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THE FORMULATION AND VALIDATION OF MATHEMATICAL MODELS OF CALCIUM METABOLISM

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A thesis submitted for the degree of Doctor of Philosophy

The City University, London.

Department of Systems Science

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The Formulation and Validation of Mathematical Models of Calcium Metabolism

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"Facts?" he repeated. "Take a drop more grog, Mr Franklin, and you'll get over the weakness of believing in facts!
Foul Play, Sir!"

Wilkie Collins, The Moonstone

ABSTRACT

This thesis presents an example of and argument for the use of mathematical models in the study of physiological systems, and specifically calcium metabolism. It is shown through the example of calcium, that the use of isomorphic models incorporating init processes soundly based upon the underlying physiology can produce useful insight concerning the structure and control mechanisms of metabolic systems. Further even though these models can be theoretically and practically unidentifiable, a high degree of empirical validity can be observed. Models such as these are fundamental to the continued development of understanding and the basis for directed physiological experimentation.

This thesis begins with a survey of the physiology of calcium metabolism, and a critical classification of existing models related to calcium. The distinction between curve fitted tracer models and those based in some way upon the underlying physiology is highlighted. The first model produced was an attempt to incorporate unit processes into a tracer model. This approach was shown to be unsatisfactory, as the uncertain identity of the model compartments hindered meaningful development.

A second model was produced from scratch according to the methodology, soundly based upon physiologically meaningful compartments, and control structures. This model was further refined using a form of structural sensitivity analysis, to reduce the complexity of the model whilst still retaining those elements needed to predict the short-term dynamics of calcium. Thus some of those elements originally included were shown to be superfluous.

This iterative approach to model development was further extended by considering extra situations, and specifically hypocalcaemic stimuli, to evolve a model, with better empirical behaviour. The validity of this model is then examined over a wide range of experimental situations, and shown to provide predictions that are consistent with available data. This validity is present despite the unidentifiability of the model, and provides useful insight into the physiological control of calcium. This approach should be valid for other physiological systems.

The feasibility of applying this approach to the long term behaviour of calcium is then investigated, leading to the production of a long term model firmly based upon unit processes at the level of the bone cells involved. This model is shown to be internally valid and is a suitable vehicle for investigation of the long term situation.

CHAPTER 1

CHAPTER 1

1. Introduction

This thesis provides an example of and an argument for the use of mathematical models in the investigation of physiological systems. The work presented focuses upon the use of complex models to provide insight and understanding of system behaviour. The very activity of formulating and validating mathematical models is shown to yield useful information concerning the structure and function of the calcium metabolic system. This is of benefit to both physiology and the methodology of explanatory modelling.

1.1 Scope For Study

Calcium is that most ubiquitous of minerals, present in almost every human tissue, and important in physiological areas as divergent as: the maintenance of both normal neuromuscular excitability and an adequate load bearing skeleton. The formation of bone, the coagulation of blood, and the maintenance of normal muscular function are all dependent upon the maintenance of the extracellular calcium concentration. The range of bodily demands for calcium suggests that the control mechanisms associated with it are potentially similarly complex.

A wide range of abnormal or disease states is found, reflecting the complexity of the control mechanisms. From a symptomatic view these include: tetany, hard calcified deposits in soft tissue, weak and brittle bones, and over excitable motor muscles. The problem of growth itself has not been mentioned, but here the skeleton's huge appetite for mineral is sated without disturbance of the critical plasma calcium concentration.

Complex control processes are rarely amenable to straightforward static experimental investigation. The underlying structural basis can only be revealed through an investigation of the dynamic behaviour of the system. It is the dynamics of calcium that are in practice of most interest, most clinical conditions resulting from disturbance of the individual's healthy steady state. Mathematical models have a significant role here, with their explicit incorporation of assumptions and ideas that are not always apparent from less precise verbal descriptions or 'models'.

The use of explanatory models in physiology and medicine has a fine tradition (cf. Fick, 1855; and Starling, 1896). The models presented here are generally more complex than those early beginnings, but similarly focus upon explanations of observable phenomena. The complexity of calcium metabolism provides a number of situations whose underlying causality is hidden by the symptom's intricacy.

1.2 Objectives

The major objective of this study is to increase knowledge and understanding of human calcium metabolism through the formulation and validation of explanatory mathematical models. It was intended that the work would have implications for both physiology and the methodology of systems modelling. Although specific areas of methodological concern were not identified at this stage, specific physiological areas and problems were. These include but were not restricted to the following:

The simulation of the short-term homeostatic control of plasma calcium. This should specifically include the response to both hyper- and hypo-calcaemic conditions, and the common occurrence of fluctuating feeding patterns.

The role of phosphate in the control of plasma calcium.

The relevance of data available from the disappearance from plasma of a radioactive calcium tracer (e.g. ^{45}Ca).

The role of parathyroid hormone in the short and long term control of calcium metabolism, and an assessment of the importance of the role of calcitonin.

The role of bone in the short-term control of plasma calcium, does it in fact have any?

Is the long term maintenance of the skeleton achieved through control mechanisms entirely separate from those concerning the plasma?

1.3 Thesis Structure

Chapter 2 first provides an introduction to the detailed physiology of calcium metabolism, the subsystems, hormones, and other minerals that are relevant.

Thus calcium physiology is introduced by considering the extracellular fluid, soft tissue, and bone as physiologically distinct as well as anatomically separated sites. The central role of parathyroid hormone is stressed, and the particular problems of hormonal measurement covered with detailed reference to PTH. Disorders of calcium metabolism are considered with particular reference to the level of plasma calcium.

Before embarking upon the modelling exercise, Chapter 3 reviews and classifies other metabolic system models according to form or style of formulation. These models are reviewed with particular reference to the generation of models that have explanatory value. The particular set of models that describes the disappearance of radioactive tracers from plasma is criticised in this respect, and one particular model is reviewed in detail.

The use of radioactive tracer disappearance data in the generation of models has its limitations, but it can be argued that information regarding the steady state distribution is provided. Chapter 4 assumed this, and describes the development of a series of models (MODEL1-5) that have a tracer model as a framework upon which are hung controller equations describing the influence of hormones and chemical species apart from calcium. It was intended that greater predictive value would be incorporated, but attempts to validate these models still lead to rejection of both the models and the approach, despite the successful reproduction of some experimental situations.

An aspect that was important in the rejection of MODEL5 was the incoherent physiological isomorphism that hampered further development and refinement : a vital aspect of an iterative approach to model development that fully incorporates the sequential formulation, identification and validation of a model. Chapter 5 extends this idea through the development of two models (MODEL11-12) that explicitly incorporate a greater degree of physiological isomorphism. The practical problems of identification and validation of non trivial non- -linear models are well illustrated as the process of model reduction is used to refine the model. This refinement concludes by indicating the superfluous nature of some of the model components.

MODEL12 as formulated in chapter 5 represents an experiment in modelling as part of the cycle of model identification and validation. Chapter 6 documents further evolution as MODEL13 through to MODEL17 are developed. The short term homeostatic control of plasma calcium is used as the major determinant of the validity of these models. The techniques of sensitivity analysis, parameter optimisation and identification are illustrated as part of this process.

Chapter 7 covers the validity of MODEL17, reviewing the meaning of validity with particular reference to the initial objectives of this study. The performance of MODEL17 is then assessed over a wide range of experimental situations including; the disappearance of a plasma calcium tracer, the administration of a calcium bolus to the plasma, the consequences of typical feeding behaviour, calcium EDTA and phosphate infusions, variations in PTH status, and long term variations in oral intake. Data from most of these situations were not used during model formulation, hence they are especially significant tests of the model validity. This performance

assessment enables the validity of the model to be critically evaluated with regard to the understanding of the short-term control of the metabolic system it provides.

Chapter 8 assesses the feasibility of using the same approach used for the formulation of MODEL17; the incorporation of physiologically based unit processes, coupled with step-wise refinement and validation in modelling the long-term behaviour of calcium. Thus a model is formulated which attempts to incorporate bone cell unit processes. Short-term homeostatic regulation is of little consequence. As this is purely a feasibility study, simulations are confined to assessments of internal (and mainly algorithmic) validity. This 'trial' model formulation enables directions to be set for future work.

Chapter 9 concludes this thesis providing: a resume of the work covered, the initial objectives, and the contributions made to physiology and the methodology of modelling. The particular methodological problems peculiar to metabolic and physiological modelling are discussed and recommendations made for further studies of this nature. In particular it is shown that a major contribution explanatory models can make is not the solution(s) to a number of questions, but rather the provision of potential questions, critical tests, and the direction of future work. Indeed the success of a model could be gauged by the extent of the ignorance uncovered. Almost of necessity more new questions are raised than definitively answered.

Appendix I is a reference section with details of the nomenclature used, and a full specification of every model referred to in this thesis.

Appendix II details the computer hardware and software used in this work, both in terms of design and the actual programs that were written and used.

CHAPTER 2

CHAPTER 2

2. Calcium Physiology

This chapter is concerned with providing an introduction to the physiology of calcium metabolism. This will be dealt with at various organisational levels from that of a single kidney cell to the whole body.

Any physiological discussion of calcium metabolism cannot be divorced from that of phosphorous (in its various phosphate ionic forms), magnesium and those substances that have hormonal influence upon the distribution of calcium. These are Parathyroid Hormone (PTH), Calcitonin (CT), and the metabolites of vitamin D3. These are all considered.

There are two possible means of organising a discussion of calcium physiology; according to distribution and body site, or according to the chemical and hormonal species that influence calcium. The early sections of this chapter draw upon the former leaving the details of hormonal action to section 2.5 onwards. The chapter ends by considering the PTH measurement difficulties that beset the physiologist, and some of the more commonly occurring clinical disorders.

2.1 The Functions of Calcium

The functions of calcium include the signalling of muscular contraction, skeletal maintenance, synaptic transfer, and other ancillary functions. The main sites and fluxes of body calcium are shown in Figures 2.1 and 2.3. Some of the lumped compartments or pools depicted are anatomically distinct whilst others are essentially conceptual (respectively, PCC, PIC, TC, FBC, GC, and ECFC, STC, EBC). In these figures the symbols associated with flows, transfers or transformations between compartments merely name rates of flow and are not rate constants. Full details of the nomenclature adopted are given in Appendix 1.

2.1.1 Muscular Contraction and Plasma Calcium Homeostasis

The supply and distribution of calcium is crucial to the functioning of mammals at two basic levels: muscular concentration itself - with a flux of calcium ions being responsible for the initiation of contraction in all vertebrate muscle, and the provision of an 'adequate' skeleton. In vertebrate skeletal muscle, calcium ions operate literally as an on off switch. It is the former and other important functions of calcium that necessitate the extremely efficient control of plasma calcium in the human body. This variable provides an excellent example of homeostatic control with total plasma calcium typically having the value of 9.5 mg/100 ml with $\pm$0.5 mg/100 ml

representing the 2.5 and 97.5 percentiles of a large 'normal' population (Kleeman et al, 1971).

2.1.2 Skeletal Maintenance

Bone consists of a collagenous matrix (with some 5% non-collagenous material) which supports the inorganic mineral. The structure has a complex vascular system, and various types of cell are intimately involved in supporting and maintaining this structure. These details are fully dealt with in section 2.4.

Bone mineral contains mainly calcium, phosphate and carbonate in a molar ratio of 10:6:1 with varying amounts of magnesium, sodium, potassium, chloride, citrate, fluoride and pyrophosphate (Armstrong and Singer, 1965). A number of chemical forms are found, leading to variations in this molar ratio. These include: secondary calcium phosphate, tertiary calcium phosphate, octocalcium phosphate, and hydroxyapatite. An adequate supply of calcium is necessary for the maintenance of this complex structure.

Although skeletal growth stops at maturity, internal remodelling continues until death. The healthy young adult remodels approximately 10 per cent of the skeleton per annum. It is important that deposition and apposition are approximately equal, and further that the nature of the mineral deposited is the same as that which is resorbed.

The maintenance of an 'adequate' skeleton is an area of major clinical interest, the loss of bone which accompanies ageing being a universal phenomenon which unfortunately does not always stay within acceptable limits. Bones can become so weak that fractures frequently occur with only minimal trauma. Women are especially at risk; at least one in ten post menopausal women can be classified as suffering from Osteoporosis, clinically manifested as fracture prone brittle bones (see Lindsay and Hart, 1978; and section 2.8.5 of this chapter).

Skeletal changes are insidious. The degenerative process that has led to a clinically diagnosable state may have been active for five or ten years. It is unlikely that treatment, if available, can reverse this degeneration in a shorter time frame. Early detection or predictive measurement is desirable, but no satisfactory metric has yet been found to identify the 10-25% of the post menopausal population at risk before degeneration occurs (see Lindsay and Hart, 1978; and Recker, Saville and Heaney, 1977).

2.1.3 Other Functions

Calcium has other physiological roles which necessitate an adequate or controlled plasma calcium level. These are the maintenance of nervous transmission, and blood clotting.

Neuronal depolorisation, which initiates and transmits all nervous signals requires an adequate concentration of calcium in the fluid surrounding the nerve. Neuro transmitter release at junctions between nerves (the synapses) is the transmission signal. This release also requires an adequate supply of calcium at the junction (see Buller, 1975).

Blood clotting is essentially dependent upon the conversion of the soluble protein fibrinogen into the insoluble protein fibrin by the enzyme, thrombin. Thrombin is formed from prothrombin only in the presence of extracellular Ca^{++} and a number of factors derived from damaged tissues, disintegrating platelets, and the plasma itself.

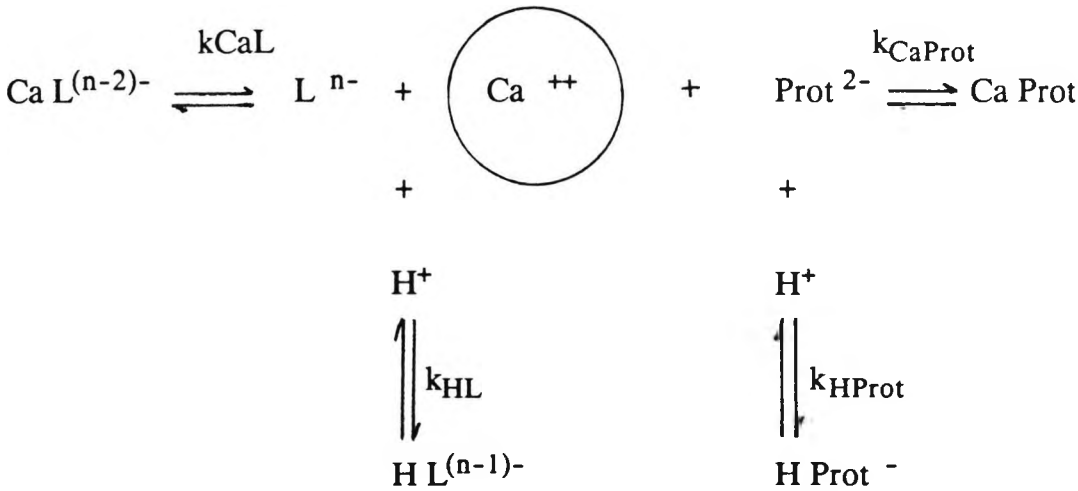
2.2 The Extracelullar Fluid

This section is the first of three, each of which focuses upon a particular body space or structure and examines mineral metabolism in this space. This section considers the whole of the extra cellular fluid space, which has a special role as the distributive intermediary for much of the mineral movement within the body.

2.2.1 Calcium Content

Although the extra cellular fluid (ECF) volume is greater than the plasma space, this discussion concentrates upon the latter. The ECF has the same constituents as plasma, but there are reduced concentrations of protein ligands.

Plasma contains approximately 10mg/100ml of calcium, but an in vitro plasma and bone mineral preparation will only support an ionised plasma content of around 2 mg/100ml. McLean and Hastings (1935) demonstrated the existence of three separately definable plasma calcium fractions. These are ionised, ligand bound or complexed, and protein bound, as shown overleaf:



'L' = Ligands including citrate, phosphate, and bicarbonate
'Prot' = proteins, major ones are albumin and globulin.

It is now accepted that it is the ionised calcium (PIC) that is the physiologically active form, and further that it is only this species that is the subject of hormonal control (via PTH and possibly calcitonin). Although at a detailed level there is open argument about the techniques and accuracy of measurement the following table gives some typical values:

	(n = 69) Normals		(n = 18) Hyper-Parathyroids	
Plasma PH	7.4	± 0.02	7.43	± 0.04
Plasma Protein (g/l)	78.5	± 5.2	73.5	± 7.0
Total Calcium (mg/100ml)	10.5	± 0.4	13.6	± 2.0
Ultrafiltrable Calcium (mg/100ml)	5.6	± 0.2	7.7	± 1.2
Ionised Calcium (mg/100ml)	4.6	± 0.1	6.4	± 1.1

(Figures from Pedersen (1972))

The equilibrium that exists between the three calcium species will provide an immediate buffer to disturbances of the ionised fraction.

2.2.2 Control Of The Plasma Calcium Level

Calcium enters the plasma through absorption from the gastrointestinal tract, bone, various 'soft' body tissues and reabsorption of the bulk of calcium that is filtered by the kidneys. It is the balance between these flows that is responsible for the maintenance of a steady plasma calcium.

At a first approximation control is achieved through variations in the amount of calcium that is removed by the kidney.

The kidneys filter the plasma at a rate (GFR - the Glomerular Filtration Rate) which for the purposes of this study can be approximated as being constant (Robertson et al, 1974). This process results in the removal of the ultrafiltrable fraction of plasma calcium (at a rate of Tubular Calcium Filtration TCF). An almost equal quantity of both plasma and calcium are reabsorbed (Tubular Calcium Reabsorption TCR), always however, with a nett loss to the urine. It is through variation in this reabsorption rate (TCR) that the kidney exerts its control over the level of plasma ionised calcium (PIC). TCR can be shown to vary directly with the level of plasma parathyroid hormone (PTH), the secretion rate of which is approximately inversely proportional to the level of PIC.

This provides a simple negative feedback on the level of ionised calcium. Figures 2.1 and 2.4 show that this is only part of the picture, both the plasma physico - chemical equilibrium effects referred to in section 2.2.1, and the other actions of PTH on plasma gut and plasma bone transfers playing a role in the overall regulation of plasma ionised calcium.

2.2.3 Phosphate Content

The plasma phosphate level is less closely controlled than calcium, typically varying between 2.4 and 5.4 mg/100 ml in adults.

Plasma phosphate can be divided into an acid insoluble fraction consisting mainly of phospholipids, and an acid soluble fraction consisting mainly of inorganic phosphate. This latter fraction will also include a small amount of organic ester phosphate. This is shown in the table below:

Phosphate Fraction	Concentration (mmol l^{-1})
Acid Soluble:	1.3
- Organic Ester	0.1
- Inorganic	1.2
Acid Insoluble (Phospholipids)	2.6
Total Phosphate	3.9

However not all of the inorganic fraction is diffusable, as shown in the table overleaf :

Inorganic Fraction	Concentration (mmol l^{-1})	% age
Free HPO42-	0.81	68
Free H2PO4-	0.2	17
Complexed MgHPO4	0.03	2
Complexed CaHPO4	0.04	3
(Total Diffusable)	(1.08)	(90)
Protein Bound	0.12	10
Total Inorganic	1.2	100

The phosphate levels in plasma are not 'controlled', but they are affected by variations in plasma calcium, and by plasma PTH levels. This latter variation is mediated by the action of PTH upon the kidney. Tubular phosphate reabsorption (TPR)) varies in inverse proportion to plasma PTH levels. It was at one time thought that phosphate had a direct effect upon PTH secretion, but this has been shown to be a result of the effect of phosphate on plasma calcium (see Walser, 1961; and Herbert et al, 1966). Thus an increase in plasma phosphate will lower plasma calcium through the precipitation of $CaHPO_4$, a straightforward solubility product reaction.

A scheme of body distribution is shown in Figure 2.2.

2.2.4 Magnesium Content

Magnesium and calcium are both group II divalent metals, and as such can behave in a similar manner, but the body distribution of magnesium is largely intra-cellular. Unlike calcium, it appears that no control mechanisms exist specifically for the homeostatic maintenance of plasma ionised magnesium (PIM). This is not to say that magnesium does not affect PTH secretion. It does, but the effect is not as marked as that of calcium (see Mayer, 1975). Similarly some of the physiological actions of calcium are mimicked by magnesium.

2.3 The Soft Tissue

In any discussion of mineral metabolism the body's soft tissue is often ignored, yet an appreciable fraction of the body' calcium (0.6%) and phosphate (14%) is found in muscle and other soft organ cells. These figures should be compared with the ECF (respectively 0.1% and 0.6%) (see Nordin, 1976b).

2.3.1 Calcium Distribution

The calcium content of body tissue other than bone varies between 1 and 10 mmol $kg^{-1}$of tissue (Widdowson and Dickenson, 1964), this large variation mainly reflecting differences in the kind and amount of connective and structural tissue. Within muscle

cells an additional space holding calcium at an appreciably higher concentration than in the ECF, and providing a reservoir of calcium to sustain the contractile properties of these cells has been reported (Hajdu and Leonard, 1975).

The bulk of the calcium within the cells is found within various organelles (e.g. mitochondria, or the sarcoplasmic reticulum, the free intracellular calcium being estimated to be around 10^{-3} mmol l^{-1} or less. This is less than one thousandth that of the ECF (Borle, 1975).

2.3.2 Phosphate Distribution

Little has been written regarding the distribution of phosphate in soft tissues, although the absolute mass involved is significant. Bearing in mind the absolute need of all energy and synthetic systems for inorganic phosphate, it is possible that the intracellular inorganic phosphate is controlled, at the expense of plasma phosphate (see Reeve and Joiner, 1973).

2.4 The Bone

This section deals with the skeleton or bone. The composition, and structure are first considered, then the process of mineral turnover and bone remodelling or maintenance are discussed. It should be borne in mind that the skeleton contains a number of types (e.g. cortical and trabecular) or classes of bone. This section does not attempt to cover their detailed differences.

2.4.1 Composition and Structure

Bone consists of an organic cartilaginous matrix with the mass of mineral deposited upon this matrix. It is a highly vascular tissue with three cell systems incorporated into its structure: osteoclasts, osteoblasts and osteocytes. It is thought to be these three cell systems that control the 'remodelling' of bones, a process that is constantly occurring (Aaron, 1976).

A distinction has been drawn between fixed and exchangeable bone calcium (FBC and EBC), but this is largely a conceptual simplification. Thus exchangeable bone calcium is considered to be that 'pool' of bone that is more readily accessible to exchange, thus providing a store of calcium able to buffer extracellular fluid and plasma ionised calcium.

Fixed bone calcium is considered to be structural bone that is relatively inaccessible to exchange with extracellular fluid calcium. In fact such exchange does take place, but at a slow, relatively constant rate.

Bone mineral contains mainly calcium, phosphate and carbonate in a molar ratio of 10:6:1 with varying amounts of magnesium, sodium, potassium, chloride, citrate, fluoride and pyrophosphate (Armstrong and Singer, 1965). The ratio is only approximate as various chemical forms are found (e.g. Hydroxyapatite and amorphous calcium phosphate).

2.4.2 Turnover and Remodelling

Turnover or remodelling is the resorption of existing bone and its replacement with new tissue. Between birth and maturity this process assumes a very special significance as a 'positive' mineral balance is maintained as the skeleton grows. Strictly speaking the term remodelling applies mainly to the growth situation as changes of shape or model are involved. In the mature adult, the shape remains the same and formation and resorption balance each other.

Matrix and mineral are removed together by osteoclastic resorption. The chemistry of resorption is complex as collagen is digested, mucopolysacharides are depolymerised, and mineral is dissolved. Between 0.5 and 5 percent of the bone surface being the subject of bone resorption at any one time.

Bone formation is a two step process: production of matrix, and its subsequent mineralisation, both under the control of osteoblasts. Around 15 percent of the bone surface is covered by osteoid seams, the as yet unmineralised layer of matrix, approximately 15 micro metres thick.

Mineralisation is thought to be a multi stage process possibly involving the sequential formation of:

1) Secondary calcium phosphate
2) Tertiary calcium phosphate
3) Octocalcium phosphate
4) Hydroxyapatite

(from Wergebal and Baylink, 1974)

This process takes at least a number of days. The active participation of bone cells must occur. If the solubility products of these four chemicals are considered, the process cannot occur without local control of the fluid at the osteoid seam (see Parfitt and Kleerekoper, 1979). They would not be able to form otherwise.

Microradiographic studies suggest that the mineralisation of newly formed matrix is around 50 to 70 percent complete within a few days (primary mineralisation, see Parfitt, 1976). A secondary mineralisation phase, probably outside cellular control

then increases mineral density to around 90 percent of maximum over 3 to 6 months, with a mineralisation of 95 percent taking a number of years to achieve.

The bone cells can be considered to be organised into functional 'remodelling' units (the Bone Remodelling Unit). The BRU successively resorbs and replaces bone over a cycle time that includes a resorptive phase of 2 to 4 weeks, and a formative phase of around 70 to 100 days. These are separated by a combined reversal phase of 2 to 4 weeks.

2.5 Parathyroid Hormone (PTH)

PTH is manufactured and secreted by the four parathyroid glands, which are located on either side of the thyroid gland. PTH holds a central position in the control of calcium metabolism over a whole range of time scales from short term plasma calcium homeostasis to the long term control of skeletal remodelling, as first demonstrated by Albright et al (1932). This section considers the synthesis, secretion, metabolism and actions of PTH.

2.5.1 PTH Synthesis

PTH is an 84 amino acid polypeptide of molecular weight 9500 Daltons, but its biological activity appears to reside in the amino terminal portion, the 1-34 (N-terminal) fragment being as potent as the intact molecule (Segre et al, 1977). Within a parathyroid gland 'chief cell', hormone synthesis is thought to occur according to the following 4 stage sequence (From Parfitt and Kleerekoper, 1979):

Step		Product	Time-Scale
1)	Messenger RNA Translation This is then directed into the cisternal spaces of the rough endoplasmic Reticulum	Pre-Pro-PTH	seconds
2)	Remove 25-Amino Acid This is transported to the Golgi region (along micro-tubules)	Pro-PTH	10-15 minutes
3)	Cleave Molecule	PTH	
4)	Secrete hormone as it is formed, or package into secretory granules and store.		

2.5.2 PTH Secretion

PTH release can occur from both stored vesicles and freshly formed hormone (see Cohn, 1975). The relationship between the two is as yet inconclusive.

2.5.2.1 Influence of Calcium

It is clear, that PTH release from the parathyroid gland is inversely related to the level of plasma ionised calcium (PIC) (Blum et al, 1974a and 1974b; and Mayer and Hurst, 1978), and to a lesser extent to the concentration of plasma ionised magnesium (PIM) (Buckle et al, 1968). Mayer (1975) has shown that magnesium is around one third as potent in mediating PTH release.

Although this inverse relationship between PIC and PTH secretion rate was at first thought to be proportional, in reality the situation is not that simple: a basal level of PTH secretion has been demonstrated even in the presence of dangerous levels of hypercalcaemia (Mayer, Habener and Potts Jr. 1976). Maximum secretion rates have been demonstrated by a number of authors (Mayer, 1975; Targovnik et al, 1971) as has the suggestion of a relation to the rate of change of PIC (derivative control) and also to the duration of the change in PIC (integral control). Interestingly, in vitro experiments have shown that the rate of Pro-PTH synthesis and cleavage is unaffected by hypocalcaemic incubation, but that the rate of intracellular PTH degradation and recycling was markedly reduced (Cohn and Hamilton, 1976). Conversely incubation in a medium of high calcium concentration increased the rate of intracellular degradation.

Changes in the number of active parathyroid gland chief cells have been demonstrated (i.e. hypertrophic and hyperplastic changes) in response to a persistently low or high plasma calcium concentration (Capen, 1971; Lee and Roth, 1975). Thus hypocalcaemic influences affect the parathyroid gland in a range of ways dependent upon time, as follows:

	Mechanism	Time-Scale
1.	Release of preformed hormone increases	seconds/minutes
2.	Intracellular degradation decreases	minutes/hours
3.	Hormone synthesis increases	hours/days
4.	Cell quiescent interval decreases	days/weeks
5.	Cell numbers increase (hyperplasia)	weeks/months

2.5.2.2 Other Influences

The following actions associated with PTH have been demonstrated to exist independently of the influence of calcium: A diurnal variation in plasma PTH has been observed with maximal plasma concentrations in the early morning. The mechanism for this variation is the subject of argument (Jubiz et al, 1972). Severe magnesium depletion (Anast et al 1976) suppresses PTH release. Adrenergic stimulation (either adrenaline or noradrenaline) increases PTH secretion (see Heath III, 1980; and Blum et al, 1977; for a full discussion of the significance of this and related observations).

Plasma phosphate variation has been shown to have no direct effect on PTH those that are observed are merely mediated via changes in plasma calcium (Sherwood et al, 1968). Changes in gastrin, secretin, cholecystokinin, glucagon, prostaglandins, and acid base balance have been reported to be of influence, but as yet no clear physiological role or independence from a PIC change has been shown for any of these (Heath III, 1980).

2.5.3 PTH Metabolism

Breakdown occurs in the kidney and the liver, but cleavage of the intact molecule can occur at various positions in the peptide chain. As some of the fragments have been shown to be biologically active at physiological concentrations (Parsons et al, 1975), and also to possess much larger half-lives than the intact molecule a detailed knowledge of the process of metabolic breakdown is desirable. In addition, because of the activity of the metabolites, measurements of the intact immunoreactive molecule may present only part of the picture.

2.5.4 Actions of PTH

The most obvious physiological action of PTH is to restore plasma calcium to an 'acceptable' level following hypocalcaemia. This effect is the result of a number of specific actions upon; bone, kidney and gut. These will be dealt with in turn. At a cellular level activation of membrane bound adenylate cyclase, and an increased cell membrane permeability to calcium are involved. All actions have been shown to require an adequate supply of $1,25(OH)_2$ VIT D3 (Parfitt, 1976, and Miravet et al, 1981).

2.5.4.1 On Bone

PTH affects all types of bone cell, providing a major influence upon the prevailing level of bone remodelling and turnover. In common with its other actions, bone cell

actions appear to be mediated via binding and activation of cell membrane adenylate cyclase receptors (Peck et al, 1977). In humans given continuous PTH infusions over 24 hours, urinary hydroxyproline excretion doubles over approximately 8 hours (Froeling and Bijvoet, 1974) and declines over a similar period upon cessation, indicating an increase in osteoclast activity. In vitro results indicate similar short term effects from an increase in osteoclast activity, and also longer term effects from an increase in their numbers.

The increased number of osteoclasts may arise by activation of pre osteoclasts arrested in the G1 or G2 stages of the proliferating cell cycle. The increased cell recruitment will provide a means for perpetuating resorption after the PTH stimulus has subsided. A rise in osteoclast numbers will inevitably lead to a rise in osteoblast numbers within a few weeks as the remodelling cycle progresses to its formative stage, and an increase in bone formation.

2.5.4.2 On The Kidney

PTH has a profound effect upon the kidney (see Froeling and Bijvoet, 1974). This action provides the fastest acting homeocalcaemic influence following hypocalcaemia. The primary effect is an alteration in tubular maximum calcium reabsorption (TMC) directly proportional to the plasma PTH level (TMP varies indirectly). Vitamin D3 metabolites appear to be necessary for the effects to occur, but an influence of more significance than facilitation is unclear. (See Parfitt and Kleerekoper, 1979).

2.5.4.3 On The Gut

One of the normocalcaemic actions of PTH is an enhancement of absorption of both calcium and phosphate. Although the effect upon phosphate absorption is less clearly demonstrated. In common with the action upon the kidney a suitable vitamin D3 status also appears to be necessary for these effects.

2.6 Other Endocrine Influences

Although PTH clearly holds a central role in the endocrine control of calcium metabolism, there are others that also have a significant effect. The two most important influences are those of vitamin D3 and calcitonin. This section considers their contribution.

2.6.1 Vitamin D

Figure 2.5 shows a basic scheme of distribution of vitamin D3. It is generally accepted that the active metabolite is 1,25 $(OH)_2$ vitamin D3 (P2D), the function of

24,25 $(OH)_2$ vitamin D3 (P3D) being, as yet, uncertain (see Deluca et al, 1975; and Colsten et al, 1975). The physiologic actions of P2D include: stimulation of intestinal calcium transport and PTH dependent bone calcium mobilisation. There is also strong evidence that P2D stimulates intestinal phosphate transport and a PTH independent bone mineral mobilisation under hypophosphataemic conditions (Deluca, 1980).

2.6.2 Calcitonin

The distribution of calcitonin (CT) is shown in Figure 2.4. Its physiological action is an inhibition of bone resorption giving it a hypocalcaemic action. Its significance for the healthy adult is unclear, however, as the hypocalcaemic action is most profound during bone growth (in infancy) or other remodelling activity (e.g. after injury) (MacIntyre et al, 1980). Intravenously administered calcitonin has negligible effect upon plasma ionised calcium in normal adult subjects (Kleeman et al, 1971).

Most significantly there have been no reported cases suffering from a lack of this hormone. Indeed total thyroidectomy has no known influence upon human calcium homeostasis, although it is likely to be more important in other mammalian species.

2.7 Measurement of PTH

From a clinical viewpoint a successful PTH assay has one requirement ; the ability to yield an accurate assessment of the functional activity of the parathyroid gland. From a modelling stance, reliable estimates of metabolite species concentration are more appropriate. This section considers the available options and their clinical usefulness.

Radioimmunoassay is the most widely used in routine PTH assays; liquid chromatography, cytochemical bioassay, and homologous bioassay have also been used.

2.7.1 Influence of Metabolites

The parathyroid gland secretes both intact hormone, and metabolites formed through proteolytic breakdown. Furthermore this breakdown has been observed to occur both in the gland and in the peripheral circulation (Cohn and MacGregor, 1981; and Mayer et al, 1979). As well as the intact molecule (1-84), three classes of metabolite are found: the C-terminal (37-84, or 34-84), the N-terminal (1-34) and various mid-region fragments. The potentially varying half-lives, antisera, and biological functions impact greatly upon the assays.

2.7.2 Radioimmunoassay Techniques

Antisera have been developed that fall into one of four groups directed respectively towards: the N-terminal region where the intact molecule is recognised; the C-terminal region where the intact hormone is recognised; a mid molecule assay recognising all mid molecule metabolites as well as the intact hormone; and finally a two stage assay using 2 antisera, that is specific to the intact molecule only.

The biologically active hormone fragment is the N-terminal region, but assays specific to this region have been found to lack specificity (Martin et al, 1980). C-terminal assays suffer from complementary advantages and disadvantages: the C-terminal fragment is biologically inactive and has a longer half-life than intact PTH or PTH(1-34).

They have however been found to be more reliable discriminators of hyperparathyroid function, because of this longer half-life. Mid region antisera are specific to PTH(43-68) or PTH(44-68), which both also fall within the C-terminal range.

2.7.3 Liquid Chromatography

High performance liquid chromatography (HPLC) has been used to both purify and analyse PTH. When used as an assay, radioimmunoassay has been used to detect PTH in the collected functions of column effluent (Zanelli et at, 1983). The technique has not been used as a routine assay.

2.7.4 Bioassay

A cytochemical assay dependent upon the degree of stimulation of glucose-6-phosphate dehydrogenase activity in the distal convoluted tubules of segments of guinea-pig kidney maintained in vitro, has been developed (Allgrove et al, 1983). This suggests that the concentration of biologically active PTH in the circulation is less than 10% of the circulating PTH measured by radioimmunoassay. The technique is cumbersome, and only used to compare other methods.

An assay that is not as cumbersome has been reported, but is not as sensitive as the cytochemical assay. This is dependent upon the measurement by radioimmunoassay of the cyclic AMP (cAMP) generated by membrane enriched renal cortex tissue. All biologically active fragments will be measured by this technique which is more practical to use than the cytochemical assay (Nissenson et al, 1981).

2.7.5 Clinical Usefulness of Assays

The primary clinical function of a PTH assay is the discrimination of cases of primary hyperparathyroidism from normals and other causes of hypercalcaemia such as a carcinoma of the lung and kidney. As indicated in the previous sections only the newer N-terminal assays can distinguish patients with primary hyperparathyroidism more than 90% of the time (Hawker et al, 1984).

Indeed it is the C-terminal and mid molecule assays that provide a clearer distinction, possible because the longer half-lives of these fragments present a picture of parathyroid function integrated over a longer time span. The 2 stage intact PTH immunoassay of Lindall et al (1983), and the cytochemical bioassay have been shown to provide extremely good discrimination, but as yet the complexity of both has precluded their routine adoption.

2.8 Clinical Disorders

This section covers some of the more commonly occurring disorders of calcium metabolism. All of the disorders discussed are characterised by an excess or lack of PTH, a lack of Vitamin D3 metabolites, or inappropriate levels of bone turnover. Further most of the symptoms are due to changes in plasma calcium. The consequences of these changes are discussed in isolation first.

2.8.1 Consequences of a High Plasma Calcium

The kidney, heart, and neuromuscular excitability are the three areas of concern. The high ionised calcium level will cause the solubility product of calcium phosphate to be exceeded in extraosseous regions, the most dangerous being the renal tubules, leading to nephrocalcinosis and eventually renal failure. Acute hypercalcaemia also causes an impairment of renal concentrating ability, sometimes leading to dehydration. The electrocardiogram is altered, and if the hypercalcaemia is severe, there is a risk of cardiac arrest. Neuromuscular excitability is depressed, muscle hypotonia may be present, and smooth muscle effects can lead to constipation and abdominal pain. Abdominal pain can also result from higher than appropriate gastric acid secretion.

2.8.2 Consequences of a Low Plasma Calcium

There are two areas of concern: neuromuscular activity will be greatly increased, and the lens of the eye is affected. The former can produce tetany or even spasm of the larynx. The effects upon the lens cause cataracts to form.

2.8.3 Hyperparathyroidism

A high circulating level of PTH is 'inappropriate' or 'appropriate'. The former under conditions which should not lead to a high PTH level, and the latter as a normal or appropriately high parathyroid gland response to unusual circumstances, e.g. prolonged hypocalcaemia.

Inappropriate PTH secretion occurs in three situations: primary-, tertiary-, or pseudo-hyperparathyroidism. The primary condition is associated with an adenoma which in 90% of cases is solitary and benign. Multiple adenomas, parathyroid chief cell hyperplasia, and the very rare parathyroid carcinoma are responsible for the rest. Features at presentation are those due to an excess of plasma calcium, and if the condition has been present for some time, the excess PTH may have affected the bone.

The tertiary condition is due to a prolonged appropriately high level of PTH secretion, due to previous hypocalcaemia, which has become autonomous when the stimulus for PTH secretion has been removed (e.g. renal transplantation). Pseudo-hyperparathyroidism is the production of PTH by non endocrine malignant tissue, which is of course autonomous from plasma calcium feedback control. It is extremely rare.

Appropriate or secondary hyperparathyroidism is always associated with low or normal plasma calcium, it is never high, and except in cases due to renal failure, the plasma phosphate may also be lowered. This can be caused by reduced exposure to sunlight, and/or reduced vitamin D intake which will reduce calcitriol levels and hence calcium absorption.

2.8.4 Hypoparathyroidism

Primary depression of PTH secretion is most often due to accidental surgical damage following thyroidectomy, either total or partial (Kleeman, et al 1971), or more rarely primary atrophy of the parathyroid gland.

Just as hypocalcaemia from any cause will lead to secondary hyperparathyroidism, so any hypercalcaemia not associated with inappropriate PTH secretion, will suppress PTH secretion. Hypercalcaemia of this form can be caused by: vitamin D excess, sarcoidosis, idiopathic infantile hypercalcaemia, thyrotoxicosis, malignant bone disease, and milk-alkali syndrome.

2.8.5 Other Disorders

These are associated with the bone or kidney. Osteoporosis occurs in a significant proportion of the population, and is characterised by a deficit in bone mass compared with a healthy control group of corresponding age and sex (see Kuhlencordt, 1976). What bone remains is qualitatively normal. The plasma calcium and phosphate are usually normal, but nonetheless bone formation and resorption go through a prolonged period of inbalance, leading to loss of trabecular and/or cortical bone. This commonly occurs in women after the menopause.

Osteomalacia is the loss of bone through the failure of mineralisation of newly formed osteoid. In infants it is manifest as rickets. Plasma calcium is of course lowered. The commonest form is secondary to vitamin D deficiency, which in the UK can be particularly common in Asian immigrants.

Vitamin D resistant rickets is associated with a disorder of renal tubular phosphate reabsorption. The resulting hypophosphataemia leads to poor bone calcification, and is relatively but not completely resistant to the administration of vitamin D. Plasma calcium levels are normal unlike the hypocalcaemia of osteomalacia.

2.9 Summary

The human physiology and metabolism of calcium have been introduced. Thus the functions of calcium in the body are first considered to illustrate the wide ranging role this metal plays in the body. The contrasting time scales of the need to maintain both neuromuscular excitability and a functional skeleton are emphasised.

The three anatomical spaces in which calcium is found were then considered, these are the extracellular fluid, the soft tissue, and the bone. The composition and differing physiology of these spaces are reviewed in addition to other relevant chemical species together with the mechanisms that control the composition of these spaces.

The physiology and metabolism of parathyroid hormone (PTH) is dealt with in detail due to its central role in the control of calcium. Other endocrine influences such as vitamin D3 and calcitonin are introduced, and the difficulties associated with hormonal measurement, in particular those of PTH are examined.

The chapter concludes by introducing the disturbance of calcium metabolism from the clinical viewpoint, in functional terms as the consequence of an altered plasma calcium concentration.

This chapter has provided an introduction to calcium metabolism as a basis for considering the models that follow. Chapter 3 will consider those models that have been formulated by others.

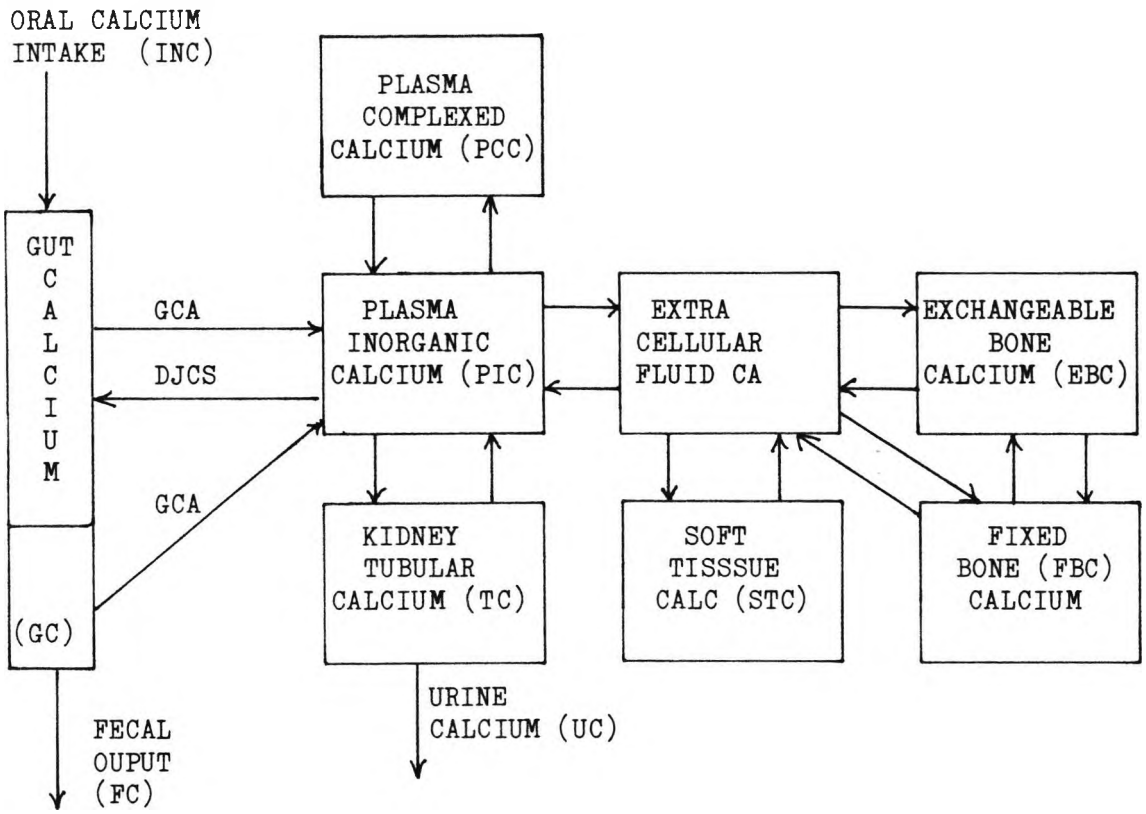


Figure 2.1 A basic scheme of calcium flux and distribution.
See Appendix I for further details of nomenclature.

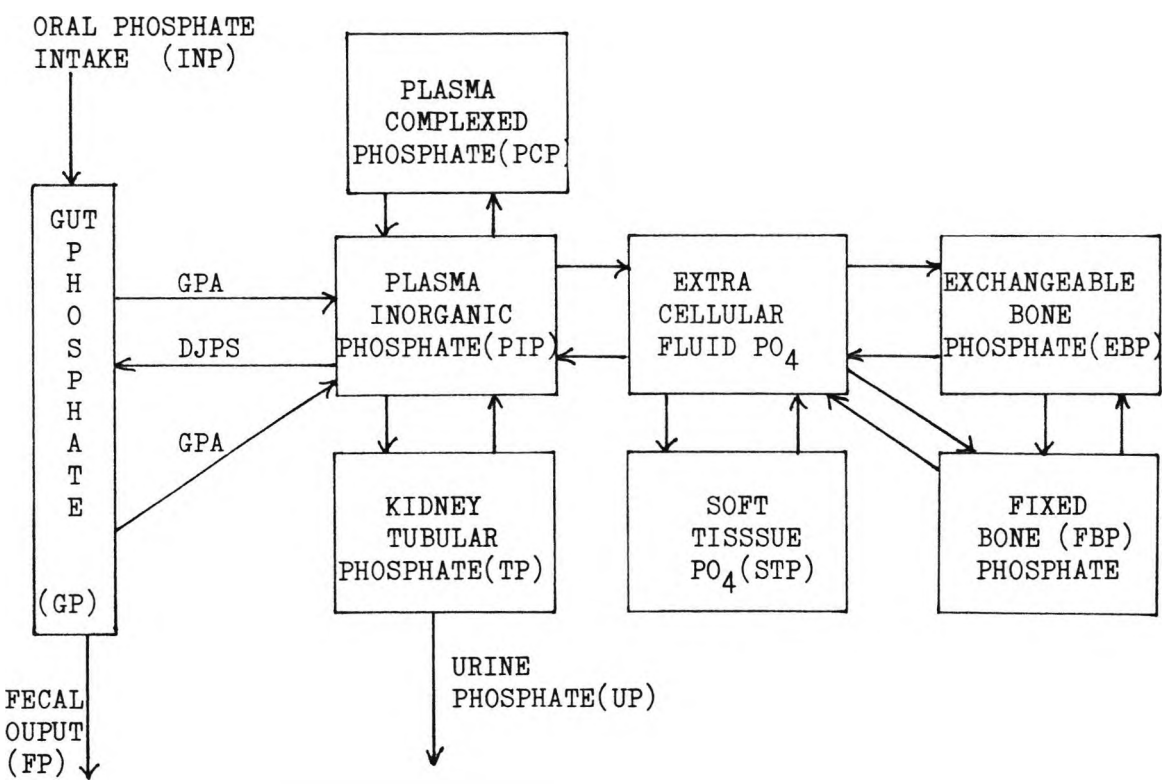


Figure 2.2 A basic scheme of phosphate flux and distribution.
See Appendix I for further details of nomenclature.

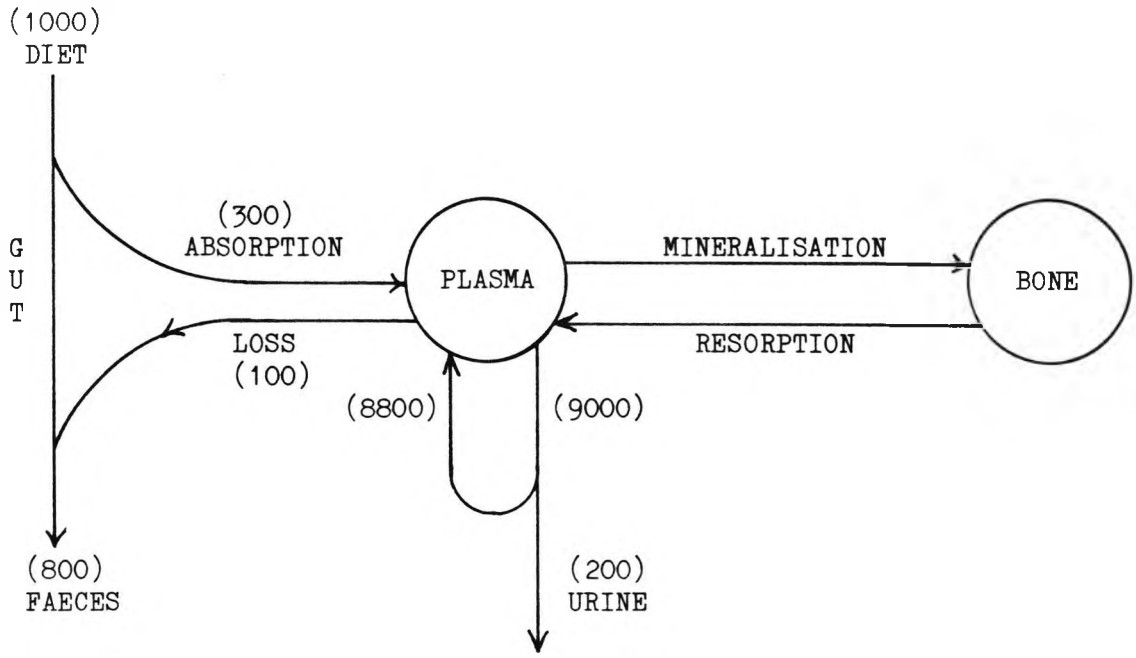


Figure 2.3 The major calcium flows in and out of plasma. All quantities have the units of mg d^{-1}. (from Nordin, 1976a).

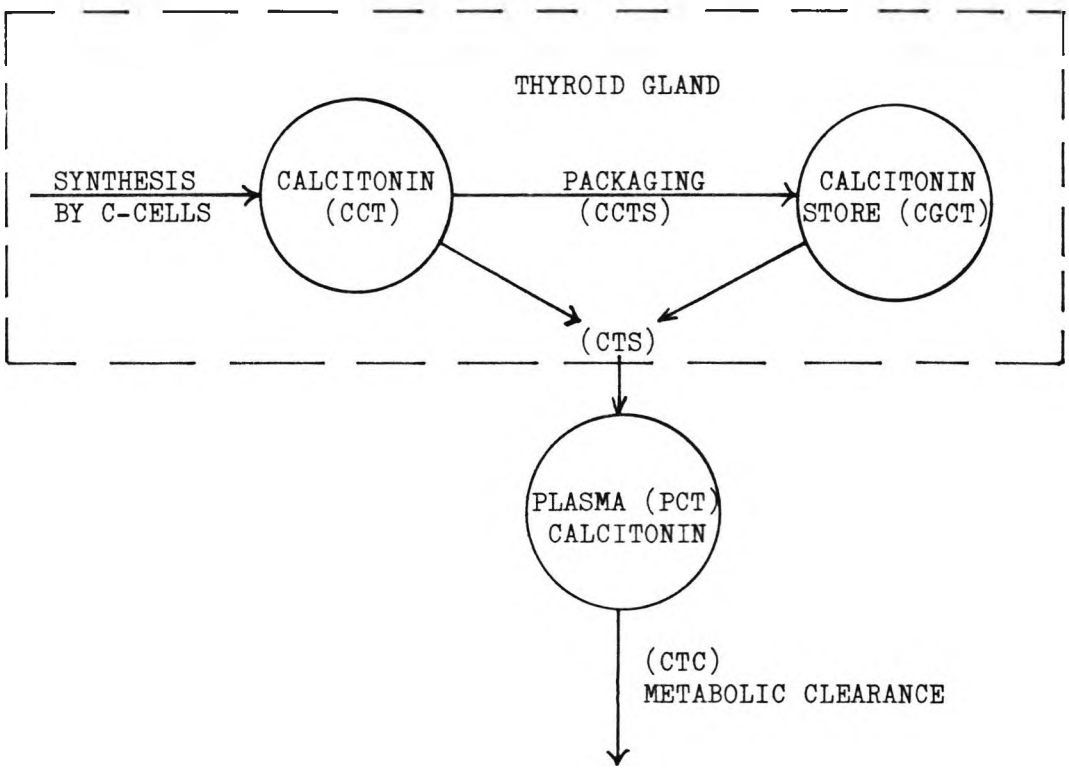


Figure 2.4 A basic scheme of calcitonin (CT) flux and distribution.

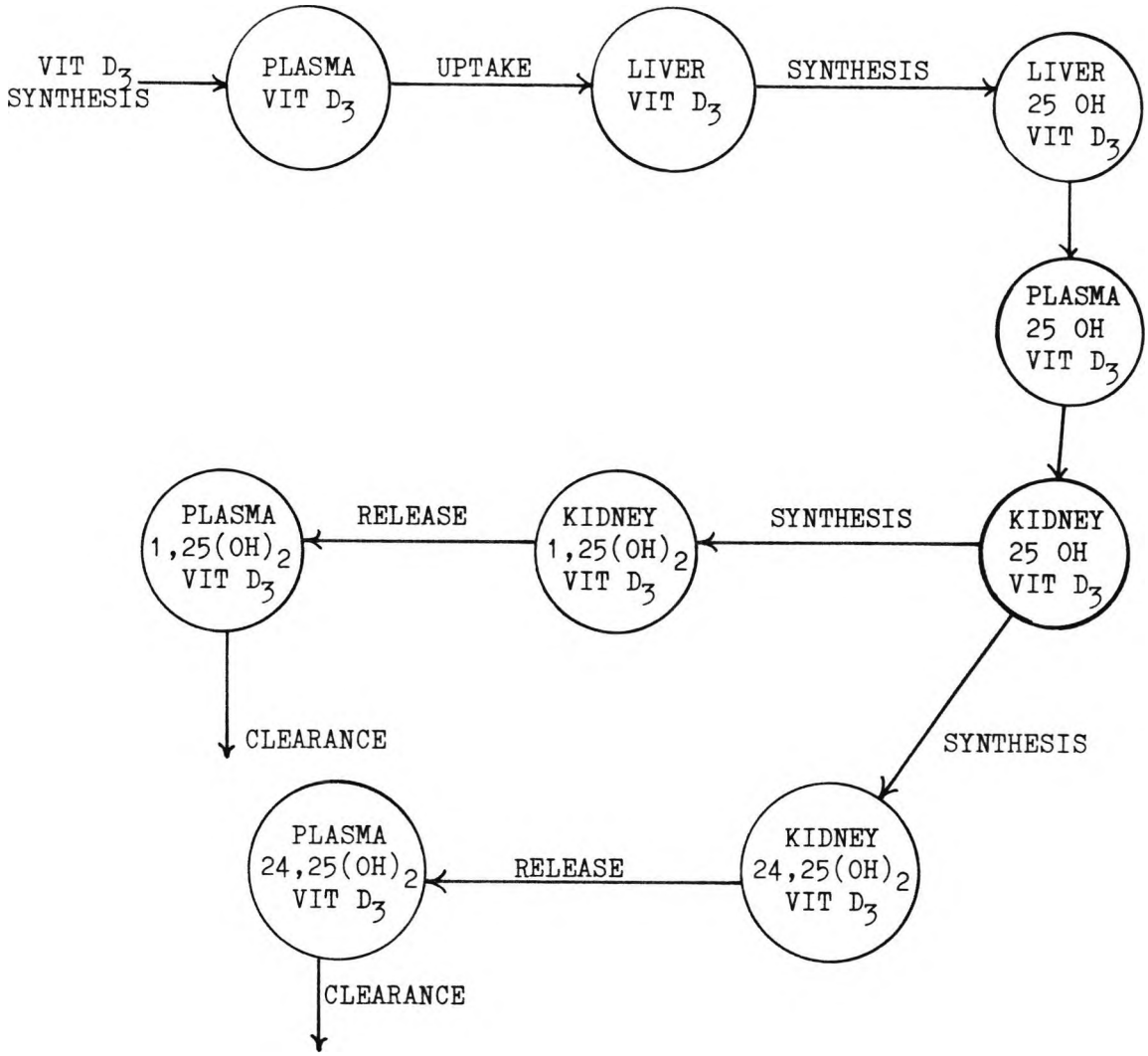


Figure 2.5 A basic scheme of the flux and distribution of vitamin D_3 and its metabolites.
See Appendix I for further details of nomenclature.

CHAPTER 3

CHAPTER 3

3. Existing Models

This chapter provides a review of the major types and classes of calcium models. As part of the review one model is considered in some detail as it will be referred to at length in the next chapter.

3.1 Model Classification

Mathematical models of physiological systems, and calcium metabolism in particular, can be classified in a number of ways. Two appropriate methods of classification are introduced; based upon representational form, and on purpose, followed by a tabular presentation of all of the models considered in this chapter.

3.1.1 According To Representational Form

Mathematical models of metabolic systems cover the range of approaches and hence forms available to the systems engineer. There are examples of deterministic and stochastic models, linear and non-linear models, and lumped and distributed models. These categories are not of course mutually exclusive and combinations of form could be used in the one model.

Existing models of calcium metabolism are mainly of two representa tional forms. The first relates to the kinetics observed following the administration of a radioactive calcium tracer (e.g. ^{45}Ca) to the organism. The second is concerned with models which seek to describe the control mechanisms involved in the homeostatic regulation of plasma calcium. The latter models can be comprehensive, are often isomorphic, and used to examine the effects of large perturbations to the organism or to describe the dynamics observed in situations where compartment or pool sizes are not constant, for example, during growth. The former are essentially numerical fits to observed kinetic behaviour, and as such are typically lacking in structural isomorphism with the real system.

The two forms involve different types of representation, different initial assumptions regarding the model, different abilities to predict, and present differing validation problems. Thus tracer models are essentially descriptive of a specific experimental situation. Control models are 'constructed' from at least an underlying set of mechanistic assumptions regarding their physiological analogue.

Most of the models considered in this chapter are 'compartmental' in that they consist of a number of suitably connected well mixed pools or compartments of

homogeneous chemical constitution. This is a simplifying assumption made to ease subsequent analysis. There are some situations in which this assumption of homogeneity may not be appropriate, and with regard to calcium metabolism, the bulk of the bone mass is often considered to be one of these.

There exists a group of models that are termed non-compartmental, as they mostly consist of whole organism or whole subsystem input-output models based on empirical input-output data. These models are typically formulated to enable a specific physiological quantity to be estimated (e.g. skeletal uptake or gut absorption rate), quantities which are sometimes termed model independent (see Reeve and Hesp, 1976; and Reeve, Hesp and Wootton, 1976). The relative merits of compartmental versus non-compartmental models are discussed by DiStefano (1982).

3.1.2 According To Purpose

If we accept the prime importance of the premise that "a valid model is one which satisfies its modelling objectives" (Leaning, 1980), an obvious classification for models of any situation is one based on the model purpose or objectives. The potential range of model objectives can be extremely wide, and indeed a number of objectives can be involved for a particular model. For this classification, it is considered appropriate to sub-divide objectives into one of three groups: descriptive, predictive, or explanatory (after Finkelstein and Carson, 1979).

Thus descriptive mathematical models are formulated to express a quantitative relationship, for the sake of economy or conciseness, or more likely a greater precision than is possible with a graphical or verbal description. If the system concerned is complex, it is possible that only a mathematical expression(s) is suitable.

A predictive model is expressly formulated to provide a forecast or prediction concerning the system, such as the effect of a drug or particular physiological event upon the organism. Models in this class have been used as diagnostic aids (e.g. Fraser et al, 1971, with particular reference to the differential diagnosis of hypercalcaemia).

Explanatory models are formulated to provide insight and understanding of system behaviour. A model of this nature can be used to test hypotheses concerning system behaviour, possibly indicating critical tests, or areas that require further research.

3.1.3 Models Considered

Models concerned with calcium at a physiological level have covered the whole range of forms, and purposes described in the previous two sections. A complete table of all calcium models considered appears in Table 3.1.

Some model forms have received a great deal of attention and others almost ignored. Thus as long ago as 1963, Heaney reviewed the subject of tracer models. The drive for this concentration upon tracers probably stems from the relative inaccessibility of the bone to direct measurement, and the possibility that an understanding of bone dynamics would have wide implications. The chemical similarity and bone seeking behaviour of such isotopes as ^{90}Sr, ^{135}Pu, and those of Radium and Uranium has also helped widen the audience of interest beyond a purely medical one.

Although generalities are harder to draw concerning control system models, it is true that the problems of identification and validation have not received enough attention. This is in contrast to the tracer models for which parameters have to be identified, but whose range of validity is necessarily limited.

Some models concern only a part of the calcium metabolic system, such as PTH, or are essentially based upon events at the level of a single cell. These include the cell model of Borle (1975), the calf PTH models of Jung et al (1982) and Hunziker et al (1977), and the calcitonin kinetic model of Huwler et al (1979). In general these are not considered in this chapter.

There are a number of models, or modelling works, that are worthy of mention, that are not covered in any detail in this chapter, and these are those that concern the long-term dynamics of bone or calcified tissue, without attempting to discuss the significance of compartments or pool sizes, as is common to tracer models. These include the stochastic models of Horsmann, Marshall and Peacock (1985), and Reeve (1986); the analysis of skeletal uptake of Groer and Marshall (1973), and the review of kinetic analyses of Jung et al (1978).

Table 3.1 Models of calcium metabolism

Author(s)	Illustration (If shown)	Form	Purpose/Principle Feature
Ackerman et al (1967)		Tracer	(Stochastic model)
Anderson et al (1962 & 1967)		Tracer	(Stochastic model)
Aubert and Milhaud (1960)		Tracer	
Aubert et al (1963)		Control	
Aubert and Bronner (1965)		Control	(Rat Ca Homeostasis)
Bauer and Ray (1958)		Tracer	(^{90}Sr tracer)
Birge et al (1969)	Figure 3.3	Tracer	(Gut Absorption)
Bronner (1973)	Figure 3.5	Control	(Rat model)
Bullermore et al (1971)		Tracer	(1 Expanding Compartment)
Burkinshaw et al (1969)	Figure 3.2	Tracer	(1 Expanding Compartment)

Coleman (1970)		Control	(Experimental verification)
Cohn et al (1965) & (1964)		Tracer	(2/3 Comps)
Copp et al (1960)	Figure 3.4	Control	(Analogue)
Glass & Nordin (1963)	Figure 3.2	Tracer	(Infinite Chain)
Gonick and Brown (1970)		Tracer	(Review & comarison)
Groer and Marshall (1973)		Tracer	(Skeletal uptake)
Gusmanns (1966)		Tracer	(Rat model)
Heaney (1963)		Tracer	(2 Comps, & Review)
Horsman, Marshall & Peacock(1985)			Stochastic Fracture incidence
Jaros et al (1979)		Control	(Explanatory)
Knop et al (1980)		Tracer	(Hyperparathyroid)
Liversey (1970)		Control	(Explanatory)
Marshall, J. (1964)		Tracer	(Power Function)
Marshall, D. and Nordin (1969)		Tracer	(Gut absorption)
Marshall, J. and Onkelinx (1968)		Tracer	(Power function)
Marshall (1967)	Figure 3.2	Tracer	(Mamillary structure)
Neer et al (1967)	Fig 3.9/10	Tracer	(4 Compartments)
Pearson (1972)	Figure 3.7	Control	(Explanatory)
Pedrolietal(1980)		Tracer	(Expanding compartment)
Phang et al (1969)		Tracer	(4 Compartments)
Powell and Valentinuzzi (1974)		Control	(Explanatory)
Reeve, E.B & Joiner (1973)	Figure 3.8	Control	(Explanatory)
Reeve, J., Hesp & Veall (1974)		Tracer	(Gut absorption)
Reeve, J., Hesp & Wootton (1976)		Tracer	(Skeletal uptake)
Reeve, J. (1986)			(Stochastic - trab. bone)
Riggs (1966)	Figure 3.6	Control	(Explanatory)
Roston (1959)		Control	(Explanatory)
Segre (1967)		Tracer	(Gut absorption)
Talmage and Grubb (1977)			(Descriptive / Explanatory)
Wajchenberg et al (1979)		Tracer	(Non-exchangeable plasma fraction)
Wise (1979)		Tracer	(Power Function)

Table 3.1 continued

3.2 Curve Fitted Tracer Models

This section describes the basis for and particular form of curve fitted tracer models. Some particular examples are briefly considered.

3.2.1 Basis of Tracer Models

Most models based upon calcium tracer kinetics have involved the use of linear compartmental analysis, where body calcium is divided into a number of conceptual pools/compartments, interlinked by exchange pathways. Assuming that each pool is of uniform specific activity changing over time, equations can be written describing the rate of change of radioactivity at each location (see Atkins, 1969; Berman et al 1962a, 1962b; Berman, 1963, 1965; Heaney, 1963). Thus:

$$M_i \frac{dS_i}{dt} = -\sum_j k_{ji} S_i + \sum_i k_{ij} S_j - k_{0,i} S_i$$

for the ith compartment of mass M_i , specific activity S_i , where k_{ij} is the rate constant for the flow of calcium from the jth compartment to the ith compartment and $k_{0,i}$ is the rate constant defining loss from the ith compartment to the environment. Figure 3.1 shows a generalised linear compartmental model as described above.

3.2.2 Use of Tracer Models

The use of radioactive tracer data generally involves the simplifying assumption that all compartmental sizes and exchange rates remain constant. Hence this approach has little predictive value regarding the nature of the feedback control mechanisms. It can be argued however, that tracer models do provide important information regarding the distribution and steady state flux of calcium. Because of this, tracer models could supply information regarding the structure of one sub-system of a control system model. This very idea is fully developed in the next chapter as MODEL1 is formulated. Obviously a control system model should be able to predict the kinetics of the tracer disappearance data.

3.2.3 Problems of Tracer Models

A significant problem encountered is the lack of a unique model for the analysis of calcium kinetics. Models have been chosen either to accommodate the experimental test data or else data have been chosen such as to be consistent with the postulate of a specific number of compartments in the model. Typical of this class are the two compartment models of Heaney (1963), and Cohn et al (1965); the four compartment

models of Bauer and Ray (1958), Aubert and Milhaud (1960), Cohn et al (1964) and Neer et al (1967) and the five compartment model of Phang et al (1969). These models have been reviewed by Marshall (1969) (See Figure 3.2).

Similarly another questionable facet of such models is; which are the appropriate exchange pathways between such compartments? Neer et al (1967) for instance could not distinguish between three different model structures (see Figures 3.9 and 3.10). Jung et al (1978) have critically examined the accuracy, errors, and usefulness of the differing methods of analysis of tracer disappearance data.

3.2.4 Other Approaches To Tracer Data

Other approaches relating to radioactive tracer kinetics include the expanding compartment models of Burkinshaw et al (1969) and Wajchenberg et al (1979), and analyses using power functions (Marshall, 1964; 1967; Marshall and Onkelinx, 1968, and Wise, 1979). Stochastic models have also been proposed in which the rate of transfer between compartments is governed by probability density functions (Ackerman et al, 1967; Anderson et al, 1962 and 1967).

Linear compartmental models describing the ingestion of calcium tracer have been proposed by Marshall and Nordin (1969) and by Birge et al (1969) (see Figure 3.3), in which the absorbed dose is calculated by utilising the mathematical process of deconvolution since direct measurement is not feasible. Details of the general approach are given in Segre (1967) and specifically applied to calcium metabolism in Reeve, Hesp and Veal (1974).

3.3 Control System Models

This section describes chronologically some of the control systems models and their particular form and features. They are split into simple or comprehensive on the basis of their complexity.

3.3.1 Simple Models

The first control system type model for calcium regulation was that developed by Roston (1959). A compartment structure was proposed corresponding to plasma ionised calcium and bone calcium. A simple proportional negative feedback from plasma calcium to bone calcium was assumed, corresponding in practice to the signal transmitted through the release of parathyroid hormone. No other effects were included in the model.

Copp et al (1960) produced an analogue computer model of the regulation of plasma ionised calcium (PIC) (See Figure 3.4). This model includes more detail than that of Roston. The urinary excretion of calcium is represented as being zero below a threshold value of plasma ionised calcium, and importantly the dependence of the rate of exchange of calcium, between plasma and bone, on bone flood flow rate was also included.

Through variation of bone blood flow and the size of the labile bone pool, the model was used to simulate the response of plasma ionised calcium to a calcium infusion in man. No hormonal regulation is included, however, nor bone resorption, intestinal dynamics or any representation of the phosphate system.

Aubert and Bronner (1965) and Bronner (1973) have proposed various models of the plasma calcium homeostatic systems in rats (See Figure 3.5). Basically, these models are similar to that of Roston (1959) but with the incorporation of a representation of gut absorption. A similar approach has been adopted by Riggs (1966), but with more feasible controller equations describing the release and action of PTH (see Figure 3.6).

Powell and Valentinuzzi (1974) and Bronner (1973) have produced models including hormonal control through calcitonin (CT) as well as PTH. In addition, the Powell and Valentinuzzi model includes control of gut absorption. Neither of these models uses the concept of exchangeable bone.

3.3.2 Comprehensive Models

The two models which attempt to include a more realistic treatment of the underlying unit processes are those of Pearson (1972) and Reeve and Joiner (1973) (See Figures 3.7 and 3.8).

That of Pearson (1972) is an extension of Liversey (1970), including the effects of PTH and CT upon renal excretion (Figure 3.7). The gut absorption equations are improved and the loss of calcium via sweating is included. Although this is a complex model, there is no provision for the action of vitamin D3 and its metabolites, and phosphate distribution is ignored.

The model of Reeve and Joiner (1973) (Figure 3.8) includes phosphate distribution. The importance of extracellular phosphate for bone calcification is emphasised. Non-linear modelling of gut absorption is provided assuming a protein binding carrier mechanism. Protein bound calcium in the plasma is included, and a soft tissue store of calcium phosphate (possibly mitochondrial) is also included. A hypothetical

additional hormone with a secretion level inversely proportional to bone calcification is postulated.

The Reeve and Joiner model is complex and was formulated to be representative of current thinking and ideas concerning calcium metabolism. It could be argued that its very complexity renders its predictions inaccessible to validation. Model predictions are however compared with observed infusion data yielding a good concordance with calcium and phosphate infusions, but a poor fit to EDTA infusion data, at the admittedly crude level of detail involved.

3.4 The Neer Model

The Neer(1967) model is examined at greater length in this section, being chosen as representing a noteworthy example of a tracer model. It is noteworthy because it explicitly attempts to overcome some of the shortcomings of previous studies, and also through the thoroughness and integrity of the data collection protocol used.

The model is a steady state tracer model. Data with which to validate this model were obtained by giving intravenous injections of ^{47}Ca radioactively labelled tracer to ten normal subjects. Serum, urinary and faecal radioactivity were measured over the period from one minute to twenty days following the injection. Data from a simultaneous metabolic balance study were analysed with the tracer data using a computer program designed to fit the rate of disappearance of tracer from serum as a sum of exponentials.

A fourth order model with exchange between the relevant compartments was found to satisfy the data, but as Figure 3.9 shows it was not possible to distinguish between three possible arrangements of compartments.

As compartment one represents the site of injection of isotope, this compartment must include the plasma volume. Since the physiological plasma volume is lower than this, part of the extracellular fluid space must be included; and although this still does not fully account for the calculated mass of compartment one, it is the best physiological analogue available. Compartments two, three and four represent progressively slower exchange with the plasma calcium. Together they comprise the labile pool of tissue calcium and 'exchangeable bone'.

Attempts were made to further identify the physiological analogue of the compartments, by radioactive counting on the thigh and ankle, demonstrating that compartments one and two predominate over the thigh, reflecting mainly soft tissue. Compartments three and four predominate over the ankle reflecting 'exchangeable bone'.

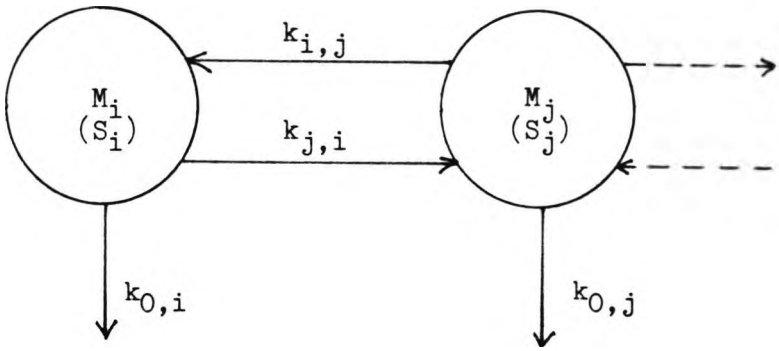
3.5 Summary

This chapter has introduced the reader to the history and extent of calcium metabolic modelling. This has include a detailed discussion of the general forms and purposes that models can take and attempt to satisfy. A general model classification was introduced for this purpose that highlighted the purpose (descriptive, predictive, or explanatory) of a model as its major taxonomic feature.

A tabulated presentation of all the models considered is used to display some of the models major features.

Each of the major representational categories considered; tracer and control system models (both simple and comprehensive) are reviewed, and one model in particular, that of Neer et al (1967) is described in some detail. This survey of calcium models prepares the way for the development of further models in subsequent chapters.

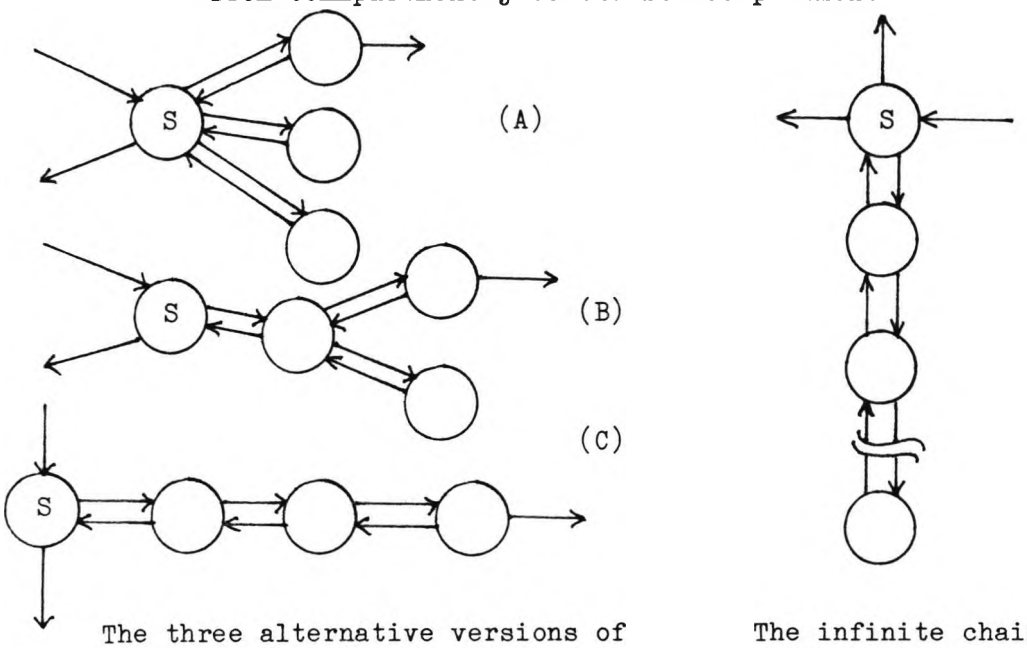
The Neer model is considered at length as a noteworthy example of a tracer model, which it is particularly important to review as chapter 4 deals with the development of MODEL1 through the extension of this model.



The following relationship holds;

$$M_i \frac{ds_i}{dt} = \sum_i k_{i,j} S_j - \sum_j k_{j,i} S_i - k_{o,i} S_i$$

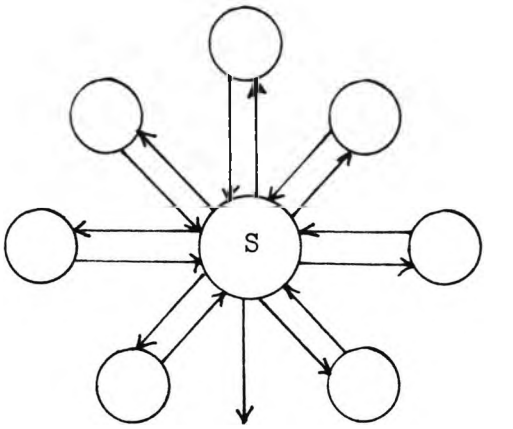
Figure 3.1 A generalised linear compartmental model, derived from tracer data.
M_i represents the mass of the ith compartment,
S_i represents the specific activity of the ith compartment,
$k_{i,j}$ represents the rate constant of transer of material from commpartment j to the ith compartment.



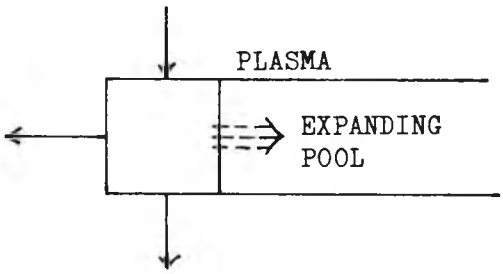
The three alternative versions of Neer et al(1967)

The infinite chain of Glass & Nordin (1963)

Figure 3.2 Examples of compartmental models of calcium kinetics, showing some of the possible arrangements of compartments. The expanding pool of Burkinshaw(1969) does not specify either the number or the arrangment, but has just one expanding compartment. The mammillary model of Marshall(1967) should be contrasted with the infinite chain of Glass & Nordin(1963) . Continued overleaf:->



The mammillary or clock model of Marshall (1967)



The expanding pool model of Burkinshaw et al (1969)

Figure 3.2 Continued, see previous page for legend.

