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Modelling amino acid metabolism

Review Article

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Summary. This article briefly summarises various aspects of building a model, before reviewing some models of protein turnover, the metabolism of selected amino acids, and urea synthesis in human subjects. Some software for use in modelling is noted. An Appendix lists terms with descriptions and definitions, which are often employed in modelling.

Keywords: Amino acids – Computers – Inborn errors of metabolism – Models – Protein synthesis– Software – Systems analysis – Urea

Introduction

Systems analysis may be defined as "the study of the interdependence of several parts of an organism". Building a model of the system is an essential part of the analysis. The advantages of modelling include the codification of facts and hypotheses about the system, the revelation of deficiencies in information about the system, and the implications of the hypotheses concerning the system. One important aim of the analysis is to establish new properties of the model, not (directly) accessible from available experimental data, and by implication of the system itself. Predictions may be made based on the analysis that are suitable for experimental verification. A positive outcome of such experiments would support the operational validity of the model. A result differing from the prediction would initiate a more or less drastically iterative remodelling. Another aim is to generate numerical values and estimates of complex functions from simple measurements. This should be of considerable importance in academic and clinical medicine, where practical and ethical restrictions often prevent important questions from being answered. Systems analysis facilitates the integration of theoretical and practical work (for a review of principal aspects of modelling, see Groth, 1977).

In contrast to the situation in electrical and mechanical engineering, modelling has only relatively recently been used in biology and medicine, reflecting the high degree of complexity of biomedical systems and previous lack of essential background information (Bier, 1989). Current examples are models of blood pressure regulation, the cardio-respiratory system, capillary permeability, carbohydrate metabolism, protein synthesis, metabolic disorders and drug metabolism. This development has been greatly facilitated by the rapid progress in computer technology.

The metabolism of amino acids and their conversion to urea are tightly controlled, for in spite of considerable fluctuations in intake of protein, their concentrations on the whole, remain remarkably constant. This implies the existence of control mechanisms (homeostasis). The history of control mechanisms goes back to before James Watt (1736–1819), engineer, who devised a 'governor' to regulate the velocity of a steam engine under variable loads. This he achieved by controlling the input of steam according to the velocity (or rotation) of the engine. James Clerk Maxwell (1831–1879), mathematical physicist, provided a theoretical and mathematical basis to the subject. Claude Bernard (1813–1878), physiologist, introduced the concept of the constancy of the *milieu intérieur*. Felix Linke (1840–1917), mechanical engineer took the next step in outlining a unifying theory of feedback control that is applicable to machines as well as to organisms. Norbert Wiener (1894–1964), mathematician, further developed these concepts and coined the word 'cybernetics' from the Greek *kubernetes*, 'steersman' to cover the developing field of research into control or feedback systems. Modelling and understanding feedback mechanisms in amino acid metabolism are closely linked. (Henn, 1987; Belknap, 1991)

There are various types of models. At one end of the spectrum, the model is designed to mimic the input/output characteristics of the system, without attempting to represent the underlying biological and physiological processes. At the other end of the spectrum, there are deterministic models based on these biological and physiological processes. They have the advantage that information obtained from animal studies may be incorporated into the human system.

This review is focussed on the application of systems analysis to amino acid metabolism in human subjects.

General principles of modeling

The following are the steps in the process of building a model of amino acid metabolism (Fig. 1). Further information may be found under Cramp (1982).

I. The problem

At the outset, it is important to define the problem or the purpose of the model. What will it be used for? What degree of accuracy is required? Two examples are given from the field of amino acid metabolism:

(i) In phenylketonuria, it would be helpful to have estimates of the concentrations of phenylalanine and its metabolites in inaccessible organs, in particu-

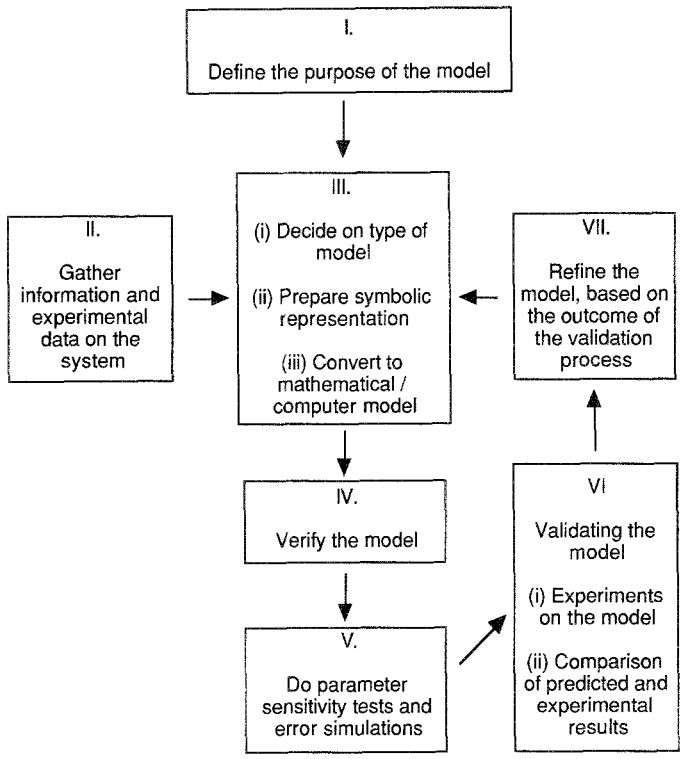

Fig 1. Building a model

lar the brain, which is the target organ, at times when the dietary treatment is being relaxed or stopped. Are there correlations between the estimated brain concentrations and psychological and intellectual changes?

(ii) Arginine is an essential amino acid in all except one of the urea cycle defects. What is the optimum supplement of arginine to give in the treatment of these disorders? If too little, then there is inadequate replacement for losses of intermediates such as citrulline in the urine, for net protein synthesis, and for stimulus of the cycle. If too much, the residual activity of the urea cycle is swamped, and toxic ammonium ions accumulate.

II. Background information

As much experimental data and information about the system are collected as possible. These might include:

(i) Information on the transport of substances across blood-brain barrier (BBB) if concentrations of metabolites in the brain are to be estimated.
(ii) Plasma concentrations of substrate and product following a stress or loading test. [See further Phenylalanine and Urea cycle, below.]
(iii) Data on the renal elimination of metabolites.
(iv) Information from animal studies.
(v) Compartmentation.

III. Building the model

There are two basic approaches:

(i) "Bottom up", for which a *minimalist* model is employed. Later, the model can be refined and expanded if necessary.
(ii) "Top down or *maximalist* model", in contrast, all possible features are incorporated into the model, with later simplification, following parameter sensitivity testing. This has the advantage that the model more closely reflects the real system, but it may not be possible to decide between different alternatives, or data may not be available to "identify the model".

