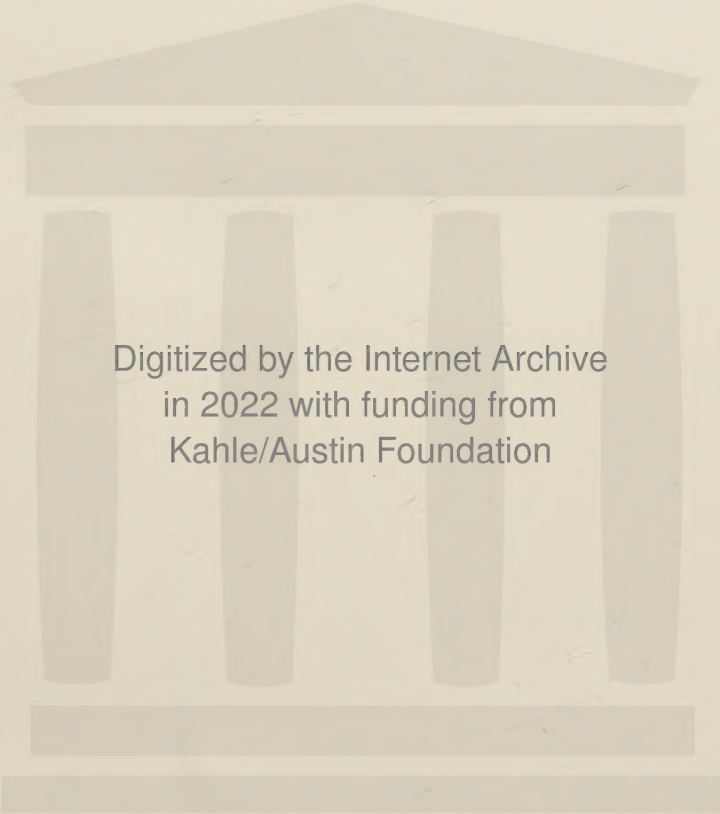

MODELING RUMINANT DIGESTION AND METABOLISM

R. L. BALDWIN



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R.L. Baldwin

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Introduction

In 1886, Lord Kelvin stated:

I often say that when you can measure something and express it in numbers, you know something about it. But when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, progressed to the level of science, regardless of what the matter may be.

(Stine, 1992)

1.1 ROLE OF RUMINANTS IN HUMAN FOOD PRODUCTION

Food-producing animals, particularly ruminants, play a key role in converting plant products humans cannot or do not choose to consume into desirable, high-quality human food. Plant resources utilized in ruminant animal production include cereal grains, forage and silage crops, pasture and range forage, crop residues, and a wide range of by-products from food processing. In discussing world food problems and the role animals should fulfill in the production of human food, numerous authors (Guirry, 1964; DeGraaf, 1967; Blaxter, 1973; Combs, 1973; Phillips, 1973; Reid, 1973; McDowell, 1975; Baldwin *et al.*, 1978; Baldwin, 1980) have identified a number of relevant issues that must be addressed to determine how much animal production may change in the future in terms of amounts of animal products produced and what resources and management systems will be utilized. Important issues are what quantities of cereal grains will be available for animal production, since much of the Earth's energy is stored in forms not edible by humans but which can be converted by ruminants to human food; effective utilization of non-arable land and rotational forage crops in food production; and plant and animal agriculture are complementary and interdependent. There is general agreement among most informed authors that products of animal agriculture will continue to contribute significantly to the world food supply. However, the key challenge of ascertaining how much animals should contribute has not been resolved. Resolution of this issue requires quantitative consideration of the above statements, and additional issues to determine optimal

balances and interactions between plant and animal agriculture in differing agricultural lands, climates and physical and societal circumstances in the world. A comprehensive model adequate for optimization of a human food production system is not available. In fact, one would have great difficulty in developing a consensus of the crop and animal product inputs that would constitute an optimal human food supply. There are many combinations of crop and animal products that can fulfill the recommended dietary allowances of the National Research Council (NRC), USDA and FAO. Some combinations of foods used to fulfill these recommendations may be better than others or may be more desirable than others for specific populations and these are often vigorously debated. Debates on an optimal human food supply are usually undertaken without consideration of the impact of participant's ideas, preferences or biases about the total amount of human food which would be available if their views were to be adopted. Our inability to undertake quantitative evaluations of impacts of competing human nutrition strategies on human food availability is due in large part to the fact that current plant and animal production models are normally restricted to single species and have not been interfaced. Development of the collage of equations and models essential to this task is probably feasible now or will be in the near future. However, the task would be a massive undertaking, heavily dependent upon the use of modern computing software and equipment. This is a long-term goal that will require the availability of advanced dynamic, mechanistic models of ruminant digestion and metabolism, which are the primary focus of this book.

Evolution of the use of mathematical models in nutrition started with definition of the laws of thermodynamics in the 1840s. The first law on conservation of energy is essential to the difference calculations commonly utilized by nutritionists. The second law, which holds in part that all forms of energy are quantitatively convertible to heat, and the law of Hess, which specifies that heat generated in an energy transaction is independent of path, are essential to the use of calorimeters by nutritionists to assess energy transactions in animals. Additional advances essential to the development of mathematical models of energy transactions in animals included the report of Lavoisier and Laplace, which established the equivalence of oxygen use and carbon dioxide and heat production between burning carbon and a fasting guinea pig. Subsequent developments in food chemistry led to formulation of the bases for direct and indirect calorimetry and were essential to the evolution of nutrition as a science. Application of the quantitative relationships and methods developed during the latter part of the 19th century led to development of the Physiological Fuel Value system for humans by Atwater and Bryant (1900), and analogous metabolizable and net energy-based systems for the feeding of animals were developed for use in agriculture early in this century by Armsby (1917), Kellner (1919) and others. These early models of animal metabolism were based upon energy and nitrogen input:output relationships established with intact animals,

and were static and factorial in nature in that animal nutrient requirements were estimated by summing separate estimates of amounts of each nutrient needed for maintenance, growth, lactation, pregnancy, etc. As knowledge of specific nutrients advanced, such as for vitamins, minerals and amino acids provisions for these were added to the several feeding systems. In addition, as further input:output data became available and improved statistical techniques for analyses of animal data were developed, parameter values in the feeding systems were refined and extended to apply more specifically to individual feedstuffs and animal types. However, the basic concepts underlying the feeding system models that were devised at the turn of the century have not been appreciably altered and are well represented in current NRC publications on the nutrient requirements of domestic animals. These aspects of the development, evolution and application of feeding system models in support of animal agriculture are presented in greater detail in Chapters 5, 6 and 7 of this publication.

1.2 WHY AN ANIMAL SCIENTIST WOULD CHOOSE TO MODEL ANIMAL SYSTEMS

The history of the field of nutrition clearly indicates that the classical scientific approach, which formulates in mathematical terms current concepts in feeding systems, was utilized in order to evaluate quantitatively the adequacy of those concepts. Thus, it is not surprising that biologists contributed significantly to development of the laws of thermodynamics (Chapter 5), and that Lavoisier is considered by many to be the father of both chemistry and nutrition. While this approach has been retained in the physical sciences throughout this century, research in nutrition diverged. Many workers continued to conduct input:output balance studies to refine and extend the applicability of the early feeding systems. Others turned their attention to identification of specific nutrient requirements of animals and intermediary metabolism. This divergence was recognized by Armsby (1917) in the following comment:

The truth is that both types of investigation are equally necessary and each aids in the interpretation of the other. The balance experiment has been especially prominent in the past, while at present attention is being directed to a greater extent to investigation of the intermediary metabolism but neither can say to the other 'I have no need of thee'.

In the 1940s and early 1950s several prominent scientists concluded that models based upon the input:output approach had a number of basic limitations that could only be accommodated through development and utilization of knowledge of underlying physiological and metabolic processes, and directed their research efforts to this end. It appears to have taken quite some time for our knowledge of the physiology of digestion and meta-

bolism of ruminants in particular, but other domestic species as well, to advance to the stage where it is appropriate to reunite the two divergent paths of nutritional research. Our view and the view to which this book is directed is that the large amount of data currently available on ruminant digestive, metabolic and physiological processes justifies attempts to construct quantitative mathematical models of these processes. The unification and summarization of concepts and data that can be achieved through application of computer supported mathematical modeling techniques increases not only our insight but also our effectiveness in utilization of existing information in the design, conduct and interpretation of experiments. The availability of mathematical models enables us to utilize many types of data in order to optimize resource allocation and use in animal agriculture; models are also useful in analyses of problems too complex for intuitive approaches.

A trivial but relevant example that applies to the gross efficiency of growth should help clarify this view that models fulfill an essential role in nutritional research and the application of research results. Consider the diagram presented in Fig. 1.1. If dietary carbohydrate is converted to acetate in the rumen and the acetate is used to synthesize body fat, the biochemical efficiency of the process would be (0.8×0.76) about 61%. If dietary carbohydrate was absorbed and used for body fat synthesis, the net efficiency would be 78%. When dietary fat and protein are used to form body fat, the respective biochemical efficiencies would be 97 and 67.5%. Similarly, the

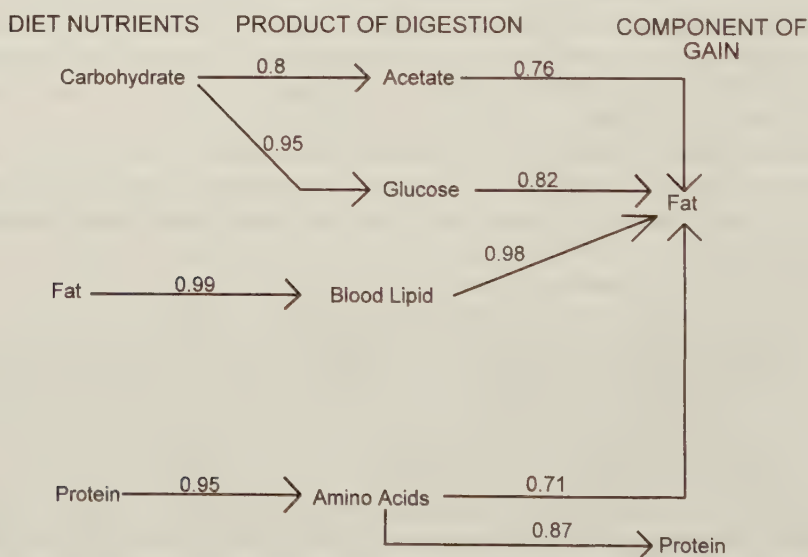


Figure 1.1 Efficiencies associated with the use of alternate nutrients for gain of body fat and protein. Estimated biochemical efficiencies of conversions are based on known metabolic pathways and are presented on arrows.

efficiency of use of dietary protein for protein gain would be 83%. According to these estimates and implied differences in either diet or patterns of nutrient use, quite a range in apparent net efficiencies of body energy gain can result. For example, in ruminant digestion and metabolism carbohydrate is fermented in the rumen to form acetate, which is used in turn to form 100 MJ of body fat. Therefore, $(100 \text{ MJ}/0.61)$ 164 MJ of dietary carbohydrate must be input. In contrast, when dietary fat is provided as a precursor of body fat, only $(100 \text{ MJ}/0.97)$, 103 MJ of dietary energy need be supplied to support the prescribed fat gain. In an animal gaining 100 MJ each of body fat and protein, respective inputs to support body energy gains with a high carbohydrate as opposed to high fat diet would be 164 MJ of carbohydrate for fat gain plus $(100 \text{ MJ}/0.83)$, 120 MJ of protein versus 103 MJ dietary fat for fat gain plus 120 MJ for protein gain, which yield respective net efficiencies of gain of $(200/284)$ 70 and $(200/223)$ 90%. Now, consider a feedlot animal in the finishing phase where, conservatively, two-thirds of energy gain is in body fat and the respective biochemical efficiencies are 67 and 92%. Thus, on a biochemical basis, considerable variance exists that can be attributed to diet and composition of gain. These observations clearly indicate that feeding systems, which do not accommodate composition of diet beyond the historical aggregate terms digestible and metabolizable energy and crude protein, are inadequate. Chemical entities in the diet, their digestion and metabolism must be accommodated in models used to refine or optimize diets in animal agriculture. However, this accommodation is not, in and of itself, adequate, because other sources of variance exist.

Based solely on biochemical arguments, we cannot account for the apparent efficiencies of gain that are observed in classical energy balance experiments. On more or less typical ruminant diets, estimated biochemical efficiencies for growth are 70–75%, while energy balance studies indicate efficiencies of growth of 45–50%. Thus, biochemical estimates of efficiencies of gain only accommodate about 50% of the total costs of weight gain. Lactation studies, similarly, indicate that biochemical costs comprise about 50% of the total costs of incremental increases in metabolizable energy intake and milk production. Additional physiological and metabolic costs associated with growth and milk production are increases in physiological work (heart work, work of muscles of respiration, etc.), increases in rates of protein turnover, increases in energy expenditures in $\text{Na}^+ \text{K}^+$ -ATPase activity associated with nutrient transport across membranes and increases in the relative weights of tissues with high energy expenditures per unit mass at the expense of tissues with low energy expenditures per unit mass. These latter sources of energy expenditures vary as functions of feed intake, diet, hormonal status and genetic potential, and not as simple functions of productivity. Classical input:output experiments cannot define and, indeed, did not lead to identification of these sources of variance in efficiencies of animal production. Understanding and, most importantly, quantitative definition of these in attempts to explain and accommodate

variance requires studies of tissue and cell metabolic functions and utilization of this information. Models are the means by which knowledge of physiology and metabolism gained through studies of organ and cell functions can be utilized to advance our quantitative understanding of animal functions, sources of variance and, finally, effective utilization of this knowledge to develop optimal animal production strategies. Progress toward development of this latter goal are emphasized in this book.

1.3 BASIC ORGANIZATION OF THIS BOOK

Theories, terminology and methods relevant to the construction, evaluation, and use of quantitative models in support of the allocation of agricultural resources and optimization of the role of animal agriculture in support of food production are discussed in Chapters 2, 3 and 4. Chapters 2–4 and 5–7 are based in large part upon graduate lectures on the application of mathematical models in nutrition and current feeding systems in use in animal agriculture, respectively.

Our original intent was to discuss basic biology relevant to ruminant digestion, growth and lactation in three chapters followed by three chapters on algebraic models of these processes. However, considerable redundancy arose, which resulted in the decision to present the biology of the several processes along with related algebraic models in Chapters 8–10. Major books on the biology of ruminant digestion, growth and lactation have been published. Therefore, only aspects of the biology of these processes applicable to either concepts underlying models or algebraic models are developed. In selecting algebraic models for inclusion in these chapters, emphasis was placed upon illustrating applications of different types of models in support of the research process.

The next sections of the book, Chapters 11–14 and 15–17, emphasize evolution of the modeling of animal functions from representations that are static and empirical to representations that are mechanistic and dynamic. Dynamic models offer a number of advantages over static models in both application in optimizing animal feeding and management strategies and in research. In the first case, dynamic models trace effects current decisions can have upon bioeconomic behavior of an animal or production system over time. The detailed dynamic models of ruminant digestion and tissue metabolism presented in Chapters 11–14 aid in analyses of metabolism in individual tissues and the formulation of improved representations of tissue metabolic functions in animal level models such as those presented in Chapters 15 and 16. A number of modeling analyses of the dynamics of ruminant digestion have been reported over the past 25 years. Discussion of these served as an opportunity to illustrate the evolution of knowledge and models of a specific set of functions over time in Chapter 11.

The metabolic functions of adipose tissue (Chapter 12), mammary gland

(Chapter 13) and liver metabolism (Chapter 14) are clearly different but they also hold a number of metabolic functions in common, including the hexose monophosphate pathway, the Embden–Meyerhoff glycolytic pathway, the tricarboxylic acid cycle, lipogenesis and β -oxidation. In order to avoid redundancy, Chapters 12–14 were written from different but complimentary perspectives to emphasize different aspects of the modeling process in terms of reasoning, data utilized and aspects of model evaluation; each tissue model has attributes that best illustrate one or more aspects of the overall modeling process. All aspects of the modeling process illustrated, of course, were applied to the three tissue models presented.

Several dynamic models of growth that differ in complexity and intent have been developed. Of these, the models of the NRC (1984), Fox and Black (1984) and Oltjen *et al.* (1985, 1986a,b) were selected for discussion in Chapter 15. All of these were developed with a goal of practical application and thus serve to illustrate the ongoing evolution of applied models wherein mechanistic and dynamic elements are being added to increase prediction accuracy and breadth of application. In addition, the sheep growth model of Sainz and Wolff (1990a,b) is presented in Chapter 15 to illustrate the use of research models to evaluate possible explanations of observed responses which are not fully understood at the intact animal level.

Chapters 16 and 17 are devoted to presentation of our current model of digestion and metabolism of a lactating cow in the format of a user-oriented modeling language, and evaluation of that model in terms of behavior, sensitivity analyses and possible use in risk analyses.

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Modeling principles and terminology

The gap between biologists and mathematicians represents a significant obstacle to the application of the combined methods of research.

Gauss (1934)

2.1 INTRODUCTION

The basic goal of biochemists, microbiologists, nutritionists and physiologists studying ruminants is to advance animal production through a greater understanding of ruminant digestion and metabolism. Most scientists favor the view that advances in our basic understanding of ruminant digestion and metabolism have contributed to improvements in animal production. We do not believe this view can be challenged. Informed, experienced and intuitive scientists have suggested many changes in feeding and management that resulted in improvements in productivity and efficiency. The existence of many successful professional consultants in the animal industry is clear evidence that supports this view. It is also clear, however, that in formulating their suggestions, agricultural scientists are not making full use of many relevant research data and proven concepts in evaluating management alternatives. Further, it is evident that agricultural scientists are often not able to predict with precision how much economic or societal benefit will result if several management changes are made. This is not surprising when one considers the complex plant and animal functions that must be considered, the tremendous mass of relevant data available and the fact that the human mind, although unique in its capacity to formulate concepts and hypotheses, is limited in its ability to trace in a quantitative fashion over time implications of the concepts utilized qualitatively in formulating recommendations (Forrester, 1971). Computers, when supplied with equation and data sets (models) representing knowledge pertaining to a system, can readily trace the quantitative and dynamic implications of available data and concepts in terms of system behavior. From this it follows that the intuitive and conceptual approaches used in the formulation of suggestions

for improvement of animal production can be complemented and extended through more effective use of computers. The promise of this approach has already been recognized and implemented. Many models, usually in the form of linear programs, are in current practical use. These range from computer programs used in ration formulation to programs used in support of management decision making. Despite the fact that these models all have limitations and must be applied with great care, they have proven very useful.

Most models in current use are based upon whole animal input:output (empirical) data, i.e. do not accommodate effects of underlying functions which can vary and influence input:output relationships; are static, i.e. do not consider effects of previous or current management upon subsequent performance; and are deterministic, i.e. do not accommodate variance. All of these characteristics of current models require the use of intuition and judgement in their application as alluded to above. These limitations frustrate animal scientists who have dedicated their careers to the study of metabolic and physiological regulatory functions that vary over time and contribute to variance in the animal population. They realize that in the absence of appropriate models, the understanding and quantitative data they have developed can only be applied in an intuitive fashion in evaluations of animal production strategies. This leads to the view that full benefits from our knowledge can be attained only when objective and quantitative means are devised to bridge the gap between our knowledge of basic function and animal performance. The techniques of systems analysis and biomathematics can be utilized to achieve this objective. We are convinced that systems analysis is the only approach available that enables rigorous quantitative evaluations of our understanding of animal functions and can lead to full utilization of this knowledge in the solution of problems in animal production.

The application of mathematical modeling and systems techniques in analyses of energy and nitrogen transactions in animals has not yet developed to the point where a number of unique and definitive contributions arising from the approach can be cited. Contributions to date have been formulation of current knowledge, evaluations of current knowledge and hypotheses for probable adequacy as explanations of the quantitative and dynamic behavior of animal systems and identification of critical areas of experimentation. Also, a rational array of philosophies, approaches and techniques have emerged.

In this book, applications of computer modeling in animal production research will be emphasized more than applications involving more effective utilization of research information in animal agriculture, because examples of the latter have been limited by a lack of effective methods for integration and quantitative evaluation of data and concepts for use at the practical level. Clearly, research advances in this area will lead to practical application; we just have not reached this point yet. In this chapter, defini-

tions, approaches and philosophy applicable to the use of modeling and systems analysis will be presented. Also, we will attempt to illustrate the fact that many applications of system analysis preceded adoption of this approach in the animal sciences. Thus, animal scientists did not have to develop the techniques and philosophy from scratch. Rather, they had to select from an array of existing approaches, those most relevant to the animal sciences (see Hall and Fagen, 1956; Gordon, 1969, 1978; Shannon, 1975; Law and Kelton, 1982; France and Thornley, 1984).

2.2 CLASSIFICATION OF MODELS

Thornley and France (1984) outlined the following scheme for classification of models:

Dynamic	or	Static
Deterministic	or	Stochastic
Mechanistic	or	Empirical

This terminology seems to have been adopted by the animal science modeling community and will be utilized as far as possible in characterizing models in this book. Short definitions of these terms are illustrative and convenient for purposes of discussion. However, ambiguities can arise which require further elaboration.

Riggs (1963) defined an empirical equation as one that has been fitted to experimental data so as to describe a relationship which has actually been observed between two or more variables. He emphasized that such equations imply nothing about the underlying reason for the relationship and thus, although a particular set of data may be faithfully described, cannot be safely applied beyond the range of values for independent variables upon which the model is based. Most current models used in formulating practical recommendations in animal agriculture are comprised primarily of empirical equations. Thus, by definition, these models must be applied with care to assure that recommendations are not made for conditions where the data used in formulating the equations do not apply. Theoretical equations were defined by Riggs (1963) as being derived not from a particular group of data but from some theory or hypothesis about the fundamental nature of the system. Thus, a well-founded theoretical equation may be able to describe all data sets and explain why observed relationships exist. When a model comprised of theoretical equations is complete, full knowledge of causal relationships within the system is implied and computed results should relate to a broad range of realities. Thornley and France (1984) suggested the term 'mechanistic' rather than 'theoretical' be utilized to describe equations formulated on the basis of concepts regarding underlying function.

The term 'deterministic' implies that all solutions of an equation or set of

equations are exact. In the animal sciences, use of this term implies that solutions apply specifically to the average animal in a population.

Rather than having all variables defined as unique values, mathematical simulation models can contain variables or have input variables defined by probability distribution functions. The term 'stochastic' refers to models containing processes defined by probability functions. Stochastic models are frequently employed to simulate population attributes (Mendelsohn, 1978; Azzam *et al.*, 1990).

Stochastic indicates accommodation of variance we do not understand and, therefore, cannot account for in the equations that comprise a model; or, that provision has been incorporated into a model to allow simulation of effects of a known cause of variation.

In the first case, a coefficient of determination may be reported to indicate what proportion of population variance is accounted for by an equation or set of equations and as a measure of our understanding or lack thereof. Multiple solutions of such a model obtained by letting parameter values vary randomly according to probability distribution function would provide an estimate of the uncertainty of a prediction.

In the second case, udder metabolic capacity, a factor known to be a primary cause of differences in the 'genetic' capacities of cows to produce milk, might be specified as a mean value $\pm$ its standard deviation. When the value for udder metabolic capacity is allowed to vary according to a probability distribution function and multiple solutions are undertaken, not only the population (deterministic) mean can be estimated, but also variance attributable to differences among cows in genetic potential. Thus, use of the term stochastic can be ambiguous and the intended meaning must be clearly stated.

The first stated definition is the one most commonly applied. With regard to this application of the term stochastic, Lucas (1964) stated that 'the basic goal of science is to reduce the use of stochasim'. By this, he meant that the challenge is to explain the sources of variance and uncertainty, and thereby reduce the necessity for use of stochastic elements in models.

The only use of stochastic elements addressed in this treatise will be of the latter type – definition of key parameters specified in mechanistic models as mean values $\pm$ their standard deviations. In this application, a random number generator is used to set the parameter value in each run in a series to simulate responses of an animal population, rather than the average animal to a specific perturbation. In this use of stochastic elements in a model, the caution of Thornley and France (1984), that a deterministic model should be formulated and evaluated before stochastic elements are introduced, must be satisfied. Otherwise, the modeler runs the risk that stochastic elements incorporated into the model reflect ignorance of the system and, as a result, compromise the scientific value of the model. For example, in developing a model of the productivity of sheep under range

conditions, a person could formulate a stochastic relationship for birth rate ($X = 0.9$, $SD = 0.5$) based upon historical range data. The model would be hard to refute given the high variance component but would have very little explanatory (scientific) power. Alternatively, a deterministic model could be formulated in which rainfall, sunlight, temperature and soil fertility determine forage growth and quality. Forage availability could, in turn, affect ovulation and birth rates. Having evaluated a deterministic version of such a model in terms of accurate simulation of specific years in specific environments one could in turn decide to introduce stochastic parameters for weather, soil fertility and the relationship between nutritive state and ovulation rate in ewes into the model. Such a model would have greater explanatory and scientific value in that contributions of these key variables to total variance in multi-year range experiments could be evaluated. Models with these attributes are also useful in risk analyses that might be undertaken in support of management decision making (see Chapter 17).

The terms dynamic and static are self explanatory. Dynamic models are based upon differential equations of the form

$$\frac{dx}{dt} = f(a, b, c) \quad (2.1)$$

and are integrated over time, usually using a numerical integration technique (see Chapter 3). Thus, dynamic models are potentially capable of tracing impacts of previous and current management decisions on subsequent performance.

Static models are usually algebraic in form and are solved for a specific set of conditions, which exist at a set point in time. Most feeding system models in current use (see Chapter 5) are static in that they predict the nutrient requirements of an animal of known body weight, sex and rate of production at a specific time. Conversely, these models can predict rate of production, e.g. gain, given inputs such as feed intake, weight and sex at a specific time. Most static models are, of necessity, factorial in nature, e.g. estimates of nutrient requirements are the sum of requirements for maintenance, activity, pregnancy, gain, etc. In estimating productivity, given a set intake of a defined feed, requirements for maintenance, activity and pregnancy are satisfied first, and then gain is estimated based upon remaining available nutrients. This approach requires acceptance of the implicit assumption that there is an absolute hierarchy in demand functions-maintenance over activity over pregnancy over gain and that there are no interactions among these. This is clearly not true, a difficulty upon which we will elaborate in subsequent chapters.

In context with the classification of models put forth as discussed above, regression equations would generally be considered static, stochastic and empirical. Linear programming models used in ration formulation require that all relationships be linear and all values specified be exact and additive.

Thus, these are considered empirical, static and deterministic. A model comprised of differential equations that captures our knowledge of underlying function would be considered mechanistic, deterministic and dynamic. Unfortunately, although this terminology seems discrete and clear, even persons who accept it can get into arguments regarding classification of a specific model. This problem is particularly acute with regard to the distinction between mechanistic and empirical.

Boulding (1956) identified 10 organizational levels for the universe, with animals located at the sixth level. Additional hierarchical levels can be nested within this organizational structure. Thornley and France (1984) presented the following formulation of the organizational hierarchy of biological systems as related to levels of aggregation, the formulation of models and further definition of the terms 'empirical' and 'mechanistic':

Level	Description of Level
$i + 1$	herd of animals
i	animal
$i - 1$	organs
$i - 2$	tissues
$i - 3$	cells

In general, the empirical modeler formulates equations at a given level of organization based upon data gathered at that level, e.g. input:output data gathered at the animal level (i) are used to describe or model animal level function. Regression equations are clearly the most common approach used in formulating such models. In relation to the remarks of Riggs (1963) summarized above, the only constraint that need be imposed upon the form of a regression equation is that it best fit the data. However, regression equations can be formulated on the basis of concepts developed from studies of lower level processes ($i - 1$, $i - 2$, $i - 3$, etc.). Further, definition of the terms theoretical and mechanistic implies that both equation forms and parameter values in mechanistic models arose from studies of lower level processes. This is a difficult criterion to satisfy. Explicit consideration of the level of analysis helps in further definition of empirical as compared to mechanistic models, but still allows some ambiguity. We are not aware of either a pure empirical model or a pure mechanistic model in use in animal agriculture today. One can argue that our current feeding systems are not empirical, because the criterion is not met that the only constraint imposed in selection of equation forms is that it best fit available data. The models commonly used in current feeding systems take the (multiple regression) form

$$\text{Energy Intake} = ax_0 + bx_1 + cx_2 \dots \quad (2.2)$$

where x_0 is taken to reflect the animal's maintenance requirement, while x_1 and x_2 are productive functions such as protein and fat gain. The coeffi-

cients a , b and c are reciprocals of the apparent efficiencies of nutrient use for maintenance and the productive functions x_1 and x_2 . This argument would hold that the equation form does indeed imply something about underlying function, i.e. that energy is used for multiple purposes and that the biochemical efficiencies of energy use for alternative functions differ. In contrast, one could argue that equation 2.2 can fit the curvilinear responses observed when intakes are varied over a wide range as well as any other equation, and that the coefficients a , b and c do not necessarily imply anything about the physiological and biochemical processes involved in maintenance and protein and fat accretion. For purposes of definition, we are going to accept the latter argument on the grounds that the concepts captured in the model were developed by inference from animal input:output data, and that a large number of data clearly indicate that a , b and c vary greatly from experiment to experiment. Thus, these coefficients do not capture a consistent set of physiological and metabolic functions associated with maintenance and growth, i.e. do not have explanatory power with regard to mechanism.

In contrast, one could argue that a feeding system model that is empirical, as defined above, could be considered mechanistic if some of the equation forms used were formulated on the basis of concepts arising from studies of lower level functions.

For example, a basically empirical feeding system for growth might contain equations in which the rate of muscle protein accretion is represented as a function of protein and DNA content that were, respectively, introduced to define biosynthetic capacity, protein degradation rate and availability of information as messenger RNA for the support of protein synthesis. Such an equation set clearly has mechanistic connotations in that the equations capture concepts developed in studies of lower level function (see Chapter 9). However, data required to define the relationships among muscle protein mass, biosynthetic capacity, protein degradation rates, muscle DNA and rates of protein biosynthesis in ruminants are extremely limited and certainly not adequate to support parameterization of the equations. Rather, the modeler is forced to deduce several of the required parameters for these equations from animal-level data.

What terminology can be employed to characterize such a model? We are clearly entering a gray zone. In this treatment, such models will be identified as largely empirical models containing mechanistic elements. Over the short term, we expect that improvements in practically oriented models such as the feeding systems will be achieved through the addition of mechanistic elements to empirical models. The AUSPIG feeding and management model of Black *et al.* (1986) and the Cornell Net Protein and Energy System (Search:Agriculture, 1990) are examples of this approach.

Another mix of terminology problems is posed by models based primarily upon equations whose forms derive from studies at levels below the target level but were parameterized using data obtained at a number of

levels of aggregation. One problem is incurred when, for example, an equation of the Michaelis–Menten form (see equation 2.3 below) is used to represent a metabolic process in a specific tissue in an animal level model.

Concentrations of metabolites in blood and extracellular tissues do not usually vary over a range large enough to justify use of an equation representing saturation kinetics nor to define apparent affinity of a tissue for a given substrate (k_s). These must be justified and defined in *in vitro* studies using tissue slices or, preferably, isolated cells. When used in a model of liver metabolism, such an equation would be considered empirical in that level $i - 2$ data were used to formulate an $i - 2$ model. When applied in simulating animal metabolism, such an equation would fit the definition of mechanistic in that $i - 2$ data were used to simulate i level function. Thus, such an equation and the k_s would generally be accepted as mechanistic components of a model of animal metabolism and function. However, metabolic rates of tissue slices and isolated cells incubated *in vitro* are usually significantly lower than those expressed *in vivo*, so a $V_{\max}$ estimate based upon *in vitro* data cannot be used to simulate the *in vivo* condition.

Other complexities intervene, but for purposes of simplicity let us assume that the liver function referred to above is urea synthesis and that all urea synthesized is excreted in urine and, thus, can be measured in animal level studies. For the Michaelis–Menten type equation

$$v = V_{\max} / (1.0 + k_s / [S]) \quad (2.3)$$

if one knows blood ammonia concentration $[S]$, rate of urea excretion in urine (v) and k_s based upon *in vitro* studies, $V_{\max}$ can be calculated simply. Obviously, this equation is mixed in type; the form fits the mechanistic criterion in that some parameter (k_s and $[S]$) values fit the mechanistic criterion and the equation could not be utilized to simulate animal function until $V_{\max}$ was scaled using whole animal data. Other scalars from whole animal experiments commonly used to parameterize otherwise mechanistic elements in animal models are heat production, protein and fat gain, and milk lactose, fat and protein secretion data.

We are not aware of models based upon mechanistic equations that have been fully parameterized on the basis of $i - n$ data, which can effectively and accurately simulate animal function. In fact, one could suggest that it is not feasible to achieve a purely mechanistic model of animal metabolism. On the other hand, most workers consider models of the type discussed above to be mechanistic, so this definition will be applied herein.

A somewhat different problem in terminology arises when one considers research models in which the equation forms adopted are based predominantly upon knowledge of underlying function and are used analytically to estimate parameter values for specific functions. In this case, animal input:output data may be used along with tissue- and even cell-level data to parameterize the model. A wide array of models designed for this pur-

pose have been formulated. Examples are presented in Chapters 8, 13 and 14, but these reflect a minuscule portion of total activity (Berman *et al.*, 1962; Matis, 1972; Carson *et al.*, 1983). Modeling efforts of this type have been referred to as top down, multi-compartmental models. A few examples are presented here to illustrate the type.

Consider a dynamic model of calcium metabolism (Ramberg *et al.*, 1975), in which measurements of calcium digestion and excretion, calcium balance, circulating Ca^{2+} , hormone and radiotracer data were used to deduce whether or not the lesion associated with milk fever is due to a limitation in calcium absorption from the digestive tract or inadequate calcium mobilization from bone. The model used to address these issues included provisions for calcium absorption, excretion, secretion in milk, deposition and reabsorption from bone, and multiple pools of calcium in the body. Clearly, equations in the model capture mechanistic relationships. On the other hand, the statistically deduced pool sizes may not reflect actual biological entities, e.g. blood Ca^{2+} , plasma Ca^{2+} , extracellular Ca^{2+} , intracellular Ca^{2+} or a definitive sum of some of these. This could lead to the suggestion that the model is empirical in the sense that input:output data were used and deduced parameters (Ca^{2+} pool size) do not reflect discrete, measurable biological entities.

We do not believe it is appropriate to attempt to classify models of this type as empirical or mechanistic. These models utilize concepts and data collected at multiple levels of animal function in support of the research process and will be referred to collectively as analytical models, since they have explanatory power and can be used to deduce parameters and properties descriptive of a system.

Whenever a series of equations describing input:output relationships at the animal or metabolic pathway levels are written to define the conversion of nutrients to products and solved to obtain a net balance, a balance model has been constructed and used. Many such models have been worked out by hand and published (NRC feeding systems; Wolin, 1960; Krebs, 1964; Hungate, 1966; Milligan, 1971; Reichl and Baldwin, 1975).

When modeling objectives include evaluations of data, it follows that balance equations were used at some point in model development. A balance model may define an energy balance relationship, such as

$$\text{HP} = \text{MEI} - \text{BEL} \quad (2.4)$$

where HP is heat production by an animal, MEI is metabolizable energy intake and BEL is body energy loss. Or a balance model may define oxidation of a mixture of nutrients

$$\text{HP} = a\text{O}_2\text{UP} + b\text{CO}_2\text{PRD} - c\text{UN} \quad (2.5)$$

where HP is heat production, O_2UP is oxygen uptake, CO_2PRD is carbon

dioxide production, UN is urinary nitrogen excretion, and a , b and c are coefficients.

Balance models are static and deterministic in nature and can often be solved without resorting to a computer. However, when they must be solved many times, it is useful to program the equations for solution by computer, e.g. this is the case for linear programming models used to formulate least-cost rations in order to evaluate the effects of specific assumptions or perturbations upon an outcome, or to estimate parameter values. Additional advantages gained are improved accuracy and, often, a reduction in simplifying assumptions which one often makes to facilitate hand calculation. Example applications of balance or analytical models will be presented in Chapters 3, 5, 6, 7, 8 and 10.

Dynamic models are utilized in analyses of the behavior of a biological system through time. These may be empirical in nature, such as the Gompertz equation, which is often used to describe increases in body weight in growing animals of a specific species or breed through time (Chapter 9), or the Wood equation, which is used to estimate milk production during the lactation cycle (Chapter 10). When one desires to estimate key parameters for a set of metabolic equations depicting regulatory processes, a dynamic, mechanistic model is clearly required. Examples of this type of analytical model are presented in Chapters 7, 8, 10 and 12–14.

The above discussion represents an attempt to clarify terminology that will be utilized in this book and is utilized, not without controversy, in oral communications and discussions of models. In later discussions, it will be emphasized that the true value to science of a model cannot be judged on the basis of the degree or mix of empirical and mechanistic elements. Rather, models should be evaluated on the basis of the relevance and importance of the objective stated in the modeling exercise and to what extent the modeling objective was achieved. As in all research, if the objective posed was inappropriate or irrelevant, the modeling exercise should be similarly characterized. If the stated modeling objective was not achieved, the modeling exercise might be considered as an unsuccessful learning experience, but nevertheless, it is not publishable. When the objective is relevant and was achieved, the research exercise should be considered a contribution to science and, therefore, publishable.

2.3 OBJECTIVES IN MODELING

Objectives dictate the general type and form of a model. Ideally, models should have the following features: (1) simplicity for manipulation and understanding by those applying the model, (2) representative enough to allow generalization to a wide range of situations and (3) sufficient complexity to accurately represent the system under study (Chorafas, 1965).

There are two levels of objectives in modeling. Generic objectives will be

discussed in this section. Specific objectives stated at the outset of each modeling study will be discussed in Chapter 3 and in connection with individual models presented in subsequent chapters.

Thornley and France (1984) stated the following:

To introduce a mathematical modeling approach into, for instance, an animal science department or institute may be viewed as simply widening the scope of intellectual approaches available to that which has been commonplace in the physical and chemical sciences for decades.

In other words, physical and chemical scientists have been developing hypotheses, converting these to mathematical statements and evaluating how well the mathematical relationships thus derived describe reality. If a system of equations fails to fit reality, they study the system further and attempt to explain why observed deviations occur. Early biologists used this approach, as will be illustrated in Chapter 4. However, for the past 60–80 years biologists have been more concerned with such things as phenomenology, the chemistry of life, the discovery of required nutrients and metabolic pathways. This was essential since one cannot undertake research modeling using mechanistic equations when knowledge of underlying function is totally inadequate.

It would seem, however, that at some time during the past two or three decades our knowledge progressed to the point where utilization of quantitative techniques in addition to statistics became appropriate for biology in general and for animal science in particular.

We think many animal scientists would agree with Thornley and France's next statement:

It has been all too easy to observe traditional and empirical agricultural scientists doing 'more of the same' type descriptive experiments, perhaps, due to the absence of a vision of a fully articulated subject area.

This statement is a strong condemnation of the agricultural sciences. The contention is that agricultural scientists haven't been attempting to formulate theories that can be mathematically evaluated and, further, that this has limited our rate of progress. Rather than trying to explain variance, agricultural scientists have been characterizing variance by doing the same types of experiments over and over in slightly differing situations – species, breeds, environments. In formulating our current research program and structuring this book, we have been cognizant of the remarks of these two men who were trained in physics and, later, became significant contributors to the agricultural sciences.

Thornley and France (1984) very strongly contend that:

Mechanistic modeling is 'hard science' in that it follows the traditional scientific method as used in the mathematical and physical sciences. Problems are examined by looking downward in the organizational

hierarchy, with the synthetic process of modeling taking one back up to the original level; reduction and analysis are followed by integration and synthesis....

Further, they contend

mathematical modelers, with attitudes derived from the physical sciences, working with creative biologists, will be able to contribute to the refinement, extension, and integration of the biological sciences to an extent that lies far beyond our present imaginings.

They are saying that if we adopt the approach classically used in the physical sciences, we should be able to move further. They do not know how far we can go utilizing that approach, but expect we will go very far.

The first step in developing a research program in modeling is to identify the objectives, or advantages, one wishes to attain. Advantages that can be achieved through modeling have been considered by various investigators (DeWit, 1970; Gordon, 1969; Rashevsky, 1961; Riggs, 1963; Yates and Schneider, 1974; Baldwin and Smith, 1971; Dent and Anderson, 1971). General objectives in development of practically oriented management models can differ significantly from those posed when modeling studies are used in support of a research program. Therefore, these will be discussed separately.

2.3.1 Objectives in applied modeling

Many mathematical models based upon empirical relationships have been developed and are very useful when applied carefully. Appropriate recognition must be given to the limitation that empirical equations are not applicable outside the range of conditions defined by the data used to parameterize the model. This problem can and has been alleviated, in part, by introduction of causal or deterministic elements. In the continuing evolution of our feeding systems, this approach has been utilized (Chapter 4). The distinguishing feature, discussed above in struggling with terminology, is that animal-level data were used to parameterize the equations. Mechanistic models apply over a wider range of conditions, but two major problems have precluded development of practically directed models based solely on mechanistic relationships.

The first is that mechanistic, dynamic models are very complex and expensive to solve iteratively as would be necessary for formulation of least-cost rations or in evaluations of alternative management strategies.

The second is that development of mechanistic models implies that our knowledge of the system is adequate and, further, that sufficient biochemical, physiological and metabolic data are available to parameterize the models. This is rarely, if ever, the case.

These observations were the basis for the suggestion made above and

reiterated here that over the short term we expect improvements in applied models to arise from incorporation of mechanistic elements into largely empirical models to broaden their applicability. Over the longer range, we expect applied models to become more and more mechanistic in nature. Several observations support this view.

First, computer capacities and speeds are increasing rapidly and solution costs and time are decreasing rapidly. The costs of iterative solutions of dynamic, mechanistic models are becoming of much less concern.

Secondly, our knowledge of underlying functions is increasing rapidly and becoming a sounder and sounder basis for development of mechanistic elements to better explain variance.

2.3.2 Objectives in research modeling

The wide range of metabolic, nutritional and physiological information that must be integrated in analyses of animal metabolism and function indicates that modeling techniques can be utilized and can contribute effectively to the research process. As knowledge continues to advance, computer simulation models become more and more essential to the study of quantitative and dynamic behavior of animal systems.

Models provide a means for integration of concepts and information regarding entities/objects which comprise the system, their inter-relationships and their regulation. Unfortunately, naive and improper applications of the modeling approach have led to doubt among many experimentalists about its usefulness in research. If a negative attitude towards mathematical models persists among experimentalists and, as a result, models are constructed without associated experimentation, the usefulness of a powerful technique will be limited. If, on the other hand, experimentalists accept and utilize systems techniques and participate in modeling studies, contributions of this methodology to the advancement of knowledge could rival and surpass the contributions of conventional statistical techniques to animal science research. Certainly, methods that complement intuition are required for analyses of the complex, dynamic properties of animals.

A comparison of model predicted and observed behavior of a system constitutes a test of the concepts and information upon which the model is based. Models that do not simulate observed behavior are either inadequate in representation, or the concepts and data which comprise the model are inadequate. This test of our understanding of a system is the central pillar upon which the application of models in support of research is based. Is our understanding of the system adequate or is it not?

More specific objectives in research modeling are presented in the following sections.

(a) Integration of existing concepts and data in a format compatible with quantitative and dynamic analyses

Ulyatt *et al.* (1976) contended that this might be the most significant advantage experimental scientists gain through exposure to a modeling exercise. The challenge associated with organization of data and concepts imposed by this objective requires that data be addressed in a fashion not normally undertaken in the course of preparation for statistical analyses and publication. The emphasis is not on whether or not there is a difference between two groups of animals, but whether available data and concepts are adequate to enable formulation and parameterization of equations that might explain the difference. This is the learning process alluded to by Ulyatt *et al.* (1976). Example data summaries used in support of the modeling process are presented in Chapter 3 and following chapters on specific models.

(b) Reduction of conceptual difficulties in analyses of interactions among elements of complex systems

If a person chooses to address patterns of nutrient use by animals (nutrient partitioning), modeling techniques must be used. Otherwise, how can concepts regarding actions of hormones upon multiple tissues and subsequent interactions among nutrients be addressed without the ability to undertake quantitative evaluations? One must have equations that represent available concepts and data and a means of solving them to undertake such evaluations. Depending upon an analyst's intuitive abilities, qualitative analyses of varying relevance can be achieved, but quantitative analyses cannot. The modeling process allows one to consider concepts and data on each separate process to develop equations and parameter values. Thus, the modeler needs to consider only one process at a time and the conceptual difficulty of the analysis is considerably reduced. When the system of equations thus developed is solved on the computer, quantitative implications of the several concepts and their interactions are revealed.

(c) Evaluation of concepts and data for adequacy in both the quantitative and dynamic domains

Evaluations of current concepts and data for quantitative and dynamic adequacy as explanations of the behavior of complex systems are absolutely essential to the research process in applied sciences. By and large, in the biological sciences, concepts and data have been evaluated in a qualitative fashion: A goes up, B goes up and C goes down. Data to the effect that A binds to a receptor that stimulates an enzyme that forms B which is, in turn, an inhibitor of the enzyme that forms C would clearly support formulation of a hypothesis that would explain or anticipate responses in C (up/down) to changes in A.

Traditionally, we have not asked whether or not the hypothesis, thus developed, is adequate to describe the quantitative and dynamic behavior of C. This is one of the questions you can ask using a model. Success in simulating behavior of C under a variety of conditions implies adequate understanding. Failure implies that our understanding of factors that influence C is inadequate and that further experimental research be directed to identify other factors that effect the enzyme that synthesizes C. Alternatively, one might suspect that the kinetic parameters used to define the effect of A upon B or B upon C were inaccurate. The nature of the model failure and further modeling analyses may provide a basis for discriminating between these two alternatives.

(d) Evaluation of alternative hypotheses for probable adequacy when current concepts are found to be inadequate, and identification of critical experiments and measurements

When an evaluation of current concepts forces the view that our conceptual frame is not adequate, we can formulate hypotheses, incorporate these into a model and ask whether or not a hypothesis, if true, would help us better explain reality. After having tested a hypothesis and finding that if it were true it would help us explain reality, it should be tested experimentally. Also, alternative hypotheses can be ranked in terms of how much they could, if true, advance our ability to explain reality, e.g. reduce errors of prediction, and thus aid in setting experimental priorities. In our experience, this is a very important benefit gained from the marriage of an experimental research program and modeling analyses. As an experimentalist, this author had a strong tendency, faced with alternative hypotheses, to give the hypothesis that presented the greatest intellectual or academic challenge the highest priority. Unfortunately, these often rank below other hypotheses in explanatory power. As a result, the use of models has caused our experimental research to become mundane and, perhaps, less intellectually challenging in nature, but more relevant in that in selecting experiments we were assured beforehand that we were focusing on the most important issues.

This objective imposes several requirements upon the characteristics of models used in support of the research process. Elements in the model have to represent specific biological entities and transactions among these; they must be mechanistic. The models have to be based on established principles and contain no hypothetical relationships. Remember that if the objective is to evaluate hypotheses, this is not possible if the model already contains hypotheses. How can you test a hypothesis with a model that is already hypothetical? Failure of a model to simulate reality must reflect a failure of established concepts, not an unfortunate choice of imaginary, untested hypotheses. To be valid in this context, numerical inputs to models must be firmly based upon experimental measurements made at appropriate levels

of organization. This requirement transcends definitions regarding empirical versus mechanistic.

This emphasizes an important interaction between experimental and modeling research. Critical experiments identified through the modeling exercise justify further study of a concept found to be inadequate. To achieve this benefit, you must have an integrated research program. You cannot make progress in modeling unless you possess some unique ability to convince experimental scientists that the experiment you propose is more important than the ones they have conceived. This is unlikely in most cases. Therefore, experimental capabilities must be available to conduct the critical experiments identified in order to gain full benefit from a modeling study. When the combined capabilities of modeling and experimental analyses are available within a group, research productivity is greatly enhanced in terms of the identification of important experiments, in the design and conduct of the experiments and, finally, in the interpretation of the data collected.

(e) Estimation of parameter values not directly measurable and interpretation of new data.

Often, while building a model, one finds that a number of parameter values required to implement the model are not available often because current technology is inadequate, i.e. we cannot measure the parameters directly with current methods. We must have a way of estimating these indirectly from data we can collect. As discussed above, models used in support of this objective are identified as analytical and balance models, which are constructed and used with a specific purpose in mind. They incorporate knowledge gained from several levels of analysis to constrain solutions such that unique and correct estimates of unknown and unmeasurable parameter values can be obtained. Methods utilized in application of such models will be elaborated upon in Chapter 4.

2.3.3 Summary of objectives in modeling

Achievement of the above objectives within an animal research program requires application of a number of modeling strategies. The specific objective of a modeling study dictates the strategy, mathematics, degrees of aggregation and empiricism utilized, and criteria used in evaluation.

The use of modeling techniques in animal science and production is based on the premise that the complex interactions within and among the environmental, digestive, physiological and metabolic elements that influence animal performance cannot be feasibly evaluated in a quantitative and dynamic fashion using traditional research approaches. Thus, the objective in modeling of combining the power of the human mind with the power of computers applies (Forrester, 1971). Modeling simply involves

formalization of current knowledge of a system in the form of equations, solution of the equations using, usually, a computer, and evaluation of the computed results. If the concepts which comprise the model are valid and adequate, computed results reflect reality. If not, computed results reveal inadequacies in current knowledge. The latter is a valid result of research modeling and can be very useful in the selection and design of experiments undertaken to advance knowledge of the system.

All uses of models mentioned above are compatible with the general goal of animal scientists, which is to advance our understanding of animal digestion and metabolism, utilize this understanding to explain quantitative variations in animal performance and, finally, to effectively apply this knowledge to the solution of problems in animal and human food production. A wide range of models can be usefully employed in support of animal agriculture. Types of models used at the applied level were discussed in this chapter along with models appropriate to the objectives of research. Applied modeling objectives will be discussed in subsequent chapters, but primary emphasis will be placed on research modeling.

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The modeling process I – objective statements, block diagrams, equation forms and parameterization

All models are wrong but some are useful.

G. E. P. Box (1979)

3.1 INTRODUCTION

A number of textbooks on modeling theory and methodology have been published (Riggs, 1963; Dent and Anderson, 1971; France and Thornley, 1984; Thornley and France, 1984; Carson *et al.*, 1983). Thus, just as was true for Chapter 2, it is inappropriate for us to undertake a comprehensive analysis of modeling methodology and available hardware and software in two chapters. We regard ourselves as users of approaches, methods, hardware and software developed by others; we truly appreciate their contributions to our research program. In this chapter, emphasis will be placed on discussion of methods and tools we believe are appropriate to modeling ruminant systems, and that have been adopted and used in modeling analyses presented in subsequent chapters. The point of view presented will be that of users, not of theoreticians or software developers.

3.2 STEPS IN MODELING

Model development is an orderly process and should advance through achievement of the following steps (Dent and Anderson, 1971):

1. Set modeling objective;
2. Construct block diagram identifying essential elements of the system and interactions among these;

3. Formulate mathematical statements;
4. Collect numerical data required to parameterize mathematical statements;
5. Solve, evaluate and verify model in terms of modeling objective. When the stated objective has not been achieved, the investigators must return to steps (2), (3) or (4) as indicated by the nature of failure of the model.

In this, modeling studies are exactly like experimental studies in that the most important step is setting the objective. The setting of a specific objective for a modeling exercise is essential to selection of elements of the system and interactions among these, which must be included in the block diagram, formulation of the equations that will comprise the model and definition of data required to parameterize the model. Most importantly, the objective statement is the criterion for adequacy with which to evaluate the model. A positive evaluation is attained when the objective is fulfilled. As stated in Chapter 2, models submitted for publication are appropriately evaluated according to two criteria: was the objective appropriate? and was the objective achieved? Sometimes, in less quantitative areas of research, a mass of experimental data collected without a specific objective in mind can be analyzed, interpreted in terms of an objective not set beforehand and yield new valuable insights. This does not happen in modeling. The modeling objective is paramount; it predetermines and guides the whole research process. In fact, without a clearly stated objective, the modeler cannot know when the study is complete.

Steps (1)–(4) of the modeling process will be presented in this chapter and the final step, model solution, evaluation and verification will be discussed in Chapter 4.

3.3 SETTING THE MODELING OBJECTIVE

The objective statement must be carefully constructed, concise and clear.

We have found that it is extremely important that several persons participate in the modeling process. The participation of experienced persons with differing backgrounds and disciplines in a modeling study speeds up the process, since no single person can be expert in all aspects of function represented in a model. Additional advantages gained when a group participates in a modeling study are more critical analyses of concepts and data; reduction of bias and naive assumptions or presumptions that single model makers might incorporate into a model; and an increase in the efficiency of data collection.

When a number of collaborators participate as a group in a modeling study, they must all understand and accept the objective statement. Nothing can destroy a collaborative arrangement more quickly than a misunderstanding regarding the objective. For example, if a participant suggests that

the kinetic properties of hexokinase be included in a model, the group would consider the suggestion seriously if the modeling objective was to evaluate our understanding of the mechanism of insulin action in a tissue. Conversely, they would probably reject the notion if the objective was to evaluate causes of variation in whole animal energy expenditures. If the modeling objective is general, e.g. to model factors that influence patterns of nutrient use in cows, there are no criteria available to accept or reject any suggestion and the modeling exercise is compromised. Unfortunately, a number of modeling exercises have been undertaken without a carefully defined objective, with the result that experimental scientists who could have been very valuable collaborators instead became disillusioned about the value of modeling research.

For purposes of discussion we pose the following modeling objectives, which are excerpts of those addressed in subsequent chapters (8, 12, 15 and 16):

1. Develop an analytical model of the metabolic pathways of rumen microbes suitable for estimation of stoichiometric parameters for fermentation of specific chemical entities in ruminant diets.
2. Modify a dynamic, mechanistic model of adipose tissue metabolism for use in extending the interpretation of available radiotracer studies and, if appropriate, the design of further experiments.
3. Develop dynamic, mechanistic elements representative of the growth process that enable improved prediction of effects of frame size upon body weight and composition at specific (finishing) weights in beef cattle.
4. Develop a dynamic, mechanistic model of digestion and metabolism in lactating dairy cows suitable for evaluation of hypotheses regarding underlying energetic relationships and patterns of nutrient use.

Objective (1) defines the type of model (analytical) and chemicals and processes that must be accommodated within the block diagram (chemical components of the diet, metabolic pathways and products of fermentation). Either by omission of the word dynamic or by knowledge – the modelers would likely know that the only significant data available for analysis are for steady-state conditions and, thus, not dynamic – the objective statement implies that static or algebraic statements should comprise the model. The same words that define the chemicals and processes to be represented in the block diagram define the input and output data that must be collected. Finally, the objective statement defines the evaluation criterion – the model must be suitable for (unique) definition of fermentation parameters.

Objective (2) presupposes that an acceptable dynamic, mechanistic model of adipose tissue metabolism exists. It requires further that provisions for simulating radiotracer data be added to that model and that the model be capable of explaining available radiotracer data. Alternatively, or in the case where available data are inadequate to further define metabolic

relationships in adipose tissue, this objective requires that the model be adequate for use in identifying and evaluating experimental designs or measurements, which when available, will advance our understanding of metabolism in that tissue.

Objective (3) requires that the block diagram, and equations derived therefrom, capture underlying knowledge of the nature of the growth process; that dynamic or differential equations be used; and that the criterion of evaluation be that the equations predict body weight and composition over time in cattle differing in mature body weights better than pre-existing models.

Objective (4) requires that the model be mechanistic in nature, and that the block diagram and equations developed therefrom accommodate rates of nutrient entry and utilization and concomitant energetic relationships over time. Further, this objective requires that the model be suitable for evaluation of hypotheses regarding nutrient use and energetic relationships.

3.4 BLOCK DIAGRAMS

Block diagrams are familiar to most biological scientists, because these have long been used to represent, in diagrammatic form, concepts of complex systems. Model makers can often adopt such diagrams as starting points. During the course of a modeling study, block diagrams are usually modified because of inadequacies identified during evaluation as indicated above for step (5) of the modeling process, e.g. if the modeling objective is not satisfied, return to step (2), (3) or (4) as indicated by the nature of model failures.

Block diagrams are presented for all models discussed in subsequent chapters and are so common in biology that only a simple example will be presented here by way of illustration. If we consider a very constrained modeling objective, such as a model of glucose-6-P (G6P) metabolism in mammary tissue, one might construct the block diagram depicted in Fig. 3.1.

Several conventions commonly used, unfortunately not without variations by ourselves or others, in the formulation of block diagrams are illustrated in Fig. 3.1. The first very commonly accepted convention is that entities enclosed in solid lined lines (G6P, F6P, TP) are considered to be state variables. These must be represented explicitly in static, algebraic models and by differential equations in dynamic models. Another convention is that entities enclosed by dashed lines must be considered in formulating, for example, stoichiometric and kinetic relationships even though they may not be represented as state variables (NADP, Ru5P). Entities in circles or ellipses can represent inputs to the model, which are specified by the user (GLC), or are sinks in which products are accumulated (LACTOSE). Solid arrows indicate specific reactions or transactions which must be repre-

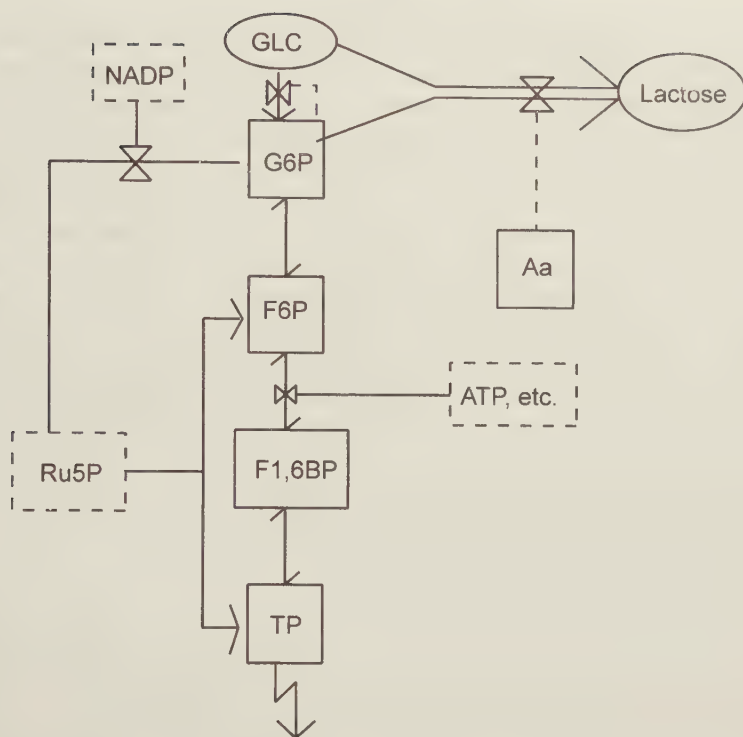


Figure 3.1 An example of a block diagram depicting glucose-6-P metabolism in mammary tissue. Codes are glucose (GLC), glucose-6-phosphate (G6P), amino acids (Aa), fructose-6-phosphate (F6P), ribulose-5-phosphate (Ru5P), fructose-1,6-bisphosphate (F1,6BP) and triose phosphate (TP).

sented by or in equations. The valve notation ($\bowtie$) and sometimes a solid dot ($\cdot$) indicate regulation by an entity connected by a solid arrow or by known effectors that might be considered explicitly (dashed line, G6P) if the initial representation of the system fails to simulate reality. For example, an initial representation of G6P metabolism based upon Fig. 3.1 was unsatisfactory. The nature of the failure indicated that the (known but not then represented) negative feedback of G6P upon the hexokinase reaction and the amino acid requirement for α -lactalbumin and, therefore, lactose synthesis had to be added.

This example emphasizes an aspect of our approach to modeling that must be stressed: the first model version and block diagrams must be as simple as possible. Model failures then force introduction of additional complexity into the model. This approach assures that preconceived notions regarding the importance of regulation are not introduced *a priori* and, as a result, the presence of all transactions and regulatory processes in a model can be defended with rigor. Given indicative failures, the dashed

lines from G6P and amino acids to their respective valves became solid lines in our adipose tissue and mammary gland models (Chapters 12 and 13). This convention does not prevent introduction into block diagrams of recognition that additional possibilities exist. Rather, this convention allows the modeler to indicate that, by choice and in the interests of simplicity, specific provisions were not introduced. This guarantees that benefits gained, particularly through addition of regulatory processes, can be better assessed and simulated after introduction of that process, e.g. progress can be assessed quantitatively. In this partially hypothetical case, consider the possibility that representation of regulation of the $F6P \rightarrow F1,6BP$ reaction was not essential to adequate simulation of G6P metabolism. If this provision had been introduced at the outset, its inclusion in the model could not have been adequately defended and the model might be unnecessarily complex.

Major considerations in formulating a block diagram are that the model conform to the modeling objective in terms of scale or level of aggregation and the time frame within which solutions will be undertaken. In making decisions about whether or not a specific entity should be included as a state variable, some interplay between steps (2), (3) and (4) is often necessary, i.e. one may have to be cognizant of the equation forms to be used and have general knowledge of numerical inputs which will be required.

Specific decisions will be discussed in later chapters, so only an example will be presented here to illustrate some of the arguments which must be considered. If an investigator chose to model the tricarboxylic acid (TCA) cycle in isolated mitochondria to determine whether or not current data were adequate to support the simulation of abrupt perturbations of this system, s/he would recognize that ADP must be represented as a state variable, because it is an important effector of the NAD-linked isocitrate dehydrogenase in mitochondria. Further, many perturbations of TCA cycle flux are mediated via ADP. Also, most simulations need only be minutes in duration, since responses in this system are very fast, requiring only seconds or minutes to occur.

In contrast, if the investigator undertakes development of a model of animal growth to enable simulations of effects of alternative feeding management strategies on rate and composition of gain, selection of ADP as a state variable would be incorrect. There are several reasons for this. First, the simulation periods dictated by this objective are months rather than minutes. The pool size of ADP in a tissue is very small relative to the total rate of energy transactions; the total ADP pool in most tissues turns over several times per minute. A rule of thumb in computer-based numerical integration is that when a pool turns over more than 30–50% per integration interval, solutions become unstable. Thus, if ADP is represented as a state variable, the integration interval required would be measured in seconds, resulting in very slow solutions. For example, if the integration interval was

1 s and the simulation period was 90 days, 7.8 million iterations would be required to obtain a solution.

Secondly, the role of ADP concentration as a regulator of oxidative metabolism is a fine control; this enables a tissue to quickly respond to changes in energy expenditures at the local level, which cause relatively small changes in ADP concentration. At the animal level, the primary determinants for oxidative metabolism are energy expenditures in functions: activity, heart work, Na^+ transport and protein synthesis. These are regulated independently of oxidative metabolism by the central nervous system, hormones and environment. A change in ADP concentration may mediate the effect of these energy expenditures upon oxidative metabolism, but need not be represented as a state variable at this level of aggregation.

It is much more efficient to sum the energy expended in animal level functions, as ATP equivalents required (ATP use) if desired, and compute the amounts of nutrients which must be oxidized to satisfy this demand. Second-by-second mediation of oxidative metabolism by ADP is a fine control, when an integration interval is minutes, hours or days as is desired in simulations of whole animal processes. In these time frames, the ADP pool turns over hundreds or thousands of times such that a 20–100% change in ambient concentration is unimportant. The important changes are in animal activity, rates of protein synthesis, etc. These are the coarse controls and successful simulations are not dependent upon whether or not rates of oxidation respond in seconds, minutes or hours. The essential result is that rates of oxidative metabolism are in balance with energy expenditures over periods of days and months.

Generalizations or specific guidelines for selection of state variables are difficult to formulate, since many decisions require judgement and experience, and must evolve during the course of a modeling study. Above, we stated one guideline that we try very hard to hold to, namely, start as simple as possible and let model failures force the addition of state variables and other details such as negative feedbacks.

Another, less definitive guideline is to consider that models at varying levels of organization ($i, i-1, i-2$, etc; Chapter 2) are hierarchical in nature. In this, models of regulatory enzymes would be expected to be very detailed if the implied objective is to explain the behavior of the enzyme. Such a model might be comprised of 20–30 differential equations (Garfinkel, 1968). In a model of metabolism in intact cells, representation of an enzyme must be simplified based upon results and insights gained in the enzyme modeling study. Fine control elements can be eliminated, since the time frame of enzyme studies is short (seconds to minutes) compared to cell level studies (hours). A model of cell metabolism might also be comprised of 20–30 differential equations, but a much broader range of metabolic transactions are represented. In a model of animal metabolism the functions of a tissue may be reduced to a few equations based upon insights gained in tissue modeling studies. This represents considerable aggregation of con-

cepts and data arising from experimental and modeling studies of enzymes, metabolic pathways, cells and tissues. The interplay between tissue and animal modeling studies has been a primary focus of our research program, and this is reflected in the organization of this book. The interplay is complex, and a number of specific aspects are considered in subsequent sections of this chapter on selection, formulation and parameterization of equations and in subsequent chapters. Therefore, only a simple example will be presented here to illustrate the interplay.

Consider the case in studies with isolated cells wherein rates of fatty acid synthesis from acetate increase as the glucose concentration in the medium increases. There are several possible explanations for this response. One is that glucose is an essential source through oxidation via the pentose cycle of NADPH_2 required for fatty acid synthesis. Another is that glucose serves as a source of α -glycerol-P (α -GP) for esterification of fatty acyl-CoA, which reduces the concentration of fatty acyl-CoA. Increased availability of α -GP would reduce the inhibition of fatty acid synthesis by fatty acyl-CoA. How does one decide how to represent this in an animal level model? Our approach has been to construct a model of metabolism in the tissue that provides for quantitative evaluations of alternative explanations. Having evaluated the relative impact of each possible explanation, the primary determinants of the observed response can be captured simply in the block diagram and equations of the animal model.

There are two approaches to the herd ($i + 1$) level of the hierarchy. One is further aggregation and simplification, which may result in oversimplification of the biology and loss of general applicability. Another approach is to identify key parameters in animal models that are significant contributors to variance within a herd. These can then be introduced as stochastic variables and multiple solutions to evaluate alternative management strategies undertaken. We prefer this latter approach (Chapter 17).

3.5 FORMULATION OF MATHEMATICAL STATEMENTS

The conversion of the block diagram to mathematical statements is usually a straightforward process. The objective statement specifies or implies the type of equations required, and the block diagram identifies the transactions and controls that must be represented. Probably, the most frustrating aspect is formulation of the mathematical statements when an equation formulated on sound theoretical grounds must be simplified, because available data are not adequate to parameterize the equation of choice. This is one of the reasons why some emphasis is placed on the fact that there must be a close interaction between the modeling and experimental components of a research program. Given this, needs for critical data can be satisfied.

3.5.1 Mass action equations (KINSYM)

The user-oriented language we adopted early in development of the modeling aspect of our research program was the kinetic simulation modeling system (KINSYM) developed by Garfinkel (1966,1968). This user-oriented language (SHARE 360D032008) was adopted by Baldwin and Smith (1971), because the mathematical formulations and symbolism were familiar to investigators of animal energetics and also because of the ease of comparing formulations within and results from simulation models to specific experimental data. In addition to ready availability, this language allows for flexibility and capacity consistent with explicit and meaningful representations of components of complex animal functions.

KINSYM is comprised of two programs, the generator and simulator. Inputs to the generator program are 'chemical' equations of the following form:

$$1.0 \text{ A} = 1.0 \text{ B} / 10.0 / 10.0 \text{ or } (10.0 (10.0 \quad (3.1)$$

The coefficients 1.0 and 1.0 represent the concentrations of the chemicals A and B. The equals sign [=] identifies a reversible reaction. Alternatively, a dash [-] would indicate an irreversible reaction. The slash [/] identifies the following number [10.0] as a rate constant or, alternatively, a flux can be identified by a left-hand parenthesis sign [(]. When a flux is specified, the input data are used in the program to calculate the corresponding rate constant. The generator converts the 'chemical' equations A to B and B to A to flux equations of the general form (in Fortran)

$$F1 = K1 * A \quad (3.2)$$

$$F2 = K2 * B \quad (3.3)$$

and formulates differential equations for each chemical entity of the general form

$$DA = -F1 + F2 \quad (3.4)$$

where DA corresponds to dA/dt . In more standard (Thornley and France, 1984) mathematical format, equations 3.2–3.4 correspond to the following:

$$U_{A,B} = K_{A,B} * A \quad (3.5)$$

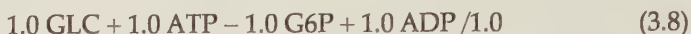
$$U_{B,A} = K_{B,A} * B \quad (3.6)$$

$$dA = U_{B,A} - U_{A,B} \quad (3.7)$$

In this convention, mass action coefficients are identified by a capital K and have a unit of day^{-1} . The generator then sorts the flux and differential equations into a Fortran-compatible subroutine (DERV) and writes a main program that sets up and dimensions arrays containing input data.

An important advantage gained by users of the generator program is that the 'chemical' equations can be written using familiar mnemonics (G6P, F6P, CoASH) and in an order consistent with the modeler's conception of

a system, rather than that forced by Fortran which requires that a variable be defined before it can be specified as an effector in an equation. For example, from a biochemical point of view one can write the reaction (KINSYM Format)



without having to define the differential equation for ATP beforehand. Fortran programming requires that the pool size of ATP be defined before equation 3.5 can be formulated. If a person is a biochemist and naive in Fortran programming, it is intimidating to have to specify differential equations for variables in the order required in a Fortran program (DERV). Another advantage gained is that the generator does the bookkeeping essential to formulation of very complex differential equations for entities such as ATP, ADP and CoASH. In one of our models of tissue metabolism, the differential equation for ATP had 26 terms; correct formulation of this equation by hand would have been very laborious and the probability of error – leaving out a term in the differential equation – is high.

The simulator component of KINSYM contains all provisions essential to solution of the equations specified in the DERV subroutines developed by the generator and outputs over time all pool sizes, fluxes, etc., requested by the user.

Rate equations (3.2 and 3.3) generated within KINSYM were all first-order equations. When modeling systems at the enzyme level, the use of first-order equations conforms to the well accepted concepts embodied in the relationship



which was used with appropriate assumptions to derive the well known Michaelis–Menten equation

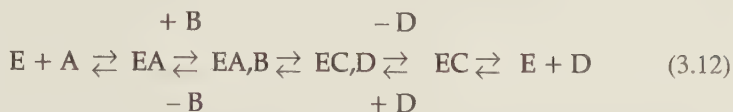
$$v = V_{\max} / (1.0 + k_m / [\text{S}]) \quad (3.10)$$

or

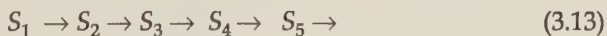
$$U_{s,p} = V_{s,p} / (1.0 + k_{s,p} / [\text{S}]) \quad (3.11)$$

which clearly defines a non-linear relationship between substrate concentration $[\text{S}]$ and velocity (v or $U_{s,p}$) that approaches a maximum velocity ($V_{\max}$ or $V_{s,p}$) at high substrate concentration. Thus, the KINSYM system allows simulation of non-linear kinetics, as does the Michaelis–Menten equation and, in fact, can produce this kinetic without resort to the simplifying assumption that the $\text{E} + \text{P} \rightarrow \text{EP}$ reaction can be ignored because the concentration of $[\text{P}]$ is close to zero in experiments designed to estimate k_m . This condition is not met in kinetic studies with intact cells.

Further, more complex reactions, such as the ordered bi – bi



can be represented readily and without resort to simplifying assumptions. This approach has been very successfully applied in studies of the kinetic properties of single enzymes and single metabolic pathways by Garfinkel (1968). In these cases, most of the parameter values required to implement the model were deduced directly from *in vitro* data with purified enzymes. However, when the goal is to model the non-linear kinetic properties of cells, tissues or animals, the required data to parameterize the large number of equations essential to this detailed approach cannot be collected because of inherent limitations in our ability to manipulate intact biological systems. Further, the complexity of the required equation sets and resulting computer solution times preclude use of this approach. Indeed, systems analysis concepts regarding the essential relationships between the scale of the system being modeled and the appropriate amount of detail incorporated into and level of aggregation in the model clearly forbid such a detailed approach. When data that can be collected on the system are not adequate to uniquely define the parameters in the model, the model will be non-unique in the sense that the same behavior could be simulated by numerous combinations of parameter values. This condition fulfills the more or less classical adage to the effect that 'if you give me nine degrees of freedom, I can simulate the behavior of an elephant and if you give me ten, I can make it wag its tail'. In research modeling, and in context with the modeling objectives stated above, this is unacceptable. Parameter values in mechanistic models must be defined by experimental data. Applied models containing first-order mechanistic elements of the form



and

$$v_{ij} = K_{ij} S_{ij} \quad (3.14)$$

are also inappropriate since

$$V_{S1,S5} = K_{S1,S5} * S_1 \quad (3.15)$$

would produce the same linear relationship and one only need collect experimental data to define one as compared to four rate constants. Also, the pool sizes of S_2 , S_3 and S_4 need not be known. Of course, when there are inputs to S_2 , S_3 and S_4 other than from S_1 or outputs other than S_5 , the additional detail and attendant experimental data would become essential.

The KINSYM system did not preclude the use of higher-order equation forms, but this required a greater understanding of the KINSYM system

and Fortran programming than an ordinary user might choose to acquire. This led to development of BIOSIM (1974), a system which retained the attributes of using biochemical equations as input, bookkeeping functions and, in addition, enabled the user to specify the equation forms desired for each biochemical transaction. This user-oriented programming language offered considerable utility, but was not generally adopted.

A number of other user-oriented programming languages have been developed. The languages most commonly adopted by modelers of metabolic systems were, first, the Continuous Simulation and Modeling Program (Fugazi, 1974) and, more recently, the Advanced Continuous Simulation Language (ACSL, 1990). Many colleagues modeling ruminant digestion and metabolism now use ACSL, as do we, thereby facilitating exchanges of models among users. Detailed descriptions of the CSMP, SAAM, ACSL and SIMUSOLVE[®] systems are presented in Chapter 4. All of the newer simulation languages require that the user specify equation forms for each metabolic transaction.

3.5.2 Other equation forms

We held rigorously to the convention that the simplest equation justified by available data be used in our early modeling studies. This approach offered the specific advantages of model simplicity and satisfied the requirement that all parameter values in a model be defined by experimental data. This led to heavy use of mass action equations in our models of tissue metabolism (Waghorn and Baldwin, 1984), ruminant digestion (Baldwin *et al.*, 1970, 1977) and animal metabolism (Smith, 1970). However, first-order equations in series can require that very short integration intervals be used during numerical integration, leading to excessive solution times even on modern computers. Such models can also produce aberrant results when extreme conditions are encountered (see discussion of lysine uptake below).

All enzymes, transporters, etc., exhibit some form of saturation kinetics. Therefore, all equations representing metabolic transactions catalyzed by single enzymes or groups of enzymes exhibit saturation kinetics and are appropriately represented by equations having this attribute. There are a number of metabolic transactions that exhibit linear kinetics under physiological conditions. Examples are rates of protein degradation (Fig. 3.2a and b) and the uptake of acetate and amino acids (Fig. 3.3a and b). In the case of protein degradation and with regard to equation 3.11, concentration of the substrate (protein) is always very high. As a result the ratio $k_{s,p}/[S]$ is always close to zero and the equation simplifies to

$$U_{s,p} = V_{s,p} \quad (3.16)$$

which can be treated as a zero-order process (Fig. 3.2a). All experimental data for short-term experiments support this representation. This is not to

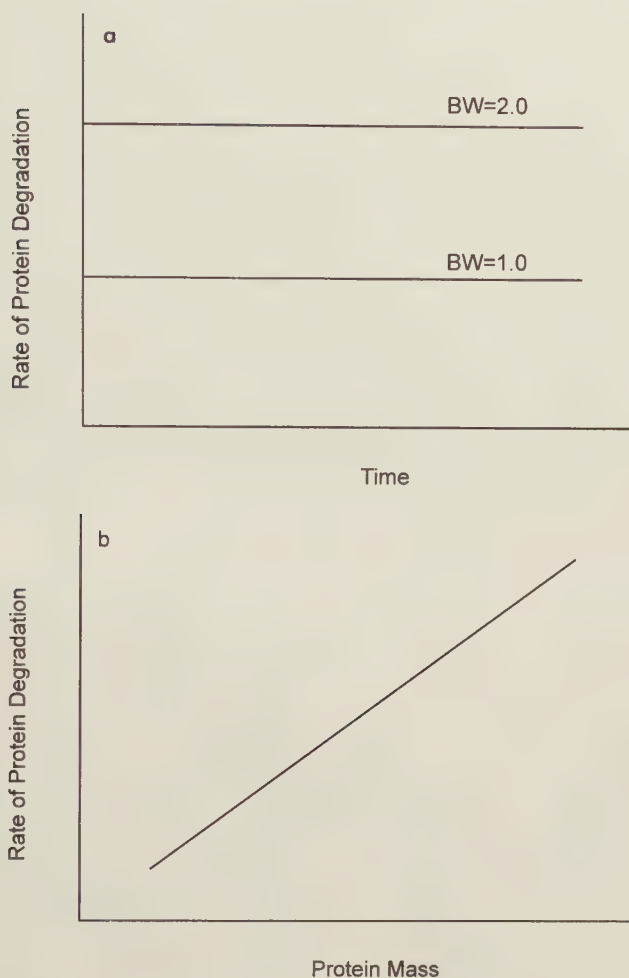


Figure 3.2 Protein degradation. In panel (a), a zero-order process appropriate to short-term studies is represented. In panel (b), changes in protein degradation rates throughout the growth cycle are represented according to equation 3.5.

say that $V_{S,P}$ cannot vary dependent upon physiological state for a specific tissue or specific class of proteins. On the other hand, experimental scientists report data on protein degradation rates as fractional degradation rates (FDR), which implies mass action kinetics. They do so, in part, to accommodate changes in protein mass and concomitant changes in degradative enzymes throughout the growth cycle (Fig. 3.2b). Decreases in FDR over time can be interpreted as reflecting differences in the mix of proteins in a tissue and their respective susceptibilities to degradation and, thus, have funda-

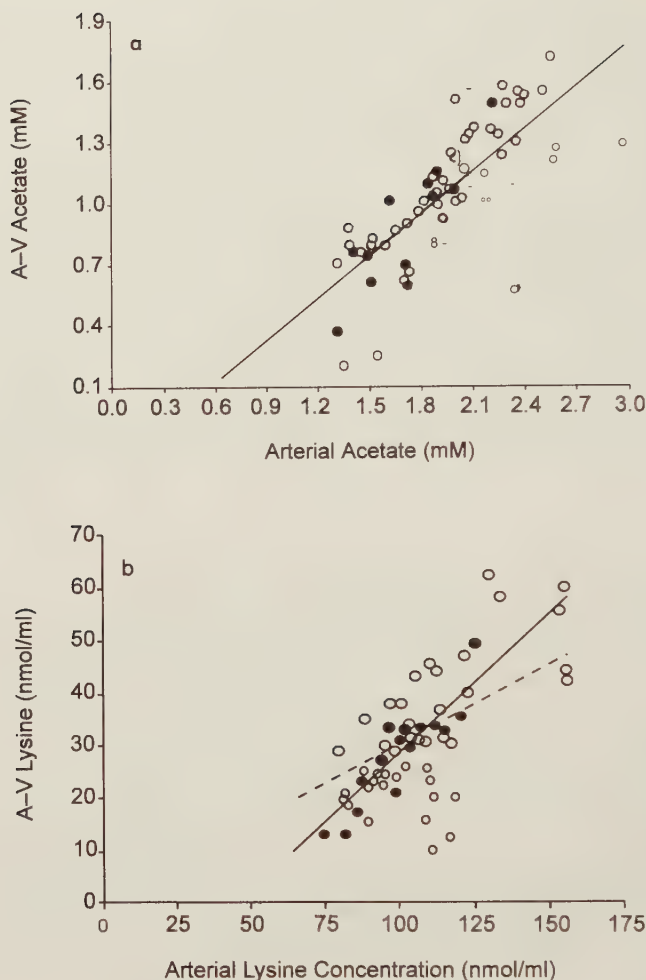


Figure 3.3 Example of best fits of equations to experimental data. In panels (a) and (b), respectively, uptakes of acetate and lysine by cow mammary tissue according to equation 3.5 are presented. In panel (b), the solid line represents a best fit and the dashed line an equation forced to a zero intercept.

mental significance that a modeler of protein turnover might well wish to capture by using first-order equations, where the rate constants reflect measurements of FDR over time.

In the case of acetate uptake by mammary tissue (Fig. 3.3a) physiological concentrations of acetate are always low relative to k_{Ac} such that equation 3.10 exhibits essentially linear kinetics, e.g. if $[Ac]$ increases 2-fold, the reaction velocity almost doubles. The apparent affinity (k_{Ac}) of mammary tissue for acetate measured *in vitro* is about 2.0 mM, while physiological concen-

trations range from 1.5 to 4.0 mM. Thus, a mass action equation for acetate uptake might be considered most appropriate, since deviations from apparent (local) linearity over this range are not likely to be detected experimentally. However, if one wishes to use a model of tissue metabolism and radiotracer data collected *in vitro* to evaluate nutrient interactions in mammary tissue and experimental conditions *in vitro* involved in the use of acetate concentrations of 10–15 mM to define these evaluations (Chapter 13), an equation form exhibiting saturation kinetics would be appropriate in the tissue model (equation 3.10). Further, if one prefers that analogous equation forms be used in tissue and animal models, one might elect to use the same equation for acetate uptake in both models in order to simplify incorporation of information gained using tissue models into a whole-animal model.

Rates of uptake of many amino acids by mammary tissue also display linear kinetics (Hanigan *et al.*, 1992). The linear equation for lysine uptake in Fig. 3.3(b) is

$$U_{\text{BLYS, TLYS}} = -25.4 + 0.54 * [\text{BLYS}] \quad (3.17)$$

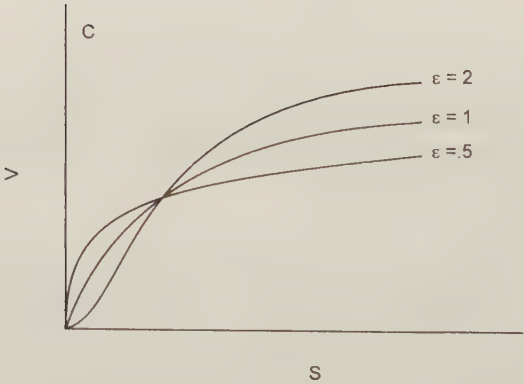
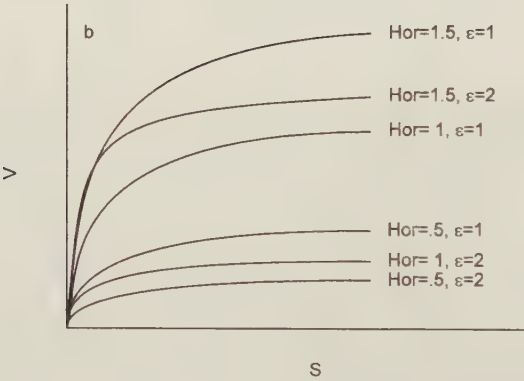
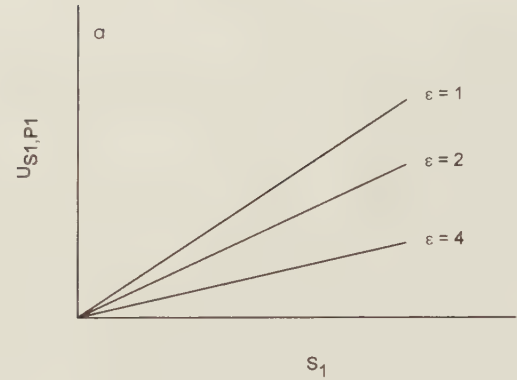
where the prefixes B and T refer to blood and tissue lysine. If a concentration below 47 μM for lysine was specified as input in a simulation of cow metabolism, a condition that might occur on poor quality diets, the mammary glands in that simulation would produce lysine (negative uptake), which is obviously incorrect because animal tissues cannot synthesize this essential amino acid. Clearly, an equation form that prevents lysine uptake by the mammary glands from going negative at low concentrations and has an intercept of zero when lysine concentration is zero is required (Chapter 13). These and other considerations have led us to utilize an array of equation forms in our models, in addition to equations 3.5, 3.11 and 3.17. The equation

$$U_{\text{S}_1, \text{P}_1} = K_{\text{S}_1, \text{P}_1} * S_1 * ([\text{S}_2] / [\text{iS}_2])^\epsilon \quad (3.18)$$

is used to denote mass action reactions where S_2 can be a second substrate or other effector of reaction rate and iS_2 is an initial or reference concentration of S_2 . The exponent (ϵ) is usually set to one, but can be set otherwise to accommodate higher order (steepness of response profile) effects (Fig. 3.4a). Use of this form is preferred over

$$U_{\text{S}_1, \text{P}_1} = K_{\text{S}_1, \text{P}_1} * S_1 * S_2^\epsilon \quad (3.19)$$

even though the two equations behave identically, because the unit of K remains day^{-1} rather than becoming $(\text{day} * \text{mole})^{-1}$; S_2 is often a coenzyme present at a very low concentration such that $K * (\text{day} * \text{mole})^{-1}$ values are very large, awkward to interpret and lead to unstable solutions. Substrate activation and inhibition can be simulated by varying the exponent. Also, use of $[\text{S}_2]/[\text{iS}_2]$ is preferred over S_2/iS_2 , since in long-term simulations, e.g. growth, the pool size of S_2 would change over time relative to iS_2 while the



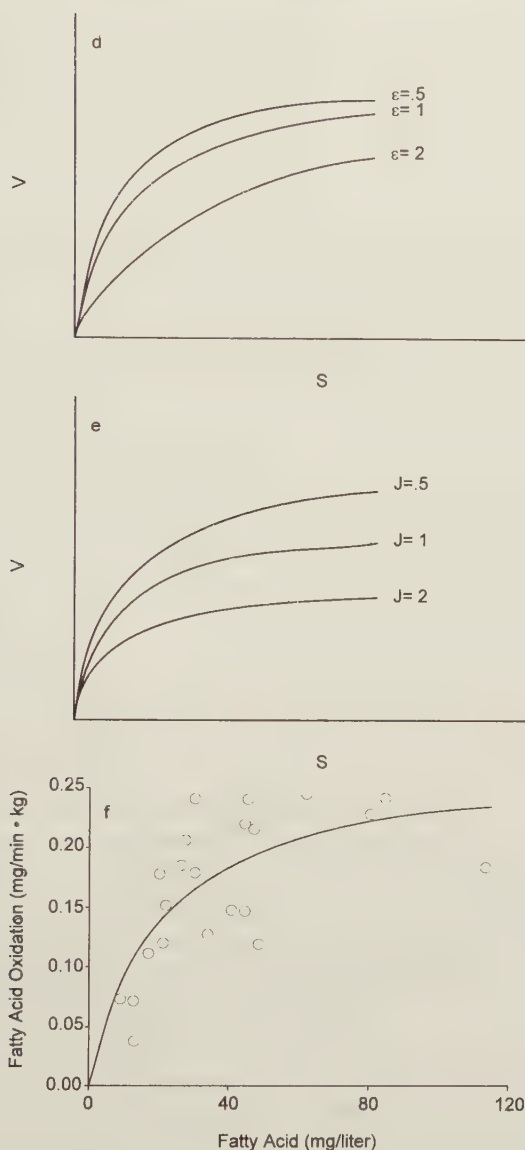


Figure 3.4 Representations of the behavior of several types of equations commonly used in modeling animal systems. Units are arbitrary. Panel (a) shows the effect of varying the exponent (ϵ) in equation 3.18 when $[S_2]/[iS_2]$ is set at 0.5. Behavior of equation 3.23 with varying values for hormone (HOR) and an exponent (ϵ) is illustrated in panel (b). Allosteric kinetics (equation 3.24) are depicted in panel (c). Effects of exponential hormone (equation 3.25) and inhibitor (equation 3.26) effects on reaction rates are presented in panels (d) and (e). As a reminder that simple equation forms such as 3.11 can yield 'best fits' to data, panel (f) represents the large variance around the relationship that can arise when these equation forms are applied to whole-animal data.

concentration of $[S_2]$ might not change relative to $[iS_2]$. Alternatively, in equation 3.18, S_2 can be a hormone or second messenger that does not participate in the reaction, but modifies responses to changes in the pool size of S_1 . The equation

$$U_{S_1, S_2} = K_{S_1, S_2} * S_1 * ([iS_2] / [S_2])^\epsilon \quad (3.20)$$

is a modification of the mass action form which provides for increases in S_2 above a reference metabolite, second messenger, or hormone concentration to inhibit the S_1 , S_2 reaction.

Equations 3.21–3.26 are modifications of the general Michaelis–Menten form which have proven useful. In these, the prefix $c_$ denotes concentration. Equation 3.21 is a simplified version of the kinetic equation for a two-substrate reaction:

$$v = V_{S,P} / (1.0 + k_{S1}/cS_1 + k_{S2}/cS_2) \quad (3.21)$$

The simplification is the elimination of the substrate interaction terms and generally is justified in higher level models on the grounds that data on intact cells, tissues, etc., have inherent variance that does not justify the higher level of resolution implied by interaction terms. This is illustrated in Fig. 3.5(a and b).

Equation 3.22 is a simplified representation of a multisubstrate reaction in which a coenzyme (CoENZ) is one of the substrates:

$$v = (V_{S,P} / \{1.0 + k_s / cS\}) * (cCoENZ/icCoENZ) \quad (3.22)$$

Adoption of this equation form requires or implies that the concentration of the coenzyme is considerably below the affinity of the enzyme for the coenzyme such that responses to changes in coenzyme concentrations are essentially linear. The ratio $(cCoENZ/icCoENZ)$ is 1.0 when the initial or reference condition ($icCoENZ$) is satisfied. In effect, this equation form is of a mixed type – partially Michaelis–Menten in form and partially mass action in form. Several considerations associated with modeling of cell and tissue metabolism led us to adopt this type of equation. The first consideration is, for example, that direct measurements of NADP concentrations in quick-frozen tissue samples are of total NADP only and not concentrations of free NADP available to bind to a given enzyme. Most often, 90–99% of NADP is bound to other enzymes at any given time (Williamson, 1980); correct values for concentrations of free NADP are not often available. Data sufficient to allow calculation of free NADP concentrations are very scarce but, in all cases, clearly indicate that ambient NADP concentrations in cells are far below k_{NADP} values for enzymes. Thus, the condition that responses to changes in coenzyme concentrations are essentially linear is met even though exact concentration estimates are not available and values specified as input are arbitrary. As a result, arbitrary values become acceptable as long as responses to changes in concentration are linear. A second consideration

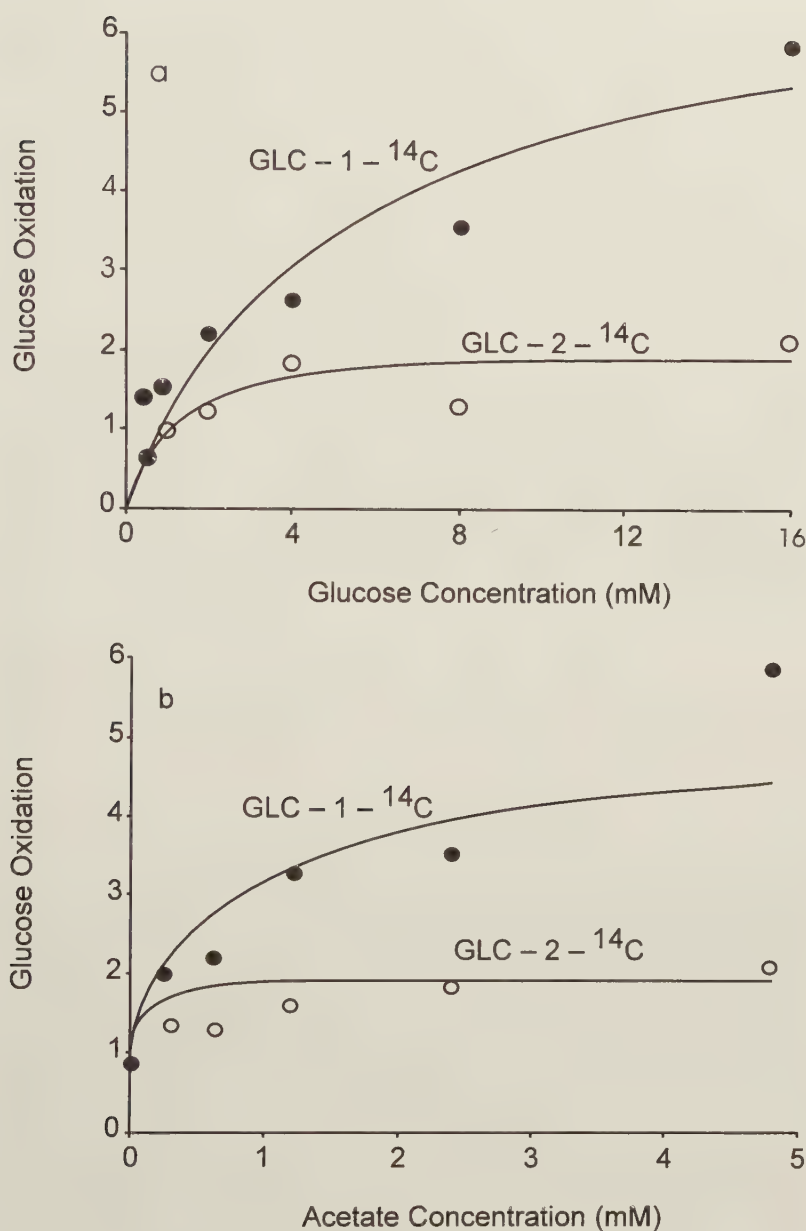


Figure 3.5 Example of best fits of equations to experimental data. In panels (a) and (b), effects of glucose and acetate concentrations upon rates of glucose oxidation according to equation 3.21 are presented. See text and Chapters 13 and 16 for further discussion.

is that the turnover rates of coenzymes are usually very high – seconds or less. If one wishes to simulate events over hours, days or years, it is inappropriate to represent these as state variables, since solution times would be intolerably long even with our best computers. The better decision is to treat these as ‘zero pools’, wherein rates of NADPH₂ use determine NADP concentration relative to a specified iNADP concentration.

The term in the numerator of equation 3.23 provides for effects of hormones on maximum enzyme activity (Fig. 3.4b) as follows:

$$v = V_{S,P} * HOR^{\epsilon} / (1.0 + k_s / cS) \quad (3.23)$$

The exponent (ϵ) in this equation allows for conditions where hormonal effects are non-linear. Equation 3.24

$$v = V_{S,P} / (1.0 + \{k_s / cS\}^{\epsilon}) \quad (3.24)$$

provides for allosteric kinetics attributable to substrate activation (Fig. 3.4c). This equation was used to fit the relationship between lysine concentration and mammary gland uptake in Fig. 3.3(b) alluded to above (see also Chapter 13). Similarly, division of k_s by HOR in equation 3.25

$$v = V_{S,P} / (1.0 + k_s / \{cS * HOR^{\epsilon}\}) \quad (3.25)$$

provides for hormonal effects on the affinity of an enzyme for its substrate and the exponent (ϵ) provides for non-linear responses (Fig. 3.4d). The term cJ/k_J in equation 3.26

$$v = V_{S,P} / (1.0 + k_s / cS + cJ/k_J) \quad (3.26)$$

allows for a chemical J to inhibit a reaction (Fig. 3.4e).

Selection of the simplest equation form for a given transaction is still a high priority. However, compromise is sometimes indicated to accommodate pragmatic issues such as improved model stability and solution speeds, avoidance of aberrant solutions, and improved compatibility with experimental data. Also, inclusion of additional biological knowledge to increase the range of applicability of a model or maintenance of compatibility between models directed at different levels of organization may require compromise. The major problem associated with adoption of more complex equations is that greater numbers of data and observations are required to parameterize the model. This issue is considered in the next chapter.

Non-esterified fatty acid (NEFA) concentrations in blood are plotted versus fatty acid oxidation rates *in vivo* in Fig. 3.4(f). The best-fit equation for these data is of the Michaelis–Menten type. Figure 3.4(f) is presented to illustrate two points that must be considered in selecting equations for a model.

The first is that when whole-animal studies of nutrient turnover and utilization are available, they can dictate equation forms that must be used

in representations of tissue functions and also constrain parameter values which can be specified. In this case, use of Michaelis–Menten kinetic arguments in tissue-level models is required by the fact that the total system exhibits saturation kinetics. Further, the sum of $V_{\max}$ estimates for individual tissues cannot exceed the $V_{\max}$ observed in the whole animal and the k_s values for the individual equations for fatty acid oxidation must, taken in aggregate, produce the k_s observed *in vivo*.

The second point illustrated in Fig. 3.4(f) is that there can be tremendous variance about the curve defined by the best-fit equation, indicating considerable animal variance. Often, detailed evaluations of the experimental data reveal underlying reasons for animal variance such as that depicted in Fig. 3.4(f). For example, one might find that two values obtained at a similar concentration of NEFA in blood differ because concentrations of acetate, an alternate substrate for oxidation, are higher in one animal than in a second one. As a result, rates of NEFA oxidation in the two animals would be expected to be lower in the first animal than in the second. This type of observation impacts the selection of equation forms for acetate and NEFA oxidation in order that the model have the capacity to simulate and explain variance due to interactions among alternative substrates as well as other differences among animals used in experimental studies.

3.6 DEVELOPMENT OF NUMERICAL INPUTS

Mathematical expressions, such as those discussed in the previous section, attempt to define true underlying relationships among processes of interest. The relational structure produced by a set of equations is represented by variables that may be predetermined or endogenous. Values for endogenous variables are determined by behavior of the system (subsystem), while predetermined values are set outside the system (subsystem) under consideration. Coefficients associated with the variables identify or establish relationships among variables. Coefficients of equations may be known with certainty (constants), e.g. descriptors of time and molecular masses and heats of combustion of chemical compounds of interest. Others must be deduced from consideration of (often) large sets of experimental data. Several aspects of the problem of model parameterization will be discussed separately in this section.

The first subsection will emphasize the development of summaries of basic data on organ weights and energy expenditures, blood and tissue metabolite pool sizes, and patterns of nutrient use. These must be known whether one is modeling at the cell, tissue, or animal level. These types of data provide essential guidance and impose important constraints upon the parameterization process. For example, the development of a model of liver function that produced more heat than the intact animal produces

would not work. Similarly, one would not want to represent a tissue as utilizing more of a nutrient than blood flowing to that tissue could supply.

The second subsection will emphasize the types of data that are required to parameterize kinetic equations (3.7–3.18) used in a model. The third subsection will develop, in part, concepts relating to the collection of data for use in analytical models to estimate parameter values that cannot be measured directly using current technology and methods.

3.6.1 Basic data summaries

It is unusual to find tabulations of data, rate constants and other numerical inputs required for implementation of a model in the literature. However, the literature does contain many relevant data. These data must be collected, analyzed, studied, evaluated, reconciled and, finally, converted to model inputs. Smith (1970) summarized a great many basic data that we still rely on for the formulation of tissue and animal models. There were some clear gaps in data available at that time.

In presentations of models in subsequent chapters, newer data will be presented that fill in these gaps. This process illustrates an important aspect of the interplay between experimental and modeling research alluded to above, i.e. the modeling exercise helps to identify critical measurements and data that must be collected; an experimental research program capable of generating these data is essential to the continued progress of modeling studies. In order to illustrate the types of basic data that must be collected and reconciled, gaps in available data that existed at that time and data summarized and reconciled by Smith (1970) are presented essentially unchanged in Tables 3.1–3.8. By way of preface to presentation of his data summaries, Smith (1970) stated the following:

Early analysis of the nutritional, physiological, and biochemical literature required for the construction of models of ruminant metabolism indicated that data currently available on ruminants were not sufficient to fully support model construction. In several cases where specific required data were not available, however, it was noted that numerous relevant data had been obtained in studies of other species.

A prominent consideration was definition of a minimum number of tissues and organ systems that should be considered in Smith's (1970) model. His modeling objective was, essentially, to assess the adequacy of available data and concepts regarding ruminant metabolism, develop a model suitable for evaluating energy expenditures in lactating cows, and develop hypotheses regarding the cause(s) of the depression in milk fat percentage when cows are fed high concentrate rations. Smith's decisions are evident in Table 3.1.

Kidney, nervous tissue and skin were selected for representation in aggregate, based upon observations that the metabolic activities, metabolite

Table 3.1 Characteristics of tissues and organ systems utilized in constructing the basal ruminant model^a

Tissue and organ systems ^b	Empty body weight (%)	Tissue mass (kg)	H ₂ O (%)	Total H ₂ O (kg)	Extracellular H ₂ O		Cell mass (kg) ^c
					Percent of total	Litres	
Skin, brain and kidney	9.6	9.12	70	6.38	50	3.19	5.93
Adipose	10.0	9.50	30	2.85	75	2.14	7.36
Gastrointestinal	6.5	6.18	76	4.70	50	2.35	3.83
Liver	1.6	1.52	72	1.09	20	0.22	1.30
Heart	0.6	0.57	78	0.45	43	0.19	0.38
Muscle	41.0	38.95	75	29.21	20	5.84	33.11
Blood	5.0	4.75	82	3.9	76	2.97	1.78
Other (skeleton, etc.)	25.7	24.41	45	10.98	—	—	—
Total	100.00	95.00	—	59.56	—	—	—

^a Altman and Ditter (1968), Bard (1961), Coffman (1963), Fales *et al.* (1962), Folkow and Halicka (1968), Ganong (1969), Khachadurian *et al.* (1966), Levy and Berne (1970), Mayerle and Havel (1969), Mellander (1970), Milnor (1968a,b), Neutze *et al.* (1968), Reis *et al.* (1969), Robinson (1968), Scott *et al.* (1962), Wade and Bishop (1962).

^b Based on 100 kg total body weight and 95 kg empty body weight.

^c Tissue mass minus extracellular H₂O

requirements and physiological functions of these tissues are relatively invariable under normal physiological conditions (Wade and Bishop, 1962; Schmidt, 1964; Cizek, 1968; Milnor, 1968a,b; Ganong, 1969).

Adipose tissue, the gastrointestinal tract, heart, liver and muscle were selected for separate representation, because these tissues perform unique and variable metabolic and physiological functions relevant to the modeling objectives. Smith (1970) represented arterial and venous blood separately, but these were combined to simplify representations in this and subsequent tables.

The skeletal system and minor tissues and glands, including several endocrine glands, were considered in aggregate (other) because their energy expenditures appear to be relatively constant (Dukes, 1955; Bard, 1961; Wade and Bishop, 1962) and data sufficient to support separate analyses were lacking.

Smith (1970) and his associates made an important decision prior to model development: in the first stages of model development, a steady-state condition would be considered. In this approach, nutrient and metabolite turnover, utilization and oxidation rates, and patterns of nutrient use could be set to conform as closely as possible to experimental data. Also, each pool in the model was constrained to input = output and, thus, some fluxes could be calculated by difference. This approach also assured that there were no errors in equations in the model and that arithmetic done to calculate individual nutrient transactions was correct, since either type of error would cause the model not to hold steady-state. Since we continue to make errors in coding and typing our equations and arithmetic errors in calculating and typing numerical inputs, we continue to use the steady-state concept in developing and testing our models and strongly recommend that others do so as well.

Based upon the decision to start with basic steady-state models of ruminant metabolism, reference animals were synthesized, in which intermediary metabolism was expressed in terms quantitatively consistent with amounts of nutrients available, nutrient and metabolite turnover and entry rates, and overall animal energy expenditures and function. The complex of data considered and integrated in the representation of a basic, steady-state model of the metabolism of a 100 kg reference ruminant are presented in Tables 3.1–3.9.

Data on tissue weights and total and extracellular water volumes for a 100 kg ruminant are in Table 3.1. These estimates were obtained from research reports and summarizations (Swett *et al.*, 1937; Brody, 1945; Barcroft, 1947; Walser and Bodenlos, 1954; Spector, 1956; Wade and Bishop, 1962; Streter and Woo, 1963; Altman and Dittmer, 1968; Cizek, 1968; Gauer *et al.*, 1970; Grace and O'Dell, 1970; Smith and Baldwin, 1974). Skin, nervous tissue and kidney comprise approximately 8.0, 1.0 and 0.6% of empty body weight, respectively. Skeleton and other tissues not selected for separate representation comprise 16.0 and 9.7% of empty body weight, respectively.

The water contents of skin, nervous tissue and kidney were calculated at 65, 75 and 75%, while skeleton and other tissues were calculated at 30 and 70%. The overall water content of 59.56% of body weight is within the normal range of 55–65% reported for adult ruminants (Swett *et al.*, 1937; Barcroft, 1947; Dukes, 1955; Spector, 1956; Kleiber, 1961; Altman and Dittmer, 1968). Estimates of tissue extracellular water content were calculated from data obtained in several species (Walser and Bodenlos, 1954; Dukes, 1955; Spector, 1956; Streter and Woo, 1963; Altman and Dittmer, 1968; Cizek, 1968; Gregersen and Chien, 1968; Levi, 1969; Grace and O'Dell, 1970), and include both blood plasma and interstitial fluids. Since most data regarding tissue metabolite levels and metabolism are reported on a tissue basis, cell mass was calculated for representation of cell functions and pool sizes, rather than considering intracellular water separately.

Data from an extensive survey of literature (see footnote to Table 3.2) regarding blood metabolite concentrations, rates of turnover and oxidation in several species, and nutritional states are presented in Table 3.2. Multiple nutritional states were considered in formulating this table, because concentrations, rates and patterns of nutrient use can vary considerably, and such data must be considered in formulating models. Most literature data are easily assigned to the nutritional states listed.

The generally greater rates of acetate oxidation and lower rates of glucose oxidation in ruminants as compared to other species clearly reflects the ruminant digestive process. However, when rates of metabolite oxidation in 'basal' animals are compared, the values are strikingly similar. It would seem that the major differences in patterns of metabolite utilization between species are an effect of diet and digestive processes; when substrates available to different species are similar, patterns of metabolite utilization are similar. This observation supports the view stated above and elaborated upon below that it is useful to consider interspecific data in formulating metabolism models.

The estimates of nutrient turnover and oxidation rates in Table 3.2 are based heavily upon radiotracer experiments. A number of reviewers have attempted to calculate the contributions of major metabolites to total animal energy expenditures based on radioisotope tracer experiments (Annison *et al.*, 1967; Leng, 1970). In most cases, summed rates of glucose, acetate, ketone body, fatty acid and triacylglycerol oxidation account for only 60–80% of total animal energy expenditures. Thus, it is difficult to construct a completely defensible table, depicting patterns of nutrient entry and use, based upon radioisotope tracer data alone.

Consideration of additional data is essential to development of realistic estimates of patterns of nutrient entry and use. These data include estimates of amounts of digestive end products absorbed by the animal, tissue metabolic and physiological functions, blood metabolite concentrations and blood flow rates to tissues, and tissue and animal energy expenditures under various conditions. The estimates presented in Table 3.2 represent

Table 3.2 Blood metabolite concentrations and rates of turnover (TO) and oxidation^{a,b}

Species	State	Glucose			Acetate			NEFA		
		concentration	TO	oxidation	concentration	TO	oxidation	concentration	TO	oxidation
Ruminant	Fed	2780 (2200-3300)	34 (21-47)	12 (9-16)	1500 (1000-2000)	220 (180-260)	130 (50-160)	370 (250-500)	28 (20-35)	7 (5-9)
	Basal	2550 (2100-2900)	27 (20-34)	11 (8-13)	800 (600-1000)	126 (115-135)	80 (75-85)	400 (300-500)	20 (18-22)	8 (7-10)
	Starved	2150 (1650-2600)	19 (15-24)	7 (5-10)	500 (400-600)	47	-	1300	75	20
	Ketotic	1780 (1390-2200)	18	7	1500 (600-2500)	110 (60-170)	20 (19-21)	950 (700-1200)	-	-
Human	Fed	4700 (4100-5300)	-	-	-	-	-	200 (150-250)	12	1.5 (1.3-1.7)
	Basal	3900 (3600-4200)	24	15	200	-	-	780 (500-1000)	23 (20-27)	12 (8-16)
Rat	Fed	4700 (4100-5300)	45 (35-55)	28 (20-40)	-	-	-	300 (200-400)	19	-
	Basal	3600	19	7	-	-	-	750 (600-900)	22 (14-34)	6
Dog	Fed	4450 (4100-4800)	39	-	-	-	-	-	-	-
	Basal	3900	30	19	180	-	-	750 (600-900)	34 (15-45)	8 (7-9)
Rabbit	Fed	4500 (3900-4950)	-	-	1000	-	-	-	-	-
	Basal	3900 (3600-4200)	-	-	-	-	-	1200	35	-

AcAc + acetone			BHBA			Total ketone body		
	concentration	TO	oxidation	concentration	TO	oxidation	concentration	TO
Ruminant	Fed	50 (30-70)	7 (6-8)	4	300 (200-500)	19	350 (230-570)	26
	Basal	50 (30-70)	-	-	300 (200-500)	20	350 (230-570)	-
	Starved	200 (100-350)	37 (35-40)	22	1000 (500-1500)	-	1200 (600-1850)	-
	Ketotic	600 (500-750)	68 (64-72)	43	3400 (2400-4400)	-	4000 (2900-5150)	-
Human	Fed	-	-	-	-	-	100 (70-140)	12
	Basal	-	-	-	-	-	-	-
Rat	Fed	75 (68-85)	- (35-55)	- (20-40)	85	-	160 (145-175)	-
	Basal	470 (400-550)	-	-	1600 (1300-1900)	-	2070 (1700-2300)	-
Dog	Fed	-	-	-	-	-	-	-
	Basal	75	-	-	200	-	275	-
Rabbit	Fed	-	-	-	-	-	-	-
	Basal	-	-	-	-	-	-	-

Table 3.2 (continued)

		LDLTG + VLDLTG		Glycerol		Lactate		Amino acids
		concentration	TO	concentration	TO	concentration	TO	
Ruminant	Fed	300 (100-500)	-	33 (25-39)	7.2 (6.5-8.0)	950 (800-1100)	60	2400 (2000-3000)
	Basal	400	-	55	-	950 (800-1100)	42	3000
	Starved	-	-	90 (80-100)	15.0	1200 (900-1500)	39	-
	Ketotic	-	-	130	18.0	-	-	-
Human	Fed	1400 (900-1900)	-	-	-	1200 (1000-1400)	-	3200 (2700-3700)
	Basal	1200 (800-1600)	1.0 (0.6-1.2)	80 (70-90)	5.0	1200 (800-1600)	-	2700 (2400-3000)
	Fed	800 (600-1000)	2.7 (2.0-3.0)	-	-	1400 (1000-1800)	-	3200 (2500-4000)
	Basal	1300	2.8	-	-	2100 (1500-2600)	-	3800 (3500-4200)
Dog	Fed	-	-	-	-	600	-	-
	Basal	-	1.7 (1.5-1.8)	45	6.0	1800 (1500-2100)	-	-
Rabbit	Fed	750	1.8	100	4.0	-	-	-
	Basal	1050	1.9	-	-	-	-	3300

^a Data tabulated from:

- Ruminant: Annison *et al.* (1963a,b, 1967), Annison and Lindsay (1961), Annison and White (1961), Baile *et al.* (1969), Barry *et al.* (1963), Bassett *et al.* (1966), Baxter *et al.* (1955), Bensadoun *et al.* (1962), Bergman (1963, 1968), Bergman *et al.* (1963, 1966, 1968, 1970), Bergman and Hogue (1967), Bergman and Kon (1964a,b), Bringe and Schultz (1969a,b), Ford (1963), Ford and Lindsay (1964), Gardner (1969), Carton and Duncan (1964), Glascock *et al.* (1966), Huber *et al.* (1969), Ichhponani and Johnson (1966), Jackson *et al.* (1968), Jacobsen *et al.* (1967), Jarrett *et al.* (1964), Jorgensen *et al.* (1965), Katz and Bergman (1969), Khachadurian *et al.* (1966), Kronfeld (1968), Kronfeld *et al.* (1959a,b, 1968), Kronfeld and Raggi (1964), Kronfeld and Simenson (1961), Leat and Ford (1966), Leat and Gillman (1963), Lindsay and Ford (1964), Linzell (1967), Long (1961), Moller and Black (1967), Moore *et al.* (1969), Palmquist *et al.* (1969), Reid (1950), Rindsig *et al.* (1969), Senel and Owen (1967), Steele (1969), Storry and Rook (1965), Thomas and Emery (1969), Varman *et al.* (1968), Varman and Schultz (1968a,b), West and Passey (1967), Yamdagni and Schultz (1969), Yousef *et al.* (1969).
- Human: Ballard *et al.* (1960), Birkenhanger and Tjabbes (1969), Bjornorp *et al.* (1969a,b), Carlson and Froberg (1967), DeLanne *et al.* (1959), Feigin *et al.* (1968), Frederickson *et al.* (1958), Friedberg *et al.* (1960), Gorman *et al.* (1967), Havel (1965), Havel *et al.* (1963), Hockaday (1961), Huckabee and Judson (1958), Issekutz *et al.* (1968), Landau and Lugibhl (1967), Long (1961), Meintzer *et al.* (1961), Nestle (1967), Nestle and Whyte (1968), Nichols *et al.* (1957), Pernow and Wahren (1962), Persson *et al.* (1967), Reaven *et al.* (1965), Rubin and Aladjin (1954), Shreeve *et al.* (1956).
- Rat: Akerblom *et al.* (1969), Baker and Schotz (1967), Bates *et al.* (1968), Bosch and Camejo (1967), Buckley *et al.* (1969), Depocas (1964), DiLuzio and Poggi (1963), Eaton and Kipnis (1969), Fain *et al.* (1965), Feller *et al.* (1950), Hill and Dvornik (1969), Krebs (1965), Lakhanpal *et al.* (1969), Laurell (1959), Long (1961), McBurney and Radnonski (1969), Olson *et al.* (1958), Radomski (1966), Sakata *et al.* (1963), Sanahuja and Harper (1963), Shipley *et al.* (1967), Simon *et al.* (1961), Simpson (1963), Williamson *et al.* (1969).
- Dog: Altzuler *et al.* (1963, 1967), Armstrong *et al.* (1961a, b), Ballard *et al.* (1960), Bates (1967), Bjornorp and Schersten (1963), Cowan *et al.* (1969), Feller *et al.* (1961), Havel and Carlson (1963), Havel and Goldfien (1961), Issekutz *et al.* (1963), Long (1961), Miller (1967), Steele *et al.* (1956), Weil *et al.* (1961), Winkler *et al.* (1964), Young (1959).
- Rabbit: Friedman and Byers (1961), Hagen (1963), Havel *et al.* (1962), Long (1961).

^b abbreviations Ac, acetate; BHBA, β -hydroxybutyrate; NEFA, non-esterified fatty acids; LDLTG low density lipoprotein triglyceride; VLDLTG, very low density lipoprotein triglyceride. Concentration expressed as $\mu\text{moles/l}$ blood; TO expressed as $\mu\text{moles} \cdot (\text{min}^3)^{-1}$; oxidation expressed as μmoles metabolite oxidized $\cdot (\text{min}^3)^{-1}$. Values in parentheses indicate range of values found.

Table 3.3 Tissues metabolite concentrations in rats and ruminants^a

Species	Tissue	Metabolite ^b ($\mu\text{mole/g wet weight tissue}$)						
		Glucose	KB	NEFA	STG	EXTG	Acetate	AA
Rat	Skin, brain and kidney	1.55	—	—	300	60	—	—
	Adipose	—	—	1.66	900	58	—	—
	Gastrointestinal	—	—	—	—	—	—	—
	Liver	5.90	2.07	—	—	23	—	10.5
	Heart	1.00	0.30	10.00	—	—	—	—
	Muscle	1.10	0.25	1.20	—	—	—	—
	Mammary	1.05	0.25	4.60	—	—	—	13.5
	Other	—	—	—	—	—	—	—
Ruminant	Skin, brain and kidney	(1.00) ^c	(0.25)	(1.00)	200	40	(1.0)	—
	Adipose	0.60	(0.25)	(2.00)	900	60	1.5	—
	Gastrointestinal	(1.00)	2.00	(1.50)	(100)	20	(2.0)	(1.0)
	Liver	12.00	0.75	(4.00)	100	18	1.2	(10.0)
	Heart	(0.70)	(0.40)	(10.00)	—	—	(1.0)	—
	Muscle	(0.90)	(0.40)	(1.50)	60	10	(1.0)	(0.80)
	Mammary	1.90	0.45	(4.60)	—	—	(1.5)	(10.0)
	Other	(0.70)	(0.25)	(1.00)	—	—	(1.0)	(10.0)
								1.30
								—

Metabolite^b

Species	Tissue	Glucogen	Glycerol	AGP	CoASH	Fatty Acyl-CoA	ADP	ATP	O ₂
Rat	Skin, brain and kidney	2.25	—	0.15	0.04	—	1.15	2.36	1.10
	Adipose	—	0.15	—	0.05	—	0.90	0.50	1.25
	Gastrointestinal	—	—	—	—	—	—	—	—
	Liver	350	—	0.30	0.17	0.25	1.20	2.75	1.25
	Heart	13.5	—	—	—	—	1.10	11.30	2.25
	Muscle	33.3	—	—	—	—	2.00	6.20	1.95
	Mammary	—	0.26	0.40	0.06	0.10	0.85	0.40	—
Ruminant	Other	—	—	—	—	—	—	—	—
	Skin, brain and kidney	—	(0.10)	(0.10)	—	—	(1.15)	(2.40)	(1.10)
	Adipose	—	0.10	(0.15)	0.05	(0.10)	0.95	0.30	(1.25)
	Gastrointestinal	—	(0.10)	(0.10)	(0.05)	(0.10)	(1.00)	(2.00)	(1.50)
	Liver	225	0.15	0.70	(0.15)	(0.25)	(1.00)	(2.50)	(1.25)
	Heart	(15)	—	—	—	—	(1.10)	10.00	(2.25)
	Muscle	69	(0.10)	(0.10)	—	—	2.00	5.00	(1.95)
	Mammary	00	(0.10)	0.20	0.05	(0.10)	0.55	0.50	(1.25)
	Other	—	—	—	—	—	(0.60)	(0.50)	(1.10)

^a Data obtained from references: Baird *et al.* (1968), Baldwin (unpublished), Baldwin *et al.* (1969), Ballard and Cheng (1969), Ballard (1970), Bergstrom and Hultman (1966), Burch *et al.* (1970), Cox and Mathias (1969), Goldberg *et al.* (1966), Halperin and Robinson (1970), Henderson *et al.* (1969), Howarth (1968), Kraupp *et al.* (1967), Kuhn (1967a,b), Long (1961), Lowry *et al.* (1964), Lumbars *et al.* (1969), Prior *et al.* (1970), Schonfeld and Kipnis (1966), Veech *et al.* (1969, 1970), Weidemann *et al.* (1969), Williamson (1966).

^b KB, ketone body; NEFA, non-esterified fatty acid; STG, storage triglyceride; EXTG, exchangeable triglyceride; AgP, α -glycerophosphate; COASH, coenzyme A; AA, amino acids.

^c Values in parentheses are estimates.

the result of an iterative process undertaken by Smith (1970) and his colleagues to reconcile these several types of data.

Data from the literature regarding concentrations of nutrients, intermediary metabolites and coenzymes in tissues of rats and ruminants are presented in Table 3.3. Literature values for metabolite concentrations in ruminant tissues were quite scarce, indicating a need for collection of additional data. Values for tissue metabolite concentrations in the rat were much more plentiful. Where comparable data were available, metabolite concentrations in tissues of the two species appeared to be quite similar. Therefore, it was considered that reasonable estimates for ruminant tissue metabolite concentrations could be derived from rat data. Many, but not all, of these estimates have been updated based upon new data in recent models of tissue metabolism.

Extracellular and tissue metabolite pool sizes for each tissue and organ system were calculated from blood and tissue metabolite concentration data in Tables 3.2 and 3.3. Volumes of extracellular fluids and tissue mass for each tissue and organ system were from Table 3.1. Results of these calculations are presented in Tables 3.4 and 3.5. We recognize that presentation of the estimates in Tables 3.4 and 3.5 is a bit redundant in that they are derived from data presented in other tables. On the other hand, these must be calculated as they represent the initial pool sizes of nutrients, which must be specified for each nutrient represented as a state variable in a model.

Table 3.4 Rates of metabolite turnover and oxidation and heat production in the basal ruminant model^a

<i>Metabolite</i>	<i>Turnover (mole/day)</i>	<i>Oxidation (mole/day)</i>	<i>Heat produced (MJ/day)</i>
Glucose	1.228	0.642	1.807
Propionate	1.380	0.564	0.854
Acetate	5.740	3.586	3.130
KB ^b	0.806	0.612	1.255
NEFA	0.908	0.364	3.657
LDLTG + VLDLTG	0.026	0.008	0.234
Glycerol	0.206	—	—
Lactate	1.866	—	—
AA → Glucose + KB (600 mmole AA/day)			0.276
Total			11.213

^a Adapted from Smith (1970). Abbreviations as in Table 3.2 and Table 3.3.

^b Includes butyrate and ketone bodies.

Estimates of rates of entry of nutrients into the several tissues (nutrient availability) are presented in Table 3.6. Estimates of cardiac output in resting ruminants were summarized by Dukes (1955). Values for oxen, sheep and

Table 3.5 Calculated tissue extracellular metabolite pool sizes for basal ruminant model^a

Tissue	H_2O (kg)	Metabolite (mmole/pool)								
		Glucose	KB	Acetate	NEFA	LDLTG + VLDLTG	Glycerol	Lactate	AA	Propionate
Skin, brain and kidney	3.19	8.14	1.12	2.55	1.28	1.44	0.175	3.03	7.66	0.080
Adipose	2.14	5.46	0.75	1.71	0.86	0.96	0.118	2.03	5.14	0.054
Gastrointestinal	2.35	5.99	0.82	1.88	0.94	1.06	0.129	2.23	5.64	0.500
Liver	0.22	0.56	0.08	0.18	0.09	0.10	0.012	0.21	0.53	0.060
Heart	0.19	0.48	0.07	0.15	0.08	0.09	0.010	0.18	0.46	0.005
Muscle	5.84	14.89	2.04	4.67	2.34	2.63	0.321	5.55	14.02	0.146
Other (skeleton, etc.)	10.98	25.83	3.55	8.10	4.05	4.56	0.557	9.62	24.31	0.253
Blood	3.67	10.58	1.45	3.32	1.66	1.87	0.228	3.94	9.96	—

^a Adapted from Smith (1970)

^b Abbreviations as in Tables 3.2 and 3.3

Table 3.6 Calculated tissue metabolite pool sizes for basal ruminant model^a

Tissue	Cell mass (kg)	Metabolites ^b (mmole/tissue cell mass)															
		Glucose	KB	NEFA	STG	EXTG	Acetate	AA	Lactate	Glycogen	Glycerol	AGP	CoA	Fatty Acyl- CoA	AMP + ADP	ATP	O ₂
Skin, brain and kidney	5.93	5.93	1.48	5.93	1186	237	5.93	-	1186	-	0.593	0.593	-	-	6.82	14.23	6.52
Adipose	7.36	4.42	1.84	14.72	6624	442	11.04	-	4.05	-	0.736	1.104	0.368	0.736	6.99	2.21	9.20
Gastrointestinal	3.83	3.83	7.66	5.75	383	77	7.66	3.83	3.83	-	0.383	0.383	0.192	0.383	3.83	7.66	4.60
Liver	1.30	15.6	0.98	5.20	130	23	1.56	13.0	1.78	293	0.195	0.195	0.195	0.325	1.30	3.25	1.63
Heart	0.38	0.27	0.15	3.80	-	-	0.38	-	0.30	5	-	-	-	-	0.42	3.80	0.86
Muscle	33.11	29.80	13.2	49.67	1987	331	33.11	464.0	331.1	2285	3.311	3.311	1.987	3.311	66.22	165.55	64.56
Other	12.48	8.74	3.12	12.48	-	-	12.48	-	-	-	-	-	-	-	7.49	6.24	13.73

^a Adapted from Smith (1970).