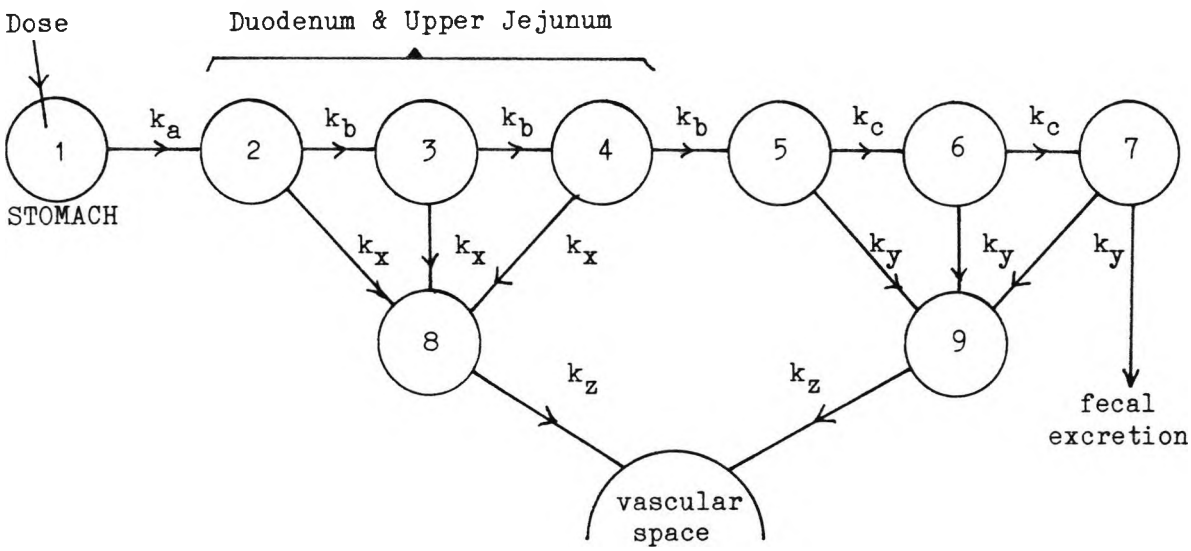


Figure 3.3 The model of Birge et al(1969) describing the absorption of orally ingested tracer (^{47}Ca).
Tracer doses were introduced both orally and intravenously and the process of deconvolution used to derive this model.
Tracer was introduced into the gut at various levels to enable a distinction to be made between the series of compartments.
k_a, k_b, k_c, k_x, k_y, are all first order rate constants.
k_z represents a delay estimated at 10 minutes.

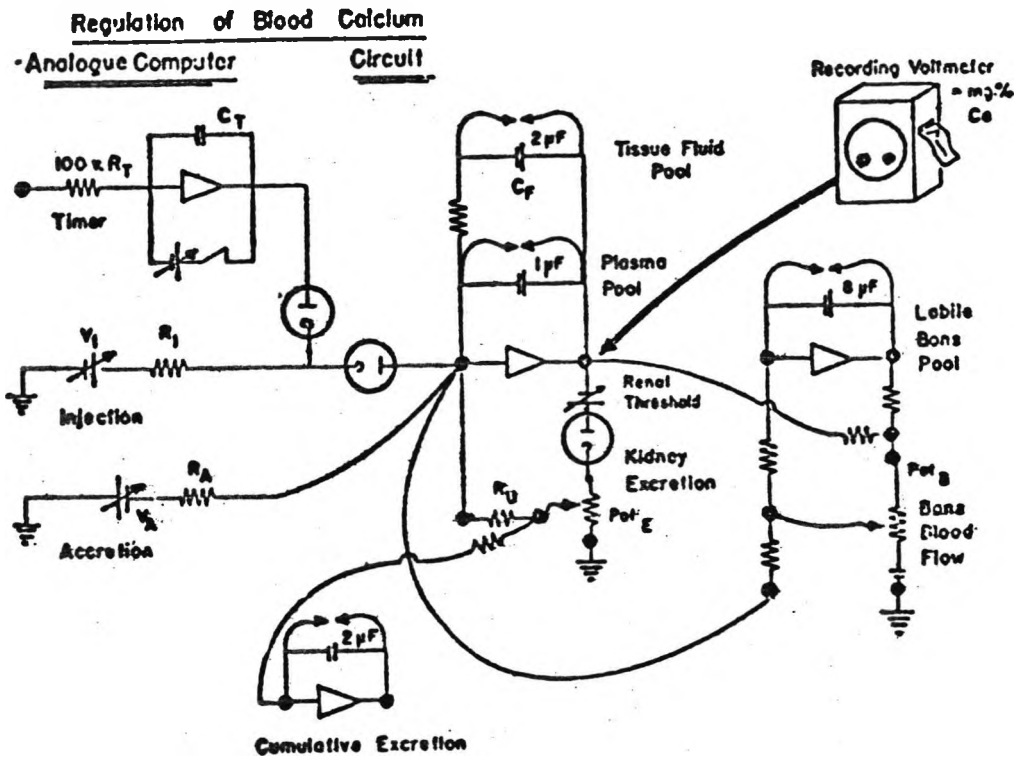


Figure 3.4 The analogue computer model of the regulation of calcaemia of Copp et al (1960). The model includes the division of plasma calcium into two pools, and more interestingly the dependence of the rate of exchange of calcium between plasma and bone on bone blood flow.

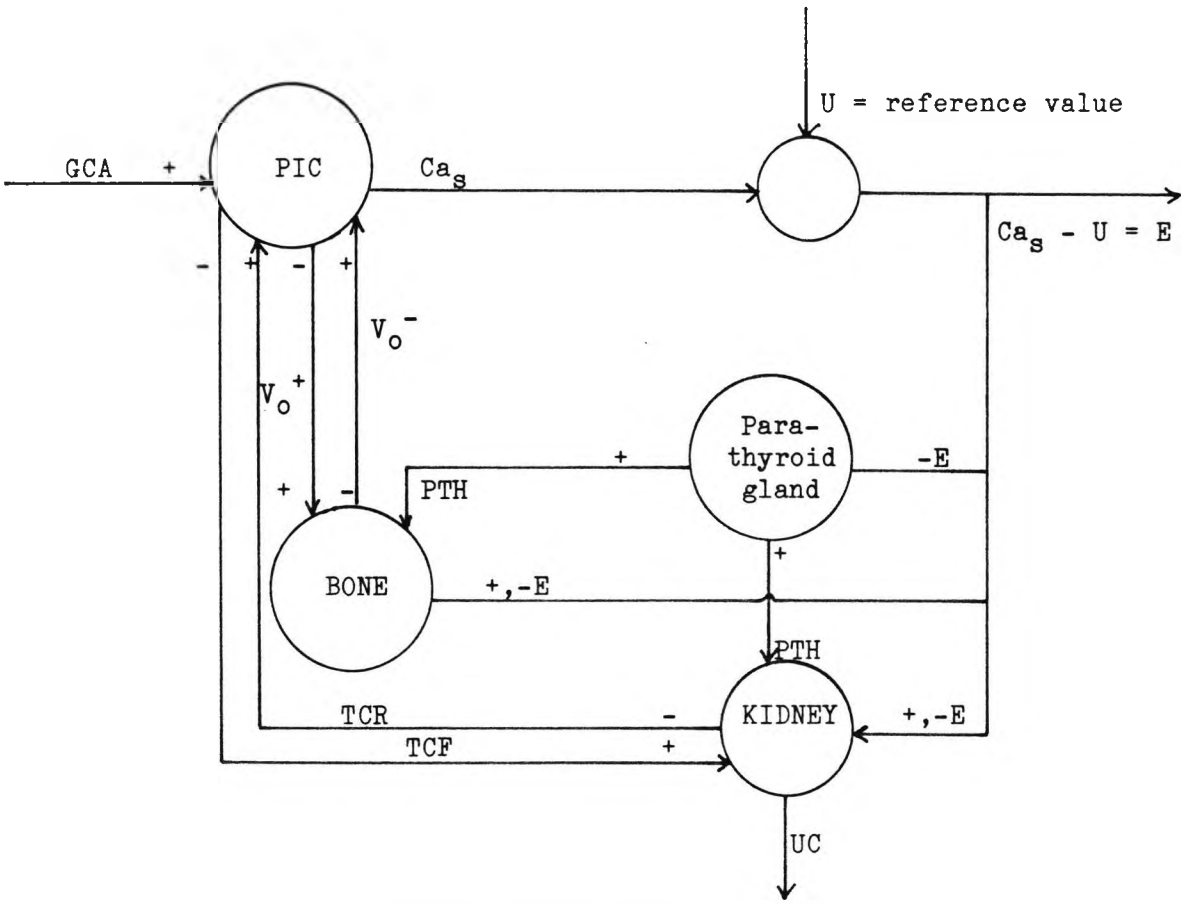


Figure 3.5 Model of Bronner (1973), with all hormonal controls apart from parathyroid hormone (PTH) omitted.

V_o^+ = calcium accretion from bone,

v_o^+ = calcium resorption from bone

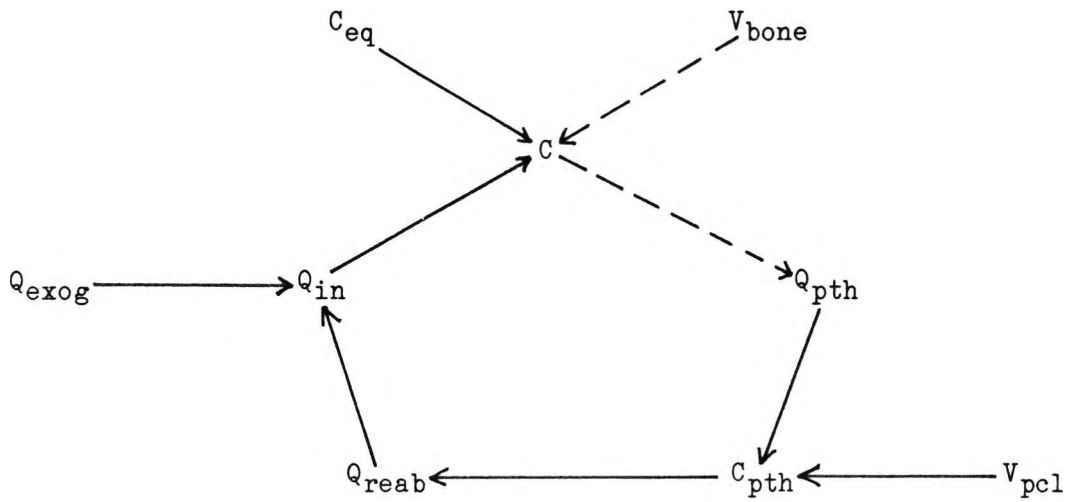


Figure 3.6 The Model of Riggs (1966).

Q_{exog}	=	Calcium infusion rate
Q_{in}	=	rate of entry of calcium into plasma
Q_{reab}	=	rate of active calcium reabsorption from bone
Q_{pth}	=	PTH secretion rate
C	=	plasma calcium concentration
C_{eq}	=	calcium concentration in plasma which is in passive equilibrium with bone
C_{pth}	=	plasma PTH concentration
V_{bone}	=	bone blood flow
v_{pcl}	=	PTH clearance rate

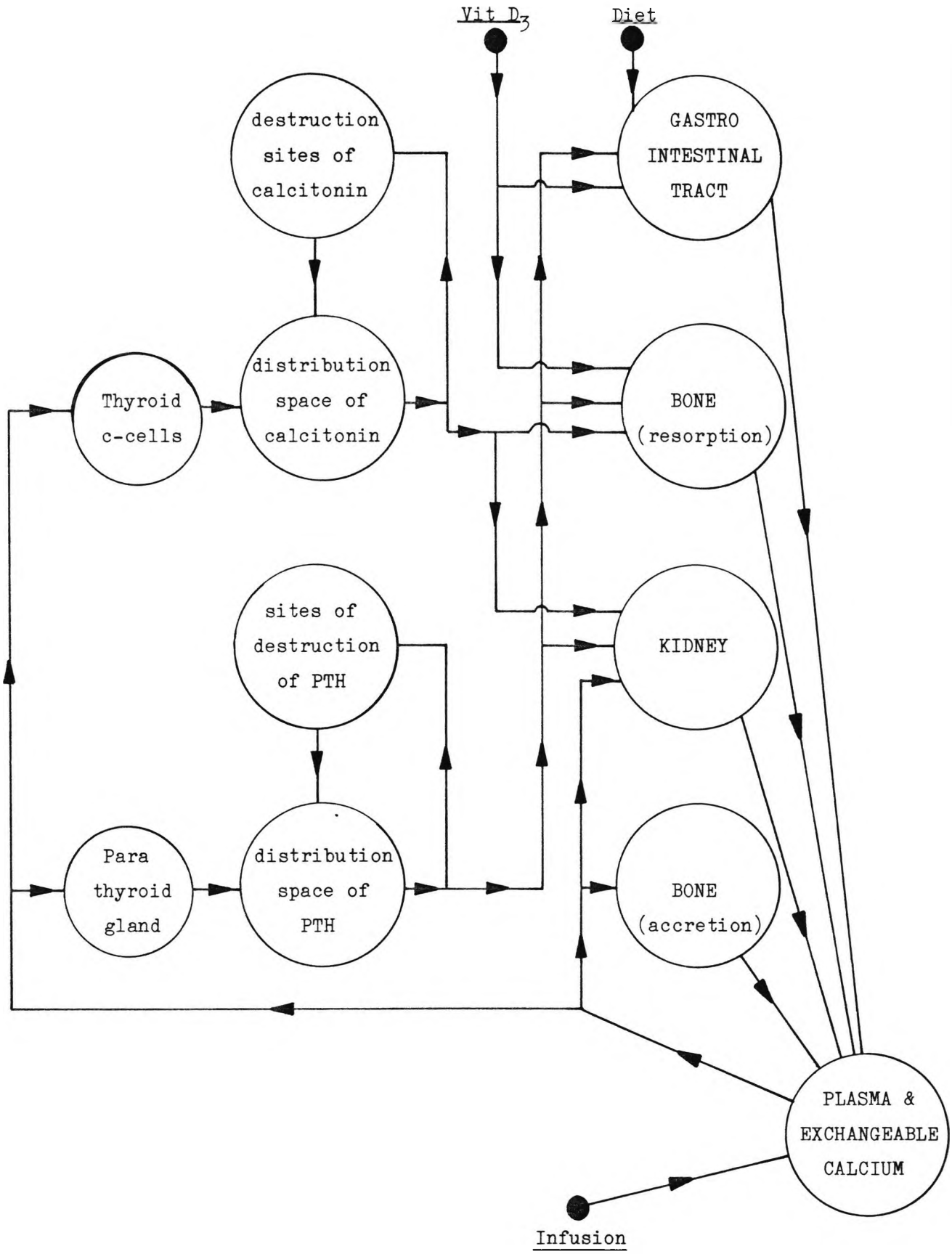


Figure 3.7 An information flow diagram of the model of Pearson (1972).

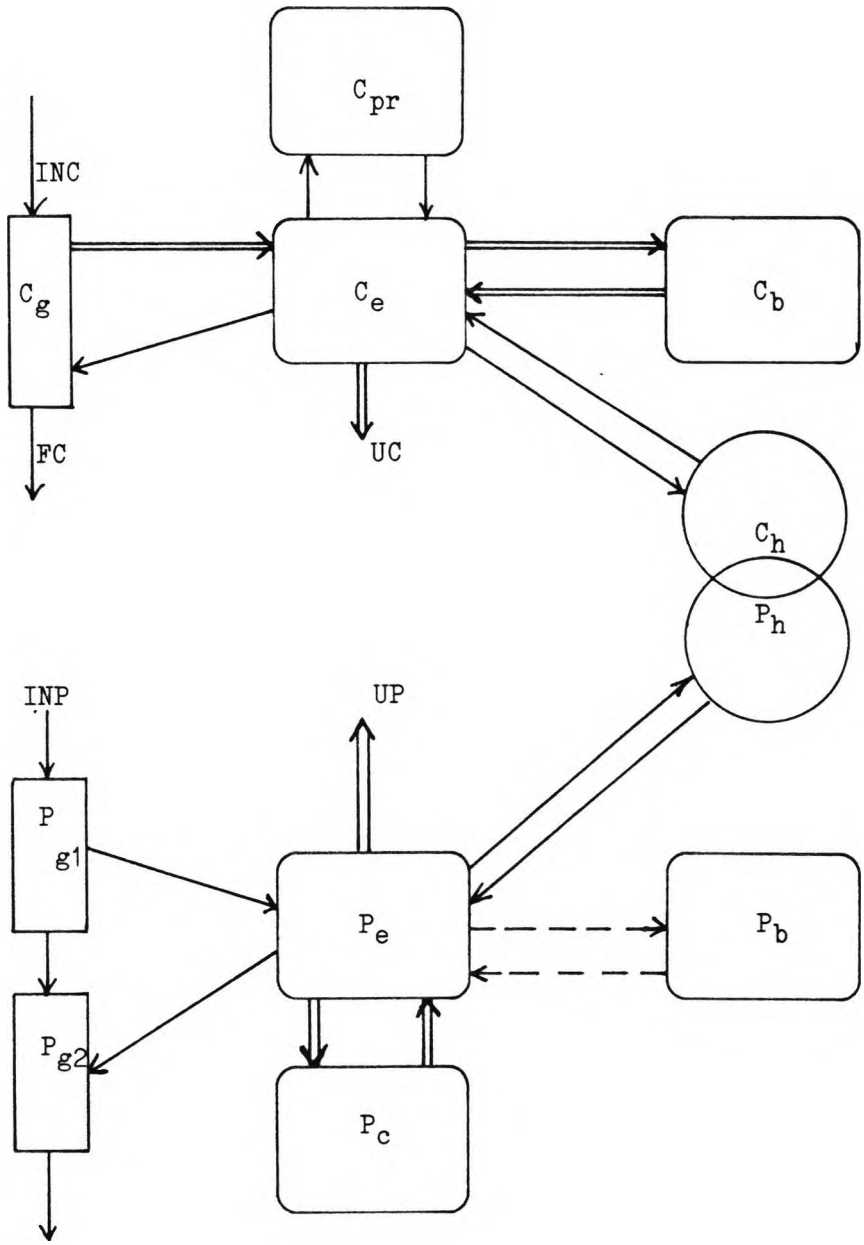


Figure 3.8 The model of Reeve and Joiner (1973).

C = Calcium
P = Phosphate

Subscripts:

g = gut
e = extracellular fluid
pr = plasma protein
c = cellular
b = bone
h = tissue hydroxyapatite

Double arrows indicate controlled flows, as opposed to flows that use first order rate constants, see Figure 3.1 for further details of the conventions associated with these.

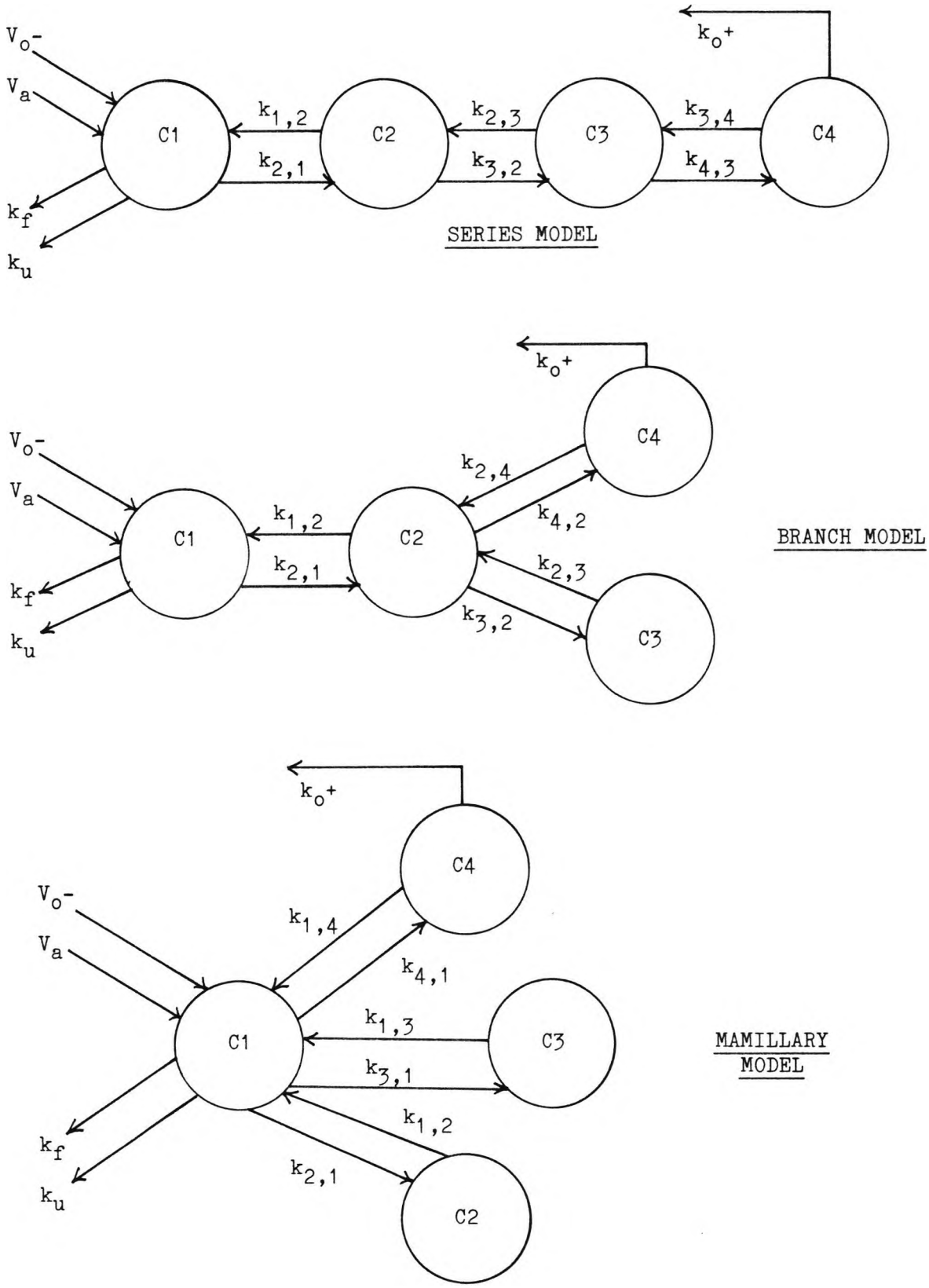


Figure 3.9 The three model structures of Neer et al (1967) that fitted the data. It was not possible to distinguish between the three alternatives on statistical grounds, each model is equally valid. The parameter values for each model are shown in Figure 3.10, overleaf.

	SERIES	BRANCH	MAMILLARY
k_f	0.13 ± 0.03	0.13 ± 0.03	0.13 ± 0.03
k_u	0.21 ± 0.09	0.21 ± 0.09	0.21 ± 0.09
k_o+	0.08 ± 0.06	0.07 ± 0.05	0.07 ± 0.04
$k_{1,2}$	25.0 ± 4	25.0 ± 4.0	30.0 ± 5.0
$k_{2,1}$	29.0 ± 7	29.0 ± 7.0	24.0 ± 5.0
$k_{2,3}$	1.4 ± 0.4	1.8 ± 0.5	
$k_{3,2}$	4.2 ± 1.3	3.4 ± 1.1	
$k_{3,4}$	0.087 ± 0.057		
$k_{4,3}$	0.3 ± 0.09		
$k_{2,4}$		0.08 ± 0.06	
$k_{4,2}$		0.81 ± 0.21	
$k_{1,4}$			30.0 ± 5.0
$k_{4,1}$			24.0 ± 6.0
$k_{1,3}$			1.6 ± 0.4
$k_{3,1}$			3.5 ± 1.1

Figure 3.10 Parameter values for the three alternative model structures of Neer et al (1967). Each value represents the mean ± 1 standard deviation. The models structures are shown in Figure 3.9. Note the change in those parameters that are common to more than one structure.

CHAPTER 4

CHAPTER 4

4. An Extension of The Neer Model (MODEL1)

Previous chapters have considered calcium physiology and previous approaches to modelling calcium metabolism. This chapter describes the development of MODEL1 through MODEL5. The approach used is borrowed from both the two main classes of models described in Chapter 3, namely tracer and control system models.

As described briefly in the previous chapter, the use of radioactive tracer data to formulate compartmental models involves the simplifying assumption that all compartmental sizes and exchange rates remain constant. Hence this approach has little predictive value regarding the nature of the feedback control mechanisms. If it is postulated, however, that a tracer model can provide information regarding the distribution of calcium, controller equations can be added to describe the hormonal effects, and this approach was adopted in the models described in this chapter.

4.1 The Framework of MODEL1

MODEL1 is based upon a 'steady state' model described by Neer et al (1967). This model was chosen as the base framework, from the gamut of available models, as the theoretical and experimental protocols used in its derivation were the most rigorous of those studied.

4.1.1 Neer Model Validation

Data with which to validate the model were obtained by giving intravenous injections of ^{47}Ca radioactively labelled tracer to ten normal subjects. Serum, urinary and faecal radioactivity were measured over the period from one minute to twenty days following the injection. Data from a simultaneous metabolic balance study were analysed with the tracer data using a computer program designed to fit the rate of disappearance of tracer from serum as a sum of exponentials.

A fourth order model with exchange between the relevant compartments was found to yield a significantly better fit to the data than a third order model. A fifth order model produced no significant improvement over the fourth. The three model forms investigated are shown in Figures 3.9 and 3.10.

4.1.2 Physiological Analogue of The Neer Model

As discussed in chapter 3, tracer models are derived from data that possibly have little relation to the physical structure of the modelled system, but as controller equations will be added to the Neer model the question of physiological significance becomes relevant.

As compartment one represents the site of injection of isotope, this compartment must include the plasma volume. Since the physiological plasma volume is lower than this, part of the extracellular fluid space must be included; and although this still does not fully account for the calculated mass of compartment one, it is the best physiological analogue available. Compartments two, three and four represent progressively slower exchange with the plasma calcium. Together they comprise the labile pool of tissue calcium and 'exchangeable bone'; further delineation is not possible.

Attempts were made to further identify the physiological analogue of the compartments, by radioactivity counting on the thigh and ankle, demonstrating that compartments one and two predominate over the thigh, reflecting mainly soft tissue. Compartments three and four predominate over the ankle reflecting 'exchangeable bone'. It was not possible to suggest any more detailed analogues.

4.2 Model Formulation (MODEL1)

This section presents the formulation of MODEL1 by considering first the overall structure, followed by a series of specific details. A block diagram is shown in Figure 4.1. A complete set of equations and steady state parameter and variable values is given in Figure 4.2 and Appendix I.

4.2.1 Basic Structural Details

As indicated in the chapter introduction MODEL1 was formulated by adding extra compartments and controller functions to the model of Neer et al (1967).

Five extra compartments were added (coded M5 to M9) as follows:

M5	Fixed Bone Calcium	(FBC)
M6	Plasma Inorganic Phosphate	(PIP)
M7	Plasma 25 OH Vitamin D3	(D1)
M8	Plasma 1,25 $(OH)_2$ Vitamin D3	(D2)
M9	Plasma Parathyroid Hormone	(PPT)

To enable the model to simulate the dynamic behaviour of calcium a number of controller functions were also incorporated. These enable the action of hormones (e.g. PTH), or the interaction between calcium and phosphate, to be modelled. These control the following processes:

PTH secretion (PTS)
Gut calcium and phosphate absorption (GCA & GPA)
oft tissue calcium accretion (STCA)
Soft tissue calcium resorption (STCR)
Urine calcium production (UC)
Urine phosphate production (UP)
Vitamin D3 metabolism (D1S & D2F)

Each addition is described individually in subsequent sub-sections.

4.2.2 Model Units and Conventions

Where possible, compartmental masses (M1 through M8) are given in units of millimoles (mmol). This has the added advantage over the use of grams (or kg) in giving direct compatibility with the description of dynamic processes in terms of concentration and thermodynamic activity. The exceptions are PTH (M9) for which ng are used; and Vitamin D3, for which 'units of activity' are used.

Where appropriate, parameters and masses are adjusted so as to correspond to those of a 70kg man. Rate constants are coded $k_{x,y}$; such that $k_{x,y}\ M_y$ is the transfer rate from compartment y to compartment x, where the mass of compartment y is M_y .

4.2.3 Fixed Bone Calcium (M5)

This compartment represents the bulk of the body's bone calcium mass. A single extra series compartment was used:

$$\dot{M5} = k_{5,4}\ M4 - k_{1,5}\ M5 \qquad (4.1)$$

If a physiologically realistic mass of fixed bone is assumed (25,000 mmol), then the mean flux through this compartment given by Neer et al (1967) at 14.2 mmol day^{-1} represents the steady state bone resorption rate. Hence:

$k_{1,5}$	=	0.568×10^{-3}	day^{-1}
$k_{5,4}$	=	0.08	"
$M5_{ss}$	=	25000	mmol

4.2.4 Phosphate (M6 & FBP)

Plasma inorganic phosphate is represented by compartment 6 (M6), soft tissue phosphate is ignored and fixed bone phosphate (FBP) is assumed to be passively and stoichiometrically related to fixed bone calcium in the ratio 2:3. This implies that the FBP mass will have a steady state mass equal to 2/3 that of FBC, the corresponding calcium compartment, although of course the associated rate constant of compartmental transfer will remain the same. Thus:

$$\dot{M6} = GPA + FBPR - UP - FBPA \quad (4.2)$$

$$\dot{FBP} = FBPA - FBPR \quad (4.3)$$

$$FBPA = 2/3\ k_{5,4}\ M4 \quad \text{mmol day}^{-1} \quad (4.4)$$

$$FBPR = 2/3\ k_{1,5}\ M5 \quad \text{" "} \quad (4.5)$$

$$FBP_{SS} = 16667 \quad \text{mmol}$$

In contrast to FBP the steady state mass of M6 is assumed to be independent of the corresponding calcium compartment (M1), and a physiologically realistic value is assumed;

$$M6_{SS} = 4.586 \quad \text{mmol}$$

4.2.5 Plasma PTH (M9)

A single compartment representation is adopted for PTH (see Figure 4.1). Given that the half life of the intact PTH molecule lies in the range 6 minutes (Parsons et al, 1975) to 12.3 minutes (Reeve, 1977), no more complex structural representation appeared to be required, although it is possible that the action of a breakdown metabolite could be important.

$$\dot{M9} = PTS - k_{0,9}\ M9 \quad \text{ng day}^{-1} \quad (4.6)$$

$$M9_{SS} = 490 \text{ ng}$$

$$k_{0,9} = 144 \text{ day}^{-1}$$

4.2.6 PTH Secretion (PTS)

Mayer (1975) has shown that the secretion rate of parathyroid hormone (PTS) varies inversely with plasma calcium, in a sigmoidal manner. Thus the parathyroid hormone secretion rate is represented as a straight line with an upper and a lower level, as follows:

$$
\begin{aligned}
PTS &= 5.3 && : \quad (M1 < 25.0) && ng\ s^{-1} \\
PTS &= 20.3 - 0.6\ M1 && : \quad (25.0 < M1 < 33.3) && \text{" "} \qquad (4.7) \\
PTS &= 0.32 && : \quad (M1 > 33.3) && \text{" "} \\
PTS_{SS} &= 0.8\ ng\ s^{-1} && = \quad 69120\ ng\ day^{-1}
\end{aligned}
$$

The lower limit to PTS represents the basal secretion rate that is observed even at extremely high levels of plasma calcium.

4.2.7 Absorption From The Gut (GCA & GPA)

1,25 $(OH)_2$ vitamin D3 (D2) is known to increase absorption from the gut of both calcium and phosphate. The following relationship for the rate of calcium absorption (GCA) was used as a suitable vehicle for the incorporation of the effect of vitamin D3, theoretically enabling all of the oral intake to be absorbed with a massive 1,25 $(oh)_2$ Vit D3 concentration.

$$GCA = IC(M8/(M8 + k_c\ M8_{SS}))\ mmol\ day^{-1} \qquad (4.8)$$

k_c = constant = 1
IC = Oral calcium intake
and GCA_{SS} = 15 mmol day^{-1}

Similarly for phosphate, the rate of absorption GPA, is given by:

$$GPA = IP(M8/(M8 + k_p\ M8_{SS}))\ mmol\ day^{-1} \qquad (4.9)$$

k_p = constant = 0.8
IP = Oral phosphate intake
and GPA_{SS} = 25.25 mmol day^{-1}

4.2.8 Soft Tissue Calcium Accretion (STCA)

Borle (1975) has suggested that extracellular phosphate controls the level of intracellular calcium stores. This has also been demonstrated in cell cultures. Because of the difficulties associated with extrapolation to the whole organism, a simple proportional relationship is assumed in the model:

$$STCA = k_s\ M6\ M1\ \ mmol\ day^{-1} \qquad (4.10)$$

k_s = constant = 6.3

4.2.9 Soft Tissue Calcium Resorption (STCR)

The data in Figure 4.3 of Reeve (1979b) relate to the effective change in a baseline rate constant of the calcium system, assuming a simple two compartment model, in response to exogenous administration of PTH, such that the circulating plasma level increases by approximately 80%. The results indicate that a 40% increase in $k_{1,2}$ is brought about by an 80% increase in plasma PTH. This was modelled by the function numbered 4.11, below:

$$STCR = k_{1,2}\, M2\, (M9 + M9_{ss})/(2*M9_{ss})\ \text{mmol day}^{-1} \quad (4.11)$$

$$STCR_{ss} = 927.5\ \text{mmol day}^{-1}$$

4.2.10 Urine Calcium Control (UC)

Regarding the kidney as a pressure fed system the filtered load of calcium and phosphate was assumed to be simply proportional to the plasma concentration, assuming a constant glomerular filtration rate (GFR). Although various curves have been drawn representing the variation in tubular reabsorption (TCR) (see Bijvoet, 1975) the kidney model was simplified by assuming that TCR is a linear function of the filtered load, and further that the magnitude of this linear function is a function of plasma PTH level (M9). Thus from Figure 4.4 :

$$UC = UFC - TCR \quad (4.12)$$

From Figure 4.5 : $$UC = a_i(PINC - 3.7) - 0.4\ \text{mg min}^{-1} \quad (4.13)$$

Where the value of a_i is dependent upon the parathyroid status and PINC is the plasma concentration of inorganic calcium. Thus from Mioni et al (1971) (Figure 4.5, and equation 4.13) the following three slopes were obtained:

a_1 = 0.1667 (hypoparathyroid)
a_2 = 0.0689 (hyperparathyroid)
a_3 = 0.112 (normal parathyroid)

A linear relationship between the slope ai and plasma parathyroid level, was then postulated to give equation 4.14:

$$a_i = 0.1667 - 0.0546\, M9 / M9_{ss} \quad (4.14)$$

where $a_i \geq 0.0689$

Converting to mmol day^{-1} and defining the two extremes of hypo- and hyper-parathyroid status respectively gives:

$$UC_{max} = 1.79\,(M1 - 12.4) - 14.4 \quad \text{hypoparathyroid}$$
$$UC_{min} = 0.74\,(M1 - 12.4) - 14.4 \quad \text{hyperparathyroid}$$

or: $UC = A\,(M1 - 12.4) - 14.4$ (4.15)

where $A = 1.79 - 0.586\,M9 / M9_{ss}$ (4.16)

and: $A \geq 0.74$

From these two extremes, two components of reabsorption are postulated; one relating to a passive tubular reabsorption, and one relating to an active component that is controlled by PTH. Hence:

$$UC = \underbrace{(0.74\,(M1 - 12.4) - 14.4)}_{\text{passive component}} + \underbrace{1.05\,(M1 - 12.4)(1 - M9/(M9 + k_{uc}))}_{\text{active component}} \quad (4.17)$$

where $(1 - M9/(M9 + k_{uc}))$ is a suitable relationship that varies between 0 and 1 as M9 varies between large and zero. Rearranging gives equation 4.18:

$$UC = 0.74M1 - 23.58 + 1.05(M1 - 12.4)(1 - M9/(M9 + k_{uc})) \quad (4.18)$$

where:

$kuc = 382$ and $UC_{ss} = 9.75$ mmol day^{-1}

4.2.11 Urine Phosphate Control (UP)

For phosphate, partly similar assumptions are made to those detailed above for urine calcium. In contrast to calcium, however, phosphate excretion was directly and independently linked to the plasma PTH level. Thus from Figure 4.6:

$$U = UFP - TPR \quad (4.19)$$

where

$$UFP = k_f\,M6 \quad (4.20)$$

Assuming that tubular phosphate reabsorption, TPR, is a linear function of PPT:

$$TPR = k_{tp} - k_{tq}\,M9 \quad (4.21)$$

Suitable parameter values were derived to give:

$k_f = 47 \quad k_{tp} = 3440 \quad k_{tq} = 6.63$

such that $UP_{ss} = 25.25$ mmol day^{-1}

4.2.12 Vitamin D3 Metabolism (M7 & M8)

Two metabolites are included: 25 OH Vitamin D3 (D1) and 1,25 $(OH)_2$ Vitamin D3 (D2), represented as a two compartments series model (see Figure 4.1). Arbitrary 'units' of vitamin D are used, and the larger metabolite (D2) was taken to be the active form (see Reeve, 1979). M7 typically exists at concentrations of 100 times that of M8. Thus:

$$M7 = k_{df} M7 - D2F*M7 \quad (4.22)$$

$$M8 = D2F - k_{0,8} M8 \quad (4.23)$$

$M7_{ss} = 100$ 'units'

$M8_{ss} = 1$ "

Deluca et al (1975) have showed an inverse relationship between the ability to synthesise 1,25 $(OH)_2$ vitamin D3 (at a rate D2F) and the level of plasma inorganic phosphate (PIP) in rats (see Figure 4.7). A similar relationship was taken from these data to describe the rate of formation of 1,25$(OH)_2$ vitamin D3. The turnover of 25(OH) vitamin D3 is approximately three to four weeks (Haddad and Rojanasathit, 1976). Hence:

$$D2F = (0.0673 - 0.00598\ M6)(M7/M7_{ss}) \text{ units day}^{-1} \quad (4.24)$$

$k_{0,8} = 0.04$ day^{-1}

$k_{df} = 0.04$ "

4.3 Model Performance

A range of situations were simulated. Model performance was lacking in some respects, and in line with the overall approach used for modelling, some of these led to model changes and the incremental development of a series of models. The resulting models are labelled MODEL1 to MODEL5.

4.3.1 MODEL1

4.3.1.1 Variation in Oral Intake

The steady state conditions of the model (MODEL1) are adequate for a steady normal diet, but the response was totally unsatisfactory when ìattempts were made to simulate dietary variation. For instance a doubling of the phosphate intake led to widely oscillating extracellular calcium (M1) and phosphate (M6) values. Non-feasible instabilities similarly arose with a reduced phosphate or calcium intake. The observed instability arises because of the stiffness of the model equations, and the inability of the Runge-Kutta integration routine used, to adequately cope with this. Although it was appropriate to use an integration routine designed for stiff systems (e.g. that of Gear, 1971), or use the traditional Runge-Kutta algorithm with a very short step length, the observed instability focused attention on those aspects of the model that were responsible for the stiffness. This is especially appropriate when it is considered that the human system is resilient to large changes in diet. The first of the resulting changes made led to MODEL2.

4.3.2 MODEL2

4.3.2.1 Examination Of Urine Phosphate Control

In MODEL1 the following expression was used for urine phosphate:

$$UP = k_f M6 - (k_{tp} - k_{tq} M9)$$

where $k_f M6$ represents tubular phosphate filtration (UFP) and $(k_{tp} - k_{tq} M9)$ represents tubular phosphate reabsorption (TPR). Thus reabsorption is not a function of the filtered load, but only of plasma PTH level, and TPR can become greater than UFP yielding negative urine losses (i.e. inputs).

The model was amended to reduce the reliance of UP upon PTH and prevent the occurrence of negative values. This was achieved by making TPR a function of both PIP (M6) and plasma PTH (M9):

$$UP = k_f M6 - (k_{tp} - k_{tq} M9\ M6/M6_{ss}) \qquad (4.25)$$

This change resulted in a more tractable system. Thus Figures 4.8 and 4.9 show the effect of increased and reduced calcium intake respectively, when the Rung-Kutta routine was used. Changes in phosphate intake (Figure 4.10 and Figure 4.11) are also solvable with only damped oscillatory behaviour being observed.

4.3.2.2 Simulation Of Infusions

Intravenous infusions of calcium, EDTA, phosphate, and PTH were simulated. The calcium and phosphate results were not unrealistic, but only a small variation in extracellular calcium was observed in the calcium infusion. The EDTA and PTH infusions were unsatisfactory with oscillatory instability, and algorithmic problems being encountered.

4.3.3 MODEL3

In an attempt to further damp the oscillating urine outputs, the possibility that PTH still had too great an influence upon phosphate tubular resorption (TPR) was explored by halving the effect of a change in PTH upon TPR to give equation 4.26:

$$UP = k_f M6 - (k_{tp} - k_{tq} M6(M9 + M9_{ss})/(2*M6_{ss})) \qquad (4.26)$$

This, however, had little effect upon any of the simulated infusions.

4.3.4 MODEL4

The slope of TPR upon PTH is assumed to be a straight line, when most published studies (see Bijvoet, 1969) show an asymptotic approach to a maximum reabsorption rate. To mimic this further model modification was carried out by progressively changing the slope of PTH upon TPR with change of M9 (plasma PTH level). Thus:

$$UP = k_f M6 - (k_{tp} - k_{tq} M6*Y'/M6_{ss}) \qquad (4.27)$$

where:

$Y' = (M9 + M9_{ss})/2$: $M9 < 490$

$Y' = (M9 + 2*M9_{ss})/3$: $490 < M9 < 510$

$Y' = (M9 + 3*M9_{ss})/4$: $510 < M9 < 530$

$Y' = (M9 + 4*M9_{ss})/5$: $530 < M9 < 560$

$Y' = (M9 + 5*M9_{ss})/6$: $560 < M9 < 600$

$Y' = (M9 + 6*M9_{ss})/7$: $600 < M9 < 620$

$Y' = (M9 + 9*M9_{ss})/10$: $620 < M9$

This mirrors the physiology of an active absorption mechanism, which would exhibit an asymptotic approach to a maximum transport rate.

Infusion simulations gave encouraging results in that the extent of the urine phosphate instability was reduced although not eliminated, demonstrating that the system stiffness was still present.

4.3.5 MODEL5

The effects of PTH have been assumed to be instantaneous in the model equations (e.g. effects upon UP and UC), whereas they are unlikely to be instantaneous in the human system. In addition it is this state variable that has the largest (or fastest) time constant of disappearance, and hence it is this variable that is primarily responsible for the system stiffness. Accordingly in addition to the modifications introduced in MODEL4 the rates of production and disappearance of PTH were lowered to one fifth of the original values. This will not affect the steady state values, only the rate of turnover, giving equation 4.28:

$$M9 = 0.2(PTS - k_{0,9}\, M9) \qquad (4.28)$$

Simulation of the same infusions as carried out with the earlier models now results in feasible values throughout the range of interest and the previously exhibited algorithmic instability (manifest as large transients) when solved with with the Runge-Kutta routine is no longer observed.

Although the modification can be thought of as a solution to a numerical analysis problem, as stated earlier stiffness of the order observed does not exist in the human system. A necessary validation step for this series of models is the removal of these transients. This last modification incorporated into MODEL5 effectively increases the time constant of disappearance of PTH by a factor of 10. Such a change can also be argued to be physiologically realistic since a degree of uncertainty exists as to the pharmacology of PTH, and in particular the biological activity activity of its metabolites (e.g. $hPTH_{1\text{-}34}$), some of which have much longer half-lives than the intact molecule.

4.4 Model Validity

The final version of the modified model, MODEL5, is able to produce generally realistic simulations of some experimental conditions. Whilst the model is partly 'physical', in that a substantial degree of physiological information has been incorporated into its formulation, it is equally in part purely 'functional' with mathematical representation provided in a manner to produce adequate responses on a 'curve fitting' basis. The primary aim of this modelling programme, however, was to provide physiological insight as to the gross system behaviour.

The linking of numerical stiffness with biological validity could be argued with, but is presented here as a necessary consequence of a valid mathematical model of a system that does not exhibit rapidly decaying transients. Thus although the lack of stiffness is a necessary condition in a similar manner to the requirement that the

model should actually be solvable, it is in fact a validity test at a higher level of complexity, comparable with the existence of a feasible steady state.

Some physiological insight is possible with MODEL5 and the necessary reduction in rate of disappearance of 'biologically active' PTH to achieve model stability is interesting in this respect, the degree of insight possible with MODEL5 is limited by the lack of a clear physiological isomorphism. This is not altogether surprising since MODEL5 has as its foundation the 'tracer' model of Neer et al (1967) wherein there was a lack of knowledge as to the precise physiological identity of the postulated calcium compartments.

4.5 Summary

A compartmental model (MODEL1) of calcium metabolism is developed by taking a tracer model (from Neer et al, 1967) and adding extra state variables and controller functions, to more fully describe the variables and functions that are thought to be important to the metabolism and control of calcium. The extra variables represent fixed bone calcium, plasma inorganic phosphate, plasma 25 OH Vit D3, $1,25(OH)_2$ Vit D3, and plasma parathyroid hormone. Controller equations representing the control of PTH secretion, gut calcium and phosphate absorption, soft tissue calcium accretion and resorption, control of urine calcium and phosphate, and vitamin D3 metabolism are added.

The performance of MODEL1 was evaluated over a range of situations. This included variations in oral intake of both calcium and phosphate, and the infusion of calcium, phosphate, EDTA, and parathyroid hormone. Instabilities in the response to simulated system inputs when used with standard numerical procedures, due to the stiffness of the model equations led to changes in the model being made, in line with the integrated approach to model formulation and development used. These changes spawned a further four models (MODEL2 to MODEL5). The need to reduce the model stiffness was viewed as more than just a condition for model solution, but rather as a necessary validity test, as the human calcium system does not exhibit rapidly varying transient behaviour of the order initially encountered.

MODEL5 is able to produce generally realistic simulations of some experimental conditions, and a necessary condition for this was the lengthening of the time constant of disappearance of plasma PTH, suggesting that the metabolites of PTH (with much longer half-lives) could be biologically important. However the model lacks a detailed physiological isomorphism with the real system and this inevitably leads to problems of detailed interpretation, caution regarding predictions, and hinders further development.

The next chapter details the development of a model to cover the short- term situation that attempts to overcome the shortcomings of MODEL1 to 5, through the explicit incorporation of a greater degree of physiological isomorphism.

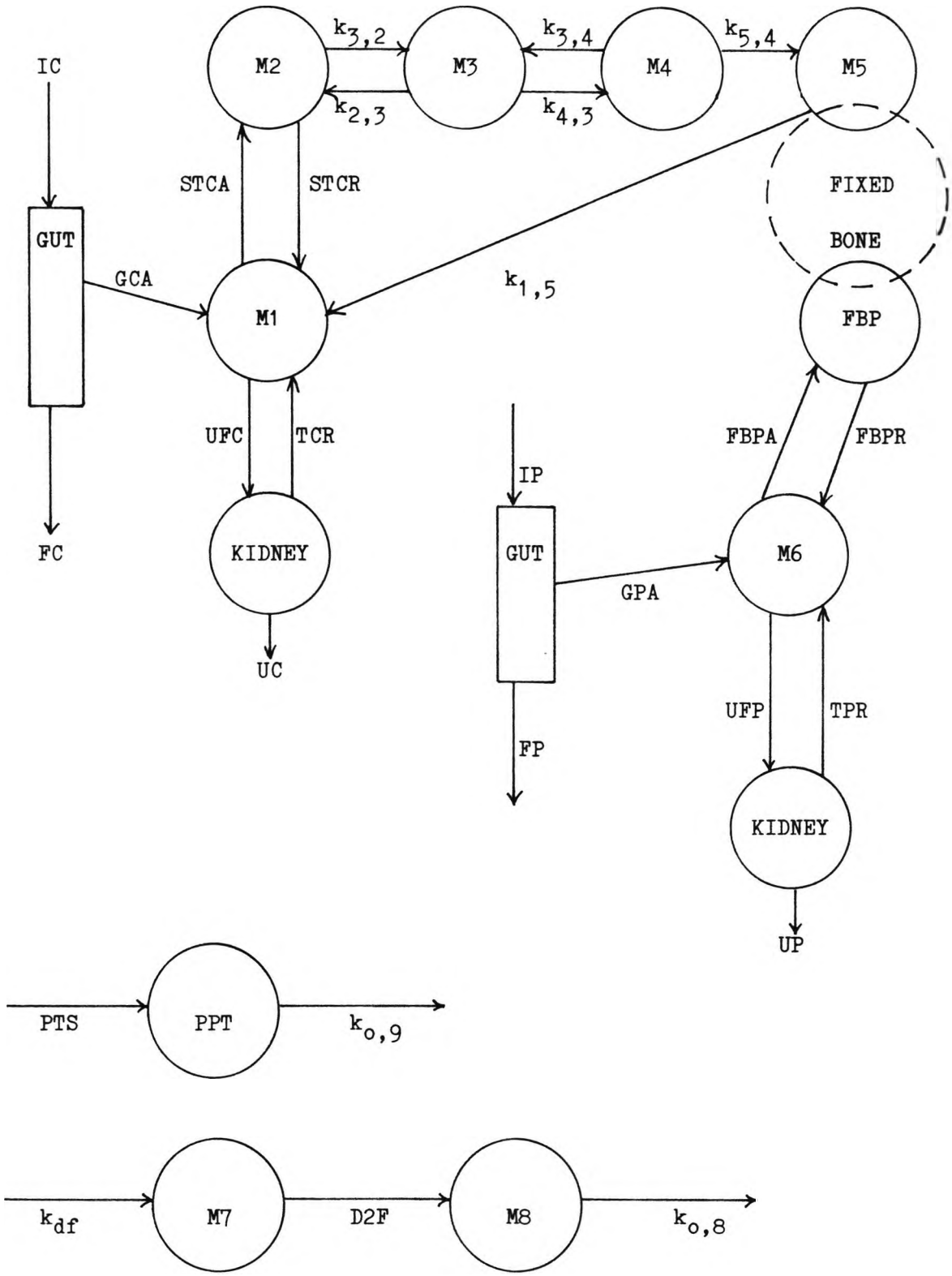


Figure 4.1 A schematic block diagram of the structure of MODEL1. Full details of the nomenclature are given in Appendix I.

State Equations (MODEL1)

$$\dot{M1} = GCA - k_{o,8}M1 + k_{1,5}M5 - STCA + STCR - UC$$

$$\dot{M2} = k_{2,3}M2 + STCA - k_{3,2}M2 - STCR$$

$$\dot{M3} = k_{3,4}M4 + k_{3,2}M2 - k_{2,3}M3 - k_{4,3}M3$$

$$\dot{M4} = k_{4,3}M3 - k_{3,4}M4 - k_{5,4}M4$$

$$\dot{M5} = k_{5,4}M4 - k_{1,5}M5$$

$$\dot{FBP} = (2/3\ k_{5,4}M4) - (2/3\ k_{1,5}M5) = 2/3\ M5$$

$$\dot{M6} = GPA + FBPR - UP - FBPA$$

$$\dot{M7} = k_{df}M7 - D2F*M7$$

$$\dot{M8} = D2F - k_{o,8}M8$$

$$\dot{M9} = PTS - k_{o9}M9$$

Undefined Functions (MODEL1)

$$UC = 0.74M1 - 23.58 + 1.05(M1 - 12.4)(1 - M9/(M9 + k_{uc}))$$

$$UP = k_f M6 - (k_{tp} - k_{tq}M9)$$

$$PTS = 5.3 \quad : \quad (M1 < 25.0)\ ng\ s^{-1}$$

$$PTS = 20.3 - 0.6\ M1 \quad : \quad (25.0 \leq M1 \leq 33.3)\ "\quad "$$

$$PTS = 0.32 \quad : \quad (M1 > 33.3)\ "\quad "$$

$$GCA = IC(M8/(M8 + k_c M8_{ss}))$$

$$GPA = IP(M8/(M8 + k_p M8_{ss}))$$

$$STCA = k_s\ M6\ M1$$

$$STCR = k_{1,2}\ M2\ (M9 + M9_{ss})/(2*M9_{ss})$$

$$D2F = (0.0673 - 0.00598\ M6)(M7/M7_{ss})$$

Figure 4.2 See overleaf for legend:

Nominal Steady State and Parameter Values (MODEL1)

IC	=	30.0	mmol day^{-1}
IP	=	45.0	" "
GCA_{ss}	=	15.0	" "
GPA_{ss}	=	25.0	" "
$STCR_{ss}$	=	927.5	" "
UC_{ss}	=	9.75	" "
UP_{ss}	=	25.25	" "
PTS_{ss}	=	69120	ng day^{-1}

$M1_{ss}$	=	32.5	mmol	$M2_{ss}$	=	35.0	mmol
$M3_{ss}$	=	90.0	"	$M4_{ss}$	=	177.5	"
$M5_{ss}$	=	25000.0	"	$M6_{ss}$	=	4.586	"
$M7_{ss}$	=	100.	'units'	$M8_{ss}$	=	1.0	'units'
$M9_{ss}$	=	490.0	ng	FBP_{ss}	=	16667.o	mmol

k_f	=	47.0	k_{df}	=	0.04
k_c	=	1.0	k_p	=	0.8
k_s	=	6.3	k_{tp}	=	3440.0
k_{tq}	=	6.63	k_{uc}	=	382.0

$k_{1,5}$	=	0.000568	day^{-1}	$k_{1,2}$	=	26.52	day^{-1}
$k_{2,3}$	=	1.476	"	$k_{3,2}$	=	4.2	"
$k_{3,4}$	=	0.0868	"	$k_{4,3}$	=	0.329	"
$k_{5,4}$	=	0.08	"	$k_{o,8}$	=	0.04	"
$K_{o,9}$	=	144.0	"				

Figure 4.2 A complete set of the equations of MODEL1, together with the nominal steady state and parameter values. The changes that were made to MODEL1 to produce MODEL2 to MODEL5 are only detailed in the text and in Appendix I.

$k_{1,2}$ Before Treatment	Change After Treatment	%age Change
0.637	+ 0.373	+ 59
0.752	+ 0.323	+ 43
0.599	+ 0.286	+ 48
0.943	+ 0.132	+ 14
0.813	+ 0.139	+ 17
0.813	+ 0.469	+ 58
0.654	+ 0.264	+ 40
Mean	+ 0.208	+ 40

Figure 4.3 The changes in $k_{1,2}$ after daily administration of PTH such that the circulating plasma level increases by approximately 80%. A simple two compartment model (extracellular fluid, and the rest of the body) was used to derive the data.

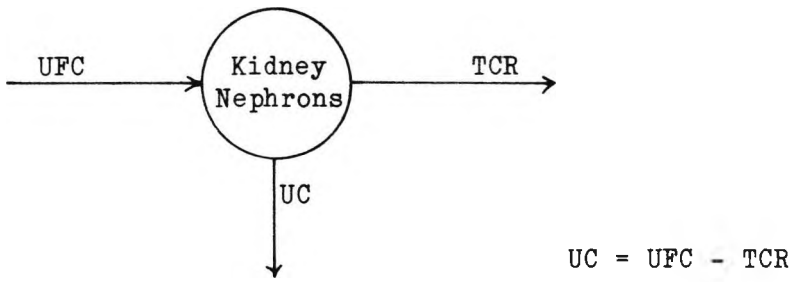


Figure 4.4 The basic scheme of kidney function used to derive the representation of urine calcium control.

UFC = Ultra Filtrable Calcium
TCR = Tubular Calcium Reabsorption
UC = Urine Calcium

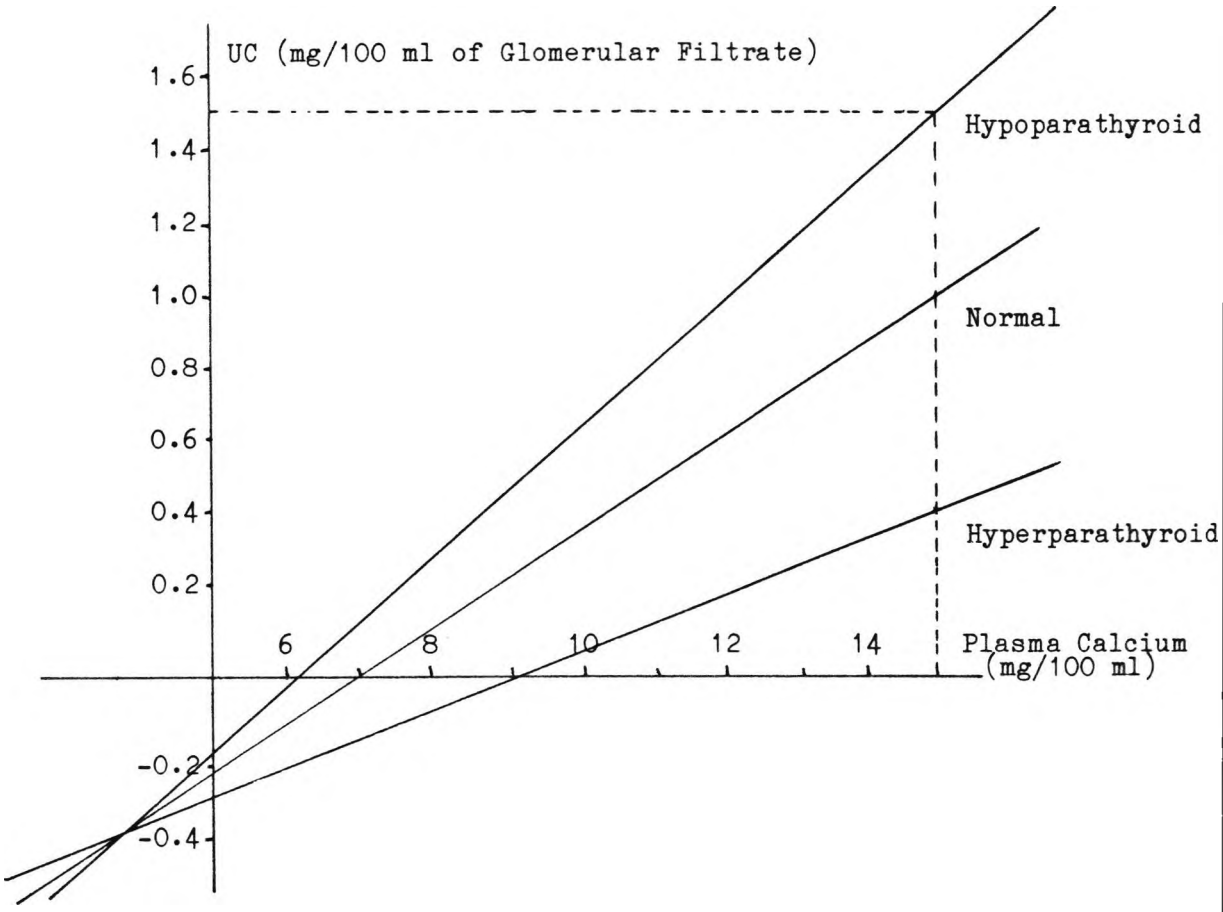


Figure 4.5 The relationship between parathyroid function and urine calcium used in the evaluation of the equations for urine calcium. Data were derived from Mioni et al (1971).

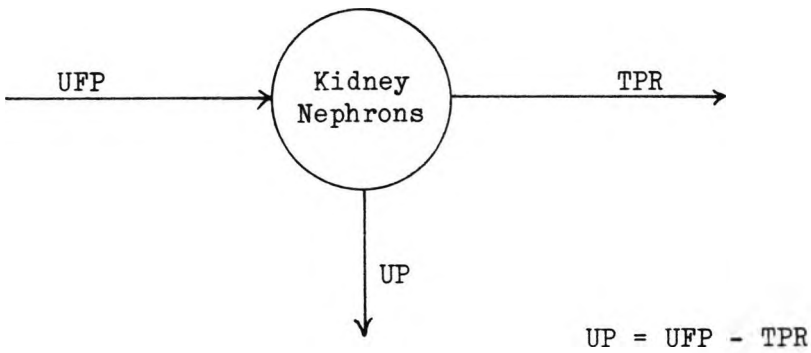


Figure 4.6 The basic scheme of kidney representation used to derive the equations describing the control of urine phosphate.

UFP = Ultra Filtrable Phosphate
TPR = Tubular Phosphate Reabsorption
UP = Urine Phosphate

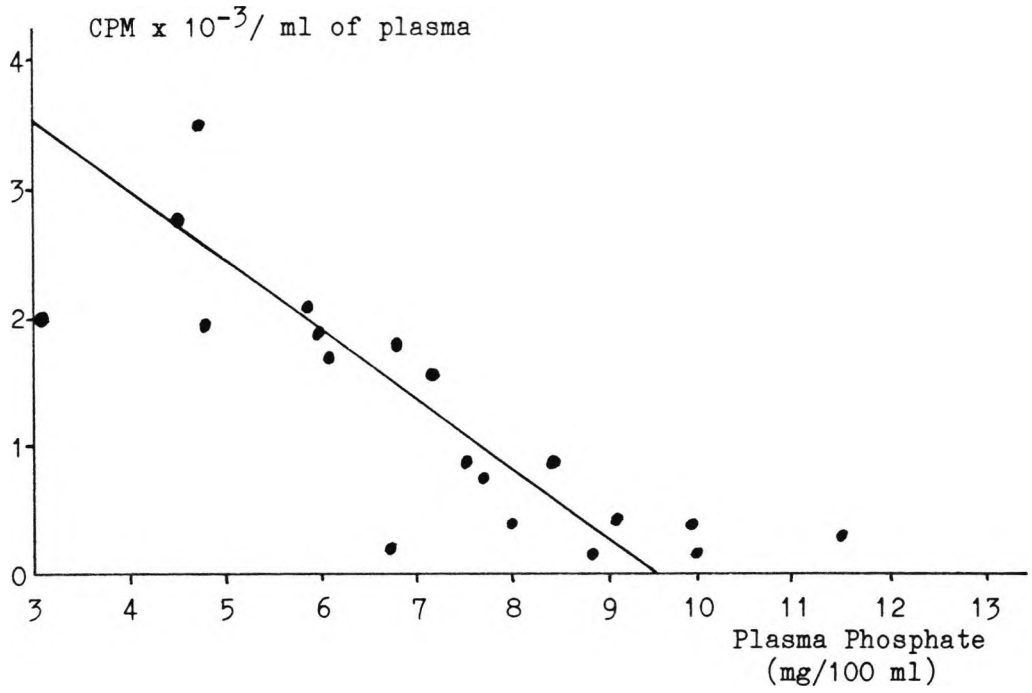


Figure 4.7 The relationship between the ability to synthesise 1,25(OH)2 Vit D3 and plasma phosphate in a group of rats. From DeLuca and Tanaka (1975).

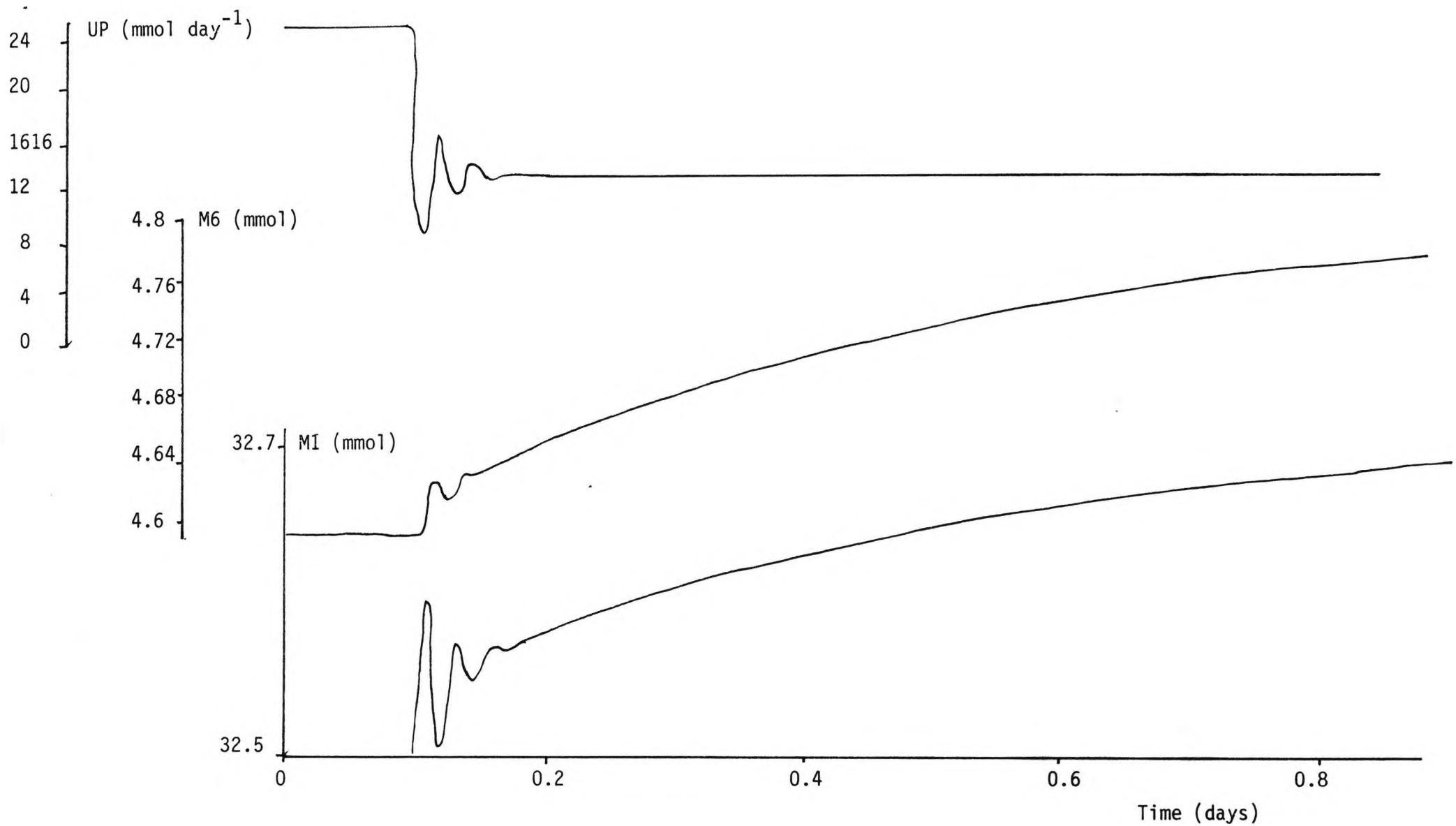


Figure 4.8 A simulation showing the effect of increasing INC to 45 mmol day^{-1}. MODEL2.

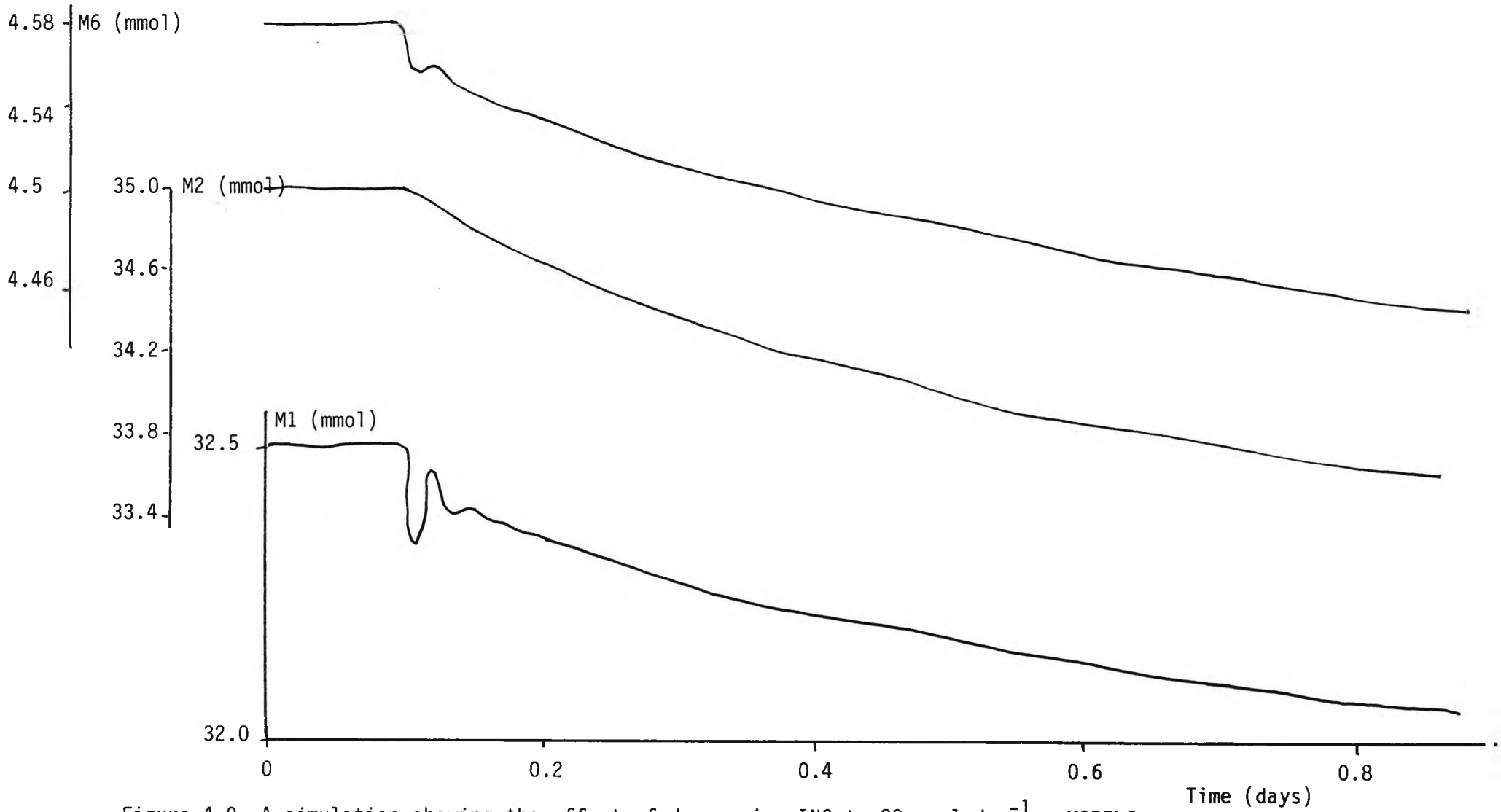


Figure 4.9 A simulation showing the effect of decreasing INC to 20 mmol day^{-1}. MODEL2.

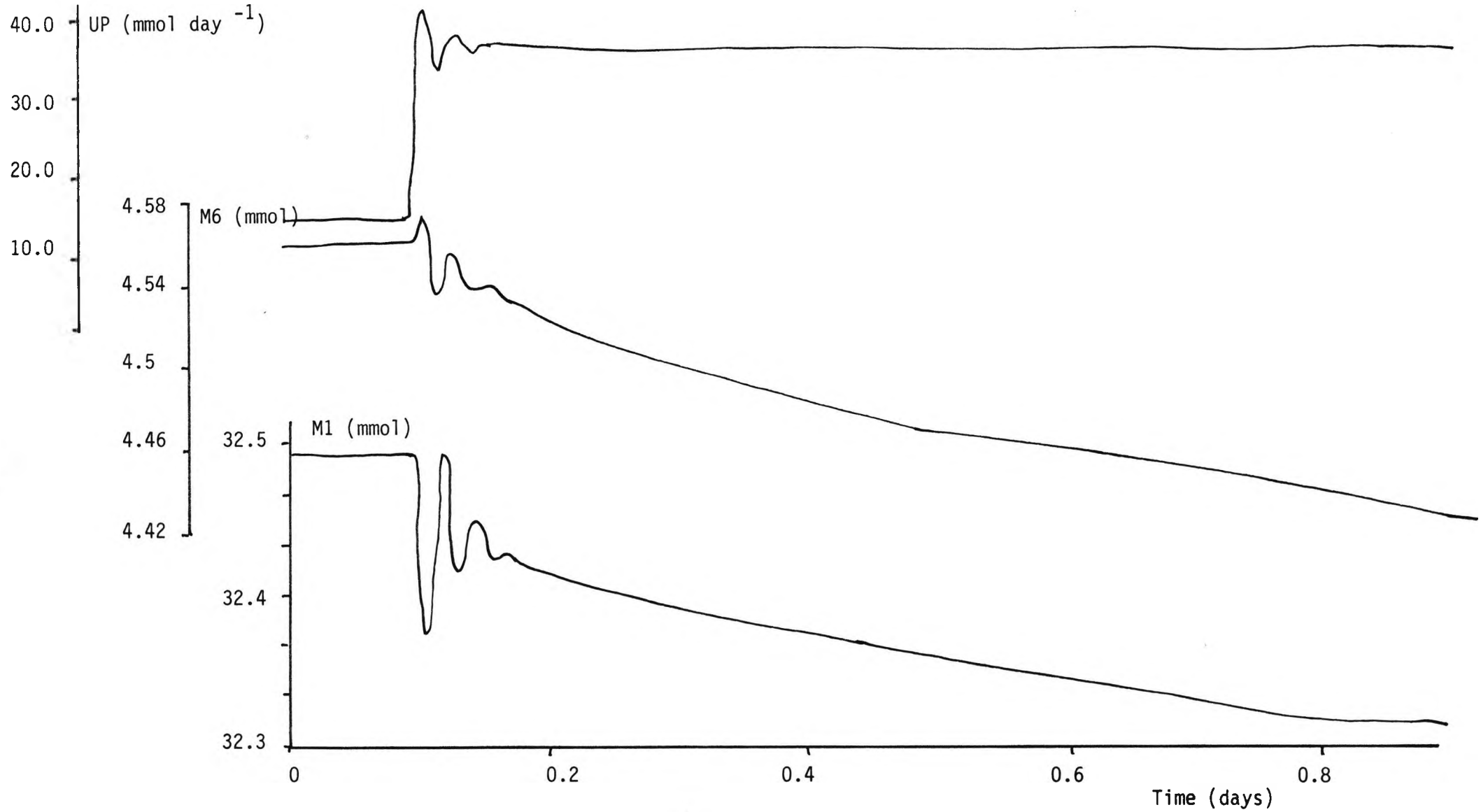


Figure 4.10 A simulation to show the effect of increasing INP to 65 mmol day^{-1}. MODEL2

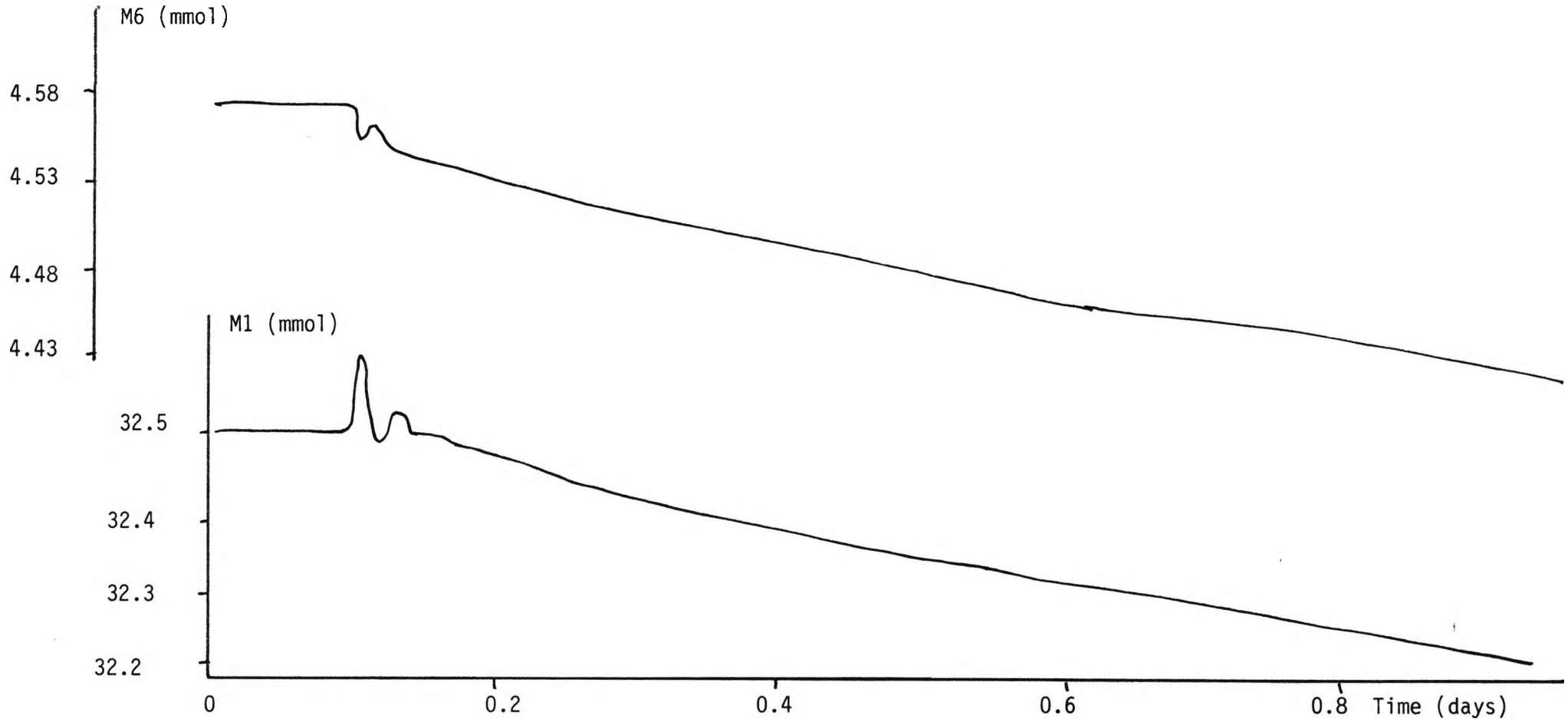


Figure 4.11 A simulation that shows the effect of decreasing INP to 15 mmol day $^{-1}$

CHAPTER 5

CHAPTER 5

5. A Short-Term Model (MODEL11)

This chapter is concerned with the development of a short-term model that attempts to overcome some of the shortcomings of MODEL5 as developed in the previous chapter.

MODEL5 was only partly based upon current physiological knowledge and although some of the simulated situations led to model responses that were in qualitative agreement with available data, quantitative validation techniques could not be easily applied due to the uncertain physiological identity of some of the model compartments.

Further models (MODEL11 & MODEL12) are developed in this chapter that have a greater degree of physiological isomorphism. The philosophy of model development used is first described, leading to the formulation of a number of sub-system models. The development of each sub-system is then considered in turn, the merits and disadvantages of sub-system alternatives being reviewed at each stage. Finally the further development of the complete model is shown. This illustrates the problems of identification and validation of large nonlinear models and suggests that the approach of model reduction used is a suitable one in these circumstances.

5.1 Modelling Philosophy

This section deals with the philosophy of model development that was used to formulate MODEL11 and its derivatives. Much has been written on this topic (see for instance, Mirham, 1972), but universally applicable 'rules' and guidelines that can be used in practice are still rare.

5.1.1 Isomorphic Models

The intention was to produce a number of 'plug-in' units that could each be identified and validated separately. Each sub-model would incorporate as much a priori information as was practicable and available.