IV. Verifying the model

By this is meant checking the mathematical accuracy of the model. Are the dimensions of the units correct? Is matter created or destroyed?

V. Parameter sensitivity testing and error simulation

These have important roles in model construction and validation, and enable the effects of experimental error and uncertainty about physiological constants on the values of calculated outcome parameters to be estimated (Hyltoft Petersen, Hjelm, and Groth, 1979; Cobelli, Ruggeri, DiStefano, and Landaw, 1985; Geiseler, Kay, Oberholzer, Seakins, and Hjelm, 1985). Possible unsuspected relationships between derived parameters may also be revealed during the course of error simulation. (Seakins, Wang and Hjelm, 1992).

VI. Validating the model

There are two steps in the validation the model.

(i) The first is the design of experiments for use on the model. An example could be the stepwise reduction in activity of a key enzyme from normal values to 50% (carrier status) to near zero activity (homozygous abnormal). Another could be a range of loading tests. Hjelm, Seakins, Kitney and Holmes (1985) used a "*Comparability test*" comparing enzyme constants obtained from three separate groups of phenylketonuric patients.
(ii) This is followed by comparison of the predicted and experimental results. Are there gross discrepancies between the two sets of data, data derived from the model and data from (real) experiments? Or are the differences relatively small, and could be explained by experimental error? See V above, and Cobelli, Carson, Finkestein and Leaning (1984).

VII. Refining the model

The model may then have to be modified according to the results obtained. Is it too complex? Is it over simplified for the purposes for which it is designed?

The process is repeated until a satisfactory model is obtained, that is, it will predict operationally inaccessible parameters, for example, concentrations of metabolites in the brain, or enzymic constants *in vivo* with an acceptable uncertainty. For a detailed application to amino acid metabolism see Matthews and Cobelli (1991) and Cobelli, Saccomani, and Matthews (1992).

Models of considerable complexity can be handled by desk top computers, but in some instances a main frame computer will be required, for example to process time-series.

Physico-chemical properties of amino acids in solution

Physico-chemical properties of physiological compounds influence their behaviour in biological systems and their metabolic impact. There are numerous properties to take into account, for example ionic character, solubility, intramolecular distribution of energy, molecular structure and possible secondary and tertiary configurations. Many of the properties vary depending on the molecular environment. Amino acids are no exception in this respect. At present little is known about primary effects of the cellular environment on the physico-chemical properties of amino acids and possible consequences *in vivo*. A good case can be made for studying the dynamic aspects of this interaction because of the complexity of the situation. Some work has been started along these lines. There are programs available for simplistic modelling of the basic structures and properties of amino acids and proteins on desk top computers [See Appendix I, Aids to modelling, (5) and (6)]. More sophisticated programs are available for main-frame computers for detailed modelling of the energy distribution in an individual amino acid, peptide or protein, or the interaction of such molecules and their environment, for example, water (Beveridge and Jorgensen, 1986). This is an important development for future modelling of the physiological behaviour of the substances in a biological matrix including detailed models of transport of these substances through cellular and subcellular membranes.

Protein turnover and synthesis

Models have been used for a long time to characterise the whole-body turnover of proteins *in vivo*. They were usually based on measures of the infusion rate of labelled amino acid at isotopic steady state. Initially, ^{15}N glycine or ^{13}C leucine were used and estimates of turnover derived from a simple compartmental model. (for review of earlier approaches see Waterlow, 1981) Later expanded models involving up to ten compartments have been developed in an attempt to overcome fundamental difficulties in modelling an extremely complex system (Bier, 1989). These earlier models were largely stochastic, that is consist of "black boxes", combined in such a way as to obtain the best fit to experimental data, rather than representing compartments with well defined meanings from a physiological and biochemical point of view. The derived estimates, to a large extent, rest on "mathematical" models, put into black boxes.

It has recently been realised that information about the metabolism of individual amino acids will have to be incorporated into these models to obtain

more accurate measures of whole body protein turnover (Bier, 1989). Despite present shortcomings, fundamental information about protein turnover *in vivo* has been established based on estimates derived from the developing series of models both under physiological and pathological conditions (see articles in book edited by Waterlow and Stephens, 1981)

Catabolism of amino acids

Whole body modelling of amino acid and protein metabolism in vivo is fundamentally difficult due to the intrinsic complexity of the system. Free amino acids only account for about 1% of the total amino acids present in the body, the composition and turnover of which vary considerable from protein to protein (Bier, 1989; Cobelli and Saccomani, 1991). The concentrations of free amino acids within cells are generally higher than in plasma. Some reactions are organ specific, for example the conversion of phenylalanine to tyrosine in normal subjects occurs only in the liver. In human subjects, blood (or plasma) and urine are the only two tissues that can be readily sampled, but the metabolism of amino acids takes place within cells; therefore indirect measures of intracellular concentrations need to be devised (Matthews and Cobelli, 1991). Furthermore, the formed elements of blood represent an additional source of heterogeneity within the circulatory compartment, and they participate in interorgan transport of amino acids. The existence of many homeostatic processes can be inferred, but little is known about their mechanisms.

Phenylalanine and phenylketonuria

Phenylalanine is an essential amino acid, and its major catabolic pathway in normal subjects is via tyrosine. The absolute fluctuation of its concentration in plasma is small, indicating the presence of important control mechanisms. The phenylalanine hydroxylating system has been extensively studied *in vitro*, but relatively little study has been done *in vivo*, and in particular in human subjects (Scriver, Kaufman, and Woo, 1989). This, together with the inborn error of amino acid metabolism, phenylketonuria (PKU), which is caused by the absence or much reduced activity of phenylalanine hydroxylase, makes modelling of phenylalanine metabolism of considerable interest. Three approaches are examined. Firstly, loading tests, where the objectives are to identify heterozygotes for PKU or to characterize phenylalanine hydroxylase activity *in vivo*; and secondly, the use of labelled L-phenylalanine, to identify carriers for PKU, to characterise variant forms of PKU, and to measure phenylalanine hydroxylase activity *in vivo* in these different conditions; and finally, a generalized model of phenylalanine metabolism, PKUSIM, where the aim has been to estimate, in patients with PKU, concentrations of phenylalanine and key metabolites in inaccessible organs, particularly, the target organ, the brain. The transport of amino acids across the BBB in phenylketonuria is considered in the section on transport.

Phenylalanine metabolism

Two approaches for the detection of carriers for PKU have been suggested, either the ratio of plasma phenylalanine to plasma tyrosine under standardised conditions, or the plasma phenylalanine response following a load of phenylalanine. Both methods result in some degree of overlap between reference subjects and obligate carriers for PKU (see review by Güttler, 1980). In an attempt to improve further the separation between these two groups, Rödjer and his colleagues (Jagenburg, Regårdh, and Rödjer, 1977; Rödjer, 1977) used *intravenous* phenylalanine, given as a bolus, to eliminate factors such as variable gastric emptying and absorption. They then measured the plasma phenylalanine concentration over the next 180 minutes at 10-minute intervals. Since the plasma phenylalanine decay (rise above fasting concentration) declined bi-exponentially, Rödjer used a two-compartmental (pharmacological) model (Fig. 2) to derive appropriate rate constants for the two groups. The results were disappointing, the discrimination between the reference group and the obligate carriers was no better than that obtained by the fasting phenylalanine/tyrosine ratio.