^b Abbreviations as in Table 3.3.

goats were 328, 210 and 282 ml blood * (kg^{0.75} * min)⁻¹. On these bases, a cardiac output of 250 ml blood * (kg^{0.75} * min)⁻¹ was utilized to calculate blood flow in the 100 kg ruminant (11 380 l/day).

Estimates of percent distribution of cardiac output and total blood flow to tissue and organ systems in the reference animal (Table 3.1) were derived from a number of sources. In our early models, nutrient entry or availability to tissues was explicitly defined as the product of blood flow rate to that tissue times the concentration of that nutrient in blood as in Table 3.6. Later, we adopted the convention that blood flow rates to tissues vary as a function of tissue energy expenditures, and that blood flow rates to tissues need not be explicitly defined in whole-animal models. Adoption of this convention greatly simplified our models of animal metabolism and is defended in Chapter 6. Nevertheless, in developing both animal and tissue models, it is important to be cognizant of the fact that blood flow rate times nutrient concentration imposes an absolute maximum on the amount of that nutrient a tissue can utilize.

Table 3.7 Tissue energy expenditures in basal ruminant model^a

<i>Tissue and organ system</i>	<i>Daily animal energy expenditure (%)</i>	<i>Heat produced (MJ/day)</i>
Skin, brain and kidney	21.7	2.44
Adipose	8.0	0.90
Gastrointestinal	7.2	0.81
Liver	22.5	2.52
Heart	10.0	1.12
Muscle	23.0	2.58
Other (skeleton, etc.)	7.6	0.85
Total	100.0	11.22

^a Adapted from Smith (1970)

In discussing data on nutrient turnover and oxidation derived from radio-tracer data as presented in Table 3.2, it was stated that these data have to be reconciled with data on animal and tissue energy expenditures and tissue preferences (affinities) for nutrients. Further, it was stated that the process of reconciling these types of data is iterative, e.g. a number of cycles of hand calculation and exercise of judgement are required. The estimates presented in Tables 3.7, 3.8 and 3.9 are a typical result of such a process. Estimated energy expenditure by the reference 100 kg ruminant in steady state in the basal state of 11.22 MJ/day was based upon 0.355 MJ/W^{0.75}.

Estimates of percentages of total energy expenditures for each tissue and organ system (Table 3.7) were calculated from a number of different types of data. Additional information utilized included the following: *in vivo*

Table 3.8 Nutrient availability to tissues of basal ruminant model^a

Tissue	Cardiac output (%)	Metabolite ^b (mole/day)					LDLTG + VLDLTG	Amino acids
		Glucose	KB	Acetate	NEFA			
Skin, brain and kidney	31.4	9.104	1.25	2.86	1.43	1.43	10.7	
Adipose	9.6	4.82	0.38	0.87	0.44	0.44	3.27	
Gastrointestinal	23.0	6.71	0.92	2.10	1.05	1.05	7.89	
Liver	27.0	7.86	1.08	2.47	1.23	1.23	9.25	
Heart ^c	4.1	1.21	0.17	0.44	0.22	0.20	1.42	
Muscle	18.0	5.23	0.72	1.64	0.82	0.82	6.15	
Other (skeleton, etc.)	9.9	2.86	0.39	0.90	0.45	0.45	3.36	

^a Adapted from Smith (1970). The primary sources of information were summarizations of tissue energy expenditures by Altman and Dittmer (1968), Barncroft (1947), Bard (1961), Brody (1945), Dukes (1955), Forster (1964), Ganong (1969), Levy and Berne (1970), Milnor (1968a,b), Spector (1956), Wade and Bishop (1962). Estimates of rates of nutrient flow to tissues based upon total cardiac output of 11 380 l/day.

^b Abbreviations as in Tables 3.2 and 3.3

^c Includes portal and arterial blood flow rates.

Table 3.9 Metabolite utilization and heat production in basal ruminant muscle model^a

Metabolite or metabolic process	Uptake (mmole/day)	Oxidation (mmole/day)	Heat production	
			kJ/day	Percent of total
Glucose	838.0	38.0	53.56	4
NEFA ^b	59.66	59.66	299.57	23
TG ^c	6.38	6.38	96.23	7.5
KB	376.98	376.98	384.93	30
Acetate	969.46	969.46	419.24	32.5
Glucose → Lactate	(400 mmole glucose/day)		35.14	3
Total			1288.67	100

^a Adapted from Smith (1970).^b NEFA or non-esterified fatty acids as tripalmitin.^c TG or triacylglycerol as β -OH-butyrate.

arteriovenous difference estimates for oxygen, carbon dioxide and metabolites; oxygen uptake and metabolite utilization rates by tissues *in vitro*; and results of tissue perfusion studies. The values presented in Table 3.7 define limits which must be imposed during the development of tissue and animal models. Rates of metabolite oxidation, utilization for synthetic processes and turnover in tissue and organ systems are limited by the tissue energy expenditure stipulated in Table 3.7 or an analogous analysis, since the sum of energy expenditures associated with these metabolic processes must equal the estimate of tissue energy expenditures and rates of metabolite turnover and oxidation in the reference animal. These latter estimates are presented in Table 3.8 and must conform as much possible with the data in Tables 3.2 and 3.7. These estimates, in turn, constrain and help define the estimates of nutrient uptakes, oxidation and heat production illustrated in Table 3.9 using muscle as an example.

During the past 20 years, a great many additional and highly relevant data concerning processes summarized in Table 3.9 have been published. These data have resulted in considerable improvements in the accuracy and confidence one can have in analogous formulations. These will be considered in subsequent chapters. Nevertheless, it is surprising, at least to us, how closely current estimates of patterns of nutrient utilization agree with those developed in 1970 and illustrated in Table 3.9.

3.6.2 Kinetic relationships

Despite limitations that must be imposed upon interpretations of kinetic relationships observed in whole animal and *in vitro* studies, such relation-

ships are very important, because they impose constraints upon properties assigned to specific nutrient transactions. Among these are the constraints that kinetic parameters assigned to tissues must yield overall estimates of apparent affinity and maximum capacity comparable to those measured in the whole animal.

In all modeling studies, problems with the availability of adequate data for unique definition of required parameters are encountered. Some of these compromise the concept that all numerical inputs to a mechanistic model must be firmly based upon experimental data. For example, Pettigrew *et al.* (1988) stated

Values for maximum reaction rates and for affinity and inhibition constants were estimated indirectly from empirical data on...

In our modeling studies, we are constantly confronted with this problem, even though our experimental research program for the past 20 years has been dedicated to the collection of required data. Problems with parameterization of mechanistic models will be addressed in many subsequent chapters and only briefly in the remainder of this chapter.

Our first modeling analysis of lactating cow metabolism (Baldwin and Smith, 1971) indicated that knowledge of adipose tissue metabolism in ruminants was totally inadequate and most limiting to formulation of an effective, mechanistic model. This remains true today (Chapter 12). This early observation led us to undertake experimental analyses of the metabolism of isolated ruminant adipocytes to establish kinetic relationships, e.g. apparent affinity and metabolic capacity estimates, metabolite interactions, and hormone actions (Yang and Baldwin, 1973a,b).

These and other experiments (Vernon, 1980) enabled us to develop a mechanistic model of cow adipose tissue metabolism (Hu, 1984), because the data enabled formulation of equations that could simulate response patterns, nutrient interactions, etc. However, the data were not adequate to identify a unique set of parameter values. We have data on input:output relationships for the tissue, knowledge of the metabolic pathways, knowledge of enzyme and metabolite levels, data on energy expenditures, information on basic responses to changes of the substrate concentration, etc. These data enabled development of a simple representation of patterns of nutrient flow within adipose tissue (Chapter 12).

At this time, some parameters in the model are fairly good, but there are a number of parameters that are not specifically defined on the basis of experimental data. Some of those that are not well defined can be probed using data from radioisotope tracer studies. Use of these data required that we write a tracer model that represents all the transformations specific tracers can undergo. Then we could undertake sensitivity analyses to see which of the (poorly defined) parameters are most central to model behavior. These analyses are very important, because one can not deduce a parameter value from measurements that are not effected when that

parameter value changes. Thus, sensitivity analyses can be used to identify measurements necessary to define unknown parameter values. Then you can run the appropriate experiments and use the data obtained, along with the model, to estimate new parameter values.

Well-defined, detailed models of metabolism in individual tissues are essential to the construction of the more highly aggregated models required for analysis of factors that determine patterns of nutrient use in animals. The tissue models are utilized to generate parameter values and kinetic relationships used in intact animal models. These processes will be illustrated in subsequent chapters.

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The modeling process II – solution algorithms, model evaluations and parameter estimation

WITH M. BOWERS AND T. JENKINS

Models provide a logical and systematic way to examine a complex problem.

Joandet and Cartwright (1975)

4.1 INTRODUCTION

The final steps in the modeling process are model solution and evaluation in terms of the objective set at the onset of the study. On the surface, this seems as if it should be a straightforward process: deceptively simple or we would not be devoting a whole chapter to the topic. The complexity arises from the plethora of modeling objectives, model types and, as a result, evaluation criteria which must be addressed. This plethora results in the need for a number of solution algorithms and a tailoring of evaluation methods to modeling objectives. When the objective of a study is development of models for direct application in industry, the primary objective of the evaluation process simply requires demonstration of the fact that the proposed (challenger) model yields predictions superior to those of a current (defender) system. Some rules apply to assure that the challenger versus defender comparison is unbiased; these will be discussed in the section on validation of models for practical application.

In the case of research models, evaluation criteria can vary greatly dependent upon the level of animal function addressed (i , $i - 1$, etc.) and the modeling objective. Development of evaluation criteria and challenge data sets can be quite complex, since accurate simulation of specific data sets is not always an adequate nor appropriate evaluation criterion. For example,

one must somehow assure that research models yield correct results for biologically correct reasons; that they yield insight and understanding; and, that they truly fulfill the stated objectives. These issues are addressed in subsequent chapters as they relate to specific models and will be addressed in a general or conceptual fashion in this chapter.

Since models may be static or dynamic, empirical or mechanistic, deterministic or stochastic, linear or non-linear in form, a number of solution algorithms are required, each with specific strengths and weaknesses. In a sense, it would be best to discuss each of these in context with specific models presented in later chapters. However, this would lead to considerable redundancy. Therefore, solution algorithms for management and research models will be discussed and contrasted in the first section of this chapter. This section will be followed by a discussion of criteria used in the evaluation and utilization of management and research models.

The evaluation and use of models for parameter estimation are treated separately in the next section of this chapter, because the solution algorithms, evaluation criteria and the use of such models as an aid in experimental design are a special case. Finally, decision support software will be discussed briefly. In subsequent chapters, we will only need identify the model, software used and relevant evaluation criteria. Then the solution algorithms used will be clear.

4.2 MODEL SOLUTION ALGORITHMS

4.2.1 Static, factorial (algebraic) models

A very common use of algebraic models in management is the use of linear programs to optimize resource allocations in terms of least cost, maximum income, etc. Many books and research papers have been written on this topic and the algorithms used will not be summarized here. The literature is comprehensive and extensive (Dent and Anderson, 1971; Bath, 1985). We simply wish to note that this was the first prominent use of computers to solve management-oriented (feeding system) models in support of animal agriculture and the first application of iterative solution techniques to optimize animal production systems.

Major problems encountered in the use of linear programs in the biological sciences in general and animal agriculture in particular have been the required assumptions that response relationships be linear and additive. Problems with linearity are generally accommodated by breaking non-linear response patterns characteristic of biological systems into a series of pseudo-linear elements. Programs capable of optimizing models containing non-linear equations are now becoming available and with ever increasing CPU speeds, are becoming economic to solve (Brooke *et al.*, 1988). Nutritionists generally agree that, under most commercial feeding condi-

tions, the linear programs in current use are robust and accurate, because the coefficients in the linear programming matrices were developed under similar feeding conditions. The same argument will be applied when non-linear optimization programs are developed, adopted and applied.

Problems with lack of additivity become prominent when one seeks to optimize, for example, feed resource allocations. In the case of grazing cattle, factors such as forage quality vary over time and, as a result, optimum interactions among nitrogen and energy supplements are neither constant nor additive (see Chapters 8 and 15). Also, in this case, subsequent benefits arising from compensatory growth must be considered (see Chapters 9 and 15). In lactating cows, condition at calving and nutrition during early lactation can have significant effects upon subsequent performance (Chapter 17). Since most ruminants grown for meat graze, and since feeding management regimes for lactating animals are quite variable, accommodation of cause-and-effect relationships expressing their effects over time is essential. This requires the development of dynamic, mechanistic models for use in support of animal agriculture *per se*, as well as in support of research. The latter is an emphasis in this book.

A person could defend the view that a number of models containing dynamic, mechanistic elements have advanced to the point where they are superior to the conventional static, (multi)factorial, largely empirical feeding-system models in current use and that, in the near future, additional superior models will become available. Note that use of the term (multi)factorial in the previous sentence implies additivity and a lack of interaction among feeds, nutrients and animal metabolic functions as emphasized in Chapters 5, 8 and 9.

We have noted in this section that linear programming models and algorithms have been important in advancing animal agriculture and that non-linear programming algorithms have been available for some time. We also note that implementation of models with non-linear equations by animal scientists has been very slow. An alternate approach to the use of non-linear optimization programs was adopted in the NRC dairy (1989) and Cornell Net Carbohydrate and Protein System (Search:Agriculture, 1990). This approach has been to link two programs. The first program uses a combination of linear and non-linear equations to estimate the requirements of a specified animal. Then, a linear program is used to formulate a least-cost ration to fulfill these requirements. This approach is an advance in the sense that some non-linear and mechanistic elements are accommodated. On the other hand, the approach is still factorial in assuming no interactions among nutrients with regard to animal functions and productivity in either the static or dynamic sense. This approach will very soon become subject to the same types of limitations we criticize current feeding systems for, e.g. they ignore nutrient interactions in the animal over time. Only dynamic, mechanistic models can overcome this limitation. Unfortunately, only limited efforts are being directed to the development of algorithms appropriate to

the use of dynamic, mechanistic models in optimizing resource utilization in animal production systems.

This brings our brief discussion of the application of optimization techniques to an abrupt close. Linear programs for optimization of resource allocations in animal agriculture have been very useful and widely applied. The development of non-linear programs for optimization of resource allocations in animal agriculture has been very slow. An alternative approach has been adoption of a two-stage technique, e.g. non-linear relationships and interactions among nutrients during the digestive process and in the animal are considered in calculating animal nutrient requirements; linear programs are then used in ration formulation. We believe this approach should and can be surpassed by development of algorithms for the use of dynamic models in the optimization of animal production systems. This approach would offer the advantages of considering favorable and unfavorable interactions among nutrients and feedstuffs during digestion, utilization of products of digestion by the animal, and consideration of effects of current feeding strategies upon subsequent animal performance. This is essential to maximization of the bioeconomic efficiencies of animal production enterprises as well as effective risk analyses.

4.2.2 Dynamic models

Using a special-purpose modeling language can greatly simplify development of a simulation model. While more general languages such as Fortran, C or Pascal are more flexible and available, they lack many features found in languages such as SAAM (Berman *et al.*, 1962; SAAM Manual, 1978), CSMP (Fugazi, 1974), SIMUSOLVE® (SIMUSOLVE®, 1990) and ACSL (ACSL, 1990). These include the availability of functions such as integration routines, free form input, macros and function generators, output controls, and graphic routines. Many languages allow organization of a model in terms and structure more familiar to domain experts, rather than computer scientists. For example, ACSL sorts model equations before translating them to Fortran, so statement order is unimportant. Automatic collection of statistical data needed for model evaluation is also found in some languages. Most languages allow the user to extend the basic functions to fit the needs of the individual modeler.

SAAM (Simulation, Analysis and Modeling Manual, 1978) is used to model biological systems, especially kinetic relationships. It allows you to fit parameters to experimental data.

ACSL, CSMP and SIMUSOLVE® are continuous system simulation languages. They are designed to help solve problems which can be described by differential equations. They are all preprocessors which translate the model into Fortran and supply external routines to handle input, integration and output. Fortran statements can be imbedded in the programs.

They include several integration methods, simulation functions and macros, and various diagnostic and debugging routines.

SIMUSOLVE® is a combination of ACSL and its own run-time language, which extends ACSL. SIMUSOLVE® will run any ACSL model as is, however it adds such features as steady-state analysis, parameter estimation against experimental data, optimization of model parameters and sensitivity analysis. With these additional features, SIMUSOLVE® is especially useful in validating and quantifying complex dynamic models. SIMUSOLVE® can also identify where additional data may be needed to reduce uncertainty in the model parameters.

4.2.3 Solving stiff equations in models of ruminant digestion and metabolism

Even though computer technology and solution speeds continue to increase rapidly, so-called stiff equations continue to pose a problem to modelers of metabolic systems. Stiff equations in this context are differential equations for state variables which have a small pool size, many inputs and outputs, and which turn over very rapidly. Stiffness is a relative rather than absolute term, especially in non-linear models. By way of example, consider metabolites such as the adenine nucleotides (ATP, ADP and AMP) which have concentrations of 1.0 μM or less, have numerous inputs and outputs, and turnover rates greater than 1.0 $\mu\text{mole/s}$. In general or with most of the numerical solution algorithms, solutions become unstable when more than one-third of a pool turns over per integration interval. Thus, stable solutions of models in which the adenine nucleotides are represented as state variables require an integration interval of 0.33×10^{-6} s or 3.3×10^7 integrations/s, 1.98×10^9 integrations/min and 2.85×10^{12} integrations/day. The differential equations for the adenine nucleotides would not be particularly stiff (see France *et al.*, 1992, for a more detailed mathematical analysis of stiffness) in the sense of requiring excessive computer resources if the modeling objective requires solutions measured in seconds, but would clearly require excessive computer resources if the modeling objective requires solutions over days, weeks or years.

The issue that arises from consideration of the above example is the fundamental principle of systems theory, which holds that scale and detail must be held in balance in every modeling exercise and that these must be defined in the statement of the modeling objective (Chapter 3). The problem is how the scale and detail concept can be applied in modeling animal systems. This issue is highly relevant when experimentalists trained as reductionists in biochemistry, nutrition and physiology, e.g. those who learn 'more and more about less and less', attempt to integrate current knowledge of cell, tissue and organismic functions using models. Our early models were totally compromised by our failure to accommodate the concept of only incorporating detail appropriate to the scale or level of the modeling

study. In retrospect, it seems that we held, however implicitly, to the view that if we understood a specific metabolic regulatory process, it was important *a priori* and must be captured in our models of pathways and of cell, organ and whole animal metabolism. This led to the development of hopelessly complex, overparameterized models whose solutions were very stiff.

In our discussion above, the relevant modeling argument is whether or not and at what level or scale should the adenine nucleotides be represented as state variables. When the modeling objective is to evaluate the adequacy of our knowledge regarding the roles of AMP, ADP and ATP in regulating key enzymes (such as phosphofructokinase-1 and the mitochondrial NAD-linked isocitrate dehydrogenase, which maintains energy charge $[(2 \times \text{ATP} + \text{ADP})/2 \times (\text{ATP} + \text{ADP} + \text{AMP})]$ in the cell), the adenine nucleotides must be represented as state variables in order to accommodate perturbations of the system, which increase or decrease energy expenditures.

In contrast, when modeling whole-animal metabolism over time frames of days, weeks or years, it is equally clear that the above noted regulatory mechanisms represent fine controls that operate second by second and that higher level functions, such as physiological and biochemical work, determine rates of oxidative metabolism and are the driving variables. Thus, the adenine nucleotides need not and, in accord with the scale and detail concept, should not be represented as state variables. Since ATP is the common intermediate in energy transactions in cells, one may choose to track ATP transactions in the cell as $\text{ATP}_{\text{use}} = \text{ATP}_{\text{formation}}$ for accounting and stoichiometric reasons as we do in our animal models (Chapter 16) but certainly not as a state variable.

Similar arguments apply to coenzymes such as NAD, NADH₂, FAD, FADH₂ and CoASH. It is useful to track the formation and utilization of these to maintain stoichiometric accuracy and assure that a model adheres to the first law of thermodynamics. We have coined the term zero pools to denote chemical entities which are explicitly considered in a model for accounting and stoichiometric reasons but are not represented as state variables, because their pool sizes essentially zero out in each integration step during long-term solutions (France *et al.*, 1992; Chapter 16).

Introduction of the concept of zero pools into our modeling approach reduced the stiffness of our models considerably and moved us toward a more appropriate balance between scale and detail, but it did not solve all stiffness-related problems.

The next problem we addressed was our tendency to represent an excessive number of intermediary metabolites explicitly as state variables. Pool sizes of intermediary metabolites are generally small and turnover rates are generally high, which contributes to stiffness. By and large, we have dealt with this problem by adopting the rule that only metabolites whose subsequent metabolism is regulated, or which regulate other transactions, should be state variables.

Another consideration developed in Chapter 12 was that intermediary metabolites essential to simulation of the metabolism of specifically radio-labeled substrates also be included as state variables, e.g. where radiotracer in one carbon of an intermediate is incorporated into one product, while other carbons in the intermediate are incorporated into another product. Thus, basic rules for inclusion of an intermediary metabolite as a state variable are:

1. Rate of formation and/or metabolism of the metabolite is subject to regulation;
2. The metabolite is a regulator of its formation, use, or of the metabolism of other substrates or intermediates;
3. The metabolite is converted to multiple products and is essential to simulation of patterns of radiotracer metabolism.

Underlying criteria for the first rule are that regulatory enzymes must catalyze a reaction that is rate limiting in a pathway; the reaction must not be in equilibrium *in vivo* and the enzyme catalyzing the reaction must be subject to regulation. Consider a reaction $A \rightleftharpoons B$ for which the thermodynamic equilibrium constant (V_{equil}) is the ratio B/A under standard conditions. Physiological conditions do not resemble those under which k_{equil} values are determined but when the ratio B/A *in vivo*, often called the mass action coefficient or k_{ma} , equals k_{equil} , one can conclude that the reaction is in equilibrium *in vivo* and, further, that the activity of the enzyme which catalyzes the reaction is in considerable excess in that tissue. When the activity of an enzyme is high enough to maintain a reaction at equilibrium *in vivo*, it cannot be the limiting enzyme in a pathway (Newsholme and Leech, 1983). In addition, it is not likely that regulation of the activity of that enzyme would influence nutrient flow through a metabolic pathway. For example, Baldwin and Yang (1974) compiled rat and cow data on enzyme and metabolite levels in the pathway of lactose synthesis. Activities (V_{max}) of phosphoglucosyltransferase, uridine diphosphoglucose pyrophosphorylase (UDPG-PPase) and uridine diphosphogalactose-4-epimerase were found to be in considerable excess of *in vivo* rates of lactose synthesis. Also, mass action coefficients calculated on the basis of metabolite levels in the mammary glands of these two species were very close to the k_{equil} for the three enzymes. In rats, the activity of UDPG-PPase was 200 times net flux through the pathway. Glucocorticoids are known to regulate the activity (amount) of UDPG-PPase. In the absence of glucocorticoids, the activity of the enzyme decreased to 50 times the net flux and the k_{ma} remained essentially equal to k_{equil} . Thus representation of intermediates in the pathway between G6P and lactose as state variables in a model of mammary gland metabolism is neither necessary nor appropriate (Fig. 3.1; Chapters 13 and 16).

A large proportion of reactions *in vivo* exhibit these properties and thus need not be considered explicitly in a model unless either the substrate or

product is a metabolic effector, or the product is further metabolized via more than one pathway. It may not be strictly necessary to represent an intermediate as a state variable on these grounds, but it is sometimes useful to do so to help in model development and presentation. For example, in Fig. 3.1 and the models discussed in Chapters 12 and 13, ribulose-5-P is represented explicitly as an intermediary metabolite in the pentose cycle, but need not be as illustrated in the liver model (Chapter 14).

Another approach to reduction of problems associated with stiff equations in a model is selection of an appropriate integration method for numerical solutions of the model. Modern continuous simulation languages such as SAAM, ACSL and SIMUSOLVE® offer the modeler a choice of several different integration algorithms for solving systems of differential equations. For our purposes the methods can be broken into two groups: fixed step methods such as Euler and Runge-Kutta (R-K), and variable step methods such as Runge-Kutta-Fehlberg (RKF), Gear and Adams-Moulton.

The R-K methods work by evaluating the derivatives at various points across the integration interval and then using a weighted combination of the derivatives to calculate the value for the next step. These methods are best described by examining the Euler method, which is a first-order R-K. The formula is

$$y(n+1) = y(n) + h f'(x_n, y_n) \quad (4.1)$$

where h is the integration step size and $f'(x_n, y_n)$ is the derivative at $y(n)$. Euler's method is not recommended for practical use, since it is not always accurate and can yield unstable solutions as compared to the higher order R-K methods.

However if, in addition to the first step, a second, trial step is taken to the midpoint of the interval and then the real step across the whole interval is computed using the values of both x and y at that point, we have a second-order R-K method. This can be extended to one of the most used algorithms, the fourth-order R-K method:

$$k_1 = h f'(x_n, y_n) \quad (4.2)$$

$$k_2 = h f'(x_n + h/2, y_n + k_1/2) \quad (4.3)$$

$$k_3 = h f'(x_n + h/2, y_n + k_2/2) \quad (4.4)$$

$$k_4 = h f'(x_n + h, y_n + k_3) \quad (4.5)$$

The new state is then calculated by the following:

$$y(n+1) = y_n + k_1/6 + k_2/3 + k_3/3 + k_4/6 \quad (4.6)$$

This integrator has proven to be the major workhorse in our dynamic models. It virtually always succeeds even though it is not necessarily the fastest integration. Major advantages of R-K methods over other methods include:

1. They are self starting; only the differential equations and initial values are needed;
2. They are explicit; no iterations are needed;
3. No results of previous steps are needed (SIMUSOLVE[®], 1990, p. 6–7).

One might think that a variable step size algorithm, such as RKF, would be faster and at least as accurate. Variable step algorithms are generally useful for models with a number of discontinuities, such as discontinuous feeding in a model of rumen function. However, they are not efficient when the driving functions in the system have high harmonics (France *et al.*, 1992).

Gear's algorithm is useful for stiff systems. Gear's primary advantage is its stiff stability. The algorithm can take larger steps once the equations requiring a small step size are no longer significant to the overall solution. However the overhead of Gear's algorithm (a linearized state transition matrix must be formed and inverted) means that most biological models with a range of time constants of two or three orders of magnitude are best solved by other methods. Both SIMUSOLVE[®] and ACSL contain several versions of Gear's algorithm.

After problems associated with scale and detail have been corrected, e.g. rapid turnover pools have been changed from state variables to zero pools as appropriate, state variables are eliminated that are neither regulatory nor subject to regulation, and the best algorithm is selected for numerical solution of the model, most models become tractable in that solution times on modern computers are acceptable. However, this is not always true, especially when a dynamic model has to be solved hundreds of times to estimate parameter values not directly measurable or to optimize management strategies (Chapter 17).

Consider two types of problems that may not be solved using the methods discussed above. Concentrations of amino acids and peptides in the rumen are very low, actually barely measurable, and turn over very rapidly. Yet amino acids and peptides, which are major effectors of microbial growth, vary throughout the day and, thus, must be represented as state variables according to the criteria listed above. Provided the observation that amino acids and peptides act similarly (Argyle and Baldwin, 1989), the problem can be partially alleviated by combining the two pools and applying a correction,

$$Aa = (\text{amino acids} + \text{peptides}) \quad (4.7)$$

$$RAa = Aa * RAaCOR \quad (4.8)$$

$$U_{RAa,Fv} = (V_{RAa,Fv} * Mi) * (1.0 + k_{RAa}/cRAa)^{-1} \quad (4.9)$$

$$cRAa = RAa / (RAaCOR * RVOL) \quad (4.10)$$

where Aa is the total pool size of amino acids and peptides in the rumen, RAa is the pool size of Aa inflated by the multiplier RAaCOR, $U_{\text{RAa,Fv}}$ is the rate of fermentation of RAa, $V_{\text{RAa,Fv}}$ is the capacity of 1.0 kg of microbes (Mi) to ferment RAa, k_{RAa} is an affinity coefficient analogous to the k_m in enzyme kinetics, cRAa is the concentration of Aa in the rumen and RVOL is rumen volume.

Assuming for the sake of argument that the differential equation for Aa is most limiting in terms of solution time and the turnover rate of Aa is three times a minute, it becomes clear that if AaCOR is set at 10.0, the integration time can be increased from 0.33 to 3.3 min using this approach. This inflation of pool size causes a slight lag in responses of Aa to perturbations of the system. However, within 30 min or 10 integration steps, the solution will adjust to agree with those observed when the 0.33 min integration step size is used. France *et al.* (1992) presented an example of effects of perturbing glucose input upon glucose oxidation and lactose synthesis with a physiological and inflated glucose pool size. If the simulation period is one day or longer, the effect of inflating the pool size is absent, since under both simulation conditions the same steady-state rates were achieved within 0.4 days (Fig. 4.1). This should be acceptable, because sampling times are 30 min or greater in most experimental studies of rumen metabolism.

An analogous approach is to slow down reversible reactions. For example, if rates in a reversible reaction such as the phosphohexose isomerase reaction are very high

$$\begin{array}{c} 100 \\ \text{G6P} \rightleftharpoons \text{F6P net flux} = 1.0 \\ 99 \end{array} \quad (4.11)$$

the rates can be reduced to

$$\begin{array}{c} 10 \\ \text{G6P} \rightleftharpoons \text{F6P net flux} = 1.0 \\ 9 \end{array} \quad (4.12)$$

and solutions speeded up without effecting the chemical equilibrium. This may not be true when considering isotopic equilibria. An alternate to this approach is to combine two pools that have a rapid turnover. In Chapters 12–14, this approach was adopted for the triose-P isomerase reaction, where the pools of dihydroxyacetone-P and glyceraldehyde-3-P were combined and named the triose-P pool.

These strategies can be very helpful in reducing the stiffness of a model and can greatly improve solution speeds. However, one must always assure that these do not compromise achievement of the modeling objective.

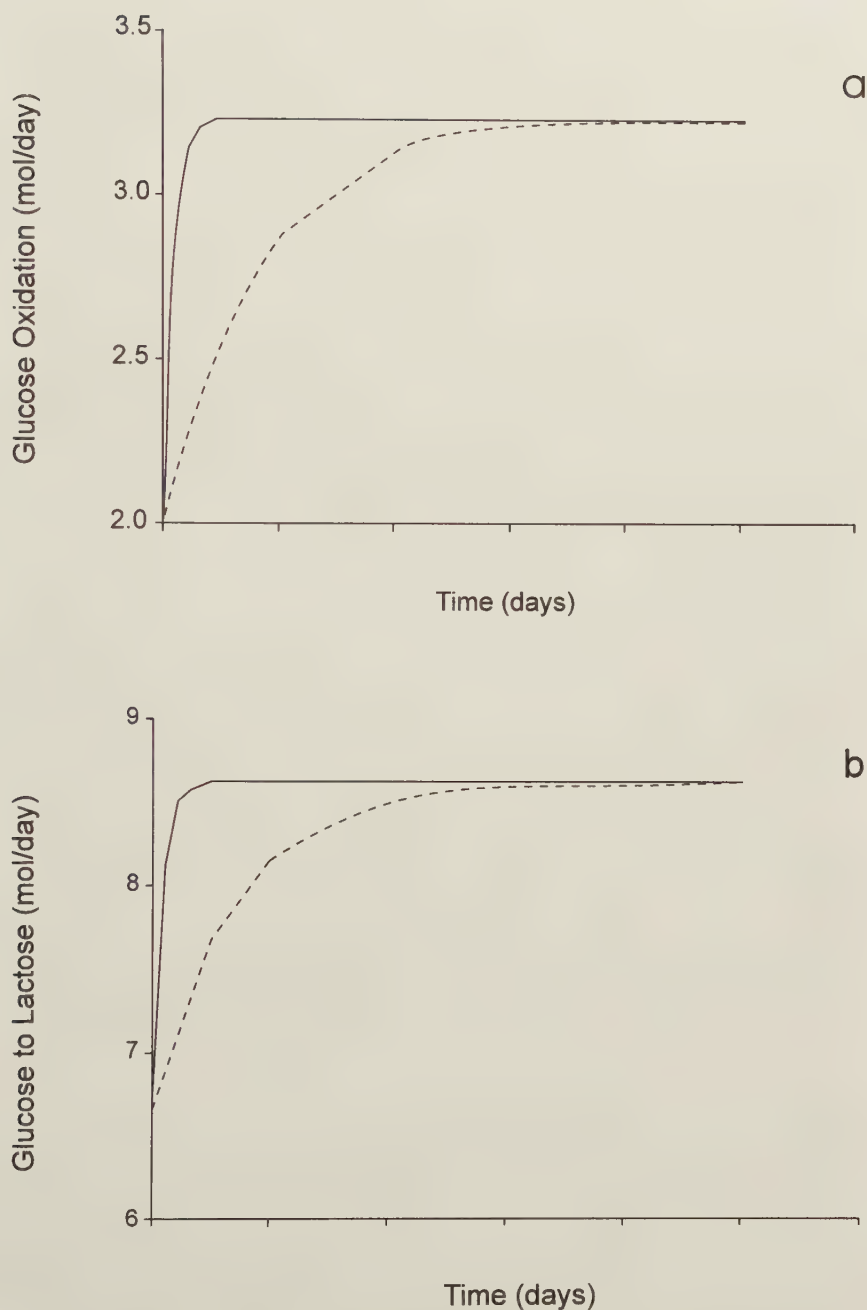


Figure 4.1 Numerical solutions to a single-pool model of glucose metabolism showing transient behavior of glucose concentration and effluxes to oxidation and milk lactose synthesis. Solid lines give correct transients and broken lines transients obtained by inflating pool sizes. Adapted from France *et al.* (1992).

4.3 EVALUATION OF MANAGEMENT AND RESEARCH MODELS

The complex nature of problems subjected to systems analyses requires that a wide range of approaches be used in model evaluations. A model must be constructed in such a way that it can be challenged experimentally by the authors of the model and by others (DeWit, 1970). If a model cannot be challenged experimentally, it cannot be adequately tested and serves no useful purpose as a tool to be used in studies of the modeled system, in decision making and in prediction. As indicated in Chapter 3 (Steps in Modeling), a common result of model evaluation is a return to steps (2), (3) or (4) of the modeling process, i.e. the model requires additional work. In accord with the advantages we hope to gain through modeling as listed in Chapter 3, this is a perfectly acceptable result that can advance the goals of a research modeler. However, if the modeling goal is prediction and practical application, failure of a model during validation is less desirable.

There are no set rules or guidelines that dictate criteria or procedures to be employed in the testing phase of model development. A well-written modeling objective is the best guide, as was emphasized in Chapter 3. The ideal testing procedure is one in which the model is used to anticipate behavior of the simulated system under circumstances not previously studied. Behavior thus predicted can then be checked in a series of experiments. If the model anticipates the experimental data well, the model might be judged valid. If model anticipation is poor, further model refinement is indicated. The conditions specified for such an experiment must be carefully selected and represent a rigorous test. It is very easy to construct experiments which do not rigorously challenge a model. For example, the range or magnitudes of independent variables selected may resemble very closely those used to generate the experimental data used in model construction. In this case, the experiments might indicate that the model generates outputs in agreement with previous experiments, but they do not constitute a valid challenge to the model. Limitations in funds, land, facilities or constraints imposed by other factors such as unacceptable treatments of the (animal) system can often preclude achievement of this ideal validation procedure. If these preclude rigorous evaluation, other approaches must be conceived and provided for when the modeling exercise is initiated and when the modeling objectives are set.

A common alternative is to divide available data into two sets; one to be used in model construction and the second to be used in testing. Such separations of data must be random. It must be assured that a wide range of independent variables are represented in the experimental data set used in model development, and that the challenge data set is at least as extensive as the development data set and truly independent. When these requirements cannot be met, the testing process is compromised.

A method we have used which fulfills these requirements has been to use a small and specific portion of available data to parameterize the model,

thereby leaving the bulk of available data for use in model evaluation. As an example, we used steady-state (continuous feeding) sheep data collected for an alfalfa diet fed at one intake to parameterize a dynamic model of ruminant digestion (Chapter 11). Appropriate challenge data sets then became alfalfa fed at different intakes and frequencies and data on all other diets. Thus, the challenge data sets were independent and very extensive as compared to the data set used to parameterize the model (Baldwin *et al.*, 1977a). This latter attribute, that the challenge data set be more extensive than the data set used in model development, is highly desired but often difficult to attain and maintain, because when models fail, data from the challenge data set must often be used to correct equations and parameters which led to the observed failure.

Another evaluation procedure can be used when the modeling objective is to evaluate the adequacy of our understanding of interactions among subunits of a system. In this case, the model would normally be developed using data and concepts derived from studies of the individual elements or subunits of the system. For example, a model of a metabolic pathway might be constructed using data from kinetic studies of the enzymes of that pathway. Evaluation of models of this type can be accomplished by comparing the simulated behavior of the model with experimental results obtained in studies of the whole system. If the model simulates the whole system, then our knowledge of the subunits of the system and interactions among these may be judged adequate. In using this approach, investigators still must ensure that data on the whole system are independent of the data used in model construction. Also, a suitable range of conditions and responses of the whole system must be tested, with special care directed to rigorous evaluation of the scaling of *in vitro* observations to enable simulations of *in vivo* conditions.

A number of additional validation approaches have been utilized. The important point to recognize is that care needs to be exercised in devising an applicable and feasible testing procedure.

4.3.1 Challenging applied models

It was noted above that the challenger versus defender concept is central to the evaluation of applied models. The modeler must demonstrate that the new model yields superior predictions of system behavior as compared to the current (defender) model. In this context, the term *superior* can have several connotations:

1. Superior may simply mean that the new model yields superior predictions of system behavior. For example, a new feeding system model might predict rates of gain of cattle based upon specific feed inputs more accurately than the current system. This was the criterion used by an NRC committee (NRC, 1976) in adopting the net energy system devised

by Lofgren and Garrett (1968) to replace the TDN system used previously (Chapter 9).

2. Superior may indicate that a new model for formulation of feeds allows the user to meet the nutritional needs of animals at less cost than conventional methods of ration formulation. The program for formulation of least cost rations for dairy cattle developed by Bath *et al.* (1972) was judged superior when it was demonstrated that cows fed the cheaper dairy rations, formulated using the model, performed as well as did those fed diets formulated using conventional methods, with the result that income over feed costs was increased.
3. Superior may indicate that the range of applicability of a model has been extended without sacrificing accuracy under normal conditions. This was the justification for adoption of the equations developed by Fox and Black (1984) by the NRC committee (1984) on the nutritional requirements of beef cattle.
4. Superior may indicate that a model has an improved front end, is more user-friendly, provides additional outputs useful to the user, or incorporates expert system elements. Addition of such provisions to an otherwise excellent model does not appear to be a high priority of many animal scientists because of the time and effort required under current conditions of limited resources. However, this implication of superior is becoming more and more important and, in many cases, essential to adoption of new models. For example, differences between the nutritional elements of the current version of the Bath *et al.* (1990) PC Dairy program and the NRC program for computation of nutritional requirements of dairy cattle (NRC, 1989) are small, but many users have adopted PC dairy because it is more user-friendly and provides more outputs of value to users. Similarly, the commercial success of the AUSPIG model of Black and Chapple (1991) can be attributed to the effort devoted to development of a user-friendly, expert system based front end or interface.
5. Superior may indicate that a new model is more satisfying on academic grounds. Usually, this reflects the presumption that if a model contains more cause-and-effect, mechanistic elements, it is inherently better than empirically based models. This view may be appropriate to evaluations of research models but does not necessarily apply to models destined for application in the animal industry. These models must be challenged just as rigorously as previous models have been challenged. Before they are adopted, it must be demonstrated that academically satisfying, mechanistic models provide more accurate predictions of system performance over a broader range of conditions than do the defender models. To adopt models on academic grounds without rigorously challenging their ability to predict performance accurately under commercial conditions can lead to disaster. The protein and nitrogen metabolism elements in the 1980 ARC system for ruminant livestock is a relevant example. The

system had to be retracted and revised. Our thoughts on the release of unproven models for use in industry are that animal scientists should serve, rather than run experiments on, the animal industry.

4.3.2 Evaluation of research models

After encoding a system of equation(s), testing of methodology and quantitative assessments of model responses should be conducted. The former assesses the correctness of the mechanics during formulation and development, e.g. were errors made in equation formulation, coding or parameterization? The latter addresses the appropriateness of the model assumptions for simulation of the system. The evaluation seeks to document or establish a close resemblance between model input:output and known response relationships of the system under consideration (Naylor and Fuiger, 1967).

Correctness of the mechanical features of the modeling activity refers to checking for errors occurring between conceptualization and execution of the simulation model (Shannon, 1975). Is there consistency between equation forms and assumptions regarding underlying functional relationships? Should scaling be incorporated and are dimensional relationships correct? Was the encoding or programming effected correctly and efficiently? These represent checks to be accomplished prior to evaluation of a model's performance.

The second phase of model evaluation encompasses parameter and model response verification. Do the simulation model responses fit criteria of acceptance? Are the directions of responses correct? Relative to synthesized or collected data sets, are systematic inconsistencies observable in the predicted responses? Are the predicted responses affected more by changes in some parameters than by others? Is the model too complex and overparameterized? To answer these types of questions about the model, the evaluation incorporates both quantitative and qualitative assessments (Van Horn, 1971).

Law and Kelton (1982) suggested several subjective evaluational steps for assessing the validity of model performance. Included among the recommendations were responses from experts to implicit and explicit assumptions used in model development, adherence to existing theory and comparison to field data. While all these approaches may serve as early checks during development of a model, the latter, evaluations relative to independent observations, represents a more stringent assessment protocol (Schellenberger, 1974).

In early stages of model testing, we characteristically use a standard set of procedures. When we are working on either an algebraic (static) or a dynamic model, we set up a reference or steady-state condition for the tissue or animal system we intend to simulate. We then calculate by hand all input:output relationships, interactions, fluxes, pool sizes, etc., for the reference state. When the reference state is simulated and the equations and

numerical values are correct and encoded accurately, the hand-calculated and simulated values will agree. For a dynamic model, if inputs are held constant over time, the model must maintain a steady state which equals the hand calculated values for the reference steady state (Chapters 8–16). This may seem trivial, but we find more errors in this phase of model testing than in any other. When we short-cut this often laborious procedure, we invariably discover errors later and must retrace our steps.

Another set of checks we incorporate into our models and monitor closely are summary equations. In these we check energy, carbon and nitrogen input:output balances. These can often be calculated in more than one way. For example, heat production in an animal can be calculated as the difference between metabolizable energy intake and energy in products (RE) such as milk and body energy. It can also be estimated by summing heat losses in all of the individual energy transactions in the modeled system. If the two estimates do not agree, there is an error in the model.

The idea of having experts evaluate the model in terms of implicit and explicit assumptions and adherence to existing theory is a good one. This is referred to as inspection testing in computer science. However, few experts not involved in the modeling exercise have the patience and time required for this exercise. We prefer to involve or consult with the experts during the process of model development.

(a) Behavioral analyses

The basic goal in behavioral analyses of research models is to assure that the model contains provisions adequate to simulation of response relationships observed in studies of the system and of discrete elements which comprise the system. Examples of behavioral analyses of several models are presented in Chapters 11–15 and 17, therefore only a simple example will be presented here by way of illustration.

In early versions of our model of mammary gland metabolism we represented glucose uptake and conversion to glucose-6-P (G6P) using first a mass action equation and later a Michaelis–Menten-type equation. In both of these representations, uptake increased as blood glucose concentration increased in behavioral analyses. This was considered acceptable behavior until more recent arteriovenous difference studies indicated that glucose uptake was not effected by blood glucose concentration (Fig. 4.2). Thus, the behavioral analysis indicated that either the model did not or could not simulate an observed response relationship.

The problem then became identification of an appropriate equation form. The same arteriovenous difference study indicated a relationship between acetate uptake and glucose uptake (Fig. 4.3). Increases in rates of mammary lipogenesis require NADPH_2 generated via the pentose cycle from G6P (Forsberg *et al.*, 1985). Also, G6P is a negative feedback effector of hexokinase (Newsholme and Leech, 1983) and a substrate for lactose synthesis and

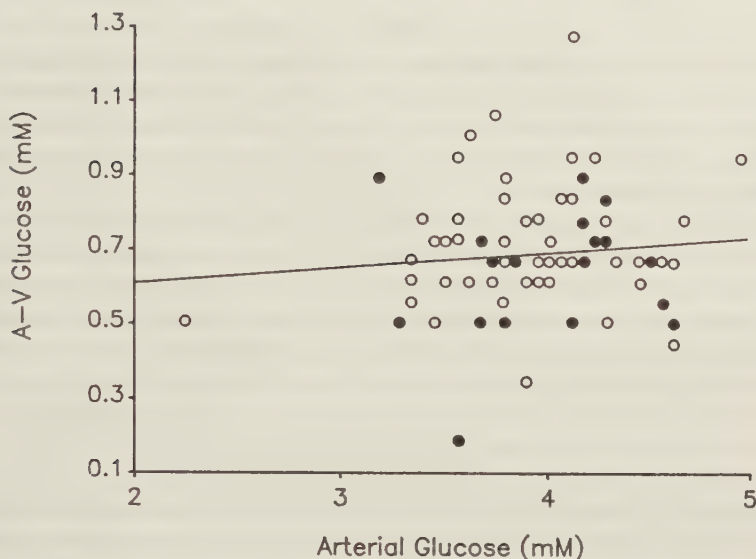


Figure 4.2 Relation between glucose A-V difference and glucose arterial concentration. BST-treated cows day 104 and 120 (●) of lactation; all cows day 34 and 69 and placebo cows day 104 and 120 (○) of lactation. Parameter estimates for regression line (—). Adapted from Miller *et al.* (1991).

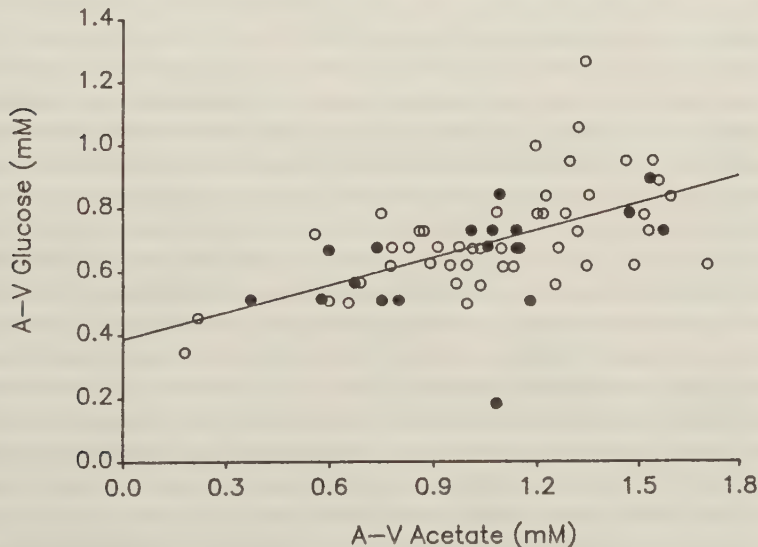


Figure 4.3 Relation between glucose and acetate A-V difference. BST-treated cows day 104 and 120 (●) of lactation; all cows day 34 and 69 and placebo cows day 104 and 120 (○) of lactation. Regression equation (—); glucose AVD = $6.93 + \text{acetate AVD} \times 5.15$, $r^2 = 0.32$, $P \leq 0.0001$. Adapted from Miller *et al.* (1991).

glycolysis via the Embden–Meyerhoff pathway. Our resolution of the problem with glucose uptake was to add G6P as a feedback inhibitor of net glucose uptake and conversion to G6P (see Fig 3.1 and Chapter 13). Behavioral analyses of effects of varying glucose concentrations using this equation and available data on response relationships indicate the new equation is adequate to simulate observed relationships.

Note that the objective of behavioral analyses is to determine whether or not the model has the capacity to simulate observed response patterns. This is a qualitative criterion which must be satisfied before undertaking sensitivity and quantitative evaluations. Actual absolute outputs need not be correct at this stage, since the model may contain best-guess parameter values.

(b) Sensitivity analysis

The term sensitivity analysis has a number of connotations dependent upon context and author. This same term is used in two different contexts in this chapter. In this section, the term refers to analyses directed to identification of equations wherein solutions are insensitive to changes in key parameters that are known to be important effectors of system behavior or, alternatively, overly sensitive to parameter values generally considered not to be major effectors of system behavior. In a later section of this chapter, the use of sensitivity analyses to determine whether or not a given data set can be used to estimate a parameter not directly measurable will be discussed.

As was the case in the brief discussion of behavioral analyses above, a number of specific examples of the use of sensitivity analyses to ascertain whether or not model solutions are appropriately sensitive to changes in values assigned to key parameters are presented in subsequent chapters. Therefore, only a couple of simple examples will be presented here by way of illustration. The first example arose from a sensitivity analysis of parameters in the ruminant digestion element in a model of a range production system (Rice *et al.*, 1974). It was considered in formulation of the model that energy density (ME/kg) of range forages limited intake and, thus, that rumen fill would determine feed intake. Since this was a model of the total range production system – soil fertility, rainfall, forage growth and intake, pregnancy, growth, lactation, etc. – the rumen digestion element was represented in a very aggregated form. One rate constant specified the rate of organic matter fermentation in the rumen and, as a result, microbial growth rates. When this rate constant was set at $14.0 \text{ g} * (\text{g microbes} * \text{day})^{-1}$, simulation results in terms of rate of gain, productivity, etc., were correct for cattle. When this value was used in simulations for sheep, rates of gain and feed intakes were about twice normal. When the rate constant was adjusted to 13.5 for sheep, simulations for sheep production were, essentially, correct. This level of sensitivity was clearly too great. Ruminant

digestion, even across species, cannot be this sensitive to one rate constant. The system is much more resilient and robust than that. The signal to the modeler here is that the system, as represented, is too highly aggregated and more (mechanistic) detail is needed. As noted in Chapter 3, our premise that one should start with the simplest model possible and let failures of the model force addition of detail clearly worked. The detail added was clearly forced by a model failure (Rice *et al.*, 1974).

It may happen in evaluations of a research model that sensitivity analyses reveal outputs that are inappropriately insensitive to particular parameters. This result poses two questions which must be addressed. The first is that current concepts are to some degree based upon intuitive and qualitative analyses of data that may not have been evaluated in the quantitative and dynamic domains. We noted above in our discussion of scale and detail that the fact that we 'know' a great deal about a given regulatory process does not necessarily mean that this mechanism is an important determinant of higher level function. It may simply be a fine control rather than a driving function.

A second consideration is the concept of substrate cycling popularized by Newsholme and Leech (1983). If one considers regulation of a specific process such as lipolysis in isolation, a 3- to 5-fold increase in fatty acid release by adipose tissue in response to an epinephrine challenge would be expected. However, in the presence of adequate glucose and insulin to support elevated rates of fatty acid re-esterification, the net increase in fatty acid release during an epinephrine challenge may be nil, indicating insensitivity. Conversely, when the insulin:glucagon ratio is low, the response in fatty acid release by adipose may be much greater than expected, because of reduced insulin concentrations and rates of fatty acid re-esterification. These types of disparate effects make it very difficult to interpret sensitivity analyses and, more important, model fatty acid release by adipose tissue for a broad range of conditions (Chapter 12) in the absence of full understanding of the system.

Assuming for the purpose of discussion that a modeler and colleagues can answer the first question in the negative, e.g. the system should be sensitive to the parameter value and is not, the question becomes what is wrong in the model and how can it be corrected. There are a myriad of solutions to this problem, dependent upon the model and level of analysis. A common observation is that the suspect element is represented in excessive detail and, as a result, is overparameterized to the extent that effects of changes in key parameters are damped out.

In an early symposium on protein digestion in the rumen and microbial growth, Preston (1982) and Waldo and Glenn (1982) presented details on several mechanistic models then in development to improve predictions of amino acid availabilities to ruminants. Both authors complimented the several modelers for advancing our conceptualization of processes that influence amino acid availabilities, but also emphasized that experimental

data required to uniquely define the many parameter values required for implementation of their models were not available. Perhaps crudely, but nevertheless appropriately, Preston (1982) pointed out that a series of (here fictitious) parameters in a series of linear equations such as $6 \times 3 \times 2 \times 4$ and $3 \times 6 \times 4 \times 2$ would yield the same result, which happened to be the same result obtained using the NRC crude protein system in which one parameter value was used, e.g. the data used to parameterize the conceptual models were the same data used to set the existing system. Without the availability of additional experimental data, mechanistic models represent no improvement over empirical models. This is, of course, why we emphasize throughout this book that independent experimental data are required to parameterize mechanistic models. Progress toward this end has been good in the area of protein digestion and metabolism in the ruminant digestive tract (Chapters 8, 11 and 16).

Additional solutions to the second question can result in equation modifications, such as addition of terms to accommodate positive and negative effectors of the transaction, adding elements to accommodate inhibitions, allosteric kinetics, threshold phenomena, model simplification (aggregation) and many others that will be alluded to in subsequent chapters.

(c) Quantitative evaluations

Verification of algebraic and dynamic relationships and encoding of these are clearly essential steps in the modeling process. Similarly, behavioral and sensitivity analyses are essential. These are the steps that assure that if a model yields simulation results in quantitative agreement with results obtained in studies of the real system or, in other words, the model accurately simulates reality and does so for the right reasons. It is easy and, in fact, enticing to stop and publish a model before comparing quantitative and dynamic simulation results with observations. 'Here it is folks, a simulation model of digestion and metabolism of the gerbil. It captures all the concepts and data available on the gerbil in a concise, comprehensive, quantitative format'. Indeed, it does and as such represents a real contribution to science.

Sometimes, this is as far as the modeler can go. For example, an economist may develop a model of how economic forces influence the decisions of politicians. In developing the model s/he is likely to have utilized all available historical data on the subject in formulating the equation forms and parameters that comprise the model. In this case, the investigator would not be able to perturb the (real) system in a systematic fashion to adequately challenge the model. Further, to show that the model is capable of simulating the data it was based upon only represents a self-fulfilling prophecy. In biology, it is equally feasible to 'use up' all existing data in formulation of a model such that quantitative analyses are equally self-fulfilling. Publication

at this point may be appropriate, because it could stimulate other workers to run experiments that truly challenge the model and its components.

On the other hand, if one or more of the rules for model formulation and parameterization put forth in Chapter 3 were followed, quantitative and dynamic evaluations of the model become feasible and appropriate. For example, a clear separation of data used in model formulation from truly independent data sets provides a venue for truly challenging the model. Similarly, formulation of a model based upon data and concepts developed in studies of level $i - 1$ to simulate challenge data sets collected in studies of level i allow quantitative and dynamic evaluation of the model. In our view, such evaluations become paramount in quantitative fields such as nutritional energetics and animal science, where the goals in research modeling are identification of critical research problems and better utilization of our basic knowledge of animal functions in advancing animal agriculture.

The first and simplest quantitative evaluation of any model is to determine whether or not an appropriate (to the modeling objective) range of inputs yield a set of outputs in conformance with experimental observations. For example, if feed intakes are varied and model estimates of rates of growth vary in conformance with experimental data, the model passes this initial challenge. If model estimates of rates of gain of cattle differing in a genetically determined trait and the simulated estimates are superior to those obtained using competitive models, the model passes a second level of challenge (Chapter 15). However, in research modeling – unless we have incorporated grave errors of omission or commission – these results are expected. What we truly seek in research modeling is additional insight. This is gained through more systematic evaluations of model outputs. Some of these will be discussed in subsequent paragraphs.

Evaluation of the total performance of the model relative to 'observed' information allows evaluation of systematic errors of prediction. Observed data are usually based upon experimentation. However, outputs from Monte Carlo simulations, where the distributional properties of input variables are used to generate 'observed' data sets, can also serve as evaluation data sets. Predictions of the simulation model are regressed on observed values for the trait(s) of interest. If the functional relationships among the components are fully understood, parameters measured without error and the relationship(s) among traits are correctly modeled, the intercept and slope estimates from the regression would equal 0 and 1, respectively. This evaluation approach provides a quantitative assessment of the model's accuracy. Figure 4.4 provides a graphical evaluation of a model's appropriateness. Predicted output values from the model are regressed upon the observed values from an independent data set. A 'prediction' from an ideal model would result in the linear relationship illustrated in Fig. 4.4. As the values for estimates b_0 and/or b_1 deviate from 0 and 1, the model becomes less than ideal for the trait of interest; this is indicated by the data points representing the lack of fit (lowered accuracy).

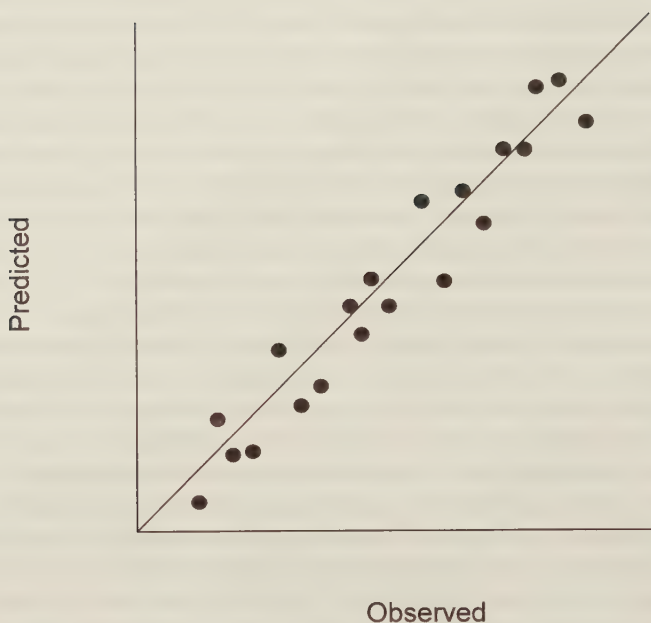


Figure 4.4 The high degree of linear relationship between predicted and observed values indicates the appropriateness of a model.

As accuracy decreases, those engaged in the modeling activity must determine the point at which the predictions of the model become of lesser value. For this reason, in subsequent chapters some emphasis will be placed upon a model's ability to explain variance or differences between experimental and model estimates. This approach is not limited to evaluation of the model *in toto*, but also for relationships within and among subcomponents of the model. Appropriateness of predictive equations relative to observed values from lower level(s) may be determined. Relative low accuracy at these sublevels documents that interrelationships or functional equations in the model require reconsideration. The modeler's response is to return to the protocol for model development (i.e. revise existing equations) or introduce new components to resolve the problem. If, after evaluation, the decision is that the functional form of the relationship is inappropriate, description of the process may require the synthesis of equation forms based on knowledge of lower level functions and the use of this information to increase applicability of the model. The model form becomes more and more mechanistic as this process is repeated.

Uncertainty exists with regard to the direct statistical evaluations of hypotheses that differences between simulation outputs and 'observed' values are not significant. Law and Kelton (1982) suggested that such testing of a hypothesis is inappropriate. The justification for this statement is that the model is only a simplification of the true system. As such, testing the null hypothesis (i.e. they are the same) is philosophically incorrect. In other words, as the model is only an approximation, the equality assumption of the hypothesis would be false. This comment is well-taken, but should not be considered as an absolute. As indicated in the quotation from Box (1979) used to introduce Chapter 3, all models are simplifications of reality – this is the nature of models. If we fully understood reality, research models would no longer be required. Similarly, further experimental research would not be appropriate, since we would have all the answers and these would all be incorporated in the 'ultimate' model. In the practical sense, an 'ultimate' model could be used to optimize the utilization of all resources available for human food production without the necessity for human intervention. Quite a number of science fiction scenarios that incorporate this concept – farming by robots – have been published, but not challenged except for implied recognition of the fact that this 'ideal' lies far in the future and is not feasible now. Science fiction has, however, presented quite a number of futuristic analyses of models of 'ultimate' and 'ideal' social systems to optimize the well-being of human society. In my (RLB) lifetime, I have seen several real-life social models fail, and in science fiction, they normally fail unless humans acquire new traits, such as ESP.

The point is that current models are simplifications of reality, but can be very useful. From the practical point of view, when a regression of predicted versus observed observations has a slope of 1.0, intercept of 0.0, a low coefficient of variation and high r^2 , the model can be useful. From the research point of view, such a result may be rewarding but must be examined carefully as to cause and effect. Also, as already emphasized a number of times, when a model fails this test, the scientist learns.

Quantitative evaluations of systematic errors should also be undertaken; systematic errors suggest the model is not a robust predictor of reality. Causes of systematic errors must be identified. Then, based upon the severity of the errors, decisions are made about the course of action for effective resolution of the problem.

A degree of uncertainty exists with parameters estimated from experimental data, since these normally contain errors attributable to measurement and sampling. Inspection of residuals (deviations of predicted from observed values) plotted against observed values is an effective evaluational method for determination of whether or not bias exists (Fig. 4.5). If the residuals truly represent errors about the observed value, then deviations would be randomly scattered about zero (i.e. the scatter illustrated in Fig. 4.5). This is true because the mean value for residuals, by definition, is zero if a model is unbiased. This approach is extremely useful in identifying

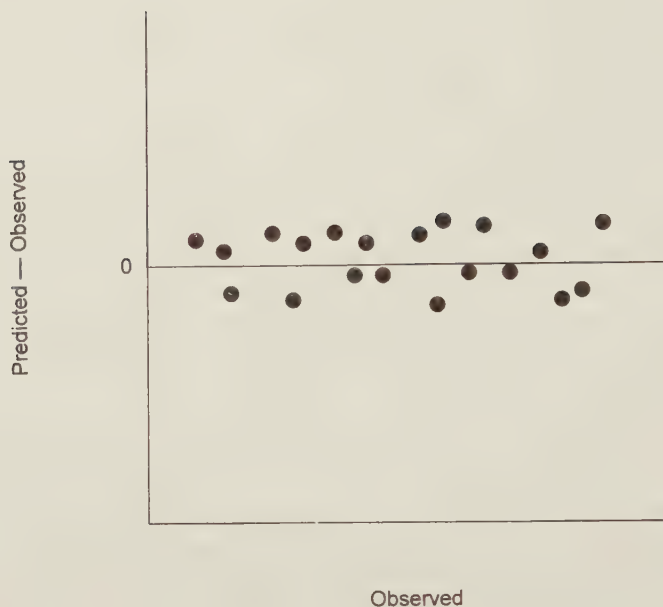


Figure 4.5 Random scatter of the residual (predicted–observed value) suggests no bias in the parameter of interest.

systematic errors and evaluating specific equations within subcomponents for inappropriate equation forms. For example, a quadratic trend observed in residuals resulting from predictions involving a linear model suggest curvilinear relationships may exist. Those involved in the modeling activity should consider fitting a non-linear equation to better describe underlying relationships.

Residuals are effective in the investigation of systematic errors. The evaluation is made by plotting the residual against other variables in the model. If the residual tends to be positive for 'small' values of the variable and negative at 'large' values, a systematic error is indicated, suggesting a reduction in the general applicability of the model.

Baldwin *et al.* (1977b) presented an analysis comparable to the theoretical evaluations presented in Figs. 4.4 and 4.5 by way of illustrating this aspect of model evaluation. They selected the model of Waldo *et al.* (1972) as a starting point (Fig. 4.6). In this representation, A and B are the respective amounts of potentially digestible and potentially indigestible cellulose in a feed, k_1 is the digestion rate constant, and k_2 is the passage rate constant. The model was defined by two differential equations:

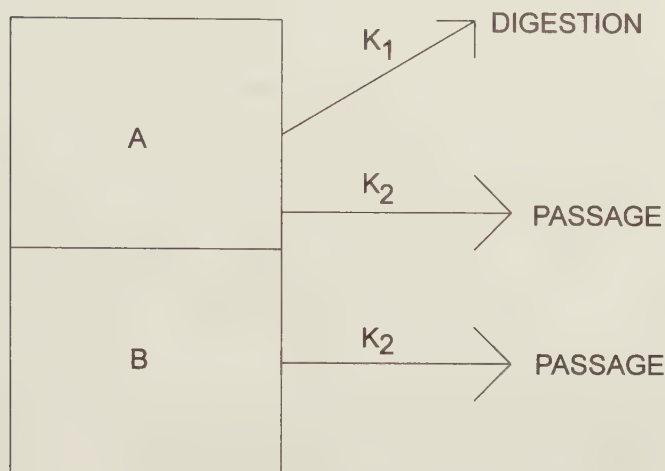


Figure 4.6 Model 1: A is potentially digestible cellulose and B is potentially indigestible cellulose; k_1 and k_2 are respective rate constants for fermentation and passage. Adapted from Waldo *et al.* (1972).

$$dA/dt = -k_1A - k_2A \quad (4.13)$$

$$dB/dt = -k_2B \quad (4.14)$$

The model incorporated several useful concepts but was not generally applicable because new rate constants had to be defined for each feedstuff considered.

This led to development of a second model (Fig. 4.7) that incorporated additional features, including the ability to vary feed input rates and frequency, division of rumen cellulose into large and small particle pools, where only small particle pools could pass from the rumen, and where rates of digestion of cellulose in the large and small particle pools differed. Ten differential equations, seven first-order rate constants and eight pool sizes had to be specified to implement this model. The critical parameters are the rate constants, because when the model is run to a steady-state condition, the pool sizes, being dependent variables, are defined by default. Of the seven rate constants, k_1 is defined as an input by the user. Thus, six rate constants (k_2 through k_7) must be estimated. Twelve data sets were used in an iterative process to estimate the 'least sum of square errors' best fit rate constants (see next section of this chapter) for the model. Then, the model was evaluated by comparing model predictions for 17 additional data sets

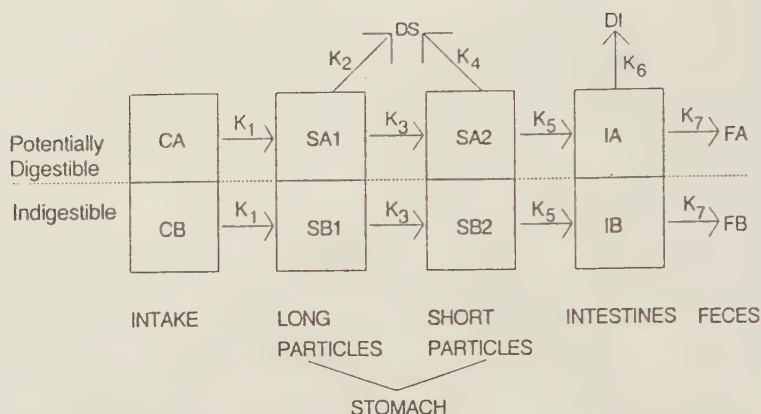


Figure 4.7 Representation of Model 2 adapted from Baldwin *et al.* (1977b): a dynamic model of cellulose digestion in the ruminant. CA and CB are artificially defined pools that, coupled with k_1 ($CA \times k_1$), determine rate of input to the rumen of potentially digestible (A) and potentially indigestible (B) cellulose. SA1 and SB1 are stomach (S) pools of large cellulose particles. SA2 and SB2 are stomach pools of small cellulose particles. IA, IB and FA, FB are intestinal (I) and fecal (F) pools. DS and DI denote amounts of cellulose digested in the stomach and intestine. k_2 and k_4 are rate constants for fermentation of cellulose; k_6 is the intestinal fermentation rate constant; k_3 is a rate constant defining rate of conversion of large to small cellulose particles; k_5 and k_7 are passage rate constants.

on cellulose digestion in sheep not used in estimating the rate constants. The results obtained are plotted in Fig. 4.8.

While it is clear that if a regression equation of observed versus predicted yields a non-zero intercept and a slope of less than one, the agreement between observed and predicted values actually looks quite good in Fig. 4.8. However, when residuals are plotted versus observed values (Fig. 4.9), it becomes abundantly clear that the model has significant bias. The lessons we learned from this limited exercise were that plots of predicted versus observed values can be misleading if not accompanied by appropriate statistical analyses; that residual plots are more revealing; and, that residual plots can be very useful in identifying sources of systematic errors of prediction (see below and Chapter 8).

As more information becomes available and is incorporated (i.e. changing parameter estimates), the influence upon behavior of the model needs re-evaluation. For these reasons, sensitivity analyses should be conducted routinely.

Parameter sensitivity may be tested with sets of inputs with known responses. Comparison of the error structures resulting from the predictions relative to the known is the test. If the predicted value remains relatively constant as the parameter of interest varies over a realistic range of values, the model is insensitive to this parameter. Substantial variation in the residual sums of squares resulting from varying a parameter suggests sensitivity.

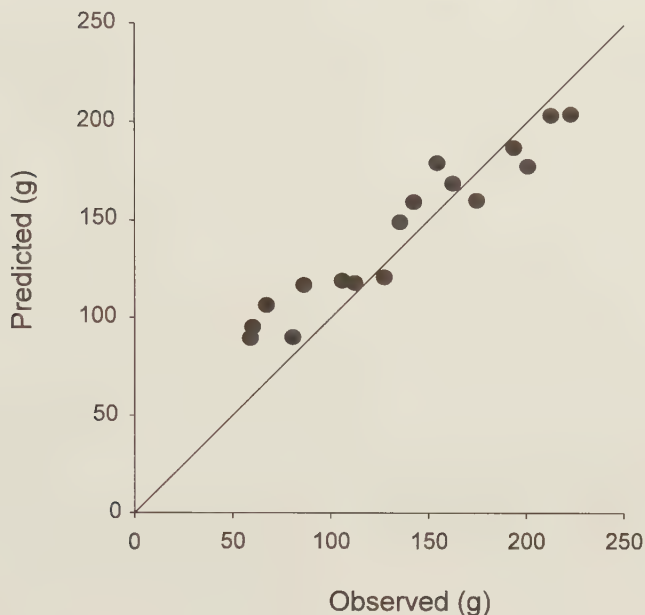


Figure 4.8 Comparison, with experimental data, of rumen cellulose digestion predicted with Model 2. The reference line corresponds to perfect agreement between model-predicted and experimental values. Adapted from Baldwin *et al.* (1977b).

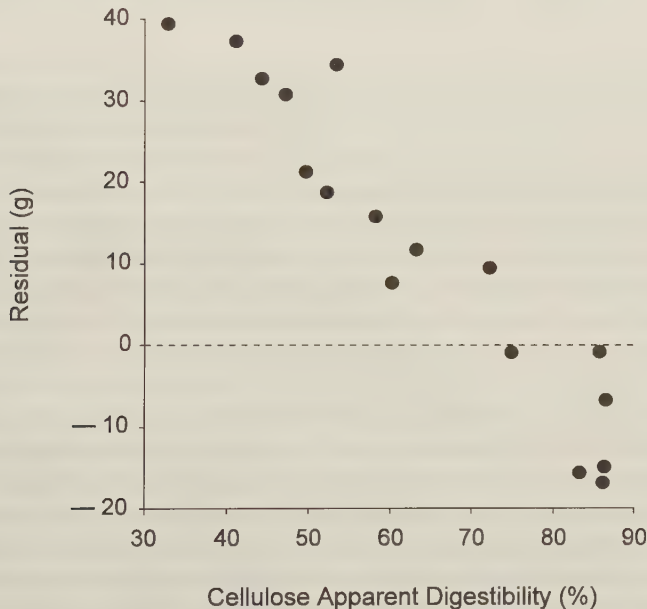


Figure 4.9 Differences between observed and predicted values for rumen cellulose digestion (residual) plotted against experimentally measured apparent cellulose digestibilities.

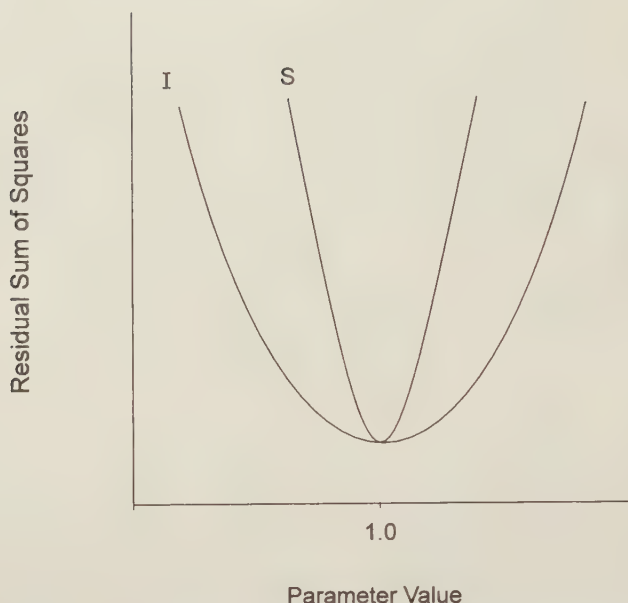


Figure 4.10 Procedure to assess level of sensitivity for model parameters. A stable relationship in error structure of model and varying parameter or input values indicates an insensitive parameter (I). The curve labeled S is indicative of a sensitive parameter, i.e. as input values vary, response observed in the error structure is relatively stable. From Baldwin *et al.* (1977b).

In Fig. 4.10, evaluation of the residuals relative to two different parameters illustrate this concept. The curve labeled S is indicative of a highly sensitive parameter relative to the curve labeled I. The error structure of curve S is affected markedly by changes in parameter estimates, while curve I is much less sensitive. Results from this comparison would suggest to those involved in model development that the parameter associated with curve S must be known and can be deduced, using an analytical model as illustrated in Chapters 8 and 15, with accuracy. Also, simplifying assumptions may be made with regard to parameters associated with curve I (France and Thornley, 1984).

A second evaluation technique compares the ratio of the standard deviation resulting from fitting to a data set relative to its parameter estimate (i.e. CV, the coefficient of variation). If several parameters are adjustable, these parameters may be ranked to evaluate the sensitivity of the model's performance to each of the parameters. Relative sensitivity of a parameter would be indicated by the value for the coefficient of variation of the i th parameter. Ranking CV associated with the respective parameters enables the modeler to identify parameters more critical for acceptable performance of the model. Consider Fig. 4.10; the CV from the curve labeled S would have a low value relative to the CV for the curve labeled I.

If a single output is identified from a model, an additional criterion for determining the sensitivity of specific parameters consists of evaluating the relative change in the response variable per change in the parameter. If the change in this ratio exceeds 1, the parameter has a large effect upon the expected response. If the value of the ratio is equal to one, the response is proportional. Determination of the sensitivities of parameters allows the modeler to be more efficient. The complexity of a model may be reduced or results may suggest incorporation of additional functional relationships if sensitivity is great.

Analytical models for parameter estimation

Tools, technology and information are available for a systematic approach to estimate more effectively nutrient requirements for domestic animal production. The application requires development of computer programs that implement models sufficiently robust so that the programs provide information critical to the decision making process. Information from these simulation models could serve as input for other models to allow the producer to meet specific goals. Colleagues in the plant production fields have developed and are applying computer simulations that are contributing to the management decision process (Maas and Arkin, 1980; Jones and Kiniry, 1986).

4.4 EVALUATION AND USE OF ANALYTICAL MODELS FOR PARAMETER ESTIMATION

Approaches to parameter estimation may be characterized by requirements to describe the process of interest or, more pragmatically, to obtain estimates of parameter values that cannot be measured using currently available experimental techniques. Inherent to estimation is correct identification of parameters for a set of relationships defined in the system, that is, to insure that solutions from the estimation procedure represent the true underlying structure of the relationship. If the relationship between an endogenous variable and a predetermined variable can be characterized by a single equation, regression analyses represent an effective means for estimation. For complex relationships requiring a series of equations for description, analytical approaches applicable to solving systems of simultaneous equations are recommended (Heady and Dillon, 1972; Johnson, 1984; Swan, 1984).

For the case where a relationship is characterized by a single equation, estimation of parameters relating the endogenous and predetermined variables is direct. Estimates from prior experimentation reported in the literature represent values determined from a knowledge base. Averaging across many studies reporting the parameter of interest is analogous to improving the precision of a mean by increasing sample size. With few or no literature estimates available, *in vivo* or *in vitro* experimentation represents the appropriate course of action. Data, normally bivariate, from experiments repre-

senting samples from a distribution are subjected to statistical analyses. Solutions for less complex relationships are readily obtainable through application of linear and non-linear regression techniques within the level of interest. The general form of the regression model is

$$Y_t = f(x_i; B) + E_t \quad (4.15)$$

where $f(x_i; B)$ is a function of ' n ' independent variables and ' p ' parameters. The assumption is made that errors (E_t) are random and uncorrelated. Regression models generally can be classified as either linear or non-linear (Abraham and Ledolter, 1983). This classification scheme considers both parameters and the independent variable(s). The form of a linear model is such that the change in the response variable is constant over the range of the predictor variable. Generally, estimative models are linear or linearizable with respect to parameters; the exceptions are those models whose derivatives are dependent upon parameter(s) in the model itself. The exponential type models used for the characterization of growth are an example of non-linear models (e.g. the Gompertz equation; Chapter 9). Transformations available for linearization of many single variable non-linear functions reduce the difficulty of parameter estimation (McCracken and Dorn, 1964). For example, parameters of power functions expressed in the logarithmic form are amenable to estimation by linear regression. Models with interactions between independent terms are non-linear. Equations such as these have to be restructured to apply solution techniques other than regression analyses.

Common algorithms developed for estimation of parameters through regression techniques commonly apply the error minimization approach of least squares as the objective. Investigation of functional relationships represents a typical application of the least squares approach. Solution of a set of normalized equations to minimize the sum of the squared deviations allows estimation of the parameter(s) of interest. After the solution, parameters should be evaluated for biological appropriateness in the equation of interest.

4.4.1 Evaluation of analytical models

(a) Behavioral analyses

Approaches used in behavioral analyses of models intended for use as analytical models are much the same as those described above for all models. The basic criteria that must be satisfied are that models have the capacity to simulate, at least qualitatively, patterns of response exhibited by the system modeled or, in other words, model responses to changed inputs such as diet, hormones, etc., conform to current experimental data in terms of direction and approximate magnitude. Further, the responses must be attributable to equation forms and parameter values that current concepts hold

are the most appropriate cause-and-effect relationships. Inferences drawn from further modeling and experimental studies may challenge currently held concepts or indicate that these are inadequate – after all, this is an objective in using models to evaluate current concepts for adequacy in the quantitative and dynamic domains – but current concepts are the most appropriate starting point.

(b) Sensitivity analyses

Analytical models require that a broader range of sensitivity analyses than those discussed above be applied. The most important additional issue that must be addressed in sensitivity analyses of analytical models is definition of experimental designs and measurements, which must be undertaken to assure that unknown or unmeasurable parameters can be uniquely 'identified'. The concept of 'identification' of parameter values estimated using analytical models, experimental data and appropriate parameter estimation algorithms (see next section) was developed in some detail by Carson *et al.* (1983). The essence of the concept is that one must assure 'before the fact' that a given set of experimental data collected for the purpose of estimating parameter values not currently available or not directly measurable are adequate to 'uniquely' define the set of parameters to be estimated. The standard statistical rules – regarding degrees of freedom ($n - 1$) or that three 'independent' measurements are required to estimate three unknowns – are generally not applicable when one seeks to probe underlying functions or even establish cause-and-effect relationships, because at these levels, 'independent' measurements are not often truly independent of one another.

The technique of multiple linear regression as applied in interpretations of many nutritional energetic studies is a good example. Using the basic model discussed in Chapter 6

$$\text{MEI} = b_1 * \text{MBS}_1 + b_3 * \text{LEG}_1 + b_4 * \text{FEG}_1 + \varepsilon_1 \quad (4.16)$$

it is often found in the covariance matrix that the correlation between protein and fat gain is 0.5 or greater. As a result, estimates of b_1 , b_3 and b_4 from experiment to experiment are quite variable and, thus, non-unique, because the true N is less than the number of actual measurements, i.e. ME intake (MEI), protein gain (LEG) and fat gain (FEG).

When more complex, mechanistic models are used analytically to estimate greater numbers of unknown parameters in equations for functions that are not independent of one another, this potential problem must be addressed directly and with rigor. Therefore, the problem of 'identification' becomes paramount in sensitivity analyses. In our experience, the most rigorous approach has been a modification of the Monte Carlo technique. In standard applications of this technique, a model is fit to a given data set using standard 'best fit' statistical procedures, which output each estimated

parameter and its standard deviation or standard error of estimate. Then the deduced parameters and their respective errors of estimation are used to generate a new data set that is used, in turn, with a parameter estimation algorithm to obtain new estimates of the unknown parameter values. If the original and new estimates of parameter values are similar, the analytical model is considered robust and the original set of deduced parameter values is considered valid. This is generally considered to be a very rigorous test of an analytical model and deduced parameter values, because very few Monte Carlo evaluations result in verification of the model and parameters deduced using that model. The reader might note by way of verification of the above statement that papers are rare in which the results of multiple linear regression analyses of nutritional energetics data were subjected to Monte Carlo analyses. The above comments are not directed against multiple linear regression techniques and the highly competent scientists who have used the technique. The emphasis is on the rigor associated with successful Monte Carlo analyses.

A major objective in research modeling is usefulness in the design and interpretation of experiments. A modification of the Monte Carlo technique can be very useful in this regard. The modification is that the proposed analytical model is solved one time using 'best guess' estimates of unknown parameters to produce what we call a deterministic data set (no variance). This data set and the proposed model are then used along with a parameter estimation algorithm to try to estimate the unknown parameter values used in the model to generate the deterministic data set. If the parameter values in the model used to generate the deterministic data set are correctly estimated, this first challenge of the model and data required to identify key parameters is satisfied. If the data set generated is as simple as possible, as it should be, the estimated parameter values will not agree with those used to generate the data set. Then, it is appropriate in sequential evaluations to add measurements to the deterministic data set until the data set is adequate to identify or uniquely define the unknown parameter values.

The next step in the analysis is to add variance to the test data set. For example, if rate of fat gain is one of the measurements to be used, one would specify the mean value and a standard deviation characteristic of experiments in which fat gain is measured as an input to a random number generator to create a data set for fat gain. After doing the same for all proposed measurements, one has generated a data set that can be used to attempt to estimate the parameters in the model (the above approach assumes all outputs/measurements are independent one of the other. When interdependence exists alternate approaches that accommodate this problem can be used). Again, if the model parameters are estimated with appropriate or desired accuracy, one can conclude that an experiment which includes the measurements used would be adequate to estimate the unknown parameters with accuracy, e.g. identify the model. Conversely, if the proposed measurements are not adequate to identify the model, additional

measurements can be added and their efficacy as identifiers evaluated until a set of measurements adequate to identification of the model is found.

We have all used this approach, at least intuitively, in designing experiments, e.g. we state (in introductions to experimental papers) that if we measure L, M, N, O and P, we should be able to deduce X, Y and Z. Also, we have intuitively selected and made measurements in experiments that, in retrospect, were not relevant to our experimental objective. Use of the modified Monte Carlo approach described above allows the addition of quantitative rigor to intuitive insight in experimental design. At one extreme, application of this approach helps in the design of efficient experiments by eliminating unnecessary measurements. At the other extreme, this approach may persuade an investigator to completely reorient his/her experimental approach on the grounds that the measurements required to identify a system are unfeasible given local resources. In this regard, there is no sense in running an experiment that is not adequate to define the unknown parameters you desire to estimate. Examples of this application are presented in Chapters 8–10 and 12–14.

4.4.2 Algorithms

For complex relationships, where the underlying structure of the model requires several endogenous and predetermined variables to describe a relationship, solutions of a set of simultaneous equations can provide estimates of the required parameters. The general system can be represented as

$$\begin{aligned} q_{11}y_{1t} + q_{21}y_{2t} + \dots + q_{M1}y_{Mt} + b_{11}x_{1t} + \dots + b_{K1} + b_{K1}x_{Kt} &= e_{t1} \\ q_{12}y_{1t} + q_{22}y_{2t} + \dots + q_{M2}y_{Mt} + b_{12}x_{1t} + \dots + b_{K2} + b_{K2}x_{Kt} &= e_{t2} \quad (4.17) \\ q_{1M}y_{1t} + q_{2M}y_{2t} + \dots + q_{MM}y_{Mt} + b_{1M}x_{1t} + \dots + b_{KM} + b_{KM}x_{Kt} &= e_{tM} \end{aligned}$$

where y_M 's represent endogenous variables, x_K 's the predetermined variables and e_{tM} the random error. For solutions to be obtained, the equations must be algebraically manipulated to express the endogenous variables in the equation of interest as a function of the predetermined variables. In terms of the simple model relationship discussed previously (equation 4.15), the dependent variables must be represented as a function of the independent variable.

For unique solutions to be obtained, the number of equations must equal the number of predetermined (independent) variables. If fewer predetermined variables than number of equations exist, parameters cannot be estimated. If more predetermined variables exist than equations, restrictions can be used to allow estimation of the parameters. Unlike relationships described by equation 4.15, least-squares estimation procedures are not the most appropriate. The best estimate (least biased) of the parameters results from application of the full maximum likelihood estimation procedure. This approach uses all the equations in the linear system in the solution for the

parameters. An additional maximum likelihood procedure, a recursive estimation, does not require solution of the full system of equations, rather it solves a single equation for the endogenous variable and has that value substituted into a later equation as a predetermined variable.

(a) Algebraic models

In general, the process of parameter estimation as used in animal modeling is a problem in function minimization or, more accurately, optimization. You are given a single function that depends on one or more independent variables. You want to find the value of these variables where the function takes on its minimum (or sometimes maximum) value. The primary problem is to find an algorithm which requires evaluating the function as few times as possible.

The problem with most algorithms used for optimization is that they can find either a global (the absolute) minimum or simply a local minimum. Virtually all algorithms used can only find local minima; methods to verify that the point found is global include (1) start from widely spaced values of the independent variables and find various local minima, and then pick the best one; and (2) find a local minima, move away by a fixed amount (something in the order of about 15–20% is usually used) and see if you then always return to the same point or to a better point. If totally unrealistic initial parameter values are specified to initiate the iterative process, the possibility of finding only local minima becomes highly likely.

Many algorithms commonly used to optimize functions require you to supply the first derivative. This step is not easy or possible in many of our models. For the most part we have used two algorithms to estimate parameters: steepest descent and the so-called downhill simplex (not to be confused with the simplex method of linear programming).

The steepest-descent method has generally been superseded by much better algorithms. The algorithm can be described as follows: starting at a point, move to the next point by proceeding in the direction of the steepest gradient while the function value sum squared error, for example, keeps getting smaller. The problem with this method is that the next step is always perpendicular to the direction traversed in the last step. This is not necessarily the direction that takes you to the minimum. The steepest-descent method will tend to get you in the general area of the minimum relatively quickly, but it will take a long time to find the actual minima of the function. Various other algorithms, called conjugate-gradient methods, are preferred; they require the calculation of first derivatives (Press *et al.*, 1989).

The simplex method is based upon the Nelder and Mead (1965) model. It requires only function evaluations and is rather robust, but sometimes it is time consuming. The algorithm is relatively simple to implement. We have found that the simplex algorithm works best for systems with four or less variables.

The general method revolves around the concept of a 'simplex', which is simply a geometrical figure in N dimensions consisting of $N + 1$ points and all the interconnecting line segments. In the case of a function with two variables, the simplex is a triangle; with three variables it is a tetrahedron. The initial points must be supplied as initial guesses of the parameter values. The algorithms then move the simplex in one of several ways – reflection, expansion or contraction. Most steps involve reflection; the point with the highest function value is moved through the opposite face of the simplex to a lower point. If possible, the algorithm will expand the simplex in one or more directions in order to take larger steps. If the simplex reaches a valley, it contracts in one direction to fit the valley and tries to move down the valley. It can also contract itself in all directions. The method generally terminates when either the step size is smaller than a specified tolerance, the simplex gets into a 'reflection' loop, or the function improvement is less than some error.

(b) Parameter estimation for dynamic models¹

The SAS procedure NLIN can be used to produce least-squares estimates of a non-linear (algebraic) model. There are four iterative methods: Gauss–Newton and Marquardt, which require partial derivatives of the model, and two variations of conjugate-gradient methods.

SIMUSOLVE[®] uses the method of maximum likelihood to calculate the probability of having obtained a given set of data, assuming that a model with those parameters is correct. Given the fact that the true values are a myth, nonetheless a model can be used to find those parameter values that are most likely to be correct. The method of maximum likelihood provides a method to calculate the probability of having obtained a given set of data, in our case the experimental values we are comparing against the model, given that the model's parameters are correct (SIMUSOLVE[®], 1990, Section 5.2). SIMUSOLVE[®] optimizes a complicated objective function derived from the model itself to find the best parameter estimates for that model. This method uses a form of an error model, since the actual measured data are of course imprecisely measured to some extent or another. Initial values are set by the modeler; some controls on the value (i.e. must be greater than zero, etc.) can also be specified. The parameters obtained by maximizing the log likelihood function are those with the highest probability of generating the acquired data. This method can be extended to test between several

1 The discussion of algorithms used in SAAM for parameter estimation was kindly supplied by Ray Boston (University of Pennsylvania). Ray also provided a valuable critical review of Chapters 2, 3, and 4.

possible models by comparing the value of the likelihood function calculated for each model.

SIMUSOLVE[®] uses two optimization methods, SEARCH and GRG. SEARCH is a version of the Nelder-Mead simplex method explained previously. GRG is a gradient-based method that uses a conjugate gradient algorithm to compute the search direction. The objective function is compared with other solutions using a maximum likelihood estimator. GRG is used for problems with one or more than three adjustable parameters; SEARCH is used for models with two or three adjustable parameters.

SAAM's (SAAM Manual, 1978) optimization facility employs a weighted least-squares objective function coupled with a modified Gauss-Newton fitting algorithm. A number of novel features enable this system to move swiftly and robustly to a model fit (Berman *et al.*, 1962). Linear parameters are fitted separately from non-linear parameters. In conjunction with each iteration of the non-linear parameter adjustment, conditional fits for linear parameters are found. The parameter decoupling achieved here greatly facilitates the fitting procedure. Both linear and non-linear adjustments can be constrained to 'sensible' regions. This prevents wild parameter value discursions (for less well identified parameters) and, thus, saves considerable computing time.

When information exists regarding the expected value and the variance of an adjustable parameter, this information can be incorporated into the fitting procedure. The user has control over both the number of iterations and fit acceptance criteria. To simplify use of the procedure for the user, partial derivatives for the non-linear adjustable parameters are approximated using forward differencing. The user can control the parameter adjustment in conjunction with this determination.

There are three preprogrammed block data weighting schemes that ease the assignment of uncertainty to data (SD, constant standard deviation; FSD, constant fractional standard deviation; and RQO, standard deviation proportional to the root of the observation). Where direct knowledge of the error of the data exists, this can be easily applied.

As provisional parameter adjustments become available, two additional refinements are applied prior to their use for the generation of new parameter values. Firstly, the adjustments (also referred to as corrections) are scaled back in a manner proportional to their variance and then a linear adjustment to these scaled values is applied based on a weighted linear regression increment. Each of these steps help to data-sensitize the parameter adjustments.

As well as catering to the fit of kinetic models to data (usually tracer based), SAAM is also able to fit linear and non-linear tracer models to data. Model solutions are obtained using a Newton-Raphson procedure (SAAM Manual, 1978) and parameters are estimated using the routine optimization approach to a precision dictated by the 'known' (or anticipated) uncer-

tainty. To prevent underidentified systems (with the attendant singular covariance matrix) from causing the program to fail during the estimation phase, SAAM uses a generalized matrix inversion procedure. Under circumstances where this procedure is invoked, the user needs to exercise caution in attaching significance to certain aspects of the results from data fitting.

Both SIMUSOLV[®] and SAAM have been used extensively and effectively in analyses of kinetic data within constraints imposed by the use of biologically rational (mechanistic) models. In many cases, the choice of one over the other for data analyses is arbitrary or based upon the experience or preference of a given investigator. In this sense, utility is in the eye of the user. This is also true of the many newer and more user-friendly windows, etc., versions of these and other competing software programs that are becoming available. Underlying principles, algorithms, etc., mainly are held in common as new software seems to evolve more quickly than theory.

Our current bias is that the approach embodied in SAAM is most appropriate when time-course (usually tracer) data are being utilized to gain insight into numbers of pools, transactions and parameter values that can be identified by a given data set and how these are most likely affected by physiological and disease states. In contrast, we believe that the SIMUSOLVE[®] approach is most appropriate when one wishes to deduce parameter values for a predefined model (based upon underlying biochemical concepts) based upon the conduct of a series of *in vivo* or, usually, *in vitro* experiments in which multiple products are formed from a number of tracers, such as 1-¹⁴C, 2-¹⁴C, 6-¹⁴C, U-¹⁴C-glucose; 1-¹⁴C, 2-¹⁴C-acetate; several ¹⁴C-labeled amino acids; etc.

4.4.3 Evaluation of deduced parameters

Once identified and derived, estimates of parameters require evaluation for accuracy and bias. Primary estimation procedures include numerical methods whose solution depends upon minimization of errors of prediction. Least squares and maximum likelihood (above) represent two of the more common methodologies. Development of computer algorithms for execution of a model frequently requires incorporation of conditional constraints based upon such features as rates, masses, or concentrations associated with specific variables. Differences in magnitude within relationships for measures such as these frequently require scaling. This scaling procedure creates an expression in relative dimensions among components of interest. A frequently acceptable method of scaling is to standardize the observed variable by the standard deviation.

The problem of verification of deduced parameter values is a very difficult and, to us, inexact process. At least, none of the authoritative texts we are aware of set forth a set of rules and methods which assure that all parameters deduced using an analytical model of a biological system are

STANDARD	DESCRIPTION	PARAMETER ESTIMATES	
DEVIATION	-----	-----	-----
	LOG LIKELIHOOD FUNCTION	INITIAL	FINAL
		K1 102.34	102.36
1.671E-03		K1 0.50103	0.50127
1.139E-08		K5 1.14067E-06	1.14006E-06

TIME	A OBSERVED	A PREDICTED	% ERROR	STANDARDIZED RESIDUAL	RESIDUAL PLOT
0.0000E+00	0.7500	0.7500	0.00	0.000E+00	
1.000	0.4141	0.4543	-9.71	-1.83	*****
1.500	0.3618	0.3536	2.26	0.467	**
2.000	0.2548	0.2752	-8.01	-1.53	*****
2.500	0.2024	0.2142	-5.83	-1.14	****
3.000	0.1512	0.1667	-10.26	-1.92	*****
4.000	9.7900E-02	0.1010	-3.16	-0.632	***
6.000	3.8900E-02	3.7059E-02	4.73	0.977	*****
8.000	1.3500E-02	1.3600E-02	-0.74	-0.152	*
10.00	4.9000E-03	4.9915E-03	-1.87	-0.379	**
12.00	1.8800E-03	1.8324E-03	2.53	0.523	**
14.00	6.8400E-04	6.7338E-04	1.55	0.320	*
16.00	2.4300E-04	2.4815E-04	-2.12	-0.429	**
18.00	9.8700E-05	9.2399E-05	6.38	1.32	*****
20.00	3.4100E-05	3.5004E-05	-2.65	-0.533	**

TIME	B OBSERVED	B PREDICTED	% ERROR	STANDARDIZED RESIDUAL	RESIDUAL PLOT
0.0000E+00	0.3108	0.0000E+00	100.00	3.50	*****
1.000	0.3615	0.2957	18.21	0.741	**
1.500	0.3615	0.3964	-9.65	-0.393	**
2.000	0.5036	0.4748	5.72	0.324	*
2.500	0.4984	0.5358	-7.50	-0.421	**
3.000	0.6392	0.5833	8.75	0.629	**
4.000	0.6724	0.6490	3.48	0.263	*
6.000	0.7201	0.7129	0.99	8.057E-02	*
8.000	0.7276	0.7364	-1.21	-9.903E-02	*
10.00	0.7486	0.7450	0.48	4.042E-02	*
12.00	0.7194	0.7482	-4.00	-0.324	*
14.00	0.6662	0.7493	-12.48	-0.935	**
16.00	0.7994	0.7498	6.21	0.559	*
18.00	0.7546	0.7499	0.62	5.281E-02	*
20.00	0.7551	0.7500	0.68	5.779E-02	*

STATISTICAL SUMMARY

WEIGHTING	MAXIMIZED LOG LIKELIHOOD	WT RESID SUM OF	WEIGHTED RESIDUAL	STANDARD ERROR OF	PERCENTAGE VARIATION
PARAMETER	FUNCTION	SQUARES	SUM	ESTIMATE	EXPLAINED
OVERALL	102.4	0.154	-142.		98.770
2.00 A	87.33	3.518E-02	-143.	6.580E-06	99.710
0.00 B	15.03	0.118	0.362	9.545E-02	68.761

CORRELATION MATRIX

	K1	K5
K1	1.000	
K5	-0.8133	1.000

VARIANCE-COVARIANCE MATRIX

	K1	K5
K1	2.7931E-06	
K5	-1.6161E-11	1.4136E-16

OPTIMIZATION METHOD: NELDER-MEAD SEARCH

SUCCESSFUL TERMINATION: STANDARD DEVIATION OF PARAMETERS
WAS LESS THAN SDPOPT.
SDPOPT = 1.00000E-04

FUNCTION EVALUATIONS FOR OPTIMIZATION = 83
FUNCTION EVALUATIONS FOR STATISTICS = 4
OPTIMIZE TIME = 2.430 SECONDS

uniquely defined. Carson *et al.* (1983) devoted a considerable portion of their book to this problem. A number of methods operate to the negative in preliminary evaluations as described, in part, above. Several of the user-oriented software systems described above provide statistical outputs as shown on p112 (from SIMUSOLVE®).

These are quite useful and informative, but we must be aware of biologically relevant error structures and sources of error. Also, all methods for parameter and model evaluation discussed in the section on evaluation of research models apply.

4.5 DECISION SUPPORT SOFTWARE

Numerical algorithms and computer programming concepts are available to enhance the decision making process. Simulation models should be developed sufficiently to allow producers to incorporate more recent information and increase the accuracy of estimates. Expert knowledge should be readily available to the producer. Once goals are defined, methods for efficient determination of the best course of action are needed. Many of these methods are available today. This section will introduce several and provide a general description of applicability.

Predicting future biological behavior is one goal of models. Regression models are precise within a limited range, whereas models based on biological mechanisms have more general application (Oltjen, 1984; Chapter 15). Though they aid in understanding, mechanistic models have generally not been used to predict animal performance.

Preliminary observations of a biological system, such as performance of a pen of cattle early in a finishing period, can be used to modify a model and thereby customize the prediction for a specific case. Model adjustment factors can be derived by a procedure based on the Bayes modification of the Kalman filter (Kalman, 1960; Kalman and Bucy, 1961). Using the Bayes procedures, information about prior behavior of a system can be integrated with current observations to predict future behavior (Allen and Jordan, 1982). These adjustments allow a mechanistic model to gain precision without sacrificing its generality.

Application of the Kalman filter to biological modeling has been limited (Meinhold and Singpurwalla, 1983). When applied to a dynamic linear system of bovine lactation (Goodall and Sprevak, 1985), it accurately estimated total milk yield after only a few observations early in lactation. It also has been used for recursive prediction of breeding values (Harville, 1979; Sallas and Harville, 1981; Hudson, 1984).

The algorithm to adjust model predictions by these methods is an attempt to make any mechanistic biological model, which accurately predicts performance of a population, useful in management to predict performance of a subset of the population based on preliminary data from that

subset. Most complex simulation models of animal performance over a wide range of conditions are quite general. They yield precise estimates but require a number of inputs that are infrequently known. Thus, their predictions in a given situation may be biased. On the other hand, simpler empirical models based on past observations usually are quite accurate and precise for a given set of management conditions. However, their application under other management conditions yields imprecise estimates. To gain both empirical and mechanistic modeling advantages, one can capture the generality of the mechanistic models and improve their accuracy by altering predictions based on past observations. The use of Kalman filters is an example of this approach. General models based on fundamental principles can be integrated with a producer's database to improve precision of forecasting future performance. As part of an integrated management information system, this procedure allows a manager to project future performance of cattle already on feed and to answer 'what if' questions about changes in his management strategy. With increasing emphasis on efficient use of information for managing modern beef cattle enterprises, this procedure should enhance planning and decision making.

Expert systems are computer programs that emulate the reasoning process of human experts in a particular area. These tools have been shown to be effective decision makers for problems that require heuristic knowledge, a set of rules derived through experience. Thus, they are also called knowledge-based systems. An expert system bears many similarities to a human expert. It has a reasoning procedure for attacking a problem and arriving at a decision. It has a base of experience, or rules, many of which are rules of thumb, by which it arrives at a decision. An expert system has the advantage of potentially containing the expertise of many of the world's greatest experts, except that it is tireless, does not retire, become ill, or take vacations. Expert systems may replace many of the other kinds of decision-aid software, because of their basic user-friendliness and their greater utility in situations where only incomplete data are available (Holt, 1985).

The integration of expert systems tools with traditional systems analysis (optimization and simulation modeling) is a relatively new and unexplored area of animal science. The potential is particularly great for agricultural production, where the equivalent of an expert's knowledge is needed for typical day-to-day problem solving and decision making. Ultimately expert systems may allow local producers with a microcomputer to utilize the knowledge of an expert in each of the diverse fields for which s/he has responsibility. Therefore, its application is appropriate for management in animal agriculture.

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Animal energetic models

Thus, to confine myself to simple ideas that everyone can grasp, I would say that animal respiration is nothing else but a slow combustion of carbon and hydrogen, which takes place in the lungs, and that from this point of view animals that respire are veritable lamps that burn and consume themselves.

by Lavoisier (Holmes, 1985)

5.1 INTRODUCTION

Basic objectives in this chapter are to review concepts in thermodynamics as they specifically relate to the field of nutritional energetics, present some early contributions to development of the field of animal energetics and trace the evolution of feeding system models in current use.

5.2 THERMODYNAMIC CONCEPTS IN NUTRITION

5.2.1 Basic units

The basic definition of energy (E) is potential capacity to perform work (w). There are many forms of energy: chemical, mechanical, kinetic, positional, electrical and heat. Nutritionists deal primarily with chemical and heat energy. The international metric system defines six basic units: meter (m) as the measure of length, kilogram (kg) as the measure of mass, second (s) as the measure of time; ampere (A) as the measure of electrical current, Kelvin (K) as the index of thermodynamic temperature and Cadela (cd) as the measure of luminous intensity. International units relevant to nutritional energetics are all based upon these basic units. For example, the Newton (N) or unit of force is defined as $\text{kg} \cdot \text{m}/\text{s}^2$, and the joule (J), which is the international unit of energy, is $\text{N} \cdot \text{m}$ or $\text{kg} \cdot \text{m}^2/\text{s}^2$.

In nutrition, the calorie was the classical unit for measurement of energy. Adoption of the International System of Units by nutritionists meant discarding the use of the calorie in favor of the joule, a more absolute unit of energy. Initially, there was considerable controversy about adoption of the

joule as the standard international unit for energy within the nutritional community. For example, Kleiber (1972) presented a strong argument against using the joule in nutrition. He suggested that the calorie be used as the basic unit for measurement of heat (basis of all nutritional measurements) and that the joule be reserved as the basic unit (common denominator) for energy. In the view he expressed, the erg (10^{-7} J) would be retained as the unit for expressing mechanical energy, the unit for expression of work would remain $\text{m} \cdot \text{kg}$ (9.81 J), the unit for electrical energy would remain $\text{w} \cdot \text{s}$ (1.00019 J) and the unit for heat energy would remain the calorie (4.184 J). Other nutritionists argued in favor of retaining use of the calorie as a measure of heat energy on practical grounds. They argued that it is much easier to teach and apply the Physiological Fuel Value (PFV) system used in human nutrition when the respective values assigned carbohydrate, fat and protein are 4, 9 and 4 kcal/g as opposed to 16.7, 37.6 and 16.7 kJ/g. This author is sympathetic to this argument in that it is easier at my age to remember that the heat of combustion of acetate is 209 kcal/mole as compared to 874 kJ/mole and that the interspecific mean for fasting heat production by young adults is $70 \text{ kcal/W}_{\text{kg}}^{0.75}$ as compared to $293 \text{ kJ/W}_{\text{kg}}^{0.75}$.

I was not involved in this controversy, but many nutritionists in the US obviously found arguments in favor of the calorie persuasive, since many American scientific journals continued to accept the calorie as a measure of energy transactions in animals for some time after other nutritional journals had adopted use of the joule (J). This has now changed, in that the joule is fast becoming the standard measure of heat energy in US journals. A fundamental reason for acceptance of the joule as the commonly used measure of energy in most of the nutritional literature is the fact that the international calorie is the so-called 15°C calorie – the amount of heat required to raise the temperature of 1 g of water from 14.5 to 15.5°C (4.1858 J). The nutritional calorie has historically been the thermochemical 17°C calorie (4.184 J), because the benzoic acid standards used in nutritional research were calibrated in thermochemical calories, i.e. calorie estimates vary dependent upon water temperature and standards used in calibration, while the joule has an absolute value. Additional arguments favoring adoption of the joule were presented by Ames (1970). In this book, the joule will be used as the unit for expressing energy. Also, the term isoenergetic rather than isocaloric will be used to describe diets of equal energy content – we have not seen the term isojoulic utilized. On the other hand, classic terms such as direct calorimetry will be retained, because new terms such as direct joulimetry have not (yet?) been introduced and we do not choose to suggest basic changes in the English language.

5.2.2 Laws of thermodynamics

Three laws of thermodynamics are fundamental to nutritional energetics

and they will be discussed briefly in the following paragraphs. Our purpose in discussing these is not to be comprehensive. Interested readers are referred to Chapters 2 and 7, respectively, in the books of Brody (1945) and Kleiber (1961) for more detail regarding the nutritional relevance of thermodynamic concepts and appropriate texts in physical chemistry for further details.

The first law of thermodynamics holds that energy can be transformed from one form to another, but can neither be created nor destroyed. This law is absolutely essential to measurements and calculations utilized in nutrition. It dictates that one accept the equality

$$\text{MEI} = \text{TE} + \text{HE} \quad (5.1)$$

where MEI is defined as energy consumed by an animal and not excreted in feces or urine or emitted in the form of combustible gases, TE is energy deposited in animal tissues and HE is heat released by the animal. Thus, if one measures two of these, the third can be calculated by difference. It is interesting to note (Lusk, 1926) that this law was formulated separately in 1842 by Robert J. Mayer, a physician, and in 1847 by Herman Helmholtz, a physiologist. It was substantiated by James Prescott Joule, a brewer, in 1843 when he determined the heat equivalent of mechanical work ($1 \text{ m} \cdot \text{kg} = 9.81 \text{ J}$). This historical observation clearly indicates the commonality among biological and physical scientists in applying quantitative techniques in their research during this period.

The second law of thermodynamics holds that all forms of energy are quantitatively convertible to heat or, alternatively, that the driving force in all systems is the tendency to 'run down' to the lowest energy form, i.e. to release energy as heat. This law along with the law of Hess, which states that heat lost in a net chemical transformation is independent of path, are the basis of all measurements made in nutritional energetics. These laws, taken together, dictate that if one measures the heat released from a gram of fat upon total oxidation to CO_2 and H_2O in a laboratory calorimeter, the result will be exactly equal to the heat released when a gram of fat is totally oxidized by an animal.

The first law of thermodynamics indicates that if the energy content of an isolated system changes by a factor of ΔE , the following equality holds (terminology employed is from Brody, 1945)

$$\Delta E = q - w \quad (5.2)$$

where q is the energy transferred to an isolated system from its surroundings and w is the energy lost to or work done on the surroundings. In this equation, energy gained by a system is positive and energy losses are negative.

If a change in energy (ΔE) content of a system occurs at constant pressure as is basically the case for biological systems, the following holds

$$\Delta E = \Delta H = q - w \quad (5.3)$$

where ΔH is the enthalpy change or change in the potential heat content of the system. Under isothermal conditions, usually the case for homeotherms, the following holds

$$\Delta G = \Delta H - T\Delta S \quad (5.4)$$

where ΔG is the Gibbs free energy content of or energy available for useful work within the system and $T\Delta S$ is the change in energy content not convertible to work in the system. Under these conditions, ΔG is the maximal value of w and $T\Delta S$ is the minimal value of q .

Example cases which illustrate equation 5.4 are that when ice melts at 0°C no useful work can be done ($\Delta G = 0$; $\Delta H = T\Delta S$); and that when glucose is oxidized, ΔH is -2.82 MJ/mole, ΔG is -2.87 MJ/mole and $T\Delta S$ is $+0.06$ MJ/mole, i.e. ΔG values of foods are very close to ΔH . Since nutrients are consumed and used by the body to perform useful work, it can be argued that nutritionists should be concerned with the ΔG values of foods or nutrients. However, nutritionists use ΔH as their basic unit in assessments of energy transactions.

Two contrasting arguments have been posed. The first is that, theoretically, it would be best to adopt ΔG as the basis for evaluating nutrients, because it is an index of useful work that can be obtained from utilization of that nutrient. It follows from this argument that ΔH is used as a basis for evaluation of feeds because it is much easier to measure. The alternative argument is that ΔH is used because it is an index of total energy transactions in an animal and that is what nutritionists are interested in.

Biochemists use ΔG extensively. Several such applications will be elaborated upon here because arguments based upon application of ΔG will be used in later chapters. Biochemists use ΔG or the Gibbs free energy equation to determine the probable direction of a biochemical reaction

$$\Delta G = -RT\ln K_{\text{eq}} + RT\ln K_{\text{ma}} \quad (5.5)$$

where for a reaction $A = B$, the equilibrium constant K_{eq} is the ratio of product to substrate (B/A) at equilibrium; and the mass action coefficient (K_{ma}) is the ratio of product to substrate (b/a) in a specific biological condition. If $K_{\text{ma}} = K_{\text{eq}}$, $RT\ln K_{\text{eq}} = RT\ln K_{\text{ma}}$ and $\Delta G = \text{zero}$, net flux in the reaction is zero and no work can be derived from the reaction. This condition is approached in many biochemical reactions in animal tissues. If K_{ma} is less than K_{eq} , ΔG is negative and the reaction will proceed in the direction A to B. Further, if ΔG is a large negative number (-50.2 kJ/mole or less), one can postulate that, if coupled appropriately, the reaction could lead to the formation of a high energy bond ($\sim P$) in ATP and, via this intermediate, provide energy for the performance of useful work. Conversely, if K_{ma} is greater than K_{eq} , the reaction will proceed B to A in that particular tissue. The most common means used to estimate ΔG values for individual reac-

tions is by measuring K_{eq} . Then, for the standard conditions of 25°C, gases at 1 atm, and reactants and products at unit activity such that $K_{ma} = 1/1$ and $RT\ln K_{ma} = 0.0$,

$$\Delta G = -RT\ln K_{eq} \quad (5.6)$$

Standard conditions are not physiological. For example, if a hydrogen ion (H^+) is involved in a reaction, unit activity would imply a pH of 0.0. Tables of free energy values presented in most physiological and biochemical texts have been adjusted to a pH of 7.0 and are denoted as $\Delta G'$ values. Even so, many other factors must be accounted for in estimating ΔG values under physiological conditions. One must know the physiological concentrations of reactants and products to calculate K_{ma} . This is not always easy when the ionic environment can complicate such estimates. For example, $\Delta G'$ values for hydrolysis of the high energy phosphate bonds of ATP (kJ/mole) can vary as follows (Long, 1961):

pH		7.0	$\Delta G'$ 7.5	
	$ATP^{4-} \rightarrow ADP^{3-} + HPO_4^{2-} + H^+$	- 36.0	- 39.7	(5.7)
	$MgATP \rightarrow MgADP + HPO_4^{2-} + H^+$	- 29.3	- 32.2	(5.8)
	$ADP^{3-} \rightarrow AMP^{2-} + HPO_4^{2-} + H^+$	- 32.6	- 35.6	(5.9)

Further, ratios of ADP/ATP *in vivo* range from 0.01 for muscle to 0.3 for liver and mammary tissue, leading to physiological estimates of ΔG for hydrolysis of ATP ranging from approximately 33.5 to 50.2 kJ/mole. For the sake of simplicity and consistency in this book, a standard value for hydrolysis of the high energy phosphate bond of 41.8 kJ/mole has been adopted. The reader is asked to recognize that this value is a variable and that adoption of any constant value is a simplification.

One approach which could be utilized in applying the concept that ΔG values should be utilized in assessing the values of nutrients to animals would be to sum the ΔG values for individual reactions in the several pathways for utilization of that nutrient in each tissue. This has been done for some major nutrients and major pathways, but the physiological data required for application of this approach are clearly extensive and would vary with nutritional and physiological status. The complexity associated with application of this approach in terms of the data and extensive arithmetic required is further compounded when one considers the chemical complexity of most feeds.

An alternative approach to summing ΔG values for reactions involving the metabolism of specific nutrients is calculation of ΔG values for the utilization specific nutrients based upon comparing heats of formation of chemical bonds in nutrients and products of their metabolism. This approach was utilized to obtain the estimate, presented above, that the ΔG for oxidation of glucose to CO_2 and H_2O is 2.87 MJ/mole for glucose. This

approach does not require extensive data on concentrations of intermediary metabolites, pH, ionic environment, etc., but the chemical complexity of feeds still requires extensive work as compared to simply measuring the heat loss associated with total oxidation of feed (ΔH) in a bomb calorimeter. A person arguing in favor of the concept that ΔG should be used as a measure of feed values on theoretical grounds might accept the use of ΔH as acceptable on the practical ground that ΔH values are much easier to obtain.

The argument presented above in favor of the use of ΔH to express feed energy values, that nutritionists should be concerned with total energy transactions in an animal and not solely with free energy transactions, also has theoretical validity. The simplest examples that favor this view are the environmental extremes of cold and heat. In a cold environment, an important property of a feed would not be only the amount of useful work the feed could provide but rather the amount of heat released upon oxidation, since heat is required to maintain body temperatures in homeotherms. In extreme heat, the converse is true, since a homeotherm may have to expend useful energy to dissipate an excessive heat load imposed by a feed.

As a young scientist, this author had considerable difficulty reconciling these opposing views. This is evidenced in publications in which the ΔG value of -2.87 MJ/mole for glucose oxidation was used (Baldwin, 1968) and others in which the ΔH value of -2.82 MJ/mole was used (Baldwin and Smith, 1974). The effect of using either one or the other of these estimates was small, since $T\Delta S$ was small relative to ΔH and ΔG . However, the indecision was clear and more learned reviewers did not question the fact that different choices were made in these publications.

Finally, a light dawned and it is now embarrassing to admit that I ever accepted the application of ΔG over ΔH values. The reasoning is simply illustrated by the following example: total glucose oxidation ($\text{glucose} + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$) releases 2.87 MJ/mole. *In vivo*, when glucose is oxidized as an energy source, ATP is an intermediate product ($\text{glucose} + 38\text{ADP} + 6\text{O}_2 + 38\text{P}_i \rightarrow 38\text{ATP} + 6\text{CO}_2 + 6\text{H}_2\text{O}$) and $(2.87 - 38 \times 0.0418)$, 1.28 MJ/mole of heat is released; and, when the ATP is hydrolyzed to support physiological work ($38\text{ATP} + \text{H}_2\text{O} \rightarrow 38\text{ADP} + 38\text{P}_i$), 1.58 MJ/mole of heat is released. Thus, when 41.8 MJ/mole is used to represent the ΔG value for the $\sim\text{P}$ bond, only $(1.58/2.87)$ or 55% of potentially useful energy in glucose is trapped in ATP and the remainder is no longer available for useful work. If a value of 50.2 kJ/mole had been adopted for the ΔG value of $\sim\text{P}$, the estimated efficiency of trapping potentially useful energy would be 66% . Efficiency values for various nutrients calculated as above vary, but there are no known biological conditions under which the efficiency of trapping of potentially useful energy in the form of $\sim\text{P}$ upon oxidation of a nutrient even closely approaches 100% . If one accepts the concept clearly established in the 1950s that the high energy phosphate bond ($\sim\text{P}$) is the central cur-

rency in energy transactions in biological systems, one must conclude that ΔG is not an appropriate index of the energetic values of feeds because the maximal potential of energy potentially available from nutrients to support work (ΔG) is never realized in biological systems and, further, that the net efficiency of use of ΔG as a source of energy for work is a variable.

These arguments lead to the convention that underlies all of the biochemically based calculations which will be presented in subsequent parts of this book. The convention simply holds that if a nutrient is oxidized by an animal in support of a process necessary to maintenance of life, it is appropriate to compute the energy expenditures associated with that process in terms of ΔH as follows:

$$\text{heat production} = \text{moles of nutrient oxidized} * \Delta H \quad (5.10)$$

where ΔH is the heat of combustion of that nutrient in MJ/mole. This concept is developed in greater detail in Chapter 6 and is presented in Fig. 6.5 and Table 6.3.

5.3 HISTORICAL DEVELOPMENT OF BASES FOR FEEDING SYSTEM MODELS

5.3.1 Early history

Many persons view the history of science as a dull recitation of names, dates and discoveries. Even major early discoveries are not exciting because they are taken as givens today. There are, however, lessons to be learned from history about how and how not to ask questions, design experiments and interpret data; about priorities, politics and science; about intellectual bias; and about the basic structure of science. These points were well illustrated by excerpts presented below from an editorial by Irving H. Page (1974), a former director of the research division of the Cleveland Clinic:

A Sense of the History of Discovery

Far too many physicians and scientists, as well as laymen, look upon the history of discovery as an entertaining pastime, a tiresome academic exercise, or merely the record of egotistical aspirations. But it is essential to understand the successes and failures of others.

Here, Page states the view we expressed above.

Alexander Fleming was favored by fortune in having a mycologist working on *Penicillium notatum* on the floor above his laboratory. Fleming's laboratory was primitive, and outside air circulated in a way that only a rugged Scotsman would tolerate. Furthermore, he failed to wash his petri dishes before he went on vacation. Using common sense, Fleming followed up on an observation that many had made

before him. He looked at the petri dishes when he returned from vacation and did something about what he saw. Had Fleming been an active committee attendee, with plenty of dishwashers and technicians, it is unlikely he would ever have noted the clear zone around the bacterial colony, because a dutiful technician would long since have destroyed the evidence even if it had been allowed to develop.

We believe that the lesson Page sought to make here is that you cannot make discoveries in the absence of first-hand contact with your research.

A second example – the discovery of liver extract for the treatment of pernicious anemia resulted from work by Whipple, Minot, and Murphy. The pathologist Whipple was trying to determine the hematopoietic efficiency of diets. The clinician Minot was broadly enough read not only to know of Whipple's basic work, but to see its applicability. This led to its clinical use by Murphy, and the award of three Nobel prizes.

The lesson here is that one must be well and broadly read in both the basic and applied sciences. This is particularly relevant in nutrition, an applied science. To read only in your own narrow area of work limits your horizons. Can authors possibly anticipate or capture the broader relevance and applicability of their work in the limited number of key words that serve as the bases for current literature search algorithms?

The discovery of insulin by Banting and Best tells another story. Even though it had been conclusively shown that the pancreas was crucially involved in diabetes, none of the major research centers did much about it. It took a young unknown surgeon with avid curiosity and an equally eager young medical student to do what should long since have been done.

Here Page seeks to show that big science is not the only way to go. Sometimes the seemingly trivial, self-evident and cheap experiment is the correct one. In current jargon, such experiments are often called elegant. At this point, Page gets a bit antiestablishment:

Note that government grants and major planning committees representing consumers, economists, sociologists, *et al.* played no part in the above discoveries.

Today many investigators fail to appreciate that knowledge of the history of discovery is vital if wise policies are to be generated. Instead the young are being taught, by example, that status and celebrity-seeking promotes careers. Serving on important committees, belonging to the right societies, associating with the right people, and seeking discreet but maximum public exposure pay handsomely – if this is the way of life that is sought.

The life depicted may go under the guise of science but is it truly consistent with the image scientists would like to project of dedicated, highly moral, hard-working, creative people attempting to advance the cause of mankind?

Government, with the power of money at its disposal, plays a critical part. The power-oriented among us find it easy to accept the hegemony of government over practice and research, *forgetting that a sense of the history of discovery is almost wholly absent in those making the policy.*

Do the policy makers and scientists in power know how great discoveries are made, or do they view science as simply another form of technology? Remember, technology advances only on the basis of what we already know. I wonder what Page would have said today on this issue, given the increasing prominence evident today of 'in group' scientists, interest groups, administrators and political considerations in the setting of research policy.

If greatness is a goal, it will take great thinking and consummate honesty to achieve it. History has shown us and formulated the guidelines.

The following treatment of development of the feeding system models proposed early in this century is highly abridged and was heavily influenced by the article, *A History of Metabolism*, by Graham Lusk (1926) and several colleagues including, most prominently, Magnar Ronning and Max Kleiber. Mag Ronning was instrumental in creating a UC Davis course, currently entitled Nutrition 202: Advanced Energy Metabolism. This course was designed, in part, to replace Max Kleiber's course on *Fire of Life* subsequent to his retirement from the University of California. Having created this new course, Mag took advantage of an opportunity to spend a year in Egypt as a Fullbright Scholar and his new course, never previously taught, was assigned to me. Max, if I may be so bold as to use the familiar, chose to attend my lectures in this course. I had emphasized biochemistry in my Ph.D. program and was not at all competent in energetics except that I had enjoyed the courses I had taken in thermodynamics. After I had more than dutifully read Lusk's article (1926), Kleiber's *Fire of Life* (1961) and Brody's *Bioenergetics and Growth* (1945), as well as the then-current NRC publications on the energy requirements of several species (including humans), I understood that '*Fire of Life*' implied something regarding the difficulty early workers had in establishing the fact that combustion was essential to life. Also, my first offering of Nutrition 202 was trial by fire. I do not believe that my condition at that time was one of publish or perish, but rather one of understand or perish.

To further aid in my initiation, Mag suggested that I pose the following question to the class, 'Why doesn't the change in body weight plus the weight of excreta equal the weight of food and drink consumed?'. I must admit that I did not have an immediate answer to this question. I have

asked this question of graduate students in the first lecture of the Nutrition 202 class for 25 years now and very few students have had an immediate answer either. I am sure that readers of this book, after an appropriate and short period of deliberation about current knowledge of chemistry and metabolism, can answer the question. On the other hand, one can relate readily to early workers who did not have the benefit of current knowledge of chemistry and metabolism and to whom the question was readily apparent, but the answer was not.

A highly relevant observation was made by Leonardo da Vinci (1452–1519). He noted that animals could not survive in an atmosphere that could not also support a flame. One hundred years later, Von Helmont (1577–1644), inventor of the term *gas*, experimented with a gas which asphyxiated animals. It evolved from burning charcoal, from fermenting wine, when vinegar was poured on lime and was found in the Grotto del Carno, a mine near Naples. This gas was later identified as carbon dioxide. Robert Boyle (1621–1678), known for the gas laws he formulated, experimented with isolated portions of air that ‘permitted flames to burn and animals to live’. He called this ‘air imminently respirable’. John Mayer in 1675, a contemporary, confirmed Boyle’s observations and pronounced that he had ‘...clear proof that...fire and animals remove particles of the same nature from the air and that combustion deprives air of its “fire air particles” so as to render the air unfit to support flame’. Mayer was clearly answering the question, which by inference based upon then existent data was that



Unfortunately, Mayer met an untimely death at 39, before George Ernst Stahl (1660–1734) arrived on the scene. Stahl developed and popularized the phlogiston theory first proposed by J. J. Beecher in 1669. This theory held that combustible materials contain a substance called phlogiston, which is released during burning, an alternative answer for the question posed above. The phlogiston theory held that certain metals, when burned, changed their form due to loss of phlogiston, but their original metallic form could be restored (rephlogisticated) by roasting along with carbon, obviously a rich source of phlogiston since it burned so readily. This led to the belief that air was rendered unfit for supporting a flame or for breathing by becoming phlogisticated. This was contrary to Mayer’s data and belief that air was *deprived* of something. The birth, growth and acceptance of the phlogiston theory lacked the support of scientific experimentation. Even so, there were many who explained their observations in conformance with the phlogiston doctrine. This theory was strongly held by many until the late 1700s, or for over 100 years. For example, Joseph Priestly (1733–1804) observed in the 1770s that mint grows in an isolated atmosphere where previously a candle would not burn. Furthermore, after the mint had grown for several days, he found that a candle could again burn. His conclusion was that growing plants absorb phlogiston that had been added to

the atmosphere by the first burning candle, such that a second candle could then burn in the dephlogisticated air.