The practicality constraint was felt to be especially important as some modellers (see for instance Guyton, 1971; and Guyton et al, 1972), have produced large compartmental models of very high order (around 100 compartments or so) and considerable complexity which may not yield any physiological insight. This question of small versus large models has been the subject of a lively debate between some authors (see for instance Guyton, 1979; and Yates, 1979). The situation is analogous

to the controversy that surrounded many of the 'world models' that have been constructed and the questionable predictions that have arisen, see for instance Forester (1971) and Meadows et al (1972) and the critiques of Charlwood and Noton (1978), and Vermeulen and Jongh (1977).

The problem with formulating a set of isolatable sub-models is that most of the data that can be used in model identification relates to a number of sub-models and rarely just one. For instance there are no data available on the calcium dynamics of the isolated bone or bone cell unit, only data concerning the response of the whole person. One is thus left to incorporate concepts and theories in sub-models together with those few data that are available, and use the complete system data to identify the overall model.

5.2 MODEL11 Formulation

This section deals with the structure that was adopted for MODEL11, showing how an isomorphic sub-system structure was arrived at. Some of the problems of formulating manageable isomorphic models are considered. Model units and the particular method of handling phosphate in this model are introduced.

5.2.1 Overall Structure

MODEL11 was conceived of in physiological terms and was intended to consist of a number of suitably connected sub-models:

(i)	Plasma + Extracellular Fluid	)	
(ii)	Kidney	)	For both calcium and
(iii)	Bone	)	phosphate
(iv)	Parathyroid Hormone		
(v)	Gut		
(vi)	Vitamin D3		

Each sub-system is dealt with in turn, although it should be noticed that both the gut and vitamin D3 systems were not fully incorporated at this initial stage of formulation. This was not essential to the construction of an adequate model as discussed in section 5.2.4. Equations are introduced through the text as particular sub-systems are dealt with. A complete model schematic diagram is shown in Figure 5.1 and set of equations in Appendix 1.

5.2.2 Model Units

As in previous models compartmental masses are expressed in mmoles, with parameters adjusted to correspond to a 70kg male. The exception is parathyroid hormone (PTH) for which the measurement problems and lack of standardisation across techniques obviate the use of molar units. PTH is hence expressed in nanograms, which does not in itself solve the standardisation problem. The ideal for PTH would be some sort of 'unit of activity', but this would also present significant problems.

5.2.3 Phosphate Sub-system

In the following sub-system descriptions, reference in the main is made only to calcium. Because of the 'passive' nature of the involvement of phosphate, its system structure is assumed to mirror that of calcium, with rate constants remaining constant but initial estimates of steady state masses being equal to two thirds of the complementary calcium value. The ratio of calcium to phosphate in bone mineral was three to two, as discussed in chapter 4.

5.2.4 Omissions From MODEL11

The initial formulation of MODEL11 involved a number of omissions from the list of plausible components. These include: gut absorption, vitamin D3, and calcitonin. Each omission was either complete, as with calcitonin and vitamin D3, or involved a reduction in the level of detail involved, as with gut absorption. Further detail concerning each of these omissions is presented below.

The gut can be regarded as a mechanism to absorb calcium and phosphate from the oral intake. Calcium absorption is not a totally 'passive' mechanism, as calcium is present in the digestive juices, and also a nonlinear relationship between calcium intake and faecal calcium exists. Thus Stanbury (1968) in reviewing 549 balance studies wrote two equations for calcium absorption. One covered a dietary intake up to 450 mg Ca day^{-1}, the other dealt with intakes greater than 550 mg Ca day^{-1}. Phosphate absorption can be regarded as linearly related to oral intake (Nordin & Smith, 1965). However it probably is not mediated by a simple passive diffusion process (Stanbury, 1971).

MODEL11 does not incorporate an explicit gut compartment, but the equations of Stanbury are straightforward, hence the use of a simple nett absorption figure was felt to be justified. This is especially so as complex variations in feeding were not initially included in the scope of investigation.

The vitamin D3 situation is more complex. As well as a number of metabolites being involved, a range of physiological actions have been implicated. A distinction between metabolites need not be of concern here, although any vitamin D3 model might consider the different actions of the metabolites. Vitamin D is implicated in the regulation of calcium absorption from the duodenum, and bone resorption. MODEL11 does not have a separate gut sub-system, and the bone effects are essentially long-term. The most important effect of vitamin D omission may be the omission of the links that exist between PTH and vitamin D3, although it has been suggested that these links are essentially passive effects of changes in intracellular calcium and phosphate (Galante et al, 1972).

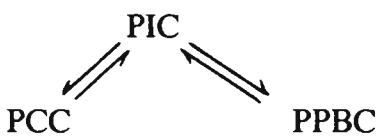
Calcitonin is not included in the list of potential sub-systems. This omission was deliberate, and based on the reasoning that calcitonin is unimportant in the adult human. It may be extremely important in other species, such as those large litter mammals the rat, or mouse, or fish living in a high calcium environment (e.g. salmon). Most importantly there is no evidence in man of calcium homeostatic problems following a total thyroidectomy (Austin and Heath, 1981).

5.3 Plasma and Extracellular Fluid

This section details the derivation of the plasma and extracellular fluid compartments (PIC, EIC, BSFC, PIP, EIP and BSFP), illustrating the particular problems that are introduced by the use of a bone fluid compartment. These compartments can be seen in Figure 5.1.

5.3.1 Ionic Forms To Be Considered

Both calcium and phosphate exist in plasma in three forms; ionised (PIC, PIP), protein bound (PPBC and PPBP), and complexed (PCC and PCP), with exchange occurring between the three species as indicated below:



These exchange dynamics are extremely rapid.

It is assumed that ionised calcium is the physiologically active and controlled form in plasma, the same being assumed for phosphate. In support of this assumption a correlation coefficient of only 0.45 has been found between ionised and total plasma calcium (see Ladenson and Bowes jr., 1973), suggesting that errors could occur if total plasma calcium was used as the controlled and measured variable. In addition homeostatic control of plasma calcium is in the main mediated through variations in

urine calcium of which 80% is of ionic origin. Thus both complexed and protein bound forms were ignored.

5.3.2 Volumes

A plasma volume of 3 litres was assumed, and steady state mass of 3.6 mmol for PIC. The total extracellular volume disregarding plasma was assumed to be 12.0 litres, this figure being derived from single tracer studies and a two compartmental analysis of the data (Reeve, 1979a). From estimates of the uptake of labelled calcium in a single passage through bone (Wootton et al, 1976) a volume ratio of 19:1 was predicted for EIC:BSFC, giving steady state masses of 13.84 and 0.7284 mmol, with associated volumes of 11.4 and 0.6 litres respectively.

5.3.3 Place Of Bone Fluid

Although BSFC is properly part of bone it is mentioned here as it is also part of the total extracellular fluid. This compartment represents a bone surface fluid layer that is effectively separate from the rest of the extracellular fluid after Neumann (1971).

Further detail is given under Bone (section 5.5).

5.3.4 Calcium Extracellular Fluid Equations

$$\dot{PIC} = INC + BSFCR + FBCR + k_{1,2}EIC - k_{2,1}PIC - UC - BSFCA \quad (5.1)$$

$$\dot{EIC} = k_{2,1}PIC - k_{1,2}EIC \quad (5.2)$$

$$\dot{BSFC} = BSFCA + k_{3,4}BSC - FBCA - BSFCR - k_{4,3}BSFC \quad (5.3)$$

5.3.5 Phosphate Extracellular Fluid Equations

$$\dot{PIP} = INP + k_{7,9}BSFP - k_{9,7}PIP + k_{7,11}FBP - k_{8,7}PIP + k_{7,8}EIP \quad (5.4)$$

$$\dot{EIP} = k_{8,7}PIP - k_{7,8}EIP \quad (5.5)$$

$$\dot{BSFP} = k_{9,7}PIP - k_{7,9}BSFP - k_{11,9}BSFP - k_{10,9}BSFP + k_{9,10}BSC \quad (5.6)$$

5.3.6 Estimation of Parameter Values

Parameter values for MODEL11 were initially derived from considering the state equations at the steady state. To make this a sufficient condition for solution, some values were taken from the tracer model of Neer (1967). These included:

$k_{1,5} = k_{7,11} = 0.00056\ day^{-1}$
representing the overall steady state bone resorption rate

from which $k_{11,9}$ and $k_{5,3}$ can be derived, as in the steady state the following applies:

$$K_{1,5}FBC = k_{5,3}BSFC$$
$$K_{7,11}FBP = k_{11,9}BSFP$$

The derivation of $k_{9,7}$ and $k_{7,9}$ involves the assumption that the corresponding calcium flux in the steady state is a simple linear function as is the phosphate flux. Then:

$$k_{9,7} = k_{3,1}$$

and

$$k_{7,9} = k_{1,3}$$

Similarly:

$$k_{10,9} = k_{4,3}$$

and

$$k_{9,10} = k_{3,4}$$

Further details of the derivation of these parameter estimates are given in sections 5.5.2 through to 5.5.5.

The plasma and extracellular fluid rate constants can be shown to obey the following relations, as phophate is assumed to mimik calcium:

$$k_{1,2} / k_{2,1} = k_{7,8} / k_{8,7} = PIC/EIC$$

Various compartmental analyses have shown a short-term disappearance rate of calcium from plasma, of 0.18 to 0.35 mmol kg^{-1} day^{-1} (Neer, 1967; Bell, 1966).

If PIC/EIC = 0.26, the daily flux is estimated as 18 mmol day^{-1} and the masses are known, the following parameters can be estimated:

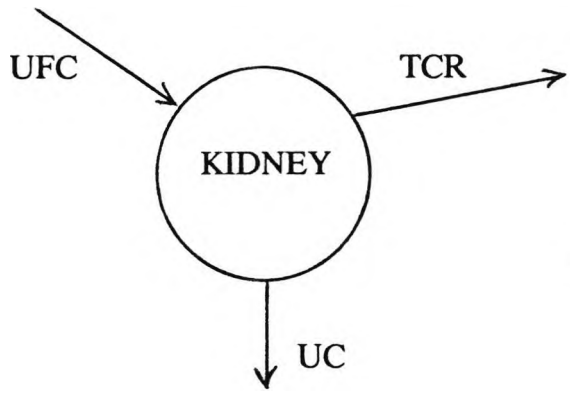
$k_{1,2}$	=	1.3006	day^{-1}
$k_{2,1}$	=	5.0	"
$k_{7,9}$	=	3.549	"
$k_{9,7}$	=	6.132	"
$k_{7,11}$	=	0.000568	"
$k_{8,7}$	=	3.871	"
$k_{7,8}$	=	1.0084	"
$k_{11,9}$	=	19.49	"
$k_{10,9}$	=	41.51	"
$k_{9,10}$	=	0.3024	"

5.4 The Kidney

The kidney plays a crucial role in the short-term metabolism of calcium. This is mediated in two ways; through variation in urine calcium output in proportion to plasma calcium changes, and through changes in the slope of this variation as the circulating level of parathyroid hormone changes. There are various ways of modelling the kidney; the three approaches considered are dealt with in this section.

5.4.1 Overall Structure Of Kidney Model

Although treated as a separate sub-system, no extra compartments are included for the kidney, merely equations describing the rate of urine production with variations in relevant state variables. In very comprehensive models it would be possible to involve extra compartments if desired, to represent separate parts of the nephron. The kidney can be represented simply as one equivalent 'giant' nephron by:



$$UC = UFC - TCR$$

and similarly

$$UP = UFP - TPR$$

Each of the approaches considered used the 'single equivalent nephron'. These are considered in sections 5.4.2, 5.4.3, 5.4.4, and compared in 5.4.5.

5.4.2 A Theoretical Approach (Option 1)

Calcium excretion is regulated by a combination of filtration and partial reabsorption, the calcium in the urine being the fraction of the filtered load which is not absorbed. The same is true of phosphate, although Bijvoet (1975) has suggested that active glomerular secretion of phosphate can occur.

Walser (1966a) provides an analysis of sodium reabsorption giving the following equation:

$$Z_o - Z = \frac{apx}{b+p} - \frac{bm}{b+p}(\log_e (Z_o/Z)) \qquad (5.7)$$

where:

Z	=	axial flow rate of solute at point x
Z_o	=	axial flow rate of solute at point x = 0
a	=	Michaelis-Menten constant for maximal reabsorption rate
p	=	Plasma osmolarity
x	=	Fractional distance along a nephron
b	=	Michaelis-Menten constant for the solute
m	=	Axial flow of unreabsorbable solute

Walser (1966b) extended this analysis to the reabsorption of other solutes (such as calcium) which do not constitute a significant fraction of plasma osmolarity, using the assumption that water reabsorption could be approximated by a linear function. If this assumption is not made, and an 'active' saturable mechanism for reabsorption of the second solute Z' is assumed (as could be the case for calcium and phosphate), then:

$$\frac{dZ'}{dx} = \frac{-a'}{1+b'/y'} = \frac{-a'Z'}{b'(Z+Z'+m)/p+Z'} \qquad (5.8)$$

It can then be shown that (Z_o' - Z') is linearly related to loge (Zo'/Z'). This would mean that with an increasing filtered load, the resorption rate will tend to a maximum value which is a function of a', the true maximum resorption rate.

If an operational maximum tubular absorption rate Tm and an associated constant Km for a minority solute are defined then resorption (r) can be expressed as:

$$r = (Z_o - Z) = Tm - Km(\log_e(Z_o/Z)) \quad (5.9)$$

The next two sections apply this approach to calcium and phosphate.

5.4.2.1 Calcium Reabsorption

Although most investigators have failed to demonstrate a tubular maximum reabsorption rate, this does not mean that the reabsorption mechanism is not obeying saturable kinetics (see Marshall, 1976) but rather that the maximum cannot safely be reached (even in those suffering from osteomalacia) without endangering the subject. Hence an equation similar to equation (5.9) can be used for calcium reabsorption:

$$UFC - UC = Tmc - Kmc \log_e (UFC/UC) = TCR \quad (5.10)$$

A curve of this form is shown in Figure 5.2.

5.4.2.2 Phosphate Reabsorption

For phosphate, unlike calcium, a tubular maximum resorption rate can be clearly demonstrated (Bijvoet, 1969). A suitable equation is the following:

$$UFP - UP = Tmp - Kmp \log_e (UFP/UP) = TPR \quad (5.11)$$

Figure 5.3 shows a curve of this form fitted to phosphate data.

5.4.3 A Single Exponential Delay (Option 2)

A simple equation bearing some similarity of shape and form to equations (5.10) and (5.11) can be written:

$$UC = UFC + Tmc(e^{-UFC/Tmc} - 1) \quad (5.12)$$

Figure 5.4 shows three curves of this form. It is however clearly difficult to obtain an adequate approximation to recorded figures over a wide enough range of feasible values of UC, Tmc and UFC.

5.4.4 A Piece-Wise Approximation (Option 3)

Three straight lines can be drawn to represent the available data extremely well (see Figure 5.5). These are for calcium and phosphate respectively:

$$UC_1 = 0.174 \ (UFC - 0.5\ Tmc) \quad (5.13)$$
$$UC_2 = 0.352 \ (UFC - 0.68\ Tmc) \quad (5.14)$$
$$UC_3 = 0.7 \ (UFC - 0.9\ Tmc) \quad (5.15)$$
$$UC = \max\ [UC_i,\ i = 1,2,3]$$

This is shown in Figure 5.6

$$UP_1 = 0.174 \ (UFP - 0.5\ Tmp) \quad (5.16)$$
$$UP_2 = 0.352 \ (UFP - 0.68\ Tmp) \quad (5.17)$$
$$UP_3 = 0.8 \ (UFP - 0.9\ Tmp) \quad (5.18)$$
$$UP = \max\ [UP_i,\ i = 1,2,3]$$

This is shown in Figure 5.7

5.4.5 A Comparison Of The Three Kidney Models (Options 1,2, & 3)

Although the theoretical approach of Walser is elegant, the equations concerned are implicit (i.e. UC = f(UC)) which brings with it severe computational difficulties.

To obtain solutions a nomogram or numerical approximation must be used. Computationally a nomogram is impractical, and the available numerical algorithms (e.g. section EO4 NAG Library Mark VI) all require either, fairly reliable initial estimates or, an interval to be specified which contains the solution. All obviously require suitable errors bounds, and error exit actions.

If one was solely interested in computing excretion rates against the other values, the problem is reasonably straightforward, but if, as is the case here, one is solving the equations (two in fact, phosphorous and calcium) as part of a set of 'model equations' which are themselves repeatedly called by a numerical integration routine the computational difficulties mount. The situation is hampered by variations in Tm and Km via plasma PTH (see section 5.4.8), making the specification of a useful initial estimate or solution interval bound impossible.

A single exponential delay is simply not accurate enough over a wide enough range even if the shape of the curve is optimised by the addition of suitable constants to the equations (see Figure 5.4).

The straight line segment approximation provides no computational problems, suffers from little error, and is soundly based upon the saturable kinetics of the Walser equations. Thus the concept of a tubular maximum resorption rate is retained and this approach was adopted. Typical curves for UP and UC are shown in Figures 5.6 and 5.7 respectively.

5.4.6 Control of Tmc and Tmp by PTH

PTH affects both TCR and TPR as part of the short-term homeostatic control of plasma calcium through variation in the tubular maximum reabsorption rate (Tmc and Tmp).

Bijvoet (1975) has shown data comparing the Tmc and Tmp of normal with hypo- and hyper- parathyroid subjects. Although the data are inherently crude (see Figure 5.8) they can be summarised as shown in Table 5.1 below:

	Tmc	Tmp (mmol day^{-1})
Hypo-PTH	217	185.8
Normal	251	130
Hypo-PTH	285	69.7

Table 5.1 The variation in Tubular Maximum reabsorption rate (Tmc and Tmp) with parathyroid status. From Bijvoet (1975).

If it is assumed that in the steady state, using current model units, that plasma parathyroid hormone PPT (Normal) = 490 ng, PPT (Hypo -) = 0, PPT (Hyper-) = 2240; i.e. a fall to zero and a four-fold rise in circulating levels of PTH respectively. This variation can be approximately modelled by:

$$Tmc = 0.06939\,(PPT) + 217.0 \quad ; PPT < 490.0 \qquad (5.19)$$
$$Tmc = 0.01943\,(PPT) + 241.48 \quad ; PPT \geq 490.0 \qquad (5.20)$$

$$Tmp = 185.8 - 0.11375\,(PPT) \quad ; PPT < 490.0 \qquad (5.21)$$
$$Tmp = 146.961 - 0.03449\,(PPT) \quad ; PPT \geq 490.0 \qquad (5.22)$$

giving steady state values of:

$$Tmp_{SS} = 130 \text{ mmol day}^{-1}$$
$$Tmc_{SS} = 251 \text{ mmol day}^{-1}$$

5.4.7 Calculation of UFC and UFP

If the GFR is assumed to be 100 ml min^{-1}, and the plasma volume 3.0 litres, we can assume that UFC = 48 x 1.2 PIC mmol day^{-1} as the filtered calcium load is 1.2 times the level of PIC (some of the non- ionised calcium is ultrafiltrable).

Similarly UFP = 41.75PIP mmol day^{-1}, the filtered phosphate load comprising approximately 87% of the ionised fraction. The GFR is assumed to be non varying.

5.4.8 Kidney Equations

The equations incorporated in MODEL11 are as follows:

Urinary calcium output rate (UC)

$$UC_1 = 0.174\ (UFC - 0.5\ Tmc) \quad (5.13)$$

$$UC_2 = 0.352\ (UFC - 0.68\ Tmc) \quad (5.14)$$

$$UC_3 = 0.7\ (UFC - 0.9\ Tmc) \quad (5.15)$$

$$UC = \max\ [UC_i,\ i = 1,2,3]$$

Urinary phosphate output rate (UP)

$$UP_1 = 0.174\ (UFP - 0.5\ Tmp) \quad (5.16)$$

$$UP_2 = 0.352\ (UFP - 0.68\ Tmp) \quad (5.17)$$

$$UP_3 = 0.8\ (UFP - 0.9\ Tmp) \quad (5.18)$$

$$UP = \max\ [UP_i,\ i = 1,2,3]$$

Tubular calcium maximum reabsorption rate (Tmc)

$$Tmc = 0.06939\ (PPT) + 217.0 \quad ;\ PPT < 490.0 \quad (5.19)$$

$$Tmc = 0.01943\ (PPT) + 241.48 \quad ;\ PPT \geq 490.0 \quad (5.20)$$

Tubular phosphate maximum reabsorption rate (Tmp)

$$Tmp = 185.8 - 0.11375\ (PPT) \quad ;\ PPT < 490.0 \quad (5.21)$$

$$Tmp = 146.961 - 0.03449\ (PPT) \quad ;\ PPT \geq 490.0 \quad (5.22)$$

Ultrafiltration Rate (UFP and UFC)

$$UFP = 41.75\ PIP \quad mmol\ day^{-1}$$

$$UFC = 57.6\ PIC \quad mmol\ day^{-1}$$

5.5 Bone

Apart from its role as a supporting structure the skeleton also acts as a reservoir of bone mineral that can be called upon, or added to, in times of need or plenty respectively. The structure of living bone is complex, far from being a dead calcified matrix it is a highly vascularised organ, replete with various types of cell.

This section details the approach that was adopted to represent this unique structure. This includes the distinction between surface and deep bone, and the concept of an independent bone surface fluid.

5.5.1 Bone Model Structure

The concept of a Bone Surface Fluid (BSF) layer surrounding bone that is physiologically separate from the bulk of the extracellular fluid is adopted. There are a number of studies that support this. If the influx and efflux of labelled calcium are studied in live chick calvaria, these cells are able to maintain a medium in which calcium and phosphate levels are far above those indicated by the passive solubility of bone mineral. Similarly plasma is supersaturated with respect to preformed bone mineral. Various ideas have been put forward to explain these observations including the existence of a bone cell 'membrane' that physically separates a bone surface fluid layer from the rest of the extracellular fluid (see: Talmage 1969; Newman and Ramp, 1971; Talmage & Grubb, 1976; Brommage et al, 1978).

Additionally a surface bone layer of 1 to 4 micron thickness is postulated (BSC and BSP) after Groer and Marshall (1973), this surface being available for the rapid exchange of mineral as distinct from the bulk of bone (the deep bone compartments; FBC and FBP) which is not as readily available for exchange. These latter two compartments are similar to the traditional distinction between 'exchangeable' and 'fixed' bone.

As detailed earlier BSFC and BSFP have steady state masses of 0.7284 mmol and 0.4856 mmol with an associated volume of 0.6 l.

BSC is assumed to have a mass of 100 mmol (from Groer & Marshall, 1973), two thirds of this value being assumed for BSP.

The masses of FBC and FBP are taken from data relating to total bone calcium and phosphate (Ingalls, 1931).

The equations relating to bone are given overleaf:

$$\dot{BSFC} = BSFCA + k_{3,4}\,BSC - FBCA - k_{4,3}\,BSFC - BSFCR \quad (5.23)$$

$$\dot{BSC} = k_{4,3}\,BSFC - k_{3,4}\,BSC \quad (5.24)$$

$$\dot{FBC} = FBCA - FBCR = k_{5,3}\,BSFC - k_{1,5}\,FBC \quad (5.25)$$

$$\dot{BSFP} = k_{9,7}\,PIP - k_{7,9}\,BSFP - k_{11,9}\,BSFP - k_{10,9}\,BSFP + k_{9,10}\,BSP \quad (5.26)$$

$$\dot{BSP} = k_{10,9}\,BSFP - k_{9,10}\,BSP \quad (5.27)$$

$$\dot{FBP} = k_{11,9}\,BSFP - k_{7,11}\,FBP \quad (5.28)$$

5.5.2 Derivation of Steady-State Bone Compartment Parameters

Parameter values were estimated as follows from the limited data that are available concerning the uptake and resorption of calcium by and from bone.

Referring to Figure 5.9, and ignoring hormonal influences as only the steady state is being considered:

PIC_{ss} = 3.6 mmol
$BSFC_{ss}$ = 0.7284 "
BSC_{ss} = 100.0 "
FBC_{ss} = 2500.0 "

$$\dot{BSFC}_{ss} = k_{3,1}PIC + k_{3,4}BSC - k_{1,3}BSFC - k_{4,3}BSFC - k_{5,3}BSFC = 0.0 \quad (5.29)$$

$$\dot{FBC}_{ss} = k_{5,3}BSFC - k_{1,5}FBC = 0.0 \quad (5.30)$$

$$\dot{BSC}_{ss} = k_{4,3}BSFC - k_{3,4}BSC = 0.0 \quad (5.31)$$

Neer (1967) has quoted a typical FBC flux of 14.2 mmol day^{-1} yielding the following values:

$k_{1,5}$ = 0.568 x 10-3 day^{-1}
$k_{5,3}$ = 19.49 day^{-1}

If:

$k_{3,4}$ = 0.3024 day^{-1} (from Groer & Marshall, 1973) then
$k_{4,3}$ = 41.51 day^{-1}

From (5.29):

$$PIC\, k_{3,1} = BSFC\,(k_{1,3} + 19.49) \qquad (5.32)$$

Hence:

$$k_{3,1} = 0.2023\, k_{1,3} + 5.414 \qquad (5.33)$$

If:

$$k_{3,1}PIC - 14.2 = 7.875 \text{ mmol day}^{-1} \quad \text{(from Marshall, 1976)} \qquad (5.34)$$

Then:

$k_{3,1}$ = 6.132 mmol day^{-1}
$k_{1,3}$ = 3.549 mmol day^{-1}

All of the steady state bone parameters are tabulated below:

$BSFC_{ss}$	=	0.7284 mmol	$BSFP_{ss}$	=	0.486 mmol
BSC_{ss}	=	100.0 "	BSP_{ss}	=	66.7 "
FBC_{ss}	=	25000.0 "	FBP_{ss}	=	16667.0 "

$k_{3,1}$	=	6.132	day^{-1}	=	$k_{9,7}$
$k_{3,4}$	=	0.3024	day^{-1}	=	$k_{9,10}$
$k_{1,3}$	=	3.549	day^{-1}	=	$k_{7,9}$
$k_{4,3}$	=	41.51	day^{-1}	=	$k_{10,9}$
$k_{5,3}$	=	19.49	day^{-1}	=	$k_{11,9}$
$k_{1,5}$	=	0.568×10^{-3}	day^{-1}	=	$k_{7,11}$

5.5.3 Non-Steady State Bone Compartment Relationships

PTH and phosphate were both assumed to influence bone calcification. The former through hormonal action, the latter via changes in the serum solubility product and passive mineralisation of bone matrix. In adhering to the bone surface fluid (BSF) concept all bone calcification is assumed to occur through the BSF, the flows represented by $k_{1,3}$ and $k_{3,1}$ being influenced by phosphate and PTH respectively. The derivation of these relationships follows.

5.5.3.1 BSFC Accretion

Borle (1972 and 1975) has demonstrated in vitro the influence of the extracellular medium phosphate concentration upon labelled calcium uptake by kidney cells. A similar saturable linear relationship was assumed to operate in the bone surface cells that control the constitution of the bone surface fluid.

$$BSFCA = k_{3,1}PIC\,(PIP/PIP_{ss}) \quad (5.35)$$

$$0.0 < BSFCA < 30 \text{ mmol day}^{-1} \quad (5.36)$$

PIP_{ss} = steady state value of PIP

5.5.3.2 BSFC Resorption

PTH is assumed to affect $k_{1,3}$ by a saturable linear relationship:

$$BSFCR = k_{1,3}BSFC(\,k_jPPT + (1-k_j)\,PPT_{ss})/PPT_{ss} \quad (5.37)$$

$$0.0 < BSFCR < 10.0 \text{ mmol day}^{-1} \quad (5.38)$$

PPT_{ss} = steady state value of PPT

and

k_j = 0.5.

This value of k_j is derived from data relating to the administration of exogenous PTH. Its effect upon bone resorption indicate that a 40% increase in the rate constant of bone resorption can be mediated by an 80% increase in the level of circulating PTH (Reeve, 1979b). This analysis assumes a simple two compartment calcium model (Plasma/ECF and Bone).

5.6 Parathyroid Hormone (PTH)

As indicated earlier PTH is the most important factor involved in the homeostatic maintenance of plasma calcium; mediating an increase in BSFCR, and a decrease in Tmc. The rate of secretion of PTH has been demonstrated to be inversely related to PIC (and magnesium to some extent; see Mayer, 1975). PTH is also implicated in long-term effects upon bone modelling/resorption (see Reeve et al, 1976; 1978; and 1980) in particular via osteocyte and osteoblast activity (Talmage, 1969; Borle, 1975), and absorption of calcium from the gut by influencing the rate of synthesis of vit D3 metabolites (Riggs and Gallagher, 1978; and Reeve, 1979). This section considers the modelling of PTH secretion distribution and clearance.

5.6.1 The Simple Approach To PTH

Many authors have shown the existence of what approximates to an inverse relation between plasma ionised calcium (PIC) and PTH secretion rate (cf: Mayer, 1975; and Mayer et al, 1976). The relationships published have all been of a sigmoidal shape, but when these are examined in the light of the attendant measurement errors and the extent of the data spread it can be inferred that a smooth curved fit to the data is not necessary. Hence a simple inverse relation with upper and lower bounds was used to represent PTH secretion:

$$PTS = 13.6 - 3.58\ PIC \tag{5.39}$$

$$5.0 \geq PTS \geq 0.3 \quad ng\ s^{-1} \tag{5.40}$$

and assuming a single compartment of distribution due to its short half-life, and first order clearance kinetics:

$$\dot{PPT} = 86400\ PTS - 125.54\ PPT \quad mmol\ day^{-1} \tag{5.41}$$

5.6.2 Other PTH Approaches

It has been suggested that an increase in PTH secretion can occur from two sources; a changed synthesis rate, and a store of PTH, and that this store is finite and only called upon at very low levels of plasma calcium (Jung et al, 1982), the plasma calcium levels concerned being so low as to provide a severe emergency for the organism. Although attractive from various angles the store was not investigated further at this stage.

5.7 MODEL11 Performance & Refinement

The response of MODEL11 to a range of simulated system inputs was examined. These enabled the model performance to be assessed and in line with the modelling approach of sequential formulation, identification, and validation used, provide a direction for further model refinement.

Model simulations were performed using an online model simulation system, written in FORTRAN, run on a Prime 550, that reported model output as a graphic display of up to 6 model outputs per screen image. A written report backup was also available. This system enabled model parameters to be varied easily, giving immediate feedback on the effect of changes. Structural model changes would involve recompilation of the model subroutines, and were more awkward to implement and test. Further details of the facilities used are given in Appendix II.

5.7.1 Initial Model Behaviour & Validity

MODEL11 was comprehensively tested over a variety of situations that are either known (i.e. data are available), or would provide useful and possibly testable model predictions, potentially enabling improvements to be made to the model. The following sub-sections discuss these situations:

5.7.1.1 EDTA Infusion
5.7.1.2 Calcium Infusion
5.7.1.3 PTH Infusion
5.7.1.4 Phosphate Infusion
5.7.1.5 Long-term reduction of oral intake (Ca and/or PO_4)

5.7.1.1 EDTA Infusion

Figure 5.10 (from Jones & Fourman, 1963) shows the changes in plasma calcium during an EDTA infusion. Figure 5.11 shows the response of MODEL11 to a simulated infusion of between minus 1.25 and minus 6.25 mmol calcium equivalent over 3 hours. This is expressed as calcium equivalent because the physiological effect of EDTA is to chelate or render inactive the calcium in plasma. Hence it is equivalent to a negative input of calcium.

The most noticeable difference apart from the shape of the Plasma Ionised Calcium (PIC) response, is the rate of recovery of PIC to the steady state level. The simulated recovery takes less than two hours. Figure 5.10 shows the observed recovery time to be approximately 12 hours. None of the other state variables show behaviour that is inconsistent with expectations. The plasma phosphate drop is consistent with the large rise in circulating PTH (mediated via a large increase in urine phosphate). The large drop in urine calcium is anticipated. All the other variables are unobservable directly, but there are no inconsistencies.

5.7.1.2 Calcium Infusion

Figure 5.12 shows the changes in plasma calcium during an infusion of calcium (from Ibbertson, et al, 1966). If this is compared with the model response shown in Figure 5.13 the following elements of mismatch can be discerned.

PIC rises too quickly, and to a level that is incompatible with life.

The rate of return of PIC is too rapid. Thus the model steady state conditions are reached in less than two hours after cessation of the infusion. The data show the actual time to be around 12-14 hours.

This mismatch could arise from a number of model assumptions. The first possibility is that PIC although undoubtedly the 'controlled' variable, is a poor predictor of total plasma calcium (the measured variable), as suggested by Ladenson and Bowes (1973); especially during the major changes in concentration involved. This lack of concordance could be because plasma protein bound and plasma complexed calcium are omitted from the model. The extra buffering action that would be provided by two extra plasma compartments with very fast exchange rates is, however unlikely to produce sufficient damping of the model response.

The second possibility is a more plausible source of error and concerns the nature of the plasma/bone exchange pathways and the manner in which PTH influences these. Thus the parameter values, control strategy, or model structure may be incorrect.

5.7.1.3 PTH Infusion

This is shown in Figure 5.16. There are only limited data available for comparison with this simulated situation. This is mainly due to the experimental difficulties associated with the administration of a 'standardised' dose of PTH. Generalisations can however be drawn, both Podbeseck (1982) and Parsons et al (1971) have shown a maximum increase in the plasma calcium of dogs of approximately 15% four hours after a single large intravenous or subcutaneous injection of hPTH. The simulated PIC response would appear to rise too little (a maximum of 11%, after the maximum sustained Plasma PTH (PPT) level has been held for 3 hours). Thus a simulated single dose could not raise PIC any further.

5.7.1.4 Phosphate Infusion

The response of MODEL11 to phosphate infusions of varying magnitudes is shown in Figure 5.17. The most important features of this response, apart from the expected rise in plasma and urine phosphate, are the drop in plasma calcium and the rise in BSFC and plasma PTH. These are the accepted effects of a phosphate infusion, indeed phosphate supplementation has long been used in the management of hypercalcaemia (Herbert et al 1966). The following table compares the responses:

	Human Data	MODEL11 Response
UC	- 35%	- 20%
PIC	- 8%	- 8%

Herbert et al did not provide measurements of PTH, and of course the physiological analogue of BSFC is inaccessible to direct measurement. The authors do argue that calcium is lost from the plasma to bone, the rise in BSFC is a loss from plasma to bone. In this connection the loss of bone weight over a longer time scale predicted by MODEL11 due to reduced absorption is interesting (see Figure 5.20).

5.7.1.5 Long-Term Reduction of Oral Intake

The model assumes a steady input of both calcium and phosphate from dietary sources. Any daily variation in gut absorption due to normal dietary patterns is ignored, variations in intake thus correspond to long-term reductions in food intake, specifically calcium and phosphate, but due to the difficulty of isolation of calcium and phosphate from an otherwise adequate diet, poor absorption is the most common real life analogue.

Three situations of this type were simulated: (See Figures; 5.18, 5.19 and 5.20) the first the general case of intake reduction, the latter two specific reductions of calcium and phosphate intake respectively. It would be expected that long-term reductions of this kind could be a causative factor behind long-term bone degeneration. Thus all the situations simulated gave rise to what is termed a negative calcium, and phosphate balance, i.e. that the bodily output exceeds input.

Perhaps the most interesting negative balance is the last of these simulations (Figure 5.20) as calcium absorption is unchanged but the reduction in phosphate intake still produces a negative calcium balance and a steadily decreasing fixed bone calcium (FBC). Thus a zero phosphate intake produces an FBC decrease of 5.8% per annum, a phosphate intake of 35% of normal yields an FBC decrease of 3.2% per annum. Decreases of this magnitude are of clinical significance.

Data are available for comparison, including those of MacFadyan et al (1965). A low calcium diet (similar to the 5.0 mmol day^{-1} shown in Figure 5.19) led to a 2% decrease in plasma calcium and a 30% decrease in urine calcium. This should be compared with the simulation shown in Figure 5.20: decreases of 8% (PIC) and 31% (UC) respectively. However if the ultrafiltrable calcium figures of MacFadyan et al are examined, the decrease is 5.9% over one day. This figure is the one that should be compared with the model response.

This agreement was considered to be very good, especially as no long term data were used in the formulation of MODEL11.

5.7.2 Model Refinement

Four approaches to the refinement of MODEL11 were explored and detailed in the following four sections:

5.7.2.1 Use Of Alternative PTH Models

Alternative PTH control strategies were examined.

(a) Variation in the slope of PTS upon PIC.
(b) An on-off controller, basically switching secretion between a minimal basal rate and 'full-on', the switching point(s) being particular levels of PIC.
(c) The presence of hysteresis in the PTH/PIC control loop i.e. that the slope of PTS upon PIC is different for a rising PIC value than a decreasing PIC value, or that the switching point in the above is different for a rising and a falling PIC.

None of the alternatives yielded an improvement in model response to any situation, and indeed instability was easily produced during simulated short-term infusions.

5.7.2.2 Model Adjustment

Manual variation of various rate constants was investigated. Attempts were made to systematise this variation by using a formal parameter estimation routine (a least-squares cost function being used together with calcium infusion data) that did not optimise the whole of the parameter vector. Instead a small number (say three) would be randomly chosen from the full set, and optimisation allowed to proceed using this portion of the vector. Hundreds of these runs were performed, the results of these giving a useful indication of model sensitivity to parametric variation. Further details of this procedure are given in chapter 6, where it was used more extensively.

Figure 5.14 shows the effect on a calcium infusion of incorporating an extra plasma fraction (plasma calcium = PIC + ECFC). It should be noted that this change has blunted the rise of the measured variable (in this case plasma calcium), note the PIC curve is unchanged. The response is still unsatisfactory, as the rate of return to the steady state now takes approximately 48 hours.

5.7.2.3 ECFCR Is A Function of PPT

It is already assumed that PTH affects the return of calcium to the plasma from BSFC. This flux is observed during an EDTA or PTH infusion, in the whole person or in a culture system (e.g. the chick calvaria data of Brommage, et al, 1978). If a similar effect occurred in the bulk of the body's cells, then a similar effect would be observed in the ECF as the BSF.

As this extra control option was considered the use of only this PTH control function was also considered for the sake of completeness. Although there is no direct evidence to support the existence of only this pathway for the PTH mediated return of calcium to plasma from bone or tissue.

Neither of these two options gave any improvement to the model response to simulated calcium or EDTA infusions.

5.7.2.4 ECFCR & ECFCA Involve Derivative Control

This option is an extension of the above combined with the suggestion that occurs in any general discussion of hormonal or metabolic regulation; that some pathways or mechanisms involve derivative control.

Derivative control yielded little improvement in model response, and instability was easily introduced. For example Figure 5.15 shows this as both the extra plasma calcium fraction of 5.7.2.2 and derivative control of ECFCR and ECFCA are introduced. This simulation should be compared with that in Figure 5.14.

5.7.3 MODEL11 Validity Conclusions

The model illustrates a number of interesting concepts, and performs extremely well when dealing with long-term variations in intake, and short-term variations in phosphate.

However MODEL11 is unable to reproduce adequately the patterns of response seen in experimental data concerning the short-term variation in plasma calcium. This is a significant shortcoming.

Since MODEL11 is theoretically unidentifiable due to the number of parameters involved, it is not appropriate to employ quantitative parameter estimation techniques. Validation can only proceed further through the use of progressive refinement and adaptive fitting using less rigorous or qualitative assessments of model performance.

If we consider that MODEL11 attempts to encompass too many components and hence exhibits structural uncertainty, it ought to be possible to perform a detailed model structure sensitivity analysis to arrive at a set of sub-models, each of which represents that part of the whole model or the 'minimal model' necessary to cope with a particular situation. This approach to model development has been described by Young (1978, and 1982) in connection with hydrodynamic models. Each of the sub-models can then be dealt with separately and may in fact be amenable to quantitative identification and validity assessment techniques. The differences in (say) two sub-models can then be examined. This approach was adopted for the short-term situation only, and is described in the next section, leading to the development of MODEL12.

5.8 The Formulation of MODEL12 by Model Reduction

The performance of MODEL11 is not satisfactory. This deficiency has been shown to be structural and not solely parametric. Thus before considering the incorporation of additional or omitted features, a process of model reduction was performed that involved progressive removal of components of the model structure, and a before/after removal comparison of the model response to two precisely defined experimental situations.

5.8.1 The Reduction Procedure

The use of an iterative model reduction procedure of this form has been described by Young (1978 and 1982) in connection with hydrodynamic models. Throughout this study the procedure was found to be extremely useful as an initial qualitative model test, especially as an interactive model simulation system complete with online graphics output was used (see Appendix II).

The reduction process can be considered to be a form of 'structural sensitivity analysis' that will enable the identification of the minimal plausible model structure that is compatible with the modelling objectives. The reduction procedure results in the unidentifiable model being rendered more tractable. The two experiments used for the analysis are the intravenous infusion of calcium and EDTA respectively; one yielding a hyper- the other a hypo-calcaemic stimulus.

The approach adopted in model formulation had essentially consisted of incorporating those controller functions and state variables that plausibly affect calcium metabolism. The overriding concern now was to arrive at that set of model variables and functions whose presence was necessary to describe the behaviour of PIC during calcium infusion, and only that set, a so called 'minimal model'. If this set

is small enough the model could be theoretically identifiable and quantitative parameter estimation techniques would assume a greater significance.

It is important to note that the rejection criteria used in each case can be viewed as "the lack of any large relative sensitivity coefficients in all the parameters concerned with a particular state variable"

This is perhaps an understatement of the rejection process as one of far more power is in fact used because it is the shape of the output response (in this case PIC) that is examined not its magnitude at a certain point, or the absolute difference between two measurement times, which can be greatly affected by the steady state differences produced when parameters are varied in order to calculate relative sensitivity coefficients. This is considered further in chapter 6 and a simulation illustrates this in Figure 6.14.

5.8.2 Model Reduction Results

The justification for including each compartment in the model was investigated, starting with all the phosphate compartments and Fixed Bone Calcium (FBC). Thus Figure 5.21 shows a comparison between the full model (MODEL11) and a reduced model (FBC, BSFP, BSP, and FBP removed). The PIC responses are essentially identical, and as it is primarily the shape of the response that is unsatisfactory these variables can be dispensed with.

Similarly Figure 5.22 involves the further removal of BSC and results in little change to the PIC response. Figure 5.23 shows the PIC response to complete phosphate removal, and as a further check, an EDTA simulation using the reduced model (Figure 5.24) compared with the full model was performed. This justifies the removal of the phosphate compartments when considering the short-term response to hypo- or hyper-calcaemic inputs.

5.8.3 Model Reduction Conclusions

Despite the physiologically reasonable structure of MODEL11, it failed to adequately simulate the short-term dynamic behaviour of plasma ionised calcium (PIC). Furthermore repeated model reduction has shown that many of the model compartments are superfluous to the simulation of these short-term (less than one or two days) dynamics of plasma calcium.

Hence the reduced version of MODEL11, MODEL12, incorporates only five of the original eleven compartments, and phosphate is not represented at all. The only compartments included are as follows:

PIC Plasma Ionised Calcium
ECFC Extracellular Fluid Calcium
BSFC Bone Surface Fluid Calcium
BSC Bone Surface Calcium
PPT Plasma Parathyroid Hormone

The compartments rejected from MODEL11 are as follows:

FBC Fixed Bone Calcium
PIP Plasma Ionised Phosphate
ECFP Extracellular Fluid Phosphate
BSFP Bone Surface Fluid Phosphate
BSP Bone Surface Phosphate
FBP Fixed Bone Phosphate

MODEL12 does still suffer from shortcomings in relation to the adequate simulation of infusion data. These shortcomings are not just a lack of fit but a failure to mimic the correct shape of the response. As discussed previously this tends to indicate a structural deficiency, and not simple parametric errors in the model.

A full definition of MODEL12 is given in Appendix I, and further details of structure are presented in section 6.1.

5.9 Summary

Two models; MODEL11 and MODEL12 have been developed, specifically to cope with the short-term behaviour of calcium, in response to the shortcomings of MODEL5. A degree of physiological isomorphism was deliberately incorporated into the two models, such that subsequent model identification and validation would not be hampered by the lack of this isomorphism

Validation and performance assessment of the first model, MODEL11, indicated that some aspects were satisfactory, including the simulation of long-term variation of oral intake, but the model performance over the short-term was lacking in a number of important respects.

A second model, MODEL12, was developed by progressively removing compartments from MODEL11, that were judged to be superfluous to the simulation of short-term behaviour. However despite the removal of many compartments found to be unnecessary to the simulation of short- term experiments, the response of MODEL12 to hypo- and hyper-calcaemic influences is still significantly lacking.

The next chapter extends this development through the further identification and validation of MODEL12, with particular regard to the short-term situation.

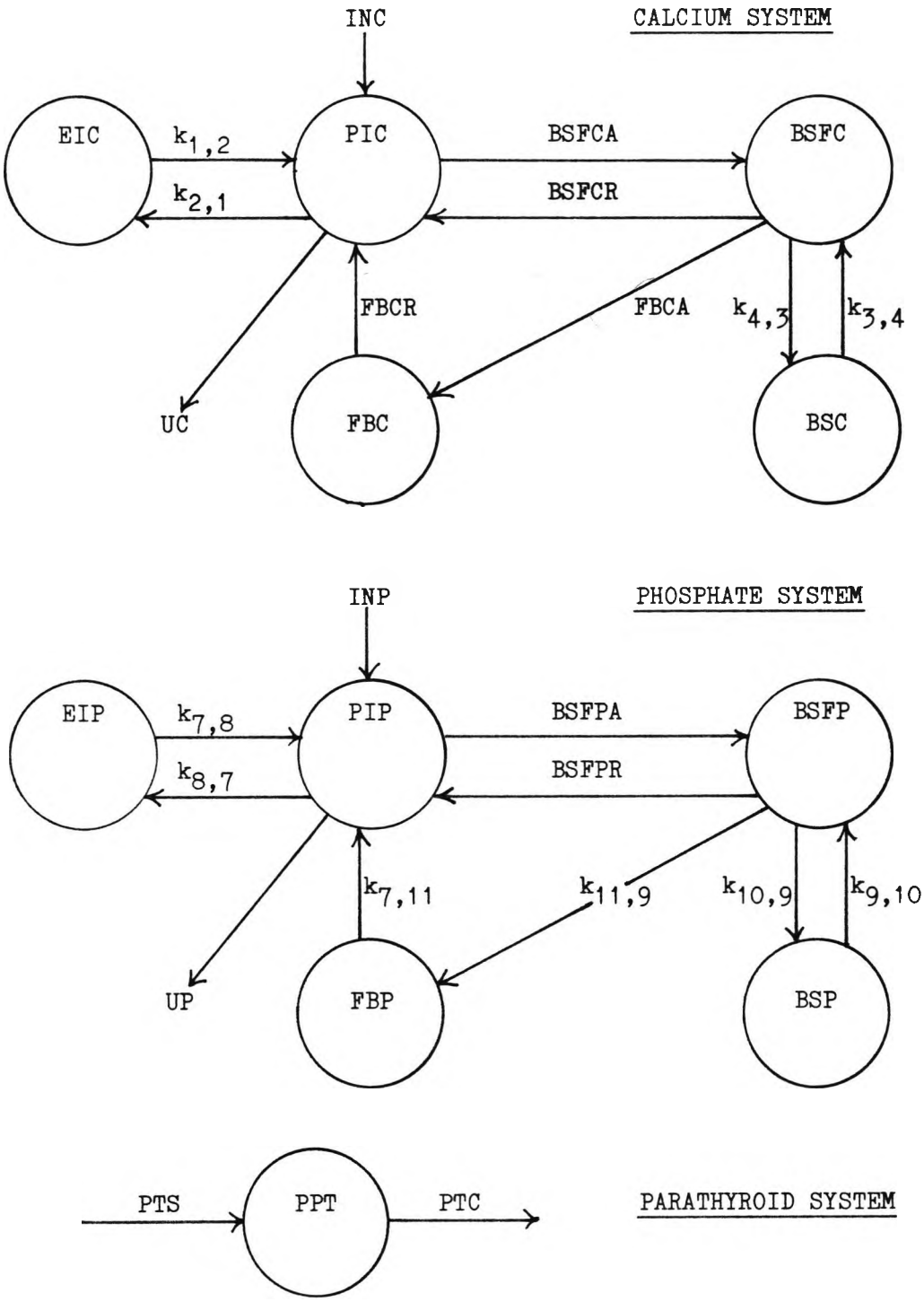


Figure 5.1 Complete MODEL11 schematic. The equations and steady state values are shown in Appendix I and at relevant points in the text. Full details of the nomenclature are also given in Appendix I.
Each box represents a defined body compartment or 'pool'.
$k_{i,j}$ represents a transport rate constant, such that the flow of material from compartment i of mass M_i to compartment j is given by $k_{j,i}M_i$.

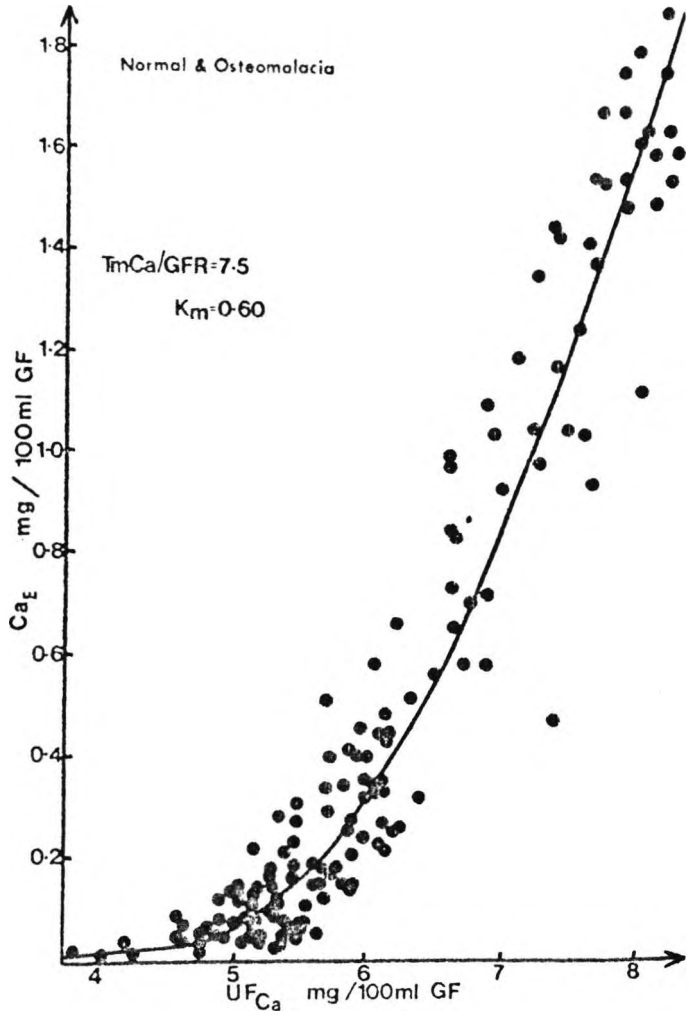


Figure 5.2

The relationship between UFC and UC during infusions of calcium in seven cases of Osteomalacia and nine normal subjects. The fitted line represents:

$$UFC - UC = TMC - KM \text{ Log}(UFC/UC) = TCR$$

Data from Marshall (1976)

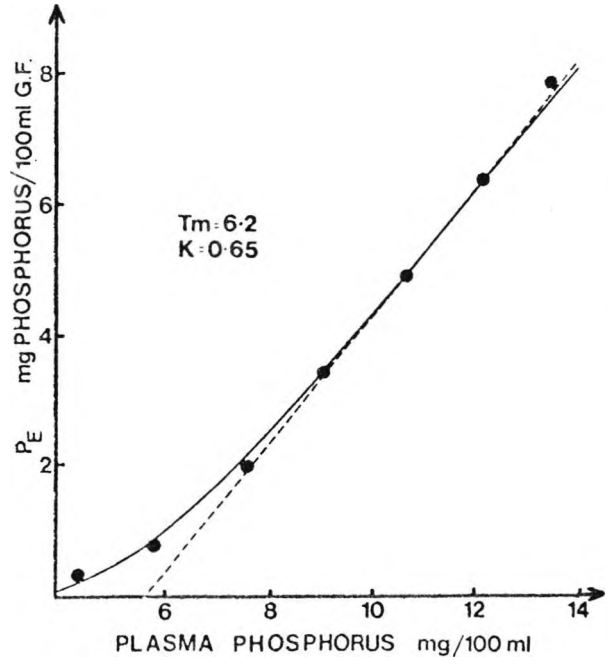


Figure 5.3

The relationship between UFP and UP. The fitted line represents:

$$UFP - UP = TMP - KM \text{ Log}(UFP/UP) = TPR$$

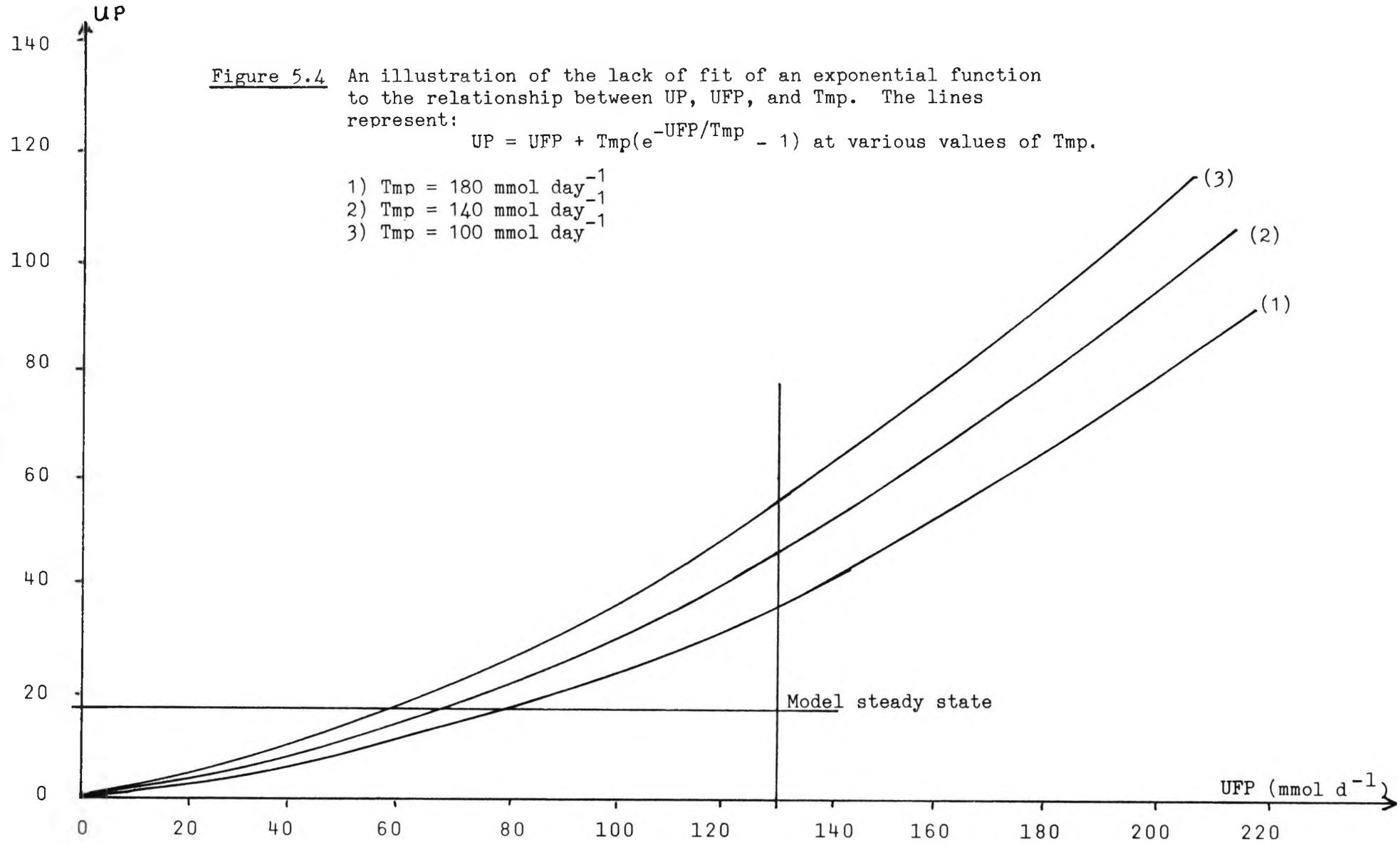


Figure 5.4 An illustration of the lack of fit of an exponential function to the relationship between UP, UFP, and Tmp. The lines represent:

$$UP = UFP + Tmp(e^{-UFP/Tmp} - 1)$$ at various values of Tmp.

1) Tmp = 180 mmol day^{-1}
2) Tmp = 140 mmol day^{-1}
3) Tmp = 100 mmol day^{-1}

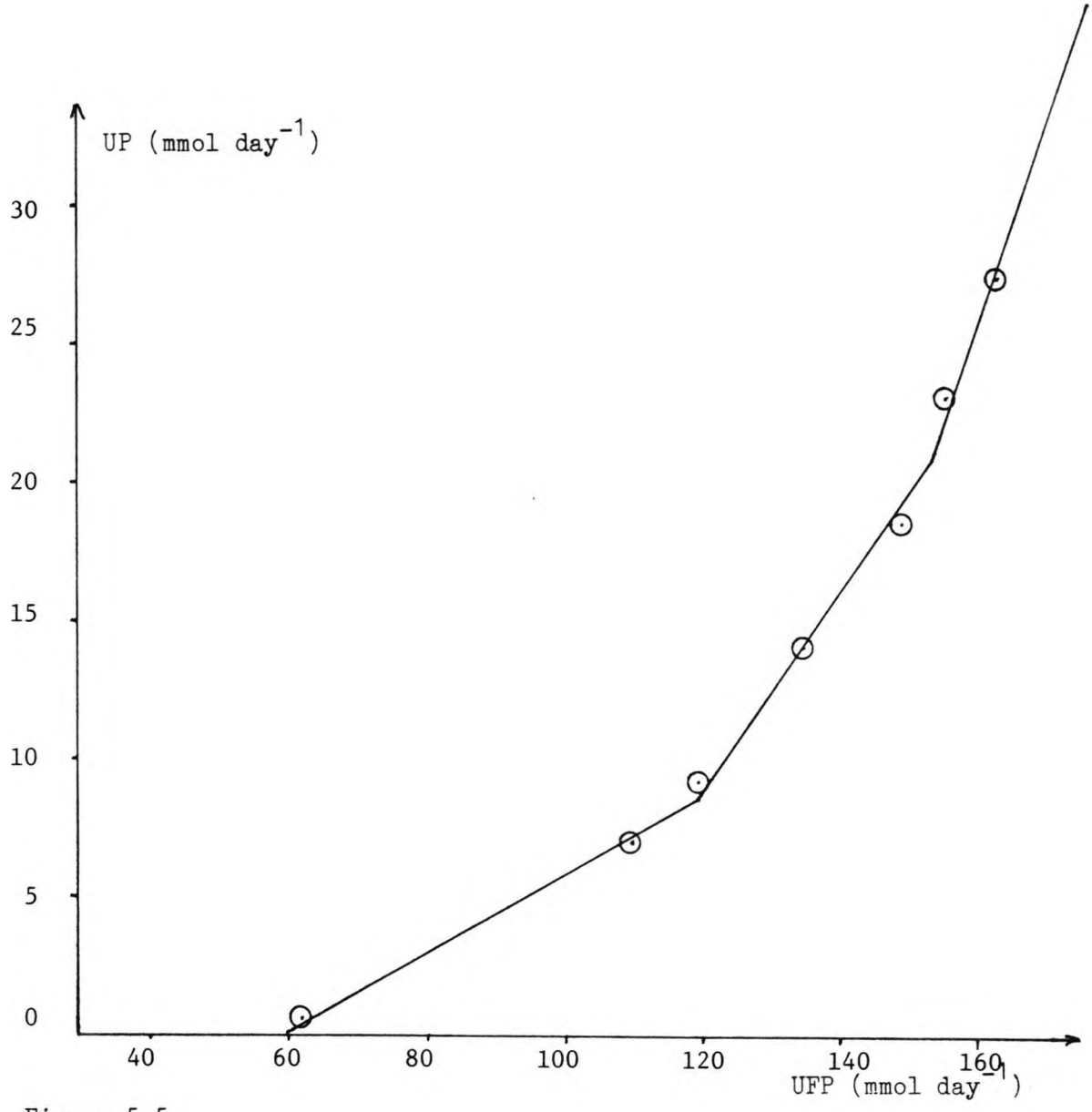


Figure 5.5

The fit of three straight lines to the variation in UP with UFP at a TMP of 140 mmol day^{-1}. The data shown are from Bijvoet (1975).

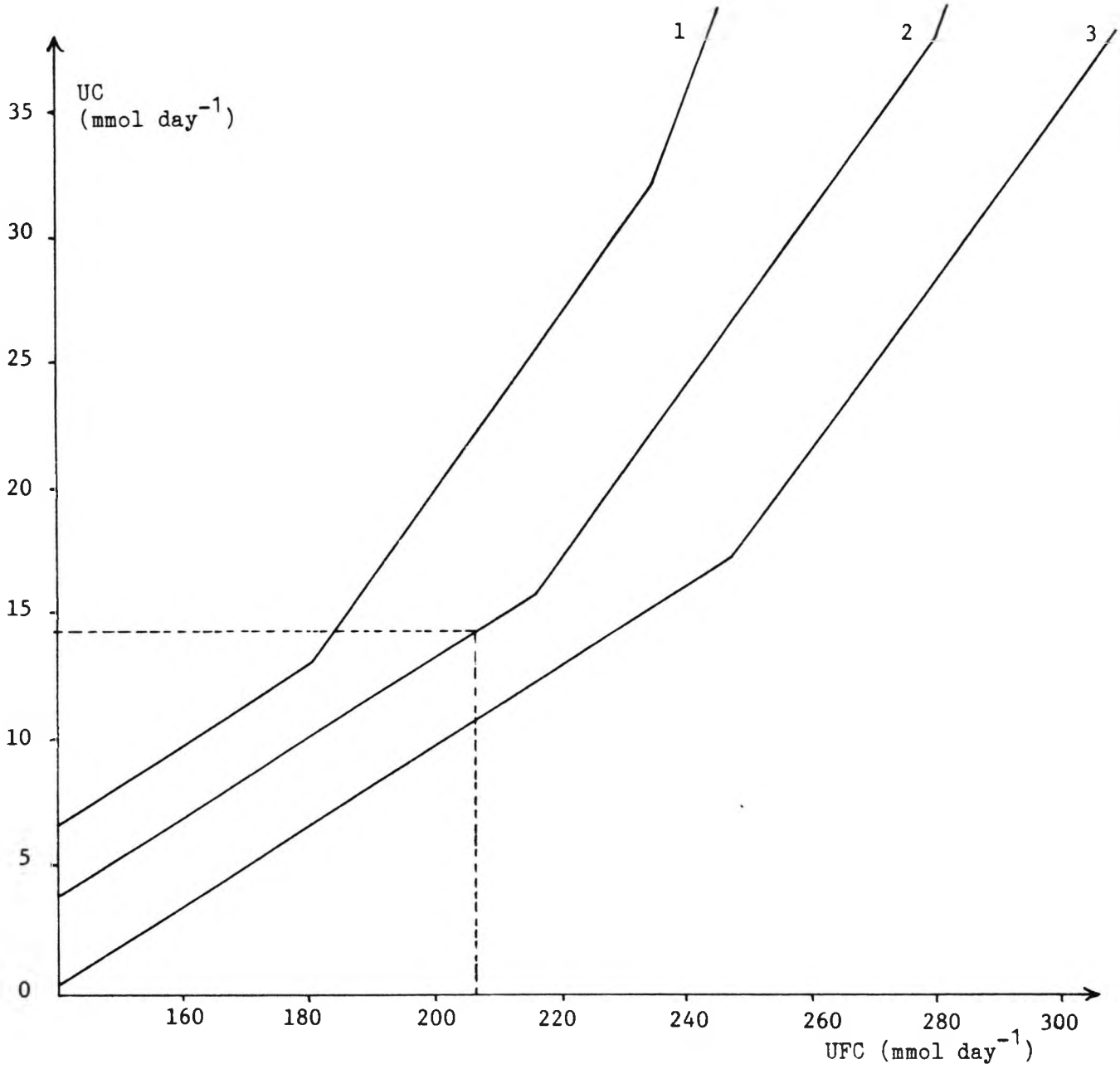


Figure 5.6

The variation in urine calcuim (UC) with TMC and UFC as used in MODEL11.

1) : TMC = 210 mmol day^{-1}
2) : TMC = 250 " "
3) : TMC = 290 " "

The dotted lines represent typical steady state values.

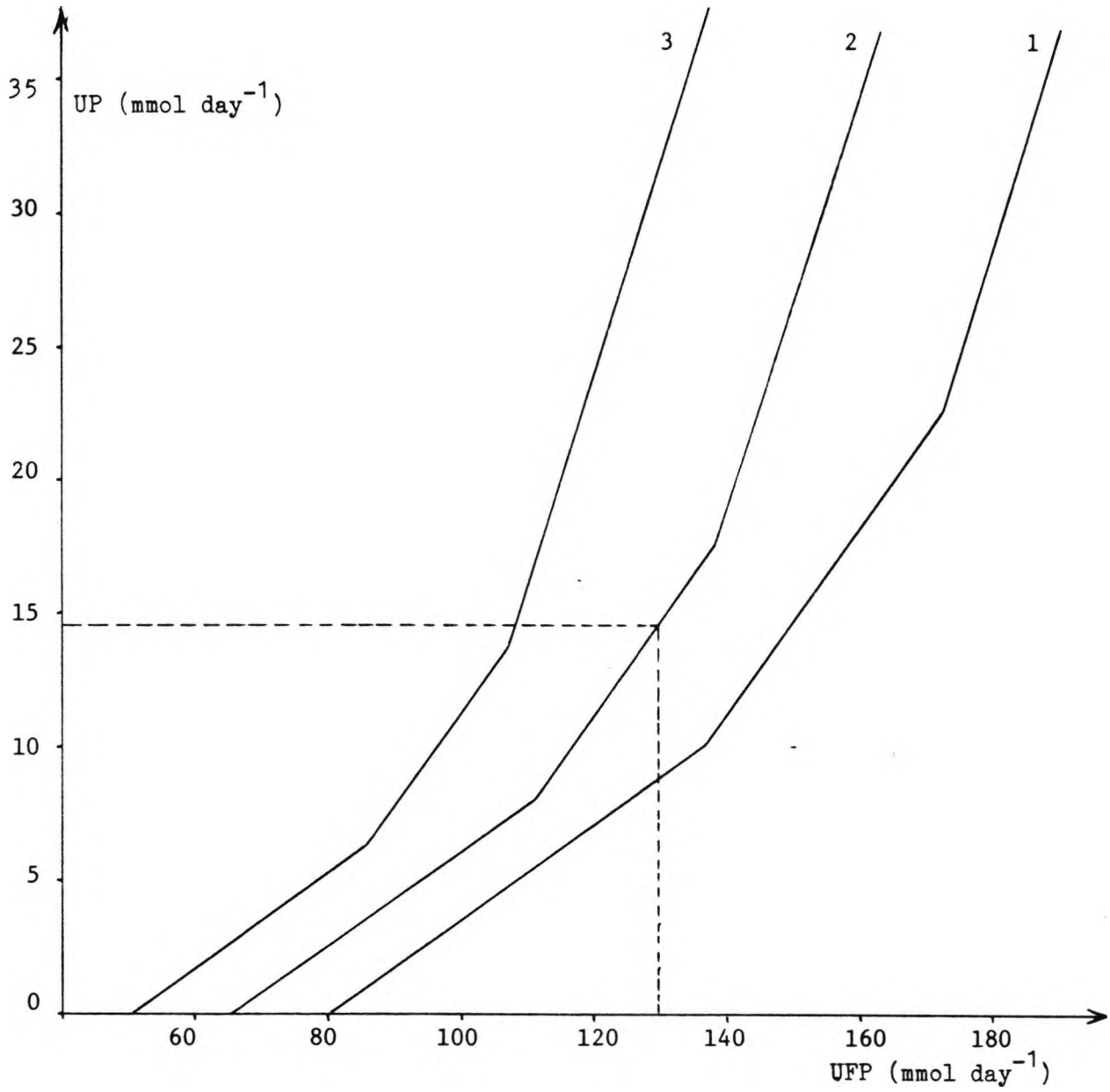


Figure 5.7

The variation in urine phosphate (UP) with TMP and UFP as used in MODEL11.

1) : TMP = 160 mmol day^{-1}
2) : TMP = 130 " "
3) : TMP = 100 " "

The dotted lines represent typical steady state values.

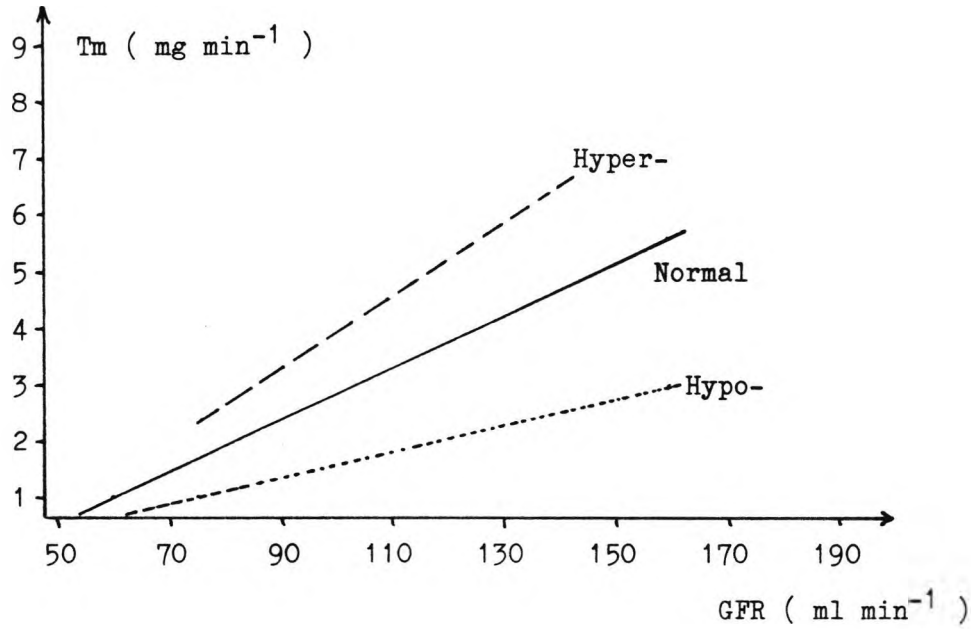


Figure 5.8 The relationship between TMP and GFR in normal, hypo-, and hyper-parathyroid subjects. The data are from Bijvoet (1975), and n = 63, 23, and 14 respectively. The actual data points are not shown for the sake of clarity, the lines representing the best fit to the data for each subject class.

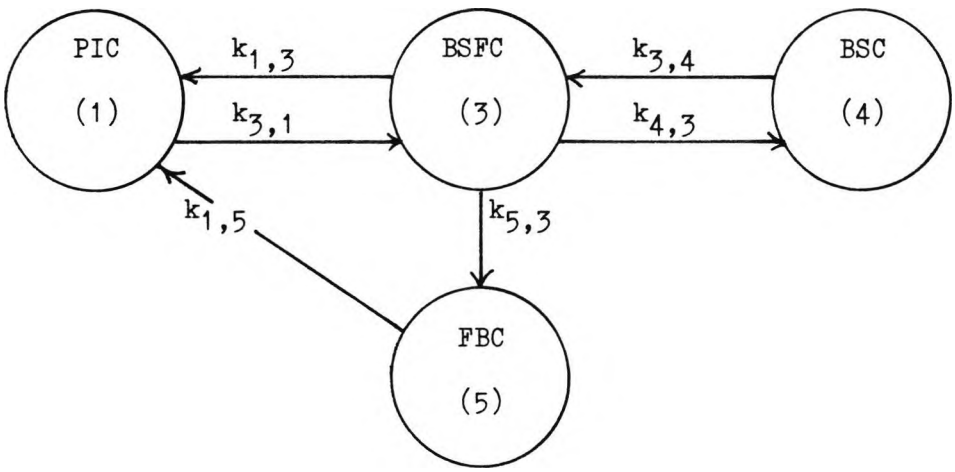


Figure 5.9 Bone and plasma calcium compartments from MODEL11. Hormonal influences are not shown.

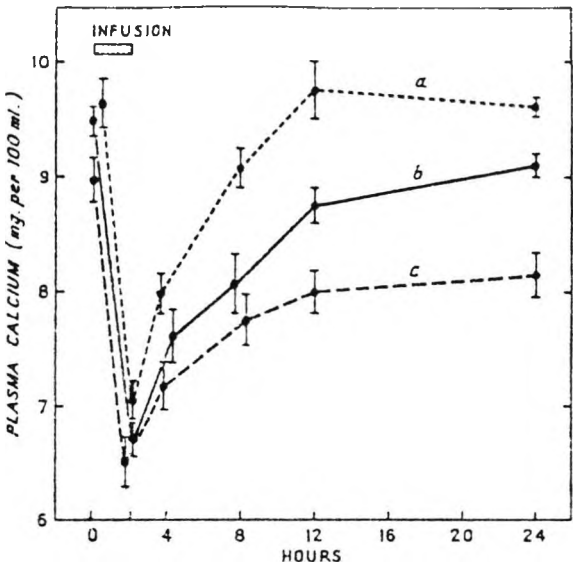


Figure 5.10 The effect on plasma calcium of an edetic acid infusion (70 mg per kg body weight in 0.5 l 5% dextrose with 20 ml of 2% procaine) over 2 hours. Mean values and standard errors are shown. From Jones and Fourman (1963).
(a) = 12 normal subjects.
(b) = 11 patients with a normal phytate test following thyroidectomy.
(c) = 7 patients with an abnormal phytate test following thyroidectomy.

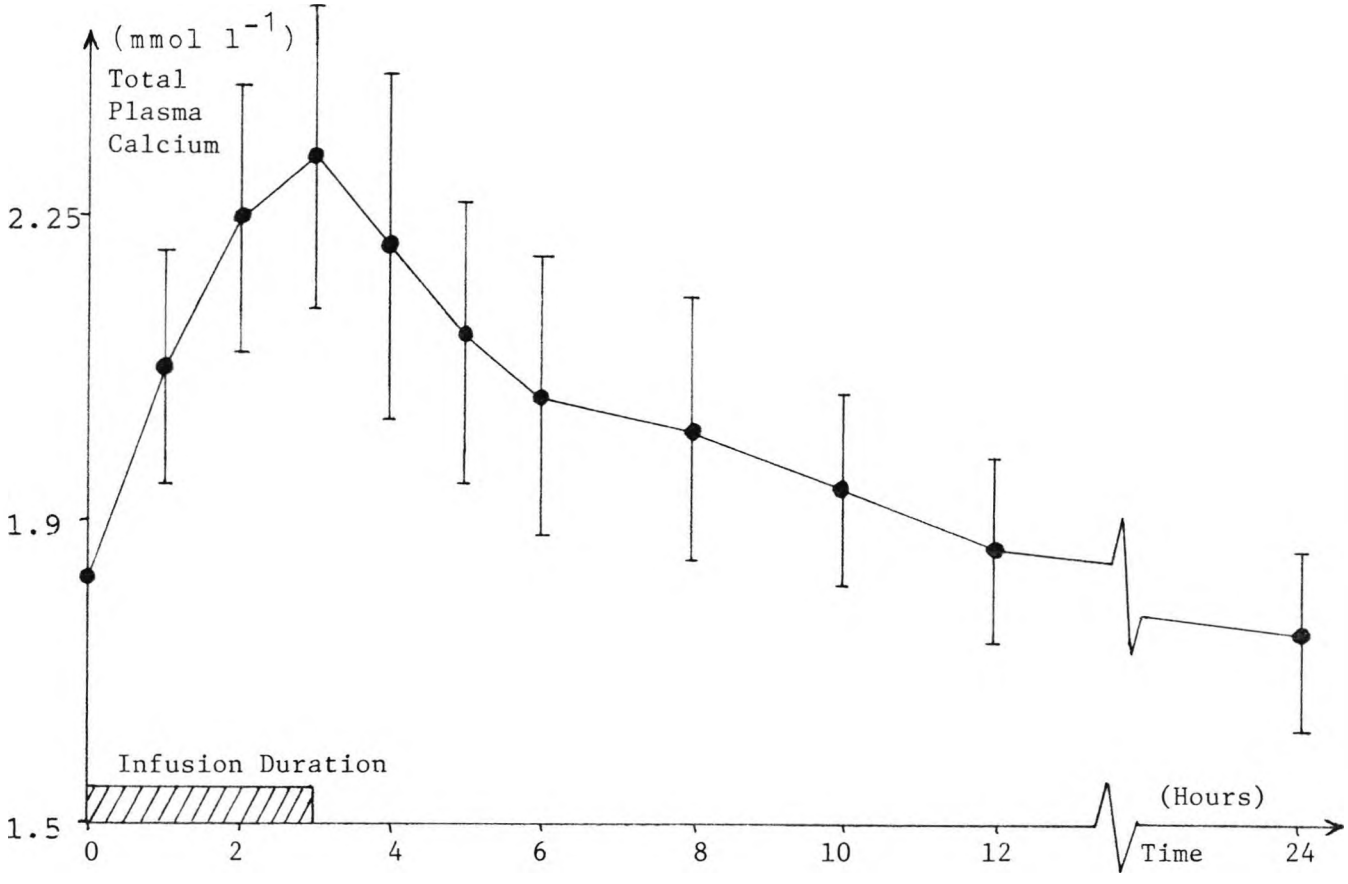


Figure 5.11 The changes in total plasma calcium as a result of an infusion of 0.3 mmol/kg body weight of calcium in 9 normal subjects. Data from Ibbertson, Roche, and Pybus (1966).