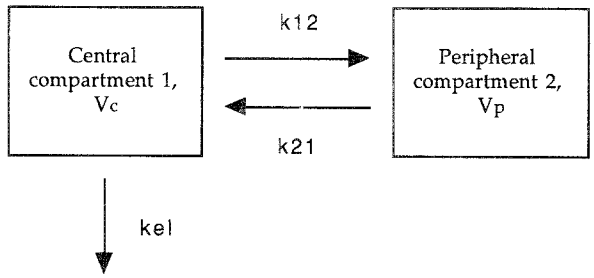

Fig 2. Two-compartmental (pharmacological) model to derive rate constants for the elimination of phenylalanine in normal subjects and obligate carriers for PKU. *kel* is the elimination rate constant for phenylalanine (Rödjer, 1977)

Possible explanations for the poor discrimination include:- (i) Inadequacy of the two-compartmental {see further below}. (ii) Non-steady state conditions at the critical first part of the curve. (iii) Poor correlation between body mass or surface area and weight of liver, where most of the conversion of phenylalanine to tyrosine takes place.

However, complete separation was achieved if the rate of elimination of phenylalanine was calculated from constant infusion loads. (Jagenburg and Rödjer, 1977; Rödjer, 1977). This approach implies that the exchange rates for phenylalanine between plasma and all other compartments are in equilibrium when the plasma concentration is in steady state and thus the infusion rates correspond to the elimination rates. If elimination of phenylalanine only takes place by hydroxylation to tyrosine, an irreversible reaction, then the infusion rate will equate to the elimination rate and provide a measure of the amount of hepatic phenylalanine hydroxylase. This assumption is reasonable in healthy individuals and heterozygotes for PKU, but would not be valid in untreated

PKU. Auxiliary pathways of phenylalanine metabolism are activated at higher concentrations of phenylalanine and used to eliminate major quantities of phenylalanine for example urinary phenylacetyl-glutamine, and higher concentrations of plasma phenylalanine (>1 mmol/L) also phenylpyruvate, phenyllactate and 2-hydroxyphenylacetate (Seakins, Ersser, and Hjelm, 1982). Lack of access to accurate data in this respect may lead to erroneous conclusions about the residual activity of the phenylalanine hydroxylating system in PKU, measured by tracer infusion studies (Thompson and Halliday, 1990).

PKUSIM

The models implicit in the previous studies on phenylalanine metabolism assume that the conversion of phenylalanine to tyrosine takes place in the central compartment, and that the contribution of the auxiliary pathways can be ignored. These considerations do not hold in PKU. The auxiliary metabolic pathways now become of major importance in the elimination of phenylalanine, and metabolism takes place in several organs (Hjelm, Kitney, Lindsey, and Seakins, 1984). The model (PKUSIM), used to characterise this situation, represents one of the first attempts to develop an organ-oriented, mixed biochemical-

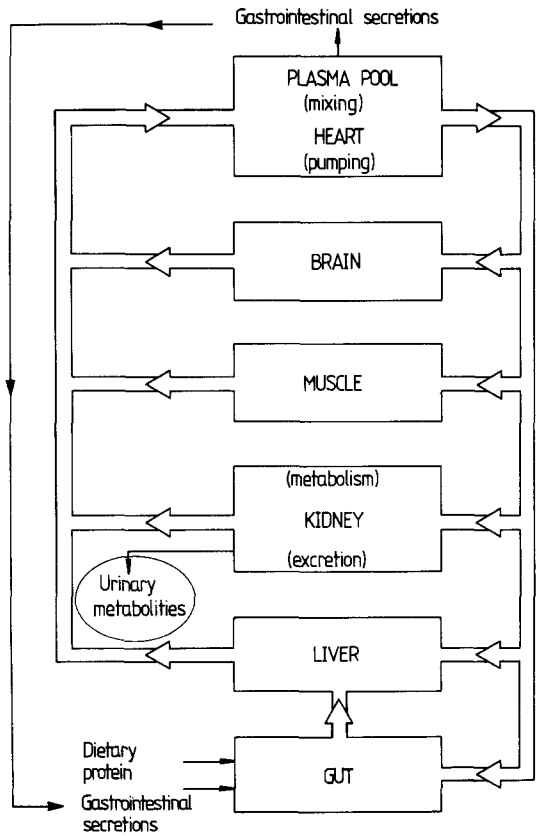

Fig 3. Biochemical-physiological model (PKUSIM) for phenylalanine metabolism (Hjelm, Kitney, Lindsey, and Seakins, 1984)

physiological model. It consists of seven *physiologically* identifiable compartments connected to blood circulation (Fig. 3). [This approach has been vindicated by recent developments of traditional compartmental models, where a ten compartmental model has been proposed for modelling leucine metabolism in order to obtain a good fit (Cobelli, Saccomani, Biolo, Tessari, Luzi, and Matthews, 1991).] With this approach, differences in the intermediary metabolism of phenylalanine and its metabolites can be accommodated *at specific organ level* as can differences in the renal handling of such metabolites. This model is *lucid* in the sense that it attempts to provide a simplified description of the true biological process.

When the concentrations of phenylalanine and its metabolites were modelled, phenylpyruvate, a metabolite thought to have neurotoxic effects, did not rise significantly until the activity of hepatic phenylalanine hydroxylase decreased to a few percent of normal (Hjelm, Seakins, Kitney, and Holmes, 1985). The model also allowed the calculation of individual values of apparent Km and Vmax in the subjects studied by Rödjer (1977). Interestingly, the values constituted two regression lines which were well separated for healthy subjects and heterozygotes for PKU (Fig. 4). Subsequent work has shown that these correlations represent a covariance between the two parameters which is an inherent property of the Michaelis-Menten equation and a function of experimental errors involved in determining substrate concentrations (plasma phenylalanine at steady state), velocity (infusion rate) in the actual situation, and standardising the results to surface area, (Seakins, Wang, and Hjelm, 1992; Wang, Kitney, Seakins, and Hjelm, 1992). This outcome illustrates that relationships discovered by modelling have to be interpreted with care and that error simulations of unexpected relationships represent an important part of validating such results.

