THE DYNAMICS OF CALCIUM TRANSFER IN MAN

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THESIS

ABSTRACT

An existing compartmental model of human calcium metabolism able to describe successfully the dynamics of stable and radioactive calcium administered both orally and intravenously, is extended to include the bone calcium and its protein matrix, collagen, the secretion and catabolism of the hormones calcitonin and parathyroid hormone, and the hormonal control of calcium excretion by the kidney. Data selected from a literature survey of the rates and calcium contents of the major intestinal secretions, and of sweat, are incorporated into the model. Allowance is made for the increased secretion of calcium into the intestine during meals.

By means of a digital computer program (CAMET2) 15 simplified simulations are carried out, corresponding to a variety of real situations including calcium and ethylenediamine tetraacetic acid infusions, the removal of the parathyroid glands, fasting, a low calcium diet and the intestinal absorption of radioactive calcium. The results emphasize the importance of renal calcium excretion in the overall control of calcium homeostasis. The roles of the skeleton, on which much emphasis has been placed in the past, and of the soft tissue calcium in buffering perturbations to the plasma calcium are considered. Common methods of calculating the total calcium secreted into the gut and of the endogenous faecal calcium are simulated and sources of error exposed. A meal subsequent to an oral tracer dose is shown to depress tracer absorption. The responses to a range of dietary calcium intake are considered.

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INTRODUCTION

Living systems have evolved with a hierachy of linked regulatory control systems necessary to the maintenance of life. One of these systems is concerned with the regulation of calcium; its distribution, chemical status and excretion. Control is known to be principally exerted through an important group of endocrinological agents - calcitonin, parathyroid hormone and vitamin D. The regulation of calcium is essential to its dual roles of mechanical support (as a component of bone mineral) and its participation in numerous intra- and extra-cellular biochemical processes, and is such that calcium is present within the one organism at local concentrations as low as 10^{-8} moles/litre and simultaneously as a calcium phosphate mineral. Breakdown in the regulation at various points results in disorders ranging from rickets in children to osteoporosis in the elderly.

A research program into calcium metabolism was begun at the University of Canterbury by Dr. W.S. Metcalf to examine the more important aspects - with a view to clarifying the causes of some of the commoner medical complaints such as osteoporosis. To date the work has been theoretical in that no direct experimental investigations have been undertaken. Rather, the medical literature has been surveyed and the best clinical data incorporated into a mathematical model describing calcium metabolism in man. The initial work in setting up the model and the development of the computer program CAMET was carried out by J.H. Livesey as research towards a Ph.D. degree. Subsequently the computer program was rewritten by A.E. McKinnon of the Electrical Engineering Department of this university

to improve its efficiency and to facilitate further additions and alterations.

In order to avoid confusion the present model, whose development from CAMET is discussed in this volume, is referred to as CAMET2. The symbol '±' is used throughout to denote one standard deviation

$$\sigma = \left(\sum_{i=1}^{n} (\overline{x} - x_i)^2 / (n - 1) \right)^{\frac{1}{2}}$$

where $\overline{x}$ is the mean of the n observations (x_i).

The purposes of modelling are four-fold : to describe the system in mathematical terms, to critically examine present methods and conclusions, to suggest new experiments, and, where possible, to use the model as a cheap and convenient (though approximate) exploratory tool. Hopefully the usefulness of this approach is illustrated in the following pages.

CHAPTER 1

A REVIEW OF THE MODEL CAMET

1.1 Over the last 50 years the kinetics of numerous metabolites have been investigated by observing the dynamics of a tracer following its introduction to the biological system. Usually the tracer is an isotopic form of an element, either radioactive or stable, although in some cases dyes and other readily detectable substances have been used (Atkins 1969). Radioactive Ca^{45} and Ca^{47}, stable Ca^{48} (detected by neutron activation analysis) and sometimes strontium and its radioactive isotopes Sr^{85}, Sr^{89} and Sr^{90}, have all been used as probes for calcium. Such experiments are dependent on the tracer not perturbing the system under investigation and on the tracer being handled in a manner similar to or identical with the unlabelled substance.

The measured specific activity s(t) of the tracer (where t is the time) in many biological systems has been found to be fitted satisfactorily by a sum of exponentials of the form

$$s(t) = \sum_{i=1}^{n} \alpha_i e^{-\beta_i t}$$

The set of α_i's and β_i's and n are required to be determined from the experimental data. Such a system

can be represented by a multicompartmental model where the substance present (in our case calcium) can be considered to be distributed between a number of distinct, completely mixed compartments, and where the rate of transfer of the substance out of a given compartment is proportional to the amount or concentration of the substance within that compartment. If the additional assumption is made that the system is in a steady state (that is, that the sizes of the metabolic pools are constant, or at least are changing negligibly during the course of the experiment) then the model consists of a set of simultaneous, linear differential equations whose solution is a sum of exponentials (Atkins 1969, Ackerman et al. 1967).

A number of multicompartmental models of calcium metabolism, of varying complexity have been published (table 1.1). Of those based on the disappearance of injected tracer calcium from the blood, that of Neer et al. (1967) is especially important to the present model, as many of the model parameters have been derived from Neer's experimental data. Also listed in table 1.1 are a number of models relating to the kinetics of ingested tracer calcium as well as others representing the regulation of calcium concentration in the blood. The non-compartmental models proposed include the use of power functions and probability density functions to describe radiocalcium turnover in man.

Table 1.1 Models of Calcium Metabolism

[A] Plasma Compartmental Models

Bauer et al. (1957)

Aubert & Milhaud (1960)

Bronner & Aubert (1965)

Cohn et al. (1965)

Neer et al. (1967)

Phang et al. (1969)

Gonick & Brown (1970)

Ramberg et al. (1970)

[B] Intestinal Compartmental Models

Birge et al. (1969)

Marshall & Nordin (1969)

[C] Regulatory Models

Roston (1959)

Copp et al. (1960b)

Aubert & Bronner (1965)

Riggs (1966)

[D] Noncompartmental Models

Marshall (1964)

Ackerman et al. (1967)

Anderson et al. (1967)

Marshall & Onkelinx (1968)

Burkinshaw et al. (1969)

As the basis of most of these models, the approximations involved, and the results achieved have already been reviewed in detail (Livesey 1970), this chapter is confined to describing the structure, derivation and capabilities of the model CAMET.

1.2 The Calcium Metabolism Model CAMET

CAMET is comprised of 11 homogeneous compartments and one unmixed compartment (fig. 1.1). An unmixed compartment is defined to be one which mixes so slowly over the duration of the tracer experiment (Neer's data was collected over twenty days) that an insignificant fraction of the material entering the compartment is found to leave it.

As the homogeneous compartments contain less than 10 grams of calcium between them out of a total calcium content of more than 1000 grams in the adult (Mitchell et al. 1945, Forbes et al. 1953), the unmixed compartment can be identified with slowly exchanging bone calcium comprising over 99% of the total body calcium. The movement of calcium to and from this compartment (R_{acc} and R_{dec}) represents the processes of bone formation (accretion), bone resorption (decretion) and also some very slow exchange where one calcium ion replaces another ion with no net change in the number of ions (Chapter 6). It is because the quantity of calcium involved in these processes is small in comparison with the amount of skeletal calcium over the experimental period that tracer entering the unmixed compartment is considered

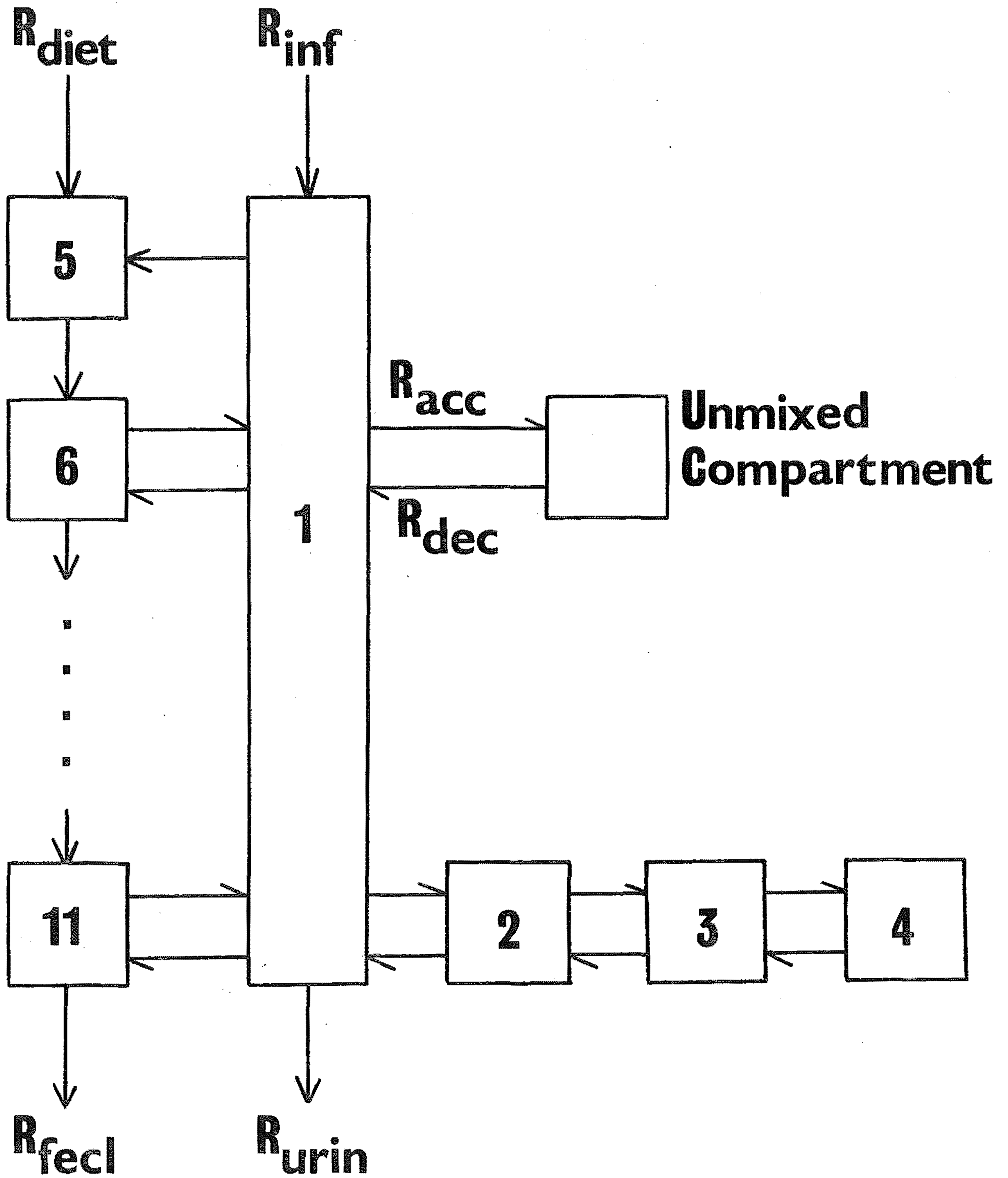

Figure 1.1 CAMET compartmental structure

lost to the system.

The remaining rapidly exchanging bone calcium and the calcium present in the soft tissues (apart from intestinal calcium) is distributed between four homogeneous compartments in the model (labelled 1 to 4 in fig. 1.1). Because simultaneous exchange processes in the bone and distributive processes occurring in the soft tissues with similar rates are unresolved by compartmental analysis, compartments 1 to 4 do not correspond in a one to one manner with any anatomical region of the body. Neer (1967) concluded that the rapidly exchanging compartments 1 and 2 were predominantly composed of soft tissue calcium while the more slowly exchanging compartments 3 and 4 were mainly exchangeable bone calcium - although no compartment could be considered exclusively either as bone or soft tissue calcium. Nevertheless as the isotope is injected into the blood and subsequent samples for analysis are taken from the blood, compartment 1 includes vascular space.

Compartments 5 to 11 represent the digestive tract from the stomach to the distal ileum. The stomach is modelled by compartment 5 and the remaining small intestine, divided by length, is represented by the other six compartments; six being the minimum number necessary to describe the experimental data (Birge et al. 1969). The duodenum can be

identified with compartment 6, the jejunum with compartment 7 and the ileum with 8 to 11 inclusive. Although the movement of water and of electrolytes, particularly sodium, potassium and chloride ions, across the walls of the colon is considerable (Fordtran & Ingelfinger 1968) little net absorption of calcium is usually considered to occur, but this conclusion has been questioned (Brooke 1958). While the colon has not been included in the model more information is required on this aspect of calcium metabolism.

1.3 Model Equations

The set of equations describing the movement of stable and radioactive calcium between and out of the model compartments and the movement of fluid in the gut is presented in table 1.2. The equations proposed by Livesey (1970) are more complex in form than those tabulated below as provision was made for the actions of calcitonin (CT), parathyroid hormone (PTH) and vitamin D. However as parameters relating to these substances, for example the concentrations of CT and PTH, could not be determined they were set equal to zero. As a result there was no hormonal regulation of any metabolic process in the CAMET simulations. Under these circumstances those parts of the equations relating to these substances have been excluded here for simplicity, without any change in the predictions of the CAMET model.

Implicit in the tracer equations is the assumption that tracer calcium is handled in a manner identical with stable calcium. This has some experimental basis (Neer et al. 1967).

A full explanation of the symbols used below and in subsequent chapters appears in Appendix I, but at this point we may note that

M_I is the quantity of calcium in compartment I.

MT_I is the quantity of tracer in compartment I.

$R_{I,J}$ is the rate of transfer of calcium from compartment I to compartment J.

$RT_{I,J}$ is the rate of transfer of tracer from compartment I to compartment J.

$\dot{x}$ is the first derivative of x with respect to time (dx/dt)

Table 1.2 Model Equations

Differential Equations

(1.1) $$\dot{M}_1 = R_{2,1} + R_{dec} + R_{abs} + R_{inf} - R_{1,2} - R_{acc} - R_{gsec} - R_{urin}$$

(1.2) $$\dot{MT}_1 = RT_{2,1} + RT_{abs} + RT_{inf} - RT_{1,2} - RT_{acc} - RT_{gsec} - RT_{urin}$$

where $R_{abs} = \sum_{I=6}^{11} R_{I,1}$ $\quad RT_{abs} = \sum_{I=6}^{11} RT_{I,1}$

$R_{gsec} = \sum_{I=5}^{11} R_{1,I}$ $\quad RT_{gsec} = \sum_{I=5}^{11} RT_{1,I}$

(1.3) $\dot{M}_2 = R_{1,2} + R_{3,2} - R_{2,1} - R_{2,3}$

(1.4) $\dot{MT}_2 = RT_{1,2} + RT_{3,2} - RT_{2,1} - RT_{2,3}$

(1.5) $\dot{M}_3 = R_{2,3} + R_{4,3} - R_{3,2} - R_{3,4}$

(1.6) $\dot{MT}_3 = RT_{2,3} + RT_{4,3} - RT_{3,2} - RT_{3,4}$

(1.7) $\dot{M}_4 = R_{3,4} - R_{4,3}$

(1.8) $\dot{MT}_4 = RT_{3,4} - RT_{4,3}$

Stomach

(1.9) $\dot{M}_5 = R_{diet} + R_{1,5} - R_{5,6}$

(1.10) $\dot{MT}_5 = RT_{diet} + RT_{1,5} - RT_{5,6}$

(1.11) $\dot{V}_5 = RF_{diet} + RF_{1,5} - RF_{5,6}$

Intestine

(1.12) $\dot{M}_I = R_{(I-1),I} - R_{I,(I+1)} + R_{1,I} - R_{I,1}$ $\quad 6 \leqslant I \leqslant 10$

(1.13) $\dot{MT}_I = RT_{(I-1),I} - RT_{I,(I+1)} + RT_{1,I} - RT_{I,1}$ $\quad 6 \leqslant I \leqslant 10$

(1.14) $\dot{M}_{11} = R_{10,11} - R_{fecl} + R_{1,11} - R_{11,1}$

(1.15) $\dot{MT}_{11} = RT_{10,11} - RT_{fecl} + RT_{1,11} - RT_{11,1}$

<u>Rate Expressions</u>

(1.16) $R_{I,J} = M_I K_{I,J}$ $\quad 1 \leqslant I \leqslant 4, \quad 1 \leqslant J \leqslant 4, \quad J = I \pm 1$

(1.17) $RT_{I,J} = MT_I K_{I,J}$ $\quad 1 \leqslant I \leqslant 4, \quad 1 \leqslant J \leqslant 4, \quad J = I \pm 1$

Non Exchanging Calcium

(1.18) $R_{acc} = K_{acc} C_1$

(1.19) $RT_{acc} = R_{acc} SA_1$

(1.20) $R_{dec} = A_1$

Urinary Excretion of Calcium

(1.21) $R_{urin} = A_6 C_1 - A_7 \qquad C_1 < (A_7 - A_{11})/(A_6 - A_{10})$

(1.22) $R_{urin} = A_{10} C_1 - A_{11} \qquad C_1 \geqslant (A_7 - A_{11})/(A_6 - A_{10})$

(1.23) $RT_{urin} = R_{urin} SA_1$

Stomach

(1.24) $RF_{5,6} = FV_5^{\frac{1}{2}}$

(1.25) $R_{5,6} = RF_{5,6}\, C_5$

(1.26) $RT_{5,6} = R_{5,6}\, SA_5$

Small Intestine

(1.27) $RF_{I,(I+1)} = RF_{(I-1),I}\,(1 - AF_{I,1}) + RF_{1,I} \qquad 6 \leqslant I \leqslant 10$

(1.28) $RF_{fecl} = RF_{10,11}\,(1 - AF_{11,1}) + RF_{1,11}$

(1.29) $V_I = B_{I,1}\, RF_{I,(I+1)}/(B_{I,2} + RF_{I,(I+1)}) \qquad 6 \leqslant I \leqslant 10$

(1.30) $V_{11} = B_{11,1}\, RF_{fecl}/(B_{11,2} + RF_{fecl})$

(1.31) $R_{I,(I+1)} = RF_{I,(I+1)}\, C_I \qquad 6 \leqslant I \leqslant 10$

(1.32) $RT_{I,(I+1)} = R_{I,(I+1)}\, SA_I \qquad 6 \leqslant I \leqslant 10$

(1.33) $R_{fecl} = RF_{fecl}\, C_{11}$

(1.34) $RT_{fecl} = R_{fecl}\, SA_{11}$

(1.35) $R_{I,1} = E_{I,1}\, C_I/(E_{I,2} + C_I) \qquad 6 \leqslant I \leqslant 11$

(1.36) $RT_{I,1} = R_{I,1}\, SA_I \qquad 6 \leqslant I \leqslant 11$

(1.37) $C_1 = M_1/V_1$

(1.38) $C_I = M_I/V_I \qquad 5 \leqslant I \leqslant 11$

(1.39) $SA_I = MT_I/M_I \qquad 1 \leqslant I \leqslant 11$

Calcium Balance

(1.40) $C_{bal} = S_{diet} + S_{inf} - S_{fecl} - S_{urin}$

1.4 Derivation of Model Parameters and Equations.

On the basis of the four term exponential function

$$s(t) = \sum_{i=1}^{4} \alpha_i e^{-\beta_i t}$$

fitted to the data from a single isotope study over twenty days following the tracer injection, Neer et al. (1967) proposed a four compartment model. The same number of compartments, with the arrangement of fig. 1.2, were adopted by Livesey, although the arrangement of the compartments is arbitrary (Berman 1963, Neer et al. 1967).

Tracer specific activities generated by Neer's model, based on the average model parameters of 10 normal men were fitted to the model in fig. 1.2. Least-squares fitting was carried out using the computer program ORGLS (Busing & Levey 1962). In this way the rate constants between the four rapidly exchanging compartments ($K_{1,2}$, $K_{2,1}$ etc.) and the rate constant K_E for the loss of calcium from compartment 1 via other routes (urinary and digestive juice calcium as well as loss to the unmixed compartment) were derived by Livesey. M_1, the mass of calcium in compartment 1 was calculated by Neer et al. (1967) from the plasma specific activity at zero time, obtained by back extrapolation from the initial measurements. With this value the set of steady state simultaneous equations

$$M_1K_{1,2} = M_2K_{2,1}$$

$$M_2K_{2,3} = M_3K_{3,2}$$

$$M_3K_{3,4} = M_4K_{4,3}$$

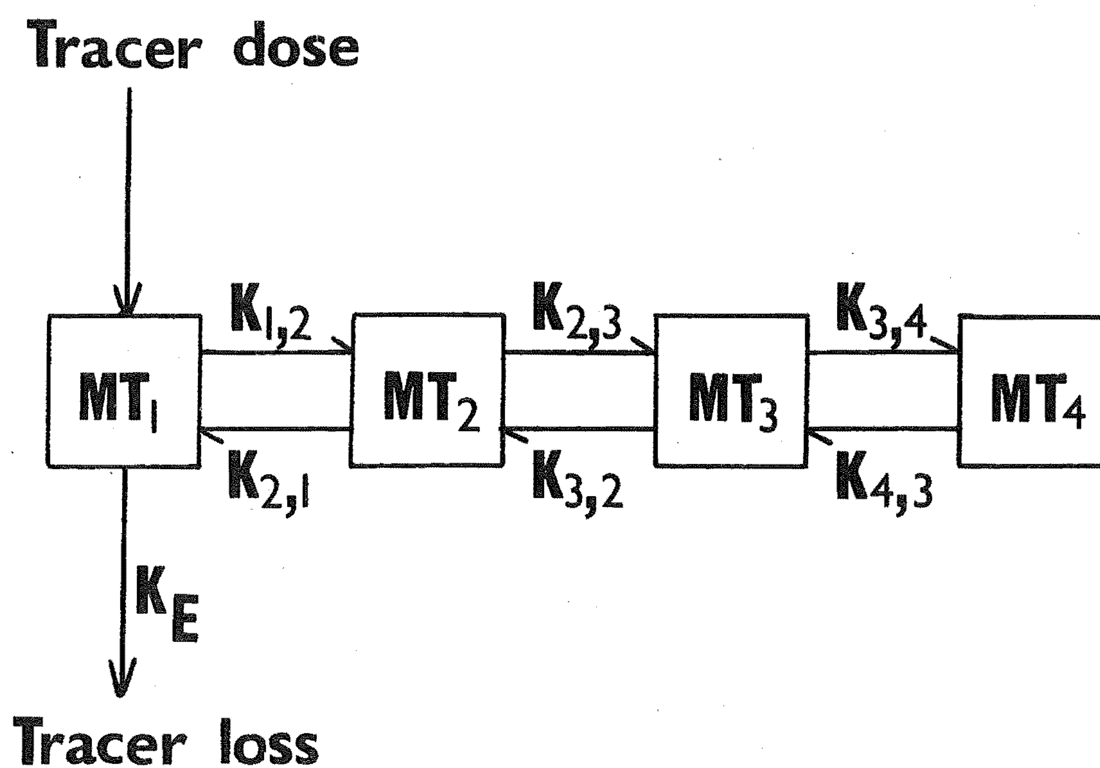

Figure 1.2 Model fitted to specific activity data of Neer et al. 1967

was solved for M_2, M_3 and M_4. These values and the rate constants for the rapidly exchanging calcium pools given by Livesey are adopted in the present work.

In contrast to the techniques of compartmental analysis employed to this point the intestinal model was built up largely on physiological grounds. The square root relationship between the volume of the stomach and its rate of emptying given by equation (1.24) is approximate (Hopkins 1966), but adequate for the purposes of the model. Each compartment from 6 to 11 represents 48 cm of the small intestine (based on a total length of 285 ± 43 cm measured by Hirsch et al. (1956) in ten subjects). To avoid confusion the terms 'calcium absorption' and 'calcium secretion' refer, in this volume, to the unidirectional flows of calcium across the intestinal membranes from the lumen to the blood and the blood to the lumen respectively. Mathematically they are defined in equation 1.2. Into the stomach and each of the intestinal compartments there is a flow of calcium $R_{1,I}$ $(5 \leqslant I \leqslant 11)$ and fluid $RF_{1,I}$ $(5 \leqslant I \leqslant 11)$ from the plasma compartment. Some of the calcium that is secreted into the gut in this way is reabsorbed but the fraction that is ultimately excreted is the 'endogenous faecal calcium'.

Recently the rate of absorption of calcium from buffered solutions of calcium gluconate in various segments of the human intestine was measured by Wensel et al. (1969). The parameters of Michaelis-Menten type equations for the rate of absorption of calcium from the gut (equation 1.35) were fitted to his data using the least squares program ORGLS.

As far as the fluid dynamics are concerned the outflow of fluid from an intestinal compartment is assumed to be always equal to the inflow minus what is absorbed (equation 1.27) which implies that the segment volume is constant, and it is further assumed that the flow is unidirectional from the stomach to the distal ileum. The absorption of fluid from each compartment, through the gut wall is calculated as a constant fraction of the inflow into the compartment (equation 1.27). Finally the equations relating to the volume of the intestinal compartments (equations 1.29 and 1.30) are based on the relationships between the fluid flow and the apparent volume of human intestinal segments (Dillard et al. 1965), which removes the restriction of constant volume, and is therefore inconsistent. The fluid flow equations are of such form as to make computation rapid, but as will be seen in Chapter 5, the inconsistency produces anomolies in the simulation of a meal.

Equation 1.40 calculates the calcium balance throughout a simulation and is defined as the total simulated excretion of calcium by all routes subtracted from the intake of calcium (dietary and/or infused calcium) since the beginning of the simulation.

1.4 CAMET Parameter Values

CAMET model parameters corresponding to the equations of table 1.2 are given below.

Table 1.3 Parameter Values

Parameter	Value	Units
A_1	0.33	m.moles/kg/day
A_6	0.09	litres/kg/day
A_7	0.17	m.moles/kg/day
A_{10}	1.10	litres/kg/day
A_{11}	2.60	m.moles/kg/day
$AF_{6,1}$	-2.5	-
$AF_{7,1}$ to $AF_{11,1}$	0.5	-
$B_{6,1}$ to $B_{8,1}$	0.004	litres/kg
$B_{9,1}$ to $B_{11,1}$	0.0025	"
$B_{6,2}$ to $B_{8,2}$	0.14	litres/kg/day
$B_{9,2}$ to $B_{11,2}$	0.12	"
$E_{6,1}$ to $E_{8,1}$	0.19	m.moles/kg/day
$E_{9,1}$ to $E_{11,1}$	0.10	"
$E_{6,2}$ to $E_{8,2}$	2.2	m.moles/litre
$E_{9,2}$ to $E_{11,2}$	5.0	"
F	4.5	$litres^{1/2}/kg^{1/2}/day$
K_{acc}	0.08	litres/kg/day
$K_{1,2}$	28.9	day^{-1}
$K_{2,1}$	25.2	"
$K_{2,3}$	3.10	"
$K_{3,2}$	1.30	"

Parameter	Value	Units
$K_{3,4}$	0.127	day^{-1}
$K_{4,3}$	0.180	"
M_1	0.45	m.moles/kg
M_2	0.52	"
M_3	1.23	"
M_4	0.87	"
M_5	0.01	"
M_6	0.8×10^{-3}	"
M_7 & M_8	0.1×10^{-2}	"
M_9 to M_{11}	0.6×10^{-3}	"
MT_1 to MT_{11}	0.0	m.moles/kg
$R_{1,5}$	0.07	m.moles/kg/day
$R_{1,6}$	0.05	"
$R_{1,7}$ & $R_{1,8}$	0.02	"
$R_{1,9}$ to $R_{1,11}$	0.01	"
$RF_{1,5}$	0.1	litres/kg/day
$RF_{1,6}$	0.018	"
$RF_{1,7}$ to $RF_{1,11}$	0.0011	"
V_1	0.18	litres/kg
V_5	0.5×10^{-3}	"

N.B. M_1 to M_{11}, MT_1 to MT_{11}, and V_5 are the initial conditions of the differential equations (1.1) to (1.15).

1.5 The Computer Program CAMET

The necessary computation required for the solution of the differential equations (1.1 to 1.15) is carried out with the computer program CAMET, written in FORTRAN by A. McKinnon, for the University of Canterbury IBM 360/44 computer. The memory requirements of the complete program as it exists for the simulations in this thesis (including the plotting subroutine PLOTER in Appendix IV) are approximately 22,000 thirty-two bit words. Current listings and/or card decks are available from Dr. W.S. Metcalf, Chemistry Dept., University of Canterbury, Private Bag, Christchurch 1, New Zealand.

1.6 Numerical Methods

The differential equations (1.1 to 1.15) of table 1.2 are solved numerically using a predictor-corrector method (Milne 1953, Ralston 1960). Briefly stated this involves the repeated solution of each equation at small steps in simulated time until the simulation is completed.

The steps involved for the solution of a differential equation

(1.41) $\dot{y} = f(x,y,...)$

(where $\dot{y}$ is the first derivative of y with respect to time) at the $(n + 1)^{th}$ step are -

(a) the prediction of y_{n+1} using

(1.42) $y_{n+1} = y_{n-1} + 2h\dot{y}_n$

(where h is the step length in time and $\dot{y}_n$ is the derivative of y at the nth step).

(b) the calculation of $\dot{y}_{n+1}$ from the differential equation (1.41) using the value of y_{n+1} from the preceeding step.

(c) the correction for y_{n+1} using the formula

(1.43) $y_{n+1} = y_n + h/2\ (\dot{y}_{n+1} + \dot{y}_n)$.

As the method is not exact the error is periodically estimated for each variable, and depending on its magnitude the step length h is lengthened or shortened if necessary. McKinnon has since improved upon the integration algorithm by the addition of a Runge Kutta starting procedure and the use of the integration routine itself to perform interpolations between previously computed points, when step length reductions are required.

Simultaneously, at each step, the cumulative totals of a number of model variables (the integrated totals since the beginning of the simulation at t = 0) are calculated. Variables summed include urinary and faecal calcium, dietary intake and total absorption for each gut compartment.

A simple trapezoidal summing is used

$$S_{n+1} = S_n + \left(\frac{R_{n+1} + R_n}{2}\right) h$$

where S_n, $S_{(n+1)}$ are the calculated sums at the nth and $(n+1)^{th}$ steps R_n, $R_{(n+1)}$ are the variable rates at the nth and $(n+1)^{th}$ steps. h is the step length as before. Though simple, the method is adequate in practice.

1.7 Model Inputs and Outputs

The use of subroutine OPTION developed by McKinnon allows any parameter to be changed before a simulation. Unless a parameter is altered in this way it is automatically set equal to the value assigned to it in table 2.2. Control parameters such as the length of the simulation and whether or not tracer calcium is to be included are also regulated with OPTION.

The computer program allows the simulated input of either tracer or stable calcium by two different routes. Oral input (R_{diet}, RT_{diet}) introduces calcium into the stomach compartment, and intravenous input (R_{inf}, RT_{inf}) directly into the plasma compartment. The duration and magnitude of such inputs are specified by the user, otherwise they are set equal to zero. As a result of the present work simulated infusions of the hormones calcitonin (CT) and parathyroid hormone (PTH) are also permitted.

The principal model output takes the form of two graphs, plotted by the computer line printer, with four variables on each graph. Model variables to be plotted are chosen by the user and additional printed output can be specified if required. The program to plot the graphs reproduced in this volume was developed as part of the current work and a listing appears in Appendix IV.

1.8 Results of Model Simulations.

The model described above was used by Livesey to simulate a variety of experiments related to calcium metabolism. In the main the parameters were those of table 1.3 although for each simulation some alterations were made (Livesey 1970). Subsequently model predictions were compared with experimental data obtained independently.

Simulation 1

The four compartment model of figure 1.2 was tested by simulating the plasma, urinary and faecal dynamics of tracer calcium following an intravenous dose. The results were compared with those of Neer et al. (1967) - an excellent fit being observed. However as the model parameters are derived from Neer's experimental data, this simulation was essentially a test of the numerical stability of the computation and of the accuracy of the fitted model parameters.

Simulations 1b & 2

The intestinal absorption of a single oral dose of calcium and tracer calcium was simulated to examine the ability of the model to combine the intestinal and plasma dynamics of tracer calcium. Two runs were made with different initial quantities of carrier calcium

in the stomach to predict the effects of different sized loads on the tracer dynamics. The larger dose simulation (1b) was based on an experiment by Jaworski et al. (1963) and the smaller dose simulation (2) on data supplied by Caniggia et al. (1963). The results of these simulations are reproduced in table 1.4. The predicted maximum tracer specific activities are satisfactorily close, Simulation 1b being within the experimental standard deviation although in Simulation 2 the result is somewhat high compared with the experiment. The predicted time at which the maximum concentration of tracer in the plasma occurs closely approximates that found in practice and the deviations found in the total faecal tracer are also acceptably small. On the other hand, as was pointed out by Livesey, the delay of 10 minutes observed by Caniggia between the ingestion of the tracer and its appearance in the plasma was absent in the simulated output. After this the experimental data and the model predictions were in agreement.

Table 1.4 Oral Tracer Calcium Simulations

Reference	No. of Subjects	Stomach Calcium Dose (m.moles/kg)	Faecal Excretion of Tracer (% of dose) and Collection Period	Max. Plasma Specific Activity (% dose/litre) and Time
Jaworski et al. (1963)	11	0.11	62 ± 4 (6 days)	1.73 ± 0.26 (2-3 hours)
Simulation 1b	-	0.12	58 (3 days)	1.6 (2.8 hours)
Caniggia et al.	5	0.035	33 ± 4 (3 days)	2.41 ± 0.15 (1.5 - 2 hours)
Simulation 2	-	0.035	26 (3 days)	3.0 (2.1 hours)

Simulation 3

The simulation of two simplified 3 meal days was carried out in this run. Three discrete doses of calcium (each of 0.114 m.moles/kg - corresponding in size to the typical calcium content of a meal) were ingested at approximately 8, 13 and 18 hours on each simulated day. Simultaneously the dynamics of a single dose of tracer calcium in the plasma (given at t = 0) over the simulated period were followed.

The circadian oscillation in the plasma calcium predicted by the model was in agreement with the experimental data of Jubiz & Tyler (1969) who discovered a minimum in plasma calcium between 4 and 6 a.m. and a maximum at 8 p.m. A similar pattern with respect to urinary calcium (Briscoe & Ragan 1966, Nordin et al. 1967) has been observed and this too was evident in the simulated output.

The upper and lower limits of the endogenous calcium secretion into the gut were calculated from the model simulation, using a method based on tracer experiments reported in the literature (Heaney & Skillman 1964). The rate calculated in this way (between 0.0456 and 0.0809 m.moles/kg/day) using the simulated excretion data was much lower than the actual simulated intestinal secretion of calcium (0.19 m.moles/kg/day), confirming the discrepancy previously noted between estimates based on physiological considerations alone and calculations based on tracer experiments. The basic cause of this disagreement between methods, as pointed out by Livesey, is that the absorption of calcium is a function of the quantity of calcium in the gut. Hence it is not possible to speak of an invariant fraction of the digestive juice calcium that is reabsorbed and recycled back to the blood stream. (Heaney & Skillman 1964).

Simulation 4

Finally to check the parameters and the equations governing renal excretion a four hour calcium retention test (Nordin & Fraser 1954, Bhandarkar & Nordin 1962, Nordin & Smith 1965) was simulated. This test involves the infusion of calcium at the rate of 2.25 m.moles/kg/day for four hours. The 24 hour urinary excretion simulated by the model was 0.154 m.moles/kg/day and by employing the method described by Nordin & Smith (1965) the calculated calcium retention was 56.3% of the infused dose (the experimental range is 44-66%, Bhandarkar & Nordin 1962) It is also worth noting that the four hour rise in plasma calcium concentration (0.72 m.moles/litre) was close to the mean experimental value (0.80

± 0.14 m.moles/litre, Nordin & Fraser 1954).

Several of these simulations (or slightly modified versions) will be repeated in the following chapters with the new model equations and parameters of tables 2.1 and 2.2. In the course of discussion of these simulations, including comparisons between the output from CAMET and CAMET2, a more detailed account will be given.

1.9 Conclusions

CAMET represents a substantial advance on the previous models in that it can simulate to within normal physiological limits both oral and intravenous tracer dynamics. The models of the first two classes listed in table 1.1 accounted for either oral or intravenous tracer dynamics, but not both. CAMET has enabled the simulation of more complex and interesting experiments, not previously attempted, so throwing light on a number of aspects of overall calcium metabolism.

The fact that hormonal control of calcium metabolism (Chapter 3) was not included in the model is worth noting. Of course to some extent the averaged effects of the hormones are built into the parameters used in the model. But in general, inexplicable deviations have not occured in any simulation to suggest unsuspected hormone action. At the same time under conditions of stress hormonal effects are well documented (Chapter 3). By the same token, hormonal control of renal excretion improves the characteristic dynamics

of some simulations and situations where the presence or absence of the hormone is crucial to the organism may also be demonstrated (Chapter 4).

Hence CAMET as it existed at the beginning of 1971 represented a fusion between the two main types of published compartmental models, but remained undeveloped in respect to the active regulation of calcium metabolism via the complex system of hormonal feedbacks. The way in which this problem has been approached, while at the same time preserving and extending the capabilities of the model is outlined in the following chapters.

CHAPTER 2

CAMET2 EQUATIONS AND PARAMETER VALUES

2.1 The equations and parameters resulting from the work described in the following chapters are given in tables 2.1 and 2.2 respectively. In table 2.1 where the equation has remained essentially unaltered from that in table 1.1, it is marked with an asterisk and in table 2.2 where parameter values between CAMET and CAMET2 are comparable the CAMET value (Livesey 1970) is also given.

Throughout this thesis units are usually in terms of kilograms, litres, moles and days, except where hormones are involved and the more conventional nanogram units are used. Extensive quantities are normalized to unit body weight. A body weight of 65 kg is adopted if there is no evidence to the contrary.

Table 2.1 Model Equations

Differential Equations

Plasma and exchanging calcium

(2.1) $$\dot{M}_1 = R_{2,1} + R_{inf} + R_{dec} + R_{abs} - R_{1,2} - R_{acc} - R_{gsec} - R_{urin} - R_{derm}$$

(2.2) $$\dot{MT}_1 = RT_{2,1} + RT_{inf} + RT_{abs} - RT_{1,2} - RT_{acc} - RT_{gsec} - RT_{urin} - RT_{derm}$$

$$\text{where } R_{abs} = \sum_{I=6}^{11} R_{I,1} \qquad RT_{abs} = \sum_{I=6}^{11} RT_{I,1}$$

$$R_{gsec} = \sum_{I=5}^{11} R_{1,I} \qquad RT_{gsec} = \sum_{I=5}^{11} RT_{1,I}$$

(2.3) $\dot{M}_2 = R_{1,2} - R_{2,1}$

*(2.4) $\dot{MT}_2 = RT_{1,2} + RT_{3,2} - RT_{2,1} - RT_{2,3}$

*(2.5) $\dot{MT}_3 = RT_{2,3} + RT_{4,3} - RT_{3,2} - RT_{3,4}$

*(2.6) $\dot{MT}_4 = RT_{3,4} - RT_{4,3}$

Stomach

*(2.7) $\dot{M}_5 = R_{diet} + R_{1,5} - R_{5,6}$

*(2.8) $\dot{MT}_5 = RT_{diet} + RT_{1,5} - RT_{5,6}$

*(2.9) $\dot{V}_5 = RF_{diet} + RF_{1,5} - RF_{5,6}$

Intestine

*(2.10) $\dot{M}_I = R_{(I-1),I} + R_{1,I} - R_{I,(I+1)} - R_{I,1} \qquad 6 \leq I \leq 10$

*(2.11) $\dot{MT}_I = RT_{(I-1),I} + RT_{1,I} - RT_{I,(I+1)} - RT_{I,1} \qquad 6 \leq I \leq 10$

*(2.12) $\dot{M}_{11} = R_{10,11} + R_{1,11} - R_{fecl} - R_{11,1}$

*(2.13) $\dot{MT}_{11} = RT_{10,11} + RT_{1,11} - RT_{fecl} - RT_{11,1}$

Hormones

(2.14) $\dot{M}_c = R_{sc} + R_{cinf} - R_{dc}$

(2.15) $\dot{M}_p = R_{sp} + R_{pinf} - R_{dp}$

Bone

(2.16) $\dot{M}_{bca} = R_{acc} - R_{dec}$

(2.17) $\dot{M}_{bcoll} = R_{coacc} - R_{codec}$

Rate Expressions and Volumes

Plasma and Exchanging Calcium

*(2.18) $R_{1,2} = M_1K_{1,2}$

*(2.19) $R_{2,1} = M_2K_{2,1}$

*(2.20) $RT_{I,J} = MT_IK_{I,J}$ $\quad 1 \leq I \leq 4, \quad 1 \leq J \leq 4, \quad I = J \pm 1$

Stomach

*(2.21) $RF_{5,6} = FV_5^{1/2}$

*(2.22) $R_{5,6} = RF_{5,6}C_5$

*(2.23) $RT_{5,6} = R_{5,6}SA_5$

(2.24) $R_{1,5} = K_cRG_5$

*(2.25) $RT_{1,5} = R_{1,5}SA_1$

Intestine

(2.26) $RF_{1,6} = 3RF_{5,6} + G_1$

(2.27) $R_{1,6} = K_c(G_2RF_{1,6} + G_3)$

(2.28) $R_{1,I} = K_cRG_I$ $\quad 7 \leq I \leq 11$

*(2.29) $RF_{I,(I+1)} = RF_{(I-1),I}(1 - AF_I) + RF_{1,I}$ $\quad 6 \leq I \leq 10$

*(2.30) $RF_{fecl} = RF_{10,11}(1 - AF_{11}) + RF_{1,11}$

*(2.31) $V_I = B_{I,1}RF_{I,(I+1)}/(B_{I,2} + RF_{I,(I+1)})$ $\quad 6 \leq I \leq 10$

*(2.32) $V_{11} = B_{11,1}RF_{fecl}/(B_{11,2} + RF_{fecl})$

*(2.33) $R_{I,(I+1)} = RF_{I,(I+1)}C_I$ $\quad 6 \leq I \leq 10$

*(2.34) $RT_{I,(I+1)} = R_{I,(I+1)}SA_I$

*(2.35) $R_{fecl} = RF_{fecl}C_{11}$

*(2.36) $RT_{fecl} = R_{fecl}SA_{11}$

*(2.37) $R_{I,1} = E_{I,1}C_I/(E_{I,2} + C_I)$ $\quad 6 \leq I \leq 11$

*(2.38) $RT_{I,1} = R_{I,1}SA_I$ $\quad 6 \leq I \leq 11$

RG_I and RF_I are constant rates - see table 2.2 and Chapter 5.

Hormones

(2.39) $R_{sc} = 0.0$ $\qquad C_1 \leqslant A_{22}$

(2.40) $R_{sc} = A_{23}(C_1 - A_{22})$ $\qquad C_1 > A_{22}$

(2.41) $R_{dc} = K_{dc}M_c$

(2.42) $R_{sp} = A_{25}(A_{24} - C_1)$ $\qquad C_1 < A_{24}$

(2.43) $R_{sp} = 0.0$ $\qquad C_1 \geqslant A_{24}$

(2.44) $R_{dp} = K_{dp}M_p$

Bone

(2.45) $R_{coacc} = A_4$

(2.46) $R_{codec} = A_1/K_c$

(2.47) $R_{acc} = K_cK_{oss}U_{mcoll}$

*(2.48) $RT_{acc} = K_{acc}C_1 \quad SA_1$

(2.49) $R_{dec} = K_{rp}R_{codec}$

Urinary Excretion of Calcium

(2.50) $R_{urin} = (A_6C_1 - A_7)/((1 + A_8C_p)(1 - A_9C_c))$

*(2.51) $RT_{urin} = R_{urin}SA_1$

Dermal Excretion of Calcium

(2.52) $R_{derm} = K_dC_1$

(2.53) $RT_{derm} = R_{derm}SA_1$

Concentrations and Specific Activities

$$^{*}(2.54) \quad C_1 = M_1/V_1$$

$$^{*}(2.55) \quad C_I = M_I/V_I \qquad 5 \leq I \leq 11$$

$$(2.56) \quad C_c = M_c/V_c$$

$$(2.57) \quad C_p = M_p/V_p$$

$$(2.58) \quad K_c = C_1/\overline{C}_1 \text{ where } \overline{C}_1 = 2.5 \text{ m.moles/litre}$$

$$^{*}(2.59) \quad SA_I = MT_I/M_I \qquad 1 \leq I \leq 11$$

Quantity of Unmineralized Bone Collagen

$$(2.60) \quad U_{mcoll} = M_{bcoll} - K_{pr}M_{bca}$$

Total Tracer Calcium

$$(2.61) \quad MT_{tot} = \sum_{I=1}^{11} MT_I \quad \text{at } t = 0.$$

Calcium Balance

$$(2.62) \quad C_{bal} = S_{diet} + S_{inf} - S_{fecl} - S_{urin} - S_{derm}$$

$$(2.63) \quad CT_{bal} = ST_{diet} + ST_{inf} + MT_{tot} - ST_{fecl} - ST_{urin} - ST_{derm}$$

Unless a parameter is specifically stated to have a new value in the subsequent simulations then it will have the value assigned to it in table 2.2. The initial values of the gut parameters which are listed (the quantity of calcium in each intestinal compartment M_5 to M_{11} and the stomach volume V_5) are the eventual steady state values obtained by running the program with no oral inputs.