Given this conclusion it is not clear what prompted Priestly to perform another significant experiment. He heated red powder of mercury and observed that this produced an air in which a candle burned very brightly and vigorously. He assumed, therefore, that mercury removed phlogiston and that this must be 'pure dephlogisticated air', as compared with normal air that was only 'partially dephlogisticated'. He wrote

My reader will not wonder that after having ascertained the superior goodness of dephlogisticated air by mice living in it and other tests above mentioned, I should have the curiosity to taste it myself... The feeling of it to my lungs was not sensibly different from that of common air; but I fancied that my breath felt peculiarly light and easy for some time afterward. Who can tell but that in time this pure air may become a fashionable article in luxury? Hitherto, only two mice and myself have had the privilege of breathing it.

Whether in primary or secondary professional order, Priestly was a clergyman, apparently with unpopular religious beliefs, for he fled England, and died in Pennsylvania at the age of 71, still believing the phlogiston theory and unimpressed by the convincing experiments of Lavoisier (below). He apparently was unaware that he would be credited as one of the discoverers of oxygen, even though oxygen had been originally discovered by Boyle 150 years earlier.

There was a great deal of activity in gas chemistry during this period. Joseph Black (1728–1799) in the mid-1750s experimented with 'Magnesia Alba, Quicklime and other Alkaline Substances' and produced 'fixed air', later to be known as carbon dioxide. Henry Cavendish (1731–1810) discovered in 1766 a gas he called 'inflammable air', two volumes of which when combined with one volume of Priestley's 'dephlogisticated air' and an electric spark disappeared, leaving only water. Thus, hydrogen had been discovered. Daniel Rutherford (1749–1819) found in 1772 that when a candle had burned in an enclosed chamber until it went out and the 'fixed air' of Black was absorbed by alkali, there remained a large volume of air that extinguished life and flame immediately. He called his air 'residual air' and had discovered nitrogen. In part, these workers were rediscovering the wheel and were ignorant of the works of Von Helmont, Boyle and Mayer of the previous century. Sidetracks and misinterpretations accepted and promoted by the establishment (as was the case with the phlogiston theory) often necessitate such rediscoveries.

Carl Wilhelm Scheele (1742–1786), a Swedish apothecary working at about the same time as Priestley, also rediscovered oxygen, which he called 'fire air' because a flame burned so brightly in it. He held that phlogiston, given off by a burning object, united with 'fire air' and that this union was heat, which could pass through glass. Based on his interpretation of this

experiment, only Scheele knows why he conducted another significant experiment in which he placed two bees in a cylinder that had been filled with 'fire air' and inverted over lye water to absorb the 'fixed air' of Black. When the bees died 8 days later, the gas in the cylinder had been almost completely replaced with the lye water.

Scheele had run the first respiration experiment and had disproved the phlogiston theory by showing that when his 'fire air' disappeared, 'fixed air' was produced, e.g. not only heat formed from the union of phlogiston and 'fire air'. Scheele had made a breakthrough observation but misinterpreted it to conform with dogma and strongly supported the phlogiston theory until his death in 1786.

In September of 1774, Priestly dined with Lavoisier in Paris and discussed his experiments with 'pure dephlogisticated' air. In October of 1774, Scheele wrote to Lavoisier and reported his experiments with 'fire air' and bees. During the Easter period of 1775, Lavoisier read a paper to the French Academy of Sciences. He reported that when red mercury was heated, a gas was evolved in which flame burned with the splendor of phosphorus and that this gas was 'air eminently respirable'. Further, he reported that the loss in weight of the red mercury was equal to the weight of the gas evolved. Clearly then, red mercury did not dephlogisticate the air, it added 'air imminently respirable'.

Two years later, Lavoisier reported that when sparrows were confined in sealed chambers 'air imminently respirable' disappeared from the atmosphere and was replaced by 'fixed air' or CO_2 , e.g. he repeated the bee experiment of Scheele. Three years later in 1780, Lavoisier and Laplace published their observation that when equivalent amounts of carbonic acid were produced by a guinea pig and by burning carbon within their ice calorimeter (Fig. 5.1), equivalent amounts of ice were melted. This established the relationship between O_2 use, CO_2 production and heat production. The breakthrough relating combustion and metabolism had been made, permitting formulation of the following equation:



In a relative sense nothing so remarkably new has been discovered in the field of energy metabolism. The discovery of ATP and the fact that high energy phosphate bonds are the central currency of energy transactions in biological systems may be comparable. The notable lesson is the fact that development, by Lavoisier, and acceptance of the theory of combustion and metabolism was severely delayed by general acceptance of the phlogiston dogma.

Lavoisier, although highly regarded by many scientists of his day, apparently was a bit abrasive. For example, in reporting his theory on combustion, he referred to the phlogiston doctrine as being an

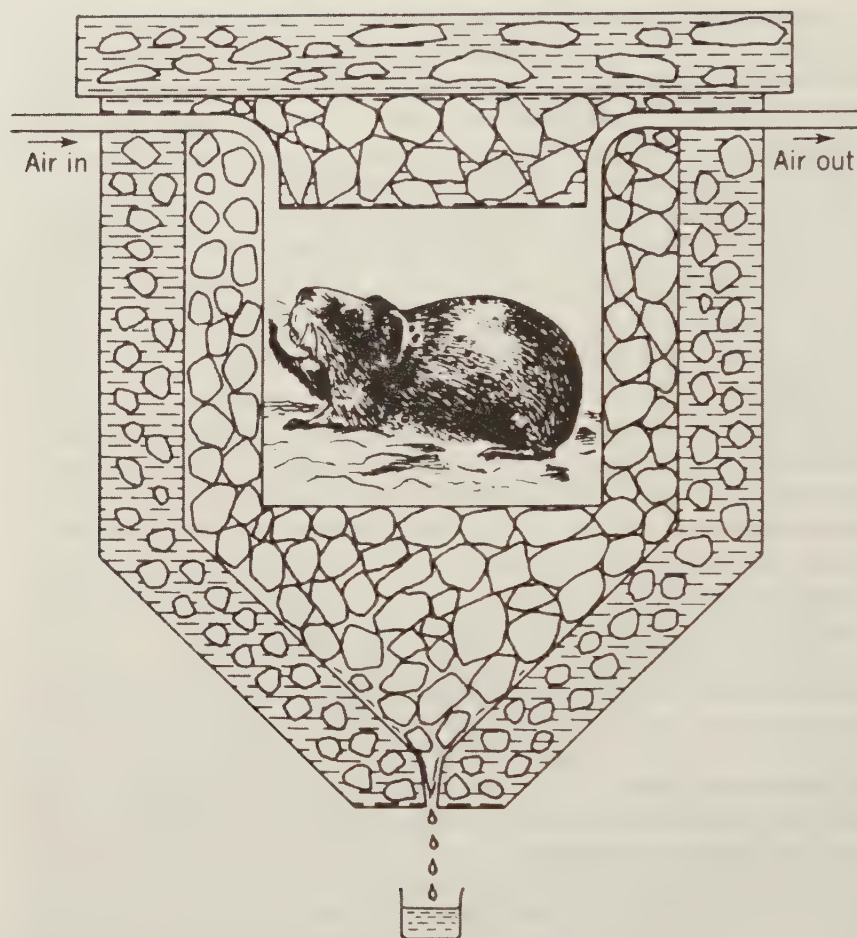


Figure 5.1 Lavoisier's calorimeter. The animal melts the ice and the water thus formed drains out of the apparatus. The weight of the water measures the animal's heat production (335 kJ of heat melt 1 kg of ice). Mixture of ice and water in jacket prevents heat flow through walls (adiabatic jacket). From Kleiber (1975) *The Fire of Life*, John Wiley, New York.

imaginary creation...scaffolding more embarrassing than useful for the continuance of the structure of the science of chemistry.

Joseph Black wrote to Lavoisier,

Having for 30 years believed and taught the doctrine phlogiston as it was understood before the discovery of your system, I for a long time felt inimical to the new system which represented as absurd that which I had hitherto regarded as sound doctrine, but this enmity, which springs only from force of habit has gradually diminished, subdued by the clearness of your proofs and the soundness of your plan.

Whether due to Lavoisier's provocative style or the inherent lethargy of science, the new theory of combustion was not accepted readily. Priestly, who lived until 1804, never accepted the theory. Scheele, who had done the first really critical experiment, wrote,

Is it impossible to convince Lavoisier that his system will not find universal acceptance.

Jean-Paul Marat, a physician and leader in the French Revolution wrote,

Lavoisier, the putative father of all the discoveries that are noised about, having no ideas of his own, snatches at those of others, but having no ability to appreciate them, he quickly abandons them and changes his theories as he does his shoes.

Of course, Marat had his own theories, which had been ridiculed by Lavoisier. Some histories hold that Marat's emnity led to Lavoisier's trip to the guillotine on a trumped up charge of levying an illegal tax on the water in tobacco. Because of his contributions, Lavoisier is credited as the 'Father of Nutrition'.

5.3.2 Partition of feed energy

After the pioneering work of Lavoisier, new objectives were appropriate for workers in nutritional energetics: establish relationships between gas exchange and heat production; devise bases for evaluation of foods that can be related directly to energy requirements and expenditures; and establish causes of energy expenditures. The laws of thermodynamics had to be established. These were developed in the 1840s as discussed above. Berthelot (1827–1907) invented the oxygen bomb calorimeter, which enabled workers to determine the gross energy contents of organic compounds, feeds, feces and urine in a reproducible and accurate fashion (Fig. 5.2). Another essential advance was development of the concept that foods should be partitioned into carbohydrates, fats and proteins because their metabolism differed. The chemists who made major contributions to development of this concept were Justus Von Liebig (1803–1873) and his students. One of Von Liebig's students, Carl Von Voit, utilized the open-circuit respiration apparatus developed by Max Von Pettenkofer (1818–1901) to do extensive energy balance experiments. The open circuit system of Von Pettenkofer (Fig. 5.3) was the prototype of modern instruments. The open circuit design of Von Pettenkofer's respirometer used outside air and measurements of CO₂ production and carbon and nitrogen balance to compute heat produced. Another respiration apparatus was the closed-circuit system of Regnault (1810–1878). In closed-circuit chambers such as that of Regnault (Fig. 5.4), air was circulated through adsorbents to remove CO₂ and water vapor, and oxygen was added to compensate for its use before the air was returned to the chamber. An advantage of the Regnault system

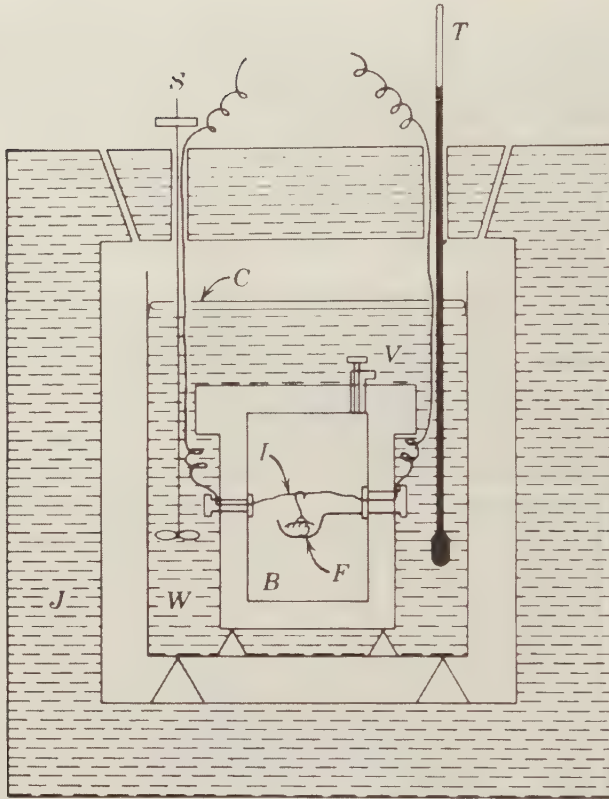


Figure 5.2 Cross-section of a bomb calorimeter. From Kleiber (1975). *The Fire of Life*, John Wiley, New York, originally from chapter by Kleiber, in Über (1950) *Biophysical Research Methods*, Interscience Press, New York.

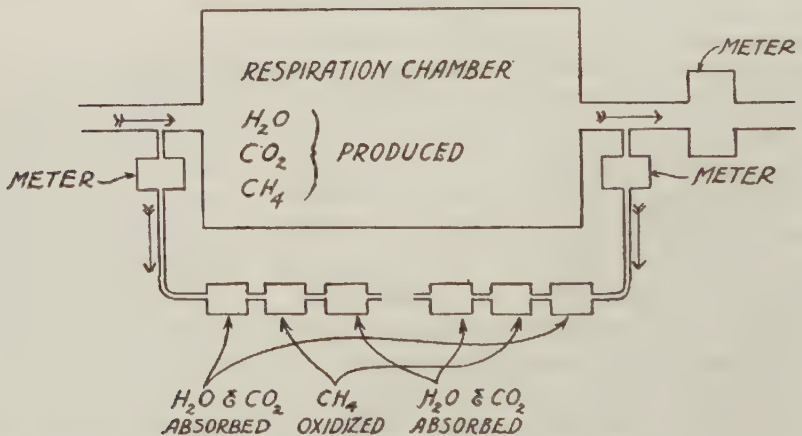


Figure 5.3 Scheme of Pettenkofer respiration apparatus. Adapted from Armsby (1917).

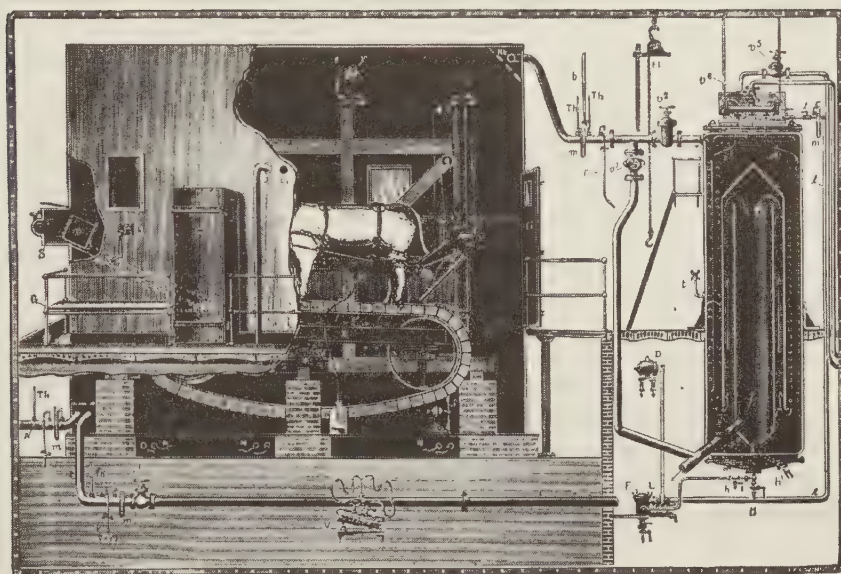


Figure 5.4 Regnault-Reiset apparatus as used by Zuntz. Adapted from Armsby (1917).

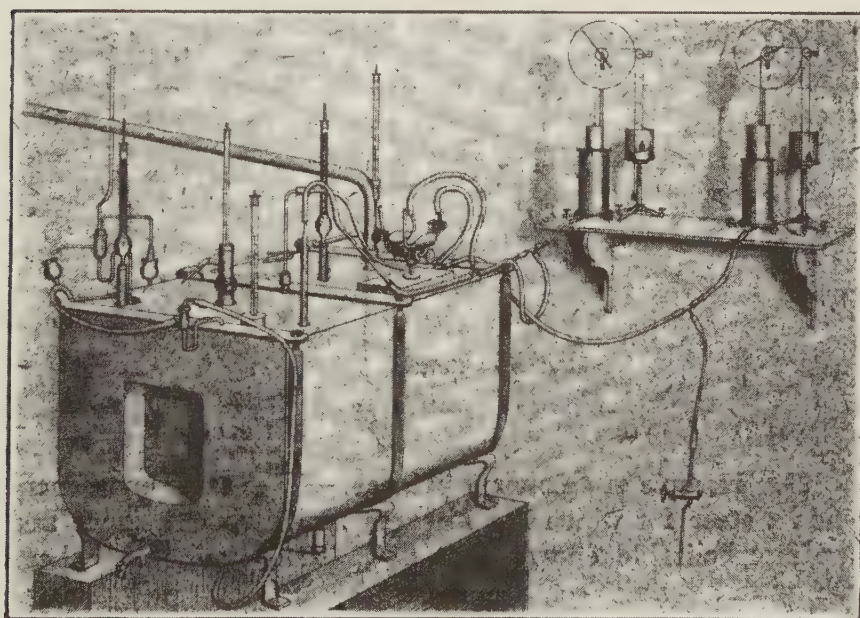


Figure 5.5 Rubner's respiration calorimeter. Adapted from Armsby (1917).

was that estimates of oxygen consumption were easy, since one only need measure the amount of oxygen added to the system.

Early use of indirect calorimetry required workers assume that the laws of thermodynamics apply to biological systems. This view was not generally acceptable to theologians at that time but essential to the use of heats of combustion determined on pure compounds in the bomb calorimeter and to establish a direct relationship between respiration data and heat production *in vivo*. The classical experiment required to establish this relationship was performed by Rubner, who used a respiration calorimeter (Fig. 5.5). In this instrument, water surrounding an open-circuit respiration chamber was used to measure heat production directly, while respiratory exchange was used to estimate heat production indirectly. Rubner's data, presented in Table 5.1, clearly established that the laws of thermodynamics apply to biological systems. What is amazing is the accuracy of the measurements reported. Modern instruments used for direct and indirect calorimetry are certainly more convenient and yield greater productivity, but one can wonder whether or not accuracy has been improved.

Table 5.1 Rubner's results with dogs, using direct methods of estimating heat and the carbon and nitrogen balance method^a

<i>Treatment</i>	<i>Heat measured (kJ)</i>	<i>Estimated from metabolism (kJ)</i>	<i>Discrepancy (%)</i>
Fasting	5460	5422	-0.7
Fasting	4565	4422	-3.1
Fat diet	6268	6318	+0.8
Meat and fat diet	16,560	16,673	+0.7
Meat diet	19,953	20,004	+0.3

^aAdapted from Blaxter (1967)

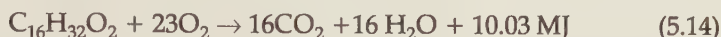
Early studies using these methods established the calculations currently used as the theoretical bases for calculations in use for interpretation of respiratory exchange data. Carbohydrate combustion



yields a respiratory quotient (RQ) of (mole CO₂ produced/mole of O₂ used) of one. The heat of combustion (ΔH_c) of glucose in a bomb calorimeter is 2.8 MJ/mole or 15.6 kJ/g. Thus [(2.8 MJ/mole of glucose) * (6 moles O₂ used or CO₂ produced)⁻¹], 469 kJ of heat are produced for each mole of O₂ used or CO₂ produced when glucose is oxidized as an energy source by an animal. Further [(469 kJ/mole glucose * (22.4 l/mole CO₂ or O₂)⁻¹], 20.92 kJ (5.0 kcal) of heat are produced per l O₂ used or CO₂ produced.

A tabular method for analogous calculations for a fat of elemental composition 76% C, 12% H and 12% O (palmitic acid) is presented in Table 5.2.

The heat of combustion of palmitic acid is 10.03 MJ/mole or 39.1 kJ/g, since its molecular weight is 256.4 g/mole. Thus, the stoichiometric equation for oxidation of palmitic acid is as follows:



Calculations analogous to those presented above for glucose indicate that for palmitic acid oxidation the RQ is 0.708 and heat released per 1 O₂ used is 27.9 kJ.

Equations for respiratory exchange and heat release associated with glucose and palmitate oxidation can be expressed as follows (Blaxter, 1967)

$$6x + 6y = 2.8 \text{ MJ} \quad (5.15)$$

and

$$23x + 16y = 10.03 \text{ MJ} \quad (5.16)$$

where x is O₂ consumed and y is CO₂ produced. Solving these as simultaneous equations yields respective values for x and y of 361 and 108.7 kJ/mole and

$$\text{Heat Release} = (16.1 \text{ kJ/l O}_2) * (\text{l O}_2 + (4.9 \text{ kJ/l CO}_2) * \text{CO}_2) \quad (5.17)$$

when a mixture of carbohydrate and fat is oxidized.

The tabular computation for meat protein in Table 5.3 indicates an RQ for meat protein oxidation of 0.83 when 100 g of meat protein is catabolized as an energy source, e.g. 83 l of CO₂ are produced and 100 l of O₂ are used (Kleiber, 1975). Also, since meat protein contains 17% N, respiratory exchange can be expressed as (100 l O₂)/(17 g N), 5.9 l O₂/g N and 4.9 l CO₂/g N. Meat protein catabolism in fasting subjects is estimated by multiplying g urinary N by 5.88 rather than the value of 6.25, which applies to the catabolism of plant proteins because the N content of meat protein is 17%, while that for plant proteins is 16%. The heat of combustion for complete combustion of meat protein in a bomb calorimeter is 23.8 kJ/g. Thus, body energy loss associated with one gram of urinary N is (1.0 * 23.8 * 5.88) or 140 kJ. However, protein is not totally oxidized by animals, because urea is the form in which N is excreted. The heat of combustion of urea (NH₂-CO-NH₂) is 636 kJ/mole or 22.6 kJ/g urea N. Thus, (140 kJ protein energy/g urinary N - 22.6 kJ/g urea N) 117.6 kJ of heat are produced per gram of urinary N lost during fasting; and heat production per 100 g meat protein catabolism (17g N * 117.6 kJ) is 2.0 MJ/100 g protein. Similarly, 20 and 24 kJ, respectively, of heat are lost per l of O₂ used and CO₂ produced during protein catabolism. The values derived above are summarized in Table 5.4. These values derived from ΔH_c and chemical balance equations can be used to estimate energy balance relationships as follows.

Example A is a fasting subject, where a basic assumption is that primary fuels available are body fat and protein. With two substrates, two measure-

Table 5.2 Tabular calculations of respiratory exchange for fat oxidation^a

Element	g%	Atomic weight	g atoms/100 g fat	Internal oxidation	Available for respiration	O ₂ required	Mole CO ₂
C	76	12	6.3	—	6.3	6.3	6.3
H	12	1	12	-1.6	10.42 ^b	2.6	—
O	12	16	0.8	-0.8	—	—	—

^aAdapted from Kleiber (1975)

^b10.4 H + 2.6 O₂ → 5.2 H₂O

Table 5.3 Respiratory exchange in meat protein oxidation^a

Element	g%	Atomic weight	g atoms/100 g protein	Urea	100 g protein—moles urea	Internal oxidation	Respiration	O ₂ Requirement ^b
C	52	12	4.30	0.6	3.7	—	3.7	3.70
H	7	1	7.00	2.4	4.6	1.6	3.0	0.75
O	23	16	1.40	0.6	0.8	0.8	—	—
N	17	14	1.20	1.2	—	—	—	—
S	1	32	(0.03)	—	—	—	—	—

^aAdapted from Kleiber (1975)

^bSee calculation of RQ in text

Table 5.4 Estimated heat production and energy losses associated by respiratory exchange and nitrogen balance data

	kJ/l CO_2	kJ/l O_2	$\frac{\text{kJ/g N}}{(\text{urinary N as uca})}$	RQ
Glucose	20.9	20.9	—	1.0
Fat	27.0	19.7	—	0.7
Protein	20.0	24.0	140 ^a or 117.6 ^b	0.8

^a Body energy loss.

^b Heat production.

ments are required: CO₂ production (1275 l/day) and urinary nitrogen losses (53 g/day). Calculations follow:

- Total carbon loss (assume all urinary N is urea).

(a) as CO ₂ : (1275 l CO ₂ /22.4 l/mole) * 12 g C/mole CO ₂	=	683 g C
(b) in urea: (0.43 g C/g urea N) * 53 g urea N	=	23 g C
TOTAL		706 g C
- Protein carbon loss.
53 g urea N lost * (5.88 g meat protein/g urea N) * (0.52 g C/g protein = 162 g protein C/day
- Fat loss.
(706 g total C loss – 162 g protein C) * (0.76 g C/g fat)⁻¹ = 715 g fat/day
- Heat production.
(117.6 kJ/g N) * 53 g urea N + 715.8 g fat * 39.1 kJ/g = 34.2 MJ/day
- Body energy loss.
(140 kJ/g N) * 53 g urea N + 715.8 g fat * 39.1 kJ/g = 35.4 MJ/day

The current international (Brouwer, 1965) regression equation for estimating body energy loss during fasting yields an estimate

$$[E_{\text{loss}} = (51.88 \text{ kJ/g C}) * (706 \text{ g C}) - (19.4 \text{ kJ/g N}) * (53 \text{ g N})] \quad (5.18)$$

of 35.57 MJ/day. Thus, the estimate based upon chemical balance and heat of combustion data is very close to that based upon direct estimates.

Example B is an animal fed a normal diet. Since three chemical entities must be considered – carbohydrate, fat and protein – three measurements are required.

data: urinary N = 0.37 g/h
 CO₂ = 9.48 l/h
 O₂ = 11.4 l/h

Calculations follow:

- Protein catabolism (all urinary N assumed urea).
 0.37 * 4.9 l CO₂/g urea N = 1.81 l CO₂
 0.37 * 5.9 l O₂/g urea N = 2.18 l O₂
 0.37 * 117.6 kJ heat/g urea N = 43.5 kJ/h due to protein oxidation
- Calculation of non-protein RQ (NPRQ).
 NPRQ = l CO₂ = 9.48 – 1.81 = 7.67 l CO₂ = 0.83
 l O₂ 11.4 – 2.18 9.22 l O₂
- Calculate proportions of CO₂ from fat (C_f) and CH₂O (C_{CHO}) using equation derived by Kleiber (1975).

$$C_f = 2.41/\text{NPRQ} - 2.41 = 49\% \quad (5.19)$$

$$C_f = 2.41/0.83 - 2.41 = 0.49$$

$$C_{\text{CHO}} = 1.0 - 0.49 = 0.51$$
- Calculate heat production.

$$\begin{aligned}
 0.49 * 7.67 \text{ l CO}_2 * 27.9 \text{ kJ/l CO}_2 &= 104.9 \text{ kJ from fat} \\
 0.51 * 7.67 \text{ l CO}_2 * 20.9 \text{ kJ/l CO}_2 &= 81.8 \text{ kJ from CH}_2\text{O} \\
 &+ 43.5 \text{ kJ from protein} \\
 \text{Total} &230.2 \text{ kJ/h}
 \end{aligned}$$

Heat production calculated using the current international (Brouwer, 1965; Blaxter, 1967) equation for normal diets is

$$\begin{aligned}
 [\text{HP} = (16.18 \text{ kJ/l O}_2) * (11.4 \text{ l O}_2) + (5.02 \text{ kJ/l CO}_2) * (9.48 \text{ l CO}_2) \\
 - (5.99 \text{ kJ/g urinary N}) * (0.37 \text{ g urinary N})] \quad (5.20)
 \end{aligned}$$

or 230 kJ/h. Thus, early workers using pure theory were obtaining estimates very close to those obtained today, even though the current multiple regression equations accommodate body and diet components other than glucose, palmitate and meat protein.

5.3.3 Development of feeding systems models

(a) Early terminology

Armsby, Atwater, Kellner and Rubner, all of whom studied with Von Voit, used the methods of direct and indirect calorimetry to develop the basic definitions and concepts that underlie feeding systems in use today. Basic definitions for the terms gross energy (GE), digestible energy (DE), metabolizable energy (ME), net energy (NE), specific dynamic action, work of digestion and thermal energy were established. Definitions quoted below are from Armsby (1917). Gross energy (GE) is the 'Amount of energy manifested as heat when the feed is completely oxidized'. Metabolizable energy (ME) is 'The energy of the feed minus the gross energy of the excreta'. This is an index of 'Energy capable of transformation within the body'. 'The net energy value of a feed for maintenance (NE_m) is measured by the loss of body energy which it prevents'. 'The expenditure of energy by the body which results from the ingestion of feed has been..., designated as work of digestion...a collective expression for the energy cost to the organism of all the various processes involved in the digestion and assimilation of feed'. The term heat increment of maintenance (HI_m) was adopted later to designate these latter energy costs. 'The net energy value of the same feeding stuff may differ according to the species of animal by which it is consumed and the purpose for which it is used'. '...Net energy values for growth...represent that portion of the feed energy supplied in excess of the maintenance requirement which the animal is able to store up in the gain mode'. '...Net energy value of feeding stuff for milk production...is that part of the feed energy supplied in excess of the maintenance requirement which is recovered in the product'. The term heat increment of production (HI_p) was adopted later to designate energy costs associated with product synthesis.

Despite this early agreement regarding evaluations of foods and feeds and their use for maintenance and productive functions, there are long-standing disagreements in terminology. Many terms have been invented and applied to describe energy transactions that occur in animals. Historical terms such as 'specific dynamic action' have appeal and are still used, even though they imply little about underlying relationships. Other terms, including 'work of digestion', have specific but incorrect implications; and still others such as 'diet induced thermogenesis' have multiple definitions corresponding to either H_dE (see below) and/or effects of plane of nutrition upon fasting heat production. A term which has multiple mathematical definitions is of little value in a quantitative science.

(b) Current terminology for energy

A subcommittee of the Committee on Animal Nutrition of the NRC (1981) developed a systematic terminology for description of energy utilization by animals that is consistent with most feeding systems in current use. This terminology is presented here because it will be utilized in this book. Ingested energy (IE) is defined as the heat of combustion of feed times the amount of feed consumed. Fecal energy (FE) is the heat of combustion of feces. This includes energy in compounds of both dietary (F_iE) and metabolic (F_mE) origin. Apparent digestible energy (DE) is IE minus FE. True digestible energy (TDE) equals DE + fecal energy of endogenous (F_eE) or metabolic (F_mE) origin. Urinary energy (UE) is energy in urine of both dietary (U_iE) and endogenous (U_eE) origin. Gaseous energy (GE) is energy in combustible gases produced in the digestive tract. Metabolizable energy (ME) equals DE minus UE and GE. True and corrected ME estimates correct for fecal and urinary energy of endogenous origin and heat losses due to fermentation (H_fE) in the digestive tract, respectfully. Recovered energy (RE) is energy recovered in tissue (TE), milk (LE), eggs (OE), conceptus (YE) and wool (VE). Heat energy (HE) is defined as energy lost as heat due to basal or fasting metabolism (H_eE); costs of digestion, absorption and assimilation of meals fed at maintenance (H_dE); costs of production ($H_pE = \Sigma H_iE, H_rE, H_oE$, etc.); and costs of voluntary activity (H_vE), thermoregulation (H_cE), and waste synthesis (H_wE). In aggregate, H_rE, H_dE, H_fE and H_wE can be referred to as heat increments (H_iE) of maintenance and production.

As with all systems of nomenclature, this system is imperfect. However, it offers the major advantage that all terms are defined mathematically, thereby reducing errors in interpretation and communication. It is compatible with terminology used in the UK and Western Europe. Also, it is compatible with partitioning apparent maintenance requirements and costs of production into component parts. In this NRC (1981) system, the classical term, gross energy intake, has been replaced by intake of energy (IE). Conventional definitions of apparent digestible and metabolizable energy are retained. The term recovered energy (RE) replaces the older, collective term

Net Energy in Product (NE_p). Use of the term RE obviates confusion between NE_x values assigned to feeds (discussed below) and energy transactions in the animal.

Partition of total heat production (HE) into meaningful physiologic or metabolic components is the most complex and controversial aspect of all systems of nomenclature. This is due in part to lack of knowledge and quantification of the physiologic and metabolic processes that contribute to total heat production; systematic differences among estimates of components of heat production that should be identical on mathematical grounds or by definition, but which differ because alternate methodologies may aggregate energy expenditures associated with somewhat different metabolic processes and, as a result, differ (Chapter 6); and the attempt to reconcile terminology with academic or research goals, as well as the pragmatic requirement that the terminology accommodate current and future feeding systems. These problems will be elaborated upon in subsequent chapters.

The terms defined above allow descriptions of energy transactions within an animal in accord with the first two laws of thermodynamics. For example, a lactating, growing animal might partition ingested energy as follows: $IE = FE + GE + UE + SE + HE + RE$, where $RE = TE + LE$; and, if desired, $HE = H_eE + H_jE + H_rE + H_cE + H_dE + H_wE + H_fE$. Further partition, e.g. of H_rE into costs of milk lactose, protein and fat synthesis, and body fat and lean synthesis, is permissible as long as the equality of input and output is satisfied.

(c) Evolution of energy systems

Ideally, a system for evaluating feeds should predict with accuracy the performance of an individual based on knowledge of (a) body weight, environment, activity and rate of production; and (b) amount of each feedstuff in the diet and its nutritive value. No system completely satisfies this need. Based upon the view that ME is a measure of the energy from a feedstuff available for transformation in the body, a number of systems generally based upon estimates of feed values and animal requirements in terms of ME were developed. These included the Physiological Fuel Value (PFV) system of Atwater, which is still in use by human and laboratory animal dieticians, and the Total Digestible Nutrient (TDN) system, for which data are still included in many NRC publications on the nutrient requirements of livestock. The current NRC systems for swine and poultry and the British Agriculture and Food Research Council (AFRC, 1990) systems for ruminants are examples of applications of this concept. Other scientists elected to evaluate feeds based upon their net energy values for specific functions such as fattening, maintenance, growth and lactation. The starch equivalent system, based upon the net energy values of feeds for fattening, was the most prominent and widely adopted example of a system based upon the net energy concept. The NRC (1984, 1989) systems for beef and dairy cattle

Table 5.5 The basis for calculating physiological fuel values per gram of human food

	Average gross energy/g (kJ)	Assumed digestion coefficient (%)	DE/g (kJ)	Assumed loss in urine (kJ)	PFV/g (kJ)
Protein	23.64	91	21.50	(1.25 * 0.91 = 4.77)	16.74
Fats	39.33	96	37.74	none	37.65
CH ₂ O	17.36	96	16.65	none	16.74

are based upon net energy values of feeds for maintenance, growth and lactation and are current examples of systems based upon the net energy concept.

The PFV system currently in use for evaluating nutritive values of foods for human consumption was developed by W.O. Atwater and his associates (Atwater and Bryant, 1900). The nutritive values employed are called PFVs and are essentially ME values determined at maintenance rates of food intake. A correction for energy losses due to gaseous products of digestion was not included but does not produce a significant error in non-ruminant species. Data used to derive the PFVs of protein, fat and carbohydrate are given in Table 5.5. The digestibility coefficients used were average values for a wide range of standard human foods at maintenance feeding. The correction applied for protein was estimated based upon the replacement value of protein for carbohydrate as an energy source in the diet. We now know that this correction factor of 4.8 kJ/g protein approximately reflects the cost of urea synthesis plus the energy content of urea excreted in urine. Formation of 1 mole of urea by the urea cycle requires 4 moles of ATP or 2 moles ATP/mole amino nitrogen. Assuming protein contains 16% N and using 74 MJ/mole ATP as the average heat loss/ATP used (basis for this value is developed in Chapter 6), the cost of formation of urea would be 1.7 kJ/g protein. Excretion of urea results in a loss of 23 kJ/g N or 3.8 kJ/g protein. Modifications of these physiological fuel values, which take into account digestibility results from more recent experiments with humans, were used to develop the standard tables of energy values of foods in use today.

In the TDN system, values for feeds were calculated from estimates of the digestible chemical components of feed as follows:

$$\text{TDN} = \text{DCP} + \text{DFIBER} + \text{DNFE} + \text{DEE} \times 2.25 \quad (5.21)$$

where TDN is expressed in pounds/pound, and DCP, DFIBER, DNFE and DEE are digestible crude protein, fiber, nitrogen free extract and ether extract. TDN requirements of animals are estimated by summing tabular values of TDN required for maintenance expressed as a function of body weight, TDN required for a given rate of gain, TDN required for production of milk expressed as a function of fat content, TDN required for activity, pregnancy, etc.

In the starch equivalent system, feed values were measured in terms of amount of energy stored in fat per kilogram of feed provided above maintenance in adult steers as compared to energy retained per kilogram starch provided above maintenance. Animal requirements were, again, estimated as a simple sum of requirements for maintenance, growth, lactation, etc.

(d) Limitations of current systems

These early systems incorporated a number of systematic errors that were addressed in development of systems currently in use by animal nutrition-

ists (see Baldwin, 1986, for a more detailed discussion). Several problems with the TDN system were recognized early. One was that maintenance requirements of animals were estimated based upon the observation of Rubner that heat production at maintenance varied across species as a function of surface area and was estimated as $W^{0.67}$. Brody (1945) and Kleiber (1932) developed interspecific relationships between fasting heat production (FHP) and body weight (Chapter 6) which led to adoption of the well known allometric equation:

$$\text{FHP} = a * W^{0.75} \quad (5.22)$$

The value of the coefficient (a) for young adults was 0.29 MJ/unit of metabolic body weight ($W^{0.75}$). It was also noted early that application of the concept of metabolic body weight within a species led to considerable variance in the coefficient (a) dependent upon age, previous plane of nutrition and physiological state (growing, lactating, pregnant, etc.). Brody (1945) called specific attention to these sources of variance. Later, workers utilizing statistical fitting routines, including Thonney *et al.* (1976), found that superior fits for the relationship between FHP and body weight within species are obtained when both the coefficient and exponent are allowed to vary. This approach reduces variance attributable to age but not to previous plane of nutrition or physiological state and, as a result, has not been widely adopted. Most current feeding systems accommodate variation in FHP and apparent maintenance requirements by adjusting the coefficient (a) dependent upon age and physiological state.

A second major problem with the TDN system was that TDN values measured for forages and concentrates fed to ruminants were not additive; TDN from forages supported lower rates of production than did TDN from concentrate. This discrepancy cannot easily be corrected, because the relative values of TDN from forages and concentrates differ for different animal functions (maintenance, growth, etc.). The differences in the true feeding values for forages and concentrates are partly due to greater relative energy losses in methane from forages as compared to concentrates. This error was accommodated early by redefinition of ME as energy in feed minus energy losses in excreta and combustible gases.

Other causes for the non-additivity of forage and concentrate TDN values are differences in heat losses during fermentation, differential changes in digestibility associated with intake, and differences in products of fermentation and their inherent efficiencies of utilization by the animal. All of these tend to vary as collinear functions of the energy density of the feed expressed as ME/kg (Baldwin, 1986). Thus, current ME-based systems incorporate a correction factor based upon energy density of the feed. For example, the estimate of efficiency of nutrient use for maintenance used to estimate the ME required for this process might vary from 0.65 to 0.80 dependent upon ME/kg feed. Similarly, the ME requirement for gain (ME) would be calculated as: $\text{ME}_{\text{required}} = \text{energy in gain}/k$, where k might vary

from 0.33 to 0.65 depending upon the composition of expected gain and ME/kg diet. ME requirements of animals in current ME-based systems are calculated as the sum of tabular values of ME required for maintenance, growth, lactation, etc. as with early systems. The tabular estimates of ME requirements for each function vary dependent upon the energy density of the feed.

The starch equivalent system was adopted widely and is still in use in Eastern Europe. In the UK and Western Europe, this system has been replaced with ME-based models. The major problem with the starch equivalent system was one of those encountered with the TDN system, i.e. that relative values assigned feeds were not additive across functions. This is because, in this system, feeds were evaluated solely on their efficiency of use for fat gain, and relative values of feeds for fattening differ from their relative values for other functions. For example, if diet fat is used to synthesize body fat, the efficiency is 94–97%. In contrast, if carbohydrate is fermented in the rumen to form acetate and the acetate is used to synthesize body fat, the biochemical efficiency is 60–65%. Thus, the SE value of starch as compared to fat is about 0.5. When diet fat and starch are used to support maintenance, the relative value of starch to fat is about 0.85. Thus, in the starch equivalent system, the value of fat for support of maintenance is significantly overestimated relative to starch. A similar undervaluation of forage relative to corn for support of maintenance is also observed.

The NRC (1984) adopted a net energy model for use in the beef cattle industry. In this, feedstuffs are assigned separate net energy values for maintenance (NE_m) and gain (NE_g). Then, animal requirements for maintenance and gain are computed, separately. Feed NE_m values are used to compute the feed requirement for maintenance and NE_g values are used to compute the feed required to support a given rate of gain. This approach overcomes the major problems with additivity of feed values discussed above for the TDN and starch equivalent systems. The system yields excellent predictions for British breeds of cattle, since their maintenance requirements and composition of gain are similar throughout the standard feeding period. Adjustments for 'frame size' were included to accommodate differences in composition of gain at a given weight in medium versus large breeds of cattle (see Chapter 12).

Chemical composition of rations is another source of variance in energy expenditures in maintenance and production and products of digestion absorbed and actually available to the animal. A number of examples that illustrated this source of variance were developed by Baldwin *et al.* (1980). Products from fermentation of soluble carbohydrates, starch, hemicellulose and cellulose differ considerably as they are fermented by different microbes (Murphy *et al.*, 1982), with the result that heat and methane losses associated with the fermentation of forages are considerably greater than with concentrate rations. Thus, the mix of absorbed nutrients from forages are used at lesser efficiencies for maintenance and production than ab-

sorbed nutrients from concentrate rations. The use of dietary energy density adjustment factors to better accommodate these sources of variance was noted above. This approach helps a great deal but does not completely eliminate the problem. This led Baldwin *et al.* (1977, 1987b) to suggest that the use of mechanistic elements that explicitly describe specific chemical entities in diets, the biochemistry of their fermentation, and pathways of utilization of the several fermentation products by the animal would better accommodate these sources of variance. An example of this approach is the following comparison of the use of glucose vs. acetate as a source of ATP for use in maintenance.

The biochemical efficiency of trapping acetate energy in ATP is less than that for glucose (Chapter 6). As a result, when acetate is used as an energy source, heat production is 118% that observed when the same amount of ATP is produced for physiological work functions via oxidation of glucose (see Chapter 6, Table 6.3). As previously discussed, an extreme example of differences in energy losses in nutrient use by the animal is that dietary fat is incorporated into the body and milk fat at an efficiency of 94–97%, while the efficiency of conversion of dietary carbohydrate, via fermentation to acetate, to body fat is only 60–65%. The importance of this difference is becoming more prominent as the use of protected fats and whole oilseeds in cattle rations has increased markedly in recent years. The models of Gill *et al.* (1989a,b) and Baldwin *et al.* (1987a,b) are examples of the application of this concept and illustrate the utility of using our knowledge of underlying function, intermediary metabolism in this case, to address problems encountered with models used in support of animal agriculture. These concepts are developed in greater detail in subsequent chapters.

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Energy requirements for maintenance and production

I can, if the worst comes to the worst, still realize that the Good Lord may have created a world in which there are no natural laws. In short, chaos. But that there should be statistical laws with definite solutions, i.e. laws that compel the Good Lord to throw the dice in each individual case, I find highly disagreeable.

Albert Einstein

6.1 INTRODUCTION

A complete description of maintenance was given by Armsby and Moulton (1925):

The concept of maintenance must involve the idea of conserving the existing status of the animal while doing no work and producing no product. There should be an exact balance between income and outgo of ash, nitrogen, hydrogen and energy, showing that there was neither a loss nor a gain of protein, fat, carbohydrate or mineral matter. Strictly there should be no translocation of material within the animal itself.

A more modest definition of maintenance is a physiological steady state in which there is no net change in body energy content or, alternatively, energy intake is adequate to support an energy balance of zero (Chapter 5). The concept of maintenance has long been a focal point in the study of nutritional energetics. Early workers used balance studies and direct heat measurements on animals to estimate the energy required as food to support daily requirements, but could only speculate on the nature of 'vital processes' involved. Specific partitioning of energy input and identification of fixed and variable energy expenditures associated with maintenance are recent advances dependent upon current knowledge of physiology and intermediary metabolism. This chapter will focus on mathematical equations used to estimate maintenance, variance in these estimates, identification and quantification of specific energy-requiring 'vital processes' and efficiencies of nutrient use in support of these functions.

The following definitions and conventions were applied in developing the discussion of maintenance requirements in this chapter: ME_m equals the metabolizable energy intake required to maintain an energy balance of zero; BMR, or basal metabolic rate, applies to humans in a postabsorptive state, in a thermoneutral environment and at rest but not sleeping; FHP equals fasting heat production of an animal in a postabsorptive state, in a thermoneutral environment and at rest, lying or standing but otherwise inactive. Fasting catabolism (FCAT) is the total energy loss of a fasting animal. BMR and FHP are, essentially, equal to H_eE , while fasting catabolism equals $H_eE + F_eE + U_eE + H_wE$ and ME_m equals $H_eE + FE + UE + H_dE + H_wE$.

6.2 EQUATIONS USED TO ESTIMATE MAINTENANCE AND COSTS OF PRODUCTION

6.2.1 Fasting heat production

Comparisons of basal metabolic rates within or between species require a common base of expression free of variability associated with body size. This variability is easily recognized and was a subject of speculation very early in the evolution of nutritional energetics as indicated in the following quotes from Brody (1945):

Basal metabolism and the 'surface law'. A most intriguing observation in basal metabolism studies is that the basal heat production per unit body weight in homeotherms decreases rapidly with increasing weight. Thus the basal metabolism per unit weight of such small animals as mice and canaries is 20 to 25 times as great as of large animals, such as cattle. This means that the basal-metabolic significance of a physical weight unit in a canary is about 25 times that in a cow. This means that simple body weight is not a suitable reference base for metabolism...Now if the heat loss is proportional to the surfaces, the heat production must likewise be proportional to the surfaces, since in homeotherms heat production must equal heat loss. Homeotherms must, therefore, have developed in the course of evolution a heat-production control to function in proportion to surface area. This, in brief, is the evolutionary logic of the 'surface law'.

This relation of heat production to linear size and to surface area was first formulated over a century ago by Rameaux and Sarrus (1838). ...To avoid reviewing many subsequent papers, it seems best to quote, in free translation, somewhat rearranged, this first formulation of the 'surface law'.

1. Assuming constancy of body temperature, there must be equality between heat loss and heat production.
2. Assuming equality of other conditions, heat loss is proportional to 'free' surface.
3. Heat production is proportional to oxygen consumption or approximately to inspired air.
4. Since heat loss and heat production are proportional to 'free' surface, and since surfaces vary with the squares of the homologous sides, it follows that oxygen consumption, heat production, and heat loss are proportional to the square of the corresponding dimensions of the animals under comparison.
5. This proportionality between oxygen consumption and free surface may be achieved in several ways, one of which is by varying the lung surface and number of respirations.

These authors also presented relations between pulse rate, pulse volume, and body size.

The more recent formulations of the 'surface law' do not appear to the present reviewer to offer improvements on the original formulation.

In equation form, one may say that surface, or heat loss, or heat production, or oxygen consumption, Y , is proportional to the square of the linear size, L :

$$Y \propto L^2 \quad (13.1A)$$

or to the $2/3$ power of volume, or to the $2/3$ power of weight, W (if the specific gravity is constant), as indicated by the following equation:

$$Y = aW^{2/3} \quad (13.1B)$$

Unfortunately, a suitable formula for estimation of true body surface has not been agreed upon and represents a major obstacle to use of surface area as a reference base. For example, when Brody (1945) examined the weight to surface area relationship for dairy cattle he found that the best fit equation was surface area = $0.15W^{0.56}$. Respective coefficients (a) and exponents (b) for relating weight to surface area in horses, swine and sheep were 0.1 and 0.64, 0.1 and 0.63, and 0.12 and 0.57. Brody (1945) continued:

Metabolically effective body size vs surface area. Since simple weight, $W^{1.0}$, is not a good reference base for basal metabolism and since metabolism tends to vary more nearly in proportion to surface area or to $W^{2/3}$, shall we then employ surface area or $W^{2/3}$ as reference base? No, for the following reasons:

1. The surface area of a living animal is not constant and cannot be measured in such manner that the results can be checked by differ-

ent investigators. Surface-area measurements are not even reproducible on a dead animal.

2. Surface area as it relates to heat loss changes with environmental temperature not only by 'rolling up' in cold weather and 'spreading out' in hot weather, but also by developing heat-conserving and heat-dissipating devices (by varying the fur or feathers, subcutaneous fat, caliber of the blood vessels and the like), thus changing the effective or 'free' surface area.
3. It is true that surface area varies with the $2/3$ power of weight, but only in geometrically similar bodies of constant specific gravity; and small and large, young and old, fat and thin, animals – especially of different species – are not geometrically similar and not of constant specific gravity.
4. Representing metabolism as a function of external surface area would imply that surface area is the 'cause' of the metabolic rate, whereas, as explained above and in other Chapters..., the immediate causative mechanism resides mostly in the neuro-endocrine system and not in the external surface area. Moreover, according to Lambert's 'law' of heat radiation, heat loss is proportional not to simple surface but to the profile of the projected area...

For these reasons it would be a gross oversimplification to accept the very vague 'surface area' as reference base. The $2/3$ power of weight, $W^{2/3}$, is a more definite reference base than area; but as explained above, there is no geometric reason for assuming that the exponent is exactly $2/3$, since animals are not geometrically similar. It seems more rational, as a matter of principle, to take as reference base not $W^{2/3}$ but W^{beta} , the value of the exponent (beta) being determined on the basis of actual data.

Kleiber (1961, 1975) reached a similar conclusion, to the effect that it is clearly advantageous for large animals to have lower basal metabolic rates per kilogram than do small animals. However, this was not taken to indicate that heat transfer determines this rate or that more accurate determinations of true body surface would improve the precision of estimates of fasting heat production (FHP).

These observations led both Kleiber (1932) and Brody (1945) to publish results of empirical analyses of relationships between heat production and body weight (BW) in an attempt to formulate a more suitable predictive equation for FHP. They reasoned that if the logarithms of these two variables could be plotted as a straight line, they could conclude that FHP is directly proportional to body weight raised to a power. Their analyses covered a wide range of species and mature body weights (Fig. 6.1). Note that the line defined by the logarithms of BW and heat production was straight. Based on his analysis, Kleiber proposed the following equation be used on an interspecific basis to estimate FHP from body weight for homeothermic species:

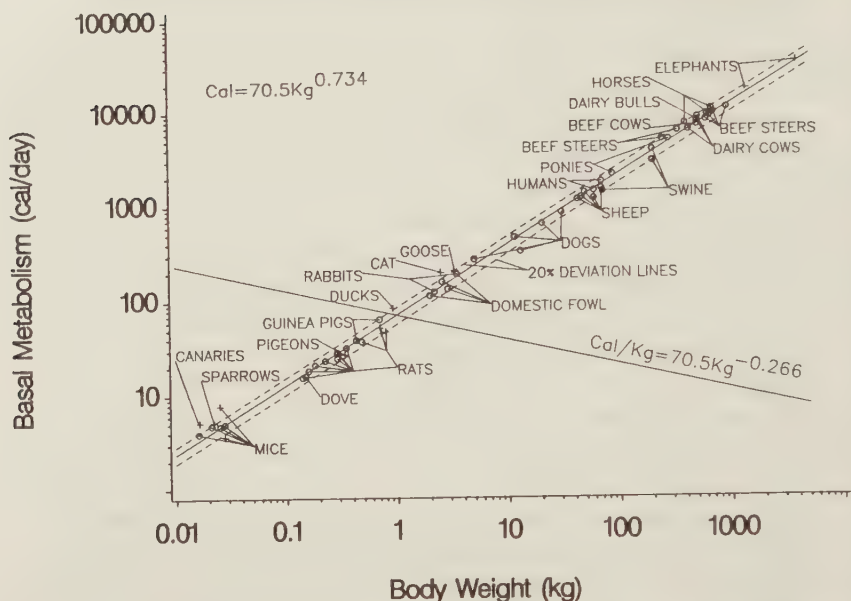


Figure 6.1 A reproduction of the figure relating body weight to basal metabolism published by Brody (1945).

$$\text{FHP} = 70W^{0.75} \quad (6.1)$$

where units on the coefficient 70 are $\text{kcal}/W^{0.75}$ (or $293 \text{ kJ}/W^{0.75}$) and W is weight in kg. Brody's analysis, in close agreement with Kleiber's, indicated an exponent of 0.73. There was no statistical difference between the two equations within the range of 'a 10-g mouse and a 16-ton super elephant' (Kleiber, 1975).

Kleiber (1975) favored use of the power of 0.75, on the grounds that it did not differ significantly from 0.73 and was much more easily calculated on a slide rule – not such a persuasive argument given today's hand calculators, but certainly persuasive in the 1940s and 1950s.

For a number of years (1930–1960) workers in nutritional energetics adopted the allometric equation form

$$\text{FHP} = aW^b \quad (6.2)$$

but exponents selected varied between 0.55 and 1.0, dependent upon the number of species and range of weights of animals involved in the study, experimental objectives and, perhaps, personal preferences. Of course, as the exponent (b) was allowed to vary, the coefficient (a) varied to correspond. This made comparisons between experiments difficult, because all relevant results from the literature had to be converted to a common base before they could be compared. In order to alleviate this problem, delegates

Table 6.1 Intraspecific allometric equations for estimation of fasting heat production^a

<i>Species</i>	<i>Mass coefficient (a)</i> <i>kJ/(W^b)</i>	<i>Mass exponent (b)</i>
Birds	372	0.64
Rabbits	241	0.82
Dogs	594	0.61
Jersey cattle		
30–150 kg	389	0.81
200–600 kg	1084	0.60
Holstein		
20–90 kg	368	0.84
100–400 kg	1234	0.56
overall	841	0.62
Ponies	841	0.62
Percheron gelding		
< 500 kg	1711	0.52
500–900 kg	33	1.18
Humans		
0–3 years	218	1.02
3–16 years	619	0.59
16–31 years	460	0.65
31–60 years	623	0.56
> 60 years	598	0.55

^aCompiled from Brody (1945)

to the 1960 Symposium on Energy Metabolism (Blaxter, 1967) voted to adopt 0.75 as the standard exponent for use in expressing heat production data. Subsequently, this standard has been adopted by many, but certainly not all, workers in nutritional energetics. Some workers continue to accept and utilize the 'surface law' and use $W^{0.667}$ even though it is clear that this exponent does not relate to the surface areas of most animal species. Poultry may approach the surface area of a ball upon which the 2/3 power is based and, perhaps, this is the reason the NRC (1984a) publication on the nutrient requirements for poultry uses this exponent. Brody's empirical analyses of birds (Table 6.1) support this.

Given the tremendous differences within and among species in subcutaneous fat, skin thermoconductivity, covering of hair, fur or feathers and mechanisms developed for thermoregulation, one must wonder whether or not surface area was the driving force in evolution as was suggested by early workers and challenged by Brody (1945). A contrasting view might be that ability to gather the fuel required to support high rates of metabolism in larger species might have also been an important evolutionary factor. The point of the above discussion is that the $W^{0.75}$ convention is based upon a pure empirical fit of data and best describes the relationship between FHP

and body weight for young adults of a wide range of species and was adopted to standardize the expression of data.

Brody (1945; see above quote) and Kleiber (1961) clearly recognized that their best-fit relationships were purely empirical and that it was inappropriate to speculate on the physiological meaning of the 0.75 exponent. The allometric equation parameters from Brody (1945) and summarized in Table 6.1 clearly indicate Brody recognized that statistical fits to this equation across body weights, ages and sexes within a species resulted in a wide range of coefficients (a) and exponents (b). Thonney *et al.* (1976) extended the analyses of Brody (1945) and Kleiber (1932) by using a very extensive data set gathered from the literature; they found that when data from all species and sexes are assumed to represent a single population, the appropriate weight exponent was very close to 0.75 (0.752–0.766). However, when best-fit relationships within species and sexes were estimated, weight exponents (excluding extremes observed for small data sets) ranged from 0.33 (human females) to 1.0 (male chickens).

These workers used the equation

$$\log \text{FHP} = b_0 + b_1 (\log W) \quad (6.3)$$

in their statistical analyses and thus allowed a non-zero intercept.

This is quite appropriate when one wishes to develop a statistically best-fit relationship within a species. Factors such as age, which are usually collinear with weight, would be expected to skew the relationship away from a zero intercept, because young animals have higher heat production rates per unit W^b than do older animals. The important fact is that best-fit analyses within species and over sexes and ages can vary greatly. These workers suggested, appropriately, that in statistical analyses of experimental data, the exponent should not be fixed at 0.75. Rather, they suggested that weight be incorporated as a covariate in statistical analyses.

Heusner (1985, 1987, 1991) has undertaken sophisticated mathematical analyses of the meaning of the allometric weight coefficient (a) and the weight exponent (b). He has found through dimensional analysis that parameters, when deduced statistically, represent a mixture of extensive properties that are size dependent, such as length, surface area, volume, and mass and intensive properties that are size independent, such as density, concentration and temperature, which vary as a function of underlying functions related to age, rate of growth, physiological state, etc. The analyses of Thonney *et al.* (1976) and Heusner (1985) are examples of application of a 'top-down' approach in analyses of input:output data. The approach has promise based upon the concept of extensive and intensive properties forwarded by Heusner. However, it is limited by our ability to identify intensive properties of animals that are captured in the mass coefficient (a) and exponent (b).

The mechanistic approach advocated in this treatise contrasts with but is completely compatible with those advocated by Heusner (1991) and Thon-

Table 6.2 Maintenance energy requirements for various animals

<i>Animal</i>	<i>Main treatments</i>	<i>Maintenance requirement (kJ/W^{0.75})</i>
Rat (weaning; 48 g)	protein (%)	
	2	361
	25	533
	35	523
	45	546
Rat (6 months; 310–350 g)	fat (%)	
	2	265
	10	263
	30	253
Pig (100–180 kg)	corn and barley	371
	barley and oats	486
Chicken (10–25 days)	protein (%)	
	6.3	715
	12.6	742
	18.9	720
	25.2	710
Chicken (3.5–4.5 kg)	4% fat, 28°C	354
	4% fat, 22°C	418
	32% fat, 22°C	398
Rabbit (9–12 months)	60% wheat + 40% hay	446
Sheep (mature)	chopped hay	383
	66% hay + 34% oats	311
Steers (mature)	66% hay + 34% oats	445
Woman (21 years, obese at 129 kg)	no exercise	309
	exercise	385
Man (19 years old, obese at 145 kg)	no exercise	305
	exercise	415

^a Adapted from summary of Reid (1974).

ney *et al.* (1976), in that the common goal has been to explain variance in FHP on the basis of physiological and metabolic factors which are influenced by age, physiological state, previous and current nutritional state, hormonal factors, etc. We agree totally with previous workers, who have pointed out that it is inappropriate to confer upon $W^{0.75}$ the term metabolic body weight – although we continue to use this term as a matter of convenience and to speculate on the underlying physiological, metabolic meaning of 0.75. We will use the exponent 0.75 in this presentation as a matter of convention and convenience. The convenience is that when a specific exponent is forced in data analyses, all variance is folded into the coefficient (*a*) and discussion is more easily focused on variance in (*a*) rather than in (*a*) and (*b*). This convenience is illustrated in Table 6.2. In the table, it is easy to identify effects of dietary-protein percentages in rats and chickens; fat percentages in rats and chickens; cereal grain used in pig feed; age in rats and

chickens; ambient temperature in chickens; species (compare mature sheep and cattle fed the same diet); and sex and exercise in humans. When both the mass coefficient (a) and exponent (b) are allowed to vary in obtaining best-fit relationships as in Table 6.1, these differences are not readily apparent and are difficult to address in discussion.

6.2.2 Estimates of maintenance requirements and costs of production

Measurements of fasting heat production such as those used to derive the equations discussed in the previous section are fairly consistent, because the methods used to obtain the estimates are relatively uniform. Usually, animals are maintained at feed intakes close to maintenance for some time before they are fasted and measurements of fasting heat production undertaken (ARC, 1980). When animals are growing, lactating, pregnant or otherwise producing at varying rates, fasting perturbs metabolism and productivity such that heat production data obtained are not representative of the normal fed state. For this reason, measurements made on producing animals in the fed state, estimates of maintenance requirements ($FHP + H_dE + U_eE + F_eE$), and efficiencies of productive functions are derived

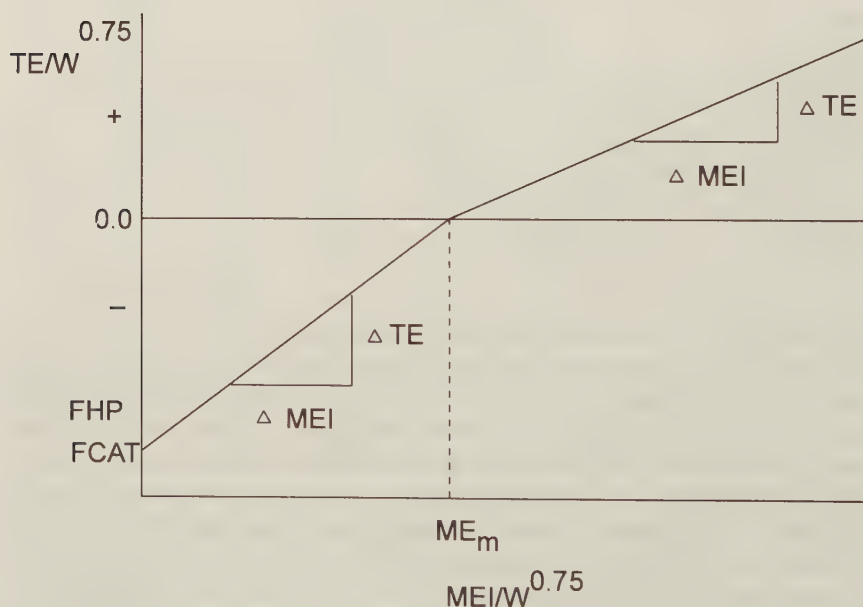


Figure 6.2 Generalized relationship between metabolizable energy intake (MEI) and tissue energy where ΔTE denotes tissue energy gain, balance or tissue energy loss. In the notation used in this figure, FHP is fasting heat production, FCAT is fasting catabolism and ME_m is the MEI required for energy balance ($\Delta TE = 0.0$).

statistically. The basic concepts, upon which statistical models are based, are presented in Fig. 6.2. Using the notation in Fig. 6.2, the following generalizations arise:

1. FHP should average $-293 \text{ kJ/W}^{0.75}$ and equal the fasting heat production in a young adult of a species previously fed approximately at maintenance as discussed above.
2. FCAT should be about $-335 \text{ kJ/W}^{0.75}$ and reflect fasting catabolism (FCAT) or total body energy loss by a fasting young adult previously fed at maintenance.
3. The ratio or slope of the line connecting FCAT and ME_m ($\Delta\text{TE}/\Delta\text{MEI}$) equals the efficiency of use of metabolizable energy for maintenance (k_m) and varies from 0.80 to 0.95, dependent upon diet and species as discussed in the next section of this chapter.
4. ME_m is the metabolizable energy intake required to maintain an adult animal fed to energy balance (TE equals 0.0) and varies from about 356 to $460 \text{ kJ/W}^{0.75}$, dependent upon diet and species. The low estimate reflects a simple-stomached species consuming a well balanced, low H_dE diet or fasting catabolism/0.95 or $(\text{H}_e\text{E} + \text{U}_e\text{E} + \text{F}_e\text{E})/0.95$. The high estimate reflects an adult ruminant animal where H_dE ($1.0 - k_m$) equals about 0.2 and H_fE equals an additional 8–10% of the ME requirement for maintenance.
5. The slope associated with the ratio $\text{TE}_g * (\text{MEI} - \text{ME}_m)^{-1}$ is the efficiency (k_g) of use of ME provided above maintenance for tissue energy gain and can vary from 0.3 to 0.8, dependant upon diet, composition of gain and other factors discussed in Chapters 7, 9 and 15.
6. Efficiency of ME use for maintenance (k_m) as compared to tissue gain (k_g) differs significantly as indicated by the slopes depicted in Fig. 6.2. This is an idealized representation, since Lofgren and Garrett (1968) have shown that when the logarithms of MEI and TE are plotted and statistically evaluated for large data sets, the regression relationship is a straight line. This implies that a better depiction of the MEI vs. TE relationship in Fig. 6.2 would be a curve rather than two straight lines. Further, this observation suggests that the mix of metabolic transactions that contribute to heat production and losses and gains in tissue energy vary continuously as opposed to discretely over a range of intakes as depicted in Fig. 6.2.

In order to undertake statistical analyses of relationships between MEI and body energy changes, the basic equations in Fig. 6.2 are normally combined and rearranged as follows:

$$\text{TE}_g = (\text{MEI} - \text{ME}_m) * k_g \quad (6.4)$$

where

$$\text{ME}_m = \text{FCAT}/k_m \quad (6.5)$$

and thus

$$\text{MEI} = \text{FCAT}/k_m + \text{TE}_g/k_g \quad (6.6)$$

Further, the allometric relationship ($y = aW^b$) discussed above can be incorporated to define FCAT as a function of metabolic body size ($\text{MBS} = W^b$), where b is usually set by convention at 0.75, to yield the following equation:

$$\text{MEI} = a * \text{MBS}/k_m + \text{TE}_g/k_g \quad (6.7)$$

For computational simplicity and efficiency, a/k_m can be defined as b_1 and $1/k_g$ as b_2 , and the equation and statistical model becomes

$$\text{MEI}_i = b_1 * \text{MBS}_i + b_2 * \text{TEG}_i + \varepsilon_i \quad (6.8)$$

where MEI_i , MBS_i and TEG_i are the metabolizable energy intake, metabolic body size and tissue energy gain of animal i ; ε_i is the random residual error specific to the i th observation; and ε has a null mean and variance. In this analysis, $b_1 * \text{MBS}_i$ is considered to represent an estimate of ME_m and b_2 is the inverse of the efficiency of ME use for gain (k_g).

Considerable discussion among scientists has occurred as a result of adoption of this model. This discussion has revolved, in part, around the fact that in energy balance experiments, metabolizable energy intake is the independent variable, e.g. MEI is varied to produce responses in energy balance, while in the statistical model MEI is treated as the dependent variable. In doing this, the analyst runs a risk that the error structures associated with treating MEI, rather than energy balance, as independent variables could differ and result in aberrant estimates of b_1 and b_2 . In reality, this source of error may be the least of our worries.

Tissue energy gain (TE), as measured, is the sum of lean (mostly protein) energy gain (LEG) and fat energy gain (FEG). Thus, Kielanowski (1965) suggested that the statistical model (equation 6.8) could be extended to obtain estimates of the partial efficiencies of lean ($1/b_3$) and fat gain ($1/b_4$) as follows:

$$\text{MEI} = b_1 * \text{MBS}_i + b_3 * \text{LEG}_i + b_4 * \text{FEG}_i + \varepsilon_i \quad (6.9)$$

The analytical model represented by equation 6.9 can be scaled to metabolic body size resulting in the following:

$$\text{MEI}_i/\text{MBS}_i = b_1 + b_3 * (\text{LEG}_i/\text{MBS}_i) + b_4 * (\text{FEG}_i/\text{MBS}_i) + \varepsilon_i \quad (6.10)$$

There is no question about the fact that these analytical models (equations 6.8–6.10) can describe carefully collected experimental data very well. The results can be utilized effectively in empirically based feeding systems such as the NRC dairy (1989) and beef cattle (1984b) systems. The problem arises when one attempts to assign biological meanings to the coefficients b_1 to b_4 . Brody (1945), Webster (1981), Fowler *et al.* (1980), Koong (1977), Roux *et al.* (1983), Canas *et al.* (1982) and Bernier *et al.* (1986), among others, have ad-

dressed this issue. Estimates of apparent maintenance requirements vary over a tremendous range from 167 to 700 kJ/W^{0.75}. Estimates for growing mice vary from 305 to 690 kJ/W^{0.75}. These differences can be explained in part on the bases of sex, age, diet, and environment (Bernier *et al.*, 1986, 1987), but problems associated with this high variance in developing feeding recommendations are clear and have led to considerable discussion.

Some workers have argued that the concept of maintenance, which is explicitly incorporated into all current feeding systems, is inadequate in view of this variance and should be discarded or replaced (Meissner *et al.*, 1980; Webster, 1981). Others argue that since we know, for example, that the apparent maintenance requirements of sheep, growing steers, and lactating cows differ greatly, there is no problem. We only need use the coefficients that apply to specific species and physiological states. This approach is compatible with Brody's (1945) observation that apparent maintenance requirements are higher in producing animals and his suggestion that the term 'working maintenance' be used to distinguish the apparent maintenance requirements of producing animals from those of non-productive animals. Unfortunately, at differing rates of productivity, apparent maintenance requirements vary continuously rather than discretely and, as a result, assignment of maintenance requirements becomes an 'expert judgement' rather than a quantitatively defined scientific fact. A third, collateral argument is that the greater apparent maintenance requirements of producing animals are really a cost of production and should be reflected in estimates of net efficiency of production rather than as costs of maintenance, i.e. maintenance requirements should be considered constant (Armstrong and Blaxter, 1984).

We accept the argument of the first group that, over the long term, we should abandon the concept of maintenance in favor of explicit consideration of physiological and metabolic functions that vary in a known cause-and-effect fashion. Heat production in a given physiological or productive state would then be accounted as a sum of energy expenditures for specific functions and could vary over time, among diets, sexes, species, etc., due to known physiological and metabolic relationships. This would obviate the argument of group three in that energy expenditures and changes therein could be attributed to maintenance, production, etc., in whatever fashion any individual desired. However, total energy expenditure and contributions of specific functions to this would remain clear, in agreement with the first group.

Collectively, we are making progress toward this goal, but have not yet achieved it and may not achieve a working system with these desired attributes for a number of years. In the interim, a compromise between the approaches of groups one and two seems most appropriate. This compromise was alluded to above and simply involves the introduction of mechanistic elements, varying in sophistication, to current feeding systems.

Koong *et al.* (1982) proposed that fasting heat production for growing rats can best be estimated using the equation

$$\text{FHP (kJ/day)} = 445 W^{(0.686 + 0.165\text{ADG})} \quad (6.11)$$

where W and average daily gain (ADG) are expressed in grams. This equation implies that true metabolic body size is a variable dependent upon rate of gain.

A similar equation form was utilized in a model developed for the pig by Black *et al.* (1986). In this, metabolic body size was based upon body protein with additive adjustments for rate of gain and environment. In the model of Fox *et al.* (1988) the following equation was used:

$$\text{NE}_m = [(a_1 + a_2) * W^{0.75} * (\text{AX}) * (\text{BE}) * (\text{L}) * (\text{NE}_{\text{MHS}})] + \text{NE}_{\text{MCS}} \quad (6.12)$$

where NE_m is the net energy required for maintenance (essentially, FCAT in Fig. 6.2), a_1 is 0.332 MJ/ $W^{0.75}$, a_2 is an acclimatization factor dependent upon previous temperature, AX is a grazing adjustment factor, BE is a breed factor, L is a tabular factor for lactation, and NE_{MHS} and NE_{MCS} are adjustments for heat (panting) and cold stress, respectively. Graham *et al.* (1976) incorporated empirically deduced maintenance adjustments for milk production, age, rate of gain, endogenous urinary energy loss, work associated with food intake and DE intake. All of these represent attempts to identify factors which contribute to variation in apparent maintenance requirements and to accommodate these using equation forms and parameter values deduced from large input:output data sets and multiple linear regression techniques.

There is a clear, statistically defined limit to progress or advantages that can be gained using the statistical approach discussed above. This limit is the number of parameters that can be deduced with certainty from a standard input:output data set. In order to better address this variance, several groups have developed mechanistic models which attempt to utilize a much broader set of measurements and data to capture cause and effect relationships underlying maintenance energy expenditures as they vary across physiological states, environmental conditions, breed, etc. A number of physiological and metabolic functions that contribute to variance in apparent maintenance requirements have been identified and at least partially characterized. These will be discussed in subsequent sections.

A similar and, perhaps, more distressing result of application of the multiple linear regression models represented by equations 6.9 and 6.11 is that not only do statistically deduced partial efficiencies of protein and fat accretion vary considerably from experiment to experiment, deduced partial efficiencies are often biologically questionable and even unfeasible. Roux *et al.* (1983) and Bernier *et al.* (1986), among many others, have addressed this issue. A major problem that has been clearly identified is the fact that when ME intake is systematically varied in animals, energy recovered in products (RE) such as lean and fat gain are highly correlated (collinear) one with the

other. Thus, a basic assumption of multiple linear regression analyses to the effect that 'independent' variables not be highly correlated is violated. Slinker and Glantz (1985) reported that

If multicollinearity is present to a harmful degree in physiological data, multiple linear regression often yields non-sensical results: parameter estimates of incorrect magnitude, incorrect sign, or high standard errors of the estimate (which quantitate the imprecision of the parameter estimates).

This case is not uncommon in energy balance experiments. For example, Bernier *et al.* (1987) reported correlations between lean and fat gain in energy balance experiments with mice of over 0.9. Canas *et al.* (1982) reported correlations between body energy loss and milk energy yield of a similar magnitude (over 0.9).

Roux and his associates (1982, 1983) and Koong *et al.* (1983), respectively, suggested that ridge regression and principal component analyses could help alleviate the problems of multiple collinearity and aid in obtaining more biologically meaningful parameter estimates from energy balance studies. Bernier *et al.* (1986, 1987) investigated these possibilities further, and readers are directed to these publications for more detailed discussions. The data of Bernier *et al.* (1986, 1987) illustrated some of the problems associated with multiple linear regression analyses and estimates of maintenance requirements and partial efficiencies of protein and fat accretion in growing animals (Fig. 6.3). The particular study was of energetic efficiencies of two lines of mice: a control line (CH) and a high growth rate line (Ch). The first part of the problem is variance. It is clear to the eye that the slopes of the regression lines between MEI and energy gain (BE) differ but not whether or not the intercepts at $BE = 0.0$ differ (the difference in intercepts is statistically significant). The visual problem in evaluating the data is due to the fact that at metabolizable energy intakes ranging from 500 to 710 $\text{kJ/W}^{0.75}$, quite a number of mice maintained energy balances close to zero. In other words, as intakes approached and fell below the apparent maintenance requirement indicated by linear regression intercepts, the mice maintained body energy balance. This observation is analogous to that made by Brody (1945) and led to his suggestion that the term 'working maintenance' be applied to producing animals. The question is, how do mice fed at 500 $\text{kJ/W}^{0.75}$ maintain energy balance as well as those fed 40% more? How do they adapt? These questions cannot be addressed using statistical inference. One must utilize our knowledge of underlying function to gain further insight, and this issue will be addressed in several ways in subsequent sections.

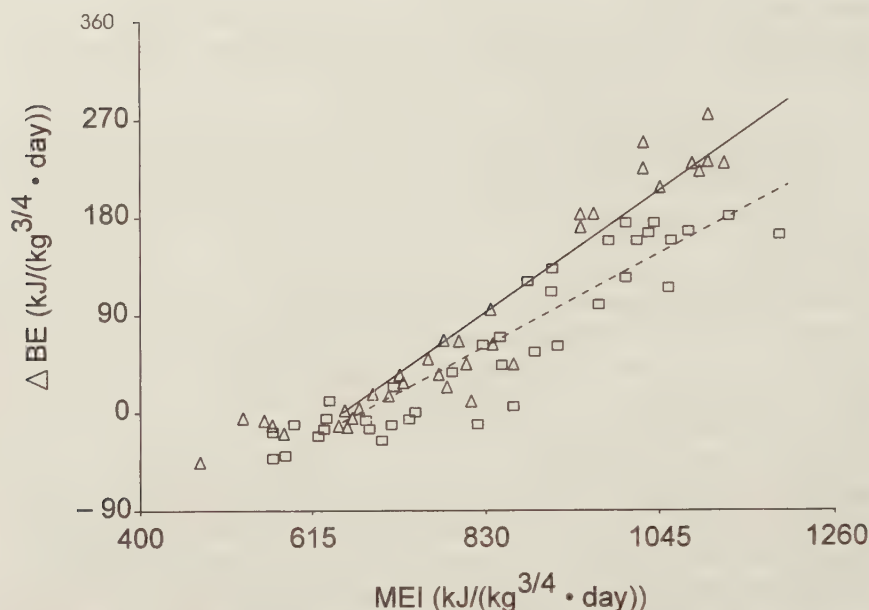


Figure 6.3 Change in total body energy (ΔBE) as a function of metabolizable energy intake (MEI) in CH ($\square$) and Ch (Δ) mice. Each symbol represents one mouse. Standard error of the parameter estimates are given in parentheses ($\pm$ SE). The regression line for CH (---) is described by $\Delta BE = 0.385 (\pm 0.026) MEI - 62.94 (\pm 5.35)$ ($x, y = 38$) with an x -axis intercept of $163.66 (\pm 3.55)$. The correlation coefficient (r) is 0.93 ($P < 0.001$). For line Ch (—), the regression equation is $\Delta BE = 0.495 (\pm 0.028) MEI - 76.88 (\pm 5.46)$ ($x, y = 37$) with an x -axis intercept of $155.36 (\pm 3.38)$. ($r = 0.95$; $P < 0.001$) The slopes of the regression equations for each line are significantly different ($P < 0.005$). The x -axis intercepts of the regression lines are also significantly different ($P < 0.01$). Adapted from Bernier *et al.* (1986).

6.3 COMPONENTS OF MAINTENANCE

6.3.1 Costs of digestion and assimilation

The primary, identifiable energy costs of digestion and assimilation of a meal are bond breakage, nutrient absorption, synthesis of digestive proteins and assimilation or storage of nutrients. These energy costs correspond to the old term, heat increment of maintenance (HI_m) and represent the difference between body energy loss when body stores are used to sustain life in fasted animals (equivalent to fasting catabolism and the old term net energy required for maintenance, NE_m). Metabolizable energy required from food for maintenance is as follows:

$$ME_m = NE_m + HI_m = H_eE + U_eE + F_eE + H_dE + H_wE \quad (6.13)$$

Prior to discussing the contributions of various functions to H_dE and H_eE ,

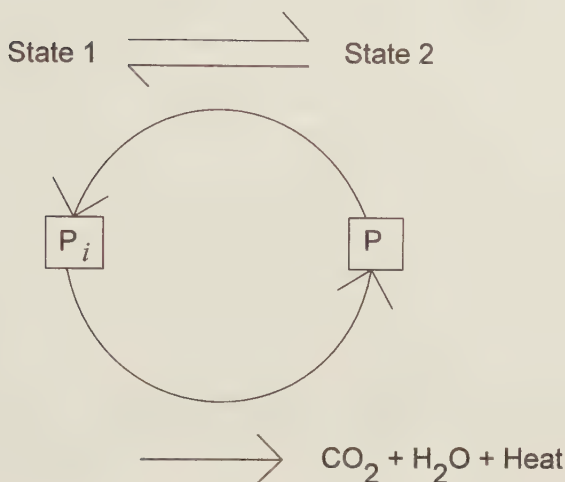


Figure 6.4 Depiction of the relationship between maintenance for an initial condition (State 1) and rates of substrate oxidation. Energy as high energy phosphate bonds is expended in returning spontaneous deterioration of the system to State 2 back to the initial condition, State 1.

the convention we will use in estimating heat losses associated with the oxidation of substrates to form the high energy phosphate bonds of ATP ($\sim\text{P}$), which are, in turn, utilized to support maintenance functions, must be developed beyond the discussion presented in Chapter 5. All estimates of energy expenditures associated with maintenance are based upon the premise that energy expended is directed to the maintenance of an initial state (1) as depicted in Fig. 6.4. When the initial state (1) is an intact protein, state 2 would be free amino acids arising from degradation of that protein. Energy for resynthesis, as $\sim\text{P}$, of the protein must be derived from oxidation of a substrate. The net result of this maintenance function is that all energy derived from the oxidation of substrates used to support the process is lost as heat. If one accepts the commonly used estimate that five high energy bonds ($\sim\text{P}$) are required to synthesize a peptide bond and glucose is used as the substrate oxidized, then



and 5/38 or 0.131 moles of glucose would be oxidized to support the process. As a result, (0.131 moles glucose * 2.82 MJ/mole), 370 kJ of heat would be released per mole of peptide bond hydrolyzed and resynthesized. This analysis presumes that one can calculate the heat loss equivalent of a high

energy phosphate bond formed from glucose and used in a maintenance process as $[2.82 \text{ MJ/mole glucose}] \div 38 \text{ moles/ATP formed per mole of glucose}$, 74.1 kJ (see Table 6.3 for additional examples) and multiply this value times the number of $\sim\text{P}$ used to perform a maintenance function to obtain an estimate of heat production. It is important to note that heat release associated with maintenance processes can vary significantly dependent upon the substrate oxidized to support that process (Table 6.3). When protein and acetate are used as energy sources, respective heat losses relative to glucose are 28 and 18% greater. This tabulation clearly illustrates the effect differing nutrients can have on apparent energy requirements. This source of variance is not considered in the following estimates relating to $\text{H}_\text{d}\text{E}$ but must be borne in mind.

Table 6.3 Heat production associated with the formation and use of high energy ATP bonds in support of maintenance processes^a

Substrate oxidized	$\sim\text{P}/\text{mole}$	Heat losses		Heat loss relative to stearate
		MJ/mole	kJ/mole $\sim\text{P}$	
Glucose	36–38 ^b	2.82	78.2–74.1	95–99
Stearic acid	146	11.35	77.7	100
Acetate	10	0.87	87.4	112
Propionate	18	1.54	85.3	110
Butyrate	27	2.19	81.2	105
Protein (100 g)	22.6	2.14 ^c	94.8	122

^aAdapted from Milligan (1971).

^bThe value of 38 represents the maximum ATP yield from glucose oxidation which will be used throughout. The value of 36 represents the ATP yield from glucose when the glycerophosphate shuttle is used to transfer reducing equivalents from the cytosol to the mitochondria.

^cHeat of combustion adjusted for energy excreted as urea when protein is oxidized.

The estimates of functions contributing to $\text{H}_\text{d}\text{E}$ in Table 6.4 were discussed and justified in detail elsewhere (Baldwin, 1968, Baldwin and Smith, 1974, 1979). Therefore, their bases will be presented only briefly here. The arguments presented relate to simple-stomached animals. These examples were adopted for simplicity and clarity in developing concepts underlying analyses of energy expenditures for specific metabolic functions. Similar estimates are developed in later chapters for ruminants but are significantly more complex and, as a result, can compromise clarity in an initial development of basic concepts.

Heat release associated with hydrolysis of the glucosidic bonds of starch can be estimated as follows:

Bond energy	=	18.0 kJ/mole of starch
Heat released from starch combustion	=	17.6 kJ/g
Percentage starch energy lost as heat	=	18 kJ/mole * 100
		162 g/mole * 17.6 kJ/g
	=	0.6%

Table 6.4 Estimated H_dE of two meals fed at maintenance^a

Process	Carbohydrate meal		Carbohydrate + fat (50:50) meal	
	short %	long %	short %	long %
Method (duration)				
Bond breakage	0.5	0.5	0.3	0.3
Absorption (active transport)	2.6	2.6	1.3	1.3
Digestive secretions	2.0	4.0	2.0	4.0
Assimilation (storage) ^b				
glucose as glycogen	1.5	1.5	1.5	1.5
glucose as fat	8.0	11.0	—	—
fat as fat	—	—	1.5	1.5
Theoretical H_dE	14.6	19.6	6.6	8.6
Observed H_dE	10–15	18–22	6–8	8–10

^aShort-term experiments conducted using indirect calorimetry while long-duration experiments involved comparative slaughter.

^bCalculations are based upon data indicating that during the period of assimilation of a carbohydrate meal, 20% of the carbohydrate is oxidized, 30% is stored as glycogen and 50% is converted to fat storage. The cost of storage of glucose as glycogen is 5% and as fat is 16% (see text). Thus $(0.3 * 15\%) = 1.5\%$ glucose stored as glycogen and $(0.5 * 16\%) = 8\%$ is the short-term cost of glucose storage as fat, etc. (see text). When a mixed carbohydrate and fat diet is stored, all glucose is stored as glycogen, and dietary fat is stored as fat.

Similar calculations for the peptide bonds of proteins and the ester bonds of triacylglycerol lead to respective estimates of 0.6 and 0.1%. During fasting, ester bonds of storage triacylglycerol are hydrolyzed during the mobilization of body fat and, as a result, contribute to fasting heat production. Thus, the estimates of energy release due to bond breakage must be corrected for the heat loss associated with fat mobilization during fasting as follows:

glucosidic bond	$0.6 - 0.1 = 0.5\%$
peptide bond	$0.6 - 0.1 = 0.5\%$
ester bond	$0.1 - 0.1 = 0.0\%$

This correction is trivial in a quantitative sense but illustrates the concept that estimates of H_dE must be unique and not confounded with processes that are components of H_eE or fasting catabolism. The energy costs of absorption of carbohydrate, lipid, and amino acids (including peptides),

respectively, can be estimated on the bases of one $\sim P$ expended per mole glucose absorbed, four $\sim P$ expended per triacylglycerol resynthesized from a monoglyceride and two fatty acids, and 0.5 $\sim P$ used for each mole of amino acid absorbed. These yield respective estimates of the percentages of energy in glucose, tripalmitin and amino acids lost as heat during absorption of 2.6, 0.9 and 1.9.

The energy costs for synthesis of digestive secretions are relatively minor, excepting costs of synthesis of digestive proteins (Milligan, 1971). The cost of synthesis of digestive proteins can be calculated from estimates of increases in rates of protein secretion (and synthesis) that occur during eating and digestion. These estimates vary from 3.5 to 7.5 g/W^{0.75}. Using a one-kg animal as a reference, 5.5 g/W^{0.75} of digestive proteins are secreted per day. At five $\sim P$ used per peptide bond synthesized, an average molecular weight of amino acids in protein of 110 g, fasting catabolism of 335 kJ/day and glucose as an energy source $[(0.0055 \text{ kg} * 5.0 * 74.1 * 100)/(0.110 * 335)]$, 5.5% of maintenance energy requirements (ME_m) is expended in digestive protein synthesis.

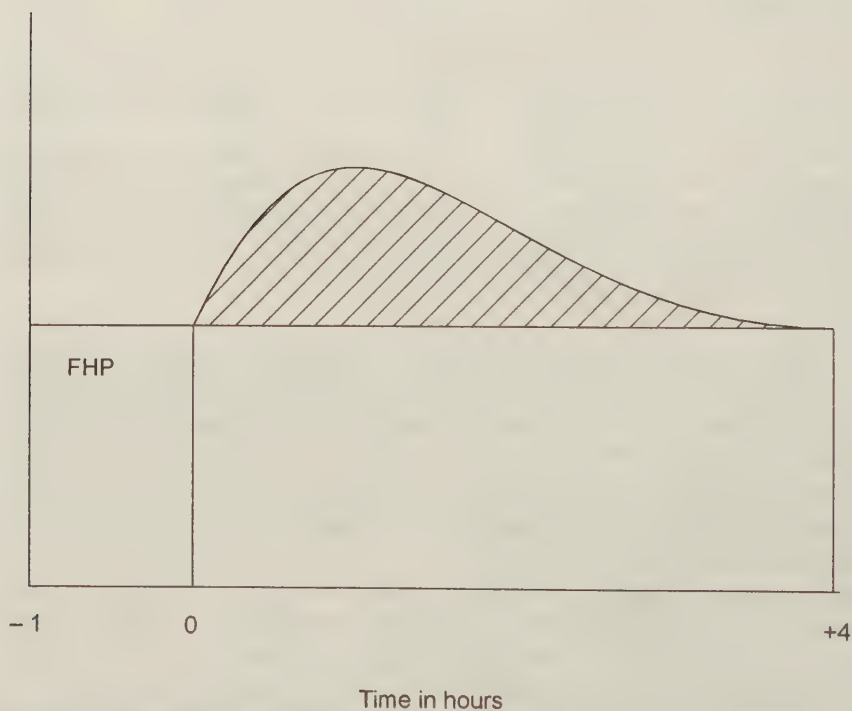


Figure 6.5 Depiction of increased heat production following consumption of a meal at time zero. Shaded portion identifies heat production attributable to costs of digestion, absorption and assimilation (H_{dE}) of food in a 'short-term' study as discussed in text.

In comparing short-term (4 h) estimates of H_dE as heat production in excess of FHP (Fig. 6.5, hatched area), it is difficult to estimate how much digestive protein is secreted and resynthesized during the monitoring period since an uncertain proportion of digestion protein synthesis occurs whether an animal eats or not. In formulating Table 6.4, it was assumed that 1.5% out of the 5.5% of the maintenance requirement expended in digestive protein synthesis occurs independent of eating ($5.5 - 1.5\% = 4.0\%$). Further, it was assumed that one-half of the remaining cost (2%) occurs during the 4 h monitoring period used in short-term studies of H_dE . For the long-term study estimate (Table 6.4), it was assumed that 4.0% out of the 5.5% of maintenance would be realized as a component of H_dE .

One of the most obvious costs associated with assimilation of a carbohydrate meal is the cost of glucose storage as glycogen. According to the formulation in Table 6.5, two ATP are expended $[(2 \times 74 \text{ kJ}/\sim P)/(2.82 \text{ MJ}/\text{mole glucose})]$, indicating that 5.2% of glucose energy is lost when it is stored as glycogen in liver and later released for use by peripheral tissues. In contrast, if such storage occurs in muscle and the glucose is later used in that tissue (without G6P hydrolysis) as a source of energy, the net cost of storage would be one ATP or 2.6%.

Table 6.5 Cost of glucose storage as glycogen

GLC + ATP	→	G6P + ADP
G6P	→	G1P
UTP + G1P	↔	UDPglucose + Pi
UDPglucose + glycogen _(n)	→	glycogen _(n+1) + UDP
UDP + ATP	↔	UTP + ADP
net: GLC + 2ATP + glycogen _(n)	→	glycogen _(n+1) + 2ADP

The problem in comparing short-term (calorimeter) estimates of H_dE with long-term (comparative slaughter) studies (Table 6.4) becomes more prominent when considering heat production associated with the conversion of glucose to fat for temporary (within a day) storage. In short-term studies, heat losses associated with carbohydrate storage as fat would be those which occur during fat synthesis as described in Table 6.6 and would equal 16.2%. Assumptions regarding sources of $NADPH_2$ used in fatty acid synthesis, C_2 transport, and the mix of metabolic pathways involved can cause this estimate to vary by about 2.0% (Milligan, 1971; Baldwin and Smith, 1979). We are most comfortable with an estimate of 16–18% in static calculations. In long-term studies, costs associated with the storage process are quite different (Table 6.7). In this case, energy losses unique to the conversion of 8.0 acetyl-CoA plus 0.5 glucose to tripalmitin, and recovery of 8.0 acetyl-CoA and 0.5 glucose upon mobilization of the stored fat for use in support of maintenance processes, are those which must be considered.

Table 6.6 Short-term cost of glucose storage as fat

1. Synthesis of palmitate

4 GLC + 8 ADP + NAD	→	8 PYR + 8 NADH ₂ + 8 ATP
8 PYR + 8 NAD	→	8 acetylCoA + 8 NADH ₂ + 8 CO ₂
8 acetylCoA + 8 OAA	→	8 citrate
8 citrate + 8 ATP	→	8 acetylCoA + 8 ADP + 8 OAA
8 OAA + 8 NADH ₂	→	8 malate + 8 NAD
8 malate + 8 NADP	→	8 PYR + 8 NADPH ₂ + 8 CO ₂
8 PYR + 8 CO ₂ + 8 ATP	→	8 OAA + 8 ADP
7 acetylCoA + 7 CO ₂ + 7 ATP	→	7 malonylCoA + 7 ADP
7 malonylCoA + 1 acetylCoA + 14 NADPH ₂	→	palmitate + 14 NADP + 7 CO ₂
0.5 glucose + 6 NADP	→	3 CO ₂ + 6 NADPH ₂
4.5 GLC + 8 NAD + 15 ATP	→	8 NADH ₂ + 11 CO ₂ + 15 ADP + palmitate
8 NADH ₂ + O ₂ + 24 ADP	→	8 NAD + H ₂ O + 24 ATP

net: 4.5 GLC + 9 ADP → 9 ATP + palmitate

Palmitate synthesis times 3 equals

13.5 GLC + 27 ADP → 27 ATP + 3 palmitate

costs of esterification of 3 palmitate

0.5 GLC + ATP + NADH ₂	→	αGP + ADP + NAD
3 palmitate + 6 -P + 3 CoASH	→	3 palmitoyl CoA
3 palmitoyl CoA + αGP	→	tripalmitin + 3 CoASH

net: 14 GLC + 17 ADP → tripalmitin + 17 ATP

output = 1 tripalmitin 31.8 MJ + 17 ATP * 0.074 MJ/mole^a = 33.0 MJ

input = 14 glucose at 2.82 MJ/mole = 39.5 MJ

Thus the H_dE for glucose stored as fat is ([39.5 - 33] * 100/39.5) = 16.2%.

^aSee Table 6.3.

These are the costs unique to the storage process, because when glucose is utilized as an energy source either directly or indirectly (via storage), it must be converted to acetyl-CoA. Similarly, acetyl-CoA formed from glucose or fat is used with the same efficiency. Thus, the long-term costs of glucose storage as fat are those associated with the conversion of acetyl-CoA to fatty acids and the oxidation of fat to reform acetyl-CoA. A major cost associated with this process is the fact that one-half of the NADPH₂ used in palmitate synthesis is recovered as FADH₂ when the fatty acid is oxidized, and oxidation of NADPH₂ via NADH₂ can yield three ~P while oxidation of FADH₂

Table 6.7 Long-term cost of glucose storage as fat

21 ATP used to convert 21 acetyl-CoA to 21 malonyl CoA
24 ATP used to translocate acetyl-CoA in the mitochondrion to acetyl-CoA in the cytosol via citrate lyase reaction
24 ATP used to complete the malate shuttle and generate NADPH ₂
6 ~P to activate palmitate to palmitoyl-CoA for tripalmitin formation
6 ~P to activate palmitate for oxidation after hydrolysis of tripalmitin
21 ~P loss associated with input of NADPH ₂ and recovery of FADH ₂
2 ATP used in converting 0.5 glucose to α -glycerol-P and conversion of glycerol back to 0.5 glucose

104 mole ~P/mole of storage tripalmitate formed and used.

@74 kJ/~P from glucose, heat loss is $(74 \text{ kJ} * 104) = 7.7 \text{ MJ/12 mole of glucose stored as tripalmitate, or } (7.7 \text{ MJ} * [2.82 * 12]^{-1} * 100) = 22.8\% \text{ of glucose energy stored as fat.}$

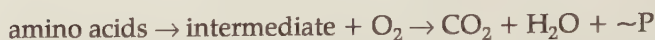
yields two ~P. In the conversion of 24 acetyl-CoA plus 0.5 glucose to tripalmitin and back to 24 acetyl-CoA and 0.5 glucose, the metabolic transactions associated with glucose storage as fat, are as represented in Table 6.7. This loss of ~P must be considered along with the other transactions depicted. The net metabolic equation for long-term storage is thus $(104 * 74 \text{ kJ/~P}) * 100/(12 \text{ GLC} * 2.82 \text{ MJ/mole}), 22.8\%$.

In contrast with the energy costs associated with glucose assimilation and storage, net costs of digestion, absorption and temporary storage of dietary fat as body fat are relatively small. Hydrolysis of dietary fat to monoglycerides and fatty acids, reformation of triacylglycerol, and hydrolysis of blood triacylglycerol to form glycerol and fatty acids, followed by re-esterification in adipose tissue, results in the use of 12 high energy phosphate bonds and a net cost of $[(12 \text{ ATP} * 77.7 \text{ kJ/~P from stearate}) * (31.8 \text{ MJ/mole tristearin})^{-1}, 2.9\%$.

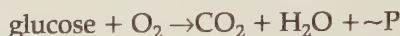
Differences in short- and long-term estimates of digestive protein secretion and resynthesis and glucose storage as fat contribute to variance in estimates of H_dE among experiments and differences due to methods – short- versus long-term experiments or indirect calorimetry versus comparative slaughter experiments.

An important aspect of the summarization in Table 6.4 is that the theoretical estimates fall well within range with experimental observations. This set of calculations was the first to indicate to us that energy expenditures observed in energy balance experiments can be accounted for using straightforward metabolic and physiological arguments.

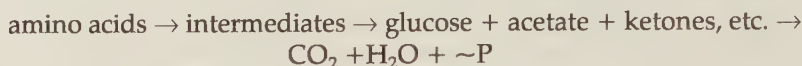
Krebs (1964) was, to our knowledge, the first to consider the H_dE of protein meals. He considered the utilization of each amino acid as an energy source relative to glucose:



as compared to



His calculations indicated that the H_dE associated with protein use as an energy source, exclusive of storage costs, would be 9%. This estimate is low as compared to experimental estimates in the 15% range, even after consideration of energy costs of temporary storage. However, when the following scheme is considered,



the estimate of H_dE increases to 15%, primarily due to costs of gluconeogenesis in liver (Baldwin and Smith, 1971). Again, identifiable metabolic and physiological costs allow us to explain H_dE .

The terms specific dynamic action/effect, work of digestion, and heat increment of maintenance as discussed in Chapter 5 were all defined mathematically as identical to H_dE :

$$\text{HE}_m = \text{fasting catabolism} + \text{H}_d\text{E}, \text{SDA}, \text{HI}_m, \text{ or } \dots \quad (6.16)$$

Alternative terms such as 'Luxus Consumption' and more recently 'diet induced thermogenesis (DIT)' have been introduced. These terms are not easy to deal with, because they are not usually defined in a consistent mathematical fashion by authors using them. These terms have come into vogue with nutritionists studying humans and rats, but are not normally used by other animal nutritionists and, thus, will only be discussed briefly here.

The terms, Luxus Consumption and DIT, arose from the observation that overnutrition does not always produce a proportionate or normally expected gain in body energy. For example, dogs fed 130% or more of their stated energy requirements for maintenance showed no significant gain after one month (Share *et al.*, 1952). Similar results were found in dogs fed 175% of their requirement, an energy excess of 155 MJ over 14 weeks (Janowitz and Hollander, 1955). The implied concept that an increase in energy expenditure in response to excess energy intake is supported by studies in humans and rats and are considered difficult to explain so the terms, Luxus Consumption and DIT, were invented. Excess energy was not simply eliminated in feces (Strong *et al.*, 1967). Changes in energy costs of voluntary activities could not account for the differences (Passmore *et al.*, 1963). Studies by Gulick on his own body weight maintenance requirements provide a historical example (Gulick, 1922). During one 41-day period, his intake averaged 11.5 MJ/day, and he lost 510 g. Assuming an energy value of 25 MJ/kg of adipose tissue, his energy loss would be 0.61 MJ/day. During a later 73 day period, his intake averaged 14.8 MJ/day, and he gained 2.88 kg or 1

MJ/day calculated as adipose tissue. This suggests an energy expenditure of 13.8 MJ/day, a value 1.7 MJ/day greater than his previous daily energy expenditures, assuming that activity was similar during both periods. This difference cannot be attributed to costs of fat deposition, while consuming a normal human diet. The lack of consistent mathematical and experimental definitions for DIT does not allow us to differentiate between energy costs associated with H_dE and H_eE .

Implications of this failure in mathematical definition of terms will be elaborated upon below in discussions of factors that underlie variations in H_eE and are clearly related to the data of Bernier *et al.* (1986) presented in Fig. 6.3 and the question posed above regarding the physiological and metabolic adaptations which account for observed differences in apparent maintenance requirements based upon differences in previous and current rates of nutrient intake. Neither statistical inference nor the mysticism associated with terms such as DIT direct attention to the quantitative metabolic and physiological causes of variance that must be addressed if we, as scientists should, seek to understand why energy expenditures vary.

6.3.2 Components of fasting heat production

Variance in fasting heat production is quite great and ranges from 170 kJ/W^{0.75} in animals subjected to prolonged undernutrition to 580 kJ/W^{0.75} in very young and highly productive (lactating) animals (Baldwin and Bywater, 1984). Obviously, a wide range of factors contribute to this variance, including age, sex, breed, species, physiological state, rate of production, hormonal environment, and previous and current planes of nutrition. There are a number of ways one can go about addressing this variance. One is the statistical approach discussed above, in which the exponent (b) is held at 0.75 and, as a result, all variance is associated with the coefficient (a). In conventional feeding systems, the coefficient (a) is varied systematically to accommodate observed differences in energy required for apparent maintenance, e.g. (a) is set at 420 kJ/W^{0.75} for adults fed at maintenance, at 481 kg/W^{0.75} for growing animals and at 565 kJ/W^{0.75} for lactating animals. This approach requires that discrete decisions be made regarding apparent maintenance requirements in growing, pregnant, lactating and maintenance-fed adults of a species, while it is clear that the apparent maintenance requirements are a continuous variable dependent upon physiological state, feed intake, etc. An alternate approach, also discussed above, is to consider variance in energy expenditures associated with apparent maintenance as a continuous function of feed intake (Koong *et al.*, 1982). An additional approach is to consider variance on a tissue-by-tissue basis and thereby attempt to understand and accommodate differences in apparent maintenance energy expenditures.

In this section, the latter two approaches will be considered, as both approaches to explaining variance have merit. Underlying this discussion

is recognition of the fact that a number of hormonal changes are associated with age, sex, feed intake and physiological state.

(a) *Whole animal functions*

Estimates of energy expenditures associated with several maintenance functions are presented in Table 6.8. The first listed functions can be considered service functions in the sense that they are performed by one tissue in support of the functions of other tissues and performance of the organism as an integrated whole. The first service function identified, Na^+ resorption by kidney, is probably most obvious, next to heart work and work of respiration. The estimates of service functions in Table 6.8 are based upon those in physiology texts such as Bard (1961) and Guyton (1956).

The estimate of energy expenditures associated with Na^+ resorption by kidney (Table 6.8) is based upon the observation that in an adult, 70 kg human male, the glomerular filtration rate in kidney indicates that 24–26 moles of Na^+ are reabsorbed each day. Further, data indicate that 65% of this resorption is by active transport and the estimated mean efficiency of the two Na^+ pumps of kidney is 2.5 Na^+ transported/ $\sim$ P expended. Thus, $[(2.5 * 0.65)/2.5] * 75 \text{ kJ}/\sim\text{P} = 489 \text{ kJ}/\text{day}$ and, at a basal metabolic rate of $(293 \text{ kJ}/\text{W}^{0.75})$, $7.1 \text{ MJ}/\text{day}$, $[(0.489 \text{ MJ}/7.1 \text{ MJ}) * 100]$, 7% of fasting heat production can be attributed to Na^+ resorption by kidney. This estimate is remarkably high in view of the fact that most estimates of the contribution of kidney to total energy expenditures in humans are in the 8–10% range.

Table 6.8 Energy expenditures in several maintenance functions^a

<i>Functions</i>	<i>BMR (%)</i>
Service	
kidney work (Na^+ transport)	6–7
heart work	9–11
nervous functions	15–20
respiration (muscle work)	6–7
Repair	
protein resynthesis	10–15
triacylglycerol resynthesis	1–2
Na^+ transport (membrane potential)	20–25

^aCalculated for 70 kg man at basal metabolic rate (7.1 MJ/day).

We will not consider energy costs for transport of other ions in discussion of service and tissue maintenance functions, but they can be significant as indicated by data tabulated by Caldwell (1968) and presented, in part, in Table 6.9.

Table 6.9 Estimated energy expenditures associated with ion transport^a

<i>Ions</i>	<i>Ions transported/~P hydrolyzed</i>
K ⁺	0.0; antiport to Na ⁺
Na ⁺	2.0 and 4.0; two pumps of different efficiencies
Ca ²⁺	1-2
Mg ²⁺	3.0
PO ₄ ³⁻	1-2
H ⁺ and HCO ₃ ⁻	10-20
Pyruvate and lactate	3-4

^a Adapted from Caldwell (1968).

The estimates of service or physiological work by heart, muscles of respiration and nervous tissue are more or less consensus estimates from a number of sources (Baldwin and Smith, 1974). The sum of the estimates of service functions listed in Table 6.8 is about 40% of total energy expenditures. Some service functions, including kidney work and nervous functions are relatively constant at a given body weight, e.g. they do not differ with physiological state and can be considered in aggregate in models of animal metabolic functions. The others, including costs of heart work and respiration, vary with food intake, activity, and rate of production, and must be considered dependent variables. It is noteworthy that these vary continuously in animals on low to high planes of nutrition and manifest as components of both maintenance energy expenditures and costs of production in regression models (equations 6.8 and 6.10). This is also true of costs of protein synthesis and ion transport (see below). This creates a real problem.

When energy expenditures associated with single functions, such as heart work or protein resynthesis, are partitioned between estimates of maintenance requirements and costs of production by a statistical model, how does one utilize or relate estimates of efficiency to direct estimates of cardiac output and protein turnover in producing animals? In response to this problem, mechanistic models are increasingly diverting away from the classical concept of depicting costs of maintenance and production separately. Rather, energy expenditures in relatively constant processes are represented in aggregate and summed with variable costs associated with physiological work, protein synthesis, ion transport, etc., in order to obtain estimates of total animal energy expenditures to be satisfied through oxidation of available nutrients.

Two important cell maintenance costs are energy expenditures in ion pumping (transport) and protein turnover. The basic method of estimating energy expenditures in Na⁺ transport is to measure oxygen uptake by tissues or cells with and without ouabain in the medium. Since ouabain is an inhibitor of the Na⁺ K⁺ -ATPase associated with the Na⁺ pump, the

percentage reduction in oxygen uptake is taken as a measure of energy expenditures in Na^+ transport. Data summarized by Summers *et al.* (1988) on energy expenditures attributable to ion transport are presented in Table 6.10 and indicate that 26% of fasting heat production is due to this single function. No data were available for components of the body that are responsible for 32% of fasting heat production. If an estimate of one-third of ouabain-sensitive respiration is used in these tissues, the estimated contribution of Na^+ transport to fasting heat production rises to about 35%. This estimate was decreased in formulating Table 6.8, to account for kidney and nervous tissue functions associated with service functions resulting in the estimate of 20–25% of energy expenditures in ion transport. Note also that Table 6.8 relates to an adult human male (higher energy expenditures in nervous tissue) while the Summers *et al.* (1988) summary in Table 6.10 relates to a generic experimental animal. Therefore, not all values are directly comparable. The fact that energy expenditures in ion transport are a significant contributor to fasting heat production, however, should be clear. Causes of variation in this important cause of energy expenditures will be considered below.

Table 6.10 Estimate of percentage fasting heat production due to Na^+K^+ -ATPase activity or ion transport^a

Tissue	Total body HP (%)	Ouabain sensitive respiration (%)	Whole body ouabain sensitive respiration (%)
Muscle	20	16	3.2
Brain	14	40	5.6
Liver	12	38	4.6
Gut	8	35	2.8
Kidney	14	70	9.8
Others	32	—	—
Sum	100		26

^aAdapted from Summers *et al.* (1988).

Most estimates (Neuberger and Richards, 1964; Chapter 7) of rates of protein turnover in humans fall between 200 and 300 g/day. Using an average molecular weight of 110 g/mole for amino acids in a protein, these estimates indicate that 1.82 to 2.73 moles of peptide bonds must be synthesized per day in an adult human male in order to maintain his protein mass. Most nutritionists seem to agree, although on different grounds (Baldwin and Smith, 1974; Lobley *et al.*, 1980; Reeds, 1989), that about five high energy phosphate bonds ($\sim\text{P}$) are hydrolyzed in the synthesis of a peptide bond. Thus, at 75 kJ/ $\sim\text{P}$, $[(1.82 * 5 * 75) * 100 * (7.1 \text{ MJ/day})^{-1}] = 9.6$ or $[(2.73 * 5 * 75) * 100 * (7.1 \text{ MJ} * \text{day})^{-1}] = 14.4\%$ of fasting heat

production (Table 6.8) can be attributed to costs of maintenance of body protein, a repair function. Costs associated with other repair functions, using triacylglycerol turnover as an example, are more modest at 1–2% (Table 6.8). It appears that repair functions in fasting adults previously fed at maintenance are in the 15% range. The sum of service (40%), ion transport (20–25%) and repair (15%) functions is in the order of 80% of basal heat production.

Milligan and McBride (1985), Summers *et al.* (1988) and Baldwin *et al.* (1980) discussed sources of variation in protein turnover and ion transport, which include age, plane of nutrition, physiological state and, for ion transport, cold adaptation. Baldwin *et al.* (1987) and DiMarco and Baldwin (1989) included provisions for variation in these functions in dynamic models of lactating dairy cows and growing steers (see Chapters 16 and 17). They found that such provisions enabled better simulations of total body energy expenditures even though only limited data were available to support parameterization of the equations used. Further data are clearly needed to support the development of improved mechanistic relationships.

(b) Tissue energy expenditures

An alternative approach to analyses of functions that contribute to fasting heat production is to undertake a tissue-by-tissue evaluation. This approach is also useful in terms of identifying and estimating sources of variation in apparent maintenance requirements. As a starting point, estimates of the relative weights, of energy expenditures by, and blood flow rates to tissues and organs systems can be considered. Results of such an evaluation derived from the literature survey of Smith (1970) are presented in Table 6.11. Several aspects of this tabulation are of particular interest. The first is the tremendous range among organs in the ratio of percent FHP/percent body weight. For example, the ratios for liver and muscle are 14 and 0.56, respectively. This indicates that heat production per unit weight of liver is $(14/0.56)$ 25 times greater than that for muscle. The range of estimates of 0.22 to 23.0 clearly indicates that the standard estimate of fasting heat production of $293 \text{ kJ/W}^{0.75}$ is a weighted average of energy expenditures per unit weights of very diverse tissues and organ systems. Thus, nutritional and physiological factors that cause changes in the relative weights of tissues can cause significant changes in the $293 \text{ kJ/W}^{0.75}$ coefficient.

Halliday *et al.* (1967) addressed the possibility that the high metabolic rates of infants could be explained on the basis of the high relative weights of brain and visceral organs in infants as compared to adults. Using the assumption that energy expenditures per unit mass of tissue do not differ between infants and adults, they estimated that over 50% of the difference in heat production per unit metabolic body weight in infants compared to adults could be explained on the basis of differences in relative organ weights.

Table 6.11 Relationships among tissue mass, energy expenditures and blood flow

	Weight as percentage EBW ^a	Percentage of cardiac output (CO)	Percentage of FHP ^a	%CO %EBW	%FHP %EBW
Nervous tissue	2.0	10	12	5	6
Liver	1.6	27	22.5	17	14
Heart	0.6	4.1	10	6.8	16
Adipose	15	9.6	8.0	0.64	0.53
Muscle	41	18	23.0	0.43	0.56
Other	27.7	9.9	5.8	0.35	0.22
Kidney	0.3	13.4	7.0	44	23
Gastrointestinal	6.5	23.0	9.0	3.5	1.4
Skin	6.3	8.0	2.7	1.2	0.42

^aEBW, empty body weight; FHP, fasting heat production.

In animals subjected to prolonged undernutrition – certainly not unusual in many animal production environments – relative liver weights can decrease from the norm of 1.6% of body weight to 1.3% or lower, while in lactating animals this value can increase to 1.8% or higher. If changes in liver weight between 1.3 and 1.8% were compensated by changes in muscle weight, a 10% change in FHP would be expected. A number of workers, including Webster (1981), Koong *et al.* (1983), Ferrell (1984), Smith and Baldwin (1974) and Canas *et al.* (1982) have evaluated these effects. Estimates of the impact of changes in the relative weights of tissue and organ systems upon apparent energy requirements for maintenance during lactation in cows (Smith and Baldwin, 1974) and rats (Canas *et al.* 1982) are presented in Tables 6.12 and 6.13. Apparent maintenance requirements expressed as ME_m increase from about 460 to 565 kJ/W^{0.75}, or 23% (NRC, 1989) in lactating cows. Based upon the assumption that energy expenditures per unit weight of tissue are not altered during lactation, which is implicit in the calculations presented in Table 6.12, 10% out of the 23% increase in FHP in lactating cows can be attributed to the change in relative organ weights associated with lactation in cows. Similar calculations (Table 6.13) indicate that a 20% increase in apparent maintenance requirements in mid-lactation rats could be explained by changes in relative organ weights. Unfortunately, due to limitations associated with the use of multiple linear regression techniques to interpret energy balance experiments with lactating rats (see Chapter 10), a rigorous estimate of the impact of lactation upon apparent maintenance requirements of rats is not available.

Koong *et al.* (1982), Ferrell (1984) and Pekas (1991) have undertaken quite a number of analyses of effects of feed intake on relative organ weights and FHP using advanced statistical techniques, including path coefficient (Ferrell, 1984) and cluster (Pekas, 1991) analyses. In these analyses, correlations

Table 6.12 Estimation of the effects of changes in tissue or organ size upon apparent maintenance requirements of non-lactating and lactating cows^a

Tissues or organ systems	Non-lactating cow				Lactating cow			
	weight of tissue (kg)	tissue percent of body weight	percent of heat production	MJ/day	MJ/kg tissue	weight of tissue (kg)	tissue percent of body weight	MJ/day ^b
Kidney and brain system	2.6	0.4	17.0	10.65	4.1	2.6	0.4	10.65
Skin	33.0	5.1	3.5	2.19	0.07	31.3	4.8	2.08
Gastrointestinal	26.4	4.1	7.2	4.51	0.17	33.9	5.2	5.79
Liver	9.1	1.4	22.5	14.1	1.55	11.4	1.8	17.66
Heart	2.6	0.4	10.0	6.26	2.41	3.2	0.5	7.71
Visceral fat	51.1	7.9	5.0	3.13	0.06	42.0	6.5	2.56
Carcass	406.3	62.5	26.0	16.28	0.04	375.2	57.7	15.07
Mammary	13.1	2.0	2.3	1.44	0.11	22.7	3.5	2.5
Other	105.3	16.2	6.5	4.07	0.04	127.7	19.6	4.92
Sum	650.0	100	100	62.63		650	100	68.94
MJ/kg ^{0.75} per day				0.46				0.506

^a Data for 700 kg Holstein cows, assumed empty body weight of 650 kg. Data adapted from Smith and Baldwin (1974).

^b Based on MJ/kg tissue in non-lactating cow and kg tissue in lactating cow.

Table 6.13 Estimation of the effects of changes in tissue and organ weights upon apparent maintenance requirements of lactating rats^a

Tissue or organ systems	Non-lactating rats		Lactating rats	
	body weight (%)	heat production (kJ)	body weight (%)	heat production (kJ)
Skin, brain, and kidney	12.0	41.88	12.0	41.84
Adipose	15.0	22.84	7.0	10.46
Gastrointestinal	4.0	8.20	5.45	11.3
Liver	3.38	38.07	4.87	54.81
Heart	0.30	19.04	0.35	22.59
Carcass and other	65.32	60.38	66.33	89.12
Mammary tissue	—	—	4.0	7.53
Total	100.0	190.41	100.0	237.65
Heat production/ $W^{0.75}$	—	418.4	—	518.8

^a Adapted from Canas *et al.* (1982). Both lactating and non-lactating rats weighed 350g; Convention used in calculations are as in Table 6.12.

Table 6.14 Regression analysis of effects of previous level of nutrition on maintenance requirement in rats and sheep^a

Species	Previous level of nutrition	Regression coefficient		R ²	Maintenance requirement	
		b ₁	b ₂		RSD	g(kg ^{0.75} * day)
Rats	high	-23.6 ± 3.3	0.596 ± 0.065	0.87	0.92	39.6
	medium	-27.6 ± 3.2	0.743 ± 0.065	0.91	0.89	37.2
	low	-27.3 ± 2.7	0.711 ± 0.054	0.85	1.11	28.8
Sheep	high	-19.6 ± 2.4	0.359 ± 0.033	0.94	49.0	54.6
	medium	-19.7 ± 1.8	0.446 ± 0.026	0.98	35.0	44.1
	low	-12.9 ± 3.6	0.423 ± 0.041	0.99	40.0	30.5
Sheep	high then low	-18.0 ± 1.9	0.401 ± 0.030	0.95	48.9	44.9
	low then high	-21.2 ± 1.7	0.358 ± 0.022	0.96	39.9	59.1

^a Adapted from Ferrell (1984).

among changes in organ weights and FHP or apparent maintenance requirements as influenced by feed intake are very high. Data from several trials tabulated by Ferrell (1984) are presented in Table 6.14. The data and regression equations used to deduce the apparent maintenance requirements presented in Table 6.14 were clearly quite good, with r^2 values of 0.85 or better. The apparent maintenance requirements of rats and sheep, previously provided high as compared to low planes of nutrition expressed as g feed required $\cdot (W^{0.75} \cdot \text{day})^{-1}$, were 38 and 79% greater, respectively. When sheep were fed to the same final body weights using a low followed by a high plane of nutrition, apparent maintenance requirements were 32% greater than those of sheep provided a high plane of nutrition followed by feed restriction. This analysis clearly indicated high contributions of, particularly, visceral organ weights to variance in fasting heat production as emphasized by Webster (1981). The cluster analyses of Pekas (1991) indicated that small intestines, kidneys, liver and pancreas were important sources of variance in FHP. Canas *et al.* (1982) summarized data from experiments with undernourished rats, growing rats, and lactating rats which suggested a logistic relationship between FHP and metabolizable energy intake and, consistent with observations discussed above, a high correlation between FHP and differences in relative organ weights.

There is no question at this point about the correlations among feed intakes, organ weights and FHP, and apparent maintenance requirements. The question has now become what portions of the changes in FHP and apparent maintenance requirements are attributable to relative changes in organ weights *per se*, and what portions are attributable to changes in tissue functions that vary in a collinear fashion with feed intake, physiological and hormonal state, and relative organ weights.

The first observations that led us to consider these relationships were those of Ismail-Beig and Edelman (1971), which indicated that differences in energy requirements due to thyroid status largely could be attributed to thyroid hormone effects upon $\text{Na}^+ \text{K}^+ \text{-ATPase}$. An early and crude study of effects of pregnancy and lactation upon energy expenditures in several tissues, undertaken in part because of the studies of Ismail-Beig and Edelman (1971) and Webster (1981), indicated to us that energy expenditures per unit weight of organ vary with physiological state. Further, these data indicated that the implicit assumption made in the formulation of Tables 6.12 and 6.13 was incorrect, in that energy expenditures per unit weight of organ were likely greater in lactation as compared to non-lactating animals. Also, the unaccounted one-half of the increase in apparent maintenance requirements in lactating cows and rats may be attributable to this change. Further evaluations support this view, which stated differently would hold that both relative organ weights and energy expenditures per unit weight of organ vary in a collinear fashion with feed intake and can explain much of the variance we observe in estimates of FHP and apparent maintenance requirements.

Summers *et al.* (1988) presented an excellent evaluation of the identities, relative magnitudes of, and variance in a number of metabolic and physiological functions. These included, both synthesis and degradation of proteins (see Chapter 7), nucleic acids and phospholipids, macromolecule and ion (Na^+ , K^+ , Ca^{2+}) transport, substrate cycling, and service functions such as heart, respiratory muscle, kidney, liver and skeletal muscle work. Some of these were discussed above, and the reader is referred to Summers *et al.* (1988) for further discussion and authoritative analyses.

In the Summers *et al.* (1988) presentation and some of our earlier analyses, the point is made that effects of feed intake and physiological state upon energy expenditures must be undertaken on a tissue-by-tissue basis, because changes at the tissue level do not occur in parallel. Several relevant analyses are presented in Tables 6.15–6.19. The estimates in Tables 6.15 and 6.16 are presented to identify and place into a quantitative context evaluations of energy expenditures in human and ruminant liver that could be undertaken in the 1970s. The ruminant gastrointestinal tract estimates (Table 6.17) are presented for the same reasons and, further, to illustrate that estimates of the contribution of the gastrointestinal tract to energy expenditures at that time were clearly too low. The limited list of functions listed in Table 6.17 certainly should not have added up to 100%.

Table 6.15 Energy expenditures in human liver functions^a

Process	kJ	Percentage of liver energy expenditure
Plasma protein synthesis (18 g/day)	65.69	4.5
Liver protein synthesis (10 g/day)	35.98	2.5
Ribosomal RNA synthesis	0.628	0.045
$\text{Na}^+ *$ (K^+) ⁻¹ transport (active)	502.08	35.0
Cori cycle and TG turnover	71.13	5.0
Amino acid degradation ^b	200.83	14.0
FA conversion to ketone bodies	60.25	4.2
Unaccounted	486.18	34.0

^aAdapted from Baldwin and Smith (1981); calculated for liver of 70 kg man expending 1422.56 kJ/day in liver function.

^bIncludes heat loss in glucose, ketone bodies and urea synthesis.

More up-to-date listings of energy expenditures in hepatocytes and muscle adapted from Summers *et al.* (1988) are presented in Tables 6.18 and 6.19. These are included to emphasize some of the uncertainty associated with accounting for energy expenditures at the tissue level when one undertakes the conservative approach adopted by these workers. We will and do contend in subsequent chapters – not in disagreement with Summers *et al.* (1988) – that the variance depicted in Tables 6.18 and 6.19 largely is accounted for on the basis of differences in the physiological and metabolic

Table 6.16 Estimated energy expenditures for several ruminant liver functions^a

<i>Process production</i>	<i>kJ/day</i>	<i>Percentage of total liver heat</i>
Protein synthesis		
liver proteins (14 g/day)	50.21	1.5–2.5
plasma proteins (20 g/day)	71.13	2.5–3.5
Glycogen turnover (20 g/day)	20.92	0.5–1.0
Gluconeogenesis ^b		
from lactate (144 g/day)	292.88	9–14
from propionate (68 g/day)	188.28	6–9
from amino acids (10–13 g/day) ^c	309.61	10–13
from glycerol (36 g/day)	50.21	2–3
Ketogenesis (acetate, FA) ^b	41.84	1–2
Triglyceride synthesis ^{b,d}	62.76	2–3
Urea synthesis (65 g/day) ^e	326.35	11–15
Ion transport	627.60	20–31
Total	2041.79	65.5–97

^aAdapted from Baldwin and Smith (1981).

^bCalculations based upon data summarized by Smith (1970). Estimates of amounts of glucose synthesized from various substrates expressed as g glucose produced per day at basal rate.

^cCalculation for gluconeogenesis and ketogenesis from amino acid mixture comparable to meat protein. Estimate includes cost of synthesis of 0.367 mole urea (115.90 kJ).

^dIncludes costs of fatty acid synthesis from acetate and incorporation of fatty acids into triglyceride.

^eBased upon determinations of urea excretion and secretion. Estimate corrected for urea synthesis accounted for under gluconeogenesis from amino acids.

Table 6.17 Evaluation of processes contributing to heat release by the gastrointestinal tract of a ruminant^a

<i>Process</i>	<i>Percentage of total GI heat production</i>	<i>kJ/24 h</i>
Ketone body formation from butyrate	5.8	58.58
Triglyceride synthesis	0.8	7.95
Digestive enzyme secretions	24.2	243.9
Protein turnover		
mucosa	28.8	290.8
other	13.2	133.0
Active transport in absorption	17.2	172.8
Ion transport	10.0	101.2
Total	100	1008.2

^aCalculated for a 100 kg ruminant 24 h post-feeding with an empty body weight of 95 kg and estimated total energy expenditure of 1.0 MJ/day

Table 6.18 Energy-consuming processes in skeletal muscle^a

Process	System	Percentage of basal metabolic rate	Reference
Protein synthesis	Isolated muscle	14.6–22.6	Reeds <i>et al.</i> (1985)
F6P/(FBP cycling ^b)	Isolated muscle	0.5	Challis <i>et al.</i> (1984)
Active transport Na ⁺ , K ⁺	Isolated muscle	16.7–45.8	Gregg and Milligan (1982a,b); Summers and Milligan (unpublished)
Ca ²⁺	SR (theoretical) ^c	0.7	Hasselbach and Oetliker (1983)
	Isolated muscle	13.1	Summers and Milligan (unpublished)
Total		45.6–82.7	

^aAdapted from Summers *et al.* (1988).^bF6P/FBP, fructose 6-phosphate/fructose 1,6-bisphosphate.^cSR, sarcoplasmic reticulum.**Table 6.19** Energy-consuming processes in hepatocytes^a

Process	Percentage of basal metabolic rate	Reference
Protein synthesis	15.1–26.5	Reeds <i>et al.</i> (1985 ^b , 1987); McBride (1986)
Substrate cycling ^c		
GL/G6P	3.0–4.6	Rabkin and Blum (1985)
Glycogen/G6P	0.1–1.8	Rabkin and Blum (1985)
F6P/FBP	8.8–14.2	Rabkin and Blum (1985)
PEP/Pyr/OAA	3.3–6.6	Rabkin and Blum (1985)
acetate/AcCoA	2.8–3.1	Rabkin and Blum (1985)
Na ⁺ , K ⁺ transport	27.9–55.3	McBride and Milligan (1985a,b)
Urea biosynthesis	8.3–12.1 ^d	Rabkin and Blum (1985)
Total	69.3–124.2	

^aAdapted from Summers *et al.* (1988).^bPerfused liver.^cGL, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; PEP, phosphoenolpyruvate; Pyr, pyruvate; OAA, oxaloacetate; AcCoA, acetyl-CoA.^dExtrapolated from Rabkin and Blum (1985), assuming P:O = 3 and 4 ~P hydrolyzed/mole urea.

states of the animals studied, e.g. protein synthesis and active transport vary as functions of feed intake and physiological state, urea synthesis as a function of diet protein intake, etc. The basic point is that energy expenditures differ by organ and function, can be estimated given appropriate experimental data, and can only be effectively evaluated across time, diets and physiological states with dynamic, mechanistic models.