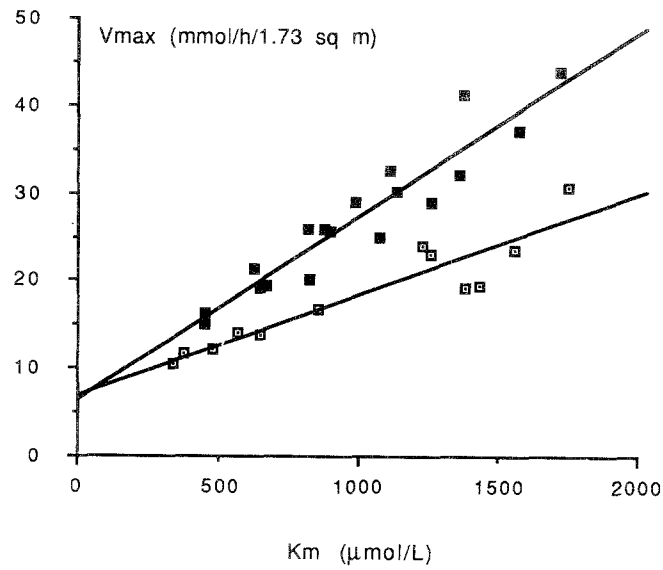

Fig. 4. Correlation between estimated apparent Km and Vmax for phenylalanine hydroxylation *in vivo* in normal subjects and obligate carriers for PKU, generated by PKUSIM (Seakins, Wang, and Hjelm, 1992; Wang, Kitney, Seakins, and Hjelm 1992)

Leucine metabolism

The essential amino acid, leucine, has been extensively used in studies of protein turnover and nutrition, but it merits modelling in its own right. Abnormalities in leucine metabolism occur in diabetes in addition to the inborn error of metabolism Maple Syrup Urine Disease (MSUD). Its metabolism differs from some of the other essential amino acids, in that it takes place predominantly in muscle and other extrahepatic tissues.

In untreated diabetes mellitus, there is muscle wasting, increased gluconeogenesis and increased concentrations of the branched-chain amino acids, leucine, isolelucine and valine. Insulin treatment restores plasma branched-chain amino acids and nitrogen balance to normal. Since these amino acids are mostly metabolised in muscle, the plasma concentration of these amino acids and their metabolism may reflect altered protein metabolism in the diabetic state. Umpleby, Boroujerdi, Brown, Carson, and Sonksen (1986) used carboxy labelled leucine, given as a bolus, to measure the rate of production, clearance and oxidation of this amino acid in reference subjects, treated and insulin-withdrawn treated diabetic patients. They devised a six-compartment model, (Fig. 5), which was mathematically identifiable and physiologically sound. Compartments 1 and 5 were fixed as the plasma and blood volumes respectively. They interpreted their results, using this model, as demonstrating that the increased plasma leucine arose from increased proteolysis, which became normal following insulin therapy; but leucine oxidation rate still remained elevated.

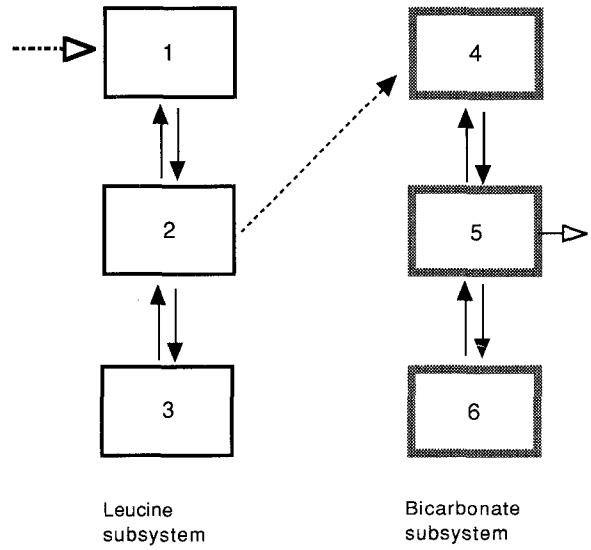

Fig. 5. Compartmental model for leucine metabolism (I). Compartments *1*, *2* and *3* form the leucine subsystem, compartment *1* is fixed as the plasma volume into which the labelled leucine is injected and sampled. Compartment *2* contains both extracellular and intracellular components and the sites of oxidation of leucine. A three-compartment bicarbonate subsystem (*4*, *5*, and *6*) is used with compartment *5* corresponding to the blood volume, from which elimination of carbon dioxide occurs (Umpleby, Boroujerdi, Brown, Carson and Sonksen, 1986)

Although this model represented a considerable advance on earlier non-compartmental models, there are three disadvantages. Firstly, protein synthesis and leucine breakdown take place intracellularly. Secondly, the interconversion of leucine and ketoisocaproate is ignored. And thirdly, the authors were not able to distinguish between the three alternative entries (compartments 1, 2 or 3) for leucine inflow from protein breakdown.

Cobelli, Saccomani, Biolo, Tessari, Luzi and Matthews (1991) used simultaneous injection of 1-C (carboxyl-) labelled leucine and a deuterated or tritiated labelled ketoisocaproate, either as a bolus when radioactive tracers were used, or as constant infusion when stable isotopes were employed. This enabled the interconversion of leucine and isocaproate and the irreversible oxidative decarboxylation of leucine to be evaluated. Concentrations of the isotopically labelled leucine and ketoisocaproate in plasma, and carbon dioxide in expired air were measured. Various physiologically-based compartmental models of increasing complexity were tested against the sets of experimental data using the conversational form, CONSAM (Berman, Beltz, Gries, Chabay, and Boston, 1983) of SAAM (Berman and Weiss, 1978). The best fit was obtained with a ten-compartment model (Fig. 6). There was some variation between subjects for the different transfer constants between the ten compartments, but variability was of the same order as that found by Rödjer (1977) in his two compartment model of phenyl-

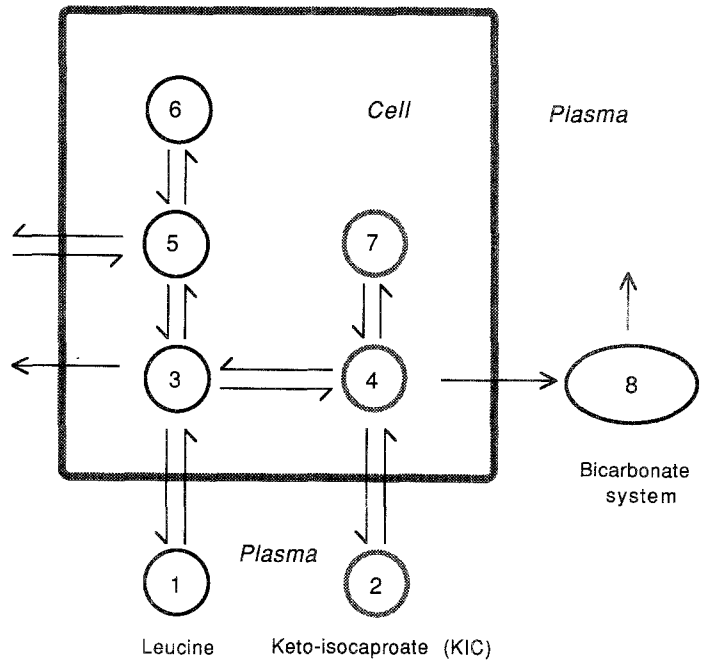

Fig. 6. Compartmental model for leucine metabolism (II). Compartments *1*, *3*, *5*, and *6* form the leucine subsystem; compartments *2*, *4*, and *7* the ketoisocaproate subsystem; the bicarbonate subsystem (*8*) comprises three compartments. Compartments *1* and *2* correspond to the extracellular space, compartments *3–7* are intracellular in which metabolism takes place: – transamination, oxidative decarboxylation of ketoisocaproate, and protein synthesis and breakdown. (Matthews and Cobelli, 1991)