Table 2.2 Model Parameters

Parameter	CAMET2	CAMET	Units
Differential Equation Initial Conditions			
M_1	0.45	0.45	m.moles/kg
M_2	0.52	0.52	"
M_3	1.23	1.23	"
M_4	0.87	0.87	"
M_5	0.28×10^{-3}	0.01	"
M_6	0.11×10^{-2}	0.8×10^{-3}	"
M_7	0.65×10^{-3}	0.1×10^{-2}	"
M_8	0.21×10^{-3}	0.1×10^{-2}	"
M_9	0.10×10^{-3}	0.6×10^{-3}	"
M_{10}	0.64×10^{-4}	0.6×10^{-3}	"
M_{11}	0.40×10^{-4}	0.6×10^{-3}	"
MT_1 to MT_{11}	0.0	0.0	"
M_c	7.1	-	ng/kg
M_p	93.0	-	ng/kg
M_{bca}	0.4242	-	moles/kg
M_{bcoll}	21.23	-	g/kg
V_5	0.187×10^{-3}	0.5×10^{-3}	litres/kg
Rate Constants			
$K_{1,2}$	28.9	28.9	day^{-1}
$K_{2,1}$	25.2	25.2	"
$K_{2,3}$	3.10	3.10	"
$K_{3,2}$	1.30	1.30	"
$K_{3,4}$	0.127	0.127	"
$K_{4,3}$	0.180	0.180	"
K_{acc}	0.078	0.078	"
K_d	0.0153	-	litres/kg/day
K_{dc}	41.7	0.0	day^{-1}
K_{dp}	36.1	0.0	"
K_{oss}	0.60×10^{-2}	-	litres/kg/day

Parameter	CAMET2	CAMET	Units
Intestinal Fluid Secretion Rates			
$RF_{1,5}$	0.041	0.1	litres/kg/day
$RF_{1,7}$	0.011	0.0011	"
$RF_{1,8}$ to $RF_{1,11}$	0.0024	0.0011	"
Intestinal Calcium Secretion Rates			
RG_5	0.062	0.07	m.moles/kg/day
RG_7	0.016	0.02	"
RG_8	0.0036	0.02	"
RG_9 to RG_{11}	0.0036	0.01	"
Duodenal Secretion Parameters			
G_1	-0.078	-	litres/kg/day
G_2	0.83	-	m.moles/litre
G_3	0.013	-	m.moles/kg/day
Fluid Flow Constants			
AF_6 to AF_{11}	0.48	0.50	-
$B_{6,1}$ to $B_{8,1}$	0.004	0.004	litres/kg
$B_{9,1}$ to $B_{11,1}$	0.0025	0.0025	"
$B_{6,2}$ to $B_{8,2}$	0.14	0.14	litres/kg/day
$B_{9,2}$ to $B_{11,2}$	0.12	0.12	"
F	3.0	4.5	$litres^{\frac{1}{2}}/kg^{\frac{1}{2}}/day$
Hormone Effect Constants			
A_1	0.58×10^{-2}	-	g/kg/day
A_4	0.58×10^{-2}	-	"
A_6	0.262	-	litres/kg/day
A_7	0.405	-	m.moles/kg/day
A_8	3.84	-	ml/ng
A_9	0.847	-	"
A_{22}	1.7	0.0	m.moles/litre

Parameter	CAMET2	CAMET	Units
<u>Hormone Effect Constants</u> (Ctd.)			
A_{23}	0.37×10^{3}	0.0	ng litre m.mole^{-1} kg^{-1} day^{-1}
A_{24}	3.0	0.0	m.moles/litre
A_{25}	0.67×10^{4}	0.0	ng litre m.mole^{-1} kg^{-1} day^{-1}
<u>Intestinal Absorption Parameters</u>			
$E_{6,1}$ to $E_{8,1}$	0.19	0.19	m.moles/kg/day
$E_{9,1}$ to $E_{11,1}$	0.10	0.10	"
$E_{6,2}$ to $E_{8,2}$	2.2	2.2	m.moles/litre
$E_{9,2}$ to $E_{11,2}$	5.0	5.0	"
<u>Volumes</u>			
V_1	0.18	0.18	litres/kg
V_c	71.0	0.0	ml/kg
V_p	155.0	0.0	"
<u>Calcium/Collagen Bone Ratios</u>			
K_{pr}	50.0	-	g/mole
K_{rp}	0.02	-	mole/g
<u>Control Parameters</u>			
D_{tmin}	0.1×10^{-4}	-	day
E_{rrhi}	0.1×10^{-5}	-	-
E_{rrlo}	0.1×10^{-6}	-	-
X_{dt}	0.5×10^{-5}	-	-

Model Inputs

R_{diet}, RT_{diet}	calcium and tracer in diet
RF_{diet}	fluid in diet
R_{inf}, RT_{inf}	calcium and tracer infusion
R_{cinf}	calcitonin infusion
R_{pinf}	parathyroid hormone infusion

All the above input rates are automatically set equal to zero unless altered by the programmer for a simulation involving one or more of these inputs.

At this point it may be noted that the features of CAMET2, discussed in subsequent chapters, which distinguish it from CAMET are as follows -

Chapter 4

(1) Introduction of the hormones calcitonin (also known as thyrocalcitonin) and parathyroid hormone.

(2) Improved control of renal excretion - including the effects of calcitonin and parathyroid hormone.

Chapter 5

(3) Many CAMET intestinal parameters are changed to conform with better or more recent information.

(4) Loss of calcium through the skin is introduced.

(5) The inclusion of the additional calcium secretion into the gut in response to a meal.

Chapter 6

(6) Reduction in the number of exchangeable calcium compartments from 4 to 2 (fig. 6.1) while maintaining the number of exchangeable tracer compartments at 4.

(7) Introduction of bone calcium and bone collagen as definite model entities.

(8) The rates of bone calcium deposition and decretion are made dependent on the corresponding collagen rates.

Each simulation is summarized on a separate page for easy reference (see list of simulations). The summary includes the simulation title, simulated inputs, parameter changes (if any) and the principal numerical results. Graphs belonging to the simulation are headed by the simulation title.

CHAPTER 3.

HORMONAL CONTROL OF CALCIUM HOMEOSTASIS.

3.1 Three substances, calcitonin (CT), parathyroid hormone (PTH) and vitamin D, together play a central role in calcium metabolism. In fact, in the absence of either PTH or vitamin D calcium metabolism becomes deranged and death may follow. In this chapter current knowledge about these substances is reviewed, and numerical data of relevance is reduced to tabular form. The equations and parameters used in Chapter 4 are based on this information. Although vitamin D is not yet included in the model (little is known about the dynamics of vitamin D metabolites) more recent experimental work is covered for completeness.

It is by now well established that the injection of PTH causes a delayed and relatively slow rise in plasma calcium, which takes from 18 to 24 hours to reach a peak and is accompanied by a fall in phosphate concentration. CT, on the other hand, causes a rapid and transient fall in plasma calcium over a period of a few hours. Its effects are particularly marked in young animals or in diseases where bone turnover is known to be elevated. The mode of precise control of calcium concentration within the body can be explained in terms of the antagonistic effects of CT and PTH. Both hormones, together with vitamin D work through a complex, integrated system of control

and feedback loops involving a number of target organs. The main control pathways are shown in figure 3.1 and the specific actions of the hormones are discussed in sections 3.6 to 3.8.

3.2 The Thyroid and Parathyroid Glands

Before the beginning of this century the parathyroid glands had been located anatomically and the drastic effect of their removal observed (section 4.5). Some time later the first extraction of PTH was accomplished (Collip 1925). It was only as recently as 1961 however, that the discovery of the hypocalcaemic hormone, calcitonin was announced (Copp et al. 1961). This followed the classical experiments of Sanderson et al. (1960) where it was shown that dogs whose thyroid and parathyroid glands had been removed (an operation termed a thyroparathyroidectomy) were less able to cope with either hypocalcaemic or hypercalcaemic stress than were control dogs. The lack of control in hypocalcaemia was correctly attributed to the absence of PTH, but no convincing explanation could be made of the impaired control in hypercalcaemia compared with dogs possessing intact glands. Since then work on CT has proceeded at a very rapid rate. At the present time as much, if not more, is known about the chemistry and dynamics of CT than about PTH.

It is now generally accepted (Bussolati & Pearse 1967, Copp 1969) that CT is secreted by specialized

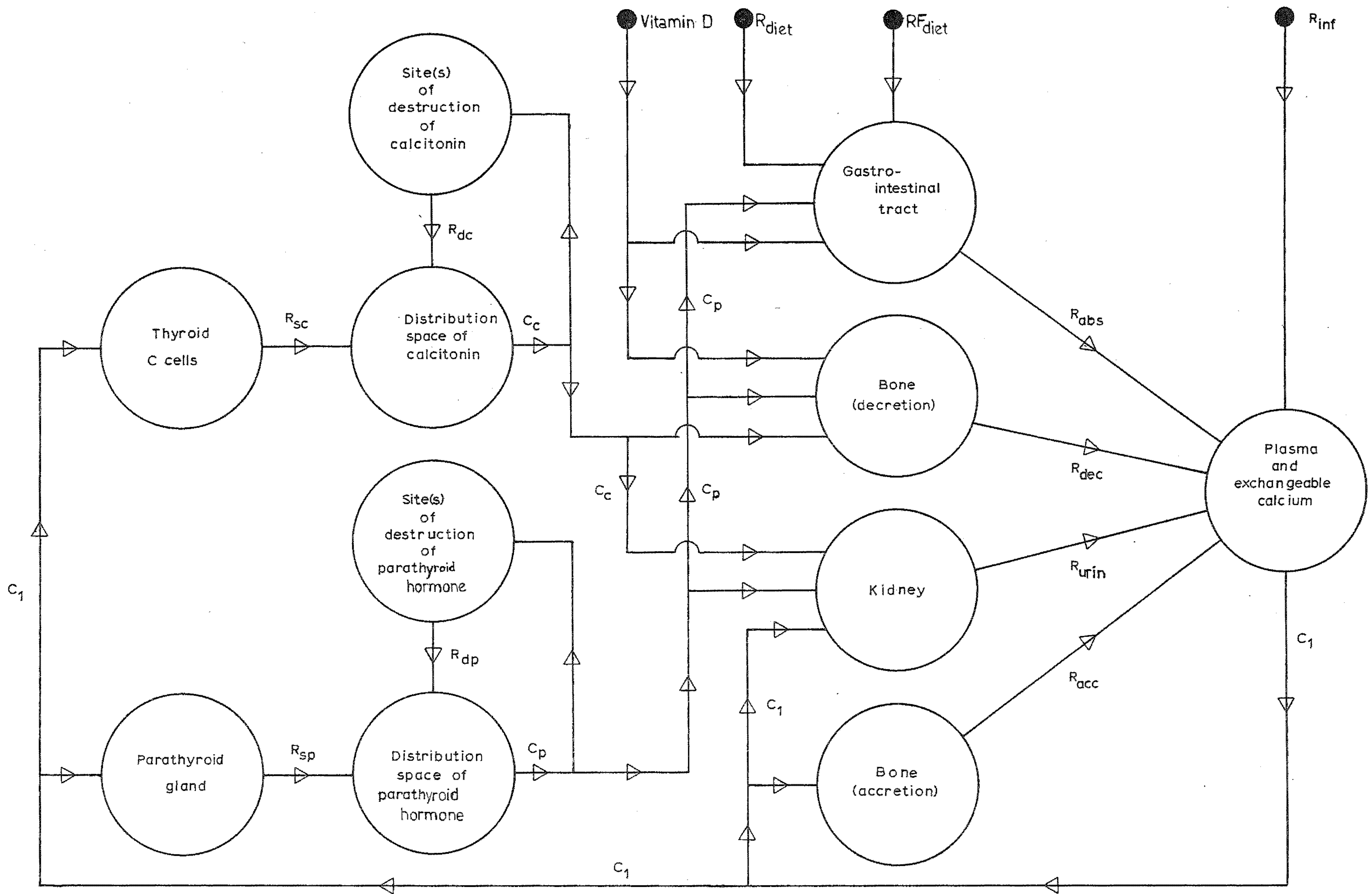

FIGURE 3.1 INFORMATION FLOW DIAGRAM OF THE CALCIUM METABOLISM CONTROL SYSTEM

cells called C cells or parafollicular cells which originate embryologically in the last branchial (ultimobranchial) cleft (Pearse & Carvalhiera 1967). Immunofluorescent techniques have demonstrated a localized concentration of CT within these cells (Bussolati & Pearse 1967). In vertebrates (other than mammals) these cells form a distinct ultimobranchial gland from which CT may be extracted (O'Dor et al 1969a). During mammalian embryonic development the C cells become dispersed and are mainly incorporated into the thyroid. In man these cells are also located in the thymus and the parathyroid glands (Galante et al. 1968). The embedded C cells are distinct morphologically, histochemically and embryonically from the surrounding tissue. The relative contribution of the C cells at different locations to the total amount of hormone secreted is uncertain. However very low or undetectable levels of CT in the peripheral plasma are found in the absence of the thyroid gland. in both man (Melvin et al. 1971) and the pig (Cooper et al. 1971). This suggests that the thyroid is the dominant source of CT in both these species.

PTH is secreted by the parathyroid chief cells situated within the parathyroid glands. In man, two pairs of glands exist (weighing about 0.1 g in all) in close association with the thyroid (Fourman & Royer 1968).

3.3 Hormone Chemistry

In the short time since the discovery of CT its chemical structure, including the amino acid sequence has been determined. Separate groups using a variety of approaches (Bell et al. 1968, Potts et al. 1968b) first resolved the structure of purified CT extracted from the pig thyroid. All of the calcitonins so far chemically analysed - including porcine, bovine, human and salmon CT - have certain structural characteristics in common. They are single chain polypeptides characterized by a 1-7 intrachain disulphide bridge, forming a 23 membered ring at the N terminus.

```
      S-----------------------S
      |                       |
H2N-Cys.Gly.Asn.Leu.Ser.Thr.Cys.Met.Leu.Gly.
     1   2   3   4   5   6   7   8   9   10

Thr.Tyr.Thr.Gln.Asp.Phe.Asn.Lys.Phe.His.Thr.Phe.
11  12  13  14  15  16  17  18  19  20  21  22
                                                O
    Pro.Gln.Thr.Ala.Leu.Gly.Val.Gly.Ala.Pro    NH2
    23  24  25  26  27  28  29  30  31  32
```

Figure 3.2 Amino Acid Sequence of Human Calcitonin

Apart from two amino acids in human calcitonin (fig. 3.2), the residues in this ring are identical in all four calcitonins. Leucine at position 9, glycine at position 28 and a prolinamide carboxyl terminal are also common features. However, with the exception of the bovine and porcine hormones which differ at only three locations, the different types of CT vary considerably in the composition and sequence of amino acid residues within the two

extremes of the molecule. This is reflected in immunological differences (section 3.4). Human, porcine and salmon calcitonin have all been synthesized with full biological potency. Relative to the mammalian calcitonins which have similar biological activities, salmon CT has a very high potency (table 3.1). The precise biochemical role of the molecule in relation to its hypocalcaemic effect remains obscure (section 3.9).

Table 3.1 Physical Properties of Calcitonin

Type	Molecular* Weight	No of amino acids	Biological** Potency	Reference
Bovine	3634	32	200	Brewer & Ronan (1969)
Human	3418	32	309	Dietrich & Rittel (1970) Tashjian & Voelkel (1970)
Porcine	3604	32	270	Bell et al. (1968)
Salmon	3427	32	3500	Gutteman et al. (1969), Niall et al. (1969), O,Dor et al. (1969b)

* calculated from amino acid composition. Similar values have been determined from gel filtration elution times (O'Dor et al. 1969a).

** as determined by biological assay on rats (section 3.4)

Parathyroid hormone is a protein hormone consisting of a straight chain polypeptide, like CT, but is almost three times as large. So far only the absolute amino acid sequence of bovine PTH has been determined (Brewer

and Ronan 1970). It consists of 84 amino acid residues, is strongly positive due to an excess of basic amino acids, particularly lysine and arginine, but contains no cystine. From the initial determination of the empirical formula of human PTH it would appear that it contains two additional amino acid residues and that significant differences occur in the proportions of some residues (O'Riordan et al. 1971). Overall however, the amino acid composition of the two hormones is similar. In fact the physical properties such as the molecular weight (~ 9600 determined by ultracentrifugation) and the charge properties of the three types of PTH studied so far (bovine, human and porcine) are almost identical. They are however immunologically distinguishable (O'Riordan et al. 1969, Woodhead & O'Riordan 1971).

In contrast to earlier views (Potts et al. 1965) it appears that the biologically active portion of the molecule is within the 34 amino acid chain at the amino terminal of the peptide. This fragment has been synthesized (Potts et al. 1971) and tested for biological effects. The synthetic peptide had an equal or greater biological activity than the natural hormone fragment but was only a quarter to one third as effective as the complete native hormone. It was suggested that the remaining carboxyl terminal chain (residues 35 to 84) of the complete molecule protects the hormone from metabolic degradation, although as yet this idea remains unproven.

Essentially pure bovine PTH has a biological potency of greater than 3000 U.S.P. units/mg (Brewer & Ronan 1970). The corresponding value for human PTH, estimated from rather impure extracts is approximately 2500 U.S.P. units/mg (Addison et al. 1971).

3.4 Hormone Assay Procedures and Units of Measurement

CT and PTH have been estimated almost exclusively by bioassay until comparatively recently. However with the availability of the pure hormones, radioimmunoassay techniques of much greater sensitivity have been developed. Immunoassays make use of the ability of animal immunity mechanisms to recognise foreign substances (antigens) and to eliminate them by developing specific proteins (antibodies) which chemically bind the antigen. Antibodies are developed against the hormone to be measured and by determining the degree of binding in an incubation mixture of antibody-hormone the quantity of hormone in an unknown sample can be calculated from a predetermined standard curve. In radioimmunoassays a small quantity of labelled hormone is added to the incubation medium to measure the extent to which the measured hormone binds to the antibody. Radioimmunoassays developed for CT and PTH are being increasingly employed both clinically and experimentally - permitting the study of their roles in disease states, their distribution and rates of disappearance from the blood and the factors controlling their production. In the mean time however, data for man has accumulated

expressed in terms of the biologic or immunologic equivalent of the hormone from cows, pigs and other species. Such results are valuable from a qualitative point of view but cannot be used to derive reliable model parameters, particularly as clear chemical and immunological differences are known to exist between extracts from different animals. The difficulty is compounded by the use of many different assay techniques. Wherever possible in choosing model parameters emphasis has been placed on experimental results expressed in gravimetric terms and involving a minimum number of implicit interspecies comparisons (Chapter 4).

Calcitonin

The earliest of the bioassays for CT (Hirsch et al. 1964) is based on the fall in plasma calcium one hour after the injection of the sample into a rat under carefully standardized conditions. This assay has since been improved (Cooper et al. 1967) and other similar assay procedures described (Kumar et al. 1965, Copp & Kuczerpa 1968, Gudmundsson et al. 1969). Sufficient sensitivity to measure the concentration of CT in normal human plasma has been achieved but only after extraction and concentration of the plasma protein fraction. The usual CT unit of measurement in biologic assays is the MRC (Medical Research Council) unit where 1/100 of an MRC unit is defined as the quantity of a standard porcine thyroid extract required to produce a fall of 0.25 m.moles/litre (1 mg/100 ml) in the plasma calcium

concentration of a 150g rat (Research Standard A for Thyroid Calcitonin, Medical Research Council Communication, February 1966). Currently standard B of a much higher potency is available. Synthetic human calcitonin has a biological activity close to 300 MRC units/mg (Tashjian & Voelkel 1970).

Since 1968 when the first radioimmunoassay for porcine CT was developed (Deftos et al. 1968), at least three radioimmunoassays have been reported for the measurement of CT in human plasma. Two of these (Clark et al. 1969, Deftos & Potts 1970) could not detect the hormone in normal plasma, but could measure elevated levels in patients with cancer of the thyroid. A much more sensitive and specific assay (Tashjian et al. 1970) which is not disturbed by the presence of CT from other species or the presence of other polypeptide hormones (including human PTH) has shown concentrations of CT in the range 0.02 to 0.40 ng/ml (in 58 normal subjects). Very high values, up to 540 ng/ml, were measured in 32 patients with thyroid carcinomas. This assay was based on the production of guinea pig antibodies to human CT and the use of labelled synthetic human CT as the tracer.

Parathyroid Hormone

The biological potency of PTH is generally expressed in USP units. One USP unit is defined as 1/100 of the amount of hormone or extract which will raise the serum

calcium concentration of mature male dogs by 0.25 m.moles/litre (1 mg/100 ml) within 16-18 hours (Collip & Clark 1925). This assay has since been replaced by others; with rats as the assay animal (Munson 1961) and an *in vitro* technique (Zanelli et al. 1969).

The first radioimmunoassay for PTH was developed for bovine PTH (Berson et al. 1963), where the assay tracer was I^{131} labelled bovine PTH. In fact a great deal of the existing knowledge about PTH and its characteristic dynamics has resulted from the use of this assay, or modifications of it (Potts et al. 1968a). The assay has been extended to measure the PTH of other species but the results for human PTH have generally been disappointing. With few exceptions most attempts to apply this assay to man have been unable, or only just able, to measure PTH in normal human serum, although high blood levels of PTH have been detected in hyperparathyroidism (Berson & Yalow 1966, Melick & Martin 1968, Reiss & Canterbury 1969). One of the reasons for this finding is that the extent of the cross reaction between the bovine PTH antibodies and human PTH is relatively small and difficult to determine (O'Riordan et al. 1969). In preliminary studies it has also been found that a PTH precursor, some twenty amino acids larger than the secreted hormone, is synthesized by the parathyroid chief cells (Sherwood et al. 1970, Kemper et al. 1972), and that this 'proparathyroid hormone' is not only chemically but also immunologically distinct from the secreted hormone (Habener et al. 1971). Hence the heterogeneous nature of parathyroid gland extracts (to which

the assay antibodies are developed) complicates the interpretation of assay results - particularly when applied to other species.

To overcome the low sensitivity of the usual bovine assay procedures for human PTH several different approaches to the problem have been published recently. Arnaud et al. (1971) described a radioimmunoassay with a lower limit of detection estimated at 0.1 to 0.2 ng/ml which was capable of measuring the PTH concentration in 94% of normal persons. The radioimmunoassay employed antiserum to porcine PTH and the measured human hormone competed with bovine PTH (which was also the tracer) for the antiserum binding sites. An even more sensitive procedure (Addison et al. 1971) employs labelled bovine PTH antibodies (rather than labelled PTH). After incubation the antibody - PTH complexes are separated and counted. This method overcomes some difficulties in the conventional assay where iodination of the PTH tends to damage the hormone, and where the labelled hormone is somewhat unstable. Quantities as small as 5 pg of bovine PTH and 8 pg of human PTH could be detected and the mean serum concentration in 30 normal persons was 0.56 ng/ml with a range of 0.1 to 1.8 ng/ml.

In table 3.2 published values for the concentrations of CT and PTH in human plasma are collected. Where CT values, measured by bioassay, are given in MRC units the corresponding value in ng/ml is also given in parentheses - assuming 300 MRC units to one milligram of hormone.

The hormone concentrations adopted in the model are 0.1 ng/mℓ for CT and 0.6 ng/mℓ for PTH (at a plasma calcium of 2.5 m.moles/litre).

Table 3.2 Human Plasma Concentrations of Calcitonin and Parathyroid Hormone

Number and status of subjects	Method*	Concentration**	Reference
Calcitonin			
1 normal	B	0.6 m MRC u/mℓ (2ng/mℓ)	Sturtridge & Kumar (1968)
1 normal		0.12 m MRC u/mℓ (0.4ng/mℓ)	
normals	R	< 2ng/mℓ	Clark et al. (1969)
6 with thyroid tumours		15-320 ng/mℓ	
10 normals	B	0.17-0.49 m MRC u/mℓ (0.5 - 1.5 ng/mℓ)	Gudmundsson et al. (1969)
58 normals	R	0.02 - 0.4 ng/mℓ	Tashjian et al.(1970)
18 with thyroid tumours		1-540 ng/mℓ	
5 with thyroid tumours	R	0.3-100ng/mℓ	Deftos et al. (1971)
Parathyroid Hormone			
normals	R	0.25-2ng/mℓ	O'Riordan et al. (1969)
normals	R	10 - 60***	Reiss & Canterbury (1969)
10 with primary hyperparathyroidism		294(190-390)	
normals	R	undetectable < 0.25ng/mℓ	Buckle (1970)

Number and status of subjects	Method*	Concentration**	Reference
20 with chronic renal failure		2.4 (0.1-5.4)ng/ml	
34 with chronic renal failure	R	8.4±1.1 ng/ml	O'Riordan et al. (1970)
30 normals	R	0.56(0.1-1.8)ng/ml	Addison et al. (1971)
1 hyper-parathyroid		15 ng/ml	
1 hyper-parathyroid		9.5 ng/ml	

* B represents bioassay, R radioimmunoassay

** where possible means and ranges are given

*** arbitrary units in terms of bovine PTH equivalents

3.5 Hormone Secretion and Disappearance

Studies with a range of peptide hormones have emphasized the rapid rate of turnover of these hormones in the blood. Calcitonin and parathyroid hormone are no exception to this observation - their rates of secretion are rapidly responsive to physiological demands and once secretion is abolished they disappear with a half-life measured in minutes.

The rates of secretion of both CT and PTH are directly controlled by the ambient calcium concentration.

Other substances may influence the rates of secretion but the calcium concentration is the principal factor in the fast acting hormonal feed back loops by which extracellular calcium is regulated. Earlier physiological experiments which had indicated this control (such as the inverse relationship between the parathyroid gland size and the dietary calcium) have been confirmed, not only by the use of biologic and radioimmunoassays, but also by *in vitro* biochemical studies. The uptake of labelled amino acids from the culture medium of parathyroid glands and the appearance of labelled, biologically active, peptide is inversely proportional to the concentration of calcium in the incubation medium (Hamilton & Cohn 1969, Au et al. 1970). By radioimmunoassay Sherwood et al. (1968) were able to show a highly significant inverse relationship between the plasma concentration of PTH and the calcaemia over a range of 1 m.mole/litre to 3 m.moles/litre in the cow. In general, infusions of the chelating agent ethylene diamine tetraacetic acid (EDTA) leading to a decrease in plasma calcium in turn leads to a marked rise in the level of circulating hormone, detectable within 3-5 minutes from the beginning of the infusion, while sustained hypercalcaemia as a result of calcium chloride infusion resulted in a complete disappearance of measurable PTH. Analogous results have been reported for humans (Buckle 1970, Arnaud et al. 1971), but because of the difficulties of measurement the results are neither as precise nor extensive as for bovine PTH.

However Arnaud et al. (1971) showed a significant negative correlation between serum calcium and circulating PTH in normals; elevated values in hyperparathyroidism despite high blood calcium, and undetectable levels in patients with hypoparathyroidism.

The suggestion that the phosphate ion is able to effect the rate of secretion of PTH (Clark 1968) lacks convincing experimental support. The increased PTH secretion associated with phosphate infusions or diets rich in phosphate is believed to be secondary to the association of ionized calcium with phosphate ions (Sherwood et al. 1968, Reiss et al. 1970). However it is quite clear that plasma magnesium is able to exert an effect on PTH secretion in a similar manner to calcium, although *in vivo* the gland appears to be more sensitive to small changes in calcium than in magnesium (Buckle et al. 1968, Targovnik et al. 1971). Another Group II metal ion, strontium, may also be active in this process - but under normal circumstances it would not be expected to play a significant physiological role (Care & Bates 1970). CT secretion rate is proportional to the concentration of calcium in blood.

The secretion of CT from surgically isolated pig thyroids *in vivo* into the effluent venous blood was directly proportional to the concentration of calcium in the perfused blood (Care et al. 1968, Cooper et al. 1971) over a range of about 1.5 m.moles/litre to 4 m.moles/litre. Maximal CT concentrations of up to

2000 - 3000 ng/ml were measured when the thyroid was perfused with hypercalcaemic blood (Cooper et al. 1971). Both hyper- and hypocalcaemic challenge were rapidly meet (within minutes) by alterations in the secretion of CT into the blood stream. Marked hypermagnesia has also been shown to elevate CT secretion from the perfused pig thyroid (Littledike & Arnaud 1971) and it was noted that equimolar concentrations of calcium and magnesium stimulated the release of comparable quantities of CT.

The ability of calcium to stimulate the secretion of CT in experimental animals also extends to humans (Tashjian et al. 1970) including those with high endogenous CT levels due to medullary thyorid carcinomas (Deftos et al. 1971, Melvin et al. 1971). Although patients suffering from chronic renal failure tend to have high PTH concentrations (O'Riordan et al. 1970), their plasma CT and calcium are within the normal limits (Chittal et al. 1971). In 17 such patients a strong positive correlation was found between plasma calcium and CT concentrations, and the data from two hypocalcaemic subjects suggested that CT secretion could be completely suppressed somewhere below 2 m.moles/litre of plasma calcium.

In addition to the calcium and magnesium cations the C cells are sensitive to molecules that are capable of activating an adenyl cyclase system. In brief, the addition of dibutyryl cyclic-3',5'-adenosine monophosphate raises the output of CT from the perfused pig thyroid; the details are reviewed elsewhere (Care & Bates 1970).

Glucagon, a hormone involved in carbohydrate metabolism, has been demonstrated to increase CT secretion in animals (Care, Bates & Gitelman 1970, Cooper et al. 1971) while in man the response is variable (Melvin et al. 1970, Tashjian et al. 1970, Deftos et al. 1971). The effect of glucagon appears to be exerted through the activation of the adenyl cyclase system. In addition it has recently been discovered that several polypeptide hormones involved in digestion, including pancreozymin and gastrin, together with other peptides possessing the same C terminal tetrapeptide, increased CT release in the pig (Care et al. 1971). The importance to be attached to this discovery is uncertain since the hormone concentrations employed were somewhat above physiological values. However, if confirmed it raises the interesting possibility of the thyroid anticipating a dietary calcium load before it is actually transferred to the blood from the gut.

From a survey of the literature it is evident that under some circumstances the relation between the plasma calcium and the secretion rates of CT and PTH breaks down. Deviations occur when there is a sudden change in the plasma calcium. Under conditions of rapidly decreasing calcium the production of PTH (Sherwood et al. 1968), and under conditions of increasing calcium or magnesium the production of CT (Littledike & Arnaud 1971) is biphasic, consisting of a higher than expected secretion rate at first, followed by a return to the predicted linear relationship.

In patients suffering from chronic hypocalcaemia resulting from malabsorption of calcium or renal disease the PTH gland hypertrophies and exceptionally high concentrations of the circulating hormone have been recorded (Arnaud et al. 1971). A further example of PTH gland overactivity has been found associated with thyroid tumours and high CT concentrations (Melvin et al. 1971), but it is not known whether subtle changes in calcium concentration or a more direct link between the tumour and the PTH gland is involved.

CT secreting cells differ from their PTH counterparts in that they contain large stores of hormone relative to the normal secretion rate. It has been estimated that the pig thyroid contains sufficient CT to maintain the normal secretion rate for about eleven days with no further synthesis (Care & Bates 1970). However chronic hypercalcaemia such as that produced by a consistently high calcium diet (Gittes et al., 1968) or an excess of vitamin D (Frankel & Yasumura 1970, Frankel & Yasumura 1971) is able to significantly reduce the quantity of CT stored within the thyroid.

The biochemical mechanisms by which CT and PTH are released into the blood are little more than conjecture at present. But the basis of present theories is that changes in plasma ionized calcium change the rate of release of stored CT while effecting the actual synthesis rate of PTH (Care & Bates 1970).

Once secreted, CT and PTH are rapidly metabolized, with the result that both hormones have short half lives. Such a rapid removal reflects uptake by target organs and degradation in the blood stream. When the results of tissue uptake of I^{131} resulting from intravenous injection of either [I^{131}]CT or [I^{131}]PTH were compared, the major distinction between the two hormones was the relatively higher liver uptake of CT and the higher uptake of [I^{131}]PTH by the kidney. Accumulation of radioactivity in skeletal muscle and, to a lesser extent, in bone were also recorded (de Luise et al. 1970). As there is no known CT effect on the liver it was suggested that this was its major site of degradation *in vivo*. The rate of plasma inactivation of CT *in vitro* has been measured on a number of occasions and found to be significant (Tashjian & Munson 1965, West et al. 1969). Porcine CT in human plasma disappears in a biphasic manner, rapidly at first and then more slowly. The slower component has a half-life of 3.8 hours (Riggs et al. 1971). On the other hand loss of activity of synthetic human CT incubated in human plasma is small - disappearing with a half life of 1.84 days (Ardaillou et al. 1970). CT from the sheep, cow and pig which are structurally very similar are more rapidly degraded than human CT and much more rapidly than salmon calcitonin which is especially resistant to inactivation (Habener et al. 1972).

After the initial cleavage of proparathyroid hormone to produce PTH within the gland (section 3.4) there appears to be further splitting of the chain into smaller fragments once the hormone is released into the blood (Sherwood et al. 1970, Habener et al. 1971). It is possible that one of these fragments is the biologically active molecule, but then the second cleavage may simply represent the first stage of the catabolism and degradation of PTH.

In vivo, the disappearance from the plasma of CT and PTH have been investigated by a number of workers, but data derived from these studies is somewhat at variance. The single half life found by some workers for CT and PTH implies a first order rate of disappearance, but recently others using more advanced techniques have described a much more complex situation for CT, in which the disappearance of CT cannot be described by a single exponential (Ardaillou et al. 1970, Riggs et al. 1971). Analysis of the experimental data leads to multiexponential equations (with two or three terms) in much the same way that tracer calcium is described (Chapter 1). Comparable studies of PTH *in vivo* have not been made. In table 3.3 the half lives of CT and PTH reported in the literature, together with volumes of distribution are summarized.

Table 3.3 The Half Lives and Volumes of Distribution of Calcitonin and Parathyroid Hormone.

Number and Species	Method* and Type of Calcitonin used	Half Life** (minutes)	Volume of Distribution (% body wt)	Reference
Calcitonin				
Man (1)	endogenous CT (B)	15	-	Cunliffe et al. (1968)
Rabbit (5)	porcine CT (R)	5-15	-	Deftos et al. (1968)
Pig (1)	labelled porcine CT	(1) 5 (2) 37	9	West et al. (1969)
Pig (2)	infused porcine CT	(1) 2-3 (2) 33-37	-	
Man (10)	labelled human CT	(1) 2.8 (2) 11.5 (3) 317.8	7.07±0.04	Ardaillou et al. (1970)
Man (10) uraemic subjects	"	(1) 4.5 (2) 22.5 (3) 1564.3	9.17±0.06	
Man (5)	labelled porcine CT	(1) 2.4±0.3 (2) 40.9±6.2	21.4±2.0	Riggs et al. (1971)
Parathyroid Hormone				
Rat (6)	labelled bovine PTH	22.3	36.4	Melick et al. (1965)
Cow (1)	bovine PTH	18	30	Sherwood et al. (1968)
Cow (3)	endogenous PTH (R)	10 - 15	-	
Man (8 hyper-parathyroids	endogenous PTH (R)	19.8(11.4-28.8)		Buckle (1969)
Man (7)	labelled bovine PTH	27.6	15.5	Melick & Martin (1969)
Man (5 nephrectomized)	"	35.8	13.6	
Man (3 chronic renal failure)	"	40.8	12.6	
Man (2 uraemic subjects)	bovine PTH (R)	(1) 5 (2) 60	10-20 litres	O'Riordan et al. (1970)

*Clearance of hormone measured by bioassay (B) or radioimmunoassay (R). **Where more than one exponential component in the disappearance curve was reported the corresp-ing half lives are given as (1),(2) or (3).

3.6 Physiological Effects of Calcitonin (CT)

By virtue of its ability to secrete CT the thyroid is able to control or blunt hypercalcaemic stress, whether caused by calcium infusion (Ibbertson et al. 1967, Kennedy & Talmage 1971), parathyroid hormone injection (Sorensen 1970) or, in the rat, even a normal dietary calcium load (Gray & Munson 1969).

Bone

As the appearance of hypocalcaemia after the administration of CT was observed in nephrectomized animals (Gudmundsson et al. 1966) and after the removal of the intestinal tract (Aliapoulious et al. 1965) it was suspected that CT must act, at least in part, on the bone. It is now generally agreed that CT acts by inhibiting bone resorption and by inhibiting the action of PTH on bone resorption (section 3.7) although the presence of PTH is not essential (Gudmundsson et al. 1966, Bell & Stern 1970). The following lines of evidence support this contention.

[A] In bone tissue culture systems the morphological and biochemical consequences of the addition of various substates may easily be determined; however interpretation of the results is sometimes difficult because of doubt about the degree of correspondence between the physiological and culture system environments. While there is some disagreement between

investigators with various tissue culture systems (Goldhaber et al. 1968), the main features observed when CT is added to the culture medium are a reduced number of osteoclasts (multinucleated bone resorption cells) and a reduced rate of release of hydroxyproline (an amino acid component of the collagen matrix of bone tissue) and of Ca^{45} that had previously been incorporated into the bone (Raisz et al. 1967, Flanagan & Nichols 1969).

[B] The specific activity of Ca^{45} injected into the plasma remains higher in animals given exogeneous CT, than in controls. However the characteristic absolute rate of disappearance remains essentially unaltered. This is interpreted to mean (in the absence of any CT action on the gut) decreased resorption of stable calcium from the bone (Hirsch 1967, O'Riordan & Aurbach 1968, Caniggia et al. 1969).

[C] The major component of the inorganic matrix of bone is the protein collagen (Chapter 6) and this protein is almost the sole source of the amino acid hydroxyproline. Hydroxyproline is not directly involved in collagen synthesis but arises from the hydroxylation of proline incorporated into the procollagen polypeptide (Grant & Prockop 1972). Hence the plasma level and the rate of urinary excretion of hydroxyproline serves as an indicator of the rate of collagen catabolism. It should be remembered however that collagen is not confined to bone but is a general connective protein distributed throughout the body, and that loss of polypeptide chains in

collagen synthesis may also contribute to hydroxyproline excretion (Flanagan & Nichols 1969).

The excretion of hydroxyproline drops sharply in animals given calcitonin by injection. The rise that is observed when PTH is injected can be negated, at least initially, by the simultaneous injection of CT (Pechet et al. 1967, Rasmussen & Feinblatt 1971).

[D] In thyroparathyroidectomized rats repeated doses of CT result in a decrease in the number of osteoclasts and an increase in the quantity of bone compared with control animals (Foster, Doyle et al. 1966). When similar experiments are carried out in animals with intact parathyroids no significant differences are observed, presumably due to increased parathyroid activity as a result of the continual bouts of CT induced hypocalcaemia (Chiroff & Jowsey 1970).

[E] In man, only in patients with diseases involving increased bone turnover does CT substantially reduce plasma calcium levels (Foster, Joplin et al. 1966, Bijvoet et al. 1968, Martin & Melick 1969). This phenomenon is most strikingly illustrated in Paget's disease which is characterized by intensive bone remodelling activity.

Another possibility that must be considered is an effect of CT on bone formation. Flanagan & Nichols (1969) found that CT had a small inhibitory effect on bone collagen synthesis, but it seems likely that CT does not play a significant role in this respect.

The removal of the ultimobranchial glands from young chickens had no significant effect on serum calcium or phosphorus or on the X-rays and microradiographs of the skeleton, over a period of 14 weeks compared with controls (Brown et al. 1969).

Intestine

Attempts to demonstrate a correlation between CT administration and changes in the movement of calcium from the gut lumen to the blood or in the opposite direction have failed (Krawitt 1967, Cramer et al. 1969). The possibility remains open however that digestive hormones may influence CT secretion (section 3.5).

Kidney

On the basis of the evidence at the time it was written the appropriate equation in CAMET2 requires an increased renal excretion of calcium when serum CT is increased. However there is considerable controversy over the effects of CT on the renal excretion of electrolytes, for a number of reasons. Firstly, there are probably genuine species differences in the reaction to CT - which are exaggerated by the wide range of dose levels, different types of CT used and the differing forms of administration. But also important (in this respect) is the general observation that where urine collections of sufficient frequency have been made (Bijvoet et al. 1968, Haas et al. 1971) the effects of CT on renal excretion of calcium

(and other electrolytes) are transient and fall away rapidly after an injection or the cessation of a CT infusion. In the case of calcium the renal effects of CT become masked by the induced hypocalcaemia and the ensuing stimulation of PTH secretion - combining to actually reduce urinary calcium below that of controls. This is particularly so in young animals where the propensity of CT to lower plasma calcium is marked. Hence measurements made over extended periods may not only fail to record the initial peak in excretion but actually find either a reduced level or a statistically insignificant change.

Aldred et al. (1970) demonstrated increased sodium, calcium and phosphate with lesser, but significant, increases in urinary potassium and creatinine and reduced magnesium excretion in rats when salmon CT was injected subcutaneously. Porcine CT was able to increase sodium and potassium excretion, although larger doses were required. Both forms of CT produced hypocalcaemia and hypophosphatemia. Cramer et al. (1969) also observed increased phosphate and reduced magnesium clearance in the dog, and others have noted the phosphaturia (excess phosphate in the urine) subsequent to CT administration (Hirsch et al. 1964, Kenny & Heiskell 1965, Milhaud & Moukhtar 1966, Robinson et al. 1966, Rasmussen et al. 1967). The fact that increased phosphate clearance occurs in animals lacking parathyroid glands rules out the possibility of PTH producing this effect (section 3.7). However part of it may simply be associated with the fall

in plasma calcium (resulting from CT administration) as Rasmussen et al. (1967) found that the phosphaturia could be reproduced simply by lowering the calcium levels in parathyroidectomized animals with EDTA.

CT has been found to decrease (Pechet et al. 1967, Rasmussen et al. 1967) and to increase (Milhaud & Job 1966, Aldred et al. 1970) urinary calcium in animals. The increased calcium excretion in rats treated repeatedly with CT over a period of three days by Milhaud & Job (1966) was not statistically significant but was achieved in the face of significantly lowered plasma calcium. In young rats, which are particularly susceptible to the calcium lowering properties of CT, Williams et al. (1972) showed that only large doses of salmon CT could counteract the hypocalcaemia to produce an increase in calcium excretion - smaller CT doses were associated with decreased values. Yet in the dog Clark & Kenny (1969) and Pak et al. (1970) could find no direct effect of CT on renal function; the increased phosphate excretion that was observed could be abolished by parathyroidectomy and was attributed to secondary hyperparathyroidism as a consequence of the decrease in serum calcium.

In man there seems to be general consensus that CT reduces tubular reabsorption of calcium and phosphate (Robinson et al. 1966, Ardaillou et al. 1967, Bijvoet et al. 1968, Martin & Melick 1969, Singer et al. 1969, Haas et al. 1971); a result that is not

dependent on the parathyroid gland as it has been observed in hypoparathyroid patients (Ardaillou et al. 1967, Haas et al. 1971). The small complicating hypocalcaemic effect of CT in adult man, compared to the smaller mammals, is probably the main reason for this consensus. It should be taken into consideration however that the dose levels involved in most of these studies were very high in comparison to physiological levels - up to several orders of magnitude greater.

The overall place of CT in calcium metabolism and the possible significance of its effect on the kidney, two factors that are not well understood are considered in section 4.4 and section 4.7.

3.7 Physiological Effects of Parathyroid Hormone (PTH)

It is known that the administration of PTH to a parathyroidectomized animal produces a characteristic sequence of events: an immediate increase (within minutes) in the excretion of phosphate by the kidney, a decrease in the calcium excretion and a simultaneous fall in the concentration of phosphorus in the plasma. There follows a gradual rise in plasma calcium which may not appear for some hours and which is accompanied by a progressive increase in the excretion of calcium in the urine. Once established, hypercalcaemia and hypercalciuria persist and their magnitudes are related to the dose of the administered hormone.

Bone

It has become clear that the action of PTH on the bone is basically a cellular process, taking the form

of increased osteoclastic bone resorption. PTH has no effect on dead bone (as estimated by the release of Ca^{45} in tissue culture systems) nor does it operate in the absence of oxygen or glucose (Tenenhouse et al. 1966). Osteolytic bone resorption has been consistently demonstrated in tissue culture after the addition of PTH to the medium (Raisz 1965, Tenenhouse et al. 1966, Goldhaber et al. 1968). Tenenhouse et al. (1966) showed that hormone concentrations of 10^{-8} moles/litre (compared with 10^{-10} moles/litre *in vivo*) gave rise to a discernable increase in calcium mobilization. From both morphological and biochemical considerations PTH leads to the removal of both the collagen matrix as well as the bone mineral, although the exact sequence of events is uncertain (Mecca et al. 1963, Stern et al. 1970). Collagen degradation is reflected in the increased presence of collagenase enzymes and in the renal excretion of hydroxyproline (Harris & Sjoerdsma 1966, Latiner 1966, Pechet et al. 1967).