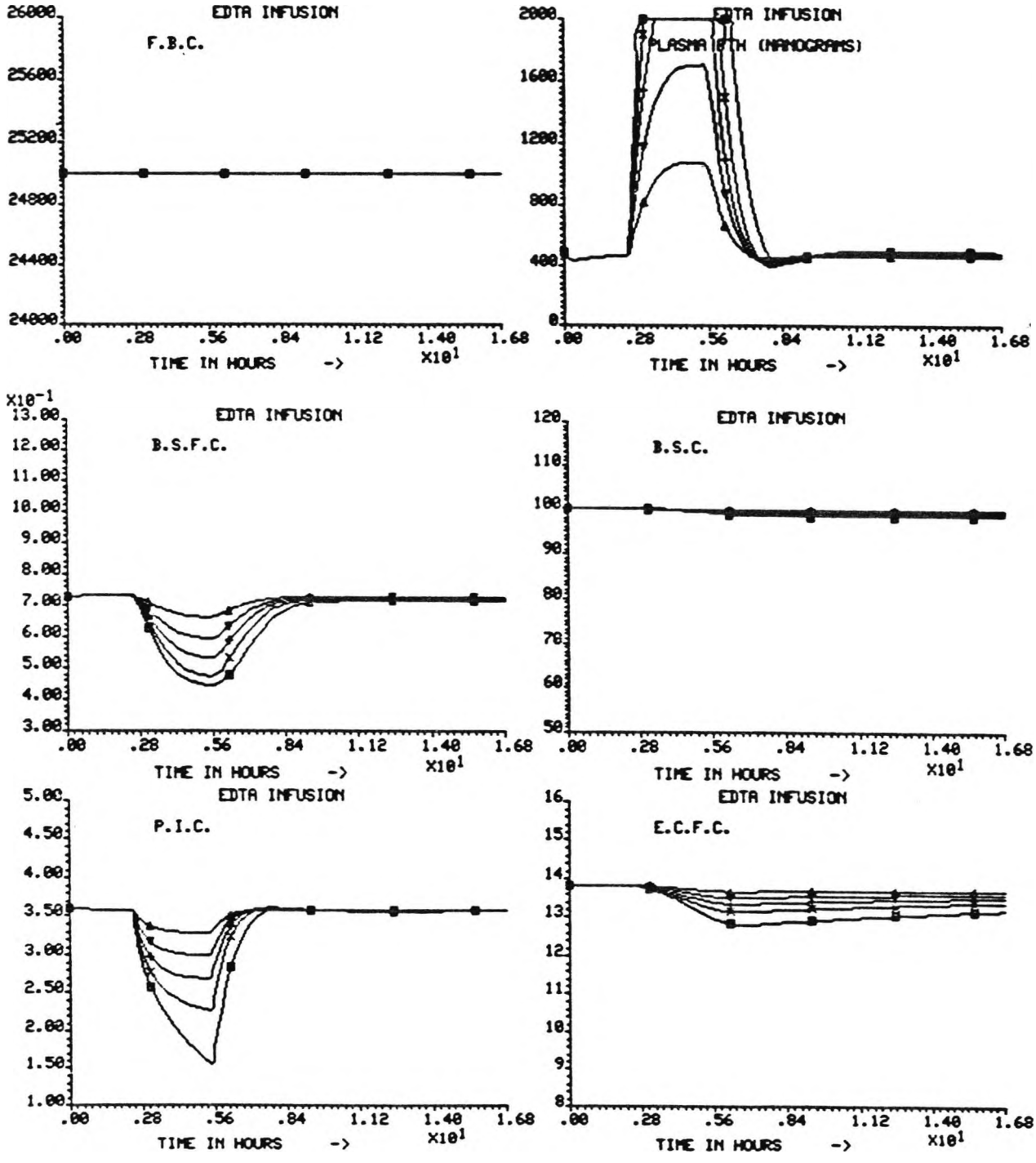
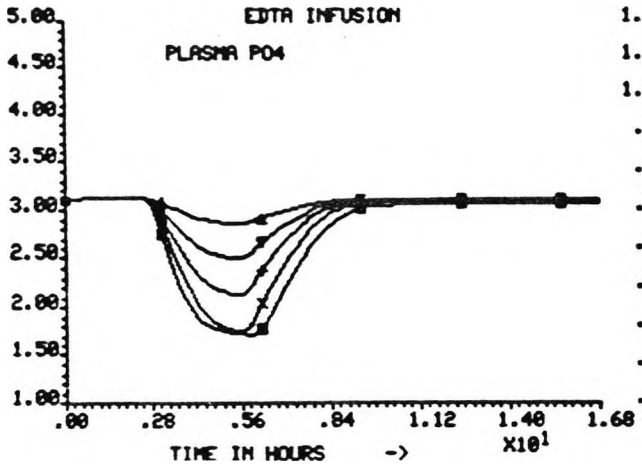
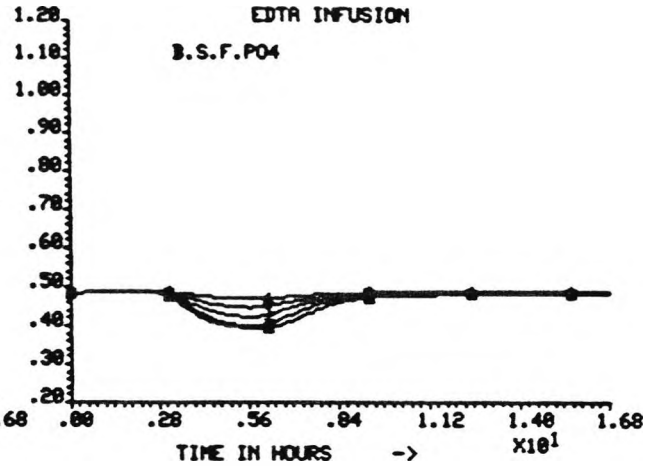
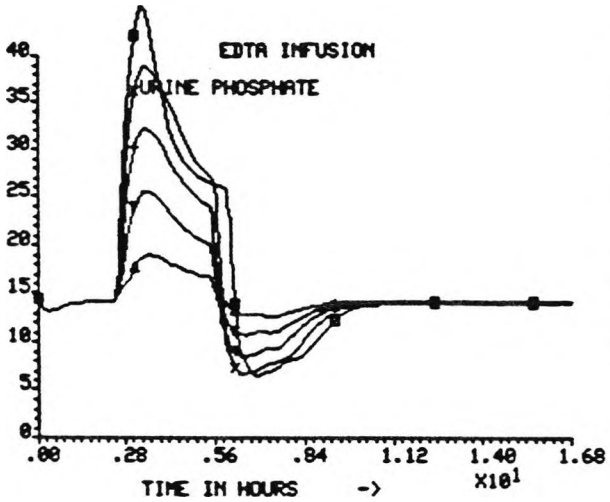


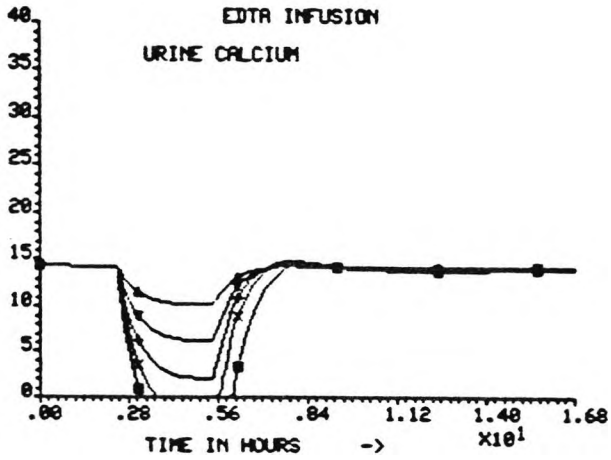
Figure 5.12 MODEL11 : EDTA Infusions of varying amounts.
- 1.25, - 2.5, - 3.75, - 5.0, - 6. 25 mmol calcium equivalant over 3 hours (2.4 to 5.4 hours on time axis).

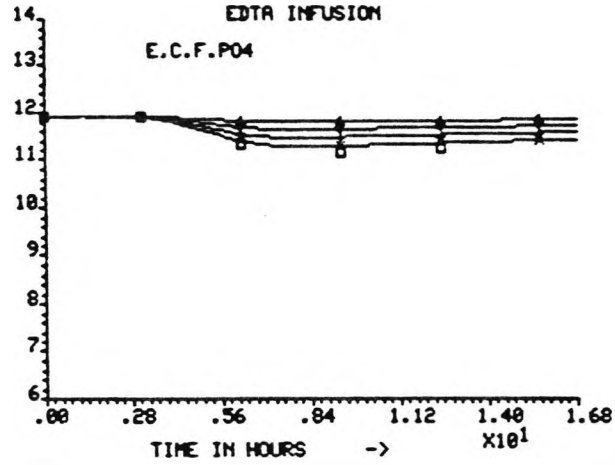
cont....

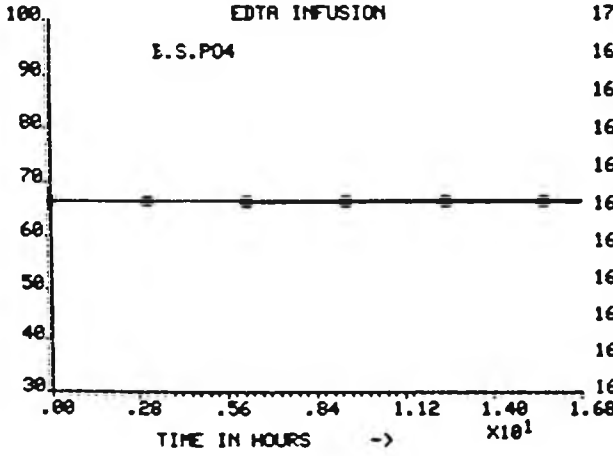

EDTA INFUSION
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹

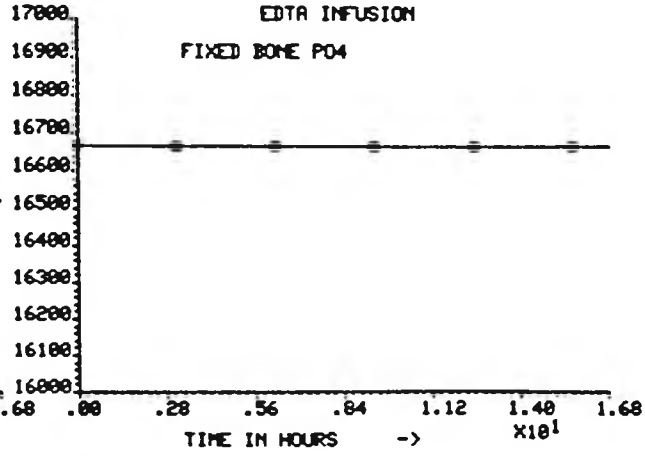

EDTA INFUSION
B.S.F.PO4
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


EDTA INFUSION
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


EDTA INFUSION
URINE CALCIUM
40
35
30
25
20
15
10
5
0
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


EDTA INFUSION
E.C.F.PO4
14
13
12
11
10
9
8
7
6
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


EDTA INFUSION
E.S.PO4
100
90
80
70
60
50
40
30
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


EDTA INFUSION
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹

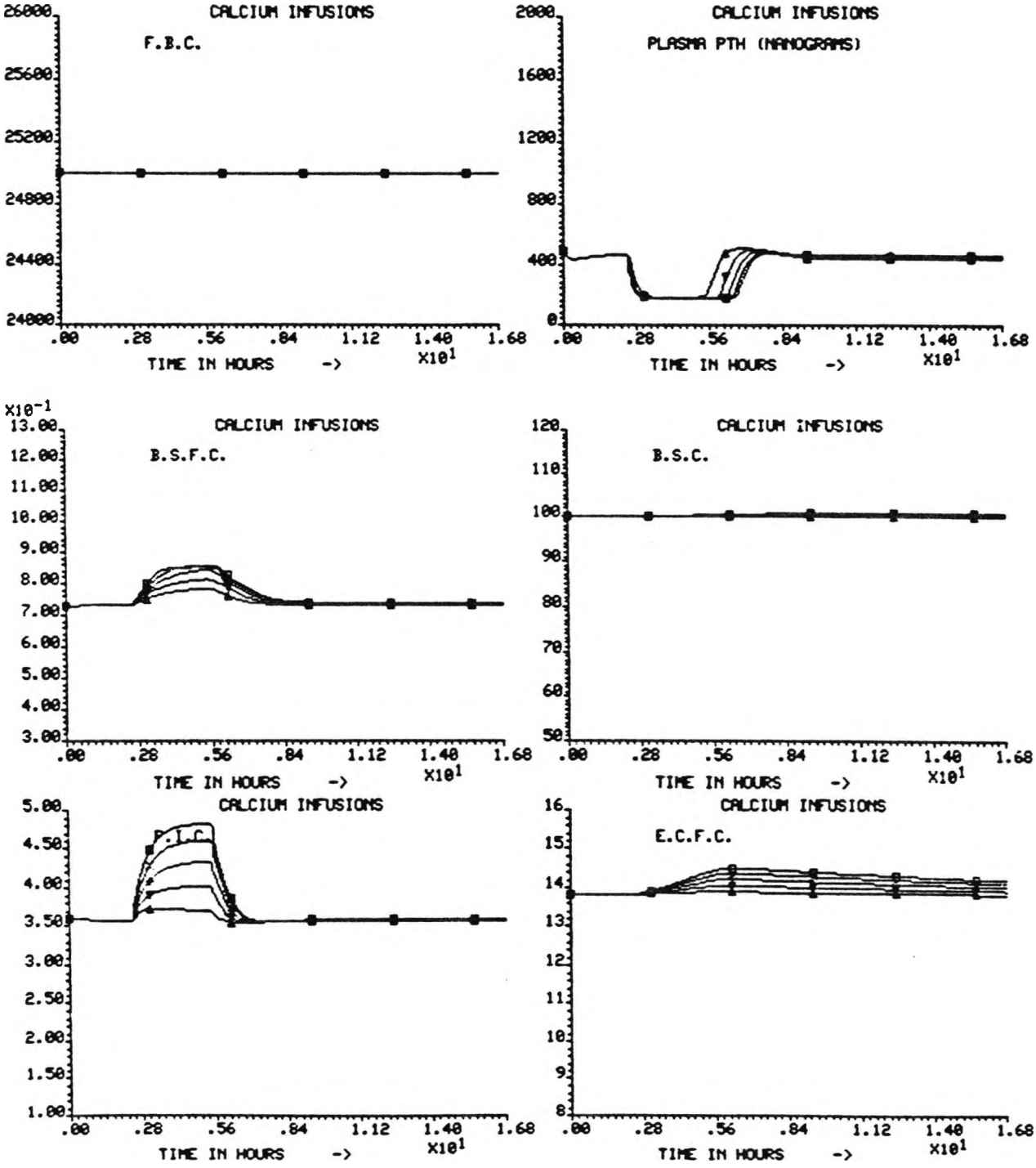
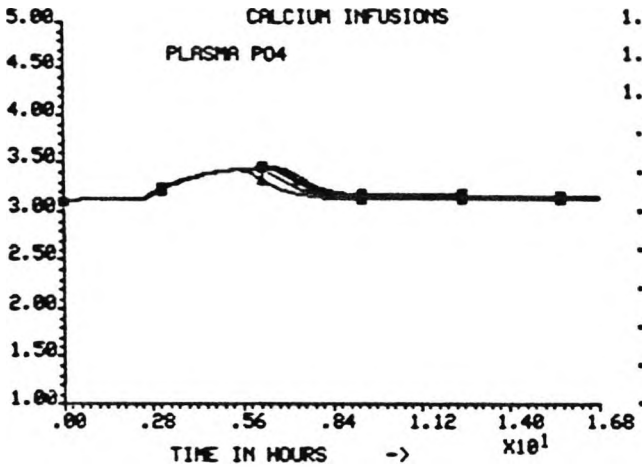
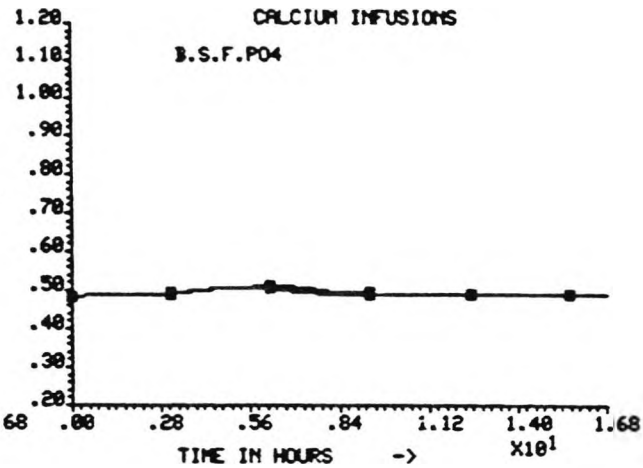
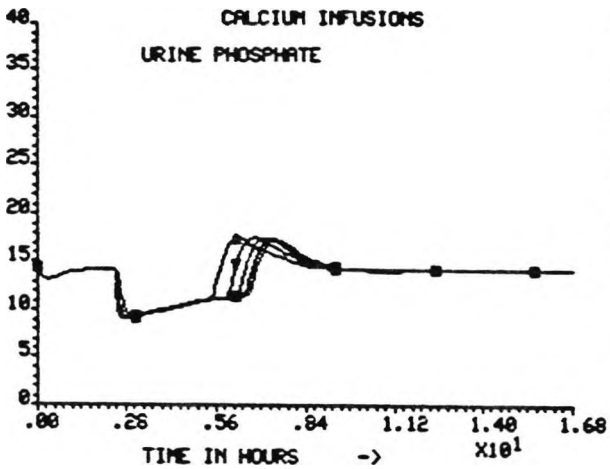


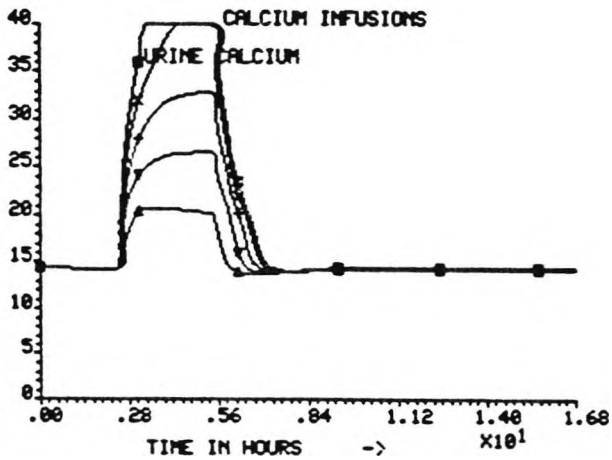
Figure 5.13 MODEL11 : Calcium infusions of varying amounts.
1.25, 2.5, 3.75, 5.0, 6. 25 m mol over 3 hours.
(2.4 to 5.4 hours on time axis).

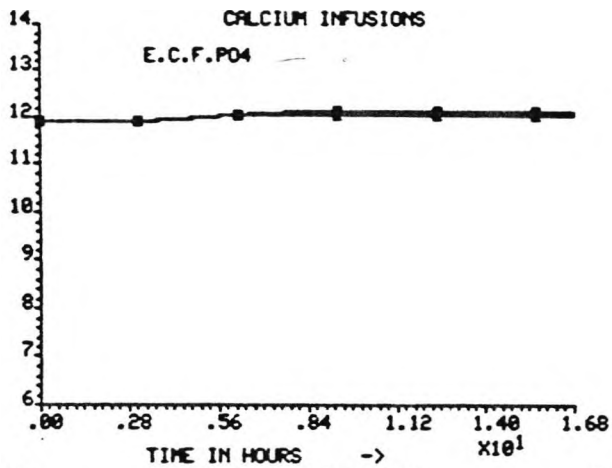
cont....

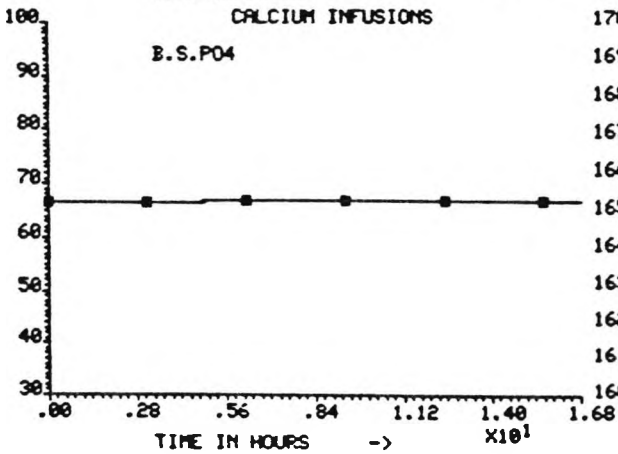

CALCIUM INFUSIONS
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹

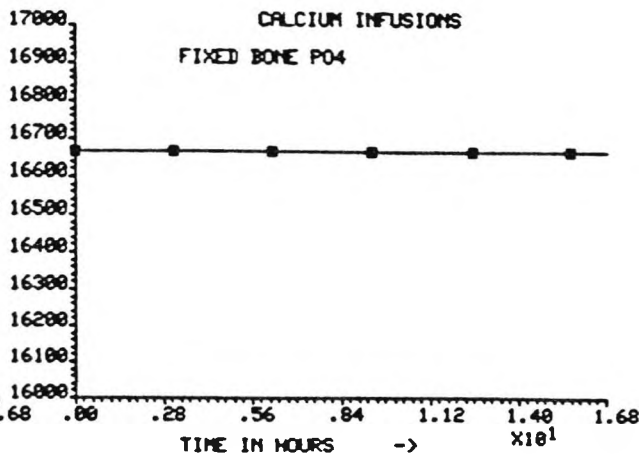

CALCIUM INFUSIONS
B.S.F.PO4
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


CALCIUM INFUSIONS
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


CALCIUM INFUSIONS
URINE CALCIUM
40
35
30
25
20
15
10
5
0
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


CALCIUM INFUSIONS
E.C.F.PO4
14
13
12
11
10
9
8
7
6
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


CALCIUM INFUSIONS
B.S.PO4
100
90
80
70
60
50
40
30
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹


CALCIUM INFUSIONS
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
.00 .28 .56 .84 1.12 1.40 1.68
TIME IN HOURS ->
X10¹

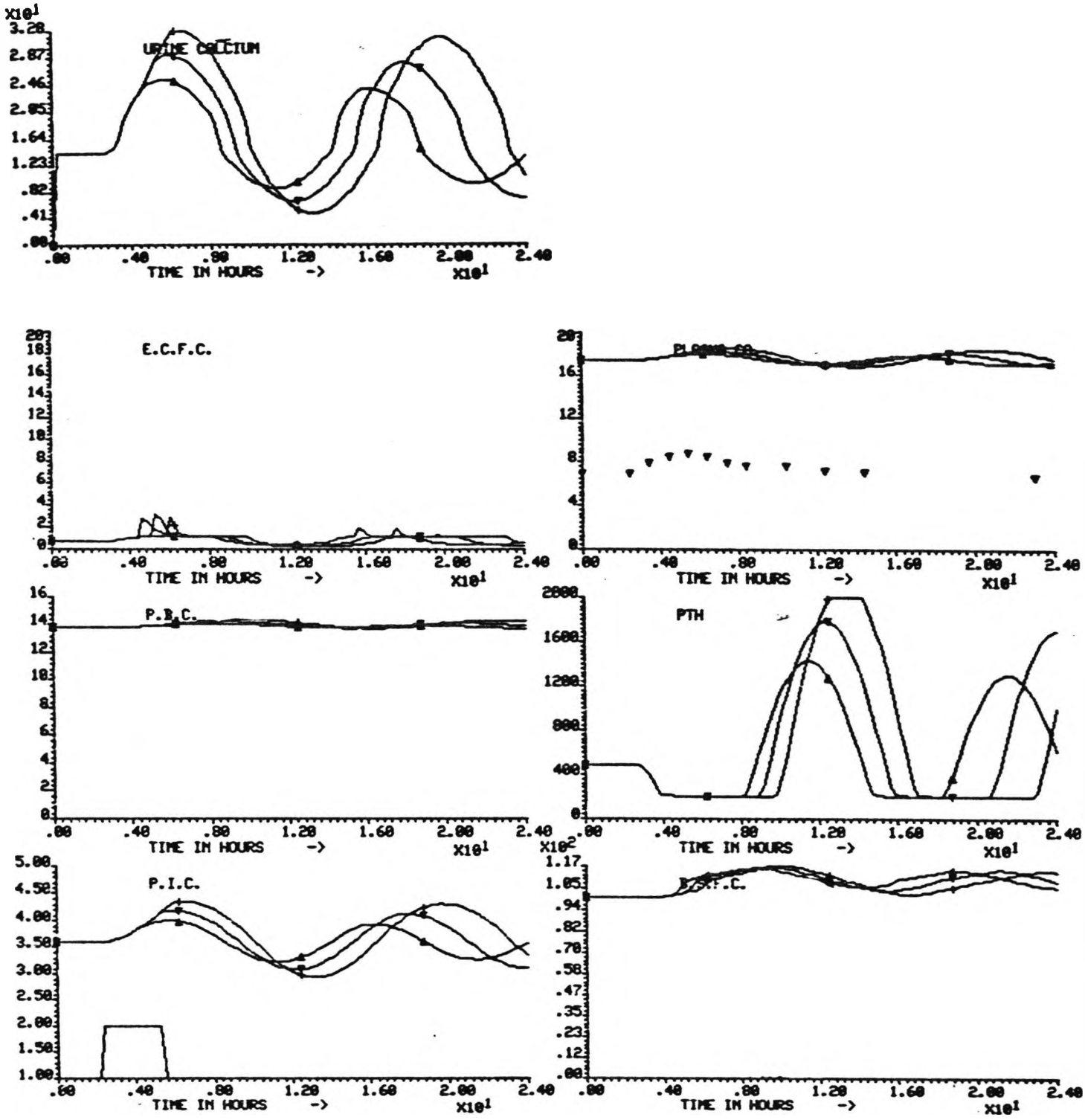


Figure 5.14 MODEL11 - A simulated calcium infusion

Model is altered such that an extra plasma calcium fraction is incorporated (Plasma Calcium = PBC + PIC), and derivitive control of $k_{1,3}$ and $k_{3,1}$ (BSFCA and BSFCR). This model is inherently unstable.

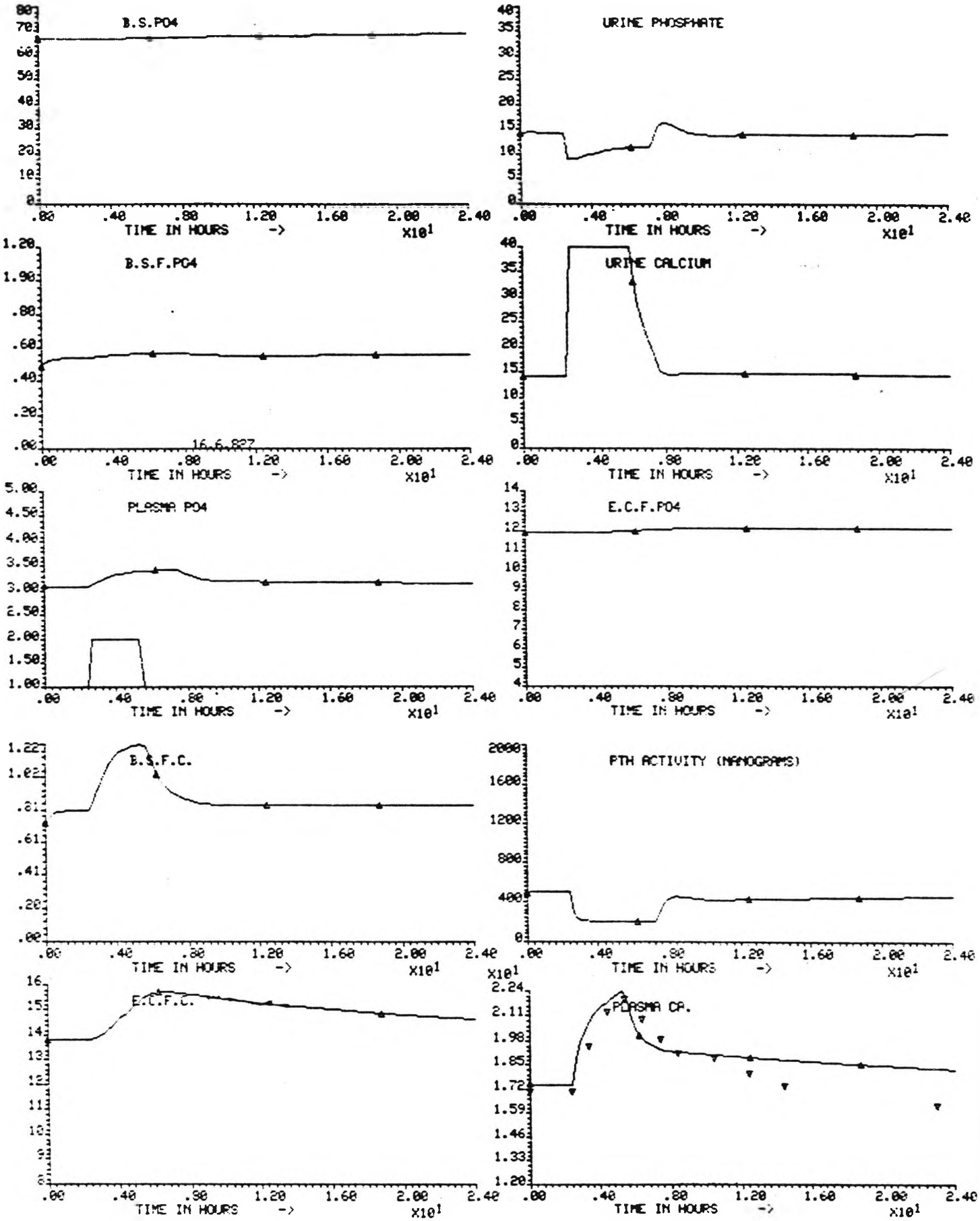


Figure 5.15 MODEL11 - A simulated calcium infusion
An extra plasma fraction is incorporated such that Plasma Calcium = PBC + PIC. Note the rate of return of Plasma Calcium to the steady state compared to the data (the triangles).

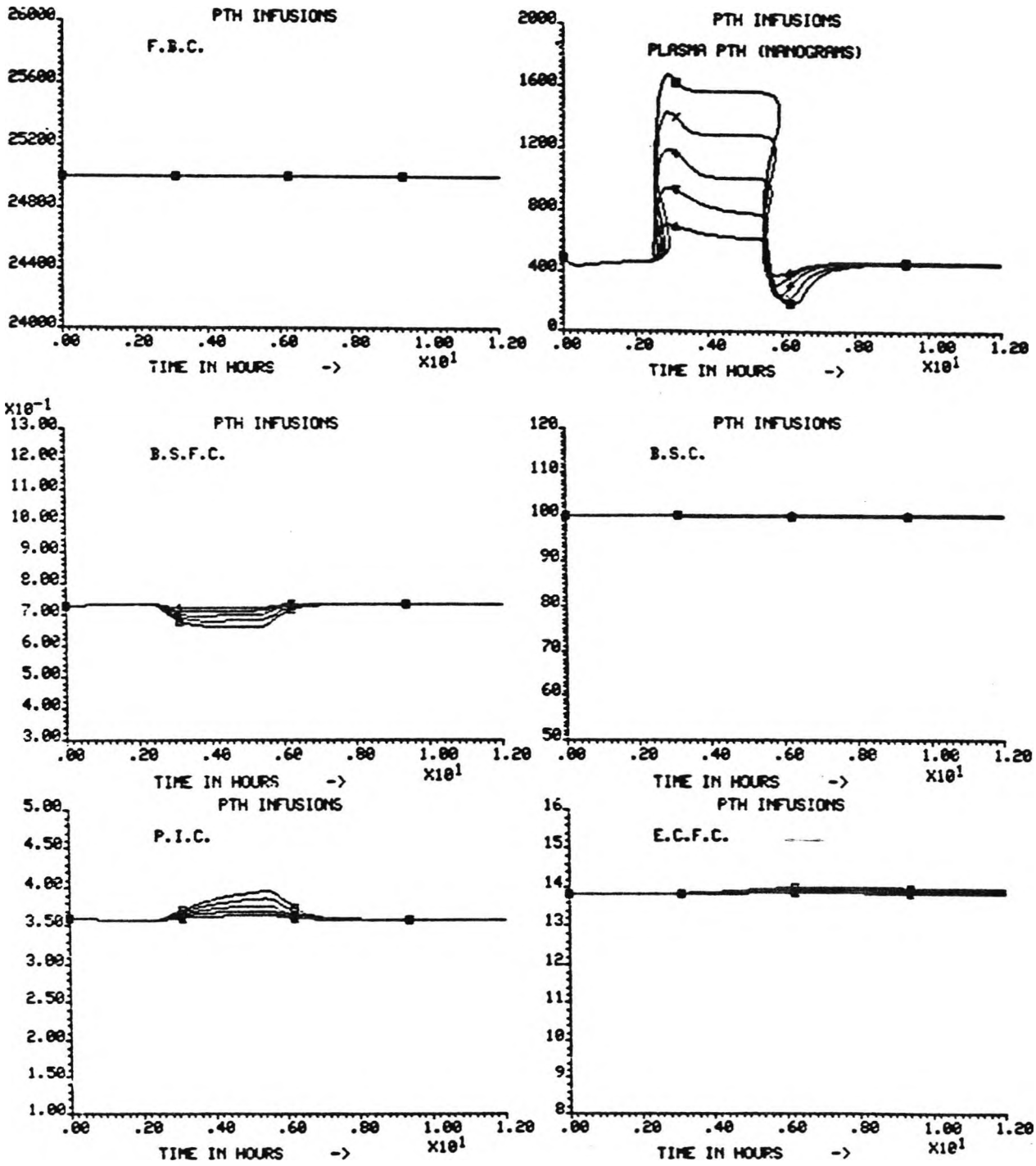
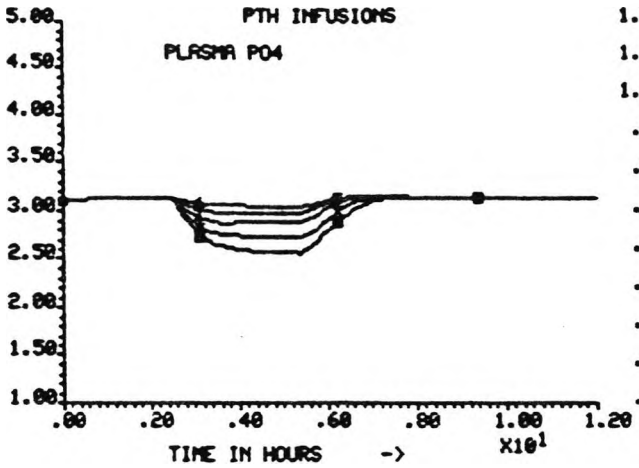
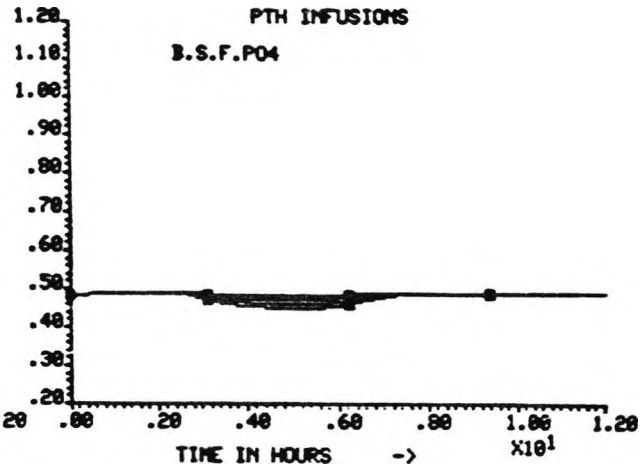
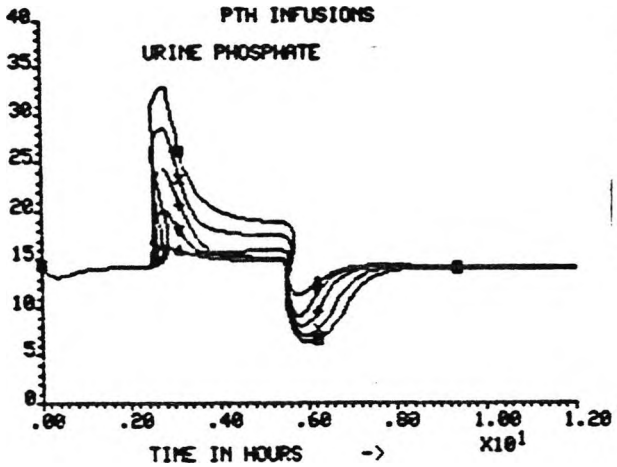


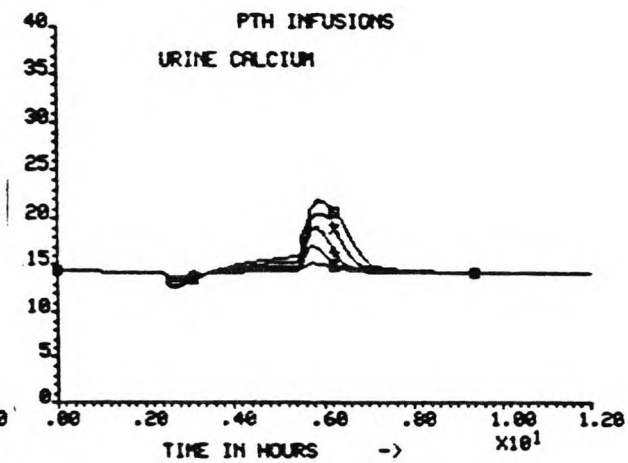
Figure 5.16 MODEL11 : PTH Infusions of varying magnitude.
5, 10, 15, 20, 25 micro-grams over 3 hours.
(between 2.4 and 5.4 hours on time axis)

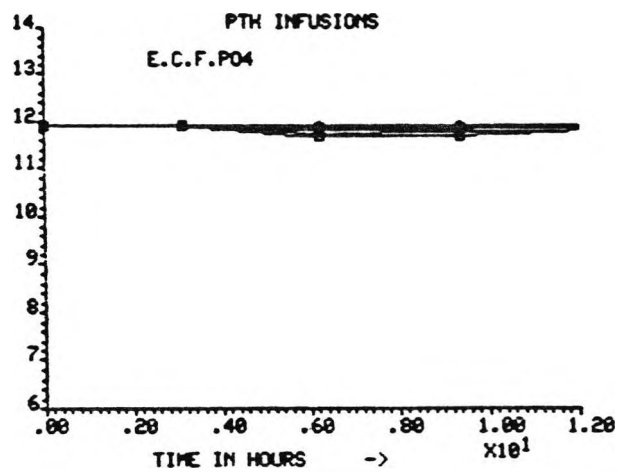
cont....

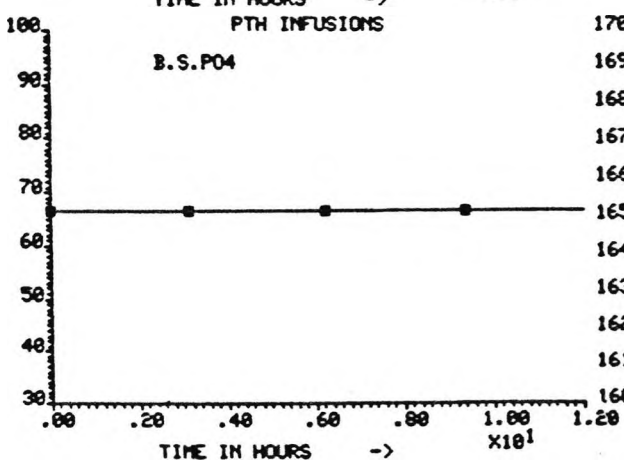

PTH INFUSIONS
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹

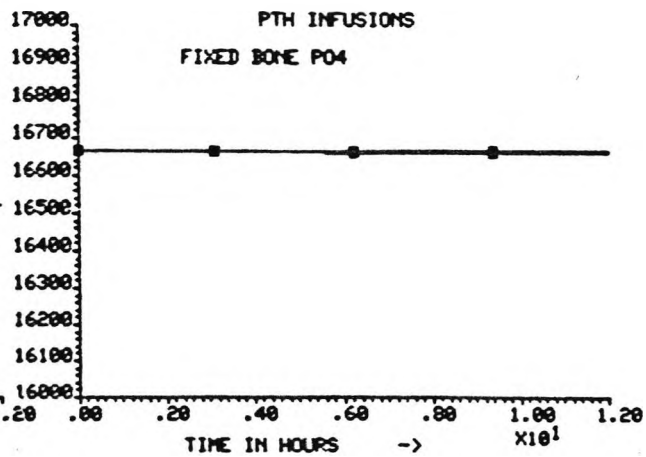

PTH INFUSIONS
B.S.F.PO4
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PTH INFUSIONS
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PTH INFUSIONS
URINE CALCIUM
40
35
30
25
20
15
10
5
0
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PTH INFUSIONS
E.C.F.PO4
14
13
12
11
10
9
8
7
6
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PTH INFUSIONS
B.S.PO4
100
90
80
70
60
50
40
30
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PTH INFUSIONS
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹

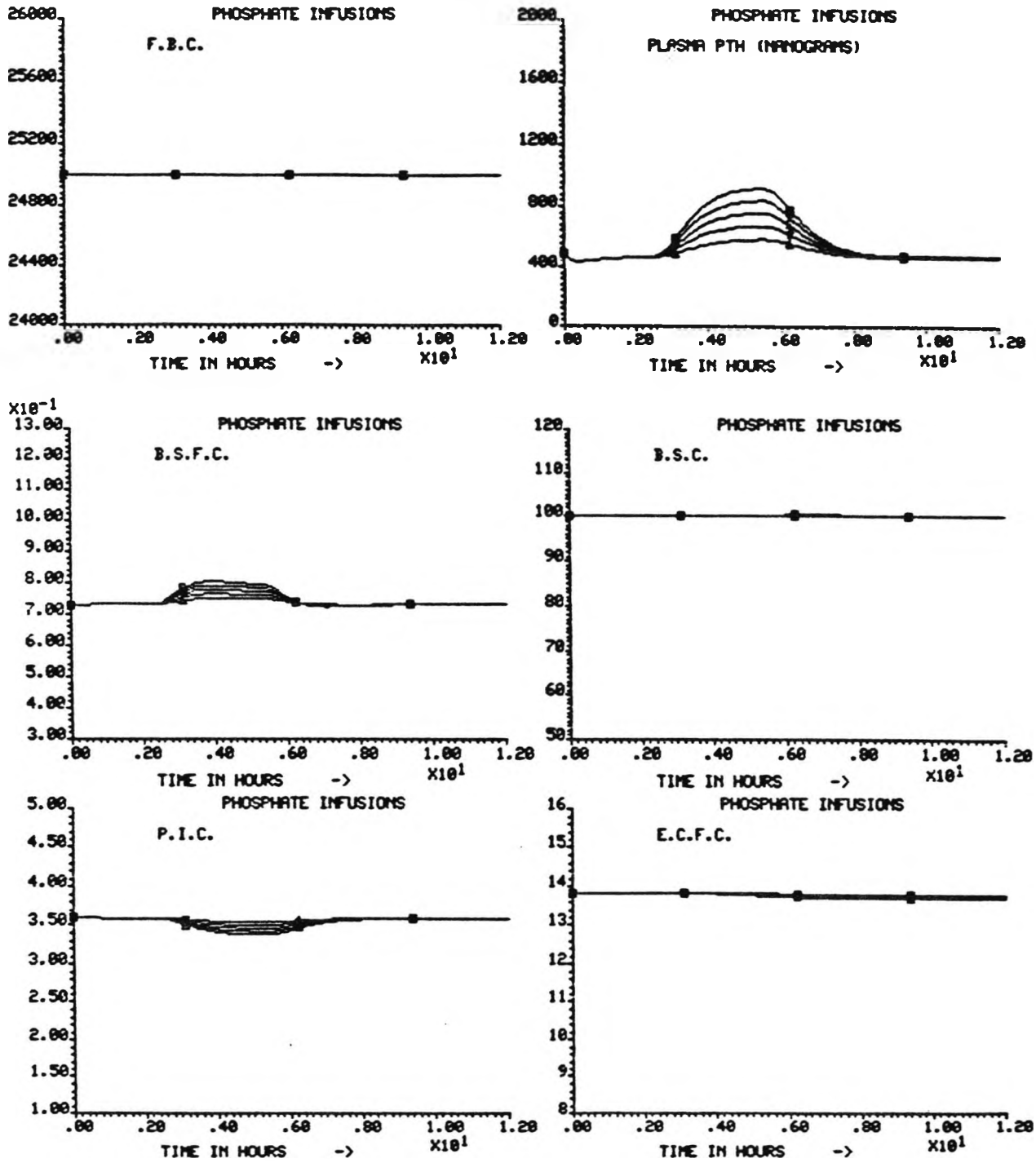
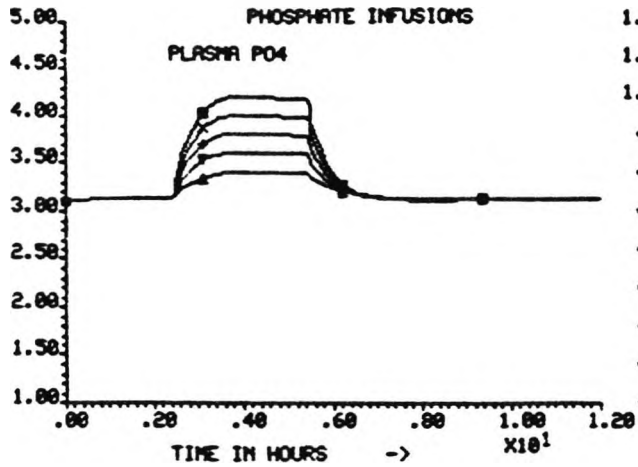
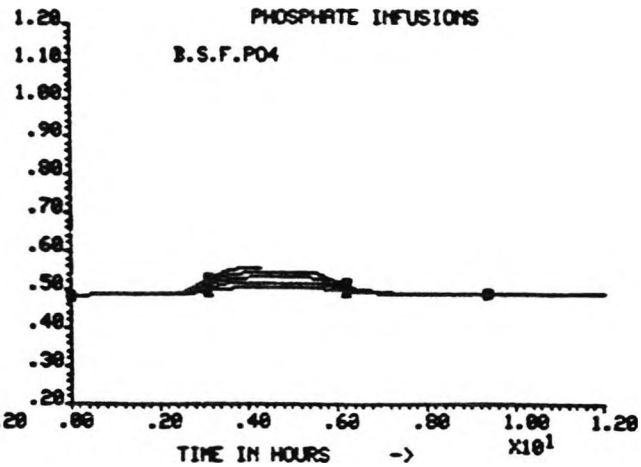
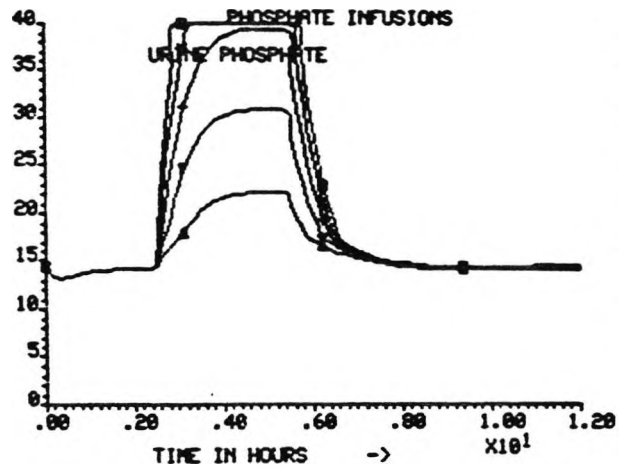


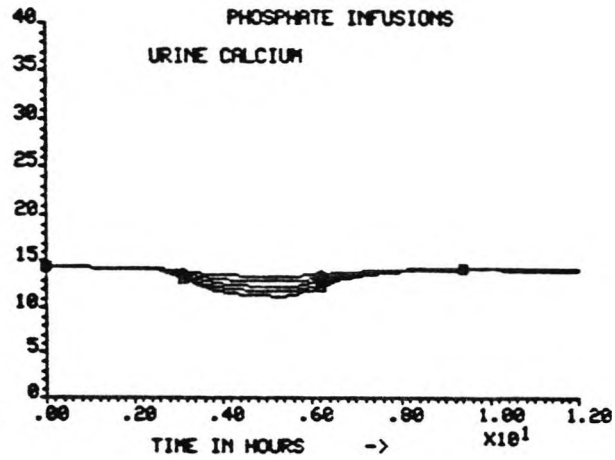
Figure 5.17 MODEL11 : Phosphate Infusions of varying amounts. 1.25, 2.5, 3.75, 5., 6.125 m mol over 3 hours. (between 2.4 and 5.4 hours on time axis).

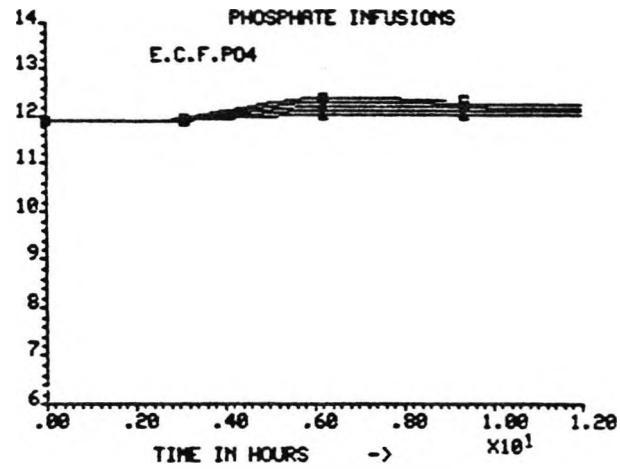
cont....

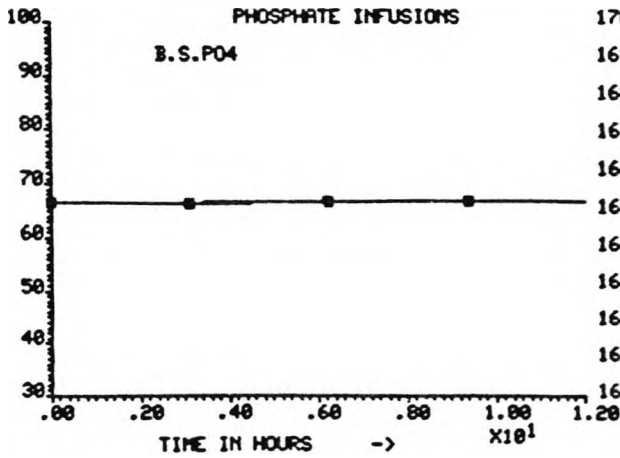

PHOSPHATE INFUSIONS
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹

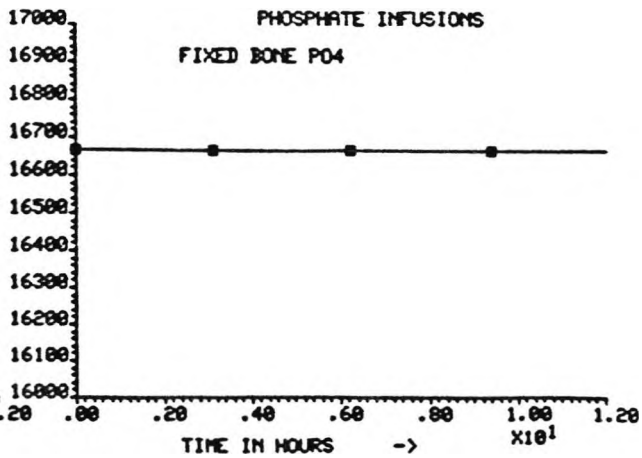

PHOSPHATE INFUSIONS
B.S.F.PO4
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PHOSPHATE INFUSIONS
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PHOSPHATE INFUSIONS
URINE CALCIUM
40
35
30
25
20
15
10
5
0
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PHOSPHATE INFUSIONS
E.C.F.PO4
14
13
12
11
10
9
8
7
6
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PHOSPHATE INFUSIONS
B.S.PO4
100
90
80
70
60
50
40
30
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹


PHOSPHATE INFUSIONS
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
.00 .20 .40 .60 .80 1.00 1.20
TIME IN HOURS ->
X10¹

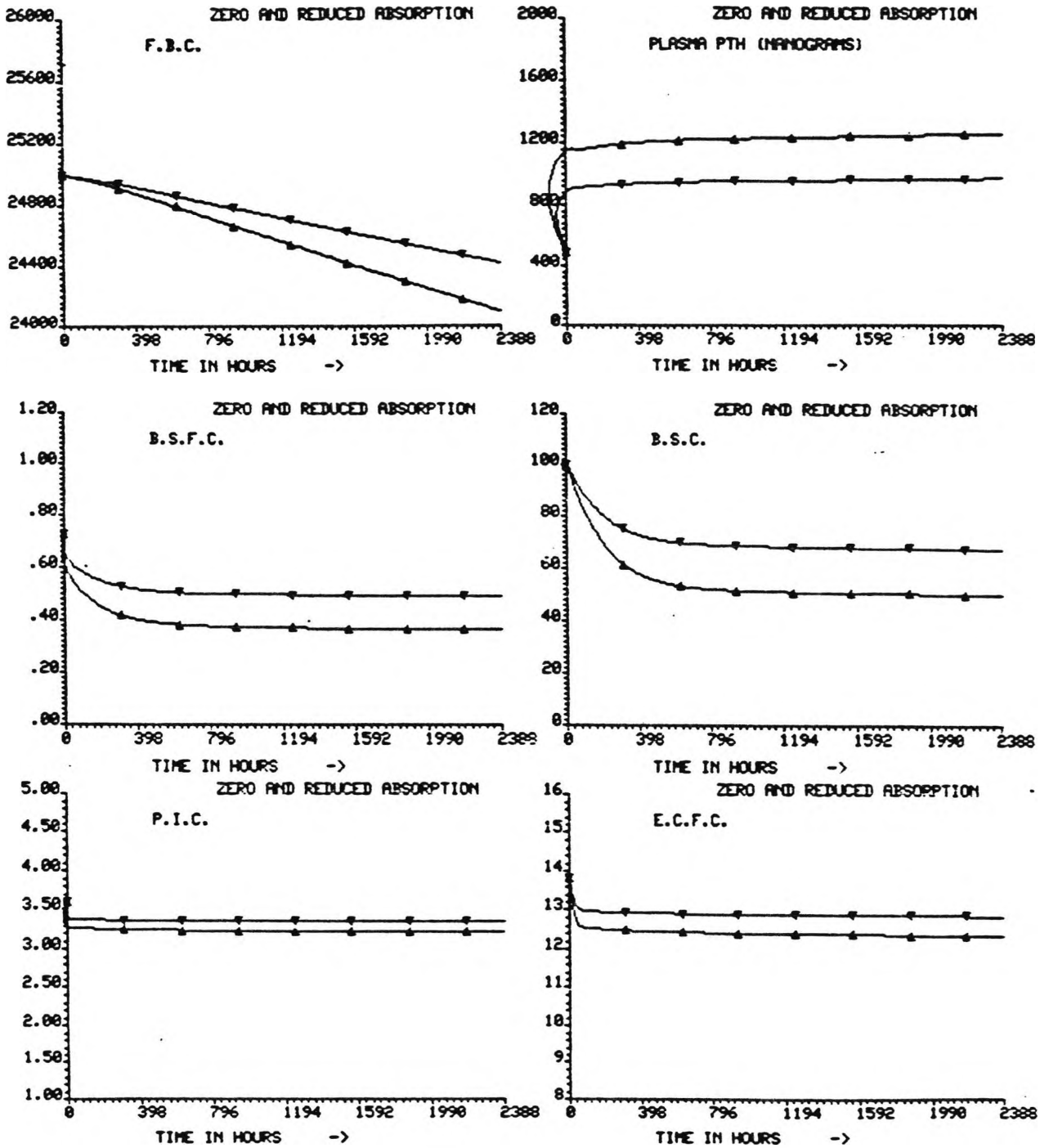
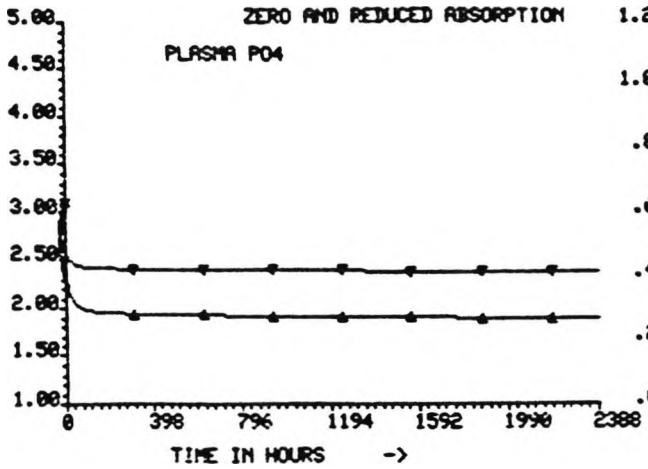


Figure 5.18 MODEL11 : Zero and reduced absorption of both calcium and phosphate, over 100 days.
INC = INP = 0.0; or = 5.0 mmol day^{-1}
In the steady state, INC = 14.33 mmol day^{-1}

cont.....


ZERO AND REDUCED ABSORPTION
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
B.S.F.PO4
1.20
1.00
.80
.60
.40
.20
.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
URINE CALCIUM
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
E.C.F.PO4
14
13
12
11
10
9
8
7
6
5
4
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
B.S.PO4
80
70
60
50
40
30
20
10
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED ABSORPTION
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->

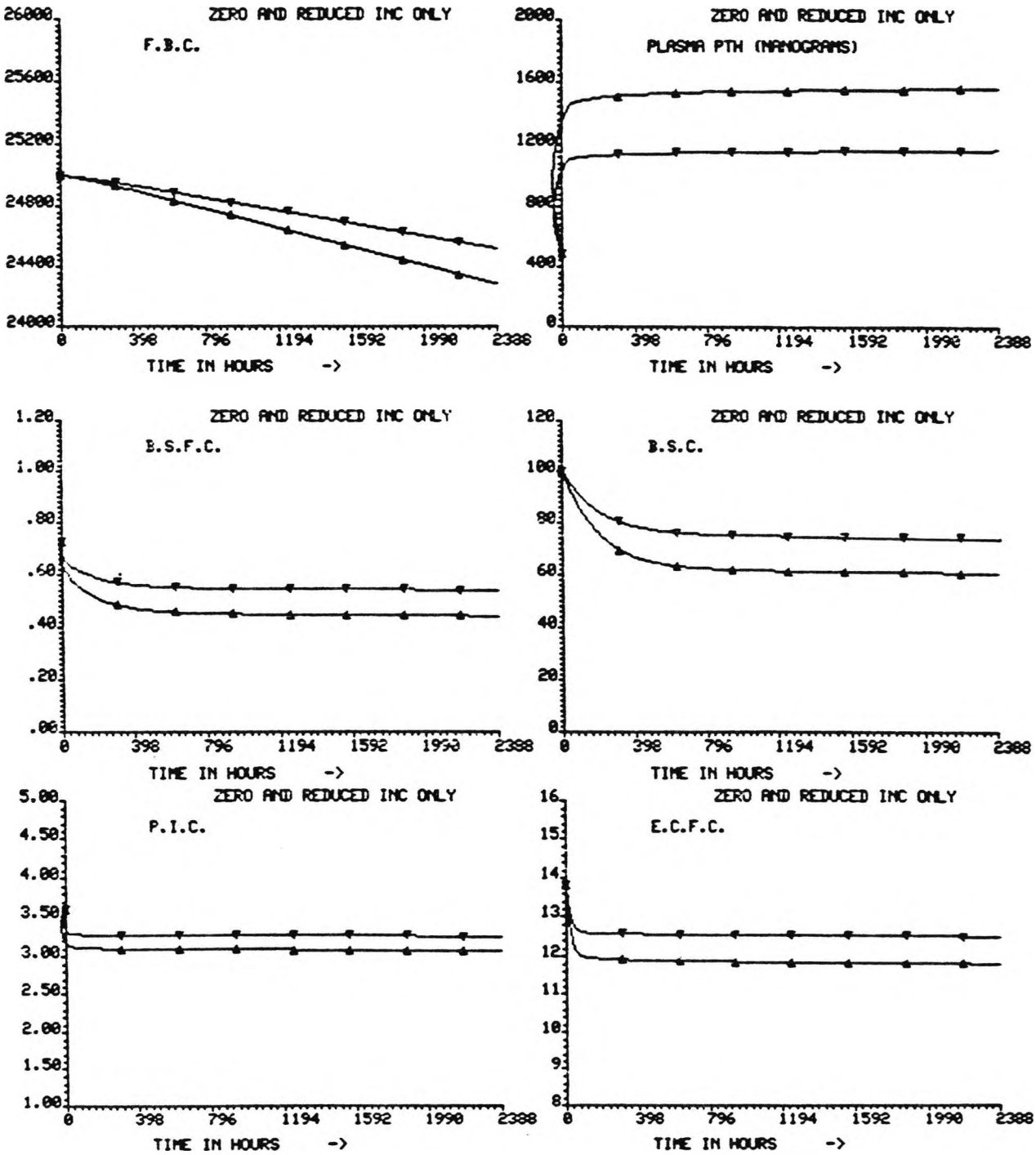
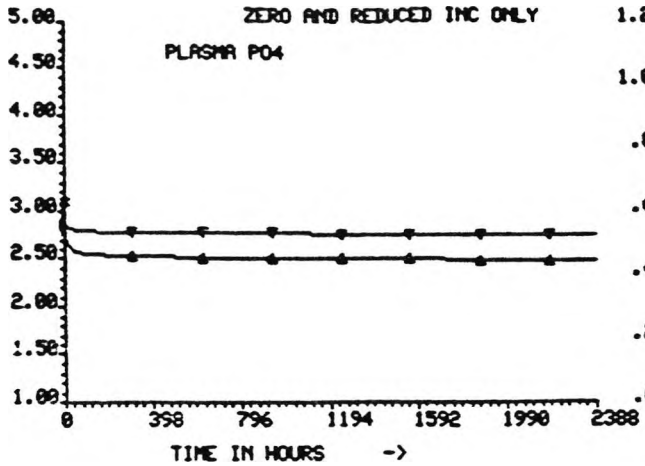


Figure 5.19 MODEL11 : Zero and reduced absorption of Calcium only. Phosphate absorption is unchanged.
INC = 0.0, or 5.0 mmol day^{-1}

cont....


ZERO AND REDUCED INC ONLY
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
B.S.F.PO4
1.20
1.00
.80
.60
.40
.20
.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
URINE CALCIUM
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
E.C.F.PO4
14
13
12
11
10
9
8
7
6
5
4
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
B.S.PO4
80
70
60
50
40
30
20
10
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INC ONLY
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->

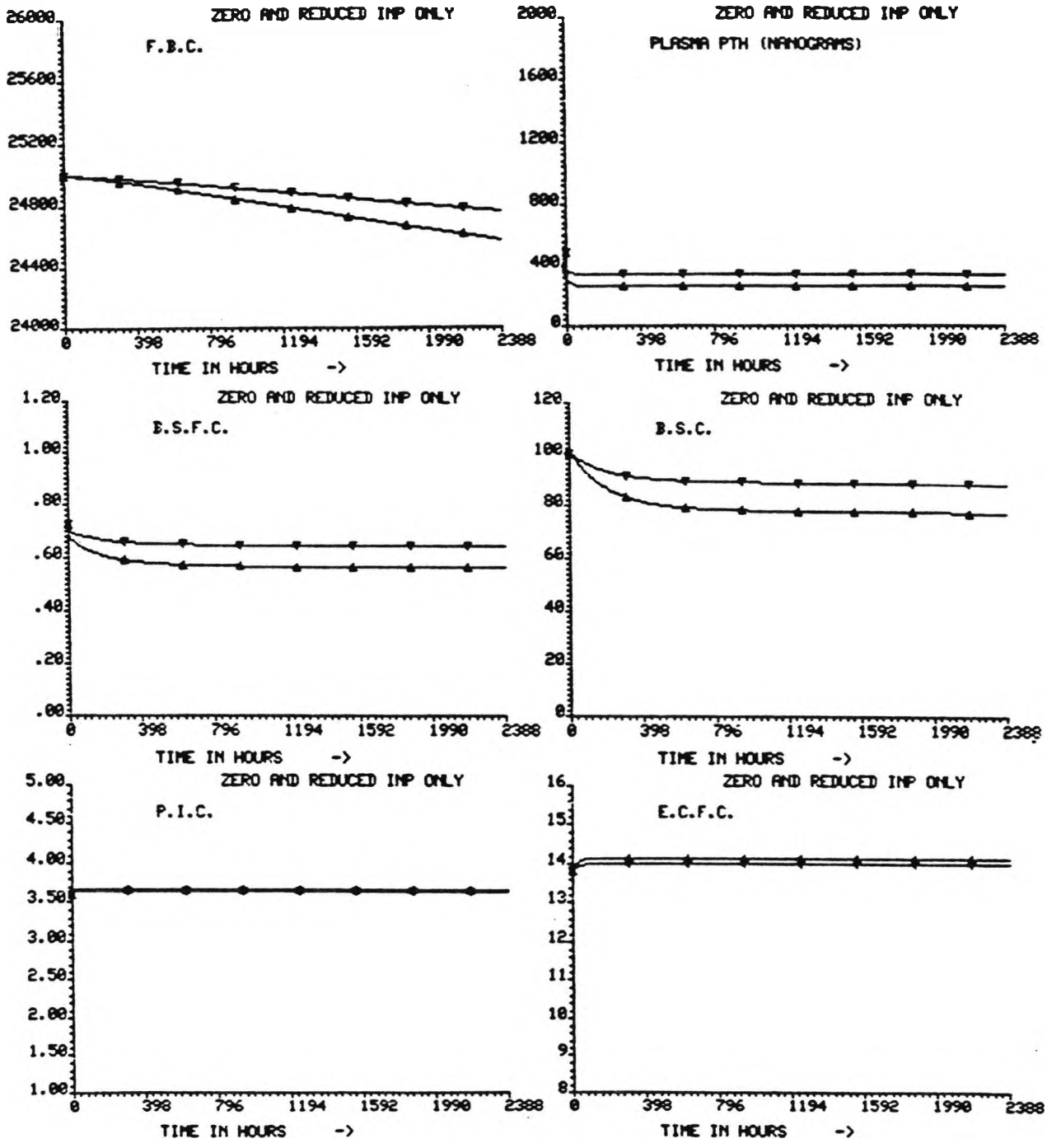
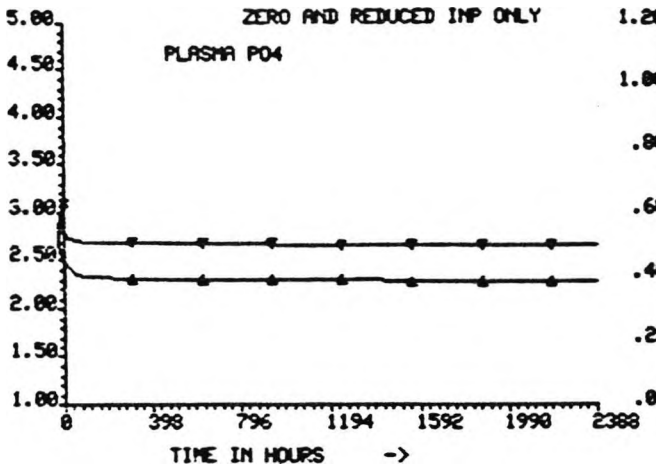


Figure 5.20

MODEL11 : Zero and reduced Phosphate absorption only.
Calcium absorption is unchangd, over 100 days.
INP = 0.0, or 5.0 mmol day^{-1}.

cont....


ZERO AND REDUCED INP ONLY
PLASMA PO4
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
B.S.F.PO4
1.20
1.00
.80
.60
.40
.20
.00
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
URINE PHOSPHATE
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
URINE CALCIUM
40
35
30
25
20
15
10
5
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
E.C.F.PO4
14
13
12
11
10
9
8
7
6
5
4
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
B.S.PO4
80
70
60
50
40
30
20
10
0
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->
ZERO AND REDUCED INP ONLY
FIXED BONE PO4
17000
16900
16800
16700
16600
16500
16400
16300
16200
16100
16000
0
398
796
1194
1592
1990
2388
TIME IN HOURS ->

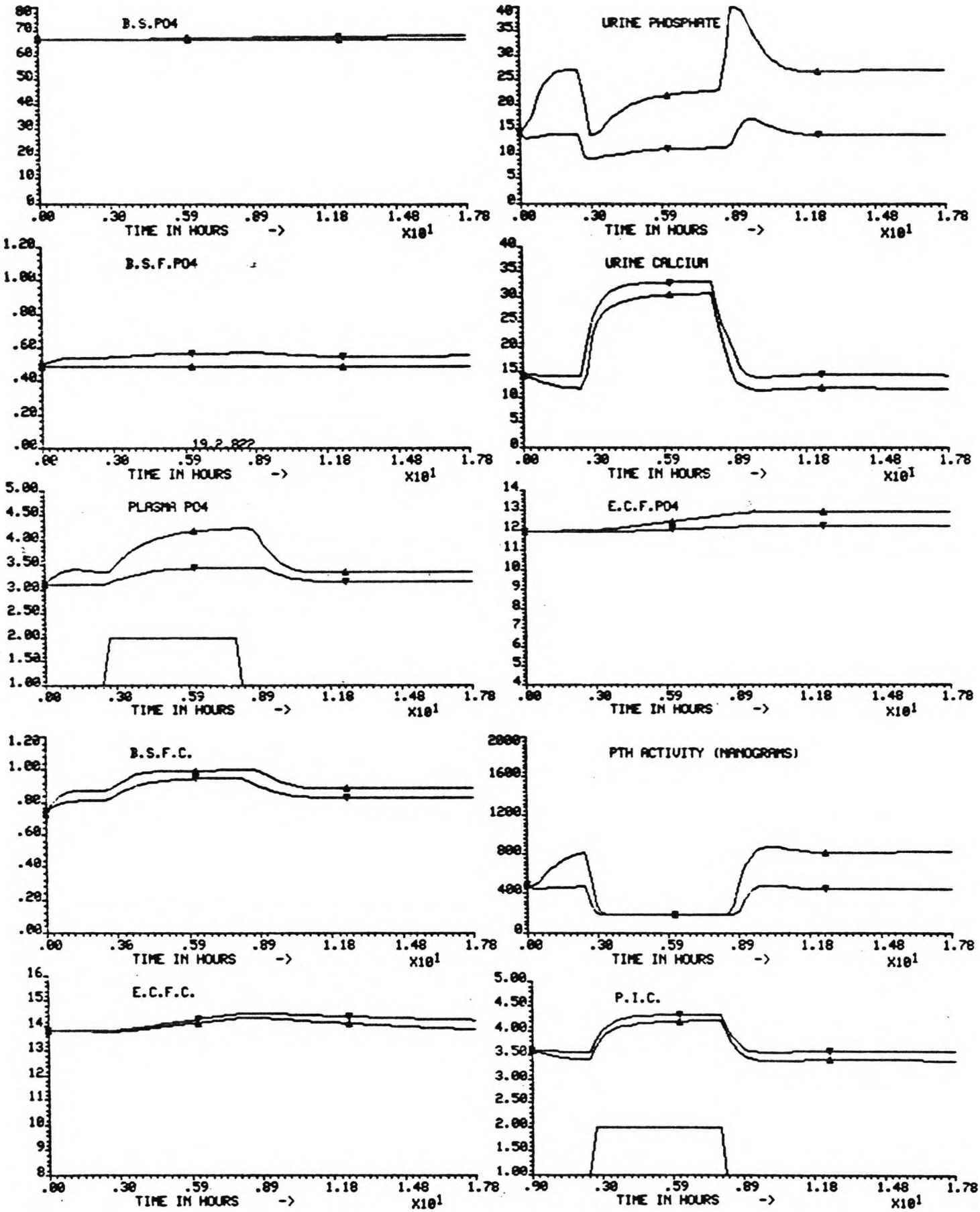


Figure 5.21 MODEL11 - A simulated calcium infusion

A reduced MODEL11 (FBC, BSFP, BSP, and FBP removed) is compared with the full MODEL11. Note the similarity of shape of the two PIC curves.

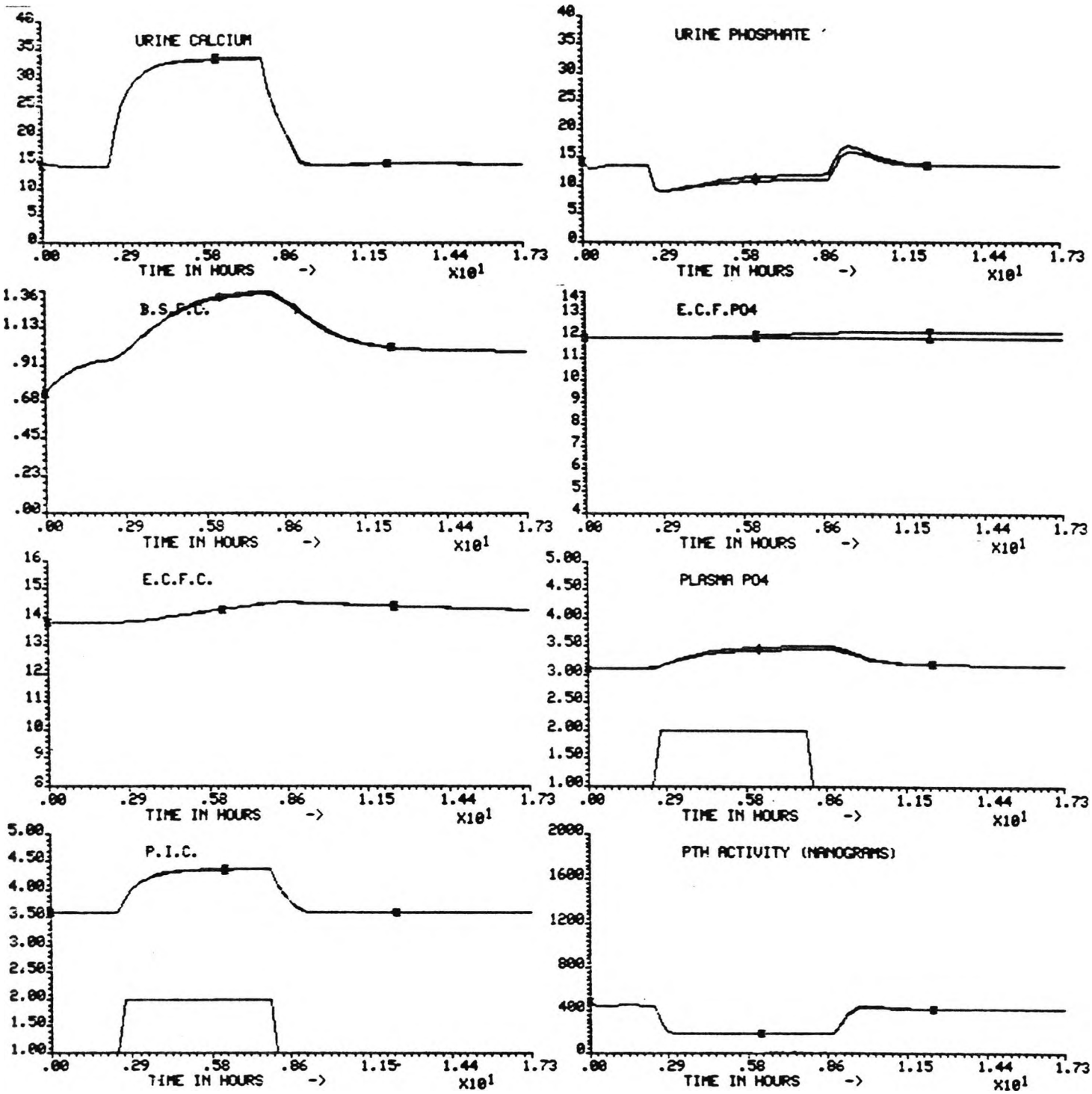


Figure 5.22 MODEL11 - A simulated calcium infusion

A reduced MODEL11 (FBC, BSC, BSFP, BSP, and FBP removed) is compared with the reduced MODEL11 shown in Figure 5.21 Note the similarity of response.

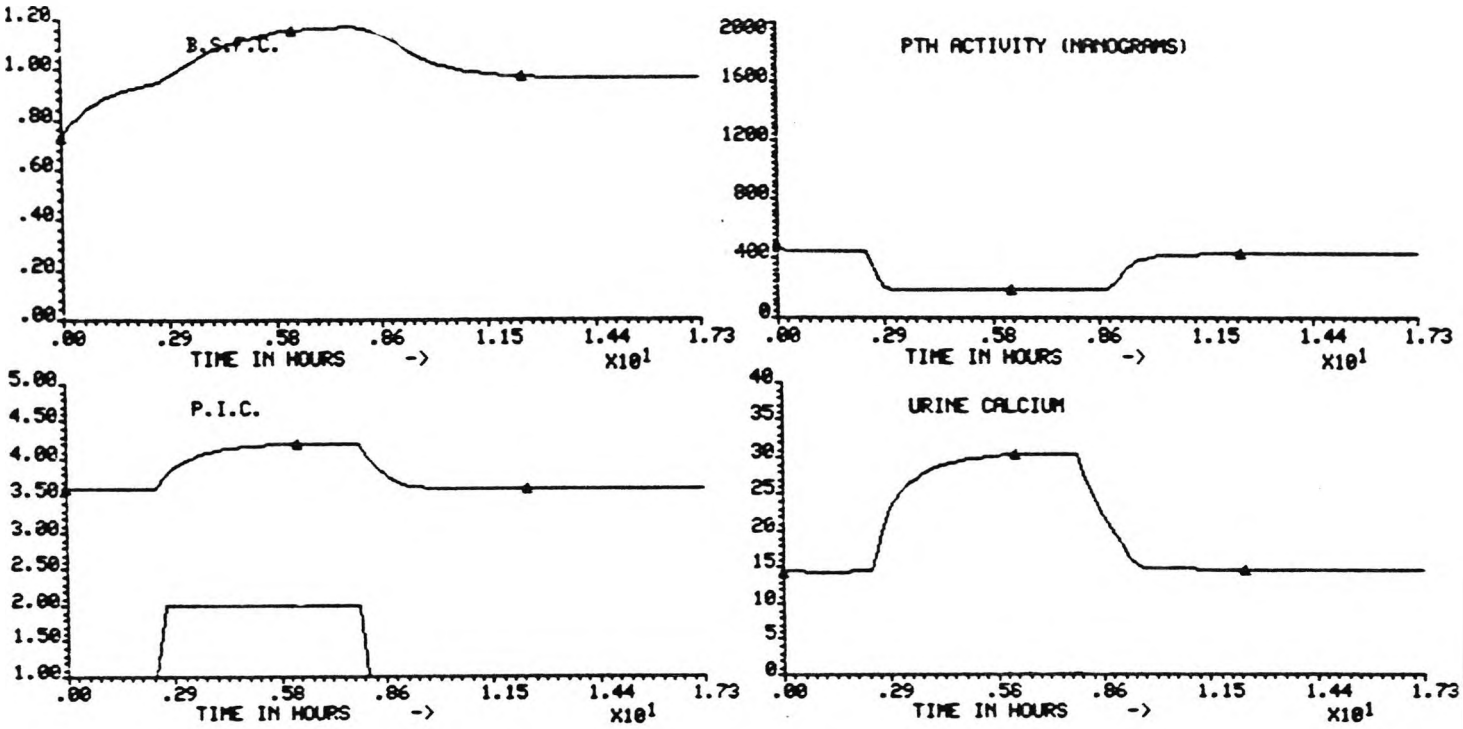


Figure 5.23 MODEL11 - A simulated calcium infusion
All the Phosphate compartments, FBC, and BSC, have been removed from MODEL11. Note that the PIC response is largely unchanged.