alanine metabolism (Fig. 2, see above). The coefficient of variations (CV) calculated from Rödjer's data (fifteen male reference subjects) for the exponential decay constants (α and β), the derived transfer constants and the elimination constant, and the peripheral and central compartments, ranged from 20–33%.

Thompson, Walter, Leonard and Halliday (1990) used continuous infusion of 1-^{13}C labelled leucine to estimate leucine oxidation in reference subjects and patients with maple syrup urine disease (MSUD). In contrast to the situation in PKU (see above) patients with MSUD demonstrated negligible oxidation of leucine. This may be linked to the fact that there is only one auxiliary pathway in MSUD, namely conversion to 2-ketoisocaproate and 2-hydroxyisocaproate, both of which are absorbed efficiently at the renal tubule.

Methionine metabolism

The essential amino acid, methionine, is not only involved in protein synthesis and is a supplementary source of cysteine, cystine, taurine, sulphate, which are important in various synthetic reactions, but it is also an intermediary in transmethylation reactions and methyl neogenesis (Fig. 7). Methylation is important in the synthesis of myelin, creatine and adrenalin, and detoxication of drugs and anutrients such as plant phenolics. There are also several inborn errors of metabolism associated with its metabolism, the most common being homo-

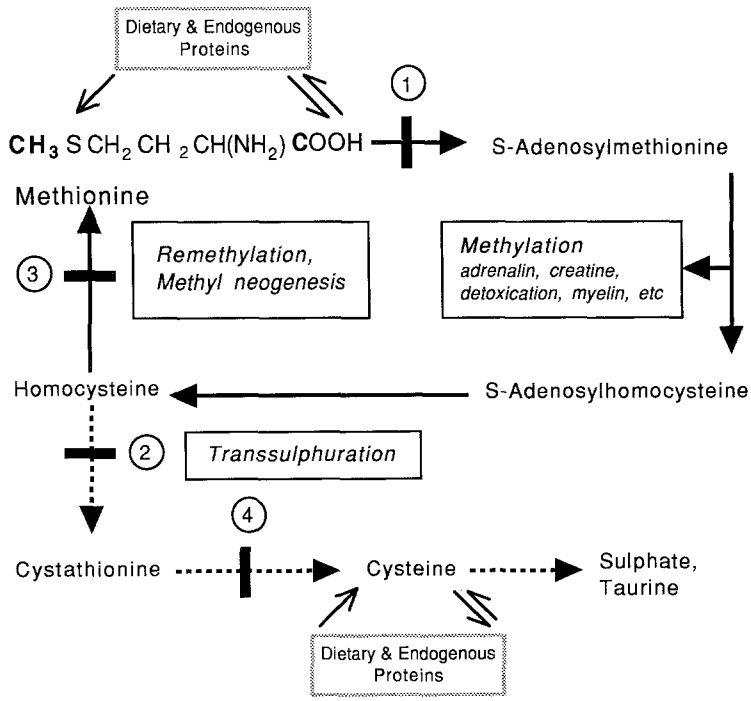

Fig. 7. Metabolism of methionine. Metabolic blocks; *1* hypermethioninaemia (methionine adenosyltransferase deficiency); *2* homocystinuria (cystathionine synthase deficiency); *3* defects in folate and B_{12} metabolism; *4* cystathioninuria (cystathionase deficiency). (Storch et al, 1988)

cystinuria. Storch, Wagner, Burke, and Young (1988) studied the metabolism of methionine labelled with [1-^{13}C] and [*methyl*-$^{2}H_3$]methionine (bis- and mono-labelled tracers) in the fed and post absorptive states, and also following a diet devoid in sulphur amino acids, or a methionine-free cystine-supplemented diet (Young, 1987). The tracer was given as a primed continuous intravenous infusion, and the isotopic compositions of plasma methionine and expired air (carbon dioxide) were measured. The isotopic data were analysed using a single compartment model, but making allowances for dilution by methionine within intracellular pools, (no correction was made for uptake and metabolism of dietary methionine by the splanchnic bed, an aspect covered in the lysine study, below). This simple modelling approach yielded information on: – protein synthesis (S) and breakdown (B), transsulphuration (irreversible conversion to cysteine, TS), transmethylation (methyl donation, TM) and remethylation (methylneogenesis, RM). In the fed state, their results indicated not only the expected increase in irreversible oxidation and diminished protein breakdown, but also an enhanced activity of the methionine cycle. TS, TM, S and B were significantly reduced in the sulphur amino acid restricted diets. The authors conclude from their studies that both methionine and homocysteine are key regulatory sites in methionine conservation. This study represents a biochemically oriented approach to modelling similar to that used in modelling urea synthesis.

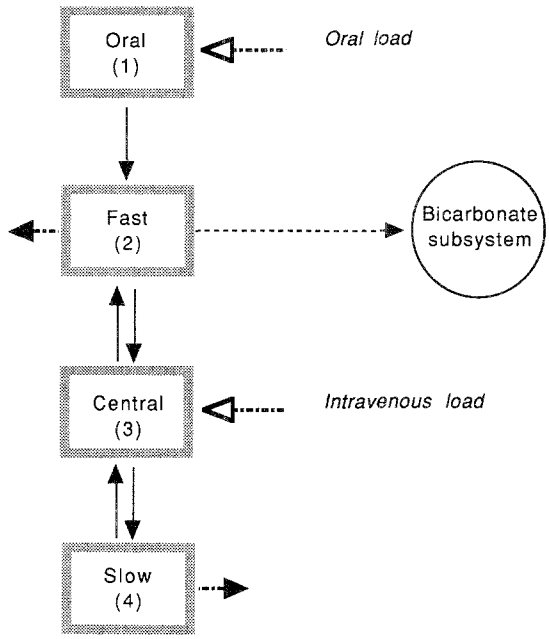

Fig. 8. Compartmental model of lysine metabolism. Compartments *1*, *2*, *3*, and *4* form the lysine subsystem: *1*, oral compartment; *2*, fast exchanging peripheral compartment; *3*, central compartment; *4*, slow exchanging compartment. Protein synthesis occurs in *2* and *4*, and catabolism of lysine from *2* to the metabolic carbon pool and bicarbonate subsystem. (Irving et al, 1986)

