 patients with a variety of disorders of calcium metabolism including hypoparathyroidism, hyperparathyroidism, idiopathic hypercalcuria, and nontropical sprue. Except for two patients with hypoparathyroidism and one patient with idiopathic hypercalcuria, all subjects were studied on a metabolic ward and consumed diets constant in calcium, phosphorous, and total calories. All subjects were maintained on a constant diet and activity regimen for a minimum of 2 wk before the initiation of the absorption studies. No patient or volunteer was studied while receiving a daily dietary intake that differed by more than 150 mg of calcium from his customary intake as estimated by careful history.

On the day of isotope administration, a standard 12 mg calcium meal consisting of cream of wheat, salt-free toast, jelly, and lemonade was given to the subject at 8:00 a.m. after an overnight fast. The meal was supplemented with a desired amount of calcium equivalent to that taken with the regular breakfast on other days, either as calcium lactate added to the lemonade or as calcium gluconate given intravenously over a 2½ hr period from 8:00 to 10:30 a.m. Unless otherwise stated, 200 mg of calcium as calcium lactate was used as the supplement. ^{45}Ca with a specific activity of approximately 150 $\mu\text{Ci}/\text{mg}$ was given orally to the subject in 50 cc of skim milk at 10:30 a.m. Each oral dose of ^{45}Ca was followed in 8–14 days by an intravenous injection of the isotope at the same time of day under identical dietary conditions, the arm or blood being monitored as after oral administration of the isotope. The appearance of the ^{45}Ca in the forearm was monitored with a large volume liquid scintillation γ -spectrometer (Armac, Packard Inst. Co., Phila., Pa.) by a modification of the technique described by Lutwak and Shapiro (12). Blood activity was determined from serial 3 cc serum samples counted in a low background γ -ray spectrometer.

To ensure exclusion of the decay product ^{45}Sc , a base level of 0.450 mev was used in both spectrometers. With the Armac, the patient was monitored for periods of 3 min over the first 2½ hr, then for four consecutive 5-min periods every 40 min for an additional 6–8 hr. The changing background radioactivity was monitored alternately with the forearm radioactivity. The background was obtained with the forearm removed from the counter chamber and the elbow of the flexed arm placed at the entrance of the chamber without otherwise altering the position of the body. As a standard, a known aliquot of the administered radioactivity was diluted to 1 liter and was monitored at hourly intervals in the Armac. Isotope decay and fluctuations in counting efficiency were corrected for by expressing the net arm counts (counts from the arm minus background) or counts from serum obtained within a given 60 min period as a per cent of the standard monitored during the same 60 min period. Finally, the data were expressed as a per cent of the administered dose. With the use of serum radioactivity, 12 blood samples were obtained at intervals sufficiently close to define the response of the administered dose. A known dilution of the administered dose was monitored as above with the low

background spectrometer, and the data were expressed as a per cent of the administered dose. With the use of the Armac, 4 μCi orally or 2 μCi intravenously were sufficient to give good counting statistics for counting periods of 3 min. Doses about two and one-half times greater were required to obtain satisfactory statistics on radioactivity in serum. Because of greater facility and lower radiation doses, the Armac was used in most of the studies reported here.

In most studies stool was collected for 6-day periods demarcated by fecal dye markers. Radioactivity determinations were made on homogenates of the pooled specimens made up to 1 liter. A standard of identical geometry and the stool sample was monitored with the large volume liquid scintillation spectrometer. Parathyroid hormone used in these studies was kindly prepared by Dr. G. D. Aurbach and Dr. Potts from hormone purified on a Sephadex column (13).

Mathematical analysis. Mathematical analysis involved two phases. The initial phase in developing a model for intestinal absorption was the derivation of a function to describe the rate of entry of labeled calcium into the plasma after an oral dose. This function was derived from measurements resulting from a unit of ^{45}Ca injection into the plasma and a unit of ^{45}Ca given orally. The mathematical procedure for this derivation is known as deconvolution, or inverse convolution. The basic formulation of convolution and deconvolution may be described as follows: let the term "response," $R(t)$, be any tracer measurement in the system after some time-dependent input, $I(t)$. In particular, let the response to a single unit injection be $W(t)$. Since, as far as the tracer is concerned, the system is linear¹ with time-independent rate constants, the response, $R(t)$, to any input, $I(t)$, can be derived in terms of the response to a single injection by the convolution integral (14, 15).

$$R(t) = \int_0^t I(\theta) W(t-\theta) d\theta,$$

where θ is a dummy variable of integration.