Gill *et al.* (1989a,b) reported extensive data and simulation analyses of energy costs associated with protein turnover and Na^+ , K^+ transport in 10 tissues in growing lambs. Protein synthesis in the several tissues was represented using equations of the form

$$\text{rate} = k_{\text{xaxp}} Q_{\text{xp}} / (1.0 + k_{\text{xa,xaxp}} / C_{\text{xa}}) \quad (6.17)$$

where $k_{\text{xaxp}} Q_{\text{xp}}$ was the maximum rate of conversion of amino acids into protein as a function of the quantity of protein (Q_{xp}), in that tissue; $k_{\text{xa,xaxp}}$ was the affinity constant of the protein synthesis system for intracellular amino acids; and C_{xa} was the concentration of amino acids. Equations for protein degradation were of the form

$$\text{rate} = k_{\text{xpxa}} Q_{\text{xp}} / (1.0 + C_{\text{xa}} / J_{\text{xa,xpxa}}) \quad (6.18)$$

where the maximum rate is defined by a constant (k_{xpxa}) and quantity of protein (Q_{xp}) and $J_{\text{xa,xpxa}}$ was defined as an inhibition constant for an effect of intracellular concentrations of amino acids on degradation rates. Transfer of amino acids into the tissue from blood was represented using a mass action equation. The equation for transfer of amino acids out of the cell had the same form as equation 6.18, with the concentration of amino acids in blood acting to inhibit efflux. Equations for ion transport provided for a basal rate and a variable rate that increased with feed intake. Simulation analyses of growing lambs fed at two intakes indicated that protein turnover accounted for 19% of the change in energy expenditures due to increases in feed intake, while increased costs in Na^+ , K^+ transport accounted for 39%. Costs associated with fat turnover and accretion accounted for 25% of the increase in energy expenditures. This left 17% of the increase in energy expenditures unaccounted for.

The point one should take based upon the above discussion is that we have identified a large proportion of energy costs associated with apparent 'maintenance' requirements and causes of variation in these. Continuing experimental and modeling analyses of these functions will soon enable nutritionists to develop systems in which 'maintenance' and 'production' need not be considered as discrete variables but rather as continuous functions of underlying processes which are known and characterized to the extent that they can be characterized mathematically, be used in the development of superior systems for optimization of resource use in animal production and, thus, replace the static, factorial systems in current use.

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Protein and amino acid models

with C. L. Ferrell and C. C. Calvert

While the carbohydrate are dominated by starch and cellulose substances remarkable for accomplishing two very different tasks with very similar materials, the protein astonish with their shear variety and versatility.

McGee (1984)

7.1 INTRODUCTION

Current estimates of protein requirements of sheep (NRC, 1985a), beef (NRC, 1984b) and dairy cattle (NRC, 1989), and for protein and amino acid requirements of poultry and swine (NRC, 1984a, 1988) are based on a factorial approach that is philosophically similar to that proposed by Mitchell and coworkers about 60 years ago (Mitchell, 1929; Mitchell *et al.*, 1932; Blaxter and Mitchell, 1948). This is illustrated in the definitions quoted below from Blaxter and Mitchell (1948):

The factorial method for the estimation of the protein requirements of animals, as exemplified by Mitchell (1929), attempts to measure separately the different components of the total requirement and to vary each component as changes in age and functional activity of the animal, as well as difference in sex, may require. Thus, if E be the minimum endogenous nitrogen loss to the animal per day, determining the maintenance requirements of protein, and G be the daily increment in tissue nitrogen during growth, the total requirement of conventional net protein by a growing animal would be, according to the procedure heretofore used,

$$R_n = 6.25 (E + G) \quad (1)$$

Similarly, the factorial evaluation of a feed (or ration) as a source of protein, as illustrated by Mitchell and Carman (1924), measures separately the content of nitrogen per 100 grams of dry matter (N), its true

digestibility (T), and the biological value of the absorbed nitrogen (BV) for some particular animal function or combination of animal functions. The 'net protein' value of a feed or ration (F_n) then becomes, per 100 grams of dry matter when T and (BV) are expressed as decimals,

$$F_n = 6.25 N \cdot T (BV) \quad (2)$$

Evidently, according to this factorial system, one pound of F_n will exactly cover a requirement for one pound of R_n , just as one therm of net energy in a ration will cover a requirement of one therm by an animal, according to the Armsby system.

Estimates of protein requirements of farm animals by the factorial method are generally lower than commonly accepted standards, at least for older animals, as pointed out by Mitchell (1929), and have not been generally accepted...

When the metabolic fecal N is brought into the factorial procedure as Blaxter and Price suggest, it will modify the expression both of the requirement of net protein and of the net protein content of the feed. Since the metabolic fecal nitrogen must be replaced in the body in the form of mucosal cells and mucus, nitrogenous constituents of the digestive juices, etc., it becomes a third item in the right-hand side of equation (1), i.e.

$$R_n = 6.25 (E + G + M \cdot D) \quad (3)$$

where M is the metabolic fecal nitrogen per 100 g of dry matter ingested, and D , the dry matter intake in hundreds of grams required to cover the energy needs of the animal.

If the coefficient of apparent digestibility of protein (A) is used in the evaluation of feeds as a source of protein rather than the coefficient of true digestibility (T), then equation (2) becomes

$$F_n = 6.25 (N \cdot A + M) (BV) \quad (4)$$

From equation (3) it appears that the net protein requirement of an animal is dependent upon the dry matter intake. However, the dry matter intake, D , required to satisfy the energy requirements of the animal is a characteristic of the ration fed and of the feeds of which it is composed, and might, therefore, better be considered a tax upon the feed rather than a variable factor in the animal's requirements. The term ($M \cdot D$) may be removed from the right-hand side of equation (3) and deducted from the net protein consumed as follows:

$$\begin{aligned} D[6.25(N \cdot A + M)(BV)] &= 6.25 [E + G + M \cdot D] \\ D[6.25(N \cdot A + M)(BV)] - 6.25(M \cdot D) &= 6.25 [E + G] \\ D\{6.25[(N \cdot A + M)(BV) - M]\} &= 6.25 [E + G] \end{aligned} \quad (5)$$

so that the net protein value of the ration and of each feed in it with its characteristic nitrogen content, N , becomes, per 100 g of dry matter,

$$Fn^1 = 6.25[(N \cdot A + M)(BV) - M] \quad (6)$$

this characteristic protein value meeting the net protein requirements for maintenance and growth above. The truly digestible (metabolizable) protein content of a feed or ration would still be $6.25(N \cdot A + M)$ and what we shall call the 'available protein' content would be

$$F_a = 6.25[(N \cdot A + M) - M/(BV)] \quad (7)$$

for each 100 g of dry matter in each case. In an analogous sense, the requirement of the growing animal for available protein would be

$$R_a = 6.25(E + G)[I/(BV)] \quad (7a)$$

7.2 CURRENT PROTEIN AND AMINO ACID SYSTEMS

In the system adopted for sheep (NRC, 1985a), estimates of protein requirements were calculated according to the following equation:

$$\text{Crude protein required (g/day)} = (\text{PD} + \text{MFP} + \text{EUP} + \text{DL} + \text{Wool})/\text{NPV} \quad (7.1)$$

where PD is the protein deposited, MFP is the metabolic fecal protein, EUP is the endogenous urinary protein, DL is the dermal loss, wool is the wool protein and NPV is the net protein value.

Values of each component of this model were empirically derived from experimental data and are included in the model as constants or as simple empirical equations. Example values or equations used to estimate each component of the model follow:

$$\text{PD (g/day)} = \text{daily gain (kg)} * (1121 - 123) * \text{NE}_g \text{ (kJ/day)} / \text{gain (g/day)} \quad (7.2)$$

$$\text{Wool} = 6.8 \text{ g/day for ewes and rams; } 3 + 0.1 * \text{PD for lambs} \quad (7.3)$$

$$\text{NPV} = 0.561 \quad (7.4)$$

The system currently in use to express the protein requirements of beef cattle (NRC, 1984b) is similar to the one adopted for sheep. The equation used is

$$\text{CP} = (F + U + S + G + C + M) / (D * BV * CE) \quad (7.5)$$

where F is the metabolic fecal protein ($N * 6.25$), U is the endogenous urinary protein ($N * 6.25$), S is the skin, hair and scurf loss, G is the tissue protein deposition, C is the protein deposition in the products of conception, and M is the protein output in milk. The denominator is true digestibility (D) times biological value (BV) times the efficiency of converting dietary protein to ruminal protein (CE). Values are assumed to be 0.90, 0.66 and 1.0% for D , BV and CE , respectively. The product, 0.594, is not con-

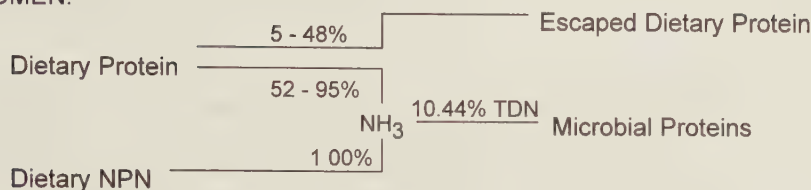
ceptually (or numerically) different from the $NPV = 0.561$ used for sheep (NRC, 1985a). The factors in the numerator are similar conceptually to factors included in the model for sheep, although most were estimated somewhat differently than for sheep.

Thus, models used to calculate the protein requirements of ruminants were based upon regression equations. Important sources of variance noted were DP/DE (DE is the digestible energy) ratio in the diet and age or composition of gain. Therefore, appropriate adjustments were made when developing tabular recommendations for DP or CP (crude protein) in cattle rations. Subsequent to this, it was recognized that the types of protein supplements used in ruminant rations can have marked effects upon performance. Specifically, it was noted that microbial growth in the rumen is not adequate to support maximal productivity. Thus, diets containing only proteins that are highly available and do not escape degradation in the rumen support neither maximum microbial growth rates nor maximum milk production. Many conventional diets have this property, such that delivery of proteins or amino acids to the lower gut to supplement those available from the rumen microbes can increase production 20–30%.

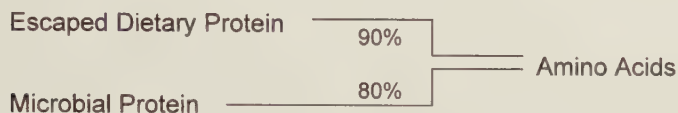
Thus, it became apparent that the physical properties of dietary proteins must be balanced to provide adequate nitrogen and protein (or amino acids and peptides) to support optimal microbial growth and, at the same time, adequate escape of dietary protein from the rumen to support optimal or maximal rates of growth and milk production. Widespread recognition of this problem led, in the 1970s and early 1980s, to extensive research and the development of, at least, 10 different models of the static, factorial type and several dynamic models directed to the study and formulation of feeding recommendations for protein (Owens and Zinn, 1982).

Most of these models were based upon the set of concepts depicted in Fig. 7.1. Dietary crude protein is partitioned into true protein that is degraded to ammonia and fatty acids by the rumen microbes, true protein that escapes rumen degradation, and non-protein nitrogen (including urea), which acts as a source of ammonia for rumen microbial growth. Microbial growth was usually calculated as a constant function of DE or ME intake or organic matter digestion in the rumen. In some cases, provisions for salivary protein and urea entry to the rumen were included. In these models, microbial protein and dietary protein that escape from the rumen are digested at differing efficiencies in the lower gut yielding an estimate of amino acid availability to the animal. Absorbed amino acids are in turn partitioned to support maintenance and productive functions, including metabolic fecal protein losses, protein accretion, milk production and amino acid degradation and gluconeogenesis. Parameter values used to implement the equations varied somewhat among models, but these differences tend to cancel out because of the factorial nature of the computation and the fact that nitrogen balance data were used to adjust many of the key parameters in the models (Owens and Zinn, 1982). Objectives in these models were to

RUMEN:



INTESTINE:



METABOLISM:

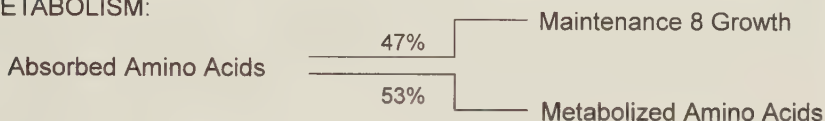


Figure 7.1 Model of metabolizable protein in the rumen and intestine and metabolism of amino acids.

assure that the nitrogen requirements of the rumen microbes were met and, further, that the amino acid requirements of the animal are met economically, by a combination of rumen microbial growth and protein escaping rumen degradation.

In view of these considerations, the National Research Council (NRC, 1985b) suggested the use of a somewhat more sophisticated approach to characterize ruminant nitrogen utilization. The conceptual framework for estimation of animals' protein needs was similar to those described previously, e.g. estimates of scurf protein loss, endogenous urinary loss, metabolic fecal nitrogen and nitrogen requirements for growth, gestation, milk production, and wool were estimated from available data by the use of empirically derived relationships. The primary conceptual difference is that an attempt was made to quantitate diet effects on protein available to the small intestine in the form of microbial protein and as protein of dietary origin that escaped ruminal degradation. In addition, amounts of nitrogen available in the rumen for microbial protein synthesis were estimated, allowing an assessment of the value of added non-protein nitrogen.

Based upon these recommendations, several important modifications were incorporated into the current NRC (1989) system for dairy cattle. Requirements are defined in terms of absorbed rather than crude protein, thereby enabling explicit consideration of fractionation of ingested protein into degraded (in the rumen) protein and undegraded protein, bacterial

and protozoal protein availability as a function of fermentation energy, and the mobilization of tissue protein in early lactation. Also, equations for estimating available protein requirements for live weight gain and milk protein yield were modified.

At this point in evolution of the protein system for ruminants, a departure from the classical empirical approach is evident. Knowledge of underlying or mechanistic relationships was introduced. Further, it is clearly evident that a number of the parameters that must be defined in the model cannot be deduced statistically from animal input:output data. Thus, the current dairy cattle system must be defined as a largely empirical system that contains key mechanistic elements but remains static and factorial in nature. This may be true, to a lesser extent, in the sheep and beef systems also. The AFRC (1990) proposals were similar to the NRC (1989) dairy system in this regard.

Protein and amino acid intake guidelines for swine (NRC, 1988) and poultry (NRC, 1984a) are expressed as amount per kilogram diet and in g/day for commonly used commercial-type diets with stated energy densities (DE or ME/kg). The values presented for protein and amino acid requirements were derived conceptually, as with the other species, from application of the factorial method. However, in development of the guidelines presented in these publications (NRC, 1984a, 1988) there was heavy and explicit reliance placed upon the experience and judgment of the nutritionists involved in interpreting large numbers of animal performance data and much less reliance on utilization of knowledge about underlying functions than is evident in the presentation of dietary recommendations for ruminant livestock.

Fox *et al.* (1988) have proposed a system that can be used along with the current NRC system for computation of the Nutrient Requirements of Dairy Cattle (1989). Separate provisions for the digestion of soluble carbohydrates, starch, and fiber and the microbes fermenting each type of carbohydrate are included in the model. Microbial growth yields are allowed to vary as a function of growth rate (driven by carbohydrate availability), microbial maintenance requirements, theoretical maximum microbial growth yield and pH. Also, growth rates/yields of microbes fermenting non-structural carbohydrates are a function of the availability of peptides and amino acids arising from the degradation of true protein in the rumen. Additional provisions to accommodate variance in microbial protein availability due to diet composition, fermentation rates and protein availability in the rumen adds considerable utility (see Chapter 8 for additional details).

Dynamic models that contain many of the same provisions as the static model of Fox and coworkers (1988) are available (Baldwin *et al.*, 1977, 1987a,b; Black *et al.*, 1980). These offer the advantage of considering the effects of changes that occur within a day and over days in protein requirements, rates of nitrogen cycling from the animal to the rumen, and vari-

ations in rumen microbial growth yields. These models will be discussed in greater detail in Chapters 8, 11 and 16.

7.3 ANALYTIC MODELS OF AMINO ACID AND PROTEIN METABOLISM

7.3.1 Amino acid flux

The relationship between amino acids and protein was described by Golden and Waterlow (1977) and Waterlow *et al.* (1978) as

$$Q = I + B + N = E + Z + M \quad (7.6)$$

where Q is the amino acid flux, I is the intake of amino acids, B is the amino acids derived from protein breakdown, N is the *de novo* synthesis of amino acids, E is the amino acid oxidation, Z is the amino acids incorporated into protein and M is the amino acids metabolized to other products, all expressed in moles per unit of time. This equation serves as a model that describes in highly aggregated form our understanding of amino acid metabolism and the relationship to protein turnover. The model has two pools, one being amino acids and the other protein. The model described by equation 7.6 dictates the measurements required to calculate amino acid flux. Either the sum of I , B and N , or E , Z and M is required to define Q . Further, it is apparent that the model describes a system in which *all* amino acid metabolism occurs relative to a single pool of amino acids and *all* protein is viewed as a single pool. Depending upon the amino acid, equation 7.6 may be simplified. First, amino acid metabolized to products other than protein (M) can be considered to be negligible relative to the amount of amino acid directed towards protein synthesis and oxidation. While this may not always be the case, knowledge of the metabolism of an individual amino acid will provide the information necessary to determine the impact of assuming $M = 0$.

A second aspect of the model open to simplification is *de novo* synthesis of amino acid (nitrogen). This term may generally be discounted for amino acids classified as nutritionally essential. When describing the metabolism of a nutritionally essential amino acid with limited ability to be converted to products other than protein, equation 7.6 may be simplified to

$$Q = I + B = E + Z \quad (7.7)$$

where Q , I , B , E and Z are as described for equation 7.6.

Based on equation 7.7, which implies that amino acid and protein metabolism is reflected by the pool sampled, several methods for estimation of amino acid flux are available. For the present case, consider the constant infusion of a radiolabeled amino acid into an appropriate vein of a given mammalian system. Constant infusion of a radiolabeled amino acid at a

given rate is associated with a concomitant increase in plasma amino acid specific radioactivity (S_p) to a plateau. The shape of the plasma amino acid specific radioactivity curve over time is described by a single exponential equation (Zilversmit, 1960; Garlick *et al.*, 1973),

$$S_p = S_{pmax} * (1 - e^{-\lambda_p t}) \quad (7.8)$$

where S_{pmax} is the value of S_p when the specific radioactivity of the amino acid in plasma has reached a plateau, λ_p is a rate constant that describes the rate at which S_p rises to a plateau and t is time. This equation, which has been used to estimate amino acid flux (Waterlow, 1967; Waterlow and Stephen, 1967), was discussed extensively by Waterlow *et al.* (1978, Chapters 6 and 8). Waterlow and Stephen (1967) calculated amino acid flux at plateau specific radioactivity as

$$Q = (I * S_n) / S_{pmax} \quad (7.9)$$

where Q is amino acid flux rate (mole/min), I is acid infusion rate (mole/min) and S_n is the specific radioactivity of the infused amino acid (Bq/mole). Several assumptions are required prior to the use of equation 7.9 as a means for estimating amino acid flux including the following:

1. Amino acids entering and leaving the plasma pool per unit time are equal, e.g. animal is in steady state.
2. The radioactivity entering and leaving the plasma pool at plateau are equal per unit time.

Given equation 7.9, which allows for experimental estimation of amino acid flux and realizing that $Q = E + Z$, rate of whole-body protein synthesis may be indirectly estimated by subtracting amino acid oxidation from flux ($Z = Q - E$). However, as with estimation of amino acid flux, the free amino acid pool must remain in steady state. Given that the assumption of steady state is accepted, one needs only an estimate of amino acid oxidation, measurement of which is fraught with its own difficulties. Then protein synthesis can be calculated by difference. If the assumption of steady state does not hold true, change in plasma amino acid concentration will likely alter plasma amino acid specific radioactivity without a concomitant change in whole-body protein synthesis.

The requirement of amino acid pool steady state limits use of this indirect approach to estimate whole-body protein synthesis. For example, effect of diet or patterns of food intake on whole-body protein synthesis would be most difficult to evaluate. The entry of dietary amino acids into the plasma pool would expand the size of this pool, resulting in a decrease in the plasma amino acid specific radioactivity. Based on equation 7.9, such a change would result in a transient decrease in the estimated flux of amino acid and, assuming that amino acid oxidation remained stable, a decrease in the estimate of whole-body protein synthesis rate. Rather, it is likely that the opposite is actually true, that whole-body protein synthesis rate will

either remain the same or increase in response to food consumption and an influx of amino acids into the system.

A second aspect of estimating whole-body protein synthesis rate indirectly from amino acid flux concerns amino acid oxidation. Any error associated with determination of oxidation rate will impact the estimate of protein synthesis rate as it is calculated as the difference between amino acid flux and oxidation. It is generally recommended that ^{14}C -amino acids be used in deference to ^3H -amino acid due to the difficulty of quantitative recovery of ^3H from oxidized amino acids. However, similar problems exist with regard to ^{14}C -amino acids in that it is difficult to insure that all $^{14}\text{CO}_2$ generated from amino acid oxidation is recovered. It is well recognized that radiolabeled carbon in bicarbonate generated from oxidation of amino acids, glucose or a fatty acid is used in fixation reactions, taken up by bone and used for urea synthesis. Thus amino acid catabolism is likely to be underestimated when based on $^{14}\text{CO}_2$ generation, with the result that protein synthesis would be overestimated. Given the requirement that the plasma amino acid pool must be in steady state and that amino acid oxidation can be accurately determined, the indirect approach to estimation of protein synthesis rate is discouraged. Further, such an approach does little to advance understanding of amino acid metabolism relative to protein synthesis in individual tissues.

7.3.2 Protein synthesis

While constant infusion of a radiolabeled amino acid to a plateau of specific radioactivity in plasma, coupled with the use of equation 7.9, is useful for estimating amino acid flux, greater use of the continuous-infusion technique has been to estimate whole-body protein synthesis or protein turnover rate. Such use is predicated on acceptance of an additional set of assumptions, which include the following:

1. Plasma amino acid specific radioactivity is representative of that of the infused amino acid throughout the body at any given time.
2. No recycling or re-entry of the radiolabeled amino acid into plasma from protein.
3. No change in sizes of the protein and amino acid pools.
4. There is a single protein pool with a given rate of synthesis and degradation (which must be equal if assumption 3 is accepted).

In this case the use of continuous infusion of a radiolabeled amino acid is not just for the purpose of estimating Q from knowledge of the plasma amino acid specific radioactivity. Rather, protein synthesis rate can be measured using precursor-product analysis of the precursor and protein pools of amino acids (Zak *et al.*, 1979). In this case the general formula for calculation of protein synthesis rate is

$$S_b/S_p = (1 - e^{-k_s t}) \quad (7.10)$$

where S_b is the specific radioactivity of the tracer amino acid that has been incorporated into protein, S_p is the specific radioactivity of the tracer amino acid in plasma, k_s is the fractional rate of protein synthesis and t is time. In the case of continuous infusion of a tracer amino acid, the equation assumes that $S_{p\max}$ is obtained immediately.

This not being true, there are several approaches for overcoming this problem. One such approach is to describe the shape of the plasma specific radioactivity curve as with equation 7.8 (see Waterlow *et al.*, 1978, Chapter 10, for a detailed discussion). Of greater concern, relative to estimation of protein synthesis rate, is the assumption that plasma amino acid specific radioactivity is representative of that of the infused amino acid throughout the body at any given time. In other words, it is assumed the specific radioactivity of the tracer amino acid in the plasma pool is similar to that of the amino acid in the precursor pool actually used to synthesize protein, the aminoacyl-tRNA pool. This pool is very small relative to total free amino acids in the cytoplasm and has a half life of two seconds or less (Airhart *et al.*, 1974). Measurement of the specific radioactivity of amino acids in the aminoacyl t-RNA pool is both technically difficult and time consuming. Most published rates of protein synthesis are based on the assumption that specific radioactivities of amino acids in the aminoacyl-tRNA (S_t), extracellular (S_e) and intracellular (S_i) pools are similar. Seldom have investigators determined S_t or attempted to eliminate or quantitate the differences of specific radioactivities between amino acid pools used to calculate protein synthesis rate. Essentially all of the published literature on the impact of physiological state (i.e. growth, pregnancy, lactation, immune response, starvation), diet or metabolic modifiers on protein synthesis are potentially compromised by the failure to measure S_t .

It appears that distinct substrate pools of free amino acids, separate from the total intracellular pool of free amino acids, are used for the charging of tRNA. Studies following the specific activity of radiotracer amino acids originating from extracellular sources (S_e) such as the media in which cells are incubated, or blood of live animals, have been used to establish the routing of extra- and intracellular amino acid towards protein synthesis. However, interpretation of these studies is difficult due to the multiple pools and the difficulty of determining S_t . In some studies, S_t was observed to be intermediate to values of S_i and S_e (Airhart *et al.*, 1974; McKee *et al.*, 1978; Airhart *et al.*, 1981; Hammer and Rannels, 1981; Watt *et al.*, 1991), which indicated charging of tRNA from a mixture of intra- and extracellular amino acids. In other systems, S_t was observed to be lower than values for S_i and S_e (Airhart *et al.*, 1981; Hildebran *et al.*, 1981; Schneible and Young, 1984; Opsahl and Ehrhart, 1987; Hall and Yee, 1989; Smith *et al.*, 1991; Barnes *et al.*, 1992), which suggests direct charging of tRNA with amino acids released from proteolysis without prior mixing with the general intracellular

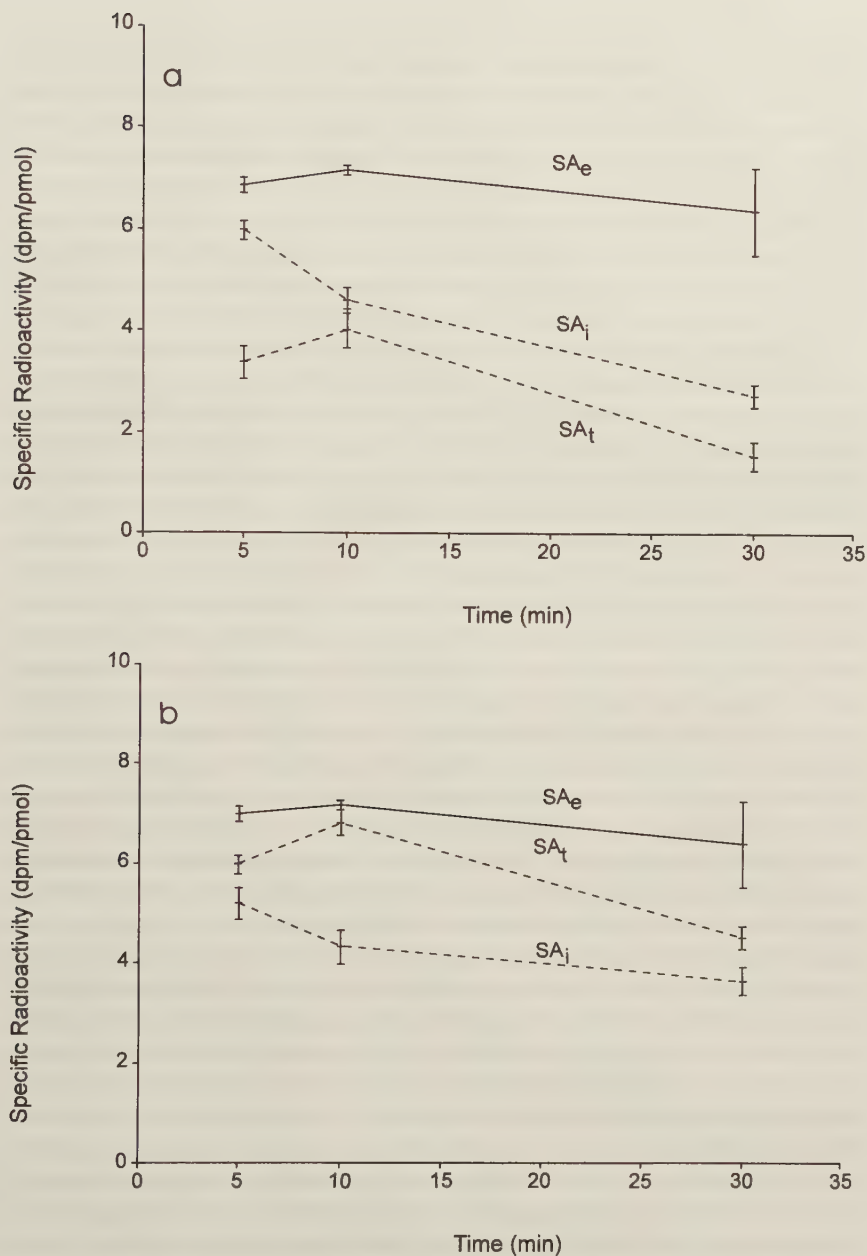


Figure 7.2 The effect of time upon intracardial administration of 400 μCi of ^3H -phenylalanine in 100 mM phenylalanine solution on the specific radioactivity of phenylalanine in the extracellular (SA_e), intracellular (SA_i) and phenylacetyl-tRNA (SA_t) pools of free phenylalanine in the gastrointestinal tract (panel a) and skin (panel b; C. Klasing, University of California, Davis, unpublished data).

pool. Furthermore, when cellular protein is labeled to a high specific activity and then the label in the intracellular and extracellular pools is washed out, S_t approaches that of protein, while S_i and S_e remain low relative to S_p and S_t . Since the only source of radiolabeled amino acid is protein, the data suggest 'channeling' of amino acids from protein degradation to tRNA charging (Barnes, 1990; Barnes *et al.*, 1992; Fig. 7.2a and b). Based on these data, it seems reasonable to suggest that tRNA is charged with amino acids transported from outside the cell and from amino acids liberated from proteolysis and not from the general pool of free amino acids in the cell.

The flooding- or loading-dose method seeks to circumvent the need to measure aminoacyl-tRNA by decreasing differences in the specific activities of the several amino acid pools. First used by Henshaw *et al.* (1971), this method combines a large dose of labeled and unlabeled amino acid (McNurlan *et al.*, 1979; Garlick, 1980). The flooding dose is used to expand the intracellular pool of amino acids such that S_i becomes equivalent to S_e (Garlick *et al.* 1980), supposedly forcing S_t to be equivalent to S_e and S_i . However, S_t is not necessarily similar to S_e or S_i , even when a flooding dose is given. Airhart *et al.* (1981) examined the effect of extracellular amino acid concentration on S_i and S_t in chick skeletal muscle cells *in vitro*. They found that a flooding concentration (5.0 mM) of radiolabeled leucine forced S_e and S_i to equality, while cells in a physiologically normal extracellular concentration (0.2 mM) of leucine had an S_i that was 41.4% of S_e . S_t was less than 0.5% of S_e at both 0.2 and 5.0 mM extracellular leucine. They concluded that expansion of the cellular amino acid pools was not successful in flooding the aminoacyl-tRNA pool with the radiolabeled amino acid and proposed that this failure was due to direct charging of tRNA with amino acids arising from protein degradation without mixing with a common intracellular pool. Similarly, Schneible and Young (1984) found that they could not force leucyl-tRNA specific radioactivities to a level equal to either S_e or S_i in cultured chick muscle cells. They concurred with Airhart *et al.* (1981), that accurate quantitation of protein synthesis requires a measurement of S_t . Recently, Smith *et al.* (1991) demonstrated that neither a tracer nor a flooding dose could eliminate the contribution of proteolysis to tRNA acylation in the brains of rats. They found that the direct contribution of protein degradation to tRNA acylation was 63 and 23% with tracer and flooding doses, respectively.

S_t changes when cells and tissues are exposed to regulatory hormones or processes that impact protein accretion. For example, S_t changed by a factor of two during the anabolic phase that accompanies the recovery of rat hindlimb muscles from immobilization (Morrison *et al.*, 1987). Kipnis and Reiss (1960) described the direct incorporation of extracellular amino acids into rat muscle protein without mixing with intracellular pools and, indirectly, indicated that S_t deviates from S_i as the result of growth hormone treatment. Thus, it appears that knowledge of S_t is requisite to accurate estimation of fractional protein synthesis rate.

Another of the previously stated assumptions that must be accepted, if radiolabeled amino acids are used to estimate protein synthesis, is that there is no recycling or reentry of the radiolabeled amino acid from protein to the amino acid precursor pool used for protein synthesis. From the previous discussion, it is obvious that some recycling does occur and, in fact, it may be that under certain conditions that recycling of radiolabeled amino acids from protein is channeled (see discussion below) directly to the precursor pool of free amino acids. The more rapidly a protein turns over, the greater the impact of this recycling upon estimates of protein synthesis.

Using the precursor-product analysis to calculate fractional rates of protein synthesis is based on the ratio of specific radioactivity of amino acids incorporated into the protein to that of specific radioactivity of the free amino acids in the precursor pool per unit of time that incorporation was allowed to take place. As time for incorporation is increased, a greater proportion of the protein pool becomes labeled. Likewise, as this time frame is increased, the specific radioactivities of amino acids leaving protein via protein degradation increases. Thus, as length of time for incorporation increases, a greater proportion of the radiolabeled amino acids incorporated into protein is simply replacing radiolabeled amino acids that left the protein as a result of protein degradation. The result of this recycling can be appreciated by considering a pool of protein in a steady state that consists of a single, fast-turnover protein. Initially, the protein contains none of the radiolabeled amino acid. As the length of exposure to the tracer amino acid increases, the specific radioactivity of the amino acid in the protein increases as does the specific radioactivity of the amino acid released from the protein due to degradation. Ultimately, the specific radioactivity of the precursor pool, the protein and the amino acid released from the protein will be equal. At this point, there is no measured rate of protein synthesis, since for every radiolabeled amino acid incorporated, a radiolabeled amino acid is released. Thus, calculated fractional protein synthesis rates are sensitive to time. Most often, protein synthesis measurements are not made on a single protein but rather a group of proteins such as the whole-body protein or the gastrointestinal protein.

The consequence of recycling has greater impact when considering a pool of mixed proteins with differing turnover times. An assumption, which was previously accepted, held that there is a single protein pool with a given rate of synthesis and degradation. Obviously, this is not true. Consider the pool of gastrointestinal proteins. This protein pool is made up of a considerable number of individual proteins, each with their own turnover rates. There is no single protein with a given rate of synthesis and degradation. Rather, there are many proteins with differing synthesis rates. Thus, as time of exposure to the radiolabeled amino acid increases, the specific radioactivities of an amino acid incorporated into each of the differing proteins increase in proportion to differences between the relative amounts of fast as compared to slower turnover proteins. The real consequence is that

as length of time increases the specific radioactivity of the amino acid released via degradation approaches that of the specific radioactivity in the pool used for synthesis, because the more rapid turnover proteins approach the specific radioactivity of the precursor pool. The final result is that calculated fractional protein synthesis rates decrease with time. This decrease, obviously, is artificial and the result of the assumptions accepted.

In general, the continuous infusion method allows for incorporation times ranging from two to several hours, whereas the flooding dose method uses a time frame of less than 30 minutes. As a result, estimates of fractional rates of protein synthesis obtained using the flooding-dose method are generally greater than those obtained with the continuous-infusion technique. Even with use of the flooding-dose method, there is a decrease in estimated synthesis rate when synthesis is calculated at various time points between 5 and 30 min (Bernier and Calvert, 1987; Fig. 7.3). The solution to this problem is unclear, yet at the same time, it is obvious. Protein synthesis must be calculated for time zero relative to exposure to the radiolabeled amino acid. This can only be accomplished with dynamic, mechanistic models that explicitly account for mixed turnover rates of protein pools and incomplete radiotracer equilibration among amino acid pools (S_i , S_e and S_t).

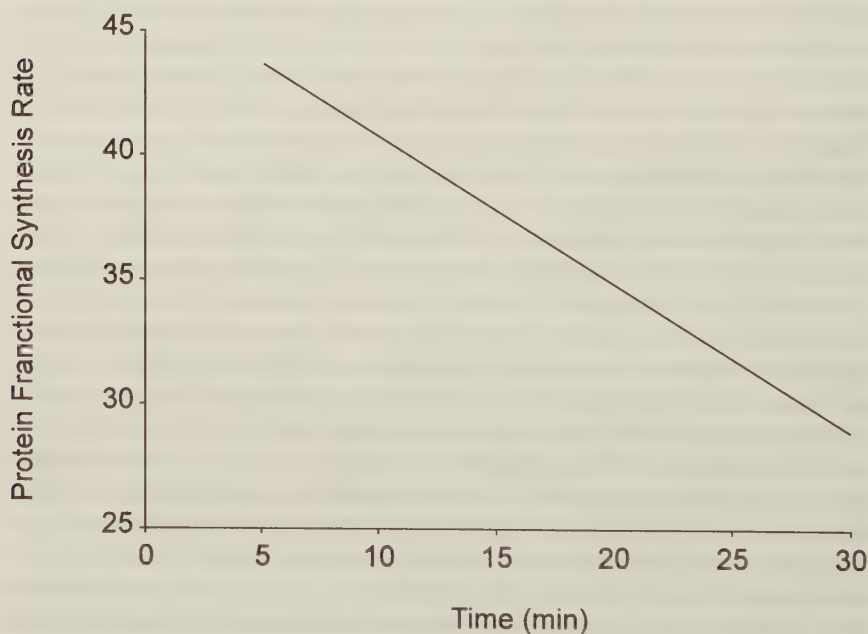


Figure 7.3 The influence of time of incorporation upon protein fractional synthesis rate (FSR) estimates in mice with a gene for rapid postweaning gain. Data were presented by Bernier and Calvert (1987), in which specific radioactivity of a radiolabeled amino acid was determined at 5, 10, 15, 20, 25 and 30 min in intracellular fluid and body protein.

7.3.3 Amino acid channeling

The implications of the disparity in amino acid specific radioactivities between compartments (i.e. S_p , S_e , S_i and S_t) must be considered. In estimating protein synthesis rate (equation 7.10) it is obvious that choice of an amino acid pool as representative of the true precursor pool of that amino acid for protein synthesis will impact the calculated synthesis rate. For example, if the free amino acid specific radioactivities are related as $S_e > S_i > S_t$ then the calculated protein synthesis rates from these pools would be related as $S_t > S_i > S_e$. If the absolute 'true' rate of protein synthesis is to be determined, the choice of the precursor pool is obviously important. The assumption that plasma amino acid specific radioactivities are representative throughout the body at any given time clearly does not hold as discussed above.

While the aspect of appropriateness of the choice of precursor pool used to calculate the protein synthesis rate is of importance, the impact of the previously discussed data upon the theoretical framework, from which protein and amino acid metabolism are discussed, is of equal importance. In an effort to explain the disparity in S_t relative to other amino acid pools and to explain the high S_t relative to S_e and S_i when the only source of labeled amino acid is that which is released from protein degradation, it can be argued that amino acids resulting from protein degradation are channeled to and used preferentially for charging tRNA. It is recognized that organization of multienzyme complexes can result in compartmentalization or channeling of specific metabolites from one enzyme to another without equilibration with other pools of that metabolite.

Metabolite channeling is an important aspect to evaluations of many metabolic pathways, including DNA synthesis, fatty acid synthesis, glycolysis, and the urea cycle (Srere, 1987; Watford, 1989; Srere and Ovadi, 1990). Channeling has the potential of increasing efficiency of a metabolic pathway by limiting loss of substrate and may serve a role in regulating substrate movement between competing pathways (Ovadi, 1991). Substrate transport through cell membranes often depends on coupling the transport protein to an enzyme that utilizes the substrate (e.g. glucose). The channeling of substrates through complex metabolic pathways often begins at the cell membrane. One prerequisite for channeling is the existence of a structural organization for the components of the pathway that can lead to the catalysis of sequential reactions without disassociation of intermediates. A second prerequisite for channeling is demonstration of distinct pools of substrates and intermediates that do not freely mix with the respective general intracellular pools. Evidence supporting these two prerequisites implicates the channeling of amino acids arising from proteolysis towards protein synthesis.

Aminoacyl-tRNA synthetases are enzymes that activate amino acids and esterify them to tRNAs. Many of the aminoacyl-tRNA synthetases exist in a

multienzyme complex. Presently, 10 of the 21 synthetase activities have been isolated from the multienzyme complex (isoleucine, leucine, lysine, methionine, arginine, proline, phenylalanine, glutamine, glutamic acid, aspartate) and it is suspected that additional enzymes of the complex were lost in the purification procedure (Schimmel, 1987; Yang and Jacobo-Molina, 1990). The enzyme complex has been identified in many cell lines as well as myoblasts (Shi *et al.*, 1991). The synthetase complex is physically associated with elongation factor eEF1 α (Sarisky and Yang, 1991), one of the primary regulators of the rate of translation. Several of the enzymes in this multienzyme complex have amino terminal hydrophobic regions that associate with lipids, presumably in one of the cell membranes (Huang and Deutscher, 1991). With gentle homogenization of chick embryos, aminoacyl-tRNA synthetases purify in the microsomal fraction (Norton *et al.*, 1965). Although it has not been shown which membrane the complex is associated with, the linkage between tRNA charging enzymes and amino acid transport across membranes is implicated. Quay *et al.* (1975) have shown that the transport of leucine is linked to and regulated by leucyl-tRNA synthetase activity.

Distinct substrate pools have also been identified. Sivaram and Deutscher (1990) provided evidence for two pools of arginyl-tRNA, one of which is free in the cytosol and is involved in the post-translational modification of proteins, and a second pool that is a component of the aminoacyl-tRNA synthetase complex. Further, neither free arginyl-tRNA or free phenylalanyl-tRNA are used for ribosomal protein synthesis, but the aminoacyl-tRNAs formed in the multienzyme complex are efficiently used (Negrutskii and Deutscher, 1991). This suggests that aminoacyl-tRNAs formed in the multienzyme complex are transferred directly from the synthetase to the elongation factor and the ribosome without mixing with the total fluid of the cell, which represents a channeling of aminoacyl-tRNA for protein synthesis.

Putative charging of tRNA from amino acids channeled directly from protein degradation is understandable both energetically and nutritionally. By directly reutilizing a significant proportion of the amino acids released from protein degradation, the cell can prevent the efflux of essential amino acids from the cell and minimize their loss to catabolic pathways. Efficient reutilization of amino acids could result in decreased energy requirements associated with their transport into the cell. Additionally, efficient channeling of amino acids arising from the diet or other tissues across the cell membrane directly to the aminoacyl tRNA synthetases would preclude the possibility of loss of the amino acid to oxidation.

7.4 DYNAMIC MODELING

In light of the previous discussion, the limitations of protein synthesis meas-

urements obtained from S_e or S_i must be resolved if truly quantitative rates of protein synthesis are to be obtained. It is documented from direct measurements that tracer amino acid specific radioactivities in extracellular, intracellular and tRNA pools can be very dissimilar. Previously discussed data strongly suggest that neither the intracellular or extracellular pools are the sole source of amino acids used to charge the tRNA pool. Thus protein synthesis rates calculated from either S_e or S_i will not provide an accurate estimate of synthesis rate. Further, it is obvious that recycling of a radiolabeled amino acid and the assumption of a single protein pool may likely have great impacts on calculated protein fractional synthesis rates.

Obviously, if one is to obtain data that truly reflect the rate of protein synthesis in any given system, accurate and precise measures of precursor specific radioactivities are essential. The relationships among the extracellular, intracellular, and tRNA pools must be described in greater detail. The complexity of amino acid pools and relationships among them preclude the interpretation of tracer data using simple empirical and static models. In order to adequately describe and analyze amino acid tracer data, dynamic (time-variant) and mechanistic (causal, theoretical) models must be developed. Such models must represent the dynamic relationships among extracellular, intracellular, tRNA and protein-bound amino acids.

A limited number of mechanistic models of nutrient utilization and protein metabolism are available. Baldwin and Black (1979) presented a mechanistic model of mammalian growth that gave good simulations of growth, but had a limited description of metabolic processes and protein metabolism. Schultz (1978) described a mechanistic model of rat energy metabolism that had a very detailed description of amino acid metabolism, but protein and lipid turnover were not described. Bernier (1986) developed a mechanistic model of nutrient utilization by mice that adequately simulates effects of feed restriction on energy expenditures and body composition, but does not adequately describe effects of restricted feeding on protein synthesis and degradation. Gill *et al.* (1989) developed a model that examined relationships between lamb growth rate and whole-body protein synthesis rates and relative contributions of individual tissues to whole-body synthesis. The model yielded reasonably close estimates of protein accretion rates and effects of energy intake on protein accretion rate relative to observed results. However, model predicted responses in protein synthesis and degradation to nitrogen retention and food intake were not in agreement with experimental data. Sainz and Wolff (1990a,b; Chapter 15) used a dynamic, mechanistic model of lamb metabolism to show that modest changes in protein synthesis and/or degradation could account for large changes in protein and fat accretion due to metabolic modifiers. This result indicates that small errors in estimation of protein synthesis could invalidate efforts to understand the impact of hormonal, nutritional and genetic manipulations of animal performance on protein metabolism. In a recent review of modeling amino acid metabolism, Hjelm and Seakins (1992) dis-

cussed several published modeling efforts relative to individual amino acids. They took the position that metabolism of individual amino acids must be incorporated into existing models of protein metabolism in order to improve accuracy of protein synthesis measurements. However, they also point out that many of the current models used to calculate protein synthesis from tracer amino acids are largely stochastic type 'black boxes', which obtain the best fit to experimental data rather than define pools that have physiological and biochemical meaning.

As described above, current methods used to measure protein synthesis are flawed by the assumptions used to calculate protein synthesis from the precursor-product relationship. This makes it difficult to evaluate discrepancies between model predictions and experimental data. Possibly, model simulations are more useful than the experimental observations. None of the models discussed above contains provisions for proteins with rapid turnover or for accurate estimation of the specific radioactivity of aminoacyl-tRNA. Thus, modeling efforts must be initiated to improve representations of protein turnover by incorporating greater detail of physiological reality at the cellular and subcellular levels.

In Chapter 13, a model of mammary gland metabolism, in which effects of the availabilities of several critical amino acids on milk protein synthesis, is presented. The approach utilized in that model may be appropriate or acceptable in the sense that most protein synthesized in mammary glands are secreted and, thus, do not turn over in mammary secretory cells. We have a version of the lactating cow model described in Chapter 16 that utilizes, essentially, the same approach as that in the mammary gland model to enable simulations of effects of limiting amino acids on protein metabolism in the lactating cow (Cant *et al.*, 1993). This approach may or may not be appropriate or adequate based upon discussions presented above. Thus, this version of the lactating cow model is not presented. Tissue level data on and models of amino acid metabolism, channeling, and rates of equilibration among extracellular, intracellular and aminoacyl-tRNA pools are essential not only to improve estimates of protein turnover within individual tissues and the animal but also to simulation of amino acid pools at the whole-animal level.

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Biology and algebraic models of ruminant digestion

According to these views, the carbon dioxide, methane, acetate, propionate and butyrate, final products in the rumen fermentation, are formed because pathways leading to them also provide the most efficient conversion of fermentable substrate into the microbial cells.

Hungate (1966)

8.1 INTRODUCTION

The first section of this chapter is devoted to presentation of a very brief overview of the rumen microbes and physiological and metabolic properties of the microbes that are accommodated, to varying degrees, in early and current models of rumen digestion. The interested reader is directed to the book, *The Rumen and its Microbes* by R. E. Hungate (1966), which is an excellent, authoritative and comprehensive treatment of the subject by a widely recognized leader in development of the field. Subsequent reviews and updates by Bryant (1977), Russell and Hespell (1981), Baldwin and Allison (1983), Czerkawski (1986), and the symposium series on the *Physiology of Digestion and Metabolism of Ruminants*, the most recent of which was edited by Milligan *et al.* (1986), are also recommended. This section is followed by presentations of representative elemental and carbon balance models of rumen microbial digestion, an analytical balance model that was used to estimate stoichiometric parameters for fermentation of chemical entities in the diet, and a discussion of the microbial growth elements of the Cornell Net Carbohydrate and Protein System (Search:Agriculture, 1990).

8.2 THE RUMEN MICROBES AND THEIR METABOLISM

Our knowledge of rumen digestion and microbial ecology developed in several stages. The first stage led to the conclusions that the rumen microbes play a major role in the digestive process, volatile fatty acids are major

products of digestion and diets effect the relative amounts of volatile fatty acids formed (Hungate, 1966). A following stage involved isolation, taxonomic identification and enumeration of the microorganisms present. Although activity in this area of inquiry continues, considerable progress had been achieved by 1958, at which time the major microbes listed in Table 8.1 had been identified (Bryant, 1959). This stage was followed in the 1960s by studies of pure cultures of predominant rumen microbes in which their physiological properties and metabolism were defined. In addition, their relevance to the rumen ecosystem and interactions among rumen microbes were evaluated. This stage is by no means complete, even though activity has been sporadic and limited during the past two decades. The final stage of understanding the rumen as an ecosystem requires quantitative and dynamic analyses of the extent to which microbes express their characteristics in mixed cultures and *in vivo*. An implied requirement for achievement of this goal is that we understand microbial interdependence and competition (Russell and Hespell, 1981). Progress in understanding these is in a very early phase and may require several decades for resolution, given the current low level of activity in the area.

Considerable research emphases have been directed to manipulation of the rumen microbiota through the formulation of diets and/or application of pharmaceutical and molecular biological techniques to either optimize the balance of digestion end-products available to the ruminant or enhance the functions of specific, presumably desirable, microbes. Some of these efforts have contributed to our knowledge of quantitative and dynamic properties of microbes in the rumen ecosystem but, unfortunately, most have not. With the exception of the model of Russell and Allen (1984) of the behavior of *Streptococcus bovis* and *Ruminococcus albus* in co-culture, no dynamic, mechanistic models of interactions between rumen microbes have been published. This may well be because of a lack of interest in modeling the system, an absolute lack of required data, a lack of opportunities to undertake the coupled modeling and experimental analyses required to make progress, or all of these. Whatever the case, this limitation clearly limits the range of models of ruminant digestion to those which consider the rumen microbes in aggregate or as functional groups. Discussions of rumen function in this and Chapters 11 and 16 will reflect this reality.

8.2.1 The rumen microbes

Several prominent microbes are listed in Table 8.1 according to the primary niche they occupy in the rumen, e.g. cellulolytic, amylolytic, etc., with an indication of their relative prominence. The classification based upon substrate specificities is consistent with that adopted by Hungate (1966), Russell and Hespell (1981) and Baldwin and Allison (1983). The primary cellulolytic microbes in the rumen are among the most restricted of rumen microbes in terms of the niche they occupy. In general, they do not ferment monosac-

Table 8.1 Major microbes of the rumen^a

Microbial species	Percentage of total isolates	Secondary substrates ^b	Net products in mixed culture ^c	Nutrient requirements ^d
(Holo) cellulolytic				
<i>Fibrosolvens succinogenes</i>	5-9	ST, Pec	Ac, Pr _s , CO ₂	BcFA, Val, NH ₃ , biotin, Pab
<i>Ruminococcus albus</i>	3-5		Ac, H ₂ , CO ₂	BcFA, NH ₃ , biotin, Pab
<i>Ruminococcus flavefaciens</i>	3-5		Ac, Pr _s , H ₂ , CO ₂	BcFA, NH ₃ , biotin, Pab
Amylo- and dextrinolytic				
<i>Bacteroides amylophilus</i>	1-10	Pec, SS	Ac, Pr _s , CO ₂	NH ₃
<i>Streptococcus bovis</i>	0-20	SS, Prt	Ac, La, CO ₂ (H ₂)	Aa, biotin
<i>Succinimonas amyolytica</i>	1-3		Ac, Pr _s , CO ₂	BcFA
<i>Succinivibrio dextrinosolvens</i>	1-13	Pec	Ac, Pr _s , La, CO ₂ (H ₂)	Aa
Saccharolytic				
<i>Bacteroides rumenicola</i>	10-19	ST, Pec, Prt	Ac, Pr, CO ₂ (H ₂)	BcFA
<i>Butyrivibrio fibrisolvens</i>	8-12	Hc, ST, Prt	Ac, Bu, La, CO ₂ (H ₂)	BcFA, NH ₃ , Aa, biotin, folic acid, pyridoxal
<i>Megasphaera elsdenii</i>	0-1	La, Prt	Ac, Pr, Bu, Val, H ₂ , CO ₂	Aa
<i>Selenomonas ruminantium</i>	4-12	ST, La	Ac, Pr, La, H ₂ , CO ₂	BcFA, Met
Hydrogen utilisers				
<i>Methanobrevibacter ruminantium</i>	<1		CH ₄	BcFA, NH ₃
<i>Vibrio succinogenes</i>	0		Pr _s , NH ₃	NH ₃

Table 8.1 (continued)

Microbial species	Percentage of total isolates	Secondary substrates ^b	Net products in mixed culture ^c	Nutrient requirements ^d
Protozoa				
<i>Isotricha</i>		ST, SS	Ac, Bu, La, H ₂ , CO ₂	
<i>Dasytricha</i> , <i>Diplodinium</i> sp.		ST, SS	Ac, Bu, La, H ₂ , CO ₂	
<i>Entodinium</i> sp.		ST	Ac, Pr, Bu, La, CO ₂ (H ₂)	(bacterial cells)
Sum	40-75			

^a Adapted from Baldwin and Allison (1983).

^b Coding for substrates: Hc, holocellulose; ST, starch; SS, soluble sugars; Pec, pectin; Prt, protein; La, lactate.

^c Coding for products: Ac, acetate; Pr, propionate; Bu, butyrate; Val, valerate and longer chained fatty acids; La, lactate. Listing H₂, CO₂ indicates organism has a hydrogenase and produces hydrogen. CO₂ (H₂) indicates organism produces formate that is converted to CO₂ + H₂ by another organism. Pr₂ codes for succinate that is converted to propionate and CO₂ by other organisms in mixed culture.

^d Coding for nutrients: BCFA, C₄-C₅ (branched chain) volatile fatty acids; Val, valerate; Aa, amino acids; Met, methionine; Pab, p-aminobenzoate.

charides and thus, are restricted to disaccharides released during hydrolysis of holocellulose as sources of carbon and energy. Often, they depend upon other rumen microbes for their supply of B vitamins, branched-chain fatty acids and ammonia for growth. The amylolytic and dextrinolytic microbial species vary the most in numbers, because starch varies greatly with diet. There are also great differences in soluble sugar content across diets. However, numbers of saccharolytic microbes do not vary as drastically as do numbers of amylolytic species. This reflects the fact that the saccharolytic microbes compete favorably for disaccharides released by the extracellular hydrolytic enzymes of the cellulolytic and amylolytic species. The high percentage of *S. bovis* isolates reflects their rapid overgrowth when animals are suddenly switched to high starch diets. Their numbers are normally low. *Megasphaera elsdenii*, *Methanobrevibacter rumantium* and *Vibrio succinogenes* are minor microbes in terms of percentage of total isolates, but are listed because of their interesting functions and biochemical characteristics. Numbers of protozoa are far below numbers of bacteria. However, their greater size compensates, such that they can comprise 50% of the total microbial mass (Bryant, 1977).

8.2.2 Rumen microbial metabolism

Generally, to establish the presence of a metabolic pathway involves identification of the product(s) of the pathway, demonstration that specific carbons of the substrate are distributed among and within products as predicted, and identification and characterization of enzymes in the pathway. The first phase of this process, e.g. identification of major products of predominant species, was largely completed prior to 1958 (Byrant, 1959). Some studies of pure cultures fermenting substrates radiolabeled in specific carbons were reported in the 1950s, but the bulk of this research was conducted in the 1960s (Baldwin, 1965; Wallnofer *et al.*, 1966). Only limited effort has been directed at identification and characterization of the enzymes of rumen microbes (Joyner and Baldwin, 1966; Palmquist and Baldwin, 1966) with the exception of specific electron transport systems and mechanisms involved in methanogenesis (Byrant, 1977; Macy and Probst, 1979; and above references).

(a) Metabolism of lipids

Mono- and digalactoglycerides, triacylglycerol (TG), and phospholipids of plant origin are hydrolyzed in the rumen. Unsaturated fatty acids released are reduced to form saturated and monoenoic fatty acids (Fig. 8.1). The carbohydrate and glycerol arising from hydrolysis of lipids are fermented to volatile fatty acids. Some emphasis has been placed upon avoiding TG hydrolysis in the rumen by treating associated proteins with formalin or heat or by feeding whole oilseeds. Another relatively recent cause of vari-

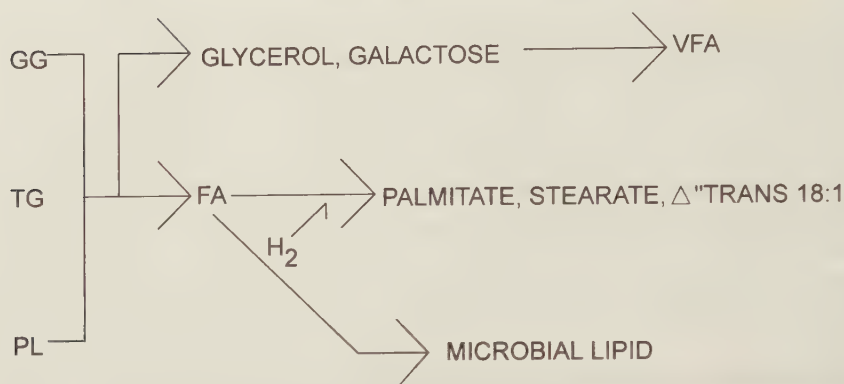


Figure 8.1 Digestion of lipids in the rumen. Mono- and digalactoglycerides are coded GG, TG codes triacylglycerol, and PL codes phospholipids.

ation from normal patterns of lipid metabolism in the rumen has been the use of insoluble (Ca^{2+}) salts of fatty acids in ruminant diets to reduce their availabilities to rumen microbes. Real and perceived benefits of these modifications have been the production of polyunsaturated animal products and improved energy availability and productive efficiencies. From the point of view of modeling rumen microbial metabolism, these modifications are important, because they can alter energy balances and methane production.

(b) Metabolism of carbohydrates

Major carbohydrates in ruminant diets include soluble sugars, cellulose, hemicellulose, pectins and starch. The first step in the fermentation of carbohydrate polymers is hydrolysis to oligo- and disaccharides. Cellulose exists in a loosely associated (amorphous) form along with hemicellulose and other polymers. Cellulose can also exist in a highly crystalline form in plants. The crystalline form is more difficult to degrade than is the amorphous form. The *Ruminococci* (Table 8.1) produce extracellular cellulase complexes that are active in the hydrolysis of denatured and amorphous cellulose but are relatively inactive against crystalline cellulose. *Fibrobacter succinogenes* produces a cellulase complex that effectively hydrolyses crystalline cellulose. This probably explains why *F. succinogenes* is the most prominent cellulolytic organism in rumen contents of animals fed diets of straw, for example, that are high in crystalline cellulose. A primary product of cellulose hydrolysis is cellobiose, which can be used by a number of saccharolytic as well as cellulolytic rumen microbes.

In contrast with cellulose, which is, largely, a polymer of β -1,4 linked glucose units, hemicelluloses are groupings of polysaccharides consisting of β -1,4 linked xylose chains containing varying amounts of arabinose,

uronic acids and galactose. Hemicellulose hydrolysis results primarily in the extracellular release of pentose disaccharides which, as is the case with cellobiose, can be utilized by saccharolytic as well as cellulolytic microbes. It appears that cellulolytic bacteria are the most important species involved in hemicellulose hydrolysis. Hemicellulose hydrolysis is gratuitous for closely associated non-cellulolytic bacteria that can use xylobiose because, as noted above, several cellulolytic microbes, most notably *F. succinogenes*, do not ferment disaccharides from hemicellulose hydrolysis (Dehority, 1973).

Considerable progress has been achieved in recent years in characterizing the multiprotein cellulose complexes of rumen microbes. Much of this progress can be attributed to interest in potential interspecific transfers of cellulose genes.

Pectins are a complex group of polysaccharides in which the basic building blocks are uronic acids. Varying amounts of the methyl esters of galacturonate and other sugars are present within the linear portion of the polymer and in side chains. At least two enzymes, a methylesterase and polygalacturonidase, are required for pectin hydrolysis. Pectin is hydrolyzed readily by rumen microbes.

Rates of starch hydrolysis by microbial amylases to maltotriose, maltose and some glucose is highly dependent upon physical form. For example, heating disrupts the crystalline structure of cereal starches and greatly increases rates of hydrolysis and fermentation. Heat treatments of starch to improve growth rates of feedlot cattle received considerable attention in the 1960s and early 1970s. Gains achieved through this research have been partially offset by the high energy costs required for treatment. At the high feed intakes characteristic of high producing dairy cattle, rumen digestion coefficients for starch can be very low at 40–50% (Herrera-Saldana *et al.*, 1990; Klusmeyer and Clark, 1991; see Chapter 17). This has led to efforts to optimize treatments of corn and sorghum grains to improve digestion in the rumen and rumen microbial growth yields. Maltose released by extracellular amylases is readily fermented by the amylolytic and saccharolytic rumen microbes.

The fermentation of mono-, di- and trisaccharides is the primary source of energy needed in the form of ATP molecules required to satisfy rumen microbial maintenance and growth requirements. The primary pathway of hexose fermentation in rumen microbes is the classical Embden–Meyerhoff (EM) pathway depicted on the left-hand side of Fig. 8.2. Net products are two ATP, two pyruvate and two NADH₂. A number of enzymes of the EM pathway have been identified in rumen microbial species (Joyner and Baldwin, 1966) and all radioisotope tracer data indicate that this is the major glycolytic pathway. Two routes of pentose fermentation have been suggested based upon radioisotope tracer data. The most prominent route appears to involve conversion of three pentose-P to two hexose-P and one triose-P via the reactions of transketolase and transaldolase (Fig. 8.2). The

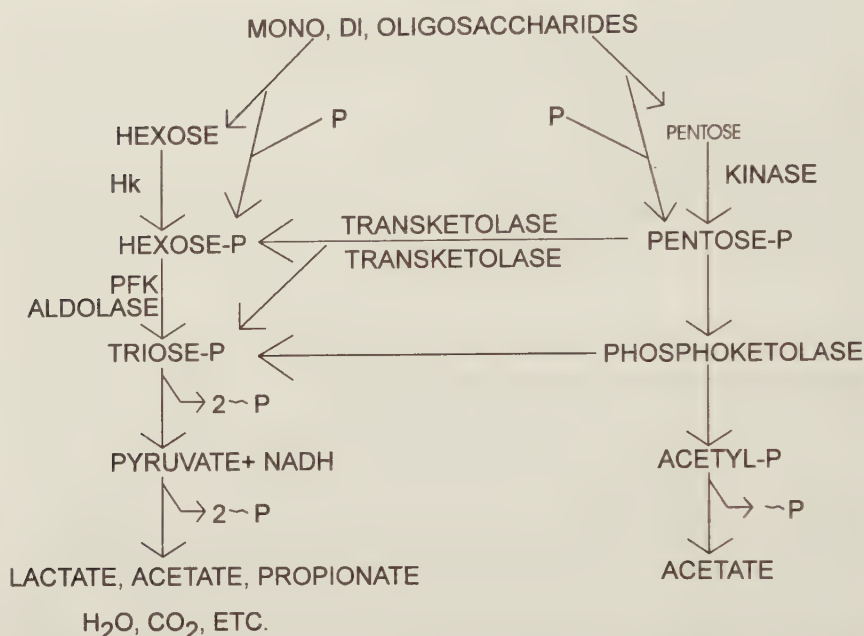


Figure 8.2 Fermentation of hexoses and pentoses by rumen microbes. Hexokinase is coded HK, PFK codes phosphofructokinase and ~P codes the high energy bond of ATP.

hexose-P and triose-P thus formed are then metabolized via the EM pathway. The other route, for which evidence is more limited (Wallnofer *et al.*, 1966), involves formation of a ketopentose-P and cleavage by phosphoketolase. The fate of pyruvate and NADH₂ formed during hexose and pentose fermentation varies greatly depending upon the microbes involved and *in vivo* and *in vitro* incubation conditions. The overriding requirement within and among organisms is that of maintaining redox balance in the system. For example, if methanogenesis is active and, as a result, the partial pressure of H₂ in the environment is low, organisms possessing a hydrogenase release the hydrogen atoms in NADH₂ as H₂. If H₂ in the external environment is high, the thermodynamics of the reaction are unfavorable and the organisms reduce pyruvate to lactate or propionate to maintain redox balance (Wolin, 1975).

Several reactions of pyruvate are illustrated in Fig. 8.3. Two mechanisms for conversion of pyruvate to acetate have been observed in rumen microbes. The most common is the pyruvate-formate lyase system, which produces formate and acetyl-CoA as immediate products. Formate thus released is converted to CO₂ and H₂, usually by another organism in the mixed rumen culture (Hungate, 1966). This is why formate is a common product of rumen microbes in pure culture but is not observed in mixed cultures. The second enzyme system is the pyruvate-ferredoxin oxidore-

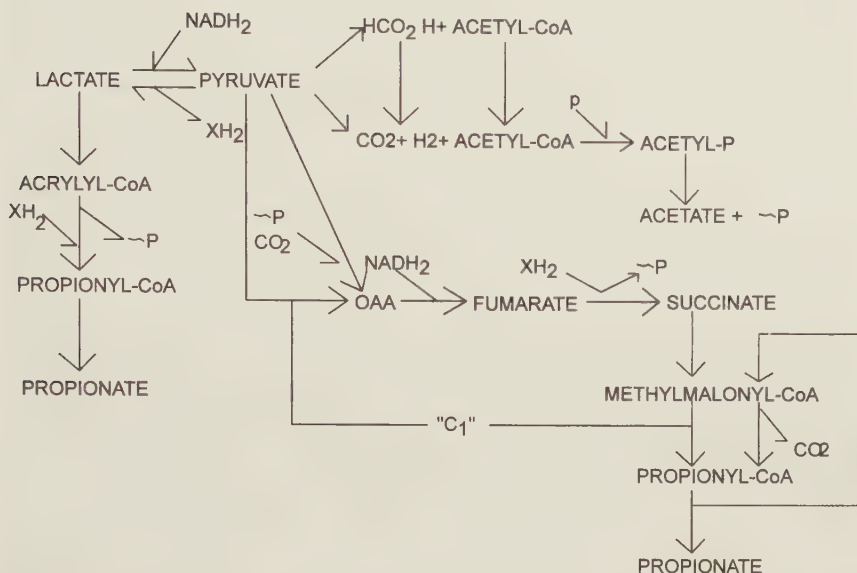


Figure 8.3 Several reactions of pyruvate in rumen microbial systems. Various electron carriers in the reduced form are coded XH_2 .

ductase that converts pyruvate to reduced ferredoxin, CO_2 and acetyl-CoA. Reduced ferredoxin is usually oxidized with the release of hydrogen gas. Acetyl-CoA is converted to acetate plus ATP by phosphotransacetylase plus acetate kinase. In modeling rumen fermentation, it is important that stoichiometric coefficients used reflect the mixture of hexoses, uronic acids and pentoses available for fermentation and the pathways via which these are fermented, because these effect the balance of products formed and yields of ATP from fermentation which are essential to estimation of microbial growth yields.

The most common and prominent pathway of pyruvate conversion to propionate is the dicarboxylic acid pathway (Baldwin, 1965). Three alternate enzymes can catalyze the carboxylation of pyruvate: phosphoenolpyruvate (PEP) carboxykinase, which converts PEP plus ADP or GDP plus CO_2 to oxalacetate (OAA) plus ATP or GTP; pyruvate carboxylase, which converts pyruvate plus CO_2 plus ATP to OAA plus ADP; and methylmalonyl-CoA carboxyltransferase, a biotin containing enzyme, which transfers a one-carbon unit from methylmalonyl-CoA to pyruvate (carboxyl transferase) concomitant with the conversion of succinate to propionate. During the fermentation of cellulose, very little recycling of carbon via the carboxyltransferase reaction is observed. This is because cellulolytic organisms (Table 8.1) produce succinate as an end product. In mixed culture, other

organisms convert succinate produced by the cellulolytics to propionate. During glucose fermentation, recycling of one carbon unit via the carboxyl-transferase reaction is prominent, reflecting the fact that the saccharolytic microbes produce propionate as an end product (Table 8.1). As noted above, it is important in the modeling context to be cognizant of the relative contributions of the several possible mechanisms for conversion of pyruvate to oxalacetate because net yields of ATP available for microbial growth differ. When the pyruvate carboxylase as compared to the PEP carboxykinase and transcarboxylase mechanisms is utilized, the ATP yield from propionate formation is reduced by one.

The alternate pathway for conversion of pyruvate to propionate is the direct reductive pathway depicted on the left side of Fig. 8.3. This pathway, which has been observed in *M. elsdenii* and *F. succinogenes* (Wallnofer and Baldwin, 1967), involves the conversion of lactate to acrylyl-CoA via the intermediate phospholactyl-CoA and reduction of acrylyl-CoA to propionyl-CoA by a NADH₂-linked (acrylyl-) crotonyl-CoA reductase (Baldwin and Allison, 1983). The contributions of the dicarboxylic and direct reductive pathways can be distinguished on the basis that radiotracer in the C-2 position of lactate is recovered equally in carbons two and three of propionate formed via the dicarboxylic acid pathway and exclusively in carbon two of propionate formed via the direct reductive pathway. The contribution of the direct reductive pathway increases from 15 to 30% as the proportion of concentrate in the diet increases (Wallnofer *et al.*, 1966). There has been considerable speculation about the possibility that electron transport associated with the reduction of fumarate to succinate and/or crotonyl-CoA reduction to butyrate could yield high energy phosphate (ATP) bonds. Growth yields of microbes grown on fumarate indicate that this may be true. However, the existence of electron transport mediated phosphorylation in rumen anaerobes via either of these pathways remains to be conclusively established (Macy and Probst, 1979). This uncertainty imposes an important limitation in modeling the system.

(c) Metabolism of compounds containing nitrogen

The primary nitrogen containing compounds in ruminant diets are soluble and insoluble proteins, nucleic acids, and urea. Metabolism of these diet components is depicted in Fig. 8.4. Critical elements in the diagram are ATP yields from carbohydrate and amino acid and peptide fermentation; relative rates of soluble and insoluble protein hydrolysis and utilization of the hydrolytic products – peptides and amino acids; effects of amino acids and peptides on microbial growth rates and yields; and total passage of dietary and microbial protein from the rumen. With regard to the first issue, amino acid and peptide fermentation, we wrote a number of years ago (Baldwin and Allison, 1983) that

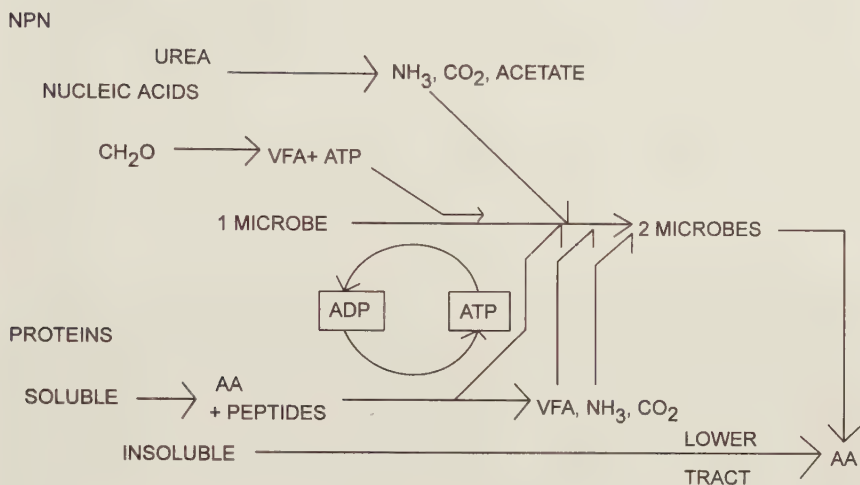


Figure 8.4 Nitrogen metabolism in the rumen.

no specific microbe occupies the 'protein fermentation' niche. Many rumen microbes possess proteolytic activity, many can utilize or ferment nutrient amino acids or peptides, but only a few minor microbes such as *M. elsdenii* can survive and grow on amino acids in the absence of carbohydrate as an energy source (Bryant, 1977). The lack of a specialized microbe has, perhaps, reduced progress in understanding factors which (1) influence rates of hydrolysis of protein or (2) patterns of amino acid metabolism in the rumen.

This limitation was alleviated by several important contributions of Chen and Russell (1989a,b, 1990, 1991). The first was isolation of three microbes from the rumen that ferment amino acids and small peptides rapidly, and grow readily with amino acids as their sole energy source. Thus, amino acid fermentation can contribute to the ATP supply for microbial maintenance and growth. A second was partial characterization of peptide and amino acid transport mechanisms and factors such as pH and ionophore effects upon amino acid transport. Finally, they demonstrated that significant amounts of peptides formed during diet protein hydrolysis can pass from the rumen and contribute to the amino acid supply available to ruminants.

Two anaerobic mechanisms for the degradation of amino acids have been identified in the rumen. The first class of mechanisms is decarboxylation yielding the corresponding amine and CO₂. This mechanism is associated with low pH and is only a minor contributor in the rumen under normal conditions (Prins, 1977). Under feedlot conditions where rumen conditions can become acidic, the production of volatile amines via this mechanism

microbial maintenance requirements, which vary dependent upon the mix of microbes in the rumen on a given diet and pH (Russell and Baldwin, 1979a,b; Russell and Strobel, 1990; Russell, 1991); and efficiency of the growth process *per se* which, as noted above, can vary dependent upon amino acid and peptide availability. Early models specified constant rumen microbial growth yields as either grams dry weight of microbes per mole of substrate fermented ($Y_{\text{substrate}}$), per mole of ATP from fermentation (Y_{ATP}) or per 100 g organic matter transformed in the rumen.

Even the superficial treatment presented above is sufficient to identify a number of variables of concern in evaluating central elements describing rumen digestion. Quantitative data required for detailed representations and parameterization of many elements are very limited. As a result, most current models are highly aggregated but do accommodate sources of variation in rumen microbial metabolism and growth to some degree. Provisions for explaining this variation will be considered below and in Chapters 11, 16 and 17, in context with discussions of specific models.

8.3 BALANCE MODELS OF RUMINANT DIGESTION

When a series of metabolic equations are solved to obtain a net balance, a balance model has been constructed. Thus, most rumen microbiologists and ruminant nutritionists have used balance models in teaching and writing (Wolin, 1960; Hungate, 1966). Examples of balance models frequently used in teaching are presented in Table 8.2. These simplified models can be used to emphasize the requirement for maintenance of redox balance in anaerobic systems, illustrate the varied metabolic functions of rumen microbes and interactions among microbes, demonstrate the concept of variable ATP yields from fermentation and characterize the general quantitative relationships of rumen fermentation, all of which have been referred to above.

It must be emphasized that these simple balance models are illustrative and do not capture all aspects of variance. For example, one might imply that a mixture of examples I and II in Table 8.2 with energy losses in methane of 0 and 30.5% are compatible with the balance equation of Hungate presented in example III. A loss of 18% of energy in substrates fermented in the form of methane can and may have led to anticipation of significant (commercial) benefits from inhibition of methanogenesis by a feed additive. Certainly, considerable efforts have been directed to inhibition of methanogenesis in ruminants with the expectation of improvements in animal productivity. The serendipitous result has been that the most successful purported 'methane inhibitors' have been the ionophores whose primary benefits are realized via alternate mechanisms of action. The estimate that 18% of carbohydrate energy is lost as methane energy (example III, Table 8.2) is inflated by the fact that reducing equivalents used in support of

Table 8.2 Three early rumen balance models

I.	Glucose conversion to acetate and propionate: $1.5 \text{ glucose} + 3\text{ADP} \rightarrow 3 \text{ pyruvate} + 6\text{H}^+ + 6\text{e}^- + 3\text{ATP}$ $1 \text{ pyruvate} + \text{ADP} \rightarrow \text{CO}_2 + \text{acetate} + 2\text{e}^- + 2\text{H}^+ + \text{ATP}$ $2 \text{ pyruvate} + 8\text{H}^+ + 8\text{e}^- + [2\text{ADP}] \rightarrow 2 \text{ propionate} [+ 2\text{ATP}]^a$ $1.5 \text{ glucose} + 6\text{ADP} \rightarrow \text{CO}_2 + \text{acetate} + 2 \text{ propionate} + 6\text{ATP}$ Efficiency = $\frac{2 \text{ propionate} + 1 \text{ acetate}}{1.5 \text{ glucose}} * 100 = 94\%$ Energy loss as heat = 6.0% (does not exclude energy trapped in ATP)
II.	Glucose conversion to acetate and methane Organism A polysaccharide $\rightarrow 1.5 \text{ glucose-P}$ $1.5 \text{ glucose-P} + 4.5\text{ADP} \rightarrow 3 \text{ pyruvate} + 6\text{H}^+ + 6\text{e}^- + 4.5\text{ATP}$ $3 \text{ pyruvate} + 6\text{H}^+ + 6\text{e}^- \rightarrow 3 \text{ lactate}$ Organism B $3 \text{ lactate} + 3\text{ADP} \rightarrow 3 \text{ Acetate} + 3\text{CO}_2 + 6\text{H}_2 + 3\text{ATP}$ Organism C $6\text{H}_2 + 1.5\text{CO}_2 + 1.5\text{ADP} \rightarrow 1.5\text{CH}_4 + 3\text{H}_2\text{O} + 1.5\text{ADP}$ $1.5 \text{ glucose units} + 9\text{ADP} \rightarrow 3 \text{ acetate} + 1.5\text{CO}_2 + 1.5\text{CH}_4 + 3\text{H}_2\text{O}$ Efficiency = $\frac{3 \text{ acetate}}{1.5 \text{ glucose}} * 100 = 61\%$ Methane loss = 30.5% Heat loss = 8.5%
III.	Standard rumen fermentation ^b $100 \text{ glucose units} \rightarrow 107 \text{ acetate} + 37 \text{ propionate} + 28 \text{ butyrate} + 59 \text{ methane} + \text{heat}$ Net efficiency of fermentation = 75% Methane loss = 18% net ATP yield ^a (depending on microbes considered) = 3.65–4.58 Heat loss = 7%

^a ATP yields can vary depending on microbe and metabolic pathway (Baldwin *et al.*, 1970).

^b Based on Hungate (1966).

microbial growth are not considered, e.g. the balance model was presented for purposes of illustration and not as a complete rumen balance model. More complex models must be used in evaluating experimental data and concepts, as well as detailed studies of quantitative aspects of ruminant digestion and metabolism. These more complex models must often accommodate the fermentation of several classes of carbohydrates by different microbes, fermentation of nutrients other than carbohydrates, the use of carbon, nitrogen and reducing equivalents for microbial cell growth, passage to and digestion of nutrients in the lower gut and use of products of digestion by the host animal. Sets of metabolic equations depicting these functions are easily developed within the constraints of available knowledge and can be solved using a hand calculator (Baldwin *et al.*, 1970). Disadvantages of hand calculation, however, are that the calculations are laborious, subject to error and usually lead to formulation of only one of many feasible solutions (Baldwin, 1970; Baldwin and Smith, 1974). Often, the values of solutions obtained by hand calculation are compromised by implicit and explicit assumptions made to simplify the calculations. Common simplifications are that nutrient use for microbial growth and microbial growth yields are constant, that patterns of nutrient flow via alternate metabolic pathways do not vary and that microbial interactions are minimal or, at best, fully understood.

At the opposite extreme, detailed dynamic simulation models can be constructed in which simplifying assumptions are not required. In Chapters 11 and 16, it is emphasized that dynamic models are difficult to construct and validate, and expensive to solve. Thus, for many analyses of data on ruminant digestion and metabolism, hand calculations might be considered too laborious or simplistic and the use of complex dynamic models either beyond the state of the art, too sophisticated for the problem, or too expensive. These views led Reichl and Baldwin (1975, 1976) to develop balance models of ruminant digestion and metabolism that are similar to those used in hand calculations but suitable for computer solution. The advantages gained by programming these for computer solution were that arithmetic errors were eliminated, simplification was not necessary, and multiple solutions could be readily obtained. By varying assumptions, effects of these on apparent input:output relationships could be evaluated. Within the context of the modeling objectives stated in Chapter 3, computerized balance models have been used to evaluate and extend data, to evaluate relationships among parameters of ruminant digestion and metabolism, and to deduce, on a statistical basis, values for parameters not amenable to experimental determination. An objective of this chapter is to address these objectives.

Two balance models were constructed by Reichl and Baldwin (1975, 1976): an elemental balance model and a metabolic pathways balance model. These were, initially, constructed to provide convenience and accuracy in calculating rumen balance relationships from complete and partial

fermentation data. Both models were reported in a matrix format suitable for solution using a linear program. Use of a linear programming matrix format was adopted at that time, because spread sheet programs were not yet available. These matrices can be easily converted to spread sheet format. The elemental balance model consists of matrix entries that represent the elemental (C, H, O, N, S and P) composition of feed nutrients digested in the rumen, rumen microbes and rumen digestion products (Reichl and Baldwin, 1975). Table 8.3 is presented to indicate values for the composition of rumen microbes used by Reichl and Baldwin (1975). These composition data have been used in many subsequent models in which stoichiometric equations for microbial growth were used.

A portion of the matrix format used to implement and solve the elemental balance model is presented in Table 8.4. Solutions were achieved by specifying feed inputs, microbial growth yields and/or amounts of rumen digestion products formed. When complete data are available, these can readily be checked for internal consistency, i.e. whether or not input equals output. When partial data are available, these can be extended using the model. For example, if data on feed digested in the rumen and amounts or proportions of fermentation products formed are known, a series of estimates of feasible microbial growth yields can be obtained. These estimates are subject to the constraint that input equals output for each element. In extending partial data, assumptions are often required. The answers obtained are, of course, no better than the assumptions. For example, an investigator may have determined amounts of volatile fatty acids (VFA), CO_2 and CH_4 produced in the rumen and may wish to estimate amounts of carbohydrate, amino acids and lipid fermented or transformed in the rumen. To do this, using the model, an assumed microbial growth yield must be introduced along with the experimental data. An advantage of using a computerized balance model is that balances for several summed microbial growth yields can be computed easily to evaluate the influence of assumed growth yields upon estimates of amounts of feed components digested in the rumen and interpret the estimates obtained accordingly. Repeated hand calculations for evaluations of such assumptions are not attractive, because of the time required.

The pathways balance model reported by Reichl and Baldwin (1975) was somewhat more complex than the elemental balance model, in that the matrix was structured to assure not only that balances between inputs and outputs be correct, but also that conversions of feed components to products occur via known metabolic pathways. Feed inputs, microbial cell yields and amounts or proportions of metabolic pathways could be computed from complete or partial data and assumptions, as described for the elemental balance model. The biosynthetic and fermentative equations that comprise the core of the pathways balance model closely reflect those developed during construction of a (naive) dynamic model of rumen fermentation published previously (Baldwin *et al.*, 1970). However, several of the

Table 8.3 Calculated composition of rumen microbes and their components^a

	Protein	Nucleic acid	Polysaccharide	Lipid	Ash
	(g/100 g dry cell)				
Bacteria or protozoa	54.46	9.08	20.16	11.54	4.76
Protozoa + storage polysaccharides	38.81	6.47	43.10	8.22	3.39
Molecular weight	115	322	162	614	—
	(mol/100 g cells)				
Bacteria or protozoa	0.474	0.028	0.124	0.019	—
Protozoa + storage polysaccharide	0.338	0.020	0.266	0.013	—
	(atoms/mol)				
	(atoms/100 g cells)				
C	5.04	9.05	6.0	30.2	
H	7.59	12.0	10.0	55.8	
O	1.77	7.0	5.0	10.0	
N	1.25	3.8	—	0.8	
S	0.03	—	—	—	
P	—	1.0	—	0.8	

^a Adapted from Reichl and Baldwin (1975).

Table 8.4 Matrix for rumen elementary balance model ^{a,b}

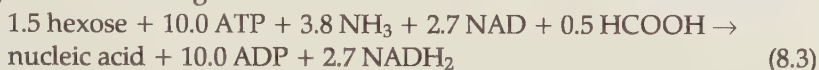
	CHO	AA	LI	H ₂ O	UREA	NH ₃	PROTO	BACT	C ₂	C ₃	C ₄	C ₅	CO ₂	CH ₄	RHS1
Z	1.0	1.0	1.0	1.0	1.0										MAX
C	-5.5	-5.09	-36.0		-1.0		3.893	3.968	2.0	3.0	4.0	5.0	1.0	1.0	0.0
H	-9.0	-7.72	-60.0	-2.0	-4.0	3.0	6.18	6.182	4.0	6.0	8.0	10.0		4.0	0.0
O	-4.5	-1.53	-5.3	-1.0	-1.0		2.072	1.672	2.0	2.0	2.0	2.0	2.0		0.0
N		-1.28	-0.1		-2.0	1.0	0.509	0.714							0.0

^a Adapted from Reichl and Baldwin (1975).

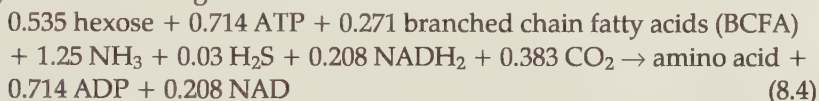
^b CHO, feed carbohydrate; AA, feed protein (average amino acid); LI, feed lipid; PROTO, protozoa; BACT, bacteria; C₂, acetate; C₃, propionate; C₄, butyrate; C₅, valerate; RHS1, right hand side no. 1. Values in mole/mole excepting values in PROTO and BACT column, which are expressed in mole/100 g cells.

equations were altered, based on a more extensive literature survey and new experimental data. Because many stoichiometric coefficients derived from the pathways balance model were used in subsequent models, the metabolic equations are summarized below. The biosynthetic reactions in the model were as follows:

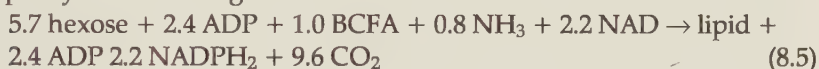
Synthesis of average nucleic acid



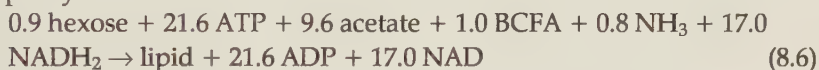
Synthesis of average amino acid



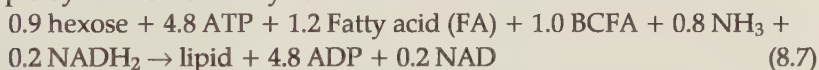
Lipid synthesis from glucose



Lipid synthesis from acetate



Lipid synthesis from fatty acids



Amino acid uptake



Protein synthesis

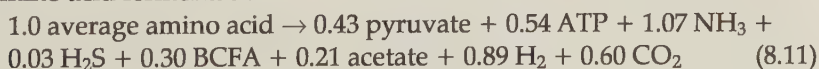


Polysaccharide synthesis



The fermentative pathways in the pathway balance model were as follows:

Amino acid fermentation



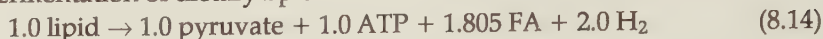
Hexose fermentation



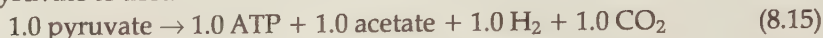
Pentose fermentation via phosphoketolase



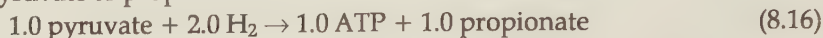
Fermentation of dietary lipid



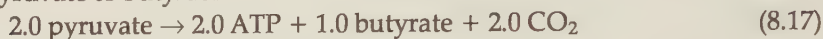
Pyruvate to acetate



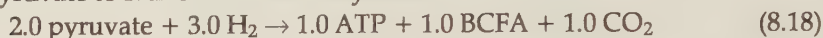
Pyruvate to propionate



Pyruvate to butyrate



Pyruvate to branched-chain fatty acids



Saturation of fatty acids



Growth of methane bacteria



Reichl and Baldwin (1975, 1976) presented a number of evaluations to illustrate both the validity and application of the elemental and pathways balance models. These will not be reiterated here, but two important lessons derived from the study will be mentioned. The first lesson is simply a pragmatic one. Whether one is evaluating experimental data or models and modeling analyses, it must be assured that the laws of conservation of energy and elements are satisfied. When the results of a balance analysis of an experiment indicate that 80% or 120% of carbon and energy input to the system are recovered in products, errors in the data must be addressed. Publications in which data indicate recoveries in products over this range are not at all uncommon, whether they are dignified by statistical estimates of experimental variance or not. Had the authors challenged their data utilizing a balance model, probable sources of error could have been identified and addressed by the authors and thereby improve the usefulness of their data. It should not be incumbent upon the 'modeler' to try to ascertain sources of quantitative errors in the reports of other scientists. Similarly, when equations for complex sets of metabolic transactions are incorporated into a model, it is very easy to make errors of commission and omission. We have incorporated enough of these errors in various models to cause us to include input:output computations into our models; when inputs of elements and energy to a system do not equal outputs, we are warned and have an opportunity to identify and correct the error, a process that can take a few hours or a few weeks. In this case, it is incumbent upon the modeler to identify and correct his or her errors. We view inclusion of input:output

relationships in analyses of our experiments and equations to monitor inputs and outputs as essential components of our research program.

A second important lesson was gained when the equations of the pathway balance model were adapted to simulate the growth requirements and metabolism of eight rumen microbes (Reichl and Baldwin, 1976). Two observations were that many feasible solutions were found, e.g. the model was non-unique. Also, during sequential solutions, considerable simplification of the rumen microflora occurred. Both of these indicated, as stated above, that our knowledge of rumen microbial functions and interactions was not adequate to simulate the rumen ecology. This led us to adopt a highly aggregated representation of rumen microbes which has been termed by ourselves and others as 'one bug models'. Adoption of this approach implies acceptance of the concept that the balance of microbial species in the rumen and their products of fermentation are largely determined by diet composition. Adoption of the 'one bug' approach to modeling the rumen fermentation (Baldwin *et al.*, 1977, 1987) does not mean that equal functions were conferred upon all microbes, since insoluble substrates such as starch, hemicellulose and cellulose can only be hydrolyzed by microbes that grew in association with each insoluble substrate (Chapter 16). Implementation of the 'one bug' approach did require that products formed from each chemical entity in the diet be defined empirically, since the microbes fermenting each chemical entity are not defined in the model. This led to formulation of the analytical model discussed in the next section.

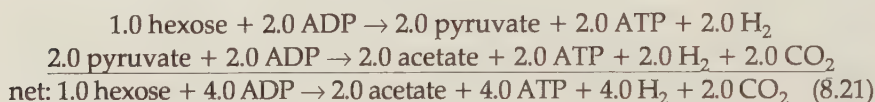
8.4 AN ANALYTICAL MODEL OF RUMEN DIGESTION

Direct experimental data on quantitative relationships among chemical components of feeds and products of rumen fermentation are very limited. For example, Wallnofer *et al.* (1966) presented data on ^{14}C -labeled VFA formed from radiolabeled glucose, xylose and cellulose, but the range of diets studied was limited and the chemical compositions of the diets were not well defined. Another problem is that it is virtually impossible to isolate radiolabeled complex carbohydrates in their native form. Thus, the results obtained using crystalline cellulose from cotton by Wallnofer *et al.* (1966) likely did not reflect the fermentation of native cellulose in forage cell walls.

An alternate approach to the problem of obtaining estimates of stoichiometric coefficients for fermentation of the chemical components of diets was proposed by Koong *et al.* (1975). In this approach, an algebraic model of the pathways of fermentation for each component of the diet was formulated in which stoichiometric coefficients could be varied systematically in sequential runs, comparing model predictions of fermentation products with experimental estimates for a number of diets until the summed squares of errors of prediction were minimized.

Koong *et al.* (1975) formulated a model of rumen digestion made up of a

set of algebraic equations for quantitatively important chemical components of ruminant feeds. For each carbohydrate utilized in the rumen ($\text{CH}_2\text{O}(\text{I})$) a portion ($\text{ALPHA}(\text{I})$) is fermented and the remainder ($1 - \text{ALPHA}(\text{I})$) is used for microbial growth, e.g. $\text{CH}_2\text{O}(1) =$ soluble sugars, $\text{CH}_2\text{O}(2) =$ cellulose, etc. $\text{CH}_2\text{O}(\text{I})$ can be converted via four different fermentation pathways to the products acetate, propionate, butyrate, or valerate. The partition coefficients $\text{A}(\text{I})$, $\text{B}(\text{I})$, $\text{C}(\text{I})$ and $\text{D}(\text{I})$, respectively, define the proportion of $\text{ALPHA}(\text{I})$ converted to acetate (A), propionate (B), butyrate (C) and valerate (D) and can be adjusted in the model during iterative solutions. The stoichiometry used for each fermentation pathway was derived from the pathway balance model (Reichl and Baldwin, 1976). In deriving algebraic equations for each fermentation pathway, metabolic equations of the form



were converted to the form (in Fortran):

$$\text{ACETF}(\text{I}) = 2.0 * \text{A}(\text{I}) * \text{ALPHA}(\text{I}) * \text{CH}_2\text{O}(\text{I}) \quad (8.22)$$

where $\text{ACETF}(\text{I})$ is acetate formed from $\text{CH}_2\text{O}(\text{I})$, 2.0 is a stoichiometric coefficient (2ACET/HEXOSE), $\text{A}(\text{I})$ is the proportion of fermented $\text{CH}_2\text{O}(\text{I})$ converted to ACET, $\text{ALPHA}(\text{I})$ is the proportion of $\text{CH}_2\text{O}(\text{I})$ fermented and $\text{CH}_2\text{O}(\text{I})$ is the amount of $\text{CH}_2\text{O}(\text{I})$ used in the rumen for microbial fermentation and growth.

Amounts of ATP, H_2 and CO_2 formed during hexose conversion were coded as follows:

$$\text{ATPF}(\text{I}) = [2 + 2 * \text{A}(\text{I}) + 2 * \text{B}(\text{I}) + 2 * \text{C}(\text{I}) + \text{D}(\text{I})] * \text{ALPHA}(\text{I}) * \text{CH}_2\text{O}(\text{I}) \quad (8.23)$$

$$= [3 + \text{A}(\text{I}) + \text{B}(\text{I}) + \text{C}(\text{I})] * \text{ALPHA}(\text{I}) * \text{CH}_2\text{O}(\text{I}) \quad (8.24)$$

$$\text{H}_2\text{F}(\text{I}) = [2 + 2 * \text{A}(\text{I}) - 4 * \text{B}(\text{I}) - 3 * \text{D}(\text{I})] * \text{ALPHA}(\text{I}) * \text{CH}_2\text{O}(\text{I}) \quad (8.25)$$

$$\text{CO}_2\text{F}(\text{I}) = [2 * \text{A}(\text{I}) + 2 * \text{C}(\text{I}) + 2 * \text{D}(\text{I})] * \text{ALPHA}(\text{I}) * \text{CH}_2\text{O}(\text{I}) \quad (8.26)$$

The portion of $\text{CH}_2\text{O}(\text{I})$ fermented ($\text{ALPHA}(\text{I})$) was calculated based on the assumption that the ratio of moles ATP generated from fermentation of $\text{CH}_2\text{O}(\text{I})$ to moles of $\text{CH}_2\text{O}(\text{I})$ available, is equal to the ratio of ATP to carbohydrate required for a unit of microbial growth, i.e.

$$\frac{\text{ALPHA}(\text{I}) [3 + \text{A}(\text{I}) + \text{B}(\text{I}) + \text{C}(\text{I})]}{1 - \text{ALPHA}(\text{I})} = \frac{\text{MGYC}}{K} \quad (8.27)$$

where MGYC is the microbial growth yield coefficient expressed as moles ATP required for growth of one gram dry weight of microbial cells. K is a constant (4.36×10^{-3}), representing moles of carbohydrate required for the growth of 1 g of microbial cells. The K value was derived from the biosyn-

thetic equations used in the pathways balance model (Reichl and Baldwin, 1975). MGYC was considered a variable in the model and was allowed to change during iterative computations to obtain the best estimate. The above equation was coded as follows:

$$\text{ALPHA(I)} = \text{MGYC} / [\text{MGYC} + \text{K}(3 + \text{A(I)} + \text{B(I)} + \text{C(I)})] \quad (8.28)$$

In addition to ATP and carbohydrate, NH_3 , H_2 , CO_2 , valerate, H_2S and acetate are used for microbial growth in the model. Amino acids derived from dietary protein in the rumen were assumed to be completely fermented. Amounts of nutrients required for microbial growth were assumed constant, as in the pathways balance model. Equations for amino acid fermentation provide for iterative computation of proportions of acetate, propionate, butyrate and valerate formed from pyruvate produced during degradation of amino acids. Other products were computed according to the pathways balance model. ATP generated from protein fermentation was used as an energy source for microbial growth. The model also accounted for hydrolysis of urea, degradation of nucleic acids, reduction of unsaturated fatty acids and growth of methanogenic bacteria. In a terminal section of the model, all digestive processes were summed to give a set of model outputs that include all digestive endproducts. These model outputs could then be compared with experimental values to obtain a sum of squared errors of prediction.

A preliminary evaluation of the approach was promising (Koong *et al.*, 1975). Subsequently, Murphy *et al.*, (1982) undertook an extensive analysis directed to estimating proportions of fermentation products formed from chemical components of ruminant diets. The original model of Koong *et al.* (1975) required experimental estimates of amounts of each fermentation product formed in the rumen for each diet used in statistical comparisons of predicted vs. observed values. The experimental data on amounts of fermentation products formed were very limited and the range of diets for which data were available was largely limited to forages. In order to deduce up to 16 unknown parameters using the analytical model, a large number of data for a wide range of diets were clearly required (Murphy *et al.*, 1982). Therefore, the model was altered so that molar proportions of acetate, propionate, butyrate and valerate produced from various diets could be used in comparisons of experimental and predicted values. The main limitation then became the identification of studies in which adequate descriptions of the chemical composition of each diet were published along with VFA data. Murphy *et al.* (1982) were able to compile two sets of data from the literature. The first set of data from 20 different publications were as results from studies with 137 animals fed basically forage diets. The second set of data were as from studies with 48, mostly concentrate, diets fed to 374 experimental animals and reported in 18 publications. In early iterative runs of the analytical model against these data sets, soluble carbohydrates, organic acids, pectin, starch, hemicellulose, cellulose and protein were con-

sidered as separate diet components. Preliminary results indicated that proportions of VFA from soluble carbohydrates, pectin and organic acids were very similar. Therefore, organic acids and pectins were included in the soluble sugar fraction by equating 2 moles of organic acids with 1 mole of hexose and 1 mole of a pectin monomer with 1 mole of hexose. This simplification can introduce small errors in carbon and hydrogen balances, but separate representations did not improve overall fits of the model to data. This aggregation was also compatible with dynamic models in which the deduced parameters are used, since all three substrates are fermented rapidly in the rumen.

An arbitrary set of initial parameter values defining proportions of each substrate fermented to various VFA and for the microbial growth yield coefficient ($1/\text{apparent } Y_{\text{ATP}}$) were input to initiate the iterative process. In sequential solutions, the parameter values were adjusted systematically using the simplex and steepest descent algorithms to minimize the weighted sums of squares of residuals (Chapter 4). Weightings of experimental data were based upon the assumptions that measurements on a number of animals are more likely to be representative than are those based upon one animal. Standard errors of the final parameter values were estimated by the jackknife procedure.

Estimated fermentation parameters for both the roughage (R) and concentrate (C) data sets are presented in Table 8.5. As noted in Chapter 4, evaluation of the uniqueness of parameters deduced using complex analytical models is difficult. A first appropriate evaluation, largely qualitative, was based upon whether or not the results are compatible with known fermentation patterns and diet-induced shifts in the relative numbers of major rumen microbes.

Amylolytic microbes in the rumen produce considerably more propionate than do cellulolytic organisms, while saccharolytic microbes are intermediate. Roughage diets are high in cellulose, intermediate in soluble sugars and low in starch. Therefore, numbers of cellulolytic and saccharolytic microbes are high and greater proportions of acetate are formed. This is due not only to cellulose fermentation, but also to the saccharolytic microbes, which compete favorably for soluble carbohydrates and the products of starch and hemicellulose hydrolysis. Similarly, propionate production is increased when cereals are fed, not only because of the high starch fermentation, but also because the fermentation products formed from other carbohydrates are altered to favor propionate. The deduced parameter values are compatible with this conventional wisdom. An initially surprising result was that the fermentation patterns of cellulose and hemicellulose differed significantly. The report of Dehority (1973), which indicated that *F. succinogenes* hydrolyzes hemicellulose and does not ferment the hydrolytic products formed, helped explain these estimates. Thus, the Murphy *et al.* (1982) estimates seem to relate well to data on the chemical composition of rations and our understanding of rumen ecology.

Table 8.5 Estimated rumen fermentation parameters^a

Substrate	Group	Acetate <i>A(l)</i> ± SE	Propionate <i>B(l)</i> ± SE	Butyrate <i>C(l)</i> ± SE	Valerate <i>D(l)</i> ± SE
Soluble carbohydrate ^b	R ^c	0.69 ± 0.06	0.20 ± 0.01	0.11 ± 0.05	0.00 ± 0.00
	C ^d	0.45 ± 0.03	0.21 ± 0.04	0.30 ± 0.04	0.04 ± 0.00
Starch	R	0.59 ± 0.04	0.14 ± 0.02	0.20 ± 0.05	0.06 ± 0.00
	C	0.40 ± 0.01	0.03 ± 0.00	0.20 ± 0.00	0.10 ± 0.01
Hemicellulose	R	0.57 ± 0.06	0.18 ± 0.03	0.21 ± 0.05	0.05 ± 0.00
	C	0.56 ± 0.01	0.26 ± 0.01	0.11 ± 0.01	0.07 ± 0.00
Cellulose	R	0.66 ± 0.10	0.09 ± 0.00	0.23 ± 0.09	0.03 ± 0.00
	C	0.79 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.09 ± 0.00
Protein	R	0.45 ± 0.07	0.30 ± 0.04	0.18 ± 0.03	0.07 ± 0.00
	C	0.36 ± 0.03	0.37 ± 0.01	0.20 ± 0.03	0.07 ± 0.00

^a Adapted from Murphy *et al.* (1982).

^b Soluble carbohydrate plus organic acids and pectin.

^c Roughage diets. Values are mean ± SE (10 jackknife runs).

^d Concentrate diets. Values are mean ± SE (8 jackknife runs).

A second appropriate evaluation, also somewhat qualitative in nature, involved comparisons of the estimated fermentation parameters in Table 8.5 with estimates derived from *in vitro* incubations of rumen microbes with ^{14}C -labeled substrates, including glucose, sucrose, xylose, hemicellulose and cellulose. With some notable exceptions that were explainable, comparisons of deduced and experimental estimates indicated good compatibility as discussed in detail by Murphy *et al.* (1982). Note that the ^{14}C data were not used in estimating the parameter values in the model and thus constituted an independent test data set.

The next test of the uniqueness of the deduced parameters undertaken was suggested in Chapter 4. In this, one of the 16 deduced parameters was set at values of above and below the deduced value and held constant during iterative runs against the data set, while the other 15 parameter values were allowed to vary freely to compensate, if possible, for errors of prediction attributable to holding the single parameter value constant. Example results are presented in Figs 8.5 and 8.6. In Fig. 8.5, the sharp 'U' shape of the curve indicates that the deduced stoichiometric coefficient for the proportion of propionate formed from soluble carbohydrate is reasonably unique (Chapter 4). Similar, though less sharply defined, relationships were obtained for starch, hemicellulose and cellulose. In contrast, the coefficient deduced for protein conversion to propionate (Fig. 8.6) was clearly non-unique. Indeed, fixed values for this coefficient actually yielded better solutions, as indicated by lower weighted sums of squares. This is likely due to the fact that the range of protein contents of the rations for which experimental data were available was very narrow (14–17% crude protein) and, hence, the data sets had very little deductive power. In our current model of rumen fermentation, independent data were used to estimate the stoichiometric coefficients for protein fermentation. With the exception of protein fermentation coefficients, the 'goodness' of deduced stoichiometric coefficients as compatible with the estimates of standard errors of estimates obtained using the jackknife procedure (Murphy *et al.*, 1982). The exception to the generalization found for protein indicates that both approaches are essential to rigorous evaluations of deduced parameters.

The next step in analyses of the deduced parameter values was examination of residual errors (Chapter 4). Application of two of the approaches to residual analyses discussed in Chapter 4 are illustrated in Figs 8.7 and 8.8. One is to plot observed versus predicted values and test the hypothesis that the slope of the relationship does not differ significantly from zero. The solid lines indicate a slope of one, and the dotted lines represent respective best-fit regression equations for roughages and concentrates. The slope of the regression equation of predicted vs. observed molar proportions of acetate did not fit the null hypothesis for roughages ($P < 0.05$), but the regression equation for concentrates did ($P > 0.05$). A similar relationship for systematic errors becomes much more evident when observed vs. residual

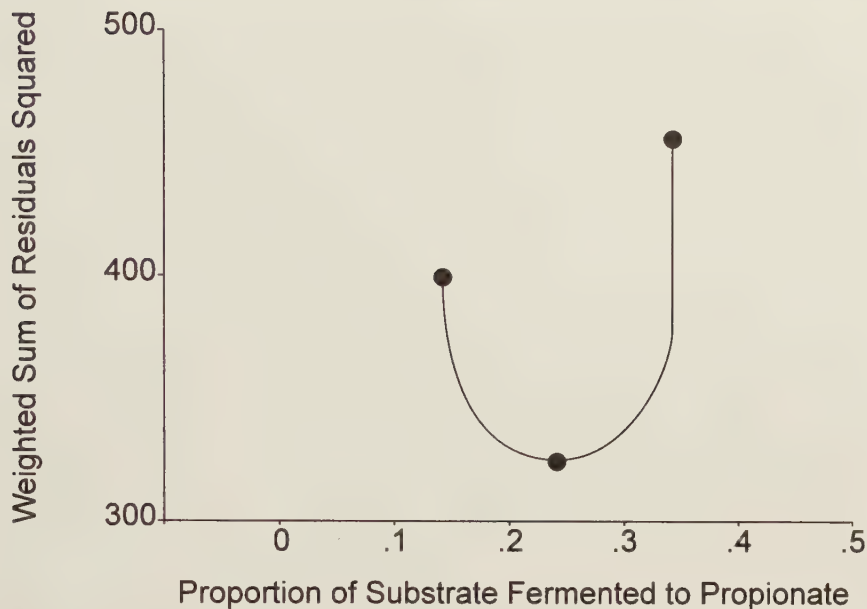


Figure 8.5 Sum of the weighted squares of residuals (WSQR) as influenced by changes in the proportion of substrate fermented to propionate away from the deduced parameter value for propionate formation from soluble carbohydrate.

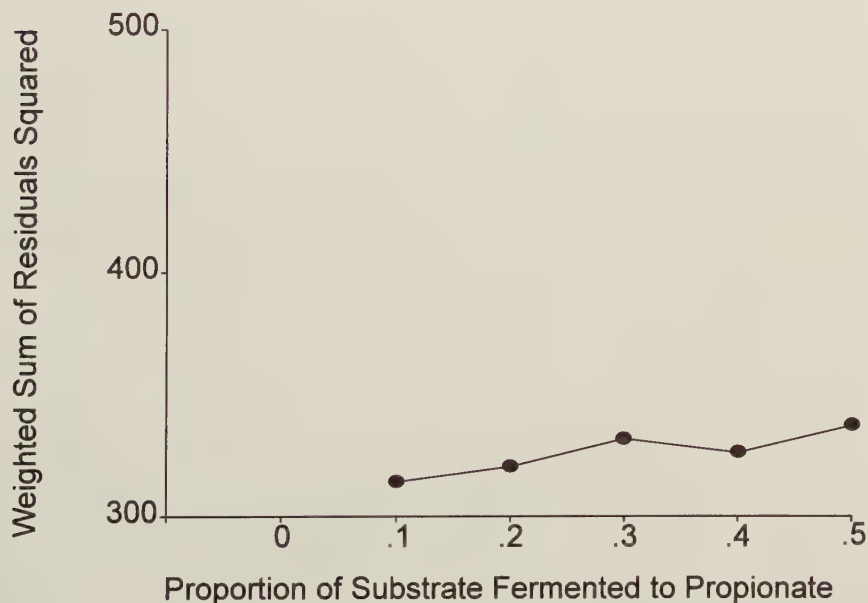


Figure 8.6 Sum of the weighted squares of residuals (WSQR) as influenced by changes in the proportion of substrate fermented to propionate away from the deduced parameter value for protein.

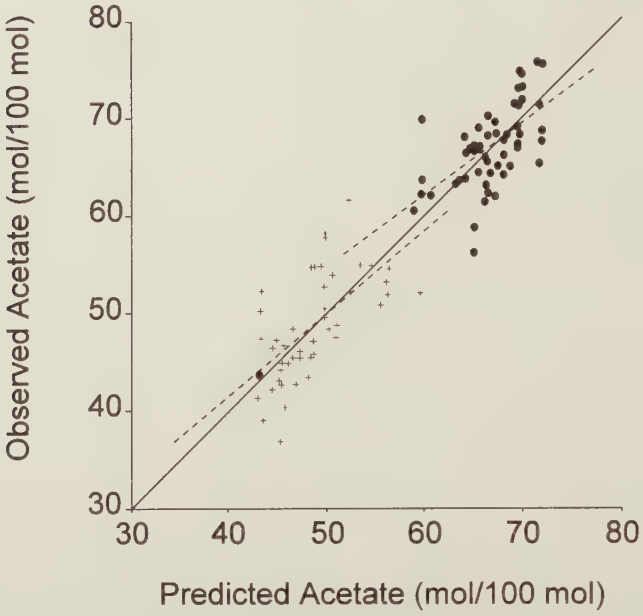


Figure 8.7 Predicted versus observed fermentation patterns across all diets for acetate.

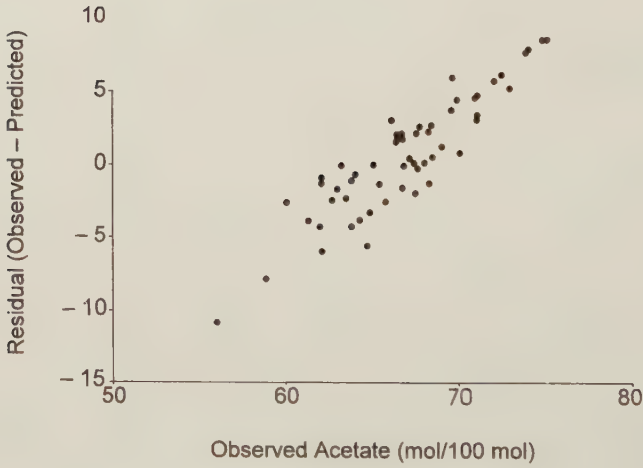


Figure 8.8 Roughage group residuals versus observed molar percent of VFA for acetate.

(observed-predicted) values for acetate in roughage fed animals were evaluated (Fig. 8.8). An obvious systematic underestimate of acetate at lower and overestimate at higher values existed. A similar systematic error in propionate estimates was also clear. Statistical analyses of the residuals indicated that lignin plus ash was a major cause of the residual errors in predictions of acetate ($r^2 = 0.21$) and propionate ($r^2 = 0.36$). When estimates were corrected using the respective regression equations, the remaining residual estimates became essentially random.

Concentrate diets exhibited the same type of systematic errors when residuals were graphed against observed molar proportions of acetate and propionate. Percentage roughage in the diet was the only statistically significant source of systematic error for the concentrate data set. Regression equations for the residuals for acetate and propionate, respectively, had r^2 values of 0.10 and 0.05. The slope for the propionate residual regression did not differ significantly from zero ($P > 0.05$), while the slope of the acetate equation did ($P < 0.05$). Subsequently, the cause of the systematic errors of prediction for fermentation products formed from high concentrate diets was attributed to effects of high concentrate diets upon ambient pH, and pH was introduced as a modifier of fermentation stoichiometric coefficients in our dynamic model of ruminant digestion (Chapter 16).

A plot of predicted versus observed molar proportions of butyrate formed on concentrate and roughage diets is presented in Fig. 8.9. Residual errors of prediction of the molar proportion of butyrate for concentrate diets were very large. For example, when predicted values for the molar proportions of butyrate were 12–14%, observed values ranged from 8–22%. No evident underlying cause in errors of prediction could be identified. Dijkstra *et al.* (1992) recently confirmed the fact that the deduced parameters for butyrate in Table 8.5 do not account for one or more sources of variance. Additional experimental work is required to identify causes of the high variance associated with predictions of proportions of butyrate formed.

In order to preserve a challenge data set for this evaluation, experimental data on total rates of production of VFA, referred to above as inadequate in number of observations and diets as a basis for estimation of parameter values, were not included in the roughage and concentrate data sets used to deduce the parameter values in Table 8.5. Thus, these data became an independent data set that could be utilized to evaluate the parameter values. Plots of observed versus predicted estimates of acetate, propionate and butyrate productions per day are presented in Figs 8.10, 8.11 and 8.12, respectively. The diets, for which experimental data are available, were all roughage diets. Thus, only the roughage-based parameter values could be used in this evaluation. Data for 47 sheep and 10 cattle and buffalo indicated r^2 values for prediction of 0.97, 0.95 and 0.95 for acetate, propionate and butyrate, respectively. Respective values for r^2 were 0.72, 0.68 and 0.46 for sheep data. Efforts directed to improve predictions over small ranges in

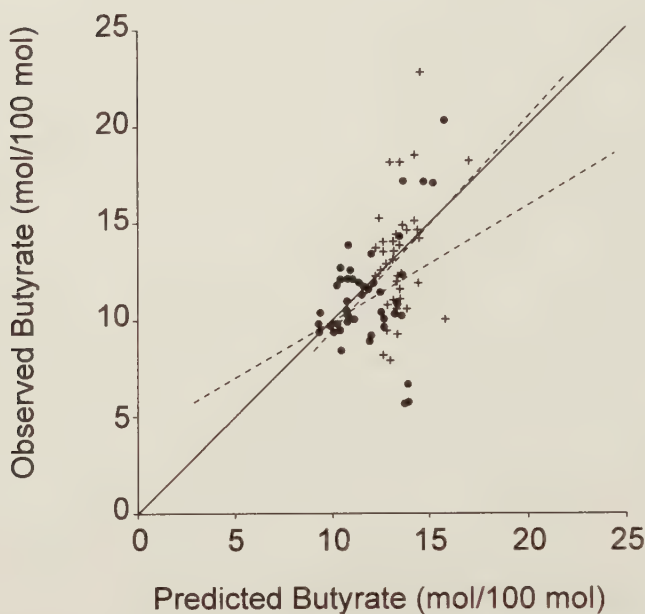


Figure 8.9 Predicted versus observed fermentation patterns across all diets for butyrate.

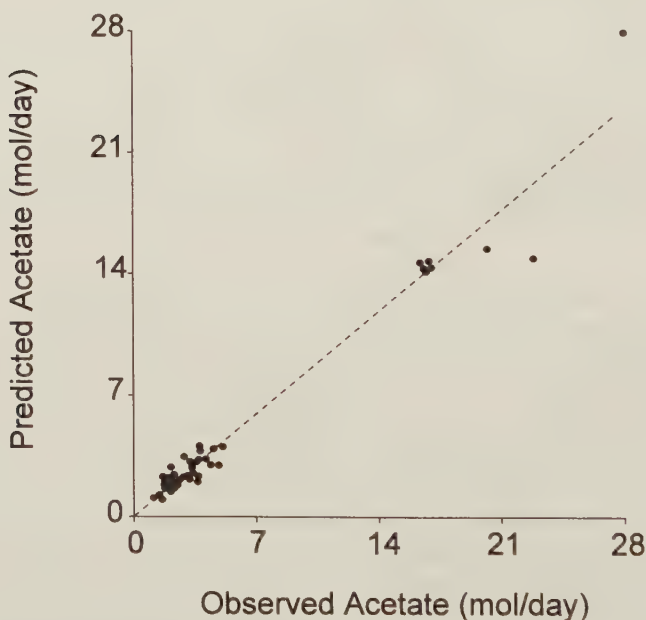


Figure 8.10 Predicted versus observed molar production of volatile fatty acids for diets for which challenge data were available for acetate.

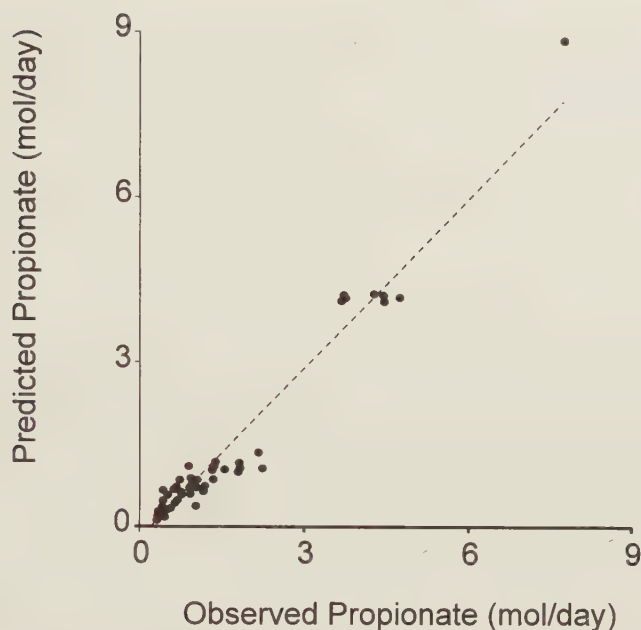


Figure 8.11 Predicted versus observed molar production of volatile fatty acids for diets for which challenge data were available for propionate.

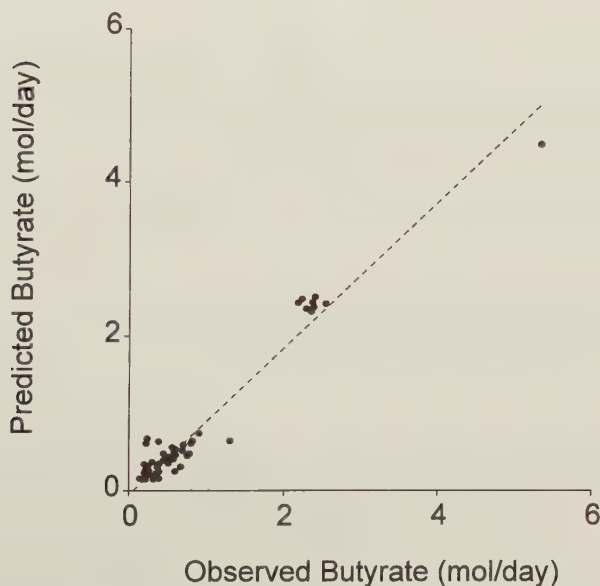


Figure 8.12 Predicted versus observed molar production of volatile fatty acids for diets for which challenge data were available for butyrate.

body weights, intakes and diets, and particularly concentrate diets, are clearly required. In our view, dynamic models that accommodate interactions among nutrients and transient changes in the rumen environment and the rumen microbes will be essential to realization of this goal. At present, with modifications noted above in regards to pH and protein, the values reported by Murphy *et al.* (1982) seem to be the best we have and the bases upon which we should build.

8.5 MICROBIAL GROWTH ELEMENTS

Russell *et al.* (1992) described development of the microbial growth element of The Cornell Net Carbohydrate and Protein System (CNCPS; Search: Agriculture, 1990) for evaluating cattle diets. In this system, inputs required to predict microbial yields are feed carbohydrate, which is divided into non-structural carbohydrate (NSC) that includes sugars, pectin and starch; structural carbohydrate (SC) which includes hemicellulose, cellulose and lignin; and feed protein. Feed crude protein is partitioned into four fractions:

1. Non-protein nitrogen;
2. Rapidly degraded soluble true protein;
3. Slowly degraded true protein;
4. Unavailable protein.

Relative amounts of each component of each feed degraded in or passed from the rumen are calculated from values tabulated for each feed ingredient for digestion rates (k_d) in the rumen (%/h) and rates of passage (k_p) from the rumen (%/h) according to the following ratios:

$$\begin{array}{l} \text{proportion of component degraded} \\ \text{in or passed from the rumen} = \end{array} \quad \frac{k_d \text{ or } k_p}{k_d + k_p} = f_d \text{ or } f_p \quad (8.29)$$

In this, all nutrients in each feed ingredient are either digested or passed from the rumen, since the fractions degraded (f_d) and passed (f_p) sum to one. The amount of each chemical component digested in the rumen is the sum of amounts of each in each feed ingredient in the diet times their respective f_d values:

$$\text{Carbohydrate fermented} = \sum_j^n f_d * \text{carbohydrate fraction in each feed ingredient in each diet} \quad (8.30)$$

Net microbial growth yields are dependent upon the amount of NSC and SC digested in the rumen; a theoretical maximum growth yield, Y_g , which is defined as maximum grams of bacteria formed per gram of carbohydrate fermented in the rumen in the absence of maintenance; a maintenance requirement; and the input of hemicellulose plus cellulose and lignin (NDF)

from forage in the diet. According to fig. 1 in Russell *et al.* (1992), the value of Y_g for bacteria fermenting SC (Y_{G1}) and NSC (Y_{G2}) is 0.4 g cells/g carbohydrate fermented. When amounts of NDF from forage in the total diet fall below 0.2, Y_G values decrease by 0.025 per percentage point decrease in forage NDF present in the diet. This provision helps capture effects of low pH values, characteristic of high concentrate diets, upon microbial growth.

Values for Y_G are used to estimate three microbial growth yield coefficients (g bacteria/g carbohydrate digested) Y_1 , Y_2 and Y_3 for the fractions of SC, sugars in NSC and starch in NSC. They are based upon the ratio of maintenance requirements of microbes fermenting SC (k_{m1}) and NSC (k_{m2}) and respective growth rates for SC-fermenting (k_{G1}), sugar-fermenting (k_{G2}) and starch-fermenting (k_{G3}) microbes for each carbohydrate fraction from each feed ingredient j :

$$1/Y_{1j} = (k_{m1}/k_{G1}) + 1/Y_{G1} \quad (8.31)$$

$$1/Y_{2j} = (k_{m2}/k_{G2}) + 1/Y_{G2} \quad (8.32)$$

$$1/Y_{3j} = (k_{m3}/k_{G3}) + 1/Y_{G3} \quad (8.33)$$

Respective values specified for k_{m1} and k_{m2} are 0.05 and 0.15 g carbohydrate*(g bacteria*h)⁻¹. Then the yield of NSC bacteria is adjusted according to a ratio of amounts of available rumen amino acids and peptides (RDPEP) and amounts of NSC sugars (RDCA) and starch (RDCB) degraded in the rumen from each feed ingredient j calculated as

$$\text{RATIO}_j = \text{RDPEP}/(\text{RDCA} + \text{RDCB} + \text{RDPEP}) \quad (8.34)$$

$$\text{IMP}_j = e^{(0.404 * \text{Ln}(\text{RATIO}_j * 100) + 1.942)} \quad (8.35)$$

where IMP is the percentage improvement in growth yield of microbes fermenting NSC from each feed ingredient j . Finally, the yield of microbes from each ingredient is calculated as

$$\text{BACT}_j = Y_{1j} * \text{RDCSC} + Y_{2j} * (1.0 + \text{IMP}_j * 0.01) * \text{RDCA} + Y_{3j} * (1.0 + \text{IMP}_j * 0.01) * \text{RDCB} \quad (8.36)$$

and total bacterial growth by summing BACT_j for all feed ingredients.