Lysine metabolism

Lysine is an essential amino acid, the catabolism of which differs significantly from the other essential amino acids in that the two amino groups do not participate in transamination reactions. Irving, Thomas, Malphus, Marks, Wong, Boutton, and Klein (1986) made use of this fact in their study of lysine and protein metabolism in young women. Their aim was to characterise the partitioning of amino acid pools and protein synthesis and breakdown under various conditions. They gave simultaneously L[$^{13}C_1$] lysine as an intravenous bolus and L-[$^{15}N_2$] lysine as an oral bolus, and analysed plasma and breath samples for labelled lysine and labelled carbon dioxide, respectively. The data were analysed in detail and used to develop a multicompartmental model of lysine and bicarbonate metabolism, (Fig. 8). The lysine subsystem consisted of three compartments (2, 3, and 4), with a fourth compartment (1) to account for the oral bolus. The central lysine pool, (3), consists primarily of extracellular fluids including plasma, and is linked to two peripheral pools, (2 and 4). The slow-exchanging pool (4) is consistent with free intracellular lysine associated with muscle. The combined data from the oral and intravenous loads indicate that the fast pool of lysine (2) is associated with the viscera, and it is from this pool that the carboxy-labelled carbon leaves.

Urea cycle

The urea cycle is the main metabolic system used in mammals to eliminate nitrogen. The immediate substrate is ammonium, which is eventually excreted as urea, a product which cannot be metabolised further (except by microorganisms in the gut flora). The five steps in the cycle and the associated enzymes are shown in Fig. 9. Inborn errors of metabolism have been described for each step in the cycle and two related enzyme systems. The initial step, producing carbamoylphosphate from ammonium, phosphate and ATP, and the final conversion of arginine to urea and ornithine are considered to be irreversible reactions. Over the years a considerable amount of information has accumulated about the kinetics of the individual steps *in vitro*, but before 1977 no attempt had been made to characterise the overall function of the cycle *in vivo*. Three different approaches are described

Kuchel, Roberts and Nichol (1977) published the first model for the urea cycle. Their model incorporates a detailed description of the kinetics of each of the five enzymic steps in terms of so-called 'ping-pong' mechanisms. This involved characterising the enzymic reactions with up to twelve rate constants, many of which had to be based on realistic assumptions. Despite the complexity of the kinetic description of the enzymic steps a good correlation was obtained between simulated and observed values for concentrations of intermediates of the urea cycle in healthy subjects and those with inborn errors of metabolism affecting the five enzymes in the urea cycle. This has greatly helped in the understanding of the kinetics of the system in abnormal situations, and in particular, the near normal production of urea in all urea cycle defects with the exception of carbamoylphophate synthetase deficiency. There is no need to

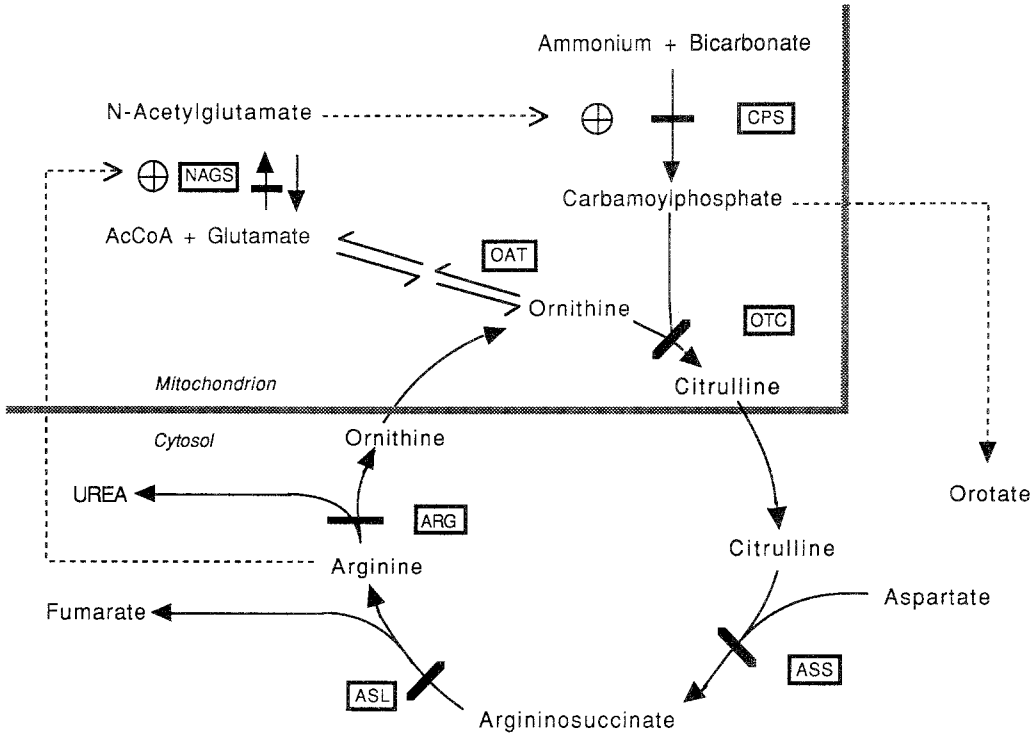

Fig 9. The compartmentation of urea synthesis. Metabolic blocks: 1. *CPS* carbamoylphosphate synthetase deficiency; 2. *OTC* ornithine transcarbamoylase deficiency; 3. *ASS* argininosuccinate synthetase deficiency (citrullinuria); 4. *ASL* argininosuccinate lyase deficiency (argininosuccinic aciduria); 5. *ARG* arginase deficiency (argininaemia); 6. *NAGS* N-acetylgluatamate synthetase deficiency; 7. *OAT* ornithine aminotransferase deficiency (gyrate atrophy of choroid and retina). (Modified from Bachman and Colombo, 1981)

invoke alternative pathways for the synthesis of urea. This study demonstrates the value of computer simulation in integrating information on individual enzymes with clinical and laboratory observations.

Bachman and Colombo (1981) developed this model by including (i) the feedback mechanism involving the synthesis of N-acetyl-glutamate, the allosteric factor for carbamoylphosphate synthesis, (ii) active transport of ornithine from the cytosol to the mitochondrion, and (iii) the mitochondrial metabolism of ornithine by ornithine keto acid transaminase, the only known pathway for ornithine elimination (Fig. 9). Their model was evaluated on a desk-top computer and gave better results than the simpler version, but there were still discrepancies, in particular, the increased excretion of orotic acid in arginase deficiency could not be explained. Their model needs further development and more experimental information, and should be used to study the related disorder of ornithine ketoacid transaminase deficiency (OAT or OKT deficiency, gyrate atrophy of choroid and retina).