Using the inverse of this technique it is possible, given the response $W(t)$ of a system to a unit impulse and its response $R(t)$ to an unknown input, to calculate the unknown input. Details of the mathematical formalism for the inverse process (deconvolution) are provided in the literature (14). Several functions for the rate of calcium absorption (rate of ^{45}Ca into plasma after an oral dose) in various clinical and physiological states are shown in Fig. 1.

In this calcium system the input is the intravenous or oral administration of the isotope. The response is the measured radioactivity in the arm (using the Armac) or the blood. Implicit in this method of analysis are the assumptions that (a) there is no difference in the metabolism of tracer calcium between oral and intravenous administration once it enters the plasma, (b) the system remains the same during the two experiments, and (c) the derived plasma input function describes the entry of ^{45}Ca into the circulation from the gastrointestinal tract after oral administration.

The second phase in the modeling involves the development of a mechanistic model to explain the characteristics of the derived input function, the entry of ^{45}Ca into plasma after

¹ A system is said to be linear if its response to a linear combination of arbitrary time-dependent inputs equals the same linear combination of individual responses. Thus, if the response to input I_1 is R_1 , and the response to input I_2 is R_2 , etc., then the response to input $(k_1 I_1 + k_2 I_2 + \dots)$ is $(k_1 R_1 + k_2 R_2 + \dots)$.

oral administration of ^{45}Ca . A compartmental model was employed for this purpose.

The deconvolution, compartmental analysis, and data fitting were performed on a digital computer using the SAAM program and the techniques developed by Berman, Shahn, and Weiss (16-18). After numerous attempts a "simplest" universal model, compatible with the results on all patients studied, was derived.

A schematic representation of the model is given in Fig. 2. The first segment (*A*) of the model behaves as a single compartment from which no absorption occurs. Segments *B* and *C* of the model represent two separate "delay" sections from which material diffuses out with rate constants λ_x and λ_y . *Z* represents another compartment-like stage through which the material passes just before it enters the circulation.

Operationally the delay segments *B* and *C* are each simulated by three compartments in series (Fig. 3). The number of compartments used to simulate delay is determined by the

shape of the function $R(t)$ and the degree of resolution required by the data. The values of the rate constants λ_1 from one compartment to the next within each delay segment are set equal to each other and represent the reciprocal mean time of transit per compartment. Thus, the total delay in a segment is $3/\lambda_1$. In the model the two segments have different delays. Diffusion paths from the delay segments into compartment *Z* is provided by λ_s from each of the compartments within the delay segments to compartment *Z*. The diffusion coefficients for the two delay segments in the model have different values.

In an attempt to correlate elements of the model with the physiology of absorption, ^{45}Ca was administered through a nasogastric tube to a normal volunteer who had constantly been receiving an 800 mg calcium diet; he was given 100 mg of calcium as calcium lactate with a standard breakfast as previously described. Under fluoroscopic control the tube in separate studies was adjusted to empty into the stomach