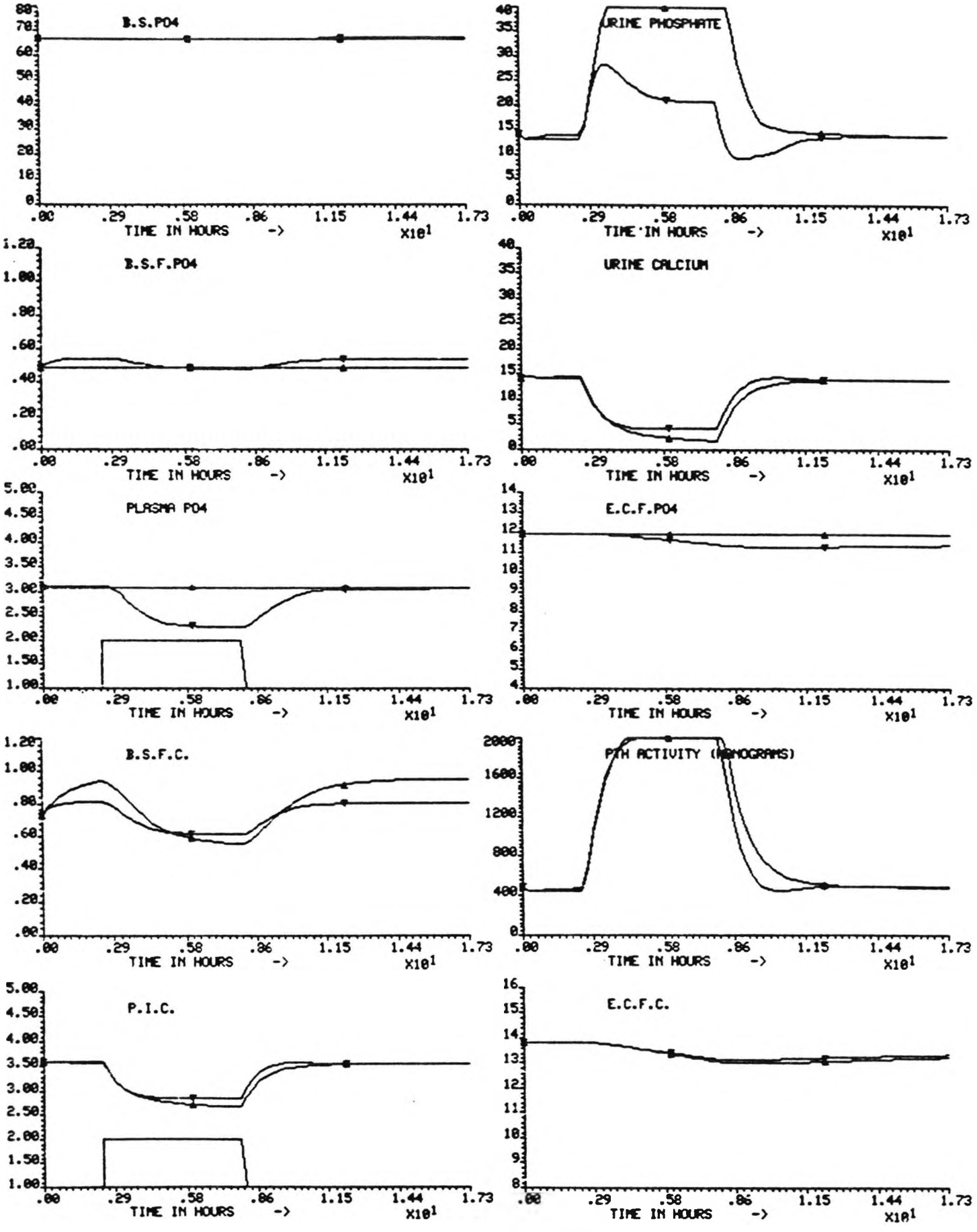


Figure 5.24 Modified MODEL11 - A simulated EDTA infusion. The full MODEL11 is compared with a reduced MODEL11 that has the following compartments removed - FBC, FBP, BSP, BSFP, ECFP, and PIP (all phosphate). Note the similarity of the PIC response.

CHAPTER 6

CHAPTER 6

6. Refinement Of The Short-Term Model (MODEL12)

This chapter considers MODEL12 as formulated in Chapter 5 and takes it through a series of further developments or refinements. The strategy, techniques and data used for this are first described in a general theoretical sense and then in the particular practical realisation that was adopted in this work. The series of models (MODEL13 - 17), all developments of MODEL12 thus produced are examined.

6.1 MODEL12

The structure of this model is shown in Figure 6.1. The model is fully described in sections 6.1.1 to 6.1.3, with a complete listing of the model equations being given in Appendix 1.

As described fully in Chapter 5, MODEL12 was derived from the eleven compartment MODEL11 through a process of model reduction that examined model performance in a range of situations, and considered in detail the structural sensitivity of the model in an experimental simulation (the intravenous infusion of calcium). This situation was chosen due to its fundamental nature - the healthy organism is often subjected to intermittent calcium supply through a typical dietary pattern. This alone was sufficient to illustrate some of the structural deficiencies of the model.

Full model details are presented below. The description and steady state masses given in Chapter 5 still applies, although some of the parameter values will have changed as a result of the parameter identification undertaken. See for instance the changes in values of parameters from MODEL11 to MODEL12. It must be realised that parameter sets realised as a result of optimisation in theoretically unidentifiable models such as MODEL12 potentially represent only one of a number of equally satisfactory parameter sets. Some of the parameters and steady state masses were however thought to be particularly significant, as they essentially recurred throughout many differing optimised parameter sets. Thus the flux between PIC and ECFC has increased twelve fold, and the nominal mass of the BSFC compartment has increased from 0.73 to 14.0 mmol.

6.1.1 MODEL12 Equations

$$\dot{ECFC} = ECFCA - ECFCR \quad (6.1)$$

$$\dot{PIC} = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC \quad (6.2)$$

$$\dot{BSFC} = BSFCA - BSFCR + BSCR - BSCA \quad (6.3)$$

$$\dot{BSC} = BSCA - BSCR \quad (6.4)$$

$$\dot{PPT} = PTS - k_{0,5}\, PPT \quad (6.5)$$

$$ECFCA = k_{2,1}\, PIC \quad (6.6)$$
$$ECFCR = k_{1,2}\, ECFC \quad (6.7)$$
$$BSCA = k_{4,3}\, BSFC \quad (6.8)$$
$$BSCR = k_{3,4}\, BSC \quad (6.9)$$
$$BSFCA = k_{3,1}\, PIC \quad (6.10)$$
$$BSFCR = k_{1,3}\, BSFC\, (PPT + k_a) / k_b \quad (6.11)$$

Urinary calcium output rate (UC)

$$UC_1 = 0.174\ (UFC - 0.5\ Tmc)$$
$$UC_2 = 0.352\ (UFC - 0.68\ Tmc)$$
$$UC_3 = 0.7\ (UFC - 0.9\ Tmc)$$
$$UC = k_c\, (\mathrm{Max}\ UC_i\ ;\ i = 1,2,3) \quad (6.12)$$

Tubular Calcium Maximum reabsorption rate (Tmc)

$$Tmc = 0.06939\ PPT + 217.0\ (\text{if } PPT < 490.0)$$
$$Tmc = 0.01943\ PPT + 241.48\ (\text{if } PPT \geq 490.0) \quad (6.13)$$

Ultrafiltration Rate (UFC)

$$UFC = 57.6\ PIC\ \mathrm{mmol\ day^{-1}} \quad (6.14)$$

Parathyroid Hormone Secretion Rate (PTS)

$$\text{Gland Activity} = k_{pt}\, (6.24 - 1.64\ PIC)\ \mathrm{ng\ s^{-1}} \quad (6.15)$$
$$0.1 < \text{Gland Activity} < 2.5$$
$$PTS = 86400.0\ (\text{Gland Activity})\ \mathrm{ng\ day^{-1}} \quad (6.16)$$

6.1.2 MODEL12 Nominal Parameter Values

$k_{1,2}$	27.2	day^{-1}	$k_{1,3}$	28.8	day^{-1}
$k_{2,1}$	61.2	"	$k_{3,1}$	112.0	"
$k_{3,4}$	0.617	"	$k_{0,5}$	57.6	"
$k_{4,3}$	4.41	"			
k_a	460		k_b	674.0	
k_c	0.385		k_{pt}	1.0	

6.1.3 MODEL12 Nominal Steady State Values

BSC_{ss}	100.0	mmol	PIC_{ss}	3.6	mmol
$BSFC_{ss}$	14.0	mmol	$ECFC_{ss}$	8.1	mmol
$PPTH_{ss}$	214.0	ng			
INC_{ss}	7.2	mmol day^{-1}			
B.W.	70	kg	Plasma volume	30 l	
ECF volume	11.53	l	BSF volume	0.61 l	
Glomerular Filtration Rate 100 ml min^{-1}					

6.2 Strategy Techniques and Data Used

The formulation, identification and validation of models has been described by various authors. As introduced in previous chapters the work presented in this thesis essentially uses the integrated approach to model formulation, identification and validation of Carson, Cobelli and Finkelstein (1983). Figure 6.2 shows a schematic illustration of this process, illustrating how the three processes; formulation, identification, and validation, involved in model development cannot be separated from each other; the model builder is always using a combination of all three. This is a similar concept to that used in some software systems development methodologies (e.g. Cohen, 1982) where analysis, design, coding and testing of different parts of one system can all occur concurrently.

To facilitate this process a number of formal techniques and data from specific short-term experimental situations are used. These include sensitivity analysis, various optimisation methods and statistical measures, and all are described in this section.

6.2.1 Sensitivity Analysis

The sensitivity of a model to small changes in its parameter values can be investigated and used in a number of ways. A parameter sensitivity matrix or, for a single model output, sensitivity vector, is used as part of some estimation algorithms (see for

instance section 6.2.2) to generate the magnitude of parameter increment at the next iteration. However for the most part the parameter sensitivity data were used to select the most appropriate sub-set of parameters from amongst the whole set of model parameters i.e. for a model of m state variables and n parameters, there are n x m sensitivity coefficients (which are actually all time varying), and from this set a sub-set n' of the parameters is chosen as the parameter set that the model is most sensitive to changes in for a given input. This sub-set n' is then varied to optimise the model response to the input in question.

Put simply the most sensitive parameter sub-set is the one that when varied will produce the greatest change in the model output of interest.

6.2.1.1 Calculation of Sensitivity Coefficient

For linear models the sensitivity coefficient can be determined analytically, but this is not possible for non-linear models. However a computationally simple approximation suffices for these cases through perturbation of each parameter P_r by a small amount DP_r

$$C_{jr} = Dx_j(t)/DP_r \tag{6.17}$$

$$Dx_j(t) = x_j(P_r + DP_r,t) - x_j(P_r,t) \tag{6.18}$$

where C_{jr} is the sensitivity of the jth state output variable x_j to variation in the rth parameter P_r.

A more useful realisation of this is the relative sensitivity coefficient, as it eliminates any imbalance in the magnitude of the state output variables. This is:

$$R_{jr} = (Dx_j(t)/x_j(t))/(DP_r/P_r) \tag{6.19}$$

where R_{jr} is the relative sensitivity of the jth state output variable x_j to variation in the rth parameter P_r.

Appendix II shows a practical realisation of this technique, and Figure 6.3 shows an example sensitivity analysis of MODEL12.

6.2.2 Optimisation Methods

All optimisation problems involve the minimisation or maximisation of a function (the objective or cost function) of several variables, possibly subject to limits on the values of the variable.

If the function to be minimised can be expressed as a sum of squared functions (a least - squares problem), then special considerations will apply.

$$\underset{x}{\text{i.e. minimise}} \quad \left[F(x) = \sum_{i=1}^{m} f_i^2(x) \right] \tag{6.20}$$

All of the optimisation methods and situations used in this program of research fall into this category. Three of the methods used were realised practically in the routines found in section E04 of the NAG numerical analysis routines library. The parameter estimation procedures used are described in the following sub-sections, and in Appendix II:

6.2.2.1 A Modified Peckham Algorithm (E04FAF)

Up to 10 parameters estimated.

6.2.2.2 A Modified Peckham Algorithm E04FAF)

3 parameters randomly chosen from the above 10 were estimated.

Progress with estimation algorithms could be inefficient with direct variation of 10 parameters. If a sub-set of three parameters was chosen at random, then estimation allowed to proceed using only the three chosen parameters, promising sets of starting values can be found. These can then be used in the other three approaches.

When estimating parameters in non-linear models, the choice of initial parameter estimates is extremely important as there is no guarantee that any minimum found is either a global minimum, or one of the best local minima that can be found. Indeed it is possible that convergance of any sort will not be achieved. The random choice of parameters to be estimated, together with a restriction upon the number of estimation iterations that are allowed to occur is very useful, and was widely used in this work. A useful adjunct to this technique is the random choice of starting parameter values within a specified (e.g. $\pm$ 10%) range to fully explore a local minimum.

6.2.2.3 A Combined Gauss-Newton and Modified Newton Approximation (E04FDF)

Up to 10 parameters estimated.

6.2.2.4 A Monte Carlo Search

In a parameter space bounded by P $\pm$ 20% for all 10 parameters.

The Monte-Carlo search, is of course the random choice (within a defined parameter space) of the parameter vector, and the subsequent system simulation, repeated many times. It has various attractions including: computational simplicity and the graphic illustration of parameter sensitivity or robustness of system response. For models of living systems the spread of results can be compared with the range of parameters values that would be found across the subject population (inter-subject variation), or with one subject at differing times (intra-subject variation). When used in conjunction with online graphical displays of system output, the visual spread of response can be a very useful and powerful indicator. Thus if Figure 6.4 is studied, the range of response can be likened to a confidence interval, although it should be remembered that it is not.

6.2.2.5 Other Aspects Of The Methods Used

The Peckham and constrained Gauss-Newton algorithms were chosen for the stated suitability for the problem at hand: unconstrained minimisation of a non-linear least squares function. The Monte Carlo search was initially used to arrive at a new starting position for the other methods, or to provide an indication of the robustness of model prediction to a specified parameter variation.

Other methods were used, again all from the E04 chapter of the NAG library. Their use was abandoned as a result of the success of the other methods, or through difficulties experienced during initial optimisation attempts. Typical difficulties included: a high degree of sensitivity to the starting vector or the chosen step length, and the use of an inordinate amount of computer time at each iteration due to difficulties in the calculation of derivatives (not all methods require derivatives to be calculated).

There are methods available that allow the specification of linear inequality or equality constraints, and while at first sight these might appear to be useful, their added complexity was found to compound the computer time problems.

6.2.3 Statistical Measures

Whenever a model or mathematical formulation is 'fitted to' or compared with observed data, the question of statistical significance is raised. This significance can be related to a number of questions: to assist in choosing one model in preference to another, as an objective metric or measure of the accuracy or 'correctness' of a model

independent of any alternative model, the accuracy of the observed data, or as the basis for a confidence interval surrounding a model prediction or an estimated parameter.

MODEL12 and all the variants that are described in this chapter are all theoretically unidentifiable. That is the model allows too many degrees of freedom for unique parameter estimates to be derived. Statistical measures of the confidence interval form, assume if the data were 'accurate' that an 'accurate' parameter vector could be derived. Thus the model output confidence interval represents the measurement variation in the data, and not some measure of the accuracy of the model response. Even if the available data were comprehensive the first pre-condition is still not met. It follows that any confidence interval derived will have only limited significance.

When dealing with unidentifiable models in addition to a paucity of data of questionable accuracy, such that reliable estimates of data variance are not available, factors other than traditional statistical criteria have to be employed to enable one model to be chosen in preference to another. The social scientist might call these 'normative or subjective values', while the physiologist might prefer 'pattern of response'. It matters not, both are concerned with non-ordinal measurements that are used in support of a choice of actions, hypotheses, or models.

Consider the two model parameter vectors A and B of models MA and MB. If the response of the models to a simulated situation is compared with the measured data, and the least squares error (EA) is greater than EB, model MA could still be chosen in preference to MB. This could certainly occur if the response of MA parallelled the data by a uniform single signed error, while that of MB fluctuated in a non-gaussian fashion on both sides of the measured data. Changes in steady state often occur in physiological models as the result of parameter estimation, leading to potentially totally erroneous estimates of least square error (see Figure 6.14 for an example of this). Even when dealing with data for which reliable estimates of variance are available, unqualified statistical conclusions can be difficult to support.

Least squares errors are used in the work presented here despite the inapplicability of conventional statistical choices. When used in conjunction with subjective estimates of response shape, measures of least squares error merely provide an extra indication of the suitability of a model structure, or parameters.

6.2.4 Data Used in Model Identification

As MODEL12 is concerned with short-term variations, data relating to the infusion of hypo- and hyper-calcaemic agents are ideal tests of model validity. Data regarding calcium infusions are available from various sources including:

Levitt et al (1958)
Bhandarkar and Nordin (1962)
Ibbertson, Roche and Pybus (1966)
O'Brien and McIntosh (1967)
Morimoto et al (1979)
Marshall (1980)

Data covering the response to an infusion of ethylene diamine tetra acetic acid (EDTA) are available from the studies of Jones and Fourman (1963). Considering each in turn:

6.2.4.1 Levitt et al (1958)

These data are mainly concerned with the effect of changes in plasma calcium upon primary electrolyte excretion (i.e. Na^+, Cl^-). The data regarding plasma calcium changes are merely illustrative, with no indication of the magnitude of the data variance given. Because of this they are of little significance for this study. They are shown in Figure 6.5.

6.2.4.2 Bhandarkar and Nordin (1962)

These data are presented as a 'test' of the proportion of the infused calcium that is retained by the skeleton during a four hour period. Plasma samples were only taken at the start and finish of the four hour period. Thus of the eight cases of osteomalacia tested, 6 showed an elevated 4 hour retention compared to normals and osteoporotics. The data are shown in Figure 6.6. It is interesting to note the similarity between osteoporotics and normals.

6.2.4.3 Ibbertson Roche and Pybus (1966)

These data concerned a 3 hour calcium infusion in normal and hypothyroid subjects. The paper concerns its conclusions with the differences between these two sets of subjects, and the possible role of calcitonin as the cause of these differences. No data on urine output are given. The data relating to the nine normal subjects are shown in Figure 6.7.

6.2.4.4 O'Brien and McIntosh (1967)

The data reported in this paper are also concerned with the role of calcitonin. A one hour infusion of calcium gluconate was given to yield the results shown in Figure 6.8. No data are given concerning the urine output or any other plasma fraction.

6.2.4.5 Morimoto et al (1979)

This study concerned itself with the influence of a very short calcium gluconate infusion upon calcitonin secretion. The infusion lasts for one minute (i.e. it is effectively a single injected bolus). The data are shown in Figure 6.9.

6.2.4.6 Marshall (1980)

Data relating to calcium infusions in 19 patients who presented with osteoporotic crush fractures of varying severity, were available. They are shown in Figures 6.10 and 6.11 (graph A). The measurement time schedule was not the same for all subjects which complicated the production of a usable mean response. This problem was overcome by taking the mean at each measurement point at which at least six measurements were available. This represented 15 measurement times.

The data were then normalised to account for the differing units used in the model (total plasma ionised calcium as opposed to total calcium), through expression as percentages of each subjects initial plasma calcium value. They are shown in this form in Figure 6.11 (graph B), and when these are converted to the model units these mean data are used in all subsequent analysis to represent a normal calcium infusion response.

Concern might be expressed about using data from osteoporotic patients and their relevance to the healthy individual. However, as described in Section 6.2.4.2, Bhandarkar and Nordin (1962) performed 4 hour calcium infusions of 15mg calcium gluconate/kg. The data collected were not as comprehensive as those from Marshall, but no difference could be seen between normals and osteoporotics, in contrast to normals and osteomalacics or stone formers.

6.2.4.7 Jones and Fourman (1963)

Jones and Fourman (1963) have described the response of plasma calcium to an intravenous infusion of EDTA (a calcium chelating agent Ethylene Diamine Tetra-acetic Acid). This was used as a test of parathyroid function. Thus recovery from the hypocalcaemia produced by EDTA would be impaired by a substandard parathyroid gland response. It is however an inherently dangerous procedure and has fallen out of favour for routine clinical assessment.

For the models under investigation the data are extremely useful as an EDTA infusion is essentially the antithesis of a calcium infusion. It is in fact modelled as a negative calcium infusion (hence the urine calcium values produced are in error). From Fourman and Royer (1968) 4g of EDTA will chelate 430 mg calcium, thus the

5g EDTA given by Jones is equivalent to -150.0 mmol calcium given over 2 hours. The data are shown in Figure 5.10.

6.3 Model Identification and Validation

This section covers the several stages of identification and validation of MODEL12. These resulted in the formulation of MODEL13-17; all variants or developments of MODEL12. Each stage represents a further iteration through the model formulation, identification, validation cycle. Thus it was thought to be important that the model was able to cope with a hypocalcaemic stimulus as well as the hypercalcaemic stimulus of a calcium infusion that was used in the initial development of MODEL12. It is the EDTA infusion (see section 6.2.4.7) that is used as the main test input to the range of models to be described.

As each model is produced, a most suitable paramter set is identified through optimisation of the model response to a simulated EDTA infusion. Appendix I refers to these parameter sets as nominal parameters, because the exact value is not of tremendous significance, rather the approximate range. Parameter confidence intervals are not given, and as elaborated earlier (section 6.2.3) are not directly relevant. The parameter range however can be explored as shown in Figure 6.12, a parameter sensitivity well will provide a graphic illustration of this range, as well as confirmation that an optimum value has been found. Full details of each model, are presented in the relevant section of Appendix I.

6.3.1 MODEL12 - The first reduced model

MODEL12 although smaller and more tractable than MODEL11 is still theoretically unidentifiable with respect to its 11 parameters on the basis of available input/output experiments. Formal parameter estimation yielded a number of local 'good fits' to the calcium infusion data. One of the most optimum is shown in Figure 6.13, which should be compared with Figure 5.23 which represents the response before parameter estimation. Interestingly all parameter vectors that gave local minima resulted in an increased BSFC steady state mass (now nominally 14.0 mmol, originally set at around 1.0 mmol), and a greatly increased calcium flux between plasma and BSFC. Figure 6.14 shows two of these local good fits clearly showing the sharp rise in mass of BSFC as the parameters sets more closely yield a PIC response that matches the data (again compare with Figure 5.23).

The calcium infusion response is useful in assessing the structural validity of the model, but it does not explicitly invoke the PTH homeostatic control mechanisms. Validation of this model component requires analysis of the response to the EDTA infusion. Major problems were experienced in attempting to realistically simulate an

EDTA infusion. A similar degree of hypocalcaemia could be reproduced, but the shape of the response was significantly in error (see Figure 6.15)

Sensitivity analyses (see Section 6.2.1) were used together with various optimisation procedures to give the best fits shown in Figure 6.16, giving a PIC least squares error of approximately 3.1.

6.3.2 MODEL13 - BSCR is a function of PTH

Consider the first alteration to MODEL12. There is some evidence that PTH affects both osteoclastic bone resorption, and the transfer of calcium from bone to blood by other mechanisms (see for instance Reeve et al, 1976). To implement this requires a change in the equation describing Bone Surface Calcium Resorption (BSCR). Thus:

$$BSCR = f(PPT) = k_{3,4}\, BSC\, (PPT + k_d)/k_e \qquad (6.21)$$

$$k_d = 64.0$$

$$k_e = 540.0$$

Optimisation of the MODEL13 response to a simulated EDTA infusion gave the values given above for kd and ke. This parameter set yielded a PIC least squares error of 3.0, representing no change from the response of MODEL12.

6.3.3 MODEL14 - ECFCR is a function of PTH

Although there is no direct physiological evidence to suggest so, this model incorporates the effect of PTH upon Extracellular Calcium Fluid Resorption (ECFCR). Thus:

$$ECFCR = f(PPT)$$

$$= k_{1,2} ECFC(PPT + k_f)/k_g \qquad (6.22)$$

$$k_f = 128.0$$

$$k_g = 86.0$$

Optimisation of the response to an EDTA infusion yielded a PIC least squares error of 1.2, representing a small improvement over MODEL12 and 13.

6.3.4 MODEL15 - BSCR and ECFCR are functions of PTH

This model variant incorporates the changes made to produce MODEL13 and those used to produce MODEL14. In other words both BSCR and ECFCR are assumed to be a function of PTH.

The various optimisation methods available were unable to provide a convergence better than that achieved for MODEL12, 13 and 14. In fact convergence was not achieved.

6.3.5 MODEL16 - BSCR is a function of PTH, BSFCR is not

MODEL12 assumed that BSFCR is a function of PTH. MODEL16 assumes that PTH only affects BSCR (the change that was incorporated in MODEL13), and not BSFCR. Thus with reference to MODEL12 the changes are:

$$BSCR = k_{3,4}\, BSC(PPT + k_h)/k_i \quad (6.23)$$

$$BSFCR = k_{1,3}\, BSFC \quad (6.24)$$

$$k_h = 480.0$$

$$k_i = 530.0$$

Again gain this variant of MODEL12 was no better at reproducing the data. A simulated EDTA infusion yielded a PIC least squares error of around 3.0, similar to that of MODEL12 and 13.

6.3.6 MODEL17 - An exhaustible store of PTH is incorporated

If the secretion mechanisms of the parathyroid gland are investigated it becomes apparent that a simple linear relationship between plasma ionised calcium and PTH secretion rate is an over simplification.

Mayer and Hurst (1978) and Jung et al (1982) have published data on the parathyroid gland secretion rate in calves. What is unique about their data is that they consist of direct measurements of parathyroid gland effluent blood. At low levels of plasma calcium a steep peak of secretion was noted, but also this peak could not be sustained. Aside from the experimental methodological difficulties associated with maintenance of plasma calcium levels this low in a live organism, this could be explained as being due to secretion at this maximal rate occurring from an exhaustible store; this store being 'separate' from the source of the rest of the secreted PTH. The data are illustrated in Figure 6.17.

MODEL17 attempts to incorporate an exhaustible store similar to that described above. Thus it is assumed that PTH release occurs from two sources; the first as in MODEL12, the second occurring during a period of increased demand (i.e. during a period of hypocalcaemia) from an exhaustible store. Thus:

$$\text{PTS} = 86400.0\ (\text{Gland Activity}) \tag{6.25}$$

$$\text{Gland Activity} = k_{pt}\ (6.24 - 1.64\text{PIC})\ \text{ng s}^{-1} \tag{6.26}$$

$$\text{Gland Activity} \geq 0.1$$

$$T_{pt} = 0.077\ \text{day} = 111\ \text{min}$$

Gland Activity > 0.66 is only supported for a time T_{pt} in any 24 hour period.

Jung et al (1982) have attempted to calculate parameter values for a similar store. Those quoted above were derived by fitting parameter values to the EDTA infusion data. MODEL17 is the same structure as MODEL12 except as quoted above.

The response of MODEL17 to a simulated EDTA infusion is shown in Figure 6.18. This both provides a good qualitative match to the data, and also yields a very much improved quantitative fit. The PIC least squares error is equal to 0.2. This compares well with MODEL14 (1.2), and MODELs 12, 13 and 16, each of which yielded least squares errors of approximately 3.0. Figure 6.19 also shows a 'best fit' of MODEL17 to a simulated calcium infusion, illustrating how this is unaffected by the PTH model changes.

The next chapter (Chapter 7) considers in more detail the validity of this model.

6.4 Summary

MODEL12 which is itself an extension of MODEL11 (the development of which was chronicled in chapter 5) is introduced, and taken through a series of further developments, as part of the integrated approach to model development used. The strategy, techniques, and data used to aid these developments are first described in the context of the juxtaposition of formulation, identification, and validation that is used in the development process (after Carson, Cobelli, and Finkelstein; 1983). Thus model parameter sensitivity analysis, and the techniques of optimisation are described with particular reference to their use and usefulness in this study. This included algorithms from Peckham, Newton, Gauss, and the somewhat crude use of monte-carlo simulation.

The problems involved in using statistical measures to infer the significance of model output predictions are discussed with reference to the choice of one model over another when both are theoretically unidentifiable. Hence the use of non-ordinal measurements, or patterns of response, is introduced as an useful inference tool when dealing with the identification and validation of these models.

The data used in the further development of MODEL12 are considered in detail. These concerned the infusion of calcium or of EDTA, providing respectively a contrasting hyper- and hypo-calcaemic stimulus. The identification and validation of MODEL12 led to a further series of models being developed; MODELs 13 to 17. The final MODEL17 is unique in that an exhaustible store of PTH is inferred to exist, and this model structure is able to mimic both the hypo-and hyper-calcaemic situations, to a degree not matched by the other models, despite the extensive use of all the available optimisation methods.

The next chapter explores in detail the performance of MODEL17, over a range of situations and data that were not used in its development. This assessement of performance enables some detailed validity conclusions to be made.

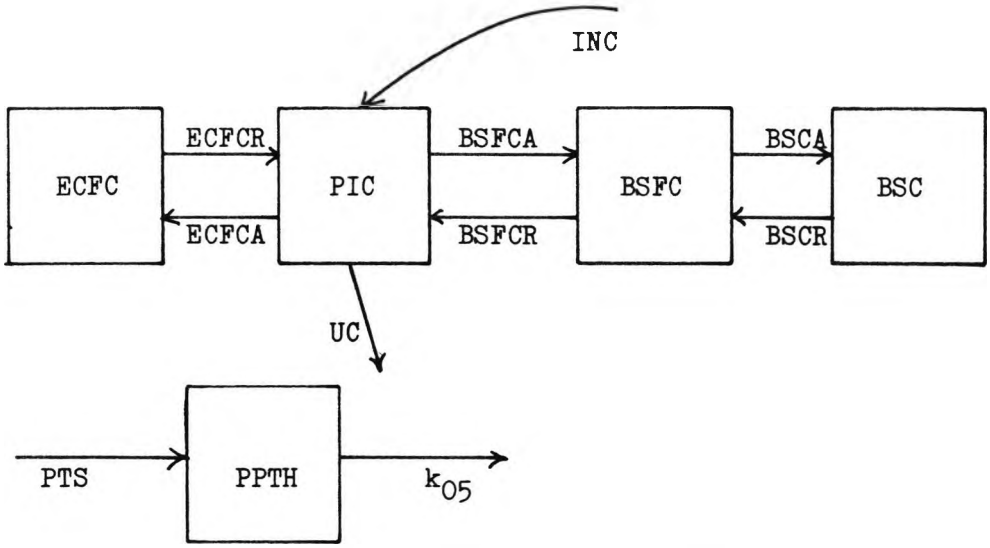


Figure 6.1 The structure of MODEL12. See Appendix I for nomenclature details, and relevant equations and relationships.

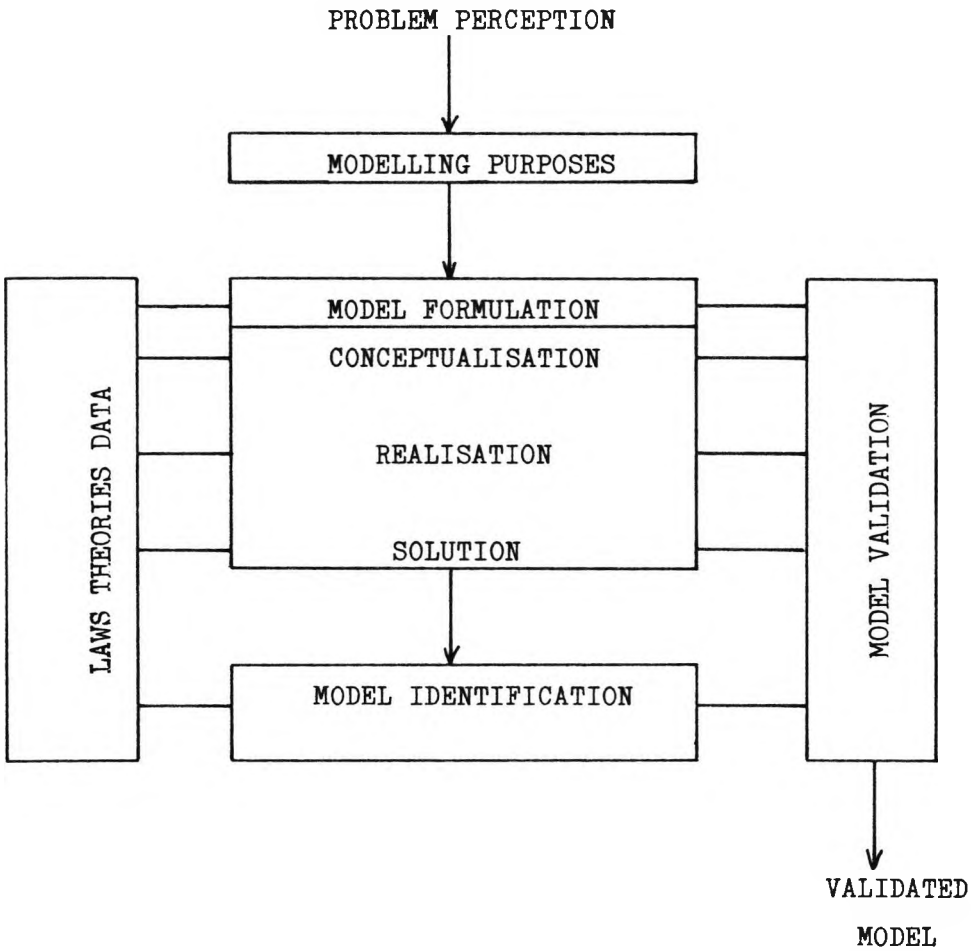


Figure 6.2 Schematic illustration of an integrated approach to model formulation, identification, and validation. From Carson, Cobelli, and Finkelstein (1983).

Parameter(P)	%age Change in Residual Error (P - 10%)	(P + 10%)
$K_{1,2}$	-1.2	1.5
$K_{2,1}$	1.1	-1.0
$K_{3,1}$	25.0	-18.0 *
$K_{1,3}$	-21.2	23.8 *
$K_{4,3}$	20.0	-17.7 *
$K_{3,4}$	-27.4	28.4 *
INC	-5.4	0.0
K_{pt}	-15.7	14.7 *
$K_{0,5}$	16.1	-14.0 *
K_c	2.4	-2.5
K_a	7.7	-18.4
K_b	11.9	-10.5

* = Most sensitive parameters.

Figure 6.3 An example sensitivity analysis of MODEL12. Parameters were adjusted individually by ± 10% and the %age change in residual error of the model PIC response compared to the EDTA infusion data of Fourman and Royer (1968).

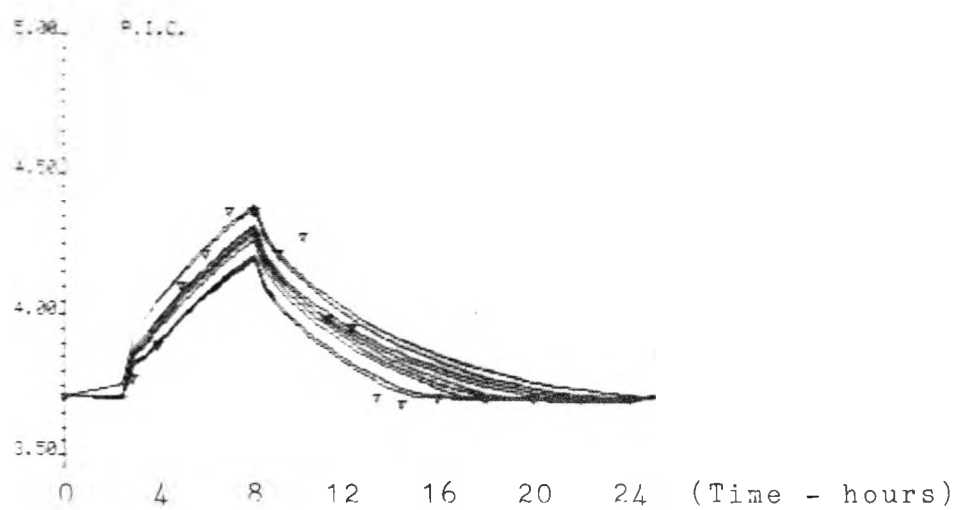


Figure 6.4 An example monte-carlo simulation that graphically illustrates the spread of response that occurs through random variation of the model parameters within a certain range.

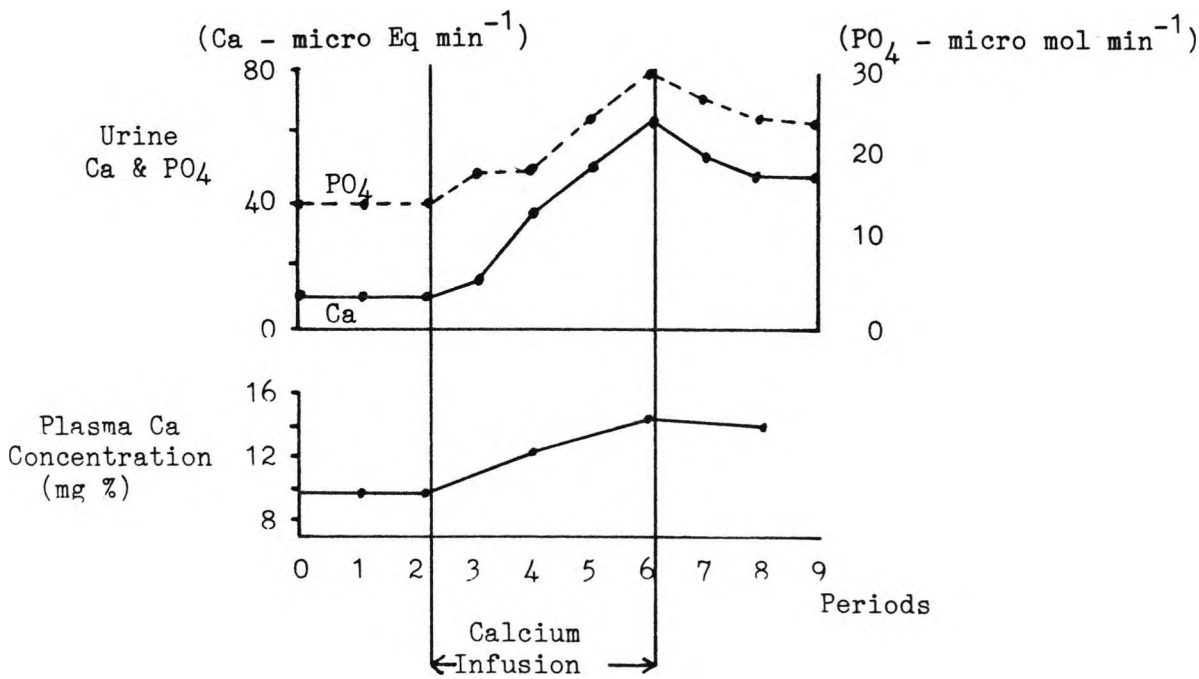


Figure 6.5 The calcium infusion data of Levitt et al (1958). Urine calcium and phosphate are shown as well as plasma calcium. The infusion rate was 0.07 mg calcium (as the gluconate) per kg body weight for 60 - 90 minutes.

Subjects	%age excretion of infused dose during the 4-hour period
Normals (n = 19)	53% : range = 44-66% (S.Deviation = 5.7%)
Osteoporotics (n = 22)	53% : range = 35-72%
Osteomalacia (n = 8)	80% : range = 60-95%

Figure 6.6 The calcium infusion data reported by Bhandarkar and Nordin (1962). Administration was 15 mg calcium gluconate per kg body weight over four hours.

Time (h)	Plasma Calcium (mEq l^{-1}) (n = 9)
0	4.8
1	5.5 ± 0.38
2	6.0 ± 0.45
3	6.2 ± 0.50
4	5.9 ± 0.57
5	5.6 ± 0.48
6	5.4 ± 0.46
8	5.3 ± 0.43
10	5.1 ± 0.31
12	4.9 ± 0.31
24	4.6 ± 0.30

Figure 6.7 The calcium infusion data from Ibbertson, Roche, and Pybus (1966). 0.6 mEq Calcium (as the gluconate) was administered over three hours. The actual standard deviation associated with the measurement technique was ± 0.08 mEq./l, which puts the inter-subject standard error shown into perspective.

Plasma Calcium (mg 100 ml^{-1})	Time (h) (n = 28)
9.35 ± 0.09	0
12.82 ± 0.1	1
11.18 ± 0.12	3
10.54 ± 0.12	5
10.24 ± 0.13	7

Figure 6.8 The calcium infusion data of O'Brien and McIntosh (1967). 10 mg calcium per kg body weight (as the gluconate) was administered over 1 hour.

Time (min)	Young		Elderly	
	Male (n = 6)	Female (n = 4)	Male (n = 6)	Female (n = 6)
0	5.1 ± 0.4	4.6 ± 0.4	4.5 ± 0.3	4.5 ± 0.4
1	6.5 ± 0.6	6.3 ± 0.5	6.9 ± 1.1	6.9 ± 0.9
2	6.8 ± 0.3	7.1 ± 2.1	6.6 ± 0.5	6.6 ± 0.4
5	6.1 ± 0.6	5.8 ± 0.6	5.7 ± 0.4	5.6 ± 0.3
15	6.1 ± 0.4	5.8 ± 0.6	5.3 ± 0.3	5.4 ± 0.2
30	6.0 ± 0.5	5.5 ± 0.9	5.3 ± 0.3	5.2 ± 0.2

Figure 6.9 The plasma calcium levels (mEq l^{-1}) reported by Morimoto et al (1979) in response to a one minute calcium infusion. A dose of 4 mg calcium (as the gluconate) per kg body weight was given over one minute.

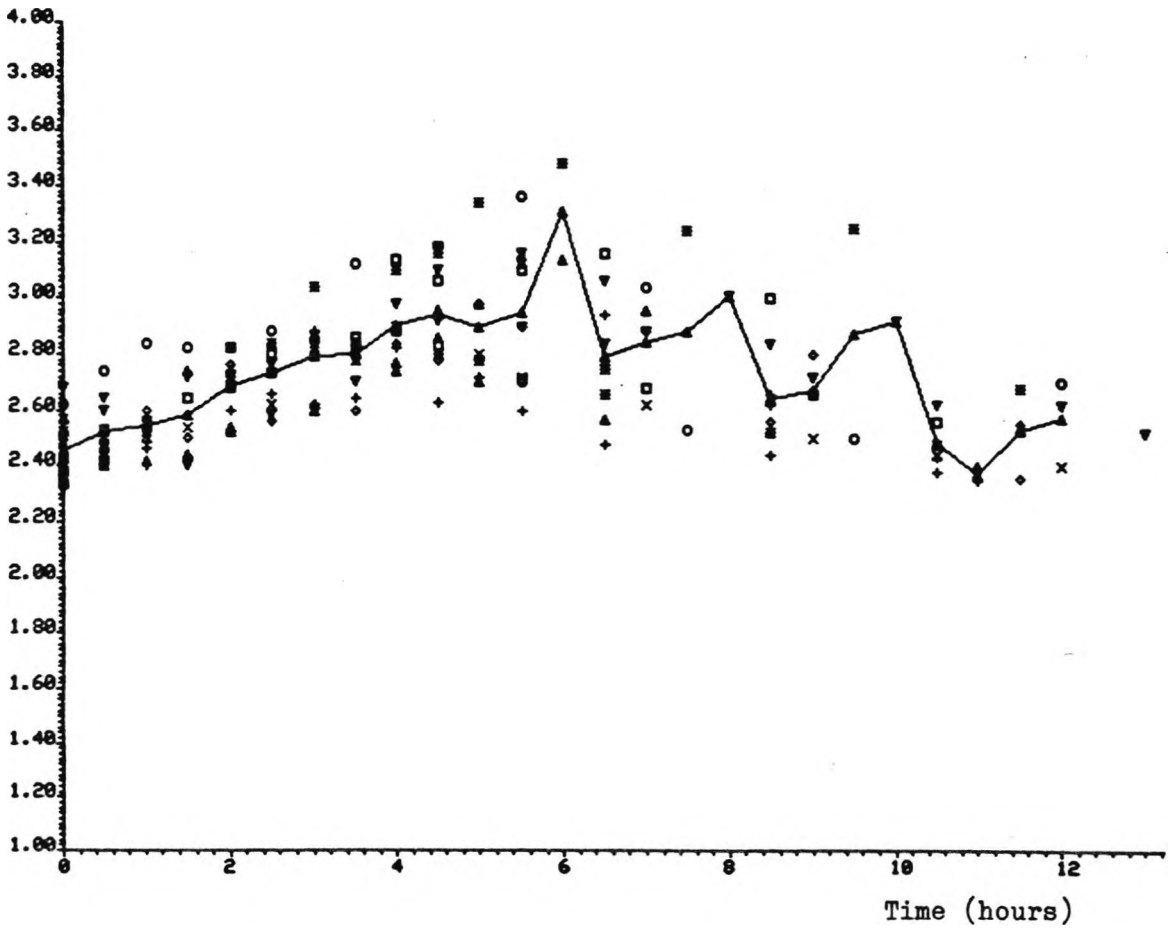


Figure 6.10 The calcium infusion data of Marshall (1980). The plotted line represents the mean at each measurement point. See also Figure 6.11.

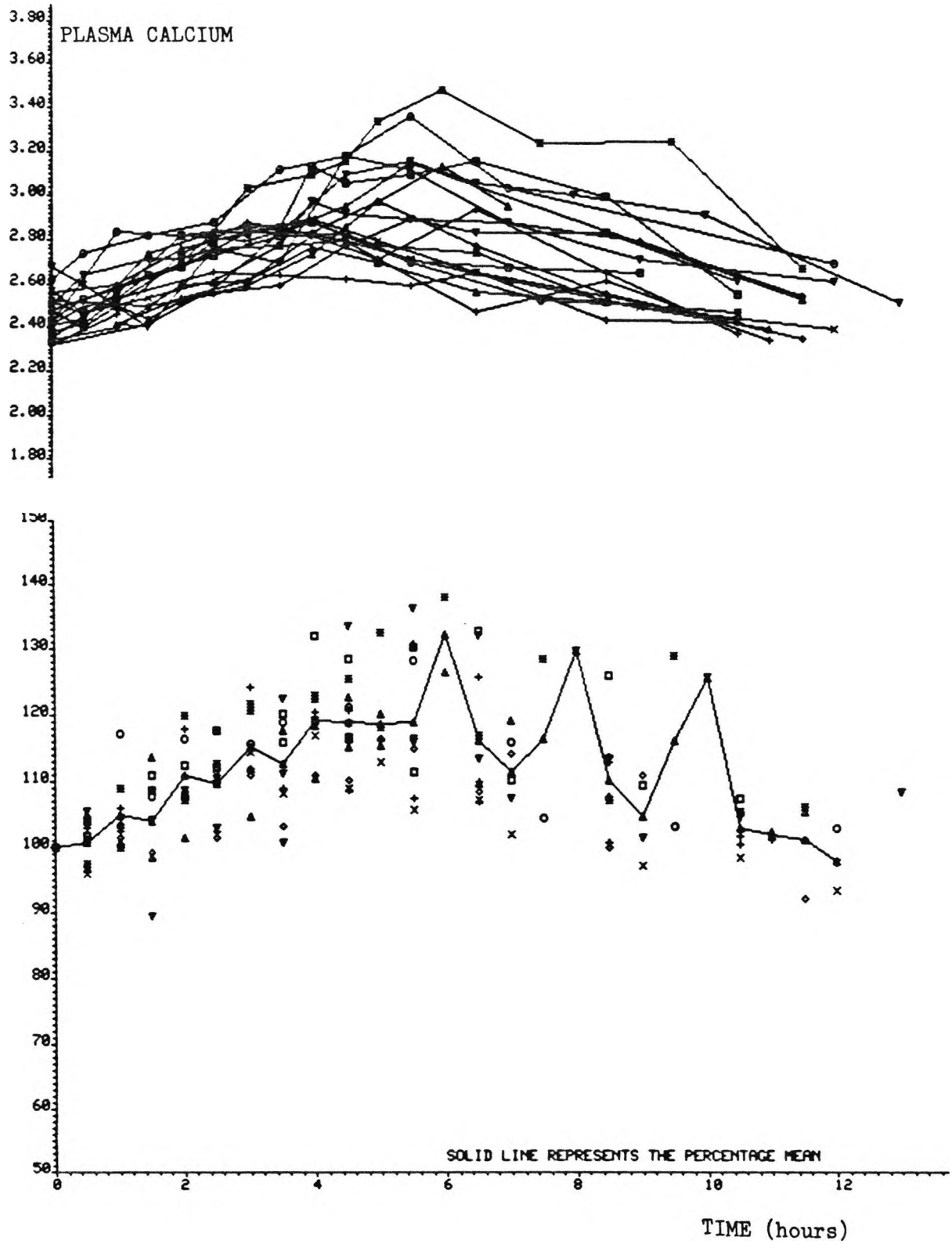


Figure 6.11 Other representations of the calcium infusion data of Marshall (1980). (A) is each subjects individual response plotted over the course of the infusion. (B) is the same data after conversion to a percentage change from each individuals' starting value, but only the maen is plotted. These latter data after conversion to model units, and elimination of those measurement times that did not have at least 6 data points, were used for model parameter estimation.

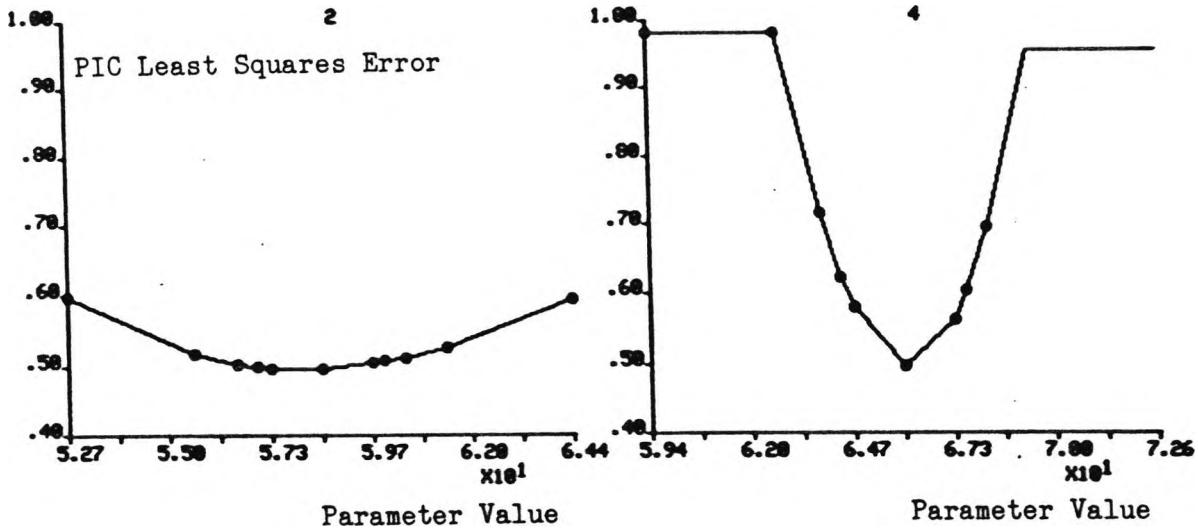


Figure 6.12 An example graphical illustration of parameter sensitivity as used to unequivocally demonstrate the existance of a local optimisation minimum. Thus the optimum parameter value within the parameter range covered is clear. It can also be seen how parameter 4 has a much higher sensitivity coefficient than parameter 2.

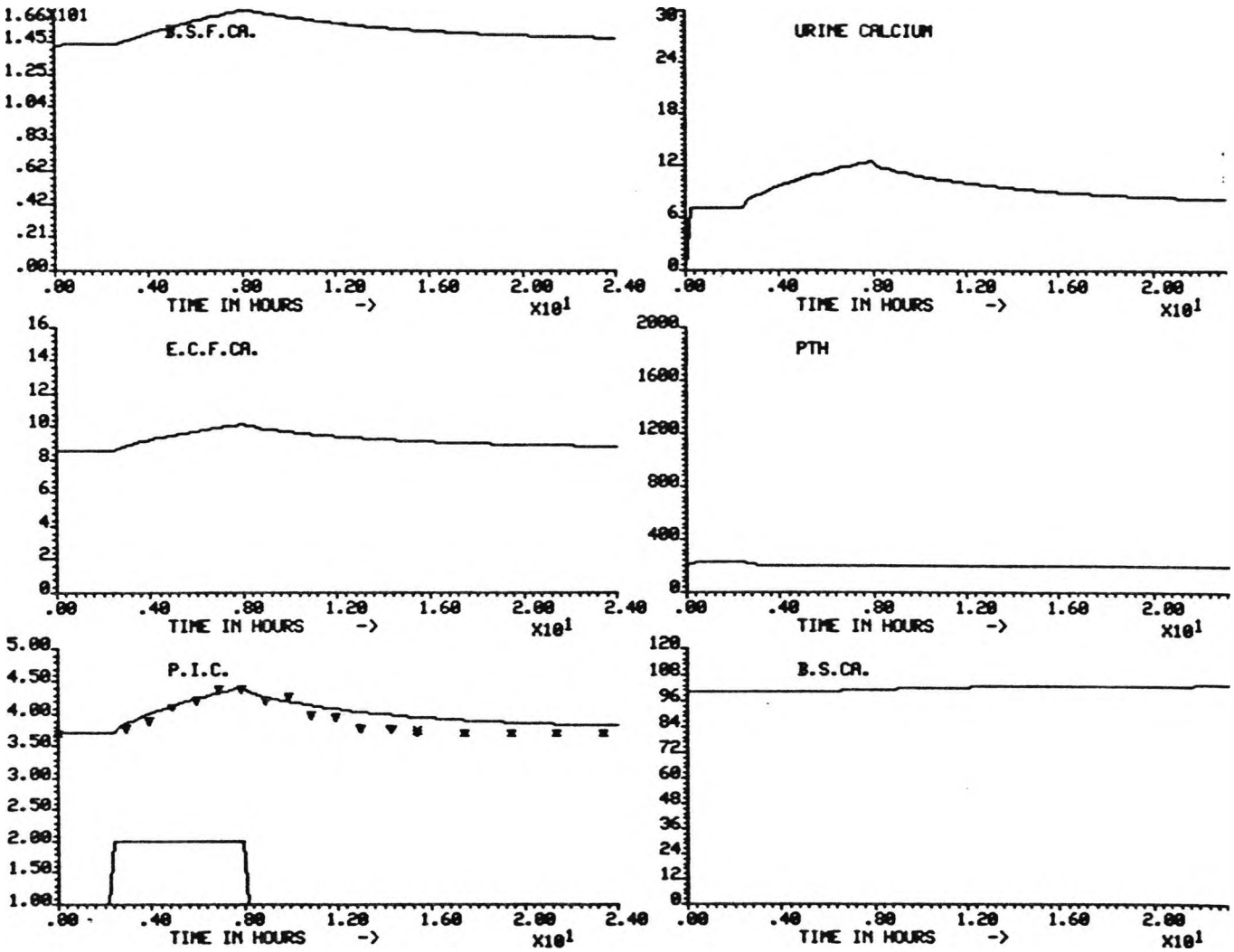


Figure 6.13 A local 'best fit' of MODEL12 to the calcium infusion data of Marshall (1980).

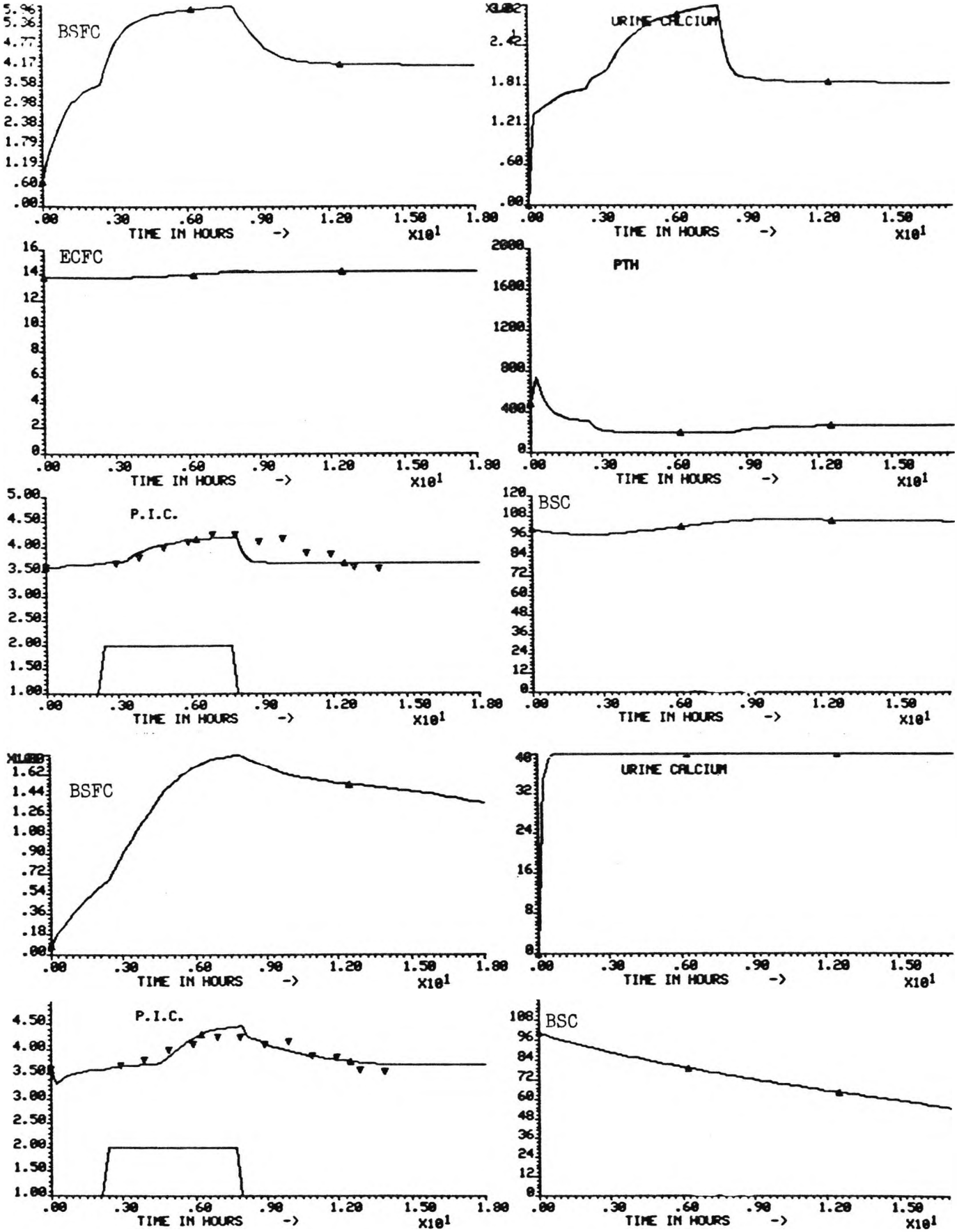


Figure 6.14 Two example simulations with parameter sets that yielded an improved calcium infusion response for MODEL12. Both of these represent part-way stages in the parameter estimation procedure(s) used. Note the steep increase in the steady state mass of bone surface fluid calcium (BSFC) in both cases. Only one of the ECFC and PTH responses is shown as they were very similiar.

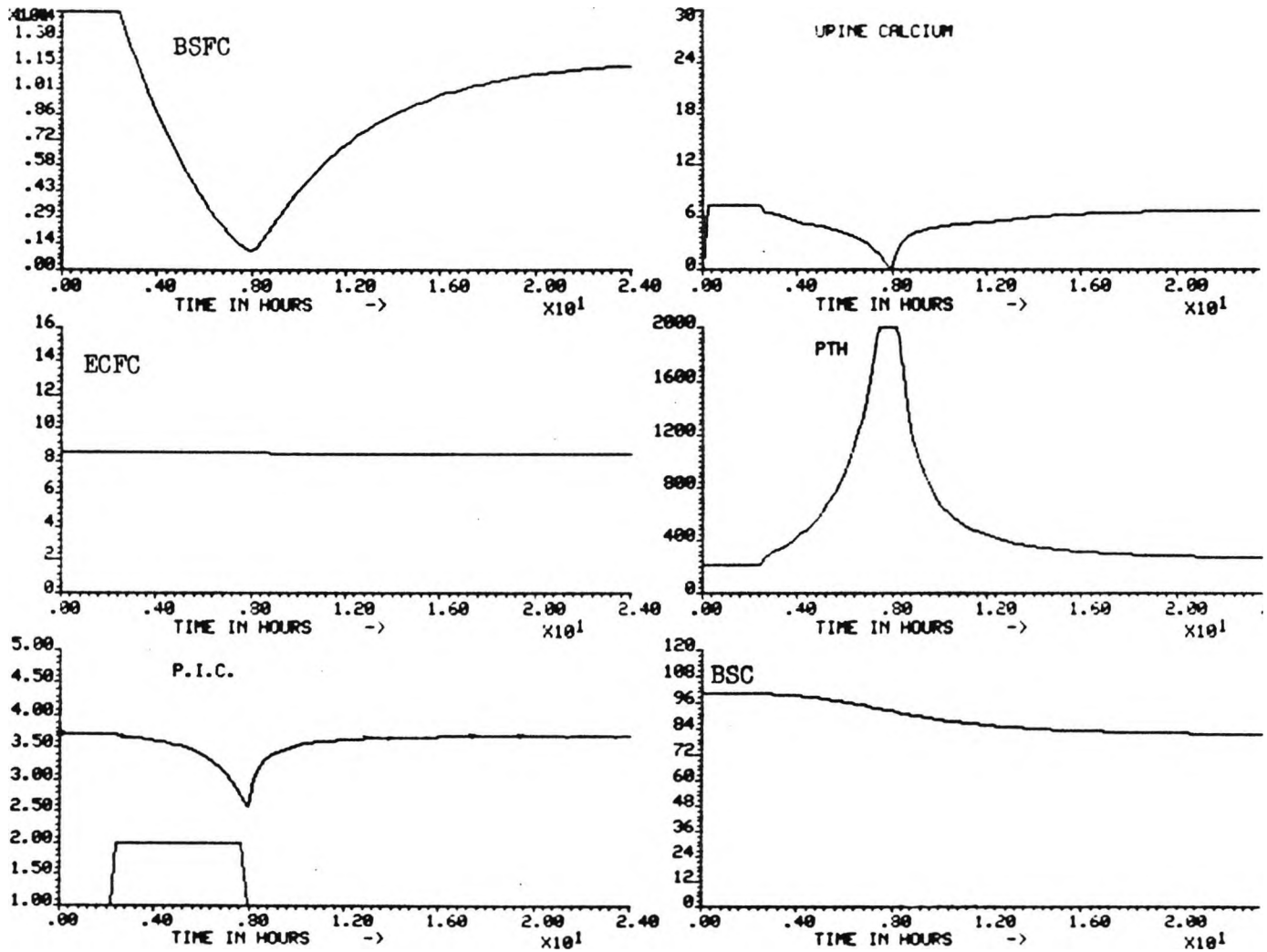


Figure 6.15 The initial unadjusted fit of MODEL12 to an EDTA infusion.

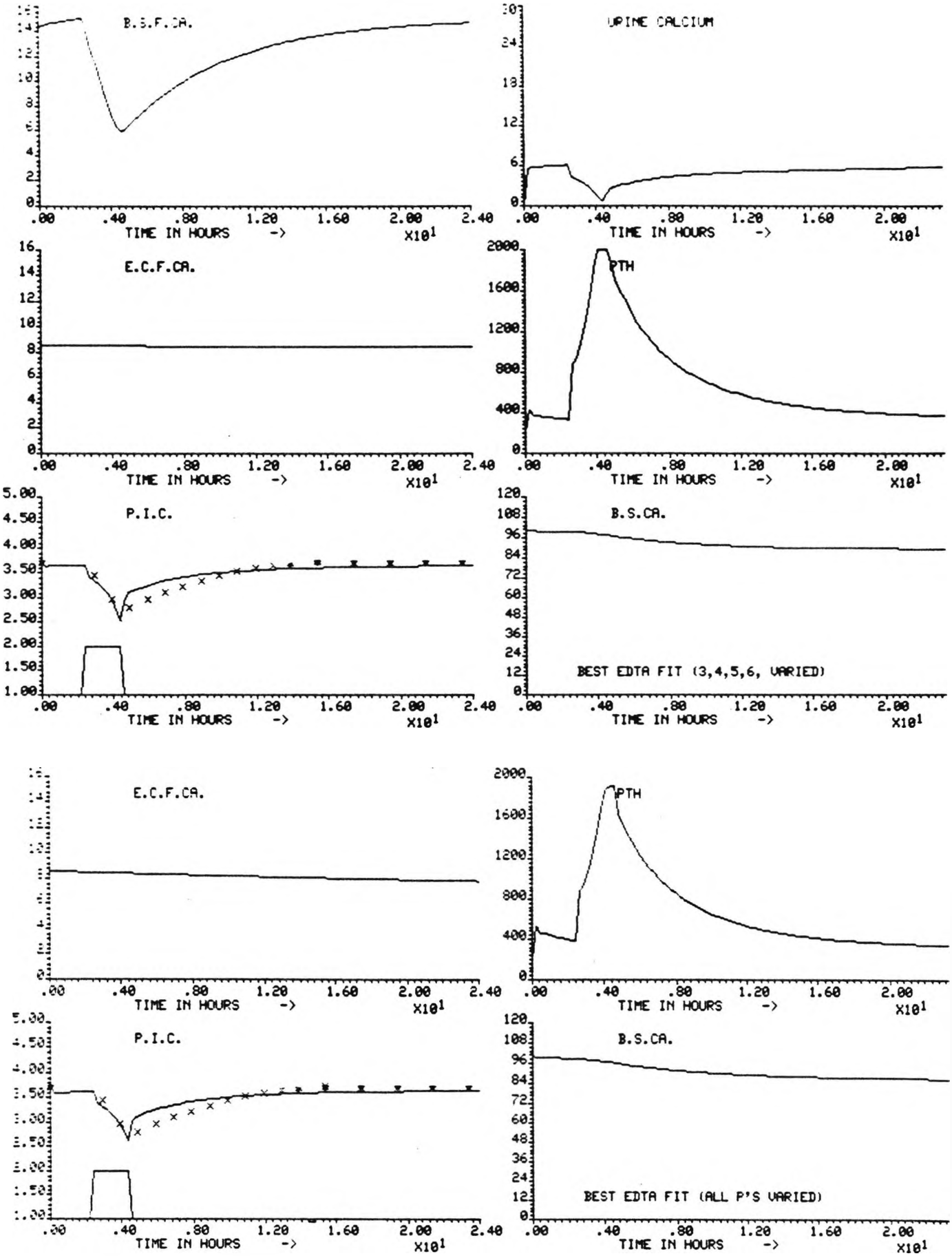


Figure 6.16 The 'best fits' that could be obtained by parameter optimisation of MODEL12 to the EDTA infusion data of Jones and Fourman (1963). Two 'fits' are shown; one deriving from optimisation of a parameter sub-set, the other from optimisation of all parameters. Compare with Figure 6.15.

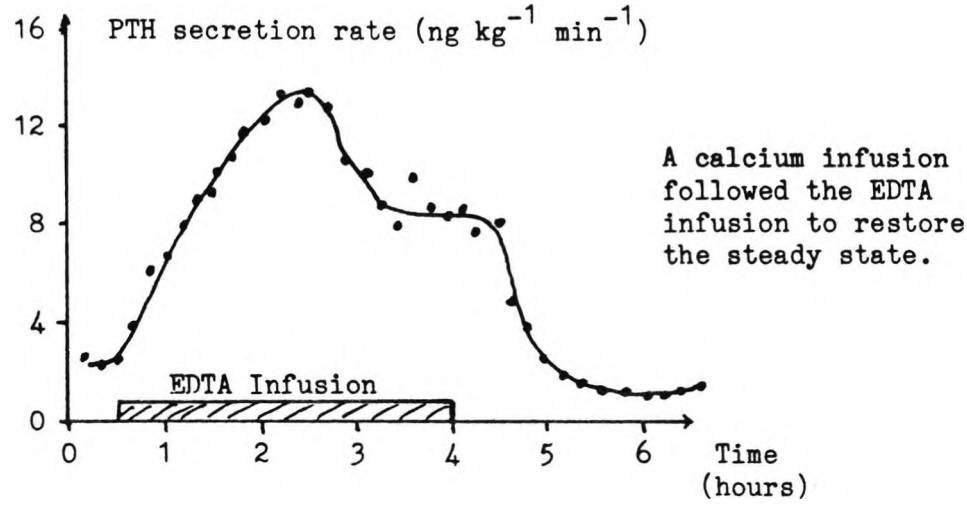


Figure 6.17 The sigmoidal relationship between plasma calcium and parathyroid hormone (PTH) secretion. The PTH secretion rate is directly measured from the parathyroid glad venous outflow. The data are from Jung et al, 1982.

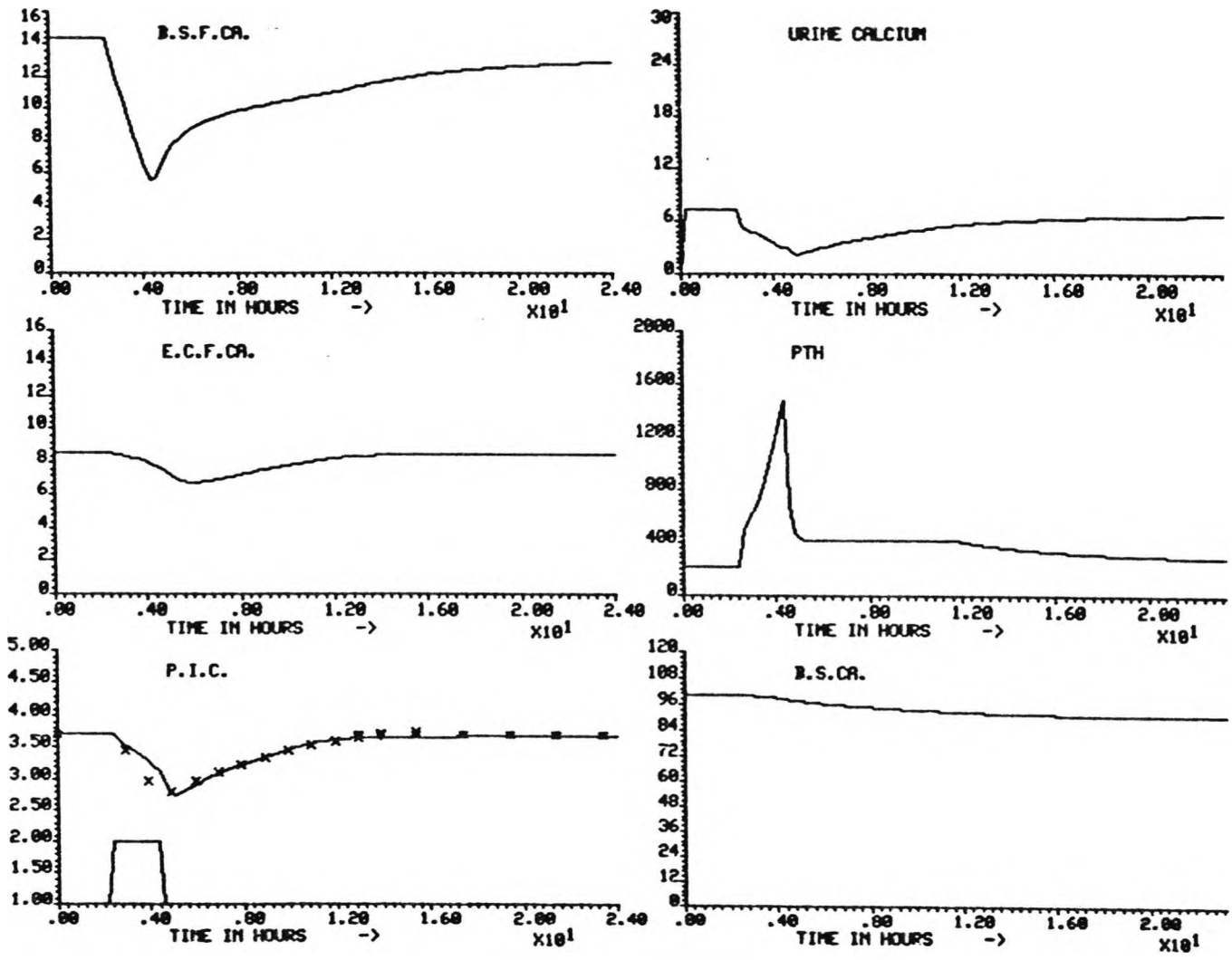


Figure 6.18 The 'best fit' of MODEL17 to the EDTA data of Jones and Fourman (1963). Compare this with Figures 6.15 and 6.16.

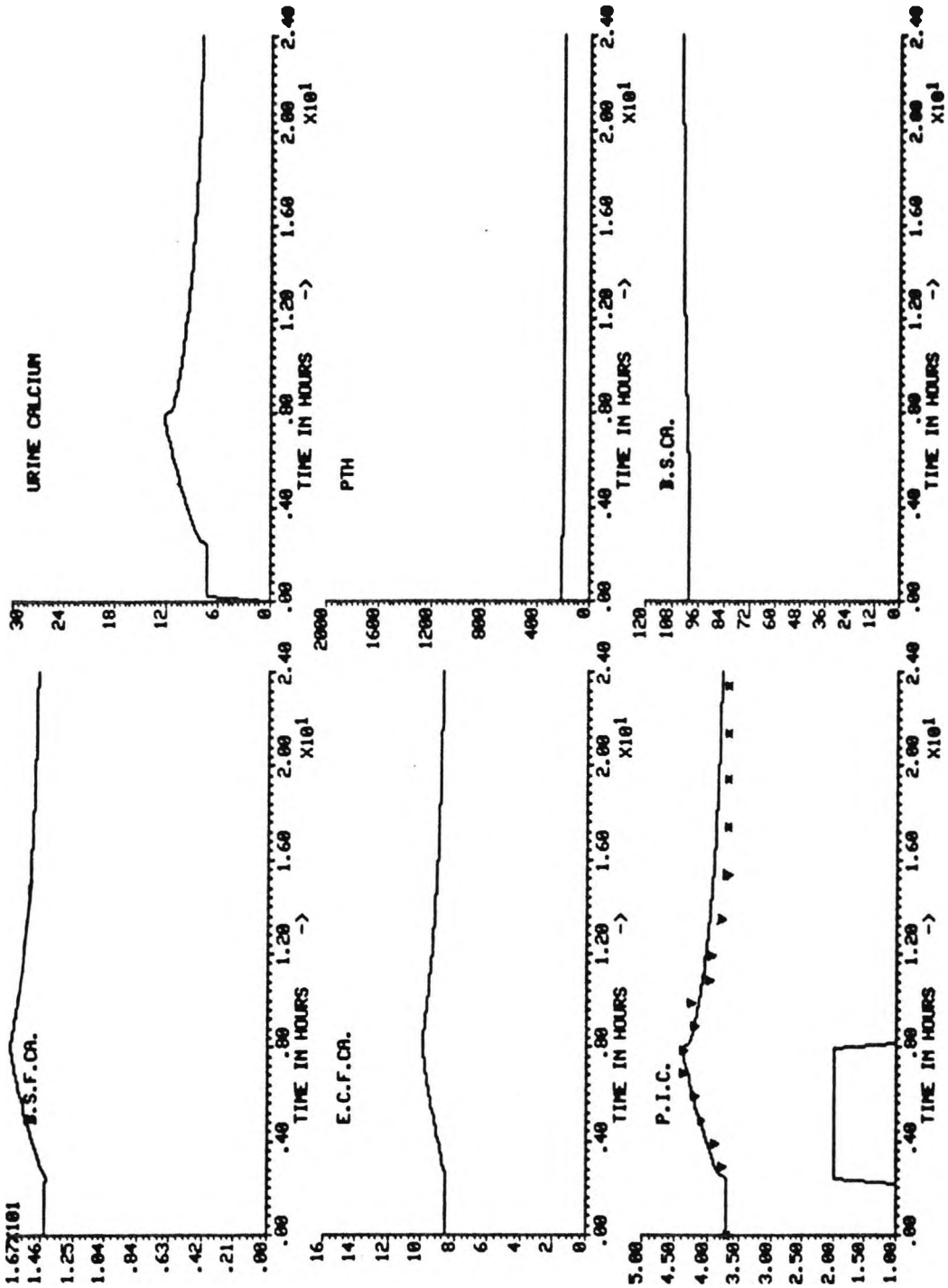


Figure 6.19 The 'fit' of MODEL17 to the calcium infusion data of Marshall (1980). Compare this with Figures 6.13 and 6.14. Parameter values are the same as those used in the EDTA infusion simulation of Figure 6.18.

CHAPTER 7

CHAPTER 7

7. The Validation of MODEL17

This chapter is concerned with the validity or 'performance' of MODEL17. As will be explained, the limits of the model will be explored, thus a comprehensive assessment of the model validity is presented which is not designed solely to 'prove' or demonstrate its 'correctness' but importantly aims as much to demonstrate areas of 'incorrectness'.

The concept of validity is first examined with particular regard to the needs of MODEL17, leading to the definition of a set of criteria or tests specific to this model, that provide a vehicle for the definition of the models 'domain of acceptance'

7.1 The Definition of Validity

Before the question of model validity can be properly considered, it is appropriate to examine and define the concept.

The concept of validity used as an attribute of a model can be described in a number of ways. The word itself - valid - is from the latin validus, meaning 'strong' or 'vigorous', suggesting that a valid model withstands criticism, is easily defended, or is not easily falsified. This is akin to the positivist view of scientific theory - 'a theory is valid until demonstrated to be false' (Popper, 1963).

7.1.1 Validity In Relation To The Development Methodology

MODEL17 and the earlier models were developed in line with the integrated modelling methodology of Carson, Cobelli, and Finkelstein (1983). The structure of this methodology was illustrated in Figure 6.2, and as this figure illustrates, it involves the sequential formulation, identification, and validation of a model; with the incorporation of theories, laws, data, and test results, in all the three stages, as appropriate. Validation is involved throughout the method and presented as the final stage of each iteration through this process, with each iteration producing an 'end deliverable' of a 'validated model'.