Actinomycin D, a potent inhibitor of RNA synthesis does not prevent the PTH stimulated release of calcium during the first 6 hours but suppresses it subsequently (Rasmussen et al. 1964, Raisz & Niemann 1967). It was suggested that the initial effect of PTH is to stimulate resorption by the existing osteoclasts and the later actinomycin D-sensitive phase of calcium resorption was due to the development and differentiation of new osteoclasts from their cellular precursors.

However this view has been questioned by Bingham et al. (1969). It was concluded on the basis of autoradiographic studies, which enabled a detailed study of the metabolism of different rabbit bone cell populations, that no significant increase in osteoclast numbers occurred until 17 hours after the PTH injection - reaching a maximum between 22 and 26 hours. In contrast plasma calcium was rising after 3 hours. From the first few hours the biochemical activity of the existing cells was increased significantly. The first measurement, 1½ hours after injection of PTH, revealed an increase in nuclear RNA synthesis. Cytoplasmic RNA reached its maximum some 7-12 hours after the beginning of the experiment and the increase in protein synthesis was roughly correlated with its appearance. Both were still elevated at 24 hours. In the main, RNA synthesis in osteoblasts (collagen synthesizing cells) was depressed compared with controls. This depression of RNA synthesis was of the order of 50% and occurred not only in the osteoblasts but also in their cellular precursors the preosteoblasts. Thus the hormone had an effect not only on the two types of mature cells but also on their cellular precursors - an effect that persisted long after the initial injection. On the basis of this experiment it was proposed that the rise in plasma calcium was mainly due to the increased activity of the existing osteoclasts on bone dissolution. The rise in their number was a secondary effect and not responsible for the initial rise in plasma calcium.

The relationship of PTH to bone formation is not well understood. Apart from the observations of Bingham et al. (1969) there have been a few reports of reduced hydroxyproline incorporation into collagen in the short term after the administration of PTH *in vitro* (Goldhaber et al. 1968, Flanagan & Nichols 1969). Prolonged exposure to high levels of PTH as in hyperparathyroidism results in increased bone formation as well as increased bone resorption (Frost 1969).

Vitamin D is now known to be important in PTH mediated bone resorption and this is considered in section 3.8.

Intestine

Of all the sites at which PTH is known to be involved least is known about its effect on the movement of calcium into the body from the gut. There is no doubt that vitamin D is the principal factor in the active process that occurs in the transfer of calcium across the intestinal membranes (section 3.8) but PTH, directly or indirectly, has a significant supplementary effect.

Clinically this relationship has been suspected for some time, mainly because of the lower than expected faecal calcium in patients with hyperparathyroidism

(Albright et al. 1932, Lafferty & Pearson 1963). Where balance and isotopic absorption studies have been carried out on patients suffering from hypoparathyroidism or hyperparathyroidism (conditions resulting from a deficiency or an excess of PTH respectively) calcium absorption has deviated significantly from the normal range. Although the numbers involved in such tests were small, hypoparathyroids tend to have low calcium absorption while hyperparathyroids have high absorption despite the elevated serosal calcium concentration (Jaworski et al. 1963, Heaney & Skillman 1964, Spenser et al. 1968, Birge et al. 1969). In addition Jaworski et al. (1963) observed a gradual decline in the absorption by one hyperparathyroid patient after removal of a parathyroid tumour. Six normal persons administered a twice daily dose of PTH increased their absorption of Ca^{47} (measured by an external radioisotope counting technique) in a carrier dose of 4.5 m.moles of stable calcium by approximately 21 ± 7% (Wills et al. 1970).

In vitro Rasmussen (1959) demonstrated that the property of an isolated rat intestinal sac to develop and to maintain a calcium concentration gradient across its walls was reduced after the rat had been parathyroidectomized. PTH action on the gut has also been further corroborated by Cramer (1963). The significantly reduced absorption of calcium from dog jejunal fistulas as a result of thyroparathyroidectomy ($p < .001$) could be restored by the administration of PTH extract to the animal.

Kidney

Under normal circumstances calcium is excreted by the kidney after over 97% of the filtered diffusible calcium is reabsorbed from the kidney nephrons (Nordin et al. 1967). PTH can alter the extent to which the calcium is reabsorbed resulting in large changes in the actual amount excreted in the urine. In 1929 Albright & Ellesworth reported that the administration of parathyroid extract to a hypoparathyroid patient at first resulted in a decreased calcium excretion subsequently followed by a rise. The significance of the initial effect was not appreciated until the 1950's when Talmage and his colleagues demonstrated in rats and mice that parathyroidectomy elicited a marked rise in urinary calcium, despite falling plasma calcium, and a corresponding fall in phosphate excretion. The converse was true when parathyroid extract was administered (Talmage & Kraintz 1954, Talmage et al. 1955). A unilateral decrease in calcium clearance (that is in urinary calcium excretion) and an increase in phosphate clearance results from the addition of PTH to the blood supply of one of the two kidneys, compared with the clearance of the control kidney (Lavender et al. 1961, Eisenberg 1968).

Earlier indirect data suggested that PTH might have the same effect on human calcium and phosphate excretion. In circumstances where stimulation of PTH secretion was thought likely, urinary excretion of calcium was low and when PTH secretion was depressed calcium excretion rose. However as the plasma calcium concentration also changes,

an action of PTH on the kidney could not be unequivocally demonstrated one way or the other. Kleeman et al. (1961) first demonstrated that PTH has the same effect in the dog and man as in rats and mice. Although his experiments were carried out with plasma calcium in a state of flux as a result of calcium infusions or removal of parathyroid glands it was concluded that at any given filtered load of calcium the greater the level of PTH the lower was the renal excretion of calcium. Soon afterwards the same result was observed in dogs where the calcium loads on the kidney were carefully controlled throughout the experiment (Widrow & Levinsky 1962).

In general the administration of PTH leads to a rapid increase in the renal clearance of sodium, potassium, phosphate and bicarbonate and a decreased clearance of hydrogen, ammonium, calcium and magnesium ions (Hellman et al. 1965, Haas et al. 1971). From the work of Haas et al. (1971) it is clear that alterations in kidney ion transport are closely linked to the current PTH status of the subject. At the cessation of PTH infusions the disturbed clearance of all measured ions changed quickly towards control values.

There is little evidence to suggest that PTH acts by altering the glomerular filtration rate (Haas et al. 1971) and, unlike in the bone, inhibitors of RNA and protein synthesis do not alter the action of this hormone on renal excretion. The primary effect of the hormone on the kidney is uncertain and it is not known whether it acts specifically on one ion such as the calcium or phosphate ion or whether it influences the handling of all ions - for example by altering membrance permeability.

The interrelationships between the calcium load, parathyroid hormone, calcitonin and renal excretion of calcium are discussed more fully in the next chapter.

3.8 Physiological Effects of Vitamin D

In the last few years there has been a rapid accummulation of knowledge relating to vitamin D chemistry and action. *In vivo* vitamin D has been shown to be converted to a number of metabolites, only some of which are believed to be biologically active (Norman et al. 1964, DeLuca 1967). 25-hydroxycholecalciferol (25-HCC) and 25-hydroxyergocalciferol have been isolated and are considered to be important biologically active derivatives of vitamins D_3 and D_2 respectively (Blunt & DeLuca 1970). More recently attention has been focussed on an even more potent biological derivative of vitamin D: 1,25-dihydroxycholecalciferol (1,25-DHCC) (Holick et al. 1971). At least part of the 10 to 12 hour delay that occurs before the administration of vitamin D_3 has any effect on intestinal transport (DeLuca 1967) is due to the conversion of the vitamin into its active metabolites probably in the liver and in the kidney.

As the action of vitamin D on both the bone and the intestine can be blocked by the prior addition of actinomycin D and other inhibitors of RNA and protein synthesis (Norman 1965, Zull et al. 1966), the vitamin is thought to ultimately involve protein synthesis. However the action of 1,25-DHCC on the intestine, unlike that of 25-HCC, is not blocked, by actinomycin D (Tanaka et al. 1971).

Bone

Vitamin D has a primary effect on bone tissue that is distinct from its action at the gut wall. In some way the rate of bone resorption is dependent on vitamin D; it is also possible that the vitamin is required in bone deposition.

The nature of vitamin D action on bone has been studied *in vitro*. It has not been convincingly demonstrated that unaltered vitamin D_2 or D_3 are able to alter significantly the rate of bone resorption in tissue culture. In contrast, 25-HCC induces a rapid and marked increase in the quantity of calcium released into the culture medium (Raisz 1965, Raisz & Trummel 1970). Increases in hydroxyproline excretion have been recorded (Pechet et al. 1967) indicating an increased rate of bone resorption following vitamin D therapy *in vivo*. However Mautalen (1970) found no significant change in this parameter.

In vitamin D deficiency hypocalcaemia may occur and bone resorption is impaired despite consequential hyperparathyroidism. It is now thought that vitamin D is important to PTH mediated resorption, but there is disagreement over the extent to which vitamin D is required. Some investigators (Harrison & Harrison 1964, Rasmussen & Fleinblatt 1971) have found that PTH cannot mobilize significant quantities of bone in the absence of vitamin D, while others claim that vitamin D, while having a synergistic effect on the action of PTH, is not essential to PTH stimulated bone resorption (Raisz & Trummel 1970).

Hypercalcaemia, hypercalciuria and changes in bone morphology resulting in increased porosity occur when

pharmacological doses of vitamin D are administered (Danowski 1962, Chen & Bosmann 1965). The increase in serum calcium can be demonstrated in rats existing on a diet lacking, or extremely low in calcium (Carlsson & Lindquist 1955); a fact which precludes the alternative explanation that stimulated intestinal absorption alone accounts for the change. The rationale behind the administration of huge doses of vitamin D in hypoparathyroidism, where serum calcium has dropped to a low level (section 4.5), is based on these effects.

The question of whether or not vitamin D has a direct effect on either bone collagen synthesis or on the subsequent mineral deposition is still largely unanswered. Canas et al. (1969) showed an increase in the rate of uptake of labelled proline into bone matrix 12 hours after vitamin D deficient rats received a dose of the vitamin, even though serum calcium had not been restored to normal. An increase in the rate of bone formation within 4 hours of the commencement of a vitamin D perfusion of a dog forelimb *in vivo* was reported by Nguyen & Jowsey (1970a). However both these reported observations may be secondary to some other vitamin action.

It is generally thought that bone mineral crystallization is only indirectly dependent on vitamin D. In rickets the serum calcium is usually in or close to the normal range although phosphate concentration is low (Fourman & Royer 1968). The calcification that occurs in rickets after vitamin D therapy is usually ascribed to the ability of the vitamin to change the concentrations of the plasma ions back

to normal levels (DeLuca 1967). However the position is probably more complex than this physiochemical explanation. Osteoid tissue of rachitic bone will not calcify *in vitro* and bone tissue in rickets is biochemically abnormal (Fourman & Royer 1968).

Kidney

There is no evidence to suggest that vitamin D in physiological quantities has any effect on the renal excretion of electrolytes. Pharmacological quantities may exert some influence - particularly in decreasing tubular reabsorption of phosphate (Ney et al. 1968). This finding does not of course rule out the possibility of more profound effects by the newly discovered vitamin D metabolites.

Intestine

It has long been known that there exists a mechanism on the intestine for the adaption of total calcium absorption to the requirements of the body and that this process involves vitamin D (Nicolaysen & Eeg-Laysen 1953, Malm 1958). The ability of vitamin D to facilitate calcium transport across the gut wall has often been demonstrated by a variety of methods. For instance Avioli et al. (1965) were able to show significantly increased Ca^{47} absorption and decreased Ca^{47} faecal excretion in patients after vitamin D therapy. Compared with the unaltered vitamin, 25-HCC acts on the gut much faster and in much smaller quantities (Olson & DeLuca 1969) while 1,25-DHCC is approximately 1000 times as potent as 25-HCC (Boyle et al. 1972).

Transport of at least part of the absorbed calcium across the intestinal membranes is now generally agreed to be an active or energy consuming process, the evidence for which is summarized elsewhere (Schachter 1970, Wasserman & Kallfelz 1970). Recently, in this connection, Wasserman and his colleagues have isolated a specific protein from intestinal tissues - the calcium binding protein (CaBP). The protein has been obtained in high purity and some of its properties have been determined. Its molecular weight is in the range 25000 to 28000 and it binds one calcium ion per protein molecule. The CaBP has been implicated in the absorption of calcium as it is absent from the tissues of rachitic animals and appears only after administration of vitamin D (Wasserman & Taylor 1966). Furthermore the rate of absorption of calcium across the intestinal membranes is highly correlated (correlation coefficient of 0.99) with the CaBP content of the duodenal mucosa (Wasserman & Taylor 1968). Thus it was shown that animals adapted to a low calcium diet by absorbing calcium more efficiently than animals on a normal diet, and that the former group had more CaBP than the normally fed animals. Although absolute evidence for the role of this protein has not yet been demonstrated, up to the present all the data suggests that it is involved in calcium absorption.

3.9 Biochemical Basis of Hormonal Action

It is beyond the scope of this review to consider in any detail the present position with regard to the biochemical mechanisms of CT, PTH and vitamin D. However it should be mentioned that subsequent to the

introduction of PTH into an organism two changes are rapidly induced.

[A] A greatly increased adenyl cyclase activity (Chase et al. 1969).

[B] A reduction in extracellular calcium concentration (Parsons et al. 1971).

The reduction in plasma calcium in the dog, following the intravenous injection of 20 USP units of PTH, was maximal at 15 minutes and of the order of 0.1 m.moles/litre (0.4 mg/100 ml) (Parsons et al. 1971). Since this was not a dilution effect caused by fluid transfer and since it is known that certain calcium-sensitive enzymes are inhibited during this phase of PTH action (Rasmussen & Tenenhouse 1968), an intracellular increase of calcium is thought to be a primary action of the hormone. Analysis of the changes in the absolute quantity of introduced tracer calcium and its specific activity provides further support for this point of view (Parsons et al. 1971). Because of the well known sensitivity of a large number of cellular enzymes to ionic calcium it is reasonable to suggest that the alterations in bone cell metabolism for example (section 3.7) follow from the increased cytoplasmic calcium.

Both the PTH mediated increase in adenyl cyclase activity and the reduction in extracellular calcium are unaffected by simultaneous treatment with CT (Robinson et al. 1972). It has been suggested that CT by shifting calcium from the cell has a similar but opposite

effect to PTH, although direct evidence on this point is lacking. Again while little is known about the primary effects of vitamin D it has been demonstrated in isolated mitochrondria systems that vitamin D is able to alter membrane permeability to calcium (Wasserman & Kallfelz 1970).

In fact all three hormones may have dual functions - the ability to alter membrane permeability in the short term, and to stimulate or depress the production of macromolecules within the cells which ultimately initiate the longer term effects discussed in section 3.6 to 3.8.

CHAPTER 4

CALCITONIN, PARATHYROID HORMONE AND RENAL EXCRETION OF CALCIUM

4.1 The equations and parameters controlling calcitonin and parathyroid hormone secretion and their subsequent disappearance together with the interaction of these hormones with the kidney are introduced in this chapter. It is evident from the preceding chapter that definitive data on many aspects of endocrinological control in man is at best sketchy. Hence the approach adopted has been to take model parameters from the experiments judged to most closely approximate normal human physiology (if in fact there is a choice) and then to test the model against independent experimental data derived from widely differing and strongly perturbed states of calcium metabolism (Simulations 1 to 5). Although the method is empirical some interesting points are illustrated, some unexpected results obtained, and some clinical experiments are suggested.

4.2 Calcitonin and Parathyroid Hormone

Linear equations to represent the relationship between the hormone secretion rates and blood calcium concentration are suggested by the experimental evidence of section 3.5. Equations of this type have been proposed in the past (Copp 1969, Livesey

1970) and are employed in CAMET2 (equations 2.40 and 2.42). Not taken into account are the small delays in the secretion of the hormones in response to physiological demands nor are changes in gland size and output as a result of chronic changes in blood calcium levels (section 3.5). However since the delay is of the order of minutes and since the model simulations are carried out over a period of a few days or less neither omission is likely to have a material effect on model predictions.

It is evident in domestic animals and, from more limited data, in man (Arnaud et al. 1971, Chittal et al. 1971) that both CT and PTH secretion are suppressed over certain ranges of blood calcium. In CAMET2 the simulated CT secretion ceases at and below a calcium concentration of 1.7 m.moles/litre (A_{22}) - obtained by extrapolation of the results of Tashjian et al. (1970) where the increases in CT concentration, consequent upon calcium infusions, were measured. The limited results of Chittal et al. (1971) are not inconsistent with this value. As no corresponding data is currently available for PTH in man, the suppression of PTH secretion found in cows (Ramberg et al. 1967, Sherwood et al. 1968) at a calcium concentration of 3.0 m.moles/litre (A_{24}) and above is adopted for man also.

An arbitrary but representative concentration of 0.1 ng/ml (at a plasma calcium concentration of 2.5 m.moles/litre) is taken for the purposes of the model since only the range (0.02 - 0.4 ng/ml) in human CT

concentration has been reported (Tashjian et al. 1970, Melvin et al. 1971). The corresponding CAMET2 value for the PTH concentration is 0.6 ng/ml selected to be close to the mean experimental value of 0.56 ng/ml in 30 normal subjects reported by Addison et al.(1971).

Both hormones are represented in the model by single pools where the rate of disappearance is proportional to the quantity of hormone in the pool (equations 2.41 and 2.44). In such a case of first order kinetics the decay rate of the hormones is described by a single exponential term.

As was noted in Chapter 3 however, recent experiments with CT have shown that its disappearance can only be described by a more complex function, such as a series of exponentials (Ardaillou et al. 1970, Riggs et al. 1971). No doubt these results reflect the rates of uptake by different target organs and perhaps degradation in the plasma. However at present little would be gained, in return for the additional computation required, to reproduce these observations (by employing a multicompartmental model for example) when virtually nothing is known about the utilization and catabolism of CT. A further source of error inherent in this representation is that the hormonal mixing time is not taken into account - but as this is of the order of 10 minutes (Melick et al. 1965) the error is probably negligible.

Given the concentration of CT and its volume of distribution (Ardaillou et al. 1970) the quantity of CT per unit body weight M_c may be calculated. Hence by substituting this value into equation (2.41)

$$R_{dc} = K_{dc} M_c$$

the first order rate constant K_{dc} may be obtained where R_{dc}, the rate of removal of CT, was given by Ardaillou et al. 1970 as the metabolic clearance rate (that is the distribution volume of blood that is irreversibly cleared of CT per unit time). Finally the parameter A_{23} in equation (2.40) can be derived from the steady state equation

$$A_{23}(C_1 - A_{22}) = R_{dc} \quad C_1 = 2.5 \text{ m.moles/litre}$$

where the rate of secretion of CT is set equal to its rate of catabolism.

Similarly for the PTH parameters, except that K_{dp} was calculated directly from the half life estimate of 27.6 minutes of Melick & Martin (1969).

The various model hormonal parameters and the references from which they were derived are collected in table 4.1 and in addition the hormone secretion rates are plotted against the plasma calcium concentration in figure 4.1 according to the equations of CAMET2.

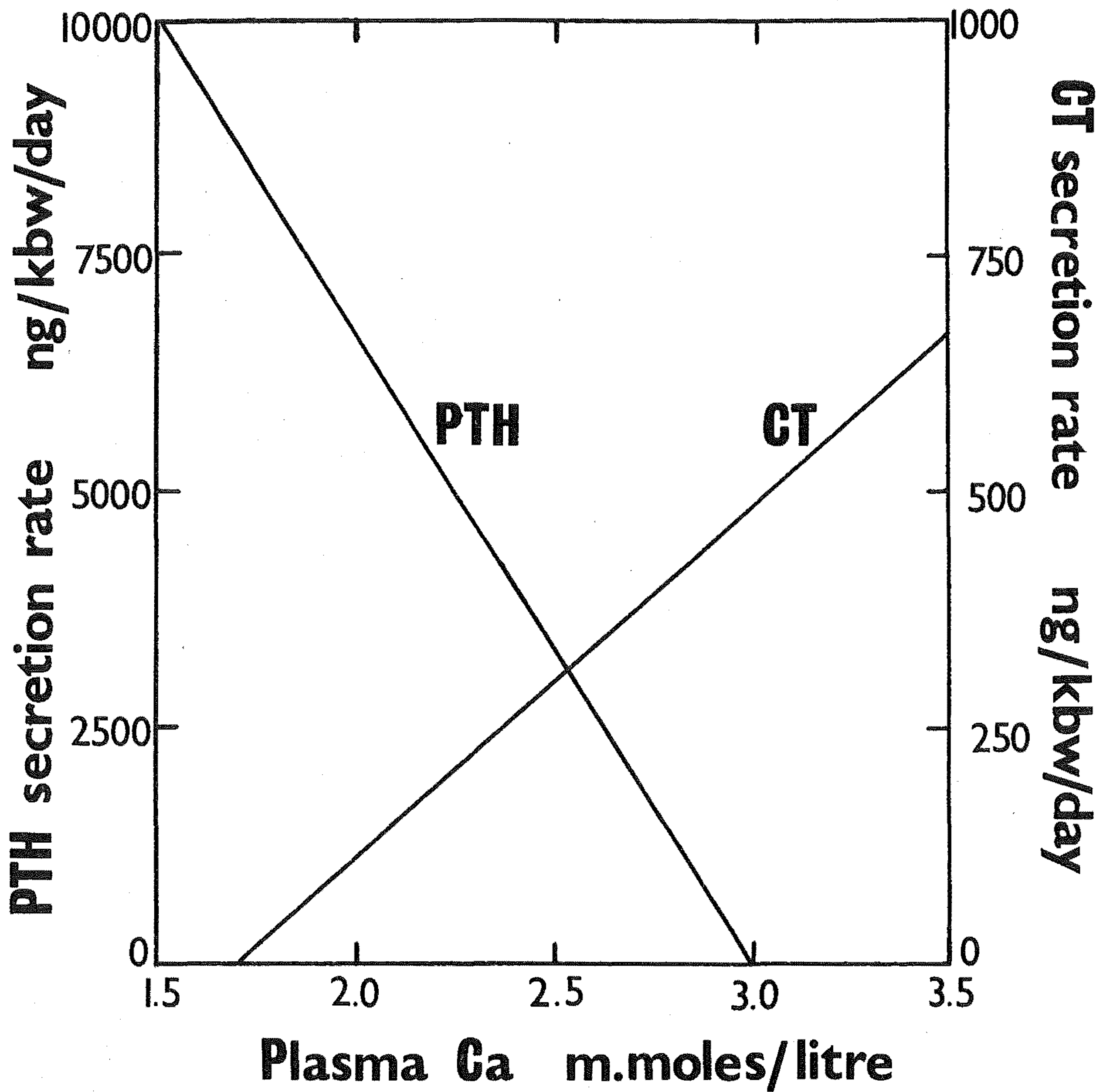

Figure 4.1 Hormonal secretion rates

Table 4.1 CAMET2 Hormonal Parameters

Parameter	CT	PTH	References*
Concentration (ng/mℓ) (C_1 = 2.5m.moles/litre)	0.1	0.6	Tashjian et al. (1970) Addison et al. (1971)
half life (minutes)	23.9**	27.6	Ardaillou et al. (1970) Melick & Martin (1969)
calcium concentration range over which hormone secretion is suppressed (m.moles/litre)	$\leqslant$ 1.7**	$\geqslant$3.0	Tashjian et al. (1970) Sherwood et al. (1968)
volume of distribution (mℓ/kg)	71	155	Ardaillou et al. (1970) Melick & Martin (1969)
secretion rates*** C_1 = 2.5 m.moles/litre (ng/kg/day)	296	3350	-

* first reference of each pair is associated with the CT value and the second with the PTH value

**calculated from the experimental results of the listed reference

***calculated from model equations

The CAMET2 values for the normal rates of secretion of CT and PTH are of the same order of magnitude as those reported for the pig normalized to unit body weight (Care & Bates 1970). The high rate of secretion of PTH relative to CT has also been noted - Care & Bates estimated that PTH secretion in the pig on a molar basis was 10 times that of CT.

4.3 Renal Calcium Excretion

According to Copp et al. (1960a) about 10 grams of ultrafiltrable calcium are filtered every day by the renal glomeruli. Since in most people, the total daily kidney excretion of calcium is of the order of 100-300 mg, over 97% of filtered calcium is reabsorbed by the renal tubules. Of the reabsorbed calcium in rats and hamsters approximately 67% is reabsorbed in the proximal convolution, 20 - 25% in the loop of Henle and 10% in the distal convolution (Lassiter et al. 1963). Since a small change in the renal reabsorption efficiency is sufficient to bring about large changes in the amount of calcium finally excreted, control at this point provides a sensitive and, as will be seen, a vital means by which overall calcium homeostasis is maintained.

The list of substances that are able, directly or indirectly, to effect the rate of renal calcium excretion is both long and varied. It includes other electrolytes such as sodium (Wills et al. 1969, Davis & Murdaugh 1970) and phosphate (Hulley et al. 1969); a number of hormones the most important of which is PTH, together with other less well established factors such as age and sex.

The magnitude of urinary calcium excretion is also dependent on the filtered load of calcium (the product of the ultrafiltrable calcium concentration and the glomerular filtration rate). Hence a change in either of these factors may bring about a change in urinary calcium excretion. However comprehensive reviews of most aspects of the renal handling of calcium have appeared elsewhere (Nordin et al. 1967, Epstein 1968).

For the present only three factors that influence renal calcium excretion are incorporated into CAMET2 - the calcium load (or more strictly the calcium concentration) and the hormones calcitonin and parathyroid hormone. Their general relationship to the kidney is shown in figure 4.2. Although direct data relating the concentrations of these three substances to urinary calcium excretion is sparse (and in the case of the hormones almost non-existent) much is known or can be safely inferred from the following experimental results or the following states of health and sickness.

[A] Hypoparathyroidism: The PTH concentration is zero or close to zero (Arnaud et al. 1971) and the CT concentration associated with the low plasma calcium levels, usual in this disorder, may also be assumed to be zero (section 3.5). Nevertheless, it should be noted that Tashjian et al. (1970) could not correlate serum CT levels with either *chronic* hypocalcaemia or *chronic* hypercalcaemia and in fact CT concentrations were usually in the normal range. Renal calcium excretion is small

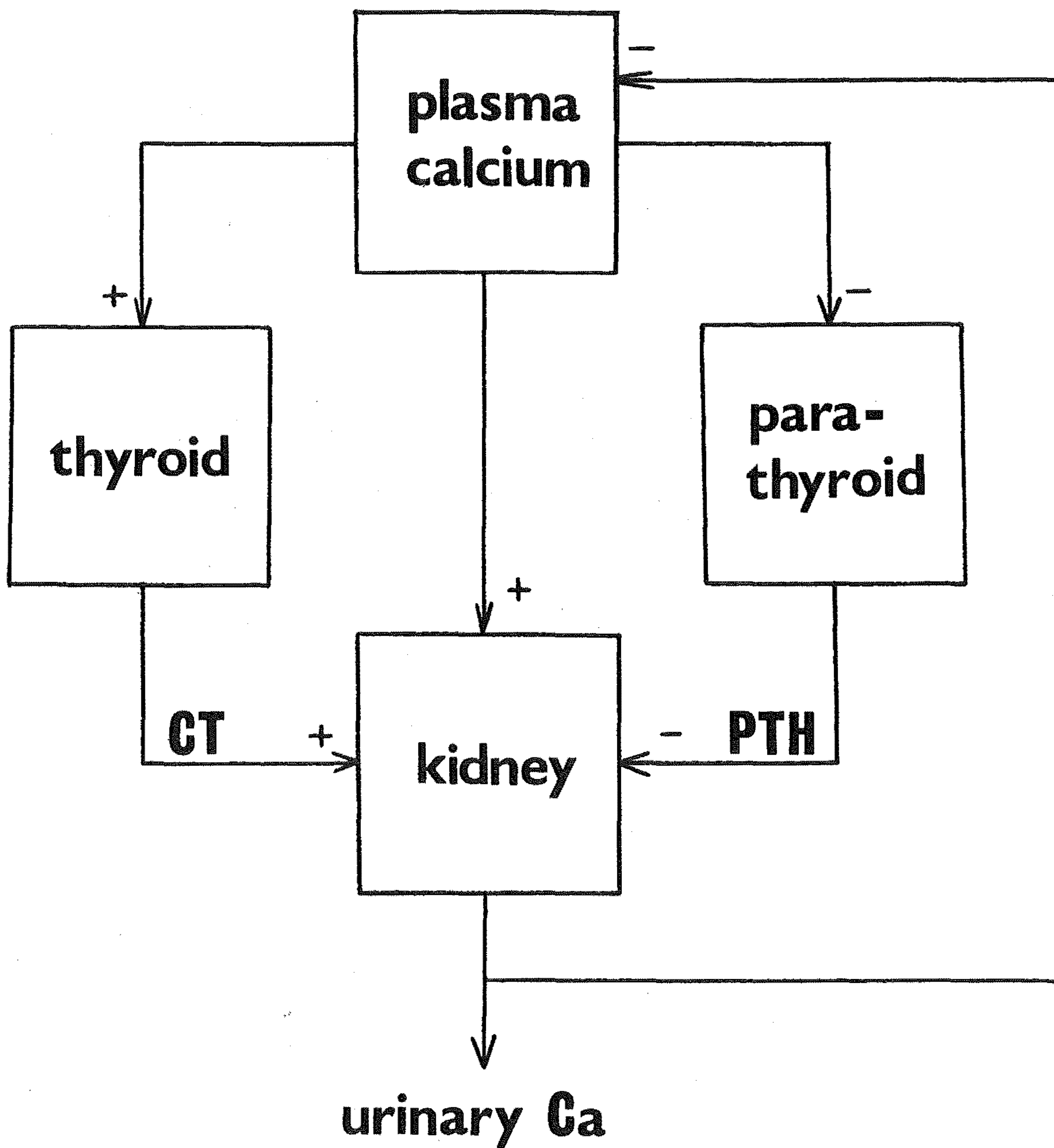

Figure 4.2 The control of renal calcium excretion

in untreated hypoparathyroids. In a group of 6 such patients a mean serum calcium concentration of 1.60 ± 0.27 m.moles/litre was accompanied by a mean urinary calcium excretion (normalized to unit body weight) of 0.014 ± 0.006 m.moles/kg/day (Lamberg et al. 1960).

[B] Calcitonin Infusion : There is some data available on the increase in calcium excretion in man during CT infusions. For the purposes of the model the experimental data of Ardaillou et al. (1967) was selected, despite the small number of subjects (4), for two reasons. First, the dose (2 MRC units infused over one hour) was small in comparison with most other experiments; secondly, relatively small urinary collection periods (¼ hour intervals) were employed. By the end of the infusion the rate of urinary calcium excretion had approximately doubled and was associated with a small drop in plasma calcium (~ 0.125 m.moles/litre). The CT and PTH concentrations associated with this experiment were predicted using the model equations. By infusing CT into young cows it has been found that there is no direct stimulus to PTH secretion by the infused CT and that any increase in PTH concentration may be predicted with confidence from the magnitude of CT-induced hypocalcaemia (Reitz et al. 1971).

However it should be emphasized at this point that the assumptions involved in applying Ardaillou's data to the model allow only a crude model approximation

of CT effects on the kidney which, as it has turned out, may be underestimated (section 4.4). For example since an infusion of 2 MRC units per hour represents about nine times the normal physiological secretion rate, saturation of the renal CT mechanism may well have occurred. Certainly much larger doses of up to 4 MRC units per kilogram body weight have not produced correspondingly larger effects (Martin & Melick 1969, Singer et al. 1969, Brown et al. 1970).

[C] Normal Daily Calcium Excretion : The ranges and means of the normal daily calcium excretion in the urine for statistically large samples of the population are known (Nordin et al. 1967). In one study reported by Nordin et al. the mean daily renal calcium excretion in 88 females was 4.6 ± 2.1 m.moles and in 104 males 6.2 ± 3.1 m.moles. The average urinary excretion of these two groups (5.4 m.moles/day) is adopted in CAMET2.

[D] Calcium Infusion : PTH secretion is suppressed (Arnaud et al. 1971) and the approximate changes in CT concentration are known (Tashjian et al. 1970) when the calcium concentration is elevated. During calcium infusions in 5 normal people, renal calcium excretion, measured over 20 - 25 minute intervals by Levitt et al. (1958), reached 0.67 ± 0.14 m.moles/kg/day associated with a calcium concentration of 3.6 ± 0.4 m.moles/litre.

The mean experimental data from each of these situations (listed below in table 4.2) was used to solve equation (2.50) for the empirical constants A_6 to A_9

inclusive. In practice there is a close temporal relationship between hormonal concentrations and renal electrolyte excretion (sections 3.6 and 3.7) so the instantaneous nature of equation (2.50) seems unlikely to cause any major errors in the simulations discussed in the present work. From a physiological point of view however the equations is unsatisfactory since it predicts negative urinary excretion rates at low calcium concentrations (< 1.55 m.moles/litre) or at high CT concentrations (> 1.18 ng/ml). On the other hand, in no CAMET2 simulation so far carried out are these concentrations reached, or even approached, and in fact they rarely occur in nature.

Table 4.2 Data Fitted to Renal Calcium Excretion Equation

	Plasma Calcium Conc. (m.moles/ litre)	CT* Conc. (ng/ml)	PTH* Conc. (ng/ml)	Urinary Ca*** Excretion (m.moles/kg/ day)	References
[A]	1.60**	0.0	0.0	0.014	Lamberg et al.(1960)
[B]	2.37	0.78	0.75	0.17	Ardaillou et al.(1967)
[C]	2.50	0.10	0.60	0.083	Nordin et al. (1967)
[D]	3.60	0.24	0.0	0.67	Levitt et al. (1958)

* hormone concentrations not given experimentally but calculated from model equations

** mean serum calcium value of 6 untreated hypoparathyroid patients (page 332 Lamberg et al. 1960). Subjects with serum calcium concentrations over 2 m.moles/litre excluded.

*** experimental results normalized to unit body weight assuming a mean mass of 65 kg.

In a situation where the determination of these hormonal and renal parameters would have been very difficult even 2 years ago it is evident that the next few years will yield better data, particularly pertaining to man, from which more reliable model constants could be derived. For the moment however, with the few exceptions noted in the following text, the present values appear to approximate satisfactorily the position as it is currently understood.

4.4 Calcium Infusions

One of the aims of the calcium metabolism project is to simulate as many routine diagnostic tests as possible - at first for normals but extending to abnormal responses as the model is developed. By this means a comparison may be made between experimental data obtained under standard conditions and the predictions of the model. Eventually such a process need not be limited to a one way comparison - the clinical test itself could be examined in the light of the predictions of a well developed model. In the present work three non-tracer tests described by Nordin & Smith (1965) are simulated. The first two (the calcium and EDTA infusion tests) are included in this chapter and the calcium deprivation test may be found in Chapter 7. As well as these tests the calcium balance is routinely calculated by CAMET2 (equation 2.62) and is given for all simulations.

The basic clinical procedure of the calcium infusion test is to measure urinary and serum calcium over the duration of a 4 hour infusion of 15mg of calcium per kilogram body weight (Nordin & Fraser 1954).

Osteomalacia, a disorder in adults characterized by large amounts of undermineralized bone matrix, can be detected by this test as additional calcium is retained in the body (presumably in the skeleton) which results in lower serum calcium and urinary excretion (Nordin & Fraser 1956). The test procedure is reproduced in Simulation 1 except that for simplicity the dietary intake of calcium is omitted. However as the experimental subjects of Nordin & Fraser (1954) were on a low calcium diet the difference is unlikely to substantially influence the outcome. The principal numerical results and the plotted output of particular interest are given on the following pages.

In table 4.3 mean increases in plasma calcium concentration (from pre-infusion control values) with standard deviations measured in 14 experimental subjects (Nordin & Fraser 1954) are compared with the CAMET2 values.

Table 4.3 Calcium Infusion Test : Plasma Calcium

Time (hours)	Changes in Plasma Calcium (m.moles/litre)	
	Nordin & Fraser (1954)	CAMET2
4	0.80 ± 0.14	0.89
8	0.55 ± 0.06	0.52
12	-	0.35
24	0.25 ± 0.15	0.08

Apart from the higher experimental value at 24 hours (which may have been due to the dietary intake) the simulated increments in plasma calcium are within one standard deviation of the experimental means. The

SIMULATION 1 CALCIUM INFUSION I

Source Program : 72150 No. : 72162-2

A simulated infusion (R_{inf}) of 15mg of calcium per kilogram body weight over 4 hours from t = 0 was carried out to test model predictions against the results of the clinical test. The associated perturbation of hormonal concentrations are also demonstrated.

References: Nordin & Fraser (1954), Nordin & Smith (1965) Arnaud et al. (1971)

Simulated Time: 1 day

Model Inputs:

Calcium Infusion* (m.moles/kg/day)	Time (days)
2.25	0.0
0.00	0.167

Results: [A] Parameters during simulation

C_1 (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	
2.50	0.0827	0.10	0.60	0.0
3.39***	0.569***	0.20	0.01	0.1675**
2.58	0.105	0.11	0.48	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{fecl}	S_{inf}	S_{urin}	
0.0609	0.130	0.102	0.0024	0.376	0.269	1.00

* R_{inf} equals 2.25 m.moles/kg/day from t = 0 until t = 0.167 days when it is set automatically to zero.

** Since CAMET2 only saves values for plotting and printing at discrete intervals the values saved at the time closest to t = 0.167 are given.

*** Maximum value.

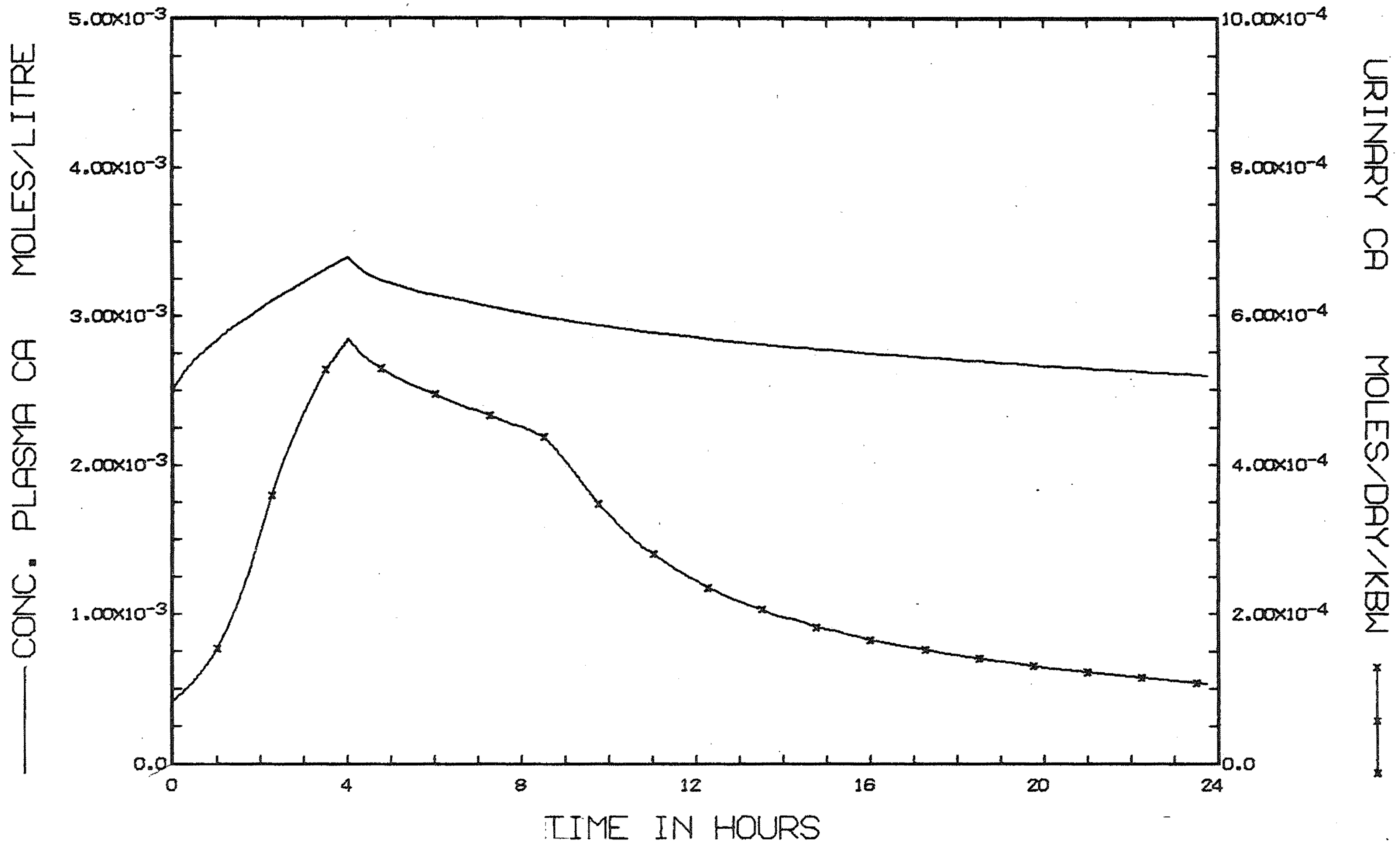

Figure 4.3 The effect of Ca infusion on plasma Ca & urinary Ca excretion

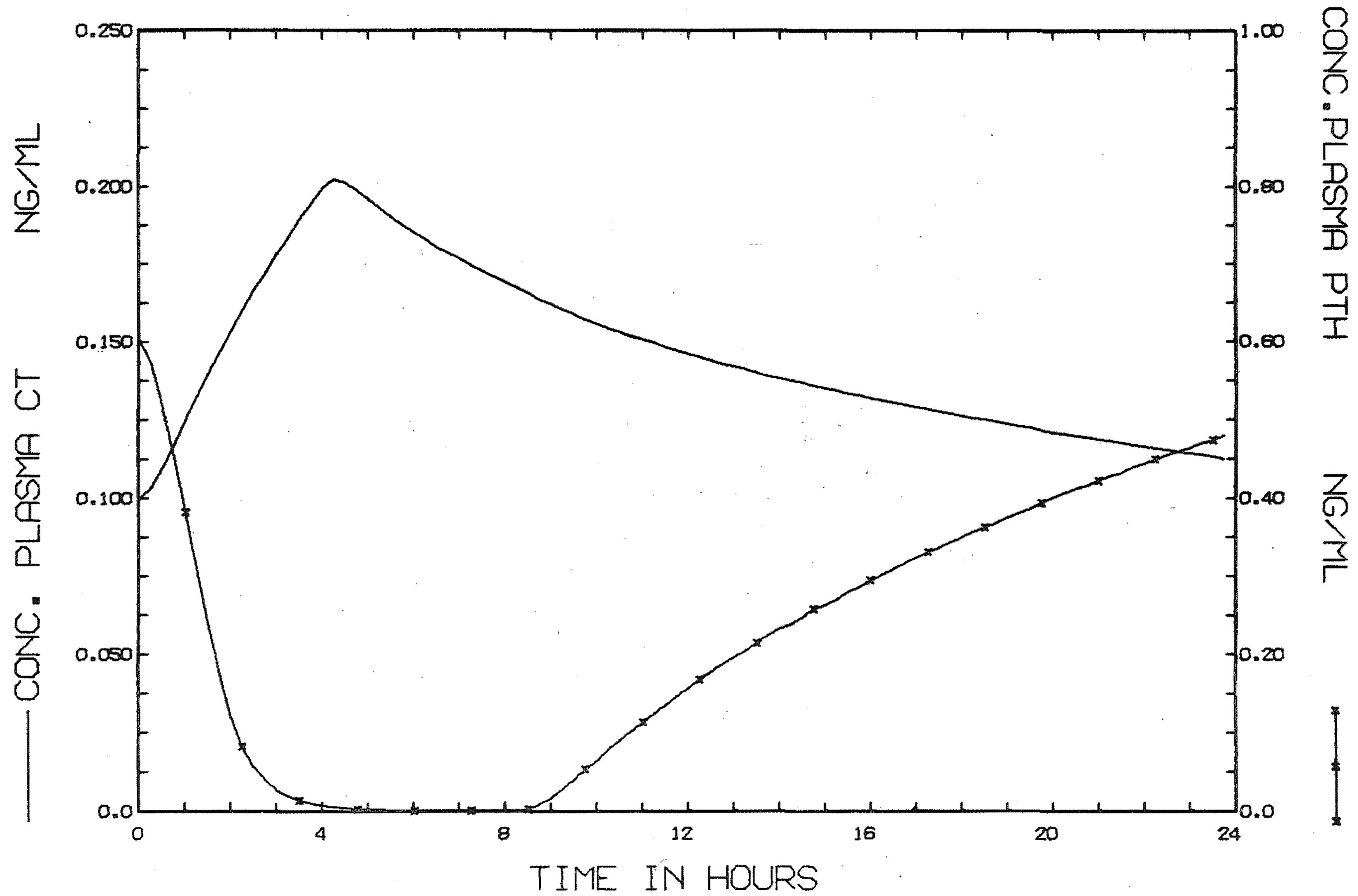

Figure 4.4 The effect of Ca infusion on plasma CT and PTH concentrations

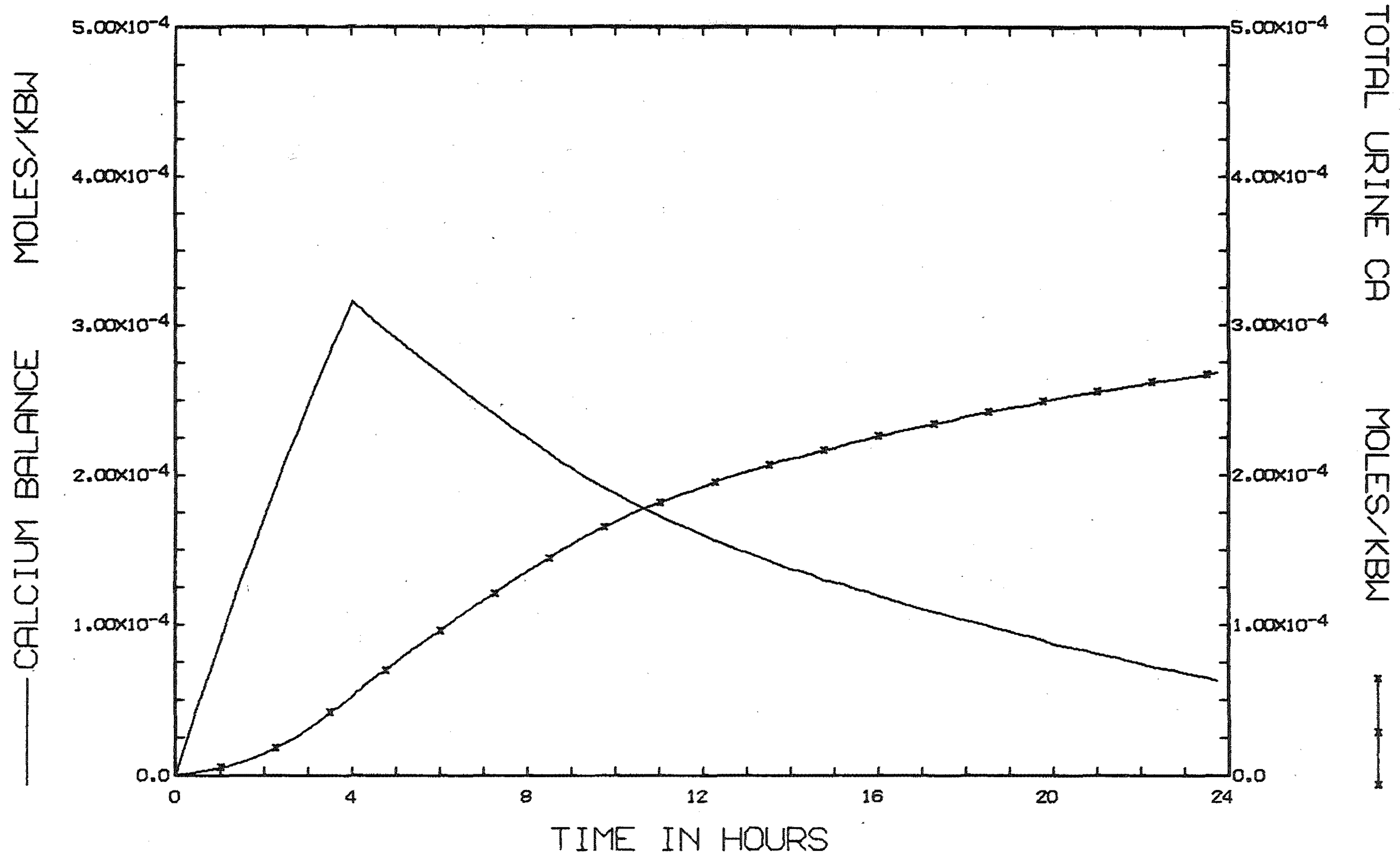

Figure 4.5 The calcium balance and the cumulative urinary calcium excretion

increased CT concentration (figure 4.4) is in line with that recorded experimentally after a calcium infusion of the same magnitude (Tashjian et al. 1970). Yet, given that the simulated rise in plasma calcium was similar to that found by Tashjian, this was to be expected since the CT secretion parameters are partly derived from Tashjian's report (section 4.2). Calcium infusions in the cow (Potts et al. 1968a) and in man (Arnaud et al. 1971) have resulted in PTH response curves approximating that plotted in figure 4.4. However in the cow the decay in PTH concentration is faster than predicted by the model - Arnaud's results for man do not permit a close comparison owing to the insensitivity of his assay at low hormone concentrations.