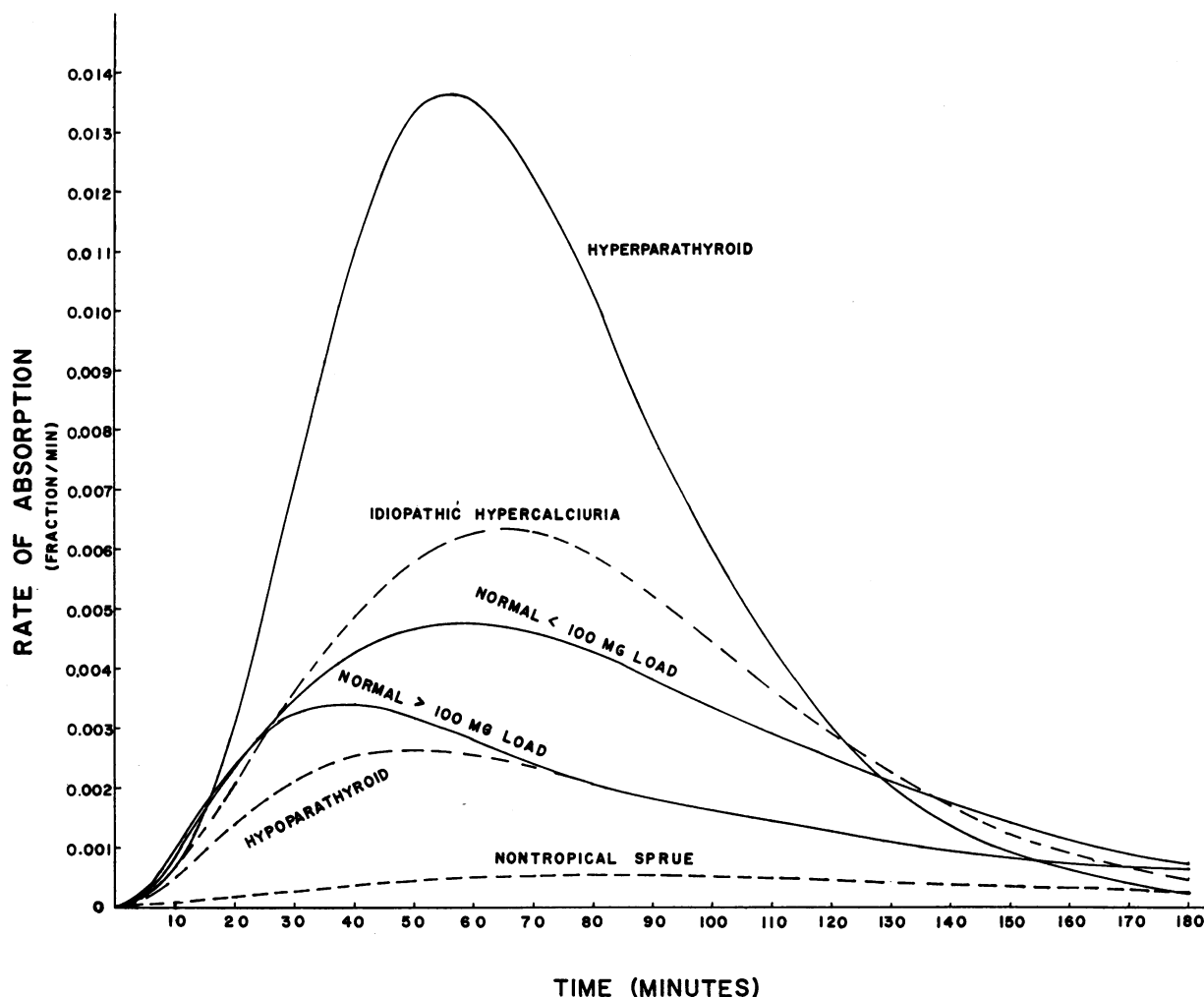


FIGURE 1 Rates of intestinal absorption of calcium (function $I(t)$) in various clinical and physiological states (averages for each of the groups studied). Functions $I(t)$ represent the rate of absorption of the calcium test dose, expressed as fraction of administered amount per minute, starting with the time of the test dose administration. The model parameter values used in generating these curves are given in Table IV.

at the cardioesophageal junction, into the second portion of the duodenum, and into the jejunum 4 cm beyond the ligament of Treitz. The ^{45}Ca and 50 mg of carrier calcium as calcium lactate were made up to 50 ml in distilled water and infused at a rate approximating stomach emptying of 5 ml/min beginning at 2½ hr after the standard breakfast. Model parameters were derived from the first study, in which the isotope was administered directly into the stomach. Changes in the model necessary to simulate the data obtained from the latter two studies then were investigated and correlated with the anatomical and physiological differences arising from the administration of the isotope at different points along the intestine.

RESULTS

Functions $I(t)$ describing rates of absorption per minute expressed as fractions of the administered dose for various clinical and physiological states were illustrated in Fig. 1. The essential characteristics of these functions are: (a) an initial delay of approximately 20 min, (b) a maximal rate of absorption which occurs at 40–60 min, and (c) completion of significant absorption at 2½ hr.