Thus, the Cornell system for microbial growth accommodates variation due to composition of feed ingredients and their digestibilities in the rumen, as well as microbial growth yields dependent upon relative rates of fermentation, growth, maintenance energy expenditures, and availabilities of amino acids and peptides (Search:Agriculture, 1990). In our view, such accommodations are essential to adequate predictions of amino acid availabilities to ruminant animals. A concern with the system as presented by Russell *et al.* (1992) is that provisions for interactions among feed ingredients are limited to effects manifested when total forage NDF in the diet falls below 20%. For example, effects of amino acids and peptides upon microbial growth are manifest only within each feed ingredient. This is probably acceptable with conventional dairy cattle rations used in the

United States. On the other hand, there are a number of diets in which interactions among feed ingredients and high carbohydrate:low protein and high carbohydrate:high true protein diets are prominent. The classical example is responses of high molasses plus urea-based diets to fish meal supplementation reported by Preston (1982), but many additional examples are available. Russell (personal communication) has indicated that in the current version of the Cornell model, a common pool of amino acids and peptides has been incorporated such that interactions among feed ingredients are now accommodated.

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Biology and algebraic models of growth¹

with R. L. Baldwin VI

Remember that 'formative substances', hormones, vitamins, genes, and similar controlling agencies, are merely mechanisms of a more deeply seated control which knits the mass of living stuff into an organism. We seek the architect, not the maker of builder's supplies.

E. W. Sinnott. Quoted from Brody (1945)

9.1 INTRODUCTION

Our understanding of growth has increased tremendously over the past 15 years, leading to identification and, in some cases, realization of new opportunities for improving the efficiency and quality of meat animal production. Two books on the basic biology of growth have been published recently (Campion *et al.*, 1989; Pearson and Dutson, 1991). These present excellent overviews and detailed analyses of the growth process, the many endocrine, paracrine and autocrine mechanisms that regulate growth, and opportunities for interventions to improve efficiency and product quality. It would be highly redundant and inappropriate to abstract these detailed treatments here, so only biology relevant to the modeling of growth will be presented in this chapter. The first section will be devoted to more or less classical, empirical mathematical analyses of the growth process. This will be followed by a discussion of nutritional models of growth and of concepts regarding the growth process, which underlie current mechanistic models of the growth process.

1 Portions of this chapter were derived from comments and writings contributed by Drs Colin Fischer and Cal Ferrel during deliberations of the NRC-CAN Subcommittee on input:output relationships.

9.2 CLASSICAL EQUATIONS FOR GROWTH

The mathematical representation and prediction of growth has been a widespread endeavor in biology and a wide range of theoretical approaches have been proposed. For nutritionists, the most accessible discussion of the area is in the work of Parks (1982), although it should be stressed that the application of nutritional ideas to theories regarding growth has been relatively modest. In nutrition, a primary aim has been to predict the deposition of nutrients, as energy or chemical entities, in the growing body. A description of increases in mass and changes in composition are thus required. Accurate descriptions of growth in terms of body components of different economic values and yields as effected by diet and alternative feeding management strategies are equally important.

Production systems that result in reproducible animal performance, such as might prevail on a single broiler farm where genetic, environment and feeding management are highly controlled and uniform, are well served by empirical descriptions of growth and body composition (Hurwitz *et al.*, 1985; Talpaz *et al.*, 1986). A more dynamic and theoretical description is required if a variety of conditions are to be considered and if effects of diet and environment on growth *per se* are to be predicted. If a desired rate of growth is defined as input, there are no problems associated with the use of empirical relationships, provided the stock are genetically capable of the desired rate of gain and the production environment is permissive. If on the other hand, a model is being used to optimize profit, productivity and/or resource utilization, the range of alternatives that must be considered may extend beyond the range of validity of simple empirical relationships and result in a need for more sophisticated equations which better accommodate cause and effect.

The concept of potential growth is a central issue in nutritional theory. Usually, it is presumed there is a genetically specified upper limit to nutritional responses, but it, or any other limit to growth, must be defined accurately, since very small increments are likely to be of economic importance. Some biotechnological developments, such as the administration of porcine somatotropin, may be viewed as agents that 'modify' genetic potential until more mechanistic descriptions of their actions become available.

The potential rate of body protein accretion (dP/dt) is often considered to be the most useful description of growth in nutritional modeling, since the composition of the fat-free body is relatively constant. For the purpose of optimizing bioeconomic efficiency and product quality, however, rates of both protein and fat accretion must be predicted accurately.

9.2.1 Allometric equations

A common model for estimating organ growth based upon overall growth and body weight has been the allometric equation

$$y = ax^b \quad (9.1)$$

where y is the weight of a specific organ or tissue, x is body weight and b is the slope of a plot of $\log y$ against $\log x$ (Brody, 1945; Stahl, 1965; Barton and Laird, 1969; Mukhoty and Berg, 1971; Lohse, 1973). This relationship has also been used to describe a number of physiological and metabolic functions in animals (Adolph, 1949; Munro, 1969). Our early research (Baldwin and Black, 1979) on growth of tissues and organ systems was undertaken after we realized that relative organ weights contribute to differences in fasting heat production (Chapter 6) and nutritional, physiological and environmental interactions, which determine patterns of nutrient utilization, growth rates and body composition. Initially, we considered the use of allometric equations for estimating tissue and organ weights in growing animals. Towards this end, we developed allometric equations for estimating organ weights in sheep using published data (Robinson, 1948; Wallace, 1948a,b; Palsson and Verges, 1952a,b; Smith *et al.*, 1952; Basset, 1960; Wardrop and Coombe, 1960; Norton, 1968; Fourie *et al.*, 1970; Jagusch *et al.*, 1970; Winter, 1971; Black and Tribe, 1973; Gabbedy, 1974; Lee *et al.*, 1975; Hilmi, 1976).

Several of these equations, together with others published for various species, are presented in Table 9.1. Both the coefficients a and exponents b vary considerably between and within species. This lack of interspecific applicability of numerical inputs to the allometric equation was not considered acceptable with respect to our modeling objective. The curves described by the allometric equations for sheep brain and liver are compared with actual observations in Figs 9.1 and 9.2. The allometric equation for brain clearly underestimates brain weight in young lambs and overpredicts it in older animals. Since nervous tissue has a high energy expenditure per unit mass relative to the body average, this systematic error was considered unacceptable for our purposes. Also, as illustrated for liver (Fig. 9.2), allometric equations are unsatisfactory for predicting weights of organs subject to large variations dependent upon nutritional or physiological status. These deficiencies of allometric equations in describing organ weights during development within a species, together with their lack of a temporal parameter, were discussed by Brody (1945), Laird (1965, 1966) and Laird *et al.* (1965).

9.2.2 Gompertz and related equations for growth

An alternative approach used widely to describe tissue growth is the Gompertz equation:

$$y = a^{-b(-kt)} \quad (9.2)$$

where y is tissue weight at time t , and a , b and k are constants deduced statistically. This equation has been shown to predict normal increases in

Table 9.1 Allometric equations for organ growth^a

Tissue	Species	Physiological state	Units of		a	b	r ²	Reference
			x	y				
Liver	sheep	growing	g	kg	39.6	0.78	0.97	—
		growing	g	kg	52.0	0.70	—	Brody (1945)
	cattle	mature	g	kg	97.0	0.66	—	Brody (1945)
		growing	g	g	0.039	1.14	—	Brody (1945)
		mature	g	g	0.28	0.69	—	Brody (1945)
	mammals	mature	g	g	0.034	0.89	—	Munro (1969)
Brain	sheep	growing	g	kg	18.4	0.51	0.95	—
	mammals	mature	g	kg	9.96	0.70	0.97	Brody (1945)
	humans	mature	g	kg	85	0.66	0.79	Stahl (1965)
	rats	growing	g	g	0.05	1.95	—	Brody (1945)
		mature	g	g	0.734	0.17	—	Brody (1945)
Heart	sheep	growing	g	kg	8.5	0.87	0.99	—
	mammals	mature	g	kg	5.9	0.98	—	Brody (1945)
	primates	mature	g	kg	4.8	0.84	—	Stahl (1965)
Muscle	sheep	growing	kg	kg	0.30	0.97	0.99	—
	mammals	mature	kg	kg	0.45	1.0	—	Munro (1969)
Gut	sheep	growing	g	kg	63.8	1.01	0.93	—
	mammals	mature	g	kg	75.0	0.94	—	Brody (1945)
	rats	growing	g	kg	114.0	1.26	—	Brody (1945)
Kidney	sheep	growing	g	kg	8.7	0.75	0.98	—
	rats	growing	g	kg	6.5	0.82	—	Brody (1945)
	mammals	mature	g	kg	7.3	0.85	—	Brody (1945)
Blood	sheep	growing	g	kg	44.1	1.07	0.99	—
	rats	growing	g	kg	62.0	0.98	—	Brody (1945)
		mature	g	kg	70.0	0.98	—	Brody (1945)
	mammals	mature	g	kg	55.0	0.99	—	Adolph (1949)

^aAllometric equations of the form $y = ax^b$, where y is organ weight and x is body weight, expressed in units stated in the table; r^2 refers to proportion of variance in plots of $\log y$ versus $\log x$ accommodated by the equation.

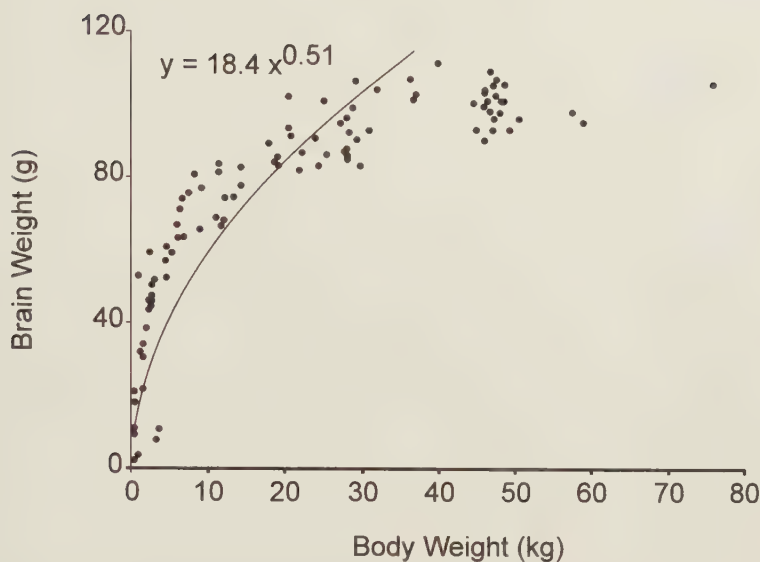


Figure 9.1 Comparison of sheep brain growth obtained from experimental observations (•) and calculated from the allometric equation.

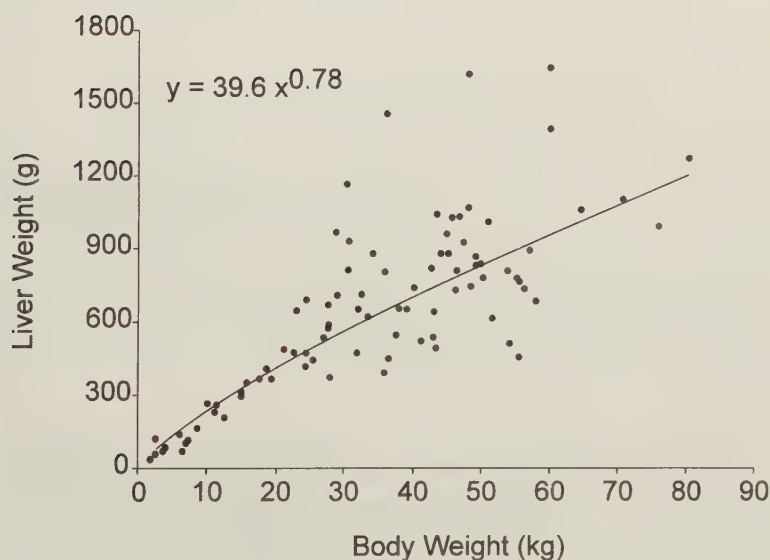


Figure 9.2 Comparison of sheep liver growth obtained from experimental observations (•) and calculated from the allometric equation.

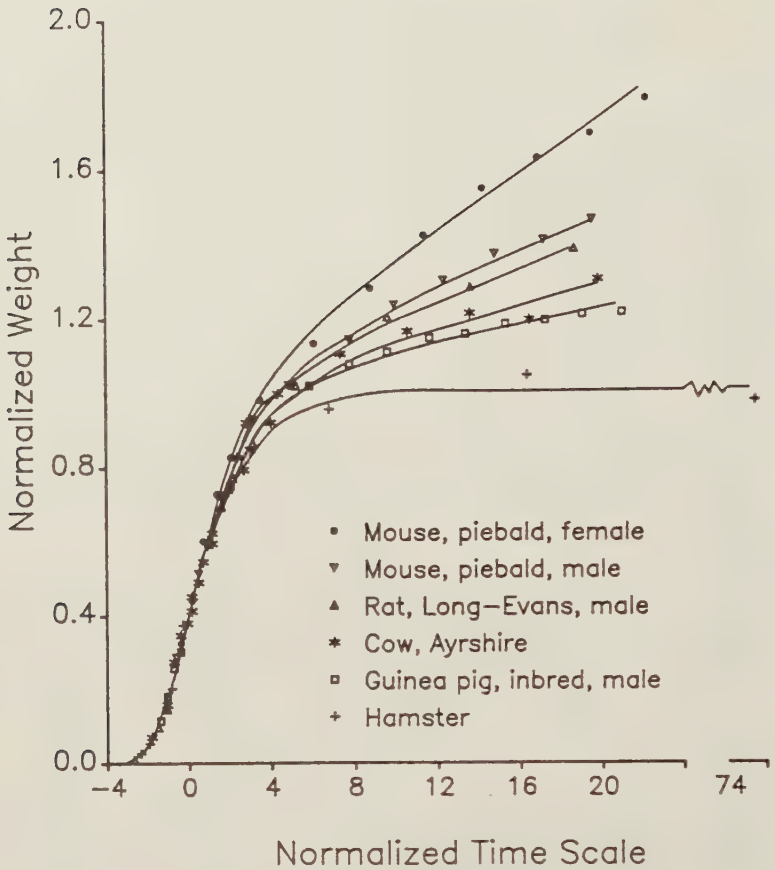


Figure 9.3 Normalized plots of compound growth curves fitted to postnatal growth data of six mammalian species. The body weights were normalized by dividing by the computed asymptote of the Gompertz portion of the curve. The time scale was normalized by fixing the inflection point of each Gompertz curve at zero and the unit for the time scale was set equal to the time in days required for the doubling of body weight immediately preceding the inflection point. The lines are the calculated compound curves giving the least squares fit to the data using the Gompertz equation. Adopted from Laird *et al.* (1965).

body and organ weights within a species from early embryonic life to maturity very well (Laird, 1965, 1966; Laird *et al.*, 1965; Fig. 9.3). The Gompertz equation offers the advantage of a temporal (t) term. However, the parameters a , b and k are not constant, and do not vary systematically across species (Table 9.2) and, as a result have no useful, underlying biological meaning (Laird, 1965; 1966). In addition, effects of nutritional state are not accommodated. Therefore, neither of these approaches fulfilled our modeling requirements.

Table 9.2 Species differences in growth constants^a

Species	Computed birth weight (W_0)	Initial specific growth rate (b)	Rate of decay of growth rate (k)
Mammals			
shrew	0.35 ± 0.03 g ^b	0.361 ± 0.047 ^b	0.144 ± 0.025 ^b
hamster	1.81 ± 0.08 g	0.225 ± 0.008	0.054 ± 0.002
mouse	1.88 ± 0.17 g	0.115 ± 0.009	0.045 ± 0.003
rat	5.60 ± 0.35 g	0.130 ± 0.004	0.033 ± 0.001
rabbit	64.2 ± 4.9 g	0.119 ± 0.007	0.029 ± 0.002
cat	93.9 ± 4.0 g	0.064 ± 0.004	0.022 ± 0.002
guinea pig	102.0 ± 4.1 g	0.036 ± 0.002	0.018 ± 0.001
dog	250.0 ± 7.9 g	0.066 ± 0.002	0.017 ± 0.001
swine	1.68 ± 0.10 kg	0.079 ± 0.003	0.017 ± 0.003
goat	3.50 ± 0.17 kg	0.026 ± 0.001	0.014 ± 0.001
cow	36.3 ± 1.2 kg	0.011 ± 0.0004	0.0044 ± 0.0002
horse	53.3 ± 3.1 kg	0.012 ± 0.001	0.0055 ± 0.0007
Birds			
quail	4.16 ± 1.6 g	0.211 ± 0.020	0.071 ± 0.007
chicken	31.2 ± 2.9 g	0.104 ± 0.004	0.026 ± 0.000
goose	45.2 ± 3.6 g	0.283 ± 0.006	0.064 ± 0.002
turkey	54.4 ± 18.0 g	0.096 ± 0.009	0.025 ± 0.000
duck	56.3 ± 11.0 g	0.187 ± 0.027	0.042 ± 0.016

^a Adapted from Laird (1966).^b Computed value $\pm$ standard error.

9.2.3 Equations scaled to degree of maturity

Taylor (1980) emphasized the value of the notion of degree of maturity, which he designates as u . Thus, maturity of protein growth is represented by the following equation

$$u = P/P_m \quad (9.3)$$

where P is the current body protein weight and P_m is the body protein weight at maturity. This enables scaling of the Gompertz function, which is widely used in nutritional growth modeling, in the form

$$\frac{dP}{dt} = B * P_m * u * \ln\left(\frac{1}{u}\right) \quad (9.4)$$

which expresses potential growth rate of protein in terms of u , a state variable, and two animal characteristics, a rate parameter, B , and the mature protein parameter P_m . Since $(P_m * B)$ is a constant for a given animal, the potential rate of protein accretion is proportional to $(u * \ln(1/u))$. This approach has been applied to the growth of broilers (Emmans, 1981) and turkeys (Emmans, 1989). More recently Moughan *et al.* (1987) defined the

potential growth of pigs in terms of (dP/dt) but made the assumption that the values are constant, for a given sex and genotype, over the growth period from 20 to 90 kg.

Roux and Meissner (1983) and Meissner and Roux (1983) used multiple measurements of the logarithms of cumulative feed intake, heat production, body protein and fat accretion in growing animals fed *ad libitum* to develop the accurate parameters for their model. Subsequently, they were able to test the model against data from restricted-fed animals. The Roux model incorporated a cumulative feed element that was adjusted to stage of development or degree of maturity. The model is based upon the log form of the Gompertz function to predict growth over time and the growth of tissues with the log of the allometric equation. Because of the addition of the cumulative feed element to the model, Roux and Meissner could test that the energy intake was accounted for by the sum of tissue energy and heat production. Thus, the inclusion of the feed intake element introduced a mechanistic component to the growth equation. However, prediction of the efficiency of feed use was still deduced empirically by observations of the log of cumulative feed intake.

Actual growth will frequently be less than potential growth for a wide variety of reasons. Some of these, such as diet composition and effects of environment, must be predicted by a model judged suitable for use in nutritional and management models. Stocking density in pens and poultry houses, and other aspects of population size, housing and subclinical disease, are the most likely reasons for growth to fall short of full potential, even if non-limiting environments are used. Although disease will probably remain outside the bounds of nutritional models, there is a critical need for theories that can predict effects of stocking densities on animal performance. Similarly, models of growth must contain provisions for carry-over effects such as compensatory growth, the use of anabolics, etc.

9.2.4 Other models of growth

Several models of tissue growth based on causal relationships have been published (Fried, 1970; Good, 1972, 1973; Mackey and Combs, 1974). In these models, pools of proliferating and non-proliferating cells, fluxes between these pools, and rates of cell death were considered. These models produce normal sigmoidal growth in a tissue and have been of assistance in identifying possible sites of action of regulators of the cell populations. Although it would be possible to introduce nutritional, metabolic and physiological elements into these models, they cannot easily accommodate changes in cell size, cell composition or component turnover rates and therefore could not simulate either differential rates of tissue weight loss during nutrient deprivation or compensatory growth following periods of nutrient restriction.

Continuous lifetime functions of food intake were described by Parks

(1982), who used data on a number of species and concluded that food intake can be described by

$$\frac{dq(t)}{dt} = \frac{q(t) - q(t^*)}{t - t^*} = \frac{C}{t - t^*} \quad (9.5)$$

where $q(t)$ is food intake (weight per unit of time) at time t , t^* is a food intake 'time-delay' factor and C is intake at maturity. Parks further suggested that deviations from expected intake, which he observed in an experiment designed to test his model, can be corrected by imposing sine waves on this equation, allowing a 'lag' between occurrence of errors and corrections for these. While this approach is suitable for describing specific data sets, it is less suitable when diet changes are considered or when the physiological state of the animal is in a constant state of change.

In nutritional modeling, body composition can be defined in terms of water, ash, protein and fat accretion. Errors arising from ignoring carbohydrate are trivial. Predictions of yield and technological and economic measures of body composition are all based upon major chemical components. The equations used for this latter purpose are quite variable. Some examples are shown in Table 9.3. Most of these preclude the possibility that nutritional treatment *per se* will change relationships among chemical and anatomical components. In most animal industries, where product composition is becoming more and more important, this limitation is becoming more and more critical.

Table 9.3 Examples of equations used to calculate yield and technological body composition from chemical components^a

Poultry^b

$$\text{skin weight} = (0.714 * (\text{live weight} - \text{storage fat weight}) * u^{0.075} + 0.25 * (\text{storage fat weight}) * u^{0.05})$$

$$\text{breast portion} = (0.325 * u^{0.05}) * \text{carcass weight} - \text{skin weight} + (0.37 \text{ skin weight})$$

$$\text{gizzard weight} = 0.016 * \text{live weight}$$

Pigs^c

$$\text{Fd} = \text{dissected fat in carcass side} = 0.559 * \text{total lipid} + 4.90$$

$$\text{dressing percentage} = 71.5 + 0.083 * \text{body weight}$$

$$\text{backfat thickness (mm)} = 75.0 * (\text{body fat weight/body weight}) - 0.95$$

^a u defined by equation 9.3.

^bEmmans (1981).

^cWhittemore and Fawcett (1976); Black *et al.* (1986).

After protein, fat is the most important component of growth, and from the points of view of evolving human nutritional standards and economic efficiency, accurate estimates of rates of fat accretion are essential. Some

early models represented fat accretion as either an allometric function of body weight or as a time-dependent, Gompertz-type function, as discussed for tissue and organ systems above. Application of these can only relate to *ad libitum* feeding conditions, where full genetic capacity can be achieved, or to the specific management and environmental conditions utilized to produce the data to which the equations were fit. A more common and generally applicable approach is to estimate fat accretion as a function of energy balance after costs of maintenance, protein accretion, thermogenesis, etc., have been accounted for, i.e.

$$E_{\text{lipid}} = [E_{\text{avail}} - (E_{\text{maint}} + E_{\text{prot}}/k_p)]k_f \quad (9.6)$$

where E_{lipid} is energy stored as fat, E_{avail} is energy available from the diet, E_{maint} is energy required for maintenance, E_{prot} is energy in protein gain, and k_p and k_f are the partial efficiencies of protein and fat accretion.

A further concern in terms of nutrient requirements for growth is the use of proteins for hair, wool, fur and feathers. The problem is most acute for feathers in poultry, for which few quantitative data are available.

9.3 NUTRITIONAL MODELS OF GROWTH

Nutritional models for growing animals should enable accurate predictions of nutrient requirements, calculation of responses of defined animals to defined feeds and calculation of optimal nutritional strategies. As discussed in previous and subsequent sections of this book, both the usefulness and limitations of using empirical, static factorial equations to calculate energy and other nutrient requirements in terms of animal functions (rate of output, maintenance, etc.) are well established in applied nutrition. Rates of evolution beyond these simple equations to more dynamic and complex models of growth have been slow and variable across species, but a set of underlying concepts can be identified that need to be developed if a wide range of circumstances are to be considered. A premise, developed in Chapter 15, is that dynamic models should enable bioeconomic analyses of the effects of alternative feeding strategies, taking into account a wide range of biological, technological and management variables.

In his book on poultry nutritional research, Morris (1986) clearly addressed these issues and presented the challenge, at least in part, that applied nutritionists must address:

It scarcely seems necessary to spend any more time or money investigating the 'nutrient requirement' for dietary energy or for dietary amino acids. Asking for statements of requirements in relation to energy and protein amounts to asking the wrong questions. Birds respond to changes in dietary energy or dietary protein concentration in a variety of ways (by changing their feed intake, their body compo-

sition or their egg size, for example) and we need good quantitative descriptions of these responses so that we can calculate optimum feeding strategies. These optima will be affected by biological responses, which can be measured in trials, but also by the prices of inputs and outputs. The optimum, calculated at any one time, has no permanent validity, being affected by changes in prices and by changes in the potential performance of the birds to be fed. What we need are better models for the prediction of responses to nutrients. The job of the poultry nutritionist in the next decade is to produce equations which can be incorporated into causal models, eliminating the fudge factors which have been used in some of the empirical models now in circulation. In some cases this will require large, multifactorial experiments to estimate response surfaces; but progress does not entirely depend upon mounting very large feeding trials. For example, an improvement in our understanding of interactions in the gut which affect the absorption of fatty acids is likely to be more helpful when predicting responses to fat supplementation of broiler diets than comparisons of feed efficiency derived by feeding many combinations and levels of fats from diverse sources.

The development of more elaborate nutritional models will tell us how to construct the most profitable diet for a flock of chickens or turkeys in a defined set of circumstances. This economic optimum will represent a compromise between the requirements of individuals in the flock, some of which could respond to higher nutrient concentrations whilst others could manage quite well on a cheaper diet.

Modeling growth requires that the following issues be considered and solved in a manner suitable to the end application. The nature of this solution may vary, for example, between models for farm use and those used to develop scientific ideas.

1. The description of growth in mass, chemical and tissue terms;
2. The differential rates of the growth of body tissues and the prediction of body composition;
3. Overhead costs associated with maintenance functions;
4. Diet composition effects on the partial efficiencies of body protein and fat accretion;
5. Genetic and environmental effects;
6. Prediction of food intake and consideration of overfeeding, underfeeding and recovery;
7. The structure of populations and variability;
8. Management structures;
9. (Bio)technological manipulations.

Within this context, the question that must be addressed in simulations of effects of nutrition on growth is, if a feed is to be given to an animal kept

in a specific environment, what will be the outcome? In seeking to answer this question, the 'driving elements' of the theory can be either:

1. That growth will follow some defined path provided the resources required are provided.
2. That feeds will be consumed at defined rates and the resulting rate of growth can be calculated.

The former construct is more applicable to animals fed *ab libitum* and requires a theory of food intake. The latter approach is more applicable to rationed animals, where feed intake is user defined. Some existing models for *ad libitum* fed animals are driven by equations for food intake (NRC, 1987). Both of these constructs require that a model enable accurate estimates of responses to feed intakes. Factors that influence responses were discussed in Chapters 5 and 6. Historical development of approaches and equations in current use will be discussed here.

Kleiber (1961) noted that the partial efficiency of energy use for maintenance is greater than that for weight gain. The feeding systems (Winchester and Hedricks, 1953; NRC, 1963) based on total digestible nutrients (TDN) in use at that time recognized only one feed net energy value. To overcome this limitation, which would require a feed's net energy value to decrease with level of feeding, Lofgreen and Garrett (1968) introduced a net energy system for growing and finishing beef cattle to the National Research Council. It separated the energy requirements for maintenance from those for weight gain, thus feed energy values for both functions were developed. The net energy requirement for maintenance was set equal to the animal's fasting heat production; the net energy requirement for gain was the energy content of the animal's gain. Hence, the net energy for maintenance content of a ration (NE_m) was set as the animal's fasting heat production divided by the amount of feed needed for body energy equilibrium. Similarly, the net energy for gain content of a ration (NE_g) was defined as the energy content of gain divided by the amount of feed fed above that required for body energy equilibrium.

The current net-energy based systems for growth of dairy and beef cattle (NRC, 1984, 1989) are quite sophisticated in the sense that significant effort has been directed to development of empirical equations to accommodate sources of variance. For example, effects of energy density upon efficiencies of ME use for maintenance and gain are computed as

$$NE_m = 5.73 ME - 0.577 ME^2 + 0.063 ME^3 - 6.90 \quad (9.7)$$

$$NE_g = 5.94 ME - 0.728 ME^2 + 0.051 ME^3 - 6.90 \quad (9.8)$$

where NE_m and NE_g are the estimated net energy values of feeds for maintenance and gain, respectively.

These accommodate ranges in efficiencies of ME use of 0.576 to 0.686 and 0.296 to 0.473, respectively, for maintenance and gain for diets ranging from

8.37 to 13.4 MJ ME/kg. Similarly, effects of body weight and rate of gain on the energy density of gain of medium-frame steers and heifers and large-frame steers (all receiving hormonal adjuvants), respectively, are

$$RE = 0.266 * W^{0.75} * EBG^{1.097} \quad (9.9)$$

$$RE = 0.328 * W^{0.75} * EBG^{1.119} \quad (9.10)$$

$$RE = 0.235 * W^{0.75} * EBG^{1.097} \quad (9.11)$$

where retained energy (RE) in MJ/day is the NE_g requirement of these animals at a given rate of empty body weight gain in kg/day (EBG).

Very large input:output data sets were required to develop these empirical equations. These systems remain static and factorial in nature, since total requirements are the sum of estimated requirements for individual functions and apply to one specific weight and rate of gain.

This system was tested under a wide variety of conditions at the California Experiment Station and in commercial feedlots, and was readily accepted for diet formulation by nutrition consultants in the beef cattle feeding industry. It overcame the criticism of previous feeding systems, which undervalued roughages for maintenance and concentrates for production. The system is useful for evaluation of performance of growing cattle and was adopted for growing beef cattle by the NRC (1976).

The net energy system described above was developed for medium-size beef breed steers given a growth stimulant and fed in a thermoneutral environment. Therefore, any effects of changes in animal type or management environment on economic benefits could not be estimated. Fox and Black (1984) recognized the deficiency of the system and accounted for observed variations in frame size, breed type and environment, as well as feed additives and non-use of growth stimulants and their respective economic costs. Also age, previous growth rate and feed intake were observed to influence animal performance. Thus, Fox and Black (1984) developed a set of adjustments based on literature data for addition to the preceding system to account for these factors and expand the information base for management decision making.

Environment and breed type were accounted for by developing adjustment factors for maintenance. A scale value of from 1 to 7 was used, with 1 defined as an outside feedlot with frequent cold stress, 5 defined as an outside lot, well-mounded and bedded during adverse weather, and 7 defined as no mud, shade, good ventilation and no chill stress. In heat stress, scale values of 6 are used for rapid, shallow breathing and 4 for deep, open mouth panting. An additional 12% adjustment for dairy animals (Holsteins) was made. Thus, net energy for maintenance is

$$0.322 * ENV * BREED * W^{0.75} \quad (9.12)$$

where ENV is $1 + 0.05$ (7-scale value) and BREED is 1 for beef animals and 1.12 for dairy cattle.

For prediction of gain, an adjustment of W in the equation for net energy for gain required was proposed. Because cattle of different frame sizes have similar body compositions at equal degrees of maturity, or proportion of mature body weight, the concept of equivalent weight was developed. This was defined as the weight at which animals of alternative frame sizes have similar body composition. This is equivalent to a 20% decrease or increase in body weight from the medium-frame standard for small- and large-frame animals, respectively. A 20% decrease or increase in body weight was assumed for heifers or bulls, respectively. Thus, a small-frame steer's weight would be multiplied by 1.25 and a large-frame animal's weight by 0.83 to reach an equivalent medium-frame weight. If animals receive no growth stimulants, equivalent weight is increased by the ratio of expected weight at 27.6% body fat (small degree of marbling) of animals given a growth stimulant to expected weight of those not given one. An additional 12% breed-type adjustment for dairy animals (Holsteins) was made. Hence, the modified equations for steers and heifers are

$$(0.05272 \text{ g} + 0.00684 \text{ g}^2) \text{ BREED} * \text{WE}^{0.75} \quad (9.13)$$

$$(0.05603 \text{ g} + 0.01265 \text{ g}^2) \text{ BREED} * \text{WE}^{0.75} \quad (9.14)$$

respectively, where WE is equivalent weight as defined above.

Ration NE_m and NE_g are adjusted for digestive stimulants and previous nutritional treatment. Feedstuff net energy values are multiplied by 1.04 for antibiotics, 1.11 for monensin and 1.06 for lasalocid. Based on scale values of 1, very fleshy and previous rates of gain of 1.05 kg/day, to 9, very thin and previous rates of gain of 0.32 kg/day, NE_m and NE_g , respectively, are multiplied by the following

$$1 - 0.047 (5\text{-scale value}) \quad (9.15)$$

$$1 - 0.105 (5\text{-scale value}) \quad (9.16)$$

Finally, Fox and Black (1984) estimate carcass quality grade (QG, Select = 8, Choice = 11, etc.) and yield grade (YG) as functions of percent carcass fat (CF). Hence,

$$\text{QG} = 2.5 + 0.23 \text{ CF} \quad (9.17)$$

$$\text{YG} = -1.7 + 0.15 \text{ CF} \quad (9.18)$$

In their system, carcass fat is calculated based on empty body fat (Garrett and Hinman, 1969), which is simply estimated from equivalent empty body weight (Simpfendorfer, 1974). Due to this direct relationship between WE and quality grade, it may be necessary to adjust QG for the effect of rate of gain in the finishing period. Many of the suggestions of Fox and Black (1984) were adopted in the latest revision of the NRC (1984). The system has been expanded, and gestation, lactation, digestion and protein requirements have been included (Fox *et al.*, 1988).

Although adjustment factors are adequate in well-defined conditions,

new situations with different sets of input variables than were used to validate the original adjustment factors may make predictions erroneous. This is due to interrelationships among variables and thus a lack of independence among adjustment factors.

9.4 CONCEPTS OF THE BASIC BIOLOGY OF GROWTH USED IN MECHANISTIC MODELS

Robinson (1971) developed the concept that growth of tissues, organ systems and whole animals occurs in three phases: hyperplasia, hypertrophy and accretion. Early pre- and postnatal growth is clearly hyperplastic in that it is due to cell proliferation in all tissues. Muscle presents a special case in that the number of fiber cells in mammals is fixed near birth. However, the number of nuclei in muscle cells continues to increase for some time as a result of satellite cell proliferation and subsequent fusion with muscle fiber cells (Allen *et al.*, 1979). Thus, a more general definition of hyperplastic might be increases in nuclei or DNA per tissue. Hypertrophic growth was defined by Robinson (1971) as, essentially, increases in protein per unit of DNA to some genetically defined maximum. This is in accord with the DNA unit size hypothesis of Cheek *et al.* (1971), which holds that each unit of DNA in each tissue of each species expresses the information required for the formation of a specific amount of cell material.

The data in Table 9.4 illustrate the relative importance of total mature tissue DNA and changes in the DNA unit (g wet weight tissue/mg DNA) across species and tissues. Tissue DNA content is clearly the primary determinant of mature organ weight. In liver, it seems to be the only major determinant, since the DNA unit appears to be constant across species in the fed state. In muscle, total DNA is also a primary determinant of mature weight, but a systematic increase in the size of the DNA unit also contributes (Table 9.4). The Robinson (1971) concept of accretion is less defined. It implies the accumulation of extracellular matrices in tissues independent of the DNA unit concept. The only clear (to us) example of accretion is the accumulation of fat in adipose tissue. Taken together the concepts developed by Robinson (1971) have become central to the development of mechanistic, often dynamic, models of growth (see Chapter 15) and underlie attempts to favorably manipulate the growth process. Comparison of the C and G mouse strains illustrates a result of an attempt to manipulate the growth process through genetic selection. In this case, the genetic selection for growth (24–39 g) was clearly selection for increases in organ DNA content, since the size of the DNA unit did not increase (Table 9.4).

As far as we can deduce, all attempts to enhance growth through manipulations of the growth hormone axis (somatotropin, antisomatomedin, growth hormone releasing hormone, insulin-like growth hormones I and II, growth hormone, transgenics, etc.); manipulations of the many endo-

Table 9.4 Relationships among body weights, tissue weights and DNA as determinants of tissue weight content^a

Species (strains)	Body weight (kg)	Liver			Muscle		
		weight (g)	DNA (mg)	g tissue/ mg DNA	weight (g)	DNA (mg)	g tissue/ mg DNA
Mouse (C)	0.024	1.5	3.13	0.48	0.014	7.78	1.8
Mouse (G)	0.039	2.3	5.35	0.43	0.021	11.67	1.8
Rat	0.160	6.7	17.0	0.40	0.072	0.042	1.7
Rabbit	1.50	45	96.5	0.59	0.675	0.251	2.7
Dog	25.5	536	1096	0.49	11.48	4.0	2.9
Steer	450	6300	16650	0.38	202.5	61.0	3.3
Horse	690	8970	22770	0.39	310.5	81.0	3.8

^a All data calculated from Munro and Gray (1969) except the mouse data, which are from Robinson and Bradford (1969). The notation mouse (C) identifies an unselected line of mice, while mouse (G) refers to a line of mice selected for rapid growth from the same parent strain.

crine, paracrine and autocrine hormones, which impact the growth process (transforming growth factors β , epidermal growth factor, fibroblast growth factor, antisense transgenics, anti-idiotypic immunization techniques, etc.); and use of anabolic agents (steroids and β -agonists) impact either hyperplasia or hypertrophy (see NRC, 1994 for detailed treatment). It is clear that if we can stimulate or prolong the period of satellite cell proliferation and fusion with muscle fibers, muscle growth and meat protein yields from domestic animals can be enhanced. Similarly, if we can either specifically increase rates of protein synthesis in muscle, without also increasing protein degradation as is often the case, or reduce rates of protein degradation, without concomitant reductions in rates of protein synthesis, rates of growth and the protein/DNA ratio would increase. Goll *et al.* (1989), for example, speculated that if the sole effect of a gene or anabolic agent was to reduce muscle protein degradation in a 454 kg steer 10% from a normal rate of 3% per day to 2.7% per day, muscle protein degradation would decrease from 0.98 to 0.88 kg/day. As a result, the rate of protein accretion would increase from 0.086 to 0.186 kg/day. This would increase rates of body weight gain from 1.2 to 2.58 kg/day and also almost double the partial efficiency of protein gain. Conversely, effects on rates of protein synthesis (i.e. a 10% increase) at constant rates of protein degradation would produce responses that are comparable but with a lesser increase in efficiency of protein gain.

Several important points regarding modeling the growth process arise from the above discussion. The first is that the historical approach of our nutritional models and feeding systems, which are based upon extensive

and expensive experimental studies of evolving populations of livestock, and attended by the use of more and more sophisticated adjuvants to manipulate the growth process, is becoming less and less tenable. The statements of Morris (1986) quoted above must be seriously considered. The often-quoted view of Blaxter (personal communication) that we must advance through utilization of 'First Principles' is becoming more and more relevant. This has led us to adopt the view espoused by Robinson (1971) that our understanding of growth and quantitative representation of the growth process and effects of endogenous and exogenous agents thereupon must accommodate the contributions of hyperplasia, hypertrophy and accretion to the growth process. Growth models that accommodate these concepts will be a primary emphasis in Chapter 15.

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Biology of lactation

with C. Ferrell

The legend tells how Zeus, wishing to immortalize Herakles, his son by a mortal woman, presented the infant to the breast of the sleeping Hera, who, had she been awake, would presumably not have co-operated. The milk continued to flow from the now-awakened goddess's breast after the infant, satiated, had stopped drinking, that which went upwards into the heavens forming the galaxy while those drops which fell downwards onto the earth gave rise to a garden of lilies.

Folley (1970)

10.1 INTRODUCTION

The biology of lactation is a very broad and interesting subject and, as such, has been reviewed extensively in both textbooks (Schmidt, 1971; Mephram, 1987) and more comprehensive and specific treatises (Larson and Smith, 1974a-c; Mephram, 1983). A review of the evolution of this field, from the mythology so well presented by Folley (1970) to characterization of (hormonal) mechanisms associated with the expression of genes for milk protein synthesis, clearly goes beyond the bounds and objectives of this book.

There are several areas where problems or limitations exist with regard to the application of classical approaches in improving our feeding systems. These include problems in accommodating variance in apparent maintenance requirements and in defining costs of productive functions due to differences in diet and product composition. Additional problems that must be addressed include effects of previous plane of nutrition on current and future performance and optimization of protein availability to producing animals.

It has been stated a number of times in this book that resolution of these problems will require greater utilization of our knowledge of underlying biological functions in our models. Significant progress in the introduction of mechanistic elements into dairy cattle feeding systems to increase the accuracy of predictions and general applicability has been made.

This continuing evolution will be illustrated in this chapter by summarizing the current protein and energy (NRC, 1989) system for dairy cattle;

identifying some current limitations; and discussing some newer systems that, although static, contain mechanistic elements, which capture our understanding of lower-level functions and, most importantly, were parameterized using data from studies of lower-level functions. This will be followed by discussion of an analytical model for evaluating nutrient transactions in rats. A rat example is used, because no comparable analytical model of nutrient transactions in ruminants is available, even though such a model would be useful.

Finally, an early balance model of cow mammary metabolism will be presented, as this was a reference or starting point for formulation of the dynamic model of cow mammary metabolism presented in Chapter 13.

10.2 RECENT EVOLUTION OF FEEDING SYSTEMS FOR LACTATING DAIRY CATTLE

10.2.1 NRC (1978) model for lactating dairy cattle

Estimates of protein requirements of domestic animals have, for many years, been based on factorial approaches similar to those proposed by Mitchell and coworkers (Mitchell, 1929; Mitchell *et al.*, 1932; Blaxter and Mitchell, 1948; Chapter 7).

In the system adopted by the NRC in 1978 for dairy cattle, estimates of crude protein (CP) requirements were calculated according to the general model

$$\text{CP required in g/day} = (U + F + S + G + C)/E_p + L/E_{pl} \quad (10.1)$$

where U was urinary N * 6.25, F was fecal metabolic nitrogen * 6.25, S was protein lost in skin secretions, scurf and hair, G was protein deposited in or associated with an increase in body weight, C was protein deposited in products of conception, and L was the sum of milk protein and the increased metabolic fecal protein losses resulting from increased feed allowances for lactation (CFDM). The terms E_p and E_{pl} are the conversion of dietary protein to absorbed amino acids times efficiencies of use of absorbed amino acids for U , F , S , G and C and lactation, respectively. E_p and E_{pl} convert the sum of the net protein requirements to crude protein (CP). Values for each component in the model were derived from experimental data and were included in the model as constants or simple equations. Values or equations used to estimate each component of the model were as follows:

$$U = 2.75 W^{0.5} \quad (10.2)$$

$$F = 0.068 * \text{fecal dry matter, or } 0.03 * \text{dry matter intake in g/day} \quad (10.3)$$

$$S = 0.2 W^{0.65} \quad (10.4)$$

$$G = \text{protein content of body weight gain} \quad (10.5)$$

$$C = 1.136 W^{0.7} \text{ for the last 60 day prepartum} \quad (10.6)$$

$$L = \text{milk in kg/day} * (1.9 + 0.4 * \text{milk fat, \%}) * 10 + 0.068 \text{ CFDM in g/day} \quad (10.7)$$

$$E_p = 0.70 \text{ for baby calves fed only liquid milk } (0.91 * 0.77) \text{ MJ/MJ} \quad (10.8a)$$

$$= 0.65 \text{ for 50 kg calves fed milk and concentrate } (0.87 * 0.75) \text{ MJ/MJ} \quad (10.8b)$$

$$= 0.56 \text{ for 75 kg calves fed mainly concentrates } (0.80 * 0.70) \text{ MJ/MJ} \quad (10.8c)$$

$$= 0.50 \text{ for 100 kg calves fed forages and concentrates } (0.75 * 0.67) \text{ MJ/MJ} \quad (10.8d)$$

$$= 0.47 \text{ for 150 kg cattle fed forages and concentrates } (0.75 * 0.63) \text{ MJ/MJ} \quad (10.8e)$$

$$= 0.45 \text{ for } 200^+ \text{-kg cattle for maintenance and growth } (0.75 * 0.60) \text{ MJ/MJ} \quad (10.8f)$$

$$E_{pl} = 0.52 \text{ for lactation } (0.75 * 0.70) \quad (10.9)$$

Equations used to predict G were developed from several data sets as functions of weight, rate of gain and sex, but were not reported (NRC, 1978).

Energy requirements (NRC, 1978) of lactating cows for maintenance, activity, pregnancy, body weight change and milk, as well as energy contents of feeds, were expressed in terms of net energy for lactation (NE_l ; Moe *et al.*, 1972). This approach was based on observations from calorimetric studies (Armstrong *et al.*, 1964; Flatt *et al.*, 1965; Moe *et al.*, 1972) that indicated ME was used at similar efficiencies for maintenance and milk production in lactating dairy cows. Equations adopted to estimate daily energy requirements were based primarily on the calorimetric data reported by Moe *et al.* (1972) and were as follows:

$$\text{maintenance} = 305 \text{ kJ } NE_l/\text{kg}^{0.75} \quad (10.10)$$

$$\begin{aligned} \text{activity} &= 0.10 * \text{maintenance for cows in stalls or drylot} \\ &\quad + 0.03 * \text{maintenance for each additional kilo-} \\ &\quad \text{meter walked, or maintenance + activity} \\ &= 335 \text{ kJ } NE_l/\text{kg}^{0.75} \end{aligned} \quad (10.11)$$

$$\begin{aligned} \text{pregnancy} &= 100 \text{ kJ } NE_l \text{ during last 4-8 weeks, or} \\ &\quad \text{maintenance + activity + pregnancy} \\ &= 435 \text{ kJ } NE_l/\text{kg}^{0.75} \end{aligned} \quad (10.12)$$

$$\begin{aligned} \text{weight change} &= -20.6 \text{ MJ } NE_l/\text{kg weight loss or } 21.4 \text{ MJ} \\ &\quad NE_l/\text{kg weight gain} \end{aligned} \quad (10.13)$$

$$\text{milk energy (MJ/day)} = (1.46 + 0.402 * \text{milk fat, \%}) * \text{milk (kg/day)} \quad (10.14)$$

In the NRC (1978) the protein and energy requirements, for example, of a 650 kg cow, 60 days postpartum, housed in drylot, producing 45 kg milk of 4% fat per day and fed a typical diet were calculated as follows:

$$U = 2.75 * 650^{0.5} = 70.11 \quad (10.15)$$

$$F = 0.03 * 5990 = 179.7 \quad (10.16)$$

$$S = 0.2 * 650^{0.6} = 9.74 \quad (10.17)$$

$$G = 0 \quad (10.18)$$

$$C = 0 \quad (10.19)$$

$$L = 45 * (1.9 + 0.4 * 4) * 10 + (0.03 * 16760) = 2077.8 \text{ g} \quad (10.20)$$

$$\text{CP required} = (70.11 + 179.7 + 9.74)/0.45 + 2077.8/0.52 = 4572.6 \text{ g/day} \quad (10.21)$$

Energy requirements were calculated as follows:

$$\begin{aligned} \text{NE}_1 \text{ for maintenance and activity} &= 0.335 * 650^{0.75} \\ &= 43.1 \text{ MJ/day} \end{aligned} \quad (10.22)$$

$$\begin{aligned} \text{NE}_1 \text{ for milk} &= (1.46 + 0.40 * 4.0) * 45 \\ &= 138.2 \text{ MJ/day} \end{aligned} \quad (10.23)$$

$$\begin{aligned} \text{Total NE}_1 &= 43.1 + 138.2 \\ &= 181.3 \text{ MJ/day} \end{aligned} \quad (10.24)$$

Predicted feed consumption is 3.5% of body weight or 22.75 kg dry matter of a diet containing 7.2 MJ NE₁/kg. Thus, predicted energy intake was 163.7 MJ/day. As a result, the cow described above would be expected to be in a daily energy deficit of 17.45 MJ/day and lose 0.85 kg body weight/day.

The approach adopted by NRC (1978) was useful for estimating protein and energy requirements of cows. It was recognized, however, that the factorial approach utilized had several inherent assumptions and limitations. The model was static (i.e. a snapshot in time) and, as such, did not address the dynamic nature of protein and energy metabolism in the ruminant. As a result, estimates of requirements were predicted for an animal at a defined weight, physiological state and rate of production in a thermo-neutral and constant production environment.

Because the system was static, nutrient requirements that change rapidly with time, such as those for pregnancy or lactation, were not easily, or accurately, predicted. For example, daily protein and energy requirements for pregnancy in the cow were estimated as 1.136 g/W_{kg}^{0.75} and 100.4 kJ NE₁

or 167 kJ ME/kg^{0.75} during the last 4–8 weeks of pregnancy, respectively. Numerous data are available including those of Winters *et al.* (1942), Brody (1938), Swett *et al.* (1948), Jakobsen (1956), Jakobsen *et al.* (1957), Moe and Tyrrell (1972a), Ferrell *et al.* (1976) and Prior and Laster (1979), which demonstrate that growth of gravid uterine tissues and nutrient requirements for pregnancy increase non-linearly with time after mating. The relationship of ME requirements for pregnancy in cows to day of gestation as determined by Moe and Tyrrell (1972b) and Ferrell *et al.* (1976) is shown graphically in Fig. 10.1, as are estimates of requirements adopted by NRC (1978, 1989).

As can be seen in Fig. 10.1, neither the pattern nor total energy needs for pregnancy were accurately represented by the allowances recommended by NRC (1978).

The system adopted by NRC (1978) implicitly assumed the philosophy of day-by-day equalization of nutrient intake and requirements, thus did not consider long-term effects of plane of nutrition on performance of the dairy cow. The system did not allow for interrelationships among previous or current nutritional status and present or subsequent performance of the animal. As a result, cumulative effects of different feeding regimens, residual or compensatory effects resulting from a previous feeding regimen, or the ability of the cow to buffer nutritional insults were not incorporated. Potential impacts of variables such as these on milk production were shown schematically (Fig. 10.2) by Broster and Broster (1984). These authors argued convincingly that variables such as those indicated above become very important in the development of feeding strategies for dairy cattle. Thus, for example, precalving plane of nutrition and nutritional status of the cow at calving may be expected to influence milk production during the ensuing lactation through compensatory effects and/or through influences on a cow's ability to buffer nutritional insults. In addition to these considerations, means of predicted responses of animals to nutrient intakes less or greater than estimated requirements become a necessity if economically optimal feeding programs are to be developed.

Components (factors) of an animal's protein requirements were considered to be additive. Protein requirements for urinary losses, metabolic fecal nitrogen and scurf protein, and protein requirements for body tissue gain, pregnancy and lactation were considered to be independent of each other. Differences in patterns of amino acids required for different physiological functions were not described. Interrelationships among animals' requirements for protein and energy or other nutrients were not incorporated into the system.

Another conceptual limitation of the NRC (1978) system was that differences among feedstuffs in the chemical and physical form were ignored. Also, the fates of dietary sources of nutrients following ingestion and interrelationships among dietary components during digestion and use of those

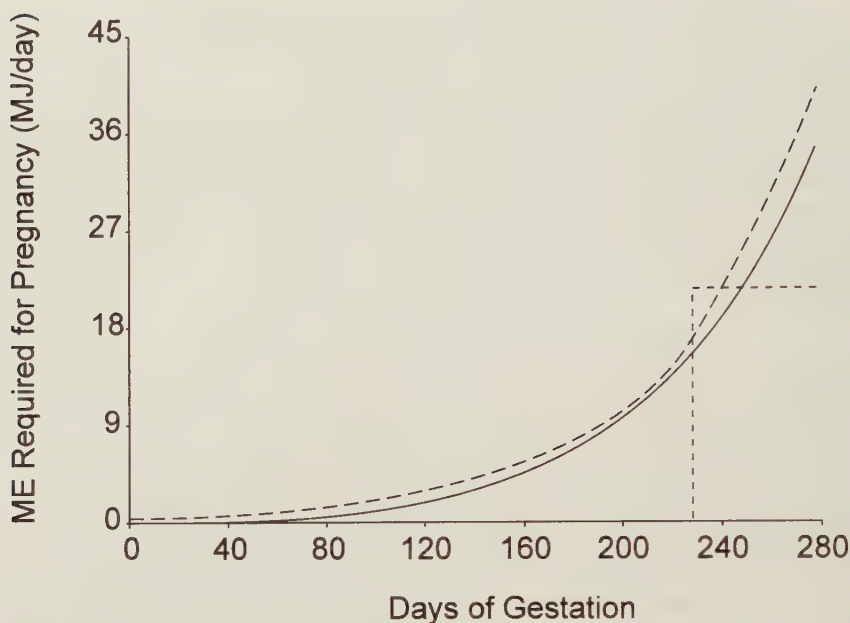


Figure 10.1 Relationship of ME requirements for pregnancy in cows, to days of gestation as determined by Moe and Tyrrell (1972b) (---), Ferrell *et al.* (1976) (—) and NRC (1978, 1979) (- - -) equations with the NRC output being at 90° angle.

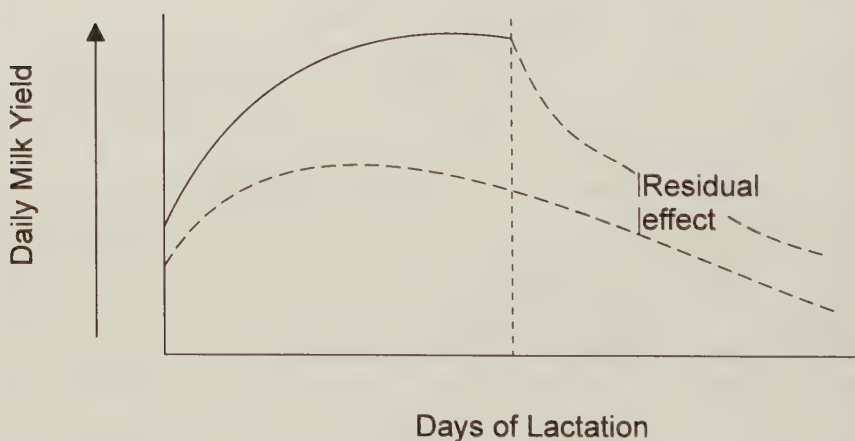


Figure 10.2 Prospective long-term effects of high (—) and low (---) planes of nutrition on milk production of the dairy cow (Broster and Broster, 1984).

components by ruminal microbes were not considered. As a result, known interrelationships between rate and extent of degradability of crude protein in the rumen and rate of availability of energy were not incorporated. Metabolic needs of the rumen microbes for nitrogenous compounds (ammonia, amino acids or peptides) or energy and interrelationships among these, as effectors of microbial fermentation patterns and growth, were not defined. Neither rate nor extent of postruminal digestion of proteins of microbial or dietary origin was addressed, nor was quantity or quality of protein/amino acids available for absorption. A parallel limitation was that influences of chemical and physical form of energy substrates on rate and extent of digestion, or digestion end products, were not defined. Influences of quantities and patterns of nutrients available for absorption on their absorption and use for maintenance or product formation (e.g. composition of body tissue gain or amount and composition of milk produced) were not incorporated into the system.

Several examples of how source or composition of dietary crude protein may influence quantity and quality of protein available for absorption and, hence, animal responses to dietary crude protein, are available (Sniffen and Robinson, 1987). Similarly, observations, such as those of Whitelaw *et al.* (1986), which indicated milk yield and/or milk protein were increased when a 'balanced diet' was supplemented by casein infusion into the abomasum, further demonstrate inadequacies of the system. The NRC (1978) did not incorporate provisions for the milk-protein-depressing-effect of high grain or high fat diets and contained no provisions for optimal use of non-protein nitrogen. Because of these types of inadequacies, relatively simple but economically and biologically important questions could not be answered quantitatively, such as, how much urea can be effectively utilized if included as a dietary component; or to what extent will milk fat or protein percentage be altered if animal fat is included at 6 versus 3% of the diet, or if corn is included at 75 versus 50% of the diet?

10.2.2 NRC (1989) model for lactating dairy cattle

The system for expressing protein and energy requirements of dairy cattle and the values of feeds to meet those requirements adopted by the NRC (1989) retained many of the concepts incorporated into the earlier system (NRC, 1978) and, thus, retained many of the limitations of the former system. The core equations regarding energy requirements were from the previous system (NRC, 1978) with some adjustments of coefficients:

$$\text{Maintenance} = 334.7 \text{ kJ NE}_1/\text{kg}^{0.75} \quad (10.25)$$

$$\text{Pregnancy} = 100.4 \text{ kJ NE}_1/\text{kg}^{0.75}, \text{ after 210 days of pregnancy} \quad (10.26)$$

$$\text{Weight change} = -20.6 \text{ MJ NE}_1/\text{kg weight loss} \quad (10.27)$$

$$= 21.4 \text{ MJ NE}_1/\text{kg weight gain} \quad (10.28)$$

Energy content of milk produced = $(1.47 + 0.4025 * \text{milk fat, \%}) * \text{kg milk}$ (10.29)

Likewise, the general equation for net protein required by dairy cattle was

$$\text{Net protein} = U + S + F + G + C + L + D \quad (10.30)$$

where U , S , F , G , C and L are as previously defined. D was defined as the protein associated with body weight loss. Values for each of these entities were estimated (in g/day) as follows:

$$U = 2.75 W^{0.50} \quad (10.31)$$

$$S = 0.2 W^{0.60} \quad (10.32)$$

$$F = 0.090 * \text{indigestible dry matter} \quad (10.33)$$

$$G = 211 - 26.2 * \text{NE}_g \quad (10.34)$$

$$C = 1.136 W^{0.70} \quad (10.35)$$

$$L = (1.9 + 0.4 * \% \text{ milk fat}) / 100 * \text{milk (kg)} \quad (10.36)$$

$$D = 256 * \text{body weight loss (kg)} \quad (10.37)$$

Required absorbed protein (AP) is calculated as follows:

$$\text{AP} = U/0.67 + S/0.67 + F + G/0.50 + C/0.50 + L/0.70 + D \quad (10.38)$$

As is evident, the concepts employed in describing the absorbed protein required are similar in the 1978 and 1989 systems. NRC (1989) addressed some of the limitations of the former system, however. A series of equations was included in the system to address the relation of ruminal nitrogen use to available energy and differences in the fates of nitrogenous compounds following ingestion. The amount of dietary protein needed as ruminally available protein (RAP) is estimated as a function of energy available in the rumen, as indicated by NE_i . Thus, bacterial crude protein (BCP) and RAP were calculated as follows:

$$\text{BCP} = 6.25(-30.93 + 11.45 \text{NE}_i) \quad (10.39)$$

$$\text{RAP} = \text{BCP}/0.90 \quad (10.40)$$

In addition, required digestible 'bypass' protein (DUP) is calculated as a function of AP and BCP, i.e.

$$\text{DUP} = \text{AP} - \text{BCP}/0.80 \quad (10.41)$$

and undegraded intake protein (UIP) is estimated as follows:

$$\text{UIP} = \text{DUP}/0.80. \quad (10.42)$$

Intake protein (IP) required is then calculated as

$$\text{IP} = (\text{RAP} + \text{UIP})/(1 + 0.15) \text{ and} \quad (10.43)$$

degradable intake protein (DIP) is calculated as

$$\text{DIP} = \text{RAP} - (0.15 \text{ IP}) \quad (10.44)$$

assuming recycled protein, i.e. rumen influx protein is 15% of intake protein. In order to estimate crude protein requirements, NRC (1989) assumes the optimal ratios of UIP and DIP to IP are 0.33 and 0.67, respectively. Excessive UIP and DIP were multiplied by 0.5 and the product is added to IP to estimate crude protein requirements. Requirements for UIP, DIP and crude protein are listed in the tables of requirements; however, only crude protein contents of feedstuffs were listed in the feed tables.

The following example is an estimate of requirements, assuming zero weight change, for the cow described in the section on NRC (1978) and was calculated by the program included with the publication (NRC, 1989):

NE ₁	=	181.6 MJ/day
Crude protein	=	4506 g/day
Degraded intake protein	=	2608 g/day
Undegraded intake protein	=	1589 g/day

Although the system adopted by NRC (1989) does not include equations to predict feed intake, the model calculated that the cow needed to consume 4.0% of her body weight (26.0 kg) of a standard diet (7.07 MJ NE₁/kg) to meet her energy and protein requirements. As an example, a diet consisting of 54.69% corn silage, 40.42% ground corn, 3.11% urea, 0.93% dicalcium phosphate, and 0.86% ground limestone on a dry basis meets all specified requirements. This diet contained 48.40% dry matter and, thus, required an 'as fed' intake of 53.72 kg. This diet is obviously impractical in terms of total dry matter intake, as fed intake and NDF intake, because these limitations are not formally incorporated into the system.

10.2.3 Cornell Net Carbohydrate and Protein System

Fox *et al.* (1988) reported a detailed description of The Cornell Net Carbohydrate and Protein System for Evaluating Cattle Diets. The model described can be generally classified as a static, empirical model of energy and protein requirements of beef and dairy cattle. Several factors are included in the model that influence nutrient requirements and that are not formally included in NRC systems for beef or dairy cattle. These include factors such as temperature, mud and activity. Also, partly because both beef and dairy cattle are included in the system, adjustments for breed or frame size are included.

The equation of Milligan *et al.* (1981) is used in the Cornell model to predict intake of lactating dairy cattle as follows:

$$\text{DMI} = 0.0185 \text{ Wt} + (0.305 * 0.4 + 0.15 * \text{milk fat, \%}) * (\text{milk, kg}) * (\text{temp adj}) \quad (10.45)$$

Temperature adjustments are included as proportions of thermoneutral intake for eight temperature ranges. Other variables are input by the user. This equation yields an estimate of 25.75 kg DMI for the cow described previously. The approach to estimation of energy and protein requirements of cattle conceptually are similar to those of NRC (1978, 1989). Energy and protein requirements are expressed in terms of metabolizable energy (ME) and protein. Energy requirements are calculated as net energy, however, and converted to ME by the use of constants. Basic net energy requirements used are those adopted by the NRC (1989), i.e.

$$NE_m = a_1 * W^{0.75} \text{ where } a_1 = 0.322 \text{ MJ } (W^{0.75} * \text{day})^{-1} \quad (10.46)$$

This estimate is adjusted for grazing activity (AX, which is calculated as a function of grazing area (GU), i.e. $AX = 1.05 + 0.03 \text{ GU}$), breed effect (BE), lactation (L), acclimatization (a_2 , which was calculated as a function of previous temperature, i.e. $a_2 = 0.003 \text{ kJ } (20 - T_p)$) and cold stress (NE_{mcs} , calculated as a function of temperature, wind and moisture relative to the animal's heat production and insulation) or heat stress (NE_{mhs}). Thus, ME required for maintenance was as follows:

$$NE_m = (a_1 + a_2) W^{0.75} (AX) (BE) (L) \quad (10.47)$$

$$ME_m (\text{MJ/day}) = (NE_m + (NE_{mcs} \text{ or } NE_{mhs}))/0.65 \quad (10.48)$$

Metabolizable protein required for maintenance (XP) is calculated as the sum of scurf protein ($2.75 W^{0.5}/0.67$), urinary protein ($0.20 W^{0.5}/0.67$) and metabolic fecal protein ($0.09 * \text{indigestible dry matter}$).

The ME for milk production (LE) is estimated as a function of the amount of milk (MM, kg) and fat percentage of milk (PQ), assuming an efficiency of utilization of ME for milk production of 0.65:

$$LE (\text{MJ/day}) = 0.1 \text{ MM} * (PQ + 3.4)/0.65 \quad (10.49)$$

The metabolizable protein requirement for milk (LP) is calculated by the use of 'energy allowable milk yield' and milk protein percentage, assuming an efficiency of 0.70.

The ME requirement for body weight gain (NG) is estimated as a quadratic function of rate of gain (WG), with adjustments for weight (W) and frame size (FA) and assuming an efficiency of utilization for gain of 0.40 according to the following equation:

$$NG (\text{MJ/day}) = ((0.234 \text{ WG} + 0.000053 \text{ WG}^2) * (W * FA)^{0.75})/0.40 \quad (10.50)$$

Metabolizable protein required for body weight gain (GP) is calculated from protein content of weight gain, assuming an efficiency of 0.60.

In order to estimate metabolizable energy (PE) and protein (WP) requirements for pregnancy, daily weight accretion rates of the fetus, cotyledon, placenta and uterus are estimated by the use of differentiated exponential equations. The resulting daily weight gains are multiplied by constants for

energy or protein for each tissue. The resulting energy and protein gains are adjusted for age of dam and for efficiencies of utilization of energy (0.125) and protein (0.50).

Total ME requirements are estimated as the sum of the previously described requirements plus an adjustment for the extra energetic cost to excrete rumen degraded nitrogen in excess of microbial needs as urea:

$$ME = ME_m + PE + LE + NG + UC \quad (10.51)$$

Likewise total metabolizable protein requirements are estimated as the sum of those needed for maintenance, gain, milk and pregnancy as follows:

$$MP = XP + GP + LP + WP \quad (10.52)$$

In the Cornell model, feed utilization is considered dependent on composition of dietary ingredients, rates of protein and carbohydrate digestion in the rumen, rates of passage of individual feedstuffs, microbial maintenance, maximum microbial yields, rate of ammonia production, intestinal digestibility and environmental conditions (see Chapter 8 for details on digestive elements).

In order to compare estimates of a diet's ability to meet energy and protein requirements of an animal from the Cornell model to those predicted by the NRC (1989) system, the following procedures were used. The cow described above (i.e. 650 kg, 5 year old cow with a body condition score of 5, at 60 days postpartum, having the potential of producing 45.4 kg of milk at 4.0% butterfat) was used as input. The diet calculated to meet the requirements of this cow (NRC, 1989) was also put into the model. The Cornell model predicted that the cow would consume 25.51 kg dry matter, but that both ME and MP of the diet were insufficient to support the indicated rate of milk production. Results from the model indicated that the cow would be able to produce 40.1 kg milk, but would gain 0.17 kg body weight per day when fed that diet. Urea equivalent was predicted to be in excess of bacterial usage, resulting in an extra cost of disposal of 8.12 kJ ME/day. The model predicted daily urea intake was 163 g in excess and rumen degradable true protein and rumen escape protein needed to be increased 0.517 and 2.36 kg, respectively.

In concept, the approaches used to estimate the animal requirements for energy and protein in the Cornell system are similar to those used by NRC (1978, 1989), except that several factors (e.g. breed, frame size, environmental effects) were included as formal adjustments. Therefore, many of the conceptual limitations of the NRC systems are inherent to the Cornell system. The approaches used to predict the ability of a diet to meet an animal's requirements are different. In particular, several mechanistic elements were incorporated for prediction of feed utilization within the gastrointestinal tract (Chapter 8). As a result of the ideas incorporated into these sections of the Cornell model, influences of the chemical and physical characteristics

of carbohydrate, and protein sources on protein availability are accommodated.

10.3 AN ANALYTICAL MODEL OF NUTRIENT TRANSACTIONS DURING LACTATION

Several major problems associated with the sole use of input:output data gathered at the animal level, along with multiple linear regression and other sophisticated statistical techniques for development of empirical static models for optimizing feeding strategies for ruminants, were discussed in Chapter 6. It was also emphasized that relative availabilities of nutrients and patterns of utilization of these for alternative processes can have significant effects upon apparent maintenance requirements and efficiencies of production. In the course of a classical type study of lactation, Canas *et al.* (1976) stated that their objectives

were to investigate the effect of lactation upon the maintenance requirements of rats, to estimate efficiency of milk synthesis in rats, and to evaluate a postulate that the variable maintenance requirements of producing animals are attributable, in part, to changes in relative weights of vital organs.

Using multiple regression analyses of data on a single diet and the model of Brody (1945), they obtained the following solution

$$MP = 799.1 + 0.899 \text{ MEI} + 3.56 \text{ BEL} \quad (10.53)$$

where MP is production of milk in kJ per unit metabolic body size ($\text{BW}_{\text{kg}}^{0.75}$); MEI is intake of metabolizable energy in $\text{kJ}/\text{BW}_{\text{kg}}^{0.75}$; and BEL is loss of body energy in $\text{kJ}/\text{BW}_{\text{kg}}^{0.75}$.

This equation implies that the maintenance requirement of lactating rats is $799.1 \text{ kJ}/\text{BW}_{\text{kg}}^{0.75}$, that the efficiency of conversion of feed ME to milk is 21.5% and that efficiency of conversion of body energy to milk is 85%. This latter efficiency is the same as the efficiency reported by Moe *et al.* (1971) for lactating cows. The implied efficiency of conversion of food to milk of only 21.5% is hard to accept because this efficiency is about 60% in cows (Brody, 1939; Moe *et al.*, 1970). The correlation matrix for the multiple regression analysis was as follows:

	BEL	MP
MEI	0.72	0.84
BEL	—	0.62

The correlation between metabolizable energy intake (MEI) and body energy loss (BEL) was 0.72. These are independent variables in Brody's (1945) equation. Multiple regression analysis depends on no error in meas-

urement of the independent variables and a low correlation between independent variables.

In view of the fact that the correlations were high, Canas *et al.* (1976) concluded that the coefficients obtained using multiple regression techniques were suspect. This led them to develop a more complex analytical model (see below) for analysis of their data. Using this model, Canas *et al.* (1976) tentatively were able to reconcile data they and others had generated in terms of known metabolic and physiological sources of variance in apparent maintenance requirements and productive efficiencies.

10.3.1 Description of an analytical model

The Canas *et al.* (1976) model served as a basis for development of an algebraic analytical model by Taylor (1985). Goals were

to utilize our knowledge of metabolic transactions and available quantitative data as a basis for identification of critical experiments to advance our quantitative understanding of energy metabolism in lactating animals.

Balance models, of necessity and as emphasized in previous and subsequent sections of this book, are factorial in nature. This requires that specific priorities in nutrient use be defined, which is, of course, a major simplification of reality. Absolute priority of one physiological or metabolic process over another does not occur in biological systems. This is a cost or limitation associated with the use of static, factorial models as analytical instruments. This limitation can be overcome by using dynamic models in analyses, but the data and computational requirements can become prohibitive, hence the simplification must sometimes be accepted. In this section, an example investigation of what one can learn or not learn using a simplified, algebraic model of energy transactions in lactating rats will be presented. The study was neither a total failure nor success and, as such, can be used to illustrate a number of concepts. In accordance with the concept of simplicity first and complexity later and as required by model limitations or failures, Taylor (1985) developed the block diagram presented in Fig. 10.3.

Two levels of functional priority were incorporated in model development. The first was that all amino acid transactions are accounted for before those for glucose and those of glucose before fat. The second level involved setting priorities for the use of each nutrient. These priorities are depicted in descending order in Fig. 10.3. For example, metabolic fecal protein is withdrawn from the amino acid pool before casein and body proteins are made. A 'reference' rat was defined to balance the flow of nutrients throughout the system. This rat was formulated based upon data from an energy balance experiment that was characterized by a 11 g loss of body weight, a

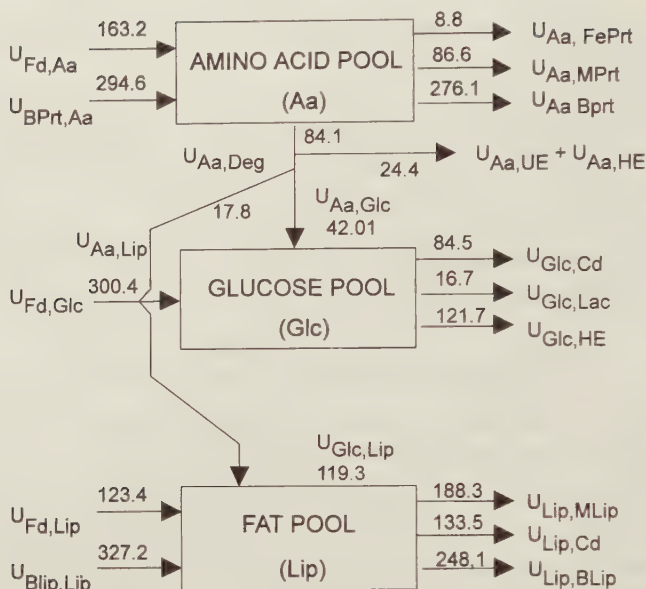


Figure 10.3 Block diagram of energy flow in a reference lactating rat. All numerical values expressed in kJ/day. Codes are feed (Fd), amino acids (Aa), body protein (BPrt), endogenous fecal protein loss (FePrt), milk protein (MPrt), urinary energy (UE), heat energy (HE) losses not associated with substrate oxidation ($U_{xx,Cd}$), glucose (Glc), carbon dioxide (Cd), milk lactose (Lac), lipids (Lip), body lipids (BLip) and milk lipids (MLip).

98.7 kJ/day loss of body energy, intake of a stock diet of 30 g/day and a rate of milk production of 291.6 kJ/day during days 7–14 of lactation (Fig. 10.3).

Inputs to the model are initial body weight, average metabolic body size ((initial body weight + final body weight)/2)^{0.75}, proportions of protein (PFdPrt), fat (PFdLip), glucose (PFdGlc) and starch (PFdSta) in the feed (FD); food intake in g/day (FI); digestion coefficients (DC) for PrT, Lip, Glc and Sta determined for the experimental diet; and heats of combustion for the diet nutrients. Inputs of nutrients from feed are computed as illustrated for protein:

$$U_{Fd,Aa} = FI * PFdPrt * DCPrT * 23.8 \quad (10.54)$$

Apparent digestible energy intake is the sum of $U_{Fd,Aa}$, $U_{Fd,Glc}$ (sum of $U_{Fd,Sta,Glc}$ and $U_{Fd,Glc,Glc}$) and $U_{Fd,Lip}$ minus metabolic fecal nitrogen losses ($U_{Aa,FePrt}$). The equation of Schneider (1935) for daily metabolic fecal nitrogen in mg ($6.79 + 1.33 * FI$ (in g)) was converted to fecal loss in kJ/(day * $W^{0.75}$)

$$U_{Aa,FePrt} = (6.79 + 1.33 * FI) * 6.25 * 23.8 / 0.309 * 1000 \quad (10.55)$$

where 0.309 was the mean $W^{0.75}$ value for Schneider's rats.

Costs of absorption (C_{ABS}) of nutrients were calculated assuming 79.5 kJ of heat released for each high energy phosphate bond utilized (Chapter 5). It was considered that one ATP was used per mole hexose or amino acid absorbed and that four ATP were used in triacylglycerol resynthesis in the intestine. Thus, 0.028, 0.028 and 0.01% of energy in carbohydrate, protein and fat are expended during absorption in the model.

Costs of bond breakage (CBB) of 0.006, 0.006 and 0.001, respectively, for starch, protein and fat hydrolysis were adopted (Chapter 6).

Amino and fatty acids arising from degradation ($U_{\text{BPrt,Aa}}$ and $U_{\text{BLip,Lip}}$) also enter the central nutrient pools. Equations for these energy transactions are as following:

$$U_{\text{BPrt,Aa}} = K1 * 0.7 * \text{IFFW} * 23.8 \text{ kJ} \quad (10.56)$$

$$U_{\text{BLip,Lip}} = K2 * \text{IFATW} * 39.3 \text{ kJ} \quad (10.57)$$

$K1$ and $K2$ can be specified from independent data or treated as unknown parameter values to be solved (see below). Since data on $U_{\text{BPrt,Aa}}$ and $U_{\text{BLip,Lip}}$ were lacking when the model was formulated, values for these rates in the reference condition (Fig. 10.3) must be considered arbitrary.

(a) Amino acid metabolism

In the initial version of the model (Canas, 1974), mass action type equations were used to represent the synthesis of milk components. Solutions were extremely sensitive to diet composition. High protein intakes caused excessive rates of protein synthesis and unacceptable variations in milk composition. These failures led to two decisions. Lactose and milk fat synthesis were made dependent variables calculated from proportion of lactose and fat to protein in rat milk, and a Michaelis-Menten type equation was substituted to compute milk protein synthesis

$$U_{\text{Aa,mPrt}} = V_{\text{Aa,mPrt}} / (1.0 + k_{\text{Aa,mPrt}} / \text{AaMlk}) \quad (10.58)$$

where AaMlk is defined as amino acids available for synthesis calculated as follows:

$$\text{AaMlk} = U_{\text{Fd,Aa}} + U_{\text{BPrt,Aa}} - U_{\text{Aa,FePrt}} \quad (10.59)$$

Equations for lactose and milk fat synthesis then became

$$U_{\text{Glc,Lac}} = 0.191 * U_{\text{Aa,mPrt}} \quad (10.60)$$

and

$$U_{\text{Lip,mLip}} = 32.38 * U_{\text{Aa,mPrt}} \quad (10.61)$$

where the coefficients 0.191 and 32.38 are the energetic ratios of lactose/protein and fat/protein in milk, respectively. This approach improved model behavior, but solutions were still overly sensitive to protein intake and yielded systematic errors. It was decided that this might reflect the fact that

not all amino acids arising from protein degradation enter the central circulation and become available to the mammary glands. This led to calculation of amino acids available for milk protein synthesis as follows:

$$\text{AaMlk} = U_{\text{Fd,Aa}} - U_{\text{Aa,FePrt}} + 0.25 * U_{\text{BPrt,Aa}} \quad (10.62)$$

The arbitrary coefficient (0.25) implies that only 25% of amino acids arising from body protein degradation are potentially available for milk synthesis. $V_{\text{Aa,mPrt}}$ and $k_{\text{Aa,mPrt}}$ can be specified, based upon independent data or become unknown parameters in analyses of experimental data (see below).

Amino acids remaining in the central pool after milk protein synthesis are available for body protein resynthesis. Body protein resynthesis was estimated as follows:

$$U_{\text{Aa,BPrt}} = V_{\text{Aa,BPrt}}(1.0 + k_{\text{Aa,BPrt}}/\text{AaBPrt}) \quad (10.63)$$

where

$$\text{AaBPrt} = U_{\text{Fd,Aa}} + U_{\text{BPrt,Aa}} - U_{\text{Aa,FePrt}} - U_{\text{Aa,mPrt}} \quad (10.64)$$

In the reference rat, body protein resynthesis was set so as to leave amino acids available for degradation and gluconeogenesis.

Amino acids available for degradation and gluconeogenesis ($U_{\text{Aa,Deg}}$) are calculated by difference:

$$U_{\text{Aa,Deg}} = \text{AaBPrt} - U_{\text{Aa,BPrt}} \quad (10.65)$$

Energy in amino acids degraded is partitioned to glucose ($U_{\text{Aa,Glc}}$; 50%), lipid precursors, such as acetate and ketone bodies, converted to fatty acids ($U_{\text{Aa,Lip}}$; 20%), urea ($U_{\text{Aa,UE}}$; 15 %) and heat ($U_{\text{Aa,HE}}$; 14%). This partitioning was based upon the amino acid composition of meat protein and the pathways of amino acid degradation summarized by Krebs (1964), which led to the following stoichiometric relationship:

$$1 \text{ mole Aa} + 1.52 \text{ O}_2 \rightarrow 0.667 \text{ CO}_2 + 1.31 \text{ MJ Glc} + 0.55 \text{ MJ Lip} + 0.40 \text{ MJ urea} + 0.358 \text{ MJ heat} \quad (10.66)$$

(b) Glucose metabolism

Total entry to the glucose pool (TGlcin) is the sum of $U_{\text{Fd,Glc}}$ and $U_{\text{Aa,Glc}}$. The first priority for glucose use is to satisfy minimum requirements for brain and kidney metabolism, etc. The equation for minimum glucose ($U_{\text{Glc,Bas}}$) developed by Canas *et al.* (1976) based upon independent data was adopted.

$$U_{\text{Glc,Bas}} = 110 * W^{0.75} \quad (10.67)$$

Production of lactose ($U_{\text{Glc,Lac}}$) has second priority. This is controlled by $U_{\text{Aa,mPrt}}$ as discussed above. Glucose remaining in the pool is available for oxidation:

$$\text{GlcOx} = \text{TGlcIn} - U_{\text{Glc,Bas}} - U_{\text{Glc,Lac}} \quad (10.68)$$

As was the case for milk and body protein synthesis above, a mass action equation for glucose oxidation was found to be inadequate. Therefore, a Michaelis–Menten type equation was incorporated

$$U_{\text{Glc,Ox}} = V_{\text{Glc,Ox}} / (1.0 + k_{\text{Glc,Ox}} / \text{GlcOx}) \quad (10.69)$$

where $V_{\text{Glc,Ox}}$ is maximum capacity for glucose oxidation and $k_{\text{Glc,Ox}}$ is the apparent affinity of the whole body for glucose oxidation. $V_{\text{Glc,Ox}}$ and $k_{\text{Glc,Ox}}$ are unknown parameter values in the analytical model. For the reference rat, the maximum oxidation rate was set at 83.7 kJ/day above basal glucose requirements. Total glucose oxidized as an energy (ATP) source is

$$U_{\text{Glc,Cd}} = U_{\text{Glc,Bas}} + U_{\text{Glc,Ox}} \quad (10.70)$$

Glucose conversion to heat ($U_{\text{Glc,HE}}$) and lipid ($U_{\text{Glc,Lip}}$) were calculated as energy losses associated with the conversion of amino acids to lipids and lipogenesis from glucose and by difference ($U_{\text{Glc,Lip}}$), respectively.

(c) Lipid metabolism

Entry to the blood lipid pool (TLipin) is as follows:

$$\text{TLipin} = U_{\text{Glc,Lip}} + U_{\text{Fd,Lip}} + U_{\text{BLip,Lip}} \quad (10.71)$$

Milk fat synthesis is calculated from milk protein synthesis, as discussed above. Remaining lipid is partitioned between fat oxidation ($U_{\text{Lip,Cd}}$) and resynthesis of body fat ($U_{\text{Lip,BLip}}$), based upon animal energy expenditures and glucose oxidation as an energy source ($U_{\text{Glc,Cd}}$)

$$U_{\text{Lip,Cd}} = a * W^{0.75} + C_{\text{ABS}} + C_{\text{Aa,mPrt}} + C_{\text{Glc,Lac}} + C_{\text{Lip,mLip}} - U_{\text{Glc,Cd}} \quad (10.72)$$

and

$$U_{\text{Lip,BLip}} = \text{TLipin} - U_{\text{Lip,mLip}} - U_{\text{Lip,Cd}} \quad (10.73)$$

where a is the maintenance coefficient, C_{ABS} is cost of nutrient absorption (discussed above); and $C_{\text{Aa,mPrt}}$, $C_{\text{Glc,Lac}}$ and $C_{\text{Lip,mLip}}$ are costs of mammary synthesis of milk protein, lactose and fat, respectively. The maintenance coefficient a enters the analytical model as an unknown parameter and was set at 464 kJ/day for the reference rat. Costs of milk protein, lactose and fat synthesis were set at 6, 3 and 7 ATP, or 477, 238 and 556 kJ/mole, respectively, for the reference rat, based upon the metabolic pathways involved (Baldwin and Smith, 1974). These can enter the analytical model as unknown parameters, but (see below) model solutions are relatively insensitive to them and, therefore, they cannot be deduced with confidence.

(d) Summary equations

The remaining equations in the analytical model are simple summations of the operative equations. Heat losses in digestion and assimilation, costs of production, gross and net efficiencies of conversion of diet and body energy to milk, maintenance requirements, losses of body fat and lean, milk production and metabolizable energy intake are outputs by the model.

10.3.2 Model characterization

General model behavior and characteristics, sensitivity analyses and investigations of the analytical values of additional measurements will be discussed in this section.

Conventional wisdom holds that when model outputs can only be compared with three experimental estimates, no more than three unknown parameter values can be estimated from data sets from multiple diets or intakes ($N = 6$) or four with multiple diets and intakes ($N = 18$ and $N = 36$) (Baldwin and Smith, 1983). Therefore, a goal in this modeling study was to identify additional experimental measurements that could be made in connection with energy balance experiments and enable unique definition of additional unknown parameter values.

(a) Behavior and sensitivity analyses

Evaluations of general behavior and characterization of the model were achieved through comparison of model outputs with data from the energy balance experiments reported by Canas *et al.* (1976) and Taylor *et al.* (1986). Approximate values for unknown parameter values were specified and model outputs compared with experimental data. Equation forms were altered until model response patterns were comparable with observed responses and unknown parameter values were adjusted until quantitative relationships agreed well with experimental data. After model behavior was found to be adequate, sensitivity analyses were undertaken to examine relationships of unknown parameter values to model outputs. Each unknown parameter was varied systematically around the values specified for the reference condition. Insignificant changes in model outputs in response to changes in parameter values were taken as indications that model outputs are insensitive to that parameter and, as a consequence, the model could not be used to deduce that parameter value. Conversely, if varying a parameter altered outputs, the model was considered sensitive to that parameter, with the conclusion that energy balance data could be used to estimate a more exact value.

The analytical model exhibited expected responses to diet changes. Increases in dietary protein resulted in decreases in body protein loss and an increase in heat from gluconeogenesis (Fig. 10.4a and b), in agreement with

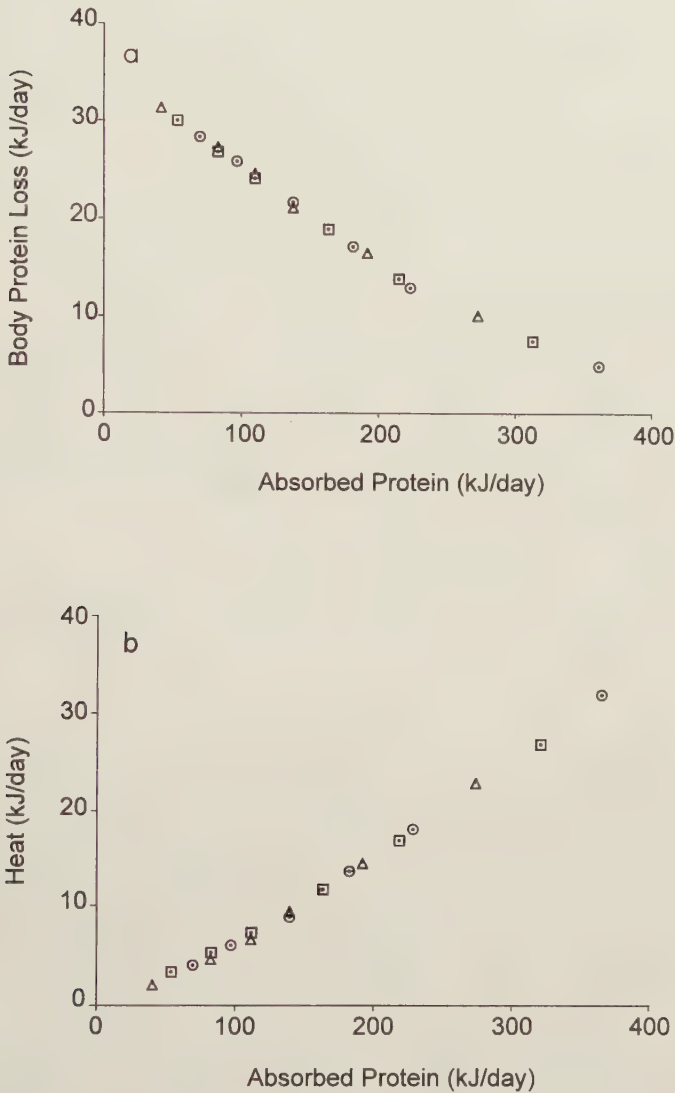


Figure 10.4 (a) Model solutions showing loss of body protein in lactating rats versus absorbed protein. Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet A(Δ) was 12% protein, 2% fat and 76% carbohydrate; diet B($\blacktriangle$) was 12% protein, 14% fat and 49% carbohydrate; diet C($\square$) was 24% protein, 2% fat and 60% carbohydrate; diet D($\blacksquare$) was 24% protein, 2% fat and 32% carbohydrate; diet E($\circ$) was 40% protein, 14% fat and 32% carbohydrate; and diet K($\bullet$) was 40% protein, 14% fat and 10% carbohydrate. (b) Model solutions showing an increase in heat losses associated with gluconeogenesis as amount of absorbed protein increases for lactating rats. Diet codes are as described above.

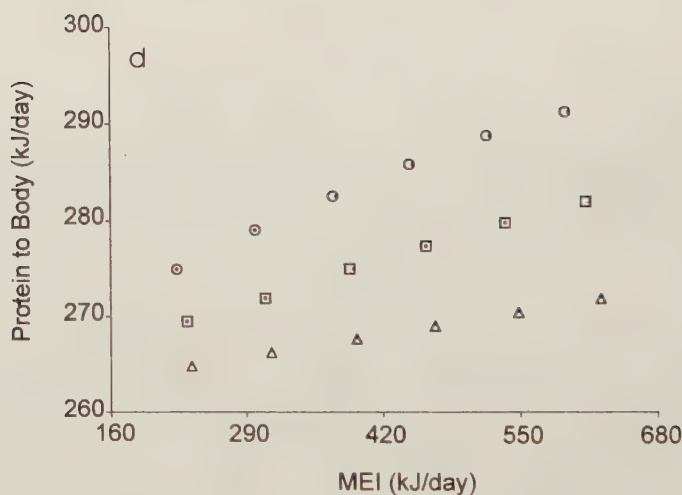
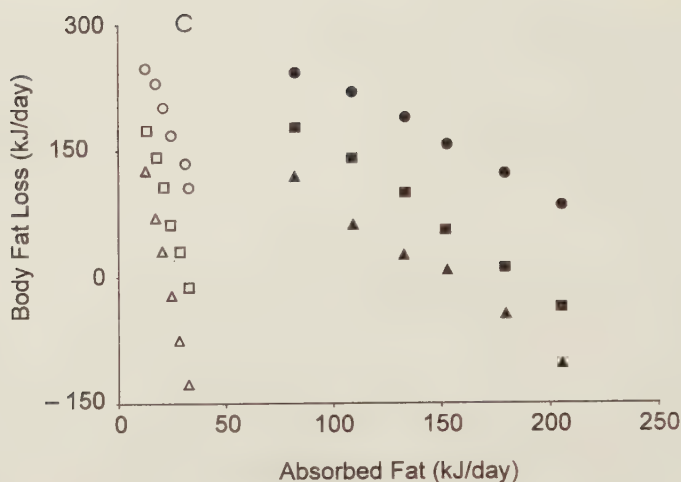


Figure 10.4 (c) Model solutions showing loss of body fat in lactating rats versus absorbed fat. Negative body fat loss is gain in body fat. Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes are as described in legend for Fig.10.4(a). (d) Relationship between body protein resynthesis and metabolizable energy intake in lactating rats. Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes as described in legend for Fig.10.4(a).

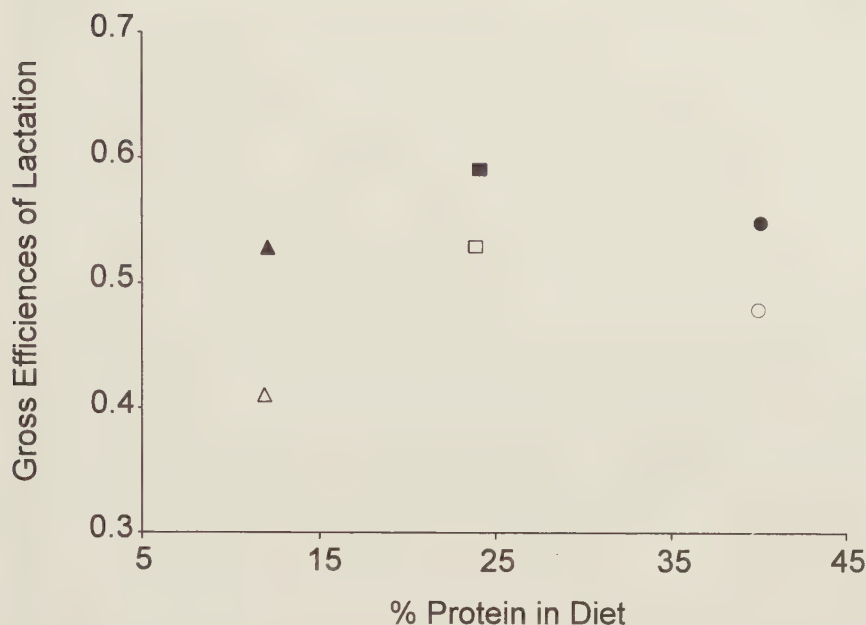


Figure 10.5 Average gross efficiencies of lactating rats from the energy balance experiment of Taylor *et al.* (1986). Diet codes are as described in legend for Fig.10.4(a)

Radcliffe and Webster (1978) and Hartsook *et al.* (1973), respectively. Loss of body fat is very sensitive to dietary fat and food intake (Fig. 10.4c). Low fat diets (diets A, C and E) led to the greatest losses of body fat at all intakes. As percentages of protein or fat in the diet increased, intake effects decreased consistent with the findings of Canas *et al.* (1976; Fig. 10.4d). Increases in MEI resulted in increases in body protein and fat synthesis (results not shown), fat oxidation decreased, and glucose oxidation increased as MEI increased (results not shown). Respiratory quotients increased for all diets as MEI increased. Theoretically, oxidation of only fat, carbohydrate or protein yield respective RQ values of 0.707, 1.0 and 0.83 (Kleiber, 1961). Model-predicted respiratory quotients were in agreement with this, declining as protein and fat increased in the diet. Milk production increased with increasing energy availability.

Gross efficiency of milk production increased as percentage of dietary protein increased. However, at high protein intakes, efficiencies decreased, presumably due to increases in heat losses in gluconeogenesis as protein energy intake increased (data not shown). Gross efficiencies of milk production in rats in the energy balance experiment of Taylor *et al.* (1986) followed that pattern, with gross efficiency being greatest for animals on the 24% protein diets (Fig. 10.5). Model-predicted results did not show this

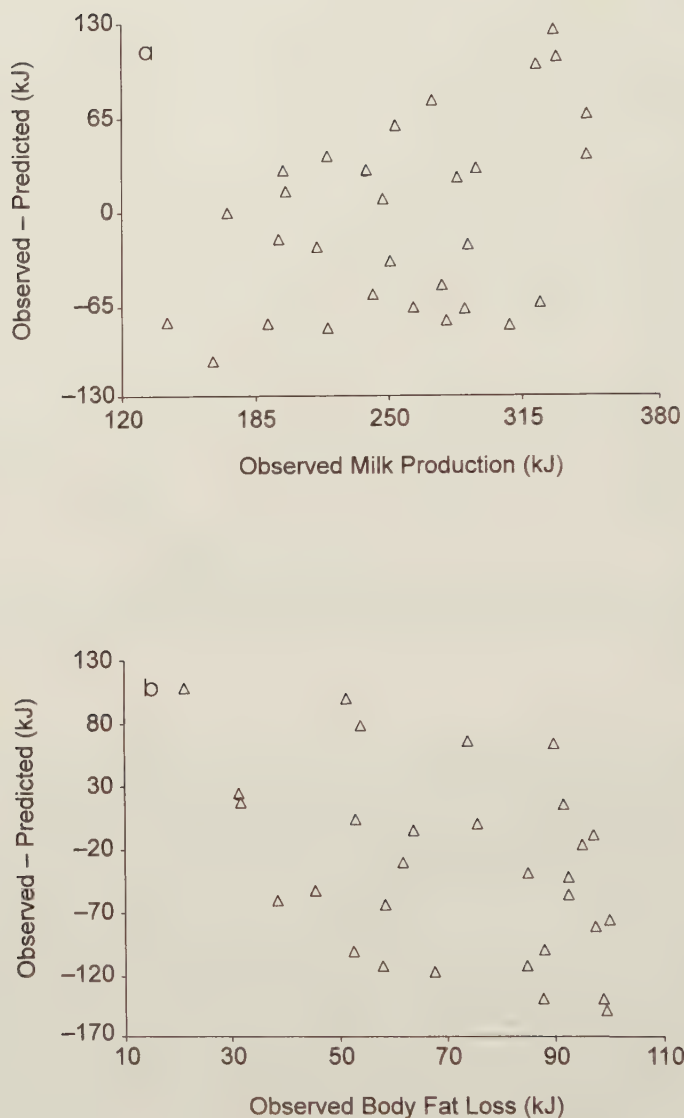


Figure 10.6 (a) Residual plot of milk production (NEp: kJ/day). Observed results are from lactating rats discussed previously (see Taylor *et al.* 1986) and predicted results are exact solutions of model. Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes are as described in legend for Fig. 10.4(a). (b) Residual plot of body fat loss (kcfat: kJ/day). Observed results are from lactating rats (see Taylor *et al.*, 1986) and predicted results are exact solutions of model. Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes are as described in legend for Fig 10.4(a).

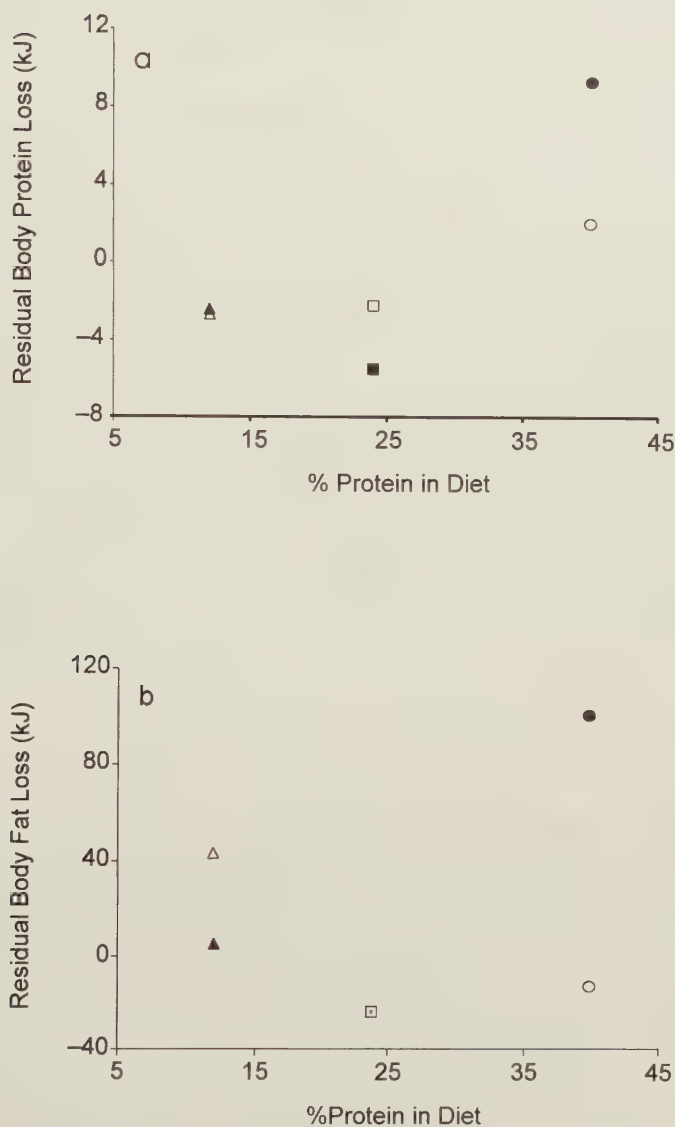


Figure 10.7 (a) Residual plot for average residual body protein loss (kcp_{rtl}). Residual is calculated by subtracting exact solution of model from data from lactating rats (see Taylor *et al.*, 1986). Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes are as described in legend for Fig 10.4(a). (b) Residual plot for average body fat loss (kcf_{atl}). Residual is calculated by subtracting exact solution of model from data from lactating rats (see Taylor *et al.*, 1986). Food intakes were 15, 20, 25, 30, 35 and 40 g/day. Diet codes are as described in legend for Fig 10.4(a).

curvilinear response. This behavior of the model is due to the heavy dependence of the model upon protein utilization and flow. Independent estimates of unknown parameter values that define protein utilization are required to correct this problem.

Residual analyses were used to compare (reference) model outputs with the experimental data of Taylor *et al.* (1986). In these, observed (experimental) results were compared with differences between observed and predicted results (Fig. 10.6 and 10.7, panels a and b). Experimental data considered included milk production and loss of body lean and fat energy. When averaged across all diets, model outputs agreed quite well with experimental data. For example, mean residuals for protein losses, fat losses and milk production were + 1.2, + 1.9 and - 0.2%, respectively, of experimental values. However, when residual values for the six diets fed at six intakes were plotted vs. observed milk production (Fig. 10.6a) and body fat losses (Fig. 10.6b), considerable scatter and significant bias were evident. Some, but not all, of the scatter in the residual values could be attributed to experimental variance, indicated by the standard errors of estimates reported by Taylor *et al.* (1986). Bias attributable to a failure of the model to correctly simulate effects of changes in dietary protein is illustrated in Fig. 10.7. At this point, it appeared that the equations which comprise the model behaved appropriately in terms of directions of observed responses, but

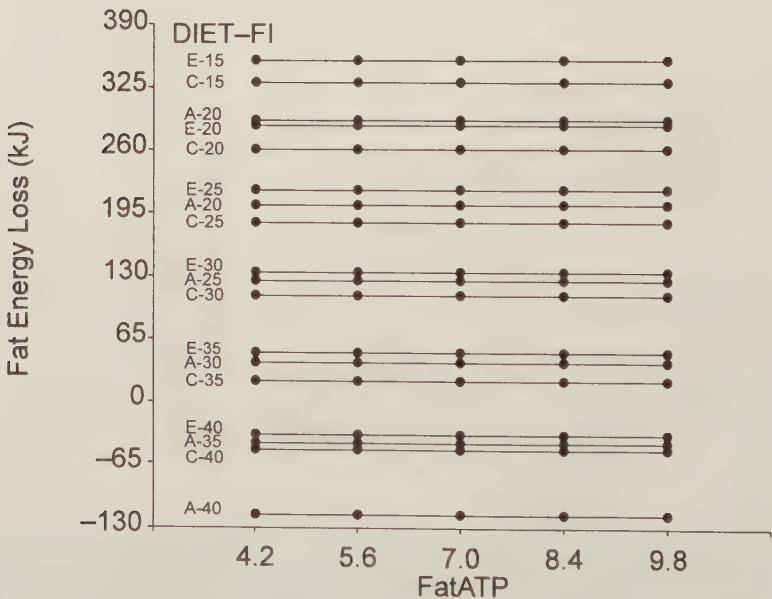


Figure 10.8 Representative results of sensitivity analyses. Relationship between number of ATP molecules specified as required for triacylglycerol synthesis and body fat loss for diets varying in protein (A = 12%, C = 24%, E = 40%) fed at varying feed intakes (FI) in grams per day.

some of the assigned parameter values were clearly incorrect. This preliminary assessment led us to undertake sensitivity analyses.

(b) Sensitivity analyses

Responses of the model to sensitivity analyses are presented in Table 10.1. Model solutions were not sensitive to changes in specified ATP requirements for protein (PrtATP), lactose (LacATP) and fatty acid (FatATP) synthesis, $V_{\text{Glc,Cd}}$, $k_{\text{Glc,Cd}}$ and k_2 . This means that data from classical energy balance studies cannot be used to estimate these unknown parameter values. Thus, they must be defined by independent data.

Unknown parameter values in the model are listed in the first column of Table 10.1. Solutions for three diets (A, C and E, see legend to Fig. 10.4) at six intakes (40, 35, 30, 25, 20 and 15 g/day) indicated that solutions were sensitive to changes in seven parameters and insensitive to six. Energy expenditures expressed as number of high energy phosphate bonds (ATP) required for peptide synthesis (PrtATP), lactose synthesis (LacATP) and fatty acid esterification (FatATP) are small relative to total energy transactions and, as a result, changes in these have only minor effects on model outputs, as shown for FatATP in Fig. 10.8. Rational changes in parameter values defining glucose metabolism ($k_{\text{Glc,Cd}}$, $V_{\text{Glc,Cd}}$) and body fat degradation (k_2) yielded similar patterns. These parameter values cannot be estimated from data from classical energy balance experiments. They must be defined on the basis of independent data or estimated based upon additional measurements made in connection with an energy balance experiment.

Example results of responses to changing parameters are presented in Fig. 10.9(a and b). Generally, when model outputs can only be compared with three experimental estimates, no more than two unknown parameter values can be estimated from data on one diet fed at one intake, three with multiple diets or intakes, or four with multiple diets and intakes. This implies that only three or four of the seven parameters to which model outputs are sensitive can be estimated from input:output data. This is also clear when one considers interrelationships among some of these parameter values. Values for $k_{\text{Aa,mPrt}}$ and $V_{\text{Aa,mPrt}}$, $k_{\text{Aa,BPrt}}$ and $V_{\text{Aa,BPrt}}$, and $k_{\text{Glc,Cd}}$ and $V_{\text{Glc,Cd}}$ appear in the same respective equations. One of each of the pairs must be defined on the basis of additional observations before the other parameter of the pair can be deduced from energy balance data. Similarly, efficiency of lipid synthesis (LipEff) and the maintenance coefficient (MCoef) are collinear variables, because as each increases body fat energy losses increase, indicating that one of these must be defined by additional data. Identification of critical experiments and measurements for unique definition of the unknown parameter values thus became the next step in this modeling analysis.

Table 10.1 Evaluation of model sensitivity to changing parameter values^a

<i>Parameter</i>	<i>Range tested</i>	<i>Effect upon model output</i>		
		<i>protein loss (kJ)</i>	<i>fat loss (kJ)</i>	<i>milk production (kJ)</i>
K ₁	0.15-0.35	-196.65 - +79.50	-209.20 - +376.56	-133.88 - +481.16
K ₂	0.25-0.6	0	0	0
K _{Aa,mPrt}	12-30	6.28 - 43.93	-251.04 - +502.08	-167.36 - +690.36
V _{Aa,mPrt}	25-55	2.09 - 33.47	-376.56 - +334.72	-92.05 - +564.84
K _{Aa,BPrt}	10-22	-138.07 - -41.84	-175.73 - +393.30	0
V _{Aa,BPrt}	35-85	-121.34 - +146.44	-334.72 - +334.72	0
k _{Glc,Ox}	45-105	0	0	0
V _{Glc,Ox}	12-28	0	0	0
LipEff	0.4-0.1	0	-271.96 - +271.96	0
MCoeff	80-180	0	-330.54 - +330.54	0
PrtATP	3-9	0	0	0
LacATP	2-6	0	0	0
FatATP	4-10	0	0	0

^a Parameter value range varied as described in text.

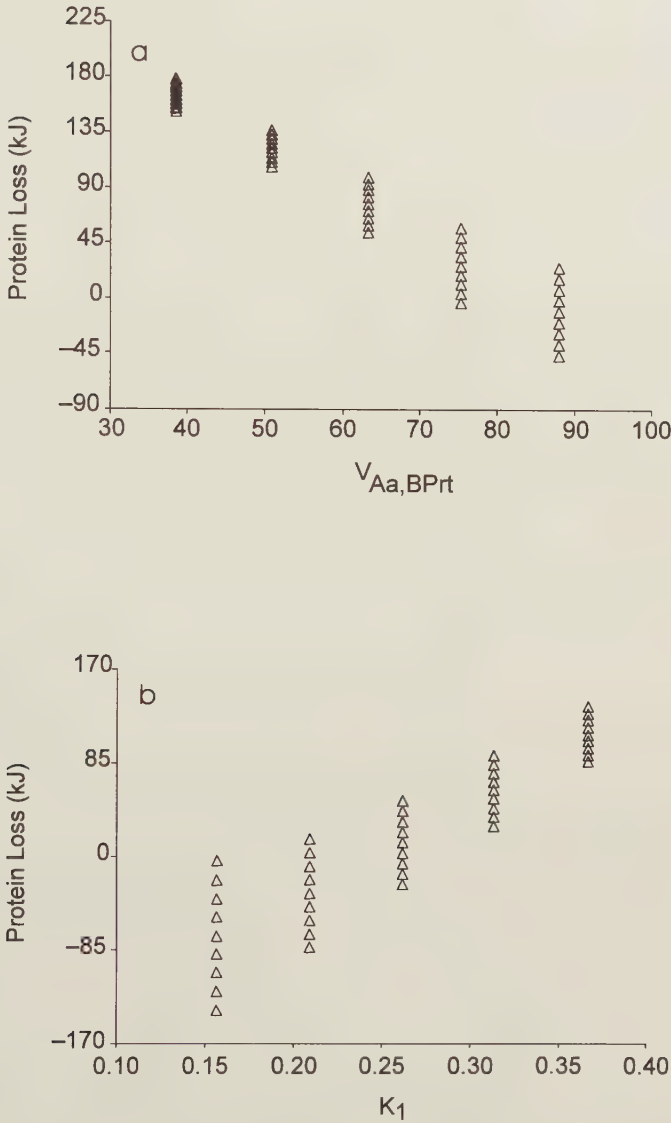


Figure 10.9 (a) Results of sensitivity analysis of response of model output body protein loss to changing unknown parameter value $V_{Aa,BPrt}$. (b) Results of sensitivity analysis of response of model output body protein loss to changing unknown parameter value K_1 .

Table 10.2 Results of iterative runs

<i>N</i>	Parameters defined by milk production, protein loss, fat loss	Parameters defined by milk production, protein loss, fat loss, body protein resynthesis, glucose transfer quotient, glucose oxidation rate, respiratory quotient, and gross efficiency
6	LipEff, $V_{Aa,mPrt}$, $V_{Aa,Bprt}$	LipEff, $V_{Aa,mPrt}$, $k_{Aa,mPrt}$, $V_{Aa,Bprt}$, $k_{Aa,Bprt}$, K_2 , MCoef ^d
18	LipEff, $V_{Aa,mPrt}$, $k_{Aa,mPrt}$, $V_{Aa,Bprt}$, $k_{Aa,Bprt}$, MCoef, K_1	LipEff, $V_{Aa,mPrt}$, $k_{Aa,mPrt}$, $V_{Aa,Bprt}$, $k_{Aa,Bprt}$, K_2 , MCoef, $k_{Glc,Ox}$, $V_{Glc,Ox}$, K_e (3) ^e
36	LipEff, $V_{Aa,mPrt}$, $k_{Aa,mPrt}$, $V_{Aa,Bprt}$, $k_{Aa,Bprt}$, MCoef, K_1 , $k_{Glc,Ox}$, $V_{Glc,Ox}$	all listed above (5)

^a Number in parentheses is number of parameters that can be deduced.

^{b,c} Parameters which are in the same equation cannot be solved together.

^d Any two can be solved together.

^e K_1 and K_2 cannot be deduced together.

(c) Identification of critical experiments

The first stage of the analysis undertaken was creation of a data set for a standard energy balance experiment using the model with the default parameter values described above. The data sets created were for three diets at one ($N = 3$) and two intakes ($N = 6$), six diets at three intakes ($N = 18$), and six diets at six intakes ($N = 36$). Then, using arbitrary starting values and an iterative parameter deduction routine and the data sets, attempts were made to deduce the parameter values used to create the data sets.

Results of iterative runs of the model against data comparable to usual energy balance experiments are shown in Table 10.2. Efficiency of lipid synthesis (LipEff) could be estimated at all times with $N = 6$, $N = 18$ or $N = 36$. $V_{Aa,mPrt}$, $V_{Aa,BPrt}$, MCoef or K_1 could be estimated when multiple diets and intakes were used ($N = 18$ or $N = 36$). When parameter values were related, deductive power was reduced. Parameters that are used in the same equation cannot be deduced together ($V_{Aa,BPrt}$ and $k_{Aa,BPrt}$ or $V_{Aa,mPrt}$ and $k_{Aa,mPrt}$).

The next phase of the study was identification of additional experimental measurements that would help uniquely define additional unknown parameter values. These measurements are not normally made in connection with energy balance experiments. However, their inclusion could be important to furthering our understanding of the system.

Iterative runs of the model were again undertaken with the addition of predicted results for added measurements. Of the many added measurements evaluated, those found to aid in 'identification' of the model were rate of body protein synthesis ($U_{Aa,BPrt}$); the glucose transfer quotient or proportion of CO_2 from glucose (fGlc); glucose oxidation rate ($U_{Glc,Cd}$); and the respiratory quotient (RQ). All of these measurements can be made in connection with energy balance experiments.

Estimates of rates of protein synthesis increased the deductive power of the model, which allowed estimation of $V_{Aa,mPrt}$, $k_{Aa,mPrt}$, $V_{Aa,BPrt}$ and $k_{Aa,BPrt}$ with data from multiple diets and intakes ($N = 6$). When the $N = 6$ data set was used, these unknown parameters could be uniquely defined singly and then deduced as pairs. Unknown parameter values in the same equation could be uniquely defined together when rates of protein synthesis were measured. Protein degradation rate was not defined with confidence. It appeared that K_1 was very sensitive to intake. These effects must be better defined in the model equations. Experimental measurement of muscle protein degradation during different periods of lactation in rats fed diets varying in protein, fat and carbohydrate content and at various levels of intake, as well as total body turnover estimates, would increase confidence in estimates of K_1 .

It was thought that $U_{Glc,Cd}$ and fGlc would add power for deduction of unknown parameters that define glucose metabolism in the model. These

additional measurements did not allow resolution of $V_{\text{Glc,Ox}}$ and $k_{\text{Glc,Ox}}$ with minimum data sets ($N = 6$). Increasing the data ($N = 18$) allowed these measurements to support unique solutions for both unknown parameters. The iterative runs could uniquely define $V_{\text{Glc,Ox}}$ and $k_{\text{Glc,Ox}}$ and three other unknown parameter values.

Addition of RQ estimates to the data set did not aid in resolution of parameters that define glucose metabolism. However, they did allow deduction of k_2 and MCoeff with confidence ($N = 6$). K_1 and K_2 could not be solved simultaneously with any of the additional measurements in the largest data set ($N = 36$). At least one of these must be independently defined. MCoeff could be solved by the addition of either RQ or gross efficiency to the data set. These would be easy unknown parameters to estimate directly in future work.

The unknown parameters that define energy expenditures in peptide bond formation, lactose synthesis and fatty acid esterification cannot be defined with confidence. Additional measurements did not aid in their resolution. Since the energy involved in these transactions is small relative to total energy expenditure in the model and to experimental error, theoretical estimates must be considered sufficient for their definition. This algebraic model of rat metabolism was selected for discussion, because it illustrates a process in which the use of a static, factorial, mixed empirical/mechanistic model to help explore/estimate factors involved in nutrient partitioning and parameter values was investigated. The results were as follows:

1. Several measurements required for identification of the model are mutually exclusive, e.g. use of multiple ^{14}C -labeled substrates. Therefore, not all unknowns can be defined in a single experiment.
2. Large numbers of animals, diets and intakes are required, and, even then, systematic errors could not be eliminated because some 'constants' change, for example, with intake and, thus, must be made dependent variables.

Results of the analysis clearly indicated that dynamic interactions must be accommodated to eliminate systematic errors. In addition, concepts and data from studies of underlying mechanisms must be utilized to advance our ability to analyze or predict energy balance relationships in lactating animals.

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Dynamic models of ruminant digestion

with J. R. Knapp

There is nothing wrong with making an assumption, a process that in a way is a hallmark of creativity and scientific intuition.

J. W. Czerkawski (1986)

11.1 INTRODUCTION

Most dynamic models discussed in this chapter were constructed to evaluate current data and concepts regarding ruminant digestion and metabolism, and identify critical research needs. Therefore, emphasis will be placed on research needs identified and the interplay between modeling and experimental aspects of a research program.

In Chapter 3, the concept that model development should proceed from the simple to the complex, with increasing complexity forced by failures of the model, was emphasized. The examples used herein were selected in part to illustrate this concept (with some notable deviations) and to represent the evolution of dynamic models of rumen digestion supported by an interplay between experimental and modeling research.

The model of Waldo *et al.* (1972) was based upon concepts advanced by Blaxter *et al.* (1956), as was discussed in Chapter 4. Therefore, it will not be discussed here, even though these were the earliest models published that introduced concepts regarding mechanistic and dynamic aspects of digestion in the rumen.

11.2 EARLY DYNAMIC MODELS

The first detailed mechanistic and dynamic model that we know of was the model of Baldwin *et al.* (1970). The stated modeling objectives were to illustrate the utility and flexibility of using mathematical models in support of research and to summarize a number of ruminant digestive and metabolic

energetic relationships. The model was written in the KINSYM user-oriented language (Chapter 3) and all equations were mass action in form (equations 3.2, 3.5 and 3.14). A major contribution to arise from the study was an updating of stoichiometric relationships for fermentation and microbial growth, which were adopted, with modifications discussed in Chapter 8, in most subsequent models of ruminant digestion. Several key provisions in the model were quite similar to those in some current models.

Three composite microbial groups were defined: a cellulolytic group (A), which encompassed known fermentation and growth properties of *Ruminococcus flavefaciens*, *Ruminococcus albus* and *Bacteriodes* (now *Fibrobacter*) *succinogenes*, plus methanogenic species; an amylolytic complex (B); and a complex (C), which included species with weak cellulolytic and very active saccharolytic activities such as *Bacteriodes rumenicola* and several *Butyrivibrio* species. Group A microbes utilized ammonia as a nitrogen source for growth, while groups B and C used ammonia and amino acids. Limitations in nitrogen availability could disrupt microbial growth. Group C microbes compete for hydrolytic products from holocellulose. This accommodated the presence of high numbers of group C microbes on high fiber diets. Based on the view that rumen microbes utilize and ferment chemicals rather than conventionally defined fiber, protein and nitrogen-free extract, description of dietary inputs to the model in chemical terms was considered necessary. Potentially digestible holocellulose, soluble carbohydrate, starch, protein and non-protein nitrogen expressed as urea were chosen to describe dietary input. Based upon application of similar reasoning to the animal, rates of production of individual volatile fatty acids (VFA) were traced. Insoluble substrates were separated into large (slow passage rate) and small (readily passed) particle pools. A number of additional factors such as rates of protein and starch solubilization were considered but were not incorporated because of a lack of data.

Mazinov and Nolan (1976) and Nolan (1975) developed and illustrated the use of a dynamic, analytical model of nitrogen metabolism in sheep. Their basic objective was to enable the interpretation and integration of ^{15}N isotope dilution experiments, in which tracers were introduced at different sites; this helped to formulate a general assessment of nitrogen metabolism in the whole animal. The submodel of nitrogen metabolism in the reticulo-rumen, omasum and abomasum (Fig. 11.1) was premised on the view that quantitative assessments of the following are necessary (Nolan, 1975):

1. The quantities of soluble and insoluble protein and non-protein nitrogen from the diet and of endogenous urea and other endogenous nitrogen compounds, and their contribution to the total pool of nitrogen compounds available for fermentation in the rumen;
2. The extent to which these nitrogen compounds are degraded to simpler compounds (i.e. peptides, amino acids and ammonia) or pass undegraded from the rumen;

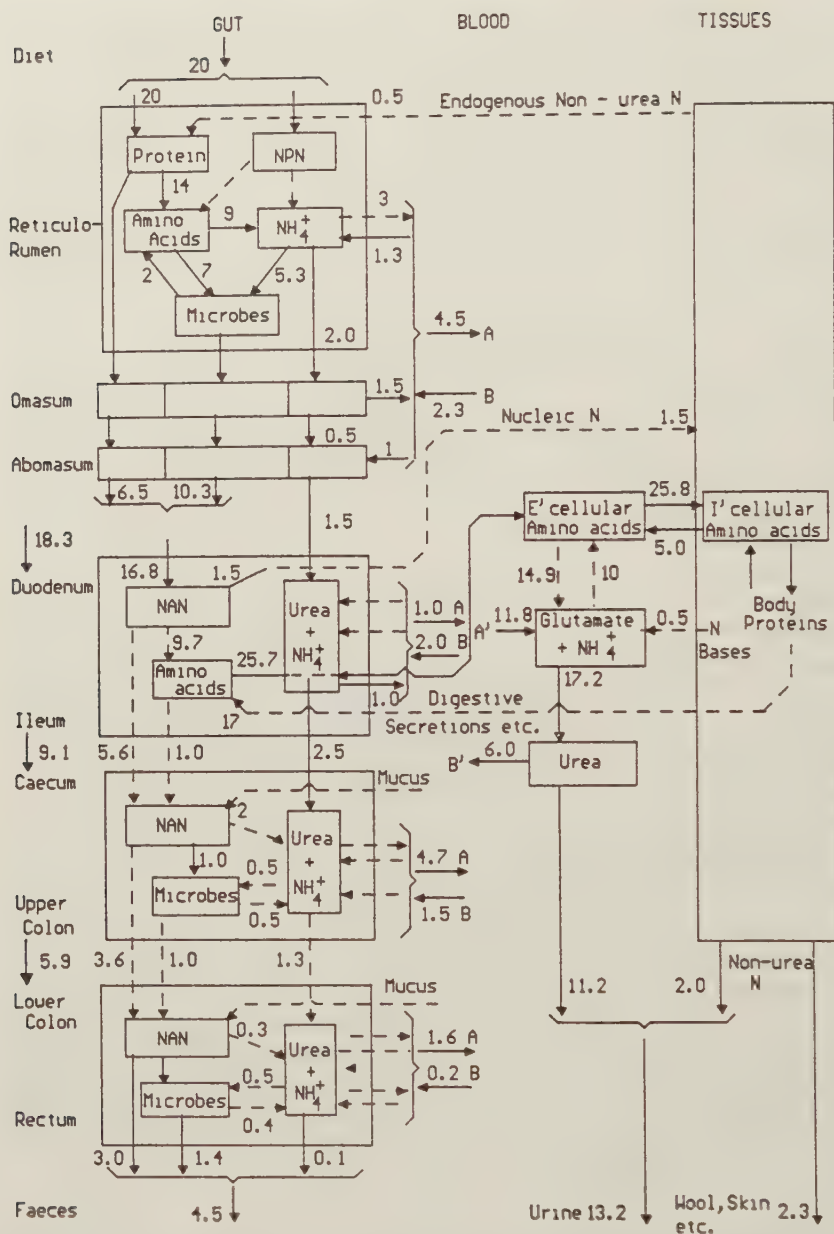


Figure 11.1 A quantitative, whole-animal model of nitrogen transactions in the sheep. Values (g N/day) are derived from various sources and, at the present time, are considered to be 'best available' estimates for sheep fed 800 g/day of lucerne chaff. A' represents the total uptake of ammonia from the gut and is made up of individual pathways, each labeled A. B' represents the total urea degradation in the gut and is made up of individual pathways, each labeled B. () pathways that appear to be quantitatively well understood, (---) more tentative pathways.

3. The extent to which peptides, amino acids, ammonia and nucleic acids are assimilated by bacteria and protozoa, and the net rate of efflux of microbial nitrogen to the small intestine;
4. The quantities of soluble nitrogen compounds absorbed through the walls of the forestomachs or their efflux in water;
5. The quantities of nitrogen recycled through pools within the rumen itself (e.g. as a result of ingestion of other microorganisms by protozoa and lysis of bacteria).

Further studies indicated that nitrogen exchange required consideration of transactions in the lower digestive tract and in the animal. Critical elements of Fig. 11.1 include separation of diet inputs into protein and NPN; explicit consideration of amino acid as well as ammonia use for microbial growth; nitrogen digestion and secretion into the gastrointestinal tract (GIT) at a number of sites; urea recycling to the GIT; turnover of extracellular and intracellular amino acids, as well as body protein; and nitrogen excretion in urine and feces. Equations used in the model were essentially mass action with lags to allow the fitting of experimental data. A very important aspect of this work was that the experimental approach of administering ^{15}N compounds at multiple sites enabled 'identification' of components of the model and computations of flux relationships among pools, as indicated by the numerical values incorporated into the model (Fig. 11.1). The experimental approach utilized by Nolan (1975) was expensive and laborious. Also, the model does not have provisions for linking fermentation rates with microbial growth and nitrogen metabolism. Perhaps these are reasons why the approach has not been expanded upon in terms of species and diets, even though most current models of nitrogen metabolism contain the same, and more, state variables. This is quite unfortunate, since most later models have been parameterized using indirect data that do not often identify the model as well as do data collected by Nolan and his colleagues 25 years ago.

Baldwin *et al.* (1977) presented a research model of ruminant digestion for use in evaluating factors that influence nutritive value. Specific objectives were the following, as quoted from Baldwin *et al.* (1977):

1. Development of a quantitative and dynamic model of the ruminant digestive process based on currently accepted and defensible concepts;
2. Identification of specific aspects of ruminant digestion where current generally accepted concepts and/or data are inadequate;
3. Development of a model that could be used to test hypotheses regarding factors affecting nutritive value.