One measure of the sharply increased urinary excretion of calcium associated with the infusion (figure 4.3) is the percentage of the infused dose excreted in any time interval (after correction for the basal level by subtracting the urinary calcium collected over the same period of a control day). For the purposes of the model the 'control day' is the fasting simulation of Chapter 7 (Simulation 9). The values calculated in this way, including the six consecutive 4 hour intervals following the commencement of the infusion, are given together with the experimental data from 21 normal subjects (Nordin & Fraser 1956) in table 4.4.

Table 4.4 Calcium Infusion Test : Urinary Calcium

Collection Period* (hours)	Urinary Calcium Excretion (% of infused dose) Nordin & Fraser (1956)**	CAMET2
0-4	15	10.4
5-8	17	18.9
9-12	10	12.3
13-16	5	6.5
17-20	4	4.0
21-24	-	2.9
0-12	41 ± 7	41.6
0-24	-	55.0

* calcium infusion over 0 - 4 hours.

** first 5 values taken from graphed results to the nearest whole number; no statistical information supplied.

As a final check on the simulated results the calcium retention - calculated by the method of Nordin & Smith (1965) which takes into account the rise in plasma calcium and the urinary excretion over the four hour infusion period - was 53%. Bhandarkar & Nordin (1962) reported a range of 44-66% with a mean of 53.3% in 19 normal subjects.

Hence, with perhaps the exception of the urinary excretion of calcium in the first 4 hours (table 4.4), the model and the biological results agree well in all respects. The lower initial urinary calcium could be the result of many factors (including a faster than predicted suppression of PTH secretion).

The most significant physiological control in hypercalcaemia not taken into account is the action of CT in depressing bone resorption (section 3.6). However the form of the equations controlling calcium deposition and removal

from the bone (equations 2.45 to 2.49) allow for changes in these rates that are respectively directly proportional and inversely proportional to the calcium concentration. Since both the plasma calcium and urinary calcium perturbations were successfully simulated it appears that these equations mimic to a sufficient extent the events at the bone with respect to the transfer of calcium. A net uptake of 0.028 m.moles/kg by the bone was predicted by the model over the 24 hour simulation. Certainly it seems unlikely that CT could have a drastically greater inhibitory effect than this figure indicates (such as a complete blockage of calcium resorption for any length of time) without becoming evident in the experimental results. In view of the small net skeletal uptake the use of the term 'skeletal retention' as a synonym for 'calcium retention' (Finlay et al. 1956, Nordin & Smith 1965) in the calcium retention test described earlier is misleading as it implies a much larger uptake by the skeleton than must actually occur in only 4 hours. The distribution of calcium to the soft tissues is probably greater than allowed for in the calculation of the calcium retention.

Of some interest is the shoulder in the curve describing the simulated renal calcium excretion at t = 8 hours (figure 4.3). This is caused by the reintroduction of PTH into the simulated system as the plasma calcium falls below 3.0 m.moles/litre; more experimental values than presently available would be required to confirm the presence of such a response *in vivo*.

The results of this simulation support the widely accepted finding that greatly increased urinary calcium excretion is the principal response to hypercalcaemic stress (McCance & Widdowson 1939, Baylor et al. 1950). Over the simulated day the renal loss of calcium was 0.27 m.moles/kg, the faecal loss was two orders of magnitude lower at 0.24×10^{-2} m.moles/kg and dermal losses were 0.044 m.moles/kg. Clearly intestinal excretion under fasting conditions, even allowing for model approximations, is insignificant compared with the total excretion by all other routes.

Since the function of CT appears to be confined to damping hypercalcaemic stress it seemed pertinent to repeat the calcium infusion test of Simulation 1, but without CT (Simulation 2). This is readily achieved mathematically by setting the quantity of CT (M_C) and its rate of secretion (R_{sc}) to zero. For the purposes of comparison the computed plasma calcium concentrations and the urinary excretion rates from Simulations 1 and 2 are plotted together in figures 4.6 and 4.7.
The main features to be observed are:

[A] A small divergence in the plasma calcium values - reaching a maximum of 0.06 m.moles/litre at 9 hours and slowly declining thereafter to 0.04 m.moles/litre at the end of the simulated day.

[B] A more striking difference in the rate of urinary calcium excretion which disappears after 9 hours at which point in time the total simulated renal calcium excretion was 0.155 m.moles/kg in Simulation 1 and 0.135 m.moles/kg in Simulation 2.

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SIMULATION 2 CALCIUM INFUSION II

Source Program : 72150 No : 72162-3

The calcium infusion of Simulation 1 was repeated but with the quantity of CT and its secretion rate set equal to zero, thereby altering the simulated renal excretion of calcium. The results of the two simulations are compared with each other and with clinical trials in hypothyroid patients.

References: Ibbertson et al. (1967), O'Brien & McIntosh (1967)

Simulated Time: 1 day

Parameter Changes: A_{23} set to 0.0*

M_c set to 0.0

Model Inputs:

Calcium Infusion (m.moles/kg/day)	Time (days)
2.25	0.0
0.00	0.167

Results: [A] Parameters during Simulation

C_1 (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	
2.50	0.0757	0.0	0.60	0.0
3.41**	0.472**	0.0	0.01	0.1675
2.62	0.105	0.0	0.44	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{fecl}	S_{inf}	S_{urin}	
0.0785	0.131	0.100	0.0025	0.376	0.251	1.00

* Setting A_{23} to zero has the effect of making the secretion rate of CT (R_{sc}) equal to zero (equation 2.40).

** Maximum value.

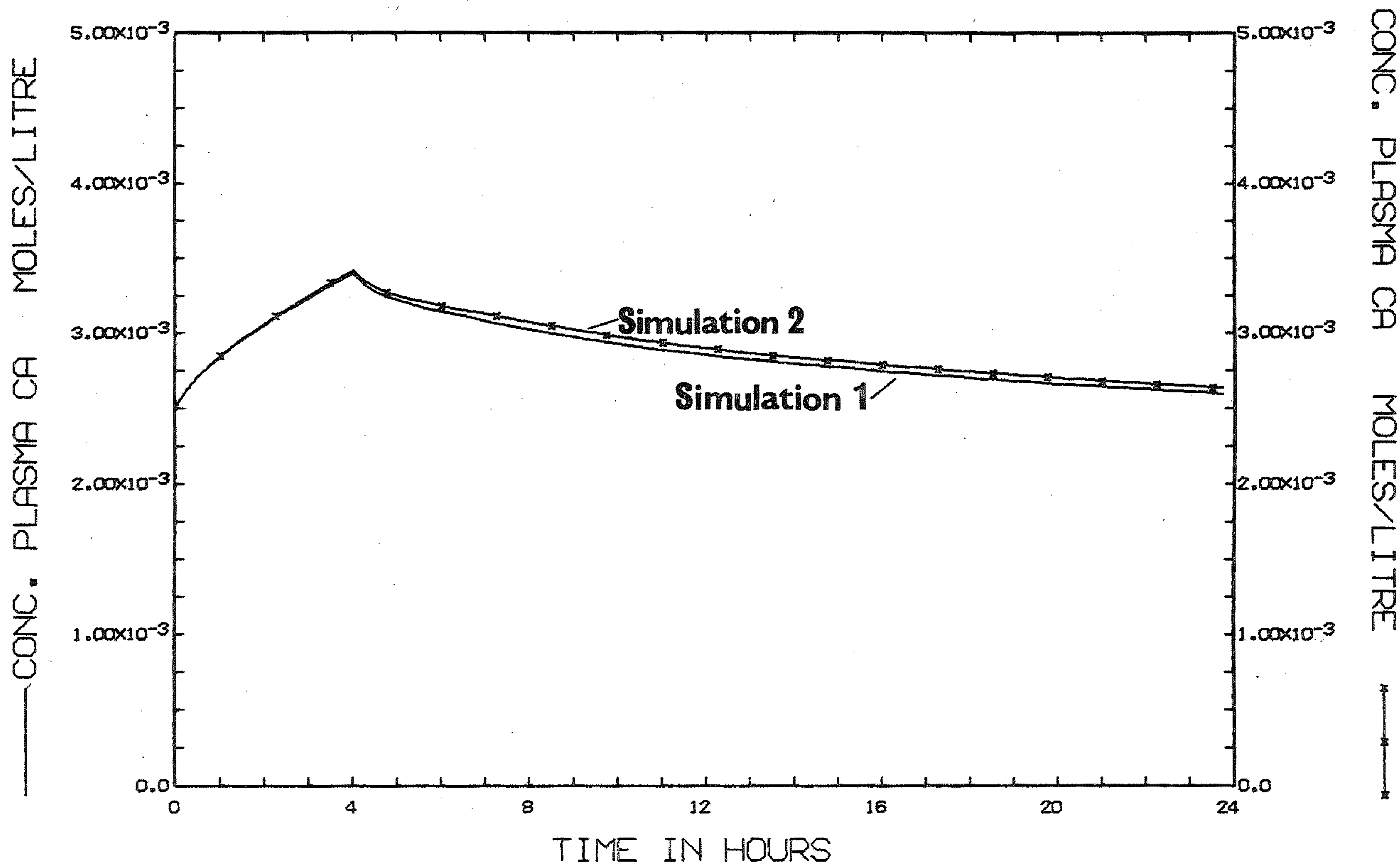


Figure 4.6 The effect of Ca infusion on plasma Ca - with & without CT

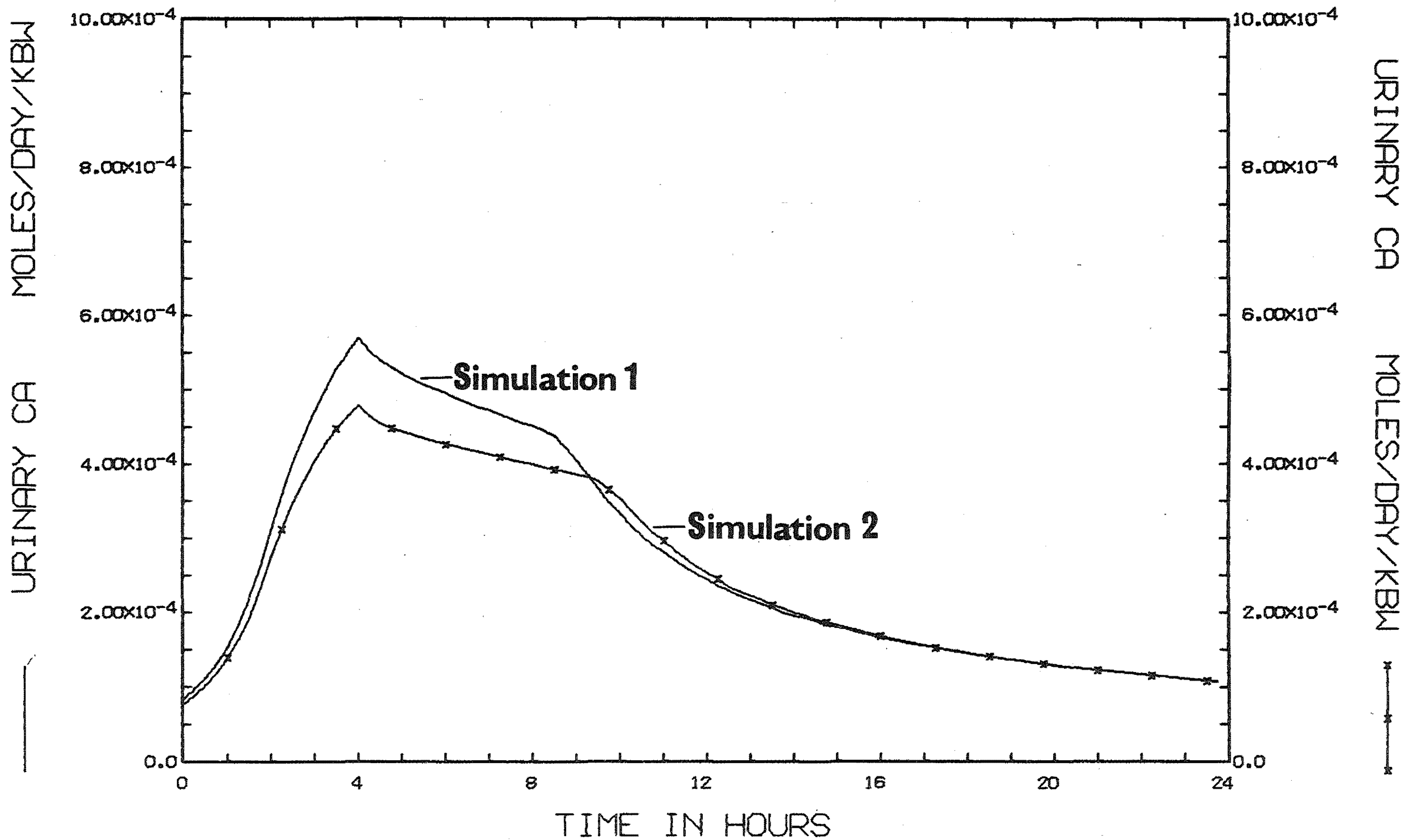


Figure 4.7 The effect of Ca infusion on urinary Ca - with & without CT

Similar *in vivo* experiments have been performed with normal and hypothyroid patients (Ibbertson et al. 1967, O'Brien & McIntosh 1967, Pharmakiotis et al. 1971). A feature common to the results of both Ibbertson et al. (1967) and O'Brien & McIntosh (1967) was the rapid divergence within a few hours of the plasma calcium levels between the hypothyroid and the control groups. Subsequently the rate of decrease in plasma calcium was similar in the two groups. In the case of Ibbertson et al. (1967) whose experimental procedure (a calcium infusion of 12 mg per kilogram body weight over three hours) most closely approaches the CAMET2 simulations a maximum difference in the calcaemia of 0.23 m.moles/litre was recorded between 6 and 7 hours decreasing to 0.15 m.moles/litre at the end of 24 hours. After the first 5 hours this difference was significant at the 0.05 level or better.

The reason for the divergence of the simulated calcium levels (figure 4.6) is the higher renal excretion of calcium in Simulation 1 which includes an effect of CT on the kidney. The sudden convergence of the plotted urinary calcium curves in figure 4.7 is caused by the reintroduction of PTH into the simulated system as the plasma calcium drops below 3.0 m.moles/litre (figure 4.4).

Whereas the pattern of events with respect to the serum calcium concentrations observed in the CAMET2 simulations bears a resemblance to the experimental situation the magnitude of the effects observed in nature is much greater than the computed quantity. However, since the CAMET2 parameters for CT were fixed upon, a report on

the renal effects of a CT infusion at an almost physiological rate has been found (Ardaillou et al. 1969). The results of this experiment indicate that the effects of CT on the kidney reabsorption of calcium may be underestimated in the model by a factor of 2 or 3. If such an effect were confirmed Ibbertson's experiment could be simulated with the same qualitative aspects relating to the serum calcium levels already discussed but with better quantitative agreement.

The question of whether CT action on the kidney or CT action on the bone is of prime importance in the reaction to hypercalcaemic stress is of some interest. Present day estimates of the normal bone resorption rate are less than 10 m.moles of calcium per day (section 6.2). In view of this it seems impossible to explain the difference in the serum calcium level between hypothyroid and normal subjects, that occurs in the first few hours of a calcium infusion, solely on the basis of a CT mediated reduction in bone resorption. Even in the improbable eventuality of a complete suppression of bone resorption during this time it is necessary to examine alternative effects of CT in the control of hypercalcaemia. Cochran et al. (1970) were able to reduce the high plasma calcium levels of 4 hyperparathyroid patients to the normal range by the slow administration of pharmacological doses of CT (4 MRC units per kilogram body weight). Significantly, the reduced plasma calcium concentration was substantially accounted for by the increased renal excretion in all 4 patients.

Taking the simulated results as a whole together with the experimental data two conclusions are tentatively drawn:

[A] CT action on the kidney may be more important than is currently recognized in meeting hypercalcaemic challenges, except (as suggested by Cochran et al. 1970) where bone turnover is elevated. Depressed bone resorption probably plays a subsidiary role in subjects free from bone disorders.

[B] The action of CT on the kidney is only fully expressed in the *absence* of PTH. This view is consistent with the well known impotence of endogenous CT to control the development of hypercalcaemia in hyperparathyroidism. Considering the direct anatagonism of CT and PTH on calcium tubular resorption (Chapter 3), this is not an unreasonable suggestion. It also follows that experiments involving the administration of CT to subjects with normal levels of blood calcium (and by implication normal levels of PTH) might not fully demonstrate the renal effects of a hormone whose physiological function is to control hypercalcaemia. Some caution must be exercised however when considering species other than man. There are good grounds for believing that in a variety of animals bone resorption may be a rather more important aspect of the homeostatic control of calcium metabolism than it is in man.

If in fact these suggestions have any physiological significance they could be readily checked out. For example similar experiments to those of Ibbertson et al. (1967), but with the addition of frequent collections and

measurements of urinary calcium over the experimental period should show significant differences between hypothyroids and normal subjects.

In passing it is evident that Simulation 2, as far as it goes, meets one important physiological criterion of hypothyroidism - a lack of noticeable effect on the blood calcium due to CT deficiency. The 8% drop in renal calcium excretion at t = 0 in Simulation 2 compared with Simulation 1 would normally cause a negligible shift in the calcium concentration of the plasma.

4.5 The Effect of the Removal of the Parathyroid Glands

Historically the discovery of the PTH glands was preceded by the observation that the removal of the thyroid was often accompanied by the onset of tetany (in severe cases characterized by physical convoltions). Later it was found that the removal of the parathyroid glands alone could produce this effect but it was not until early in this century that the link between tetany and subnormal serum calcium concentration was discovered (Fourman & Royer 1968). Since then the experiment has been carried out many times, invariably resulting in the development of hypocalcaemia and a drastically reduced renal phosphate excretion. In 414 determinations of the plasma calcium in 96 normal fasted dogs Copp (1960) found that the average serum calcium concentration was 2.47 ± 0.14 m.moles/litre (9.89 ± 0.54/100 ml) while the corresponding value from 196 determinations in 27 parathyroidectomized dogs was 1.46 ± 0.29 m.moles/litre

(5.84 ± 1.15 mg/100 ml). In man a drop in the level of calcium to between 1.5 and 2.0 m.moles/litre is usual (Lamberg et al. 1960, Danowski 1962).

The effect of the operation on renal excretion of calcium in the rat was first demonstrated by Talmage and his colleagues (Talmage & Kraintz 1954, Talmage et al. 1955) and in the mouse by Buchanan et al. (1959). In all cases it was found that urinary calcium excretion rose sharply at first, simultaneously with *declining* plasma calcium levels, only falling later as the calcium concentration became progressively lower. In other animals including the dog (Kleeman et al. 1961) and the hamster (Biddulph et al. 1970) the same phenomenon has been observed. The calciuria following parathyroidectomy in the hamster is particularly acute. Ten times the quantity of calcium was excreted in the 7 hours following parathyroidectomy compared with controls. The fall in extracellular calcium was more than accounted for by this loss (Biddulph et al. 1970, Biddulph 1972).

Obviously experimental data for man immediately following total parathyroidectomy is rare although some exists for hyperparathyroid patients after the removal of parathyroid tumours (Canary et al. 1962). The high concentration of peripheral PTH drops rapidly after the operation (Buckle 1969) and Canary et al. (1962) measured a fall in plasma calcium from high to normal levels in about a day. This was accompanied by sharply reduced

renal phosphate excretion and a rising level of extracellular phosphate. One patient who was accidently totally parathyroidectomized suffered a fall in the calcium concentration from 3.0 m.moles/litre to 1.8 m.moles/litre over the 48 hours following the operation. Unfortunately as the renal handling of calcium was not considered as a contributory factor either to the original high calcium levels or in the events subsequent to the operation the urinary calcium was not measured.

Parathyroidectomy can be simulated with CAMET2 by setting the rate of secretion of PTH to zero (Simulation 3). The consequences are seen in figure 4.8 and 4.9. In less than 4 hours the PTH concentration declines to a negligible value. This is associated with a threefold rise in the rate of urinary calcium excretion which thereafter falls with the calcium concentration. However even at 48 hours the rate of excretion (0.077 m.moles/kg/day) is only slightly lower than at t = 0 (0.083 m.moles/kg/day). Normally a similar drop in calcium concentration for any other reason causes a very sharp reduction in urinary calcium excretion (Simulation 4).

Naturally the model simulation is a simplification of the complex physiological changes that must occur with the loss of the parathyroid glands. The changes in bone and intestinal metabolism are not considered; neither are the direct effects of the hypocalcaemia (including the renal excretion of other electrolytes and the glomerular filtration rate (Kaufman & DiScala 1971)). In

SIMULATION 3 PARATHYROIDECTOMY

Source Program : 72150 No : 72162-4

The physiological consequences of the removal of the parathyroid glands were simulated by CAMET2. The rate of secretion of PTH was set equal to zero with considerable impact on both simulated renal calcium excretion and the serum calcium level.

References: Canary et al. (1962), Biddulph et al. (1970)

Simulated Time : 2 days

Parameter Changes : A_{25} set to 0.0*

Results : [A] Parameters during Simulation

C_1 (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_p (ng/ml)	Time (days)
2.50	0.0827	0.60	0.0
2.42	0.243**	0.01	0.126
2.02	0.127	0.0	1.00
1.90	0.0958	0.0	1.50
1.84	0.0770	0.0	2.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{derm}	S_{fecl}	S_{urin}	
-0.344	0.198	0.283	0.0633	0.0033	0.278	2.00

* Setting A_{25} to zero has the effect of suppressing PTH secretion (R_{sp}) (equation 2.42).

** Maximum value

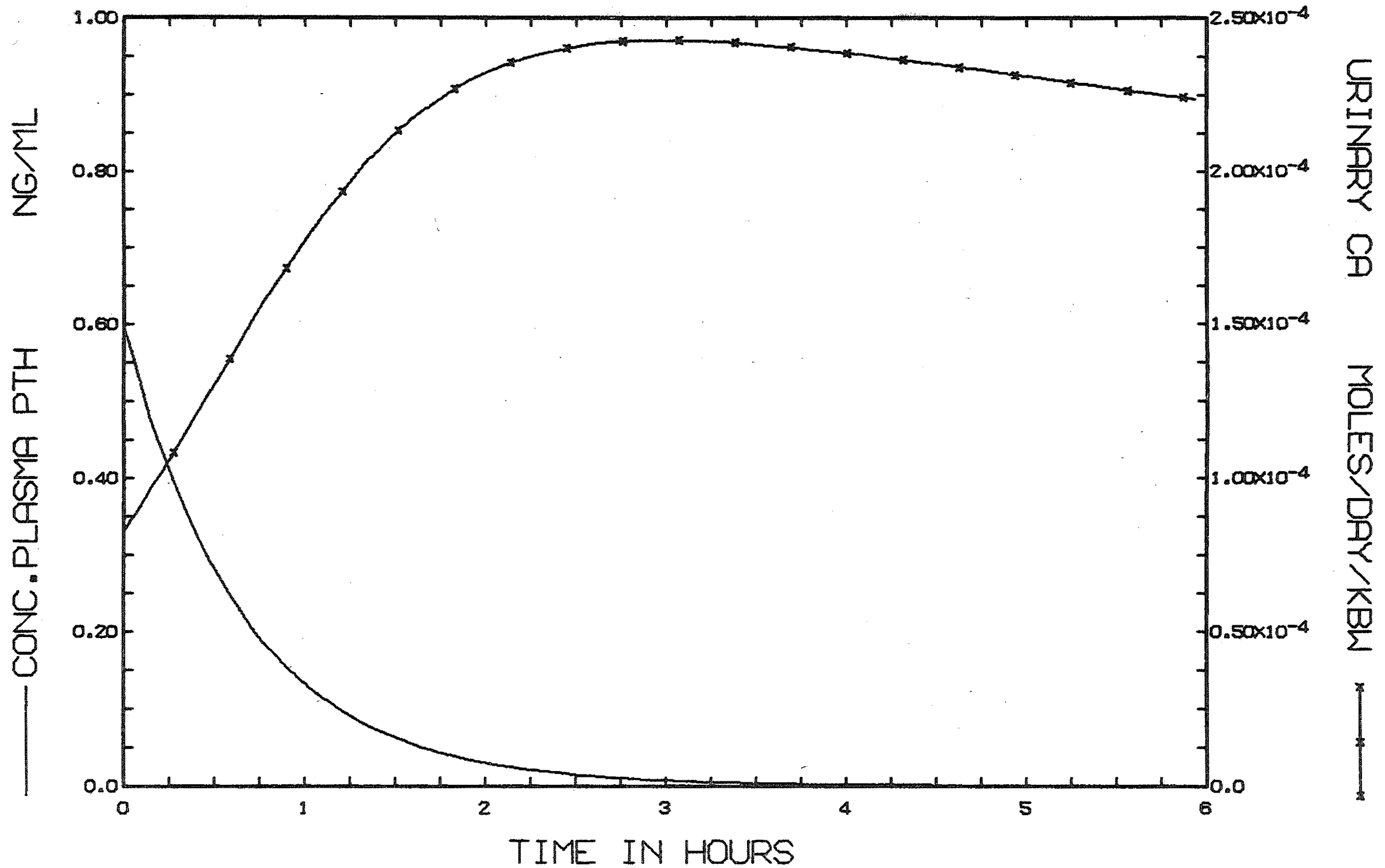


Figure 4.8 PTH concentration & urinary calcium excretion after parathyroidectomy

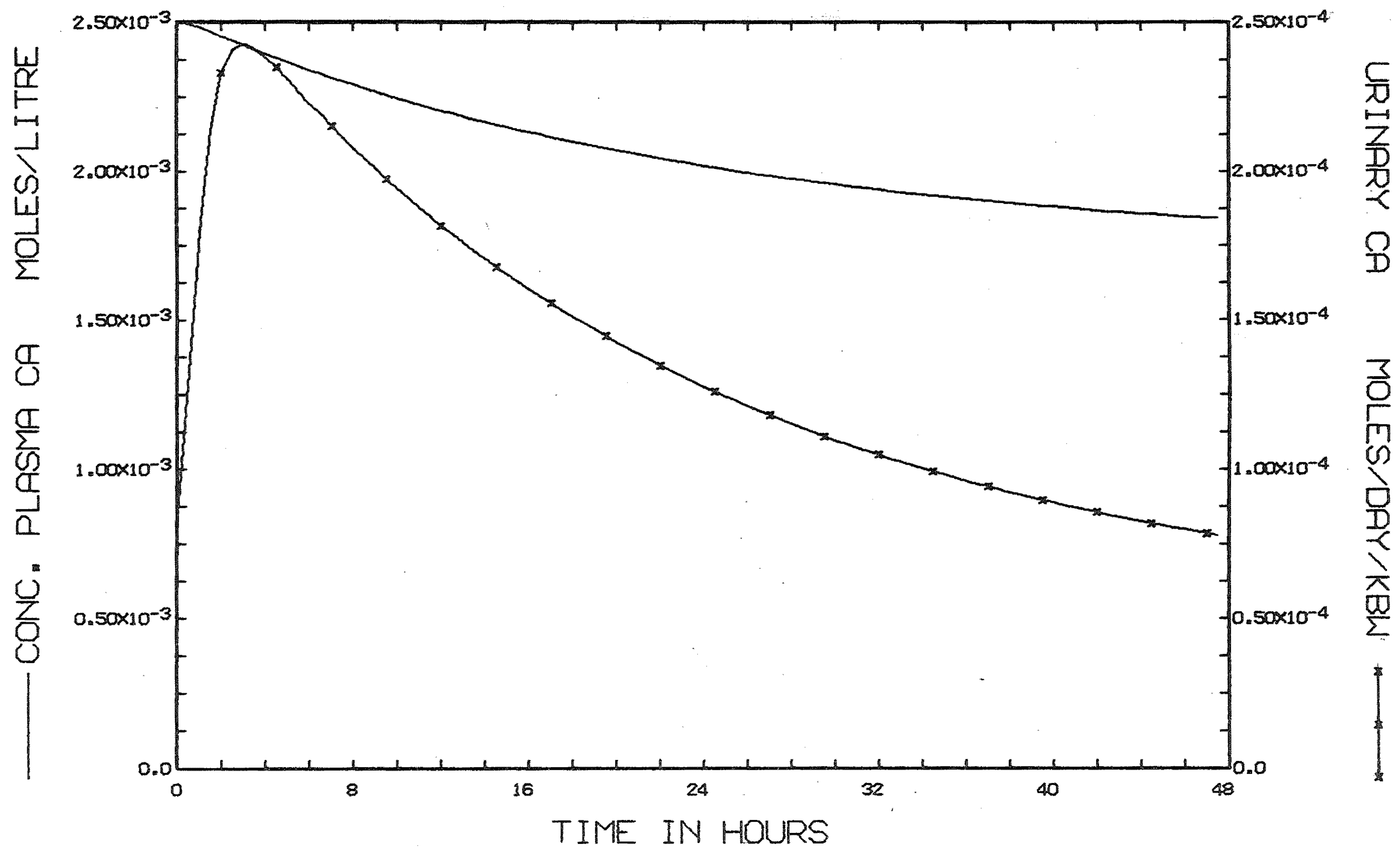


Figure 4.9 Plasma Ca concentration & urinary Ca excretion after parathyroidectomy

hypoparathyroids bone remodelling is depressed as assessed by microradiography, tetracycline labelling and other direct methods of estimating bone turnover rate (Jowsey 1968, Frost 1969, Nguyen & Jowsey 1970b). While the immediate consequence of the withdrawal of PTH is to depress bone resorption, in the long term the rate of bone formation also appears to fall and the skeleton is restored to an approximate balance (Fourman & Royer 1968). The present model bone equations do not satisfy these observations. Since collagen resorption is inversely proportional to the calcium concentration (equation 2.46) rather then linked to the PTH status the rate of resorption in Simulation 3 rises rather than falls. However the amount of unmineralized collagen does increase in accordance with observation, qualitatively at least (Jowsey 1968).

If stable levels of plasma calcium are maintained and if dermal losses of calcium are ignored, then a useful generalization to bear in mind is that renal calcium excretion represents net bone resorption plus net intestinal absorption. This being so the intestinal absorption is low in the absence of PTH considering that the urinary excretion of calcium is generally below 2 m.moles/day in untreated hypoparathyroids (if the skeleton is in calcium balance). This is in accord with direct physiological evidence (section 3.7). On a simulated diet CAMET2 would fail to exhibit the low urinary calcium excretion characteristic of this complaint because the inflows into the plasma compartment would not be reduced as they are in nature.

Notwithstanding the simplified nature of this simulation, the reproduction of the major events with respect to calcium is evidence that PTH involvement in the tubular reabsorption of calcium is of primary importance in the maintenance of high blood calcium levels. Of the simulated net negative calcium balance (-0.34 m.moles/kg) over 80% was accounted for by the simulated urinary calcium (0.28 m.moles/kg). The restoration of plasma calcium levels to normal is difficult for two reasons, firstly because of the relatively large inflow of calcium required and secondly, of more importance, is the prompt excretion of calcium by the kidney that occurs in the absence of PTH. Only by large continuous infusions of calcium (up to 37.5 m.moles over 12 hours) was Eisenberg (1965) able to achieve and maintain a normal calcium concentration in hypoparathyroids in the face of greatly increased renal calcium excretion. In reality the fall of plasma calcium is further compounded by a lowered gut absorption but this is an effect subsidiary to the loss of calcium in the urine.

4.6 EDTA Infusions

The two simulations discussed in this section, both involving the simulated infusion of ethylenediamine - tetraacetic acid (EDTA), illustrate the strengths and weaknesses of the present model.

The infusion of EDTA which complexes calcium and renders it physiologically inactive is used to diagnose parathyroid insufficiency (Fourman & Royer 1968). Rapid excretion of the EDTA-calcium complex by the kidney follows (Levitt et al. 1958). Normally, in humans with intact parathyroids the fall in the concentration of ionized plasma calcium

resulting from an EDTA infusion is recovered within 12 hours (Jones & Fourman 1963). In the presence of parathyroid insufficiency the ability to restore plasma calcium is impaired: compared with controls the concentration of non-complexed calcium is still decidedly low even at 24 hours (Jones & Fourman 1963). The simulated 2 hour decline in the calcium level (figure 4.10) was 0.61 m.moles/litre - a value identical to the mean found by Jones & Fourman in 12 subjects with neither thyroid nor parathyroid disease. An experimental range of 0.48 to 0.75 m.moles/litre was reported. However 4 hours from the beginning of the EDTA infusion the simulated and the experimental values begin to diverge. In the clinical trials serum calcium returned to the normal range (even slightly overshooting the preinfusion value) after 12 hours. By comparison the model results are quite different. Although a small rebound in the plasma calcium occurred (essentially completed in the hour after the infusion was stopped) a steady state value of 2.0 m.moles/litre was reached and maintained for the remainder of the simulation (figure 4.10). In fact this value represents the natural equilibrium calcium concentration of CAMET2 (with no oral or intravenous calcium inputs) where the outflow of calcium (in the simulated renal, intestinal and dermal losses) equals the inflow from the bone: it has however no physiological significance.

SIMULATION 4 EDTA INFUSION

Source Program : 72150 No : 72162-1

The infusion of ethylenediamine tetraacetic acid (EDTA) was simulated by a negative calcium infusion of the same magnitude but opposite sign to the rate at which calcium is chelated by an EDTA infusion of 70 mg/kg over 2 hours.

References : Copp (1960), Jones & Fourman (1963)

Simulated Time : 1 day

Model Inputs :

Calcium Infusion (m.moles/kg/day)	Time (days)
-2.27	0.0
0.0	0.0833

Results : [A] Parameters during Simulatior

C_l (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	
2.50	0.0827	0.10	0.60	0.0
1.89*	0.0173	0.042	1.13	0.0842
2.00	0.0217	0.037	1.20	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{derm}	S_{urin}	
-0.250	0.0960	0.145	0.0309	0.0238	1.00

* minimum value

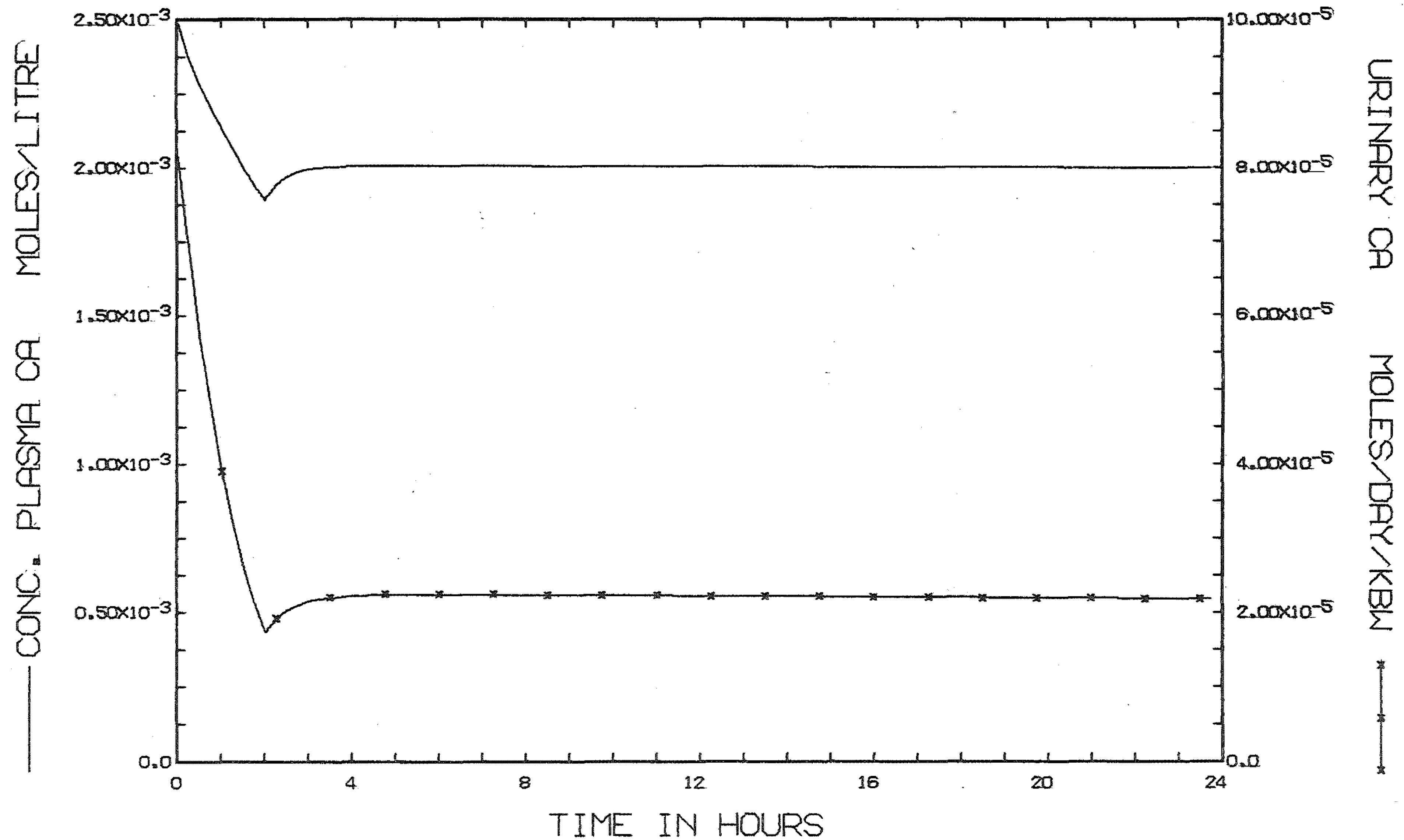


Figure 4.10 Plasma Ca & urinary Ca during & after an EDTA infusion - 70mg/kbw

While the result in clearly abnormal by the criteria of Jones & Fourman (1963) it is strikingly reminiscent of the observations of Copp (1960) in a parathyroidectomized dog infused with EDTA. The small increase in non-complexed plasma calcium at the end of the infusion followed by a persistent plateau was also evident in Copp's experiment and similar results in the parathyroidectomized dog (Alexander 1965) and the cow (Ramberg et al. 1967) have been noted. Of particular interest was the demonstration by Alexander (1965) of the ability to reduce plasma calcium in the dog to progressively lower but stable plateau values by sequential infusions of EDTA.

To ensure that the lack of action of PTH on the kidney, as in parathyroidectomized animals, does not materially alter the simulated EDTA-induced changes in plasma calcium concentration, Simulation 4 was repeated but with the addition of a simulated parathyroidectomy (as in the experiment of Copp 1960) immediately prior to the commencement of the EDTA infusion (Simulation 5). As can be seen from figure 4.11 the changes in plasma calcium are similar to those in Simulation 4, but as the calcium level at the end of the EDTA infusion is now *above* the equilibrium point for hypoparathyroidism a slow decline in the calcium concentration occurs. As in the simulated parathyroidectomy (Simulation 3) this is the result of greatly enhanced renal excretion of calcium. In this connection it is noted that in the animal experiments that have been mentioned the plasma calcium was reduced very much more severely, to below the normal hypoparathyroid range, and in several cases a slow but steady rise was evident in the plateau concentration.

SIMULATION 5 EDTA INFUSION WITH PARATHYROIDECTOMY

Source Program : 72150 No : 72162-5

To check that the simulated total removal of the parathyroids, as carried out by Copp (1960) prior to EDTA infusion, had no major effect on the results of Simulation 4 the rate of PTH secretion was set equal to zero and the simulated EDTA infusion commenced at 0.01 days.