In correlating elements of the model with the physiology of absorption, portions of the intestine were excluded by the introduction of the isotope with a nasogastric tube at various points along the small intestine. The calculated absorption of the isotope when delivered directly to the stomach, the second portion of the duodenum, and the jejunum 4 cm distal to the ligament of Treitz was 47%, 40% and 32% respectively. The latter two responses were successfully simulated by an input function feeding into the second and third compartments

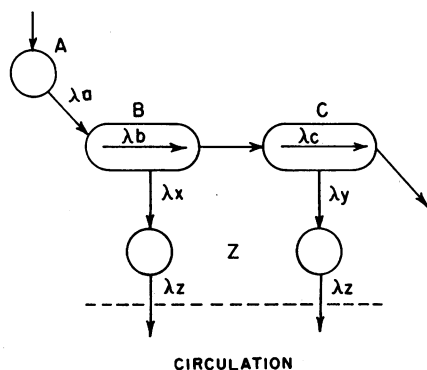


FIGURE 2 Schematic representation of the calcium absorption model. Its interpretation with respect to physiologic counterparts is as follows: A is the stomach from which calcium is transferred at a rate λ_a to B without calcium absorption occurring from A. B is a segment of the gut corresponding to the duodenum and first portion of jejunum from which absorption occurs at a rate λ_b . C is a segment of the gut distal to B from which absorption occurs at a rate λ_c and from which calcium passes outside the system. Although only superficially delineated, Z may be attributed in part to the intestinal wall by reflecting the rate of transfer λ_z from the intestinal wall into the general circulation. See text for details of the physiologic interpretation.

TABLE I
Calcium Absorption Determined in the Same Subject
under Identical Experimental Conditions

Study†	Variable model parameters*					% absorbed‡
	A	B	C	X	Y	
1	0.080	0.097	0.033	0.0014	0.0019	35
2	0.093	0.097	0.035	0.0023	0.0016	32

* Variable model parameters refer to the λ of the components of model designated in Fig. 2 and are expressed as fractional transfer per minute.

† The two studies, a month apart, were performed after 415-mg oral loads of calcium lactate given to the same patient constantly taking a diet containing 800 mg of calcium.

‡ The per cent absorbed is derived directly by integration of the function $I(t)$.

respectively of the first segment at the rate of 10% of the administered dose per min for 10 min. It was necessary, however, in the curve fitting of both studies to introduce an additional compartment to establish a delay of approximately 10 min. The additional compartment is represented in Fig. 2 by compartment Z. Other than the site of entry and the delay, no alteration of model parameters defined by the introduction of isotope into the stomach was necessary.

The values for the per cent of calcium absorbed as derived from the model were compared in the same patients with the values derived from the radioactivity recovered in the feces as collected in 6-day pools demarcated by fecal dye markers. The observed fecal radioactivity was corrected for endogenous fecal excretion by data obtained from the intravenous administration of the isotope.

Estimates of absorption based on stool data were consistently higher by 1–10% than estimates from the model (Fig. 4). The reproducibility of the technique in the same subject under identical dietary conditions over an interval of 1 month is illustrated in Table I. The deviation from the mean of the two determinations was 4.4% and is considered to be within normal physiological variation.

In the investigation of the various parameters which could possibly influence the rate of absorption of the test dose, it was noted that in addition to the amount of carrier calcium given with the ^{45}Ca , the amount of calcium in a meal 2½ hr before the administration of the isotope was also of significance. This effect of calcium loading before the administration of the isotope was studied in a group of healthy volunteers between the ages of 47 and 55 studied while receiving continuously an 800 mg calcium diet. Different calcium loads as calcium lactate were added to the standard 12 mg calcium meal given 2½ hr before isotope administration. All sub-