This chapter represents the final stage in this sequence, and is concerned with a final assessement of MODEL17 representing the end deliverable of a process that produced 'part deliverables' of MODEL11 through to MODEL16. The processes of identification and validation were involved in the rejection of these part deliverables. These intermediate models were rejected, or improvements were felt to be desirable, because they were effectively in some way invalid.

7.1.2 Validity As The Domain Of Acceptability

Notwithstanding the positivist view, there have been various definitions of validity proposed including: "a valid model is one which satisfies the purpose for which it was intended" (Leaning, 1980), and it is the variants of this definition that are of concern here. Thus Leaning (1980); Carson, Cobelli, and Finkelstein (1983); and Flood (1985), have all tended to view model validity as a multi-dimensional concept for which the yes/no, valid/invalid answer is wasteful. The word waste is used deliberately because useful knowledge or theory could be dismissed on the basis of a shortcoming or falsification that may apply to only a limited domain. The corollary is then also true, a model can be viewed as valid for a particular domain of application, or purpose, or population, or accuracy; and then the process of validation centres on the adequate definition of this domain.

7.1.3 Validity Criteria

Validity as used in this thesis adopts the definition of Leaning (1980), regarding the purpose of the model as determining the critical aspects of the criteria or 'tests' to be used to assess the validity of the model. This consideration has given rise to a number of definitions or taxonomies of model validity related to the range of potential purposes that a model can attempt to satisfy. Similarly a taxonomy or even hierarchy of validation methodologies (Leaning, 1980), and of validity criteria (Carson, Cobelli, and Finkelstein, 1983) have been defined; all related to the purpose or aim of the model in question.

Carson, Cobelli, and Finkelstein (1983) have defined two forms of validity criteria:

> Internal - concerned with the structure and representational validity of the model. This is split into 'consistency' and 'algorithmic' validity.
>
> External - concerned with model performance and comparison with the external world system that the model represents. This is split into; 'empirical', 'theoretical', 'pragmatic', and 'heuristic' validity.

These will each be considered in more detail.

7.1.3.1 Internal Criteria

The internal criteria will often necessarily be met as an intrinsic consequence of the process of model development. Thus model consistency refers to consistency with accepted theory or knowledge regarding the system, and also mathematical consistency with regard to parameter values, units, and ranges. Algorithmic validity

criteria are concerned with the extent to which the model is solvable, can it actually provide numerical predictions?

7.1.3.2 External Criteria

External criteria are largely concerned with factors outside of the model itself, and referring back to Figure 6.2; these are: purpose, theory, and data. For an examination of the validity of a model after an involved process of development these four criteria are the most important and stringent. Considering each in turn:

An 'empirically' valid model should generate predictions that correspond to the available data. For biological models this is often poses problems because of the paucity of data available, the fact that many data are fundamentally inaccessible in the living organism, and also the questionable accuracy of the data themselves. Despite these potential problems empirical validity is the concern of most modelling.

Theoretical validity requires the model to be consistent with accepted theories or models. Any shortcomings in this direction will be apparent as 'assumptions' used in model formulation and development, or the subject of learned argument!

Pragmatic validity is potentially the most important set of criteria for many models, being concerned with the degree of satisfaction of the initial objectives. Thus empirical validity criteria may have to be met before pragmatic validity can be considered. Measures of effectiveness can be defined for specific model objectives, and it may to be possible to categorise these.

Heuristic validity concerns the capacity or potential of the model for scientific explanation, insight, hypothesis testing, or discovery. This criterion is difficult to define measures of effectiveness for, but as the process of scientific discovery or explanation is ongoing, a measure of effectiveness is inappropriate.

7.1.4 The Choice Of Validation Criteria For MODEL17

As introduced in section 7.1.3, specific step by step or prescriptive validation methodologies have been proposed for the validation of various types of model. These inlude the alpha, beta, gamma, delta, epsilon validation methodologies of Leaning (1980); that are respectively designed to cope with: model-data comparison, empirical validation of well founded models, theoretical-empirical validation of models which are not totally empirically based, pragmatic validation of models with utilitarian objectives, and heuristic validation of innovative models.

The prescriptive methodologies can all be regarded as examples, which should be tailored to suit the model under consideration, on the basis that the differences between models and their objectives can be more important than their similarities.

Thus it is appropriate to consider the features of MODEL17 that are relevant to its validation. The model:

> Is an example of a theoretical-empirical model that was developed with a combination of theoretical knowledge concerning the underlying system, together with some empirical data.
>
> Was developed to provide explanatory insight regarding system behaviour over the short-term.
>
> Is theoretically unidentifiable because of the number of parameters involved. This implies a degree of uncertainty with respect to both parameters and model structure.
>
> Is the result of a comprehensive model reduction programme, designed to improve model testability.
>
> Has been empirically fitted to the data from two important experimental situations (hypo- and hyper-calcaemia), although still with the attendant parameter uncertainty.

The above factors combine to suggest that MODEL17 is a 'mature' model, that is a significant degree of validity assessement has been involved during its development, such that further tests will have to display a similar or greater complexity. The following validity assessements, divided into internal and external criteria were considered appropriate in the circumstances:

7.1.4.1 Internal Criteria

The model equations are solvable, the parameter values can be varied around their nominal values as would be expected to occur in the human population, an acceptable steady state is observed, and the model is essentially stable in that small perturbations are corrected and a (potentially new) steady state is reached. All of this has been shown through the development process as the intermediate models have been discarded.

It is appropriate to consider the extent to which these criteria are consistent with the modelling objectives, as it is probable that the domain of validity is affected. The most important of these with regard to internal criteria to be considered is the possibility of fitting MODEL17 to an individual person and not the archetypal 'normal' 70 kg man. The resultant condition that must be met (apart from repeatedly fitting the model individually to data from a range of people) is that the model performance must not show an undue sensitivity to variation in the parameter values within physiologically reasonable limits. This will be considered under external criteria, as it is inextricably linked with a pragmatic purpose for model use.

As traditional internal criteria are met in full, the only remaining question as indicated above is the ability to cope with the expected inter-subject variation in model parameters; or expressed another way: can the model be fitted to the majority of 'normal' individuals?

7.1.4.2 External Criteria

Although never ending, as within a range of application a models' validity can be repeatedly confirmed through the use of more exacting tests, certain external empirical criteria are readily available. The nature of the appropriate pragmatic criterion of explanatory usefulness cannot of course be specified, but has been met during model development, and confirmation of further empirical validity will only serve to reinforce any heuristic insight so far revealed or yet to be apparent from the further testing.

The following empirical tests are readily available, and are described in the next sections: simulation of the disappearance of a ^{45}Ca plasma tracer, adequate simulation of sequential hypo- and hyper-calcaemic infusions with a single parameter set, the fitting of one parameter set to the previous three situations (tracer, hypo-, and hyper-calcaemic), a phosphate infusion, simulation of the attainment of hyper- and hypo-parathyroid states, including parathyroid removal, administration of a very short-term calcium bolus to the plasma, the simulation of normal feeding habits and cycles, and possibly PTH infusions and long-term variations in oral input of calcium.

Following empirical testing, consideration of parameter sensitivity and significance of the observed range of parameter variation are considered. Thus some parameters are known a priori with a degree of confidence, having been the subject of a significant amount of research effort, whilst others are to some extent products of MODEL17 only, and indeed are unbservable, and only an a posteriori assessement of their significance could be useful.

7.2 MODEL17 Performance

This section is concerned with assessing the performance of MODEL17 using the test situations and criteria outlined in the previous section. This performance will be of primary importance in the subsequent definition of the domain of model acceptance, and as such it is desirable for the tests to be as wide and varied as possible.

Full details of MODEL17 are presented in Appendix I, and an illustration of its structure is shown in Figure 6.1.

7.2.1 A Combined Calcium and EDTA Infusion

Data from these two situations have been widely used in the development of MODEL17, and the data themselves have been reviewed in chapter 6. Thus Figures 6.18 and 6.19 separately show the best fits that could be obtained to an EDTA and a calcium infusion respectively. No attention has however been paid to the derivation of a single parameter set that gives the best fit to a combined EDTA and calcium infusion. Figure 7.1 shows this very situation, where an EDTA infusion is followed by a calcium infusion, and the model response has been optimised over the full time course.

Optimisation over the whole time course of the dual simulation gave the following as a suitable nominal parameter set:

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	66.0	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.603	day^{-1}	$k_{0,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}	T_{pt}	=	0.077	day^{-1}

Parameters changed to achieve this are $k_{1,3}$ (+130 %) with the infusion magnitudes = + 28.5 (Ca), and = - 150 (EDTA). The significance of this change is unclear, and may be little.

A significant test of the model's response is the examination of the residual errors that occur over the time course of the simulation. Thus it is desirable for the errors to be normally distributed over time such that no systematic deviation can be detected. Figure 7.2 shows the distribution of the residuals over the course of this combined or sequential simulation, and although they are mostly within $\pm$ 5% of the data magnitude at each measurement point, they are not normally distributed over time, with some of the larger errors occurring at the major switching points.

These points represent; the cessation of the calcium infusion, and the cessation of the EDTA infusion. However the largest error of all is observed some three hours after the cessation of the calcium infusion, and it should be considered that the model was optimised for a minimum total PIC sum of squares residual error; and not by hand (and eye) for a normal spread of residuals, which may have yielded a different result.

7.2.2 ^{45}Ca Tracer Disappearance

Although the validity and usefulness of tracer models has been questioned in earlier chapters, the data still represent a significant empirical test of model validity. A model designed to predict the behaviour of calcium should be able to adequately simulate these data.

Data were available from Caius (1981) following the intravenous administration of ^{45}Ca, and are shown in Figures 7.3, 7.4, and 7.5. These data are rather different to those encountered in straightforeward measurement of the physical variable, as they vary between 1.0 and 0.0 (fractional plasma specific activity of the administered dose). Although the measurement error may not perfectly be a direct function of the magnitude of the measurement - the larger measurements might have a smaller percentage error - the use of this assumption enables suitable weights to be easily chosen for each measurement point and hence the error function. This assumption of equal percentage error enables each data point to be of equal significance to the parameter fitting routine. A more sophisticated procedure could take account of the actual observed variance at each measurement point to derive a suitably different weight for each point, but as measurements were only available from 7 subjects, this was not appropriate. The mean data together with the derived weights are shown in Figure 7.4, with the first measurement being given a weight of 1.0.

The data were fitted to the model with the aid of the same optimisation routines used to fit MODEL17 in chapter 6, and details are given in section 6.2.2 with more detail in Appendix II. To set the model up for fitting of tracer data certain assumptions need to be made, as the model is in the steady state, but of course the fractional specific activity is not replenished from outside of the model. Thus most of the equations (only the linear functions) can be used as they stand, but those functions such as the urine output equations that incorporate non-linear dynamics must be 'fixed' in their steady state form despite the steadily falling tracer plasma calcium. It is of course only the radioactive percentage of a small initial dose (that was itself a small fraction of the total plasma calcium) that is falling, it being assumed that the overall plasma calcium is essentially 'steady'.

The good fit shown in Figure 7.6 is obtained with the following parameter set:

$k_{1,2}$	=	3.152	day^{-1}	$k_{1,3}$	=	104.71	day^{-1}
$k_{2,1}$	=	5.289	day^{-1}	$k_{3,1}$	=	32.66	day^{-1}
$k_{3,4}$	=	0.24	day^{-1}	k_c	=	0.1	
$k_{4,3}$	=	1.23	day^{-1}	T_{pt}	=	0.077	day^{-1}

These represent large parameter changes from those established for the single and combined EDTA and Ca infusions. If these values are then used in a normal dynamic simulation, a new steady state is reached within 20 days of:

PIC	=	3.69	ECF	=	6.13	BSFC	=	2.42
BSC	=	13.9	PPT	=	282.0			

These steady state changes are possibly of significance, but perhaps of more interest is the further observation that the calcium and EDTA infusion data can be fitted with these parameters, with a halved calcium infusion rate (13 mmol day^{-1}) and the EDTA infusion rate reduced by 75% to minus 34.0 mmol day^{-1}. These will both be considered in section 7.3 following this performance assessement.

7.2.3 Phosphate Infusion

Figure 7.7 shows a phosphate infusion implemented via a single extra phosphate compartment, that in turn affects BSFCA, the flow of calcium from plasma to BSFC (bone surface fluid calcium). No responsiveness of urine phosphate to PTH is included, urine phosphate being solely a function of plasma phosphate. Thus:

$$BSFCA = BSFCA*PO4/PO4_{ss}$$

Phosphate infusion has been shown to produce a small hypocalcaemia, which has been thought to be a consequence of a direct action upon PTH secretion by plasma phosphate. This is not the only explanation, rather the hypocalcaemia could result from maintenance of a solubility product with calcium, and this further produces an increase in the plasma levels of PTH, which, apart from remedying the hypocalcaemia should also increase the urine output of phosphate, and restore plasma homeostasis. Eisenberg(1970) has shown some limited data from this experimental situation, they are however rather limited in detail, with non-normal subjects being used, so that direct comparisons are not appropriate. The hypocalcaemia shown in Figure 7.7 demonstates that the model is adequate, showing no contradictory behaviour, and nothing more. In addition the lack of a urine phosphate function that is responsive to both plasma phosphate and PTH, contributes to the artificiality of the simulation.

7.2.4 Hyperparathyroidism

Figure 7.8 shows the simulated attainment of an autonomous doubled PTH secretion rate, and the following 2 days. This was modelled by fixing the PTH secretion rate as double the normal value, as might occur in a primary hyperparathyroidism in which the PTH secretion rate remains high and unresponsive to changes in plasma calcium. This method of implementation will give the same results as a model which retains sensitivity to plasma calcium changes, but has a doubled secretion rate at any given level. The question of exactly why the gland is hyper-active and any other effects that might be invoked in such circumstances are circumvented.

When the simulation is allowed to proceed for 50 days (not shown), a new steady state is reached; PIC (+ 6%), BSC (- 16%), BSFC (- 20%), UC (+ 20%), and an unchanged ECFC. These are in line with the observed effects of primary hyperparathyroidism, apart from the reduced 'bone' compartment masses. Some of the changes may not be as severe as in an individual, but hyperparathyroidism also usually involves at least a doubled calcium absorption rate (through the effect of the increased PTH upon the gut absorption mechanism), and this 'extra' calcium was missing in this simulation. If included the plasma calcium would have been higher (despite any resultant increase in urine calcium), and the bone compartments would have seen a higher turnover with possible increases in mass, as has been observed clinically (Knop et al, 1980).

7.2.5 Hypoparathyroidism

The converse of the previous section is now illustrated - the simulation of parathyroid gland removal or malfunction, with PTH secretion dropping to one eigth of normal, and the subsequent attainment of the steady state, over the following 2, 10, and 100 days. The model responses are shown in Figures 7.9, 7.10, and 7.11. The method of implementation as in the previous section obviates concern about the manner in which the reduced PTH secretion is produced, but as discussed in Chapter 2 a common cause of reduced PTH secretion was and can still be accidental surgical removal during thyroidectomy.

It can be seen that the response is not the simple attainment of a new steady state, but considering each variable in turn: PIC drops sharply and erratically in the first 10 hours to a low of minus 20 %, then climbs over the next 20 days to a new steady state that is some 5 % below the normal steady state. The urine calcium mirrors the plasma calcium at a slightly reduced rate for the PIC concerned, while BSC and BSFC both rise to new steady states between 23% and 30% higher over the 20 day period.

The following table from Wade et al (1965) shows the post-operative plasma calcium from 10 patients with an inadequate parathyroid response after thyroidectomy, as such defined as post-operative hypo-parathyroidism. Daily measurements are not available but in every case the plasma calcium is higher at 3 months than at 5 days post-operation, in line with the simulation.

	Post operative time Plasma Ca (mg 100 ml^{-1})		
	5 Days	3 Months	Difference
	9.42	9.24	0.18
	8.8	10.0	1.2
	8.69	9.1	0.41
	8.91	9.95	1.04
	9.03	9.14	0.11
	9.9	10.05	0.15
	7.23	9.45	2.22
	8.65	10.55	1.9
	8.53	8.66	0.13
	8.19	8.95	0.76
Mean	8.753	9.509	0.925 = 10%

The recovery of plasma calcium towards the more normal values observed above is exactly in line with that shown by MODEL17, it is unfortunate that data for the period in between 1 to 5 days post-operation was not available. However it should be made clear that the motive behind the above study was the assessement and improvement of operative technique, and not data collection. Also that techniques have improved such that the incidence of these cases has been reduced (see Wade et al, 1965; and Michie et al, 1965).

7.2.6 Short Term Calcium Bolus

Jaros (1979) has published data concerning the effects of a short-term calcium bolus, physically realised as a one minute calcium gluconate infusion in a young pig, and not a human. A simulation of MODEL17 in this situation is shown in Figure 7.12.

It was suggested that this calcium bolus causes a small hypo-calcaemic response, but when examined closely the the extent of this is in fact very small. The study goes on further to postulate the necessary role of calcitonin to explain the observed small overshoot in the return to normo-calcaemia. MODEL17 does not include calcitonin, and as is argued PTH can be seen to play no part in the observed simulation. The model does not of course display an overshoot, but the response shown is in total

agreement in magnitude and time course, but without the overshoot. In contrast to this work Morimoto et al (1979) studied the effect of a 1 minute calcium bolus in humans as a potential test of calcitonin response, and they do not report the existance of an overshoot; although their data do agree with that of Jaros (and hence the model) in magnitude and time-course. As ever in modelling metabolic systems care must be taken in transferring data from the animal model to the human. This is of particular significance in this situation, the pig is a large litter mammal which is unlikely to behave the same as a adult human.

7.2.7 Feeding Cycles (3 meals per day)

MODEL17 assumes that absorbed calcium is delivered to the plasma in a continuous stream, whereas in reality the pattern of delivery will always tend to mimic the subjects feeding pattern. For the majority of experimental subjects this will invariably mean some variant of three meals a day, even though 24 hour or overnight fasts are commonly used as part of data collection protocols. If the curves produced by Birge et al (1969) of absorption patterns are studied it can be seen that a simple delivery pattern can be used to mimic this, the exact shape of the delivery curve being unimportant for the simulation here.

This simulation represents a significant test of model validity, vis a vis: the data were not used in model formulation or identification, the situation is straightforward to simulate, and the desired model response is expressed extremely simply in a qualitative fashion, and not as a set of measurements - 'the model PIC, ECFC, and BSFC stability should not be disturbed by a cyclical and potentially irregular gut calcium absorption regime'.

Figure 7.13 shows a five day simulation of a cyclical delivery pattern, and a whole range was performed over periods of up to 50 days. All the longer term simulations clearly show the retained stability of the key model variables, and the range of movement of these figures. BSFC shows the greatest variability, regularly oscillating by $\pm$ 7.5 %, and PIC varying between $\pm$ 3.5%. These figures are well within the physiologically expected intra-subject range of variation and demonstrate the inherent stability of the model.

7.2.8 PTH Infusion

PTH infusions have been remarkably difficult to standardise, as can be expected from the lack of standardisation of hormone measurements themselves. When PTH has been regularly used in a therapeutic manner the situation is further complicated by suggestions that the administration regime is of significance. Podbesek (1982) gave

regular subcutaneous and intravenous injections of hPTH1-34 to greyhounds which resulted in rises in plasma calcium as would be expected. But the history of administration was found to be of importance - i.e. a sensitisation or desensitisation process was operating. Attempts to fit the model to these data were not explored in detail, but the model does of course display a hypercalcaemic response to simulated PTH administration.

7.2.9 Long-Term Variations in Oral Input

No simulations were specifically performed with long-term variations in intake for MODEL17, but the starting model (MODEL11) was investigated at some length in section 5.7.1.5. The agreement with observed data in situations involving reductions of phosphate or calcium (mediated through low intake or low gut absorption) was extremely good, demonstrating clinically significant changes in FBC (effectively total body calcium). There is no reason to suppose that this situation has changed were the simplified model to be 're-assembled' back to a structure similiar to that of MODEL11, although the exact influence of the parameter changes made cannot be predicted with certainty.

7.3 Validity Conclusions

Validity is a function of the modelling purpose, intentions and objectives. These were considered in section 7.1, where the further distinction between internal and external validity criteria was discussed. Section 7.2 covered a variety of further 'tests' of model performance that touched on internal but mainly external validity. This section will examine and draw conclusions about the validity and acceptability of MODEL17 in the light of these 'tests'. The 'tests' are tabulated in section 7.3.1 below, together with a subjective relative performance 'score' for each.

7.3.1 Performance Summary

As the table below clearly illustrates MODEL17 shows good qualitative agreement in the majority of test simulations performed. Rigid quantitative agreement is however lacking. This is not surprising considering the extent to which the model is unidentifiable. Particular consideration should be given to those tests not used as part of the iterative model formulation process: tracer disapppearance, hyper- and hypo-parathyroid states, administration of a calcium bolus, normal western feeding behaviour, and long-term variations in absorbed calcium.

	Qual	Quan	Comments
Ca Infusion	+ + +	+ +	Data limitations
EDTA Infusion	+ + +	+ +	Data limitations
CA + EDTA	+ + +	+ +	Data limitations
Tracer	+ + +	+ + +	Good data and good fit
CA + EDTA + Tracer	+ + +	+	Unique set does not exist
PO4 Infusion	+	+	Not surprising
Hyper-PTH	+ +	+ +	Good agreement - some data
Hypo-PTH	+ +	+ +	Good agreement - some data
Ca Bolus	+ + +	+ +	Good agreement - some data
Feeding Patterns	+ + +	+	Test is only qualitative
PTH Infusion	+		Limited data
Long Term Variations	+ + +	+	Good agreement - some data

Accepting that internal validity criteria have been met in full by virtue of the the successful simulation of a range of situations, it is appropriate to refer back to the types of external validity criteria introduced in section 7.1.3.2. Thus the model is 'theoretically' valid insofar as much accepted theory has been incorporated in the model, and a high degree of heuristic validity can be discerned from the insight shown during the development phases, and the specific set of tests described in section 7.2. This enables a measure of pragmatic validity to be ascribed to the model as the derivation of insight was a primary objective. The details of the empirical tests have demonstrated shortcomings in one or more of the parameters, model structure, and available data. These will be covered in turn:

7.3.2 Parameter Sensitivity

MODEL17 is of of a greater complexity than allows identifiability - there are likely to be numerous suitable parameter vectors that fit the available data - but plausible solutions have been found. The plausibility of these can be looked at by examining the sensitivity of the parameters. Importantly MODEL17 is physiologically based, and the parameters used are consistent with this physiology and it is fully reasonable to explore these parameters without the guarantee of uniqueness.

The sensitivity of the parameters was explored both as part of the individual simulation tests, and specifically as part of the optimisation process associated with (say) fitting the model to calcium and EDTA infusion data. So called 'parameter well' diagrams were produced to explore the significance of the fitted parameter set, all were seen to clearly show the existence of a 'well'. The existance of this 'well' was not affected by any of the parameter changes made during optimisation procedures used. Formal parameter sensitivity analysis (as described in section 6.2.1) as an aid to parameter optimisation, through selection of the most sensitive parameter set was

only partly used in this study. However it has to be said that identification of the parameter set that gives rise to the greatest change in model response was not difficult, visual checks using parameter well graphical displays facilitating this activity..

The range of potential values is satisfactory, thus a 5% change in the value of any parameter will not destroy the model response. This was tested in a number of ways, all involving the random assignment of 'new' values to parameters within an assigned range, and a subsequent simulation. One of these covering ten simulations is shown in Figure 7.14 and it can be seen that the shape of the response is not affected. This is important as the human population is likely to display a similiar inter-subject range of values in most parameters. Numerous sets of ten simulations of this kind were generated and the results were always positive, with the shape of the response maintained.

The feasiblity of totally covering a given parameter space was considered, but even with (say) only one second per simulation, a possible 3 values for each parameter (P_i, $P_i + dp_i$, $P_i - dp_i$), and 10 parameters, 59,049 positions in parameter space exist, which would take approximately 17 hours computer time. This was infeasible and in a formal sense abandoned. However a number of sets of sequential simulations of 1000's of parameter sets drawn from $P_i \pm dp_i$ for all parameters ($dp_i = 0.1P_i$) were generated. The residual sum of squares error for the sequential Ca and EDTA infusion was calculated and one set is displayed in a histogram in Figure 7.15. This clearly shows the inherent stability of the model with some 90% of the parameter sets yielding a sum of squares errors (SES) less than 4.4. This should be compared with the best manual SES for the 2 simulations together of 0.57.

The significance of parameter values and the changes made during empirical model fitting, can be assessed by considering the meaning of each the model parameters. Thus each k_{ij} is a baseline rate constant, most of which are unobservable, and in this situation a 'new' value is as valid as an 'old' one. The parameters falling in this category are : $k_{1,2}$; $k_{2,1}$; $k_{1,3}$; $k_{3,1}$; $k_{3,4}$; $k_{4,3}$; and $k_{0,5}$. Of more significance in this respect is the change in steady state values caused by changes in the parameters, but again the unobservability of every compartment except for PIC leads to the same conclusion - the steady state values are reasonable. In fact BSFC has been reduced significantly back towards the value assigned during the initial formulation of MODEL11 and 12 - now 2.42 mmol and not the physiologically more unreasonable value of 14.0 mmol (see section 6.1).

The infusion rates, initially set at 28 and - 150 mmol day^{-1} were taken from the data sources given, and it is realistic to assume that the calcium infusion concerned might give rise to an effective ionised calcium infusion rate of around half the calculated

value. The much larger reduction in the EDTA infusion rate when trying to arrive at a universal parameter set is much harder to justify. On similar grounds some reduction could be expected, but the extent of this is unknown, some experimental titration in a plasma substitute is called for.

It is interesting to consider the tracer simulation and the observation (section 7.2.2) that the parameter k_c had to be reduced to 0.1 (from 0.385), to produce the best fit. This parameter represents a direct multiplier upon the urine calcium output rate. However faecal loss was incorporated in the tracer simulation, but not in the standard model at a rate of 0.14 mmol day^{-1}, and if the cumulative urine output figures from the simulation are compared to the those observed (not shown); a further reduction in k_c is called for. If k_c is set at 0.04 (rather than 0.1) a good fit (SES = 0.0071) is still obtained with the following parameter set:

$k_{1,2}$	=	2.695	day^{-1}	$k_{1,3}$	=	98.24	day^{-1}
$k_{2,1}$	=	4.969	day^{-1}	$k_{3,1}$	=	32.73	day^{-1}
$k_{3,4}$	=	0.103	day^{-1}	$k_{4,3}$	=	1.412	day^{-1}

These changes from those fitted in 7.2.2 are minor, and yield new steady state variables within 40 days of:

PIC	=	3.67	ECF	=	6.77	BSFC	=	2.4
BSC	=	33.0	PPT	=	325.0			

Again this steady state is very close to that given in section 7.2.2, with good calcium and EDTA infusion fits with the same reduced values as previously given.

The PTH parameters pose interesting questions: their physiological significance. Specifically the meaning of T_{pt}, the time at which PTH secretion greater than a certain amount is supported in a given 24 hour period. This structure was arrived at through consideration of the physiological structure of the parathyroid gland cells. As such it is a logical represention of the size of the store. This parameter could have been varied as part of hypo- and hyper-parathyroid simulations as it could be related to the etiology of these conditions, but was not.

The model was not fitted to an individual but could easily have been were suitable data available. The parameter sensitivity data covered in this section strongly suggesting that the model parameters would not have to be varied very much between individuals.

7.3.3 Uncertainty of MODEL17 Predictions

Because the model is unidentifiable statistically based confidence limits are inappropriate. Even if the model was identifiable the volume of data available is small leading to further problems when defining expected model output ranges, and hence defining confidence limits on the basis of this observed variance. It is possible that statistical measures could be appropriate for a defined important sub-set of the model parameters. This form of assessement was not however used

Various statistically based optimisation procedures were used, (see Roberts, 1977 and Appendix II), but of course the derivation of confidence limits could not be used. It could have been possible as an academic excercise to use generated artificial data of known uncertainty, and fitted the model to a 'standard or perfect' person, and then examined spread when parameters were varied about what was thought to be reasonable inter-individual limits.

Confidence limits surrounding predictions present particular problems associated with the purpose of the limits. Limits are derived from estimates of the measurement variance, and assume that the model is a correct representation of the real world situation. Thus the limit represents the expected limit of measurement error perturbation. Although this could be useful, when dealing with identifiable models, it is inappropriate for the unidentifiable models considered in this thesis. A more appropriate question and hence approach, is the expected limit of model response perturbation given estimates of the parameter variance between individuals. As numerous model parameters do not have a direct physiological analogue - they are products of the model structure and are inaccessible to direct measurement - the inter-individual variance can only be estimated on the basis of a priori estimates from related knowledge. Thus the observation that random parameter changes within $\pm$ 10% will not destroy the shape of the simulated model responses to hypo- and hyper-calcaemic stimuli (see Figure 7.14) is all important.

7.4 Summary

The validity of MODEL17 has been explored in this chapter. This investigation of validity began with fundamental consideration of the nature of validity. The distinction between internal and external criteria was made enabling suitable validity criteria to be defined for MODEL17. The concept of external validity was shown to be important for this model, being concerned with empirical, pragmatic, and heuristic validity. Thus MODEL17 was intended to provide heuristic insight to calcium metabolism, and the means to satisfy this was argued to be the iterative refinement of knowledge as part of the development methodology used; and the further feedback available from direct empirical testing of the model. This direct empirical testing concentrated on situations that were not used in the initial model formulation, specifically; administration of radioactive calcium to the plasma and its subsequent disappearance, the fitting of sequential calcium and EDTA infusions with a combined parameter set.

The use of an incremental approach to model development with careful testing in as wide a range of situations as possible, was shown to provide a model which appears to be a valid representation of the overall processes associated with short-term calcium dynamics. This validity has been demonstrated despite the deliberate omission of a number of structural elements, importantly the omission of phosphate and calcitonin, confirming the assumption that these are not relevant to the short-term dynamics of calcium. The wide range of representational tests performed, showed the model structure to be valid, and of course further confirmed the validity of the PTH model used.

The model was shown to be inherently stable, with parameter variations around the 'fitted' values not ruining the shape of response in both hyper- and hypo-calcaemic simulated infusions. This suggests that the model could be fitted to an individual person, were the data available to do so.

The unidentifiablity of the model inevitably meant that the model parameters displayed some uncertainty. The use of statistical measures was shown to be inappropriate for models of this class. Parameter changes had to be made to accommodate each new situation simulated as a consequence, but it can be argued that because each new test was successfully simulated this demonstrated the validity of the model. Indeed each new test adequately simulated increases confidence that the model will cope with yet further tests.

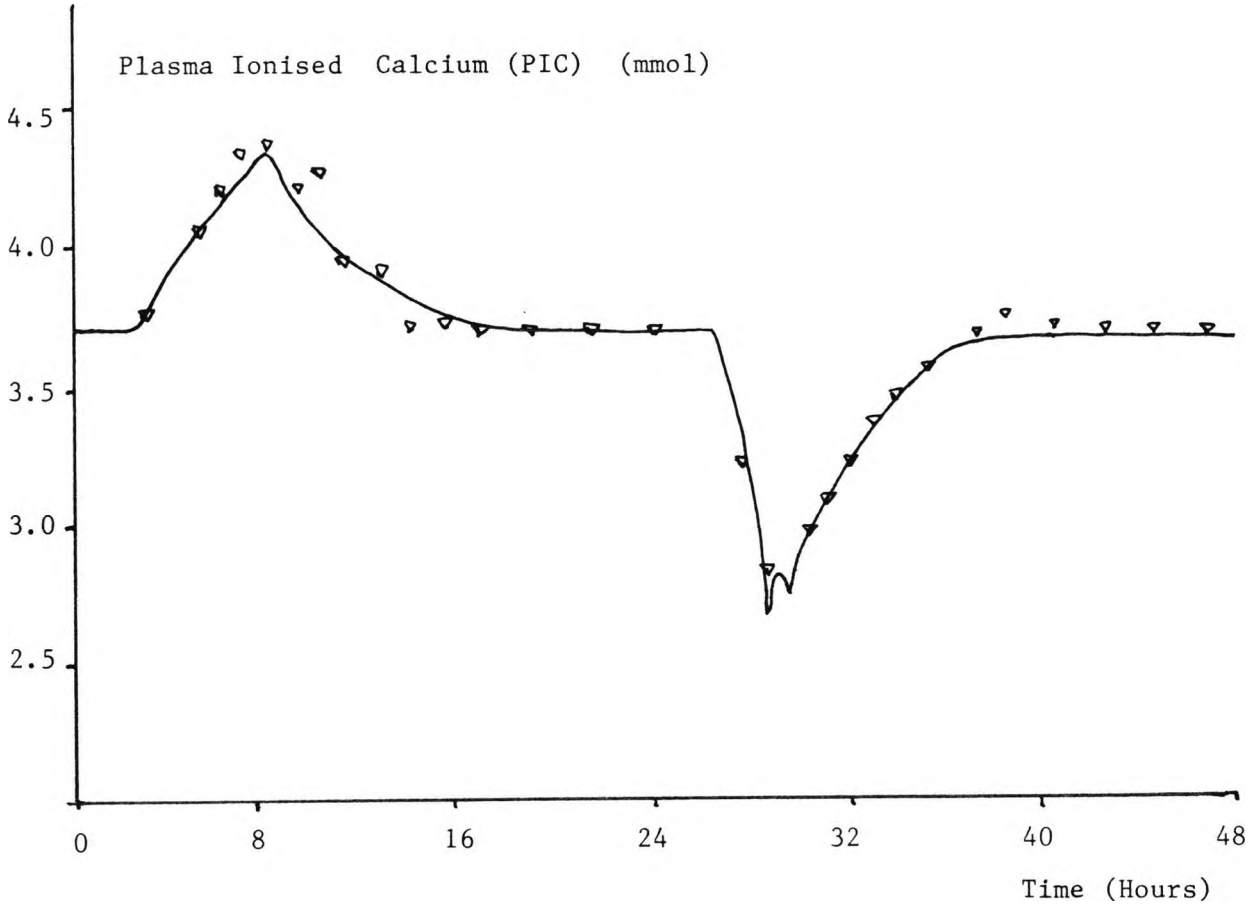


Figure 7.1 The response of MODEL17 to a simulated calcium and EDTA infusion. Simulation details are as given in Figures 6.18/19, but of course applied sequentially. The data used to fit the model response are marked.

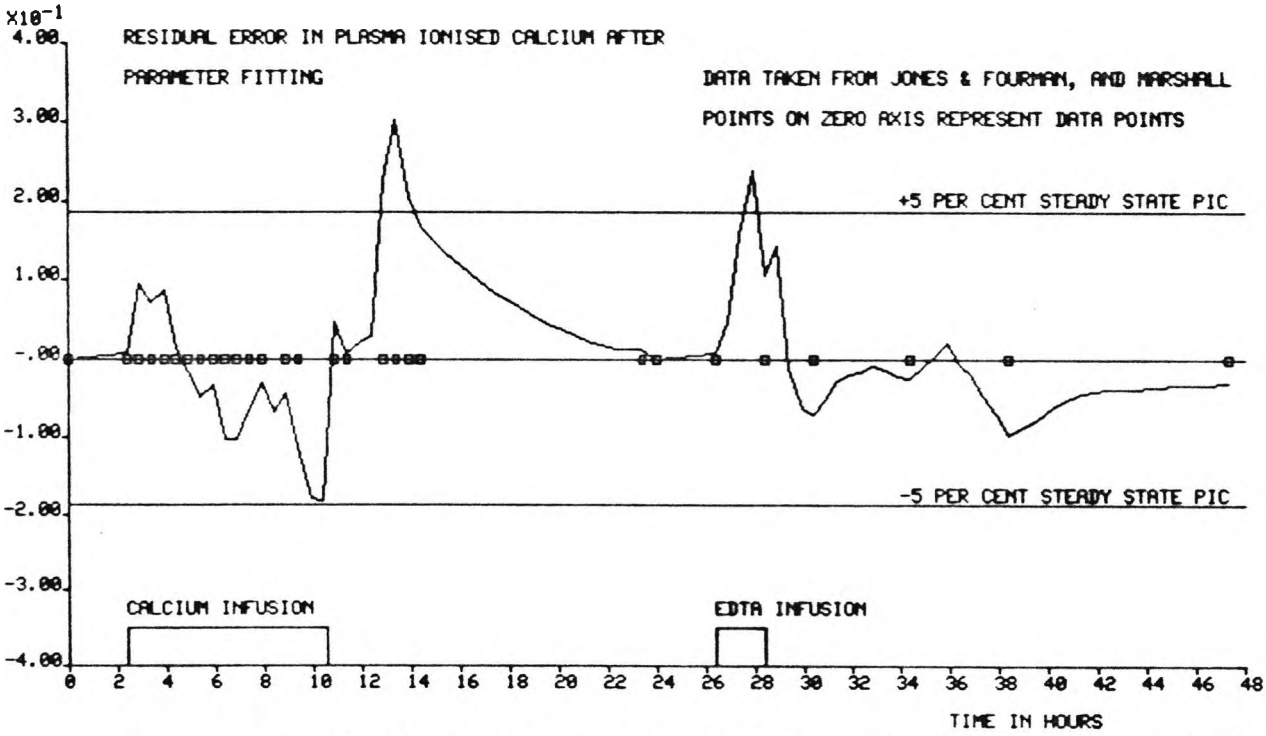


Figure 7.2 The distribution of residual errorsduring the course of the infusion shown in Figure 7.1.

Subject 1		Subject 2		Subject 3	
Time (days)	Count	Time (days)	Count	Time (days)	Count
.0032	.868	.0029	.99	.0078	.969
.0041	.779	.0036	.97	.0107	.908
.0057	.751	.0043	.958	.014	.815
.0079	.707	.0057	.9	.0174	.825
.0109	.652	.0078	.851	.0218	.746
.0143	.782	.0106	.723	.0281	.729
.0176	.581	.0141	.667	.0419	.643
.022	.551	.0176	.612	.0585	.585
.0283	.526	.021	.584	.0702	.585
.0423	.467	.024	.577	.0835	.564
.0572	.432	.0314	.514	.1444	.47
.0699	.433	.042	.485	.1944	.42
.081	.424	.0623	.468	.2521	.377
.159	.359	.0697	.414	.3528	.321
.204	.318	.0862	.373	.425	.316
.248	.303	.179	.324	.506	.298
.331	.266	.269	.272	.954	.235
.412	.255	.359	.248	1.141	.212
.531	.240	.479	.23	1.496	.185
.979	.185	.598	.207	1.969	.174
1.215	.179	.719	.192	2.331	.159
1.552	.16	.939	.151	2.974	.133
1.986	.138	1.428	.135	3.974	.114
2.431	.133	1.928	.11	4.967	.102
2.975	.123	2.946	.096	5.962	.0919
3.987	.106	3.958	.078	6.96	.0791
4.996	.0865	4.948	.068	7.377	.0828
5.992	.0833	5.952	.05		
6.375	.0644	6.927	.053		
7.375	.0636	7.926	.054		

Figure 7.3 ^{45}Ca Tracer disappearance data from Caius (1981). The data shown are the plasma specific activity expressed as a fraction of the administered dose. They are continued overleaf for three further subjects; the seventh is not shown for space reasons.

Subject 4		Subject 5		Subject 6	
Time (days)	Count	Time (days)	Count	Time (days)	Count
0.0057	.927	0.0042	.953	0.0042	.837
0.0077	.836	0.0056	.866	0.0055	0.759
0.0104	.719	0.0077	.768	0.0077	0.658
0.014	.689	0.0104	.66	0.0104	0.664
0.017	.59	0.014	.596	0.0139	0.58
0.020	.626	0.0179	.536	0.0175	0.548
0.024	.572	0.0209	.566	0.0209	0.535
0.031	.514	0.0241	.526	0.0278	0.493
0.0417	.455	0.0313	.497	0.0419	0.438
0.0555	.469	0.0418	.465	0.0577	0.423
0.0677	.431	0.0556	.469	0.696	0.423
0.0833	.36	0.0695	.44	0.0842	0.34
0.123	.336	0.0833	.401	0.1215	0.34
0.187	.296	0.128	.334	0.1865	0.285
0.254	.275	0.183	.297	0.246	0.241
0.333	.238	0.244	.287	0.317	0.225
0.419	.22	0.333	.244	0.384	0.224
0.536	.201	0.414	.219	0.538	0.189
0.93	.156	0.509	.212	0.962	0.165
1.147	.145	0.973	.169	1.174	0.146
1.439	.127	1.194	.145	1.306	0.136
1.932	.119	1.459	.134	1.482	0.126
2.519	.0989	1.979	.116	1.959	0.1174
2.948	.0928	2.462	.101	2.138	0.1106
3.947	.0759	2.97	.0922	2.438	0.115
4.952	.0691	3.977	.0668	2.959	0.106
5.944	.0658	4.968	.0526	3.966	0.0923
6.947	.0567	5.484	.0486	4.98	0.0778
7.333	.0519	6.965	.0383	5.96	0.0681
				6.96	0.0656
				7.424	0.0552

Figure 7.3 - continued, see previous page for details.

Time	Sp. Activity	Weight
0.0042	0.87976	1.0
0.0062	0.80921	1.06
0.0082	0.75298	1.16
0.01	0.71	1.22
0.014	0.66343	1.33
0.018	0.59586	1.47
0.021	0.589	1.46
0.025	0.56241	1.54
0.032	0.52416	1.66
0.042	0.48057	1.79
0.052	0.46542	1.87
0.068	0.43651	2.0
0.085	0.39995	2.15
0.14	0.34427	2.5
0.2	0.305	2.84
0.3	0.26526	3.26
0.4	0.24099	3.52
0.5	0.22762	4.0
0.9	0.18202	4.63
1.2	0.1579	5.3
1.5	0.14463	5.79
1.9	0.12984	6.38
2.4	0.11690	7.21
2.9	0.10611	8.0
3.9	0.08724	9.7
4.9	0.07584	11.0
5.9	0.06736	12.6
6.9	0.05899	14.4
7.5	0.05711	14.9

Figure 7.4 The mean data and weights used to assign an equal contribution of each measurement point to the least squares residual error criterion used to fit the tracer data of Caius, 1981 (see Figure 7.3), n = 7.

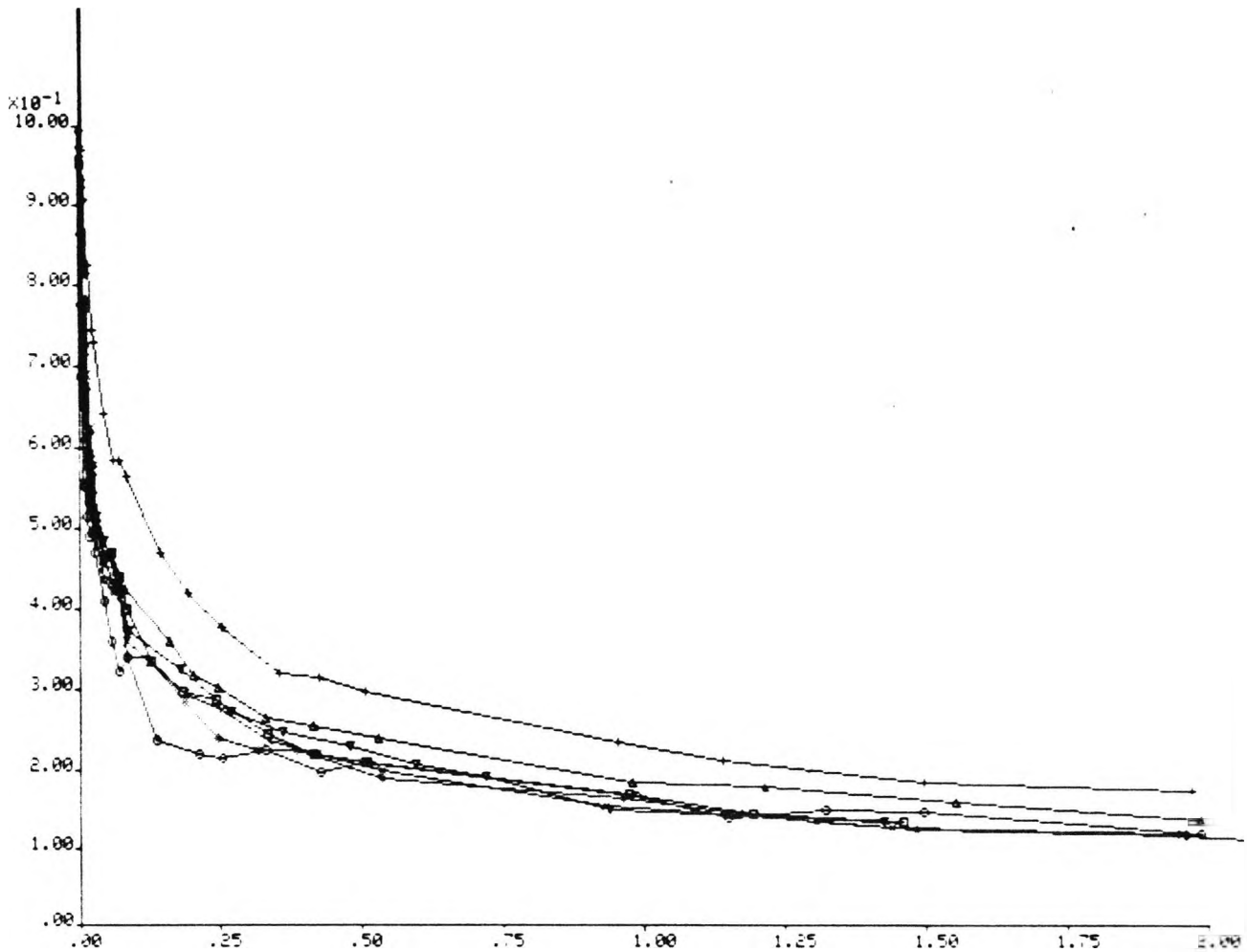


Figure 7.5 The tracer disappearance data of Caius (1981), shown so as to illustrate the variability between subjects, n = 8.

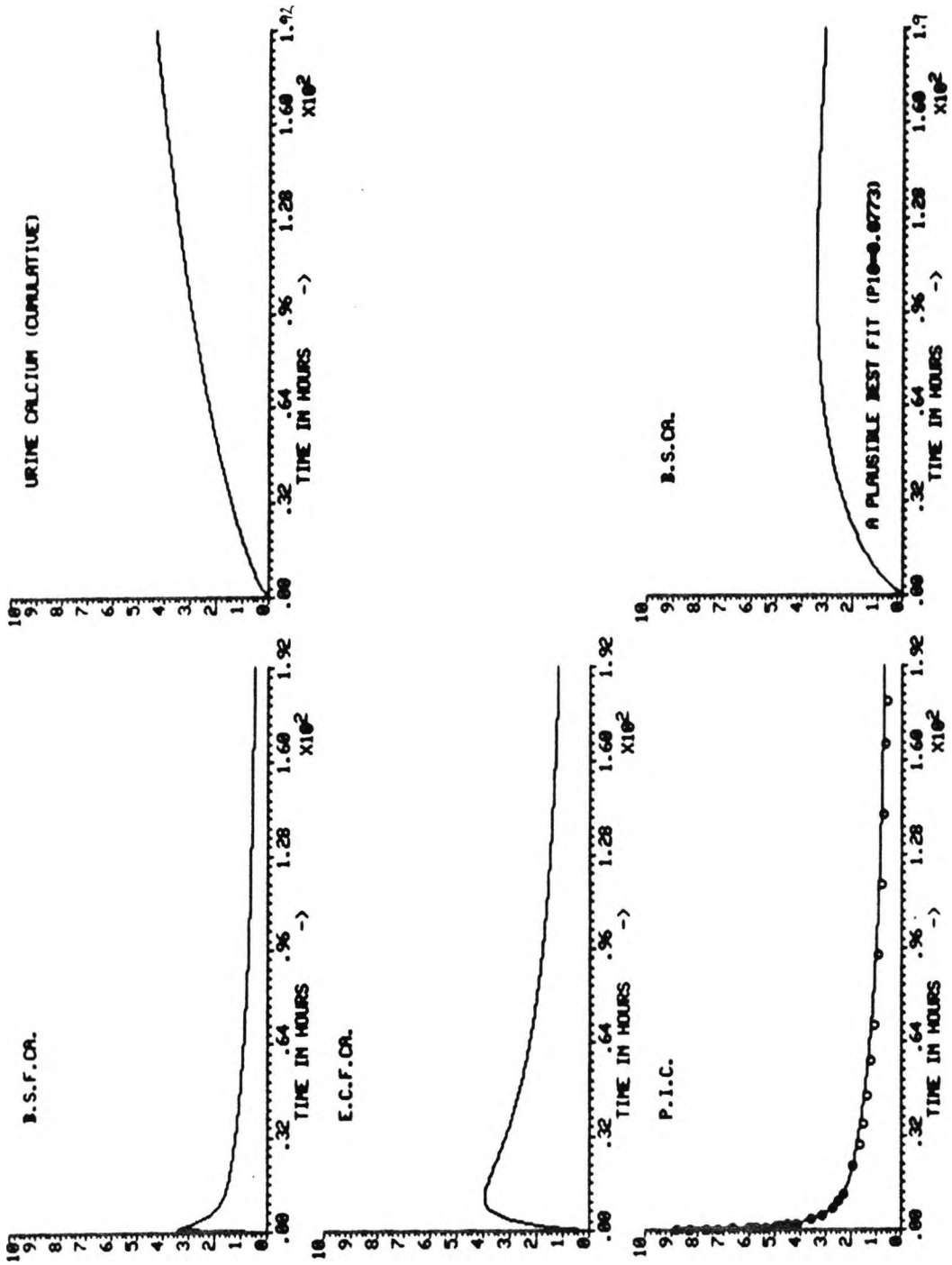


Figure 7.6 The response of MODEL17 to the simulated administration of ^{45}Ca to the plasma. The observed data from Caius(1981) are shown in Figures 7.3 and 7.5.

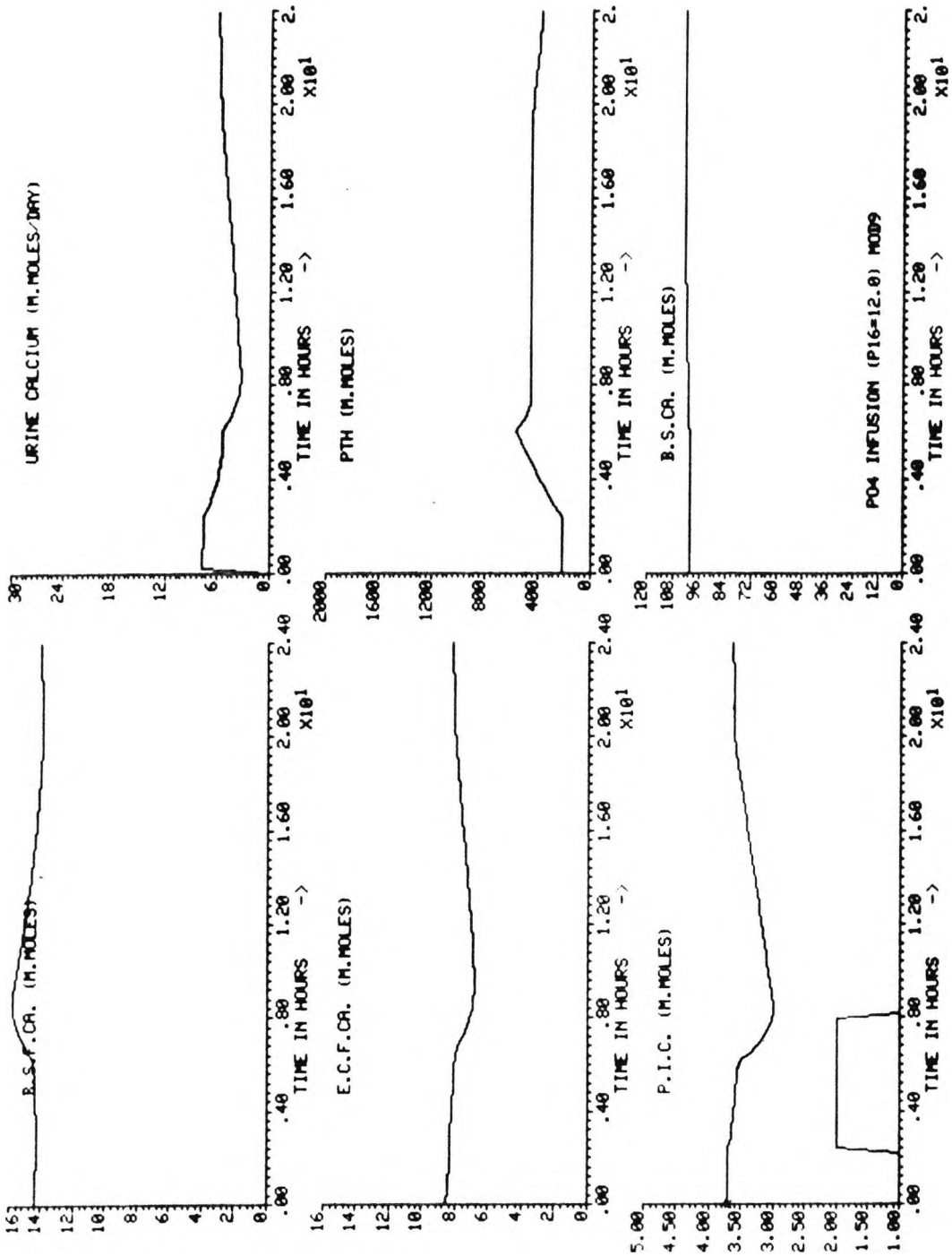


Figure 7.7 The response of MODEL17 to a simulated phosphate infusion. This is modelled via a single extra phosphate compartment that affects the flow of calcium from plasma to bone surface fluid calcium (BSFC).

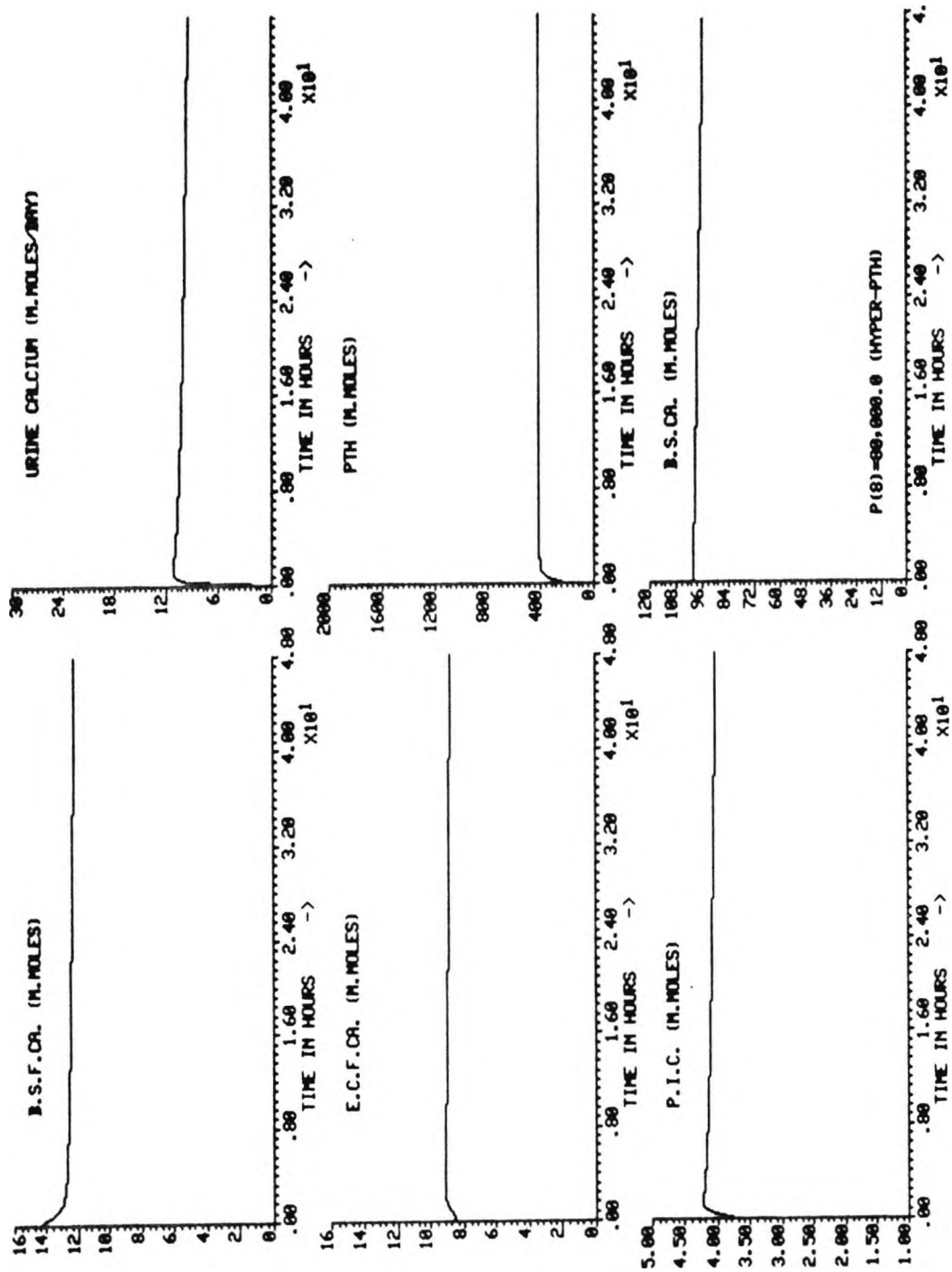


Figure 7.8 The response of MODEL17 to a simulated autonomous doubling of the steady state PTH secretion rate. This is designed to illustrate the attainment of hyperparathyroidism. The duration is 2 days.

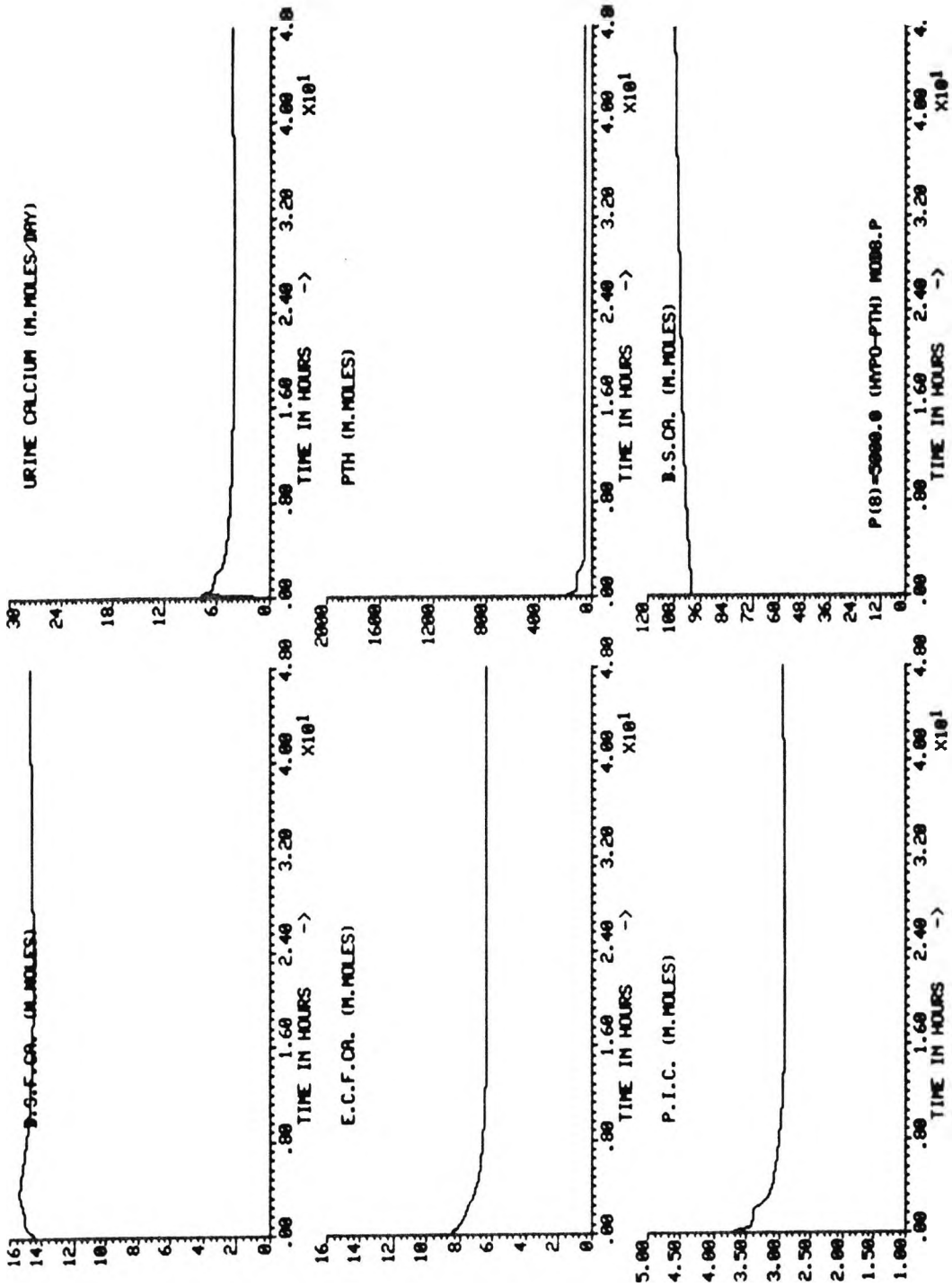


Figure 7.9 The response of MODEL17 to a simulated dropping of PTH secretion to one eigth of its normal value. This represents the attainment of a hypoparathyroid state. The time course represents 2 days.

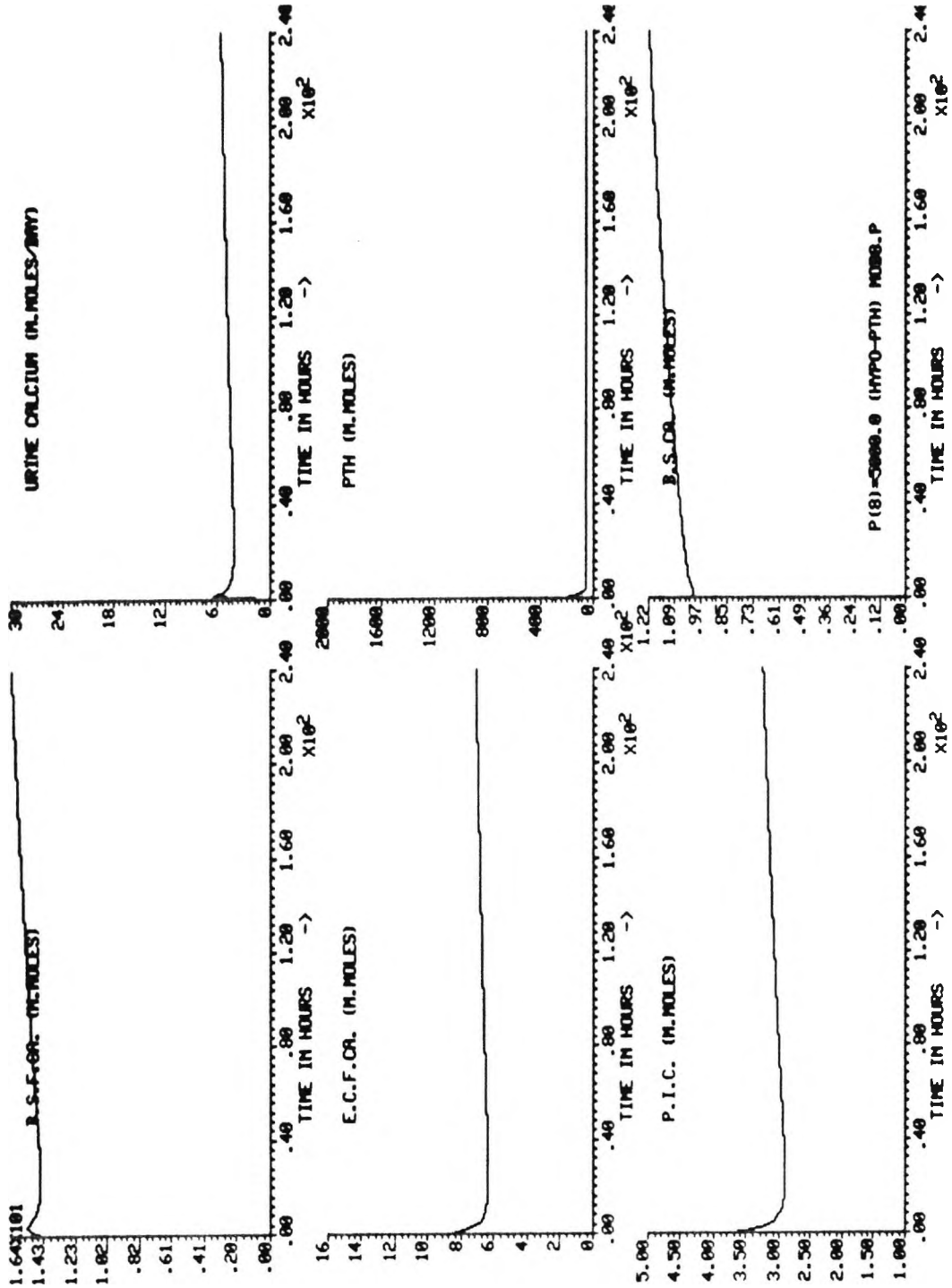


Figure 7.10 The response of MODEL17 to a simulated dropping of PTH secretion to one eigth of its normal value. This represents the attainment of a hypoparathyroid state. The time course represents 10 days.

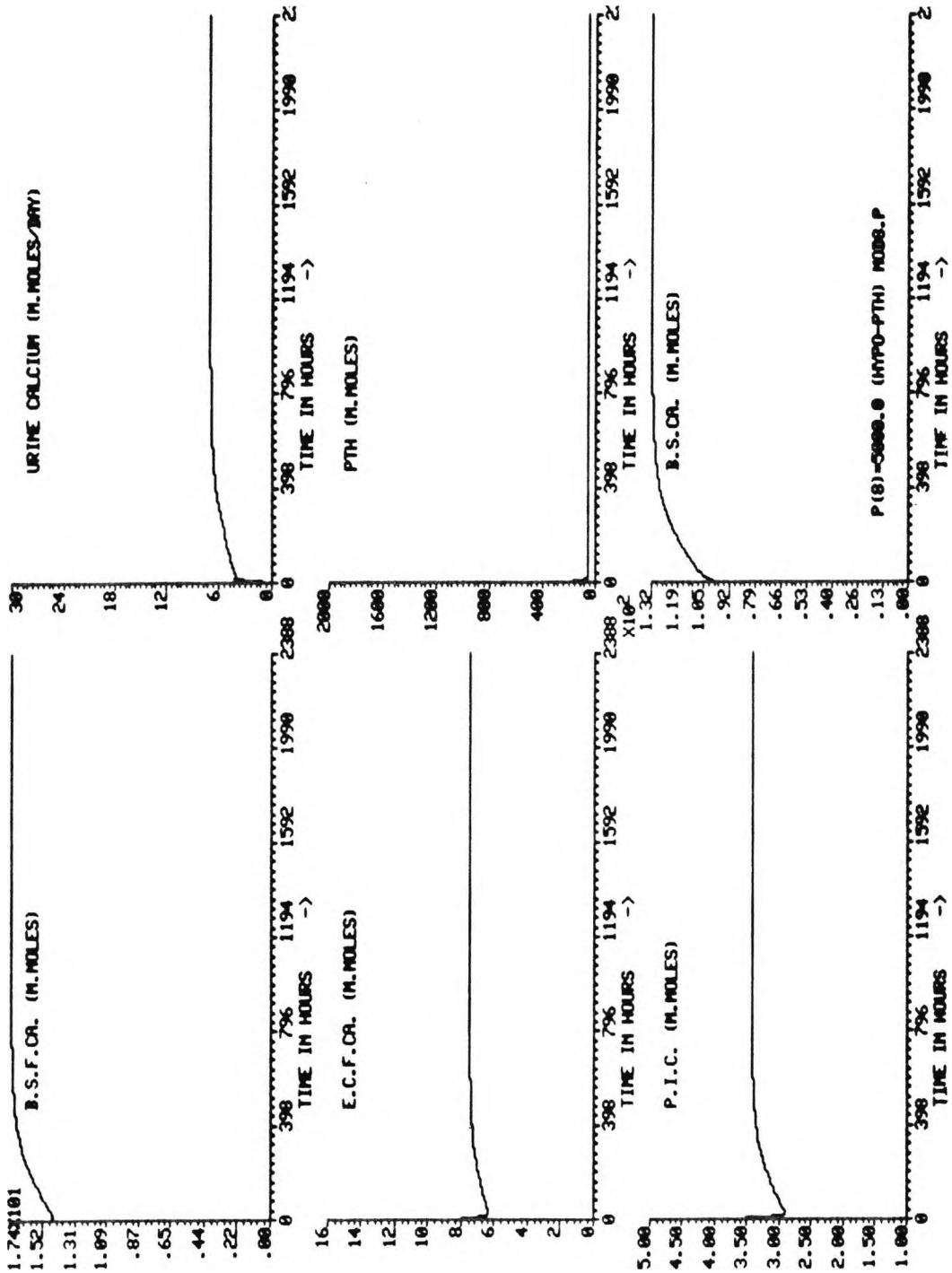


Figure 7.11 The response of MODEL17 to a simulated dropping of PTH secretion to one eigth of its normal value. This represents the attainment of a hypoparathyroid state. The time course represents 100 days.

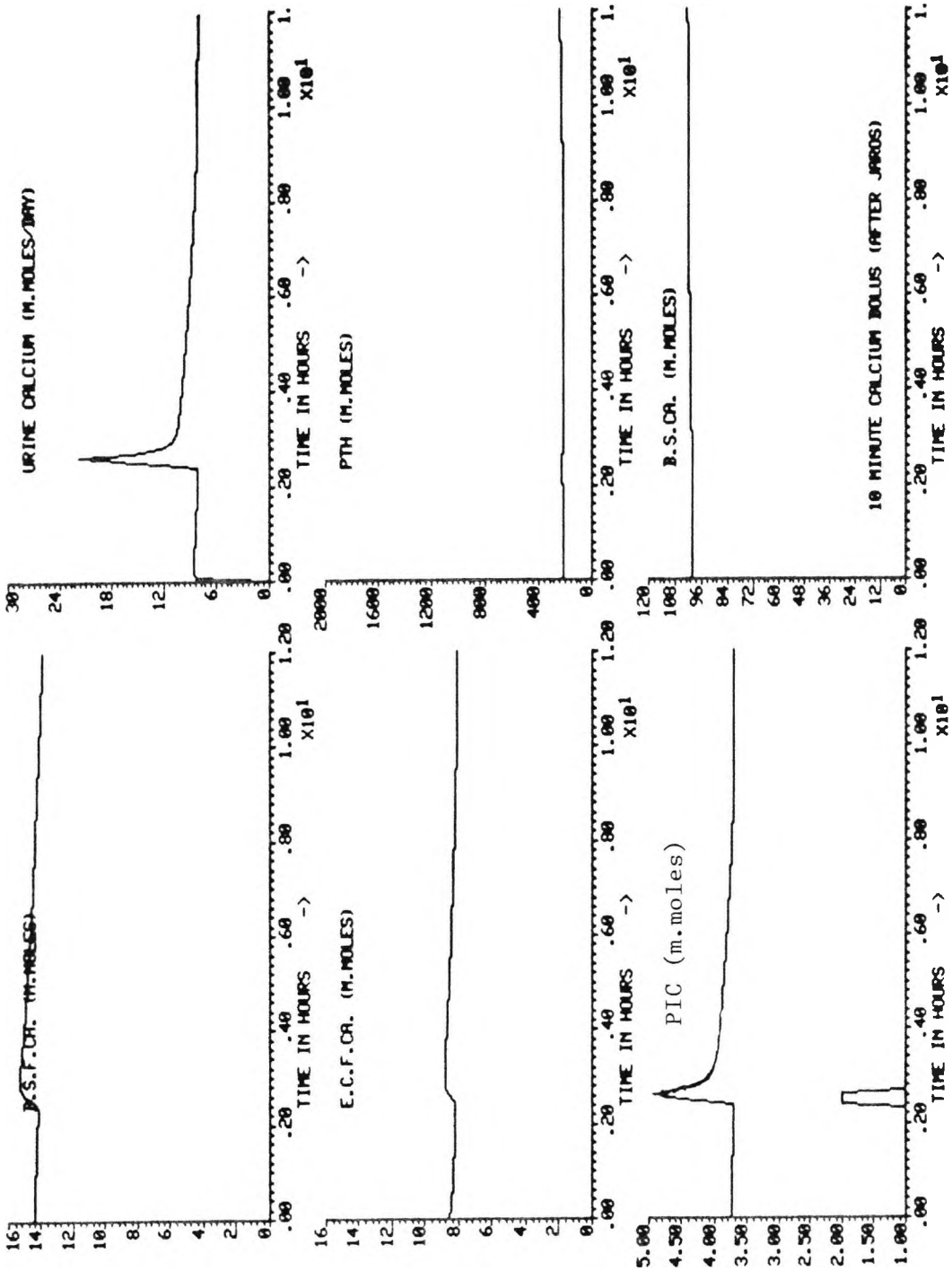


Figure 7.12 The response of MODEL17 to a simulated administration of a short-term calcium bolus to the plasma, over a ten minute period.

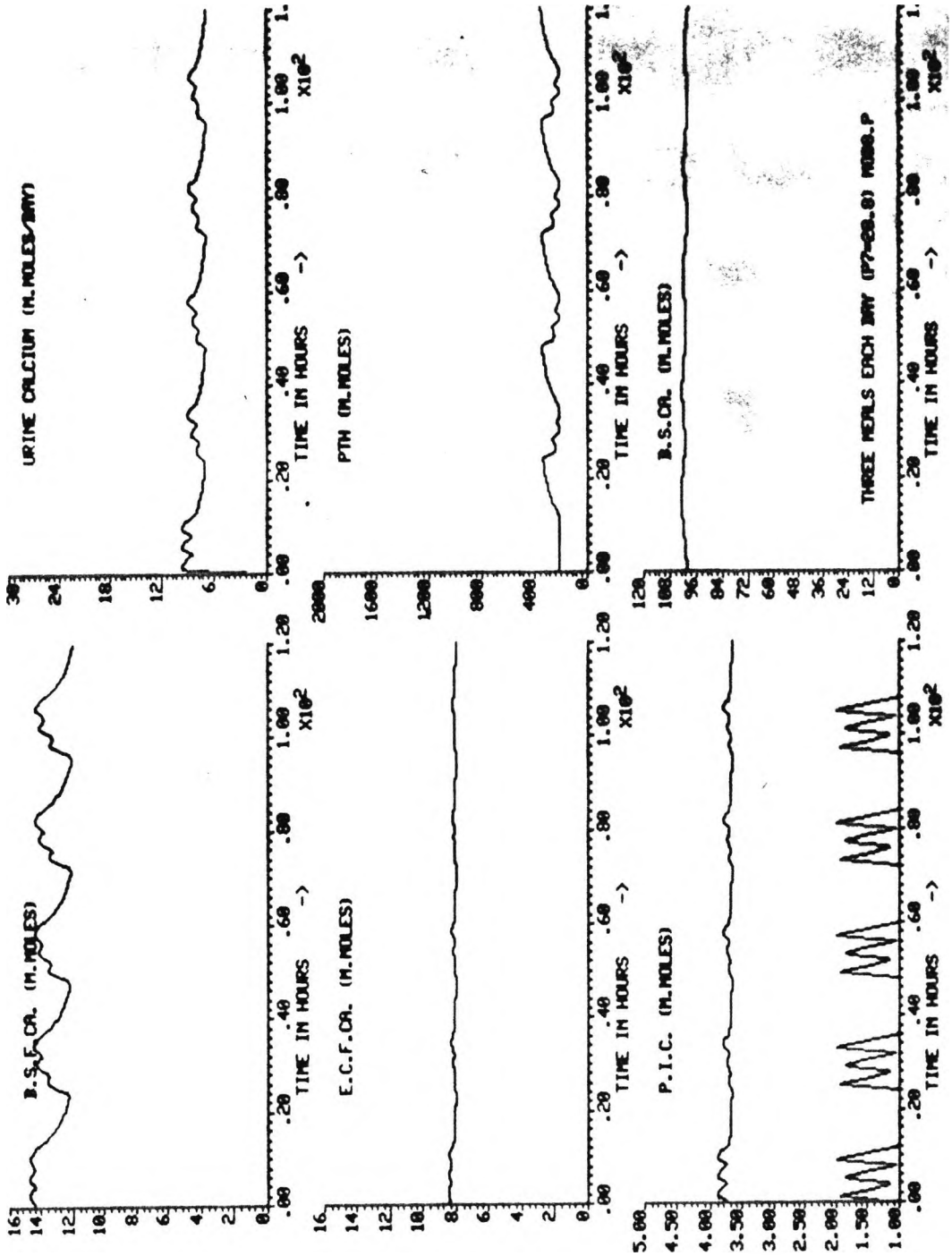


Figure 7.13 The response of MODEL17 to simulated daily variations in absorbed calcium, as would be expected from a normal western diet. The lower graph drawn on PIC shows the variation in rate of absorption. Values are adjusted to give the standard steady state intake. Time course = 5 days. Note the inherant stability of all variables.