Groth and de Verdier (1982) developed a general model of urea production in humans to assist in the interpretation of changes in serial plasma urea concentrations occurring in pathological conditions such as internal haemorrhage or

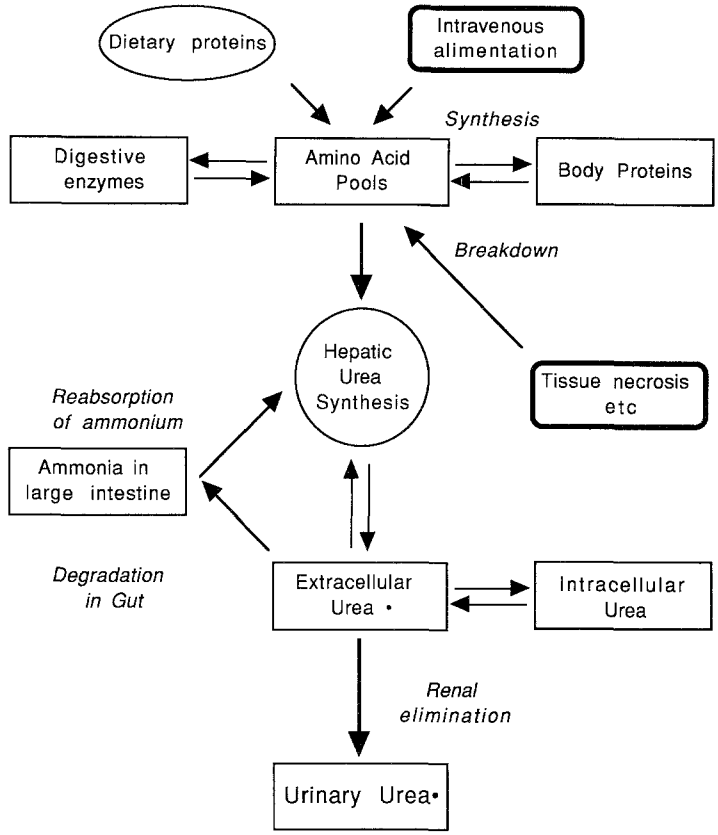

Fig. 10. A generalised model for urea metabolism following trauma. Plasma and urine can be sampled. (Adapted from Groth and de Verdier, 1982)

during intravenous amino acids or blood transfusions (Fig. 10). They assumed that the rate of synthesis of urea was proportional to the amount of free amino acids, lumped into one pool. The models was tested against clinical data from a patient who had sustained multiple fractures. The authors found good agreement between the calculated plasma urea concentrations and those found. This example illustrates that quite complex models of urea synthesis can be developed with good predictive power.

A model for characterising the production of urea (and hence the elimination of nitrogen *in vivo*) was devised by Geiseler, Kay, Oberholzer, Seakins, and Hjelm (1985). Because urea is freely and rapidly distributed in body water, a simple model involving only one compartment could be used. Alanine, given orally or intravenously, was used as the nitrogen source because of its high solubility, non-toxic properties, and rapid metabolism. Model-based parameters, used to characterise the kinetics of the production rate of urea, were derived from serial measurements of plasma urea. They included the maximum production rate of urea and the time at which that production occured after the alanine load. (Kay, Seakins, Geiseler, and Hjelm, 1986). Loading tests on mothers of children with deficiency of ornithine-carbamoylphosphate transferase (OCT) deficiency and healthy subjects have shown that, (i) the urea cycle has a very large capacity for

eliminating nitrogen, which cannot be saturated even under extreme conditions in healthy subjects, (ii) mothers of children with OCT deficiency have a decreased capacity to eliminate nitrogen. This provides a basis for a standardised test to identify carriers for OCT deficiency (Kay, Oberholzer, Seakins, and Hjelm, 1987). The model-based parameters have also been used to show that mild hyperammonaemia in patients with fits and treated with sodium valproate is caused by an inhibition of the synthesis of carbamoyl-phosphate by the drug. This mechanism may have contributed to the fatal outcome in women treated with valproate who were also carriers for OCT deficiency (Hjelm, de Silva Seakins, Oberholzer, and Rolles, 1986). Finally, evidence has been obtained of mitochondrial dysfunction in Rett's syndrome, a unique neurological syndrome in girls, using the model-based parameters of urea synthesis (Thomas, Oberholzer, Wilson and Hjelm, 1990).

Transport of amino acids

The systems involved in transporting amino acids across cell membranes and into subcellular particles such as mitochondria and lysozomes are usually quite complex and would be well suited for modelling and simulations studies, particularly as there are several inborn errors of amino acid transport, for example, cystinuria and cystinosis. There are, however, very few examples of modelling of membrane transport.

Blood brain barrier (BBB)

The brain, being an inaccessible organ for direct measurement, is a good candidate for modelling. Most models to date have concentrated on various aspects of glucose metabolism, and only few models have been developed for the uptake and metabolism of amino acids and their transport into the cerebrospinal fluid. In the study by Hommes, (1989) the mutually competitive transport of amino acids across the BBB barrier in PKU, tyrosinaemia type II, and histidinaemia has been modelled. These three inborn errors of metabolism are associated with an elevation of a single amino acid; but only untreated PKU is associated with severe mental retardation. Hommes's results indicated that flux control for both tyrosine and tryptophan is regulated by their brain hydroxylases, followed by the carrier mediated transport systems. Hommes concluded, from his simulation studies, that the effects at the BBB alone do not provide an adequate explanation of permanent brain dysfunction in PKU.

In a subsequent study, Hommes and Lee (1990) modelled the transport of the eight large neutral amino acids which use the large neutral amino acids transporter of the BBB. The fate of the amino acids was followed from blood to interstitial space, to cell and through metabolism, and in particular the hydroxylation of tyrosine and tryptophan. They concluded from their simulations, that interference with the transport across the BBB of tyrosine and tryptophan, as well as the flux through tryptophan hydroxylase leading to the synthesis of 5-hydroxytryptamine – a neurotransmitter – do not contribute to the cause of permanent mental retardation in PKU. However, the addition of dietary ty-

rosine to increase the plasma tyrosine concentration in PKU, may have a beneficial effect for the synthesis of neurotransmitters derived from tyrosine.

Conclusions

This review aims to provide background knowledge on modelling amino acid metabolism for those with a special interest in this area, and to stimulate them to incorporate this new and powerful technique in their own work. It does not pretend to be comprehensive either with regard to theoretical aspects or to the practical applications as documented in the literature. The authors would welcome correspondence about important omissions and constructive criticisms and readers' experience in the use of software in modelling amino acid metabolism. They would like to indicate that a symposium focussed on modelling amino acid metabolism is planned for the 3rd International Congress on Amino Acids and Analogues to be held in 1993.