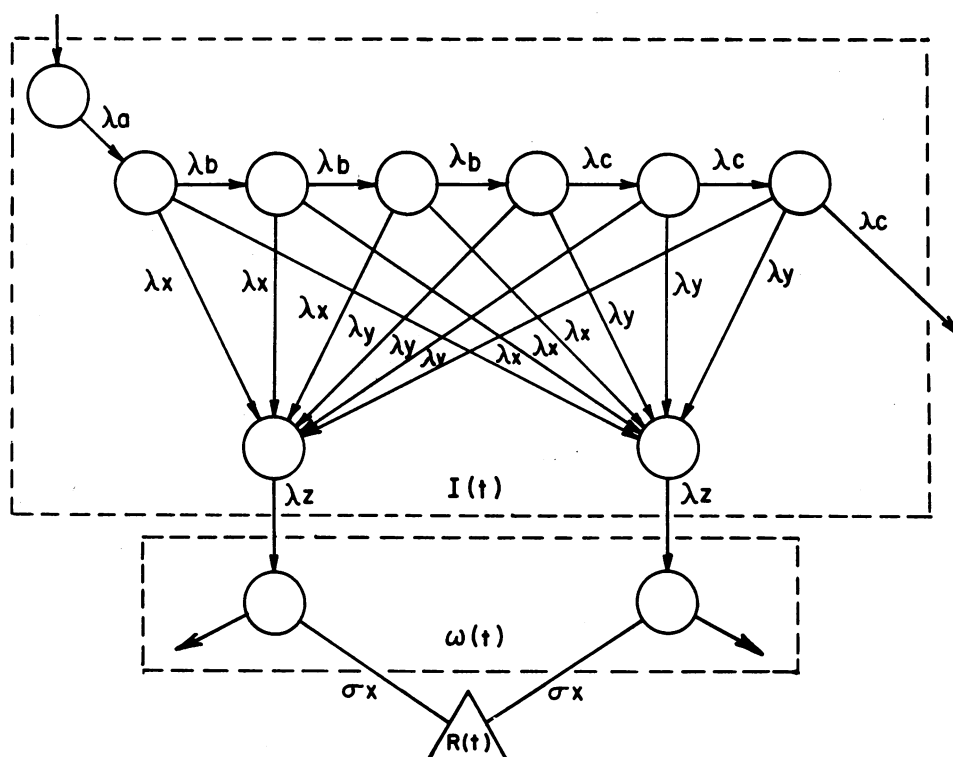


FIGURE 3 Calcium absorption model: the form of the solution of $I(t)$ used by the computer program SAAM. Circles represent compartments or components of delay series. The triangle represents the composite function $R(t)$ which contains the data obtained from the observed response to the input. Arrows are flow pathways expressed as fractional transfer per minute. The interrupted lines (σ_x and σ_y) connecting the triangle $R(t)$ to compartments of $W(t)$ are the fraction of $W(t)$ measured by $R(t)$. The lambdas with identical subscripts are equal.

jects were studied with two or more different calcium loads. Per cent absorption was found to decrease as the calcium load increased. The data also suggest that even small loads of calcium (less than 100 mg) significantly decrease per cent calcium absorption 2½ hr later (Fig. 5).

The significance of isotope dilution in the intestinal tract by the oral calcium load in comparison to the effect of an intravenously administered load simulating the absorption from the oral load was investigated in a patient fully adjusted to an 800 mg calcium diet. This equivalent intravenous load (180 mg) was determined from the per cent absorption (54%) of a 330 mg oral calcium load, as determined in a separate study in which the isotope was given with a 330 mg calcium load after an overnight fast. Furthermore, the intravenous load was given in a rate pattern which simulated the shape of the absorbed oral load as well as its magnitude. The results (Table II) indicate that there was no significant difference in suppression of the rate of absorption of the test dose by either the oral calcium load or its equivalent given intravenously. Furthermore, the rates of ab-

sorption as delineated by the model were quantitatively similar.

The role of parathyroid hormone in regulating calcium absorption was investigated in three patients with post-operative hypoparathyroidism. One of these patients (M.A.) was being treated with 50,000 U of vitamin D₃ and consequently had a higher base line level of calcium absorption. 400 U of parathyroid hormone were infused intravenously at a constant rate over a 4 hr period. ⁴⁵Ca was administered 2½ hr after a 250 mg calcium load and at 2 hr (in D.M. and M.A.) and 3 hr (M.K.) after initiation of parathyroid hormone infusion. No apparent stimulation of calcium absorption was observed during a 4–5 hr period after the initiation of the parathyroid hormone infusion (Table III). Maximal reduction of tubular reabsorption of phosphate was observed within this time interval. Serum calcium also increased (although within the limits of error for the technique).

Patient D.M. who was not receiving vitamin D was given the same preparation of parathyroid hormone intramuscularly over a 3 wk period. 200 U in a vehicle of 15% gelatin was given twice daily. Urine calcium in-

creased from an average of 106 to 550 mg/24 hr, and serum calcium increased from an average of 8.1 to 11.5 mg/100 ml. After urine and serum calcium seemed to reach a plateau, the patient was given the oral and intravenous ^{45}Ca . In contrast to the previous observation, intestinal calcium absorption increased twofold to a level comparable with patients with primary hyperparathyroidism (Table III).