References: Copp (1960)

Simulated Time: 1 day

Parameter Changes: A_{25} set to 0.0

Model Inputs:

Calcium Infusion (m.moles/kg/day)	Time (days)
0.0	0.0
-2.27	0.01
0.0	0.0933

Results: [A] Parameters During Simulation

C_1 (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	
2.50	0.0827	0.10	0.60	0.0
2.50	0.105	0.10	0.42	0.01
1.88	0.0841	0.04	0.02	0.0947
1.84	0.0784	0.02	0.0	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{derm}	S_{urin}	
-0.317	0.0921	0.151	0.0295	0.0969	1.00

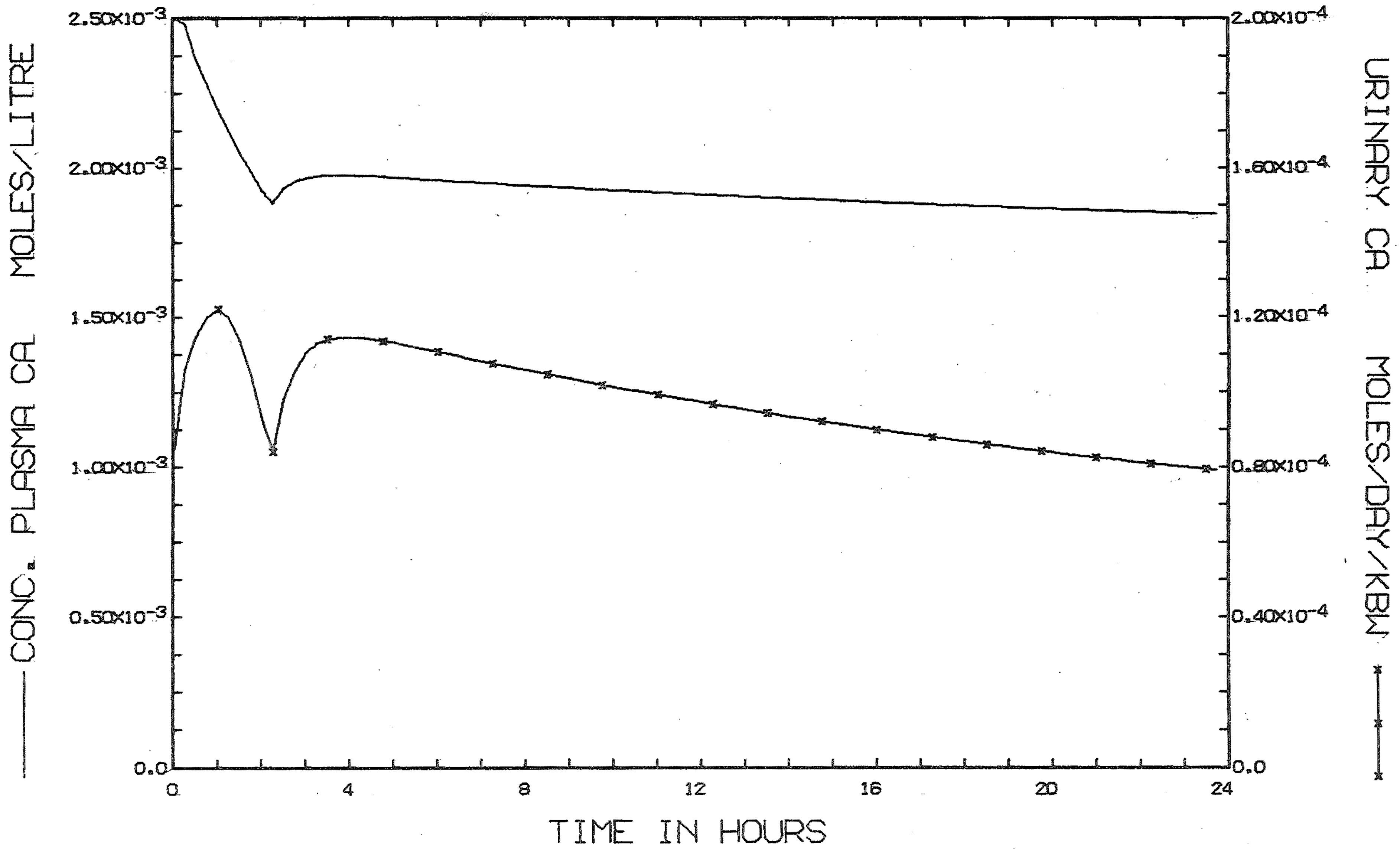


Figure 4.11 Effect of parathyroidectomy & an EDTA infusion on plasma & urinary Ca

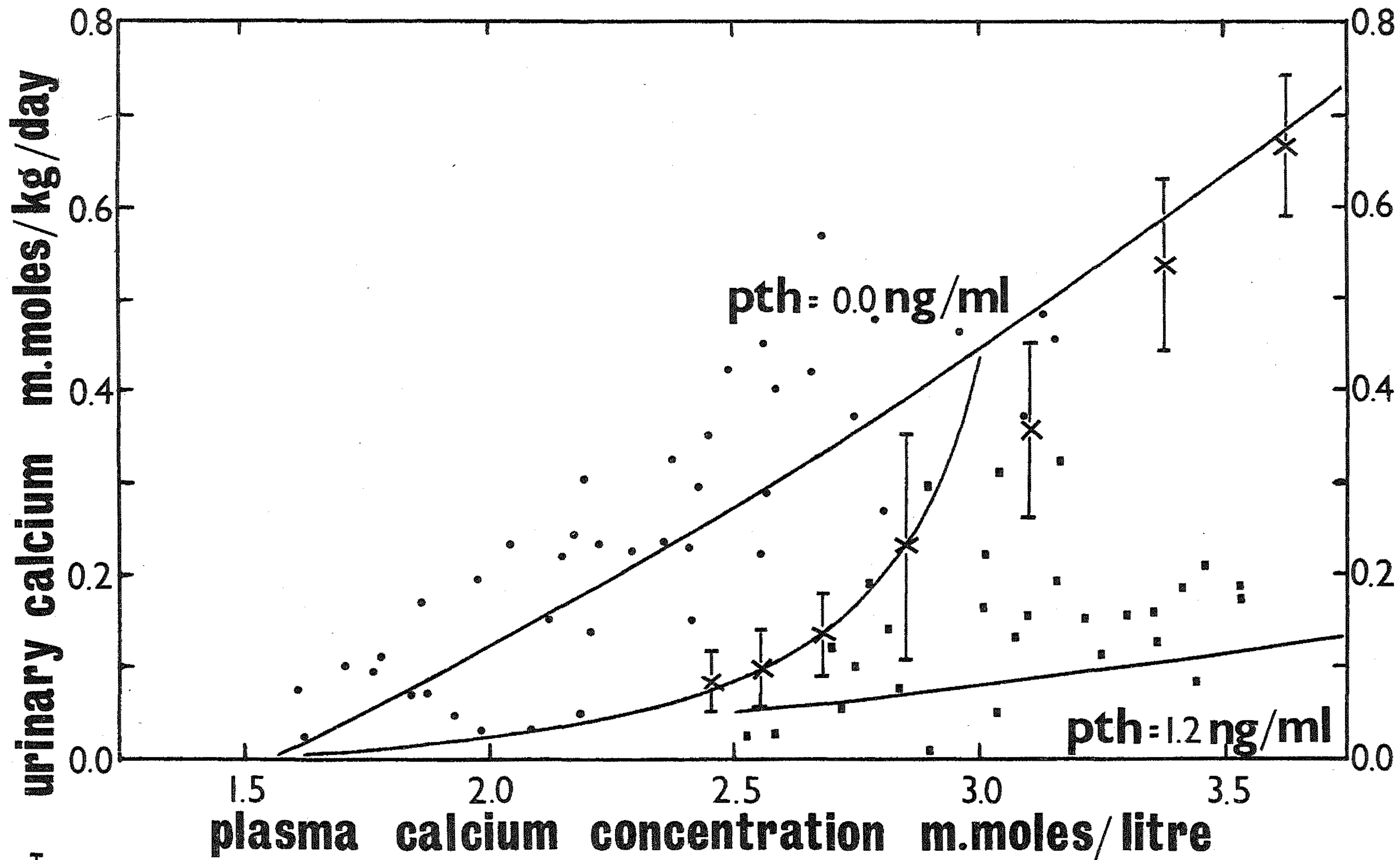
Considering that the subjects of Jones & Fourman (1963) were on a low calcium diet (4 m.moles/day), intestinal absorption cannot account for the recovery in plasma calcium after EDTA infusion. Further to this, CT secretion is suppressed under conditions of hypocalcaemia and in animals the rise in serum calcium is abolished by previous parathyroidectomy. Hence the increase in serum calcium must come from greatly increased bone resorption. As approximately 3 hours elapse before the effects of PTH on the bone are detectable in the blood (section 3.7) the good initial quantitative agreement between experiment and model prediction is not surprising. However the non-inclusion of the action of PTH on the bone is evidently a serious drawback to the present model.

Finally, it is concluded on the basis of all the simulations so far discussed that the idea of buffering of plasma calcium either by labile bone calcium reserves (McLean 1957, Ortner & von Endt 1971) or by a physiochemical equilibrium between bone crystal and serum calcium (Neuman & Neuman 1958, Nordin et al. 1965) need not be invoked to explain homeostatic control of calcium metabolism. The buffering that does occur would appear to be principally that of PTH mediated cellular resorption where both bone matrix and crystal are removed - but only after a considerable delay following the initial stimulus. On the other hand there is some evidence to suggest the existence of a quantity of unmineralized calcium able to cushion, to a limited extent, perturbations in the calcium of the extracellular fluids. Since this question is of

considerable importance to the modelling of calcium metabolism it is further pursued in Chapter 6.

4.7 The Place of the Kidney in Calcium Homeostasis

As a further check on the validity of the model parameters and equations controlling renal calcium excretion, CAMET2 predictions are compared with the data of Peacock et al. (1969) who investigated the urinary calcium excretion over a range of blood calcium concentrations (by the administration of either oral or intravenous calcium loads). Each point on the CAMET2 theoretical curve (figure 4.12) represents the steady state rate of urinary calcium excretion at the particular calcium concentration and the corresponding predicted hormone concentrations. In addition the renal calcium excretion was calculated and plotted over the range of calcium concentrations, first assuming that no PTH was present ($C_p = 0.0$) as in hypoparathyroidism, and secondly for a constant PTH concentration of 1.2 ng/ml (continuous autonomous secretion). Superimposed on the graph are the experimental values of Peacock et al. (1969) for normals (with means and standard deviations indicated) together with some representative points from both hypo- and hyperparathyroid patients. However since the experimental values were originally expressed as mg of calcium per 100 ml of glomerular filtrate (thereby partially correcting for extraneous influences) a direct comparison with the model results is not possible. To meet this situation, the experimental value for urinary calcium excretion at 2.55 m.moles/litre plasma calcium was set equal to the value predicted by CAMET2 and the remaining experimental points scaled accordingly.



normal mean and standard deviation ▪ hyperparathyroid • hypoparathyroid

Figure 4.12 See text for details

The discrepancy arising between the experimental mean and the CAMET2 value at the calcium concentration of 3.1 m.moles/litre is attributable to several causes, apart from possible inaccuracies in the model. Each experimental value of Peacock et al. (1969) represents the urinary calcium determined in a number of subjects whose blood calcium concentration extended over a small range about the mean plotted value. Hence while the CAMET2 equations predict a zero concentration of PTH at and above 3.0 m.moles/litre, PTH was probably present in at least some of the clinical subjects in the experimental sample - especially if measurements were made while the calcium concentration was increasing. It is noteworthy that the standard deviations around this range of plasma calcium concentration are especially large.

It may be seen from the diagram that a steep rise in urinary calcium excretion occurs with even a modest elevation in plasma calcium above 2.5 m.moles/litre - as was demonstrated in the calcium infusion of Simulation 1 (figure 4.3). Below 3.0 m.moles/litre the rate of urinary calcium excretion in hypoparathyroids (where no PTH is present) is considerably greater than in normal subjects with the same calcium concentration. The dramatically increased rate of urinary excretion of calcium in the simulated parathyroidectomy (Simulation 3) represents the transition between the normal and the hypoparathyroid curves at a plasma calcium concentration of 2.5 m.moles/litre.

A recommended treatment for hypoparathyroidism is the daily administration of large doses of vitamin D, often in combination with a high dietary intake of calcium (Fourman & Royer 1968). Usually this treatment is effective in partially restoring the calcium concentration in blood, but only at the expense of much higher than normal urinary excretion. Whether or not a small vitamin D - mediated increase in the resorption efficiency of the renal tubules contributes to the rise in calcium levels is a debatable point. While it is no longer believed as was once thought (Litvak et al. 1958, Gordan et al. 1962) that pharmacological doses increase the renal excretion of calcium there is very little experimental data on which to base any assessment. The experiment of Bernstein et al. (1963) is often cited as evidence against such an effect but only three persons were involved in the experiment, one of whom showed some evidence of a lowered calcium loss in the urine as a result of vitamin D treatment. In another limited study Gran (1960a) showed a much reduced calcium excretion in hypoparathyroid rachitic dogs once vitamin D was administered, and interestingly the calcium binding protein (section 3.8) has appeared in the kidney of rachitic chicks following the commencement of a vitamin D supplemented diet (Taylor & Wasserman 1967). Should it turn out that vitamin D and its metabolites have no effect, direct or indirect, on the tubular resorption of calcium and the present concept of renal responsibility for the low calcium level in hypoparathyroids is correct then vitamin D therapy in this disorder represents little

more than treatment of the symptoms.

The importance of the kidney control of calcium excretion is again evident in hyperparathyroidism. A high level of endogenous PTH increases the efficiency of tubular reabsorption of calcium (figure 4.12) resulting in the development of a high calcium concentration in the blood and a rise in the renal calcium load until the point is reached where the calcium flow into the blood from the intestine and the bone again equals the outflow in the urine. The condition is aggravated by increased bone resorbtion and intestinal absorption of calcium (section 3.7) which is reflected in the higher urinary calcium excretion commonly observed in hyperparathyroidism (Sutton & Watson 1969). While the well known increase in bone resorption associated with this disorder is a real effect it now appears that bone formation is also increased, although not to the same extent (Jowsey 1968, Frost 1969, Merz et al. 1971). This is in accordance with the observation that demineralization of bone in long-standing hyperparathyroidism is unusual (Gudmundsson & Woodhouse 1971).

Overall then it appears that the widespread emphasis in the literature on bone, particularly PTH controlled bone resorption, as the most important factor in the control of calcium homeostasis and central to the development of hypoparathyroidism and hyperparathyroidism, is misplaced. B.E.C. Nordin and his colleagues have arrived, by experiment, at conclusions similar to those expressed above (Peacock et al. 1969, Cochran et al. 1970). Their conclusions are strongly supported by the present work.

To summarize, the main conclusions of this chapter are:

[A] The kidney represents the major organ in calcium homeostasis and because of its ability to react quickly to changing conditions and to excrete large quantities of calcium (in relation to the flows from the bone and the intestine) is the major arbiter in determining the calcium concentration of the blood. So long as the calcium concentration is stable this represents an equilibrium point at which the inflow equals the outflow.

[B] The PTH induced increase in the tubular resorption of calcium is essential to the maintenance of the plasma calcium concentration at around 2.5 m.moles/litre. The drop in the calcium level after parathyroid gland removal is largely attributable to the removal of this constraint on the renal excretion of calcium.

[C] The idea is advanced that CT action on the kidney may be important in the reaction to hypercalcaemic stress and that its action on the bone is of lesser importance in the presence of normal skeletal metabolism.

[D] While the modelling of bone by CAMET2 is only at a preliminary stage it would appear that bone plays a small role in controlling perturbations in calcium concentration. Yet whereas the kidney reacts quickly in excreting excess calcium or conserving existing calcium it cannot, by itself, *restore* a subnormal blood calcium level to the normal range. In this eventuality the action of PTH on the bone is essential to make up any deficiency in diet or absorption.

Intestinal and Dermal Calcium Secretion

5.1 Only two significant transfers of calcium around the body remained unaccounted for by CAMET: the calcium in the secretions that are emptied into the alimentary canal in response to a meal and the calcium in the sweat. In this chapter the literature on these flows is reviewed and new equations and parameters are introduced to account for them. The evidence for and against a relationship between the calcium concentration in gut secretions and that in the blood is examined in section 5.5 and the model will be shown to predict (in accord with some physiological evidence) that a far larger proportion of the calcium secreted in response to a meal containing calcium appears in the faeces as 'endogenous faecal calcium' than of that secreted at other times.

5.2 Dermal Excretion of Calcium

Calcium is lost from the body in three ways; from the gut as endogenous faecal calcium, from the kidney, and through the skin. For persons in temperate climates the latter route is usually the least important, but is not negligible. Indeed, during heavy physical work or in extreme climatic conditions it may well be greater than the combined loss by other routes. Under such conditions estimates of over 1 litre per hour water loss in man (Lee 1964) and an instantaneous rate of calcium loss of up to 50 m.moles/day (Consolazio et al. 1962) have been made.

The loss of water, with its dissolved electrolytes, occurs through the sweat glands as an active secretory process concerned with body temperature regulation. But apart from this a further loss occurs directly through the skin (insensible perspiration). From considerations of water balance alone, under temperate conditions and in the absence of visible sweating, the total dermal loss of water is approximately 1 litre per day - most of which is believed to be insensible perspiration (Hernández-Péon 1960). Schwartz et al. (1964) estimated that the volume of sensible sweat in a controlled hospital environment, with minimal physical activity, as not greater than 300 ml/day. The total water loss is considerably greater in tropical climates, reaching a range of 2.5 - 3.8 litres/ day in one study (Khogali et al. 1970).

Reliable measurements of the total daily excretion of calcium through the skin are not yet available. Research is hampered in that sweat production is unevenly distributed over the body (Weiner 1945) and that the concentration of calcium in sweat also appears to vary from one position to another (Johnson et al. 1969). In addition interpretation of the published experimental results is made more difficult by the fact that most experiments are carried out under conditions designed to stimulate sweat production, but which are not typical of the normal environment. It has however been established that the calcium concentration in sweat is inversely related to the rate of sweating (Mitchell & Hamilton 1949, Johnson et al. 1969). The results of such experiments are tabulated below.

Table 5.1 Dermal Calcium Losses

Reference	Sweat Ca Conc. (m.moles/litre)	Rate of Sweating (m l/hour)	Total Daily Ca Loss (m.moles)	Experimental Method and Conditions
Mitchell & Hamilton (1949)	1.8 (1.5-2.1)	91 (62-146)	3.7 (1.8-5.8)	10 determinations with 4 subjects exposed for 7.5 hours at 29°C. Relative Humidity 50-52%* **.
	0.7 (0.6-0.8)	769 (655-897)	12.1 (8.7-15.4)	As above but 38°C, 68-69% relative humidity.
Johnston et al. (1950)	(0.34-1.00)	308		4 subjects at 36°C.
Robinson & Robinson (1954)	(0.25-2.0)			Collected references
Altman (1961)	(1.25-2.50)			83 determinations
	1.25 (0.1-3.6)			210 determinations
Harden (1964)	0.7±0.1	69±13		14 control subjects***.
	2.0±0.4	115±15		11 thyrotoxicosis patients
	0.65±0.15	83±16		6 treated thyrotoxicosis patients
Consolazio et al. (1966)				12 normal subjects* **
	0.89	353		3.5 hours at 38.5°C
	0.66	402		7 hours at 38.5°C
	0.69	297		12 hours at 38.5°C
	1.07	168		12 hours (sleeping) 33.1°C
	0.83±0.08	232	4.6±0.4	mean of 12 hour periods

Table 5.1 Dermal Calcium Losses

Reference	Sweat Ca Conc. (m.moles/litre)	Rate of Sweating (ml/hour)	Total Daily Ca Loss (m.moles)	Experimental Method and Conditions
Isaksson et al. (1967)			3.0(0.5-9.1)	13 subjects, 21 determinations****
			3.4(0.5-9.1)	data from 13 of the above determinations that were most complete.
Rasmussen (1968)			2.0-3.0	estimate
Johnson et al. (1969)	0.45 0.35 0.28	603 800 979		arm sweat collected under conditions of increasingly high thermal stress*

*total calcium collected and measured

**sweating rate determined from loss of body weight

***thermally stimulated sweat collected over two hours and calcium concentration determined directly

****patients with varying disorders including two with osteoporosis and one with osteomalacia. Calcium in sweat determined indirectly from sweat calcium to potassium ratio and the total potassium dermal loss derived from balance studies. Comfortable hospital environment.

Measurements of the normal total loss of calcium through the skin range from 0.3m.moles per day in the elderly living in an air-conditioned metabolic ward (Gitelman & Lutwak 1963) to 9.1 m.moles per day in a patient on vitamin D therapy and a high calcium intake (Isaksson et al. 1967). Overall however, the results reported by Isaksson indicate a mean daily loss of the order of 2.5 m.moles (100 mg).

Little data is available on alterations in sweat calcium consequent on alterations in calcium metabolism. Harden (1964) found the total calcium excretion and the concentration in sweat to be significantly higher in thyrotoxicosis patients than in either controls or treated patients (table 5.1). He suggested that this might be due to a decreased resorption of calcium in the sweat tubules as a result of hypoparathyroidism or alternatively to the increased load of calcium as a result of the high ultrafiltrable calcium in the plasma. A significant linear correlation was demonstrated between the concentration of sweat calcium and the urinary calcium/creatinine ratio ($\rho < 0.02$). In its ability to reabsorb minerals the sweat tubule is similar to the kidney nephron (Dobson 1969, Schultz 1969) and both are known to be subject to a wide variety of neural, endocrine and pharmacological agents.

On the basis of this limited evidence the equation used to represent dermal calcium loss in CAMET2 is

$$R_{derm} = K_{derm}\, C_1,$$

where $R_{derm} = 2.5$ m.moles/day at $C_1 = 2.5$ m.moles/litre.

Given that the volume of fluid lost every day through the skin is of the order of 1 litre, then a loss of 2.5 m.moles of calcium per day means that the calcium is present in a concentration of 2.5 m.moles/litre. This is somewhat higher than the concentrations recorded in table 5.1, and is also at variance with the reported absence of calcium from insensible sweat (Mitchel & Hamilton 1949). At these comparatively low rates of secretion the concentration of calcium in the sweat does not appear to be known. However it should be noted that a mean loss of 2.5

m.moles per day which is assumed in CAMET2 could well be an overestimation for humans in sedentary occupations.

The mean quantity of calcium lost through the skin under normal circumstances is a question of some practical importance to calcium balance techniques where this loss is usually ignored. It deserves further investigation.

5.3 Intestinal Secretion of Calcium

Under the following headings the physiology of each major intestinal flow with respect to calcium is summarized. The corresponding numerical data is shown in table 5.2.

Saliva

The secretion of saliva is continuous in the waking state averaging 0.5 ml/minute, but it may reach a stimulated maximal value of 4.7 ml/minute (Altman 1961). Saliva is a mixture of secretions principally from three major gland pairs: the submaxillary, parotid and the sublingual glands. The concentration of calcium in saliva, as well as differing between animal species, differs with the gland type and the rate of secretion of saliva.

There is general agreement among the authors referred to in table 5.2 that the concentration of calcium in saliva is close to 1.5 m.moles/litre under basal conditions (that is secretion in the absence of any known stimulus). A fall in the concentration occurs to about 1 m.mole / litre with increasing saliva production (Schneyer & Schneyer 1967). However part of this observed decrease is due to the changing relative contributions of the various glands to the total flow. The increasing proportion of parotid saliva, of a relatively low calcium concentration, means that the relationship observed between the flow rate

and the observed concentration is not necessarily true of any one component flow. As with the sweat glands, increased secretion of saliva is associated with increased sodium and chloride concentrations and a relatively stable potassium concentration.

Total human saliva production is estimated to be around 750 ml/day (Schneyer & Schneyer 1967) calculated assuming 8 hours sleeping at 0.05 ml/minute, 2.5 hours eating at 2 ml/minute and the remainder of the day at 0.5 ml/minute. In ruminants the quantity of calcium associated with the salivary flow is considerable on account of the copious production of saliva in these animals - one estimate puts the daily volume of saliva produced in sheep at over 10 litres (Storry 1961).

Gastric Juice

The relationships between gastric juice electrolytes and their various cellular origins are matters of intense investigation and controversy among physiologists. Like saliva, gastric juice is a composite fluid made up from numerous secretions from different cell types reacting in different ways to pharmacological and physical stimuli. It differs in that the different cells are interspersed - making it impossible to collect separate cellular secretions for analysis. An added difficulty in man, where the conventional animal techniques are out of the question, is the problem of contamination of the gastric juice with saliva or duodenal contents. Apart from saliva, the collection of all alimentary canal secretions is complicated by possible contamination with other fluids, and is

a major source of the wide ranges in calcium concentration exhibited in table 5.2.

The total output of hydrochloric acid, potassium and calcium increase proportionally but differently (although the concentrations of calcium and sodium decrease rapidly) as the secretion rate rises (Long 1961, Moore & Makhlouf 1968). It is thought, partly on the basis of the inverse correlation between calcium and hydrogen ion concentrations observed as the output of the acid secreting (pariental) cells increases, that calcium is predominantly secreted by non-pariental cells in the stomach (Hunt & Wan 1967).

The total non-fasting gastric fluid production is considerable, reaching 3 litres per day (Malm 1958). Basal secretion is continuous but decreases at night and with age (Long 1961). The concentration of calcium is 1.5 m.moles/litre decreasing to about 0.6 m.moles/litre at high rates of production (Moore & Makhlouf 1968).

Bile

Bile differs from other gut secretions in the remarkably high calcium concentrations that can occur - up to 32 m.moles/litre (Diamond 1967). This does not reflect a high concentration of calcium in the bile continuously secreted by the liver, but is due to the resorption of water that takes place in the gall bladder (Wheeler 1967).

Apart from its digestive function bile is an important excretory route for many degradation products of metabolism - including vitamin D metabolites (Avioli et al. 1967). It is concentrated in the gall bladder and under the stimulus of food is expelled into the duodenum (Hallenbeck 1967).

In this way the requirements of continuous excretion of waste products and the periodic nature of digestion are reconciled.

The gall bladder concentrates up to half of the hepatic bile produced daily to within 10 - 20% of its original volume (Diamond 1967). The total hepatic production of bile is 1500 (1000 - 2000) mls in a day (Doubilet & Fishman 1961): the concentration of calcium in it is about 1.5 m.moles/litre (Altman 1961, Briscoe & Ragan 1965).

Pancreatic Juice

The human pancreas secretes into the duodenum considerable quantities of alkaline juice in response to meals through a common outlet with the bile duct. This flow may reach up to 4.7 ml/minute (Janowitz 1967). The predominant ion is bicarbonate whose concentration increases to about 100 m.moles/litre in stimulated flows (Hansky 1967). From a direct intubation study involving 20 normal persons Hansky reported that the concentration of calcium in pancreatic juice, under basal conditions, was 0.75 ± 0.20 m.moles/litre and 0.58 ± 0.13 at high rates of secretion.

In dogs the calcium content of pancreatic juice does not appear to be secreted in the fluid containing the bicarbonate (Janowitz 1967) so its relatively low concentration may be a dilution effect. A low concentration of calcium in the presence of a high concentration of bicarbonate lessens the risk of calcium precipitation, although in hyperparathyroidism with concomitant hyper-

calcaemia calcium deposition in the pancreatic duct is sometimes observed (Hansky 1967).

Small Intestinal Wall Secretion

Of all the gut secretions, least quantitative information is available on the activity of the intestinal glands lining the wall of the small intestine. This is partly due to the difficulty of obtaining access without resort to surgery and partly due to the simultaneous local absorption by the intestinal villi.

In general most experiments with isolated gut loops have shown relatively small fluid secretion rates (Gregory 1962), although in at least one such experiment the duodenal secretion of dogs was considerable even after the initial transient flow had ceased (Landor et al. 1955). Local mechanism stimulation is the only definitely accepted stimulus to such secretion although nervous control may also be involved (Gregory 1962).

As far as can be judged from the available evidence the concentration of calcium in secretions produced by mechanical stimulation in the dog is about 1.5 m.moles/litre (de Beer et al. 1935). Altman (1961) cites a similar value for man although in this case contamination from above the collection point may have been significant.

Table 5.2 Flow Rates and Calcium Concentrations of Intestinal Secretions

Reference	State of Secretion	Calcium Concentration* (m.moles/litre)	Flow Rate* (mℓ/minute)
Saliva			
Altman (1961)	basal	1.5(1.3-2.4)	0.57(0.1-1.8)
	stimulated		1.9 (0.4-4.8)
Schneyer & Schneyer (1967)	nocturnal		0.05
	basal	1.5	(0.33-0.5)
	stimulated	1.0	2(1.5-5)
Gastric Juice			
Malm (1958)	daily	(1.1-2.3)	(2000-3000)mℓ/day
	ranges	(0.4-3.8)	2500 "
Hunt (1959)		1.6	
	non-fasting**		0.89
	fasting**		0.59
Long (1961)	fasting	(0.2-2.4)	1.2(0 -2.9)
	nocturnal		0.8(0.2-1.7)
	after meal		1.7(0.2-3.6)
Altman (1961)	fasting	1.8(1.0-2.4)	1.0(0.7-9.5)
Moore & Makhlouf (1968)	basal	1.5(1.12-1.79)	0.6(0.3-0.7)
	stimul-ated***	0.6(0.49-0.66)	(3.5-4.2)
Bile			
Malm (1958)		(1.0-3.5)	(250-900)mℓ/day
Altman (1961)	gall bladder	(2.5-3.5)	
	hepatic bile	(1.0-2.3)	(2.6-15.0)mℓ/kg/day
Long (1961)	gall bladder	8.3(3.0-12.8)	
Doubilet & Fishman (1961)	daily total		1500(1000-2000) mℓ/day
Briscoe & Ragan (1965)	****	1.7(1.3-2.1)	1002 mℓ/day (1 subject)
Diamond (1967)	gall bladder	(25-32)	
	hepatic bile	6	

Reference	State of Secretion	Calcium Concentration (m.moles/litre)	Flow Rate* (ml/minute)
Pancreatic Juice			
Malm (1958)		(1.1-1.6)	(600-1770) ml/day
Altman (1961)	basal	(1.1-1.6)	1.0(0.02-5.20)
Doubilet & Fishman (1961)	daily total		2500(1500-4000) ml/day
Long (1961)	fasted stimulated fasted non-fasting	(0.1-3.6) (0.6-0.9)	 0.6(0.0-1.7) (17-20)ml/kg/day
Hansky (1967)	basal stimulated	0.75±0.20 0.58±0.13	0.8 3.0
Janowitz (1967)		1.7±0.3	up to 4.7
Intestinal Secretions			
Altman (1961)	90cm beyond pylorus 120cm " " 160cm " "	1.33(1.3-1.4) 2.0 (1.3-3.2) 1.9 (1.3-2.5)	 0.56(0.18-1.05) 0.37(0.28-0.47)
Gregory (1962)	14 determinations in 3 dogs	1.6(0.8-2.7)	

* means are given where possible with ranges in parentheses

** nocturnal values

*** values for maximum rate of secretion

**** total bile collected and measured from 3 subjects over a week,only one of whom had an intact gall bladder.

5.4 CAMET2 Intestinal Parameters and Equations

Equations 2.26 and 2.27 represent respectively the duodenal fluid ($RF_{1,6}$) and calcium ($R_{1,6}$) secretions where the constants G_1, G_2 and G_3 were selected to give physiological values to the secretions both before and during a meal. The use of a separate equation for the fluid flow $RF_{1,6}$ replaces the representation of this flow in CAMET as a negative fluid absorption. For the sake of simplicity and in the absence of detailed data the duodenal fluid flow during the course of a simulated meal represents the fluid secretion over and above basal levels by *all* intestinal flows. As in CAMET this is achieved by making $RF_{1,6}$ dependent on the rate of stomach emptying. The volume of stimulated intestinal juice secreted in response to a simulated meal is three times the meal volume (Borgström et al. 1957). With equation 2.27 the calcium carried in this flow is now included in the model - with important consequences (section 5.6).

The effect of a simulated meal on the total intestinal secretion of calcium and the concentration of calcium in the duodenal secretion ($R_{1,6}/RF_{1,6}$) is illustrated in figure 5.1. Although the total calcium secreted rises rapidly, the concentration of calcium falls in accordance with observation.

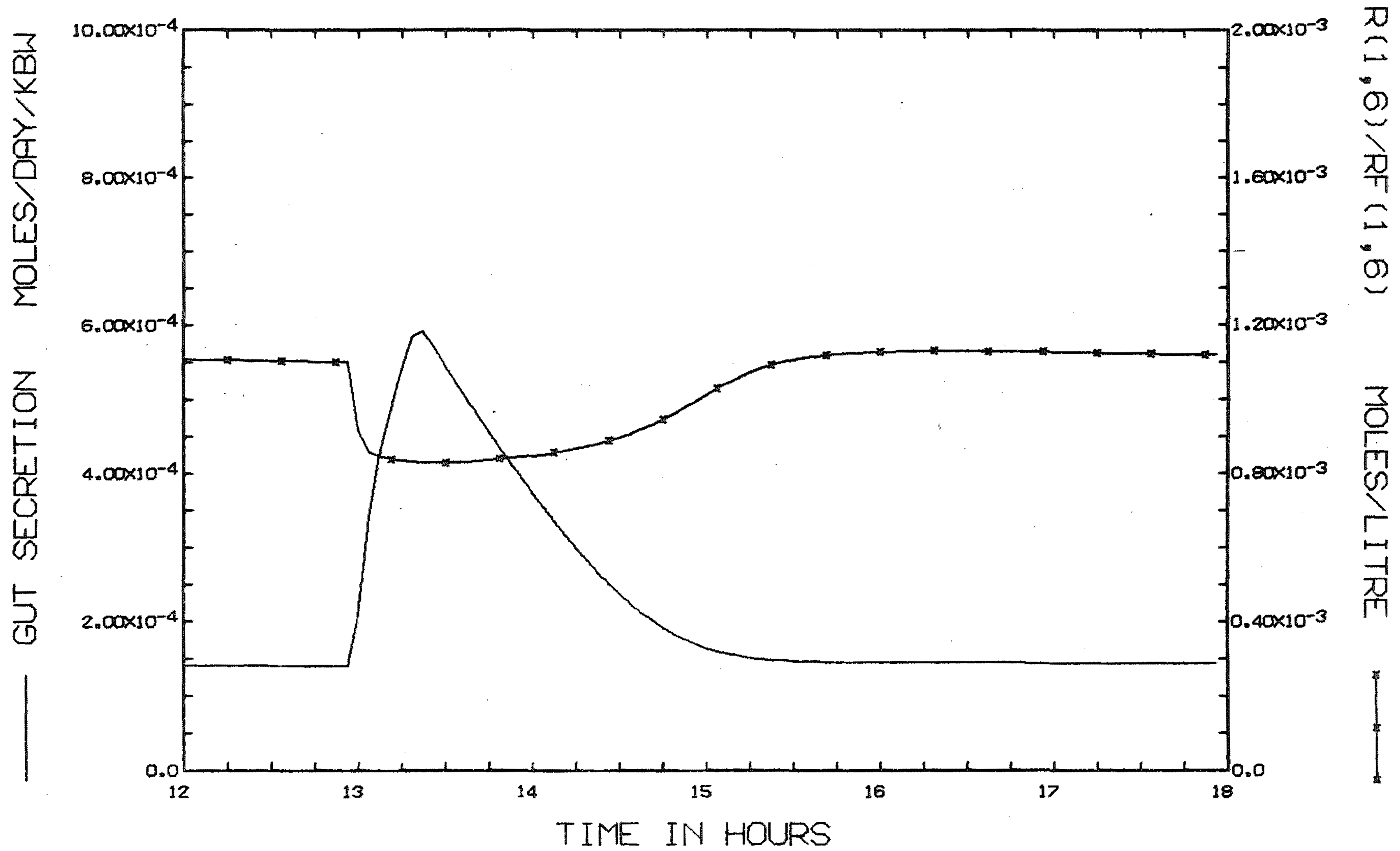


Figure 5.1 Secretion rate & concentration of Ca in response to meal

On the basis of the data in the preceding section, model parameters were chosen for the intestinal fluid flows $RF_{1,I}$ (I = 5, 11) and the associated calcium $R_{1,I}$ (I = 5,11) (table 5.3). The rates of the intestinal secretions $R_{1,7}$ to $R_{1,11}$ (as well as part of $R_{1,6}$) were derived from the data of Wensel et al. (1969). They measured the movement of calcium across the intestinal wall in both directions over different 50 cm segments of the small intestine, using an intubation perfusion method. While the data is extremely variable and may not represent basal conditions the experiment is the most complete investigation of calcium absorption and secretion made to date in this region of the human gut. Intestinal wall secretions are assumed to be 1.5 m.moles/litre in calcium (table 5.2) and on this assumption the fluid flow rates were calculated. The new values eliminate the unphysiologically high calcium concentrations in these secretions implied in CAMET.

Table 5.3 CAMET2 Intestinal Secretions

Compartment and Secretions Represented	Calcium Flow* (m.moles/kg) day)	Fluid Flow* (litres/kg/ day)	Ca Concentration (m.moles/litre)
5 saliva gastric juice	0.062	0.041	1.5
6 bile pancreatic juice intestinal wall	0.050	0.045	1.1
7 intestinal wall	0.016	0.011	1.5
8 " "	0.0036	0.0024	1.5
9 " "	0.0036	0.0024	1.5
10 " "	0.0036	0.0024	1.5
11 " "	0.0036	0.0024	1.5
Totals	0.14	0.11	1.3**

* basal secretion rate
** mean concentration (m.moles/litre)

The total basal secretion may be somewhat higher than is the case in nature as no account is taken of the lower nocturnal secretion (table 5.2), but this is of little consequence to model predictions as virtually all secreted calcium is reabsorbed during the almost steady state conditions that exist between meals.

In the course of the present work several anomalies relating to the modelling of intestinal calcium absorption have become apparent. While for the moment no attempt has been made to correct them, they are noted here for future reference.

The simulated fasting flow of fluid from the distal ileum (RF_{fecl}) is 0.36 ml/minute - in agreement with experimental estimates of less than 1 ml/minute (Karr & Abbott 1935, Whalen et al. 1966). In nature however the flow is sporadic and subject to considerable variability in magnitude between

subjects. Apparently, efficient absorption of both the fluid and the calcium secreted into the gut under resting conditions occurs *in vivo*. The basal salivary flow alone is of approximately the same size, and carries the same quantity of calcium (section 5.3), as physiological measurements of the distal ileal flow. The concentration of calcium in the simulated resting flow from the ileum is only 0.25 m.moles/litre or about 1/6th of that found experimentally. Studies on the absorption of calcium from solutions recycled through dog gut loops have indicated that calcium absorption does not occur when the calcium concentration of the intestinal contents falls below 0.50 - 0.75 m.moles/litre (Cramer & Dueck 1962). However, despite the low calcium concentrations in the intestinal compartments under basal conditions, over 98% resorption of the simulated intestinal juice calcium occurs as a consequence of the model equations. In view of the number of approximations involved in the intestinal model (Livesey 1970), the cause of this high absorption (which is also evident, but to a lesser extent, at high calcium concentrations) is not clear.

The inconsistency in the fluid equations mentioned in Chapter 1 is apparent in figure 5.2 where the calcium concentration in compartment 7 and the total absorption of calcium from the gut are plotted over the course of a simulated meal. The instantaneous flow of fluid throughout the gut as the stomach empties, implied by equations 2.29 and 2.30, causes a transient drop in the concentration of calcium in compartments 6 to 11 which persists until the associated dietary calcium reaches

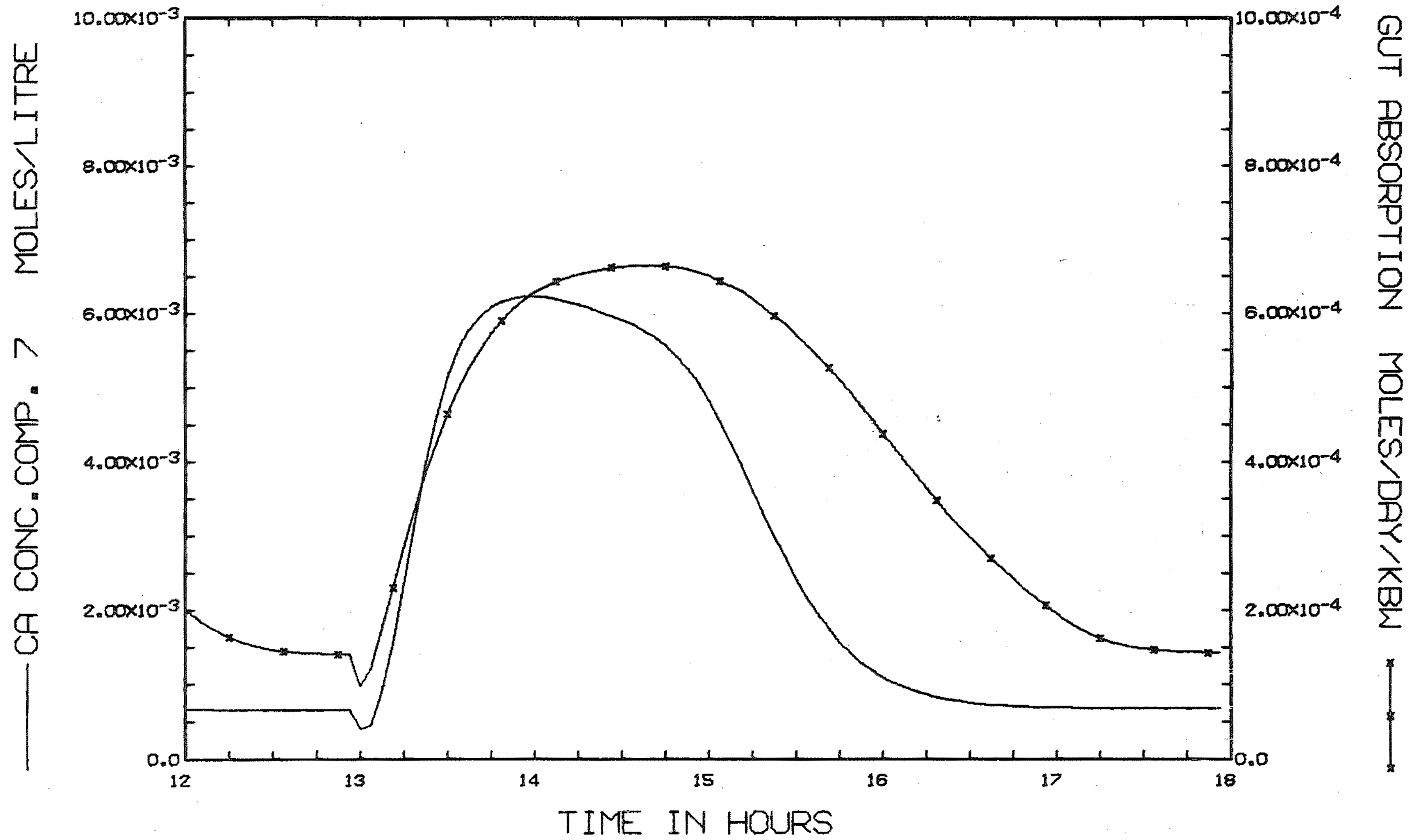


Figure 5.2 Jejunal Ca concentration & rate of gut absorption during meal

these compartments.

As a consequence of the fixed plasma volume (V_1) the inflow of calcium with the digestive juices in response to a meal causes a slight reduction in the plasma calcium concentration immediately following the simulated ingestion of a meal - before increased calcium absorption is adequate to compensate for this loss (figure 7.2). This is contrary to expectation as the calcium in the intestinal juices is less concentrated than it is in the plasma.

Hence the fluid dynamics in both the intestinal and plasma compartments together with the equations controlling the absorption of calcium require further attention if some of the present simulations (Chapter 7) are to be improved and more complex situations reproduced. A basic source of difficulty in this regard is that CAMET2 is required to simulate the oral intake of calcium in two distinct forms: as a solution (common in clinical experiments such as tracer absorption studies) and as part of the normal dietary intake. The handling of solids and liquids by the digestive tract is different, especially by the stomach (Hunt & Pathak 1960, Toyama & Blickman 1971). How this affects calcium absorption is not certain, but it is probably important.

5.5 The Plasma Calcium Concentration and Digestive Juice Calcium.

Two conflicting schools of thought exist on the relationship between the concentration of calcium in the serum and that appearing in digestive juices.

The evidence advanced against any change in the calcium content of digestive juices is twofold. Firstly there is the data of Cramer (1963) on the perfusion of distilled water through isolated intestinal loops of a dog. The amount of calcium recovered from the perfused fluid was constant over a range of 1.5 to 3.75 m.moles/litre of plasma calcium. Secondly, the lack of an increase in faecal calcium after repeated daily intravenous infusions of calcium (McCance & Widdowson 1939, Baylor et al. 1950) has been cited as support for this point of view. This type of evidence is not convincing however, as the hypercalcaemia associated with infusions is transient and would not be expected to increase faecal calcium to a significant extent, especially as the endogenous calcium forms only a small part of the total (Heaney & Skillman 1964). The experiment of Baylor et al. (1950), involving the twice daily intravenous administration of calcium totalling 550 mg, was simulated to check this point (for one three meal day only). The rate of secretion of calcium into the intestinal compartments was allowed to vary with the plasma calcium concentration. In table 5.4 the results of this simulation together with those of Simulation 11, involving no infusions, are given.