Again quoting Baldwin *et al.* (1977), for this purpose, such a model should be comprised of mechanistic rather than empirical equations and include the following features:

1. Applicability to all ruminant diets and feeding regimens with no changes in basic functional forms (equations), rate constants, or parameter values other than those required to describe feed input and the physiological status of the animal;
2. Accommodation of current concepts regarding physical, chemical, biochemical, physiological and microbiological factors that affect nutritive value and interactions amongst these;
3. Manageability in terms of size, i.e. fulfil the objective without incorporating non-essential elements or detail;
4. Outputs in a form comparable with a wide range of experimental data.

and incorporate the following concepts:

1. The relative rates of fermentation in and passage from the rumen are central determinants of extent of digestion in the rumen, partition of digestion between the stomach and intestine, and amounts and patterns of nutrients absorbed by the animal, i.e. the rumen dominates digestion in a determinant fashion;
2. Both the physical and chemical properties of feedstuffs influence rates and patterns of rumen availability, utilization and passage of nutrients;
3. Microbes fulfil an essential, integrative and variable role in ruminant digestion;
4. Interaction between the animal and digestive processes strongly influence digestion rates and patterns;
5. Estimates of most rate constants and other essential numerical inputs can be obtained or deduced from experimental data with animals fed frequently (so as to simulate a steady-state system);
6. Stoichiometric relationships in rumen fermentation can be calculated based upon available knowledge of metabolic pathways.

The bases for these concepts are self evident and have been discussed in many comprehensive reviews (Hungate, 1966; Phillipson, 1970; McDonald and Warner, 1975; Russell and Hespell, 1981; Baldwin and Allison, 1983; Dobson and Dobson, 1988).

The first concept simply suggests that rumen function should be emphasized in the model. The second dictates that feed components be defined in terms of solubility, particle size and chemical composition. Twelve groups of chemical components were selected for explicit consideration, based upon chemical and physical properties and utilization by different groups of rumen microbes (Table 11.1). Also, definition of these separate chemical entities is essential to estimate energetic and stoichiometric relationships in accord with the first law of thermodynamics and maintenance of carbon and redox balances, a feature not accommodated by higher levels of aggregation of chemical inputs. For example, combining soluble sugars and organic acids, starch and pectin, and hemicellulose and cellulose as single entities does not satisfy this criterion, because chemical compositions (C, H

Table 11.1 Specification and coding of feed input and composition

<i>Component</i>	<i>Letter codes</i>	<i>Heat of combustion (Mcal/kg)</i>	<i>mMW^a</i>	<i>p^d</i>
Feed	FEED, FD, F	—	—	—
Organic matter	OM	—	—	0.901
Soluble carbohydrates	SC	16.48	0.162	0.071
Organic acids	OA	10.50	0.134	0.060
Starch	ST, SST ^b	17.36	0.162	0.040
Pectin	PEC, SPEC ^b	15.06	0.187	0.095
Hemicellulose	HC, HC1, HC2, HC3 ^c	17.82	0.132	0.092
Cellulose	CEL, C1, C2, C3 ^c	17.36	0.162	0.232
Lipids	LIP, TG, LCFA	35.14	0.885	0.043
Soluble proteins	SP, SAA	23.85	0.115	0.100
Insoluble proteins	IP	23.85	0.115	0.100
Nonprotein nitrogen	SN	23.85	0.322	0.025
Lignin	LIG, LIG1, LIG2, LIG3	34.73	—	0.063
Ash	ASH, SASH, ASH1, ASH2, ASH3	—	—	0.099

^aMolecular weights used in model for chemical components.

^bPrefix S as in SST codes for soluble hydrolytic products and soluble ash.

^cSuffixes 1, 2 and 3 as in HC1, HC2 and HC3 code for particles differing in size and/or physical characteristics. In general, particle size decreases as the numerical value of the suffix increases. Thus, HC1 codes large particle hemicellulose and HC3 codes hydrolytic products of hemicellulose. Physical damage of plants due to chewing and rumination, resulting in increased availability, is also accommodated in this notation.

^dProportions (g/g DM) of organic matter and each chemical component in the lucerne chaff used as reference diet in constructing the model.

and O), microbial specificities and fermentation patterns of these can differ significantly.

The third concept requires that microbes be represented explicitly in the model. Microbes were divided into two groups – FREE in rumen fluids and BOUND or associated with particulate, insoluble substrates. FREE microbes pass with fluids from the rumen and microbes bound to small particles pass with these particles; microbes bound to large particles do not pass from the rumen. FREE microbes ferment soluble nutrients, while bound microbes ferment hydrolytic products formed from insoluble substrates and grow in association with the insoluble substrate fermented. These provisions allow specification of separate sets of stoichiometric coefficients for soluble nutrients, starch, pectin, hemicellulose, cellulose and protein (see Chapter 8). Growth of microbes in association with insoluble substrates also produces an appropriate lag in their hydrolysis and fermentation.

Concept four requires that animal functions such as rumination, salivation, etc., be represented. Concept five essentially restricts the data set used

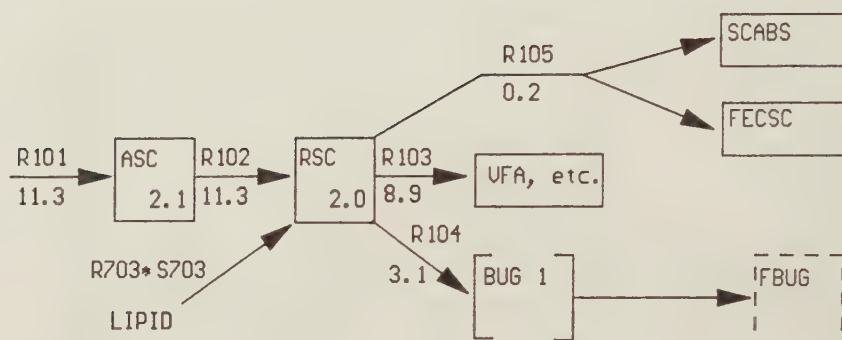


Figure 11.2 Block diagram of the soluble carbohydrate (SC) sub-unit. Entities enclosed in square boxes such as ACS and RSC are state variables. Numerical values in boxes are initial pool sizes expressed in mmole. These also equal steady-state pool sizes when the reference lucerne diet is fed continuously at the rate of 37.9 g DM/h. Reactions or transformations are identified by arrows and R101, R102, etc. Rates of these reactions in mmole/h are presented below the arrows. Brackets such as those surrounding BUG1 identify results of intermediate calculations. Entities enclosed in square boxes of dashed lines identify state variables accounted in other sub-units of the model. Entities enclosed in rectangular boxes identify outputs of the model which are usually computed only each simulated day. Coding conventions are defined in Table 11.1 and its footnotes. The prefixes are defined as follows: A_ means available, B_ is small particle pool, BS_ is hydrolytic products, LG_ is lower gut, FEC_ is excreted, FBUG is free microbial pool, BUG# is microbes formed during fermentation, BUGR# is initial microbial colonization, BUG1 is the grams of microbes formed from each substrate, R_ is rumen fluid; SCABS is the absorption of soluble carbohydrate from the intestine and VFA is volatile fatty acids.

in model development to continuously fed sheep (actually fed only one diet, alfalfa, at one intake). This allows all other diets, intakes and feeding patterns to be used in model evaluations. The final concept accepts stoichiometric coefficients from rumen balance models discussed previously (Chapter 8).

Three basic block diagrams were used to describe soluble nutrients (SC, OA, SP, SAA, SN); insoluble, rapidly available nutrients (ST, PEC, LIP); and insoluble, particulate material (HC, CEL, IP, LIG). The block diagram for soluble carbohydrates, SC, is presented in Fig. 11.2 as an example of the first

group. SC enters the rumen (reaction R101) and is available for digestion (ASC), after solubilization (R102), to become rumen-soluble carbohydrates (RSC). SC can also be formed from glycerol, which is formed as an end product of microbial lipid hydrolysis (R703 * S703). RSC have three fates: passage to the lower digestive tract (R105) and absorption by the animal (SCABS) or elimination in feces (FECSC); fermentation to VFA (R103); or incorporation into microbes (R104).

Pectin (PEC) metabolism is represented in Fig 11.3 as an example of the second group of nutrients. Pectin differs from SC in that it is less soluble and associated with particulate material. Dietary pectin (APEC) must be reduced in size by microbial action (R402) or rumination (R409/M4) to form BPEC, which is either hydrolyzed (R406) or passes to the lower gut (R405). Again, the hydrolytic products (BSPEC) can be fermented (R407) or incorporated into microbes (R408). Thus, the second group of nutrients differ from the first in that they must be solubilized before hydrolysis and fermentation.

Metabolism of insoluble, particulate nutrients is represented by hemicellulose in Fig. 11.4. Dietary hemicellulose (AHC) enters a large (HC1) or a small (HC2) particle size pool. HC2 is more available to microbes than HC1 and can more easily become associated with microbes (BHC2). The other fate of HC2 is passage to the lower gut (LGHC). The large particle hemicellulose (HC1) also becomes associated with microbes (BHC1) and a reduction in particle size to BHC2 can occur via rumination (R512) or microbial action (R513). However, HC1 cannot pass to the lower gut unless it is reduced in particle size to HC2. BHC3 represents the hydrolytic products of hemicellulose degradation which can be fermented to VFA (R516) or incorporated into microbes (R517).

Several concepts regarding microbial growth were introduced into this model. The equation for microbial growth was given as the commonly known function of ATP availability and YATP (yield of microbial cells/mole ATP). Additionally, the concept of ATP use for microbial maintenance (ATPM) was introduced. Also, dependence of microbial growth rates and yields on amino acid availability as derived from Maeng and Baldwin (1976) and Maeng *et al.* (1976) was incorporated. Thirdly, an equation describing a limitation of microbial growth by low ammonia concentrations was formulated, based on the data of Hogan (1975).

The model was coded in the CSMP user language. Equations for nutrient transactions were all mass action in nature. Most parameter values incorporated into the model were estimated using a static model, based upon data from studies of 40 kg sheep fed 910 g DM/day of a lucerne diet in equal amounts at hourly intervals. This was considered the reference base for simulations of the steady-state condition used in initial stages of model evaluation (debugging, etc.). Many of the data used were unpublished at the time but were reported separately. Essential aspects of these data are depicted in Figs 11.2–11.4 and tables discussed in subsequent chapters. In

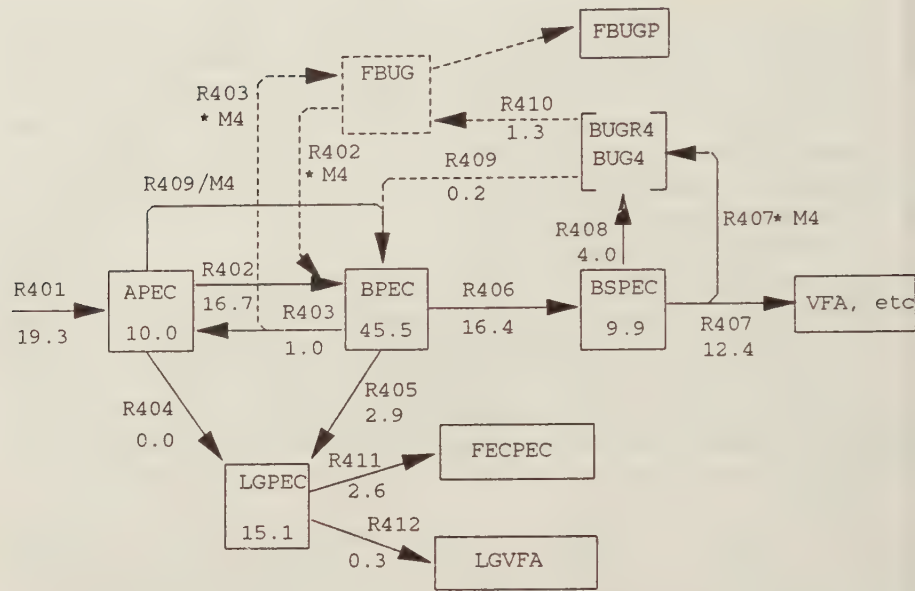


Figure 11.3 Block diagram of the pectin subunit (PEC). Coding and representation conventions are presented in Table 11.1, Table 11.1 footnotes, Fig. 11.2 legend and as follows; FBUGC is free bug passage.

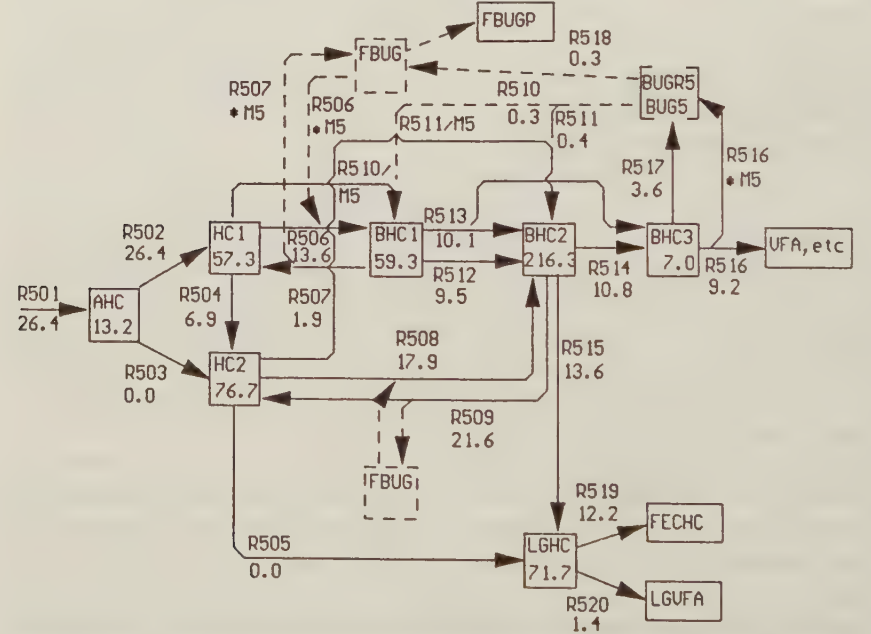


Figure 11.4 Block diagram of the hemicellulose subunit (HC, HC1, HC2 and HC3). Coding and representation conventions are presented in Table 11.1, Table 11.1 footnotes, Fig. 11.2 legend.

addition, relevant data from Hogan and Weston (1967), Hungate *et al.* (1971), Thomson *et al.* (1972) and Egan *et al.* (1975) were considered in estimating some parameter values. The reference lucerne diet had an OM apparent digestibility of 65% and produced rumen DM contents of approximately 480 g, and water and DM passage rates of 4.5–5.0 l and 500–550 g/day, respectively. Approximately 60% of particulate DM in the rumen was in the small particle pool, i.e. less than 1.0 mm (Ulyatt *et al.*, 1976).

Numerical inputs descriptive of stoichiometric relationships of fermentation and microbial growth were calculated according to concept six from above and are similar to those reported by Baldwin *et al.* (1970), Reichl and Baldwin (1975), Koong *et al.* (1975) and Baldwin *et al.* (1977). Molecular weights and heats of combustion used in the model were obtained from standard references. Given the constraints inherent to the use of a very specific data set to parameterize the model and the almost exclusive use of mechanistic elements, evaluations of the model and the concepts that comprise it were quite promising (Baldwin *et al.*, 1977; Murphy *et al.*, 1986). However, a number of specific failures of the model, including an inability to simulate the digestion of poor (straw) and very high (concentrate) quality diets, helped identify several critical limitations in then current data and concepts:

1. Water dynamics including salivation;
2. Factors involved in particle size reduction and passage;
3. Effects of diet and pH on patterns of VFA formed from the several chemical constituents in the diet (see Chapters 8 and 16);
4. Nitrogen metabolism in the rumen and exchange between the rumen and the animal.

Several improvements were incorporated into the rumen model (Murphy *et al.*, 1986) after it was described in detail (Baldwin *et al.*, 1977). These changes were undertaken to correct specific omissions and weaknesses in the model and were based, largely, on experimental data collected in response to conceptual and data problems identified in the course of the first modeling analysis. These will be discussed briefly by way of characterizing important model elements.

In the first version of the model, dry matter was distributed among three pools – soluble and large and small particle pools. When the digestion of low-quality forages was simulated, rumen digestion coefficients for hemicellulose and cellulose were systematically underestimated, because residence time in the rumen was too short with only two particle-size pools. Studies of particle-size distribution and passage (Reid *et al.*, unpublished) provided the data required for implementation of a third, intermediate-size particle pool which, although hydrated and more available to microbes than were large particles, was too large to pass from the rumen. The inter-

mediate pool size concept was introduced into the hemicellulose and cellulose subunits.

When the original model was constructed, data on rumination patterns in continuously fed sheep were not available. Waghorn and Reid collected data (unpublished), which along with previous data, allowed analyses (Murphy *et al.*, 1982) leading to formulation of an empirical equation for rumination that was incorporated into the model. The first term in the equation recognized the effect of cell-wall content upon rumination; a second term reflected a cosine-like pattern of rumination evident in the data. The periodicity and amplitude of the cosine-like rumination pattern varies systematically with feeding frequency (Murphy *et al.*, 1982).

A major problem with the initial model was correct simulation of rumen ammonia concentrations across diets and feeding frequencies. Baldwin and Denham (1979) examined several hypotheses regarding this inadequacy. Basically, they found that rate of insoluble protein hydrolysis and degradation was the most sensitive variable affecting rumen ammonia nitrogen. Representation of this was improved by explicit recognition of large and small particle pools for insoluble protein and addition of a provision that only microbes closely associated with small particles hydrolyze insoluble protein. This allowed accommodation of effects of rumen fill, passage and rumination and the concept of colonization. These changes improved model behavior markedly. However, effects of feeding frequency were still not simulated adequately. For example, predictions of rumen ammonia nitrogen in animals fed once daily differed by as much as 50% from experimental estimates at the same time of day. If the rate constant for ammonia absorption from the rumen deduced from continuous feeding data were adjusted, once-a-day feeding was adequately simulated. However, this type of change was not compatible with concept five above. These observations imply that factor(s) other than those recognized, at that time, influence protein hydrolysis and ammonia absorption.

Two additional improvements in the model are worthy of note. The first was addition of rumen volatile fatty acid concentrations as dynamic variables. The early version estimated total production per day, but not rumen concentrations over time. This addition, along with incorporation of the improved stoichiometric parameters developed using the balance model (Chapter 8), greatly expanded the number of literature data that could be used to challenge the model.

The final improvement was numerical. In construction of the first model, data needed for estimation of a number of rate constants were very limited and those data sets available were often quite variable. A premise in modeling is that experimental data are correct. When data available are limited and variable, the modeler is tempted to select the datum that works best and can never be certain whether s/he has resisted this temptation. Mechanistic models are complex in explicitly expressing causal as compared to empirical relationships. If the computation of numerical inputs – rate con-

stants and pool sizes – is not firmly based on experimental data, the model can become non-unique, i.e. when a model is complex with many equations and numerical inputs, many combinations of numerical inputs can produce satisfactory outputs. Adequate experimental data are required to prevent this. When confidence regarding uniqueness is low, the model and evaluations and conclusions derived therefrom are suspect. The very complete study of Ulyatt *et al.* (1984) helped satisfy this need for the Murphy *et al.* (1986) revision of the rumen model. All numerical inputs to the model were evaluated carefully based upon these data and adjustments, where required, were made; there were very few in fact. One could then believe that when the model behaved well, the result reflected the adequacy of the conceptual frame upon which it was based. Conversely, when the model failed, this could be taken as an indication that the conceptual frame was inadequate rather than a problem with non-unique parameter values.

As noted above, concepts regarding the dynamics of nitrogen metabolism in the rumen required improvement. Other aspects that required further study were water dynamics, which are poorly represented in most current models, particle size reduction through chewing, rumination and other factors, and factors influencing passage rates across species.

The Baldwin *et al.* (1977) model of rumen digestion was essentially a third-generation model. It was firmly based on sound data and concepts and simulated data for a wide range of diets and feeding frequencies in a very satisfactory fashion. It can fail and the specific nature of the failure usually identifies an aspect of digestion not fully understood.

At the same time that Baldwin *et al.* (1977) were working on the model discussed above, Black *et al.* (1981) were also developing a dynamic model of ruminant digestion. Their objective was to integrate current concepts of processes occurring in the rumen in equation forms compatible with their existing model of growth (Black *et al.*, 1981) and allow the prediction of growth and production in the whole animal. A flow diagram for the model is presented in Fig. 11.5. This model, although more aggregated than that of Baldwin *et al.* (1977), captured many of the same key concepts, including nitrogen recycling from the animal to the rumen via saliva, microbial maintenance requirements, use of amino acids and ammonia as sources of nitrogen for microbial growth, independent consideration of soluble and insoluble substrates, etc. Major differences were uses of empirically deduced equations, estimates of potentially degradable protein and α - and β -hexose polymers, and rates of hydrolysis as inputs to the model. For example, plasma urea nitrogen (PLASUN) was estimated from urinary nitrogen excretion (UN) using the regression equation

$$\text{PLASUN} = 0.49 + 1.33 \text{ UN} - 0.019 \text{ UN}^2 \quad (11.1)$$

while the fractional rate of degradation of β -hexose (BHXDK in h^{-1}) was estimated using the equation

and form, these two models provided valuable insights regarding the types of data and additional inquiry needed to advance our understanding of quantitative and dynamic aspects of ruminant digestive function.

11.3 CURRENT DYNAMIC MODELS

Three recent models were selected as representative of current, mechanistic and dynamic models of ruminant digestion. The first model was published by Danfaer (1990). This was selected because the objective was to develop a research model that could eventually evolve into a model for practical use in animal agriculture. The second model evolved from the Baldwin *et al.* (1987) study and was initially devised to formulate nutrient inputs to a lactating cow model; it has since been improved in a number of regards. The final model selected was that of Dijkstra *et al.* (1992) and Neal *et al.* (1992). These models share a number of common attributes and in this illustrate a convergence that appears to be taking place and may indicate future directions in the evolution of dynamic models of ruminant digestion. To a significant extent, the three models trace their origins to the analysis of France *et al.* (1982), entitled *A Mathematical Model of the Rumen*. All three models have been described in detail (Danfaer, 1990; Dijkstra *et al.*, 1992; and Chapter 16 and 17 of this book) and thus, are available for use and comparison by all interested workers. Evaluation criteria differed in the three cases, but all of these are relevant and reveal important limitations in our current understanding of digestion in the ruminant.

A very important departure from previous approaches evident in all three of these models is that most of the equations used exhibit saturation or Michaelis–Menten type kinetics, rather than the first-order, mass action arguments used previously. There are a number of good reasons for the departure. First, almost all metabolic systems are saturable such that when substrate availability is high, the system approaches a maximum reaction velocity. Also, as nutrient availabilities vary over a physiological range, patterns of nutrient use can vary in a fashion not adequately simulated using mass action kinetic equations. Another important reason is that models based upon equations that exhibit saturation equations are more stable or robust in their behavior when numerical methods are used to solve large equation sets. We have clearly accepted these advantages. However, a major disadvantage arises that will often be very difficult to address. This problem is parameter estimation. In early models, and indeed, in formulation of parameter estimates for these three models, a static model of flux rates observed in a reference, steady-state system was utilized heavily. This is adequate and appropriate to parameterizing a series of mass action equations, since for

$$R_{i,j} = k_{i,j} * A_i \quad (11.3)$$

the rate R_{ij} and the pool size of A_i are usually known and calculation of k_{ij} is simple algebra. In contrast, when

$$R_{ij} = V_{ij} / (1.0 + k_{ij} / cA_i) \quad (11.4)$$

an additional data fact is required, because we have two data facts (R_{ij} and cA_i ; rate and concentration) and two unknowns (V_{ij} and k_{ij} ; $V_{\max}$ and apparent affinity constant), either an additional data fact or an assumption is required to parameterize the equation. Common assumptions that can often be defended in general terms are that V_{ij} is very high relative to observed rates and need not be specified accurately (solutions insensitive to this parameter) or that k_{ij} and cA_i are about equal under standard conditions. However, such assumptions cannot always be invoked and assumptions must always be explicitly stated. We are not pointing this out as a criticism of the newer models, although all three of the models contain parameter estimates which can be challenged on these grounds, but rather to emphasize that further evolution of these models toward the goal of general applicability will require generation of new types of data – kinetic data in addition to steady-state data. In presentation of all three of these models, discussions of parameterization and types of data required are included. Further, selection of critical data required will become more and more dependent upon behavioral and sensitivity analyses using current, continuously evolving models.

A very troubling area of parameter estimation is detailing feed inputs to the models. In all three models, these inputs are based upon chemical and physical properties of the feedstuffs. There are notable differences in aggregation of inputs between the three models, though. The most detailed feed composition inputs are required by the Baldwin *et al.* model (Chapter 16). A drawback of this approach is that such complete analyses of the diet or feedstuffs utilized in a given diet are rarely available. Often, the information is not provided for diets fed in experiments that are used to parameterize or challenge the model. At times, the lack of data on diet composition has prevented the simulation and use of critical experimental data. However, tables of feed composition and the expertise of nutritionists can be used to estimate the required dietary inputs. This dilemma points to one area of needed research – more extensive, detailed analyses of feedstuffs. The model of Baldwin *et al.* (Chapter 16) is sensitive to these inputs. Dijkstra *et al.* (1992) also noted that data on diet composition affects the accuracy of model simulations.

The models of Danfaer (1990) and Dijkstra *et al.* (1992) utilize feed fractions that are composites of several chemical entities. For example, Danfaer (1990) combines organic acids, pectin, lactate and starch into a fermentable sugars pool, and hemicellulose and cellulose into a fermentable cell-wall carbohydrate pool. Dijkstra *et al.* (1992) combine hemicellulose, cellulose and lignin into a neutral detergent fiber (NDF) pool. By adopting this approach, these modelers have eliminated the possibility of implementing

carbon and redox balances and accounting for energetic expenditures/relationships.

Additionally, Danfaer (1990) and Dijkstra *et al.* (1992) define fermentable/degradable and unfermentable/undegradable fractions of protein, starch and NDF. These inputs are set based upon *in vitro* fermentation rates and *in vivo* degradation rates of feeds in nylon bags. By utilizing such data to set the input data, consideration of important biological processes that contribute significantly to (explainable) variance in the digestion process is avoided. In this sense, the models become partially self-fulfilling and dependent upon the accuracy of data used to define inputs on digestibility or degradability that can be suspect. Systematic errors associated with *in vitro*- and *in sacco*-based estimates of *in vivo* measurements of rumen digestion are well known. Model elements that accommodate microbial growth and passage effects (hopefully) correct these systematic errors, but further evaluations are required to assure that this is true.

In utilizing parameters that can be adjusted to accommodate the given output of a biological situation, the modelers are identifying areas where the current chemical and/or physical parameters are inadequate to define the reactions and relationships occurring in ruminant digestion. Also, while it is recognized that deficiencies exist in all three models with respect to predicting nutrient outflow from the rumen and, ultimately, nutrient availability to the animal, these deficiencies give emphasis and direction as to where research and modeling efforts should be concentrated.

Dijkstra *et al.* (1992) identified several areas in earlier models 'as being insufficiently represented'. These included 'aspects relating to microbial recycling, microbial substrate preference, uncoupled microbial fermentation, effect of pH on microbial activity and VFA and ammonia absorption' and 'variation in microbial chemical composition, as influenced by microbial species and nutrient availability'.

The concept of recycling microbial matter was captured by Dijkstra *et al.* (1992) by including model elements defining protozoa as a subfraction of the amylolytic microbial pool. Inclusion of protozoa in the model allows for microbial matter recycling within the rumen by protozoa engulfment. It also provides for equations describing the retention and lysis of protozoa, lactate metabolism by protozoa and effects of pH on protozoal growth.

To address substrate preferences, Dijkstra *et al.* (1992) represented the microbial population as two pools, an amylolytic pool, whose substrates are starch, water soluble carbohydrates and lactate, and a fibrolytic pool, whose substrates are structural carbohydrates. This is very similar to the strategy used by Baldwin *et al.* (1987; Chapter 16). By including substrate specificity of microbes, inappropriate and excessive fermentation of structural carbohydrates is prevented under conditions of high starch fermentation rates in these rumen models. Problems of parameterization associated with more than one microbial pool are discussed in Chapter 16.

Effects of pH on cellulose and hemicellulose hydrolysis were imple-

mented by Argyle and Baldwin (1988) by modifying the rate constant for these reactions in a linear manner below pH 6.2 (Chapter 16). The authors recognized that the equation should be sigmoidal in form, but insufficient data were available at that time to parameterize such an equation. In addition to pH effects on microbial action of degradable fiber (Fd), Dijkstra *et al.* (1992) also implemented pH effects on protozoa and absorption of VFA and ammonia by modifying the maximal velocity of the respective equations as a function of pH minimum and period of time below a set pH.

It has long been recognized that the proportion of polysaccharides in microbes is variable (Hespell and Bryant, 1979; Storm and Orskov, 1983; Czerkawski, 1986). This concept is captured in Dijkstra *et al.* (1992) by allowing storage polysaccharides (As) to vary as a function of the concentration of hexose available to amylolytic microbes (Ha) and amylolytic microbes themselves (Ma). This is an important concept and represents a potential advance in current models. Unfortunately, it is difficult to tell how they parameterized the equation. In the amylolytic storage polysaccharides section they state that 'rate constants involved will be described in the hexose section', but they were not discussed in that section.

These models of ruminant digestion (Baldwin *et al.*, 1977; Danfaer, 1990; Dijkstra *et al.*, 1992; Neal *et al.*, 1992; Chapter 16) are the current state of the art. They have been built upon biological concepts laid out in earlier generations of mathematical models, and have been further refined both biologically and mathematically. Some aspects of ruminant digestion have been aggregated or removed dependent on their significance to overall digestion, a determination that was made as part of the modeling exercises. Other aspects of ruminant digestion have been added to the models to improve model behavior and predictions. The overall behavior of the models is considered good, however, it is recognized that several areas exist where model predictions are poor. These include predicting cellulose and hemicellulose digestion in high concentrate diets and predicting the yield of VFA. Errors in estimates of butyrate production present a particularly difficult problem as discussed in Chapter 8.

Current models are more heavily parameterized than earlier ones due, in large part, to the adoption of Michaelis-Menten type equation forms. Many assumptions have been made in estimating the parameters for these equations. We began this chapter by quoting J.W. Czerkawski as to the positive aspects of making assumptions. Making assumptions and testing them is an integral part of modeling exercises and the modeling/research cycle. The fact that assumptions are made should not be forgotten in the process, though. In the words of Czerkawski (1986), '...the danger lies within, that others will not realize that assumptions have been made'.

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Dynamic models of ruminant adipose tissue metabolism

With P. S. Miller

The differences that exist between species with respect to carbon source, pathway of co-factor generation and possible limiting steps in fatty acid biosynthesis point up the unique adaptations that the ruminant undergoes as a result of changes in metabolite availability.

Bauman and Davis (1975)

12.1 INTRODUCTION

Adipose tissue fulfils a central role in the metabolism of growing and lactating animals. For this reason, quite a number of studies of adipose tissue metabolism in ruminants have been undertaken over the past 20 years. These studies were reviewed by Allen *et al.* (1976), Bauman (1976), Vernon (1980), Baldwin and Smith (1983) and Smith and McNamara (1990), among others. Since these are readily available, references herein will be restricted to those that support specific decisions made in the course of model development. In growing animals, adaptations in adipose tissue metabolism that occur during the growth cycle, during periods of undernutrition and due to differences in genetic background are clearly important as these influence patterns of nutrient use and the composition and efficiency of gain. The role of adipose tissue during lactation is clearly variable and critical. In early lactation, when cows are in negative energy balance, triacylglycerol or stored fat is mobilized from adipose tissue to provide energy and fatty acids for milk fat synthesis. Changes in adipose tissue function supportive of lactation include elevated lipolytic rates, decreased rates of fat synthesis and decreased uptake of blood triacylglycerol. Lipolytic rates and capacity remain elevated throughout lactation and provide for continued mobilization of fatty acids. In late lactation, lipogenic capacity increases, leading to replenishment of fat reserves if feed intake is adequate. If the balance between lipogenesis and lipolysis in late lactation is shifted to excessive fatty acid synthesis and triacylglycerol retention, animals can fatten at the

expense of lactation. For example, high concentrate diets increase lipogenesis (Opstvedt *et al.*, 1967) and decrease lipolytic rates (Yang and Baldwin, 1973b) in lactating cow adipose tissue. This leads to fattening and depressed milk fat yields. Thus, the role of adipose tissue in lactating cows is quite variable, dependent on the animal's energy balance, genetic potential and diet.

Modeling adipose tissue function throughout the growth cycle and lactation is quite challenging even though considerable data are available. Major objectives in the modeling studies summarized in this chapter have been to evaluate the adequacy of available information on adipose tissue metabolism, to extend the interpretation of existing data, and to help identify key data and concepts that require further study and refinement.

Smith (1970; see also Chapters 3 and 16) set forth to develop and parameterize a dynamic, mechanistic, deterministic model of the metabolism of a lactating dairy cow. This model was comprised of elements representing metabolism in various body tissues, including liver, adipose tissue, the gastrointestinal tract, heart, muscle, mammary glands, etc. Early evaluations of model behavior demonstrated that equations describing lipid synthesis, turnover and mobilization in adipose tissue were not adequate to simulate whole animal lipid kinetics and lipid availability to the mammary glands for triacylglycerol synthesis. This result led to the *in vitro* studies of adipose metabolism of Yang and Baldwin (1973a,b), in which measurements of patterns of substrate oxidation and conversion to products, substrate interactions, and responses to physiological concentrations of insulin and epinephrine were evaluated. These studies in conjunction with other investigations of the physiology and biochemistry of ruminant adipose tissue (Baldwin *et al.* 1969, 1973; Vernon, 1976, 1980) were utilized in the development and documentation of a dynamic, mechanistic model of adipose tissue metabolism in the cow (Baldwin *et al.*, 1976; Hu, 1984).

A considerable amount of *in vitro* experimentation in ruminant adipose tissue has involved the use of radiolabeled metabolites. Therefore, a vast collection of data is available for the purpose of evaluating the model and extending current understanding of equation behavior, provided provisions for simulating the metabolism of radiolabeled substrates are included in the model, as herein. Initial versions of the radiotracer elements of the model were unstable and CPU requirements for solutions were high. The model has been revised several times. The current version is more robust and has become a powerful tool for evaluating underlying concepts of adipose tissue metabolism in the dairy cow, even though several elements require better definition based upon experimental and modeling research.

In this chapter, several stages in the evolution of the adipose tissue model will be discussed. In addition, the current model and results of behavioral and sensitivity analyses will be presented.

12.2 EVOLUTION OF STEADY-STATE BALANCE MODEL

12.2.1 Reference balance model

The basic reference lactating dairy cow described by Baldwin and Smith (1971) was used as a basis for development of the initial input:output balance model for adipose tissue. Briefly, the reference animal was a 550 kg lactating cow, in energy balance, fed a 60% concentrate: 40% alfalfa ration with 15% crude protein and producing 30 kg of milk per day. The calculated energy requirement of the animal was 183.5 MJ/day. Her heat production was 96.4 MJ/day. Energy expenditure in adipose tissue was estimated at 5.44 MJ/day, or 5.6% of total energy expenditures. Further details regarding the adipose tissue element are in Table 12.1. Based upon these estimates and compilations of input:output relationships discussed in Chapter 3, the initial set of input:output estimates (Baldwin *et al.*, 1976) presented in Table 12.2 were formulated. These were later revised by Hu (1984) and used with only slight changes by Miller (1990). Specific considerations that led Hu (1984) to modify the original input:output estimates were that rates of lipogenesis from acetate and net release of fatty acids in adipose tissues from lactating cows in mid-lactation and, essentially, in energy balance were lower (Baldwin *et al.*, 1973; Yang and Baldwin, 1973a,b) than originally estimated based upon indirect data. Similarly, lipogenesis from ketone bodies was found to be minimal in cow adipose tissue. Rates of triacylglycerol hydrolysis and re-esterification were found to be higher than originally estimated. Thus, glycerol release was increased. These changes – reduced acetate uptake, elimination of ketone body uptake, reduced fatty acid release and increased glycerol release – fall within the constraints imposed by the nutrient entry and oxidation estimates summarized in Chapter 3. Yang and Baldwin (1973a) observed significant losses of lactate into media by ruminant adipocytes incubated with glucose. This may or may not reflect

Table 12.1 Reference conditions and standard units used in adipose model development

-
- | | |
|----|---|
| 1. | 550 kg cow (500 kg empty body) |
| 2. | 75 kg adipose |
| 3. | 58.1 kg intracellular mass |
| 4. | 16.9 kg extracellular mass |
| 5. | Pool sizes: moles |
| 6. | Fluxes: mole/day |
| 7. | Assume 1:1 conversion of kg:l |
| | Therefore, |
| | 58.1 l intracellular volume |
| | 16.9 l extracellular volume |
| | concentration:mole/l |
| 8. | Steady state: i.e. all state variable pool sizes remain constant. |
-

Table 12.2 Input:output estimates for adipose tissue metabolism in a reference lactating dairy cow

	Baldwin et al. (1976)	Hu (1984)	Miller (1990)
Input			
glucose	3.10	3.5	3.53
acetate	18.5	13.85	14.16
KB	2.05	—	—
TG → FA (as FA)	0.18	—	—
Output			
FA	2.13	0.95	0.98
glycerol	1.69	3.0	3.21
lactate	—	2.4	2.22

the *in vivo* situation, but provision for this had to be added (Table 12.2) to the model to enable simulation of patterns of glucose metabolism observed using radiotracers *in vitro*.

The steady-state, balance relationships of Miller (1990; Table 12.2) and knowledge of metabolic pathways in ruminant adipose tissue translate into the block diagram presented in Fig. 12.1. Boxes enclosed by solid lines denote state variables. Broken-line boxes represent input variables (e.g. glucose and acetate) which, once specified, remain constant throughout each simulation. Broken-line boxes also identify accumulators (e.g. extracellular lactate, glycerol and extracellular fatty acids), which are set to zero prior to each simulation. These sum specific fluxes over time are used to monitor model behavior and are not effectors of model behavior. Two-, three- and four-letter abbreviations (Table 12.3) are used to name state variables, inputs and accumulator pools. Initial pool sizes (moles) for state variables, inputs and accumulator pools are enclosed in each box of Fig. 12.1. State variables and pools not explicitly represented in Fig. 12.1 are given in Table 12.4. Solid lines represented in Fig. 12.1 denote biochemical transactions among pools. The arrow heads indicate the net direction of each metabolic transaction. Numerical values next to each line represent the rate (moles/day) of each transaction.

Movement of material in and out of each pool is written as a differential equation: $dS_1/dt = \text{flux in} - \text{flux out} = \text{change in pool } S_1 \text{ with respect to time}$. If the reference steady-state conditions depicted by the fluxes in Fig. 12.1, which were used to parameterize the model, are specified as input, then $dS_1/dt = 0$ and flux in = flux out. Differential equations for each state variable are given in Table 12.5. The primary bases for selection of state variables in the model were that metabolites be involved in regulatory processes and transactions, and transactions be explicit that involve rearrangements in the distributions of radiotracers within metabolites. Early model versions were written in KINSYM and, thus, were solely based upon mass

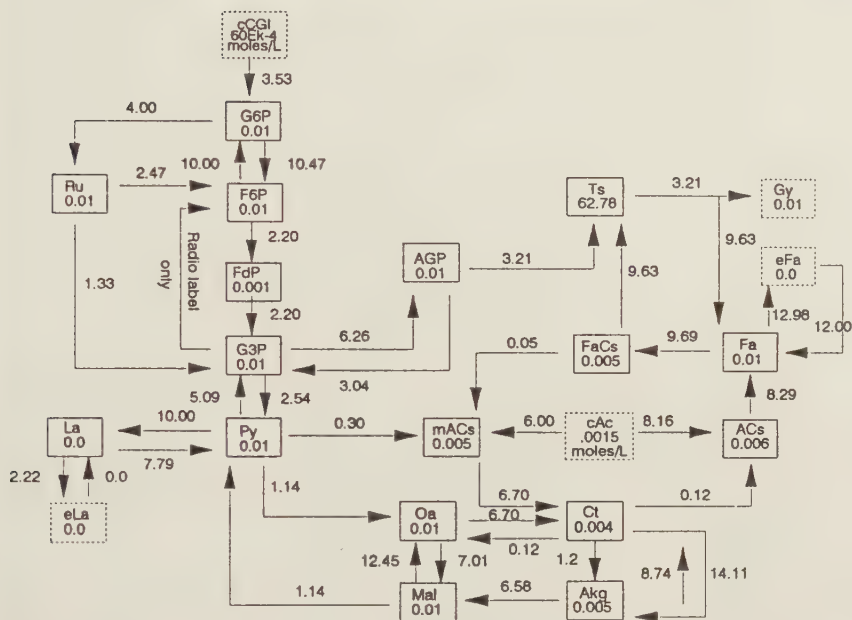


Figure 12.1 Block diagram of the adipose model. Fluxes (arrows) in mole/day. Pool sizes (boxes; mole/l). Boxes enclosed with a broken line denote accumulator variables (i.e. Gy, eLa, eFa) or constant concentrations (i.e. cG1, cAc). Codes are given in Tables 12.3 and 12.4.

action kinetics (equations 3.2 and 3.5). These versions were unstable, costly to solve and could not simulate observed data, which indicated saturation kinetics. Also, early models were over-parameterized as discussed in Chapter 3. Thus, mass action equations were systematically replaced with more highly aggregated equations, usually, exhibiting saturation kinetics.

12.2.2 Equations of the model

The data of Yang and Baldwin (1973a) clearly indicate that rates of conversion of glucose to CO₂ and triacylglycerol to glycerol exhibit saturation kinetics and are dependent upon glucose, insulin and acetate concentrations (Figs 12.2–12.5). This led to adoption of equation forms 3.21–3.26. Equations for the $U_{\text{Gl,G6P}}$ and $U_{\text{G6P,Ru}}$ transactions adopted were (Table 12.6):

$$U_{\text{Gl,G6P}} = V_{\text{Gl,G6P}} / (1.0 + k_{\text{Gl,G6P}} \{k_{\text{ins}} / [\text{INS}]\}^{\text{eins1}} / [\text{Gl}] + [\text{G6P}] / j_{\text{G6P}}) \quad (12.1)$$

$$U_{\text{G6P,Ru}} = V_{\text{G6P,Ru}} / (1.0 + k_{\text{G6P,Ru}} / \text{G6P} + k_{\text{Np,Ru}} / \text{Np}) \quad (12.2)$$

where $k_{\text{ins}} = 1.0$, $\text{INS} = 1.0$, $\text{eins1} = 0.125$ and $j_{\text{G6P}} = 1.72\text{E}-4$ in the reference state. The term $\{k_{\text{ins}} / [\text{INS}]\}^{\text{eins1}}$ in equation 12.1 provides for the effect of insulin on the apparent $k_{\text{Gl,G6P}}$ observed by Yang and Baldwin (1973a) and

Table 12.3 Abbreviations used in Fig. 12.1

<i>Abbreviation</i>	<i>Definition</i>	<i>Units</i>
cGl	glucose concentration	mM
G6P	glucose-6-phosphate	mole
Ru	ribulose-5-phosphate	mole
F6P	fructose-6-phosphate	mole
FdP	fructose diphosphate	mole
G3P	glyceraldehyde-3-phosphate	mole
AgP	glycerol-3-phosphate	mole
Py	pyruvate	mole
La	lactate	mole
eLa	extracellular lactate	mole
mAcCs	mitochondrial acetyl-CoA	mole
Oa	oxaloacetate	mole
Ct	citrate	mole
Akg	alpha-ketoglutarate	mole
Mal	malate	mole
cAc	acetate	mM
AcCs	cytosolic acetyl-CoA	mole
Fa	fatty acid	mole
eFa	extracellular fatty acid	mole
FaCs	fatty acyl CoA	mole
Ts	triacylglycerol	mole
Gy	glycerol	mole

Table 12.4 Abbreviation for state variable and zero pools not represented in Fig. 12.1

<i>Abbreviation</i>	<i>Definition</i>
At	ATP
Ad	ADP
Ndh	NADH
Nd	NAD
Fdh	FADH
Fd	FAD
Nph	NADPH ₂
Cs	cytosolic CoASH
mCs	mitochondrial CoASH
Cd	carbon dioxide
eCd	extracellular carbon dioxide
TAtp	At + Ad
TNdh	Ndh + Nd
TFdh	Fdh + Fd
TNph	Nph + Np

Table 12.5 Differential equations for adipose model state variables

State variable		Differential equation
G6P	DG6P	$= U_{G1,G6P} - U_{G6P,Ru} - U_{G6P,F6P} + U_{G6P,F6P}$
Ru	DRu	$= U_{G6P,Ru} - U_{Ru,F6P}$
F6P	DF6P	$= U_{G6P,F6P} - U_{F6P,G6P} - U_{F6P,FdP} + 0.667 * U_{Ru,F6P}$
FdP	DFdP	$= U_{F6P,FdP} - U_{FdP,G3P}$
G3P	DG3P	$= U_{FdP,G3P} - U_{G3P,AgP} + U_{AgP,G3P} - U_{G3P,Py} + U_{Py,G3P} + 0.33 * U_{Ru,F6P}$
AgP	DAGP	$= U_{G3P,AgP} - U_{AgP,G3P} - 0.33 * U_{FaCs,Ts}$
Py	DPy	$= U_{G3P,Py} - U_{Py,G3P} + U_{La,Py} - U_{Py,La} - U_{Py,mAcCs} + U_{Py,Oa} - U_{Mal,Py}$
mAcCs	DmAcCs	$= U_{Py,mAcCs} + U_{Ac,mAcCs} + U_{Fa,CsmA} - U_{Oa,Ct}$
Oa	DOa	$= U_{Py,Oa} + U_{Mal,Oa} - U_{Oa,Mal} - U_{Oa,Ct} + U_{Ct,Oa}$
Ct	DCt	$= U_{Oa,Ct} - U_{Ct,Oa} - U_{CtP,Akg} + U_{Akg,CtP} - U_{Ct,Akg}$
Akg	DAkg	$= U_{CtP,Akg} - U_{Akg,CtP} + U_{Ct,Akg} - U_{Akg,Mal}$
Mal	DMal	$= U_{Akg,Mal} - U_{Mal,Py} - U_{Mal,Oa} + U_{Oa,Mal}$
AcCs	DACs	$= U_{Ct,Oa} - U_{AcCs,Fa} + U_{Ac,AcCs}$
Fa	DFa	$= 0.125 * U_{AcCs,Fa} + 3.0 * U_{Ts,Fa} - U_{Fa,FaCs} - U_{Fa,eFa} + U_{eFa,Fa}$
FaCs	DFaCs	$= U_{Fa,FaCs} - U_{Fa,CsmA} - U_{FaCs,Ts}$
Ts	DTs	$= 0.33 * U_{FaCs,Ts} - U_{Ts,Fa}$
At ^a	DAT ^a	$= -U_{G1,G6P} - U_{F6P,FdP} + 2.0 * (U_{G3P,Py} - U_{Py,G3P}) - U_{Py,Oa} - U_{Ct,Oa} + U_{Akg,Mal} - 0.875 * U_{AcCs,Fa} - 2.0 * U_{Fa,FaCs} - 2.0 * U_{Ac,AcCs} - 2.0 * U_{Ac,mAcCs} + 0.3 * U_{Ndh,At} + 2.0 * U_{Fdh,At} + U_{Und,Ad}$
Ndh ^a	DNdh ^a	$= U_{AgP,G3P} - U_{G3P,AgP} + U_{G3P,Py} - U_{Py,G3P} - U_{Py,La} + U_{La,Py} + U_{Py,mAcCs} + U_{Ct,Akg} + U_{Akg,Mal} + U_{Mal,Oa} - U_{Oa,Mal} + 7.0 * U_{FaCs,Ma} - U_{Ndh,At}$
Fdh ^a	DFdh ^a	$= U_{Akg,Mal} + 7.0 * U_{FaCs,Ma} - U_{Fdh,At}$
Nph ^a	DNph ^a	$= 2.0 * U_{G6P,Ru} + U_{CtP,Akg} - U_{Akg,CtP} + U_{Mal,Py} - 1.75 * U_{AcCs,Fa}$
Cd	Dcd	$= U_{G6P,Ru} + U_{Py,mAcCs} - U_{Py,Oa} + U_{Ct,Akg} - U_{CtP,Akg} - U_{Akg,CtP} + U_{Akg,Mal} + U_{Mal,Py} - U_{Cd,eCd}$

^aDifferential equations are not written for Ad, Nd, Fd or Np as these are calculated by difference: Ad = TATp - At; Nd = TNdh - Ndh; Fd = TFDh - Fdh; Np = TNph - Nph.

depicted in Figs 12.2–12.4. It was assumed that ATP (At) is not limiting in the hexokinase reaction, so no term to reflect the participation of ATP in this reaction is included in equation 12.1.

The effect of acetate upon glucose oxidation is presumably mediated by an increase in rate of fatty acid synthesis from acetate (Fig. 12.6), which increases rates of NADPH₂ (Nph) utilization and the availability of NADP (Np) for the glucose-6-P dehydrogenase and 6-P-gluconate dehydrogenase reactions ($U_{G6P,Ru}$). Thus, provision for this linkage was incorporated as a ratio ($k_{Np,Ru}/Np$) in equation 12.2. Physiological concentrations of G6P are high relative to $k_{G6P,Ru}$, while free (not bound to enzymes) Np concentrations are low relative to $k_{Np,Ru}$ and so Np is the dominant effector in the

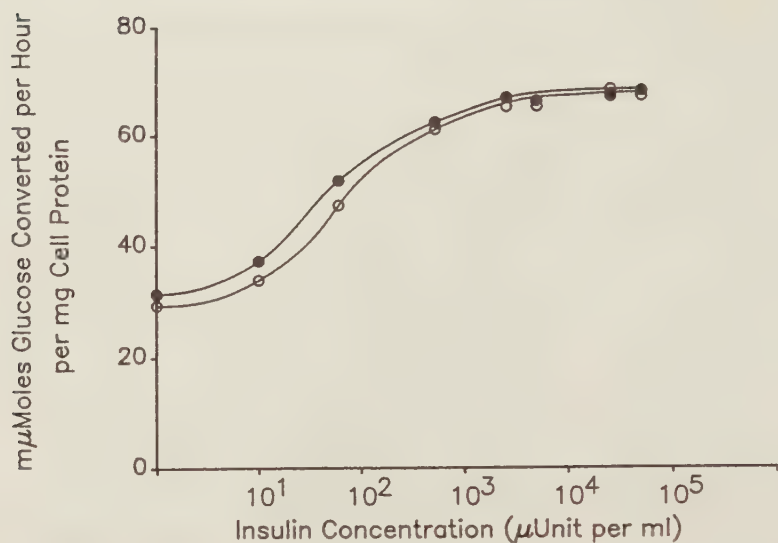


Figure 12.2 Insulin dose-response relationship on conversion of glucose to carbon dioxide (o) and lipid (●). Fat cells isolated from pooled tissues of steers were incubated in 2 ml of medium containing a U-¹⁴C-glucose (1.25 mM) with different concentrations of insulin for 120 min at 38°C. Each point is the mean $\pm$ SEM ($n=3$)

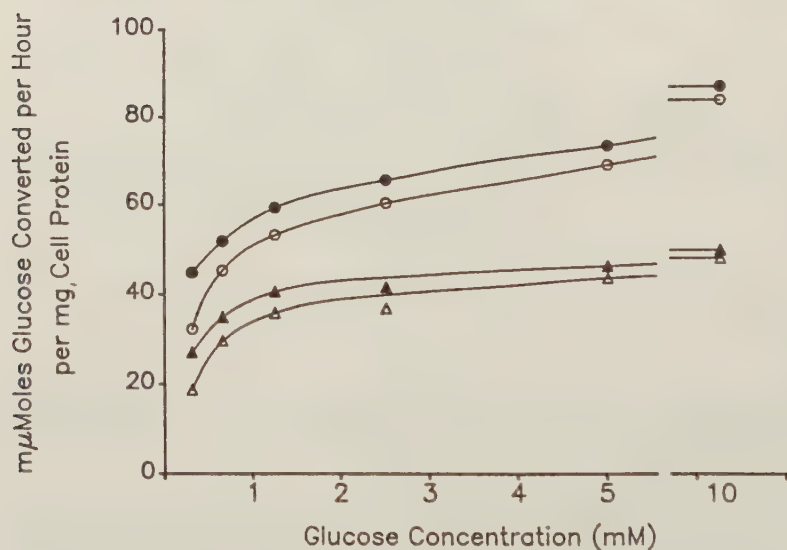


Figure 12.3 Effects of extracellular glucose concentrations and insulin on the conversion of glucose to carbon dioxide (Δ - ▲) and lipid (o - ●). Fat cells isolated from pooled adipose tissues of five steers were incubated in 2 ml of medium containing different concentrations of U-¹⁴C-glucose with or without insulin (10 units/ml) for 120 min at 38°C. Each point represents mean of triplicated samples. Solid symbol, insulin; open symbol, no insulin.

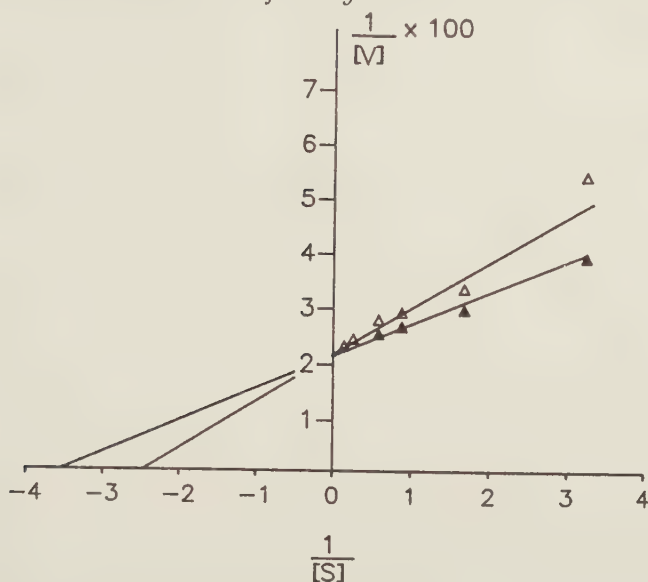


Figure 12.4 Double reciprocal plots of extracellular glucose concentrations against rates of glucose conversion to carbon dioxide ($\triangle$ - $\blacktriangle$) and effect of insulin. Solid symbol, insulin; open symbol, no insulin. $[V]$ = μ mole glucose converted/h/mg cell protein; $[S]$ = glucose concentration, mM.

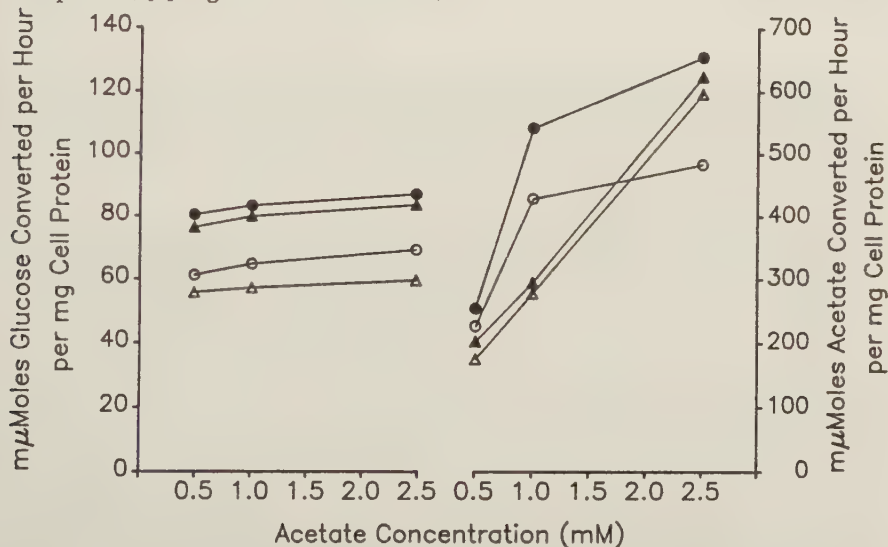


Figure 12.5 Effects of extracellular acetate concentrations and insulin on the conversion of glucose and acetate into carbon dioxide ($\triangle$ - $\blacktriangle$) and lipid ($\circ$ - $\bullet$). Isolated fat cells from pooled adipose tissues of steers were incubated in 2 ml of incubation medium containing glucose (2.5mM) and different concentrations of acetate with or without insulin (10 munits/ml). $U\text{-}^{14}\text{C}$ -glucose and $I\text{-}^{14}\text{C}$ -acetate were used as tracers. Incubation conducted for 120 min at 38°C . Each point represents mean of triplicated samples. Solid symbol, insulin; open symbol, no insulin.

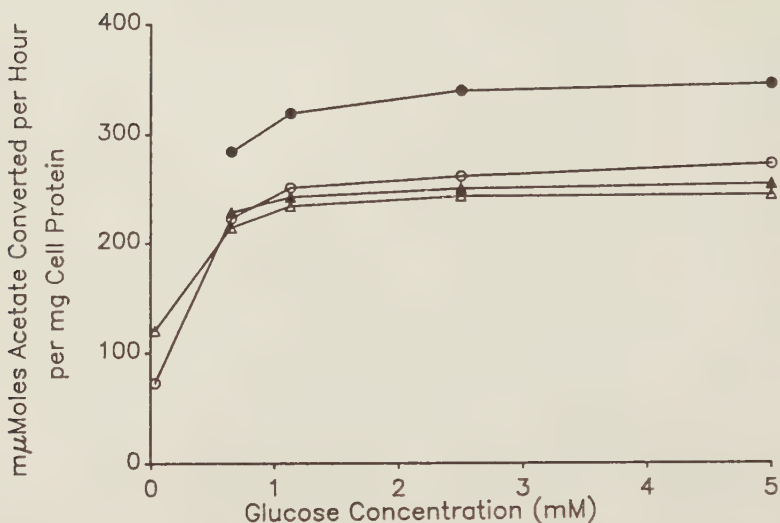


Figure 12.6 Effects of glucose and insulin on conversion of acetate to carbon dioxide ($\triangle$ — $\blacktriangle$) and lipid ($\circ$ — $\bullet$). Fat cells from pooled adipose tissues of steers were incubated in 2 ml of medium containing $1\text{-}^{14}\text{C}$ -acetate (2.5 mM) and different concentrations of glucose with or without insulin (10 munits/ml) for 120 min at 38°C . Each point represents mean of triplicated samples. Solid symbol, insulin; open symbol, no insulin.

$U_{\text{G6P,Ru}}$ transaction. The linkage between lipogenesis and glucose uptake and phosphorylation was completed by adding provision for negative feedback of G6P on hexokinase ($[G6P]/j_{G6P}$). This feedback is well characterized in other species and tissues (Newsholme and Leech, 1983), but not for ruminant adipose tissue.

Several considerations led to decisions regarding whether or not an intermediary metabolite should be identified explicitly as a state variable and whether or not a mass action equation is adequate to represent a reaction or series of reactions. One, noted above, is that when radiolabeled carbon atoms in different positions within an intermediate are converted to different products or different positions within products, that intermediate must be represented as a state variable so as to enable simulation of radiotracer data. A second consideration is the situation wherein once a metabolite is committed to a pathway, the following reactions are not rate limiting or subject to regulation. In this case, intermediates in the following reactions need not be represented. Explicit representation of Ru as an intermediate in the pentose cycle and aggregation of following reactions of this pathway as $U_{\text{Ru,F6P}}$ is an example of application of these two considerations. Once Ru is formed, the remaining reactions of the pathway that lead to the formation of two-thirds of a mole of F6P and one-third of a mole of G3P are considered to be non-limiting. Further, radiolabeled carbon atoms in Ru are converted to F6P or G3P or both, dependent upon their position in Ru (see below).

Table 12.6 Substrate and parameter estimates for fluxes listed in Table 12.4

Flux	Equation form ^a	V_{max}	S_1^b	K_1^c	S_2	K_2	S_3	K_3	S_4
$U_{G1,G6P}$	12.1	10.6	G1	6.0E-4	-	-	-	-	-
$U_{G6P,Ru}$	12.2	20.0	G6P	1.0E-2	Np	.01	-	-	-
$U_{Ru,F6P}$	3.5	-	Ru	4.0E+2	-	-	-	-	-
$U_{G6P,F6P}$	3.5	-	G6P	1.0E+3	-	-	-	-	-
$U_{F6P,G6P}$	3.5	-	F6P	1.0E+3	-	-	-	-	-
$U_{F6P,FdP}$	3.5	-	F6P	2.2E+2	-	-	-	-	-
$U_{FdP,G3P}$	3.5	-	FdP	2.2E+2	-	-	-	-	-
$U_{G3P,AgP}$	3.11	12.5	G3P	1.7E-4	-	-	-	-	-
$U_{AgP,G3P}$	3.11	6.1	AgP	1.7E-4	-	-	-	-	-
$U_{G3P,Py}$	3.11	10.2	G3P	1.7E-4	-	-	-	-	-
$U_{Py,G3P}$	3.11	5.1	Py	1.7E-4	-	-	-	-	-
$U_{Py,La}$	3.19	-	Py	1.0E+5	Ndh	-	-	-	-
$U_{La,Py}$	3.19	-	La	7.8E+4	Nd	-	-	-	-
$U_{La,eLa}$	3.5	-	La	2.2E+2	-	-	-	-	-
$U_{Oa,Ct}$	12.30	-	Oa	1.3E+5	mAcCs	-	-	-	-
$U_{Ct,Oa}$	12.40	-	Ct	6.2E+3	Cs	-	-	-	-
$U_{Ct,AkG}$	12.70	-	Ct	3.0E+2	-	-	-	-	-
$U_{Ctp,AkG}$	12.50	84.7	Ct	6.9E-5	Np	-	-	-	-
$U_{AkG,Ctp}$	12.60	52.4	AkG	8.6E-5	Nph	-	-	-	-
$U_{Py,mAcCs}$	12.8	-	Py	6.0E+5	Nd	-	mCs	-	-
$U_{Py,Oa}$	12.9	-	Py	1.1E+2	-	-	-	-	-
$U_{Mal,Py}$	12.10	-	Mal	3.3E+4	Np	-	-	-	-
$U_{AkG,Mal}$	3.14	-	AkG	6.8E+8	Nd	-	Fd	-	Ad
$U_{Oa,Mal}$	3.14	-	Oa	7.0E+4	Ndh	-	-	-	-
$U_{Mal,Oa}$	3.14	-	Mal	1.2E+5	Nd	-	-	-	-
$U_{Fa,CsmA}$	3.19	-	FaCs	2.0E+3	mCs	-	-	-	-
$U_{Ac,mAcCs}$	12.11	18.0	Ac	1.5E-3	mCs	5.0E-5	-	-	-

Table 12.6 (continued)

Flux	Equation form ^a	V_{max}	S_1^b	K_1^c	S_2	K_2	S_3	K_3	S_4
$U_{Ac,AcCs}$	12.12	32.6	Ac	1.5E-3	Cs	5.0E-5	At	1.8E-2	-
$U_{AcCs,Fa}$	12.13, 12.14	33.2	AcCs	1.0E-4	Nph	3.5E-3	At	1.8E-2	-
$U_{Ts,Fa}$	12.15, 12.16	6.4	Ts	6.3E+1	-	-	-	-	-
$U_{Fa,eFa}$	3.50	-	Fa	1.3E+3	-	-	-	-	-
$U_{eFa,Fa}$	fixed	-	-	-	-	-	-	-	-
$U_{Fa,FaCs}$	3.21	29.1	Fa	1.7E-4	Cs	5.0E-3	-	-	-
$U_{FaCs,Ts}$	3.21	48.2	FaCs	5.0E-3	AgP	3.0E-2	-	-	-
$U_{Nd,hAt}$	3.19	-	Ndh	2.7E+5	Ad	-	-	-	-
$U_{Fd,hAt}$	3.19	-	Fdh	4.9E+5	Ad	-	-	-	-
$U_{Und,Ad}$	3.50	-	At	2.0E+2	-	-	-	-	-
$U_{Cd,eCd}$	3.50	-	Cd	1.7E+3	-	-	-	-	-

^a Equation numbers defined in text or in Chapter 3.

^b First substrate, S_1 ; S_2 to S_4 , second through fourth substrate.

^c Rate or affinity constants: Michaelis-Menten affinity constant units = mole/l, mass-action rate constant units = day⁻¹; for values, 6E-4 would be 6.0×10^{-4} .

Thus, Ru was identified as a state variable and a mass action equation was considered adequate to represent this portion of the pentose cycle (Table 12.6).

Additional considerations were applied in the decision to represent F6P as a state variable. As was discussed in Chapter 3, enzyme activity and metabolite measurements must be utilized with care and should not be the sole basis for estimating fluxes. On the other hand, these data can provide guidance. A number of enzyme activity estimates are presented in Table 12.7 and an unfortunately very limited number of metabolite data are in Table 12.8. Metabolite measurements are more difficult and tedious than are most enzyme assays but, in many ways, are equally or more informative. The metabolite data in Table 12.8, which represent effects of extreme diets, provided significant guidance in modeling cow adipose tissue metabolism. Important elements of guidance provided by the metabolite data were that:

1. An insulin effect (associated with the 100% concentrate diet) upon $U_{\text{Gl,G6P}}$ was essential, since the increase in glucose concentration (48%) cannot explain the 266% increase in G6P concentration.
2. Energy charge does not appear to influence the phosphofructokinase reaction since the concentration of F16BP was not altered, even though the energy charge $\{(ADP + 2ATP)/2(AMP + ADP + ATP)\}$ was quite different for the two experimental conditions.
3. α -glycerol-P concentration influences fatty acid esterification (this rate should be elevated in cows fed high concentrate diets).
4. Concentrations of dicarboxylic acids of the tricarboxylic acid cycle are elevated by concentrate diets.
5. The NAD/NADH₂ ratio or redox state of adipose tissue as indicated by the pyruvate/lactate ratio is not influenced by diet.

These observations provided insight in development of the model and must be considered constraints to which the model must conform in behavioral and sensitivity evaluations.

The activity estimates for the hexokinase, G6P dehydrogenase and 6-P gluconate dehydrogenase reactions in Table 12.7 are below the V_{max} estimates presented for $U_{\text{Gl,G6P}}$ and $U_{\text{G6P,Ru}}$ in Table 12.6. These were adjusted upward to accommodate data on actual flux rates and responses obtained *in vitro*. In the case of the phosphohexose isomerase reaction, it is clear that the activity of this enzyme (76 moles/day) is in considerable excess of the net flux (0.47 moles) and the forward and reverse velocities depicted in Fig. 12.1. The forward and reverse velocities for this reaction were reduced to 10 moles/day, because *in vivo* concentrations of G6P (Table 12.8) and F6P calculated from K_{equil} for the reaction are not saturating as in *in vitro* enzyme assays.

In addition, these rates were arbitrarily lowered relative to estimates of velocity obtained using Michaelis–Menten kinetic arguments and the en-

Table 12.7 Adipose tissue enzyme activities^a

Enzyme	Tissue activity ^b	
	units/g tissue	total activity ^c
Hexokinase (EC 2.7.1.1)	0.48	5.52
G6P dehydrogenase (EC 1.1.1.49)	0.12	12.96
6-P-gluconate dehydrogenase (EC 1.1.1.44)	0.07	7.57
Phosphohexose isomerase (EC 5.3.1.9)	0.91	76.13
Phosphofructokinase (EC 2.7.1.11)		
Aldolase (EC 4.1.2.13)	0.22	23.76
Triose-P isomerase (EC 5.3.1.1)	17.85	1493.40
Glyceraldehyde-3-P dehydrogenase (EC 1.2.1.12)	0.49	40.99
α -Glycerol-P dehydrogenase (EC 1.1.1.8)	0.28	30.24
Pyruvate dehydrogenase (EC 1.2.4.1)	0.01	0.83
Pyruvate carboxylase (EC 6.4.1.1)		
Lactate dehydrogenase (EC 1.1.1.25)	7.0	585.65
Citrate synthetase (EC 4.1.3.7)		
ATP-citrate lyase (EC 4.1.3.8)	0.02	2.30
NADP-isocitrate dehydrogenase (EC 1.1.1.42)	1.76	147.25
Malate dehydrogenase (EC 1.1.1.37)	5.80	626.40
Malic enzyme (EC 1.1.1.40)	0.05	5.40
Acetyl-CoA carboxylase (EC 6.4.1.2)	0.03	2.51
Fatty acid synthetase (EC 6.2.1.3)	0.10	10.80
Acetyl-CoA synthetase (EC 6.2.1.1)	0.08	6.70

^aAdapted from Hu (1984).^bUnits in $\mu\text{mole}/\text{min}$ ^cTotal activity in mole/58.1 kg adipose tissue/day.**Table 12.8** Metabolite concentrations in abdominal adipose tissue in lactating dairy cows^a

	Diet ^b	
	hay ($\mu\text{mole}/\text{kg}$)	concentrate ($\mu\text{mole}/\text{kg}$)
Glucose	614.0	914.0
G6P	9.0	24.0
F-1,6-BP	1.0	1.0
Triose-P	3.7	5.3
α -Glycerol-P	175.0	240.0
Phosphoenolpyruvate	15.5	19.5
Pyruvate	25.5	27.5
Lactate	551.0	676.0
Malate	17.0	44.0
α -Ketobutyrate	10.0	15.0
Glutamate	498.0	1440.0
(ADP + 2ATP)/2(AMP + ADP + ATP)	0.47	0.66

^aFrom Baldwin *et al.* (1986).^bDiet of 100% alfalfa hay or 100% concentrate (milk fat depressing ration) to 10 lactating dairy cows in a double reversal study. Tissue samples by biopsy.

zyme and metabolite data from Tables 12.7 and 12.8. This was done to prevent the rapid turnover rates of G6P and F6P from forcing reduced integration intervals and, thus, increased solution times during model development as discussed in Chapter 4. Since phosphohexose isomerase is clearly non-limiting (as indicated by its high activity), one might ask why F6P is identified as a state variable. Conventional (algebraic) methods for estimating pentose cycle flux are based (see discussion of radiotracers below), in part, on the simplifying assumption that the specific radioactivities of G6P and F6P are the same, i.e. phosphohexose isomerase activity is high enough to attain isotopic equilibria between these two intermediates, even though there are data that indicate this may not always be so. Because of this, we chose to represent F6P explicitly so that the model would enable examination of this simplifying assumption once adequate radiotracer data become available. Given the observation that enzyme activity is in considerable excess, however, the assumption that mass action kinetics apply (Table 12.6) seems appropriate at this time.

It is widely recognized that the phosphofructokinase ($U_{F6P,FbP}$) reaction is a primary regulatory site that determines rates of hexose-P conversion via the Emden–Meyerhoff pathway to pyruvate and lactate (Newsholme and Leech, 1983). Many effectors of this enzyme have been identified including ATP (–), AMP (+), citrate (–), fructose-2,6-BP (+) and CAMP (+). In several earlier versions of the adipose tissue model, provisions for regulation of this reaction were incorporated. However, there are no data available on the ruminant adipose tissue enzyme and concentrations of the several activators and inhibitors. The limited metabolite data in Table 12.8 do not implicate ATP and AMP as effectors, but expression of adipose data on a $\mu\text{mole/kg}$ basis is clearly suspect in this regard. Thus, we could neither justify nor parameterize a complex equation. This led us to revert to the simple mass action form (Table 12.6), which allows carbon to flow down the glycolytic pathway at low glucose concentrations as observed by Yang and Baldwin (1973a) and reduce excessive cycling through the pentose pathway (Vernon, 1980).

Aldolase activity (Table 12.7) is in considerable excess of $U_{FbP,G3P}$ and so a mass action equation (Table 12.6) was considered adequate. Similarly, the activity of triose-P isomerase is extremely high, therefore the triose-P (TP) products of the aldolase reaction, G3P and dihydroxyacetone-P, were considered together as a TP pool (Fig. 12.1), even though radiotracer data (see below) may eventually require separate representation. Activities of α -glycerol-P dehydrogenase ($U_{TP,AgP}$, $U_{AgP,TP}$) and of enzymes in the $U_{TP,Py}$ and $U_{Py,TP}$ transactions, most notably the glyceraldehyde-3-P dehydrogenase reaction (Table 12.7), are in considerable excess of observed fluxes and were thus maintained as mass action equations in early revisions. However, concentrations of intermediates and, as a result, fluxes among these became very elevated when experiments in which low glucose concentrations were

simulated. This was probably because cofactors that participate in these reactions such as ATP, ADP, NAD and NADH were not represented in the mass action equations due to a lack of appropriate data. The simplest solution to this problem was to convert to Michaelis-Menten type equations (equation 3.7). To date, this resolution has appeared to be adequate. This is not to say that additional data and simulation analyses will not require and provide for the parameterization of more complex representations.

Exchange rates between oxaloacetate (Oa) and citrate (Ct) required special consideration, since the conversion of Oa and mitochondrial acetyl-CoA (mAcCs) to Ct is a mitochondrial reaction (citrate synthetase), while the hydrolysis of Ct to Oa and cytoplasmic acetyl-CoA (AcCs) is a cytosolic reaction (ATP citrate lyase). In early versions, this problem was resolved by not including the ATP-citrate lyase ($U_{Ct,Oa}$) reaction in the model, even though ATP-citrate lyase activity was known to be present (Table 12.7). This decision was justified on the grounds that Yang and Baldwin (1973a) had observed that less than 5% of ^{14}C recovered in the fatty acids of triacylglycerol was formed from U- ^{14}C -glucose. However, Vernon (1980) reported significant rates of ^{14}C -lactate incorporation into fatty acids in ruminant adipose tissue. These observations required that provisions for simulation of ^{14}C -lactate metabolism be added to the model. A reaction to provide for an exchange between extracellular and intracellular lactate ($U_{eLa,La}$) was added. This is implemented as a mass action equation based upon a logical IF statement, which provides that when the initial extracellular concentration of eLa is greater than 10^{-6} mM, eLa and intracellular La can exchange. Improved representations of controls on pyruvate metabolism and the ATP citrate lyase reaction were also added.

Problems associated with this latter addition were significant. The pool sizes of Oa, coenzyme A (Cs) and acetyl-CoA are small in most tissues, have not been measured in cow adipose tissue and, certainly, their intracellular distributions are not known. Thus, only one Oa pool could be rationally incorporated into the model. The exchange between Oa and Ct and potential for transfer of mitochondrial acetyl-CoA (mAcCs) to the cytosol were represented as follows:

$$U_{Oa,Ct} = K_{Oa,Ct} * Oa * mAcCs \quad (12.3)$$

$$U_{Ct,Oa} = K_{Ct,Oa} * Ct * Cs \quad (12.4)$$

Similarly, separate state variables for mitochondrial and cytosolic Ct and Akg are not represented. As a result, both the NAD (mitochondrial)- and NADP (cytosolic)-linked isocitrate dehydrogenases use substrates from the same pools, which implies that these pools are in rapid equilibrium. The cytosolic NADP-linked isocitrate dehydrogenase (Table 12.6) is very active (Table 12.7) and is represented as a reversible reaction:

$$U_{CtP,Akg} = V_{CtP,Akg} / (1.0 + k_{CtP,Akg} / [Ct] + k_{Np,Akg} / Np) \quad (12.5)$$

$$U_{\text{Akg,CitP}} = V_{\text{Akg,CitP}} / (1.0 + k_{\text{Akg,CitP}} / [\text{Akg}] + k_{\text{Nph,CitP}} / \text{Nph}) \quad (12.6)$$

The net direction of this reversible reaction is highly dependent upon availability of NADP (Np), since citrate (and isocitrate) concentrations in ruminant tissues are high. The mitochondrial ADP-linked isocitrate dehydrogenase reaction was considered irreversible and highly dependent upon energy status of the cell as indicated by the pool size of ADP (Ad):

$$U_{\text{Cit,Akg}} = K_{\text{Cit,Akg}} * \text{Cit} * (\text{Ad}/i\text{Ad}) \quad (12.7)$$

The importance of Ad as an effector of this reaction rate was discussed in detail by Newsholme and Leech (1983) and presumably relates to ruminant adipose tissue mitochondria.

The mitochondrial pyruvate dehydrogenase ($U_{\text{Py,mAcCs}}$) and carboxylase ($U_{\text{Py,Oa}}$) reactions are critical to simulation of lactate metabolism in ruminant adipose tissue. Additional information is clearly needed to justify and refine representations of these reactions in the current model. Most of the reasoning behind equations for these reactions arose from information gathered in different tissues and species. There are no data on the kinetics of the pyruvate dehydrogenase reaction in ruminant tissues other than the observation in cow mammary tissue that high concentrations of acetate inhibit lipogenesis from lactate (see Chapter 13). In other species, mitochondrial acetyl CoA (mAcCs) has been shown to inhibit this reaction. Cosubstrates are known to effect reaction rates, therefore a mass action equation was used (Table 12.6):

$$U_{\text{Py,mAcCs}} = K_{\text{Py,mAcCs}} * \text{Py} * \text{Nd} * \text{mCs} * \text{imAcCs}/\text{mAcCs} \quad (12.8)$$

Similarly, mAcCs is known to stimulate the pyruvate carboxylase reaction in all species and tissues studied (Newsholme and Leech, 1983) so the following mass action equation was used:

$$U_{\text{Py,Oa}} = K_{\text{Py,Oa}} * \text{Py} * \text{mAcCs}/\text{imAcCs} \quad (12.9)$$

Under normal physiological conditions in ruminant tissues the malic enzyme reaction ($U_{\text{Mal,Py}}$) is considered to be only a minor contributor of NADPH_2 for fat synthesis, even though its activity in adipose tissue is greater than those of ATP citrate lyase and acetyl-CoA carboxylase (Table 12.7). In the absence of glucose and acetate to contribute NADPH_2 via the pentose cycle and the NADP-linked isocitrate dehydrogenase, e.g. lactate as a sole substrate, the capacity for this reaction is such that it could contribute significant NADPH_2 for fatty acid synthesis. Lacking data to the contrary, a mass action equation was incorporated as follows:

$$U_{\text{Mal,Py}} = K_{\text{Mal,Py}} * \text{Mal} * \text{Np} \quad (12.10)$$

After mitochondrial acetyl-CoA is committed to the tricarboxylic acid cycle and the balance between the conversion of citrate (via isocitrate) to α -ketoglutarate (Akg) (by the NAD- and NADP-linked isocitrate dehydro-

genases) is determined by the relative concentrations of ADP and NADP, subsequent reactions do not appear to be rate-limiting in most tissues. In recognition of this, the reactions between Akg and Mal are represented in aggregate by a mass action equation (Table 12.6). This implies complete randomization of radiotracers between positions one and four and two and three in the symmetrical dicarboxylic acids of the tricarboxylic acid pathway. The activity of malate dehydrogenase is very high (Table 12.7), which suggests rapid equilibration rates between the Oa and Mal pools dependent upon the NAD/NADH₂ ratio and, therefore, that the two pools could be aggregated into one. However, previous experiences in simulating interactions between very small (Oa) and very large (Mal) pools suggest that although chemical equilibria are achieved, radioisotopic equilibria may not be achieved if turnover of the small pool, Oa in this case, is very high. This can effect ¹⁴C-glucose and ¹⁴C-lactate rates of conversion to products. For this reason, the pools were represented separately and their exchange rates by simple mass action equations.

Mitochondrial acetyl-CoA (mAcCs, Fig. 12.1) can be formed from pyruvate (equation 12.8), fatty acyl-CoA (FaCs) and acetate from extracellular fluids or media (Ac). The rate of oxidation of FaCs to form mAcCs ($U_{FaCs,mAcCs}$) in the reference state is very low and allows for some substrate cycling, but would only become important when acetate was very low and, as a result, mCs became very high, e.g., in a starving state. A mass action equation was used to represent this series of reactions (Table 12.6). The activation of acetate for oxidation in mitochondria

$$U_{Ac,mAcCs} = (mV_{Ac,mAcCs} * Ad/iAd)/(1.0 + k_{Ac,mAcCs}/[Ac] + k_{mCs,mAcCs}/At) \quad (12.11)$$

is dependent upon the energy state of adipocytes relative to their (initial) reference state (Ad/iAd) and the availability of acetate and mitochondrial coenzyme A (mAcCs). Equation 12.3 provides for mAcCs entry to the tricarboxylic acid cycle.

The equation for activation of acetate in the cytosol for fatty acid synthesis

$$U_{Ac,mAcCs} = V_{Ac,AcCs}/(1.0 + k_{Ac,AcCs}/[Ac] + k_{Cs,AcCs}/Cs + k_{At,AcCs}/At) \quad (12.12)$$

indicates that concentrations of all substrates can be significant effectors of reaction rates and implies the following: acetate is the primary substrate; ATP acts as an index of energy charge, e.g. fatty acid synthesis should be low when energy availability is low; and Cs is a substrate feedback effector when AcCs is high due to low acetyl-CoA carboxylase activity.

Insulin acts upon three reactions in the model (Table 12.6) – the $U_{Gl,G6P}$ reaction discussed above, $U_{AcCs,Fa}$ and $U_{Ts,Fa}$. The $U_{AcCs,Fa}$ reaction introduced the potential for a sigmoidal relationship ($\epsilon \neq 1.0$; equation 12.1) as an optional feature should further data indicate that linear effects of insulin upon the reaction are not adequate. The potential for a sigmoidal effect of

insulin upon $U_{\text{AcCs,Fa}}$ was introduced to enable simulation of acute (phosphorylation) effects of insulin on acetyl-CoA carboxylase activity

$$U_{\text{AcCs,Fa}} = V_{\text{AcCs,Fa}} / (1.0 + k_{\text{AcCs}} / [\text{AcCs}] + k_{\text{Nph,Fa}} / \text{Nph} + k_{\text{At,Fa}} / \text{At}) \quad (12.13)$$

$$V_{\text{AcCs,Fa}} = 14 + V_{\text{AcCs,Fa}} F / (1.0 + \{k_{\text{ins2}} / \text{INS}\} \varepsilon_{\text{ins2}}) \quad (12.14)$$

where $V_{\text{AcCs,Fa}} F = 38.3$, INS , k_{ins2} and $\varepsilon_{\text{ins2}} = 1.0$ in the reference state. There are no direct data on ruminant adipose tissue to support an effect of insulin on $V_{\text{AcCs,Fa}}$, but data on adipose tissues of other species indicate that insulin does cause phosphorylation of acetyl-CoA carboxylase at sites that increase activity of the carboxylase (Kim, 1983). These data, coupled with the observed 30% increase in rate of acetate incorporation into lipids at high acetate concentrations (Fig. 12.5), support equation 12.14. Further, the increase in $U_{\text{Gl,G6P}}$ due to insulin indicated by the data in Fig. 12.3 was only 10% and thus would not likely cause a 30% increase in rate of lipogenesis. A confounding observation is the 37% increase in α -glycerol-P concentration on the high concentrate diet (Table 12.8). This could lead to a reduction in fatty acyl-CoA concentrations in the cell and, thereby, reduce inhibition of acetyl-CoA carboxylase by fatty acyl-CoA (Newsholme and Leech, 1983). Further data may justify or cause adjustment of equation 12.14.

Another consideration regarding equations 12.13 and 12.14 is the implicit assumption that acetyl-CoA carboxylase is the limiting enzyme in the lipogenesis pathway. Estimates of the respective activities of acetyl-CoA carboxylase and the fatty acid synthetase complex in Table 12.6 support this view, but enzyme activity data on complex enzymes such as these two can always be held suspect. All three substrates for the pathways are represented as effectors of rate in equation 12.13. Since the $\text{NADPH}_2/\text{NADP}$ ratio is very high in most tissues, its effect upon the reaction rate would be expected to be minimal and, perhaps, need not have been specified. Acetate and, as a result, AcCs availability (Fig. 12.5) and energy charge, indicated in this case by ATP concentration (Table 12.8), clearly can be significant effectors.

The most difficult equation sets to parameterize are those associated with substrate cycles (Newsholme and Leech, 1983; Chapter 3) and, of these, the one we have had the most difficulty with in growing and lactating animals is the cycle of triacylglycerol turnover in adipose tissue. As depicted in Fig. 12.1, lipolysis yields nine moles of fatty acids per day, of which 0.98 moles are released to extracellular fluids and 9.69 moles of fatty acid from lipolysis plus lipogenesis from acetyl-CoA are converted to FaCs , of which 0.05 moles are oxidized and 9.64 moles esterified to form storage triacylglycerol. According to Fig. 12.7, a relatively small change in epinephrine concentration in the physiological range could cause a 20% change in lipolytic rate, from 9.0 to 10.8 moles/day. If this change was not accompanied by an increase in the rate of fatty acid re-esterification, which is not likely, fatty acid release would increase from 0.98 to 2.78 moles/day. With regard to the

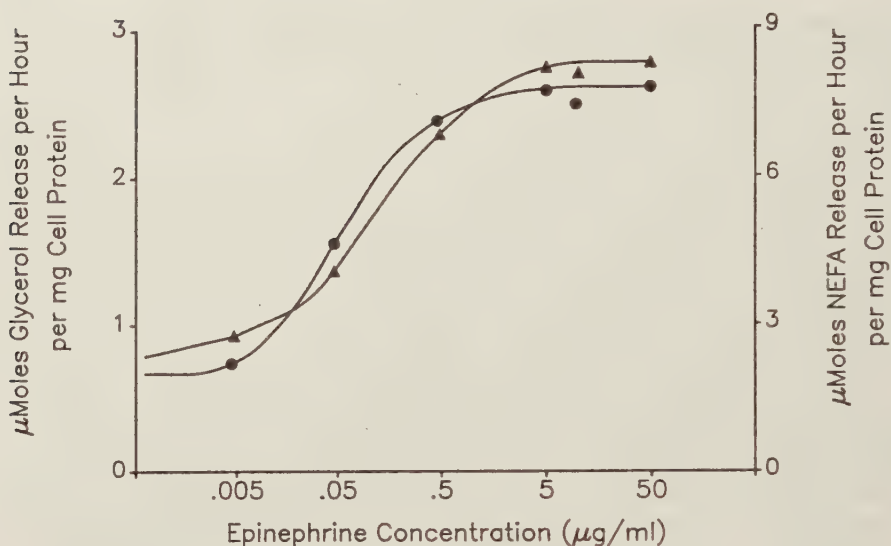


Figure 12.7 Dose-response relationship reflecting effect of epinephrine on *in vitro* glycerol (●) and non-esterified fatty acid (NEFA; ▲) release from isolated fat cells. Fat cells were prepared from adipose tissue of a non-lactating cow and incubated in 3 ml of medium with different concentrations of epinephrine for 120 min at 38°C. Each point represents the mean of triplicated samples.

highly responsive nature of this substrate cycle, particularly the opposing actions of insulin, glucagon and the catecholamines that regulate the system, the representation in this current model of ruminant adipose tissue must be viewed as naive, almost arbitrary and totally inadequate.

In short, the most important functions of adipose tissue – energy storage and mobilization dependent upon energy status and need – are not adequately represented. There are several reasons for this, the most important being a lack of knowledge and quantitative data required to develop and parameterize an appropriate set of equations. A second reason is that we understood this to be true beforehand and did not include accurate simulation of this substrate cycle and net release of fatty acids from adipose tissue in our objective statement. Rather, we emphasized development of a model that could be utilized to evaluate the adequacy of current knowledge of acetate, lactate and glucose metabolism in adipose tissue. This enabled utilization of available radiotracer data to better parameterize equations relating to the metabolism of these substrates. Considerable tedious and very difficult work will be required before this substrate cycle can be modeled. A side effect of this deficiency is that model elements dealing with net energy storage or mobilization in whole-animal models will remain, at best, speculative in nature.

The foregoing discussion was presented to emphasize an important area

of investigation that has received little attention and to point out that the representations of rates of lipolysis and fatty acid release and re-esterification respond to the needs of the current modeling objective and not to a longer-term desire to understand and model this substrate cycle. Requirements imposed at this stage were that the model support further interpretation of available radiotracer data. The equations for triacylglycerol hydrolysis

$$U_{Ts,Fa} = V_{Ts,Fa}/(1.0 + k_{Ts,Fa}/Ts) \quad (12.15)$$

and

$$V_{Ts,Fa} = 4.0 + V_{Ts,Faf}/(1.0 + INS/J_{ins3}) \quad (12.16)$$

contain a provision for high insulin levels to reduce the activity of the lipolytic enzyme complex ($V_{Ts,Fa}$) about one-third below the reference value of 6.42, since $V_{Ts,Faf}$ equals 4.84 and INS and J_{ins3} equals 1.0 in the reference state. No provision for activation of this enzyme complex by catecholamines via the cyclic AMP system was included, based upon the presumption that catecholamines are not normally added to *in vitro* radiotracer incubations designed to examine patterns of glucose, acetate and lactate metabolism. If such studies were to be evaluated, appropriate provisions would have to be added. The value for $k_{Ts,Fa}$ was set at 62.78 (equation 12.5) and equal to the pool size of Ts . Since the maximum rate of hydrolysis is 6.42 moles/day, during short-term (*in vitro*) experiments $U_{Ts,Fa}$ is essentially constant except for possible insulin effects. Similarly, $U_{Fa,eFa}$ was represented by a mass action equation (Table 12.6). Influx of Efa ($U_{eFa,Fa}$) was fixed at 12 moles/day, based upon the assumption that the eFa pool remains essentially constant during *in vitro* incubations. The equations for $U_{Fa,FaCs}$ and $U_{FaCs,Ts}$ exhibit saturation kinetics with Cs as the dominant effector of rates of fatty acid activation, e.g. the Fa pool is large and relatively constant, and α -glycerol-P is the dominant determinant of the rate of Ts synthesis. This latter provision accommodates effects of insulin and high glucose concentrations on Fa esterification rates, based upon their effects upon glucose uptake which presumably cause an increase in α -glycerol-P concentration (Table 12.8).

Rates of $NADH_2$ (Ndh) and $FADH_2$ (Fdh) oxidation are dependent upon their respective pool sizes and cellular energy charge as reflected by ADP (Ad ; Table 12.6). The final two equations in Table 12.6 are mass action in form and accommodate, respectively, energy expenditures in ruminant adipose tissue due to protein turnover and ion transport, facts which are considered to be relatively constant such that they can be aggregated ($U_{Und,Ad}$).

State variable pool sizes used in Fig. 12.1 were derived, in part, from Baldwin *et al.* (1968). Due to the high turnover rates of several metabolites, some pool sizes were increased to maintain model stability and reduce CPU requirements (see Chapter 4). Appropriate corrections to equation parameters were made so that fluxes remain equivalent to values calculated prior

to pool size adjustment. A similar approach was used to maintain equivalent fluxes when equations were changed from a mass action to Michaelis-Menten form. However, some apparent inconsistencies arose during this latter process and require mention.

Some of these inconsistencies arose from the number of different persons who participated in development of the model, a lack of data on adipose tissue metabolite concentrations and from the difficulty inherent in estimating metabolite concentrations in adipose tissue. In a model based solely upon mass action equations, it is clear that rates should be calculated as a rate constant times pool size. It would not be useful to estimate rates as a rate constant times concentrations of a metabolite times pool mass or volume. When Michaelis-Menten type equations are used, it seems best that the $V_{\max}$ represent the metabolic capacity of the total tissue and that the affinity constants and substrates be expressed as concentrations, e.g. moles/l or moles/kg, because these values are readily compared with standard methods of expression. However, with adipose tissue in particular, calculations of metabolite concentrations are difficult. If the tissue is 80% fat, should one conclude that the volume of tissue on which a phosphorylated intermediate was 0.2 l/kg of tissue, since about one-half of the non-fat volume is extracellular, 0.1 l/kg of tissue? Further, when one estimates a probable pool size for a metabolite or coenzyme from data on other tissues when no adipose tissue data are available, is it appropriate to dignify this estimate by using both pool size and concentration? Clearly, arguments among modelers working together and in sequence, among modelers and reviewers, etc., are preordained by these dilemmas. No clear resolution is possible.

Some differing decisions regarding this problem are evident in our ruminant adipose tissue model. By way of example consider equations 12.1 and 12.2. Glucose, insulin and G6P concentrations are inputs to equation 12.1. Glucose and insulin concentrations in media are controlled by the investigators in *in vitro* studies and can be measured *in vivo*, and so the use of concentration values is appropriate. For G6P, however, a problem is evident. Concentration of G6P is used in equation 12.1 and pool size is used in equation 12.2. As long as the same assumptions regarding volume of distribution are used in estimating [G6P] and j_{G6P} for equation 12.1 and G6P and $k_{\text{G6P,Ru}}$ for equation 12.2, simulation results will not differ, because the ratios of these is the important factor. The pool size of (free) Np is not known for adipose tissue and, further, is likely to be so small that it would have to be inflated a thousand times or more to reduce CPU requirements for solution of the model. In view of this, it would be inappropriate to use concentration.

The foregoing discussion emphasizes the fact that the model presented in this chapter is a working model and, therefore, that care must be taken while evaluating individual equations and parameter values in the model to recognize whether or not they relate to pool sizes or concentrations and, further, that the individual decision did not compromise behavior of the model.

12.3 RADIOISOTOPE TRACER ELEMENTS

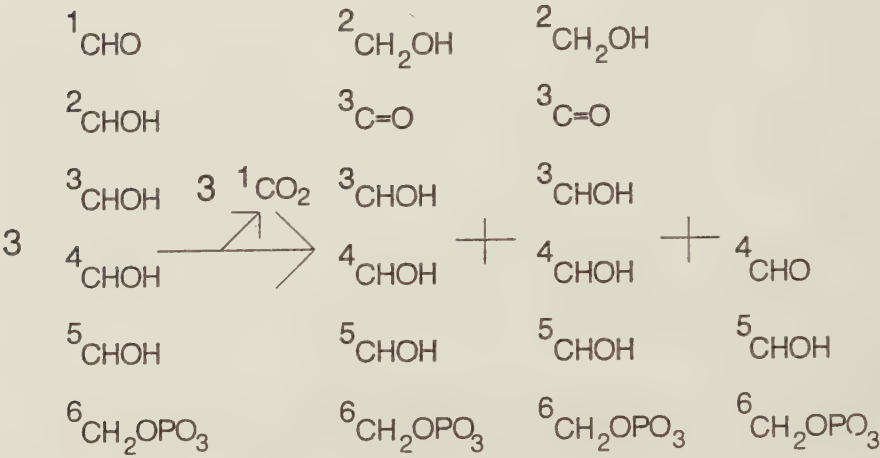
Radioisotopes have been used extensively to study whole-animal and tissue metabolism. A general objective within our research modeling program, as eluded to in our modeling objective statement for this model, has been the development of radiotracer elements adequate to extend interpretations of available radiotracer data and aid in the design and interpretation of further experiments. With regard to ruminant adipose tissue, an important issue has been quantitative assessments of the contributions of the hexose monophosphate pathway (pentose cycle), the NADP-linked isocitrate dehydrogenase and the malic enzyme to the formation of NADPH₂ for use in fatty acid synthesis, as well as the effects of diet, physiological state and substrate availability upon their relative contributions.

12.3.1 Algebraic equations

A number of algebraic equations have been developed to estimate the contribution of the pentose cycle. Thus, it seems appropriate that these be discussed briefly prior to presenting the modeling approach we have adopted. These methods were discussed in detail by Abraham *et al.* (1957), Katz and Wood (1960), Wood *et al.* (1963), Katz *et al.* (1966) and Katz and Wals (1972). All approaches are based upon the use of a glucose tracer within which a single carbon is labeled and the distribution of individual carbon atoms in products of the pathway is measured (Fig. 12.8a). Use of the term hexose monophosphate shunt in Fig. 12.8(a) is purposefully restrictive. Three moles of hexose-P (G6P) enter the pathway and 2 moles of hexose-P (F6P) and 1 mole of a triose-P (TP) are formed. Nothing is implied about the subsequent fate of products of the pathway. When one considers the subsequent metabolism of the products, confusion arises. Consider Fig. 12.8(b), where fluxes on arrows are expressed in moles/day. Dawes and Holms (1958) suggested that this diagram should be interpreted as indicating that total entry to the G6P pool was 120 moles/day, 100 moles from glucose and 20 as a result of recycling of F6P from the hexose monophosphate shunt and thus completing a cycle termed the pentose cycle. Of the 120 moles entering the G6P pool, 30 entered the hexose monophosphate shunt and, thus, they held that (30/120) 25% of G6P was metabolized via the pentose cycle. In contrast, Katz and Wood (1960) held that the net entry of G6P newly formed from glucose would be 100 moles/day, while 20 arose from the pentose cycle. Thus, they contended that the net (30–20) entry of glucose into the pathway was (10/100), or 10%. This estimate is clearly compatible with the fact that 90 out of 100 moles of glucose used were converted to triose-P via the phosphofructokinase and aldolase reactions, which are unique to the Emden–Meyerhoff glycolytic pathway. The latter definition was generally, but not universally, adopted and will be used herein.

A critical assumption in the derivation of all algebraic equations devised

a



b

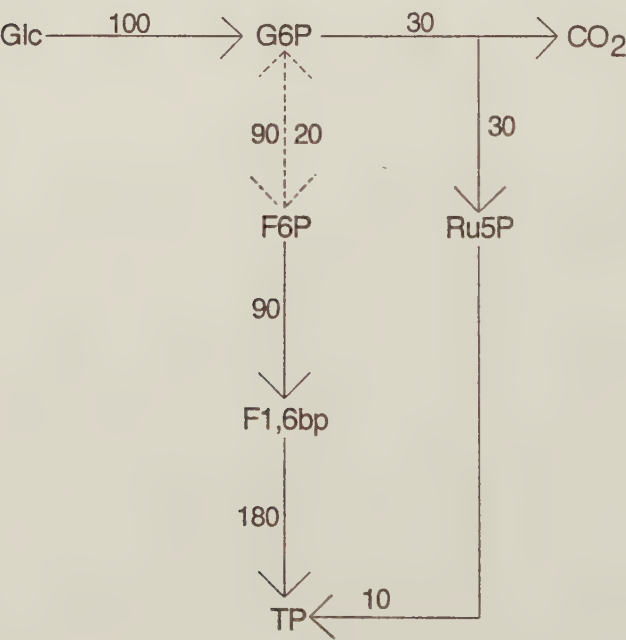
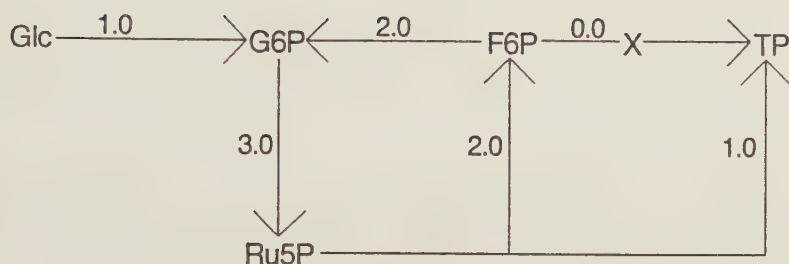


Figure 12.8 (a) Carbon flow in the hexose monophosphate pathway. (b) Pentose cycle and hexose monophosphate shunt recycling in mole/day.

c



d

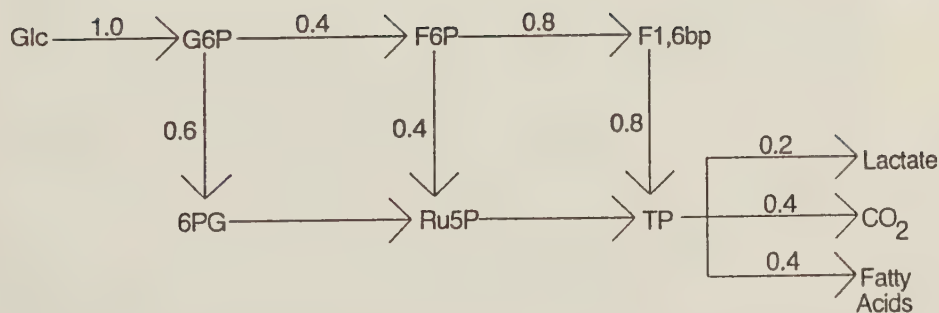


Figure 12.8 (c) Metabolism of glucose via pentose cycle. (d) A hypothetical diagram assuming no recycling of hexose monophosphate and specific activities of G6P equal to specific activities of glucose.

to estimate the contribution of the pentose cycle or hexose monophosphate shunt is whether or not the phosphohexose isomerase (G6P $\rightleftharpoons$ F6P) reaction is in isotopic equilibrium. In formulating algebraic equations, one of two simplifying assumptions must be made. The first is that isotopic equilibrium is achieved and a true (pentose) cycle is achieved. The second is that F6P formed from Ru5P does not recycle back to G6P (hexose monophosphate shunt or HMP). With regard to the first assumption, consider Fig. 12.8(c) where glucose is metabolized only via the pentose cycle. The specific activity (DPM/mole) of the 1-¹⁴C-G6P pool is one-third that of the 1-¹⁴C-glucose pool:

$$Q = \text{dilution factor} = 1.0/(1.0 + 2 * PC) \quad (12.17)$$

Q is not a simple linear function of PC flux, since at proportions of glucose metabolized via the PC of 0.25, 0.50 and 0.75, respective Q values are 0.66, 0.50 and 0.40. The concept of a dilution factor must be applied to glucose specifically labeled in carbons 1, 2 and 3. For glucose labeled in position 2 and 100% metabolism via the pentose cycle, the specific activities (SA) of carbons 1, 2 and 3 in glucose-6-P would be 0.66, 0.33 and 0.33, respectively, and the total SA of G6P would be 1.33 as compared with 2-¹⁴C-glucose (Fig. 12.8a). These estimates do not consider the fact that glyceraldehyde-3-P (G3P) acts as a substrate in the conversion of Ru5P to F6P and that radiotracer incorporated via ¹⁴C-G3P could further increase the SA. If one adopts the assumption of no recycling (HMP), the specific activities of all carbons in G6P are assumed to equal their specific activities in glucose. Consider the fluxes in Fig. 12.8(d), where the specific activity (SA) of glucose is 2000 CPM/mole and fluxes are in $\mu\text{moles/hour}$. In this, EM + PC = 1.0; EM = 0.8; PC = 0.2; and $Q = [1.0/(1.0 + 2.0PC)]$ 0.72. Thus, conversion of 1-¹⁴C-glucose (Gl1) to lactate ($U_{\text{Gl1,La}}$) would be

$$\begin{aligned} U_{\text{Gl1,La}} &= \text{SA}(2000) * \text{EM}(0.8) * Q(0.72) * N(0.2) \\ &= 230 \text{ DPM} \end{aligned} \quad (12.18)$$

while 6-¹⁴C-glucose conversion to lactate would be

$$\begin{aligned} U_{\text{Gl6,La}} &= \text{SA}(2000) * [\text{EM}(0.8) + \text{PC}(0.2)] * N(0.2) \\ &= 400 \text{ DPM} \end{aligned} \quad (12.19)$$

The ratio of rates of conversion of 1-¹⁴C-glucose to lactate:6-¹⁴C-glucose to lactate would be

$$U_{\text{Gl1,La}}/U_{\text{Gl6,La}} = 230/400 = \text{EM} * Q/(\text{EM} + \text{PC}) \quad (12.20)$$

and, substituting from above one computes the following:

$$\begin{aligned} U_{\text{Gl1,La}}/U_{\text{Gl6,La}} &= [(1.0 - \text{PC})/(1.0 + 2.0\text{PC})]/[(1.0 - \text{PC}) + \text{PC}] \text{ and} \\ &= (1.0 - \text{PC})/(1.0 + 2.0\text{PC}) \end{aligned} \quad (12.21)$$

Thus,

$$\begin{aligned}
 U_{\text{Gl1,La}}/U_{\text{Gl6,La}} &= 230/400 = 0.575 = (1 - \text{PC})/(1 + 2\text{PC}) \\
 1.0 - \text{PC} &= 0.575 + 2.0 * 0.575 * \text{PC} \\
 \text{PC} &= 0.425/2.15 = 0.2
 \end{aligned}$$

and

$$\text{EM} = 1.0 - \text{PC} = 0.8$$

Equation 12.21 applies to all products of triose-P metabolism, including acetate, alanine, the glycerol moiety of triacylglycerol and fatty acids, if the inherent assumptions are satisfied that isotopic equilibria are achieved in the phosphohexose isomerase and triose-P isomerase reactions; and that there are no non-triose-P (NTP) pathways of hexose-P metabolism, such as lactose synthesis in mammary tissue and glycogen synthesis in liver. Based upon the same set of assumptions and parallel reasoning, an equation for estimating the relative contributions of the EM and PC pathways from the ratio of $U_{\text{Gl1,La}}/U_{\text{Gl6,La}}$ can be derived. From Fig. 12.8(d),

$$\begin{aligned}
 U_{\text{Gl1,CO}_2} &= \text{SA (2000)} * Q(0.72) * [\text{EM}(0.8) * N(0.4) + 3.0 * \text{PC}(0.2)] \\
 &= 1324.8 \text{ CPM}
 \end{aligned} \tag{12.22}$$

$$U_{\text{Gl6,CO}_2} = \text{SA (2000)} * [\text{EM}(0.8) + \text{PC}(0.2)]N = 800 \text{ CPM} \tag{12.23}$$

and

$$U_{\text{Gl1,CO}_2}/U_{\text{Gl6,CO}_2} = Q(\text{EM} * N + 3\text{PC}) / (\text{EM} + \text{PC})N \tag{12.24}$$

However, when $1.0/(1.0 + 2.0\text{PC})$ is substituted into the equation for Q , N does not cancel out as it did above and, as a result, three unknowns – EM, PC, N – remain. Thus, an additional measurement is required to solve the equation. The easiest additional measurement is of total radiolabeled glucose utilization. Then the specific yields (SY) or ratio of radiolabel recovered in product to total radiolabel used can be calculated as follows:

$$\text{SY}_{\text{Gl1,CO}_2} = \text{CPM in CO}_2/\text{CMP in Gl1 used} = 1324.8/200 = 0.662 \tag{12.25}$$

and

$$\text{SY}_{\text{Gl6,CO}_2} = \text{CPM in CO}_2/\text{CMP in Gl6 used} = 800/2000 = 0.4 \tag{12.26}$$

Since $(\text{EM} + \text{PC})N = N$ and $\text{SY}_{\text{Gl6,CO}_2} = N$,

$$(\text{SY}_{\text{Gl1,CO}_2} - \text{SY}_{\text{Gl6,CO}_2})/(1.0 - \text{SY}_{\text{Gl6,CO}_2}) = 3.0\text{PC}/(1.0 + 2.0\text{PC}) = 0.43 \tag{12.27}$$

and $\text{PC} = 0.2$.

When one assumes that the hexose monophosphate pathway (HMP) operates as a shunt, i.e. radiotracer incorporated in F6P does not recycle into G6P, derivation of equation 12.28 for any triose-P product (Fa used in example)

$$\text{PC} = (U_{\text{Gl6,Fa}} - U_{\text{Gl1, Fa}})/U_{\text{Gl6,Fa}} \tag{12.28}$$

is straightforward (Abraham *et al.*, 1957). Using the tracer recoveries in lactate for $U_{G11,La}$ and $U_{G16,La}$ of 320 and 400 DPM as calculated above, the estimate of the HMP based upon equation 12.28 becomes 0.425. Conversely, if one used the respective $U_{G11,La}$ and $U_{G16,La}$ values of 320 and 400 and equation 12.21, the estimate of PC is 0.077. Many arguments have raged regarding which approach is correct. The respective equations used clearly define the range of possible estimates but in truth, none of the assumptions implied in the equations is likely to be correct under all conditions. An approach that avoids the necessity of simplifying assumptions is required. Development of such an approach has been one of our objectives.

The assumptions of phosphohexose isomerase (PHI) and triose-P isomerase (TPI) equilibria have both been challenged. In adipose tissue, the ratio of U_{G11,CO_2} to U_{G16,CO_2} should equal the ratio of 1- ^{14}C -fructose to 6- ^{14}C -fructose ($U_{G11,CO_2}/U_{G16,CO_2}$) oxidation, since no alternate pathways (aldolase b in liver and lactose synthesis in mammary tissue) exist. Under control conditions *in vitro*, the respective ratios of $U_{G11,CO_2}/U_{G16,CO_2}$ and $U_{Fru1,CO_2}/U_{Fru6,CO_2}$ were similar at 2.2 and 2.1, which supports the hypothesis of radiotracer equilibrium in the PHI reaction. However, in the presence of insulin, the respective ratios were 16.2 and 8.7, which indicates that when rates of lipogenesis are high, the assumptions may not hold.

The assumption of isotopic equilibration among the triose-Ps holds that estimates of the contribution of the pentose cycle based upon $U_{G11,La}$ and $U_{G16,La}$ should equal those based upon the ratio of the rates of conversion of G11 and G16 to the glycerol moiety of triacylglycerol ($U_{G11,Tgg}/U_{G16,Tgg}$). This is an important consideration since 1- ^{14}C -glucose is converted to 3- ^{14}C -dihydroxyacetone-P by the aldolase reaction, while 6- ^{14}C -glucose is converted to 3- ^{14}C -glyceraldehyde-P. Dihydroxyacetone-P is the immediate precursor of α -glycerol-P for fatty acid esterification and glyceraldehyde-3-P is the central intermediate of the Embden-Meyerhoff pathway. Cahil *et al.* (1959) observed that at low to average rates of lipogenesis and high rates of fatty acid esterification, estimates of the contribution of the pentose cycle based upon $U_{G11,La}/U_{G16,La}$ as compared with $U_{G11,Tgg}/U_{G16,Tgg}$ differed 3- to 5-fold, clearly suggesting that isotopic equilibrium may not occur in the triose-P isomerase reaction as is assumed in the above equations and in representing the triose-phosphates as a single pool in this model.

Above, it was noted that a critical assumption in equations 12.21 and 12.28 is that the contributions of NTP pathways of hexose-P metabolism be low. This is generally true for adipose tissue, but not for liver and mammary tissue. In liver, proportions of G6P formed from extracellular glucose converted to glycogen can be quite high. Similarly, depending upon species, 50–80% of glucose taken up by the mammary glands can be converted to lactose and one-half of this glucose is incorporated via the hexose-P pool. It may have been most appropriate to discuss the influence of NTP pathways

upon estimates of pentose cycle flux in the liver and mammary model chapters, but such discussion is most efficient here in context with the background equations. The problem with NTP pathways is that the dilution factor (Q) becomes

$$Q = (1.0 + \text{NTP}) / (1.0 + 2.0\text{PC} + \text{NTP}) \quad (12.29)$$

and, as a result, a direct estimate of NTP becomes essential to solution of equations that estimate the contribution of the pentose cycle. If NTP is ignored, estimates of PC can be 2-fold higher than they should be. In mammary tissue, for example, more accurate estimates can be obtained using the hexose monophosphate shunt (no recycling) assumption of Abraham *et al.* (1957). Clearly, estimates of NTP pathways are as significant as lactose and glycogen synthesis and are essential to interpretation of radiotracer data. Unfortunately, in the majority of experiments reported in the literature these critical measurements were not reported, perhaps because earlier methods for such experiments were laborious and time consuming. These data are essential to the interpretation of data on liver and mammary tissue, whether the equations of Katz and Wals (1972) or our current models are to be used to develop the estimates. Fortunately, more convenient methods for these measurements are now available.

As emphasized above, this discussion of equations that estimate pathways of carbohydrate metabolism is highly abbreviated and readers are directed to references cited for details. Further, many additional algebraic equations for the design and interpretation of radiotracer experiments are available and were not referred to herein. In all of these algebraic equations, simplifying assumptions are critical and controversial. Also, in these algebraic equations only a limited number of data can be utilized. For example, in the equations on glucose metabolism presented above, only data from $1\text{-}^{14}\text{C}$ - and $6\text{-}^{14}\text{C}$ -glucose conversion to single products can be utilized. Based upon Fig. 12.8(a) it is clear that data on $\text{U-}^{14}\text{C}$ -, $2\text{-}^{14}\text{C}$ - and $3\text{-}^{14}\text{C}$ -glucose metabolism and recovery in multiple products would yield additional insight. In estimating patterns of propionate metabolism in liver (Chapter 14), it is equally clear that data on $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate oxidation and conversion to ^{14}C -glucose and on rates of oxygen uptake would be very useful in deducing both rates of gluconeogenesis and flux through the tricarboxylic acid cycle in this tissue.

12.3.2 Dynamic radiotracer elements

Algebraic approaches cannot utilize full data sets effectively. Dynamic, mechanistic models of tissue and animal metabolism used in conjunction with the SIMUSOLVE® (Chapter 4) system allow utilization of all appropriate data sets in analyses of patterns of nutrient utilization. The computers are available and inexpensive and the required software exists. We need no longer be constrained by simplified static equations that utilize limited data.

We must start to use the computer power currently available to further our research.

Two considerations must be addressed in development of a tracer model:

1. Account for radiolabel in each carbon of each metabolite;
2. No discrimination between labeled and unlabeled pools.

As discussed above, labeling patterns of intermediary metabolites must be accounted for in order to simulate recycling of label, e.g. from G6P via Ru and F6P back to G6P and randomization of label between C-2 and C-3 of dicarboxylic acids of the tricarboxylic acid pathway. The second criterion was a troublesome limitation in earlier model versions. When models were comprised of mass action equations only, concentrations of radiotracers used as substrates were characteristically set to 1% of the concentration of the unlabeled substrate. Tracer equations used the same rate constants as those for the unlabeled substrates. The effect of having 101 units of substrate in radiotracer simulations as compared with 100 in the absence of tracer was considered minimal. However, the very small radiotracer pools caused problems during solution. The models were very stiff, requiring the use of very small integration intervals.

When Michaelis-Menten type equations were incorporated into these models, steps had to be taken to assure that the radiotracer equations behave the same as the equations for unlabeled metabolites. One approach could have been to set the affinity constants (k_s) in tracer equations to 1% of those in the unlabeled equations. This would work for labeled substrates, but the specific activities (DPM/mole) of metabolites derived from the radio-labeled substrates are not always 0.01. Another approach would be to set the concentrations of substrate and tracer equal so that equal k_s values could be used. This, of course, would require that tracer metabolism not be represented in differential equations for coenzymes, ATP, ADP, etc., such that behavior of the unlabeled portion of the model not be compromised. The problem with differences in the specific activities of derived metabolites differing from 1.0 would remain. A variation of this approach that accommodates differences in specific activities was adopted. In this, the following equations for the metabolism of individually labeled carbons in glucose-6-P ($G6P_i$) are

$$d_{G6P_i}/dt = SA_{Gl_i} * U_{Gl_i,G6P} - SA_{G6P_i} * U_{G6P,Ru} - SA_{G6P_i} * U_{G6P,F6P} + SA_{F6P_i} * U_{F6P,G6P} \quad (12.30)$$

$$SA_{Gl_i} = Gl_i/Gl \quad (12.30a)$$

$$SA_{F6P_i} = F6P_i/F6P \quad (12.30b)$$

and, for a uniformly labeled glucose tracer

$$SA_{Gl_i} = 0.167 * SAGl \quad (12.30c)$$

where i refers to carbon atoms 1–6 in glucose.

The differential equation for $G6P_i$ (equation 12.30) is a function of fluxes computed in the unlabeled model ($U_{G1,G6P}$, etc.) and the specific activities (SA_{G1} , etc.) of individual carbons of labeled metabolites entering or leaving the pool. Advantages of this approach are that the SA of any radiolabeled substrate can be set to unity without altering the behavior of the unlabeled model, and solutions of the radiotracer elements are stable, efficient and representative. Differential equations for radiotracers in the model follow.

In following chapters on radioisotope metabolism in liver and mammary tissue, representations of the radiotracer elements are not presented as they have already been published. To illustrate the approach, all of the equations will be presented in this chapter. Specific activities of individual carbons of all metabolites are calculated as S_i/S , where S_i denotes radiolabel in carbon i of the labeled substrate and S is the pool size of the substrate at time t . The differential equations for radiolabeled metabolites follow:

Ribulose-5-phosphate (Ru_i):

$$D_{RU1} = SA_{G6P2} * U_{G6P,Ru} - SA_{Ru1} * U_{Ru,F6P} \quad (12.31a)$$

$$D_{RU2} = SA_{G6P3} * U_{G6P,Ru} - SA_{Ru2} * U_{Ru,F6P} \quad (12.31b)$$

$$D_{RU3} = SA_{G6P4} * U_{G6P,Ru} - SA_{Ru3} * U_{Ru,F6P} \quad (12.31c)$$

$$D_{RU4} = SA_{G6P5} * U_{G6P,Ru} - SA_{Ru4} * U_{Ru,F6P} \quad (12.31d)$$

$$D_{RU5} = SA_{G6P6} * U_{G6P,Ru} - SA_{Ru5} * U_{Ru,F6P} \quad (12.31e)$$

Fructose-6-phosphate ($F6P_i$):

$$D_{F6P1} = SA_{Ru1} * 0.667 * U_{Ru,F6P} + SA_{G6P1} * U_{G6P,F6P} - SA_{F6P1} * U_{F6P,G6P} - SA_{F6P1} * U_{F6P,FbP} \quad (12.32a)$$

$$D_{F6P2} = SA_{Ru2} * 0.667 * U_{Ru,F6P} + SA_{G6P2} * U_{G6P,F6P} - SA_{F6P2} * U_{F6P,G6P} - SA_{F6P2} * U_{F6P,FbP} \quad (12.32b)$$

$$D_{F6P3} = SA_{Ru1} * 0.333 * U_{Ru,F6P} + SA_{Ru2} * 0.333 * U_{Ru,F6P} + SA_{G6P3} * U_{G6P,F6P} - SA_{F6P3} * U_{F6P,G6P} - SA_{F6P3} * U_{F6P,FbP} \quad (12.32c)$$

$$D_{F6P4} = SA_{Ru3} * 0.667 * U_{Ru,F6P} + 0.333 * SA_{G3P1} * U_{Ru,F6P} + SA_{G6P4} * U_{G6P,F6P} - SA_{F6P4} * U_{F6P,G6P} - SA_{F6P4} * U_{F6P,FbP} \quad (12.32d)$$

$$D_{F6P5} = SA_{Ru4} * 0.667 * U_{Ru,F6P} + 0.333 * SA_{G3P2} * U_{Ru,F6P} + SA_{G6P5} * U_{G6P,F6P} - SA_{F6P5} * U_{F6P,G6P} - SA_{F6P5} * U_{F6P,FbP} \quad (12.32e)$$

$$D_{F6P6} = SA_{Ru5} * 0.667 * U_{Ru,F6P} + 0.333 * SA_{G3P3} * U_{Ru,F6P} + SA_{G6P6} * U_{G6P,F6P} - SA_{F6P6} * U_{F6P,G6P} - SA_{F6P6} * U_{F6P,FbP} \quad (12.32f)$$

Fructose-1,6-bisphosphate (FbP_i):

$$D_{FbP1} = SA_{FaP1} + U_{F6P,FbP} - SA_{FbP1} * U_{FbP1,G3P} \quad (12.33a)$$

$$D_{FbP2} = SA_{FaP2} + U_{F6P,FbP} - SA_{FbP2} * U_{FbP1,G3P} \quad (12.33b)$$

$$D_{FbP3} = SA_{FaP3} + U_{F6P,FbP} - SA_{FbP3} * U_{FbP1,G3P} \quad (12.33c)$$

$$D_{FbP4} = SA_{FaP4} + U_{F6P,FbP} - SA_{FbP4} * U_{FbP1,G3P} \quad (12.33d)$$

$$D_{FbP5} = SA_{FaP5} + U_{F6P,FbP} - SA_{FbP5} * U_{FbP1,G3P} \quad (12.33e)$$

$$D_{FbP6} = SA_{FaP6} + U_{F6P,FbP} - SA_{FbP6} * U_{FbP1,G3P} \quad (12.33f)$$

Triose-phosphate (TP_i):

$$D_{TP1} = SA_{Ru3} * 0.333 * U_{Ru,F6P} - 0.333 * SA_{TP1} * U_{Ru,F6P} + SA_{FbP4} * U_{FbP,TP} - SA_{TP1} * U_{TP,AgP} + SA_{AgP1} * U_{AgP,TP} - SA_{TP1} * U_{TP,Py} + SA_{Py1} * U_{Py,TP} \quad (12.34a)$$

$$D_{TP2} = SA_{Ru4} * 0.333 * U_{Ru,F6P} - 0.333 * SA_{TP2} * U_{Ru,F6P} + SA_{FbP5} * U_{FbP,TP} - SA_{TP2} * U_{TP,AgP} + SA_{AgP2} * U_{AgP,TP} - SA_{TP2} * U_{TP,Py} + SA_{Py2} * U_{Py,TP} \quad (12.34b)$$

$$D_{TP3} = SA_{Ru5} * 0.333 * U_{Ru,F6P} - 0.333 * SA_{TP3} * U_{Ru,F6P} + SA_{FbP6} * U_{FbP,TP} - SA_{TP3} * U_{TP,AgP} + SA_{AgP3} * U_{AgP,TP} - SA_{TP3} * U_{TP,Py} + SA_{Py3} * U_{Py,TP} \quad (12.34c)$$

 α -Glycerol-phosphate (AgP_i):

$$D_{AgP1} = SA_{FbP3} * U_{FbP,TP} + SA_{TP1} * U_{TP,AgP} - SA_{AgP1} * U_{AgP,TP} - 0.333 * SA_{AgP1} * U_{FaCs,Ts} \quad (12.35a)$$

$$D_{AgP2} = SA_{FbP2} * U_{FbP,TP} + SA_{TP2} * U_{TP,AgP} - SA_{AgP2} * U_{AgP,TP} - 0.333 * SA_{AgP2} * U_{FaCs,Ts} \quad (12.35b)$$

$$D_{AgP1} = SA_{FbP1} * U_{FbP,TP} + SA_{TP3} * U_{TP,AgP} - SA_{AgP3} * U_{AgP,TP} - 0.333 * SA_{AgP3} * U_{FaCs,Ts} \quad (12.35c)$$

Pyruvate (Py_i):

$$D_{Py1} = SA_{TP1} * U_{TP,Py} - SA_{Py1} * U_{Py,TP} - SA_{Py1} * U_{Py,La} + SA_{La1} * U_{La,Py} - SA_{Py1} * U_{Py,mAcCs} - SA_{Py1} * U_{Py,Oa} + SA_{Mal1} * U_{Mal,Py} \quad (12.36a)$$

$$D_{Py2} = SA_{TP2} * U_{TP,Py} - SA_{Py2} * U_{Py,TP} - SA_{Py2} * U_{Py,La} + SA_{La2} * U_{La,Py} - SA_{Py2} * U_{Py,mAcCs} - SA_{Py2} * U_{Py,Oa} + SA_{Mal2} * U_{Mal,Py} \quad (12.36b)$$

$$D_{Py3} = SA_{TP3} * U_{TP,Py} - SA_{Py3} * U_{Py,TP} - SA_{Py3} * U_{Py,La} + SA_{La3} * U_{La,Py} - SA_{Py3} * U_{Py,mAcCs} - SA_{Py3} * U_{Py,Oa} + SA_{Mal3} * U_{Mal,Py} \quad (12.36c)$$

Lactate (La_i):

$$D_{La1} = SA_{Py1} * U_{Py,La} - SA_{La1} * U_{La,Py} - SA_{La1} * U_{La,eLa} \quad (12.37a)$$

$$D_{La2} = SA_{Py2} * U_{Py,La} - SA_{La2} * U_{La,Py} - SA_{La2} * U_{La,eLa} \quad (12.37b)$$

$$D_{La3} = SA_{Py3} * U_{Py,La} - SA_{La3} * U_{La,Py} - SA_{La3} * U_{La,eLa} \quad (12.37c)$$

Extracellular lactate (eLa_i):

$$D_{eLa1} = SA_{La1} * U_{La,eLa} \quad (12.38a)$$

$$D_{eLa2} = SA_{La2} * U_{La,eLa} \quad (12.38b)$$

$$D_{eLa3} = SA_{La3} * U_{La,eLa} \quad (12.38c)$$

Mitochondrial acetyl-CoA (mAcCs_i):

$$D_{mAcCs1} = SA_{Py2} * U_{Py,mAcCs} - SA_{mAc1} * U_{Oa,Ct} + 0.5 * SA_{AcU} * U_{Ac,mAcCs} + SA_{Ac1} * U_{Ac,mAcCs} \quad (12.39a)$$

$$D_{mAcCs2} = SA_{Py3} * U_{Py,mAcCs} - SA_{mAc2} * U_{Oa,Ct} + 0.5 * SA_{AcU} * U_{Ac,mAcCs} + SA_{Ac2} * U_{Ac,mAcCs} \quad (12.39b)$$

Citrate (Ct_i):

$$D_{Ct1} = SA_{Oa4} * U_{Oa,Ct} - SA_{Ct1} * U_{Ct,Oa} - SA_{Ct1} * U_{Ct,Akg} - SA_{Ct1} * U_{Ctp,Akg} + SA_{Akg1} * U_{Akg,Ctp} \quad (12.40a)$$

$$D_{Ct2} = SA_{Oa3} * U_{Oa,Ct} - SA_{Ct2} * U_{Ct,Oa} - SA_{Ct2} * U_{Ct,Akg} - SA_{Ct2} * U_{Ctp,Akg}$$

$$U_{Ctp,Akg} + SA_{Akg2} * U_{Akg,Ctp} \quad (12.40b)$$

$$D_{Ct3} = SA_{Oa2} * U_{Oa,Ct} - SA_{Ct3} * U_{Ct,Oa} - SA_{Ct3} * U_{Ct,Akg} - SA_{Ct3} * U_{Ctp,Akg} + SA_{Akg3} * U_{Akg,Ctp} \quad (12.40c)$$

$$D_{Ct4} = SA_{mAc2} * U_{Oa,Ct} - SA_{Ct4} * U_{Ct,Oa} - SA_{Ct4} * U_{Ct,Akg} - SA_{Ct4} * U_{Ctp,Akg} + SA_{Akg4} * U_{Akg,Ctp} \quad (12.40d)$$

$$D_{Ct5} = SA_{mAc1} * U_{Oa,Ct} - SA_{Ct5} * U_{Ct,Oa} - SA_{Ct5} * U_{Ct,Akg} - SA_{Ct5} * U_{Ctp,Akg} + SA_{Akg5} * U_{Akg,Ctp} \quad (12.40e)$$

$$D_{Ct6} = SA_{Oa1} * U_{Oa,Ct} - SA_{Ct6} * U_{Ct,Oa} - SA_{Ct6} * U_{Ct,Akg} - SA_{Ct6} * U_{Ctp,Akg} + SA_{CdU} * U_{Akg,Ctp} \quad (12.40f)$$

α -Ketoglutarate (Akg_i):

$$D_{Akg1} = SA_{Ct1} * U_{Ct,Akg} + SA_{Ct1} * U_{Ctp,Akg} - SA_{Akg1} * U_{Akg,Ctp} - SA_{Akg1} * U_{Akg,Mal} \quad (12.41a)$$

$$D_{Akg2} = SA_{Ct2} * U_{Ct,Akg} + SA_{Ct2} * U_{Ctp,Akg} - SA_{Akg2} * U_{Akg,Ctp} - SA_{Akg2} * U_{Akg,Mal} \quad (12.41b)$$

$$D_{Akg3} = SA_{Ct3} * U_{Ct,Akg} + SA_{Ct3} * U_{Ctp,Akg} - SA_{Akg3} * U_{Akg,Ctp} - SA_{Akg3} * U_{Akg,Mal} \quad (12.41c)$$

$$D_{Akg4} = SA_{Ct4} * U_{Ct,Akg} + SA_{Ct4} * U_{Ctp,Akg} - SA_{Akg4} * U_{Akg,Ctp} - SA_{Akg4} * U_{Akg,Mal} \quad (12.41d)$$

$$D_{Akg5} = SA_{Ct5} * U_{Ct,Akg} + SA_{Ct5} * U_{Ctp,Akg} - SA_{Akg5} * U_{Akg,Ctp} - SA_{Akg5} * U_{Akg,Mal} \quad (12.41e)$$

Malate (Mal_i):

$$D_{Mal1} = 0.5 * SA_{Akg2} * U_{Akg,Mal} + 0.5 * SA_{Akg5} * U_{Akg,Mal} - SA_{Mal1} * U_{Mal,Oa} + SA_{Oa1} * U_{Oa,Mal} - SA_{Mal1} * U_{Mal,Py} \quad (12.42a)$$

$$D_{Mal2} = 0.5 * SA_{Akg3} * U_{Akg,Mal} + 0.5 * SA_{Akg4} * U_{Akg,Mal} - SA_{Mal2} * U_{Mal,Oa} + SA_{Oa2} * U_{Oa,Mal} - SA_{Mal2} * U_{Mal,Py} \quad (12.42b)$$

$$D_{Mal3} = 0.5 * SA_{Akg3} * U_{Akg,Mal} + 0.5 * SA_{Akg4} * U_{Akg,Mal} - SA_{Mal3} * U_{Mal,Oa} + SA_{Oa3} * U_{Oa,Mal} - SA_{Mal3} * U_{Mal,Py} \quad (12.42c)$$

$$D_{Mal4} = 0.5 * SA_{Akg4} * U_{Akg,Mal} + 0.5 * SA_{Akg5} * U_{Akg,Mal} - SA_{Mal4} * U_{Mal,Oa} + SA_{Oa4} * U_{Oa,Mal} - SA_{Mal4} * U_{Mal,Py} \quad (12.42d)$$

Oxaloacetate (Oa_i):

$$D_{Oa1} = SA_{Py1} * U_{Py,Oa} - SA_{Oa1} * U_{Oa,Ct} + SA_{Ct4} * U_{Ct,Oa} + SA_{Mal1} * U_{Mal,Oa} - SA_{Oa1} * U_{Oa,Mal} \quad (12.43a)$$

$$D_{Oa2} = SA_{Py2} * U_{Py,Oa} - SA_{Oa2} * U_{Oa,Ct} + SA_{Ct3} * U_{Ct,Oa} + SA_{Mal2} * U_{Mal,Oa} - SA_{Oa2} * U_{Oa,Mal} \quad (12.43b)$$

$$D_{Oa3} = SA_{Py3} * U_{Py,Oa} - SA_{Oa3} * U_{Oa,Ct} + SA_{Ct2} * U_{Ct,Oa} + SA_{Mal3} * U_{Mal,Oa} - SA_{Oa3} * U_{Oa,Mal} \quad (12.43c)$$

$$D_{Oa4} = SA_{Py4} * U_{Py,Oa} - SA_{Oa4} * U_{Oa,Ct} + SA_{Ct1} * U_{Ct,Oa} + SA_{Mal4} * U_{Mal,Oa} - SA_{Oa4} * U_{Oa,Mal} \quad (12.43d)$$

Cytosolic acetyl-CoA (AcCs_i):

$$D_{AcCs1} = SA_{Ct5} * U_{Ct,Oa} - SA_{AcCs1} * U_{AcCs,Fa} + 0.5 * SA_{AcU} * U_{Ac,AcCs} + SA_{Ac1} * U_{Ac,AcCs} \quad (12.44a)$$

$$D_{AcCs2} = \frac{SA_{AcCt4} * U_{Ct,Oa} - SA_{AcCs2} * U_{AcCs,Fa} + 0.5 * SA_{AcU} * U_{Ac,AcCs}}{SA_{AcCs} + SA_{Ac2} * U_{Ac,AcCs}} \quad (12.44b)$$

Fatty acids (Fai):

$$D_{Fa1} = SA_{AcCs1} * U_{AcCs,Fa} \quad (12.45a)$$

$$D_{Fa2} = SA_{AcCs2} * U_{AcCs,Fa} \quad (12.45b)$$

Triacylglycerol (Tsi):

$$D_{Ts1} = 0.333 * SA_{AgP1} * U_{Fa,CsTs} \quad (12.46a)$$

$$D_{Ts2} = 0.333 * SA_{AgP2} * U_{Fa,CsTs} \quad (12.46b)$$

$$D_{Ts3} = 0.333 * SA_{AgP3} * U_{Fa,CsTs} \quad (12.46c)$$

Carbon dioxide (CdU):

$$D_{CdU} = SA_{G6P1} * U_{G6P,Ru} + SA_{Py1} * U_{Py,mAcCs} - SA_{CdU} * U_{Py,Oa} + SA_{Ct6} * U_{Ct,Akg} + SA_{Ct6} * U_{Ct,pAkg} - SA_{CdU} * U_{Akg,Ctp} + SA_{Akg1} * U_{Akg,Mal} + SA_{Mal4} * U_{Mal,Py} - SA_{CdU} * U_{Cd,eCd} \quad (12.47)$$

The Advanced Continuous Simulation Language (ACSL, 1990) was used to generate model outputs presented below.