Table IV summarizes the results obtained from studies of patients with various disorders of calcium metabolism and normal volunteers.

DISCUSSION

A mathematical model of calcium absorption was developed, and data supporting its physiological interpretation are presented. This analysis provides a quantitative description of the kinetics of calcium absorption and a method for the investigation of factors influencing calcium absorption. The mathematical treatment of the experimental observations made in these studies requires the critical assumption that the physiological conditions which determine the weighting function as defined by the intravenous administration of the isotope are unaltered at the time of the oral administration of the isotope 10–14 days earlier. This assumption requires that the conditions of the intravenous study with respect to the time of administration, calcium load, and dietary intake be carefully duplicated in the oral administration of the isotope. It is also necessary that the subject be sufficiently adjusted to a given daily dietary calcium intake before the initiation of the two studies.

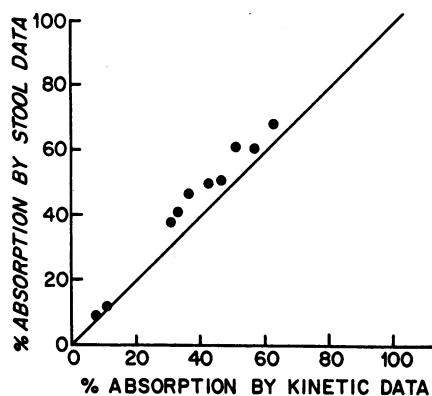


FIGURE 4 Comparison of calcium absorption data obtained by stool analysis with the data obtained from the physiologic model. Calcium absorption was measured by the recovery of ^{45}Ca in a 7 day stool collection after the oral administration of the isotope. The per cent of the administered dose retained by the patient was corrected for endogenous fecal excretion. These results represented by the closed circles were compared in the same patient to the results obtained at the same time from the kinetic data represented by the straight line. Each point represents a different patient.

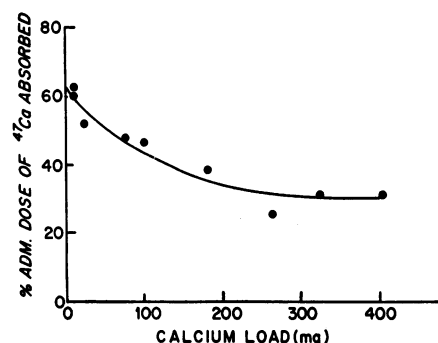


FIGURE 5 The effect on an oral calcium load on calcium absorption. Four patients were studied at two or more different oral calcium loads given 2–3 hr before the administration of ^{45}Ca . Calcium load was given as calcium lactate dissolved in lemonade.

Except possibly for adjustment to a constant level of calcium intake, these requirements would be eliminated by the simultaneous administration of the intravenous and oral doses by use of two different calcium isotopes.

Partial identification of the physiological counterpart to the model was done by evaluating the response to the introduction of the isotope by tube directly into the gut at various levels. The response resulting from exclusion of the gut proximal to the second portion of the duodenum and proximal to the ligament of Treitz was simulated in the model by altering only the site of entry of the input in the model.

Despite the exclusion of compartment A by the input, a delay of approximately 10 min was still needed in order to fit the curves of the simulated data to the curves of the observed experimental data. As the model was originally constructed, the entire delay (20 min) was described by the first compartment (A), since one could not distinguish between an early and late delay. These studies therefore serve to delineate some delay at A,

TABLE II
Comparison of the Effect of an Oral and Intravenous Load of Calcium on Subsequent Calcium Absorption

Study†	Variable model parameters*					% absorbed
	A	B	C	X	Y	
330 mg, oral	0.040	0.107	0.013	0.0042	0.00037	33
180 mg, i.v.	0.086	0.107	0.014	0.0040	0.00034	30

* The model parameters refer to those designated in Fig. 2.

† Absorption of calcium was studied in the same patient after an oral load of 330 mg and then after an equivalent intravenous load of 180 mg. The equivalent intravenous dose of calcium was determined from the amount of calcium absorbed from the 330 mg of calcium given orally.