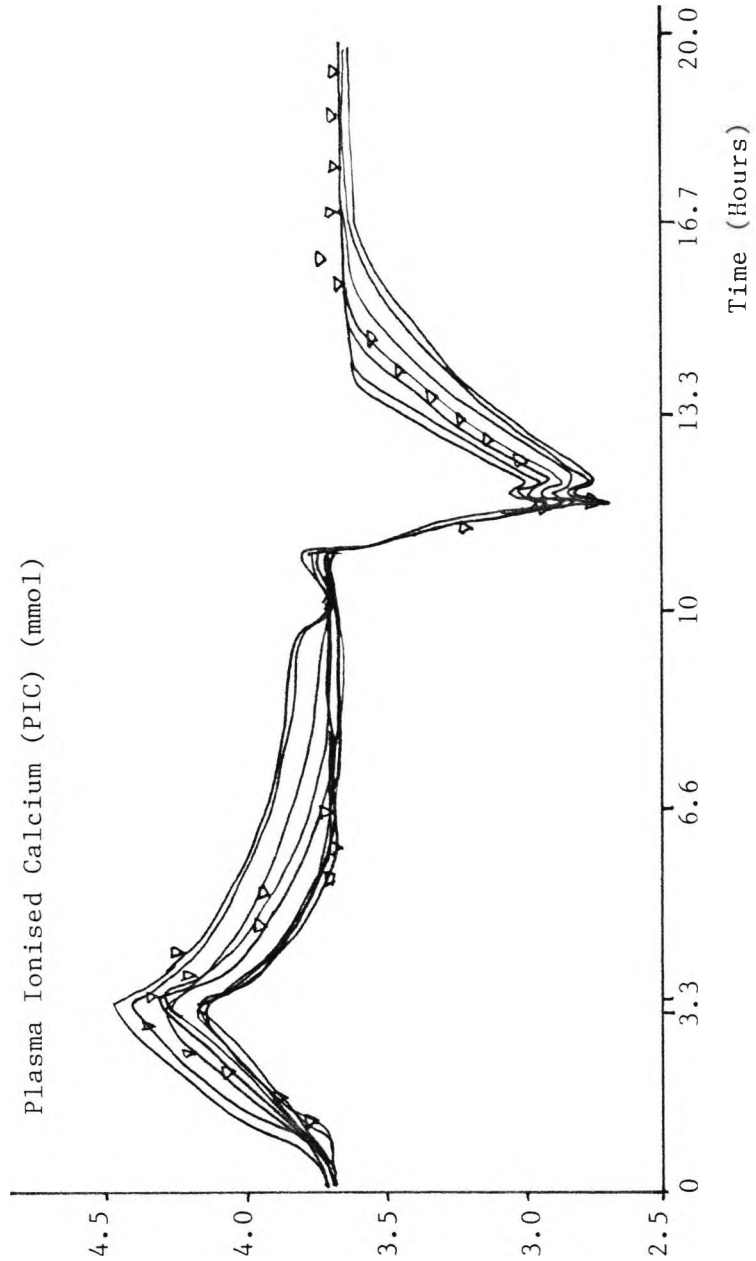


Figure 7.14 Sequential calcium and EDTA infusion applied to MODEL17 in a number of separate simulations, each with model parameters randomly varied by ± 10%. The model response still retains the distinctive shape. Some of the change from the 'fitted' response is due to steady state changes resulting from the parameter variation, and is unimportant.

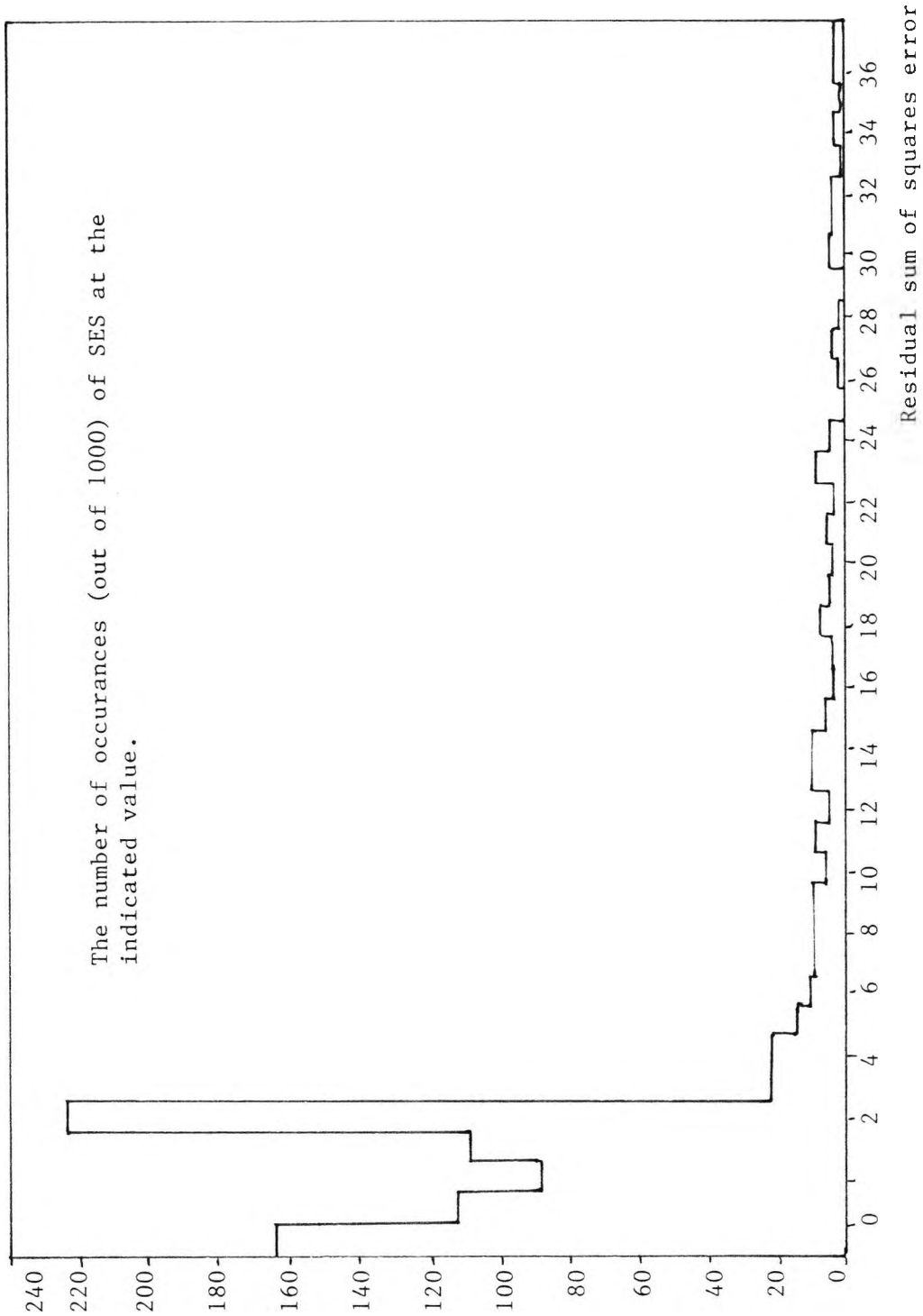


Figure 7.15 A histogram to illustrate the frequency of occurance of a particular sum of square residual error (SES), when MODEL17 was given simulated sequential calcium and EDTA infusions, and the model parameters were each selected to be = (P_i, $P_i + dp_i$, $P_i - dp_i$); where $dp_i = 0.1P_i$. Note the majority display a SES less than 4.0.

CHAPTER 8

CHAPTER 8

8. A Long-Term Model (MODEL20)

There are strong clinical reasons for the production of a valid long- term model, as first indicated in chapter 2 - the changes that lead to a clinically diagnosable osteopenia (usually presentation with long bone or vertebral crush fractures) are likely to have taken a number of years to develop. Treatment (if available) is unlikely to proceed in the reverse direction any faster, thus early identification of the population at risk is desirable,and explanatory models could help in this respect.

The two model series (MODEL1 - 5, and MODEL11 - 17) developed in previous chapters have focussed in the main on the short-term dynamics of plasma calcium as the major model output, although some long-term situations were simulated successfully with MODEL11. This chapter considers the feasibility of formulating a further model specifically designed to simulate the long-term dynamics of calcium (MODEL20) using the same strategy of formulation; the explicit incorporation of physiologically based unit processes. The intention is that this could be followed by step-wise refinement and validation using available data, as was used in previous chapters. Thus plasma calcium will not be the prime model output of interest, rather the changes associated with bone calcium.

8.1 Preliminary Considerations

This section covers the philosophy of formulation, objectives, and conventions used in MODEL20. The relevance of previous models to this one is introduced.

8.1.1 Philosophy Of Formulation

The objectives and structures of some of the previous models considered in this work (MODEL5, 11, 12, and 17) and their area of application overlap with the aims associated with MODEL20. Thus MODEL5 and 11 both include structures concerned with the long-term dynamics of calcium, and a range of simulated long-term situations were presented for MODEL11, some of which showed very good agreement with experimental data (see section 5.7.1.5).

However the level of detail involved in the formulation of these models was in the main geared to a shorter time-scale than is applicable here. Thus MODEL17 was concerned with changes that occur over a few hours, with successive experimental measurements potentially available every few minutes. MODEL20 will be concerned with data measurement and prediction intervals of the order of days, weeks and even months. Despite this it is appropriate to 'borrow' structural aspects of the other

models, but the equations associated with them may have to be simplified to prevent undue weight being given to short-term considerations.

To enable structural formulation to be firmly based upon unit processes, various signed signal and material flow diagrams were drawn as the physiology of the long-term processes was unravelled. Figure 8.1 shows one such diagram, and the separate sections under 8.2 introduce the physiology of bone that is relevant to its understanding.

8.1.2 Model Objectives

To assess the feasiblity of modelling the long-term behaviour of calcium (from one week to a number of years) in the human, by using the integrated modelling approach to building physiological systems models based upon unit-process, used for the previous model series (MODEL1 - 5, and MODEL11 - 17). The model need only aim to demonstrate algorithmic validity, as only the feasibility of the approach to the long-term situation is under investigation. A more rigorous assessement of validity could occur as part of a separate study, which would of course involve the use of the model as an isomorphic 'test-bed' for hypothesis testing, and potential clinical application. This would of course require suitable data.

8.1.3 Units & Conventions

These are no different from the previous models, except for the choice of the week as the standard unit of time. Although questionable from a fundamental viewpoint (are they 'well mixed'?), compartments are used and masses are given in mmol. Rate constants where used are quoted in wk^{-1}, and unlike before k_i has no link with compartment or state variable i. Population numbers and percentage measures are used in a similiar manner to physical compartment masses to represent particular bone cell variables, and obviously to some extent the particular steady state numbers used for these are totally arbitary.

The nomenclature remains very similiar, with some new variables being defined. These are shown in individual sections, and in the listing of MODEL20 in Appendix II.

8.2 Bone Physiology

It is desirable to formulate an isomorphic model that explicitly incorporates causal explanations of behaviour and not just curve fitted responses, and the detailed physiology of bone is of great importance. This was introduced in section 2.4, with additional information in this section.

The following aspects will be covered:

Bone cells - Osteocytes
- Basic multicellular unit
- Bone remodelling cycle

Process of Mineralisation

Effect of the menopause upon bone

Hormonal influences - Oestrogen
- PTH
- Calcitonin

8.2.1 Bone Cells

There are three readily identifiable types of cell found in the living bone. These are osteocytes, osteoblasts, and osteoclasts. Only one of these - the osteocyte - appears to function independently, the other two form part of a BMU (basic multi-cellular unit) or BRU (bone remodelling unit) and will be considered together.

8.2.1.1 The Osteocyte (CYTE)

The osteocytes lie on the bone surface, or are buried within the matrix communicating with the surface fluid and each other via canaliculi. It is possible that they can promote the activation of osteoclasts, and hence initiate a remodelling cycle, by secreting collagenase which by dissolving the collagen layer will allow the osteoclasts to have access to mineralised bone.

Apart from this facilitatory action surface osteocytes are involved in the active resorption, and through inactivity, the passive accretion of the surface bone layer of mineral (that without a supporting matrix or osteoid), in response to short term changes in activation through variation in plasma PTH. This homeostatic action is connected with the maintenance of bone surface fluid composition, which includes partial or semi-complete isolation of the surface fluid from the plasma.

The buried osteocytes are still involved in the maintenance of the bone surface mineral (that which is devoid of osteoid) as they communicate with the surface fluid in the same way as the surface osteocytes. It is possible that they have a particular role in the maintenance of the bones' mechanical integrity.

8.2.1.2 The Basic Multi-cellular Unit (BMU or CLAST and BLAST)

This is a collection of cells that exist and behave as one functional unit only for the duration of one remodelling cycle. The cycle is shown overleaf:

A group of osteoclasts are formed and become active on a bone surface, literally tunnelling or digging a hole or lacuna in the bone mineral (the matrix is of course removed as well). Duration approx 2 to 6 weeks. The activity (i.e. the rate of tunnelling) can vary.

The reversal phase, during which the osteoclasts become inactive and further resorption is stopped. The duration is normally 2 to 4 weeks.

The formative phase, during which osteoblasts are formed and become active. They first form a matrix, which is subsequently mineralised under the control of local osteoblasts. In so doing the 'hole' is filled in. During this phase some of the osteoblasts can become osteocytes, buried in the secreted mineral, or on the surface. At any one time around 30% of the BMU's that are in this phase are not active, or 'resting'. These rests can last for a number of days. The duration of the whole phase ('rests' included) is approx 18 weeks.

PTH, calcitonin, thyroid hormone, and oestrogen, can all influence the cycle by affecting the degree of activation, and hence the numbers of BMU's that are initiated, or by affecting the activity of a cell type (i.e. speed of tunnelling).

Upon the completion of an individual BMU cycle, it is thought that only 30% of the osteoblasts will have survived to transform into buried or surface osteocytes.

The trigger for the initiation of a cycle is unknown, and nett over- filling and over-resorbing can both occur. The former can only occur if there is space for this to happen (i.e. only on a surface, it cannot happen as part of the tunnelling of haversian remodelling).

As BMU activity is a process that only occurs on a bone surface and by haversian tunnelling with a lower probability, the extent of remodelling of a bone is a function of the ratio of surface area, and hence BMU population, to volume. Thus trabecular bone has a higher area/volume ratio than cortical bone, and a significantly greater rate of turnover. These differ by a factor of around four.

8.2.2 Effect Of The Menopause

For a long-term model this is epecially important due to the magnitude of the changes that can be seen. Bone resorption can be doubled, while initially formation remains the same, at least after an artificial menopause (Stepan et al, 1984). In a significant proportion of the affected population formation increases only slowly to match the increased resorption, which may account for the observed bone loss at this

time, would be expected to contribute significantly to the later development of long bone or crush fractures.

These events are likely to be invoked through changes in the three phases of the remodelling cycle. Thus the reversal phase can be extended, the resorptive phase lengthened or speeded up, and the formative phase could be slowed down.

8.2.3 The Process Of Mineralisation

Matrix or osteoid is formed by the BMU and initially mineralised under cellular control to around 70% mineralisation within a week. Following this subsequent mineralisation to 90% takes a few months, and is a totally passive process. The fullest mineralisation to around 95% takes a few years.

8.2.4 Hormonal Action

All hormones affecting bone act through their action on bone cells, or via gross plasma or extracellular changes that thus affect baseline bone activities. It is the former that are of interest, the latter are well catered for in the structures presented for MODEL17.

8.2.4.1 Calcitonin (CT)

Thought to slow bone resorption through slowing the activity of osteocytes, such that local exchangeable pools of bone surface mineral are increased at the expense of the bone surface fluid.

8.2.4.2 Parathyroid Hormone (PTH)

PTH has a number of actions that at first can appear to be conflicting. With specific regard to bone, and ignoring the wider physiological affects, the following are of interest:

> A positive effect upon BMU recruitment, and hence an initial increase in bone resorption, which may or may not be followed by a subsequent change in bone formation (see below).
>
> A positive effect upon osteoclast activity at the level of the individual BMU.
>
> A positive effect upon the lifespan of osteoblasts, without changing their activity level. By itself this will provide an increase in bone formation.

A positive effect upon the resorptive activity of osteocytes from local exchangable pools of bone surface mineral.

The manner in which PTH exerts these effects is the subject of debate, and importantly from the modelling viewpoint the PTH measurement of physiological importance is unclear. Thus the pattern of parathyroid gland secretion and not just the mean circulating level may be important.

8.2.4.3 Oestrogen (OEST)

Thought to positively affect the secretion of calcitonin, and could partly account forr the events typically seen at the menopause. Thus a drop in oestrogen levels will lead to a drop in calcitonin secretion, and a tendancy toward an increase in osteocytic bone resorption. This is in line with observations.

8.3 MODEL20 Structure

This section covers the detailed structure and formulation of MODEL20, with the development of each part of the model being covered in turn. Figures 8.2, and 8.3 provide the full schematic and equations of MODEL20. To simplify the model and especially subsequent simulations, the model was formulated only for trabecular bone, with cortical bone being omitted. This enables the number of state equations to be drastically reduced, as it is considered that for many but not all model 'spaces' their behaviour is similar.

Many parameters had to be estimated with no real basis for this estimation. In these cases parameters were adjusted to give a stable steady state to the model. However the real purpose of formulating this model was to explore these very parameters.

8.3.1 Overall Structure

Four compartmental variables are included, these are:

Plasma Ionised Calcium (PIC)
Trabecular Bone Surface Fluid Calcium (TBSFC)
Trabecular Bone Surface Calcium (TBSC)
Trabecular Bone Calcium (TBC)

Passage of mineral between these spaces is a function of Osteocyte, Osteoclast and Osteoblast activity on the separate surfaces of these spaces. These 'cell activities' are modelled as time variant state variables in the same manner as the physical compartmental variables shown overleaf:

Osteoclast Population (CLAST)
Osteoblast Population (BLAST)
Osteocyte Population (CYTE)

Activity factors for the cell populations are also included.

8.3.2 Plasma Calcium (PIC)

A single compartment (PIC) is used,

$$PIC = GCA - UC - TBSFCA + TBSFCR \quad (8.1)$$

$$UC = PIC * (k_1 - (k_5 * PPTH / PIC_{ss}))$$

$UC_{ss} = GCA_{ss} = 50.0$ mmol wk^{-1} (from MODEL17)

$PIC_{ss} = 3.6$ mmol (from MODEL17)

$k_1 = 403.0$

$k_5 = 13.82$

8.3.3 Gut Absorption

This is not modelled separately but treated as a constant that can be varied if needed. k_4 is used for this value in the model.

$GCA_{ss} = 50.0$ mmol wk^{-1} (from MODEL17)

8.3.4 Bone Surface Fluid Calcium

The trabecular surface is assumed to represent 70 % of the available mineral area such that the trabecular masses are also set at 70 % of the total available, which is assumed to be 14 mmol, from MODEL17. It can be argued that this figure is physiologically too high, but this value was arrived at for a significant number of the simulations fitted to MODEL17.

$$TBSFC = (TBSFCA + TBSCR + TBCR) - (TBSFCR + TBSCA + TBCA) \quad (8.2)$$

$TBSFCA = k_3 * PIC$

$TBSFCR = k_2 * TBSFC$

$TBSFC_{ss} = 10.0$ mmol

$k_2 = 200.0$

$k_3 = 555.5$

8.3.5 Bone Surface Calcium

Steady state values were again taken from MODEL17, and the 70% / 30% relation between trabecular and cortical bone assumed, hence:

$TBSC_{ss}$ = 70.0 mmol

$$\dot{TBSC} = TBSCA - TBSCR \quad (8.3)$$

Bone surface calcium accretion is assumed to be a passive process, linearly dependent upon bone surface fluid calcium (BSFC). Conversely resorption from the bone surface resorption is an active process, controlled by the relative activity level of the osteocyte population. Hence:

$$TBSCA = k_6 * TBSFC \quad (8.4)$$

$$TBSCR = k_7 * TBSC * TCYTEACT / TCYTEACT_{ss} \quad (8.5)$$

k_6 = 25.0

k_7 = 3.57

8.3.6 Bone Calcium

For bone calcium a relative mass was used, i.e. TBC_{ss} = 100 %

$$\dot{TBC} = TBCA - TBCR \quad (8.6)$$

Accretion is assumed to be dependent upon both the oseoblast population and it's activity. Resorption to be dependent upon the osteoclast population and its' activity. Hence:

$$TBCA = k_{18} * TBLASTACT * BLAST \quad (8.7)$$

$$TBCR = k_{17} * TCLASTACT * CLAST \quad (8.8)$$

k_{18} = 33.33

k_{17} = 33.33

8.3.7 Osteoclast Population

The cell population state equations are easily defined. The rate of change is = formation rate - death rate. Hence:

$$\dot{TCLAST} = TCLAST\text{-}FORM - TCLAST\text{-}DTH \quad (8.9)$$

$TCLAST_{ss}$ = 500.0

However osteoclast formation is assumed to be directly related to the relative PTH level, and the death rate to be directly related to the population level:

$$TCLAST\text{-}FORM = k_{11} * PTH / PTH_{ss} \quad (8.10)$$
$$TCLAST\text{-}DTH = k_{15} * TCLAST \quad (8.11)$$
$$TCLASTACT = k_9 = 1.0$$
$$k_{11} = 125.0$$
$$k_{15} = 0.25$$

8.3.8 Osteoblast Population

Similarly for the osteoblast population:

$$TBLAST = TBLAST\text{-}FORM - TBLAST\text{-}DTH \quad (8.12)$$
$$TBLAST_{ss} = 500.0$$

However osteoblast formation is assumed to be linearly related to the osteoclast formation rate, and the death rate a linear function of the population:

$$TBLAST\text{-}FORM = k_{12} * TCLAST\text{-}FORM \quad (8.13)$$
$$TBLAST\text{-}DTH = k_{16} * TBLAST \quad (8.14)$$
$$TBLASTACT = k_{10} = 1.0$$
$$k_{12} = 0.25$$
$$k_{16} = 0.0625$$

8.3.9 Osteocyte Population

Similarly for the osteocyte population:

$$TCYTE = TCYTE\text{-}FORM - TCYTE\text{-}DTH \quad (8.15)$$
$$TCYTE_{ss} = 100.0$$

With osteocte formation linearly related to the osteoblast formation rate, and death directly related to the population:

$$TCYTE\text{-}FORM = k_{13} * TBLAST\text{-}FORM \quad (8.16)$$
$$TCYTE\text{-}DTH = k_{14} * TCYTE \quad (8.17)$$
$$TCYTEACT = k_8 * PPTH / PPTH_{ss}$$
$$TCYTEACT_{ss} = k_8 = 1.0$$
$$k_{13} = 0.32$$
$$k_{14} = 0.1$$

8.3.10 Parathyroid Hormone

Parathyroid hormone was considered to have an important role in bone cell activity, as was clarified in section 8.2.5.2. This was modelled simply with the steady state = 100 % and not as a physical mass, as follows:

$$\dot{PPTH} = PTS - k_{20} * PPTH \quad (8.18)$$

$$PTS = 1000 * (k_{20} - k_{21} * PIC) \quad (8.19)$$

If $PTS < k_{19}$: $PTS = k_{19}$

If $PTS > k_{22}$: $PTS = k_{22}$

$k_{20} = 62.0$

$k_{21} = 15.0$

8.3.11 Other Hormones

No other hormones were initally incorporated in the model. There is of course scope to do so in the future.

8.4 The Behaviour Of MODEL20

The behaviour of MODEL20 was not explored, merely tested to establish internal consistency, and algorithmic validity. That a suitable steady state was reached was established, and a dedicated simulation system written that could be used to drive further simulations, and hence investigate the assumptions and paramters built into the model, or build in new ones.

8.4.1 Simulation Method

Unlike previous models, all simulations were performed on an IBM PC, or a derivative of these machines. Although the same machine power is not available in these smaller machines as in the Prime 550 mini, some useful work can be done, without resorting to a 386 chip based machine. As stated previously to facilitate the numerical integration, the model was simplified by incorporating only the trabecular bone spaces. This reduced the number of equations to be solved. A simulation system was written in Turbo-Pascal that used a Runge-Kutta integration routine.

PC's and their clones are readily available in most clinical, research, and teaching environments, potentially widening access to the model. Standardisation across main-frame and mini computing environments is still extremely poor compared to the portability of an MSDOS runnable 5.25"/3.5" disk. If models of this nature are ever to be used in a clinical environment, this approach to simulation should be used.

8.5 Summary

A long-term model MODEL20 has been formulated, as part of assessing the feasibility of using the approach used for the short-term models described in earlier chapters. That is the incorporation of physiologically based unit-processes into an isomorphic model structure.

The model was found to be valid within the intial area of concern - demonstration of internal and algorithmic consistency. Problems were experienced when attempting to derive suitable parameter values for the model. As has been said elsewhere the relative inaccessibility of the bone structures leaves many parameters to be estimated with little foundation.

The model itself provides a suitable 'test-bed' to further investigate the consistency of the physiological assumptions incorporated in the model. These assumptions covered the interaction between differing bone cell species, parathyroid hormone, and the concept of a bone surface fluid compartment that is effectvely separate from the rest of the extra-cellular fluid. This requires further study.

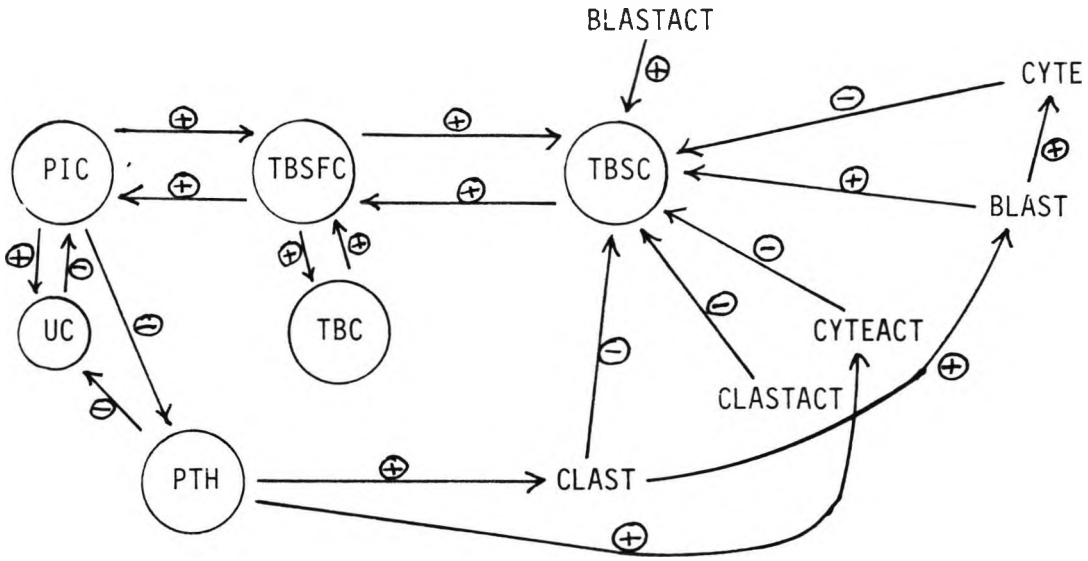


Figure 8.1 A signed digraph of information flow graph, used to illustrate the structure of MODEL20.

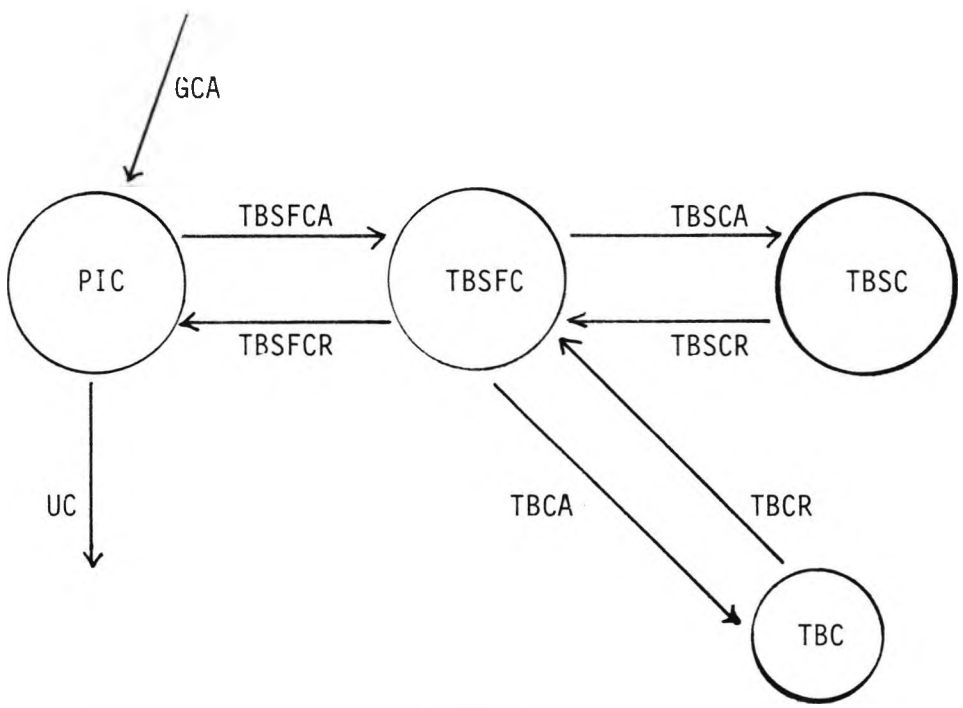


Figure 8.2 The structure of MODEL20. All the bone cell population variables are not shown, but only the physical compartments and calcium flows. It is inappropriate to show these on a diagram of this for, Figure 8.1 above makes this clearer.

$$\dot{PIC} = GCA - UC - TBSFCA + TBSFCR$$

$$\dot{TBSFC} = (TBSFCA + TBSCR + TBCR) - (TBSFCR + TBSCA + TBCA)$$

$$\dot{TBSC} = TBSCA - TBSCR$$

$$\dot{TBC} = TBCA - TBC$$

$$\dot{TCLAST} = TCLAST\text{-}FORM - TCLAST\text{-}DTH$$

$$\dot{TBLAST} = TBLAST\text{-}FORM - TBLAST\text{-}DTH$$

$$\dot{TCYTE} = TCYTE\text{-}FORM - TCYTE\text{-}DTH$$

$$\dot{PPTH} = PTS - k_{20} * PPTH$$

<u>Related Equations</u>

$$UC = PIC * (k_1 - (k_5 * PPTH / PIC_{ss}))$$
$$TBSFCA = k_3 * PIC$$
$$TBSFCR = k_2 * TBSFC$$
$$TBSCA = k_6 * TBSF$$
$$TBSCR = k_7 * TBSC * TCYTEACT / TCYTEACT_{ss}$$
$$TBCA = k_{18} * TBLASTACT * BLAST$$
$$TBCR = k_{17} * TCLASTACT * CLAST$$
$$TCLAST\text{-}FORM = k_{11} * PTH / PTH_{ss}$$
$$TCLAST\text{-}DTH = k_{15} * TCLAST$$
$$TCLASTACT = k_9 = 1.0$$
$$TBLAST\text{-}FORM = k_{12} * TCLAST\text{-}FORM$$
$$TBLAST\text{-}DTH = k_{16} * TBLAST$$
$$TBLASTACT = k_{10} = 1.0$$
$$TCYTE\text{-}FORM = k_{13} * TBLAST\text{-}FORM$$
$$TCYTE\text{-}DTH = k_{14} * TCYTE$$

<u>Figure 8.3</u> MODEL20 equations, complete with initially assumed parameter values. Continued overleaf....

$TCYTEACT = k_8 * PPTH / PPTH_{ss}$

$PTS = 1000 * (k_{20} - k_{21} * PIC)$

If $PTS < k_{19}$: $PTS = k_{19}$

If $PTS > k_{22}$: $PTS = k_{22}$

<u>Parameter Values</u>

$UC_{ss} = GCA_{ss} = 50.0$ mmol wk^{-1}

$PIC_{ss} = 3.6$ mmol

$TBSFC_{ss} = 10.0$ mmol

$TBSC_{ss} = 70.0$ mmol

$TBC_{ss} = 100$ %

$TCLAST_{ss} = 500.0$

$TBLAST_{ss} = 500.0$

$TCYTE_{ss} = 100.0$

$TCYTEACT_{ss} = k_8 = 1.0$

$k_1 = 403.0$

$k_2 = 200.0$

$k_3 = 555.5$

$k_4 = GCA_{ss} = 50.0$ mmol wk^{-1}

$k_5 = 13.82$

$k_6 = 25.0$

$k_7 = 3.57$

$k_{11} = 125.0$

$k_{12} = 0.25$

$k_{13} = 0.32$

$k_{14} = 0.1$

$k_{15} = 0.25$

$k_{16} = 0.0625$

$k_{17} = 33.33$

$k_{18} = 33.33$

$k_{20} = 62.0$

$k_{21} = 15.0$

<u>Figure 8.3</u> Continued.

CHAPTER 9

CHAPTER 9

9. Conclusions

This thesis is concerned with the examination of the control and dynamics of a complex metabolic system, to generate insight and increase understanding through the applicaion of dynamic system models. The insight that can arise from a modelling programme such as this is a pre-requisite before models of physiological processes can be exploited as aids in the management of patients.

Models offer potential application not only in research but also in relation to education and patient care. However if models are to be used for education, clinical care and support, they need to have a sound relation to the underlying physical and physiological processes. Hence this investigation has focussed upon the role of models to enhance understanding of the underlying processes as an end in itself, with the long-term thought that this is a necessary precursor to future clinical application. A traditional 'black box' approach to modelling could not hope to fulfill this need, but these models may be used as aids in the validation of isomorphic models.

This study has yielded useful insight into the dynamic behaviour of calcium and related substances. The methodological problems and questions encountered have implications for other studies of this nature. The conclusions will be presented through an intial review of the work covered

Chapter 3 dealt with the models of other authors, classifying these into two broad base sets. An important illustration was the lack of attention that any of these authors had given to the range and validity of the predictions that arise from these models. Thus 'tracer' models were shown to be indicative, almost solely, of what was measured - 'the rate of elimination of a radioactive calcium isotope from the plasma space'.

An attempt to improve upon the tracer models shortcomings was presented in Chapter 4, leading to the development of the MODEL1-5 series. An integrated approach to formulation, identification and validation was used, quickly leading to the abandonment of this model series. The lack of a physiological isomorphism for those parts of the model that were derived from the original tracer model, inhibited further meaningful iterations through the modelling methodology cycle. Significantly the model did suggest that the effective half-life of the physiologically active PTH molecule/metabolite(s) could be an order of magnitude greater than was originally incorporated in the model (3 minutes).

As a result of the perceived inadequacies of an approach that places too much credence upon a tracer model, another model was developed (MODEL11), again using the integrated methodology. This model differed significantly from the first, as a degree of physiological isomorphism was explicitly incorporated. Thus compartmental masses were initially set at 'best' estimates or reliable measurements.

Following algorithmic (are solutions possible?) and algebraic validation, a range of test situations were simulated. Although dealing extremely well with long-term variations in dietary intake, and short-term variations in phosphate; MODEL11 was unable to adequately reproduce the short-term variation in plasma calcium that can be observed. A process of reduction using detailed model structure sensitivity analysis was then used to identify the minimal plausible model structure that was compatible with the adequate simulation of the response to a hyper- and a hypo-calcaemic stimulus.

This process demonstrated that a very much reduced model (MODEL12) was sufficient to adequately describe the observed short-term dynamics of plasma calcium. Model compartments removed included all the phosphate compartments, and the compartment representing the bulk of the bone calcium (FBC). Unfortunately the model was still theoretically unidentifiable, such that unique parameter estimates could not be derived. This lack of identifiability is not solely due to the model complexity, but also of course, a function of the available data, or in this case its paucity.

The further application of the integrated methodology to MODEL12 led, through five further models, to the production of MODEL17. Again it was the detailed dynamics of plasma calcium that were of primary concern, and most importantly the detailed involvement of Parathyroid Hormone (PTH). MODEL17 demonstrates that by involving PTH release from two sources: one in proportion to the level of hypocalcaemia, the other from an exhaustible store of PTH that is only called upon when a significant hypocalcaemia is reached, the response to an EDTA infusion is easily reproduced. Significantly all other control options investigated could not mimic this response.

A clear physiological conclusion is indicated by MODEL17 regarding PTH release. The validity or accuracy of this conclusion, and others of lesser impact is of central concern for a modelling program such as this. Models as complex as MODEL17 derived through adaptive fitting that essentially involves subjective assessments of 'shape of response', are theoretically unidentifiable. That is a unique solution does not exist, and a number of parameter sets can be expected to provide a given response. It is argued and demonstrated in depth in Chapter 7, that rigorous measures of statistical validity are not the only metric available. Further that model

validity and hence the significance of model predictions, is a multi-faceted metric that must consider what data is available, and any and all predictions made by the model, before validity conclusions are drawn.

MODEL17 by prompting the question of validity points to a useful role of the application of systems modelling techniques; the production of testable predictions regarding systems behaviour. Complex physiological systems are ideal candidates for modelling of this nature. Thus suitable experiments that will add to or lessen a models' validity are continually produced during the application of an integrated approach to modelling. The experimental resources do however need to be available to fully realise the benefits.

Another conclusion regarding modelling methodology, and the role of statistical validity is provided in Chapter 7. Namely that experimental data of the comprehensiveness expected by the physical systems engineer will rarely be feasible to obtain from human subjects, although some animal experimentalists (see Rodon et al, 1967) have displayed remarkable ingenuity and imagination in the pursuit of definitive data. It is the constraints and limitations imposed by the model subject, that force the acceptance of less rigorous, but better than non-existent concepts of validity. Thus validity becomes a measure of usefulness or merit, linked with a defined domain of application, which is always intimately linked with the initial modelling objective.

The further use of the integrated methodology to derive models based upon physiological unit processes was explored in Chapter 8 in the development of MODEL20, as a prototype 'long term model'. The approach was shown to be feasible, with internal validity criteria being satisfied, and proposals for future model development being made. Again the lack of data and physical inaccessibility of the major components of the system was especially highlighted as a problem in this long-term situation, with bone itself physically inaccessible, and the long time taken for changes to manifest themselves hampering data collection.

Implications for physiology from this study include the suggestion of significant long-term bone changes as a result of dietary alterations in MODEL11. The suitability of a dual release mechanism for PTH that will provide the necessarily stronger hyper-calcaemic stimulus when it is most needed (during those times when the hypocalcaemia becomes life threatening). Calcitonin and Phosphate are shown to be unnecessary for the adequate simulation of short-term control of plasma ionised calcium. The physiologically based compartments are shown to be valid for the adequate simulation of tracer calcium disappearance from the plasma, although this validity is not uniquely empirical, a number of parameter vectors will give valid simulations. The compartments used in MODEL17 included two 'connected' with

bone; BSFC, and BSC. These were a necessary part of the adequate simulation of the gamut of tests put through MODEL17, with high transfer rates being seen in some situations.

The possibility that MODEL17, a unit based model can provide a level of validity which can be independently verified, and hence given further development, potentially lead to a clinical application, has been demonstrated. Any further clinical application would be most needed in a long term situation, and the feasiblity of this is demonstrated through the prototype MODEL20, although this model does require further development.

The methodology of modelling physiological models such as to obtain the most benefit has received attention from a number of authors, with varying levels of 'prescriptiveness' or formality. The studies described here have used variants of these as appropriate, it being the aim that matters not exactly how one got there. Modelling and indeed medicine itself is a practical activity, and many of the more formalised methods were found to be wanting, for the unidentifiable models and parameter uncertainty encountered in this study. Hence the usefulness of 'patterns of resonse' and modelling or optimisation 'by eye', as opposed to stricter quantitative measures or predictors.

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APPENDIX I

APPENDIX 1

1. Nomenclature Incorporated in the Models

Symbol	Variable
BSC	Bone surface calcium
BSCA	Bone surface calcium accretion
BSCR	Bone surface calcium resorption
BSFC	Bone surface fluid calcium
BSFCA	Bone surface fluid calcium accretion
BSFCR	Bone surface fluid calcium resorption
D2F	1,25 $(OH)_2$ vitamin D3 formation rate
EIC	Extracellular ionised calcium
ECFC	Extracellular fluid calcium
ECFCA	ECF calcium accretion
ECFCR	ECF calcium resorption
FC	Faecal calcium
FBC	Fixed bone calcium
GC	Gut calcium
GCA	Gut calcium absorption rate
GPA	Gut phosphate absorption rate
IC	Oral intake of calcium
INC	Nett calcium intake to the plasma
PBC	Protein bound calcium
PIC	Plasma ionised calcium
PINC	Plasma inorganic calcium
PIP	Plasma inorganic phosphate
STC	Soft tissue calcium
STCA	Soft tissue calcium accretion
STCR	Soft tissue calcium resorption
TCR	Calcium tubular resorption rate
TMC	Calcium maximum tubular resorption rate
UC	Urine calcium
UFC	Calcium ultrafiltration rate
PPT	Plasma Parathyroid Hormone
PTH	Parathyroid Hormone
PTS	Parathyroid Hormone Secretion Rate

2. MODEL5

2.1 Equations

Extracellular Fluid & Soft Tissue Calcium

$$\dot{M1} = GCA - k_{0,8}M1 + k_{1,5}M5 - STCA + STCR - UC$$

$$\dot{M2} = k_{2,3}M2 + STCA - k_{3,2}M2 - STCR$$

Exchangeable Bone Calcium

$$\dot{M3} = k_{3,4}M4 + k_{3,2}M2 - k_{2,3}M3 - k_{4,3}M3$$

$$\dot{M4} = k_{4,3}M3 - k_{3,4}M4 - k_{5,4}M4$$

Fixed Bone Calcium & Phosphate

$$\dot{M5} = k_{5,4}M4 - k_{1,5}M5$$

$$\dot{FBP} = (2/3\ k_{5,4}M4) - (2/3\ k_{1,5}M5) = 2/3\ M5$$

Plasma Inorganic Phosphate

$$\dot{M6} = GPA + FBPR - UP - FBPA$$

Vitamin D3 Metabolites

$$\dot{M7} = k_{df}M7 - D2F*M7$$

$$\dot{M8} = D2F - k_{0,8}M8$$

Plasma Parathyroid Hormone

$$\dot{M9} = 0.2(PTS - k_{09}M9)$$

Urine Calcium (UC)

$$UC = 0.74M1 - 23.58 + 1.05(M1 - 12.4)(1 - M9/(M9 + k_{uc}))$$

Urine Phosphate (UP)

$$UP = k_f M6 - (k_{tp} - k_{tq} M6 * Y'/M6_{ss})$$

$$Y' = (M9 + M9_{ss})/2 \quad : M9 < 490$$
$$Y' = (M9 + 2*M9_{ss})/3 \quad : 490 \leq M9 < 510$$
$$Y' = (M9 + 3*M9_{ss})/4 \quad : 510 \leq M9 < 530$$
$$Y' = (M9 + 4*M9_{ss})/5 \quad : 530 \leq M9 < 560$$
$$Y' = (M9 + 5*M9_{ss})/6 \quad : 560 \leq M9 < 600$$
$$Y' = (M9 + 6*M9_{ss})/7 \quad : 600 \leq M9 < 620$$
$$Y' = (M9 + 9*M9_{ss})/10 \quad : 620 \leq M9$$

PTH Secretion (PTS)

$$PTS = 5.3 \quad : \quad (M1 < 25.0) \quad ng\ s^{-1}$$
$$PTS = 20.3 - 0.6\ M1 \quad : \quad (25.0 \leq M1 \leq 33.3) \quad " \ "$$
$$PTS = 0.32 \quad : \quad (M1 > 33.3) \quad " \ "$$

Gut Absorption (GCA & GPA)

$$GCA = IC(M8/(M8 + k_c M8_{ss}))$$
$$GPA = IP(M8/(M8 + kpM8_{ss}))$$

Soft Tissue Calcium Accretion (STCA)

$$STCA = k_s\ M6\ M1$$

Soft Tissue Calcium Resorption (STCR)

$$STCR = k_{1,2}\ M2\ (M9 + M9_{ss})/(2*M9_{ss})$$

Vitamin D3 Metabolism

$$D2F = (0.0673 - 0.00598\ M6)(M7/M7_{ss})$$

2.2 Nominal Steady State and Parameter Values

IC	=	30.0	mmol day^{-1}
IP	=	45.0	" "
GCA_{SS}	=	15.0	" "
GPA_{SS}	=	25.0	" "
$STCR_{SS}$	=	927.5	" "
UC_{SS}	=	9.75	" "
UP_{SS}	=	25.25	" "
PTS_{SS}	=	69120	ng day^{-1}

$M1_{SS}$	=	32.5	mmol
$M2_{SS}$	=	35.0	"
$M3_{SS}$	=	90.0	"
$M4_{SS}$	=	177.5	"
$M5_{SS}$	=	25000.0	"
$M6_{SS}$	=	4.586	"
$M7_{SS}$	=	100.	'units'
$M8_{SS}$	=	1.0	'units'
$M9_{SS}$	=	490.0	ng
FBP_{SS}	=	16667.0	mmol

k_f	=	47.0	k_{df}	=	0.04
k_c	=	1.0	k_p	=	0.8
k_s	=	6.3	k_{tp}	=	3440.0
k_{tq}	=	6.63	k_{uc}	=	382.0

$k_{1,5}$	=	0.000568	day^{-1}
$k_{1,2}$	=	26.52	"
$k_{2,3}$	=	1.476	"
$k_{3,2}$	=	4.2	"
$k_{3,4}$	=	0.0868	"
$k_{4,3}$	=	0.329	"
$k_{5,4}$	=	0.08	"
$k_{o,8}$	=	0.04	"
$K_{o,9}$	=	144.0	"

3. MODEL11

3.1 Equations

Plasma & Extracellular Fluid Calcium

$$\dot{PIC} = INC + BSFCR + FBCR + k_{1,2}EIC - k_{2,1}PIC - UC - BSFCA$$

$$\dot{EIC} = k_{2,1}PIC - k_{1,2}EIC$$

$$\dot{BSFC} = BSFCA - FBCA - BSFCR - k_{4,3}BSFC + k_{3,4}BSC$$

Plasma & Extracellular Fluid Phosphate

$$\dot{PIP} = INP + k_{7,9}BSFP - k_{9,7}PIP + k_{7,11}FBP - k_{8,7}PIP + k_{7,8}EIP$$

$$\dot{EIP} = k_{8,7}PIP - k_{7,8}EIP$$

$$\dot{BSFP} = k_{9,7}PIP - k_{7,9}BSFP - k_{11,9}BSFP - k_{10,9}BSFP + k_{9,10}BSP$$

Bone Calcium

$$\dot{BSC} = k_{4,3}BSFC - k_{3,4}BSC$$

$$\dot{FBC} = FBCA - FBCR = k_{5,3}BSFC - k_{1,5}FBC$$

Parathyroid Hormone

$$\dot{PPT} = PTS - 125.5\ PPT$$

Bone Phosphate

$$\dot{BSP} = k_{10,9}BSFP - k_{9,10}BSP$$

$$\dot{FBP} = k_{11,9}BSFP - k_{7,11}FBP$$

Urine Calcium (UC)

$UC_1 = 0.174\ (UFC - 0.5\ Tmc)$
$UC_2 = 0.352\ (UFC - 0.68\ Tmc)$
$UC_3 = 0.7\ (UFC - 0.9\ Tmc)$
$UC = \text{Max}\ UC_i\ ;\ i = 1,2,3$

Urine Phosphate (UP)

$UP_1 = 0.174\ (UFP - 0.5\ Tmp)$
$UP_2 = 0.352\ (UFP - 0.68\ Tmp)$
$UP_3 = 0.8\ (UFP - 0.9\ Tmp)$
$UP = \text{Max}\ UP_i\ ;\ i = 1,2,3$

Tubular Calcium Maximum Reabsorption (Tmc)

$TMC = 0.07949\ PPT + 217.0 \quad \text{if } PPT < 490.0$
$TMC = 0.019429\ PPT + 241.5 \quad \text{if } PPT > 490.0$

Tubular Phosphate Maximum Reabsorption (Tmp)

$Tmp = 185.8 - 0.11376\ PPT \quad \text{if } PPT < 490.0$
$Tmp = 146.96 - 0.03449\ PPT \quad \text{if } PPT > 490.0$

Ultrafiltration Rate (UFC & UFP)

$UFP = 41.75\ PIP\ \text{mmol day}^{-1}$
$UFC = 57.6\ PIC\ \text{mmol day}^{-1}$

Bone Calcium Control

$BSFCA = k_{3,1}PIC\ (B.PIP - PIP_{ss}\ (B-1))/PIP_{ss}$

$0.0 \leq BSFCA \leq 30.0\ ;\ B = 1$

$BSFCR = k_{1,3}BSFC\ (k_jPPT - PPT_{ss}\ (K - 1))/\ PPT_{ss}$

$0.0 \leq BSFCR \leq 10.0\ ;\ k_j = 0.5$

Parathyroid Hormone Secretion (PTS)

$$PTS = 86400.0\ (13.6 - 3.58PIC)$$

$$25920.0 \leq PTS \leq 432000.0 \quad ng\ day^{-1}$$

3.2 Nominal Parameter Values

$k_{1,2}$	=	1.3006	day^{-1}	$k_{2,1}$	=	5.0	day^{-1}
$k_{1,5}$	=	0.000568	"	$k_{3,4}$	=	0.3024	"
$k_{4,3}$	=	41.51	"	$k_{1,3}$	=	3.549	"
$k_{3,1}$	=	6.132	"	$k_{5,3}$	=	19.49	"
$k_{7,9}$	=	3.549	"	$k_{9,7}$	=	6.132	"
$k_{7,11}$	=	0.000568	"	$k_{8,7}$	=	3.871	"
$k_{7,8}$	=	1.0084	"	$k_{11,9}$	=	19.49	"
$k_{10,9}$	=	41.51	"	$k_{9,10}$	=	0.3024	"

Subject Weight	=	70	kg
Plasma Volume	=	3.0	l
Extracellular Volume	=	11.53	l
BSF Volume	=	0.61	l
Glomerular Filtration Rate	=	100	$ml\ min^{-1}$

3.3 Nominal Steady State Values

PIC	=	3.6	mmol	PIP	=	3.1	mmol
EIC	=	13.84	"	EIP	=	11.91	"
BSFC	=	0.7284	"	BSFP	=	0.4856	"
BSC	=	100.00	"	BSP	=	66.7	"
FBC	=	25000.00	"	FBP	=	16667.00	"
PPT	=	490.00	ng				

PTS	=	0.712	$ng\ s^{-1}$	=	61517.00	$ng\ day^{-1}$	
INC	=	14.33	$mmol\ day^{-1}$	INP	=	14.43	$mmol\ day^{-1}$
TMP	=	130.1	" "	TMC	=	251.0	" "
UC	=	14.33	" "	UP	=	14.43	" "
UFC	=	207.4	" "	UFP	=	129.4	" "

4. MODEL12

4.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC (PPT + k_a) /k_b

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}

0.1 $\leq$ Gland Activity $\leq$ 2.5

PTS = 86400.0 (Gland Activity)

4.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}				
k_a	=	460		k_b	=	674.0	
k_c	=	0.385		k_{pt}	=	1.0	

4.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

5. MODEL13

5.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC(PPT k_d)/k_e
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC.(PPT + k_a) /k_b

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}

0.1 $\leq$ Gland Activity $\leq$ 2.5

PTS = 86400.0 (Gland Activity)

5.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}				

k_a	=	460	k_b	=	674.0
k_c	=	0.385	k_{pt}	=	1.0
k_d	=	64.0	k_e	=	540.0

5.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

6. MODEL14

6.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC(PPT + k_f)/k_g
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC.(PPT + k_a) /k_b

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}

0.1 $\leq$ Gland Activity $\leq$ 2.5

PTS = 86400.0 (Gland Activity)

6.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}				

k_a	=	460	k_b	=	674.0
k_c	=	0.385	k_{pt}	=	1.0
k_f	=	128.0	k_g	=	86.0

6.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

7. MODEL15

7.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC(PPT + k_f)/k_g
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC(PPT + k_d)/k_e
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC.(PPT + k_a) /k_b

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}

0.1 $\leq$ Gland Activity $\leq$ 2.5

PTS = 86400.0 (Gland Activity)

7.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}				

k_a	=	460	k_b	=	674.0
k_c	=	0.385	k_{pt}	=	1.0
k_d	=	64.0	k_e	=	540.0
k_f	=	128.0	k_g	=	86.0

7.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

8. MODEL16

8.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC(PPT + k_h)/k_i
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}

0.1 $\leq$ Gland Activity $\leq$ 2.5

PTS = 86400.0 (Gland Activity)

8.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}				

k_a	=	460	k_b	=	674.0
k_c	=	0.385	k_{pt}	=	1.0
k_h	=	480.0	k_i	=	530.0

8.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

9. MODEL17

9.1 Equations

$\dot{ECFC}$ = ECFCA - ECFCR

$\dot{PIC}$ = INC + ECFCR - ECFCA - BSFCA + BSFCR - UC

$\dot{BSFC}$ = BSFCA - BSFCR + BSCR - BSCA

$\dot{BSC}$ = BSCA - BSCR

$\dot{PPT}$ = PTS - $k_{0,5}$PPT

ECFCA = $k_{2,1}$ PIC
ECFCR = $k_{1,2}$ ECFC
BSCA = $k_{4,3}$ BSFC
BSCR = $k_{3,4}$ BSC
BSFCA = $k_{3,1}$ PIC
BSFCR = $k_{1,3}$ BSFC (PPT + k_a) /k_b

Urine Calcium (UC)

UC_1 = 0.174 (UFC - 0.5 Tmc)
UC_2 = 0.352 (UFC - 0.68 Tmc)
UC_3 = 0.7 (UFC - 0.9 Tmc)
UC = k_c (Max UC_i ; i = 1,2,3)

Tubular Calcium Maximum Reabsorption (Tmc)

TMC = 0.06939 PPT + 217.0 (if PPT < 490.0)

Tmc = 0.019429 PPT + 241.5 (if PPT $\geq$ 490.0)

Ultrafiltration Rate (UFC)

UFC = 57.6 PIC mmol day^{-1}

Parathyroid Hormone Secretion (PTS)

Gland Activity = k_{pt} (6.24 - 1.64 PIC) ng s^{-1}
PTS = 86400.0 (Gland Activity)

0.1 $\leq$ Gland Activity $\leq$ 0.66 at any time
Gland Activity > 0.66 is only supported for a time T_{pt} in any 24 hours period.

9.2 Nominal Parameter Values

$k_{1,2}$	=	27.2	day^{-1}	$k_{1,3}$	=	28.8	day^{-1}
$k_{2,1}$	=	61.2	day^{-1}	$k_{3,1}$	=	112.0	day^{-1}
$k_{3,4}$	=	0.617	day^{-1}	$k_{o,5}$	=	57.6	day^{-1}
$k_{4,3}$	=	4.41	day^{-1}	T_{pt}	=	0.077	day
k_a	=	460		k_b	=	674.0	
k_c	=	0.385		k_{pt}	=	1.0	

9.3 Nominal Steady State Values

BSC	=	100.0	mmol	PIC	=	3.6	mmol
BSFC	=	14.0	mmol	ECFC	=	8.1	mmol
PPT	=	214.0	ng				
INC	=	7.2	mmol day^{-1}				
B.W.	=	70	kg	Plasma vol	=	3.0 l	
ECF vol	=	11.53	l	BSF vol	=	0.61 l	

Glomerular filtration rate = 100 ml min^{-1}

10. MODEL20

10.1 State Equations

$\dot{PIC} = GCA - UC - TBSFCA + TBSFCR$

$\dot{TBSFC} = (TBSFCA + TBSCR + TBCR) - (TBSFCR + TBSCA + TBCA)$

$\dot{TBSC} = TBSCA - TBSCR$

$\dot{TBC} = TBCA - TBC$

$\dot{TCLAST} = TCLAST\text{-}FORM - TCLAST\text{-}DTH$

$\dot{TBLAST} = TBLAST\text{-}FORM - TBLAST\text{-}DTH$

$\dot{TCYTE} = TCYTE\text{-}FORM - TCYTE\text{-}DTH$

$\dot{PPTH} = PTS - k_{20} * PPTH$

10.2 Related Equations

$UC = PIC * (k_1 - (k_5 * PPTH / PIC_{ss}))$

$TBSFCA = k_3 * PIC$

$TBSFCR = k_2 * TBSFC$

$TBSCA = k_6 * TBSF$

$TBSCR = k_7 * TBSC * TCYTEACT / TCYTEACT_{ss}$

$TBCA = k_{18} * TBLASTACT * BLAST$

$TBCR = k_{17} * TCLASTACT * CLAST$

$TCLAST\text{-}FORM = k_{11} * PTH / PTH_{ss}$

$TCLAST\text{-}DTH = k_{15} * TCLAST$

$TCLASTACT = k_9 = 1.0$

$TBLAST\text{-}FORM = k_{12} * TCLAST\text{-}FORM$

$TBLAST\text{-}DTH = k_{16} * TBLAST$

$TBLASTACT = k_{10} = 1.0$

$TCYTE\text{-}FORM = k_{13} * TBLAST\text{-}FORM$

$TCYTE\text{-}DTH = k_{14} * TCYTE$

$TCYTEACT = k_8 * PPTH / PPTH_{ss}$

$PTS = 1000 * (k_{20} - k_{21} * PIC)$

If $PTS < k_{19}$: $PTS = k_{19}$

If $PTS > k_{22}$: $PTS = k_{22}$

10.3 Parameter Values

UC_{ss} = GCA_{ss} = 50.0 mmol wk^{-1}
PIC_{ss} = 3.6 mmol
$TBSFC_{ss}$ = 10.0 mmol
$TBSC_{ss}$ = 70.0 mmol
TBC_{ss} = 100 %
$TCLAST_{ss}$ = 500.0
$TBLAST_{ss}$ = 500.0
$TCYTE_{ss}$ = 100.0
$TCYTEACT_{ss}$ = k_8 = 1.0

k_1 = 403.0
k_2 = 200.0
k_3 = 555.5
k_4 = GCA_{ss} = 50.0 mmol wk^{-1}
k_5 = 13.82
k_6 = 25.0
k_7 = 3.57
k_{11} = 125.0
k_{12} = 0.25
k_{13} = 0.32
k_{14} = 0.1
k_{15} = 0.25
k_{16} = 0.0625
k_{17} = 33.33
k_{18} = 33.33
k_{20} = 62.0
k_{21} = 15.0

APPENDIX II

APPENDIX II

Software Details

This appendix covers the software that was used, both those commercial products, and bespoke software that was written.

General Points

The computing needs of this project fell into two broad camps; numerical integration, and parameter optimisation. In a practical sense the two were often used together, with significant use being made of online systems with user friendly interfaces to facilitate the process of model development, normally supplying graphical output as a matter of course.

All 'main-frame' or 'mini' based functions were implemented in FORTRAN, and all the ready written routines used were also either written in or 'callable from' FORTRAN. Initially use was made of a CDC mainframe installation, as all functions could be described as 'number crunching' and a significant expenditure of resources could be expected. Graphic output was generated on micro-film, and on a crude line-printer system, but turn around was slow, with program and model development being cumbersome.

The later availability of a PRIME 550 mini computer led to the development of most functions on this machine, taking advantage of on- line program development in a UNIX like environment (PRIMOS), and user friendly graphic display facilities (GINO & GINOGRAF). Although obviously lacking in power compared to the CDC machine, the ready access, quick turnaround, speed of program development, and on-line facilities more than made up for this. The on-line access, and graphical displays were especially useful in the adaptive fitting of the unidentifiable models as these needed a 'hands-on' approach using a visual assessment of the next step or parameter values to be tried.

Some limited use was made of a DEC20 system running TOPS, again with GINO graphics, but this will not be described separately, the level of support given to graphics in this environment was distinctly lacking. A PC based simulation system was written in TURBO PASCAL to facilitate the development of the long-term models, and although this did not compare with the Prime system, it was sufficiently powerful to adequately function as a development tool.

Functions Implemented

Many routines from the NAG library were used to facilitate the process of numerical integration, and also the automated search for a minimum least squares residual error when fitting models to available data. These were largely from two particular sections of the library; E04, and D02.

Program listings are included for the following:

HANDOPT1 - enabled the output residual error response of a model to be displayed, and invite the user to change parameters as required.

A graphical routine that in fact drew Figure 7.1.

E04FITE5.FTN - A comprehensive simulation system. Parameters can be changed, randomly chosen within certain specified limits, control printing of results, output the results to a file for subsequent graphing.

TRACFIT9.P - Another version of the above system that was set up to specifically fit the plasma tracer disappearnce data used in chapter 7.

TEMP36 - Simulation system set up for tracer data, and statistical assessment of the fitted response, complete with sensitivity analysis. Not actually used to justify the results. See Roberts, 1977.

TEMP31 MOD12P.FTN - Simulation system set up as above, but for calcium and EDTA infusions. As well as the parameter variation options, the results are graphed, with variable plotting axis, variable integration routines, and an automatic entry to a model variation routine, and not just a parameter variation routine.