(a) Behavior analyses

Simulations were conducted to examine patterns of metabolite utilization, substrate interactions, radioisotope conversion to products and effects of insulin on adipose tissue metabolic activities. The goal of these behavior analyses was to evaluate the model and determine whether or not, as written, it can simulate response patterns observed in experiments with ruminant adipose tissue.

Figures 12.9–12.11 illustrate the patterns of metabolism of $1\text{-}^{14}\text{C}$, $2\text{-}^{14}\text{C}$, $6\text{-}^{14}\text{C}$ and $\text{U-}^{14}\text{C}$ -glucose metabolism. Rates of $^{14}\text{CO}_2$ production from all glucose tracers exhibit saturation kinetics as expected (Fig. 12.9). At saturation the differences in magnitude of $^{14}\text{CO}_2$ production from the four glucose tracers are compatible with experimental data (Yang and Baldwin, 1973a). Production of $^{14}\text{CO}_2$ is highest from $1\text{-}^{14}\text{C}$ -glucose, since all of carbon 1 is converted to CO_2 in a single pass through the hexose monophosphate shunt (pentose pathway). The conversion of $2\text{-}^{14}\text{C}$ -glucose to $^{14}\text{CO}_2$ requires recycling through the pentose cycle. Therefore, the ratio of $2\text{-}^{14}\text{C}$ to $1\text{-}^{14}\text{C}$ -glucose conversion to $^{14}\text{CO}_2$ is an index of the extent of carbon recycling through the pentose pathway. Glucose labeled in the 6 position is not oxidized in the pentose cycle and is only converted to $^{14}\text{CO}_2$ via entry into the tricarboxylic acid (TCA) cycle. Since the activity of ruminant adipose tissue pyruvate dehydrogenase is low in the presence of acetate, conversion of $6\text{-}^{14}\text{C}$ -glucose to $^{14}\text{CO}_2$ is low. Uniformly labeled ^{14}C -glucose oxidation is between 1- , 2- and $6\text{-}^{14}\text{C}$ -glucose conversion to $^{14}\text{CO}_2$ and provides an estimate of overall glucose oxidation.

Figures 12.10 and 12.11 show the effect of low (INS = 0.01) and high (INS

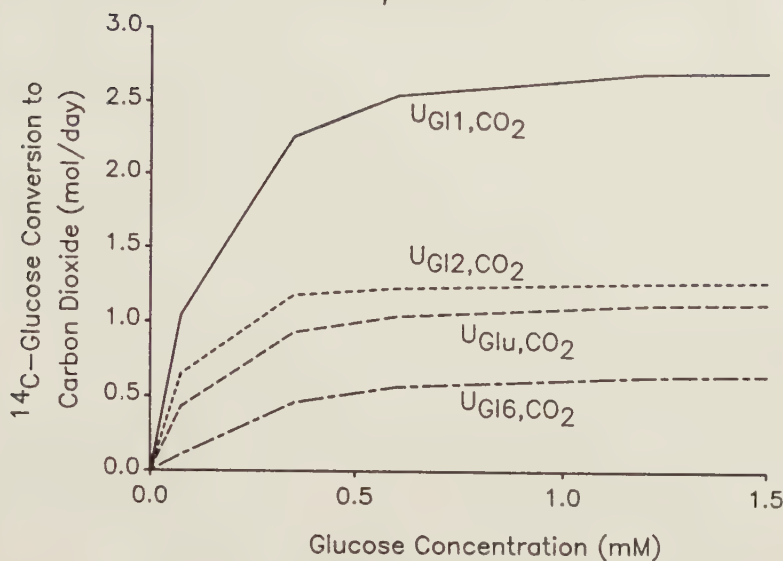


Figure 12.9 Radiolabeled ^{14}C -glucose conversion to extracellular ^{14}C -carbon dioxide ($^{14}\text{CO}_2$) with respect to glucose concentration. $U_{\text{GI1},\text{CO}_2}$, $U_{\text{GI2},\text{CO}_2}$, $U_{\text{GI6},\text{CO}_2}$ and $U_{\text{GIu},\text{CO}_2}$, respectively, denote the production of $^{14}\text{CO}_2$ from $1\text{-}^{14}\text{C}$ -, $2\text{-}^{14}\text{C}$ -, $6\text{-}^{14}\text{C}$ - and uniformly labeled (U)- ^{14}C -glucose. Conditions of simulations were: insulin multiplier (INS) = 1.0, acetate concentration = 1.5mM, and specific activities of $1\text{-}^{14}\text{C}$ -, $2\text{-}^{14}\text{C}$ -, $6\text{-}^{14}\text{C}$ - and U- ^{14}C -glucose were 1.0.

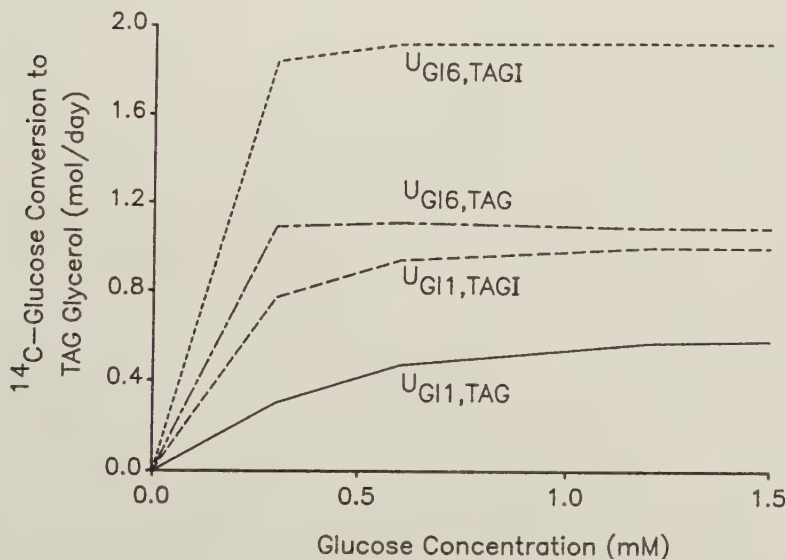


Figure 12.10 Radiolabeled ^{14}C -glucose conversion to triacylglycerol (TAG) ^{14}C -glycerol with respect to glucose concentration. $U_{\text{GI1},\text{TAG}}$ and $U_{\text{GI6},\text{TAG}}$ denote, respectively, the production of TAG ^{14}C -glycerol from $1\text{-}^{14}\text{C}$ - and $6\text{-}^{14}\text{C}$ -glucose at low insulin (insulin multiplier (INS) = 0.01). $U_{\text{GI1},\text{TAGI}}$ and $U_{\text{GI6},\text{TAGI}}$ denote TAG ^{14}C -glycerol production at high insulin (INS = 100). Acetate concentration was 1.5mM, and specific activities of $1\text{-}^{14}\text{C}$ - and $6\text{-}^{14}\text{C}$ -glucose were 1.0.

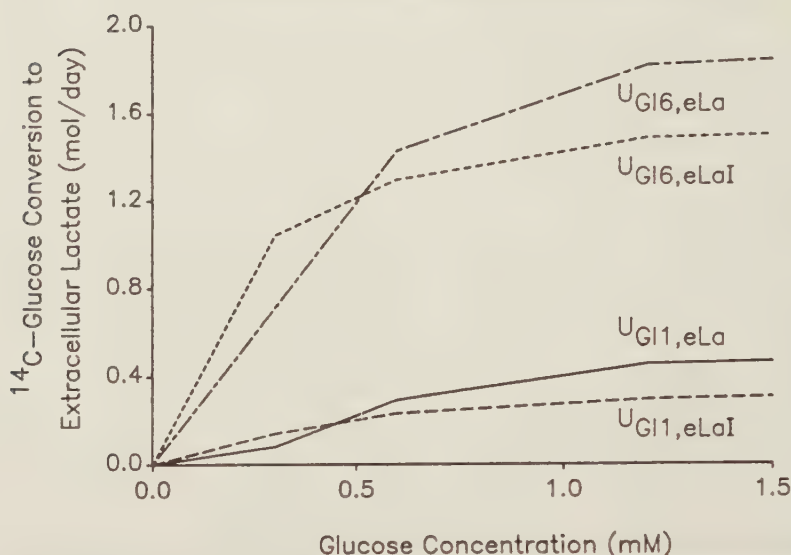


Figure 12.11 Radiolabeled ^{14}C -glucose conversion to ^{14}C -extracellular lactate with respect to glucose concentration. $U_{G11,eLa}$ and $U_{G16,eLa}$ denote, respectively, the production of ^{14}C -extracellular lactate from 1- ^{14}C - and 6- ^{14}C -glucose at low insulin (insulin multiplier (INS) = 0.01). $U_{G11,eLaI}$ and $U_{G16,eLaI}$ denote ^{14}C -extracellular lactate production at high insulin (INS = 100). Acetate concentration was 1.5 mM, and specific activities of 1- ^{14}C - and 6- ^{14}C -glucose were 1.0.

= 100) insulin concentrations on rates of 1- and 6- ^{14}C -glucose conversion to triacylglycerol (TAG) ^{14}C -glycerol and ^{14}C -extracellular lactate with respect to glucose concentration. The rates of 6- ^{14}C -glucose conversion to TAG ^{14}C -glycerol and ^{14}C -extracellular lactate are greater than for 1- ^{14}C -glucose, since a high proportion of 1- ^{14}C -glucose label is lost via the pentose pathway and results in low specific activities in this position in G6P and F6P (equation 12.17). Insulin increases the relative rate of ^{14}C -glucose conversion to TAG ^{14}C -glycerol (Fig. 12.10) and reduces the formation of ^{14}C -extracellular lactate (Fig. 12.11). At saturating concentrations of glucose, insulin increased 1- ^{14}C - and 6- ^{14}C -glucose conversion to TAG ^{14}C -glycerol equally (67 and 72%). Also, at high insulin concentrations, proportionally more glucose is metabolized via the Embden-Meyerhoff glycolytic pathway as compared with that utilized for NADPH₂ production. As a result, high insulin decreased the respective rates of 1- ^{14}C - and 6- ^{14}C -glucose conversion to ^{14}C -extracellular lactate by 50 and 20% (Fig. 12.11). The increase in total CO₂ production at high insulin levels (161%; $U_{Sij,Cd}$ versus $U_{Sij,CdI}$; Fig. 12.12) was substantially greater than the increase in total NADPH₂ production (58%; $U_{Np,NpH}$ versus $U_{Np,NpHI}$; Fig. 12.12), which indicated differential increases in the requirements for energy and reducing equivalents at greater rates of lipogenesis.

The rates of 1- ^{14}C - and 6- ^{14}C -glucose conversion to $^{14}\text{CO}_2$ were essentially zero order with respect to extracellular acetate concentrations (Fig. 12.13)

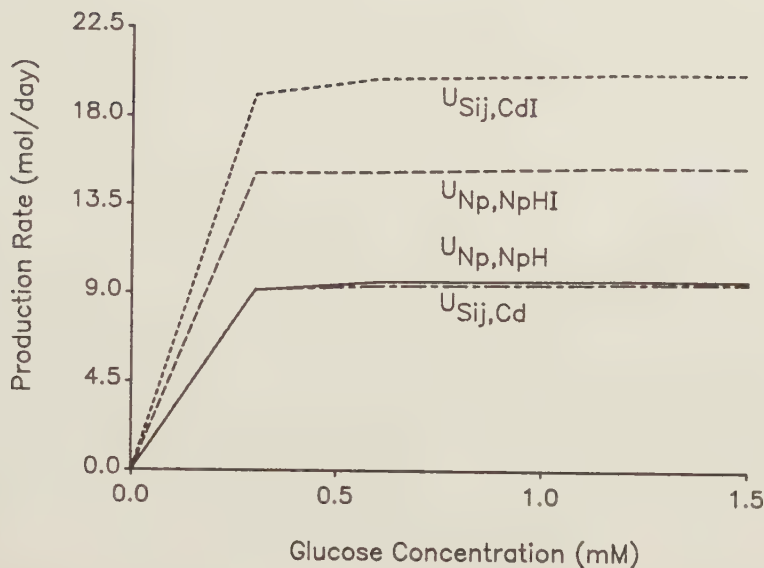


Figure 12.12 Total NADPH_2 ($U_{\text{Np,NpH}}$) and carbon dioxide ($U_{\text{Sij,Cd}}$) production at low insulin (insulin multiplier (INS) = 0.01) and at high insulin (insulin multiplier (INS) = 100); $U_{\text{Np,NpHI}}$, $U_{\text{Sij,CdI}}$ with respect to glucose concentration. Acetate concentration was 1.5mM.

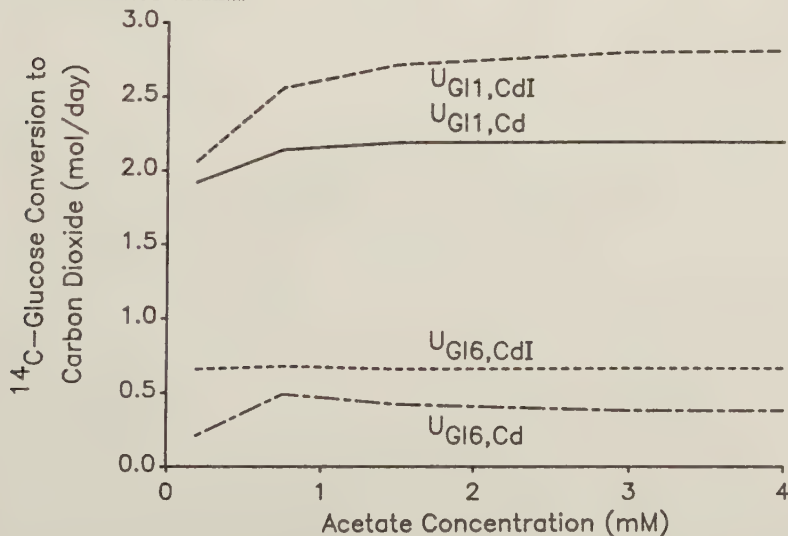


Figure 12.13 Radiolabeled ^{14}C -glucose conversion to extracellular $^{14}\text{CO}_2$ with respect to acetate concentration. $U_{\text{Gl1,Cd}}$ and $U_{\text{Gl6,Cd}}$ denote, respectively, 1- ^{14}C - and 6- ^{14}C -glucose oxidation at low insulin (0.01). $U_{\text{Gl1,CdI}}$ and $U_{\text{Gl6,CdI}}$ denote, respectively, 1- ^{14}C - and 6- ^{14}C -glucose oxidation at high insulin (100). Glucose concentration was 0.6 mM.

over the physiological range (1.5–4.0 mM) except for the production of $^{14}\text{CO}_2$ from 1- ^{14}C -glucose at high insulin levels. These relationships illustrate the high demand for NADPH_2 for fatty acid synthesis when acetate and insulin are present and are in agreement with the pattern reported by Yang and Baldwin (1973a, Fig. 12.5).

Triacylglycerol ^{14}C -glycerol formation from 1- ^{14}C - and 6- ^{14}C -glucose decreased 40 and 32%, respectively, between 0.1 mM (subphysiological) and 2.0 mM (physiological) acetate concentrations (Fig. 12.14). The decrease in TAG ^{14}C -glycerol formation was due to a reduction in the fatty acid pool size. The glucose concentration of 0.6 mM limits fatty acid synthesis in the model due to reduced NADPH_2 production via the pentose pathway. The fatty acid pool size was further reduced by an increase in movement of fatty acids into the extracellular pool ($U_{\text{Fa,eFa}}$). The conversion of 6- ^{14}C -glucose to TAG ^{14}C -glycerol was maintained at high insulin because of an increase in the SA of the α -glycerol-P pool. Comparable experimental data are not available. These results are presented to illustrate the possibility that such an experiment could yield critical insight regarding the dynamics of the triglyceride hydrolysis, lipogenesis and the fatty acid re-esterification substrate cycle.

Insulin reduced the conversion of 1- ^{14}C - and 6- ^{14}C -glucose to ^{14}C -extracellular lactate (Fig. 12.15). These responses reflect increased recycling through the pentose pathway and an increase in the specific activity of α -glycerol-P. Rates of NADPH_2 production increased as acetate concentration increased (Fig. 12.16). Total rates of NADPH_2 and CO_2 production were sensitive at acetate concentrations in the physiological range (1.0–3.0 mM). The reduced sensitivities in production rates of CO_2 , TAG glycerol and extracellular lactate at the low insulin was a consequence of limited flow of glucose carbon through the Embden–Meyerhoff glycolytic pathway.

Figures 12.17–12.20 collectively illustrate model behavior in terms of ^{14}C -acetate conversion to $^{14}\text{CO}_2$, ^{14}C -extracellular lactate and ^{14}C -fatty acids with respect to acetate concentrations. Patterns of response, where comparable, are compatible with Figs 12.5 and 12.6 and other data published by Yang and Baldwin (1973a). Acetate labeled in the 1 position is converted to $^{14}\text{CO}_2$ after one spin of the TCA cycle, while 2- ^{14}C -acetate is converted to $^{14}\text{CO}_2$ in subsequent turns. Therefore, the production of $^{14}\text{CO}_2$ from 1- ^{14}C -acetate should be greater than that from 2- ^{14}C -acetate with U- ^{14}C -acetate being intermediate, as illustrated in Fig. 12.7. Figures 12.18 and 12.19 illustrate that the conversion of ^{14}C -acetate to CO_2 and extracellular lactate are increased by insulin, and that these conversion rates are proportionally more sensitive to insulin at low acetate concentrations. The increase in $^{14}\text{CO}_2$ production observed with high insulin can be attributed to a 2- to 3-fold increase in the flux into and through the TCA cycle. The increase in ^{14}C -extracellular lactate formation resulted from a 3-fold increase in $U_{\text{Mal,Py}}$, reflecting the effect of insulin upon rates of fatty acid synthesis illustrated

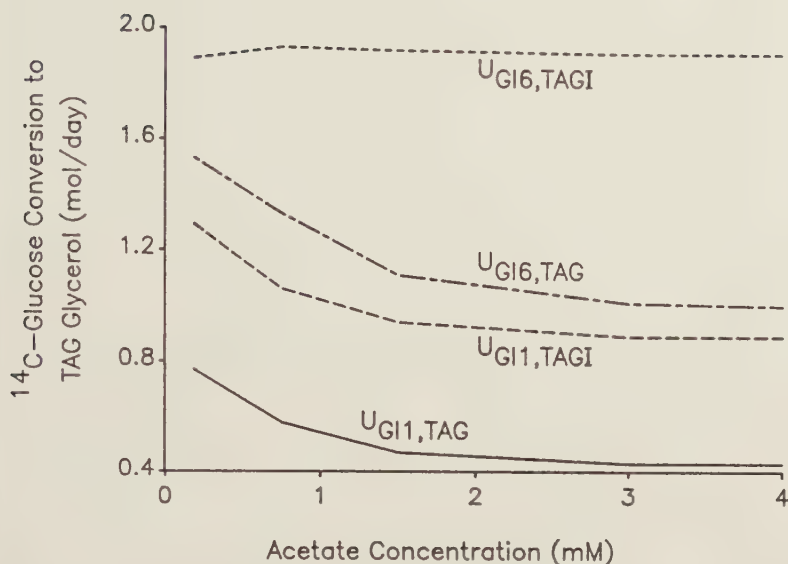


Figure 12.14 Radiolabeled ^{14}C -glucose conversion to triacylglycerol (TAG) ^{14}C -glycerol with respect to acetate concentration. $U_{\text{GI1,TAG}}$ and $U_{\text{GI6,TAG}}$ denote, respectively, incorporation of 1- ^{14}C - and 6- ^{14}C -glucose at low insulin (0.01). $U_{\text{GI1,TAGI}}$ and $U_{\text{GI6,TAGI}}$ denote incorporation at high insulin (INS = 100). Glucose concentration was 0.6mM.

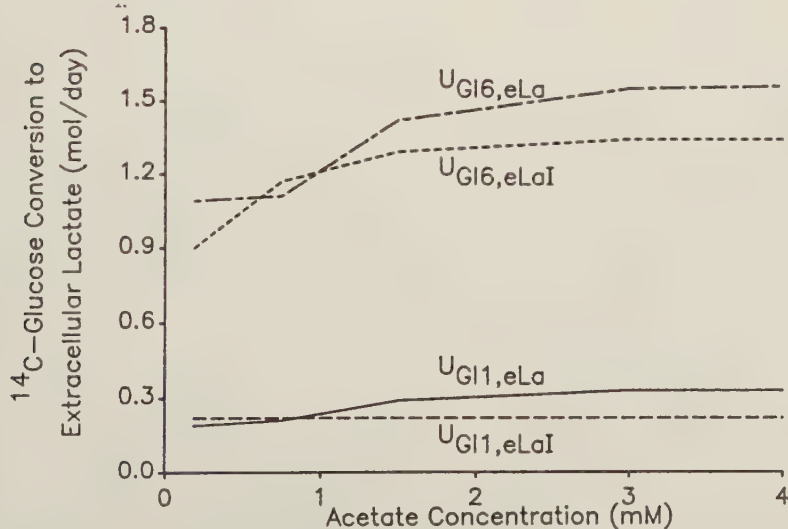


Figure 12.15 Radiolabeled ^{14}C -glucose conversion to ^{14}C -extracellular lactate with respect to acetate concentration. $U_{\text{GI1,eLa}}$ and $U_{\text{GI6,eLa}}$ denote, respectively, the production of ^{14}C -extracellular lactate from 1- ^{14}C - and 6- ^{14}C -glucose at low insulin (0.01). $U_{\text{GI1,eLaI}}$ and $U_{\text{GI6,eLaI}}$ denote, ^{14}C -extracellular lactate production at high insulin (100). Glucose concentration was 0.6 mM.

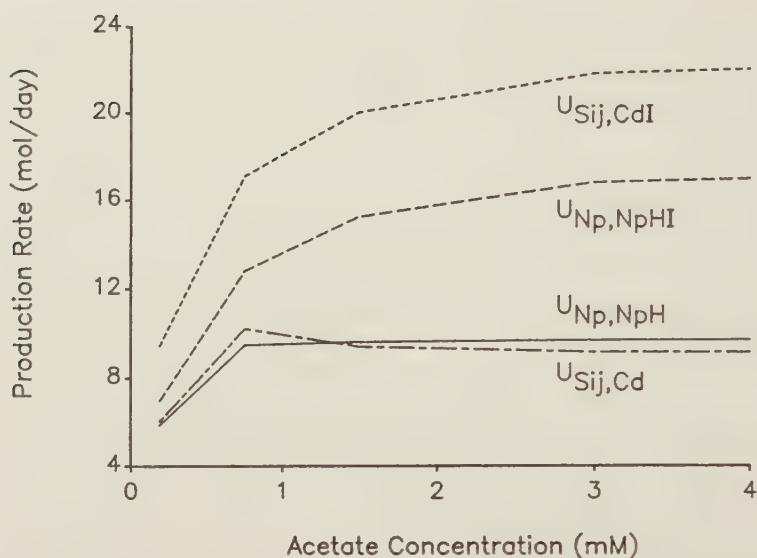


Figure 12.16 Total NADPH_2 ($U_{\text{Np,NpH}}$) and carbon dioxide ($U_{\text{Sij,Cd}}$) production at low insulin (0.01) and high ($U_{\text{Np,NpHI}}$, $U_{\text{Sij,CdI}}$) insulin (100) at varying acetate concentration. Glucose concentration was 0.6 mM.

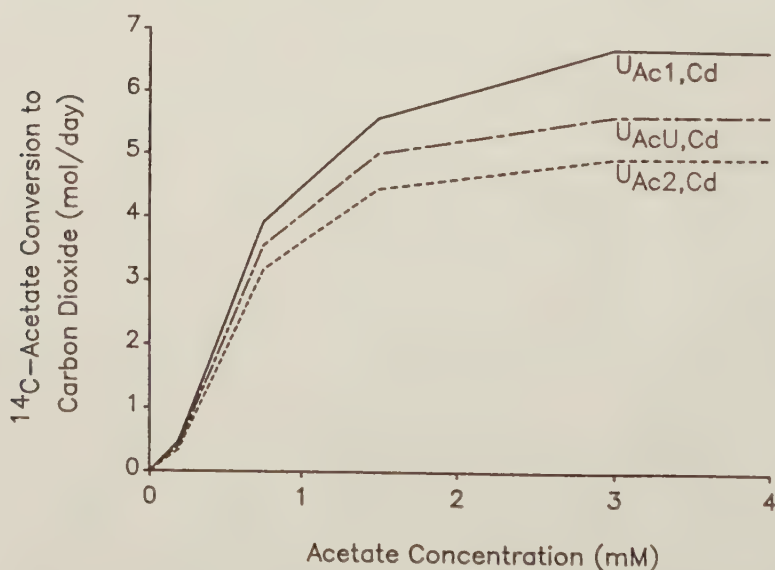


Figure 12.17 Radiolabeled acetate conversion to ^{14}C -carbon dioxide with respect to acetate concentration. $U_{\text{Ac1,Cd}}$, $U_{\text{Ac2,Cd}}$ and $U_{\text{AcU,Cd}}$, respectively, denote the production of $^{14}\text{CO}_2$ from $1\text{-}^{14}\text{C}$ -, $2\text{-}^{14}\text{C}$ - and uniformly (U)-labeled acetate. Insulin multiplier was 1.0 and glucose concentration was 0.6 mM.

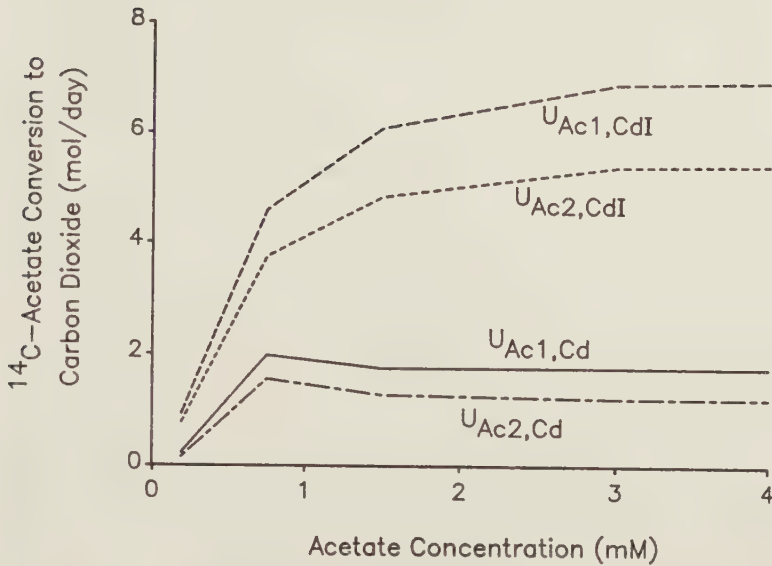


Figure 12.18 Radiolabeled acetate oxidation as effected by acetate concentration and insulin. $U_{\text{Ac1,Cd}}$ and $U_{\text{Ac2,Cd}}$ denote, respectively, $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate oxidation at low insulin (0.01) and $U_{\text{Ac1,CdI}}$ and $U_{\text{Ac2,CdI}}$ denote oxidation rates at high insulin (100). Glucose concentration was 0.6 mM.

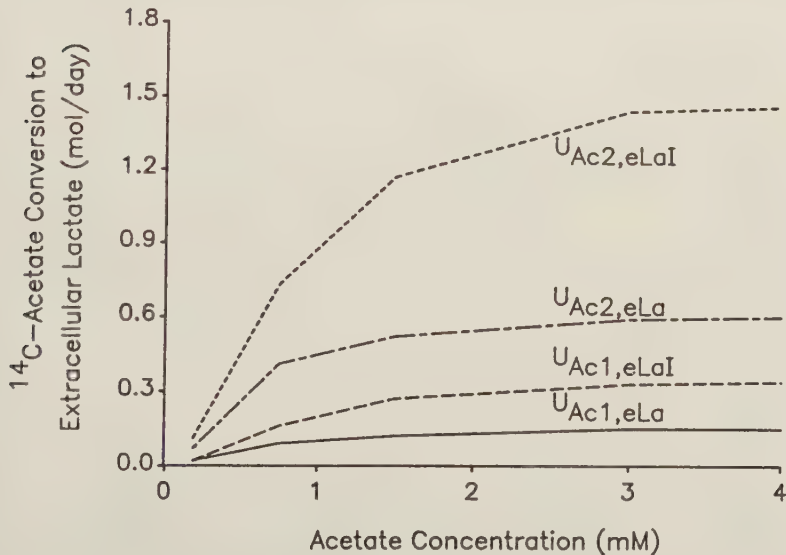


Figure 12.19 Radiolabeled acetate conversion to ^{14}C -extracellular lactate with respect to acetate concentration. $U_{\text{Ac1,eLa}}$ and $U_{\text{Ac2,eLa}}$ denote, respectively, the production of ^{14}C -extracellular lactate from $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate at low insulin (insulin multiplier (INS) = 0.01). $U_{\text{Ac1,eLaI}}$ and $U_{\text{Ac2,eLaI}}$ denote ^{14}C -extracellular lactate production at high insulin (INS = 100). Glucose concentration was 0.6 mM. The specific activities of $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate were set at 1.0.

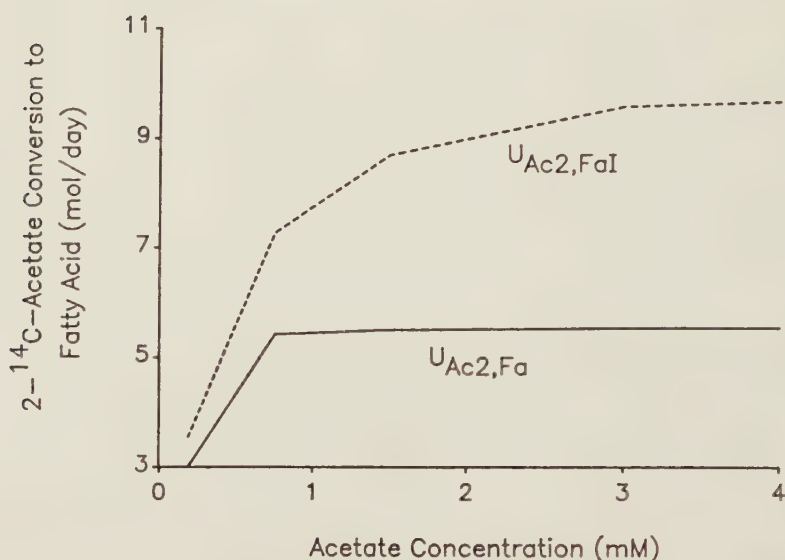


Figure 12.20 Radiolabeled 2- ^{14}C -acetate conversion to ^{14}C -fatty acid with respect to acetate concentration. $U_{\text{Ac2,Fa}}$ and $U_{\text{Ac2,FaI}}$ denote rates of ^{14}C -fatty acid production at low and high insulin, respectively (low insulin, $\text{INS} = 1.0$; high insulin, $\text{INS} = 100$). Glucose concentration was 0.6 mM. The specific activity of 2- ^{14}C -acetate was set at 1.0.

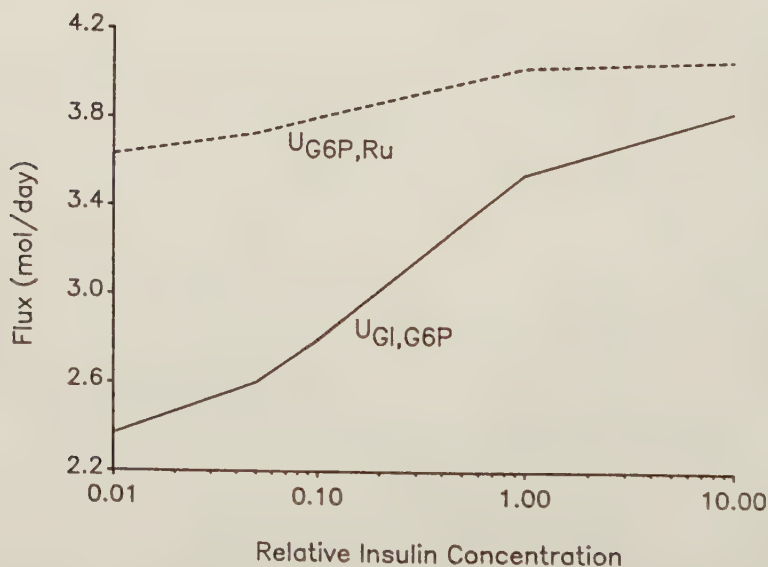


Figure 12.21 Fluxes of glucose to glucose-6-phosphate ($U_{\text{Gl,G6p}}$) and glucose-6-phosphate to ribulose ($U_{\text{G6p,Ru}}$) with respect to insulin (insulin multiplier (INS)). Glucose concentration was set at 0.6 mM and acetate concentration at 1.5 mM.

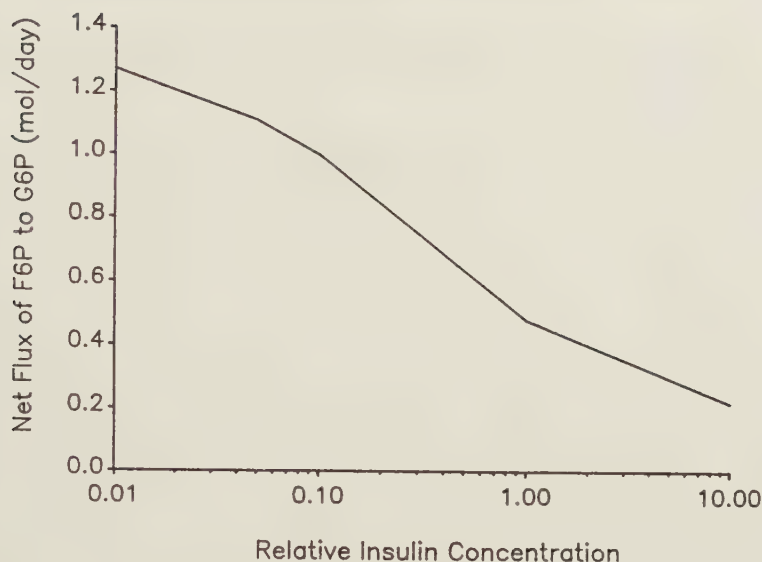


Figure 12.22 Net flux of fructose-6-phosphate to glucose-6-phosphate ($U_{F6P,G6P} - U_{G6P,F6P}$) with respect to relative insulin concentration. Glucose and acetate concentrations set at 0.6 mM and 1.5 mM, respectively.

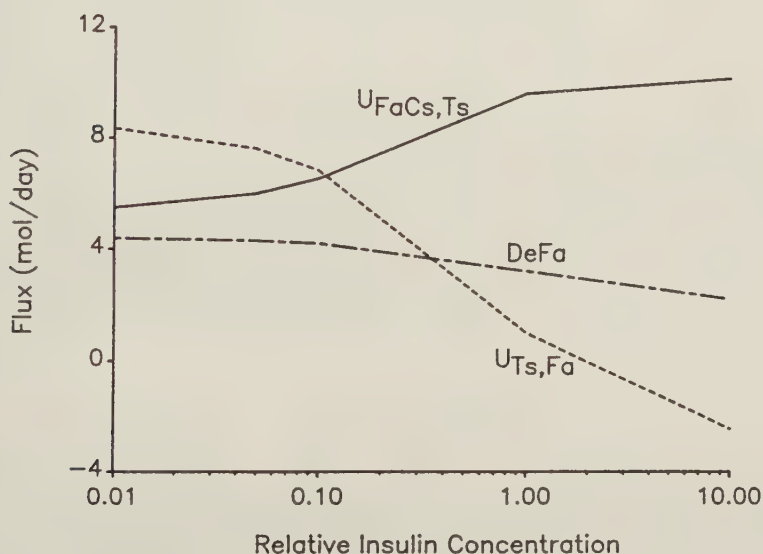


Figure 12.23 Triglyceride synthesis ($U_{FaCs,Ts}$), lipolysis ($U_{Ts,Fa}$) and net (DeFa) intra- and extracellular fatty acid exchange ($U_{Fa,eFa} - U_{eFa,Fa}$) with respect to relative insulin concentration. Glucose concentration was set at 0.6 mM and acetate at 1.5 mM.

in Fig. 12.20 and the concomitant increase in NADPH_2 required. The increase in ^{14}C -fatty acid synthesis reflects a 2-fold increase in acetyl-CoA carboxylase activity.

The above evaluation of model behavior emphasized interactions among insulin and extracellular concentrations of glucose and acetate with respect to the production of CO_2 , TAG glycerol, extracellular lactate and fatty acids. Consequently, the specific pattern of metabolite transactions within the model as determined by insulin level is of interest. Figures 12.21–12.24 are provided to illustrate not only model behavior, but the dependency of lipogenesis and glucose utilization on insulin in the model. Glucose flux through hexokinase ($U_{\text{Gl,G6P}}$) and G6P entry into the pentose pathway ($U_{\text{G6P,Ru}}$) exhibit kinetics with respect to insulin (Fig. 12.21) similar to those reported by Yang and Baldwin (1973a). The behavior of equation 12.1 showed the k_s of hexokinase to be sigmoidally related to insulin concentration. The reference level of insulin in the model is 1.0 which, in context with Fig. 12.21, illustrates that at steady-state conditions, glucose utilization via the glycolytic and pentose pathways approaches saturation. Additionally, glucose flux through hexokinase increases proportionally more than G6P entry into the pentose pathway and can be explained by the additional requirement of glucose for production of α -glycerol-P (TAG precursor) in addition to the synthesis of NADPH_2 . Figure 12.22 reveals that at low insulin concentration, recycling of carbon via the phosphoglucosomerase reaction is increased, but at high insulin concentration, more glucose moves down the glycolytic pathway. Thus, at low insulin concentrations, glucose is being conserved, which is consistent with the high priority for glucose homeostasis in the ruminant animal. In addition to altering the affinity of hexokinase for glucose in the model, insulin also increases the V_{max} of acetyl-CoA carboxylase (equation 12.14) and decreases the V_{max} for lipolysis (equation 12.16). TAG synthesis increased as relative insulin concentration increased, while lipolysis and fatty acid release decreased (Fig. 12.23). Rates of fatty acid re-esterification increased with insulin concentration with the result that the reduction in fatty acid release was reduced more than lipolysis.

(b) Sensitivity analyses

The purpose of examining the sensitivity of the model to changes in key model parameter values is 2-fold: to determine whether or not model behavior is sensitive to changes in model parameters to which current concepts suggest the model should be sensitive; and to identify experiments and measurements required to improve estimates of key parameters that cannot be measured experimentally.

Sensitivity analyses were performed on the $k_{\text{Gl,G6P}}$ of hexokinase, V_{max} of acetyl-CoA carboxylase ($V_{\text{AcCs,Fa}}$) and the $k_{\text{Ac,AcCs}}$ for the cytosolic acetyl-CoA synthetase (Figs 12.24–12.30).

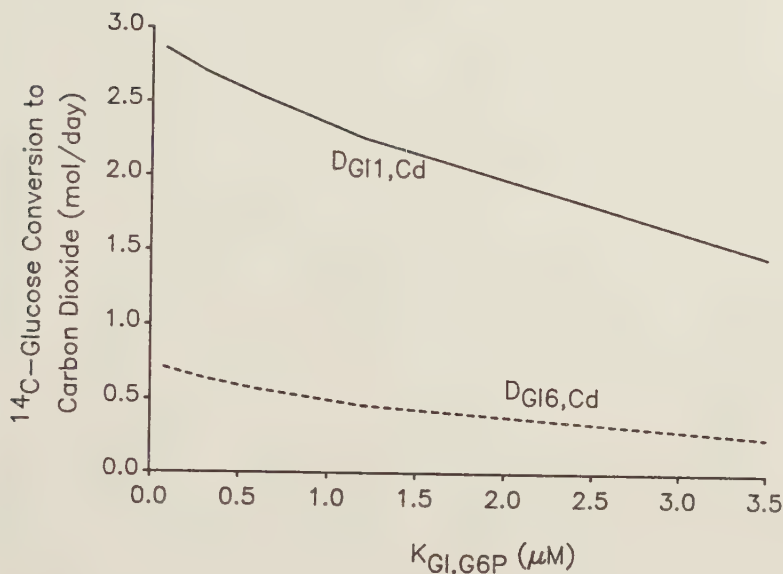


Figure 12.24 Effect of changes in $K_{GI,G6P}$ on radiolabeled ^{14}C -glucose conversion to $^{14}CO_2$. $D_{GI1,Cd}$ and $D_{GI6,Cd}$ denote the production of $^{14}CO_2$ from $1-^{14}C$ - and $6-^{14}C$ -glucose, respectively. For this simulation relative glucose concentration, glucose concentration and acetate concentration were set to 1.0, 0.6 and 1.5 mM, respectively. Specific activities of $1-^{14}C$ - and $6-^{14}C$ -glucose were set to 1.0.

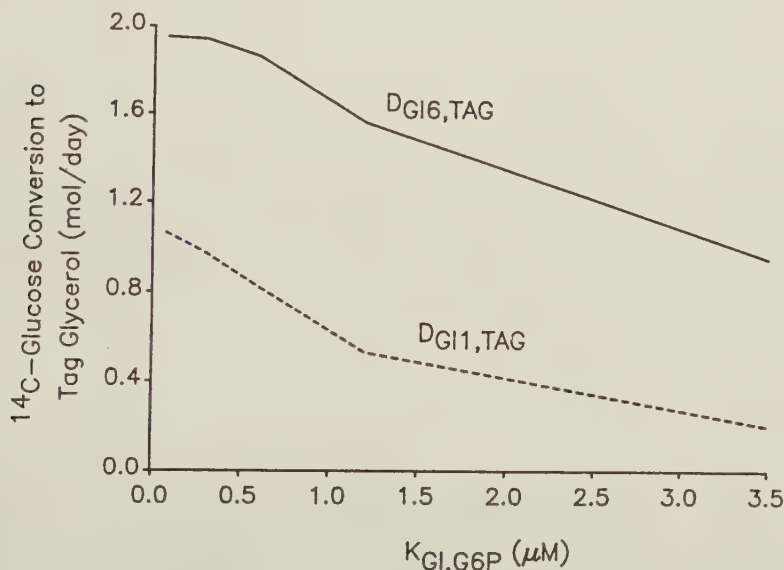


Figure 12.25 Radiolabeled ^{14}C -glucose conversion to triacylglycerol (TAG) ^{14}C -glycerol as effected by $K_{GI,G6P}$. $D_{GI1,TAG}$ and $D_{GI6,TAG}$ denote, respectively, the production of TAG ^{14}C -glycerol from $1-^{14}C$ - and $6-^{14}C$ -glucose. Relative insulin concentration, glucose concentration and acetate concentration were set to 1.0, 0.6 and 1.5 mM, respectively.

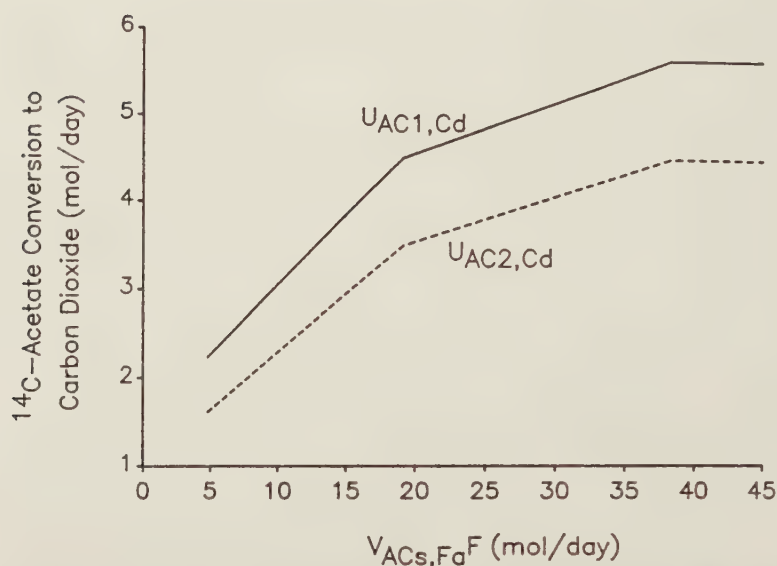


Figure 12.26 Radiolabeled ^{14}C -acetate oxidation with respect to the $V_{\max}$ of acetyl-CoA carboxylase $V_{ACs,FaF}$. $U_{AC1,Cd}$ and $U_{AC2,Cd}$ denote, respectively, $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate recovery in $^{14}\text{CO}_2$. Respective insulin, glucose and acetate concentrations were 1.0, 0.6 and 1.5 mM. The specific activities of $1\text{-}^{14}\text{C}$ - and $2\text{-}^{14}\text{C}$ -acetate were set at 1.0.

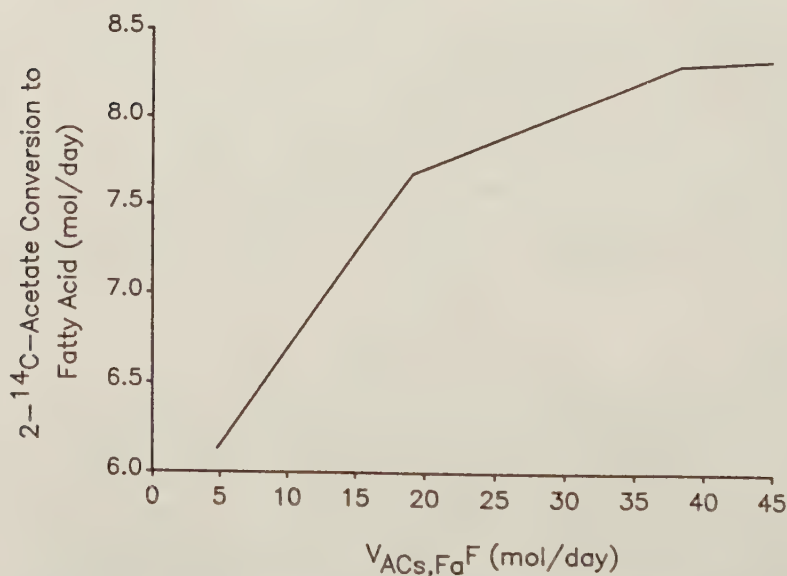


Figure 12.27 Radiolabeled $2\text{-}^{14}\text{C}$ -acetate conversion to ^{14}C -fatty acid with respect to $V_{ACs,FaF}$. Conditions as described for Fig. 12.26.

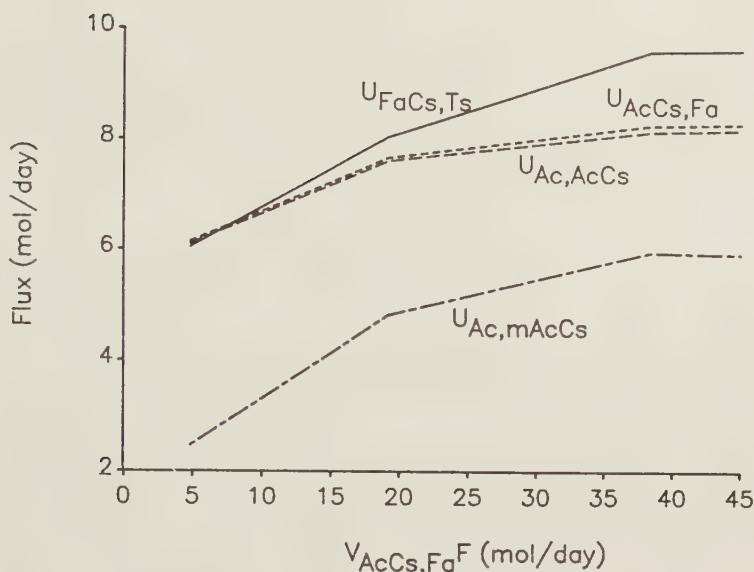


Figure 12.28 Effect of acetyl-CoA carboxylase on rates of fatty acid esterification ($U_{FaCs, Ts}$), activation of acetate in the mitochondria ($U_{Ac, mAcCs}$) and cytosol ($U_{Ac, AcCs}$), and fatty acid synthesis ($U_{AcCs, Fa}$). Simulation conditions as described in legend to Fig. 12.26.

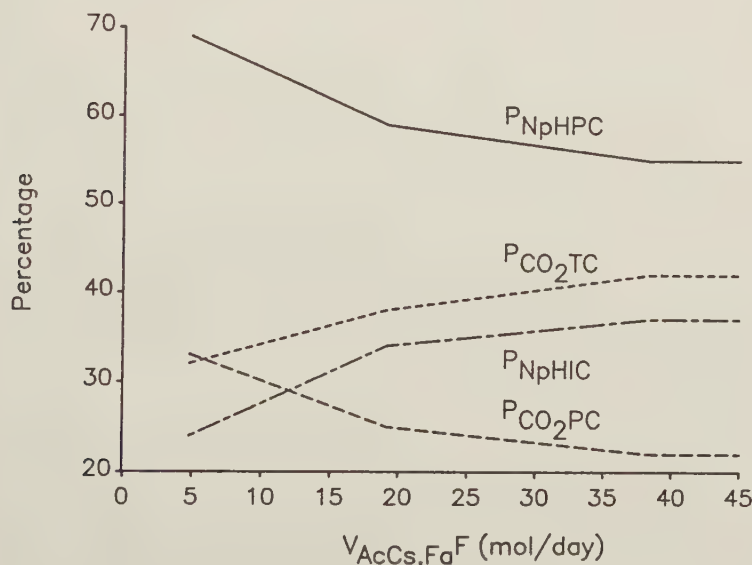


Figure 12.29 Percentages of $NADPH_2$ production from the pentose pathway ($P_{Np, HPC}$) and NADP linked isocitrate dehydrogenase ($P_{Np, HIC}$), and of CO_2 from the pentose pathway ($P_{CO_2, TC}$) and tricarboxylic acid cycle ($P_{CO_2, PC}$) with respect to the V_{max} of acetyl-CoA carboxylase ($V_{AcCs, Fa} F$). Simulation conditions as described in legend for Fig. 12.26.

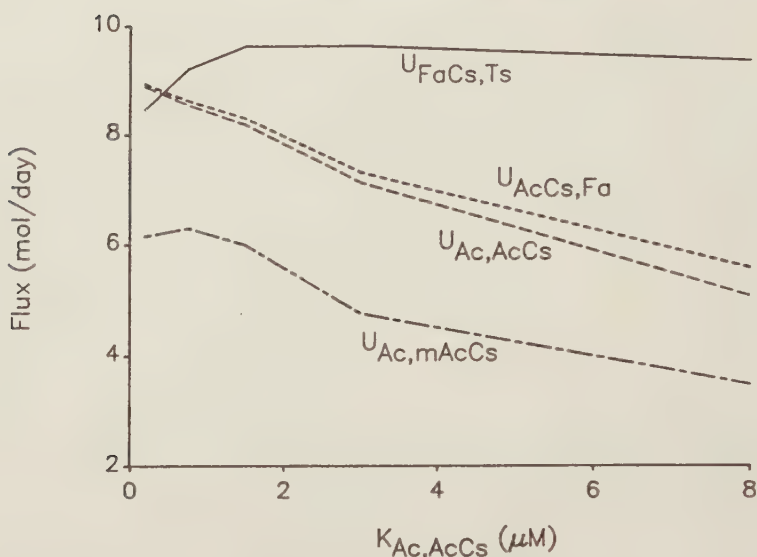


Figure 12.30 Rates of triacylglycerol ($U_{FaCs,Ts}$), mitochondrial ($U_{Ac,mAcCs}$) and cytosolic ($U_{Ac,AcCs}$) acetyl-CoA, and fatty acid ($U_{AcCs,Fa}$) synthesis with respect to the affinity of cytosolic acetyl-CoA synthetase for acetate ($k_{Ac,AcCs}$). Simulation conditions as defined for Fig. 12.26.

Both 1- ^{14}C - and 6- ^{14}C -glucose oxidation to $^{14}CO_2$ were reduced as $k_{Gl,G6P}$ increased just as available data suggest should happen (Fig. 12.24). A similar pattern is observed in ^{14}C -glucose conversion to TAG ^{14}C -glycerol (Fig. 12.25) and ^{14}C -extracellular lactate (data not shown). Thus, rates of 1- ^{14}C - and 6- ^{14}C -glucose conversion to CO_2 , TAG and extracellular lactate potentially can be employed to estimate $k_{Gl,G6P}$. The entry of G6P into the pentose pathway was less sensitive to changes in $k_{Gl,G6P}$ than either the phosphorylation of glucose or the esterification of fatty acids. Entry into the pentose pathway was maintained by the recycling of carbon through F6P to G6P. The sensitivity of fatty acid esterification to $k_{Gl,G6P}$ demonstrates the dependency of triacylglyceride synthesis on both α -glycerol-P formation and the generation of reducing power (pentose pathway flux).

The forms of the equations for fatty acid synthesis (equation 12.13 and 12.16) do not allow for directly varying $V_{AcCs,Fa}$. Therefore, $V_{AcCs,Fa}F$ was varied (5–78 moles/day) to simulate changes in $V_{AcCs,Fa}$. The reference value for $V_{AcCs,Fa}F$ was 38.8 moles/day. An overview of Figs 12.26–12.29 demonstrate that these respective model outputs are not sensitive to changes in $V_{AcCs,Fa}F$ above the model reference value, but are sensitive to lower values. The rates of oxidation of 1- ^{14}C - and 2- ^{14}C -acetate to $^{14}CO_2$ showed typical saturation kinetics with respect to $V_{AcCs,Fa}$, which reflect the use of acetate as a source of $NADPH_2$ and energy for lipogenesis (Fig. 12.26). These demands are so pronounced in the model that the percent increase in the

entry of acetate into the TCA cycle ($V_{Ac,mAcCs}$) was greater (142%) as $V_{AcCs,FaF}$ values increased from 5 to 38 moles/day (Fig. 12.27) than the increase in rates of the cytosolic acetyl-CoA synthetase ($U_{Ac,AcCs}$; 38%), acetyl-CoA carboxylase ($U_{AcCs,Fa}$; 38%) or TAG synthesis ($U_{FaCs,Ts}$; 63%) reactions (Fig. 12.28). The model (Fig. 12.29) exhibited some interesting behavior with respect to $V_{AcCs,FaF}$. The percentages of $NADPH_2$ and CO_2 produced via the pentose cycle decreased as $V_{AcCs,FaF}$ increased, while the percentages of $NADPH_2$ from isocitrate dehydrogenase and CO_2 from the TCA cycle increased relative to total production rates. The increased flux through the TCA cycle increased the availability of isocitrate for the production of $NADPH_2$ via isocitrate dehydrogenase, thereby limiting the availability of NADP for conversion to $NADPH_2$ in the pentose pathway.

Increasing the affinity constant ($k_{Ac,AcCs}$) for cytosolic acetyl-CoA synthetase (Fig. 12.30) reduced rates of activation of acetate ($U_{Ac,AcCs}$ and $U_{Ac,mAcCs}$) for lipogenesis ($U_{AcCs,Fa}$). The reduction in $U_{Ac,AcCs}$ is, of course, a direct result of the decreased affinity constant. The reduction in $U_{Ac,mAcCs}$ reflects the reduction in energy expenditures due to the reduced rates of lipogenesis ($U_{AcCs,Fa}$), which is caused by the reduced availability of substrate (AcCs). Triacylglycerol production was not affected significantly by altering $k_{Ac,AcCs}$, because the decrease in fatty acid synthesis was compensated by an increase in fatty acid re-esterification due to an increased availability of cytosolic CoA. Glucose entry into the Embden-Meyerhoff and pentose pathways were not affected by varying $k_{Ac,AcCs}$. The reduced use of $NADPH_2$ in lipogenesis was compensated by a decrease in the net flux of the NADP-linked isocitrate dehydrogenase (results not shown).

12.3.3 Conclusions

The ruminant adipose tissue model presented in this chapter was developed over the course of the past 20 years. Our objectives in describing model behavior and the results of sensitivity analyses of key model parameters were to illustrate that model behavior corresponds to experimental results and that solutions are sensitive to key parameters and, thus, can be used as an analytical model to improve estimates of these parameters as appropriate experimental data become available. The evaluations of the model went beyond literature comparisons inasmuch as the behavior of many key regulatory pathways and fluxes are not obtainable from the literature. Therefore, the framework for a series of experimental designs is maintained in the context of examining the intermediary relationships that contribute to simulation outputs.

By no means do we suggest that this model is the definitive representation of ruminant adipose tissue metabolism. Rather, we believe the model can serve as a template to refine our understanding of ruminant adipose tissue biochemistry and physiology. Many metabolic challenges still await this model, such as describing effects of exogenous hormone (i.e.

bovine somatotropin) administration and documentation of changes in nutrient uptake and release over the course of lactation. Insights gained from simulations of adipose tissue function using this model will be useful in formulating improved adipose tissue elements for more highly aggregated models of whole-animal metabolism (Chapter 16).

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Dynamic models of ruminant mammary metabolism

with M. D. Hanigan

A major reason for being interested in the mammary glands is the wish to know how milk is made, and the starting point in that investigation is to ask. From what is it made?

Linzell (1974)

13.1 INTRODUCTION

The first modeling study we undertook was to develop a model of cow mammary gland metabolism. An important reason for attempting to model metabolism in this tissue was that we had a great deal more data available upon which we could base the model (Baldwin and Yang, 1974) as compared to other ruminant tissues. In this study, we made almost all of the mistakes alluded to in Chapters 3 and 4: a clear modeling objective was not set; far too much detail was incorporated and, as a result, the model was grossly overparameterized and intractable during solution. The exercise was never completed because no criteria for evaluation were set. The only result of this study that could be published was (retrospectively) a questionable figure that illustrated the dynamics of radiotracer metabolism in a review by Baldwin and Smith (1971). Nevertheless, a great many relevant data were collected and organized in an appropriate form. Also, we learned a great deal about modeling and mammary gland metabolism in the process.

A second attempt at modeling cow mammary gland metabolism was published by Waghorn and Baldwin (1984). Several important insights arose from this study. The first was recognition of limitations in available data on amino acid availability to and metabolism in mammary tissue. For example, Wohlt *et al.* (1977) had clearly demonstrated that cow mammary glands can catabolize significant quantities of essential amino acids, but data on amounts of amino acids available for catabolism in excess of requirements for protein synthesis were not adequate to define equations for

amino acid catabolism in the model. In fact, some very tenuous assumptions were required to reconcile arteriovenous difference estimates of uptakes of essential amino acids and their secretion in milk protein. A second observation was that available data on kinetic relationships in mammary tissue were not adequate to support the formulation of equations of greater complexity than the mass action type. The final important result of this study was our first significant use of modeling analyses in the design of experiments. This result is illustrated in part in Table 13.1. In this table, the necessity of collecting specific radiotracer data in order to parameterize a model is illustrated. For example, rate constants central to model behavior require that measurements of incorporation of ^{14}C -glucose in carbons 1, 2 and 6 into lactose, triacylglycerol glycerol and CO_2 were required to define rate constants associated with glucose-6-phosphate metabolism when experimental variance was 20% and when variance was 40%, only 62% of the rate constants could be defined with confidence. Two sets of experimental analyses arose from the Waghorn and Baldwin (1984) study. The first set was the kinetic studies of mammary gland metabolism reported by Forsberg *et al.* (1984, 1985a,b). The second set of experimental data needed were arteriovenous estimates of nutrient uptakes by mammary glands of cows. Available arteriovenous difference data were sparse and largely restricted to low-producing cows (15 kg milk/day; Waghorn and Baldwin, 1984). Also, available data sets were incomplete in that uptakes of only a few nutrients were measured in individual experiments. Results of a fairly comprehensive arteriovenous study of nutrient uptakes by cow mammary glands were reported by Miller *et al.* (1991a,b) and Hanigan *et al.* (1991, 1992). These studies along with some others identified below provided the bases for formulation of our current model of cow mammary metabolism and will be discussed in some detail in this chapter. These data were essential to development of fluxes depicted in the block diagram (Fig. 13.1) and equations forms used in the model.

Table 13.1 Effect of measurements and variance upon definition of rate constants for glucose-6-phosphate metabolism^a

Measurements	Percent correct answers at variance of	
	20%	40%
All	100	62
No lactose	87	62
No glycerol	87	37
CO_2 , glycerol only	50	50
No Glc-1- ^{14}C	50	12
No Glc-2- ^{14}C	75	62
No Glc-6- ^{14}C	100	62
No Glc-1 or 2- ^{14}C	25	0

^a Adapted from Waghorn and Baldwin (1984).

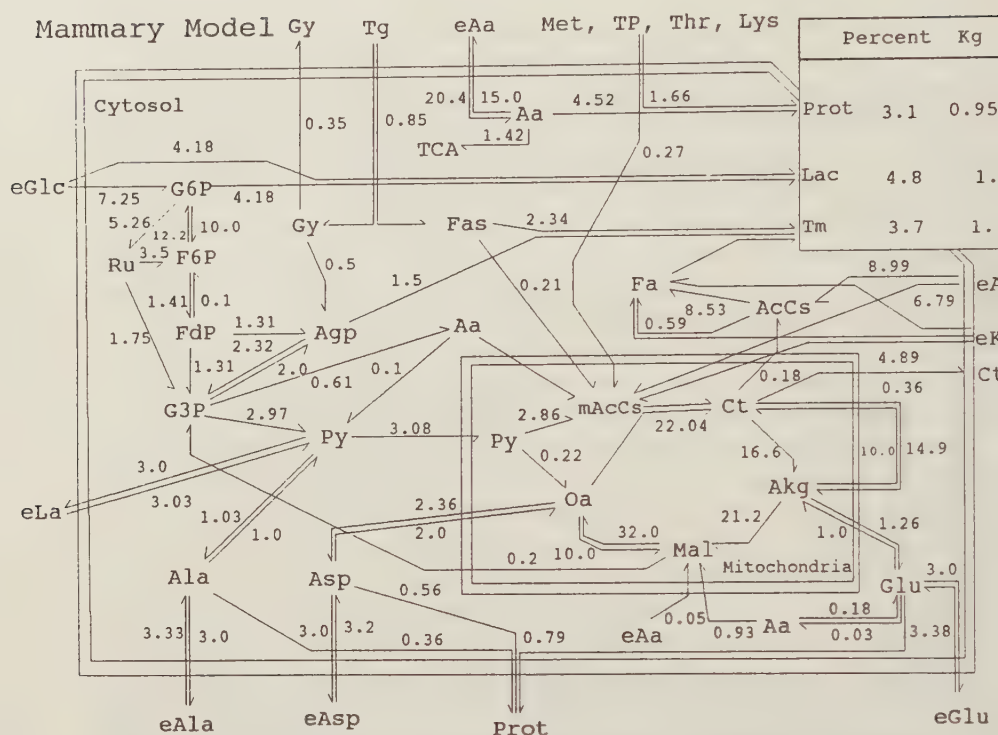


Figure 13.1 Block diagram of the mammary model. Cytosolic and mitochondrial spaces are bounded by solid double lines. Pools are identified by two-, three- or four-letter codes. Arrows between pools indicate fluxes and numbers associated with the arrows are the fluxes at steady-state concentrations (M/day). Extracellular space is that area outside of the cytosolic space. Pool sizes of extracellular nutrients do not change through a particular simulation, while pool sizes of intracellular nutrients and products do change dependent upon the relevant inputs and outputs (fluxes) to the pool. Abbreviations are milk fat (Tm), cytosolic acetyl-CoA (AcCs), mitochondrial acetyl-CoA (mAcCs), and given in Tables 13.2 and 13.3.

The complete, detailed model along with radiotracer elements has been published in detail (Hanigan and Baldwin, 1994). Therefore, only selected elements of the model will be presented here to illustrate points relevant to the objectives of this book. The first, as pointed out in several chapters, is that careful analyses of input:output data are essential during model development. Another essential point is that input:output analyses of individual experiments are much more powerful than mean values usually presented to summarize an overall study. The final goal of modeling illustrated in this chapter is a brief discussion of the use of a mechanistic model to aid in the design of additional experiments.

13.2 DEVELOPMENT OF MODEL INPUTS AND INITIAL PARAMETERS

General considerations in model development were derived from data summaries of Smith (1970) and Baldwin and Yang (1974); very few data relevant to the aspects addressed by these authors have been generated since those summaries were published. Total mammary gland size was set at 25 kg with an intracellular volume of 17.3 kg. Milk production was set to approximately 30 kg/day with respective percentages of lactose, protein, NPN and fat of 4.5, 2.8, 0.3 and 4.2%. Concentrations of nutrients in blood flowing to the mammary glands and intracellular concentrations of metabolites in the reference state are presented in Tables 13.2 and 13.3. Uptakes of nutrients per day (Table 13.4) and milk yield and composition (Table 13.5) used for the reference state in formulation of the model were derived from data published by Hanigan *et al.* (1991), Miller *et al.* (1991a,b) and Brown *et al.* (1989). The reference data on inputs and outputs are for a fictitious average cow. In the publications of Hanigan *et al.* (1991) and Miller *et al.* (1991a,b), equations relating mammary arteriovenous differences (mammary gland uptakes) to nutrient concentrations in blood were presented. Effects of stage of lactation and milk production on availabilities of nutrients to the udder and resultant uptakes of nutrients were emphasized. These major sources of variance are reflected in the standard deviations in Table 13.4 and Table 13.5, which are often more than 100% of the mean value.

Table 13.2 Extracellular concentrations of metabolites adopted to simulate the reference state^a

Metabolite code ^b	Chemical name	Concentration (mM)
eAa	amino acids	2.99
Ac	acetate	1.80
eAla	alanine	0.26
eAsp	aspartate + asparagine	0.16
Glc	glucose	3.00
eGlu	glutamate	0.12
Kb	ketone bodies	0.60
La	lactate	0.77
eLys	lysine	0.01
eMet	methionine	0.02
Tg	TAG + NEFA + glycerol	0.25
eThr	threonine	0.01
TP	tyrosine + phenylalanine	0.12

^aValues were derived from blood metabolite data presented by Smith (1970), Miller *et al.* (1991a), Hanigan *et al.* (1992).

^bThe prefix e indicates concentration in the extracellular as distinct from the intracellular space; TAG, triacylglycerol; NEFA, non-esterified fatty acids.

Table 13.3 Steady-state concentrations of metabolites in mammary tissue^a

Metabolite code	Chemical name	Concentration (mM)
Aa	amino acids	2.99
AcCs	cytosolic acetyl-CoA	0.02
Ad	ADP	0.33
Agp	α -glycerol phosphate	0.21
Akg	α -ketoglutarate	0.036
Ala	alanine	0.27
Asp	aspartate	0.99
At	ATP	0.75
Cd	carbon dioxide	0.98
Cs	cytosolic CoA	0.065
Ct	citrate	20.2
F6P	fructose-6-phosphate	0.03
FaCs	fatty acyl-CoA	0.12
Fd	FAD	0.12
Fdh	FADH ₂	0.12
Fbp	fructose-1,6-bisphosphate	0.01
G3P	glycerol-3-phosphate	0.14
G6P	glucose-6-phosphate	0.07
Glu	glutamate	4.05
Gy	glycerol	0.98
mAcCs	mitochondrial acetyl-CoA	0.02
Mal	malate	0.51
mCs	mitochondrial CoA	0.065
Nd	NAD	0.12
Ndh	NADH	0.12
NH ₃	ammonia	0.21
Np	NADP	0.12
Nph	NADPH	0.12
O ₂	oxygen	4.2
Oa	oxaloacetate	0.002
Py	pyruvate	0.046
Ru	ribulose-5-phosphate	0.05

^aDerived from data summarized by Baldwin and Yang (1974)

As emphasized a number of times in this book, a mechanistic model must have the capacity to accommodate this observed variance. However, as we have also emphasized, the quality of the experimental data must be challenged carefully by calculating input:output data for each individual arteriovenous study, e.g. the model should not be required to accommodate suspect data. These issues are addressed in the following section.

13.2.1 Evaluation of input:output data

Input:output data must be very rigorously evaluated during formulation of

Table 13.4 Uptakes of nutrients per 24 h by lactating mammary glands^a

Nutrient	n	Uptake ^b	SD
Acetate	58	18.42	7.09
Alanine	63	0.445	0.465
Amino acid ^c	63	7.16	3.75
Aspartate/asparagine	63	0.265	0.397
Glucose	57	12.21	5.02
Glutamate	63	0.499	0.617
Glycerol	58	0.033	0.363
β-hydroxybutyrate	57	7.25	5.70
Lactate	58	0.023	5.408
Lysine	63	0.696	0.238
Methionine	63	0.163	0.096
Non-esterified fatty acids	58	0.494	0.905
Threonine	63	0.377	0.179
Triacylglycerol (kg/day)	58	0.502	0.259
Tyrosine/phenylalanine	63	0.579	0.181
Total energy (MJ/day)	56	118.4	38.3

^aMaterials and methods for measurements of nutrients were published previously (Hanigan *et al.*, 1991; Miller *et al.*, 1991a,b, Brown *et al.*, 1989).

^bUnits are mole/day except where units are specified.

^cAmino acid uptake refers to the aggregated pool of amino acids as defined in the model under the amino acids section.

Table 13.5 Composition of milk^a

Component	n	Yield ^b	SD
Milk	63	30.68	6.02
Energy in milk (Mcal * day ⁻¹)	62	23.14	4.42
Total solids	63	3.93	0.73
Protein	63	0.87	0.17
NPN	62	0.01	0.002
Lactose	63	1.37	0.29
Fat	62	1.29	0.29

^aDetails regarding the diet and cows used were presented by Brown *et al.* (1989).

^bUnits are kg/day except where units are specified.

a model. The example we will utilize in illustrating appropriate evaluation criteria is drawn from the above mentioned study reported by Hanigan *et al.* (1991) and Miller *et al.* (1991a,b). In the study, a total of 21 multiparous, lactating dairy cows whose previous lactational performances ranged from 6400–13 200 kg per 305 day lactation, were used. Mammary gland arterio-venous difference (A–V) studies were conducted on days 34, 69, 104 and 125 of lactation. On days 70–125 of lactation, 10 cows were injected with 40

mg/day of recombinant bovine growth hormone (bST). Several A-V difference studies were not completed due to technical difficulties, such as loss of a catheter. Two cows did not complete the study because of leg injuries. Effects of loss of observations due to these difficulties are reflected in the nitrogen values in Tables 13.4 and 13.5.

As with all A-V difference studies of input:output experiments, blood flow estimates for calculations of individual nutrient uptakes are critical. In this study (Tables 13.6 and 13.7), these were based upon A-V differences of methionine, tyrosine plus phenylalanine, threonine and lysine (Hanigan *et al.*, 1991) using the Fick principle (Davis and Bickerstaffe, 1978) and the equation

$$BF = (MAa * 0.96)/A-V Aa$$

where BF is the blood flow (l/l milk), MAa is moles of the amino acid (Aa) of interest secreted in milk protein (moles/l milk) and A-V Aa is the A-V difference of the amino acid of interest (moles/l blood). The amino acid composition of milk protein was based upon published cDNA sequences for each of the major milk proteins (Jenness, 1985). It was assumed that 96% of the true protein secreted was synthesized in the gland. In addition to the estimates of blood flow calculated for each amino acid, minimum blood flows required to supply energy equivalent to energy in milk, nitrogen equivalent to nitrogen in milk protein and glucose required for lactose synthesis in the gland were calculated for each A-V study. These six individual estimates of flow were used to screen for aberrant values within each A-V study. Aberrant estimates of blood flow were discarded according to the following criteria:

1. an individual flow estimate resulted in uptakes of nitrogen, energy in metabolites, or glucose that were less than nitrogen, energy, or lactose secreted in milk; or
2. an individual blood flow estimate based upon one amino acid was greater than two standard deviations away from the mean of the four amino acid-based estimates for that A-V study.

Using these criteria, 23 of the 304 amino acid-derived blood flow estimates were discarded. In no case were all estimates for an A-V study discarded. Remaining estimates of blood flow calculated from the individual amino acids were averaged by A-V study and used in formulation of the balance data presented in Tables 13.6 and 13.7.

In Tables 13.6 and 13.7, milk energy was calculated using the equation

$$NE_L \text{ (MJ/kg of milk)} = 1.469 + (0.402 * \% \text{ fat})$$

and energy values used for nutrients were taken from the literature (Blaxter, 1989; *Handbook of Chemistry and Physics*, 1968–1969).

Statistical analyses (Tables 13.6 and 13.7) were performed using the gen-

Table 13.6 Energy and nitrogen balance data for control and bST-treated mammary glands during lactation^a

	Control				bST		SEM	P ^c	P ^d
	week 5 ^b	week 10	week 15	week 18	week 15	week 18			
Blood flow ^e (l)	13431.0	11600.0	10802.0	10849.0	13037.0	11179.0	495.0	0.18	0.38
BF: Milk (l/l)	807.0	777.0	741.0	786.0	830.0	766.0	34.0	0.74	0.73
Acetate (mole)	10.2	8.9	10.4	7.5	9.1	7.9	0.48	0.61	0.78
BHBA ^f (mole)	5.9 ^a	3.6 ^b	2.6 ^b	1.4 ^b	3.7 ^{ab}	1.8 ^b	0.34	0.002	0.47
Glucose (mole)	7.6 ^a	5.8 ^b	-5.4 ^b	5.3 ^b	5.8 ^{ab}	5.1 ^b	0.33	0.09	0.92
NEFA (mole)	0.55 ^a	0.16 ^{bc}	0.05 ^b	-0.06 ^b	0.54 ^{ac}	0.16 ^{bc}	0.054	0.003	0.02
TAG ^g (kg)	0.21	0.26	0.30	0.28	0.19	0.31	0.172	0.14	0.38
Lactate (mole)	0.24	-0.84	-0.60	1.41	1.05	0.22	0.362	0.43	0.83
Glycerol (mole)	-0.028	0.019	-0.008	0.027	0.139	0.020	0.026	0.82	0.34
Urea (mole)	1.69	0.12	-0.44	-0.64	-0.37	-0.46	0.45	0.18	0.93
Aa (mole)	4.5	4.6	3.7	4.2	5.6	4.0	0.24	0.31	0.22
Aa energy (Mcal)	3.4	3.4	3.0	3.2	4.0	3.0	0.15	0.37	0.35
Aa N (mole) _h	6.7	6.5	7.1	6.5	8.4	6.2	0.39	0.88	0.66
A-V energy ⁱ (MJ)	17.4 ^a	13.4 ^b	12.3 ^b	11.6 ^b	14.8 ^{ab}	12.4 ^b	0.60	0.02	0.36
Milk energy (MJ)	12.8 ^{ab}	11.3 ^{cde}	10.6 ^{cde}	9.8 ^{cd}	12.8 ^{bc}	11.7 ^{ac}	0.26	0.006	0.02
Milk Glc (mole)	4.5 ^a	4.1 ^{ab}	3.9 ^{ab}	3.4 ^b	4.0 ^{ab}	3.6 ^b	0.10	0.02	0.64

^aMeans within a row not sharing a common superscript differ ($P < 0.05$); values are expressed as units per 12 h.

^bWeek postpartum.

^cProbability that values for weeks 5 and 10 are equal to values from weeks 15 and 18 of control cows.

^dProbability that values for weeks 15 and 18 of control cows are equal to values from weeks 15 and 18 of treated cows.

^eBlood flows were calculated from uptakes of tyrosine plus phenylalanine, methionine, lysine, and threonine and output of these same amino acids in milk using the equation: BF = Mammino acids * (A-V of amino acids)¹ * 0.96, where BF, Mammino acids and A-V represent blood flow, milk amino acids and arteriovenous difference, respectively. Values presented are the mean of the four individual calculations.

^f β -Hydroxybutyrate.

^gTriglyceride.

^hA-V energy = uptake of total energy by the udder.

ⁱCalculated as grams of milk lactose divided by the molecular weight of glucose in lactose.

Table 13.7 Effects of stage of lactation and bST treatment on the proportions of energy derived from energy and nitrogen sources by lactating mammary glands^a

	Control					bST					P ^c	P ^d	P ^e	P ^f
	week 5 ^b	week 10	week 15	week 18	week 15	week 18	week 15	week 18	week 15	week 18	SEM			
Acetate	12.12 ^a	14.56 ^{ab}	17.21 ^{bc}	13.61 ^{ac}	13.07 ^a	13.61 ^{ac}	13.07 ^a	13.61 ^{ac}	14.46 ^{ab}	0.53	0.12	0.81	0.28	0.28
BHBA ^f	15.30 ^a	13.26 ^{ab}	10.30 ^{bc}	5.80 ^{cde}	11.67 ^{abe}	5.80 ^{cde}	11.67 ^{abe}	5.80 ^{cde}	8.05 ^{bd}	0.77	0.05	0.08	0.89	0.89
Glucose	28.75	30.05	30.07	30.67	25.95	30.67	25.95	30.67	27.93	0.92	0.68	0.37	0.26	0.26
NEFA	8.78 ^a	1.87 ^b	-0.34 ^b	-1.66 ^b	8.78 ^a	-1.66 ^b	8.78 ^a	-1.66 ^b	3.78 ^{ab}	1.01	0.04	0.67	0.04	0.04
TAG ^g	10.94 ^a	17.10 ^{bc}	21.48 ^b	21.78 ^b	12.64 ^{ac}	21.78 ^b	12.64 ^{ac}	21.78 ^b	21.12 ^b	0.95	0.008	0.24	0.20	0.20
Lactate	0.45	-4.40	-1.46	3.83	1.69	3.83	1.69	3.83	0.54	1.35	0.18	0.21	0.96	0.96
Glycerol	0.06	0.03	-0.01	0.07	0.35	0.07	0.35	0.07	0.08	0.06	0.51	0.41	0.21	0.21
CHO-lipid ^h	77.70	72.60	76.75	73.25	73.82	73.25	73.82	73.25	75.00	1.23	0.52	0.99	0.60	0.60
Aa	21.01	27.25 ^b	23.74 ^{ab}	27.60 ^{ab}	26.49 ^{ab}	27.60 ^{ab}	26.49 ^{ab}	27.60 ^{ab}	25.95 ^{ab}	1.18	0.83	0.64	0.56	0.56

^aMeans within a row not sharing a common superscript differ ($P < 0.05$); values are expressed as a percent of total energy uptake per 12 h.

^bWeek postpartum.

^cProbability that values for weeks 5 and 10 are equal to values from weeks 15 and 18 of control cows.

^dProbability that values for weeks 5 and 10 are equal to values from weeks 15 and 18 of treated cows.

^eProbability that values for weeks 15 and 18 of control cows are equal to values from weeks 15 and 18 of treated cows.

^f β -hydroxybutyrate.

^gTriglyceride.

^hAggregate energy supplied to the udder as carbohydrate and lipid.

eral linear models and non-linear procedures of SAS (1985). Treatment and effects by week were tested using the model

$$Y_{ij} = u + \alpha_i + E_{ij}$$

where Y_{ij} is an observation of period i ($i = 1, 2, \dots, 6$), u is a constant common to all observations, α_i is the fixed effect common to period i and E_{ij} is a random residual particular to period i with null mean and variance σ^2 . Period was considered a function of week. Changes in the proportion of total energy supplied by an individual nutrient (Table 13.7) were tested using the model

$$Y_{ij} = u + \alpha_i + \beta X_{ij} + E_{ij}$$

where X_{ij} is the A-V of total energy. These values were evaluated as ratios (i.e. A-V of glucose, A-V of total energy, etc.) to facilitate interpretation. Standard errors of means were calculated from model mean square errors. Reported probabilities and r^2 (see figures discussed below) were calculated from Type III sums of squares and corrected total sums of squares, respectively.

The nitrogenous components of milk varied with stage of lactation ($P < 0.05$), but fat, lactose, ash and total solids were not affected by stage of lactation ($P = 0.71$, $P = 0.38$, $P = 0.55$ and $P = 0.33$, respectively, Tables 13.6 and 13.7). Both protein and non-protein nitrogen (NPN) percentages increased as lactation progressed ($P < 0.005$). When average blood flows calculated from individual essential amino acids were compared, flows estimated from methionine were sometimes greater on average than flows estimated from the other four amino acids. In a smaller number of cases, flows estimated from tyrosine plus phenylalanine were greater than estimates from methionine. The differences in blood flows determined from uptake of each amino acid were not significant. Therefore, flows determined by individual amino acids were averaged for each animal. Using these blood flows, amino acid balances across udders and removal of each amino acid from blood per 12 h minus output of that amino acid in milk per 12 h were calculated. Uptakes of tryptophan, histidine, methionine, tyrosine, phenylalanine, leucine and threonine were essentially equal to their use in milk protein synthesis ($\pm$ coefficient of variation; Hanigan *et al.* (1991)). Uptakes of lysine, valine, isoleucine and arginine were greater than required for milk protein synthesis, with uptakes of isoleucine exceeding output in milk by 49 mmole/l^{-1} of milk. Cysteine balance was near zero when cystathionine uptake was considered. While methionine can be converted to cysteine in the udder, previous work has not demonstrated significant fluxes through this pathway (Verbeke *et al.*, 1967).

Mean blood flows per liter of milk were greater than are generally accepted. The overall mean was 1.58 times greater than the often stated generalization of 500 l blood flow/l milk secreted, which was established basically using goat data and data on low-producing cows (Linzell, 1974), but less

than the adjustment factor of 1.75 that Waghorn and Baldwin (1984) used to bring amino acid uptake and output by the udder into balance. Hanigan *et al.* (1991) reported that average whole blood A-V of methionine and tyrosine plus phenylalanine were 25 and 19% greater than plasma A-V differences, respectively. The combination of blood flow and red blood cell transport of amino acids apparently explains the inequalities in amino acid balance across the cow udder evaluated by Waghorn and Baldwin (1984). Furthermore, using the average milk production for this study of approximately 30 kg/day, the equation of Kronfeld *et al.* (1968) predicts a mean blood flow of 653 l/l milk. Although this value is less than the value reported here, the data range for uncompromised cows in that experiment was 4.9–27.9 kg milk/day, which is considerably less than the range of 18.6–44.6 kg/day observed for this study.

Because several assumptions must be considered when the Fick principle is used, a discussion of possible errors in this method is appropriate. The assumptions are that:

1. Samples obtained from arterial and venous sources are representative of arterial and venous flows to and from the tissue and can be measured accurately.
2. Nutrients used to calculate blood flow are transferred quantitatively to products that are measured.
3. The system under study is in steady state.

Several possibilities for error exist due to the site of sampling, i.e. coccygeal and subcutaneous abdominal veins. Emery *et al.* (1965) initially validated the use of the coccygeal vein as indicative of arterial blood. Subsequent A-V studies conducted on hindlimbs of growing cattle demonstrated extraction percentages for methionine, threonine, lysine and tyrosine plus phenylalanine that ranged from a low of 0.22% for tyrosine plus phenylalanine to a high of 9.7% for methionine. The mean for the group was 3.7% (Danilson *et al.*, 1987). Except for methionine, these differences were not significantly different from zero. Although the tail must extract some nutrients, it is unlikely that the tail has a higher rate of metabolism than the growing hindlimb. Therefore, errors associated with determinations of blood flows using blood obtained from coccygeal veins rather than arterial blood obtained from the pudic artery are probably less than 4%.

Metcalf *et al.* (1991) did not observe dilutions of venous outflow from the udder by non-mammary blood, but others have observed reduced apparent blood flows to cow udders when external pudic veins were clamped as compared to when veins were unclamped (Heap *et al.*, 1988; Rasmussen, 1965). They suggested that this was due to dilution of venous outflow from the udder by blood from non-mammary sources and that it could result in a 20% error if blood flows were determined when pudic veins were not clamped. However, it is not clear whether subcutaneous abdominal veins are able to carry all the venous outflow from the udder without some

reduction in flow. To obtain a blood flow of 500 l/l milk for cows described herein and still account for observed A-V differences of amino acids, venous outflow would have had to have been diluted with arterial blood by 36%.

Davis *et al.* (1988) observed cardiac outputs in Jersey cows producing 16 kg/day of 45.8 l/min; 15.4% of total cardiac output was directed to the udder. Treatment with bST or thyroxine resulted in substantial increases in total cardiac output and a disproportionate amount of that increase was directed to the udder. When these observations are extended to estimate flow rates required to support the greater rates of milk secretion observed (Brown *et al.*, 1989), the estimates obtained indicate that approximately 20% of total cardiac output, or 12.17 l blood/min, would flow through the udder. Given this estimate, it seems unlikely that a dilution as large as 36% could occur in high-producing cows.

Although amino acids were measured in whole blood rather than in plasma, thereby negating errors associated with red blood cell transport of amino acids (Hanigan *et al.*, 1991), the udder may possibly extract a portion of its essential amino acids in the form of small peptides. Using *in vitro* techniques and reduced glutathione labeled with ³⁵S-cysteine, Baumrucker *et al.* (1981) demonstrated that cysteine from glutathione was incorporated into milk protein. Concurrently, Pocius *et al.* (1981) observed significant A-V differences for glutathione in a limited number of samples. However, when A-V differences for glutathione were determined on a large number of samples ($n = 192$), the mean A-V differences for glutathione was not significantly different from zero (Cant *et al.*, 1993a). Furthermore, a series of investigations, using perfused goat mammary glands and homologous blood perfusates containing labeled methionine, valine or phenylalanine, demonstrated that specific activities of methionine, valine and phenylalanine in milk protein were similar to specific activities of the corresponding amino acids in perfusates (Verbeke *et al.*, 1967, 1972). Although these observations do not rule out the possibility of peptide uptake by mammary glands, they suggest that little α -amino nitrogen found in milk is derived from blood peptides. Further, calculations of minimum blood flows required to supply energy, glucose and nitrogen secreted in milk presented below support this hypothesis.

A final consideration is the accuracy of measurements of arterial and venous concentrations of metabolites utilized to calculate blood flows and the stability of those concentrations. Methionine has the largest error associated with its A-V differences, particularly in high-producing cows, due to the very low concentrations of methionine in venous blood. However, when separating amino acids by PITC derivatization (Hanigan *et al.*, 1991), tyrosine, phenylalanine, threonine and lysine are cleanly resolved and are present in concentrations that can be determined accurately. To compensate for variability in amino acids concentrations over short periods, blood samples were collected every 20 min over the 12 h experimental period.

When 20 min samples were pooled by hour, concentrations of key metabolites in samples were very consistent throughout the sampling period (Hanigan *et al.*, 1991).

Based upon the considerations discussed above, we believe that estimates of blood flows using the essential amino acids tyrosine plus phenylalanine, methionine, threonine and lysine appear to be accurate. These estimates are supported by energy and nitrogen balance data presented below.

Uptakes of specific nutrients (mole/12 h) are presented in Table 13.6. Effects of stages of lactation and bST were evaluated. Other nutrients, including cholesterol and cholesterol esters, were considered. Their net contributions to mammary energy and N balances were not significant (Miller *et al.*, 1991b), therefore these data were not included. There were no differences by stage of lactation or bST treatment for uptakes of acetate, triacylglycerol (TAG), lactate, glycerol, essential, non-essential and total amino acids energy, and nitrogen in amino acids, or blood urea nitrogen. Uptakes of β -hydroxybutyrate (BHBA) declined ($P = 0.002$) as lactation progressed, while treatment with bST resulted in BHBA uptakes in week 15 that were similar to week 5 uptakes. Uptakes of glucose declined as lactation progressed ($P < 0.05$) and were proportional to observed reductions in glucose secreted as milk lactose. Uptakes of non-esterified fatty acids (NEFA) were positive at week 5 of lactation, declined to one-third by week 10, and were negative in week 15 and 18 for control cows. Treatment with bST increased ($P = 0.02$) NEFA uptakes in week 15 and 18 to levels equivalent ($P = 0.97$) to week 5 and 10 of lactation, respectively, and paralleled blood NEFA concentrations under these several conditions. Others have observed similar effects of bST on glucose, NEFA and BHBA uptakes by mammary glands (McDowell *et al.*, 1987; Fullerton *et al.*, 1989).

As stated above, a primary objective in this section of the chapter is to illustrate considerations relevant to quantitative evaluations of energy and nitrogen balance relationships with which an acceptable reference condition can be formulated for model development using lactating cow mammary glands as an example. Another objective is identification of sources of variance in patterns of nutrient availability that a model of mammary gland metabolism must be able to accommodate. Similarly, the model of cow metabolism presented in Chapter 16 should have the capacity to simulate both effects of stage of lactation upon nutrients available to mammary glands and resultant effects on metabolism. Excepting glucose, extractions of nutrients by cow mammary glands per unit of blood irrigating the glands are a function of their respective concentrations in blood. These relationships generally are not affected by bST treatment (Davis and Bickerstaff 1978; Davis *et al.*, 1988; Hanigan *et al.*, 1992; Miller *et al.*, 1991a,b). A number of factors influence blood nutrient concentrations, including diet and the negative energy balances associated with early lactation and early stages of

bST treatment. These changes in concentrations of nutrients are reflected in changes in patterns of nutrient uptake by the udder (Tables 13.6 and 13.7). For example, the proportion of energy supplied to the udder by NEFA and BHBA declined as lactation progressed ($P = 0.04$ and $P = 0.05$, respectively) and bST treatment reversed the decline in energy available as NEFA ($P = 0.04$). The proportion of energy supplied by acetate in control cows increased from week 5 to 15 then declined to values near those of week 5 by week 18. Conversely, with bST treatment, the proportion of energy supplied by acetate did not differ from the proportions observed during weeks 5 and 10. Energy supplied by TAG increased as lactation progressed ($P = 0.008$), whereas bST treatment caused an initial reduction in energy supplied by TAG; this proportion returned to control values by week 18. These observations indicate that significant changes in blood nutrient concentrations alter the balance of nutrients available to the mammary glands for milk synthesis. The general effects of stage of lactation and bST treatment upon mammary nutrient uptakes summarized in Tables 13.6 and 13.7 are compatible with consensus as discussed by Davis and Bickerstaff (1978), Davis *et al.* (1988), Hanigan *et al.* (1992), Miller *et al.* (1991a,b) and in other publications.

The data presented in Tables 13.6 and 13.7 are unique in that they are complete enough to allow quantitative assessments of nutrient availability to the udder and energy and nitrogen balances under a range of conditions. It is not feasible to present the data for each individual A-V study. Our compromise was to group the data according to stage of lactation and bST-treatment and depict observed variance in Figs 13.2–13.5. Aggregation of the data as in Tables 13.6 and 13.7 is an unwarranted simplification of a complex set of interrelationships. Plotting the data as in Figs 13.2–13.5 has the limitation that individual A-V studies are not identified; therefore compensating changes in availabilities and uptakes of alternate nutrients cannot be traced. This is why it is essential that individual sets of data be evaluated for internal consistency and energy and nitrogen balance. In our view, these data on individual experiments can only be captured in a dynamic, mechanistic model of cow mammary gland metabolism. On the other hand, Tables 13.6 and 13.7 represent the most complete summary available of nutrient and nitrogen balances for the cow udder (Linzell, 1974).

Overall efficiencies of conversion of metabolite energy to milk energy, glucose to milk lactose and nitrogen to milk nitrogen were 87 ± 3 , 73 ± 3 and $87 \pm 12\%$, respectively, based upon the data in Table 13.7. These efficiencies of conversion were not significantly altered by treatment or stage of lactation. Previous observations of efficiencies of conversion of energy in blood nutrients to energy in milk in ruminants were 85% (Blaxter, 1989), which agrees quite well with this estimate. Fullerton *et al.* (1989) observed an overall mean for conversion of glucose to lactose of 64%, which is somewhat

lower than our estimate. Waghorn and Baldwin (1984) derived a conversion factor of 88% from values presented in the literature. As mentioned above, these workers probably overestimated the efficiency of glucose utilization due to their use of an estimate for blood flow of 500 l/l milk. The efficiency of nitrogen utilization appears to be low. These estimates were based upon uptakes of individual amino acids and blood urea nitrogen as reported by Hanigan *et al.* (1992). Uptakes of individual amino acids (e.g. isoleucine) in considerable excess of requirements for milk protein synthesis could result in production and release of significant amounts of NH_3 and could explain part of the apparent underestimate of nitrogen utilization, since the A-V differences for NH_3 were not determined.

Results of simple linear regression analyses are presented in Figs 13.2–13.5 to illustrate general relationships and variance associated with these. Relationships clearly exist between milk production or production of individual milk components and uptakes of acetate, amino acids, glucose and energy. However, the relationships have limited explanatory power since r^2 values ranged from 0.19 to 0.33. Acetate uptakes per 12 h ranged from 5 to 16 moles at rates of milk production between 16 and 17 kg milk/12 h (Fig. 13.2). Clearly additional factors act to determine uptakes of acetate at a given rate of milk production. Uptake of glucose has been shown to explain a portion of the variability observed in uptakes of acetate (Forsberg *et al.*, 1984; Miller *et al.*, 1991a). One could expect uptakes of NEFA, BHBA and TAG to influence uptakes of acetate by substituting as sources of energy and carbon for milk fat synthesis. Nutrients that contribute to the acetyl-CoA pool such as NEFA, TAG, BHBA, lactate and ketogenic amino acids such as isoleucine (Hanigan *et al.*, 1992); and nutrients that alter acetate requirements for generation of NADPH_2 such as glucose, would be expected to influence both acetate oxidation and use in lipogenesis. High NEFA uptakes at higher rates of milk production and the slope of less than 1.0 (0.63) for the relationship between milk production and acetate uptake (Fig. 13.2) are consistent with this suggestion.

Similar types of considerations apply to the formulation of possible metabolic explanations for the variance illustrated in Fig. 13.3. In the case of amino acid energy uptake and milk production, variable uptakes of several non-essential amino acids and uptakes of, in particular, branched-chain amino acids in excess of needs for milk protein synthesis, could contribute considerable variance over and above that associated with nitrogen uptake versus nitrogen output ($87 \pm 12\%$).

A major ($73 \pm 3\%$) use of glucose is for lactose synthesis. Inversion of the regression equation (Fig. 13.4) relating milk glucose output and blood glucose uptake yields a slope of 0.76 ± 0.35 as compared to the overall estimate of 0.73 ± 0.03 , which suggests that the efficiency of conversion of blood glucose to milk lactose is not significantly altered by level of production. On the other hand, Miller *et al.* (1991a) reported a regression relationship be-

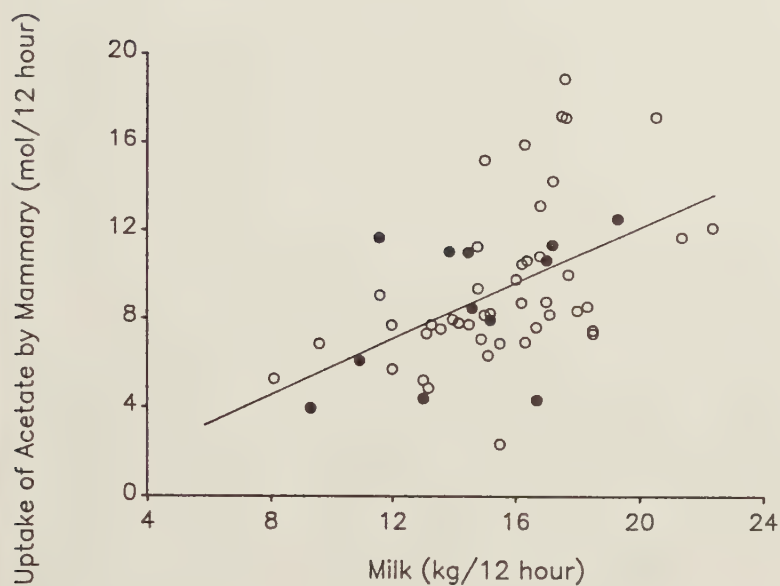


Figure 13.2 Acetate uptake by lactating mammary glands of bST-treated (●) and untreated cows (○) regressed on milk production. The regression equation describing the line was: $Y = 0.63 X - 0.58$; $P = 0.0001$, $r^2 = 0.26$.

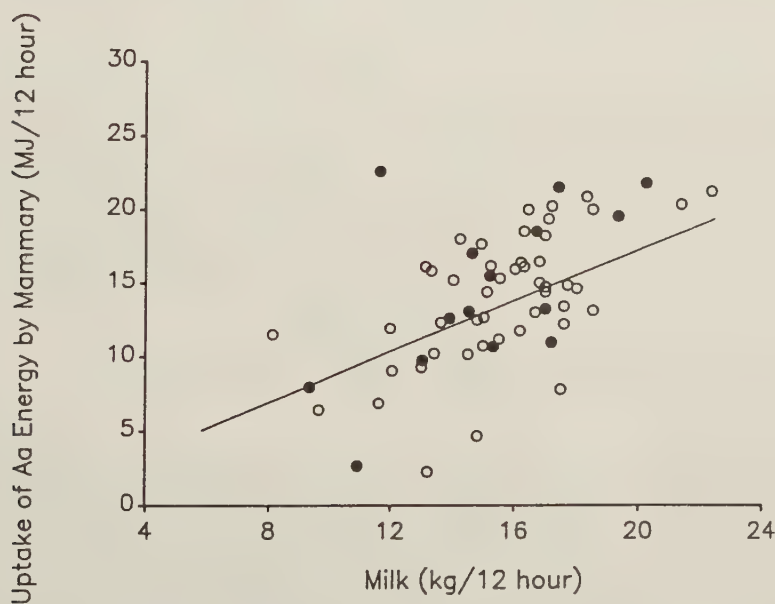


Figure 13.3 Uptake of amino acid (Aa) energy by lactating mammary glands of bST-treated (●) and untreated cows (○) regressed on milk production. The regression equation describing the line was: $Y = 0.21X - 0.006$; $P = 0.0001$, $r^2 = 0.33$.

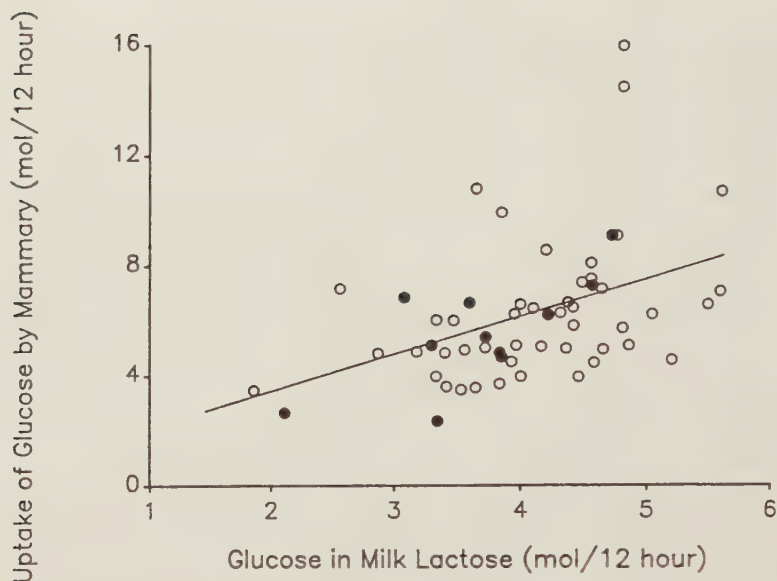


Figure 13.4 Glucose uptake by lactating mammary glands of bST-treated (●) and untreated cows (o) regressed on lactose production. The regression equation describing the line was: $Y = 1.31X + 0.78$; $P = 0.0005$, $r^2 = 0.19$.

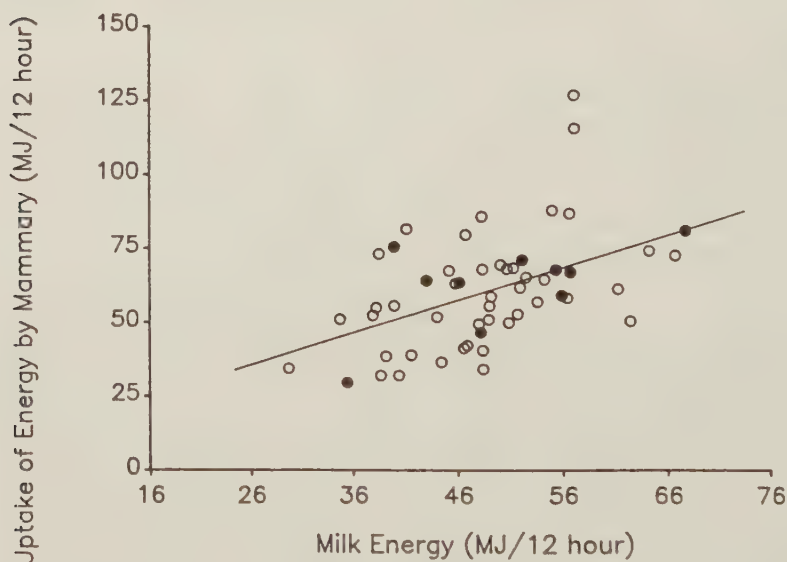


Figure 13.5 Uptake of total energy by lactating mammary glands of bST-treated (●) and untreated cows (o) regressed on milk energy. The regression equation describing the line was: $Y = 1.06X + 1.93$; $P = 0.0002$, $r^2 = 0.24$.

tween acetate availability and glucose extraction with an r^2 of 0.35, which clearly indicates that interactions among metabolites can contribute significantly to observed variance in uptake per unit of milk produced.

The overall estimate of efficiencies of conversion of energy in nutrients taken up by the udder to milk energy presented above was 0.87 ± 0.03 . Inversion of the equation relating energy in milk to energy in nutrients taken up by the udder (Fig. 13.2) yields a slope of 0.94 ± 0.26 , which suggests that high-producing cows are slightly more efficient at converting energy in nutrients to energy in milk as compared to cows of lower milk production. On theoretical grounds, a major contributor to variance in apparent efficiency of nutrient energy conversion to milk energy is due to the fact that blood lipids can be converted to milk fat fatty acids at an efficiency of 97%, while the efficiency of acetate conversion is 72% (Baldwin, 1968).

Obviously, simultaneous consideration of uptake of all these nutrients and rates of milk production create a dimensionality problem. This problem is further complicated by the existence of significant correlations among uptakes of some of the nutrients (Hanigan *et al.*, 1992; Miller *et al.*, 1991a). These complexities are not likely to be accommodated in multivariate analysis because of data requirements for resolution of multidimensional relationships using this approach. Conversely, it is possible these data are comprehensive enough to parameterize a mechanistic model once relative fluxes within the model have been set using available radiotracer data. This approach should help accommodate problems associated with correlations among regressors, thereby reducing the amount of data required for parameter estimation. In either case, it should be clear that provisions for accommodation of the causes of variation in patterns of nutrient uptake and use must be included in a mechanistic, analytical model of mammary gland metabolism. Our current model of mammary gland metabolism is directed to these objectives and is presented in abbreviated form in the next section of this chapter.

13.3 DESCRIPTION OF A MODEL OF MAMMARY GLAND METABOLISM

A block diagram of the model is presented in Fig. 13.1. Due to its complexity, the block diagram does not identify state variables in enclosed boxes nor are feedback or other effectors identified. Fluxes for the reference steady state are presented on arrows between metabolites. The cytoplasmic and mitochondrial membranes are identified by double lines. Codes used to identify specific nutrient and intermediary metabolites are presented in Tables 13.2 and 13.3 along with their concentrations in the steady-state condition used in model construction. Uptakes of nutrients per day in the reference state are presented in Table 13.4. Development of these estimates

was discussed in the previous section. Data on milk production and composition for the reference state are presented in Table 13.5. Based upon the input:output relationships defined in Tables 13.4 and 13.5, steady-state fluxes of intracellular metabolites were balanced in the following order:

1. Lactose synthesis.
2. Glycolysis.
3. Lipogenesis and lipolysis.
4. Amino acid and protein metabolism.
5. Tricarboxylic acid (TCA) cycle.

ATP use for functions not defined explicitly in the model (i.e. $\text{Na}^+ \text{K}^+$ -ATPase activity) were aggregated and identified as undefined (subscript Und; $\text{At,Ad}_{\text{Und}}$). This flux was adjusted to achieve reference rates of acetate and ketone body uptake.

Differential equations describing individual fluxes in the model are of the general form

$$\frac{d(\text{pool}_B)}{dt} = \text{inputs } (U_{A,B}) - \text{outputs } (U_{B,C})$$

where $U_{A,B}$ represents an input flux (mole/day) of metabolite or substrate A to metabolite or product B and $U_{B,C}$ represents an output flux (mole/day) of metabolite B to metabolite or product C. Equation forms used to describe fluxes ($U_{A,B}$, $U_{B,C}$) were discussed in Chapter 3 and are presented in Table 13.8. Equation forms 1 and 2 are based on mass action kinetics, where $K_{A,B}$ represents the mass action constant (mole/day), S_1 (mole/l) represents the primary substrate, S_n represents secondary or tertiary substrates, C_1 (mole/l) represents the reference or initial concentration of S_1 and C_n (mole/l) represents reference concentrations for secondary or tertiary substrates. Equation form 2 has provisions for an inhibitor (I ; mole/l) of enzyme activity that is associated with a reference concentration for I (C_i). Substrate concentrations for mass action equations were defined with respect to a reference concentration to simplify expression of $K_{A,B}$, i.e. $K_{A,B}$ is equal to $U_{A,B}$ at reference inputs. To clarify that this was an approach taken to simplify model construction rather than a classification of this parameter as a true rate constant, reference substrate and inhibitor concentrations for mass action equations were denoted as C_n and C_i in Table 13.8.

Equation forms 3–6 are based on saturation kinetic arguments. Form 3 is the standard Michaelis–Menten equation, where $V_{A,B}$ represents the maximal rate of conversion of substrate A to product B (mole/day) and k_n ($n = 1\text{--}3$) represents the apparent affinity constant for S_n . Form 4 has an exponent applied to the primary substrate/affinity constant ratio. This allows for a sigmoidal response with respect to concentration of the primary substrate. Form 5 has provisions for a competitive inhibitor (j_i) that is associated with I . By use of an exponent, form 6 exhibits sigmoidal kinetics with respect to the concentration of I .

Table 13.8 Equation forms for metabolic transactions

Form number	General equation form ^a
1	$U_{A,B} = K_{A,B} * (S_1/C_1) * (S_n/C_n)$
2	$U_{A,B} = K_{A,B} * (S_1/C_1) * (S_n/C_n) * (C_i/I)$
3	$U_{A,B} = V_{A,B} / (1 + (k_1/S_1) + (k_n/S_n))$
4	$U_{A,B} = V_{A,B} / (1 + (k_1/S_1)^E + (k_n/S_n))$
5	$U_{A,B} = V_{A,B} / (1 + (k_1/S_1) + (k_n/S_n) + (I/j_i))$
6	$U_{A,B} = V_{A,B} / (1 + (k_1/S_1) + (k_n/S_n) + (I/j_i)^E)$
7	$U_{A,B} = V_{A,B} * (I/j_i)^A / (1 + (k_1/S_1) + (k_2/S_2)^E)$

^a_n = substrate or affinity constant number if additional substrates are used in the equation. See text and Chapter 3 for further discussion of equation forms.

Rate constants associated with these equations are presented in Table 13.9. In general, affinity constants were initially set equal to substrate concentration. This approach was taken for two reasons: (1) biological affinity constants are often in the range of substrate concentrations, and (2) an arbitrary assignment of initial parameters would not bias initial evaluations of model behavior and sensitivity and thus compromise use of the Forsberg *et al.* (1984, 1985a,b) data in initial evaluations and subsequent stages of model development. Several affinity constants were adjusted to values greater or less than the reference substrate concentration based on initial behavioral and sensitivity analyses, e.g. if model evaluations indicated that solutions were not sensitive to the k_s = substrate concentration assumption, the k_s was not adjusted. If sensitivity to the assumption was high and prevented simulations consistent with experimental data, adjustments were undertaken.

In order to enable adjustment of the model to different rates of milk production, a factor (U_{enz}) was applied to V_{max} values for key equations involved in the uptake and conversion of nutrients to milk lactose, protein and fat. The ability to adjust the model to different levels of milk production is important when using data sets derived from cows differing significantly in genetic potential or stage of lactation from the reference production of 30 kg/day. Equations identified as rate limiting steps are denoted in Table 13.9 by an asterisk. The reference value for U_{enz} is 1.0 at the reference milk production of 30 kg/day.

Model equations were written in ACSL (1990; Chapter 4). Table 13.9 along with Fig. 13.1 are almost adequate to reformulate the model. For example, the differential equation for G6P can be written based upon Fig. 13.1 as follows:

$$DG6P = U_{\text{Glc,G6P}} - U_{\text{G6P,Lac}} - U_{\text{G6P,Ru}} + U_{\text{F6P,G6P}} - U_{\text{G6P,F6P}} \quad (13.63).$$

The respective equation forms for $U_{\text{Glc,G6P}}$, $U_{\text{G6P,Lac}}$, $U_{\text{G6P,Ru}}$, $U_{\text{F6P,G6P}}$ and $U_{\text{G6P,F6P}}$ are 5, 3, 3, 1 and 1 (Tables 13.8 and 13.9). The associated parameters

Table 13.9 Parameter values for metabolic transactions^a

Transaction ($U_{A,B}$) ^b	Equation form	Substrates/inhibitors					Parameter values						Equation number
		S_1	S_2	S_3	S_{j1}	$V_{A,B}$	$K_{A,B}$	k_1/C_1	k_2/C_2	k_3/C_3	j_1/C_j	ϵ/A	
Aa,Deg	1	Aa					1.42	2.99E-3					13.1
Aa,eAa	3	Aa				30.0		2.99E-3					13.2
Aa,Py	3	Aa				0.2		2.99E-3					13.3
Ac,AcCs*	3	Ac	Cs			107.86		1.8E-2	6.5E-5				13.4
Ac,mAcCs	3	Ac				19.79		1.8E-3	6.5E-5				13.5
AcCs,Tm*	5	AcCs	mCs			102.32		4.04E-5	8.24E-4	1.16E-4	2.89E-5		13.6
Agp,G3p	1	Agp	Agp	Nph	FaCs		2.32	2.06E-4	1.16E-4				13.7
Akg,Ctp*	3	Akg	Nd			120.0		3.64E-5	1.16E-3				13.8
Akg,Glu	1	Akg	Nph	Ndh			1.26	3.64E-5	2.06E-4	1.16E-4			13.9
Akg,Mal	1	Akg	NH ₃				21.22	3.64E-5	1.16E-4				13.10
Ala,eAla	3	Ala	Nd					2.67E-4					13.11
Ala,Py	5	Ala			Glu	6.0		2.67E-4			4.05E-3		13.12
Asp,eAsp	3	Asp				6.0		9.91E-4					13.13
Asp,Oa	2	Asp	Nd		Glu		2.0	9.91E-4			4.05E-3		13.14
At,Ad _{und}	1	At					160.31	7.50E-4					13.15
Cd,eCd	1	Cd					51.44	9.83E-4					13.16
Ct,Akg*	3	Ct	Nd			49.75		2.02E-2	1.16E-4				13.17
Ct,Milk	1	Ct					0.36	2.02E-2					13.18
Ct,Oa	1	Ct	At	Cs			0.2	2.02E-2	7.50E-4	6.5E-5			13.19
Ctp,Akg*	3	Ct	Np			44.69		2.02E-2	1.16E-4				13.20
eamino acids, amino acids	3	eamino o acids				40.81		2.99E-3					13.21

Transaction ($U_{A,B}$) ^b	Equation form	Substrates/inhibitors				Parameter values						ϵ/A	Equation number
		S_1	S_2	S_3	S_{j1}	$V_{A,B}$	$K_{A,B}$	k_1/C_1	k_2/C_2	k_3/C_3	j_1/C_j		
eAla,Ala	3	eAla				6.67		2.60E-4					13.22
eAsp,Asp	3	eAsp				6.40		1.65E-4					13.23
eGlu,Glu	3	eGlu				6.76		1.20E-4					13.24
eLys,Lys*	4	eLys				117.0		1.9E-3				1.76	13.25
eMet,Met*	4	eMet				117.0		1.35E-4				1.56	13.26
eThr,Thr*	4	eThr				117.0		2.45E-3				1.77	13.27
eTp,Tp*	4	eTP				117.0		8.5E-4				2.65	13.28
F6P,G6P	1	F6P					12.19	3.0E-5					13.29
F6P,Fbp*	7	F6P	At			4.24		3.0E-5	7.5E-4		7.5E-4	4.0/1.5	13.30
FaCs,mAcCs	1	FaCs	Nd				0.21	1.16E-4	1.16E-4				13.31
FaCs,Tm*	3	FaCs	Agp			14.04		1.16E-4	8.24E-4				13.32
Fbp,F6P	1	Fbp					0.1	1.04E-5					13.33
Fbp,G3P	1	Fbp					1.31	1.04E-5					13.34
G3P,amino acids	1	G3P					0.61	1.43E-4					13.35
G3P,Agp	1	G3P	Ndh				2.0	1.43E-4	1.16E-4				13.36
G3P,Py*	3	G3P	Nd			8.60		1.43E-4	1.16E-4				13.37
G6P,F6P	1	G6P					10.0	7.51E-5					13.38
G6P,Lac	3	Glc	G6P			12.54		3.0E-3	7.51E-5			TAa _{MikPh}	13.39
G6P,Ru*	3	G6P	Nd			110.46		7.51E-4	1.16E-3				13.40
Glc,G6P*	5	Glc		G6P		21.75		3.0E-4			7.51E-5		13.41
Glu,amino acids	3	Glu				0.06		4.05E-3					13.42
Glu,Akg	2	Glu	Nd	NH ₃			1.0	4.05E-3	1.16E-4		2.06E-4		13.43
Glu,eGlu	3	Glu				6.0		4.05E-3					13.44
Gy,Agp	1	Gy					0.5	9.83E-4					13.45

Table 13.9 (continued)

Transaction ($U_{A,B}$) ^b	Equation form	Substrates/inhibitors					Parameter values						Equation number
		S_1	S_2	S_3	S_{II}	$V_{A,B}$	$K_{A,B}$	k_I/C_1	k_2/C_2	k_3/C_3	j_I/C_j	ϵ/A	
Gy,eGy	1	Gy					0.35	9.83E-4					13.46
Kb,Tm*	3	Kb	Agp			3.57		6.0E-4	8.24E-4				13.47
Kb,mAcCs	3	Kb	Nd	mCs		19.58		6.0E-4	1.16E-4	6.5E-5			13.48
La,Py	3	La	Nd			9.09		1.73E-4	1.16E-4				13.49
Mal,G3p	1	Mal	Np				0.2	5.09E-4	1.16E-4				13.50
Mal,Oa	1	Mal	Nd				32.10	5.09E-4	1.16E-4				13.51
Ndh,Nd _A TP	3	Ndh	Ad			375.36		2.31E-4	3.28E-4				13.52
NNH ₃ ,Mik	1	NH ₃					0.65	2.06E-4					13.53
Oa,Asp	1	Oa	Glu				2.36	2.14E-6	4.05E-3				13.54
Oa,Ct	1	Oa	mAcCs				21.85	2.14E-6	2.02E-5				13.55
Oa,Mal	1	Oa	Ndh				10.0	2.14E-6	1.16E-4				13.56
Py,Ala	3	Py	Glu			3.08		4.62E-5	4.05E-3				13.57
Py,La	3	Py	Ndh			9.0		4.62E-5	1.16E-4				13.58
Py,mAcCs	6	Py	Nd	Cs	mAcCs	14.31		4.62E-5	1.16E-4	6.5E-5	2.02E-5	1.0	13.59
Py,Oa	4	Py	mAcCs			0.33		4.62E-5	2.02E-5			1.0	13.60
Ru,F6P	1	Ru					5.26	5.78E-5					13.61
Tg,FaCs*	3	Tg	Cs			3.4		5.0E-4	6.5E-5				13.62

*Adapted from Hanigan and Baldwin (1994).

^bTransactions superscripted with an * have EUnz as a non-competitive activator of V_{max} .

are in Table 13.9. Nutrient uptakes, initial pool sizes and concentrations are in Tables 13.2, 13.3 and 13.4. Scaling factors used to facilitate solutions (Chapter 4) and a considerable number of additional details including stoichiometric parameters were presented in Hanigan and Baldwin (1994) and are not repeated here. In view of the recent detailed publication (Hanigan and Baldwin, 1994), only a few selected equations will be discussed to illustrate underlying considerations in formulation of the model.