TABLE III
*The Influence of Parathyroid Hormone on Calcium Absorption**

	M. K.		M. A.		D. M.		PTH, i.m.
	Control PTH, i.v.		Control PTH, i.v.		Control PTH, i.v.		
% absorption	28	26	57	33	28	27	74
Serum calcium, <i>mg/100 ml</i>	7.6	8.2	8.9	9.3	7.6	8.1	11.5
% TRP†	92	66	80	68			

* Three patients with postoperative hypoparathyroidism were studied after the standard low calcium meal during a 4 hr parathyroid hormone infusion of 400 U. M. K. and M. A. were maintained on 25,000–50,000 U of vitamin D per day respectively throughout the studies. D. M. was maintained on supplemental calcium alone without vitamin D. See text for detail of protocol.

† Tubular reabsorption of phosphate.

and some at Z, a point beyond the level of intubation. Since no other changes in the model were necessary to fit these studies, one can propose that the portion of the model proximal to the input describes that portion of the gut excluded by the nasogastric tube. The delay at A, therefore, is postulated to be the rate of transit from a region of nonabsorption, the stomach, into the first portion of the duodenum. The delay Z was added to the model and postulated to reflect in part the transit time across the intestinal mucosa to the general circulation. Although not measured, we have assumed that a delay of similar magnitude is also operational in the last series segment of the model.

In the physiologic interpretation of the model, the assumption is made that the kinetics of the ⁴⁵Ca introduced into the circulation through a peripheral vein are not significantly altered by the introduction of the isotope through the portal circulation. It is reasonable that there is in fact a small delay in the kinetics of the isotope imposed by the hepatic circulation which would be manifested to a lesser extent in the kinetics of the isotope introduced into the peripheral circulation. This

delay if present would appear in the absorption model and is probably imbedded in the delay at Z (Fig. 2). Since the hemodynamics of such a delay are relatively constant and independent of calcium metabolism, the interpretation of the model for other subjects would be similar. As new information from perturbations of the absorption process is obtained, further refinements of the model may be possible.

The proposed model is not unique, so that a number of different combinations of components and connections between components are possible. It is the simplest mathematical construct that we could devise from the data and that is also consistent with general concepts of intestinal absorption. In general, the model as presented consists of three segments in series, with diffusion paths or absorption from the second and third segments. Although some physiological identification of the model components may be possible, there need not be a simple one to one correlation between an element of the model and a discrete unit of intestinal anatomy and(or) physiology. Thus for example, it is unlikely that rate of calcium absorption along the gut is discontinuous occurring

TABLE IV
Studies of Calcium Absorption in Normal Subjects and Patients with Disorders of Calcium Metabolism

Number of patients	Diagnosis	Calcium intake*	Model parameters‡					% administered dose absorbed§		
			A	B	C	X	Y	B	C	Total
6	Normal control, Ca load < 100 mg	800	0.063	0.114	0.0329	0.0035	0.0034	15.5 ± 2.5	36.7 ± 5.6	52.2 ± 5.4
4	Normal control, Ca load > 100 mg	800	0.072	0.100	0.023	0.0032	0.010	16.5 ± 2.3	16.3 ± 2.9	32.8 ± 2.4
2	Nontropical sprue	1800	0.050	0.100	0.030	0.00028	0.00033	2.5 ± 0.2	7.0 ± 1.0	9.5 ± 2.2
3	Hyperparathyroidism	800	0.055	0.101	0.032	0.0031	0.0551	16.0 ± 4.2	66.3 ± 7.3	82.3 ± 9.0
3	Hypoparathyroidism	1800	0.051	0.098	0.027	0.0029	0.00088	12.0 ± 3.5	15.0 ± 3.6	27.0 ± 1.0
4	Idiopathic hypercalciuria	800	0.061	0.102	0.048	0.0029	0.0067	15.7 ± 1.8	42.7 ± 8.7	58.5 ± 4.3

* Daily calcium intake in milligrams.

‡ Model parameters refer to those in Fig. 2 and are expressed as the mean of the designated patient group.

§ Derived absorption in segments B and C and total absorption are expressed as the mean ± SE for the patient groups.

at only two rates. It is of significance, however, that all attempts to simulate the absorption function with less than two independent rates were unsuccessful. The mathematical approach presented here need not be restricted to the study of calcium absorption. Segre (19) has successfully studied the kinetics of intestinal absorption of a variety of drugs using a similar mathematical approach.