Table 5.4 The Effect of Infused Calcium on Faecal Excretion

Simulation	Total Intestinal Secretion (m.moles/kg)	Total Faecal Calcium (m.moles/kg)
6	0.216	0.141
11	0.198	0.133

SIMULATION 6 THREE MEAL DAY WITH CALCIUM INFUSIONS

Source Program : 72150 No : 72167-2

A three meal day with two separate calcium infusions totalling 0.21 m.moles/kg was simulated to examine the effects on the total faecal excretion of calcium. The relatively small increase in the parameter, while illustrating the lack of influence that intestinal secretions have in controlling calcium levels, also shows that increased intestinal secretion may occur during hypercalcaemia without being detected experimentally.

References : Baylor et al. (1950)

Simulated Time : 1 day

Model Inputs : [A] Fluid and calcium oral inputs as in Simulation 11.

[B] Calcium Infusion (m.moles/kg/day) Time (days)

Calcium Infusion (m.moles/kg/day)	Time (days)
0.0	0.0
2.54	0.375
0.0	0.416
2.54	0.542
0.0	0.583

Results : [A] Parameters during simulation

C_1 (m.moles/litre)	R_{fecl}	R_{gsec} (m.moles/kg/day)	R_{urin}	
2.50	0.0021	0.143	0.0827	0.0
3.01*	0.184	0.492	0.251	0.58
2.76	0.0023	0.158	0.178	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{fecl}	S_{gsec}	S_{inf}	S_{urin}	
0.118	0.141	0.216	0.209	0.159	1.00

*Maximum plasma calcium concentration

The original point made by Baylor that the gut did not represent a significant route for the excretion of calcium from the plasma is supported by these results. However, considering the small number of subjects (3), the variability exhibited between the experimental control periods, and the small increase (6%) in the faecal calcium as a result of the simulated infusions, the experiment cannot be said to reveal, one way or the other, whether additional calcium is secreted into the gut during hypercalcaemia.

A dependence of the calcium in intestinal juices on plasma calcium has been directly demonstrated in animals (Gran 1960b). In hyperparathyroid humans with hypercalcaemia a significantly elevated calcium concentration in the basal gastric secretion has been observed, although no significant difference could be detected in stimulated secretion compared with normal controls (Christiansen & Aagaad 1971). Hansky (1967) also measured almost double (1.33 m.moles/litre) the mean normal calcium concentration (0.75 ± 0.2 m.moles/litre) in two patients with hypercalcaemia associated with hyperparathyroidism. However the position is more complex than a simple change in the calcium concentration as it appears that the volume of gastric secretions (and perhaps other secretions) is related to the calcaemia (Murphy et al. 1966, Hotz et al. 1971). In man both the volume and the acid output of gastric secretion increases with increased plasma calcium.

For instance, Murphy et al. (1966) measured an increase from 1.6 ± 1.1 m.moles/hour to 10.0 ± 6.8 m.moles/hour in the basal gastric acid secretion of 8 subjects following a calcium infusion.

Gran (1960b) calculated the total excretion of calcium into the intestinal tract of rats using the data from balance and tracer experiments. Although the equations he used were approximate, the calcium excretion depended linearly on the concentration of ionized calcium in the plasma. Using similar methods Toverud (1964) observed the same phenomenon in rats whose blood calcium was first lowered by parathyroidectomy and then raised by parathyroid hormone injections.

Thus apart from the experience of Cramer (1963), using intestinal fistulas under conditions differing considerably from the normal, the evidence suggests that there is a connection between the gut calcium secretions and the calcium concentration of the plasma. If the calcium in this fluid is simply the result of bulk transfer of plasma water together with its ultrafiltrable calcium from one side of a membrane to another then this is a reasonable conclusion. It is still true if there is a greater resistance to the flow of calcium than to water.

It is assumed here that the linear relationship observed by Gran (1960b) for ionized calcium also holds for the total calcium (C_1). Accordingly, in equations 2.24, 2.27 and 2.28 the rate of calcium secretion into the gut compartments is multiplied by a factor of $C_1/\overline{C}_1$,

where $\overline{C}_1$ equals 2.5 m.moles/litre. Considering the available physiological evidence it is doubtful that this is more than a rough approximation. Furthermore ionized calcium is not a simple function of the total calcium. The ultrafiltrable calcium, with respect to a cellophane membrane, does however form a remarkably constant 60% (56 - 62%) of the total serum calcium ($p \ll 0.001$) over a range in serum calcium of 1.25 to 3.25 m.moles/litre (5 to 13 mg/100 ml) at constant body temperature and pH (Robertson & Peacock 1968). Ionized calcium comprised 82.7 ± 2.9 % of the ultrafiltrable calcium.

Except perhaps where the simulated blood calcium is markedly outside the normal range, as in some disease states, this addition makes a negligible difference to the model dynamics. In disease, particularly where PTH is involved, other effects on calcium absorption complicate the interpretation of changes in the quantity of endogenous faecal calcium (section 3.7). Alterations in the quantity of secreted intestinal calcium is also a factor to be taken into consideration in tracer experiments where the calcium absorption is being measured-as the results are sensitive to such changes (section 7.3).

5.6 Endogenous Faecal Calcium

The endogenous faecal calcium is derived from the various digestive secretions - after much of the secreted calcium is reabsorbed and recycled back to the body. Until the advent of tracer calcium investigations the endogenous fraction of the total faecal calcium was impossible to determine (except in the special case of fasting) because it is indistinguishable from the unabsorbed dietary calcium.

In order to calculate the simulated endogenous faecal calcium, a three meal day (as in Simulation 11) was simulated but MT_1 (the quantity of tracer in the plasma compartment) was set equal to 1.0×10^{-3} m.moles/kg and the appropriate differential equation (2.2) was altered to keep MT_1 constant throughout the simulation. By this means the interpretation of the results is made much easier, since the specific activity in the plasma compartment (SA_1) only fluctuates ±3% as the quantity of stable calcium alters in response to the simulated calcium intake. Hence the quantity of endogenous faecal calcium (S_e) is calculated as

$$SA_1 = \frac{MT_1}{M_1} = \frac{ST_{fecl}}{S_e} \qquad (1)$$

where M_1 represents the quantity of calcium in compartment 1 and ST_{fecl} the total amount of faecal tracer over the simulated day. In Simulation 7 S_e was 0.036 m.moles/kg or 92 mg on the basis of a 65 kg mass.

The intestinal tracer excretion, plotted in figure 5.3 is concentrated at the simulated meal times and is associated with the unabsorbed fraction of the dietary calcium. As the model does not include the colon, neither the mixing of the intestinal contents nor the transit delay of a few days that occurs in the large intestine are simulated. Although the rate of excretion into the gut of calcium (and hence tracer calcium) increases by approximately fourfold during a simulated meal (figure 5.1) this only accounts for a small part

Source Program: 72150 No : 72203-1

The dynamics of urinary and faecal tracer excreted from the body during a simulated three meal day are demonstrated. The manner in which calcium is handled by the kidney and the gut gives rise to errors in the conventional methods of calculating the total calcium secreted into the gut (S_{gsec}) and, to a lesser extent, in calculating the endogenous faecal calcium (S_e).

Reference: Heaney & Skillman (1964)

Simulated Time: 1 day

Parameter Change: MT_1 set to 1.0×10^{-3} m.moles/kg*

Equation Change: (2.2) $\dot{M}T_1 = 0.0$**

Model Inputs: Fluid and Calcium oral inputs as for Simulation 11.

Results: Accumulated Totals (m.moles/kg)

[A] Stable Calcium

C_{bal}	S_{acc}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	Time (days)
0.001	0.115	0.127	0.197	0.313	0.0779	1.00

[B] Tracer Calcium ($\times 10^3$)

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST_{urin}	Time (days)
0.661	0.433	0.0790	0.444	0.358	0.175	1.00

* Since the model is mathematically linear with respect to tracer calcium the numerical value of the initial quantity of tracer calcium is arbitrary.

** This change has the effect of keeping MT_1 constant (at 1.0×10^{-3} m.moles/kg) throughout the simulation.

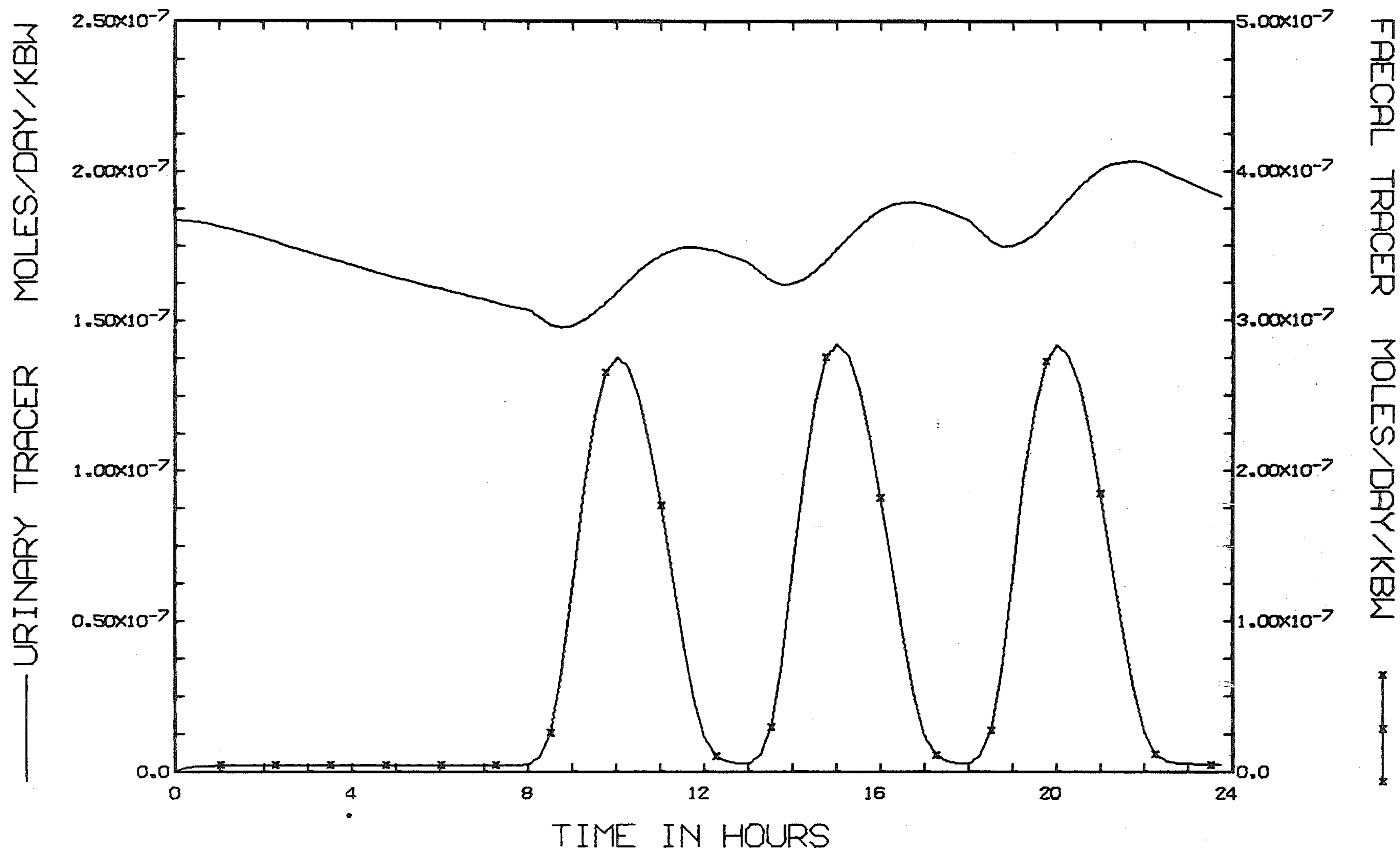


Figure 5.3 Urinary & faecal tracer excretion : Stable plasma specific activity

of the increased faecal tracer excretion that is observed. The simulated effect is however exaggerated to some extent by the abnormally high absorption parameters of the model, particularly evident at low calcium concentrations (section 5.4). By comparison the rate of excretion of urinary tracer is much more uniform over the day (figure 5.3).

This pattern of tracer excretion is important in that it means that relations such as (1), where the average specific activity is used, or its equivalent

$$S_e = ST_{fecl}/(ST_{urin}/S_{urin}) \quad (2)$$

(where ST_{urin} denotes the total urinary tracer excretion and S_{urin} the stable urinary calcium) commonly used to calculate the endogenous faecal tracer are not as exact as has been assumed. Consider a situation analogous to Simulation 7 except that the tracer calcium is not kept constant but allowed to decay, as in nature, by distribution and excretion (Simulation 8). This produces a high specific activity in the urine over the first 8 hours, but as virtually all tracer calcium excreted into the gut is reabsorbed in the absence of dietary intake, it is not until the first simulated meal that any appreciable tracer dose is delivered to the colon (figure 5.4). By this time however the plasma specific activity has declined appreciably (Simulation 15). When the simulated excretion data of Simulation 8 was substituted into equation (2) a value of 0.029 m.moles/kg for the endogenous faecal tracer was obtained - or 19%

SIMULATION 8 TRACER EXCRETION II

Source Program : 72150 No : 72219-1

Simulation 7 was repeated but the initial dose of plasma tracer was allowed to change under the model equations. The stable calcium dynamics (and hence the quantity of endogenous faecal calcium) in the two runs were identical. Yet when conventional equations are applied to calculating the quantity of endogenous faecal calcium, using the simulated excretion data, a significant difference between the calculated values from Simulations 7 and 8 is apparent.

Reference: Heaney & Skillman (1964)

Simulated Time: 1 day

Parameter Change: MT_1 set to 1.0×10^{-3} m.moles/kg

Model Inputs: Fluid and calcium oral inputs as in Simulation 11.

Results: Accumulated Totals (m.moles/kg) Time (days)

[A] Stable Calcium

C_{bal}	S_{acc}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	Time (days)
0.001	0.115	0.127	0.197	0.313	0.0779	1.00

[B] Tracer Calcium ($\times 10^3$)

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST_{urin}	Time (days)
0.914	0.115	0.0174	0.111	0.0931	0.0461	1.00

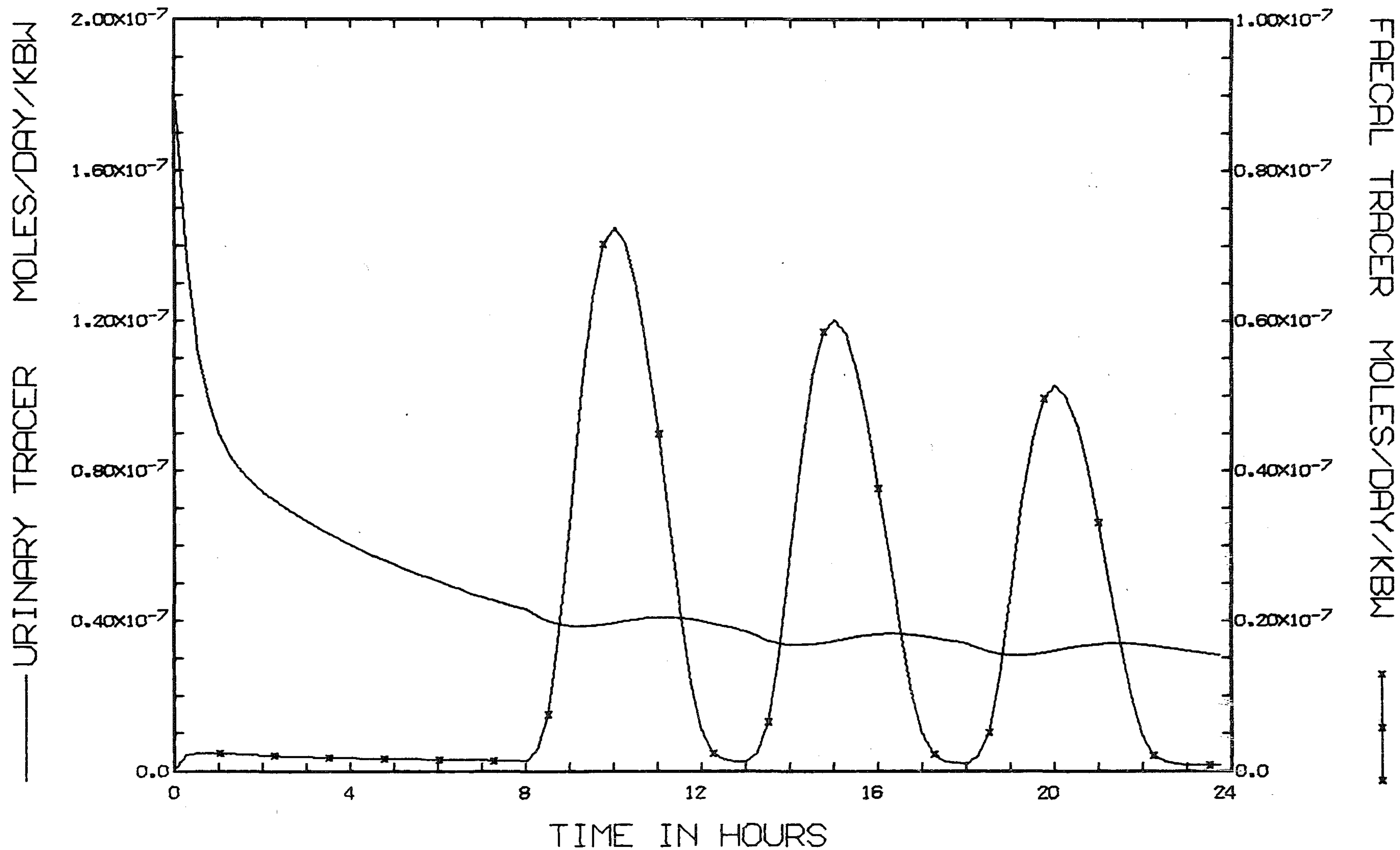


Figure 5.4 Urinary & faecal tracer excretion : Decaying specific activity

less than the accurate value of 0.036 m.moles/kg from Simulation 7. Basically this discrepancy is caused by the strong bias of the endogenous faecal calcium to reflect the plasma specific activity *during* meals. Whether or not this observation is important experimentally would depend on the experimental regime, but under some circumstances it could conceivably cause appreciable errors.

Livesey (1970) demonstrated that the method of Heaney & Skillman (1964) in calculating the total calcium secreted into the gut per day (S_{gsec}), using measurements of the endogenous faecal calcium, seriously underestimated the actual quantity secreted. Without going into details (see Heaney & Skillman 1964, and Livesey 1970) it was assumed that, at the most, the fraction of endogenous calcium secreted into the digestive tract that was reabsorbed was the same as that of the dietary calcium. Thus it was considered that S_{gsec} lay between the endogenous faecal calcium corrected for this absorption, and the measured endogenous faecal calcium assuming that no absorption occurred. On this basis Heaney & Skillman (1964) calculated from 9 determinations in 7 normal subjects a range

$$0.045 \leq S_{gsec} \leq 0.078 \quad \text{m.moles/kg.}$$

When the excretion data of Simulation 7 was substituted into the same equations relatively good agreement was obtained.

$$0.036 \leq S_{gsec} \leq 0.092 \quad \text{m.moles/kg}$$

However, unlike the experiment *in vivo*, the true value of S_{gsec} is known in the case of Simulation 7 and is

equal to 0.20 m.moles/kg. This value is well outside the calculated range. As was pointed out by Livesey a cause of this disagreement is that the fraction of the dietary calcium absorbed by the gut is not constant as was assumed, but is a function of many factors - including the intestinal content of calcium. Another source of error, related to the foregoing, is that the possibility of a greater fractional absorption of the secreted calcium than of the dietary calcium was not considered. From an examination of both intestinal physiology (section 5.4) and the CAMET2 results, it is clear that very efficient reabsorption of calcium occurs in the absence of dietary calcium. Physiologically this type of behaviour is seen in experiments such as that of DeGrazia et al. (1965) who found that by increasing the load of stable calcium carrier associated with an oral tracer dose from 10 to 200 mg, the absorption of the tracer fell from 80 to 26%.

Essentially the method of Heaney & Skillman (1964) provides an approximate estimate of the amount of calcium emptied into the intestinal lumen during meals.

CALCIUM AND THE BONE

6.1 In this chapter the processes by which calcium and its radioactive isotopes are deposited and removed from the skeleton are briefly examined. Equations are introduced to cover the growth and removal of both the mineral phase and the protein matrix of bone. Further transfer of calcium between the body fluids and bone mineral occurs by isoionic calcium exchange. Since processes that require an exact exchange cannot contribute to the regulation of plasma calcium most of the exchange processes in CAMET governing the dynamics of stable calcium are removed. They are retained for tracer calcium because exchange is effective in removing it from blood.

6.2 Bone: Structure and Turnover

Bone is comprised of two distinct but interdependent phases - the inorganic bone mineral and the organic matrix. The nature of these phases is discussed in this section; more detailed coverage may be found in the excellent reviews that have appeared recently on both the mineral (Eanes & Posner 1970) and the matrix of bone (Schiffmann et al. 1970, Grant & Prockop 1972).

The major ions in human bone mineral, expressed as a percentage weight of the total dry fat-free bone, are calcium (22.5), phosphate (10.3), sodium (0.52), magnesium (0.26), carbonate (3.5), chloride (0.11) and fluoride (0.054) with lesser amounts of other

elements (Zipkin 1970). The crystallographic structure of bone mineral is not known. The measurements available are consistent with a structure similar to, although not identical with, the naturally occuring hydroxyapatite ($Ca_5 (PO_4)_3OH$), whose unit cell is a right rhombic prism containing twice the stoichiometric number of atoms (Kay et al. 1964). Certain features of bone mineral indicate that it differs from the natural or synthetic hydroxyapatite and chief among these is the non-stoichiometric ratio of the constituent ions. The variation in the content of minor elements from one sample to the next is considerable, but even the calcium and phosphate contents fluctuate about the expected value. In part, this variability is believed to be caused by substitutions within the lattice (for example chloride and fluoride in the hydroxyl positions). Surface adsorption and crystal lattice defects have also been put forward as contributary causes. Bone crystals are extremely small. Recent X-ray diffraction studies have put the maximum dimension at about 100 Å (Posner et al. 1963). However, somewhat larger dimensions have been found using the electron microscope and it has been suggested that end-to-end fusion of the crystals would accommodate both results (Molnar 1959).

The presence of a separate non-crystalline or amorphous mineral phase, in proportions markedly affected by even mild preparative methods, further complicates the structure of bone mineral. Evidence for the existence of the amorphous mineral comes from X-ray diffraction, electron microscopy and studies of the precipitation and chemical properties of calcium phosphate minerals (Termine &

Posner 1967, Eanes & Posner 1970). The non-diffracting amorphous granules range in diameter from 60 to 200 Å (Molnar 1959) and their appearance in developing bone tissue, seen under the electron microscope as a fine film, precedes that of the hydroxyapatite. Unlike the hydroxyapatite they have no apparent structural relationship to the collagen matrix of bone. The subsequent development of the hydroxyapatite crystals is in part controlled by, and at the expense of, the amorphous phase (Quinaux & Richelle 1967, Termine & Posner 1967). Yet, even in mature bone tissue the amorphous mineral accounts for about 40% of the total inorganic content of bone (Harper & Posner 1966).

The organic matrix of bone is primarily the protein macromolecule collagen. Generally it is quoted as forming 90 - 96% of the total organic fraction of bone but recently Dequeker & Merlevede (1971) could find only 80% collagen in the dry organic matrix of human trabecular bone. The basic subunit of collagen is the tropocollagen molecule, which in turn consists of 3 polypeptide chains of about 1000 amino acid residues. The polypeptides, each of a molecular weight of about 95,000, are wound about each other in a helical structure for most of their length. By the formation of crosslinks between the chains the tropocollagen molecule, 3000 Å long and 15 Å in diameter, is formed (Grant & Prockop 1972). Two of the three chains are identical (the α_1 chains) but the third differs in amino acid composition (α_2).

Until recently it had been thought that all the collagen found within an animal was identical but chicken cartilage collagen has been found to differ from previously investigated chicken collagen in that it consists of three identical polypeptide chains of a new variety (α_1 type II) (Miller & Matukas 1969). The collagen microfibrils are built up by the ordered arrangement of the tropocollagen molecules with their long axes parallel to that of the fibre. The whole structure is strengthened by an increasing degree of covalent bonding between the tropocollagen sub-units. When viewed under the electron microscope, the alignment of certain charged regions of neighbouring tropocollagen molecules, lying parallel to one another in the microfibril, gives rise to a distinct banded periodicity of 680 Å. Significantly, the same distance separates the crystals in the early stages of nucleation and growth. Another interesting feature is the presence of 'holes' at the end of each tropocollagen molecule separating it from its immediate neighbours along the axis of the microfibril. Each hole is 410 Å long with the diameter of the tropocollagen molecule (Grant & Prockop 1972).

There exist a number of theories to account for the deposition of bone mineral on the organic matrix but, as yet, none are wholly satisfactory.

[A] Extracellular biological mechanisms are supposed to exist, which raise the calcium and phosphate concentrations to levels sufficient to cause spontaneous precipitation of bone mineral.

[B] The presence of regular features in the collagen fibres and the apparent structural association between the collagen and the bone crystals has given rise to much speculation that the development of the bone mineral is controlled in some way by the collagen matrix, and even that the holes in the matrix represent nucleating sites (primary heterogeneous nucleation). The minimum ion product of calcium and phosphate ([Ca] x [PO_4]) (where [PO_4] is the sum of inorganic phosphorus in its several forms) required for the spontaneous precipitation of hydroxyapatite *in vitro* is about 4(m.moles/litre)^2; under certain conditions and in the presence of foreign bodies this may be reduced to 2.8 (m.moles/litre)^2 - and still further in the presence of collagen (Fleisch 1964). The normal concentration of ionic calcium is about 1.3 m.moles/litre and inorganic phosphate about 1 m.mole/litre. Once seeded the crystal growth can continue even in the presence of relatively low ion concentrations (Katz 1971). Thus while the idea of collagen nucleation takes into consideration the undersaturation of body fluids in calcium and phosphate with respect

to crystal seeding, in order to account for histological changes observed in the matrix before calcification commences it has been proposed that nucleation follows the alteration or the removal of inhibiting ground substances.

[C] There are theories which, while incorporating elements of [A] and [B], propose that the amorphous bone salt is deposited before crystals form. The hydroxyapatite is thought to develop by primary heterogeneous nucleation at specific sites on the collagen, perhaps followed by secondary nucleation (the self-seeding of crystals) but with the amorphous granules supplying material for the developing, thermodynamically favoured, crystals. These theories are fully in accord with the observed appearance and subsequent decrease in the quantity of amorphous mineral in calcifying bone tissue (Quinaux & Richelle 1967, Termine & Posner 1967).

However, since the concentrations of calcium and phosphate necessary to initiate spontaneous precipitation of amorphous granules are greater than exist in biological fluids it is necessary to postulate some form of localized concentration of ions. Indeed, *in vitro*, the amorphous calcium salts are *more* soluble than hydroxyapatite - although at sufficiently high ion concentrations the precipitation of the amorphous granules are kinetically favoured over the hydroxyapatite crystals. With increasing experimental support, the idea has been put forward (Eanes & Posner 1970, Lehninger 1970) that the bone cells are responsible for the creation of the

conditions under which amorphous salt precipitation occurs. Active transport of the ions to the mineralization front or even the formation of amorphous granules within osteoblast mitochondria, in the first instance, with subsequent extracellular deposition have been suggested as possible mechanisms. Certainly the osteoblasts are intimately associated with areas of osteoid tissue undergoing calcification (Arnott & Pautard 1967) and mitochondria have a remarkable capacity to concentrate calcium and phosphate from an external solution (Lehninger 1970, Wasserman & Kallfelz 1970).

Notwithstanding the lack of knowledge of the processes involved in the deposition of mineral in bone there is a general consensus that elaboration of the collagen matrix takes place before the bone salts are precipitated. This observation forms the basis of the equations governing bone formation in the calcium metabolism model. The rather unphysiological concept of an unmixed pool in CAMET (figure 1.1) is replaced in CAMET2 by two related pools: the bone calcium (M_{bca}) and the bone collagen (M_{bcoll}). Analysis of dry, fat-free human skeletons has shown that close to 1/3 is organic and 2/3 is inorganic in nature (Trotter & Peterson 1955). A slightly smaller proportion of mineral (57.1%) has been reported by Zipkin (1970) as the mean of results taken from the literature for individual bones. Ninety per cent approximately, of the organic fraction is collagen (Eastoe 1956) and 22.5 (19.3 - 25.6)% of the total dry fat-free bone is

calcium (Zipkin 1970). The relative proportions of the two phases of bone change little with age. The organic content of trabecular bone per unit weight does not change with age or porosity (Dequeker & Merlevede 1971) but the quantity of mineral per unit weight of bone may however increase slightly with age, at the expense of water. Partly, this is brought about by the plugging of Haversian canals with amorphous salts (Arnold 1960, Jowsey 1960). In this respect osteoporotic bone, while of a lower density overall, is not significantly different from normal age-matched bone. Thus, with some experimental justification, the proportions of collagen and calcium in mature mineralized bone are considered constant in CAMET2 : given one the other may be calculated using the proportionality constants K_{pr} and K_{rp} derived from the above data. Further it is assumed in CAMET2, in order to greatly simplify the computation, that the bone collagen is either fully mineralized or not mineralized at all. Hence in the model the unmineralized collagen (U_{mcoll}) is that part of the bone collagen pool that is not calcified by the bone calcium

$$U_{mcoll} = M_{bcoll} - K_{pr} M_{bca}$$

Of course in nature there exists a continuum in the degrees of osteon calcification, but the rapid initial mineralization of newly synthesized collagen to about 75% of the maximum, and the slow turnover of adult bone results in a very small quantity of unmineralized collagen. At any one time only a few percent of all osteons in adult bone are in the process of formation (Jowsey 1960).

From an estimate based on tetracycline labelling experiments (Frost 1969) the rate of bone turnover is taken as 10% of the total skeleton per year. R_{coacc}, the rate of bone collagen synthesis, is set equal to 0.0058 g/kg/day and is constant throughout all the simulations. The many factors that influence this rate (Rasmussen 1968) have still to be investigated and included in the model as it is developed to cover long term aspects of calcium metabolism.

The rate of collagen resorption (R_{codec}) is also set equal to 0.0058 g/kg/day (at a calcium concentration of 2.5 m.moles/litre) to give overall skeletal balance. It should be noted however that after the age of 40, or thereabouts, these rates become unbalanced in favour of bone resorption (Garn et al. 1967). Frost (1969) puts resorption at 10% of the skeleton per year and formation at 9.7% in the adult. Over long periods of time such an imbalance can result in the bone disorder osteoporosis - or insufficient bone mass. In CAMET2 the rate of collagen resorption is made inversely proportional to the calcium concentration (equation 2.46) since it appears, on current evidence, to be sensitive to changes in the calcaemia - probably through the relatively rapid action of CT (section 3.6). The much greater effect of PTH in increasing bone resorption, emphasized by its absence in section 4.6, remains to be incorporated.

The rate of bone collagen deposition and removal are made to control the corresponding calcium rates in the model. Thus formally, the rate of calcium decretion from the bone compartment is represented as following the removal of the collagen matrix although, for all practical purposes, the simulated removal of both the matrix and the calcium is simultaneous.

$$R_{dec} = K_{rp} R_{codec}$$

On this point the physiological evidence is in conflict: some investigators favour the view that the mineral is removed first since unmineralized collagen is more susceptible to degradation by collagenase (Stern et al. 1970). Whatever the mechanism and the sequence of bone resorption there is much evidence to support a close relationship between the turnover of the two main components of the skeleton. Apart from the almost unchanging proportions of collagen and calcium in bone the biochemical studies of Firschein (1967) demonstrated that a direct relationship between the turnover of collagen and mineral exists under normal circumstances. Deductions about the turnover of one component may hence be made from a knowledge of the other. In passing it is noticeable that while there are pathological conditions such as rickets where collagen is laid down but not mineralized, there appears to be no documented instances of selective mineral removal from bone leaving the collagen matrix intact.

The CAMET2 equation for the deposition of calcium in bone (R_{acc}) is

$$R_{acc} = K_c \, K_{oss} \, U_{mcoll}$$

where $K_c = C_1/\overline{C}_1$

K_{oss} denotes a rate constant

U_{mcoll} the quantity of unmineralized collagen

Thus the rate of deposition of calcium is made directly proportional to the quantity of unmineralized collagen. *In vitro* this is the case (Wadkins 1968) and might reasonably be expected *in vivo*. The rate of calcium accretion is also made directly dependent on the calcaemia. There is an impaired deposition of mineral in hypocalcaemia of whatever cause. However it is probable that in nature the relationship is not linear. In hyperparathyroidism, for example, the width of uncalcified osteoid tissue is larger than normally observed despite high serum calcium (Jowsey 1968). The phosphate concentration (which in hyperparathyroidism is usually low) is also important. In fact a linear relationship between the osteoid tissue width and the calcium-phosphorus product of the blood has been demonstrated ($p < 0.001$) (Jowsey 1968). Richelle et al. (1966) have proposed second order kinetics to describe the appearance of calcium in the collagen matrix.

$$R_{acc} \propto (Ca_{max} - Ca)^2$$

where Ca is the mass of calcium per unit volume and Ca_{max} the maximum level of bone calcification. Estimates of the time taken for collagen mineralization to the 75% level vary from a few hours to a week. The rate constant

K_{oss} which controls this rate in CAMET2 (and also includes the proportionality constant K_{rp}) was arbitarily selected to fall within this range - such that in the presence of stable plasma calcium a quantity of unmineralized collagen would be half-filled with mineral in 2.31 days.

The differential equations (2.16) and (2.17) describe the changes in the quantities of bone calcium and bone collagen respectively. For a 65 kg mass the initial quantity of bone calcium amounts to 1103g and bone collagen 1380g. These values were choosen to reflect typical calcium and collagen contents of the adult skeleton (Trotter & Peterson 1955) and in a ratio such that skeletal balance at t = 0 is achieved.

$$R_{acc} = R_{dec} = 0.116 \text{ m.moles/kg/day}$$

$$R_{coacc} = R_{codec} = 0.0058 \text{ g/kg/day}$$

The rate of calcium turnover is determined by the rate of collagen turnover and at 0.116 m.moles/kg/day is equivalent to 302 mg/day. Such a mineralization rate is close to the 250 mg/day predicted by Burkinshaw & Marshall (1971) from their calcium metabolism model comprised of a continuously expanding exchangable pool to account for exchange, but is only half the corresponding rate in CAMET which included a considerable element of slow exchange.

Naturally, the two bone pools alter negligibly over the course of the short term simulations described in the present work, but eventually it is hoped to extend the model to examine long term processes such as those

involved in the development of rickets and osteoporosis. It is evident that the bone still represents the most underdeveloped aspect of the present model. A comprehensive model will need to incorporate the time delayed action of PTH in removing the calcium from the skeleton, and the factors that alter the rate of bone formation, and it must explicitly include the action of CT on bone resorption. Another aspect that requires consideration is the apparent semi-autonomous coupling of the bone formation and resorption rates that maintains a degree of skeletal homeostasis over long periods of time in the face of chronic stimulation of either formation or resorption.

6.3 Calcium Exchange

Some of the exchange properties of synthetic hydroxyapatite crystals in aqueous suspension have been examined (Neuman & Neuman 1958, Pak & Bartter 1967). It was found (Pak & Bartter 1967) that the uptake of Ca^{45} by suspended hydroxyapatite crystals could be described (as the fraction of the initial isotope dose remaining in solution) by

$$Ca^{45}_{soln,t} = 0.240e^{-\alpha_1 t} + 0.085e^{-0.177t} + 0.054e^{-0.0043t} + 0.700e^{-0.000014t}$$

where the rate constants are expressed as the fractional change in radioactivity per minute. In like manner the release of Ca^{45} into solution from prelabelled crystals (expressed as the fraction of the original tracer remaining in the crystals) was

$$Ca^{45}_{cryst,t} = 0.400e^{-\alpha_1 t} + 0.076e^{-0.126t} + 0.059e^{-0.0059t} + 0.523e^{-0.000015t}$$

The exchange dynamics up to 65 hours were covered - but the rapidity of the initial uptake and release of radiocalcium did not permit the determination of α_1. The isoionic nature and the reversibility of the exchange (indicated by the numerical similarity of the above relations) were demonstrated. Evidence was also produced suggesting that the last exponential term described intracystalline exchange, a process known to be slow; the remaining terms covering exchange in the hydration shell and on the surface of the hydroxyapatite.

Similar responses (a fast initial uptake of radiocalcium followed by a much slower phase) are observed consequent to intravenous tracer administration in man (Simulation 15). However, ion exchange is more complex when the living organism is considered since the transport component of bone ion exchange is important. Only 5% of the cardiac output of blood passes through the skeleton and, in general, the crystal surfaces are much less accessible to the medium than synthetic hydroxyapatite suspended in solution. Rowland (1966) demonstrated, among other things, that the degree of exchange labelling of bone is strongly dependent on the proximity to vascular channels. Bone further than about 2000 Å from any vascular surface was lightly

labelled; detectable by autoradiography only after 24 hours or more. On the other hand, the uptake of isotope by the exposed surfaces was rapid and isotope disappearance from these surfaces paralleled that in the blood. Heavy labelling of bone formation and bone resorption sites was also observed. Neuman et al. (1968) examined the kinetics of calcium exchange by recycling a Ca^{45} solution through a column of hydroxyapatite crystals. From the results and from what is known about the skeletal blood flow it was estimated that to achieve full equilibration between blood and bone in man a period greater than 125 days would be required (in nature of course equilibration never occurs).

In CAMET it was assumed that the movement of stable calcium could be described by a multicompartmental model based on tracer calcium dynamics. While this is true for the steady state the assumption is not well founded if perturbations to the plasma calcium occur, since it implies that calcium is free to redistribute itself throughout all 4 exchangable compartments. This is contrary to expectation however as much of the movement between the pools represents ion exchange, a process which, by definition, cannot effect a net transfer of calcium. Thus if any rates of transfer between compartments 1, 2, 3 and 4 (figure 1.1) are strictly exchanges of calcium ions, then for any such rates $R_{I,J} = R_{J,I}$ but $RT_{I,J} \neq RT_{J,I}$. In the equations adopted now the following processes are taken

to be strict exchanges

$$R_{2,3} = R_{3,2}$$

$$R_{3,4} = R_{4,3}$$

but $R_{1,2}$ is allowed to differ from $R_{2,1}$ so permitting a net flow of stable calcium between pools 1 and 2. It seems safe to assume that the slow transfer of calcium to and from compartments 3 and 4 represents largely (and perhaps solely) ion exchange (Neer et al. 1967). The addition of these constraints to the model has considerably improved its predictions where large perturbations to the plasma compartment calcium are simulated over periods of greater than 4 hours (Chapter 4). The compartmental structure of CAMET2 for stable calcium is shown in figure 6.1.

6.4 Homeostasis of Plasma Calcium

The apparent lack of a physiological equilibrium, noted in Chapter 4, between blood and bone calcium important to the regulation of plasma calcium finds strong support in the perfusion experiments of Rodan et al. (1967). The blood circulation of the hind limb of anaesthetized dogs was isolated and pumped through an external loop. Calcium added to the circulation resulted in a stable, but elevated, concentration after initial distribution had occured. The rapid renal excretion of calcium that normally blunts hypercalcaemia stress (section 4.4) is not possible in the isolated limb and no local homeostasis mechanisms with a half life of less than 20 hours could be detected. After Ca^{47} was added to the hyper-

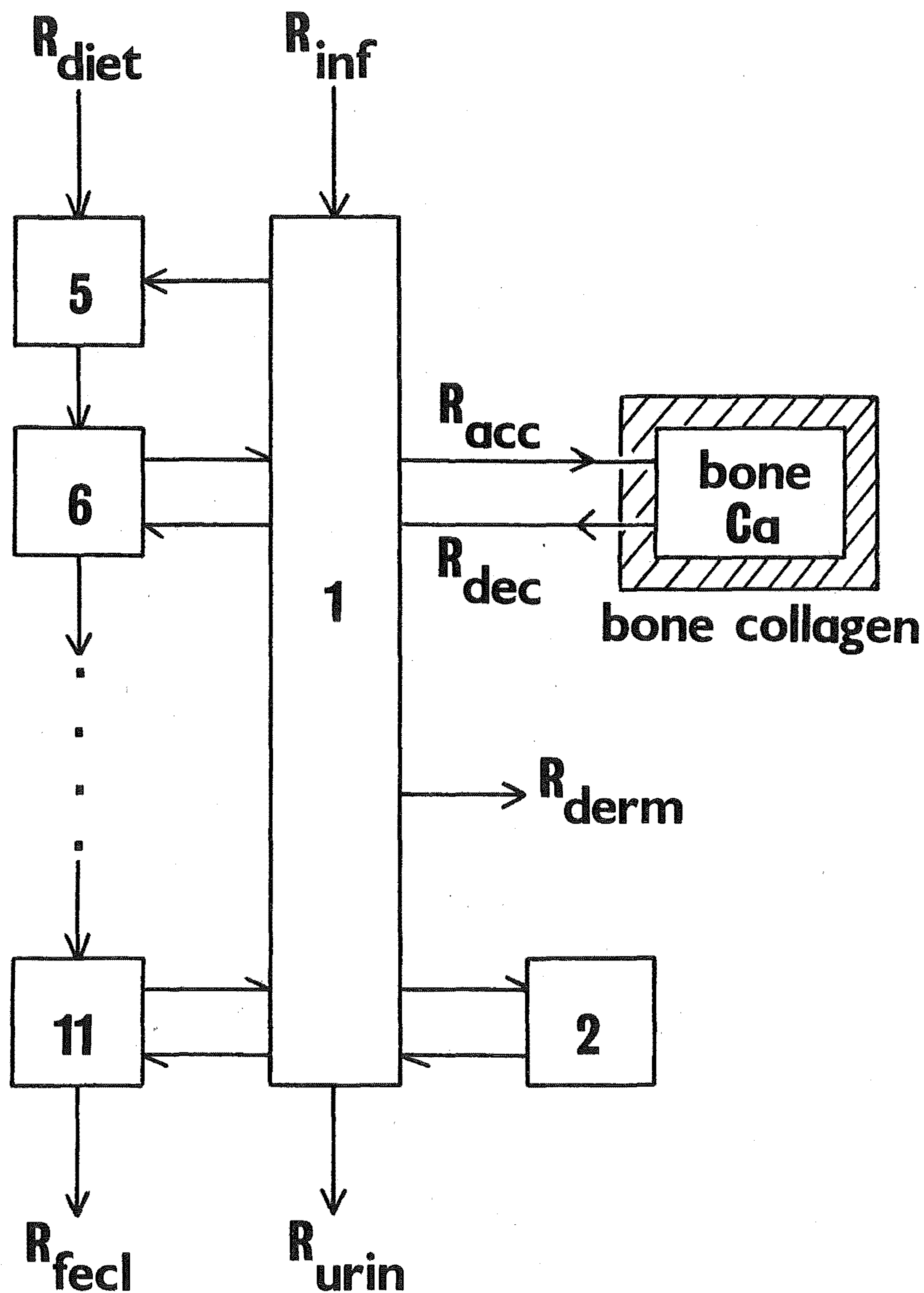


Figure 6.1 CAMET2 compartmental structure for stable Ca

calcaemic limb the specific activity of the plasma (in counts/ml) decayed in the same manner as in a control limb.

Support for the commonly held view that plasma calcium is largely regulated by some form of physiochemical equilibrium between calcium in bone and calcium in blood rests largely on the *in vitro* properties of bone mineral (Nordin 1957, Neuman & Neuman 1958, MacGregor & Nordin 1962, MacGregor & Brown 1965). For instance, Nordin (1957) showed that calf bone powder could maintain physiological calcium and phosphate concentrations when the pH of the solution was in the range 6.6 - 6.8. When this work was extended using human bone powder similar results were obtained (MacGregor & Nordin 1962). Although these results are interesting it has yet to be proved that such effects operate in living bone tissue either by the active regulation of the pH at bone surfaces or by a passive equilibrium between blood and bone.

It must, however, be recognized that there is a limited cushioning of perturbations in the calcium concentration of extracellular fluids which has not yet been explained. Perhaps evidence of this is most clearly seen in the infusion of EDTA to parathyroidectomized animals where a slow but limited upwards adjustment in the plasma calcium concentration occurs following the end of an EDTA infusion (section 4.6). The experiments of Rodan et al. (1967) showed that the same type of response was also obtained in the isolated limb as in the entire parathyroidectomized animal. They concluded that the additional calcium mobilized from outside the extracellular space

amounted to about 1/1000 of that in the limb bone.