"Men of Experiment are like the Ant, they collect and use; the Reasoners resemble Spiders, who make Cobwebs out of their own Substance. But the Bee takes a middle Course, it gathers its Material from the Flowers of the Garden and of the Field, but transforms and digests it by a Power of its own."

From Novum Organum (1620) by Francis Bacon (1561–1626).

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Appendix I. Aids to modelling

Historically, modelling was made possible by the introduction of main frame computers, allowing a large number of calculations to be carried out in a short period of time (Belknap, 1991). The main frame computer is still going to be a major tool in the development of more complicated models including those of amino acid metabolism. However, desk top computers are now in many instances sufficiently powerful to handle quite complex models. Below are listed some examples.

(1) STELLA (Structured Thinking Experiential Learning Laboratory with Animation) is a computer simulation software designed to exploit fully the user-friendly features and graphical interface of the Macintosh computer. Before the development of STELLA, computer simulation and modelling could only be carried out using main-frame computers. Not only did the investigator need to have a clear understanding of the system he wished to simulate, he also needed to be a computer expert. STELLA is eminently suited to studying compartmental models, (Washington, Washington and Wilson, 1990). The emphasis is on the modelling process, not on the mathematical structure underlying the simulation; this enables the user to identify important features and components in the model. Some twenty functions are available, these can be combined so that models of considerable complexity are easily built. Of particular value is a 'graphical converter' which can be used to enter empirical relationships,

for example the relationship between drug concentration and heart rate or chemotherapeutic action (Washington, Washington, and Wilson, 1990). STELLA may be used for parameter sensitivity testing, either entering the different values manually, or using STELLA Stack, which is a HyperCard interface. Its main disadvantages are that it cannot be used to fit curves to experimental data, or be used in 'deconvolution', (for example, in deducing the urea synthesis function given the plasma urea response curve).

(2) Hakman and Groth (1991) have described an interactive knowledge-based simulation software package, KBSIM (Interactive Knowledge-Based SIMulation). KBSIM system integrates quantitative simulation with symbolic reasoning techniques, under the control of a user interface managment system, using a relational database management system for data storage and inter-process communication. The system requires a Hewlett-Packard workstation, with 8 Mbyte of RAM and a 570 Mbyte hard disk.

(4) Chicago Kinetic Simulator (CKS-1) is used in conjunction with Mathematica which is a general software system for mathematical and other applications, and can be used on various systems including Macintosh and IBM computers. (Belknap, 1991). Although harder to use than STELLA, it has potentially more power than STELLA, particularly when further developed for use with Mathematica 2.0. Deconvolution should be possible.

(5) SAAM (Simulation, Analysis and Modelling), was designed over thirty years ago by Berman (Berman and Weiss, 1978) and can run of a variety of computers. Cobelli et al. (1991) used it on their analysis of data on leucine kinetics. Belknap (1991) gives a critical evaluation.

(6) Chem3D Cambridge Scientific Computing and

(7) Ball and Stick, Cherwell Scientific, are basic programs for modelling molecules.

Appendix II. Glossary of terms used in biomedical systems analysis

Aggregation, the joining together of physiologically related compartments for computational purposes (minimal models).

"Black box" approach (sometimes described as Non-compartmental analysis) constructs a model which mimics the input/output characteristics of the real systems without attempting to represent the underlying physiological or biological mechanisms. This contrasts with the "White box" approach which is equivalent to that employed in developing physiological and *deterministic* models. Classical *pharmacokinetic modelling* is equivalent to a "Translucent box" approach.

Block diagram is the schematic representation of the different parts of the system being modelled.

Compartment (or pool) refers to a discrete part of a metabolic system that contains a substance, of endogenous or exogenous origin, (assumed to be) at a uniform concentration.

- Mathematical, for example, central or peripheral
- Physiological compartment is a biological space enclosed by cell membranes, eg. plasma, liver.

Deterministic process/method is equivalent to the evaluation of a system by compartmental analysis when the structure of the interior of the system and accompanying internal kinetics are explicitly defined.

Error simulation (Monte Carlo Analysis) enables the effects of analytical error or physiological uncertainties on derived parameters to be estimated, and possible relationships between these parameters uncovered. It is usually assumed that the analytical errors are Gaussian in distribution, either constant absolute error, or constant relative error (constant coefficient of variation, CV), but other distributions may be employed as appropriate.

Feedback occurs when the output influences or controls the input to the system thus maintaining a steady state. An historic example is the governor on a steam engine. Feedback may be either positive or negative.

A model is **Identifiable** if experimental data can generate unique values of all the parameters of the model. If not, then either the model will need to be simplified or alternative experiments devised.

The process of **Identification** consists of the experimental determination of the model both in terms of its structure and the values of the parameters.

Impulse A mathematical concept to describe a bolus injection or brief perturbation of the system.

Input-Output models see Black box.

Linearization. The equations governing the transfer of metabolites from one compartment to another, or the conversion to another substance, are complex. Thus doubling the input will not normally double the output (non-linear). However, for small perturbations or for tracer amounts, complex non-linear equations can be replaced by linear approximations.

Non-steady state see steady-state.

Parameters are quantities which are constant in a particular case, but which vary in different cases, for example enzyme activities.

In **Parameter sensitivity testing** the effects of varying the values of different constants (for example rates, Michaelis-Menten constants, compartmental size) on the outputs of the model are determined. There is often a inbuilt relationship between derived parameters. This needs to be remembered in the planning of the study and in the interpretation of the computer simulation. The technique is part of the validation process. (See also Error simulation).

Steady-state/dynamic steady-state Equilibrium reached during constant infusion of substance, either labelled or unlabelled. The term is also applied to stationary state for unlabelled traces.

Stochastic methods/systems "Stochastic" is derived from the Greek word for a target, and with respect of systems analysis implies that the internal configuration of the system is unknown, see "Black box". Stochastic systems are the opposite to deterministic systems (qv).

Systems analysis or Holistic approach. The analysis of a complex entity with emphasis on the inter-relationships between the constituent parts.

Validation (Latin validus = strong). Models are essentially hypotheses or theories which are tested by subjecting them to critical experiments designed to falsify them. They are accepted to the extent to which they are not falsified (Sir Karl Popper). In the validation of physiological models a variety of test signals

and measurements is available. However, in biological models the range of test signals and measurements is limited. Input can be (a) a single bolus injection, (b) continuous infusion with measurements at steady state. Oral administration either for testing metabolic or pharmacological models, whilst convenient for the subject/patient, adds the further complication of the variable effect of intestinal absorption. Measurements can include concentration of substrate and metabolites in plasma and urine as well as pharmacodynamic parameters.

Verification (Latin verus = true) refers to the mathematical/arithmetical correctness of the model.

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