MODEL20 - The pascal routines used to run MODEL20 on an IBM compatible PC. All routines written in Turbo Pascal.

```
C HANDOPT.P1
C ----------
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /PHIL/ Y(70),AMT(35),YA(70),P(18),E1,ERS(70),
     1XIC(5),WT(70),NXX,NSS
      COMMON /DE/ PI(18),IMER
      DIMENSION YAZC(35),YAZE(35),YAZ2(35),
     1PR(1),IP(10),PRI(1)
      DATA P/26.7,58.52,112.,66.,4.5,.595,7.2,42730.,58.8,.415,28.5
     1,57.6,.675,3.15,0.34,-150.0,0.0,.0744/
      DATA XIC/3.71,8.57,14.4,100.,214./
      DATA YAZC/3.6,3.6,3.673,3.75,3.79,3.92,4.,4.08,4.11,4.22,4.26,
     14.26,4.26,4.19,4.11,4.14,4.17,4.14,3.88,3.89,3.85,3.82,
     13.6,3.5,3.58,10*3.6/
      DATA YAZE/3.6,3.6,3.36,3.112,2.87,2.625,2.71,2.79,2.88,2.96,
     13.02,3.07,3.13,3.19,3.24,3.3,3.36,3.41,3.44,3.47,3.49,3.53,
     23.55,3.58,3.61,3.64,3.63,3.61,7*3.6/
      DATA WT/.001,34*1.,.001,34*1./
      DATA AMT/0.,2.4,2.9,3.4,3.9,4.4,4.9,5.4,5.9,6.4,6.9,7.4,
     17.9,8.4,8.9,9.4,9.9,10.4,10.9,11.4,11.9,12.4,12.9,13.4,13.9,
     114.4,15.4,16.4,17.4,18.4,19.4,20.4,21.4,22.4,23.4/
      DATA PR/1./
      NY=1
      NS=70
      NSS=35
      NP=1
      E1=0.000000001
      CALL FOPEN(6,9,'HANDOPT.D',2,IERR)
      DO 2 I2=1,NSS
         K2=I2+NSS
         YA(I2)=YAZC(I2)+0.1
         YA(K2)=YAZE(I2)+0.1
         IF(I2.EQ.1)GOTO 1
         AMT(I2)=AMT(I2)/24.0
1        CONTINUE
2        CONTINUE
      NXX=5
      NYY=NY
      IPN=10
C   OUTPUT THE INITIAL RESPONSE
C   ---------------------------
3     CONTINUE
4     FORMAT(2X,'*********************************************')
      WRITE(1,5)(P(I),I=1,18)
5     FORMAT('P(1)= ',6(F9.5,1X)/'P(7)= ',6(F9.5,1X)/'P(13)=',6(F9.5
     1,1X))
C   ASK IF THE PARAMETERS ARE O.K.
C   ------------------------------
      WRITE(1,6)
6     FORMAT('ENTER Y FOR PARAMETER CHANGE, N FOR NONE')
      READ(1,7)K2
7     FORMAT(A1)
      IF(K2.EQ.1HN)GOTO 12
      WRITE(1,8)
8     FORMAT('ENTER THE NUMBER TO BE CHANGED'/
     1'THEN THE KEY NUMBER WITH ITS NEW VALUE'/
     2'EACH ON SEPARATE LINES')
      READ(1,9)I20
9     FORMAT(I2)
      DO 11 I=1,I20
         READ(1,10)I19,PIP
10       FORMAT(I2,G14.8)
         P(I19)=PIP
11       CONTINUE
      GOTO 3
12    CONTINUE
      S=0.0
      CALL FUNCT(IFLAG,NP,PR,S)
      SLO=S
13    FORMAT('WEIGHTED SQUARE RESIDUALS = ',F15.6)
      WRITE(1,13)SLO
C  ask if the data needs to be written?
C  ------------------------------------
      WRITE(1,14)
14    FORMAT('ENTER Y FOR DETAILED DATA, N FOR NONE')
      READ(1,15)K1
15    FORMAT(A1)
      IF(K1.EQ.1HN)GOTO 3
      WRITE(6,16)
16    FORMAT(6X,'MODEL',10X,'DATA',11X,'ERROR',10X,'ERROR SQUARED')
      DO 17 J6=1,NS
         ST=ERS(J6)*ERS(J6)
         WRITE(6,18)Y(J6),YA(J6),ERS(J6),ST
17       CONTINUE
      GOTO 3
18    FORMAT(2X,4(F12.7,3X))
      CALL FCLOSE(6,IERR)
      STOP
      END
```

```
      SUBROUTINE FUNCT(IFLAG,NP,PR,TSALL)
C     ------------------------------
C     OPTIMISATION SUBROUTINE WHICH CALCULATES WEIGHTED RESIDUALS
C     E(I) AS LEAST SQUARE FUNCTIONS OF MODEL PARAMETERS
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      DIMENSION PR(NP),X(5)
      DIMENSION W1(5,40)
      COMMON /PHIL/ Y(70),AMT(35),YA(70),P(18),E1,ERS(70),
     1XIC(5),WT(70),NXX,NSS
      COMMON /DE/ PI(18),IMER
      EXTERNAL MODEL
      NXN=NXX
      DO 1 I=1,18
         PI(I)=P(I)
1        CONTINUE
      TSALL=0.0
      DO 7 K3=1,2
         IF(K3.EQ.2)PI(11)=P(16)
         IF(K3.EQ.2)PI(15)=0.1833
         IMER=1
2        DO 3 I5=1,NXX
            X(I5)=XIC(I5)
3           CONTINUE
         T=AMT(1)
         K4=0
         IF(K3.EQ.2)K4=NSS
         DO 6 I6=1,NSS
            I9=I6+K4
            TNEXT=AMT(I6)
            IF(TNEXT.EQ.0.0)GOTO 4
            IFAIL=1
            E2=E1
            CALL DO2BAF(T,TNEXT,NXN,X,E2,MODEL,W1,IFAIL)
            IF(IFAIL.EQ.0)GOTO 4
            WRITE(6,5)I9,T,IFAIL,E2
4           Y(I9)=X(1)
5           FORMAT('****',2X,'WARNING : I6=',I3,3X,'T= ',F7.4,3X,'IFAIL=
     + ',I3,2X,'E2 =',F10.8)
C     CALCULATE THE ERROR IN THE MODEL OUTPUTS , ERS(IERS).
C     ----------------------------------------------------
            ERS(I9)=(Y(I9)-YA(I9))*WT(I9)
            TSALL=ERS(I9)*ERS(I9)+TSALL
6           CONTINUE
7        CONTINUE
      TSALL=TSALL*0.1
      RETURN
      END
```

```
      SUBROUTINE MODEL(T,X,F)
C     ----------------------
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /DE/ PI(18),IMER
      DIMENSION X(5),C(5),F(5),D1(6)
      D1(1)=PI(1)*X(2)
      D1(2)=PI(2)*X(1)
      D1(3)=PI(3)*X(1)
      D1(4)=((X(5)+(PI(14)-1.)*214.)/(PI(14)*490.))*X(3)*PI(4)
      D1(5)=X(3)*PI(5)
      D1(6)=PI(6)*X(4)
1     C(4)=13.6-3.58*X(1)
      IF(C(4).LT.0.3)C(4)=0.3
      IF(C(4).LE.PI(13))GOTO 2
      X1=X(1)
      CALL PTH(T,X1,PTS)
      C(4)=PTS
2     CONTINUE
C     C(5)=TMCALCIUM
      C(5)=0.06939*X(5)+217.0
      IF(X(5).GE.490.0)C(5)=0.019429*X(5)+241.48
      UFC=PI(12)*X(1)
      C(3)=0.174*(UFC-C(5)/2.0)
      UC1=0.352*(UFC-C(5)*0.68)
      IF(UC1.GT.C(3))C(3)=UC1
      UC2=0.7*(UFC-C(5)*0.9)
      IF(UC2.GT.C(3))C(3)=UC2
      IF(C(3).LE.PI(17))C(3)=PI(17)
      C(3)=PI(10)*C(3)
      UC=C(3)
      INC=PI(7)
      YINF =0.0
      IF(T.GE.0.1.AND.T.LE.PI(15))YINF=1.0
      F(1)=INC+D1(1)+D1(4)-D1(2)-D1(3)-UC+YINF*PI(11)
      F(2)=D1(2)-D1(1)
      F(3)=D1(3)+D1(6)-D1(4)-D1(5)
      F(4)=D1(5)-D1(6)
      F(5)=PI(8)*C(4)-PI(9)*X(5)
      RETURN
      END
      SUBROUTINE PTH(T,X1,STP)
C     ------------------------
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /DE/ PI(18),IMER
      IF(IMER.GT.2)GOTO 1
      TINIT=T
      IMER=3
1     CONTINUE
      TPTS=T-TINIT
      IF(TPTS.GT.PI(18))GOTO 2
      STP=13.6-3.58*X1
      RETURN
2     STP=PI(13)
      RETURN
      END
```

```
      DIMENSION PMT(29),PMX(29),X(70),AMTINF(9),XINF(9)
      DIMENSION AMT(35),AMT2(70)
      DATA AMT/0.,2.4,2.9,3.4,3.9,4.4,4.9,5.4,5.9,6.4,6.9,7.4,
     17.9,8.4,8.9,9.4,9.9,10.4,10.9,11.4,11.9,12.4,12.9,13.4,13.9,
     114.4,15.4,16.4,17.4,18.4,19.4,20.4,21.4,22.4,23.4/
      DATA PMT/0.,2.4,2.9,3.4,3.9,4.4,4.9,5.4,5.9,6.4,6.9,7.4,
     17.9,8.9,9.4,10.9,11.4,12.9,13.4,13.9,14.4,23.4,24.,
     226.4,28.4,30.4,34.4,38.4,47.4/
      DATA PMX/29*0.0/
      DATA AMTINF/0.,2.4,2.41,10.55,10.56,26.4,26.41,28.39,28.4/
      DATA XINF/-.4,-.4,-.35,-.35,-.4,-.4,-.35,-.35,-.4/
      CALL S5600
      CALL FOPEN(6,9,'HANDOPT.D',1,IERR)
      READ(6,1)(X(I),I=1,70)
1     FORMAT(32X,F12.7)
2     READ(1,3)NSYM
3     FORMAT(I2)
      IF(NSYM.EQ.1)GOTO 4
      CALL PICCLE
4     CONTINUE
      DO 5 I=1,35
         AMT2(I)=AMT(I)
         I1=I+35
         AMT2(I1)=AMT(I)+24.
5        CONTINUE
      CALL WINDO2(0.0,350.,0.0,200.)
      CALL AXIPOS(1,20.,20.,300.,1)
      CALL AXIPOS(1,20.,20.,150.,2)
      CALL AXISCA(3,24,0.,48.,1)
      CALL AXISCA(3,8,-0.4,0.4,2)
      CALL AXIDRA(1,1,1)
      CALL AXIDRA(-1,-1,2)
      CALL AXIPOS(1,20.,95.,300.,1)
      CALL AXIDRA(0,0,1)
      CALL AXIPOS(1,20.,60.,300.,1)
      CALL AXIDRA(0,0,1)
      CALL AXIPOS(1,20.,130.,300.,1)
      CALL AXIDRA(0,0,1)
      CALL GRAPOL(AMT2,X,70)
C     DRAW THE INFUSION POINTS
C     ========================
C
      CALL GRAPOL(AMTINF,XINF,9)
C
C     DRAW THE ACTUAL DATA POINTS
C     ===========================
C
      CALL GRASYM(PMT,PMX,29,NSYM,0)
C     LABEL THE INFUSION POINTS
C     =========================
C
      CALL GRAMOV(2.4,-.33)
      CALL CHAHOL('CALCIUM INFUSION*.')
      CALL GRAMOV(26.4,-.33)
      CALL CHAHOL('EDTA INFUSION*.')
C     LABEL THE WHOLE GRAPH
C     =====================
      CALL GRAMOV(2.4,0.4)
      CALL CHAHOL('RESIDUAL ERROR IN PLASMA IONISED CALCIUM AFTER*.')
      CALL GRAMOV(2.4,0.35)
      CALL CHAHOL('PARAMETER FITTING*.')
      CALL GRAMOV(26.,0.35)
      CALL CHAHOL('DATA TAKEN FROM JONES * FOURMAN, AND MARSHALL*.')
      CALL GRAMOV(26.,0.3)
      CALL CHAHOL('POINTS ON ZERO AXIS REPRESENT DATA POINTS*.')
      CALL GRAMOV(36.,-.48)
      CALL CHAHOL('TIME IN HOURS*.')
C
C     LABEL THE %AGE AXIS
C     ===================
C
      CALL GRAMOV(34.,-0.18)
      CALL CHAHOL('-5 PER CENT STEADY STATE PIC*.')
      CALL GRAMOV(34.,0.195)
      CALL CHAHOL('+5 PER CENT STEADY STATE PIC*.')
C
      IF(NSYM.EQ.99)GOTO 6
      GOTO 2
C
6     CONTINUE
      CALL DEVEND
      CALL FCLOSE(6,IERR)
      STOP
      END
```

```
C   THIS IS E04FITE5.FTN
C   ====================
C   SET UP FOR A EDTA INFUSION
C   --------------------------
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /GDNT/ PI(18),Y(1,35),AMT(35),YA(1,35),P(18),E1,
     1XIC(5),WT(1,35),EPS,ALF,DP(8),NXX,NY,NS,MAXIT,IPRNT,NPCH(8)
     2,MPIN
      DIMENSION ERS(35),AJAC(35,8),V(8),X(5),YAZ(35),YAZ2(35),
     1W(1000),PR(8),PRI(8), A1(8,35),A2(8,8),A3(8,1),A4(8,8)
     2,IPSET(18)
      DATA P/27.2,61.2,112.,28.8,4.41,.603,7.2,39640.,57.6,.385,
     1-150.0,57.6,0.0,3.15,0.1833,214.,428.,1./
      DATA NPCH/3,4,5,6,8,9,16,17/
      DATA XIC/3.71,8.57,14.4,100.,214./
      DATA YAZ/3.6,3.6,3.36,3.112,2.87,2.625,2.71,2.79,2.88,2.96,
     13.02,3.07,3.13,3.19,3.24,3.3,3.36,3.41,3.44,3.47,3.49,3.53,
     23.55,3.58,3.61,3.64,3.63,3.61,7*3.6/
      DATA WT/0.001,34*1./
      DATA AMT/0.,2.4,2.9,3.4,3.9,4.4,4.9,5.4,5.9,6.4,6.9,7.4,
     17.9,8.4,8.9,9.4,9.9,10.4,10.9,11.4,11.9,12.4,12.9,13.4,13.9,
     114.4,15.4,16.4,17.4,18.4,19.4,20.4,21.4,22.4,23.4/
      DATA PR/8*1.0/
      DATA DP/8*0.1/
      IPRNT=2
      NY=1
      NX=5
      NS=35
      MPIN=2
      NP=8
      E1=0.000001
      NRUN=0
      IRUN=0
      MAXIT=20
      EPS=0.001
      ALF=0.001
      CALL FOPEN(6,10,'E04FITE5.D',2,IERR)
      DO 2 I2=1,NS
         YA(1,I2)=YAZ(I2)+0.1
         IF(I2.EQ.1)GOTO 1
         AMT(I2)=AMT(I2)/24.0
1        CONTINUE
2        CONTINUE
```

```
C   OUTPUT THE INTIIAL PARAMETER CHANGE MENU
C   ----------------------------------------
3     WRITE(1,104)
      WRITE(1,105)
      READ(1,106)N1
      GOTO(7,11,15,17,21,24,48,28,26),N1
7     WRITE(1,108)E1
      WRITE(1,146)
      READ(1,147)K33
      IF(K33.EQ.1HN)GOTO 3
      IF(K33.EQ.1HS)GOTO 48
      WRITE(1,109)
      READ(1,110)E1
      GOTO 7
C   ==============================================
11    WRITE(1,112)(XIC(I),I=1,NX)
      WRITE(1,146)
      READ(1,147)K33
      IF(K33.EQ.1HN)GOTO 3
      IF(K33.EQ.1HS)GOTO 48
      WRITE(1,113)
      READ(1,114)(XIC(I),I=1,NX)
      GOTO 11
C   ======================================
15    WRITE(1,116)(DP(I),I=1,NP)
      WRITE(1,146)
      READ(1,147)K33
      IF(K33.EQ.1HN)GOTO 3
      IF(K33.EQ.1HS)GOTO 48
      WRITE(1,113)
      READ(1,114)(DP(I),I=1,NP)
      GOTO 15
C   ================================
17    WRITE(1,118)MAXIT
      WRITE(1,146)
      READ(1,147)K33
      IF(K33.EQ.1HN)GOTO 3
      IF(K33.EQ.1HS)GOTO 48
      WRITE(1,119)
      READ(1,120)MAXIT
      GOTO 17
C   ========================
21    WRITE(1,122)(NPCH(I),I=1,NP)
      WRITE(1,146)
      READ(1,147)K33
      IF(K33.EQ.1HN)GOTO 3
      IF(K33.EQ.1HS)GOTO 48
      WRITE(1,113)
      READ(1,123)(NPCH(I),I=1,NP)
      GOTO 21
```

```
C    ============================
24     WRITE(1,125)(PR(I),I=1,NP)
       WRITE(1,146)
       READ(1,147)K33
       IF(K33.EQ.1HN)GOTO 3
       IF(K33.EQ.1HS)GOTO 48
       WRITE(1,113)
       READ(1,114)(PR(I),I=1,NP)
       GOTO 24
C   ==============================
26     WRITE(1,127)MPIN
       WRITE(1,146)
       READ(1,147)K33
       IF(K33.EQ.1HN)GOTO 3
       IF(K33.EQ.1HS)GOTO 48
       WRITE(1,119)
       READ(1,120)MPIN
       GOTO 26
C     ===================================
28     WRITE(1,129)
       READ(1,120)IRUN
       WRITE(1,130)
       READ(1,120)K50
       READ(1,'20)(IPSET(I),I=1,K50)
       WRITE(1,131)(IPSET(I),I=1,K50)
       WRITE(1,146)
       WRITE(1,132)
       DO 33 I=1,NP
          PRI(I)=PR(I)
33        CONTINUE
       READ(1,147)K33
       IF(K33.EQ.1HN)GOTO 3
       IF(K33.EQ.1HS)GOTO 34
       GOTO 28
C    START THE DETERMINATION OF A RANDOMLY CHOSEN PARAMETER SET
C    ==========================================================
C    PARAMETERS USED ARE IRUN, K50, AND IPSET(I).
C    --------------------------------------------
34     IF(NRUN.EQ.IRUN)STOP
       DO 35 I=1,NP
          NPCH(I)=0
          PR(I)=PRI(I)
35        CONTINUE
       NRUN =NRUN+1
       WRITE(6,136)NRUN,IRUN,(P(I),I=1,18),(IPSET(I),I=1,K50)
       WRITE(1,137)NRUN,IRUN
```

```
C     CHOOSE THE CURRENT PARAMETER SET
C     ================================
       IF(NRUN.GE.2)GOTO 40
       ZSTEP=1.0/FLOAT(K50+1)
       WRITE(1,138)
       READ(1,139)KY
       ZP=RND(KY)
40     DO 44 I=1,NP
41        ZP=RND(0)
          K13=0
          K10=INT(ZP/ZSTEP)
          IF(K10.EQ.0)GOTO 41
          IF(I.EQ.1)GOTO 43
          I4=I-1
          DO 42 J=1,I4
             IF(K10.EQ.NPCH(J))K13=1
42           CONTINUE
          IF(K13.EQ.1)GOTO 41
43        NPCH(I)=IPSET(K10)
44        CONTINUE
       WRITE(1,145)(NPCH(I),I=1,NP)
       WRITE(6,145)(NPCH(I),I=1,NP)
48     CONTINUE
       IERS=NY*NS
       NXX=NX
       NSS=NS
       NYY=NY
       K= NP + 3 + NP/3
       IW= 2*IERS + 4*NP + IERS*NP+(NP*NP+NP)/2 + K*(2+IERS+2*NP)
       WRITE(6,149)NX,NY,NP,NS,K,IERS,IW
       WRITE(1,149)NX,NY,NP,NS,K,IERS,IW
       CALL GIDENT(PR,NYY,NSS,NX,NP,ERS,IERS,AJAC,IW,W,V,S,IFAIL,A1,A2,A3
      +,A4,NRUN)
       IF(IFAIL.EQ.0)GOTO 52
       WRITE(1,151)
       WRITE(6,150)
       WRITE(6,151)
       WRITE(6,150)
52     CONTINUE
       IF(NRUN.LT.IRUN)GOTO 34
       CALL FCLOSE(6,IERR)
104    FORMAT('THE FOLLOWING PARAMETERS MAY BE CHANGED:'
      1/'  * 1 * INTEGRATION ERROR TEST'
      2/'  * 2 * MODEL INITIAL CONDITIONS'
      3/'  * 3 * PARAMETER SCALE FACTORS'
      4/'  * 4 * MAXIMUM NUMBER OF ITERATIONS'
      5/'  * 5 * THE PARAMETERS (P) THAT ARE TO BE ESTIMATED'
      6/'  * 6 * INITIAL PR(I) VALUES'
      7/'  * 9 * CONTROL PRINT OUTPUT')
105    FORMAT(/'ENTER 1,2,3,4,5, OR 6 TO DISPLAY THE RELEVANT EXISTING
      1VALUES'/'ENTER 7 TO PROCEED WITH THE ESTIMATION'
      2/'ENTER 8 TO RANDOMLY CHOOSE A PARAMETER SET')
```

```
106   FORMAT(I1)
108   FORMAT('E1 = ',F10.8)
109   FORMAT('ENTER NEW ERROR TEST')
110   FORMAT(G13.6)
112   FORMAT('INITIAL MODEL CONDITIONS ARE:'/
     18(1X,F9.4))
113   FORMAT('ENTER THE NEW VALUES EACH ON SEPARATE LINES')
114   FORMAT(G13.6)
116   FORMAT('SCALE FACTORS ARE:',6(F8.6,1X))
118   FORMAT('MAXIT =',I4)
119   FORMAT('ENTER THE NEW VALUE')
120   FORMAT(I4)
122   FORMAT('ELEMENTS OF P TO BE ESTIMATED ARE:',6(I3,2X))
123   FORMAT(I3)
125   FORMAT('INITIAL PR(I) VALUES ARE:',6F10.6)
127   FORMAT('MPIN = ',I3
     1/'IF MPIN = 1, MODEL RESULTS WILL BE PRINTED EVERY ITERATION'
     2/'IF MPIN = 2, MODEL RESULTS WILL NOT BE PRINTED.')
129   FORMAT('ENTER THE NUMBER OF SEPARATE RANDOMLY CHOSEN'
     1/'PARAMETER SETS THAT SHOULD BE RUN, EACH OF THESE'
     2/'WILL BE SUBJECT TO THE PREVIOUSLY SET MAXIMUM'
     3/'NUMBER OF ITERATIONS')
130   FORMAT('ENTER THE TOTAL NUMBER, THEN THE  ELEMENTS OF ARRAY P'
     1/'THAT THE PARAMETER SETS SHOULD BE CHOSEN FROM')
131   FORMAT('PARAMETER SETS WILL BE CHOSEN FROM THE FOLLOWING'
     1/18(1X,I2))
132   FORMAT('YOU CANNOT START THIS MODE OF OPERATION FROM MAIN MENU'
     1/'IT HAS TO BE STARTED FROM HERE BY ENTERING S')
136   FORMAT('RUN NUMBER ',I3,' OUT OF ',I3,' RUNS.'
     1/'FROM THE ARRAY P(I):'
     2/9(F12.6,1X)/9(F12.6,1X)
     3/'SPECIFICALLY THE FOLLOWING ELEMENTS'
     4/18(2X,I3))
137   FORMAT('THIS IS RUN NUMBER ',I3,'OUT OF ',I3)
138   FORMAT('ENTER A SEED FOR THE RANDOM NUMBER GENERATOR'
     1/'AN INTEGER GREATER THAN ONE, AND LESS THAN 9999')
139   FORMAT(I4)
145   FORMAT('THE FOLLOWING PARAMETER SET WILL NOW BE ESTIMATED'
     1/'NUMBERS ',10(2X,I3))
146   FORMAT(/'**  ENTER C TO CHANGE'
     1/'**        N TO RETURN TO MENU'
     2/'**        S TO START ESTIMATION')
147   FORMAT(A1)
149   FORMAT(1X,3HNX=,I2,3HNY=,I2,3HNP=,I3,3HNS=,I3,3H K=,I3,5HIERS=,I5
     1,3HIW=,I6)
150   FORMAT(2X,'*********************************************')
151   FORMAT(23H0*** GIDENT FAILURE ***)
      STOP
      END
```

```
      SUBROUTINE GIDENT(PR,NYY,NSS,NX,NP,ERS,IERS,AJAC,IW,W,V,S,IER,
     1A1,A2,A3,A4,NRUN)
C     ==================================================================
C        IDENTIFICATION OF NONLINEAR DYNAMIC SYSTEM WITH SEVERAL
C        OUTPUT SIGNALS
C        NY   = NO OF SEPARATE OUTPUT SIGNALS (INPUT)
C        NS   = NO OF SAMPLES PER OUTPUT SIGNAL (INPUT)
C        NX   = NO OF STATE VARIABLES (INPUT)
C        NP   = NO OF MODEL PARAMETERS (INPUT)
C        ERS  = IERS ARRAY OF WEIGHTED RESIDUALS (OUTPUT)
C        IERS = DIMENSION OF ERS (INPUT) (IERS=NY*NS)
C        AJAC = IERS,NP JACOBIAN MATRIX (OUTPUT)
C        W    = IW ARRAY OF WORKING STORE (OUTPUT)
C        IW   = DIMENSION OF W (INPUT) (IW=2*IERS+4*NP+IERS*NP+
C               0.5*(NP*NP+NP)+K*(2+IERS+2*NP) WHERE K IS LARGEST
C               INTEGER LESS THAN NP+3+NP/3)
C        V    = NP ARRAY OF SCALING FACTORS (INPUT)
C        S    = SUM OF SQUARE WEIGHTED RESIDUALS (OUTPUT)
C        IER  = ERROR FLAG SET = 0 IF SUCCESSFULL (OUTPUT)
C        A1   = NP,IERS ARRAY OF WORKING STORE
C        A2   = NP,NP ARRAY OF WORKING STORE
C        A3   = NP,1 ARRAY OF WORKING STORE
C        A4   = NP,NP ARRAY OF WORKING STORE
C        IOPT = OPTION ON WEIGHTING SET = 1 IF SCALAR, = 0 IF TIME
C        USER TO SUPPLY TWO SUBROUTINES MODEL AND OUTP VIZ.
C        MODEL(DERX,X,T)
C        WHERE DERX = NX ARRAY OF DERIVATIVES, X = NX ARRAY OF STATES
C        AND T = CURRENT TIME. PURPOSE IS TO COMPUTE DERX GIVEN X
C        OUTP(T,X,Y)
C        WHERE Y = NY ARRAY OF CURRENT MODEL OUTPUTS. PURPOSE IS TO
C        COMPUTE Y GIVEN X
C        THE FOLLOWING NAMED COMMON BLOCK IS ALSO USED (ALSO SUPPLIED
C        BY USER
C        COMMON /GDNT/ PR( ),X( ),XIC( ),YA( , ),Y( , ),E( , ),C(9, ),
C                      AA(9, ),G( , ),IP( ),R( ),F( ),EBND,DT,EB( ),
C                      NXX,NIS,WT( , ),TFIN,NSS,YY( ),NYY
C        n.b. some variable names will have changed, but positions will
C             be the same
C        PR   = NP ARRAY OF MODEL PARAMETERS
C        X    = NX ARRAY OF STATE VARIABLES
C        XIC  = NX ARRAY OF INITIAL CONDITIONS ON X
C        YA   = NY,NS ARRAY OF DESIRED OUTPUTS
C        Y    = NY,NS ARRAY OF MODEL OUTPUTS
C        E    = NY,NS ARRAY OF ERRORS
C        C    = 9,NX ARRAY OF WORKING STORE
C        AA   = 9,NX ARRAY OF WORKING STORE
C        G    = NX,NX ARRAY OF WORKING STORE
C        IP   = NX INTEGER ARRAY OF WORKING STORE
C        R    = NX ARRAY OF WORKING STORE
C        F    = NX ARRAY OF WORKING STORE
C        EBND = INTEGRATION ERROR BOUND
C        DT   = SAMPLING INTERVAL
C        EB   = NX ARRAY OF ERROR BOUNDS
C        NXX  = NUMBER OF STATE VARIABLES (NXX=NX)
C        NIS  = NUMBER OF INTEGRATIONS PER SAMPLE
C        WT   = NY,NS ARRAY OF WEIGHTING COEFFICIINTS
C        TFIN = FINAL CPU TIME
C        NSS  = NUMBER OF SAMPLES (NSS=NS)
```

```
C        YY    = NY ARRAY OF CURRENT MODEL OUTPUTS
C        NYY   = NUMBER OF OUTPUTS (NYY=NY)
C      ------------------------------------------------------------
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      DIMENSION ERS(IERS),AJAC(IERS,NP),V(NP),A1(NP,IERS),
     1PR(NP),W(IW),     A2(NP,NP),A3(NP,1),A4(NP,NP)
      EXTERNAL FUNCT,MONIT
      COMMON /GDNT/ PI(18),Y(1,35),AMT(35),YA(1,35),P(18),E1,
     1XIC(5),WT(1,35),EPS,ALF,DP(8),NXX,NY,NS,MAXIT,IPRNT,NPCH(8)
     2,MPIN
      WRITE(1,1)
      EPS=0.001
      ALF=0.001
C     INITIAL RESPONSE AND DATA CHECK
C     ------------------------------
      IF(NRUN.GE.2)GOTO 12
C     ONLY DO THIS RUN ONCE.
C     ----------------------
      WRITE(6,3)
      WRITE(6,4)NY,NS
      WRITE(6,5)
      WRITE(6,6)
      WRITE(6,7)MAXIT,IPRNT,EPS,ALF,DP(1),(PR(II),II=1,NP)
      WRITE(6,2)
      CALL FUNCT(IERS,NP,PR,ERS)
      S=0.0
      DO 8 I=1,IERS
8        S=S+ERS(I)**2
      SINIT=S
      WRITE(6,0)
      WRITE(6,2)
      DO 9 I=1,NS
         ERT=Y(J,I)-YA(J,I)
         ERP=ERT*100./YA(J,I)
         WRITE(6,10)(Y(J,I),YA(J,I),J=1,NY),ERT,ERP
9        CONTINUE
      WRITE(6,2)
C     OPTIMISATION
C     ------------
12    IFAIL=1
      IF(IPRNT.NE.0)WRITE(6,13)NRUN
      EP=EPS
      AL=ALF
      IPNT=IPRNT
      S=SINIT
      MAXT=MAXIT
      DO 14 I1=1,NP
         V(I1)=DP(I1)
14       CONTINUE
      CALL E04FAF(IERS,NP,PR,ERS,S,EP,AL,V,W,IW,FUNCT,MONIT,IPNT,MAXT,IF
     +AIL)
      IF(IFAIL.EQ.0)GOTO 16
C     ERROR MESSAGE
C     -------------
      WRITE(6,15)MAXT
      IER=1
      GOTO 17
16    IER=0
C     FINAL  RESPONSE
C     ---------------
17    CONTINUE
      CALL FUNCT(IERS,NP,PR,ERS)
      WRITE(6,22)
      WRITE(6,2)
      WRITE(6,18)S
      WRITE(6,19)
      DO 21 J7=1,NP
         WRITE(6,20)J7,PR(J7)
21       CONTINUE
      IF(MPIN.EQ.2)RETURN
      WRITE(6,19)
      WRITE(6,23)
      WRITE(6,2)
      DO 24 J=1,NS
         WRITE(6,25)J,AMT(J),(YA(K,J),K=1,NY)
         WRITE(6,26)(Y(K1,J),K1=1,NY)
24       CONTINUE
      RETURN
C     SENSITIVITY ANALYSIS
C     --------------------
      WRITE(6,27)
      WRITE(6,28)(I,PR(I),A3(I,1),I=1,NP)
      WRITE(1,28)(I,PR(I),A3(I,1),I=1,NP)
C
1     FORMAT(10HIN GIDENT )
2     FORMAT('*****************************************************',
     1'*****************************************************')
3     FORMAT('**0DATA CHECK**')
4     FORMAT('**0NUMBER OF OUTPUT SIGNALS ** =',I2/'**0 NUMBER OF SAMPLE
     1S** =',I2)
5     FORMAT('**INITIAL RESPONSE**')
6     FORMAT(14X,'MAXIT   IPRNT',4X,'EPS',7X,'ALF',5X,'DSTEP',
     15X,'PR(1)',5X,'PR(2)',5X,'PR(8)',5X,'PR(4)',5X,'PR(5)',5X,
     2'PR(6)')
7     FORMAT(13X,I4,4X,I4,9F10.3)
10    FORMAT(10X,'* ',F15.6,' * ',F15.6,' * ',F10.6,F8.4)
11    FORMAT(44H0INITIAL SUM OF WEIGHTED SQUARE RESIDUALS = ,F15.6)
13    FORMAT('**OPTIMISATION MONITOR ** RUN NUMBER ',I3,'**')
15    FORMAT(15H0*** MORE THAN ,I5,15H ITERATIONS ***)
18    FORMAT(42H0FINAL SUM OF WEIGHTED SQUARE RESIDUALS = ,F15.6)
19    FORMAT(8X,'***************************')
20    FORMAT(8X,'**P(',I1,') = ',F15.6,'**')
22    FORMAT(/'**FINAL RESPONSE AND PARAMETERS**')
23    FORMAT('*SAMPLE*',1X,'MEAS. TIME*',5X,'*  OUTPUT1',2X,
     1'*  OUTPUT2',2X,'*  OUTPUT3',2X,'*  OUTPUT4',2X,'*  OUTPUT5',2X,
     2'*  OUTPUT6')
25    FORMAT('* ',I4,' *',3X,F6.3,'  *DATA *',6(F10.4,' *'))
26    FORMAT('*',6X,'*',11X,'*MODEL*',6(F10.4,' *'))
27    FORMAT(22H0ACCURACY OF ESTIMATES)
28    FORMAT(/5H PAT ,I2,1X,9HPARAM.NO.,8X,5HVALUE,8X,9HTOLERANCE//
     1(1H ,9X,I2,6X,2F15.6))
      RETURN
      END
```

```
      SUBROUTINE MONIT(IERS,NP,PR,S,ITERC,SING,LIM)
      IMPLICIT DOUBLE PRECISION(A-H,O-Y)
C     ==========================================
      LOGICAL SING,LIM
      DIMENSION PR(NP)
      WRITE(6,1)ITERC,S,(PR(I),I=1,NP)
      IF(SING)WRITE(6,2)
      IF(LIM)WRITE(6,3)
1     FORMAT(7H ITER =,I5,7H SES = ,F15.6/14H PARAMETERS = ,8F8.5)
2     FORMAT(26H *** LINEAR DEPENDENCE ***)
3     FORMAT(33H *** PARAMETER CHANGE LIMITED ***)
      RETURN
      END
C
      SUBROUTINE FUNCT(IERS,NP,PR,ERS)
C     ==============================
C     OPTIMISATION SUBROUTINE WHICH CALCULATES WEIGHTED RESIDUALS
C     E(I) AS FUNCTIONS OF MODEL PARAMETERS
      IMPLICIT DOUBLE PRECISION(A-H,O-Y)
      DIMENSION PR(NP),ERS(IERS),X(5)
      DIMENSION W1(5,40)
      COMMON /PHIL/ PI(18),Y(1,35),AMT(35),YA(1,35),P(18),E1,
     1XIC(5),WT(1,35),EPS,ALF,DP(8),NXX,NY,NS,MAXIT,IPRNT,NPCH(8)
     2,MPIN
      EXTERNAL MODEL
      WRITE(1,1)
      DO 3 J=1,NP
         DO 2 I=1,18
            IF(J.EQ.1)PI(I)=P(I)
            IF(NPCH(J).EQ.I)PI(I)=P(I)*PR(J)
2           CONTINUE
3        CONTINUE
         NXN=NXX
         DO 4 I5=1,NXN
            X(I5)=XIC(I5)
4           CONTINUE
         T=AMT(1)
         DO 6 I6=1,NS
            TNEXT=AMT(I6)
            IF(TNEXT.EQ.0.0)GOTO 6
            IFAIL=1
            E2=E1
            CALL D02BAF(T,TNEXT,NXN,X,E2,MODEL,W1,IFAIL)
            IF(IFAIL.EQ.0)GO TO 6
            WRITE(6,7)I6,T,IFAIL,E2
5           Y(1,I6)=X(1)
6           CONTINUE
C
C     CALCULATE THE ERROR IN THE MODEL OUTPUTS , ERS(IERS).
C     ----------------------------------------------------
            DO 9 I2=1,NS
               DO 8 I=1,NY
                  I3=((I2-1)*NY)+I
                  ERS(I3)=(Y(I,I2)-YA(I,I2))*WT(I,I2)
8                 CONTINUE
9              CONTINUE
1     FORMAT(10X,'IN FUNCT')
7           FORMAT('****',2X,'WARNING : I6=',I3,3X,'T= ',F7.4,3X,'
     1            IFAIL= ',I3,2X,'E2 =',F10.8)
      RETURN
      END
```

```
C     TRACFIT9.P
C     **********
C     **********
C     THIS IS TO FIT THE PARAMETERS OF MOD9 (WELL AT LEAST SOME OF THEM)
C     TO THE MEAN TRACER DATA. VARIOUS ASSUMPTIONS WILL BE MADE IN THE
C     PROCESS
C     ****************************
C     ****************************
C
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /PHIL/ Y(1,30),AMT(30),YA(1,30),P(18),E1,TS,
     1XIC(5),WT(1,30),IPRNT,NXX,NY,NPCH(7),NSS,ITER
      DIMENSION ERS(30),X(5),
     1W(800),PR(7),IP(10),PRI(7)
      DATA P/26.7,58.52,112.,66.,4.5,.595,3*0.,.415,0.
     1,57.6,0.0,0.675,4*0.0/
      DATA NPCH/1,2,3,4,5,6,10/
      DATA XIC/1.0,3*0.0,1.0/
      DATA WT/0.,1.,1.1,1.2,1.22,1.33,1.47,1.46,1.54,1.66,1.79,1.87,
     12.,2.15,2.5,2.84,3.26,3.52,4.,4.63,5.3,5.79,6.4,7.2,8.,9.7,
     211.,12.6,14.4,14.9/
      DATA PR/7*1.0/
      DATA YA/1.,0.88090
     1,0.82831
     1,0.76313
     1,0.71932
     1,0.66500
     1,0.60225
     1,0.59600
     1,0.57235
     1,0.53657
     1,0.49312
     1,0.47463
     1,0.44707
     1,0.41471
     1,0.35458
     1,0.31318
     1,0.27486
     1,0.25080
     1,0.22754
     1,0.19026
     1,0.16592
     1,0.15242
     1,0.13896
     1,0.12291
     1,0.11060
     1,0.09159
     1,0.07974
     1,0.06929
     1,0.06115
     1,0.05902/
      DATA AMT/0.,0.00420
     1,0.00620
     1,0.00820
     1,0.01000
     1,0.01400
     1,0.01800
     1,0.02100
     1,0.02500
     1,0.03200
     1,0.04200
     1,0.05200
     1,0.06800
     1,0.08500
     1,0.14000
     1,0.20000
     1,0.30000
     1,0.40000
     1,0.50000
     1,0.90000
     1,1.20000
     1,1.50000
     1,1.90000
     1,2.40000
     1,2.90000
     1,3.90000
     1,4.90000
     1,5.90000
     1,6.90000
     1,7.50000/
      IPRNT=2
      NY=1
      TS=0.0
      NX=5
      NS=30
      NSS=30
      NP=7
      ITER=1
      E1=0.000001
      CALL FOPEN(6,10,'TRACFIT9.D',2,IERR)
      NXX=NX
      NYY=NY
      IW=800
      WRITE(6,1)NX,NY,NP,NS,IW
1     FORMAT(1X,3HNX=,I2,3HNY=,I2,3HNP=,I3,3HNS=,I3,
     1,3HIW=,I6)
2     FORMAT(2X,'**********************************************')
      WRITE(6,3)
3     FORMAT('**INITIAL RESPONSE**')
      WRITE(6,6)(PR(II),II=1,NP)
      WRITE(6,2)
      WRITE(6,4)(P(I),I=1,18)
4     FORMAT(' P(I) = ',9(F10.4,1X)/8X,9(F10.4,1X))
      WRITE(6,2)
      WRITE(6,5)(NPCH(I),I=1,NP)
5     FORMAT(5X,'THE ABOVE CORRESPOND TO THE FOLLOWING PARAMETERS'
     1/5X,15(2X,I4))
6     FORMAT(9F10.3)
      WRITE(6,2)
      CALL LSFUN1(NS,NP,PR,ERS)
7     WRITE(6,8)TS
8     FORMAT(44H0INITIAL SUM OF WEIGHTED SQUARE RESIDUALS = ,F15.6)
      WRITE(6,2)
      WRITE(6,9)
9     FORMAT(6X,'MODEL',10X,'DATA',8X,'WT ERROR',7X,'WT ERROR SQUARED'
     1,2X,'PERCENT ERROR')
```

```
      K4=1
      DO 10 J6=1,NS
         ST=ERS(J6)*ERS(J6)
         PS=100.0*(Y(1,J6)-YA(1,J6))/YA(1,J6)
         WRITE(6,11)Y(1,J6),YA(1,J6),ERS(J6),ST,PS
10       CONTINUE
11    FORMAT(2X,4(F12.7,3X),7X,F6.2)
C        OPTIMISATION
C        ============
C
12    IFAIL=1
      S=0.0
      CALL E04FDF(NS,NP,PR,S,IP,10,W,IW,IFAIL)
      IF(IFAIL.EQ.0)GOTO 14
C        ERROR MESSAGE
270
C        ============
      WRITE(6,13)IFAIL
13    FORMAT('*** IFAIL = ',I3)
14    CONTINUE
      CALL FCLOSE(6,IERR)
      STOP
      END
      SUBROUTINE LSFUN1(NS,NP,PR,ERS)
C     ==============================
C     ==============================
C
C        OPTIMISATION SUBROUTINE WHICH CALCULATES WEIGHTED
C        RESIDUALS
C        E(I) AS LEST SQUARE FUNCTIONS OF MODEL PARAMETERS
C
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      DIMENSION PR(NP),ERS(NS),X(5)
      DIMENSION W1(5,40)
      COMMON /PHIL/ Y(1,30),AMT(30),YA(1,30),P(18),E1,TS,
     1XIC(5),WT(1,30),IPRNT,NXX,NY,NPCH(7),NSS,ITER
      COMMON /DE/ PI(18),IMER
      EXTERNAL MODEL
      DO 2 J=1,NP
         DO 1 I=1,18
            IF(J.EQ.1)PI(I)=P(I)
            IF(NPCH(J).EQ.I)PI(I)=P(I)*PR(J)
1           CONTINUE
2        CONTINUE
      NXN=NXX
      ITER =ITER+1
      IMER=1
3     DO 4 I5=1,NXN
         X(I5)=XIC(I5)
4        CONTINUE
      T=AMT(1)
      DO 6 I6=1,NSS
         TNEXT=AMT(I6)
         IF(TNEXT.EQ.0.0)GOTO 5
         IFAIL=1
         E2=E1
         CALL D02BAF(T,TNEXT,NXN,X,E2,MODEL,W1,IFAIL)
         IF(IFAIL.EQ.0)GOTO 5
         WRITE(6,7)I6,T,IFAIL,E2
5        Y(1,I6)=X(1)
6        CONTINUE
7     FORMAT('****',2X,'WARNING : I6=',I3,3X,'T= ',F7.4,3X,'IFAIL= '
     1,I3,2X,'E2 =',F10.8)
C     CALCULATE THE ERROR IN THE MODEL OUTPUTS , ERS(IERS).
C     ----------------------------------------------------
      TS=0.0
      DO 8 I2=1,NSS
         ERS(I2)=(Y(1,I2)-YA(1,I2))*WT(1,I2)
         TS=TS+ERS(I2)*ERS(I2)
8        CONTINUE
      IF(IPRNT.EQ.2)GOTO 14
      IF(TS.GT.SLO)RETURN
      SLO=TS
      WRITE(6,9)(PR(I),I=1,NP)
      WRITE(6,10)TS,ITER
      WRITE(1,10)TS,ITER
9     FORMAT('P(I) =',6(F12.7,1X)/6X,6(F12.7,1X))
10    FORMAT('0**SES = ',F15.8,3X,'** ITER = ',I10)
      DO 11 I3=1,NP
         IF(PR(I3).LT.0.0)NOUN=10
11       CONTINUE
      IF(NOUN.NE.10)GOTO 12
      WRITE(6,13)
      STOP
12    CONTINUE
13    FORMAT('ONE OF THE PARAMETERS IS NOW NEGATIVE...STOP...')
      RETURN
14    IPRNT=1
      SLO=TS
      RETURN
      END
C
      SUBROUTINE MODEL(T,X,F)
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /PHIL/ Y(1,30),AMT(30),YA(1,30),P(18),E1,TS,
     1XIC(5),WT(1,30),IPRNT,NXX,NY,NPCH(7),NSS,ITER
      COMMON /DE/ PI(18),IMER
C      THIS IS MOD8
C     THE BASIC FOUR COMPARTMENT MODEL, WITH NO DIFFERENTIAL CONTROL.
      DIMENSION X(5),C(5),F(5),D1(6)
      D1(1)=PI(1)*X(2)
      D1(2)=PI(2)*X(1)
      D1(3)=PI(3)*X(1)
      D1(4)=(214.0/490.0)*X(3)*PI(4)
      D1(5)=X(3)*PI(5)
      D1(6)=PI(6)*X(4)
      UC=PI(10)*21.3*X(1)/3.71
      INC=-0.1*X(1)
      F(1)=INC+D1(1)+D1(4)-D1(2)-D1(3)-UC
      F(2)=D1(2)-D1(1)
      F(3)=D1(3)+D1(6)-D1(4)-D1(5)
      F(4)=D1(5)-D1(6)
      F(5)=0.0
      RETURN
      END
```

```
C     TEMP36
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /PHIL/ PI(18),Y(1,30),AMT(30),YA(1,30),P(18),E1,
     1XIC(5),WT(1,30),EPS,ALF,DP(6),NXX,NY,NS,MAXIT,IPRNT,NPCH(6)
     2,MPIN
      DIMENSION ERS(30),AJAC(30,6),V(6),X(5),
     1W(1000),PR(6),PRI(6), A1(6,30),A2(6,6),A3(6,1),A4(6,6)
     2,IPSET(18)
      DATA DP/6*0.1/
      DATA P/6.6,6.15,34.4,126.,2.15,.4,3*0.,.2,0.
     1,57.6,0.0,0.675,3*0.0,-0.14/
      DATA NPCH/1,2,3,4,5,6/
      DATA XIC/1.0,3*0.0,1.0/
      DATA WT/0.,1.,1.1,1.2,1.22,1.33,1.47,1.46,1.54,1.66,1.79,1.87,
     12.,2.15,2.5,2.84,3.26,3.52,4.,4.63,5.3,5.79,6.4,7.2,8.,9.7,
     211.,12.6,14.4,14.9/
      DATA PR/6*1.0/
      DATA YA/1.,0.88090
     1,0.82831
     1,0.76313
     1,0.71932
     1,0.66500
     1,0.60225
     1,0.59600
     1,0.57235
     1,0.53657
     1,0.49312
     1,0.47463
     1,0.44707
     1,0.41471
     1,0.35458
     1,0.31318
     1,0.27486
     1,0.25080
     1,0.22754
     1,0.19026
     1,0.16592
     1,0.15242
     1,0.13896
     1,0.12291
     1,0.11060
     1,0.09159
     1,0.07974
     1,0.06929
     1,0.06115
     1,0.05902/
      DATA AMT/0.,0.00420
     1,0.00620
     1,0.00820
     1,0.01000
     1,0.01400
     1,0.01800
     1,0.02100
     1,0.02500
     1,0.03200
     1,0.04200
     1,0.05200
     1,0.06800
     1,0.08500
     1,0.14000
     1,0.20000
     1,0.30000
     1,0.40000
     1,0.50000
     1,0.90000
     1,1.20000
     1,1.50000
     1,1.90000
     1,2.40000
     1,2.90000
     1,3.90000
     1,4.90000
     1,5.90000
     1,6.90000
     1,7.50000/
      IPRNT=2
      NY=1
      NX=5
      NS=30
      MPIN=2
      NP=6
      E1=0.000001
      NRUN=0
      IRUN=0
      MAXIT=20
      EPS=0.001
      ALF=0.001
      CALL FOPEN(6,11,'TRAC2FIT9.D',2,IERR)
C   OUTPUT THE INTIIAL PARAMETER CHANGE MENU
C   ========================================
1     WRITE(1,2)
2     FORMAT('THE FOLLOWING PARAMETERS MAY BE CHANGED:'
     1/'  * 1 * INTEGRATION ERROR TEST'
     2/'  * 2 * MODEL INITIAL CONDITIONS'
     3/'  * 3 * PARAMETER SCALE FACTORS'
     4/'  * 4 * MAXIMUM NUMBER OF ITERATIONS'
     5/'  * 5 * THE PARAMETERS (P) THAT ARE TO BE ESTIMATED'
     6/'  * 6 * INITIAL PR(I) VALUES'
     7/'  * 9 * CONTROL PRINT OUTPUT'
     8/'  *10 * INITIAL P(I) VALUES')
      WRITE(1,3)
3     FORMAT(/'ENTER 1,2,3,4 ....ETC TO DISPLAY THE RELEVANT EXISTING
     1VALUES'/'ENTER 7 TO PROCEED WITH THE ESTIMATION'
     2/'ENTER 8 TO RANDOMLY CHOOSE A PARAMETER SET')
      READ(1,4)N1
4     FORMAT(I2)
      GOTO(5,9,13,15,24,27,51,31,29,19),N1
C
5     WRITE(1,6)E1
6     FORMAT('E1 = ',F10.8)
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,7)
7     FORMAT('ENTER NEW ERROR TEST')
      READ(1,8)E1
```

```
8     FORMAT(G13.6)
      GOTO 5
C
C  ==================================================
9     WRITE(1,10)(XIC(I),I=1,NX)
10    FORMAT('INITIAL MODEL CONDITIONS ARE:'/
     18(1X,F9.4))
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,11)
11    FORMAT('ENTER THE NEW VALUES EACH ON SEPARATE LINES')
      READ(1,12)(XIC(I),I=1,NX)
12    FORMAT(G13.6)
      GOTO 9
C
C  ========================================
13    WRITE(1,14)(DP(I),I=1,NP)
14    FORMAT('SCALE FACTORS ARE:',6(F8.6,1X))
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,11)
      READ(1,12)(DP(I),I=1,NP)
      GOTO 13
C
C  ==================================
15    WRITE(1,16)MAXIT
16    FORMAT('MAXIT =',I4)
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,17)
17    FORMAT('ENTER THE NEW VALUE')
      READ(1,18)MAXIT
18    FORMAT(I4)
      GOTO 15
C
C  ============================
19    WRITE(1,20)(P(I),I=1,18)
20    FORMAT('P(1) =',5(1X,F12.6)/
     1'P(6) =',5(1X,F12.6)/
     2'P(11)=',5(1X,F12.6)/
     3'P(16)=',5(1X,F12.6))
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      IF(K33.NE.1HC)GOTO 19
      WRITE(1,22)
      READ(1,26)K87
      DO 21 I=1,K87
         READ(1,23)K86,PNEW
         P(K86)=PNEW
21       CONTINUE
      GOTO 19
```

```
22    FORMAT('ENTER THE NUMBER THAT ARE TO BE CHANGED'/
     1'THEN EACH ON A NEW LINE'/
     2'EACH ONES NUMBER FOLLOWED BY ITS NEW VALUE')
23    FORMAT(I2,1X,G13.6)
C
C  ==============================
24    WRITE(1,25)(NPCH(I),I=1,NP)
25    FORMAT('ELEMENTS OF P TO BE ESTIMATED ARE:',6(I3,2X))
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,11)
      READ(1,26)(NPCH(I),I=1,NP)
26    FORMAT(I3)
      GOTO 24
C
C  ============================
27    WRITE(1,28)(PR(I),I=1,NP)
28    FORMAT('INITIAL PR(I) VALUES ARE:',6F10.6)
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,11)
      READ(1,12)(PR(I),I=1,NP)
      GOTO 27
C
C  ==============================
29    WRITE(1,30)MPIN
30    FORMAT('MPIN = ',I3
     1/'IF MPIN = 1, MODEL RESULTS WILL BE PRINTED EVERY ITERATION'
     2/'IF MPIN = 2, MODEL RESULTS WILL NOT BE PRINTED.')
      WRITE(1,49)
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 51
      WRITE(1,17)
      READ(1,18)MPIN
      GOTO 29
C
C  ==================================
31    WRITE(1,32)
32    FORMAT('ENTER THE NUMBER OF SEPARATE RANDOMLY CHOSEN'
     1/'PARAMETER SETS THAT SHOULD BE RUN, EACH OF THESE'
     2/'WILL BE SUBJECT TO THE PREVIOUSLY SET MAXIMUM'
     3/'NUMBER OF ITERATIONS')
      READ(1,18)IRUN
      WRITE(1,33)
33    FORMAT('ENTER THE TOTAL NUMBER, THEN THE  ELEMENTS OF ARRAY P'
     1/'THAT THE PARAMETER SETS SHOULD BE CHOSEN FROM')
      READ(1,18)K50
      READ(1,18)(IPSET(I),I=1,K50)
      WRITE(1,34)(IPSET(I),I=1,K50)
34    FORMAT('PARAMETER SETS WILL BE CHOSEN FROM THE FOLLOWING'
     1/18(1X,I2))
      WRITE(1,49)
```

```
      WRITE(1,35)
35    FORMAT('YOU CANNOT START THIS MODE OF OPERATION FROM MAIN MENU'
     1/'IT HAS TO BE STARTED FROM HERE BY ENTERING S')
      DO 36 I=1,NP
         PRI(I)=PR(I)
36       CONTINUE
      READ(1,50)K33
      IF(K33.EQ.1HN)GOTO 1
      IF(K33.EQ.1HS)GOTO 37
      GOTO 31
C
C   START THE DETERMINATION OF A RANDOMLY CHOSEN PARAMETER SET
C   ==========================================================
C  PARAMETERS USED ARE IRUN, K50, AND IPSET(I).
37    IF(NRUN.EQ.IRUN)STOP
      DO 38 I=1,NP
         NPCH(I)=0
         PR(I)=PRI(I)
38       CONTINUE
      NRUN =NRUN+1
      WRITE(6,39)NRUN,IRUN,(P(I),I=1,18),(IPSET(I),I=1,K50)
39    FORMAT('RUN NUMBER ',I3,' OUT OF ',I3,' RUNS.'
     1/'FROM THE ARRAY P(I):'
     2/9(F12.6,1X)/9(F12.6,1X)
     3/'SPECIFICALLY THE FOLLOWING ELEMENTS'
     4/18(2X,I3))
      WRITE(1,40)NRUN,IRUN
40    FORMAT('THIS IS RUN NUMBER ',I3,'OUT OF ',I3)
C    CHOOSE THE CURRENT PARAMETER SET
C    ================================
      IF(NRUN.GE.2)GOTO 43
      ZSTEP=1.0/FLOAT(K50+1)
      WRITE(1,41)
41    FORMAT('ENTER A SEED FOR THE RANDOM NUMBER GENERATOR'
     1/'AN INTEGER GREATER THAN ONE, AND LESS THAN 9999')
      READ(1,42)KY
42    FORMAT(I4)
      ZP=RND(KY)
43    DO 47 I=1,NP
44       ZP=RND(0)
         K13=0
         K10=INT(ZP/ZSTEP)
         IF(K10.EQ.0)GOTO 44
         IF(I.EQ.1)GOTO 46
         I4=I-1
         DO 45 J=1,I4
            IF(K10.EQ.NPCH(J))K13=1
45          CONTINUE
         IF(K13.EQ.1)GOTO 44
46       NPCH(I)=IPSET(K10)
47       CONTINUE
      WRITE(1,48)(NPCH(I),I=1,NP)
      WRITE(6,48)(NPCH(I),I=1,NP)
48    FORMAT('THE FOLLOWING PARAMETER SET WILL NOW BE ESTIMATED'
     1/'NUMBERS ',10(2X,I3))
49    FORMAT(/'**  ENTER C TO CHANGE'
     1/'**        N TO RETURN TO MENU'
     2/'**        S TO START ESTIMATION')
50    FORMAT(A1)
51    CONTINUE
      IERS=NY*NS
      NXX=NX
      NSS=NS
      NYY=NY
      K= NP + 3 + NP/3
      IW= 2*IERS + 4*NP + IERS*NP+(NP*NP+NP)/2 + K*(2+IERS+2*NP)
      WRITE(6,52)NX,NY,NP,NS,K,IERS,IW
      WRITE(1,52)NX,NY,NP,NS,K,IERS,IW
52    FORMAT(1X,3HNX=,I2,3HNY=,I2,3HNP=,I3,3HNS=,I3,3H K=,I3,5HIERS=,I5
     1,3HIW=,I6)
53    FORMAT(2X,'*********************************************')
      CALL GIDENT(PR,NYY,NSS,NX,NP,ERS,IERS,AJAC,IW,W,V,S,IFAIL,A1,A2,A3
     +,A4,NRUN)
      IF(IFAIL.EQ.0)GOTO 55
      WRITE(1,54)
      WRITE(6,53)
      WRITE(6,54)
      WRITE(6,53)
54    FORMAT(23H0*** GIDENT FAILURE ***)
55    CONTINUE
      IF(NRUN.LT.IRUN)GOTO 37
      CALL FCLOSE(6,IERR)
      STOP
      END
C
      SUBROUTINE GIDENT(PR,NYY,NSS,NX,NP,ERS,IERS,AJAC,IW,W,V,S,IER,
     1A1,A2,A3,A4,NRUN)
C     ==================================================================
C        IDENTIFICATION OF NONLINEAR DYNAMIC SYSTEM WITH SEVERAL
C        OUTPUT SIGNALS
C
C        NY   = NO OF SEPARATE OUTPUT SIGNALS (INPUT)
C        NS   = NO OF SAMPLES PER OUTPUT SIGNAL (INPUT)
C        NX   = NO OF STATE VARIABLES (INPUT)
C        NP   = NO OF MODEL PARAMETERS (INPUT)
C        ERS  = IERS ARRAY OF WEIGHTED RESIDUALS (OUTPUT)
C        IERS = DIMENSION OF ERS (INPUT) (IERS=NY*NS)
C        AJAC = IERS,NP JACOBIAN MATRIX (OUTPUT)
C        W    = IW ARRAY OF WORKING STORE (OUTPUT)
C        IW   = DIMENSION OF W (INPUT) (IW=2*IERS+4*NP+IERS*NP+
C               0.5*(NP*NP+NP)+K*(2+IERS+2*NP) WHERE K IS LARGEST
C               INTEGER LESS THAN NP+3+NP/3)
C        V    = NP ARRAY OF SCALING FACTORS (INPUT)
C        S    = SUM OF SQUARE WEIGHTED RESIDUALS (OUTPUT)
C        IER  = ERROR FLAG SET = 0 IF SUCCESSFULL (OUTPUT)
C        A1   = NP,IERS ARRAY OF WORKING STORE
C        A2   = NP,NP ARRAY OF WORKING STORE
C        A3   = NP,1 ARRAY OF WORKING STORE
C        A4   = NP,NP ARRAY OF WORKING STORE
C        IOPT = OPTION ON WEIGHTING SET = 1 IF SCALAR, = 0 IF TIME
C
C        USER TO SUPPLY TWO SUBROUTINES MODEL AND OUTP VIZ.
C
C        MODEL(DERX,X,T)
C        WHERE DERX = NX ARRAY OF DERIVATIVES, X = NX ARRAY OF STATES
C        AND T = CURRENT TIME. PURPOSE IS TO COMPUTE DERX GIVEN X
C
```

```
C        OUTP(T,X,Y)
C        WHERE Y = NY ARRAY OF CURRENT MODEL OUTPUTS. PURPOSE IS TO
C        COMPUTE Y GIVEN X
C
C        THE FOLLOWING NAMED COMMON BLOCK IS ALSO USED (ALSO SUPPLIED
C        BY USER
C
C        COMMON /GDNT/ PR( ),X( ),XIC( ),YA( , ),Y( , ),E( , ),C(9, ),
C                      AA(9, ),G( , ),IP( ),R( ),F( ),EBND,DT,EB( ),
C                      NXX,NIS,WT( , ),TFIN,NSS,YY( ),NYY
C
C        PR   = NP ARRAY OF MODEL PARAMETERS
C        X    = NX ARRAY OF STATE VARIABLES
C        XIC  = NX ARRAY OF INITIAL CONDITIONS ON X
C        YA   = NY,NS ARRAY OF DESIRED OUTPUTS
C        Y    = NY,NS ARRAY OF MODEL OUTPUTS
C        E    = NY,NS ARRAY OF ERRORS
C        C    = 9,NX ARRAY OF WORKING STORE
C        AA   = 9,NX ARRAY OF WORKING STORE
C        G    = NX,NX ARRAY OF WORKING STORE
C        IP   = NX INTEGER ARRAY OF WORKING STORE
C        R    = NX ARRAY OF WORKING STORE
C        F    = NX ARRAY OF WORKING STORE
C        EBND = INTEGRATION ERROR BOUND
C        DT   = SAMPLING INTERVAL
C        EB   = NX ARRAY OF ERROR BOUNDS
C        NXX  = NUMBER OF STATE VARIABLES (NXX=NX)
C        NIS  = NUMBER OF INTEGRATIONS PER SAMPLE
C        WT   = NY,NS ARRAY OF WEIGHTING COEFFICIINTS
C        TFIN = FINAL CPU TIME
C        NSS  = NUMBER OF SAMPLES (NSS=NS)
C        YY   = NY ARRAY OF CURRENT MODEL OUTPUTS
C        NYY  = NUMBER OF OUTPUTS (NYY=NY)
C
C      ==========================================================
C
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      DIMENSION ERS(IERS),AJAC(IERS,NP),V(NP),A1(NP,IERS),
     1PR(NP),W(IW),     A2(NP,NP),A3(NP,1),A4(NP,NP)
      EXTERNAL FUNCT,MONIT
C
      COMMON /PHIL/ PI(18),Y(1,30),AMT(30),YA(1,30),P(18),E1,
     1XIC(5),WT(1,30),EPS,ALF,DP(6),NXX,NY,NS,MAXIT,IPRNT,NPCH(6)
     2,MPIN
      WRITE(1,1)
1     FORMAT(10HIN GIDENT )
2     FORMAT('*****************************************************',
     1'*****************************************************')
      EPS=0.001
      ALF=0.001
C
C      INITIAL RESPONSE AND DATA CHECK
C      ===============================
C
      IF(NRUN.GE.2)GOTO 16
C      ONLY DO THIS RUN ONCE.
      WRITE(6,3)
3     FORMAT('**0DATA CHECK**')
      WRITE(6,4)NY,NS
4     FORMAT('**0NUMBER OF OUTPUT SIGNALS ** =',I2/'**0 NUMBER OF SAMPLE
     1S** =',I2)
      WRITE(6,5)
5     FORMAT('**INITIAL RESPONSE**')
      WRITE(6,6)
6     FORMAT(14X,'MAXIT   IPRNT',4X,'EPS',7X,'ALF',5X,'DSTEP',
     15X,'PR(1)',5X,'PR(2)',5X,'PR(6)',5X,'PR(4)',5X,'PR(5)',5X,
     2'PR(6)')
      WRITE(6,7)MAXIT,IPRNT,EPS,ALF,DP(1),(PR(II),II=1,NP)
7     FORMAT(13X,I4,4X,I4,9F10.3)
      WRITE(6,2)
      WRITE(6,8)(P(I),I=1,18)
8     FORMAT('* P(I) = * '/
     16(2X,F12.6)/6(2X,F12.6)/6(2X,F12.6))
      CALL FUNCT(IERS,NP,PR,ERS)
      DO 12 J3=1,NY
         WRITE(6,9)J3
9        FORMAT('** OUTPUT NUMBER = ',I3)
         WRITE(6,10)
10       FORMAT(6X,'MODEL',10X,'DATA',8X,'WT ERROR',7X,'WT ERROR SQUARED
     +',2X,'PERCENT ERROR')
         ST=0.0
         DO 11 J6=1,NSS
            ST=ERS(J6)*ERS(J6)
            PS=100.0*(Y(J3,J6)-YA(J3,J6))/YA(J3,J6)
            WRITE(6,13)Y(J3,J6),YA(J3,J6),ERS(J6),ST,PS
11          CONTINUE
12       CONTINUE
13    FORMAT(2X,4(F12.7,3X),7X,F6.2)
14    WRITE(6,15)ST
15    FORMAT(44H0INITIAL SUM OF WEIGHTED SQUARE RESIDUALS = ,F15.6)
      WRITE(6,2)
C
C        OPTIMISATION
C        ============
C
16    IFAIL=1
      IF(IPRNT.NE.0)WRITE(6,17)NRUN
17    FORMAT('**OPTIMISATION MONITOR ** RUN NUMBER ',I3,'**')
      EP=EPS
      AL=ALF
      IPNT=IPRNT
      S=SINIT
      MAXT=MAXIT
      DO 18 I1=1,NP
         V(I1)=DP(I1)
18       CONTINUE
      CALL E04FAF(IERS,NP,PR,ERS,S,EP,AL,V,W,IW,FUNCT,MONIT,IPNT,MAXT,IF
     +AIL)
      IF(IFAIL.EQ.0)GOTO 20
C
C        ERROR MESSAGE
C        =============
C
      WRITE(6,19)MAXT
19    FORMAT(15H0*** MORE THAN ,I5,15H ITERATIONS ***)
      IER=1
      GOTO 21
20    IER=0
C
```

```
C        FINAL  RESPONSE
C        ===============
C
21    CONTINUE
      CALL FUNCT(IERS,NP,PR,ERS)
      WRITE(6,26)
      WRITE(6,2)
      WRITE(6,22)S
22    FORMAT(42H0FINAL SUM OF WEIGHTED SQUARE RESIDUALS = ,F15.6)
      WRITE(6,23)
23    FORMAT(7X,'***************************')
      DO 25 J7=1,NP
         WRITE(6,24)J7,PR(J7)
24       FORMAT(7X,'**P(',I1,') = ',F15.6,'**')
25       CONTINUE
      IF(MPIN.EQ.2)RETURN
      WRITE(6,23)
26    FORMAT(/'**FINAL RESPONSE AND PARAMETERS**')
      WRITE(6,27)
      WRITE(6,2)
27    FORMAT('*SAMPLE*',1X,'MEAS. TIME*',5X,'*  OUTPUT1',2X,
     1'*  OUTPUT2',2X,'*  OUTPUT3',2X,'*  OUTPUT4',2X,'*  OUTPUT5',2X,
     2'*  OUTPUT6')
      DO 28 J=1,NS
         WRITE(6,29)J,AMT(J),(YA(K,J),K=1,NY)
         WRITE(6,30)(Y(K1,J),K1=1,NY)
28       CONTINUE
29    FORMAT('* ',I4,' *',3X,F6.3,'  *DATA *',6(F10.4,' *'))
30    FORMAT('*',6X,'*',11X,'*MODEL*',6(F10.4,' *'))
      RETURN
C
C        SENSITIVITY ANALYSIS
C        ====================
      WRITE(6,31)
31    FORMAT(22H0ACCURACY OF ESTIMATES)
      WRITE(6,32)(I,PR(I),A3(I,1),I=1,NP)
      WRITE(1,32)(I,PR(I),A3(I,1),I=1,NP)
32    FORMAT(/5H PAT ,I2,1X,9HPARAM.NO.,8X,5HVALUE,8X,9HTOLERANCE//
     1(1H ,9X,I2,6X,2F15.6))
      RETURN
      END
```

```
      SUBROUTINE MONIT(IERS,NP,PR,S,ITERC,SING,LIM)
      IMPLICIT DOUBLE PRECISION(A-H,O-Y)
C     ==========================================
C     ==========================================
C
      LOGICAL SING,LIM
      DIMENSION PR(NP)
      WRITE(6,0)ITERC,S,(PR(I),I=1,NP)
      WRITE(1,0)S,ITERC
0     FORMAT('* SES = ',F12.8,2X,'ITERATION NUMBER = ',I4)
0     FORMAT(7H ITER =,I5,7H SES = ,F15.6/14H PARAMETERS = ,8F10.5)
      IF(SING)WRITE(6,0)
0     FORMAT(26H *** LINEAR DEPENDENCE ***)
      IF(LIM)WRITE(6,0)
0     FORMAT(33H *** PARAMETER CHANGE LIMITED ***)
      RETURN
      END
```

```
C  Temp31   MOD12P.FTN (MOD9.P)
C
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /DE/ P(18),IMER
      COMMON /D2/ CARR(6,251),ANN(2,251)
      COMMON /ANAL/ C(5),M9,KKK,YINF,N5,K14(5)
      COMMON /PLOT/ CBAX(5,7,251),TMAX
      COMMON /ABT/ ZXPOS,ND,ZDNAX1,ZDNIN1,I1INT,Z1,ZT2,Z3,Z4,
     1ZX3(251),Z2(5,251),ITITLE(36),M7,M10,ZINF(251),MS(5),Z9,
     2MES1(21),DAX(7),DIIN(7),ZDATA3(35),ZDATA1(35),ZDATA2(35),MINT(7)
      DIMENSION DX(6),X(6),DIN(6),TIME(251),YPV(5),W1(6,40)
      INTEGER KPV(5),K33
      EXTERNAL MODEL
      DATA YPV/5*0.0/
      DATA KPV/16,16,16,16,16/
      DATA DAX/5.,16.,16.,120.,2000.,30.,5./
      DATA DIIN/1.,0.,0.,0.,0.,0.,0./
      DATA MINT/8,8,8,10,5,5,8/
      DATA X/3.69,6.13,2.42,13.9,250.,3.0/
      DATA MES1/2HSA,2HMP,2HLE,17*2H  ,2H*./
      DATA MS/1,10,3*0/
      DATA P/26.7,58.52,112.,66.,4.5,.595,7.2,42730.,58.8,.415,28.5
     1,57.6,.675,3.15,0.34,0.0,2.0,.0744/
      DATA ZDATA2 /3.6,3.6,3.673,3.75,3.79,3.92,4.,4.08,4.11,4.22,
     14.26,4.26,4.26,4.19,4.11,4.14,4.17,4.14,3.88,3.89,3.85,3.82,
     23.6,3.5,3.58,10*3.6/
      DATA ZDATA1/0.,2.4,2.9,3.4,3.9,4.4,4.9,5.4,5.9,6.4,6.9,7.4,
     17.9,8.4,8.9,9.4,9.9,10.4,10.9,11.4,11.9,12.4,12.9,13.4,13.9,
     114.4,15.4,16.4,17.4,18.4,19.4,20.4,21.4,22.4,23.4/
      DATA ZDATA3/3.6,3.6,3.36,3.112,2.87,2.625,2.71,2.79,2.88,2.96,
     13.02,3.07,3.13,3.19,3.24,3.3,3.36,3.41,3.44,3.47,3.49,3.53,
     23.55,3.58,3.61,3.64,3.63,3.61,7*3.6/
      CALL FOPEN(6,6,'MOD9.D',2,IERR)
      WRITE(6,1)IERR
1     FORMAT(15H  IERR =      ,I2)
      IF(IERR.NE.0)STOP
      DO 2 I4=1,35
         ZDATA2(I4)=ZDATA2(I4)+0.1
         ZDATA3(I4)=ZDATA3(I4)+0.1
2        CONTINUE
      M4=0
      M10=1
      IMER=1
      M5=6
      E1=0.00000001
      M6=18
      M7=1
      M8=5
      DO 3 I=1,M5
         DIN(I)=X(I)
3        CONTINUE
      CALL S5600
      CALL ERRMAX(100)
4     CONTINUE
      M9=0
5     M9=M9+1
      GOTO(6,7,8,9,10),M9
6     CONTINUE
      I9=KPV(1)
      P(I9)=YPV(1)
      GOTO 11
7     CONTINUE
      I9=KPV(2)
      P(I9)=YPV(2)
      GOTO 11
8     CONTINUE
      I9=KPV(3)
      P(I9)=YPV(3)
      GOTO 11
9     CONTINUE
      I9=KPV(4)
      P(I9)=YPV(4)
      GOTO 11
10    CONTINUE
      I9=KPV(5)
      P(I9)=YPV(5)
11    CONTINUE
      JP=0
      DO 12 I=1,M5
         X(I)=DIN(I)
12       CONTINUE
      KESUL=10
      KESEL=1
      KESAL=10
      TMAX=1.0
      HO=0.01
      KKK=1
      T=0.0
13    CONTINUE
C
C     WRITE ALL PARAMETERS TO THE TERMINAL,
C     ************************************
C
      WRITE(1,14)
14    FORMAT(20X,'    ')
      WRITE(1,51)
      WRITE(1,45)
      WRITE(1,51)
      WRITE(1,42)(P(I),I=1,M6)
      WRITE(1,43)TMAX,HO,E1,M7
15    CONTINUE
      WRITE(1,46)
      WRITE(1,48)
16    READ(1,52)K33
      IF(K33.EQ.1HY)GOTO 18
      IF(K33.EQ.1HN)GOTO 37
      IF(K33.EQ.1HG)GOTO 25
      IF(K33.EQ.1HC)GOTO 85
      WRITE(1,17)
17    FORMAT(10X,'BAD ENTRY, TRY AGAIN')
      GOTO 15
18    WRITE(1,19)
19    FORMAT(10X,'ENTER NO. OF PARAMETERS TO BE ALTERED THEN RETURN')
      READ(1,20)K29
20    FORMAT(I2)
      WRITE(1,21)
      WRITE(1,22)
      WRITE(1,23)
```

```
21    FORMAT(20X,'ENTER KEY NO. OF PARAMETER TO BE CHANGED')
22    FORMAT(20X,'FOLLOWED BY ITS NEW VALUE, THIS MUST')
23    FORMAT(20X,' INCLUDE A DECIMAL POINT, AND RETURN')
      DO 24 K99=1,K29
         READ(1,44)K34,PZ
         IF(K34.EQ.19)TMAX=PZ
         IF(K34.EQ.20)HO=PZ
         IF(K34.EQ.21)E1=PZ
         IF(K34.EQ.22)M7=INT(PZ)
         IF(K34.LE.18)P(K34)=PZ
24       CONTINUE
      GOTO 13
25    WRITE(1,26)
      WRITE(1,27)
26    FORMAT(10X,'PRESENT PLOTTING AXIS ARE:')
27    FORMAT(10X,'VARIABLE',2X,'MINIMUM',5X,'MAXIMUM',3X,'INTERVALS')
      WRITE(1,28)(I,DIIN(I),DAX(I),MINT(I),I=1,13)
28    FORMAT(14X,I2,4X,F10.4,2X,F10.4,4X,I2)
      WRITE(1,29)
29    FORMAT(10X,'ENTER THE NUMBER OF VARIABLES AFFECTED')
      READ(1,30)K45
      IF(K45.EQ.0)GOTO 33
30    FORMAT(I2)
      WRITE(1,31)
      WRITE(1,32)
31    FORMAT(10X,'ENTER THE VARIABLE, MINIMUM, MAXIMUM, AND RETURN')
32    FORMAT(10X,'THE REQUIRED NUMBER OF TIMES')
      DO 33 I=1,K45
         READ(1,34)K46,Q1,Q2,IQ3
         DIIN(K46)=Q1
         DAX(K46)=Q2
         MINT(K46)=IQ3
33       CONTINUE
34    FORMAT(I2,2G10.4,I2)
      GOTO 13
35    FORMAT(11I2)
36    FORMAT(10X,'X( ',I2,' ) = ',F13.6)
37    WRITE(1,51)
      WRITE(1,41)
      WRITE(1,51)
      WRITE(1,38)
38    FORMAT(10X,'ENTER REFERENCE MESSAGE (MAX = 40)')
      READ(1,40)(MES1(I),I=1,20)
      WRITE(1,39)
39    FORMAT(10X,'O.K.')
40    FORMAT(20A2)
41    FORMAT(20X,'*PARAMETERS REMAIN AS ABOVE*')
42    FORMAT('P(1)= ',1X,4(F12.5,2X)/'P(5)= ',1X,4(F12.5,2X)/
     1'P(9)= ',1X,4(F12.5,2X)/'P(13)= ',4(F12.5,2X)/'P(17)= ',4(F12
     2.5,2X)/)
43    FORMAT(5X,'KEY 19  TMAX= ',F7.3/5X,'KEY 20  TSTEP= ',F8.6/5X,
     1'KEY 21  ERROR TEST= ',F12.10/5X,'KEY 22  NO. OF RUNS= ',I2)
44    FORMAT(I2,G13.6)
45    FORMAT(20X,28H* INITIAL MODEL PARAMETERS */)
46    FORMAT(10X,'IF CHANGE IN ONE PARAMETER IS DESIRED ENTER Y')
47    FORMAT(20X,'OTHERWISE ENTER N  TO START SIMULATION')
48    FORMAT(10X,'OTHERWISE ENTER N')
49    FORMAT(20X,'IF THE MODEL IS TO BE CHANGED ENTER CM')
50    FORMAT(20X,'FOLLOWED BY CO CMOD')
51    FORMAT(20X,28H****************************)
52    FORMAT(A1)
C
      WRITE(6,56)
      WRITE(6,53)(X(I),I=1,M5)
53    FORMAT(7HX(I) = ,10H**********,/(6(3X,F12.5))//)
      WRITE(6,56)
      WRITE(6,54)M9
54    FORMAT(15X,28H** RATE CONSTANT SET NUMBER ,I2,1X,2H**)
      WRITE(6,55)(P(I),I=1,M6)
55    FORMAT(6(2X,F12.5)///6(2X,F12.5)///6(2X,F12.5)///)
56    FORMAT(9X,104H*****************************************************
     1*****************************************************)
C
      DO 57 I=1,M8
         C(I)=0.0
57       CONTINUE
      ANN(1,1)=0.0
      ANN(2,1)=0.0
      DO 58 I=1,M5
         DX(I)=0.0
58       CONTINUE
C
      IMER=1
59    IF(KKK.GT.250)GOTO 73
      DO 61 I=1,M5
60       CARR(I,KKK)=X(I)
61       CONTINUE
      ANN(1,KKK)=C(3)
      IF(C(3).GT.40.)ANN(1,KKK)=40.0
      TIME(KKK)=T
      ZINF(KKK)=YINF+1.0
      IF(T.GE.TMAX)GOTO 75
C
C
      E2=E1
      T1=T+HO
      IFAIL=0
      K101=IDINT(P(18))
      GOTO(62,63,64),K101
62    CALL D02BAF(T,T1,M5,X,E2,MODEL,W1,IFAIL)
      GOTO 65
63    CALL D02CAF(T,T1,M5,X,E2,MODEL,W1,IFAIL)
      GOTO 65
64    CALL D02EAF(T,T1,M5,X,E2,MODEL,W1,40,IFAIL)
65    CONTINUE
      IF(IFAIL.LT.1)GOTO 69
C
C
      IF(IFAIL.LE.2)GOTO 67
      WRITE(6,66)
66    FORMAT(20X,' Y HAS NOT BEEN ASSIGNED A PROPER VALUE')
      GOTO 80
67    WRITE(6,56)
      WRITE(6,68)IFAIL,T
68    FORMAT(1X,'FAILURE OCCURRED, ERROR MODE  ',I2,5X,'  AT T=  ',
     1F10.4//)
      WRITE(6,56)
      GOTO 80
```

```
69      KKK=KKK+1
        JP=JP+1
        IF(JP.LT.10)GOTO 72
C
        IF(KESEL.EQ.10)GOTO 72
        WRITE(6,70)T,(X(I),I=1,M5)
70      FORMAT(2X,6H**T = ,F6.4,6(5X,F12.5)/15X,6(5X,F12.5))
C
71      JP=0
72      GOTO 59
C
73      WRITE(6,74)
74      FORMAT(30X,'DATA ARRAYS FULL')
C
75      WRITE(6,76)
76      FORMAT(30X,16H END OF DATA SET)
        WRITE(6,56)
C
        NC=M5-1
        DO 78 I=1,NC
           DO 77 J=1,KKK
              CBAX(M9,I,J)=CARR(I,J)
77            CONTINUE
78         CONTINUE
        DO 79 J=1,KKK
           CBAX(M9,6,J)=ANN(1,J)
           ZX3(J)=24.0*TIME(J)
79         CONTINUE
80      IF(M9.LT.M7)GOTO 5
81      CONTINUE
        CALL DEVPAP(370.,260.,0)
        ND=KKK
        CALL PLOTER(NC)
        WRITE(1,82)
        WRITE(1,83)
        WRITE(1,49)
82      FORMAT(20X,'DO YOU WISH TO RUN SOME MORE SIMULATIONS?')
83      FORMAT(20X,'ENTER Y OR N AS APPROPRIATE')
        READ(1,84)K35
84      FORMAT(A1)
        IF(K35.EQ.1HY)CALL PICCLE
        IF(K35.EQ.1HY)GOTO 4
        IF(K35.EQ.1HC)GOTO 85
        GOTO 88
85      CONTINUE
        WRITE(1,50)
        WRITE(1,86)
        WRITE(1,87)
86      FORMAT(20X,'AFTER FINISHING EDITING TYPE FILE, ')
87      FORMAT(20X,'THEN CO CCROM TO REACH SIMULATION MODE')
88      CALL DEVEND
        CALL FCLOSE(6,IERR)
        STOP
        END
```

```
        SUBROUTINE PLOTER(NC)
        IMPLICIT DOUBLE PRECISION (A-H,O-Y)
        COMMON /PLOT/ CBAX(5,7,251),TMAX
        COMMON /ABT/ ZXPOS,ND,ZDNAX1,ZDNIN1,I1INT,Z1,ZT2,Z3,Z4,
       1ZX3(251),Z2(5,251),ITITLE(36),M7,M10,ZINF(251),MS(5),Z9,
       2MES1(21),DAX(7),DIIN(7),ZDATA3(35),ZDATA1(35),ZDATA2(35),MINT(7)
        ND=ND-1
        IF(ND.GT.250)ND=250
        TMAX=ZX3(ND)
        Z4=0.12*TMAX
        Z1=0.2*TMAX
        J2=0
        DO 8 J=1,6
           J2=J2+1
           ZYPOS=15.
           IF(J2.EQ.2.OR.J2.EQ.5.OR.J2.EQ.8.OR.J2.EQ.11)ZYPOS=100.
           IF(J2.EQ.3.OR.J2.EQ.6.OR.J2.EQ.9.OR.J2.EQ.12)ZYPOS=185.
1          ZXPOS=20.
           IF(J2.EQ.4.OR.J2.EQ.10.OR.J2.EQ.5.OR.J2.EQ.11.OR.J2.EQ.6.OR.J2.
       +EQ.12)ZXPOS=200.
           DO 3 I=1,M7
              DO 2 K=1,ND
                 Z2(I,K)=CBAX(I,J,K)
2                CONTINUE
3             CONTINUE
           ZDNAX1=DAX(J)
           ZDNIN1=DIIN(J)
           I1INT=MINT(J)
           DO 5 I2=1,M7
              DO 4 I3=1,ND
                 IF(Z2(I2,I3).GT.ZDNAX1)ZDNAX1=Z2(I2,I3)
                 IF(Z2(I2,I3).LT.ZDNIN1)ZDNIN1=Z2(I2,I3)
4                CONTINUE
5             CONTINUE
6          Z3=ZDNIN1-0.15*(ZDNAX1-ZDNIN1)
           Z9=ZDNIN1+0.1*(ZDNAX1-ZDNIN1)
C       RUN-TIME VARIABLE FREEZING
           DO 7 I2=2,5
              IF(J.EQ.MS(I2))J2=J2-1
              IF(J.EQ.MS(I2))GOTO 8
7             CONTINUE
           ZT2=ZDNIN1+0.9*(ZDNAX1-ZDNIN1)
           CALL POLT(J,TMAX,ZYPOS,J2)
8          CONTINUE
        RETURN
        END
        SUBROUTINE POLT(K7,TM1,ZYPOS,K8)
        IMPLICIT DOUBLE PRECISION (A-H,O-Y)
        DIMENSION Z6(251)
        COMMON /ABT/ ZXPOS,ND,ZDNAX1,ZDNIN1,I1INT,Z1,ZT2,Z3,Z4,
       1ZX3(251),Z2(5,251),ITITLE(36),M7,M10,ZINF(251),MS(5),Z9,
       2MES1(21),DAX(7),DIIN(7),ZDATA3(35),ZDATA1(35),ZDATA2(35),MINT(7)
        Z7=TM1
        CALL AXIPOS(0,ZXPOS,ZYPOS,160.,1)
        CALL AXIPOS(0,ZXPOS,ZYPOS,70.,2)
        CALL AXISCA(3,6,0.,Z7,1)
        CALL AXISCA(3,I1INT,ZDNIN1,ZDNAX1,2)
        CALL AXIDRA(2,1,1)
```

```
      IF(K8.EQ.7)GOTO 1
      GOTO 2
1     PAUSE
      CALL PICCLE
      CALL AXIDRA(2,1,1)
2     CONTINUE
      CALL AXIDRA(-2,-1,2)
      CALL GRAMOV(Z1,ZT2)
      GOTO(3,4,5,6,7,8),K7
3     CALL CHAHOL('P.I.C. (M.MOLES)*.')
      GOTO 9
4     CALL CHAHOL('E.C.F.CA. (M.MOLES)*.')
      GOTO 9
5     CALL CHAHOL('B.S.F.CA. (M.MOLES)*.')
      GOTO 9
6     CALL CHAHOL('B.S.CA. (M.MOLES)*.')
      GOTO 9
7     CALL CHAHOL('PTH (M.MOLES)*.')
      GOTO 9
8     CALL CHAHOL('URINE CALCIUM (M.MOLES/DAY)*.')
9     CALL GRAMOV(Z1,Z3)
      CALL CHAHOL('TIME IN HOURS     ->*.')
      IF(K8.EQ.4.OR.K8.EQ.8)GOTO 10
      GOTO 11
10    CALL GRAMOV(Z4,Z9)
      CALL CHAHOL(MES1)
11    CALL PENSEL(1,0.,0)
      DO 13 K5=1,M7
         DO 12 I=1,ND
            Z6(I)=Z2(K5,I)
12          CONTINUE
         CALL GRAPOL(ZX3,Z6,ND)
13       CONTINUE
      IF(K7.NE.1)GOTO 14
      CALL GRASYM(ZDATA1,ZDATA2,35,2,1)
      CALL GRASYM(ZDATA1,ZDATA3,35,4,1)
      CALL GRAPOL(ZX3,ZINF,ND)
14    CONTINUE
      RETURN
      END
```

```
      SUBROUTINE MODEL(T,X,F)
C      THIS IS MOD8
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /DE/ P(18),IMER
      COMMON /ANAL/ C(5),M9,KKK,YINF,N5,K14(5)
      DIMENSION X(6),F(6),D1(6)
      D1(1)=P(1)*X(2)
      D1(2)=P(2)*X(1)
      D1(3)=X(6)*P(3)*X(1)/3.
      D1(4)=((X(5)+(P(14)-1.)*214.)/(490.*P(14)))*P(4)*X(3)
      D1(5)=X(3)*P(5)
      D1(6)=P(6)*X(4)
1     C(4)=13.6-3.58*X(1)
      IF(C(4).LT.0.3)C(4)=0.3
      IF(C(4).LE.P(13))GOTO 2
      X1=X(1)
      CALL PTH(T,X1,PTS)
      C(4)=PTS
2     CONTINUE
C     C(5)=TMCALCIUM
      C(5)=0.06939*X(5)+217.0
      IF(X(5).GE.490.0)C(5)=0.019429*X(5)+241.48
      UFC=P(12)*X(1)
      C(3)=0.174*(UFC-C(5)/2.0)
      UC1=0.352*(UFC-C(5)*0.68)
      IF(UC1.GT.C(3))C(3)=UC1
      UC2=0.7*(UFC-C(5)*0.9)
      IF(UC2.GT.C(3))C(3)=UC2
      IF(C(3).LE.0.0)C(3)=0.0
      C(3)=P(10)*C(3)
      UC=C(3)
      CIN=P(7)
      PIN=6.0
      YINF =0.0
      IF(T.GE.0.1.AND.T.LE.P(15))YINF=1.0
      F(1)=CIN+D1(1)+D1(4)-D1(2)-D1(3)-UC+YINF*P(11)
      F(6)=PIN-P(17)*X(6)+YINF*P(16)
      F(2)=D1(2)-D1(1)
      F(3)=D1(3)+D1(6)-D1(4)-D1(5)
      F(4)=D1(5)-D1(6)
      F(5)=P(8)*C(4)-P(9)*X(5)
      RETURN
      END
      SUBROUTINE PTH(T,X1,STP)
      IMPLICIT DOUBLE PRECISION (A-H,O-Y)
      COMMON /DE/P(18),IMER
      IF(IMER.GT.2)GOTO 1
      TINIT=T
      IMER=3
1     CONTINUE
      TPTS=T-TINIT
      IF(TPTS.GT.P(18))GOTO 2
      STP=13.6-3.58*X1
      RETURN
2     STP=P(13)
      RETURN
      END
```

```
program model20;
***************
type
   real_array = array[1..25] of real;

var
   k:        real_array;
   x:        real_array;
   result:   array[1..50] of real_array;
   t,dt,tmax,error:        real;
   TCR,UFC,UC:             real;
   TBCR,TBCA,PTS:          real;
   TBSFCR,TBSFCA,TBSCA,TBSCR:       real;
   TCYTEACT,TCLASTACT,TBLASTACT:    real;
   TCYTE_FORM,TCLAST_FORM,TBLAST_FORM:   real;
   TCYTE_DTH,TCLAST_DTH,TBLAST_DTH:      real;
   TCYTEACT_ss,TCLASTACT_ss,TBLASTACT_ss,PTH_ss,PIC_ss:    real;

   npar,nvar:   integer;
   x_val:       integer;
   time_step:   integer;
   loop1:       integer;
   interval:    integer;
   v,dv,y:      real_array;
   col:         array[1..10] of byte;

procedure read_parameters;
begin
npar:=23;
dt:=1.0;
tmax:=20.0;
t:=0.0;
error:=0.0001;
interval:=200;
k[1]:=403.0;
k[2]:=200.0;
k[3]:=555.5;
k[4]:=68.8;       { gut calcium absorption rate }
k[5]:=13.82;
k[6]:=25.0;       { passive bone surface accretion to TBSC)
k[7]:=3.57;       { index of bone surface backflow to BSFC)
k[8]:=1.0;        { index of tcyteact }
k[9]:=1.0;        { index of tclastact)
k[10]:=1.0;       { index of tblastact)
k[11]:=125.0;     { clast formation rate }
k[12]:=0.25;      { blast formation rate }
k[13]:=0.32;      { cyte formation rate  }
k[14]:=0.1;       { cyte death rate, no way to estimate though)
k[15]:=0.25;      { clast dth rate)
k[16]:=0.0625;    { blast dth rate)
k[17]:=33.33;     { trab bone resorption rate constant)
k[18]:=33.33;     { trab bone accretion rate constant
k[19]:= 2000.0;   { basal secretion rate)
k[20]:= 62.0;     { intercept of PTS upon PIC)
k[21]:= 15.0;     { slope of PIC upon PTS)
k[22]:= 20000.0;  { maxm PTH secretion rate)
k[23]:= 80.0;     { PTH loss rate constant)
end;
```

```
procedure read_variables;
begin
nvar:=8;
x[1]:=3.6;        { plasma ionised calcium }
x[2]:=10.0;       { trab bone surface fluid calcium }
x[3]:=70.0;       { trab bone surface calcium }
x[4]:=100.0;      { trab bone calcium }
x[5]:=100.0;      { trab bone osteocyte population }
x[6]:=500.0;      { trab osteoclast population }
x[7]:=500.0;      { trab osteoblast population }
x[8]:=100.0;      { plasma parathyroid hormone }
TCYTEACT_ss := 1.0;
TCLASTACT_ss:= 1.0;
TBLASTACT_ss:= 1.0;
PTH_ss      := 100.0;
PIC_ss      := 3.6;
PTS         := 8000.0;
UC          := 68.8;
end;

procedure save_results;
var
   fp:    text;
   loop1,loop2: integer;

begin
assign(fp,'a:results.dat');
rewrite(fp);
for loop2:=1 to 50 do
   for loop1:=1 to nvar do
      writeln(result[loop1,loop2]);
close(fp);
end;

procedure display_results;
begin
    writeln(time_step:3,' ',t:3:3,'    ',
   {result[3,time_step]:4:4,' ',}
   {result[4,time_step]:4:4,' ',}
    result[1,time_step]:4:4,' ',
    result[2,time_step]:4:4,' ',
    result[3,time_step]:4:4,' ',
    result[4,time_step]:4:4,' ',
    result[5,time_step]:4:4,' ',
    result[6,time_step]:4:4,' ',
    result[7,time_step]:4:4,' ',
    result[8,time_step]:4:4);
    writeln('uc = ',UC:4:4,' PTS = ',PTS:4:4)
end;
```

```
procedure model(nvar: integer; var v:real_array);
begin
(v[1] = PIC   , 3.6 )
(v[2] = TBSFC , 10.0 out of a total of 14.0 mmol )
(v[3] = TBSC  , 70.0 out of a total of 100.0 mmol )
(v[4] = TBC   , 100 percent = half of total bone calcium )
(v[5] = TCYTE , 100 percent as includes buried cytes so prop to bone
                volume)
(v[6] = TCLAST, 500 out of 1000 )
(v[7] = TBLAST, 500 out of 1000 )
(v[8] = PPTH  , 100 percent of normal )

(TCYTEACT  = trab osteocyte activity, 1.0 )
(TCLASTACT = trab osteoclast activity, 1.0 )
(TBLASTACT = trab osteoblast activity, 1.0 )

TBSFCR   := k[2]*v[2];
TBSFCA   := k[3]*v[1];
UC       := V[1]*(k[1] - ((k[5]*v[8])/PIC_ss));
    if UC < 0.0 then
       UC:= 0.0;
PTS      := k[20] - (k[21]*v[1]);
PTS      := PTS*1000.0;
    if PTS < k[19] then
       PTS:= k[19];
    if PTS > k[22] then
       PTS:= k[22];

TCLASTACT:= k[9];
TBLASTACT:= k[10];
TCYTEACT := (k[8]*v[8])/PTH_ss;
TBSCA := k[6]*v[2];
TBSCR := k[7]*v[3]*(TCYTEACT/TCYTEACT_ss);
TBCR  := k[17]*(TCLASTACT*v[6]);
TBCA  := k[18]*(TBLASTACT*v[7]);
TCLAST_FORM:= (k[11]*v[8])/PTH_ss;
TBLAST_FORM:= k[12]*TCLAST_FORM;
TCYTE_FORM := k[13]*TBLAST_FORM;
TCYTE_DTH  := k[14]*v[5];
TCLAST_DTH := k[15]*v[6];
TBLAST_DTH := k[16]*v[7];

dv[1]:= (TBSFCR + k[4]) - (UC + TBSFCA);
dv[2]:= (TBSFCA + TBSCR + TBCR) - (TBSFCR + TBSCA + TBCA);
dv[3]:= TBSCA - TBSCR;
dv[4]:= TBCA  - TBCR;
dv[5]:= TCYTE_FORM - TCYTE_DTH;
dv[6]:= TCLAST_FORM - TCLAST_DTH;
dv[7]:= TBLAST_FORM - TBLAST_DTH;
dv[8]:= PTS - (k[23]*v[8]);
end;
```

```
procedure runge_kutta;
(calculates variable values at the next time step)
var
   v2,v3,v4:          real_array;
   loop1,loop2:       integer;
   a,b,c,d:           real_array;
   h:                 real;

begin
h:=dt/interval;
for loop1:=1 to interval do
   begin
   model(nvar,v);
   for loop2:=1 to nvar do
      begin
      a[loop2]:=h*dv[loop2];
      v2[loop2]:=v[loop2]+a[loop2]/2;
      end;
   model(nvar,v2);
   for loop2:=1 to nvar do
      begin
      b[loop2]:=h*dv[loop2];
      v3[loop2]:=v2[loop2]+b[loop2]/2;
      end;
   model(nvar,v3);
   for loop2:=1 to nvar do
      begin
      c[loop2]:=h*dv[loop2];
      v4[loop2]:=v3[loop2]+c[loop2];
      end;
   model(nvar,v4);
   for loop2:=1 to nvar do
      begin
      d[loop2]:=h*dv[loop2];
      y[loop2]:=v[loop2]+(a[loop2] + b[loop2]*2 + c[loop2]*2 +
d[loop2])/6;
      end;
   for loop2:=1 to nvar do
      v[loop2]:=y[loop2];
   end;
end;
```

```
begin
read_parameters;
read_variables;
clrscr;
{write initial x values into results array}
{initgraphic;}
{drawborder;}
for loop1:=1 to nvar do
   result[loop1,1]:=x[loop1];
time_step:=1;
{plot_data;}
{iterate until t is greater than tmax}
display_results;
repeat
   time_step:=time_step+1;
   t:=t+dt;
   for loop1:=1 to nvar do
      v[loop1]:=result[loop1,time_step-1];
   runge_kutta;
   for loop1:=1 to nvar do
      result[loop1,time_step]:=y[loop1];
   {plot_data;}
   display_results;
   until t >= tmax;
{readln;}
{leavegraphic;}
end.
```