It is assumed, without proof, that the second exchangable calcium pool of CAMET (figure 6.1) represents the calcium able to buffer changes in the extracellular fluids (largely contained in compartment 1). The anatomical origin of this labile calcium is uncertain. However Rodan et al. (1967) reported that as a result of EDTA administration to the perfused limb that the total calcium in the circulation rose by almost 50% (inclusive of EDTA-bound calcium) but the slow disappearance of Ca^{47} (expressed as counts/ml) was unaltered. Such a result suggests that the calcium mobilized by the EDTA was not in equilibrium with the tracer injected some 2½ hours earlier. If this is the case, then the representation of this calcium in CAMET2 as a rapidly exchangeable pool may be misleading. One plausible explanation is that the additional calcium in the plasma represents some of the considerable quantity of calcium (amounting to 3-4 g) associated with the soft tissues; including the walls of the blood vessels (Stoclet & Cohen 1966), bound to membranes (Gent et al. 1964) and associated with muscle tissue (Shanes 1963). By comparison the total calcium in the extracellular fluids, including the plasma calcium, amounts to little more than 1 g (Rich et al. 1961). In view of the very small concentration of ionic calcium in the cytoplasm of cells (on the basis of reports in the literature Rasmussen (1970) estimates between 10^{-2} and 10^{-5} m.moles/litre) this source can be ruled out. Only scattered evidence is available on the nature and extent of bound calcium in soft tissues but in at least one investigation the binding of calcium to the erythrocyte

(red blood cell) membrane has been found to be a function of the calcium activity of the bathing medium (Gent et al. 1964). To determine the importance of this passive buffering in calcium homeostasis then considerably more information about the distribution, binding and transport of calcium in the various soft tissues of the body is essential.

CALCIUM TRANSPORT IN MAN

7.1 The plasma calcium concentration is maintained at a remarkably constant level in the face of large, periodic inputs of calcium from the diet. This is brought about by the co-ordinated control of the transport of calcium by the intestine, kidney and bone. While considerable progress has been made in the understanding of the nature of the control processes in calcium metabolism (Chapter 3), detailed knowledge about the fine regulation of the serum calcium is lacking. A number of CAMET2 simulations involving minimal perturbations to the plasma calcium are examined in this chapter.

7.2 The Intake of Calcium from the Diet

Simulation 9 is a one day fast. Plasma calcium and urinary excretion decline steadily (figure 7.1) As is found (Heaton & Hodgkinson 1963) the increased urinary calcium due to meals disappears, although in real life factors not yet included in the model (Chapter 4) produce fluctuations not found in Simulation 9. Presumably the serum calcium, which is also responsive to dietary intake (Carruthers et al. 1964, MacFadyen et al. 1965), falls also, thereby reducing the renal loss of calcium.

However, in the experience of local investigators and from the scattered documented data that has been collected (Duncan et al. 1948, Scheck et al. 1966), prolonged fasting results in a gradual increase in

SIMULATION 9 FASTING

Source Program : 72150 No : 72183 - 1

The model was run for one day with no inputs of any sort. Plasma calcium fell by 9% and the rate of urinary calcium excretion by 42% compared with the initial values.

Simulated Time: 1 day

Results: [A] Parameters during simulation

C_1 (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	Time (days)
2.50	0.0827	0.10	0.60	0.001
2.37	0.0602	0.084	0.75	0.50
2.28	0.0476	0.073	0.86	1.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{dec}	S_{derm}	S_{fecl}	S_{urin}	Time (days)
-0.100	0.111	0.122	0.0364	0.0019	0.0621	1.00

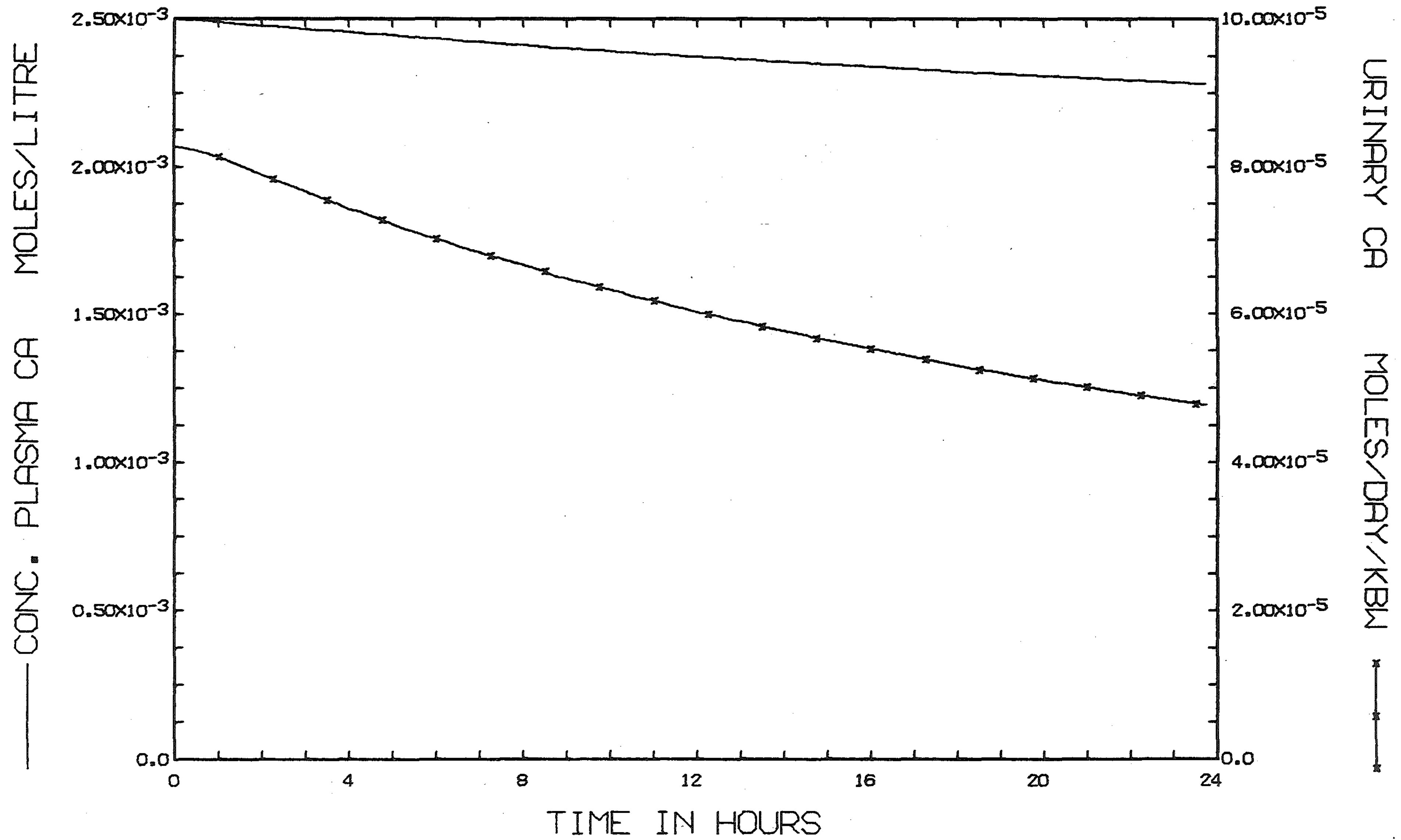


Figure 7.1 Plasma Ca concentration and urinary Ca excretion during fasting

urinary calcium to levels comparable with or in excess of control levels. The lowered serum pH developed by fasting subjects is probably responsible: metabolic acidosis has been implicated with increased urinary calcium (Duarte & Blande 1965, Nordin et al. 1967). A direct interrelationship between the renal transport mechanisms of the hydrogen and calcium ions is possible or, alternatively, it might be that the lowered pH produces this effect by inducing a shift in the partition of plasma calcium between its bound and unbound forms. The fraction of ultrafiltrable calcium increases significantly even with small decreases (0.25 pH units) in the pH (Robertson & Peacock 1968). This would reasonably be expected to produce an increase in the urinary excretion of calcium, an increase in PTH secretion and ultimately an increase in bone resorption - as must exist for the maintenance of the high rate of urinary calcium excretion during fasting.

Generally speaking, the loss of calcium in the urine is relatively insensitive to the dietary intake of calcium (Knapp 1947, Peacock et al. 1967). At least 3 mechanisms are responsible - the saturable nature of the intestinal calcium transport, the ability of PTH to maintain the plasma calcium by increasing bone resorption when short falls in dietary intake occur and, over longer periods of time, the adaption of the gut to a lowered level of calcium intake by increased absorption efficiency. Nevertheless, if the dietary calcium is reduced severely enough a fall

in the urinary calcium can be induced. Restricting the intake of calcium to below 250 mg per day forms the basis of the calcium deprivation test (MacFadyen et al. 1965, Nordin & Smith 1965) used in determining whether or not hypercalciuria is intestinal in origin. A low daily calcium diet of 0.081 m.moles/kg (210 mg) was simulated for 2 days (Simulation 10). The effects on serum and urinary calcium on the firs simulated day are compared to the results of Simulation 11 (dietary calcium of 0.250 m.moles/kg) and the experimental results of MacFadyen et al. (1965) derived from 27 subjects.

Table 7.1 The Effect of a Low Calcium Diet on the Serum Concentration of Calcium and its Renal Excretion

	MacFadyen et al. (1965)		CAMET2	
	Plasma Ca* (mg/100ml)	Urinary Ca (mg/day)	Plasma Ca* (mg/100ml)	Urinary Ca (mg/day)
Normal Dietary Ca	9.46 ± 0.24	198.8 ± 110.7	9.87	203.1
Low Dietary Ca	9.29 ± 0.22	135.7 ± 71.7	9.60	158.6**
Difference between means	0.17	63.1	0.23	44.5
Significance of difference	$p < 0.01$	$p < 0.02$	-	-

* mean of plasma calcium concentrations at 9.30 a.m., 12.30, 2.30, 4.30 p.m.

** urinary calcium from 8 a.m. on day 1 to 8 a.m. on day 2

Source Program : 72150 No : 72167-3

The calcium deprivation test described by Nordin & Smith (1965) was simulated to examine the effects of a low calcium diet on model predictions, especially the urinary calcium excretion. Three low calcium meals were simulated at the same times each day as in Simulation 11.

References: MacFadyen et al. (1965), Nordin & Smith (1965)

Simulated Time: 2 days

Model Inputs*:

Oral Calcium (m.moles/kg/day)	Oral Fluid (litres/kg/day)	Time (days)
0.0	0.0	0.0
1.8	0.513	0.333
0.0	0.0	0.348
1.8	0.513	0.541
0.0	0.0	0.556
1.8	0.513	0.750
0.0	0.0	0.765

Results : [A] Parameters during Simulation

C_1 (m.moles/litre)	C_{bal} (m.moles/kg)	R_{urin} (m.moles/kg/day)	
2.50	0.0	0.0827	0.0
2.37	-0.0581	0.0605	1.0
2.31	-0.0992	0.0509	2.0

[B] Accumulated Totals (m.moles/kg)

S_{acc}	S_{diet}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	
0.223	0.162	0.0681	0.379	0.472	0.121	2.0

*inputs repeated on second simulated day.

It seems likely that little or no net absorption of calcium occurs on 200mg of calcium in the food, even after adaption to the regime occurs (Malm 1958). Although the experimental ranges are wide this reduction in dietary intake results in a small, but statistically significant fall in serum calcium and a fall in the renal excretion of calcium. As in fasting the PTH interaction with the bone prevents a further drop in the serum calcium. A similar reduction in the plasma calcium was simulated by the model - resulting also in a reduction in the urinary calcium. Experimentally, a significant correlation ($p < 0.005$) was demonstrated between the change in the plasma calcium and the change in urinary excretion. Unlike the fasting situation there was no tendency for the urinary calcium to rise over 9 days of low calcium intake in 11 subjects (MacFadyen et al. 1965). Potentially, a detailed study of the endocrinological and physiological changes resulting from the imposition of low calcium diets should yield considerable data on calcium homeostasis in health. Fasting appears to be a separate case.

Next the model was used to simulate the consequences of 3 discrete oral calcium and fluid inputs, separated in time and of a suitable magnitude to represent the dietary ingestion of calcium in a 3 meal day, beginning at midnight (Simulation 11). The calcium content of each simulated meal (0.083 m.moles/kg) was chosen such that an approximate calcium balance was maintained over the simulated day (figure 7.4). The principal results are collected in table 7.2 in both m.moles/kg and in mg assuming a 65 kg mass.

SIMULATION 11 THREE MEAL DAY

Source Program : 72150 No : 72167-1

A simplified 3 meal day was simulated with three, 22 minute long, calcium and fluid inputs-corresponding to meals at 8 a.m., 1 p.m. and 6 p.m. In the light of the considerable body of evidence that has accumulated on the characteristics of normal human calcium metabolism the model predictions are considered.

Simulated Time: 1 day

Model Inputs:

Oral Calcium (m.moles/kg/day)	Oral Fluid (litres/kg/day)	Time (days)
0.0	0.0	0.0
5.5	0.513	0.333
0.0	0.0	0.348
5.5	0.513	0.541
0.0	0.0	0.556
5.5	0.513	0.750
0.0	0.0	0.765

Results: [A] Parameters during simulation

C_l (m.moles/litre)	R_{urin} (m.moles/kg/day)	C_c (ng/ml)	C_p (ng/ml)	
2.50	0.0827	0.10	0.60	0.0
2.39*	0.0636	0.088	0.71	0.355
2.55**	0.0922	0.11	0.56	0.875
2.50	0.0857	0.10	0.58	1.00

[B] Accumulated totals (m.moles/kg)

S_{acc}	S_{dec}	S_{diet}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	
0.115	0.117	0.250	0.133	0.198	0.315	0.0781	1.00

*minimum plasma calcium
**maximum plasma calcium

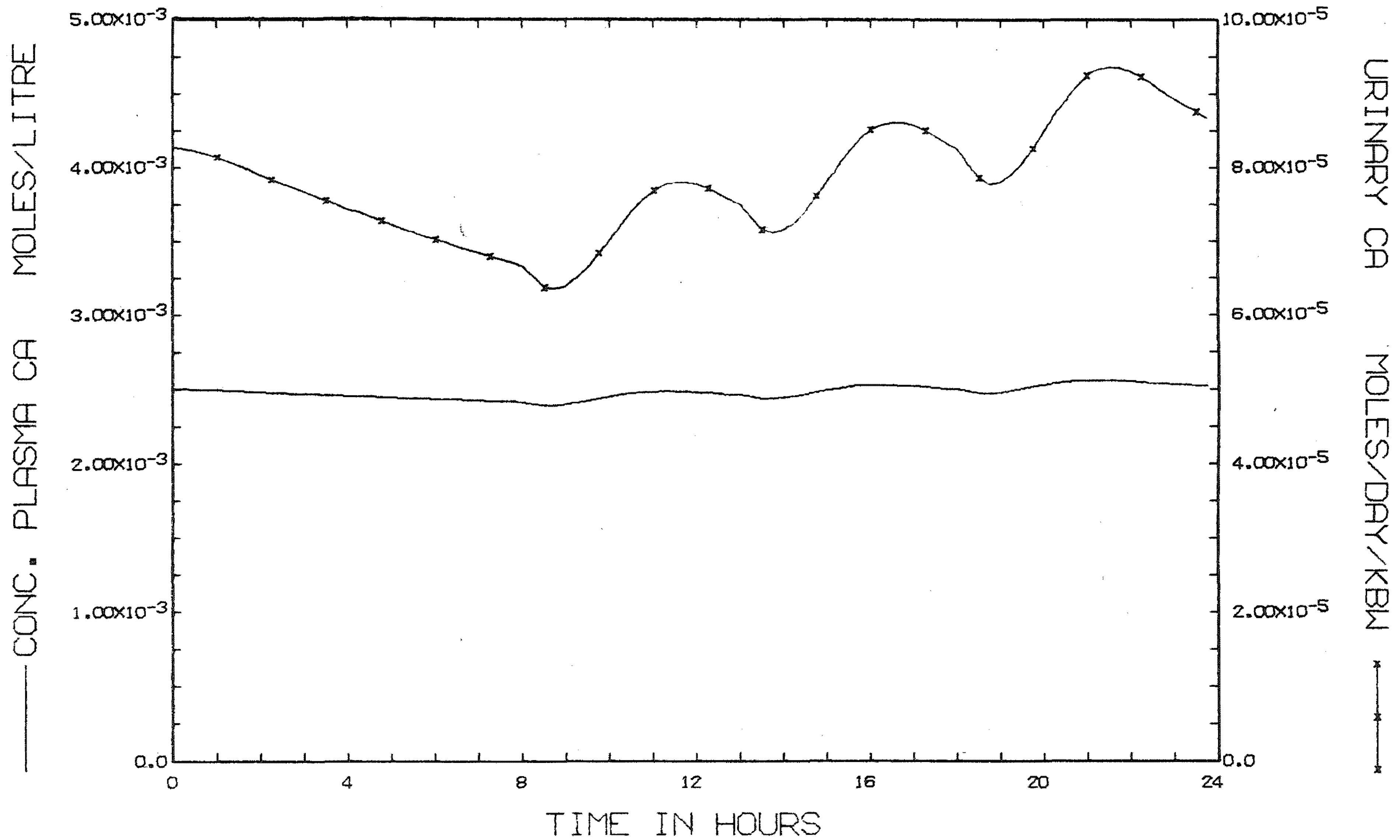


Figure 7.2 Plasma Ca & urinary Ca in response to dietary intake

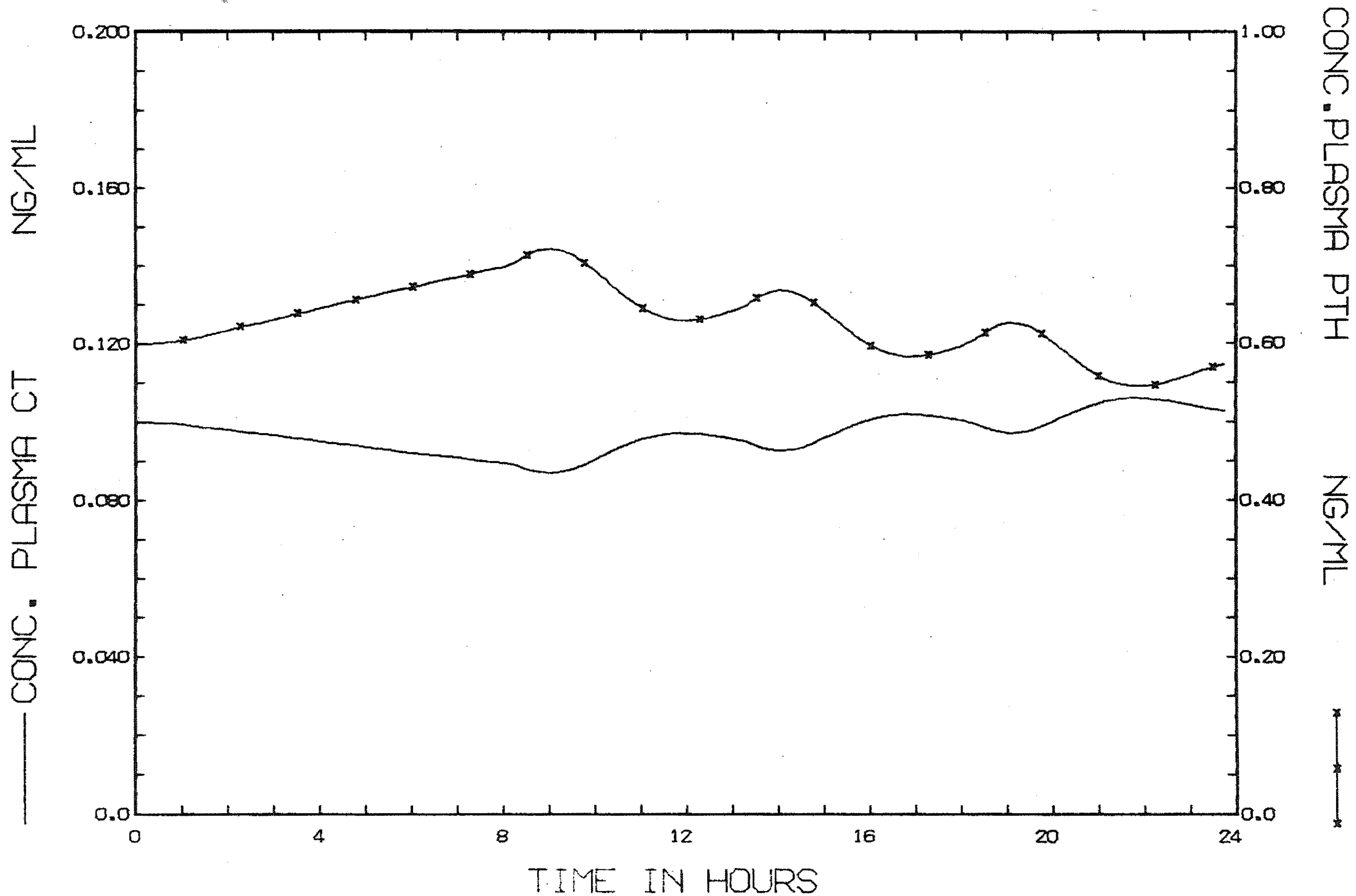


Figure 7.3 Plasma concentrations of CT and PTH in response to dietary Ca

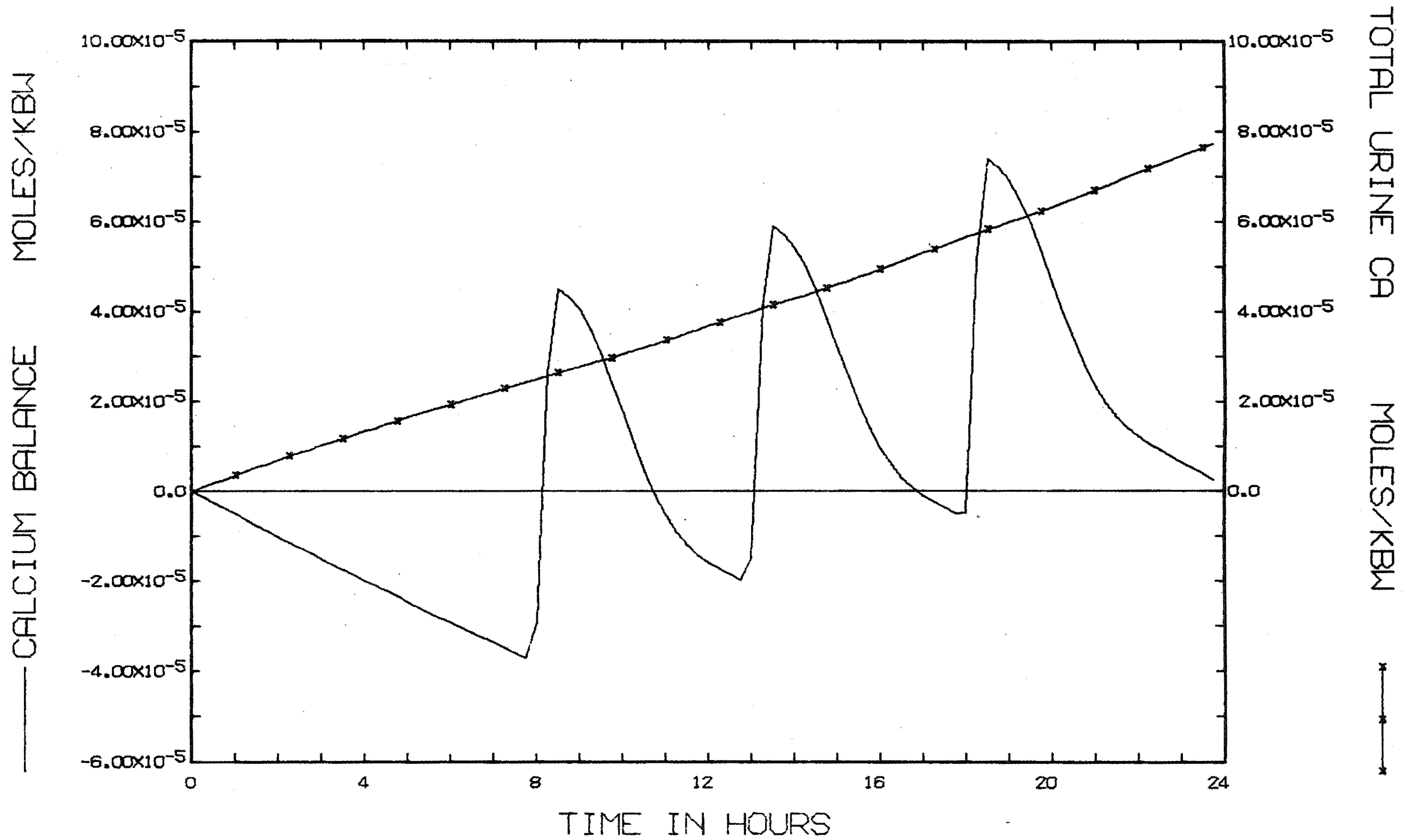


Figure 7.4 Ca balance & cumulative urinary Ca over a three meal day

Table 7.2 Calcium Transfers in a Simulated Three Meal Day

Calcium Transfer	m.moles/kg	mg
bone accretion	0.115	299
bone decretion	0.117	305
dermal loss	0.0379	98
dietary intake	0.250	651
faecal loss	0.133	346
intestinal absorption	0.315	818
intestinal secretion	0.198	515
urinary loss	0.0781	203
skeletal balance	-0.0020	-6
calcium balance	+0.0010	+3

All the listed physiological parameters are acceptable in the light of values recorded in the literature for normal humans. However the net intestinal absorption of calcium is greater than would usually be found for a dietary intake of 651 mg/day. The linear regression of faecal calcium (y) on dietary calcium (x) derived by Malm (1958) for the calcium intake range from 39 to 1010 mg per day

$$y = 0.594x + 64 \text{ mg}$$

predicts a faecal calcium of 451 mg compared with the simulated 346 mg. On the other hand biological variation is considerable and such a result is not physiologically unreasonable (Malm 1958). Hyperabsorption is a consistent feature of all the model simulations involving intestinal absorption and should be corrected as soon as possible. The overall calcium balance of Simulation 11 respresents a

considerable improvement over the -0.050 m.moles/kg predicted in the corresponding CAMET simulation where the simulated dietary intake (0.342 m.moles/kg) was substantially higher than in the present simulation.

Regular daily cycles characterize many biological quantities, of which the serum calcium is one. The fluctuations are small (of the order of ± 3%) (Briscoe & Ragan 1966, Jubiz & Tyler 1969) but nonetheless statistically significant. In agreement with this data the model predicts a minimum plasma calcium of 2.39 m.moles/kg at 8 a.m. and a maximum plasma calcium of 2.55 m.moles/kg at 9 p.m. (figure 7.2). This circadian rhythm in the plasma calcium induces similar cycles in 3 other model parameters of interest : the serum concentrations of CT and PTH and the rate of renal calcium excretion (figures 7.2 and 7.3). A circadian rhythm in the rate of urinary calcium excretion has been experimentally established. The highest loss is during the day (Heaton & Hodgkinson 1963, Carruthers et al. 1964, Loutit 1965, Briscoe & Ragan 1966). Again however, the rate of individual renal calcium excretion is subject to more erratic changes than produced by CAMET2 and Heaton & Hodgkinson (1963) found the peak in calcium excretion associated with breakfast, with a subsidiary rise in the evening.

Measured by bioassay, CT concentration rises during the day (Gudmundsson et al. 1969, Fraser & Wilson 1971) - although in the latter case the rise was not statistically

significant. Contrary to model predictions (figure 7.3) Arnaud et al. (1971) have found a strong diurnal rhythm in PTH activity with the highest concentration at 8 p.m. and a minimum at 8 a.m. On the surface this is a surprising result since the variation in the total calcium concentration would be expected to produce the opposite effect. Either there is some other important factor involved in the regulation of PTH secretion or the assumption that PTH secretion is linearly related to the total plasma calcium, at and near the normal concentration, is invalid.

7.3 Intestinal Calcium Absorption

Over the last 10 years many reports have appeared in the literature concerning the absorption of radio-calcium from the intestine. The kinetics of the isotope appearance in the plasma and the maximum concentration attained have been used as indications of the absorption efficiency in health and in various disorders involving abnormal calcium absorption. The experimental procedures have differed markedly - for example in the magnitude and chemical form of the stable calcium carrier as well as the dietary regime of the subjects. All these factors influence the results. Livesey's calculations agreed with the experimental finding that the higher the load of stable calcium carrier the lower is the fraction of the tracer dose that is absorbed (DeGrazia et al. 1965, Nordin 1968). In Simulation 13 the effect of a meal 2 hours after an oral tracer dose is examined. The initial conditions (300 mg of carrier calcium and 150 ml of fluid

in the stomach compartment) are those described by Jaworski et al. (1963) whose subjects were fasted overnight before the dose of tracer and for 2 hours after it. The beginning of Simulation 13 corresponds to 10 a.m. with the first simulated meal at t = 0.083 days (2 hours). The meals were omitted in Simulation 12, but otherwise the two runs were identical. The results of these simulations with the data of Jaworski et al. (1963) are collected in table 7.3 and the predicted plasma specific activities and the faecal excretion of tracer from each simulation are plotted together in figures 7.5 and 7.6.

Table 7.3 Effect of Simulated Meal After the Oral Ingestion of Tracer Calcium

	Simulation* 12	Simulation* 13	Jaworski et al. (1963) **
Maximum Plasma Tracer Concentration (% dose/litre)	2.18	1.96	1.73 ± 0.26
Increase in Stable Calcium Concentration (m.moles/litre)	0.11	0.09	0.18 ± 0.09
Faecal Tracer Excretion (% dose)	41.5	53.0	62.2 ± 4.1
Urinary Tracer Excretion (% dose)	4.4	4.8	6.2 + 1.5

** 8 subjects, 11 determinations. Excreted tracer collected over 6 days.

* 3 day simulation

Source Program : 72150 No : 72203-2

The intestinal absorption of a single dose tracer calcium was simulated. Apart from the 300 mg of carrier calcium associated with the tracer dose no dietary calcium and no fluid intake was simulated.

Reference: Jaworski et al. (1963)

Simulated Time: 3 days

Parameter Changes: M_5 set to 0.115 m.moles/kg

MT_5 set to 1.0×10^{-3} m.moles/kg

F set to 4.5*

V_5 set to 0.0028 litres/kg

Results: [A] Parameters during simulation

C_1 (m.moles/kg)	MT_1 (% dose/litre)	R_{abs} (m.moles/kg/day)	Time (days)
2.50	0.0	0.14	0.0
2.61**	2.18**	0.44	0.121
2.35	0.83	0.13	1.02
2.10	0.54	0.12	3.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	
-0.326	0.325	0.0595	0.400	0.455	0.161	3.00

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST^{***}_{urin}	
0.516	0.131	0.415	0.0965	0.681	0.044	3.00

*allows for faster stomach emptying of solutions (Livesey 1970)

**maximum value

***all tracer totals $\times 10^3$.

Source Program : 72150 No : 72203-3

Simulation 12 was repeated but with simulated dietary fluid and calcium intake over the three day period. The first meal input occurred at 0.083 days (2 hours).

Model Inputs*:

Oral Calcium (m.moles/kg/day)	Oral Fluid (litres/kg/day)	Time (days)
0.0	0.0	0.0
5.5	0.513	0.083
0.0	0.0	0.098
5.5	0.513	0.291
0.0	0.0	0.306
5.5	0.513	0.875
0.0	0.0	0.890

Results: [A] Parameters during simulation

C_1 (m.moles/litre)	MT_1 (% dose/litre)	R_{abs} (m.moles/kg/day)	
2.50	0.0	0.14	0.0
2.59***	1.96***	0.54	0.087
2.52	0.67	0.24	1.02
2.47	0.40	0.34	3.00

[B] Accumulated Totals (m.moles/kg)

C_{bal}	S_{acc}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	
-0.123	0.348	0.506	0.610	0.964	0.256	3.00

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST^{**}_{urin}	
0.402	0.105	0.530	0.112	0.581	0.048	3.00

*repeated on each simulated day
**all tracer totals × 10^3.
***maximum value

ORAL CALCIUM TRACER II

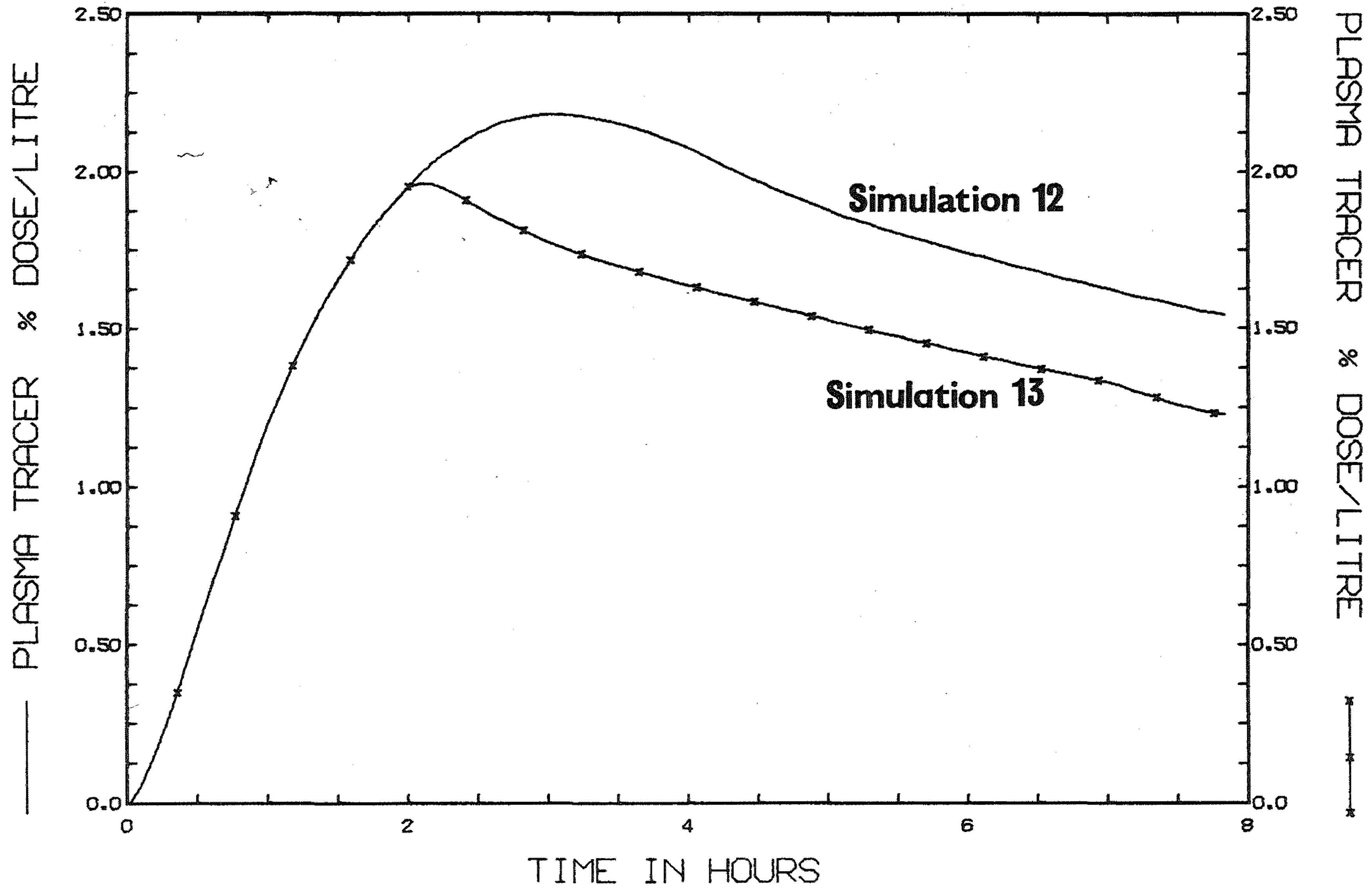


Figure 7.5 Effect of dietary Ca on the absorption of a tracer Ca dose

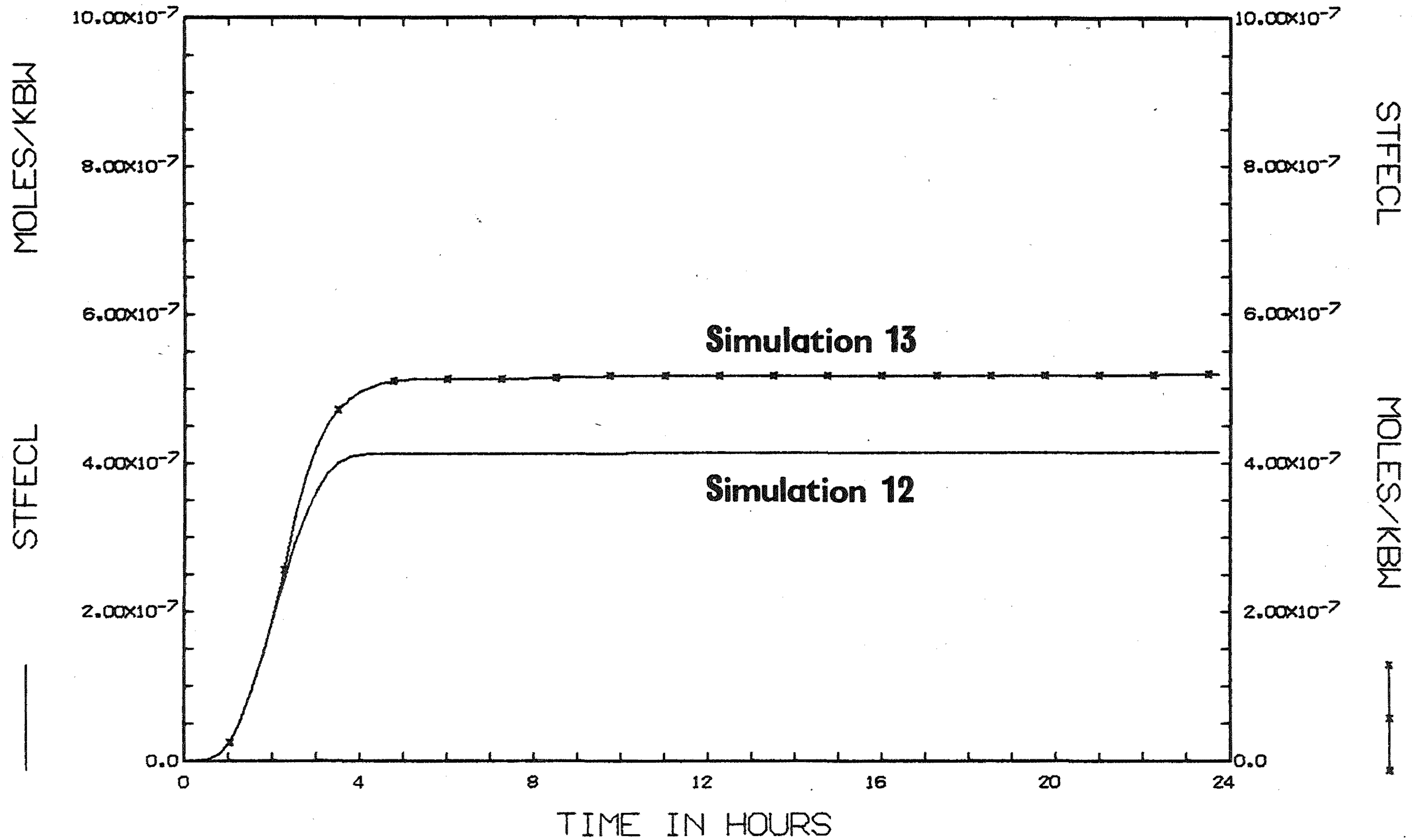


Figure 7.6 Effect of dietary Ca on the faecal excretion of a tracer dose

The effect of the meal in Simulation 13 on the absorption of calcium is significant. While it cannot be certain that Jaworski's results were influenced in this way it would seem advisable to maintain fasting for 4 hours following the administration of oral tracer. Interestingly Birge et al. (1969) found a reduction in the absorption of tracer calcium caused by a meal given 2½ hours *previously*; the extent depending on the calcium content of the meal. An action of PTH in producing this effect was sought but not found. It is possible that undigested food still in the intestinal lumen may have been partially responsible.

CAMET2 was also used to simulate the oral intake of radiocalcium as described by Mautalen et al. (1969) under carefully standardized conditions (Simulation 14). The tracer dose was given in 125 ml of solution associated with 200 mg of carrier calcium as calcium chloride. Measurements of the plasma specific activity in 24 normal subjects were taken over the subsequent 48 hours and the results are compared with those of CAMET2 in table 7.4. The simulated rate of tracer absorption and its appearance in the plasma are plotted in figures 7.7 and 7.8.

Table 7.4 The Appearance of Orally Ingested Tracer Calcium in the Plasma

Time (hours)	Plasma Specific Activity (% dose/litre)	
	CAMET2	Mautalen et al. (1969)
1.0	1.63	1.55 ± 0.62
1.5	2.23	1.97 ± 0.75
2.0	2.58	2.12 ± 0.75
3.0	2.74	2.13 ± 0.62
4.0	2.48	2.00 ± 0.58
6.0	2.05	1.69 ± 0.48
24.0	0.97	0.96 ± 0.27
48.0	0.73	0.71 ± 0.23

Source Program : 72150 No : 72260-1

The intestinal absorption of a Ca^{47} dose in solution with 200 mg of stable calcium was simulated under the conditions described by Mautalen et al. (1969). No dietary intake was permitted for at least 4 hours.

Reference: Mautalen et al. (1969)

Simulated Time: 3 days

Parameter Changes: M_5 set to 0.0772 m.moles/kg
MT_5 set to 1×10^{-3} m.moles/kg
F set to 4.5
V_5 set to 0.0021 litres/kg

Model Inputs*:

Oral Calcium (m.moles/kg/day)	Oral Fluid (litres/kg/day)	Time (days)
5.5**	0.513**	0.0
0.0	0.0	0.015
5.5	0.513	0.208
0.0	0.0	0.223
5.5	0.513	0.417
0.0	0.0	0.432

Results: [A] Parameters during simulation.