Since absorption rate actually depends not only on the magnitude of the calcium preload but probably also on the time interval between it and the time of administration of tracer, it is quite likely that the rate "constants" of the model are time and (or) concentration dependent. There is no way to resolve this possible time dependence from the data obtained in the presented experiments. The present model may require modification if time and (or) concentration dependence of parameters are introduced.

Determination of the percent absorption of the administered isotope by the model was consistently 1–10% lower but in good agreement with the values obtained by methods based on recovery of radiocalcium in the stool. The direction of this difference probably can be accounted for by minor losses of stool in collecting and processing and by continued (or delayed) excretion of the isotope beyond the first 6 days of collection (20). We cannot exclude, however, the possibility that part of this difference is due to a physiological mechanism not included in our assumptions such as, for example, an enterohepatic cycling of calcium. Further studies are necessary in this area.

Assuming the physiological interpretation of the model to be correct, then in normal subjects studied while receiving an 800 mg calcium diet and after a calcium load of less than 150 mg, the rate of absorption by the duodenum is approximately three times that of the remainder of the gut. However, if the rate of absorption is expressed per unit length of gut, and if the transit time through that portion of the gut involved in calcium transport is constant, then the rate of absorption per unit length by the duodenum is approximately nine times that of the remaining gut.

In vitro studies with rat intestine (21) have demonstrated that maximal absorption of calcium occurs in the duodenum by an active transport process. Kimberg, Schachter, and Schenker (22) have also demonstrated that although active transport occurs predominantly in the duodenum of the rat, the jejunum and ileum were also capable of active transport under conditions of low calcium intake for a period of 15–23 days. The observations reported here would suggest that a similar but more sensitive mechanism applies in man. Not only does significant calcium transport occur distal to the duodenum in human subjects, but this region, as illus-

trated in Table IV, shows the greatest response to calcium loading and in hyperparathyroidism may account for 75% of the total absorption. These data suggest that the gut distal to the duodenum is more sensitive to factors controlling calcium absorption under normal physiological conditions. Regulation of calcium absorption, therefore, is probably mediated primarily through a portion of the gut distal to the duodenum.

The suppression of absorption by calcium loading $2\frac{1}{2}$ hr before the administration of the isotope in the absence of significant isotope dilution within the lumen of the gut suggests that increased concentration of extracellular calcium suppresses intestinal mucosal transport (a) directly or (b) indirectly through a hormonal feedback mechanism such as the parathyroid axis. The action of parathyroid hormone on intestinal transport of calcium was investigated and was found to have no stimulatory effect during the first 4–5 hr after initiation of the hormone infusion. In contrast, the maximal effect on renal phosphate transport as measured by the per cent of tubular reabsorption of phosphate (TRP) occurred during the 3rd and 4th hr after the initiation of the infusion. The absence of stimulation and (in M.A.) suppression of calcium absorption acutely may in part be related to an elevation of plasma calcium by action of parathyroid hormone peripheral to the gut. A stimulatory effect on absorption, however, was observed during prolonged intramuscular administration of comparable doses of hormone despite significant hypercalcemia. Thus, the action of parathyroid hormone on the gut to increase calcium absorption is delayed in its onset. If regulation of calcium absorption by parathyroid hormone were dependent on *de novo* synthesis of a protein this might account for the observed delay in onset of action. It is even possible that the action of parathyroid hormone on the intestine may be effected through synthesis of a calcium-binding protein, similar to that described by Wasserman and Taylor (23), which could be involved in active transport of calcium across intestinal mucosa.

The rapid and dramatic suppression of calcium absorption in response to a calcium load as compared with the delayed stimulation of absorption by parathyroid hormone suggests the possibility that rapid or fine regulation of absorption is mediated through a direct effect of circulating calcium on mucosal transport, and coarse regulation is mediated through the action of parathyroid hormone on intestinal transport.

Data presented in Table IV suggest that discrimination of abnormally enhanced intestinal absorption of calcium from normal is improved by partially suppressing calcium absorption in the normal subject with a calcium load. This suppression had the effect of narrowing the range for normal subjects from 25–60% to

25-40%. Thus, pathological hyperabsorption of calcium in this series of patients was clearly delineated both quantitatively and qualitatively from intestinal absorption of calcium in normal subjects.

ACKNOWLEDGMENT

This work was supported in part by grant AM-11674 from the National Institute of Arthritis and Metabolic Diseases.

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