C_1 (m.moles/litre)	MT_1 (% dose/litre)	ST_{urin}^{***} (m.moles/kg)	
2.50	0.0	0.0	0.0
2.59	2.76****	0.0047	0.111
2.42	0.97	0.0386	1.02
2.40	0.60	0.0690	3.00

[B] Accumulated Totals (m.moles/kg)***

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST_{urin}	
0.592	0.155	0.308	0.156	0.846	0.0690	3.00

* repeated on each simulated day

** initial simulated meal omitted on day 1

*** $\times 10^3$

**** maximum value

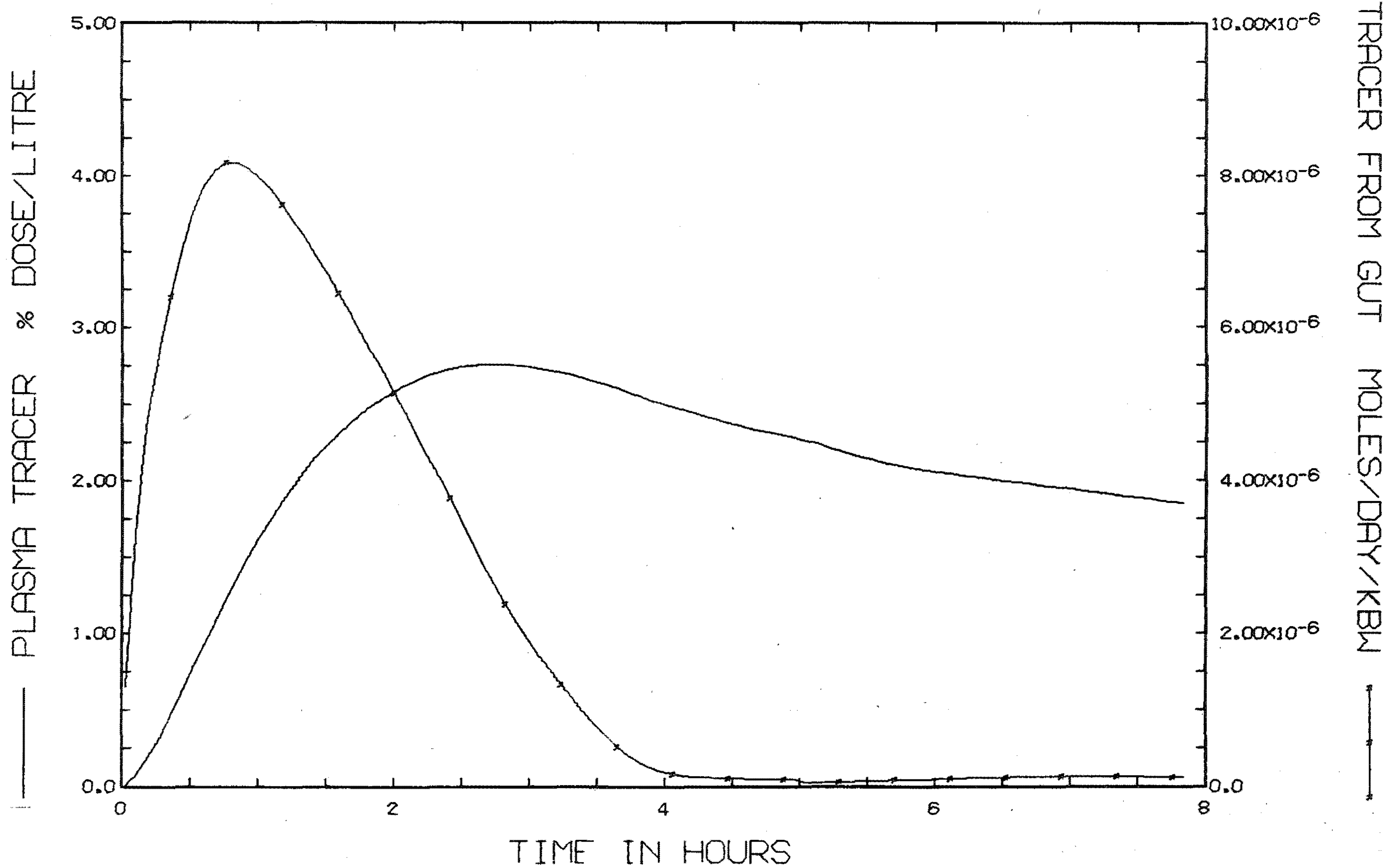


Figure 7.7 Plasma specific activity & rate of absorption of an oral tracer dose

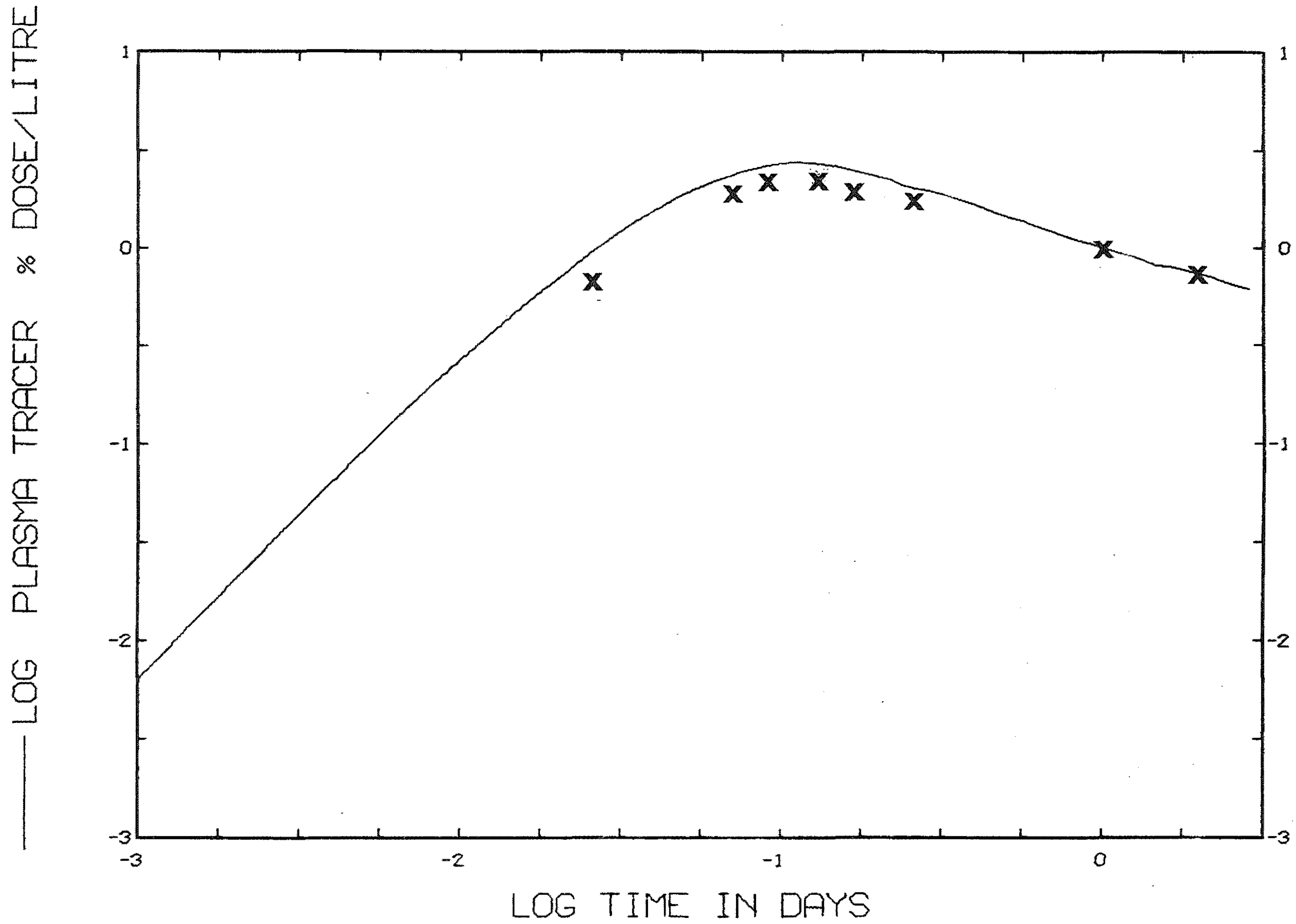


Figure 7.8 Plasma specific activity of tracer Ca & experimental points

All the predicted values are within one standard deviation of the experimental results, although only just so for the maximum values.

Since the tracer calcium is removed from the plasma (by distribution and exchange) at a rate comparable to its entry, its specific activity in the plasma is a reflection of both the rate of absorption and the rate of removal. There exists therefore a considerable overlap in the maximum plasma specific activity ranges of different disease groups (Jaworski et al. 1963, Carson et al. 1970). To a degree this has been overcome by the use of double isotope studies (Ca^{45} and Ca^{47}) - one injected intravenously and the other given orally. Under the basic assumption that calcium entering the plasma from the intestine is handled in the same way as that introduced directly, the tracer absorption as a function of time may be computed using deconvolution techniques (Birge et al. 1969, Szymender et al. 1972). The absorption curve generated by the present model (figure 7.7) is qualitatively similar to those computed by deconvolution - apart from the initial delay of 15 - 20 minutes (Birge et al. 1969) which is not predicted by CAMET2. It was found experimentally that only part of this delay was due to the stomach transit time. On the other hand when tracer was given in the middle of a meal the delay was not apparent (Szymender et al. 1972). Indeed, the maximum rate of absorption was, on average, 15.6 ± 13.1 minutes after administration - ranging as low as 4 minutes.

Currently the intestinal model is being reconstructed to give it a closer correspondence to physiological reality and to eliminate some of the present deficiences noted here and in section 5.4.

7.4 The Distribution and Excretion of Intravenous Calcium Tracer

To ensure that the model is still able to reproduce the data of Neer et al. (1967) on which the exchangable pools are based (Chapter 1) an intravenous tracer simulation was carried out (Simulation 15). This simulation differs from Simulation 1 of Livesey (1970) in that the entire model was used and in that it included simulated meals. The inclusion of meals has become essential, as a result of the changes described in Chapter 5, for an acceptable loss of tracer via the intestine. The beginning of the simulation corresponds to the experimental injection time (2 hours after breakfast) and the total time simulated was 3 days.

The decaying plasma tracer (MT_1) computed by CAMET2 (expressed as the per cent of the original dose in the plasma compartment) is tabulated below with the data generated by the model of Neer et al. (1967) based on the average measured variables of 10 subjects over 20 days. Considering that Neer's data includes a 5-10% scatter factor the simulated results are in good agreement.

SIMULATION 15 INTRAVENOUS CALCIUM TRACER

Source Program : 72150 No : 72260-2

The dynamics of a single intravenous injection of tracer calcium were simulated. The predictions are compared with the experimental results of Neer et al. (1967) to check that the ability of CAMET to reproduce this experiment has been conserved in the development of the present model.

Reference: Neer et al. (1967)

Simulated Time: 3 days

Parameter Change: MT_1 set to 1.0×10^{-3} m.moles/kg

Model Inputs:

Oral Calcium (m.moles/kg/day)	Oral Fluid (litres/kg/day)	Time (days)
0.0	0.0	0.0
5.5	0.513	0.124
0.0	0.0	0.139
5.5	0.513	0.333
0.0	0.0	0.348
5.5	0.513	0.916
0.0	0.0	0.931

Results: Accumulated Totals (m.moles/kg/day)

[A] Stable Calcium

C_{bal}	S_{acc}	S_{fecl}	S_{gsec}	S_{sab}	S_{urin}	
0.0167	0.347	0.369	0.595	0.920	0.240	3.00

[B] Tracer Calcium ($\times 10^3$)

CT_{bal}	ST_{acc}	ST_{fecl}	ST_{gsec}	ST_{sab}	ST_{urin}	
0.828	0.219	0.0390	0.224	0.181	0.0904	3.00

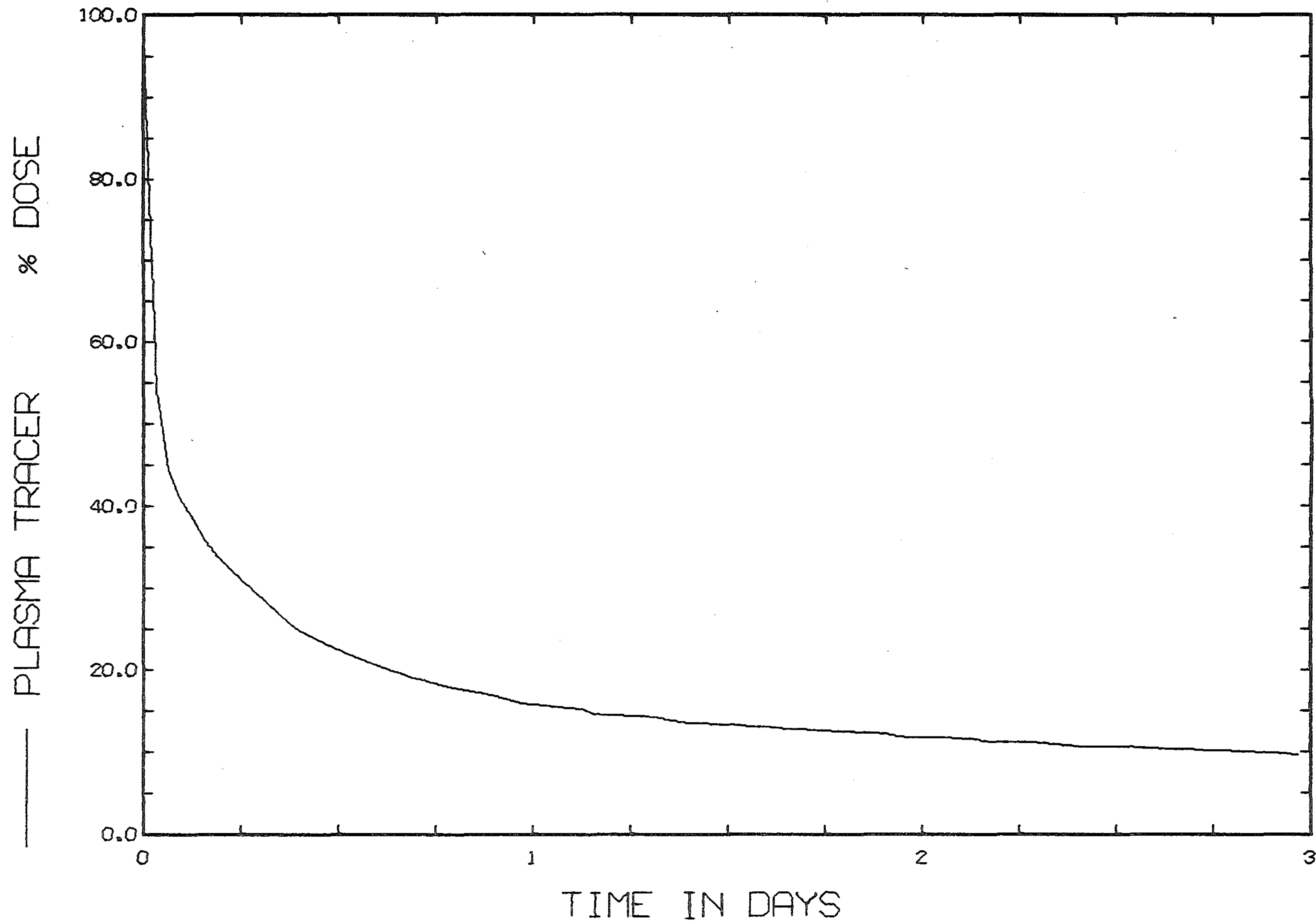


Figure 7.9 Decay in the plasma specific activity of an intravenous tracer dose

Table 7.5 The Specific Activity of Plasma Tracer Following Intravenous Injection

Time (days)	% Dose in Plasma Compartment	
	CAMET2	Neer et al. (1967)
0.0014	95.9	96.07
0.0028	92.3	96.46
0.0035	90.5	87.75
0.0056	85.4	86.45
0.0077	81.1	83.07
0.0105	76.0	70.33
0.014	70.7	75.27
0.0315	54.0	57.07
0.126	38.4	40.95
0.189	33.8	36.66
0.5	22.5	22.88
1.5	13.3	14.30
3.0	9.4	10.45

The cumulative urinary tracer by t = 2.884 days was 8.86% as compared with the value of 10.2% reported by Neer et al. (1967) after a similar interval of time (2.875 days). In addition 3.90% of the original tracer dose excreted in the faeces and 4.30% in the sweat were predicted after 3 days by the model.

Simulation 15 serves also as a check on the computational stability and accuracy of the integration procedure since the results are known (Neer et al. 1967). At the conclusion of the simulation, after 16,593 interative steps, 99.78% of the original tracer dose was either still present in the model pools or was accounted for in the simulated excretion. Such a result is a necessary (though not sufficient) condition that the numerical procedure is correct.

All the CAMET2 simulations represent considerable simplications of the natural system and correspondence between the model predictions and the experimental results is not in itself proof either of the correctness of the model itself or the conclusions drawn. However, in some respects, this simplicity has advantages over the experimental situation where the plasma calcium is a function of a large number of complexly interrelated variables, difficult if not impossible to control or measure, and about which assumptions must be made in the interpretation of the results. The present model is approaching the point where collaboration with investigators in the field of calcium metabolism will be of mutual benefit, especially in the collection of suitable data, the suggestion of new experiments and as a basis for the testing of ideas.

The scope and self-consistency of the present model would be improved by the following additions and alterations:

[A] The effects of CT and PTH on bone resorption.

[B] The reconstruction of the existing intestinal model and the correction of the absorption parameters.

[C] An examination of the effects of small changes in the plasma calcium on hormonal secretion rates and renal calcium excretion.

[D] A study of the distribution of calcium in the soft tissues of the body and their contribution to the homeostasis of calcium.

[E] The introduction of vitamin D and its action on intestinal absorption.

[F] A variable plasma volume and its effects on the glomerular filtration rate.

Beyond this there is considerable scope for the incorporation of the principles of chemistry, physics and engineering in the replacement of some of the present empirical structure of the model which, while describing the system and permitting worthwhile deductions about calcium metabolism as a whole, does not directly add to the understanding of the basic mechanisms involved. For example, calcium exchange with bone and the different chemical forms of calcium in the plasma could be profitably investigated along these lines.

LIST OF SYMBOLS USED IN THE CAMET2 MODEL

The following upper case symbols are used in the model equations to denote concentrations, rates etc.

C	concentration
F	fluid flow variable
M	quantity
R	rate
S	cumulative quantity
SA	specific activity
T	tracer calcium

Extensive quantities are normalized to unit body weight.

$\overline{C}_1$	denotes the mean value of the plasma calcium - in this model taken as 2.5 m.moles/litre
$\dot{x}$	represents the first derivative of x with respect to time (dx/dt)

The symbols used throughout the present work to represent the parameters and variables of the model are as follows:

A_I	empirical constant
AF_I	the fraction of the fluid entering the intestinal compartment I, from compartment (I-1), that is absorbed into the body
$B_{I,1}$ $B_{I,2}$	parameters controlling the volume of the intestinal compartment I
C_I	the concentration of calcium in compartment I
C_{bal}	the calcium balance
C_c	concentration of calcitonin
C_p	concentration of parathyroid hormone

D_{tmin}	the minimum allowable step length
$E_{I,1}$ $E_{I,2}$	parameters controlling the absorption of calcium from intestinal compartment I
E_{rrhi}	the maximum value of the truncation error above which the step length is decreased.
E_{rrlo}	the minimum value of the truncation error below which the step length is increased
F	parameter controlling the rate of stomach emptying
G_I	duodenal secretion parameter
$K_{I,J}$	first order rate constant for the transfer of calcium from compartment I to compartment J
K_{acc} K_d K_{dc} K_{dp} K_{oss}	rate constants
K_c	$C_1/\overline{C}_1$
K_{pr} K_{rp}	proportionality constants between the bone collagen and the bone calcium
M_I	quantity of calcium in compartment I
M_{bca}	quantity of bone calcium
M_{bcoll}	quantity of bone collagen
M_c	quantity of calcitonin
M_p	quantity of parathyroid hormone
MT_{tot}	quantity of tracer calcium in model compartments at $t = 0$
$R_{I,J}$	rate of the unidirectional flow of calcium from compartment I to compartment J

R_{abs} rate of calcium absorption from the intestinal compartments

R_{acc} rate of calcium deposition in the bone

R_{cinf} rate of calcitonin infusion

R_{coacc} rate of collagen synthesis

R_{codec} rate of collagen destruction

R_{dc} rate of calcitonin catabolism

R_{dec} rate of calcium resorption from the bone

R_{derm} rate of loss of calcium in the sweat

R_{diet} rate of ingestion of dietary calcium

R_{dp} rate of parathyroid hormone catabolism

R_{fecl} rate of excretion of calcium in the faeces (strictly the rate of flow of calcium into the colon)

R_{gsec} rate of calcium excretion into the intestinal lumen

R_{inf} rate of intravenous calcium infusion

R_{pinf} rate of infusion of parathyroid hormone

R_{sc} rate of secretion of calcitonin

R_{sp} rate of secretion of parathyroid hormone

R_{urin} rate of loss of calcium in the urine

RG_{I} basal secretion rate of calcium into the intestinal compartment I

RT_{acc} rate of tracer uptake by the bone (including incorporation in new bone as well as long term exchange)

S_{acc}	cumulative skeletal uptake of calcium by bone formation
S_{dec}	cumulative resorption of bone calcium
S_{derm}	cumulative loss of calcium in the sweat
S_{diet}	cumulative ingestion of dietary calcium
S_{fecl}	cumulative excretion of faecal calcium
S_{gsec}	cumulative intestinal calcium secretion
S_{inf}	cumulative intravenous calcium infusion
S_{sab}	cumulative absorption of calcium from the intestinal compartments
S_{urin}	cumulative excretion of urinary calcium
SA_I	specific activity of tracer calcium in compartment I
U_{mcoll}	quantity of unmineralized collagen
V_I	volume of compartment I
V_c	volume of distribution of calcitonin
V_p	volume of distribution of parathyroid hormone
X_{dt}	initial step length

APPENDIX II

ABBREVIATIONS AND DEFINITIONS

Accretion rate: Calcium uptake by the skeleton due to new bone growth

CaBP: calcium binding protein

calcaemia: the total plasma calcium concentration

calciuria: the calcium in the urine

compartment: a quantity of a substance with identical transport and metabolic kinetics

CT: calcitonin (also known as thyrocalcitonin)

decretion rate: calcium removal from the skeleton due to bone resorption

1,25-DHCC: 1,25-dihydroxycholecalciferol

EDTA: ethylenediamine tetraacetic acid

25-HCC: 25-hydroxycholecalciferol

hyperparathyroidism: a condition resulting from an excess of circulating PTH

hypoparathyroidism: a condition resulting from a deficiency of circulating PTH

nephrectomy: the surgical removal of the kidney

osteoblast: bone cells associated with bone formation

osteoclasts· multinucleated bone cells associated with bone resorption

osteoid: unmineralized bone matrix

osteon: the basic structural unit of compact bone comprising a central Haversian canal surrounded by concentric layers of bone lamellae

parathyroidectomy: the surgical removal of the parathyroid glands

phosphateamia: the total inorganic phosphorus concentration (in all its various forms) in the plasma

phosphaturia: the inorganic phosphorus in the urine

PTH: parathyroid hormone

S_e: the endogenous faecal calcium

thyroparathyroidectomy: the surgical removal of both the thyroid and the parathyroid glands

unmixed compartment: a compartment that mixes so slowly that in the duration of measurements an insignificant fraction of the material entering the compartment is found to leave it

APPENDIX III

SOURCES OF MODEL PARAMETERS

A complete list of the CAMET2 parameters is appended below together with the literature references from which they are directly derived (if any) and the section in which each parameter is considered. Some are derived from other model parameters or, where precise data is lacking, are choosen arbitarily to fall within physiological ranges. Sections marked with an asterisk refer to the work of Livesey (1970).

Parameter	Section	Reference
A_1	6.2	Frost (1969)
A_4	6.2	Frost (1969)
A_6 to A_9	4.3	Levitt et al. (1958) Lamberg et al. (1960) Ardaillou et al. (1967) Nordin et al. (1967)
A_{22}	4.2	Tashjian et al. (1970)
A_{23}	4.2	-
A_{24}	4.2	Sherwood et al. (1968)
A_{25}	4.2	-
AF_6 to AF_{11}	5.3*	-
$B_{6,1}$ to $B_{11,1}$	5.3*	Dillard et al. (1965)
$B_{6,2}$ to $B_{11,2}$	5.3*	Dillard et al. (1965)
$E_{6,1}$ to $E_{11,1}$	5.3*	Wensel et al. (1969)
$E_{6,2}$ to $E_{11,2}$	5.3*	Wensel et al. (1969)
F	5.1*	Hunt & Pathak (1960)
G_1 to G_3	5.4	Altman (1961) Doubilet & Fishman (1961) Diamond (1967) Hansky (1967) Schneyer & Schneyer (1967) Moore & Makhlouf (1968) Wensel et al. (1969)

Parameter	Section	Reference
$K_{1,2}$ to $K_{4,3}$	5.2*	Neer et al. (1967)
K_{acc}	5.2*	Neer et al. (1967)
K_d	5.2	Isaksson et al. (1967)
K_{dc}	4.2	Ardaillou et al. (1970)
K_{dp}	4.2	Melick & Martin (1969)
K_{oss}	6.2	-
K_{pr}, K_{rp}	6.2	Trotter & Peterson (1955) Eastoe (1956) Zipkin (1970)
M_1 to M_4	5.5*	Neer et al. (1967)
M_5 to M_{11}	2.1	-
M_c	4.2	-
M_p	4.2	-
M_{bca}	6.2	Trotter & Peterson (1955)
M_{bcoll}	6.2	Trotter & Peterson (1955)
$RF_{1,5}$	5.4	Altman (1961)
$RF_{1,6}$ to $RF_{1,11}$	5.4	-
$RG_{1,5}$	5.4	Altman (1961) Schneyer & Schneyer (1967) Moore & Makhlouf (1968)
$RG_{1,6}$ to $RG_{1,11}$	5.4	Wensel et al. (1969)
V_1	5.2*	-
V_5	2.1	-
V_c	4.2	Ardaillou et al. (1970)
V_p	4.2	Melick & Martin (1969)

SUBROUTINE PLOTER

Up to 4 graphs, each with either 1 or 2 model variables plotted against time, may be produced by means of PLOTER. Any model variable may be saved during the course of a simulation and subsequently plotted, both directly by the line printer (subroutine PLOT) and by using PLOTER to prepare a deck of cards forming the input to the University of Canterbury PDP11 X-Y plotter system. Parameters controlling the plotting (length of graph, variables plotted etc) are defined automatically in subroutine CONPAR but may be changed if necessary by use of subroutine OPTION (section 1.7). Log abscissa and/or log ordinate may be specified. There are no restrictions on the starting or ending times of the graph so long as these lie within the simulated time range. The function of each of the plotting subroutines (which are specific to the University of Canterbury Computer Centre) called by PLOTER are as follows -

AINIT : initializes plotter pen

AORIG : defines graph origin

AGRID : draws box with ticks

ASCA : draws a scale of integers

ALAB : draws a label of alphanumeric characters

ALINE : draws straight lines between a series of points

AEND : indicates the end of the plot

<u>Library Subroutines called</u> -

ALOG10

MAX0, AMAX1, AMIN1

<u>CAMET2 Subroutines called</u> -

LABEL : acts as a list of labels for model variables of interest

```
****SUBROUTINE PLCTER*********************************************
*                                                                *
*                           NOTATICN                             *
*                           ========                             *
*                                                                *
*                                                                *
*     BMAX(I)        MAXIMUM ORDINATE VALUE OF THE ITH VARIABLE  *
*     BMIN(I)        MINIMUM ORDINATE VALUE OF THE ITH VARIABLE  *
*     C(800)         ARRAY OF ORDINATE VALUES TC BE PLOTTED      *
*     I&J            COUNTERS                                    *
*     INC            INCREMENT IN SUCCESSIVE SCALE NUMBERS       *
*     ISCA(12)       ARRAY CONTAINING ORDINATE SCALE CHARACTERS  *
*     IXINC          INCREMENT IN X DISPLACEMENT                 *
*     IYINC          INCREMENT IN Y DISPLACEMENT                 *
*     JC             MAXIMUM NO OF ORDINATE SCALE NUMBERS GREATER *
*                    THAN ZERO                                   *
*     JD             MAXIMUM NO OF ORDINATE SCALE NUMBERS LESS   *
*                    THAN ZERO                                   *
*     JGRAPH(I,J)    IDENTIFIES ITH VARIABLE (BY REALS ARRAY NO) *
*                    ON THE JTH GRAPH                            *
*     JJHIGH         GRAPH HEIGHT IN 1/100THS OF INCH            *
*     JJLONG         GRAPH LENGTH IN 1/100THS OF INCH            *
*     JLAB(31)       ARRAY CONTAINING THE VARIABLE LABEL AND     *
*                    THE UNITS OF MEASUREMENT                    *
*     JLABEL(40)     ARRAY CONTAING THE TITLE OF THE GRAPH       *
*     JLINE(I)       NO OF VALUES OF THE ITH VARIABLE SAVED FOR  *
*                    PLOTTING                                    *
*     JLOGT(I)       EQUALS 1 IF ITH VARIABLE IS TO HAVE LOG     *
*                    ABSCISSA                                    *
*     JLOGV(I)       EQUALS 1 IF ITH VARIABLE IS TO HAVE LOG     *
*                    ORDINATE                                    *
*     JLONG(J)       LENGTH OF THE JTH GRAPH IN INCHES           *
*     JNORM(J)       IF NOT EQUAL TO 1 BOTH VARIABLES ON THE JTH *
*                    GRAPH ARE SCALED TO THE MAXIMUM ABSOLUTE    *
*                    ORDINATE VALUE                              *
*     JPLOT(I,J)     IF 0 INHIBITS THE PLOTTING OF THE ITH       *
*                    VARIABLE ON THE JTH GRAPH                   *
*     JSTORE(I,J)    ALLOWS THE ITH VARIABLE ON THE JTH GRAPH TO *
*                    BE DISTINGUISHED FROM THE SAME VARIABLE     *
*                    PLOTTED ELSEWHERE                           *
*     NL             MAXIMUM NO OF ORDINATE SCALE LABELS         *
*     NO             NO OF VARIABLES ON THE GRAPH (EITHER 1 OR 2) *
*     SCALE(I)       SCALE FACTOR FOR THE ITH VARIABLE           *
*     TFACT          DIFFERENCE BETWEEN SUCCESSIVE ORDINATE      *
*                    SCALE NUMBERS                               *
*     TGRAPH(100,2)ARRAY OF TIMES ASSOCIATED WITH THE ORDINATE   *
*                    VALUES                                      *
*     TLABEL(15)     ARRAY CONTAINING THE UNITS OF TIME          *
*     TMAX           GRAPH FINISHING TIME                        *
*     TMIN           GRAPH STARTING TIME                         *
*     X(100)         ARRAY CONTAINING ABSCISSA VALUES TO BE      *
*                    PLOTTED                                     *
*     XOR            ORIGIN VALUE OF X                           *
*     XSCAL          ABSCISSA SCALE                              *
*     Y(100)         ARRAY CONTAINING ORDINATE VALUES TO BE      *
*                    PLOTTED                                     *
*     YOR            ORIGIN VALUE OF Y                           *
*     YSCAL          ORDINATE SCALE                              *
*                                                                *
*                                                                *
******************************************************************
C
C
      SUBROUTINE PLCTER
C
C
C     NON-EXECUTABLE STATMENTS
      COMMON REALS(5000),INTS(500)
      COMMON/GRAPH/C(800),TGRAPH(100,2),VMAXA(4,2),VMINA(4,2),JLABEL(40)
C
      LOGICAL*1 LOGLAB(16),TLABEL(15),JHOLD(7,4),ZERO(3),ISCA(12),JPOW(3
     1),LOG(3),JLABEL,VTAG(15),UTAG(15),JLAB(31)
      DIMENSION IGRAPH(4,2),ILINE(2),ILOGT(2),ILOGV(2),TSTART(2),X(100),
     1Y(100),KSTORE(4,2)
      DIMENSION AFACT(4),BMAX(2),BMIN(2),JGRAPH(2,4),JK(2),JLINE(2),JLOG
     1T(2),JLOGV(2),JLONG(4),JNORM(4),JPLOT(2,4),JPOS(2),JW(2),JCOM(2),S
     2CALE(2),YORIG(2),YSC(2),YC(3),YS(2),JSTORE(2,4),JA(2),JB(2)
C
      DATA ISCA/'      X10   '/,JHOLD/'MINUTESHOURS  DAYS    MONTHS '/,LO
     1G/'LOG'/,LOGLAB/'LOG TIME IN DAYS'/,TLABEL/'TIME IN        '/,ZERO
     2/'0.0'/,JLAB/'                               '/
      DATA AFACT/1440.,24.,1.,0.0333/,YS/-10.,90./,YC/90.,40.,-10./
C
      EQUIVALENCE (IGRAPH(1,1),INTS(51)),(ILINE(1),INTS(97)),(ILOGT(1),I
     1NTS(69)),(ILOGV(1),INTS(67)),(TSTART(1),REALS(1013)),(KSTORE(1,1),
     2INTS(73))
      EQUIVALENCE(JGRAPH(1,1),INTS(221)),(JPLOT(1,1),INTS(229)),(JLONG(1
     1),INTS(237)),(JNORM(1),INTS(241)),(JSTORE(1,1),INTS(245))
      EQUIVALENCE(X,REALS(4800)),(Y,REALS(4900))
      EQUIVALENCE (VTAG(1),JLAB(1)),(UTAG(1),JLAB(17))
C
C
      DO 600 J=1,4
C
C
C     PLOT INHIBITION
      IF(JPLOT(1,J).EQ.0.AND.JPLOT(2,J).EQ.0) GO TO 600
```

```
C
C     INITIALIZE GRAPHING PARAMETERS
    2 JCOM(1)=0
      JCOM(2)=0
      DO 3 JL=1,2
      IF(JPLOT(JL,J).EQ.0) GO TO 3
      DO 3 I=1,2
      DO 3 JN=1,4
      IF(JGRAPH(JL,J).NE.IGRAPH(JN,I)) GO TO 3
      IF(JSTORE(JL,J).NE.KSTORE(JN,I)) GO TO 3
      JK(JL)=I
      JCOM(JL)=1
      JLINE(JL)=ILINE(I)
      JLOGT(JL)=ILOGT(I)
      JLOGV(JL)=ILOGV(I)
      JPOS(JL)=JN+(I-1)*4
      BMIN(JL)=VMINA(JN,I)
      BMAX(JL)=VMAXA(JN,I)
    3 CONTINUE
      NO=JCOM(1)+JCOM(2)
C
C     CHECK FOR ERRORS
      IF(NO.EQ.0) GO TO 592
      IF(.NOT.(JCOM(1).EQ.0.AND.JCOM(2).EQ.1)) GO TO 7
      JGRAPH(1,J)=JGRAPH(2,J)
      JGRAPH(2,J)=0
      JPLOT(1,J)=1
      GO TO 2
    7 IF(NO.EQ.2.AND.(JLOGT(1).NE.JLOGT(2).OR.JLOGV(1).NE.JLOGV(2)))NO=1
C
C     INITIALIZE GRAPH,DEFINE LENGTH AND ORIGIN
      JJ=JLONG(J)*100+600
      CALL AINIT(JJ)
      CALL AORIG(300,200)
      IF(JLOGV(1).EQ.1) GO TO 15
C
C     SET UP ORDINATE SCALES
      DO 14 I=1,NO
      AL=AMAX1(BMAX(I),-BMIN(I))
      IF(AL.NE.0.0) GO TO 12
      SCALE(I)=1.0
      GO TO 14
   12 JN=ALOG10(AL)+.5
      IF(AL.LT.1.0)JN=ALOG10(AL)-.5
      SCALE(I)=10.**JN
      IF(AL/SCALE(I).GT.1.)SCALE(I)=SCALE(I)*2.
      IF(AL/SCALE(I).GT.1.)SCALE(I)=SCALE(I)*2.5
      IF(AL/SCALE(I).LT.1.)SCALE(I)=SCALE(I)/2.
      IF(AL/SCALE(I).GT.1.)SCALE(I)=SCALE(I)*2.
      IF(SCALE(I)-BMIN(I).LT.SCALE(I)*.25)SCALE(I)=SCALE(I)*2.
   14 CONTINUE
      IF(JNORM(J).EQ.1.OR.NO.EQ.1) GO TO 15
      SCALE(1)=AMAX1(SCALE(1),SCALE(2))
      SCALE(2)=SCALE(1)
C
C     SET UP TIME SCALE
   15 TMAX=(NO-1)*AMAX1(TGRAPH(JLINE(1)-1,JK(1)),TGRAPH(JLINE(2)-1,JK(2)
     1))+(2-NO)*TGRAPH(JLINE(1)-1,JK(1))
      TMIN=(NO-1)*AMIN1(TSTART(JK(1)),TSTART(JK(2)))+(2-NO)*TSTART(JK(1)
     1)
      IF(JLOGT(1).EQ.1) GO TO 180
      TMAX1=TMAX-TMIN+.002
      IF(TMAX1.LE..0833)M=1
      IF(TMAX1.GT..0833.AND.TMAX1.LE.2.)M=2
      IF(TMAX1.GT.2..AND.TMAX1.LE.48.)M=3
      IF(TMAX1.GT.48.)M=4
      TMAX=AFACT(M)*TMAX
      TMIN=AFACT(M)*TMIN
      ITRAN=3.3219*ALOG10(TMAX1*AFACT(M)/6.)+.99
      INC=2**ITRAN
      IF(INC.GT.8)INC=10
      ILO=(TMIN+.74*INC)/INC
      ILO=ILO*INC
      IF(ILO+.02.LT.TMIN)TMIN=ILO
      JJ=(ILO-TMIN)*4./INC+.95
      XOR=ILO-(JJ*INC)/4.
      NX=(TMAX-XOR)*4./INC+.95
      IF(JLOGV(1).EQ.1) GO TO 181
C
C     CALCULATE NUMBER OF LABELS ON EACH ORDINATE SCALE
  143 JA(1)=0
      JA(2)=0
      JB(1)=0
      JB(2)=0
      DO 161 I=1,NO
      IF(BMAX(I).GT.0.0)JA(I)=BMAX(I)/SCALE(I)*5.0+.99
      IF(BMIN(I).LT.0.0)JB(I)=-BMIN(I)/SCALE(I)*5.0+.99
      IF(JA(I).GE.JB(I))JA(I)=5
  161 IF(-BMIN(I).GT.BMAX(I))JB(I)=5
      JC=MAX0(JA(1),JA(2))
      JD=MAX0(JB(1),JB(2))
      NL=JC+JD+1
      JN=4*(NL-1)
      IF(JN.GT.30)JN=JN/2
      GO TO 195
C
C     SET UP LOG TIME SCALE
  180 ILO=ALOG10(TMIN)-.999
      JO=ALOG10(TMAX)+.999
      JP=(JO-ALOG10(TMAX))*4.
      IF(JO-ILO.LE.1)JP=0
      NX=(JO-ILO)*4-JP
      IF(JP.GE.1)JO=JO-1
      N=JO-ILO+1
      IF(JLOGV(1).EQ.0) GO TO 143
```

```
C
C       SET UP LOG ORDINATE SCALE
  181   K=(NO-1)*AMAX1(BMAX(1),BMAX(2))+(2-NO)*BMAX(1)+.99
        JL=(NC-1)*AMIN1(BMIN(1),BMIN(2))+(2-NO)*BMIN(1)-.99
        IF(K.LT.0)K=K-1
        JS=K-JL
        IF(JS.LE.10)JY=1
        IF(JS.GT.10.AND.JS.LE.20)JY=2
        IF(JS.GT.20.AND.JS.LE.48)JY=4
        IF(JS.GT.48)JY=10
        NL=JS/JY+1.99
        JN=(NL-1)*2
        IF(JL.LT.0)K=JL
C
C       SET UP AND PLOT GRAPH GRID
  195   IXINC=JLONG(J)*100/NX
        JJLONG=NX*IXINC
        IYINC=700/JN
        JJHIGH=JN*IYINC
        CALL AGRID(0,0,NX,JN,IXINC,IYINC,1,2)
C
C
C       *PLOT GRAPH SCALES*
C       TIME SCALE
        IF(JLOGT(1).EQ.1) GO TO 207
        N=(NX-JJ)/4.+1.01
        IX=JJ*IXINC-40
        IXINC=4*IXINC
        XOR=XOR/AFACT(M)
        XSCAL=(NX*INC*100.)/(JJLONG*AFACT(M)*4.)
        CALL ASCA(IX,-22,IXINC,0,ILC,INC,N,1,2)
        GO TO 209
C
C       LOG TIME SCALE
  207   IXINC=4*IXINC
        XOR=ILO
        XSCAL=NX*25./JJLONG
        CALL ASCA(-45,-25,IXINC,0,ILO,1,N,1,2)
C
C       ORDINATE SCALE LABELS
  209   IF(JLOGV(1).EQ.1) GO TO 216
        JP=JJHIGH/(NL-1)
        DO 279 I=1,NO
        REWIND 8
        JQ=6
        K=0
        N=0
C
C       FIND FORMAT FOR SCALE NUMBERS
        IF(SCALE(I).LT..09.OR.SCALE(I).GT.999.) GO TO 213
        JL=2
        JS=1
        IF(SCALE(I).LT..9) GO TO 221
        JL=1
        JS=2
        IF(SCALE(I).LT.9.9) GO TO 221
        JL=3
        IF(SCALE(I).GT.99.)JS=1
        GO TO 221
C
C       SCALE NUMBERS INVOLVING POWERS OF 10
  213   JL=1
        JS=2
        K=ALOG10(SCALE(I))
        IF(SCALE(I).LT.1.)K=K-1
        SCALE(I)=SCALE(I)*10.**(-K)
        IF(SCALE(I).GT.9.)JS=1
        WRITE(8,214)K
  214   FORMAT(I3)
        REWIND 8
        READ(8,215)(JPOW(L),L=1,3)
  215   FORMAT(3A1)
  216   IF(K.LT.10.AND.K.GE.0)N=1
        IF(K.LT.0.AND.K.GT.-10.OR.K.GE.10)N=2
        IF(K.LE.-10)N=3
        IF(JLOGV(1).EQ.1) GO TO 280
        JQ=N+9
        JZ=(3-N)*10
C
C       SET UP SCALING AND PLOTTING PARAMETERS
  221   TFACT=SCALE(I)/5.
        SCALE(I)=-JB(I)*TFACT
        IY=(JD-JB(I))*JP-5
        YORIG(I)=-JD*TFACT*10.**K
        YSC(I)=(100.*(NL-1)*TFACT*10.**K)/JJHIGH
        JD=JB(I)+1
        JM=JA(I)+JB(I)+1
        IF(SCALE(I).LT.0.0)JS=JS-1
        IF(I.EQ.1)IX=-JQ*10-5
        IF(I.EQ.2)IX=JJLONG-JS*10+5
        JW(I)=(JQ-JS)*10
C
C       WRITE ORDINATE SCALE NUMBERS ON DISC
        REWIND 8
        DO 239 JJ=1,JM
        GO TO(235,233,237),JL
  233   WRITE(8,234)SCALE(I)
  234   FORMAT(F6.3)
        GO TO 239
  235   WRITE(8,236)SCALE(I)
  236   FORMAT(F6.2)
        GO TO 239
  237   WRITE(8,238)SCALE(I)
  238   FORMAT(F6.1)
  239   SCALE(I)=SCALE(I)+TFACT
C
```

```
C       READ SCALE NUMBERS FROM DISC AND DRAW THEM
        REWIND 8
        JX=IX-JZ+90
        DO 249 JJ=1,JM
        READ(8,240)(ISCA(L),L=1,6)
  240   FORMAT(6A1)
        IF(JJ.NE.JQ) GO TO 242
        JN=(I-1)*(JJLONG+5)-35*(2-I)
        IF(I.EQ.2.AND.JB(2).GT.0)JN=JN+10
        CALL ALAB(JN,IY,ZERO,3,1,2)
        GO TO 249
  242   CALL ALAB(IX,IY,ISCA,JQ,1,2)
        IF(JQ.LE.6) GO TO 249
        JY=IY+5
        CALL ALAB(JX,JY,JPOW,3,1,2)
  249   IY=IY+JP
C
C       DRAW ZERO ABSCISSA
        IF(JC*JD.EQ.0.OR.I.EQ.2) GO TO 279
        X(1)=0.
        X(2)=JJLONG
        Y(1)=JJHIGH-4*JC*IYINC
        Y(2)=Y(1)
        CALL ALINE(X,Y,2,0.,0.,100.,100.,1)
  279   CONTINUE
        GO TO 315
C
C       DRAW LOG ORDINATE SCALE
  280   IYINC=IYINC*2
        DO 284 I=1,2
        IF(I.EQ.1)IX=-55
        IF(I.EQ.2)IX=JJLONG+N*10-45
        YORIG(I)=JL
        YSC(I)=(JS*100.)/JJHIGH
        JW(I)=50
  284   CALL ASCA(IX,-5,0,IYINC,JL,JY,NL,1,2)
C
C
C       *LABELLING OF GRAPH*
C       ORDINATE LABELS
  315   DO 323 I=1,NO
        CALL LABEL(JGRAPH(I,J),VTAG,UTAG)
        IF(I.EQ.2) GO TO 322
        IX=-JW(1)-40
        IY=100
        IF(JLOGV(1).EQ.1)IY=180
        X(1)=IX-10.
        X(2)=X(1)
        CALL ALINE(X,YS,2,0.,0.,100.,100.,1)
        IF(JLOGV(1).EQ.1)CALL ALAB(IX,100,LOG,3,2,4)
        CALL ALAB(IX,IY,JLAB,31,2,4)
C
C       GRAPH TITLE
        IX=(JJLONG-1200)/2
        CALL ALAB(IX,760,JLABEL,40,3,2)
        GO TO 323
  322   IX=JJLONG+JW(2)+40
        X(1)=IX+10.
        X(2)=X(1)
        X(3)=X(1)
        IF(JLOGV(2).EQ.1)CALL ALAB(IX,800,LOG,3,2,0)
        CALL ALAB(IX,720,JLAB,31,2,0)
        CALL ALINE(X,YC,3,0.,0.,100.,100.,3)
  323   CONTINUE
C
C       ABSCISSA LABEL
        IF(JLOGT(1).EQ.1) GO TO 360
        DO 351 I=1,7
  351   TLABEL(I+8)=JHOLD(I,M)
        IX=(JJLONG/20.-14)*10
        CALL ALAB(IX,-70,TLABEL,15,2,2)
        GO TO 401
C
C       LOG ABSCISSA LABEL
  360   IX=((JJLONG/20)-16)*10
        CALL ALAB(IX,-70,LOGLAB,16,2,2)
C
C
C       *PLOT VARIABLES*
  401   DO 489 I=1,NO
        YOR=YORIG(I)
        YSCAL=YSC(I)
        JP=JLINE(I)-1
        IF(JLOGT(1).EQ.1) GO TO 408
        DO 407 JL=1,JP
  407   X(JL)=TGRAPH(JL,JK(I))
        GO TO 410
  408   DO 409 JL=1,JP
  409   X(JL)=ALOG10(TGRAPH(JL,JK(I)))
  410   JN=(JPOS(I)-1)*100
        DO 430 JL=1,JP
  430   Y(JL)=C(JL+JN)
        CALL ALINE(X,Y,JP,XOR,YOR,XSCAL,YSCAL,1)
C
C       DRAW CROSSES ON PLOT OF SECOND VARIABLE
        IF(I.EQ.1) GO TO 489
        JP=JP/5
        DO 461 K=1,JP
        X(K)=X(5*K)
  461   Y(K)=Y(5*K)
        CALL ALINE(X,Y,JP,XOR,YOR,XSCAL,YSCAL,2)
  489   CONTINUE
C
C       TERMINATE PLOT
        CALL AEND
        GO TO 600
  592   WRITE(6,593)JGRAPH(1,J),JGRAPH(2,J)
  593   FORMAT(//T20,'REALS(',I4,') AND REALS(',I4,') WERE NOT SAVED'//)
  600   CONTINUE
        RETURN
        END
```

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