Several comprehensive studies have been conducted on mammary tissue using various radiolabeled tracers (Forsberg *et al.*, 1984; 1985a,b). In order to use these data, differential equations were constructed for individual carbons in intermediates in the model using the approach discussed in Chapter 12 for adipose tissue metabolism.

13.3.1 Model equations

In general, Michaelis–Menten equation forms were used for all rate-limiting steps, while mass action equations were used for non-rate-limiting steps.

The differential equation for glucose-6-phosphate (G6P) was presented above (equation 13.63). Rates of extraction of Glc have been shown to be regulated by feedback inhibition by G6P (Newsholme and Leech, 1983). In early versions of the model, provision for this feedback was omitted but glucose uptake relationships were not adequately simulated. Therefore, G6P inhibition was included in equation 13.41 (Table 13.9). Pentose phosphate cycle flux is catalyzed by a series of reactions. Glucose-6-P dehydrogenase (G6PD) catalyzes the first step in this pathway and the availability of NADP (Np) is thought to be rate limiting in this reaction (Baldwin and Yang, 1974). Six-phosphogluconolactone, the immediate product of the G6PD reaction, is subsequently converted to ribulose-5-phosphate. Since the first reaction in the pathway appears to be rate limiting, the reactions are described in aggregate by equation 13.40. While the kinetic properties of any aggregated equation of this type are influenced by each enzyme involved, the basic properties of the transaction should be dominated by the rate-limiting enzyme and, in our view, the equation can be parameterized (adequately) using either current or new data on flux through the pathway. Initially, Np was included in equation 13.40 as a substrate with an affinity constant that was four times greater than the concentration of Np. Sensitivity analyses indicated that this led to incorrect simulation results. The affinity constant was subsequently set to 20 times the concentration of Np (Table 13.9). Note that the concentration of NP in the table is total concentration and not the 'free' concentration which is not known but is in the 10^{-8} to 10^{-9} mole/l range, thereby justifying a k_s value far below the concentration specified in the model.

Lactose production is catalyzed by lactose synthetase, a complex of two proteins – UDP galactosyl transferase and α -lactalbumin (Ebner and Schan-

bacher, 1974). The requirement for α -lactalbumin creates a link between rates of protein and lactose synthesis. This mechanism is captured in equation 13.39 in Table 13.9 by using the ratio

$U_{TAa, MilkPrt}/\text{Reference Flux}$

as a multiplicative activator of V_{max} , where $U_{TAa, MilkPrt}$ is the flux of total amino acids to milk protein (mole/day) and the Reference Flux is 7.90 mole/day.

Moles of lactose formed per day are determined by the flux, $U_{G6P, Lac}$. Glucose required for lactose synthesis is equal to $2 * U_{G6P, Lac}$ and total uptake of Glc is equal to the sum of $U_{Glc, G6P}$ and $U_{G6P, Lac}$. The differential equation for F6P is as follows:

$$\frac{dF6P}{dt} = U_{Fbp, F6P} + (0.667 * U_{Ru, F6P}) + U_{G6P, F6P} - U_{F6P, G6P} - U_{F6P, Fbp} \quad (13.64)$$

Inputs from Fbp, G6P and Ru are described by equations 13.33, 13.38 and 13.61, respectively. Outputs to G6P and Fbp are described by equations 13.29 and 13.30, respectively. As mentioned above, effluxes from the ribulose-5-P pool are handled in aggregate. The portion of this efflux contributing to the F6P pool is 0.667 mole/mol. Equation 13.38 represents the enzyme phosphofructokinase-1 (PFK). Adenosine triphosphate (At) serves as a substrate for PFK and acts as an allosteric inhibitor of the reaction (Baldwin and Yang, 1974; Newsholme and Leech, 1983). There have been extensive studies of PFK in other tissues but the enzyme has not been studied in cow mammary tissue. Thus, simplicity is required at this time. At was added to the numerator of equation 13.38 as a ratio of the initial or reference concentration of At divided by the concentration of At and to the denominator as a substrate. Exponents of 6 and 4 were applied to the ratios in the numerator and denominator, respectively. These exponents result in a response where PFK is highly inhibited at reference At concentrations. As At concentrations fall, PFK flux increases until At becomes limiting as a substrate at low concentrations not likely to be encountered in either reality or simulations of normal conditions, e.g. within physiological range.

Low activities of fructose-1,6-bisphosphatase activity have been observed in mammary homogenates and incorporation of radiolabel from acetate into lactose has been observed (Wood *et al.*, 1957a,b). The appearance of radiolabeled carbons derived from La, Ac, Tg or Kb in lactose may occur through the incorporation of G3P into the lower half of F6P in the pentose cycle (Baldwin and Yang, 1974) or through conversion of Fbp to F6P via fructose-1,6-bisphosphatase. The labeling pattern observed by Wood *et al.* (1957a) indicates that both pathways are active. Therefore, $U_{Fbp, F6P}$ was included.

The differential equation for fructose-1,6-bisphosphate metabolism is as follows:

$$\frac{dFbp}{dt} = U_{F6P,Fbp} - U_{Fbp,F6P} - U_{Fbp,G3P} \quad (13.65)$$

Input is from F6P and outputs are to F6P and G3P (equations 13.30, 13.33 and 13.34). Cleavage of Fbp by aldolase yields one mole each of glyceraldehyde-3-P and dihydroxyacetone-P. These are treated in aggregate as G3P based upon observations that indicate rapid equilibration between these two metabolites due to high triose-P isomerase activity. No regulation of these reactions has been reported.

Conversion of G3P to Agp is partially dependent on redox state (equation 13.36). Agp formed from G3P and glycerol is used solely for fatty acid esterification ($U_{FaCs,Tm}$). The importance of isolating triacylglycerol as an indicator of pentose cycle flux was discussed in Chapter 12. In the case of mammary tissue, this indicator of metabolic pathways is partially compromised by the presence of glycerol kinase ($U_{Gy,Agp}$). The conversion of G3P to pyruvate (Py) via the lower half of the glycolytic pathway is treated in aggregate in equation 13.37 (Fig. 13.1). The conversion of Mal to phosphoenolpyruvate (PEP) via Oa and, subsequently, to G3P ($U_{Mal,G3P}$) was included in the model to accommodate observed uptakes and calculated rates of degradation of amino acids (Hanigan and Baldwin, 1994) and observed radiolabeling patterns when La was used as a radiotracer (Forsberg *et al.*, 1985b). The aggregation of several reactions into the $U_{G3P,Py}$ flux necessitated entry of Mal directly into the G3P pool as opposed to an intermediate PEP pool, since PEP is not defined in the model. The implicit assumption in aggregation of these transactions is that radiolabel equilibrates fairly rapidly between PEP and G3P, which may not be true.

The differential equation for pyruvate metabolism (Py) is as follows:

$$\frac{dPy}{dt} = U_{Aa,Py} + U_{Ala,Py} + U_{G3p,Py} + U_{La,Py} - U_{Py,Ala} - U_{Py,La} - U_{Py,mAcCs} - U_{Py,Oa} \quad (13.66)$$

Inputs of amino acids, Ala, G3P and La are described by equations 13.3, 13.12, 13.37 and 13.49, respectively. Outputs to Ala, La, mAcCs and Oa are described by equations 13.57, 13.58, 13.59 and 13.60, respectively.

Activities of pyruvate dehydrogenase and pyruvate carboxylase have been shown to be responsive to changes in mAcCs (Newsholme and Leech, 1983). This mechanism is captured by addition of mAcCs as an activator and as an inhibitor in equations 13.60 and 13.59, respectively. However, sensitivity of these enzymes to mAcCs has not been studied in mammary tissue. Exponents (default values are one) were included in these equations to adjust sensitivity when data become available.

Amino acids not explicitly defined in the model were aggregated into the amino acids pool. Removal of individual amino acids for milk protein synthesis was considered prior to calculation of stoichiometries associated with

routes of utilization of other amino acids. Calculations associated with the amino acids pool were described in detail in Hanigan and Baldwin (1994).

Representing lactate metabolism in mammary as well as other tissues (Chapters 12 and 14) is essential, because lactate is often used as a radiotracer of metabolism (Forsberg *et al.*, 1985b; Allen *et al.*, 1976) while, on the other hand, can be a significant product of the metabolism of other radiotracers (Yang and Baldwin, 1973; Knapp *et al.*, 1992). Lactate metabolism is very difficult to represent, because shifts in the ratio of lactate to pyruvate *in vivo* and, certainly, *in vitro* can have or reflect very significant changes in intracellular redox states and the NAD/NADH₂ ratio which, in turn, influences many metabolic events. In view of the complexity of possible interactions that cannot be resolved based upon current data, the representation of lactate metabolism in the model is very simplistic. A single, extracellular pool of lactate (eLa) was incorporated and, essentially, an equilibrium between eLa and Py was assumed, e.g., $U_{eLa,Py}$ are almost equal and the exchange rate is very high (Fig. 13.1).

The differential equation for mitochondrial acetyl-CoA (mAcCs) is

$$\frac{dmAcCs}{dt} = U_{Aa,mAcCs} + U_{Ac,mAcCs} + (U_{FaCs,mAcCs} * 8) + (U_{Kb,mAcCs} * 2) + U_{Lys,mAcCs} + U_{Py,mAcCs} + U_{TP,mAcCs} - U_{Oa,Ct} \quad (13.67)$$

where $U_{Aa,Deg}$ is described by equation 13.1. Computation of the moles of mAcCs generated per mole of amino acids degraded (0.506) and Lys, and TP (tyrosine plus phenylalanine) degradative fluxes are described in Hanigan and Baldwin (1994). Inputs of Ac, FaCs, Kb and Py are described by equations 13.5, 13.31, 13.48 and 13.59, respectively. Output to Ct ($U_{Oa,Ct}$) is described by equation 13.55. It was assumed that fatty acid chain length was 16 carbons, resulting in the generation of eight moles of mAcCs per mole of FaCs oxidized. It is not clear if this is an appropriate assumption, since a large proportion of preformed fatty acids used for milk fat synthesis are 18 carbons in length.

In an early version of the model, the mitochondrial and cytosolic acetyl-CoA (mAcCs,AcCs) pools were represented as a single pool. It was soon found that these pools must be treated separately to adequately simulate radiotracer metabolism. In addition, the dynamics of the mAcCs pool are very important to regulation of pyruvate dehydrogenase ($U_{Py,mAcCs}$; equation 13.59) and pyruvate carboxylase ($U_{Py,mAcCs}$; equation 13.60) reactions.

Citrate (Ct) is an important intermediary metabolite, since it is not only the first intermediate in the tricarboxylic acid pathway but is also secreted into milk ($U_{Ct,milk}$). Also, by virtue of its rapid equilibration with isocitrate, it is an important source of NADPH₂ for support of fatty acid synthesis. The aggregate equations for the interconversion of citrate and Akg via the NADP-isocitrate dehydrogenase (NAD-ICDH) reaction are notated as $U_{Ctp,Akg}$ and $U_{Akg,Ctp}$ in equations 13.20 and 13.8, respectively. Activities of

NADP-ICDH are high in mammary tissue of ruminants, suggesting that a significant proportion of Nph required for synthesis of milk triacylglycerol is generated by NADP-ICDH (Bauman and Davis, 1974). It was arbitrarily assumed in the reference state that 30% of Nph for fat synthesis was generated by the NADP-ICDH catalyzed reaction, while 70% was generated via the pentose phosphate pathway. This proportionality is variable; we hope the mammary gland model will help characterize causes in variance in sources of NADPH₂ and help improve the representations used in our whole animal model (Chapter 16).

The variable transactions associated with α -ketoglutarate (Akg) metabolism are the NADP-ICDH reaction and a net conversion to glutamate ($U_{\text{Glu,Akg}}, U_{\text{Akg,Glu}}$) as indicated in Fig. 13.1. The primary fate of Akg is conversion to malate (Mal, $U_{\text{Akg,Mal}}$).

The stoichiometric relationships for amino acids and amino acids to the mitochondrial malate pool (Hanigan and Baldwin, 1994) and definition of a single malate pool, which assumes a rapid rate of equilibration between malate and oxaloacetate (Fig. 13.1), are simplifications of reality. Data on other tissues clearly indicate that both cytosolic and intramitochondrial pools of Mal exist and are important determinants of metabolic transactions. Further, radiotracer data indicate that rates of isotopic equilibration between Oa and Mal can have very significant effects upon radiotracer metabolism. For example, when ¹⁴C-bicarbonate is used as a tracer, ¹⁴C enters the Oa pool via the pyruvate carboxylase reaction, and one might anticipate that the specific activity of Oa, or a derivative such as Asp, could be used as an indicator of the proportion of Oa formed via this reaction, especially in view of the fact that the Oa pool is very small and turns over rapidly. However, if the rate of equilibration of radiotracer between Oa, Mal, fumarate and succinate is high, as is true in mammary tissue, the apparent pool size of Oa becomes large (Baldwin and Yang, 1974). Due to aggregation in this representation, randomization of label associated with the supposed rapid equilibration among dicarboxylic acids is assumed in the Mal pool. Since radiotracer in Oa can cycle back to Mal from citrate via the TCA cycle, the specific activity of Oa can become significantly greater than that directly attributable to the pyruvate carboxylase reaction. From the standpoint of deducing parameters based upon radiotracer data, these observations present an advantage but impose requirements with regard to experimental design. Additional data may well require either changes in parameters in the current model or inclusion of additional detail.

The differential equation for cytosolic acetyl CoA (AcCs) metabolism is as follows:

$$\frac{d\text{AcCs}}{dt} = U_{\text{Ac,AcCs}} + U_{\text{Ct,Oa}} - U_{\text{AcCs,Tm}} - (U_{\text{Kb,Tm}} * P_{\text{AcCs,Tm}}) \quad (13.68)$$

Inputs of Ac and Ct are defined by equations 13.4 and 13.19, respectively. The affinity constant for Ac was set to 10 times the reference concentration,

equation 13.4. This produces the linear response in acetate uptake reported by Miller *et al.* (1991a) and in Fig. 13.2 based on A-V studies. Normally, flux through the citrate cleavage reaction ($U_{\text{Ct,Oa}}$) is low in mammary tissue (Baldwin and Yang, 1974). However, when lactate is provided as substrate in the absence of acetate, rates of lipogenesis from lactate can be quite high (Forsberg *et al.*, 1985b). Thus, provision for transfer of mAcCs, formed from lactate via pyruvate dehydrogenase and transferred to the AcCs pool via citrate and citrate lyase ($U_{\text{Ct,Oa}}$), was essential. In the model, as *in vivo*, acetate reduces the use of lactate as a source of carbon for fatty acid synthesis (Forsberg *et al.*, 1985b) by increasing the concentration of mAcCs, which inhibits the pyruvate dehydrogenase reaction ($U_{\text{Py,mAcCs}}$; equation 13.59). Forsberg *et al.* (1985b) also suggested that high concentrations of acetate reduce the availability of coenzyme A for use in the citrate lyase reaction. This is consistent with the high concentration of citrate in milk from ruminants ($U_{\text{Ct,Mlk}}$). Use of AcCs by acetyl-CoA carboxylase and condensation with BHBA for fatty acid synthesis is described by equations 13.6 and 13.47, respectively.

Milk fat from cows contains significant quantities of fatty acids that range in chain length from four to 18 carbons. Some of these are synthesized *de novo* in the mammary gland and some are derived from blood lipids. For simplicity, Hanigan and Baldwin (1994) aggregated the fatty acids into three pools. The first pool included short-chain fatty acids, ranging from four to eight carbons in length. These were considered to be synthesized in the gland from BHBA and acetate via, essentially, a reversal of β -oxidation with crotonyl-CoA reductase, an NADPH₂-linked enzyme (Nandedkar *et al.*, 1969) catalyzing the final reduction step. For example, the reduction of BHBA to form butyrate would require one NADPH₂. The average chain length in this group was 5.03 carbons. The second pool consists of fatty acids with chain lengths from 10 to 16 carbons, which are synthesized *de novo* in the gland via the acetyl-CoA carboxylase and fatty acids synthetase reactions. Average chain length in this pool was set at 14 carbons, based upon the assumption that 50% of palmitate in milk was synthesized in the gland and 50% was derived from blood lipids. The third pool consists of fatty acids that arise from blood lipids and range in chain length from 16 to 18 carbons. In the reference state, 48% of fatty acids in milk fat is synthesized in the gland. The mean chain length of fatty acids in milk fat is 14.3 carbons, yielding a molecular weight for milk fat of 732 g/mole.

Fatty acids synthesized *de novo* were represented as being transferred directly to milk fat (T_m) rather than entering the FaCs pool to prevent recycling of radiolabel via β -oxidation ($U_{\text{FaCs,mAcCs}}$) and to circumvent problems with determining the average chain length of fatty acids oxidized to mAcCs in mitochondria (Fig. 13.1).

It was noted above one of the limitations of the Waghorn and Baldwin (1984) mammary gland metabolism model and the lactating cow model

presented in Chapter 16 is that amino acids were considered in aggregate. As a result, these models cannot respond to dietary supplementation of critical amino acids. A motivation in design of the A-V studies discussed earlier in this chapter was to collect data to enable elimination of this limitation. In the current mammary gland model, availabilities of tyrosine plus phenylalanine (TP), methionine (Met), lysine (Lys) and threonine (Thr) can limit milk protein synthesis. Uptakes of Lys, Met, Thr and TP are described by equations 13.25, 13.26, 13.27 and 13.28, respectively. The equations are as described by Hanigan *et al.* (1992). Maximal milk protein yields from each of these EAa are calculated according to the equation

$$\text{MlkPrt}_{\text{EAa}} = U_{\text{eEAa,EAa}}/P_{\text{EAa,MlkPrt}} \quad (13.69)$$

where $\text{MlkPrt}_{\text{EAa}}$ equals the moles of total amino acids that could be converted to milk protein given uptakes of each EAa ($U_{\text{eEAa,EAa}}$), and $P_{\text{EAa,MlkPrt}}$ represents the molar proportion of each EAa in milk protein and equals 0.07, 0.0221, 0.0456 and 0.0726 for Lys, Met, Thr and TP, respectively. A logic routine is used to determine which of these EAa is most limiting for milk protein production. The most limiting EAa sets the protein synthesis rate ($U_{\text{TAa,Prt}}$). Uptakes of Met, Lys, Thr and TP in excess of needs for protein synthesis are catabolized according to the following generalized equation:

$$U_{\text{EAa,Deg}} = U_{\text{eEAa,EAa}} - (U_{\text{TAa,Prt}} * P_{\text{EAa,MlkPrt}}) \quad (13.70)$$

This approach circumvents the need for additional differential equations describing intracellular pools for each of these EAa. While this approach is a great simplification of the mechanisms regulating milk protein synthesis and amino acid metabolism, data are not available to parameterize more complex equations nor has it been demonstrated that more complex equations are warranted.

Differential equations for glutamate (Glu), aspartate plus asparagine (Asp), alanine (Ala), and remaining amino acids including arginine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, ornithine, proline, serine, tryptophan and valine are included in the model. In these equations, amino acids taken up by the udder are either incorporated into milk protein or degraded. In addition, Glu, Asp and Ala can be synthesized in the gland for incorporation into protein or can act as carriers of amino nitrogen, arising from degradation of other amino acids, from the udder. This later provision accommodates the negative A-V differences for these amino acids observed *in vivo* (Hanigan *et al.*, 1992). Another reason for treating Ala, Asp and Glu separately is that they can and have been used as radiolabeled substrates to probe the metabolism of Py, Oa and Akg, respectively.

Stoichiometries associated with metabolism of the amino acids pool were presented by Hanigan and Baldwin (1994). Removal of Ser, Gly, Pro and Cys from blood was inadequate to support observed rates of milk protein synthesis. Therefore, Ser, Gly and Pro were considered to be synthesized from Arg/Orn in calculating the stoichiometric coefficients used. The appar-

ent shortage of Cys was within the standard error of measurement of Cys A–V differences (Hanigan *et al.*, 1991). Therefore, Cys uptake was assumed to be zero. Uptakes by the udder of Arg, Gln, Ile and Val were greater than required for milk protein synthesis and were prominent contributors to estimated stoichiometric coefficients for amino acid catabolism. Removal of His, Leu and Trp was nearly equal to outputs in milk protein. Therefore, degradation of these amino acids was assumed to equal zero.

Hanigan and Baldwin (1994) presented differential equations for a number of entities not explicitly identified in Fig. 13.1 but which are central determinants of model behavior *per se* and important in monitoring model behavior. These include equations for ATP, coenzyme A, NADH₂, NADPH₂, FADH₂, oxygen uptake, and lactose, milk protein, milk fat and carbon dioxide production. All fluxes in transactions associated with these entities are functions of reactions depicted in Fig. 13.1. For example, ATP use in the hexokinase reaction equals $U_{\text{Glc,G6P}}$. For the sake of brevity, these equations will not be repeated. However, they are essential to computation of model outputs that can be compared to experimental data such as the respiratory quotient, heat production, energetic efficiencies, milk yield and milk composition.

13.3.2 Model evaluations

(a) Behavioral analyses

As noted many times in previous chapters, behavioral analyses determine whether or not equation forms in the model are adequate to simulate observed response patterns. Emphasis is placed on determining whether or not the model has the capacity to simulate observed response patterns, and only limited emphasis is placed on quantitative relationships. Behavioral analyses for this model were conducted using Glc labeled in the 1 and 2 positions, Ac labeled in the 1 position, and La labeled in the 1 and 2 positions. Labeled carbon flows to various products were compared to response patterns presented by Forsberg *et al.* (1984, 1985a,b).

Glucose uptake and oxidation were responsive to Glc concentration (Fig. 13.6a). This is consistent with *in vitro* data (Forsberg *et al.*, 1985b) and may be consistent with *in vivo* data. Miller *et al.* (1991a) did not observe a relationship between Glc concentration and Glc extraction per unit volume of blood perfusing the udder. However, glucose concentrations ranged from approximately 3–5 mM in those experiments. Model predictions of glucose uptake over a similar range of glucose concentrations varied from 10 to 12 mole/day, which is equivalent to a 20% increase in uptake. Uptakes of this magnitude are well within the variance observed by Miller *et al.* (1991a). The relationship between Ac and Glc uptake (Fig. 13.6b) was consistent with the data of Miller *et al.* (1991a). However, the simulated magnitude of this in-

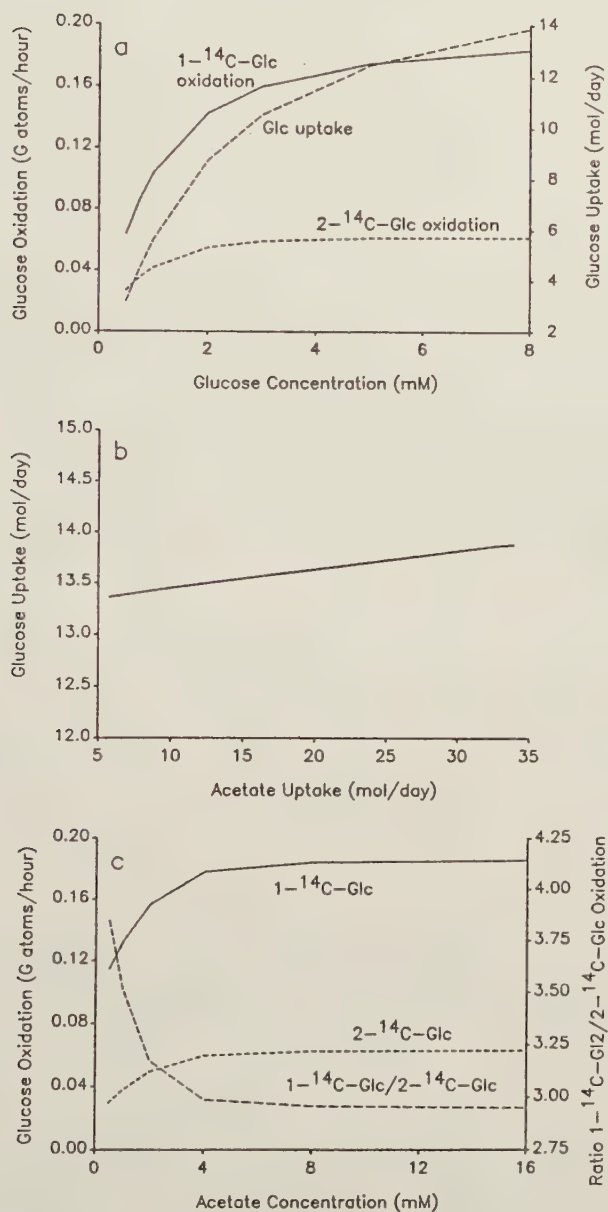


Figure 13.6 Pattern analyses of the mammary model. Abbreviations are as follows: Ac1, 1- 14 C-acetate; Glc1, 1- 14 C-glucose; Glc2, 2- 14 C-glucose; La1, 1- 14 C-lactate; La2, 2- 14 C-lactate; Oxid, oxidation; Cd, carbon dioxide; Ct, citrate; FA, the fatty acid portion of milk triacylglycerol; Gy, the glycerol moiety of milk triacylglycerol. (a) Relationships among glucose concentrations and glucose uptake and oxidation. Acetate concentrations were 8 mM. (b) Relationship between total uptake of acetate and total uptake of glucose. (c) Relationships among acetate concentration and rates of oxidation of Glc1 and Glc2. Glucose concentrations were 16 mM.

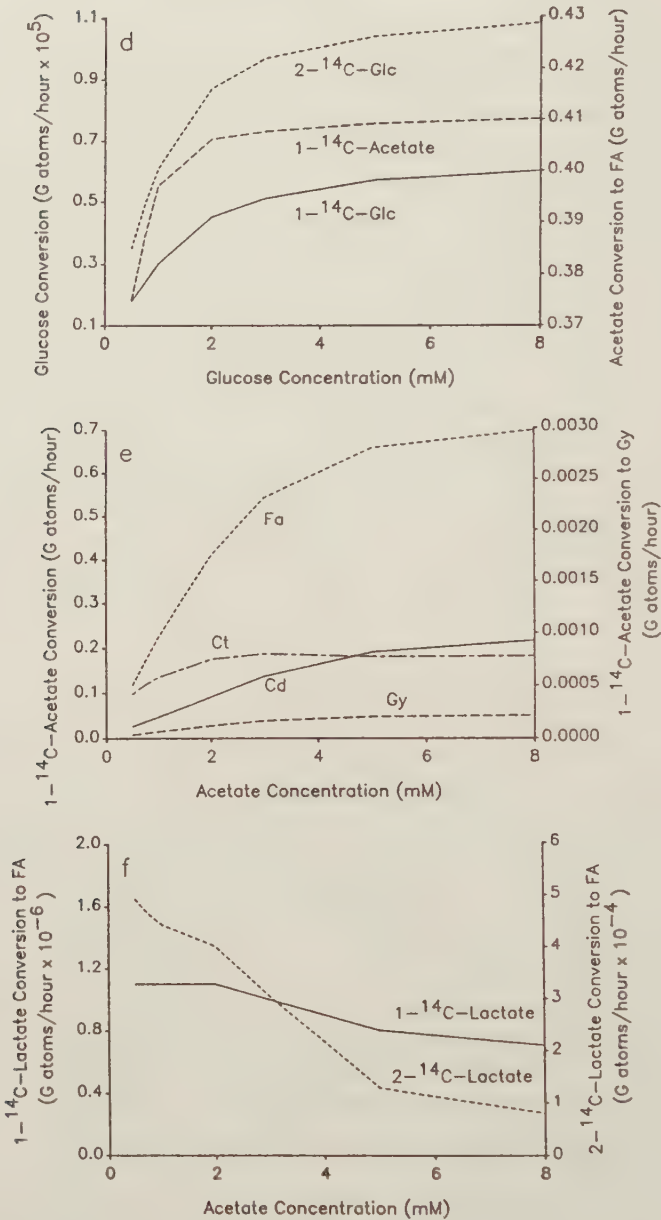


Figure 13.6 (d) Relationships among glucose concentration and glucose and acetate conversion to FA. Acetate concentrations were 8 mM. (e) Relationships among acetate concentration and conversion to FA. Acetate concentrations were 16 mM. (f) Relationships among acetate concentration and La1 and La2 conversion to FA. Lactate concentrations were 30 mM. Adapted from Hani-gan and Baldwin (1994).

teraction was much smaller than observed, which suggests that changes in equations or parameters may be required to simulate the observed relationship. This suggestion is supported by the observation that rates of 1-¹⁴C-Glc and 2-¹⁴C-Glc oxidation were not as responsive to changes in Ac concentrations as have been observed (simulation results not shown).

Relative rates of oxidation of 1-¹⁴C-Glc and 2-¹⁴C-Glc are indicative of pentose cycle activity. As Ac concentration increased from 0.5 to 16 mM, Glc oxidation increased and the ratio of 1-¹⁴C-Glc to 2-¹⁴C-Glc (Glc1/Glc2) oxidation decreased from approximately 4 to 2.9 (Fig. 13.6c). Forsberg *et al.* (1985a) observed an increase in Glc oxidation as Ac concentrations increased, but they also observed an increase in the oxidation ratio, which was not simulated correctly.

Patterns of conversion of 1-¹⁴C-Glc and 2-¹⁴C-Glc to fatty acids of milk fat were consistent with observations of Forsberg *et al.* (1985a; Fig. 13.6d).

Patterns of 1-¹⁴C-Ac oxidation and conversion to fatty acids, citrate and glyceride-glycerol with respect to Ac concentrations were consistent with data presented by Forsberg *et al.* (1984; Fig. 13.6e). Conversion of 1-¹⁴C-Ac to fatty acids was also responsive to Glc concentration as observed by Forsberg *et al.* (1984).

In general, all other patterns of precursor/product relationships were consistent with data presented by Forsberg *et al.* (1984, 1985a,b). The only notable exception was the conversion of 2-¹⁴C-La to Cd and Ct with respect to Ac concentration. As Ac concentration increased in simulations, 2-¹⁴C-La conversion to Ct declined (Fig. 13.6f). This observation is not consistent with Forsberg *et al.* (1985b).

(b) Sensitivity analyses

It is highly unlikely that a model of this complexity will be appropriately parameterized as initially conceived. Adjustment of many parameters may be required to yield simulations that are quantitatively correct. These adjustments include changes in the $V_{\max}$ estimates and the affinity and inhibition constants for various substrates and inhibitors, as well as changes in fluxes in alternative metabolic pathways. While many of the pathway fluxes are uniquely defined by the input:output data (Tables 13.4 and 13.5), several are not. As previously mentioned, the proportion of Nph generated via the pentose cycle and NADP-ICDH cannot be uniquely defined by input:output data. Behavioral analyses should yield insight into the appropriate fluxes where input:output data were inadequate for definition. Sensitivity analyses provide insight into which parameters the model is sensitive to. This knowledge can then be used to estimate the parameters in question using existing data and an optimization routine. Alternatively, when available data are not adequate to define parameters in question, results of sensitivity analyses can be used as an aid in designing experiments. This

information is also useful in interpreting treatment effects in radioisotope tracer experiments.

In the sensitivity analyses presented in Fig. 13.7, fluxes were held constant at reference concentrations of inputs. For example, when evaluating the sensitivity of fatty acid and milk fat synthesis (Tm) to parameters associated with that pathway, the net production of Tm was set to the reference rate of 1.1 kg/day when extracellular concentrations of nutrients were equal to reference nutrient concentrations. This approach assured that the model remained balanced. In order to achieve this, changes in fluxes caused by changes in affinity constants were offset by adjustments in $V_{\max}$. In Fig. 13.7(a), effects of this approach are illustrated. As $k_{Ac,AcCs}$ was changed from $1.8e-5$ to $1.8e-1$, the responsiveness of $U_{Ac,AcCs}$ to Ac concentrations changed dramatically, but the flux remained (almost) constant when Ac concentration was 1.8 mM. In some cases, such as $U_{Ac,AcCs}$, data may be adequate to define both $V_{\max}$ and the affinity constant for Ac. However, in most cases data are inadequate to define both parameters, which requires that $V_{\max}$ be arbitrarily fixed.

Initial sensitivity analyses indicated that the conversion of $1-^{14}C$ -Ac to Tm was less responsive to Ac concentrations than reported by Forsberg *et al.* (1984). Changes in rates of conversion of $1-^{14}C$ -Ac to Tm were examined while varying $k_{AcCs,Tm}$ and $k_{Ac,AcCs}$. Conversion of $1-^{14}C$ -Ac to Tm was sensitive to changes in $k_{Ac,AcCs}$ (Fig. 13.7a) but was not sensitive to changes in $k_{AcCs,Tm}$ (Fig. 13.7b, equation 13.6). This was caused by limitations in the quantity of Cs available, since decreases in $k_{AcCs,Tm}$ resulted in increases in the size of the AcCs pool. This increase in pool size sequestered Cs and thereby limited Cs availability for Ac activation. Results of this sensitivity analysis were incorporated into the model and are reflected in parameters in Table 13.9.

While patterns of Ac oxidation were appropriate, the proportion of radiolabel recovered in products deviated from observations made by Forsberg *et al.* (1984). For example, the proportion of $1-^{14}C$ -Ac recovered in Tm in simulations was approximately two to three times greater than the proportion recovered in Cd at all concentrations of Ac (Fig. 13.6e). Forsberg *et al.* (1984) observed approximately equal recoveries in Cd and fatty acids. This observation suggested that rates of Ac oxidation are higher or rates of *de novo* lipid synthesis lower than those predicted by the model. One could assume that this observation is unique to *in vitro* measurements, since rates of Ac oxidation and *de novo* lipid synthesis in the model were calculated from *in vivo* observations (Table 13.2). When rates of Ac oxidation were doubled and the undefined use of At was adjusted upward to accommodate higher rates of Ac oxidation, the model could simulate the Forsberg *et al.* (1984) observations. Similarly, if simulated rates of *de novo* lipid synthesis were reduced, Ac incorporation into fatty acids declined.

A significant number of cells are damaged when tissue is sliced for *in vitro* experiments. This damage would allow loss of functional mitochondria into

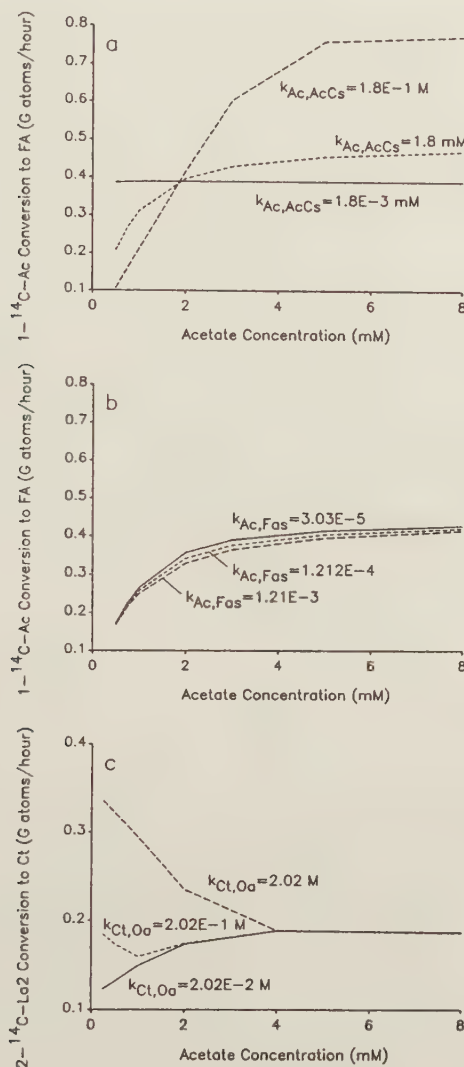


Figure 13.7 Sensitivity of the mammary model to various parameters. Abbreviations are as defined for Figure 13.6. (a) Effects of altering the affinity constant for acetate (k_{AcCs} ; equation 13.4) on rates of Ac1 conversion to FA. (b) Effects of altering the affinity constant for acetyl-CoA (k_{AcFaCs} ; equation 13.6) on rates of Ac1 conversion to FA. (c) Effects of altering the affinity constant for Ct (k_{CtOa} ; equation 13.19) on rates of La2 conversion to Ct. For this analysis, equation 13.19 was converted from a mass action to a saturable equation (Table 13.8; Equation Form 3); the affinity constant for Ct in equation 13.19 was set to $6.5E-3$; the affinity constant for mAcCs in equation 13.19 was set to $1.8E-2$; the affinity constant for lactate in equation 13.49 was increased to $15.48E-3$; the affinity constant for pyruvate in equation 13.59 was set to $9.24E-4$; the flux $U_{Ct,Oa}$ was increased to 2 mol/day; and, in order to maintain model balance, corresponding adjustments were made in the fluxes $U_{Ac,mAcCs}$, $U_{Ac,AcCs}$ and $U_{mAcCs,Ct}$. Lactate concentrations were 30 mM.

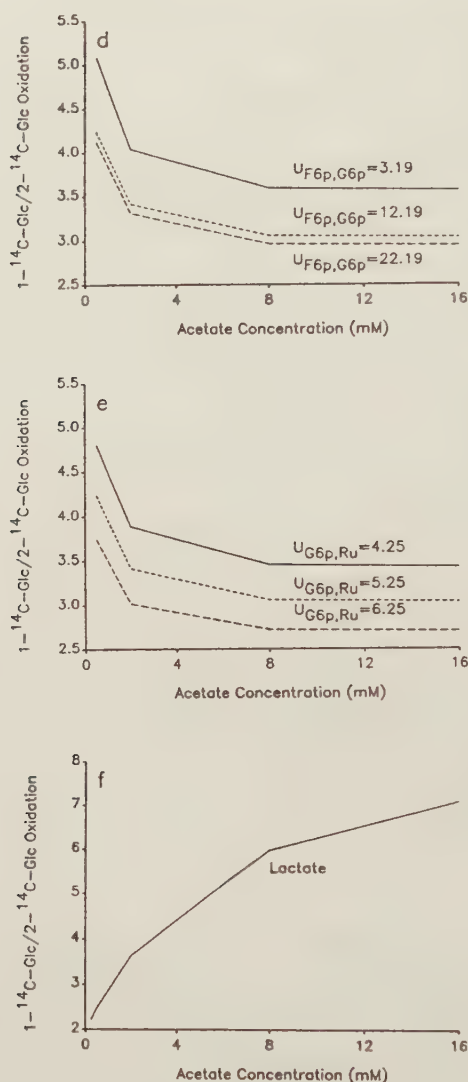


Figure 13.7 (d) Effects of increasing substrate cycling between fructose-6-phosphate and glucose-6-phosphate on the ratio of Glc1 and Glc2 oxidation. Corresponding changes were made in the fluxes $U_{F6p,G6p}$ (F6PG6P) and $U_{G6p,F6p}$ such that model balance was maintained. Glucose concentrations were 16 mM. (e) Effects of changes in rates of NADPH production associated with the fluxes $U_{G6p,Ru}$ (G6PRu) and $U_{Ctp,Akg}$. Initial changes were made to the flux G6PRu as denoted in the figure and compensatory changes were made in glycolytic, tricarboxylic acid and cytosolic isocitrate dehydrogenase pathways such that model balance was maintained. Glucose concentrations were 16 mM. (f) Effects of increasing rates of efflux from α -glycerol-phosphate to glycerol (Gy) or adding acetate as an activator of lactose synthesis (Lac). Glucose concentrations were 16 mM.

media and may result in rates of Ac oxidation that are concentration dependent on, but not coupled to, intracellular energy requirements. *De novo* lipid synthesis could be expected to be more tightly linked to proper cell function as individual components of the system, such as acetyl-CoA carboxylase, are very unstable when intracellular organization is disrupted. The possibility that rates of radiolabel incorporation into milk fatty acids *in vitro* are low cannot be discounted. Additional data are required to better define these components of the model.

Lactate conversion to fatty acids is regulated by mAcCs feedback on the pyruvate dehydrogenase and ATP-citrate lyase ($U_{Ct,Oa}$) reactions as noted above. The activity of ATP-citrate lyase, the rate limiting reaction of the aggregated flux $U_{Ct,Oa}$, is very low in mammary tissue of ruminants as compared to tissue from simple-stomached species (Bauman and Davis, 1975). If one converts observed activities of ATP-citrate lyase to a whole-udder basis, the potential maximal flux is about 7 moles Ct/day. Sensitivity analysis indicated that rates of Glc and La incorporation into Tm were sensitive to changes in $U_{Ct,Oa}$. Forsberg *et al.* (1985b) observed that La label recovered in Ct increased 3- to 4-fold and label recovered in fatty acids decreased 3- to 4-fold as Ac concentration was increased. As Forsberg *et al.* (1985b) suggested, the increase in label recovered in Ct is apparently due to declining concentrations of cytosolic CoA as Ac concentration rises. The model predicts that cCs concentrations would decline 10- to 12-fold. This decline in cCs results in a similar decline in rates of La radiolabel incorporation into Tm when the flux $U_{Ct,Oa}$ is defined as a mass action equation and set to 0.2 mole/day at steady state. In order to accommodate observed rates of incorporation of La-derived radiolabel into Tm when Ac is not present as a substrate, observed declines in La-derived radiolabel incorporation into Tm as Ac concentration increases and observed increases in La-derived radiolabel incorporated into Ct as Ac concentration increases (Forsberg *et al.*, 1985b), $U_{Ct,Oa}$ was converted to a Michaelis-Menten form with affinity constants set to approximately 100 and 10 times reference cCs and Ct concentrations, respectively. The steady state flux was increased to approximately 2 mole/day (Fig. 13.7c). These changes resulted in rates of 2- 14 C-La conversion to Tm at saturating La concentrations in the absence of Ac of approximately one-third of observed rates of radiolabel incorporation into Tm when 1- 14 C-Ac was used as a radiotracer at saturating concentrations. These model outputs are consistent with the observations of Forsberg *et al.* (1985b).

Sensitivity analyses were conducted to determine if key parameters affected 1- 14 C-Glc/2- 14 C-Glc (Glc1/Glc2) oxidation ratios. These analyses indicated that the Glc1/Glc2 oxidation ratio was sensitive to changes in rates of equilibration of radiolabels between F6P and G6P ($U_{F6P,G6P}$; Fig. 13.7d); to changes in rates of G6P entry to the pentose pathway ($U_{G6P,Ru}$; Fig. 13.7e); NADP-ICDH transactions (Fig. 13.7e); and to changes in affinity constants for Glc and G6P in the $U_{Glc,G6P}$ and $U_{G6P,Lac}$ fluxes (data not shown). The

decrease in the Glc1/Glc2 ratio due to increases in the rates of $U_{F6P,G6P}$ and $U_{G6P,F6P}$ at all concentrations of acetate was expected, since the specific activity in $1\text{-}^{14}\text{C}\text{-G6P}$ arising from $2\text{-}^{14}\text{C}\text{-G6P}$ via the pentose cycle and $1\text{-}^{14}\text{C}\text{-F6P}$ formed via this pathway should increase as rates of recycling via $U_{F6P,G6P}$ increase. Similarly, the ratio should decrease as the demand for NADPH_2 increases to support increased rates of fatty acid synthesis due to increased acetate availability. The same set of factors operate to cause a decrease in the Glc1/Glc2 ratio as flux through the glucose-6-P dehydrogenase reaction complex ($U_{G6P,Ru}$) increased at all acetate concentrations and as acetate concentration and rates of fatty acid synthesis increased (Fig. 13.7b). These changes did not alter the slope of the Glc1/Glc2 oxidation ratio with respect to Ac concentrations. In all cases, Glc1/Glc2 oxidation ratios declined as Ac concentrations increased. However, the changes influenced effects of acetate upon rates of Py conversion to mAcCs (data not shown) and relative rates of conversion of Glc1 and Glc2 to Lac (Fig. 13.7f). In this case, the increase in ratio indicates that a higher proportion of $1\text{-}^{14}\text{C}\text{-Glc}$ available is oxidized via the pentose cycle as acetate availability increases, which results in less flow down the Emden-Meyerhoff pathway to lactate and pyruvate.

The implicit linkage between G6P use in lactose synthesis and Ac concentration resulted in simulations that were similar to observations, i.e. the continuous asymptotic decrease in oxidation ratios throughout the Ac concentration range tested closely resembled experimental data (Forsberg *et al.*, 1985a). Forsberg *et al.* (1985a) observed an increase in the apparent affinity of mammary slices for Glc in the aggregated processes of Glc conversion to Lac, glyceride-glycerol and Cd as acetate concentration increased. These changes were considered indicative of increased rates of conversion of Glc to each of these products at physiological Glc concentrations. As noted above, increased rates of oxidation and conversion of Glc to glyceride-glycerol associated with increased Ac concentrations are consistent with increases in rates of *de novo* lipid synthesis. Apparent changes in mammary gland affinity for Glc required for the support of lipid synthesis could be (and are in the model) manifested through reductions in G6P concentration and reduction in the feedback inhibition of G6P on Glc uptake. However, the model, as currently defined, does not simulate as strong a link between Ac availability and Glc conversion to Lac as was observed by Forsberg *et al.* (1985b). Acetate does act to spare Glc by providing energy for reducing power and At production (data not shown), but this implicit link is not as responsive to Ac concentration as has been observed. It is not clear at this time whether an explicit mechanism linking Ac concentration or availability and Lac synthesis is required to explain observed changes in apparent affinity for Glc use for Lac synthesis or whether optimization of parameters associated with Glc and Ac oxidation will be adequate. Any improvement in the link between Glc metabolism and Ac availability would also increase

the responsiveness of Glc uptake with respect to Ac uptakes as observed by Miller *et al.* (1991a,b).

Based upon the behavioral and sensitivity analyses presented and additional studies not presented, we believe the current model of mammary gland metabolism is close to adequate for estimation of parameter values that cannot be measured directly.

Data are required to define parameters associated with Glc and G6P metabolism and, possibly, adjust current equation forms to include rates of conversion of U-, 1-, 2- and 6-¹⁴C-Glc at varying concentrations of Glc and other nutrients to Cd, Lac, triacylglycerol-glycerol and Ct. In addition, data on the metabolism of additional ¹⁴C probes of carbohydrate metabolism may be required. Waghorn and Baldwin (1984) also identified these data as pertinent to clarification of relative fluxes through the pentose cycle, lactose synthesis and glycolysis. The studies reported by Forsberg *et al.* (1984, 1985a,b), Hanigan *et al.* (1991, 1992) and Miller *et al.* (1991a,b) have been very useful in helping to define the current model. Also, recent data published by Cant *et al.* (1991, 1993a,b) and in process (Middleton *et al.*, unpublished) will help as well. The deduced parameters could explain only 60% of variance, which indicates either a deficiency in the model, the adequacy of data used to estimate the parameter values or, most probably, both.

13.3.3 Parameter estimation

We had hoped that we could use the data of Forsberg *et al.* (1984, 1985a,b) to illustrate the use of SIMUSOLVE[®] to estimate parameter values associated with G6P metabolism. Two problems arose. The first was that the relationships reported by these workers were used in model development and no longer constitute an independent data set. The second was that the model as currently constituted cannot simulate all experimental data, as indicated by model failures discussed above. We hope the additional experimental data gathered by Middleton *et al.* (unpublished) will help in this regard. However, we expect further experimental data will be required to help better define G6P metabolism.

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Dynamic models of liver and viscera metabolism

with H. C. Freetly

Under basal conditions, as much as $\frac{1}{6}$ to $\frac{1}{4}$ of the cardiac output flows through the vessels of the intestines and through the spleen, finally coursing back into the portal venous system and then through the liver. ... This is called the portal circulatory system.

Guyton (1956)

14.1 INTRODUCTION

Liver performs a number of functions that are important to lactation, including gluconeogenesis from propionate and amino acids, ketogenesis, formation of very low density triacylglycerol, clearance of lipoprotein cholesterol and cholesterol esters, urea synthesis, etc. In the course of providing this wide range of service functions, liver energy expenditures are high and amount to approximately 24% of total energy expenditures in lactating dairy cows (Chapter 6). In formulating a mechanistic model of lactating dairy cows (Chapter 16), it is clearly essential that input:output relationships and metabolic and hormonal factors that influence these be fully understood. In the early 1980s, Waghorn (1982) undertook an extensive evaluation of available data on ruminant liver metabolism. Based upon this literature survey, Waghorn (1982) was able to account for only 60% of energy inputs to liver indicated by outputs in the form of products such as glucose and heat. Waghorn (1982) formulated a provisional model of liver metabolism but considerable uncertainty existed. Subsequently, a number of *in vitro* studies using isolated hepatocytes and *in vivo* studies were reported. In the latter case, which were studies using portal drained viscera, hepatic input:output techniques were prominent. Results of these studies led Freetly *et al.* (1993) to undertake development of a model of liver metabolism in a lactating cow. The objective of the study was 'to develop a mechanistic model of ruminant liver metabolism, based on known metabolic pathways, which was capable of predicting whole organ metabolism and simulating ^{14}C -radiotracer kinetics'. Since this model has already been pub-

lished in detail, full details will not be presented here. As with the mammary gland metabolism model (Chapter 13), interested persons can most easily recreate the model by requesting a copy of the model from the authors.

In the above modeling objective, improved interpretations of results of *in vitro* studies that utilized radiotracers were considered important so as to advance our knowledge of underlying metabolic relationships. Therefore, equations to track radiotracers were incorporated into the model of Freetly *et al.* (1993). Provisions for this are completely analogous to those described in Chapter 12. In this chapter, the basic structure of the model and strategies used in selecting equation forms for key metabolic transactions will be emphasized. Also, results of behavioral and sensitivity analyses will be presented along with several challenges to the model based upon independent data sets.

14.2 OVERALL STRUCTURE AND NOTATION

The model was developed to describe liver metabolism in the standard reference Holstein cow producing 30 kg of milk per day described in Chapters 12 and 13. Liver weight was set at 10.6 kg based on the regression equations developed by Smith and Baldwin (1974). The block diagram for the model is presented in Fig. 14.1. The model was written in ACSL (Chapter 4). The model can also be solved using SIMUSOLVE® for the purpose of estimating parameters and comparing models. Chemical entities in the model are expressed in moles, mass in kilograms, volume in liters and time in days. Broken-line boxes in Fig. 14.1 represent inputs to the model. Boxes enclosed by solid lines identify state variables described by differential equations. Numbers on the arrows interconnecting state variables are fluxes (mole/day) in the reference state and differential equations for state variables in the reference state equal zero.

As in Chapters 11–13, rates of conversion of substrate to product are denoted as substrate going to product ($U_{A,B}$). Codes for metabolites, pool sizes and substrate concentrations in the reference state are presented in Tables 14.1 and 14.2. As noted in Chapter 3, enzyme data cannot be used directly or without reservation in formulating a tissue model but do provide useful guidance. Ruminant liver enzyme activities are summarized in Table 14.3. Seventeen equations forms were used to describe fluxes in the model (Table 14.4). Transactions, equation forms, substrates, inhibitors and activators in the model are summarized in Table 14.5. Equations forms 14.1–5 are mass action equations or modifications thereof (Chapter 3). $K_{A,B}$ (day^{-1}) is the fractional rate constant for the mass action argument. The ratio S_2/iS_2 in equation 14.2 provides for a linear response in reaction rates as the pool size S_2 changes relative to the initial or reference concentration (iS_2). When this ratio is raised to a power other than one as specified by ϵ in equation 14.4, the slope of the response is altered. The inverse ratio iS_2/S_2 provides that

Table 14.1 Steady-state concentrations of metabolites

Metabolite	Chemical name	Concentration (mole/l)	Reference ^a
AD	ADP	2.36E-4	9
AGP	α -glycerol phosphate	4.75E-4	2
AK	α -ketoglutarate	1.24E-4	2
AL	alanine	2.12E-3	2
AS	aspartate	1.05E-3	2
AT	ATP	2.36E-4	9
CC	cytoplasmic acetyl-CoA	1.07E-4	3,7
Cs	CoASH	1.10E-5	8
CT	citrate	3.37E-4	2
DC	oxaloacetate + malate	5.44E-4	2
FA	free fatty acids	1.36E-3	5,6
FD	FAD	2.36E-4	9
FDH	FADH	2.36E-4	9
FP	fructose-6-phosphate	8.75E-4	4
GP	glucose-6-phosphate	2.45E-4	4
GU	glutamate	4.17E-3	2
IC	isocitrate	2.36E-4	9
MC	mitochondrial acetyl-CoA	1.07E-4	3,7
nC	carbon dioxide	3.10E-2	1
ND	NAD	2.36E-4	9
NDH	NADH	2.36E-4	9
NP	NADP	2.36E-4	9
NPH	NADPH	2.36E-4	9
PC	propionyl-CoA	2.36E-4	9
PE	phosphoenolpyruvate	1.52E-4	2
PY	pyruvate	3.80E-5	2
TP	glyceraldehyde-3-phosphate + dihydroxyacetone phosphate	4.50E5	2

^a1, Altman and Dittmer (1974); 2, Baird and Heitzman (1970); 3, Baird *et al.* (1972a); 4, Baldwin, unpublished; 5, Brumby *et al.* (1975); 6, Reid *et al.* (1977); 7, Snoswell *et al.* (1978); 8, Baird *et al.* (1972b); 9, assigned concentration.

when S_2 increases above the reference concentration, reaction rates decrease as by a competitive inhibitor at concentrations significantly below k_i .

Equations 14.6–14.17 describe saturation kinetic arguments; most of these are Michaelis–Menten in form. When multiple substrates are used, interaction terms were omitted; available data are normally not adequate (due to variance) to define these. Equations 14.10 and 14.16 produce sigmoidal kinetics (Chapter 3). For a given reaction ($U_{A,B}$), $V_{A,B}$ (mole/day) describes maximum metabolic capacity, k_n is the affinity constant for substrate S_n , and j_n is the constant of a competitive inhibitor.

In cases where *in vivo* values were not available for affinity or inhibition constants (k_n and j_n), these were set equal to steady-state concentrations of metabolites. Maximum capacities ($V_{A,B}$) were calculated based on flux rates

Table 14.2 Steady-state concentrations of extracellular substrates

Metabolite	Chemical name	Concentration (mole/l)	Reference ^a
eAC	acetate	2.50E-3	5,6
eAL	alanine	1.82E-4	8
eAS	aspartate	6.40E-5	8
eBNH ₃	ammonia	7.50E-2	4
eBU	butyrate	8.00E-6	5
eFF	free fatty acid	1.36E-3	4
eGL	glucose	3.00E-3	2,4
eGN	glutamine	1.80E-4	8
eGU	glutamate	1.80E-4	8
eGY	glycerol	1.00E-4	1
eIB	isobutyrate	2.00E-5	5
eIV	isovalerate	2.30E-5	5
eLA	lactate	5.11E-4	4
ePR	propionate	3.79E-4	2,3
eUL	undefined lipids	1.36E-3	7
eVA	valerate + 2-methylbutyrate	5.60E-5	5

^a1, Baird *et al.* (1979); 2, Baird *et al.* (1975); 3, Lomax and Baird (1983); 4, Reynolds *et al.* (1988a); 5, Reynolds *et al.* (1988b); 6, Snoswell *et al.* (1978); 7, assigned concentration; 8, assigned based on Table 14.5

ate, isobutyrate, valerate and 2-methylbutyrate, butyrate, isovalerate, acetate, free fatty acids, carbon dioxide, alanine, aspartate, glutamate, and other amino acids. These are identified in Fig. 14.1 by dashed boxes. The model also allows ¹⁴C to be used as a tracer of each of the carbons of each substrate except for other amino acids, long-chain fatty acids, valerate and 2-methylbutyrate and isobutyrate. Long-chain fatty acids can be labeled only in the first carbon.

The nine outputs from the model are glucose, lactate, acetate, free fatty acids, carbon dioxide, ketone bodies, triacylglyceride, glutamate and protein. All of these except ketone bodies, triacylglyceride and protein act as both substrates and products. Ketone bodies, triacylglycerol and export protein are terminal products that require metabolism in peripheral tissues before re-entering the liver.

The differential equation for glucose-6-phosphate (GP) is as follows:

$$\frac{dGP}{dt} = U_{eGL,GP} + U_{FP,GP} - U_{GP,RU} - U_{GP,eGL} - U_{GP,FP} \quad (14.54)$$

Respective inputs to GP from glucose and fructose-6-phosphate are defined by equations 14.20 and 14.33 (Table 14.5). Data on glucose uptake by ruminant livers are very sparse, but we elected to represent the reaction as obeying saturation kinetics with an affinity constant characteristic of glucokinase even though hexokinase appears to be much more prominent in ruminant liver than is glucokinase (Table 14.3). This decision is compatible

Table 14.3 Enzyme activities in bovine liver^a

Enzyme	m/kg WW/day ^b
Glucokinase	<0.04
Hexokinase	0.09
Glucose-6 phosphate	36.20
Phosphoglucomutase	48.10
Glucose-6-phosphate dehydrogenase	2.11
6-phosphogluconate dehydrogenase	3.25
Phosphofructokinase	5.43
Fructose-1,6 diphosphatase	14.34
Aldolase	8.55
Glyceraldehyde-3-P dehydrogenase	89.64
Pyruvate kinase	17.22
Pyruvate carboxylase	22.22
Citrate synthetase	3.46
Malate dehydrogenase	226
Lactate dehydrogenase	232
Glutamate dehydrogenase	37.66
Acetyl-CoA synthetase	0.63
Propionyl-CoA synthetase	5.18
Butyryl-CoA synthetase	3.22
Phosphoenolpyruvate carboxykinase	19.40
Phosphoenolpyruvate carboxylase ^c	18.20
Glutamate pyruvate transaminase	1.65
Glutamate oxaloacetate transaminase	188
β -hydroxy butyric acid dehydrogenase	0.40
Citrate lyase	0.073
NADP malate dehydrogenase	0.168
Propionyl CoA carboxylase	11.52

^aReferences: Hanson and Ballard (1967), Hibbitt and Baird (1967), Howarth *et al.* (1968), Ballard *et al.* (1964), Heitzman (1969), Butler and Elliot, (1970), Baird and Heitzman (1970), Baird *et al.* (1972b), Ash and Baird, (1973), Baird and Young (1975), Treacher and Collis (1977), Baldwin (unpublished).

^bActivities are expressed as moles of product formed or substrate utilized per kg liver wet weight (WW) per day at 37°C.

^cReverse of PEP carboxykinase.

Table 14.4 Equation forms of metabolic transactions

Equation number	General form
1	$U_{A,B} = K_{A,B} * S_1$
2	$U_{A,B} = K_{A,B} * S_1 * (S_2/iS_2)$
3	$U_{A,B} = K_{A,B} * S_1 * (iS_2/S_2)$
4	$U_{A,B} = K_{A,B} * S_1 * (S_2/iS_2)^{\epsilon}$
5	$U_{A,B} = K_{A,B} * S_1 * A^{\epsilon}$
6	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1])$
7	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + k_2/[S_2])$
8	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + k_2/[S_2] + k_3/[S_3])$
9	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + k_2/[S_2] + k_3/[S_3] + k_4/[S_4])$
10	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + k_2/[S_2] + (k_3/[S_3])^{\epsilon})$
11	$U_{A,B} = V_{A,B}/(1 + k_1 * A^{\epsilon}/[S_1])$
12	$U_{A,B} = V_{A,B}/(1 + k_1 * A^{\epsilon}/[S_1] + k_2/[S_2])$
13	$U_{A,B} = V_{A,B} * A^{\epsilon}/(1 + k_1/[S_1])$
14	$U_{A,B} = V_{A,B} * (S_1/k_1)^{\epsilon}/(1 + k_2/[S_2] + k_3/[S_3])$
15	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] * [S_2]/j_2)$
16	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + (k_2/[S_2])^{\epsilon} + [S_3]/j_3)$
17	$U_{A,B} = V_{A,B}/(1 + k_1/[S_1] + (k_2/[S_2] + k_3/[S_3] + [S_4]/j_4 + [S_5]/j_5)$

with the fact that glucose uptake is very low as compared to net efflux in ruminant liver (Fig. 14.1). Simple mass action kinetics seem to be adequate for $U_{GP,FP}$ and $U_{FP,GP}$, since the reaction is in chemical equilibrium in ruminant liver. Outputs defined by equations 14.35, 14.36 and 14.37 are to glucose, fructose-6-phosphate and ribulose-5-phosphate (via 6-P-gluconolactone), respectively. The key reaction in the $U_{GP,eCl}$ transaction is the glucose-6-phosphatase reaction. Activity of the enzyme is regulated in non-ruminants by glucocorticoids, but the data of Ely and Baldwin (1976) indicate this is not true in ruminants and activity of the enzyme is very high (Table 14.3). Therefore, no provision for regulation of this reaction was included. Data indicating either acute or chronic regulation would clearly lead to a change in this representation.

The flux $U_{GP,RU}$ is a function of GP and NP. Enzyme activities are in considerable excess of estimated flux (Table 14.4 and Fig. 14.1). The ratio of NP/initial NP is raised to a power ϵ ($= 2$) to increase sensitivity to NP. NP is a product of fatty acid synthesis ($U_{CC,FA}$). NADPH required for fatty acid synthesis is generated by $U_{GP,RU}$ and $U_{IC,AK}$. In the reference state, 60% of NADPH is from $U_{GP,RU}$ and 40% comes from $U_{IC,AK}$. One would hope that further experimental analyses will enable representation of this (variable) proportionality in a more satisfactory fashion, but currently available data do not support even the current representation.

The differential equation for fructose-6-phosphate (FP) is as follows:

$$\frac{dFP}{dt} = U_{GP,FP} + U_{TP,FP} * 0.5 + U_{GP,RU} * 0.667 - U_{FP,GP} - U_{FP,TP} \quad (14.55)$$

Table 14.5 Metabolic transactions, equation forms, substrates, inhibitors and activators in the model of liver metabolism^a

Transaction (U _{A,B})	Equation number	Substrate and activators						
		S ₁	S ₂	S ₃	S ₄	J ₁	J ₂	A
AGP,TP	14.1	TP	NDH	—	—	—	—	—
AK,DC	14.2	AK	ND	Cs	—	—	—	—
AK,GU	14.3	AK	ND	—	—	—	—	—
AL,PY	14.4	AL	NDH	—	—	—	—	—
AS,DC	14.5	AS	ND	—	—	—	—	—
BNH ₃ U	14.6	BNH ₃	—	—	—	—	—	—
CC,FA	14.7	CC	—	—	—	—	—	ANAHOR
CT,AK	14.8	CT	AD	—	—	NDH	—	—
CT,IC	14.9	CT	—	—	—	—	—	—
CT,PY	14.10	CT	MC	—	—	—	—	—
DC,AS	14.11	DC	NDH	—	—	—	—	—
DC,CT	14.12	DC	—	—	—	CT	—	—
DC,PE	14.13	DC	—	—	—	—	—	CATHOR
eAC,CC	14.14	eAC	Cs	AT	—	—	—	—
eAC,MC	14.15	eAC	Cs	AT	—	—	—	—
eAL,AL	14.16	AL	—	—	—	eAL	—	—
eAS,AS	14.17	AS	—	—	—	eAS	—	—
eBU,MC	14.18	eBU	Cs	AT	ND	—	—	—
eFF,FA	14.19	eFF	AT	Cs	—	—	—	—
eGL,GP	14.20	eGL	—	—	—	—	—	—
eGN,GU	14.21	eGN	—	—	—	—	—	—
eGU,GU	14.22	eGU	—	—	—	—	—	—
eGY,AGP	14.23	eGY	AT	—	—	—	—	—
eIB,PC	14.24	eIB	Cs	AT	—	—	—	—
eIV,MC	14.25	eIV	Cs	AT	ND	—	—	—
eLA,PY	14.26	eLA	ND	—	—	—	—	—
ePR,PC	14.27	ePR	Cs	AT	—	—	—	—
eUL,FA	14.28	eUL	AT	Cs	—	—	—	—
eVA,PC	14.29	eVA	Cs	AT	—	—	—	—
FA,eFF	14.30	FA	—	—	—	—	—	—
FA,MC	14.31	FA	Cs	ND	AT	—	—	—
FA,TG	14.32	FA	—	—	—	—	—	—
FP,GP	14.33	FP	—	—	—	—	—	—
FP,TP	14.34	FP	AD	—	—	—	—	CATHOR
GP,eGL	14.35	GP	—	—	—	—	—	—
GP,FP	14.36	GP	—	—	—	—	—	—
GP,RU	14.37	GP	NP	—	—	—	—	—
GU,AK	14.38	GU	ND	—	—	—	—	—
GU,eGU	14.39	GU	—	—	—	—	—	—
IC,CT	14.40	IC	—	—	—	—	—	—
IC,AK	14.41	IC	NP	—	—	—	—	—
MC,eAC	14.42	MC	—	—	—	—	—	—
MC,KB	14.43	MC	NDH	—	—	—	—	—
PC,DC	14.44	PC	AT	—	—	—	—	—
PE,PY	14.45	PE	AD	—	—	—	—	CATHOR
PE,TP	14.46	PE	NDH	AT	—	—	—	—

Transaction ($U_{A,B}$)	Equation number	Substrate and activators						
		S_1	S_2	S_3	S_4	I_1	I_2	A
PY,AL	14.47	PY	ND	—	—	—	—	—
PY,DC	14.48	MC	PY	AT	—	—	—	—
PY,eLA	14.49	PY	NDH	—	—	—	—	—
PY,MC	14.50	PY	Cs	ND	—	MC	NDH	—
TP,AGP	14.51	TP	NDH	—	—	—	—	—
TP,FP	14.52	TP	—	—	—	—	—	CATHOR
TP,PE	14.53	TP	AD	ND	—	—	—	—

^aCodes are defined in Tables 14.1 and 14.2 except RU (ribulose-5-P) and UR (urea) which are defined in the text.

Respective inputs to FP from glucose-6-phosphate, ribulose-5-phosphate and triose phosphate are defined by equations 14.36, 14.37 and 14.52. FP is converted to glucose-6-phosphate and triose phosphate.

The $U_{TP,FP}$ flux describes moles of TP going to FP as 2 moles of TP forming 1 mole of FP. In contrast with the models of adipose tissue (Chapter 12) and mammary gland metabolism (Chapter 13), the net flux from TP to FP considerably exceeds the reverse flux (Fig. 14.1). The reciprocal regulatory mechanisms associated with the phosphofructokinase and fructose-1,6-bisphosphatase reactions in liver tissues from non-ruminant species are well characterized but have not been characterized in ruminant liver. For this reason, regulation of these reactions is represented very simply. The amount of FP formed from ribulose-5-phosphate is calculated by multiplying $U_{GP,RU}$ flux by a stoichiometric coefficient (0.667).

The differential equation for triose phosphate (TP) is as follows:

$$\frac{dTP}{dt} = U_{GP,RU} * 0.333 + U_{AGP,TP} + U_{PE,TP} + U_{FP,TP} * 2.0 - U_{TP,FP} - U_{TP,AGP} - U_{TP,PE} \quad (14.56)$$

The triose phosphate pool includes glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which implies that the two pools are in chemical and isotopic equilibrium because of the very high activity of triose phosphate isomerase in ruminant tissue. Respective inputs to TP from (a function of the $U_{GP,RU}$ flux), α -glycerol phosphate, fructose-6-phosphate, ribulose-5-phosphate and phosphoenolpyruvate are described by equations 14.1, 14.34, 14.37 and 14.46. Triose phosphates are converted to fructose-6-phosphate, α -glycerol phosphate and phosphoenolpyruvate.

The flux from phosphoenolpyruvate (PE) $U_{PE,TP}$ is an aggregated step that incorporates the reactions catalyzed by glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, 2-phosphoglycerate phosphatase and enolase. Equation 14.46 incorporates effects of substrates

required by these enzymes, including PE, NDH and AT. As described earlier for the contribution of ribulose-5-phosphate to FP, the contribution of RU to TP is $0.333 * U_{GP,RU}$. Outputs from the TP pool are described by equations 14.51, 14.52 and 14.53.

As described for $U_{PE,TP}$, $U_{TP,PE}$ is an aggregation of a number of reactions. Saturation kinetics were employed in representations of these, because mass action equations used in early versions yielded unstable solutions.

The differential equation for α -glycerol phosphate (AGP) is as follows:

$$\frac{dAGP}{dt} = U_{GY,AGP} + U_{TP,AGP} - U_{AGP,TP} - U_{AGP,TG} \quad (14.57)$$

Respective inputs to AGP from glycerol and triose phosphate are described by equations 14.23 and 14.51. The glycerol kinase reaction, $U_{GY,AGP}$, was represented as obeying saturation kinetics, even though the V_{max} of this reaction is currently in doubt and possibly rate limiting but, more importantly, could have a highly significant effect on radiotracer metabolism (Looney *et al.*, 1987). α -Glycerol phosphate is converted to triose phosphate and is used in triacylglycerol synthesis. Outputs are described by equations 14.32 and 14.1. The availability of fatty acids is the primary determinant of reaction rate and experimental data do not deviate from linearity, therefore $U_{AGP,TG}$ is set to 0.33 times $U_{FA,TG}$ based on the assumption that liver makes only triacylglycerol.

The differential equation for phosphoenolpyruvate (PE) is as follows:

$$\frac{dPE}{dt} = U_{TP,PE} + U_{DC,PE} - U_{PE,PY} - U_{PE,TP} \quad (14.58)$$

Inputs to PE from dicarboxylic acids and triose phosphates are described by equations 14.13 and 14.53. Phosphoenolpyruvate conversion to pyruvate and triose phosphates are defined by equations 14.45 and 14.46. The basis for using equations 14.53 and 14.46 for $U_{TP,PE}$ and $U_{PE,TP}$ was noted above. Data on regulation of the phosphoenolpyruvate carboxykinase ($U_{DC,PE}$) reaction in ruminant liver are limited to reports of activity (Table 14.3) and *in vitro* studies by Aiello and Armentano (1987), Aiello *et al.* (1989) and Looney *et al.* (1987), which indicate that this enzyme can be limiting to gluconeogenesis in ruminant liver. Therefore, provisions for hormonal regulation were incorporated into equation 14.13, even though data currently available do not support parameterization of the equation, i.e. ϵ was set to one. The primary function of ruminant liver is gluconeogenesis, which implies that $U_{PE,PY}$ should not occur. However, Knapp *et al.* (1992) observed significant conversion of dicarboxylic acids (DC) to lactate, so a provision for $U_{PE,PY}$ at low rates was incorporated into the model.

The differential equation for pyruvate metabolism (PY) is as follows:

$$\frac{dPY}{dt} = U_{PE,PY} + U_{eLA,PY} + U_{AL,PY} + U_{CT,PY} + U_{AA,PY} - U_{PY,eLA} - U_{PY,AL} - U_{PY,MC} - U_{PY,DC} \quad (14.59)$$

Respective inputs to PY from alanine, citrate, lactate, phosphoenolpyruvate and amino acids are defined by equations 14.4, 14.10, 14.26, 14.45 and 14.68b. Pyruvate conversions to alanine, dicarboxylic acid, lactate and mitochondrial acetyl-CoA are described by equations 14.47, 14.48, 14.49 and 14.50. Arguments relevant to the representations of the pyruvate dehydrogenase ($U_{PY,MC}$) and pyruvate carboxylase ($U_{PY,DC}$) reactions were presented in Chapters 12 and 13.

The differential equation for dicarboxylic acid metabolism (DC) is as follows:

$$\frac{dDC}{dt} = U_{PY,DC} + U_{AS,DC} + U_{AK,DC} + U_{PC,DC} + U_{AA,DC} - U_{DC,AS} - U_{DC,PE} - U_{DC,CT} \quad (14.60)$$

Succinyl-CoA, succinate, fumarate, malate and oxaloacetate are represented in aggregate in the DC pool. It is generally considered that these metabolites interchange and equilibrate rapidly. The differential equations for ^{14}C (not presented) reflect randomization of radiotracer in the symmetrical intermediate succinate. These simplifications should not compromise the radiotracer model as long as isotopic equilibria among these metabolites is achieved. The size of the dicarboxylic acid pool was set to the sum of the oxaloacetate and malate pools since data on the other pools are not available. Respective inputs to DC from α -ketoglutarate, aspartate, propionyl-CoA, pyruvate and amino acids are defined by equations 14.2, 14.5, 14.44, 14.48 and 14.68c. Dicarboxylic acid conversions to aspartate, citrate and phosphoenolpyruvate are defined by equations 14.11, 14.12 and 14.13.

The differential equation for citrate metabolism (CT) is as follows:

$$\frac{dCT}{dt} = U_{DC,CT} + U_{IC,CT} - U_{CT,AK} - U_{CT,IC} - U_{CT,PY} \quad (14.61)$$

Inputs to CT from mitochondrial acetyl-CoA and isocitrate (IC) plus DC are described in equations 14.12 and 14.40. Output of CT to α -ketoglutarate assumes rapid equilibrium between intramitochondrial citrate and isocitrate such that the NAD-linked isocitrate dehydrogenase reaction, which is dependent upon cell energy status as reflected by AD and redox state (NDH), is the dominant reaction. The rates of the $U_{IC,CT}$ reaction in liver are relatively low as compared to adipose and mammary tissues (Chapters 12 and 13), which reflects the fact that rates of lipogenesis in liver are low in ruminants. However, it was considered appropriate that provision for NADPH₂ generation via the NADP-linked isocitrate dehydrogenase be included in the model. Since citrate lyase is present in ruminant liver, provi-

sion for citrate conversion to pyruvate plus cytoplasmic acetyl-CoA was incorporated (equation 14.10).

The differential equation for cytoplasmic isocitrate (IC) is as follows:

$$\frac{dIC}{dt} = U_{CT,IC} - U_{IC,CT} - U_{IC,AK} \quad (14.62)$$

The exchange between intramitochondrial and extramitochondrial citrate and isocitrate was discussed above, as was the presence of the cytosolic NADP-linked isocitrate dehydrogenase.

The differential equation for α -ketoglutarate (AK) is as follows:

$$\frac{dAK}{dt} = U_{CT,AK} + U_{IC,AK} + U_{GU,AK} + U_{AA,AK} - U_{AK,DC} - U_{AK,GU} \quad (14.63)$$

α -Ketoglutarate is formed from citrate and isocitrate as discussed above. The rate of equilibration of AK with glutamate ($U_{AK,GU}$ and $U_{GU,AK}$) is rapid (Fig. 14.1). During amino acid catabolism, AK can be formed from other amino acids ($U_{AA,GU}$). A large proportion of α -ketoglutarate formed in the model enters the DC pool.

The differential equation for propionyl-CoA metabolism (PC) is as follows:

$$\frac{dPC}{dt} = U_{ePR,PC} + U_{eVA,PC} + U_{eIB,PC} + U_{AA,PC} - U_{PC,DC} \quad (14.64)$$

Respective inputs to PC from isobutyrate, propionate, fatty acids, other volatile acids and amino acids are defined in equations 14.24, 14.27, 14.29 and 14.68e. Since data are not available on propionyl-CoA concentration, a concentration was assigned. The driving variables in the differential equation for PC are inputs to the pool. No regulation of this reaction other than biotin deficiency has been reported.

The differential equation for mitochondrial acetyl-CoA (MC) is as follows:

$$\begin{aligned} \frac{dMC}{dt} = & U_{PY,MC} + U_{eAC,MC} + 8.0 * U_{FA,MC} + 2.0 * U_{eBU,MC} + U_{eIV,MC} + \\ & U_{eVA,PC} + U_{AA,MC} - U_{DC,CT} - U_{MC,KB} - U_{MC,eAC} \end{aligned} \quad (14.65)$$

Respective inputs to MC from acetate, butyrate, isovalerate, other volatile acids, fatty acids, pyruvate and amino acids are described by equations 14.15, 14.18, 14.25, 14.29, 14.31, 14.50 and 14.68f. Outputs to citrate, acetate and ketone bodies are described by equations 14.12, 14.42 and 14.43. Bases for selection of the several equation forms are either self-evident or were discussed above.

The differential equation for cytoplasmic acetyl-CoA (CC) is as follows:

$$\frac{dCC}{dt} = U_{CT,PY} + U_{eAC,CC} - U_{CC,FA} \quad (14.66)$$

The differential equation for fatty acid metabolism (FA) is as follows:

$$\frac{dFA}{dt} = U_{eFF,FA} + 0.125 * U_{CC,FA} + U_{eUL,FA} - U_{FA,MC} - U_{FA,eFF} - U_{FA,TG} \quad (14.67)$$

Respective inputs to the fatty acid pool from cytoplasmic acetyl-CoA, free fatty acids and undefined lipids are described by equations 14.7, 14.19 and 14.28. Outputs to free fatty acids, mitochondrial acetyl-CoA and triacylglycerol are described by equations 14.30, 14.31 and 14.32. Estimates of free fatty acid uptake based upon arteriovenous (A-V) difference data and estimates of the capacity of liver for *de novo* synthesis of fatty acids do not account for free fatty acid oxidation ($U_{FA,MC}$) and triacylglycerol synthesis ($U_{FA,TG}$). As a result, the model could not be balanced on the basis of available data. It was noted above that Waghorn (1982) was unable to reconcile input on data available at that time with outputs, which included heat, triacylglycerol, glucose, etc. With regard to hepatic lipid metabolism, he stated 'most investigations of hepatic lipid metabolism were concerned with only plasma FFA [free fatty acids] and TG [triglycerides]'.

There do not appear to be any published data relating to hepatic extraction or turnover of cholesterol esters or phospholipids in cattle, although Bergman *et al.* (1970) showed that 10% of chylomicron ($P < 1.006$) cholesterol and cholesterol esters are removed during passage through the sheep liver. Cholesterol esters and phospholipids may provide fatty acids for triglyceride synthesis and may also be involved in other aspects of hepatic metabolism that cannot be documented in ruminants at the present time. We also stated above that new data from portal drained viscera and hepatic A-V studies, such as those published by Huntington and Reynolds (1987) and Reynolds *et al.* (1988a,b), among others, led Freetly *et al.* (1993) to undertake development of the model described herein. However, the problem with balancing fatty acid metabolism remains. This problem was resolved for the purpose of model formulation by creating an undefined lipid pool (eUL; Fig. 14.1) from which fatty acids are drawn ($U_{eUL,FA}$). One might presume that cholesterol esters and phospholipids from lipoprotein fragments cleared by liver constitute a major portion of eUL. No data are available to either reject or accept this supposition.

Data are not available to rigorously parameterize the model for protein synthesis and metabolism. However, omission of such provisions would clearly lead to erroneous solutions in the radiotracer portion of the model. Thus, these components were included, even though a number of assumptions were required.

Synthesized proteins fall into two categories: liver and export proteins. Estimates of protein turnover rates in liver and of synthetic rates of export proteins were made to account for ^{14}C incorporation into proteins and to allow calculation of energy expenditures in protein synthesis. Hibbitt and Baird (1967) reported nitrogen contents of 26.9–32.3 mg/g wet weight in the

livers of normal lactating cows. Based upon these estimates, a protein content of 20% for liver was assumed. Lobley *et al.* (1978) reported fractional synthesis rates (FSR) for liver protein of 0.24/day and 0.38/day in growing heifers and 0.144/day in a mature non-lactating cow. Later, Lobley *et al.* (1980) reported FSR in growing heifers and a non-lactating cow of 0.104/day and 0.074/day, respectively. These values were lower than earlier measurements, leading Lobley and colleagues to suggest that the actual FSR is between values reported in these studies. An intermediate FSR of 0.15/day was adopted for use in model development. This FSR yields a turnover of liver protein of 318 g/day in the reference animal.

Export protein synthesis rates for albumin, globulins and fibrinogen were adopted from Waghorn (1982), who established a regression equation relating albumin synthesis and body weight using data from multiple species of $0.42 \text{ g/kg BW}^{0.75}$. Based upon this relationship, albumin synthesis was set at 54 g/day for model development. This estimate indicates an FSR of 6.5%/day for albumin.

Fibrinogen and globulins are synthesized and exported from liver. Assuming an FSR similar to that of albumin for globulins and fibrinogen, total synthesis would be 47 g/day. Thus, total export protein synthesis was estimated to be 101 g/day in the reference state, which yields the estimate that total hepatic protein synthesis is $(318 + 101 \text{ g/day})$, 419 g/day.

Only limited data are available on whole blood amino acid profiles in portal blood and uptake by the livers of lactating cows (Baird *et al.*, 1975; Reynolds *et al.*, 1988a). In addition, there may be a problem in accounting for peptides absorbed from the intestine and taken up from portal blood by liver (Webb, 1990). For the purposes of model construction, an arbitrary amino acid profile was set for portal blood (Table 14.6). It was assumed that amino acids were not preferentially removed by liver and that entry of a given amino acid into the liver was proportional to its concentration. In the reference state, 5.328 mole/day ($U_{AA,UP}$) of amino acids are removed from blood.

Amino acids available for catabolism come from amino acids removed from blood plus those arising from protein degradation minus amino acids used for protein synthesis:

$$U_{AA,CAT} = U_{AA,UP} + U_{PT,AA} - U_{AA,PT} - U_{AA,ePT} \quad (14.68a)$$

Products of amino acid catabolism enter the PY, DC, AK, PC, MC and KB pools. Conversions to PY, DC and AK were adjusted to accommodate explicit representations of AL, AS and GU metabolism as follows:

$$U_{AA,PY} = (0.133 * U_{AA,CAT}) - U_{AL,CAT} \quad (14.68b)$$

$$U_{AA,DC} = (0.133 * U_{AA,CAT}) - U_{AS,CAT} - U_{AN,CAT} \quad (14.68c)$$

$$U_{AA,AK} = (0.327 * U_{AA,CAT}) - U_{GN,CAT} - U_{GU,CAT} \quad (14.68d)$$

Table 14.6 Estimated amino acid composition of blood and protein in the ruminant

Amino acid	Molar proportion of amino acids
Alanine	0.0396
Arginine	0.0379
Aspartate	0.0587
Cystine	0.0209
Glutamine	0.0827
Glutamate	0.0827
Glycine	0.0293
Histidine	0.0220
Isoleucine	0.0512
Leucine	0.0773
Lysine	0.0618
Methionine	0.0229
Phenylalanine	0.0360
Proline	0.1014
Serine	0.0660
Threonine	0.0397
Tryptophan	0.0070
Tyrosine	0.0383
Valine	0.0696

The above amino acids were selected for separate representation because they have been used as radiotracers to study liver metabolism. Conversions to PC, MC and KB were set as simple functions of stoichiometric relationships for catabolism of the mixed amino acids taken up by liver (Table 14.6; Krebs, 1964):

$$U_{AA,PC} = 0.206 * U_{AA,CAT} \quad (14.68e)$$

$$U_{AA,MC} = 0.283 * U_{AA,CAT} \quad (14.68f)$$

$$U_{AA,KB} = 0.012 * U_{AA,CAT} \quad (14.68g)$$

NADH, ATP, carbon dioxide and ammonia produced during amino acid catabolism and prior to entry into metabolite pools are also calculated (Freetly *et al.*, 1993).

The differential equation for alanine metabolism (AL) is as follows:

$$\frac{dAL}{dt} = U_{eAL,AL} + U_{PY,AL} + U_{PT,AL} - U_{AL,PY} - U_{AL,PT} - U_{AL,ePT} \quad (14.69)$$

Inputs to AL are from extracellular alanine, pyruvate and protein (PT).

The differential equation for aspartate (AS) is as follows:

$$\frac{dAS}{dt} = U_{eAS,AS} + U_{eAN,AS} + U_{DC,AS} - U_{AS,DC} - U_{AS,PT} - U_{AS,ePT} \quad (14.70)$$

Inputs to AS are from extracellular aspartate, dicarboxylic acids and protein.

The differential equation for glutamate metabolism (GU) is as follows:

$$\frac{dGU}{dt} = U_{eGU,GU} + U_{eGN,GU} + U_{AK,GU} + U_{PT,GU} - U_{GU,AK} - U_{GU,eGU} - U_{GU,PT} - U_{GU,ePT} \quad (14.71)$$

Inputs to GU are from extracellular glutamate, extracellular glutamine, α -ketoglutarate and protein.

The differential equation for urea (UR) is as follows:

$$\frac{dUREA}{t} = (U_{BNH_3,UR} + U_{AA,NH_3})/2 + U_{ARG,UR} \quad (14.72)$$

Sources of nitrogen for urea (UR) synthesis are blood ammonia and amino acid catabolism. Reynolds and colleagues (1986b, 1988a) reported net hepatic urea release rates of 9.26 and 9.38 mole/day. In the reference state, 8.9 mole/day of urea are released. Since model estimates of urea production account for only amino acid and blood ammonia nitrogen, urea production is underestimated to the extent that other nitrogen sources contribute.

Limited studies of oxygen consumption by liver have been performed in dairy cattle. Baird *et al.* (1975) reported liver oxygen consumption to be 48.0 mole/day in 400 kg cows yielding 12 kg milk/day. Reynolds *et al.* (1988b) reported rates of oxygen consumption of 73.5 mole/day in livers of 645 kg cows yielding 32.2 kg milk/day in agreement with earlier estimates of 74.0 mole/day in 660 kg cows (Reynolds *et al.*, 1986a; Huntington and Reynolds, 1987). Given the relationships developed by Smith and Baldwin (1974), liver weights of the cattle studied by Baird *et al.* (1975) and Reynolds *et al.* (1988b) would be 7.72 and 10.68 kg, respectively, which indicate respective oxygen consumption rates of $9.66 \text{ ml } (100 \text{ g} \cdot \text{min})^{-1}$ and $10.47 \text{ ml } (100 \text{ g} \cdot \text{min})^{-1}$. For model construction, a value of $10.0 \text{ ml } (100 \text{ g} \cdot \text{min})^{-1}$ was used.

Baird *et al.* (1975) reported liver carbon dioxide production to be 43.1 mole/day with an RQ of 0.90. Reynolds *et al.* (1988b) reported carbon dioxide production to be 51.5 mole/day with an RQ of 0.70. An RQ of 0.80 was used to estimate carbon dioxide production rates in the course of model development.

Carbon dioxide can act as both a substrate and a product. In the model, carbon dioxide (CD) is not represented as an explicit effector of reaction rates. However, provision for ^{14}C -carbon dioxide use as a radiotracer was incorporated. The differential equation for intracellular carbon dioxide is as follows:

$$\frac{dCd}{dt} = U_{IC,AK} + U_{GP,RU} + U_{PY,MC} + U_{CT,AK} + U_{CT,PY} + U_{AK,DC} + U_{DC,PE} + U_{AA,CD} - U_{PY,DC} - U_{PC,DC} - U_{nC,eCD} \quad (14.73)$$

Coenzyme pool sizes are very small, their differential equations are com-

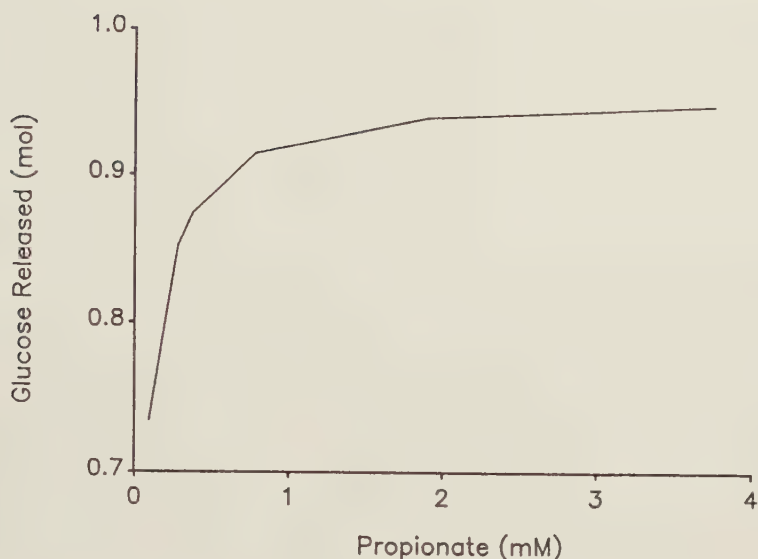


Figure 14.2 Behavioral analyses. Simulated effects of propionate concentration on rates of glucose release from ruminant liver. Simulations were of 90 min *in vitro* incubations similar to those reported by Mesbah and Baldwin (1983).

plex and their rates of turnover are high with respect to other metabolites. As discussed in Chapter 4, pool sizes specified for NAD^+ , NADH , NADP^+ , NADPH , FAD^+ , FADH , ADP and ATP were artificially increased in the model in order to decrease computer time required to solve the model without compromising solutions. Freetly *et al.* (1993) presented the differential equations for these coenzymes, which will not be repeated here.

14.2.2 Model evaluation

(a) Behavioral analyses

Behavioral analyses were conducted using a 90 min simulation time. This assured that the model reached steady states characteristic of nutritional and physiological states specified as inputs. As discussed in Chapter 12, patterns of radiotracer use were used as indicators of model behavior. The specific activities of substrate radiotracers were set to one in the behavioral analyses to facilitate convenient interpretations of results obtained (Figs 14.2 and 14.3, panels a–d).

The condition used for model formulation was the reference steady state. This strategy allows differential equations to be set to zero, thereby simpli-

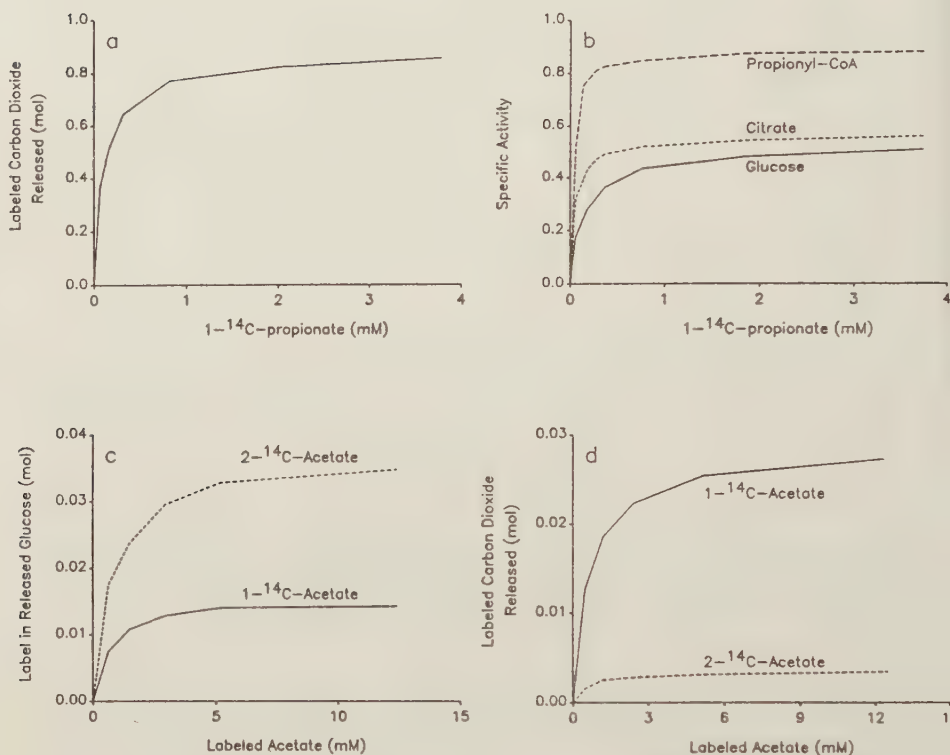


Figure 14.3 Behavioral analyses. Simulations were conducted for 90 min and ^{14}C -substrates were set to a specific activity of one. (a) Labeled carbon dioxide release with increasing concentrations of $1-^{14}\text{C}$ -propionate. (b) Specific activities of glucose, propionyl-CoA and citrate with increasing concentrations of $1-^{14}\text{C}$ -propionate. (c) Glucose released from $U_{\text{GP, eGL}}$ with increasing concentrations of 1- or $2-^{14}\text{C}$ -acetate. (d) Labeled carbon dioxide released with increasing concentrations of 1- or $2-^{14}\text{C}$ -acetate.

fying computation of numerical inputs to the model. Behavioral analyses determined whether or not the equations and parameter values based on the reference steady-state condition behave in a reasonable manner when perturbed with inputs other than those used in model development. Since the equations upon which the model is based reflect our current knowledge of underlying biology and data available for formulation of numerical inputs, failure of the model to mimic behavior of liver indicates that one or more of the equation forms were incorrect or that data used to formulate parameter inputs were inadequate. The most obvious behavioral analyses are to vary substrate concentrations and monitor effects upon formation of

products. In the model, the equations in all pathways for conversion of substrates to products include equations that represent saturation kinetics. Thus, overall behavior of the model is restricted to simulation of non-linear responses to increasing substrate concentrations. This behavior is evident in Fig. 14.2, where glucose production approaches saturation as propionate concentrations increase. This behavioral relationship is consistent with data on isolated hepatocytes (Looney, 1985) and liver slices (Mesbah and Baldwin, 1983).

Once simulated relationships for conversion of substrates to products were evaluated and found to be reasonable in terms of observed behavior, simulations of patterns of ^{14}C -radiotracer metabolism with different substrates became appropriate (Fig. 14.3, panels a-d).

Rates of $1\text{-}^{14}\text{C}$ -propionate oxidation to carbon dioxide with increasing propionate concentrations are presented in Fig. 14.3(a). The limited data of Looney *et al.* (1985) suggest a curvilinear relationship as well. Relative differences in the specific activities of glucose, propionyl-CoA and citrate formed from increasing levels of $1\text{-}^{14}\text{C}$ -propionate are presented in Fig. 14.3(b). As the concentration of $1\text{-}^{14}\text{C}$ -propionate increased, the contribution of propionate to each pool increased. The rapid increase in the specific activity of propionyl-CoA at low concentrations of $1\text{-}^{14}\text{C}$ -propionate is due to increased entry relative to alternate sources of propionyl-CoA, such as branched-chain volatile fatty acids, valerate and amino acids. Rates of entry of these latter sources are also the reason why the asymptote in the specific activity is about 0.9 rather than 1.0. This implies that at saturating concentrations of propionate about 10% of propionyl-CoA arises from other sources. The observation that the specific activity of glucose is one-half that of propionyl-CoA simply reflects randomization of ^{14}C between the 1 and 4 positions of the dicarboxylic acids and loss of one of these carbons in the conversion of oxaloacetate to phosphoenolpyruvate. The higher specific activity of citrate relative to glucose reflects the cycling of carbon from propionate in the tricarboxylic acid (TCA) cycle.

Relative rates of incorporation of 1- and $2\text{-}^{14}\text{C}$ -acetate into glucose and carbon dioxide are functions of flux through the TCA cycle and rates of removal of intermediates from the pathway. Simulation results are presented in Fig. 14.3(c and d). Radiolabel from $1\text{-}^{14}\text{C}$ -acetate is lost in carbon dioxide in the second turn of the TCA cycle, while conversion of $2\text{-}^{14}\text{C}$ -acetate to carbon dioxide requires three or more turns of the TCA cycle. As a result, $2\text{-}^{14}\text{C}$ -acetate incorporation into glucose would be expected to be greater than $1\text{-}^{14}\text{C}$ -acetate incorporation due to a higher specific activity in oxaloacetate while conversion of $1\text{-}^{14}\text{C}$ -acetate to $^{14}\text{CO}_2$ would be expected to exceed that from $2\text{-}^{14}\text{C}$ -acetate. The simulations presented in Fig. 14.3(c and d) are consistent with these known and expected response patterns. Models for analyses of the relative rates of carbon flow through the TCA cycle as opposed to rates of gluconeogenesis were proposed by Weinman *et al.* (1957), Thompson (1971), Wiltrout and Satter (1972), Hetenyi (1982)

and Goebel *et al.* (1982). They were all based to some degree on data such as those generated in the simulations presented in Fig. 14.2 and 14.3(a–d). Steinhour and Bauman (1988) improved upon these analytical models by accounting for alternate (to gluconeogenesis) routes of radiotracer removal from the TCA cycle. These models have been very useful in extending interpretations of data. However, they do not have the capacity to use full data sets in the parameter estimation process when the data sets include measurements made at varying concentrations of substrates in an attempt to develop or parameterize kinetic relationships. Radiotracer elements were included in the liver model to enable the latter type of analyses. Figure 14.3(c) depicts the appearance of acetate carbons in glucose as acetate concentration increases. Incorporation of 2-¹⁴C-acetate into glucose is greater than that of 1-¹⁴C-acetate (Fig. 14.3c), while the converse is true for incorporation into carbon dioxide (Fig. 14.3d).

These and many more behavioral analyses (not presented) agree with patterns reported in the literature. Where literature values were not available, the behavioral analyses were consistent with the known biology of ruminant liver metabolism.

(b) Sensitivity analyses

Sensitivity analyses are an important part of model construction and evaluation and can be divided into two major categories. The first is to test sensitivity of model outputs to alternative equation forms, degrees of aggregation, etc. These analyses are generally undertaken during model formulation to determine what elements of the biological system must be represented and at what level of complexity. Results of these types of analyses are reflected in the equations adopted (Table 14.5). The second type is to evaluate effects of varying parameters, such as affinity constants, on model behavior. Based on this type of sensitivity analysis, a decision can be made on a given parameter. If the model is insensitive to changes in the parameter value, that parameter can be eliminated from the model if it is not required to represent a biological function essential to function of the whole model. In this way, simplification of the model can occur. Conversely, if the model is sensitive to a parameter, the analysis indicates the importance of accurate estimation of that parameter. If such a parameter is not firmly defined by experimental data, collection of data to accurately define the parameter is the next logical step.

Results of some of the sensitivity analyses conducted during evaluation of the model are presented in Fig. 14.4. ATP acts as a substrate and regulator of many key reactions in the model. Figure 14.4(a) illustrates sensitivity in carbon dioxide production to changes in the amount of ATP required for basal metabolic processes ($U_{AT,MP}$). Varying $U_{AT,MP}$ mimics variations in energy expenditures in the tissue. The results indicate that carbon dioxide production increases as ATP use increases until a maximum is reached.

Over this portion of the curve, simulated reaction rates in the TCA cycle increase, while there is a minor decline in carbon dioxide production from the pentose cycle due to a decrease in long-chain fatty acid synthesis. The simulated decline in carbon dioxide production after reaching this maximum results from a sharp decrease in amounts of dicarboxylic acid entering gluconeogenesis and a lesser rate of increase in TCA cycle flux. This analysis demonstrates that an accurate estimate of ATP use is critical to model behavior.

Input:output data indicate a net release of acetate from liver, while isotope studies show significant rates of ^{14}C -acetate incorporation into liver metabolites and products. A sensitivity analysis was conducted to determine sensitivity of the ^{14}C -radiotracer portion of the model to estimates of $U_{\text{eAC},\text{MCs}}$ and $U_{\text{MCs},\text{eAC}}$ while net flux to eAC was held constant (Fig. 14.4b). It is clear that accurate estimates of these flux rates are required when acetate is used as radiotracer and that apparent rates of $1\text{-}^{14}\text{C}$ -acetate oxidation are more sensitive than are those of $2\text{-}^{14}\text{C}$ -acetate. An inverse relationship was evident when ^{14}C -label incorporation into glucose was estimated (figure not shown).

The conversion of pyruvate to dicarboxylic acid represents a potential regulatory step in gluconeogenesis (Looney *et al.*, 1987). Sensitivity to changes in affinity for pyruvate ($k_{\text{PY},\text{DC}}$) in this reaction ($U_{\text{PY},\text{DC}}$) was tested (equation 14.48). As the affinity constant increased, there was a small decline in glucose production. The specific activities of glucose produced using $2\text{-}^{14}\text{C}$ -lactate and $1\text{-}^{14}\text{C}$ -propionate as substrates are presented in Fig. 14.4(c). When $2\text{-}^{14}\text{C}$ -lactate was used as a radiotracer, the specific activity of glucose declined, but when $1\text{-}^{14}\text{C}$ -propionate was used as a radiotracer, there was a slight increase in specific activity. These results suggest that as $k_{\text{PY},\text{DC}}$ increases, the proportion of glucose derived from propionate increases while the proportion derived from pyruvate decreases. The data in Fig. 14.4(c) also show how the model can be used to select a tracer to enable estimation of a given parameter in the model. The relative sensitivity of glucose production to changes in $k_{\text{PY},\text{DC}}$ is greater for $2\text{-}^{14}\text{C}$ -lactate than for $1\text{-}^{14}\text{C}$ -propionate, which suggests that $2\text{-}^{14}\text{C}$ -lactate be used to estimate $k_{\text{PY},\text{DC}}$ when the specific activity in glucose is measured.

Most radiotracer studies assume rapid mixing of the tracer in the tissue and that the specific activities of all metabolites are stable. Figure 14.4(d and e) shows the simulated production of ^{14}C -labeled carbon dioxide and glucose from $1\text{-}^{14}\text{C}$ -propionate over time. Both curves lag briefly before they become linear. This lag is more exaggerated for glucose than for carbon dioxide. Neither of these lags can be examined experimentally, because their deviation from linearity is small with respect to experimental error. They do suggest, however, that specific activities of intermediate pools are not stable during initial stages of *in vitro* incubations. Simulated specific activities of α -ketoglutarate and phosphoenolpyruvate over time using $1\text{-}^{14}\text{C}$ -propionate as tracer are depicted in Fig. 14.4(f). The time required for

the specific activities of metabolites to reach a steady state appears to be unique for each pool and varies with different radiotracers (data not shown). The time required for α -ketoglutarate to reach a constant specific activity was greater than that for phosphoenolpyruvate. While the appearance of ^{14}C -label in carbon dioxide over time seems nearly linear, the relative contribution of each of the carbon dioxide-producing reactions changes until all metabolites come to stable specific activities. This simulation suggests that calculations based on assumptions that stable specific activities are instantaneously obtained may be incorrect and the shorter the incubation time, the greater the potential error. For example, at low concentrations of substrates, Looney *et al.* (1987) used incubation periods as short as 15 min. This sensitivity analysis demonstrates how the model can suggest modifications in experimental design.

(c) Independent data challenge

Data sets used to evaluate a model must not have been used in model construction. Limited data were available for model parameterization and, as a result, all available *in vivo* data were used. However, a number of *in vitro* ^{14}C -radiotracer data were not used in model construction. These data sets were used to challenge the model. Some comparisons of experimental data and model outputs for radiotracers were presented in the behavioral analysis section.

Experimental and simulated substrate interactions were tested. As noted in the behavioral analyses section, glucose production with varying concentrations of lactate or propionate agree with literature values. *In vitro* data have shown that additions of amino acids stimulate glucose production. Simulation runs indicated that additions of amino acids to limited incubation media increased 2- ^{14}C -propionate conversion to glucose and decreased propionate oxidation. Aiello and Armentano (1987) observed an increase in propionate incorporation into glucose but no difference in its oxidation. The simulated difference in carbon dioxide production is within the error term reported by Aiello and Armentano (1987). Looney *et al.* (1987) added aspartate to a limited medium containing propionate and demonstrated a subsequent increase in glucose production. The model predicted similar behavior when aspartate was added to a medium containing propionate.

Potential interactions of propionate with other volatile fatty acids that form CoASH esters were tested. Addition of valerate to limited media with 2- ^{14}C -propionate has been shown to increase label incorporation into glucose and decrease its appearance in carbon dioxide (Aiello and Armentano, 1987). The same result was simulated when valerate was added to the limited media. Previous studies have shown that butyrate added to limited media containing propionate decreased glucose synthesis (Aiello *et al.*, 1989; Looney, 1985). Aiello *et al.* (1989) also observed no increase in carbon dioxide from 2- ^{14}C -propionate with butyrate addition. Aiello *et al.* (1989)

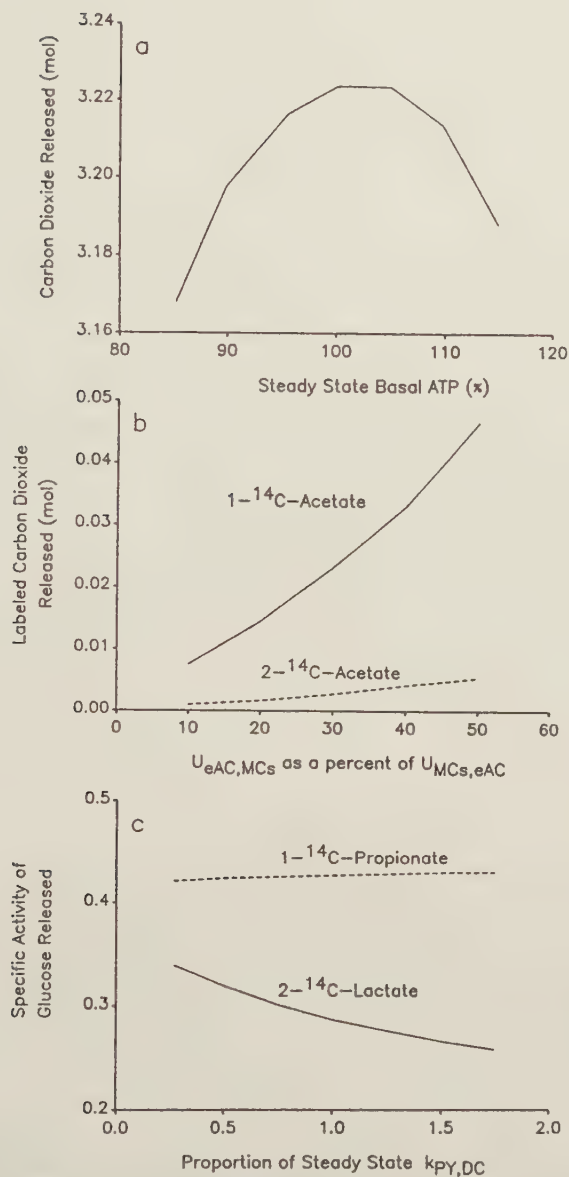
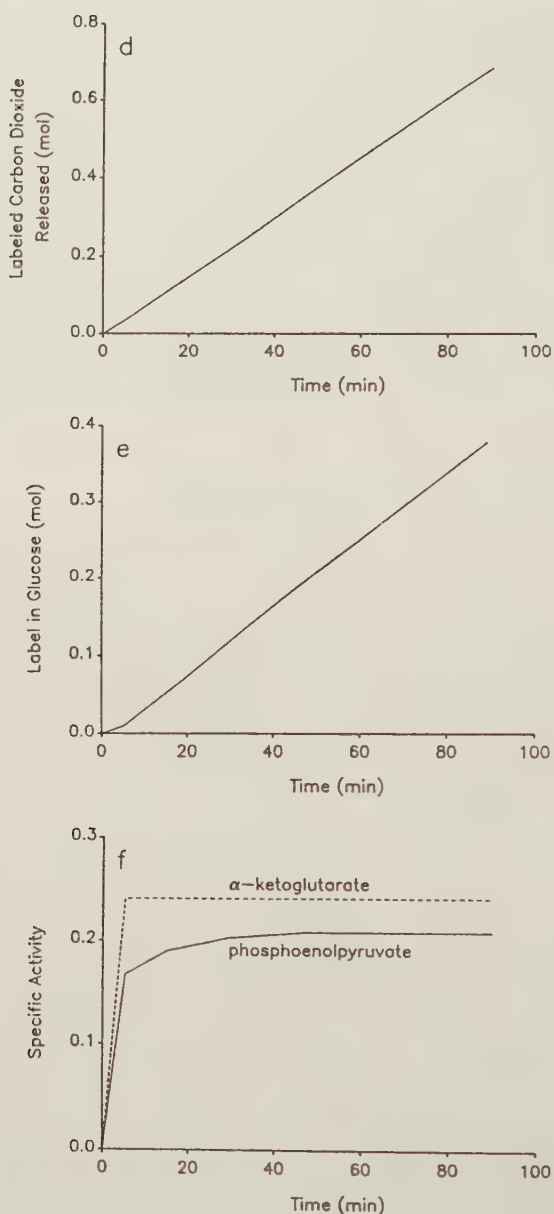


Figure 14.4 Sensitivity analyses. Simulations were conducted for 90 min at steady-state substrate concentrations with specific activities of ^{14}C -tracers set at one. (a) Carbon dioxide released from carbon dioxide-producing reactions as basal ATP expenditures were varied as a percentage of steady-state basal ATP expenditures. (b) Labeled carbon dioxide released from carbon dioxide-producing reactions when 1- or 2- ^{14}C -acetate is used as a tracer and $U_{eAC,MCs}$ increases as a percentage of $U_{MCs,eAC}$ while the net acetate flux is held constant. (c) Specific activity of glucose released from $U_{GP,eGL}$ as the affinity constant for pyruvate ($k_{PY,DC}$) in the $U_{PY,DC}$ activity (equation 14.48) changes as a proportion of the steady state value when 2- ^{14}C -lactate and 1- ^{14}C -propionate are used as tracers.



(d) Moles of ^{14}C -carbon released by the $U_{\text{GP,eGL}}$ flux with increasing simulation time and $1\text{-}^{14}\text{C}$ -propionate used as a tracer. (e) Labeled glucose released with increasing simulation time and $1\text{-}^{14}\text{C}$ -propionate used as a tracer. (f) Specific activity of α -ketoglutarate and phosphoenolpyruvate with increasing simulation time and $1\text{-}^{14}\text{C}$ -propionate as a tracer.

argued that the effect is not the result of competition for substrates like CoASH and ATP, but rather an inhibition of one of the enzymes required for propionate conversion to succinyl-CoA. A sensitivity analysis was undertaken to test this hypothesis. Affinity constants and maximum capacities for uptake of butyrate were varied to test the hypothesis that inhibition was the result of substrate competition. The maximum depression of 2-¹⁴C-propionate incorporation into glucose achieved with rational parameter values was 5%. Reported decreases in 2-¹⁴C-propionate incorporation into glucose by butyrate were 32–42% (Aiello *et al.*, 1989; Aiello and Armentano, 1987). This decrease suggests that butyrate or one of its metabolites may inhibit propionate entry. Until the mechanism of this inhibition is elucidated, equations required to simulate this response cannot be formulated.

The model predicts a sevenfold higher incorporation of 1-¹⁴C-acetate than 2-¹⁴C-acetate into carbon dioxide at maximum capacity (Fig. 14.3d). The model also predicts a twofold greater rate of 2-¹⁴C-acetate incorporation into glucose than for 1-¹⁴C-acetate at maximum capacity (Fig. 14.3c). This is consistent with the work of Knapp *et al.* (1992). Simulation results compare favorably with data presented by Knapp *et al.* (1992) for oxidation of 1-¹⁴C-lactate and 2-¹⁴C-lactate. More ¹⁴C-carbon dioxide is derived from 1-¹⁴C-lactate than from 2-¹⁴C-lactate at maximum capacity. Increasing concentrations of lactate from K_s to capacity result in increased oxidation of 1-¹⁴C-lactate to ¹⁴C-carbon dioxide but not an increase in the oxidation of 2-¹⁴C-lactate to ¹⁴C-carbon dioxide.

(d) Conclusions

The current mechanistic model of ruminant liver metabolism presented herein is based on current knowledge of underlying biochemical functions. The model integrates input:output and ¹⁴C-radiotracer data. Behavioral analyses and challenges with independent data sets suggest that model behavioral patterns are correct. Sensitivity analyses have identified additional research that is required to further our knowledge of liver metabolism and in doing so has illustrated the usefulness in the research process of models of this type. Integration of input:output and ¹⁴C-radiotracer elements has resulted in a new tool for analysis of radiotracer data.

It is clear that additional research must be conducted to fully understand patterns of nutrient use in ruminant liver. Additional information is required to accurately assess the contribution of specific amino acids to gluconeogenesis. Similarly, rates and composition of lipid uptake and release are required. Sensitivity analyses have shown that data on flux rates that provide potential points of exit for radiotracer are required. Examples of these include acetate and lactate exchange.

Integration of the existing data provides insight into liver function and experimental design. This model serves primarily to guide research in ruminant liver metabolism in an organized fashion, integrate information to

insure improved understanding of function of the organ as a whole and improve representations of liver function in models of ruminant metabolism.

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Mechanistic, dynamic models of growth

with J. W. Oltjen and R. D. Sainz

While the organs and tissues of the young rat are known to increase in size with age, little is known of the role played by the component cells in this increase. There is evidence that cells enlarge and new cells are added, but we do not know to what extent the enlargement and proliferation of the cells cause the growth of organs and tissues.

Enesco and Leblond (1962)

15.1 INTRODUCTION

Routine methods for predicting animal performance for use in livestock feeding management (Chapter 5) are changing. Static systems of feed requirements based upon static, algebraic equations and tabular estimates of requirements are beginning to be replaced by dynamic simulation models able to characterize animal responses to a wider range of input conditions than those accommodated in traditional systems. These are characterized by an expanding set of management options such as limit feeding, sorting into uniform groups and the potential use of new growth adjuvants such as the adrenergic agents or recombinant bovine somatotropin, as well as consideration of individual animal differences and variable maintenance requirements. Further, feed intakes and transition states not handled by static systems may be simulated. The objective in this chapter is to illustrate the contributions specific dynamic models can make in better predicting animal performance and formulating rations for growing cattle and sheep. These models are based on the hypothesis that specific descriptions of governing biology coupled with proper applications of mathematical modeling tools can result in greater accuracy and wider application than is usual with the older, traditionally defined, empirical approaches. Thus, emphasis in the first part of this chapter will be on dynamic, biological relationships that influence nutrient use and animal performance. Others (Emmans, 1981; Whittemore, 1986; Black *et al.*, 1986; Black and Chapple, 1991) have described dynamic models of swine and poultry production and their use as

livestock management tools. Therefore, dynamic models of cattle growth, response surfaces generated by these models, and associated management and economic analyses that become feasible will be contrasted with systems based upon set requirements and tables. The final portion of the chapter will be devoted to an illustration of how dynamic, mechanistic models can be used to evaluate hypotheses regarding mechanisms of action of growth adjuvants and, as a result, provide for accommodation of changes in nutritional input:output relationships without the conduct of experiments essential to formulation of new empirical equations.

15.2 BEEF GROWTH MODELS

15.2.1 Description of models

Oltjen *et al.* (1986b) proposed use of a more general model of beef cattle growth and composition. Because cattle feeding programs range from utilization of low quality range forages to high energy feedlot diets, including multi-phase systems where several rations of widely varying energy content are fed, accurate predictions of composition of gain are necessary for proper evaluations of animal values throughout the growing period. That is, market price is related to body composition, either as a discount for fat animals at preslaughter weights, or at maximum for an optimum fat content for slaughter-weight animals. Oltjen *et al.* (1986b) developed a dynamic model based on concepts regarding cell number and size to predict growth and net protein synthesis, and then integrated the model into the Lofgreen and Garrett (1968) system described in Chapter 9 to estimate gain of fat and lean tissue (Fig. 15.1).

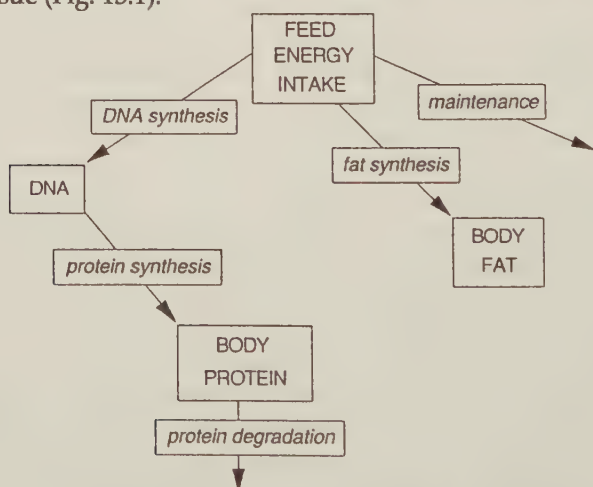


Figure 15.1 Block diagram of the model of Oltjen *et al.* (1986b).

Three premises that apparently determine growth in mammalian systems (quoted from Baldwin and Black, 1979; Chapter 9) are used in the model:

1. The primary genetic determinant of organ size is the final amount of deoxyribonucleic acid (DNA). It follows that a difference in organ size between species or between strains within a species is due largely to the amount of DNA observed in that organ in normally grown adults. This premise arises from the observations that DNA per diploid nucleus is constant in animals and that a difference in cell number and not cell size is largely responsible for variations in organ weight between mature individuals of different species (Munro and Gray, 1969) or strains within a species (Robinson and Bradford, 1969).
2. Each unit of DNA specifies, on a genetically defined basis for each tissue and each species, information required for the ultimate formation of a specific amount of cell material. This premise embodies the DNA unit concept discussed by Cheek *et al.* (1971) and overcomes any complications that may arise from polyploidy. Whether information specified by a unit of DNA leads to the formation of cell material is dependent upon the nutritional and physiological status of the animal. This premise allows for observed differences in cell size and composition between tissues and across species of mature animals.
3. The specific activities, expressed as units per gram of tissue, of enzymes or groups of enzymes responsible for tissue growth and general metabolism vary as an exponential function of organ size (Lin *et al.*, 1959). Also, the kinetic properties of enzymes are reasonably constant across species.

The adequacy of equations based upon these three premises was evaluated by simulating effects of (1) starvation on development of rat liver, (2) nutrient restriction and refeeding on rat liver and brain, (3) normal intake on nine tissues in two strains of mice, rats, sheep and pigs, and (4) normal intake on brain in several other species, including man. This led to the conclusion that these premises provide appropriate and adequate bases for mechanistic and dynamic representations of the basic processes that drive the growth process and influence growth rates.

The concepts and equation forms used by Baldwin and Black (1979) served as the starting point for Oltjen *et al.* (1985, 1986a,b). Initially they developed a model of whole-body protein accretion for normal, uninterrupted growth (Oltjen *et al.*, 1985) using rat data, since serial data on whole-body DNA were not available for cattle. The model depicted in Fig. 15.1 was dynamic, thus differential equations were integrated to estimate gain (or loss) of DNA and body protein (PROT). The following equations provided an adequate fit of normal, postweaning rat growth (Oltjen, 1983). They are shown here with coefficients for a beef steer of mature body weight of 750 kg (Oltjen *et al.*, 1986a)

$$dDNA/dt, \text{ g/day} = K1 (\text{DNAMX} - \text{DNA}) \text{ NUT1} \quad (15.1)$$

$$d\text{PROT}/dt, \text{ kg/day} = \text{SYNTHESIS} - \text{DEGRADATION} \quad (15.2)$$

$$\text{SYNTHESIS} = K2 * \text{DNA}^{0.73} \text{ NUT2} \quad (15.3)$$

$$\text{DEGRADATION} = K3 * \text{PROT}^{0.73} \quad (15.4)$$

where K1, K2 (0.0461) and K3 (0.143) are rate constants; NUT1 and NUT2 are nutritional constants set to 1 for normal *ad libitum* intake; and DNAMX is normal DNA content at maturity, calculated to be 385 g based on relationships derived by Baldwin and Black (1979) in the absence of direct data at that time. Parameters were estimated using a non-linear least squares fit of observed body protein gains in 53 groups of feedlot steers (Oltjen *et al.*, 1986b). For animals of different mature size (A'), rate constants are adjusted by the size scaling factor proposed by Taylor (1980; also see Chapter 9). Based on standardized age and rate variables, this implies that the rate of some function (r') in an animal of similar degree of maturity, but different in mature body weight (A') than another (A), is proportional to the ratio of their mature body sizes to the 0.73 power

$$r' = r (A'/A)^{0.73} \quad (15.5)$$

where r is the rate in the animal of mature size A . Hence,

$$K1 = 0.00429 (A'/750)^{-0.27} \quad (15.6)$$

but K2 and K3 are not changed. Also, K2 is increased 4% for cattle with hormonal adjuvants based on observed additional rates of protein gain.

Next, effects of energy intake on growth were added using the NUT1 and NUT2 terms, which were based on a data set that contained initial and final empty body weights and compositions, as well as metabolizable energy intakes (MEI), for over 1000 animals. Data were summarized from comparative slaughter trials to evaluate feed net energy values (W. N. Garrett, University of California, personal communication). A ratio P was defined as MEI/MEINORM, where MEINORM (MJ/day) is the pattern of ME intake that supports normal growth of a reference steer (Oltjen *et al.*, 1986b) in the above equations (NUT1 and NUT2 equal 1) and

$$\text{MEINORM} = (1.83 - 1.094 \text{ EBW}/A') \text{ EBW}^{0.75} \quad (15.7)$$

where EBW is empty body weight (kg). Rates of DNA accretion and PROT synthesis are respectively adjusted as follows:

$$\text{NUT1} = -0.7 + 1.7 P \quad (15.8)$$

$$\text{NUT2} = 0.83 + 0.2 P/(0.15 + P) \quad (15.9)$$

Finally, daily empty body fat gain (DFAT) is calculated as the net energy available after daily feed intake (FI, kg/day) is used for maintenance (MAINT) and protein gain (DPROT, kg/day)

$$\text{MAINT, MJ/day} = a_m \text{EBW}^{0.75} (A'/750) - 0.02 \quad (15.10)$$

$$\text{DFAT, kg/day} = ((\text{FI} - \text{MAINT}/\text{NE}_m) \text{NE}_g - 23.18 \text{DPROT})/39.27 \quad (15.11)$$

where a_m is 0.359, and 23.18 and 39.27 are the energy content (MJ/kg) of protein and fat gains, respectively (Garrett and Hinman, 1969). Empty body weight is the sum of fat and fat-free body masses, where fat-free body mass is $\text{PROT}/0.2201$ (Garrett and Hinman, 1969). Since the model requires initial estimates of whole-body DNA, protein and fat, empirical relationships between these and animal weight, mature size and condition score are used to set beginning values for model implementation.

The model was evaluated first with respect to its ability to predict growth and composition of steers as affected by nutrition, initial condition, frame size and use of growth adjuvants. Lack of bias and relatively small deviation between predicted and observed values for weight gain and body composition are desirable characteristics of a model. Using two independent data sets, the model predicted empty body weight and fat content with standard deviations of predicted minus observed of 14 and 10 kg, respectively (Oltjen *et al.*, 1986b). Systematic error, or a tendency of models to consistently under (or over) predict some variable as another variable increases, is undesirable model behavior. No systematic biases were evident with respect to composition, frame size, or energy intake (Fig. 15.2). However, fat gain was under-predicted ($P < 0.01$) at high feed energy concentrations (Fig. 15.3). No adjustment for variable maintenance requirements could be identified. Baldwin and Bywater (1984) have shown other factors that affect energy expenditures are normally accounted for within the definition of maintenance. When dynamic properties and stability of the model were investigated (Oltjen *et al.*, 1986a), prediction intervals (integration step size) of up to 7 days resulted in little increase in error if the coefficient of variation of feed intake was less than 15%. If the interval was 1 day, the intake coefficient of variation could be as high as 40% with no loss of fit.

Although Oltjen *et al.* (1986a,b) accounted for variations attributable to initial body composition and mature size, their model does not always yield acceptable estimates of fat gain. This is not unexpected, since fat accretion is computed after energy requirements for maintenance and protein gain are satisfied. Thus, any errors in estimates of maintenance or protein gain result in biased fat-gain predictions. Further, feed energy available for fat accretion is not used at the same net efficiency as for energy gain of protein, as the net energy system assumes (Berschauer *et al.*, 1980). For example, of the major metabolites used for fat synthesis, fatty acids are the most efficient precursor (93–96%), followed by glucose (80–85%) and, finally, acetate (65–75%; Chapter 6). Thus, for diets of similar ME content, the one resulting in absorption of more long-chain fatty acids will support faster energy gains when the composition of gain is relatively greater in fat. This is why the

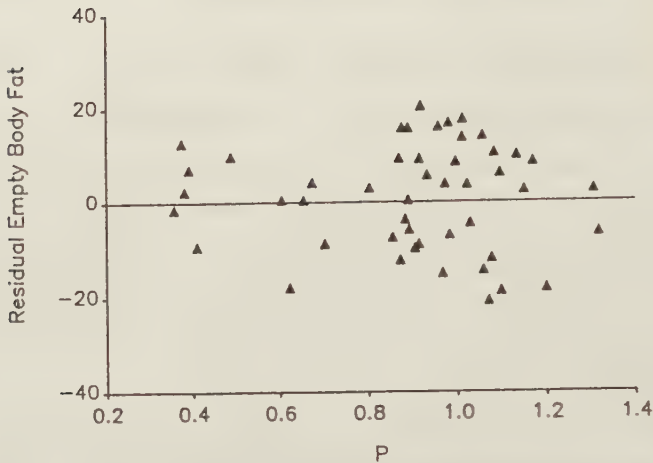


Figure 15.2 Relationship of empty body fat residual (kg) and nutritional level (P) when the exponent of maintenance requirement is 0.75 in model.

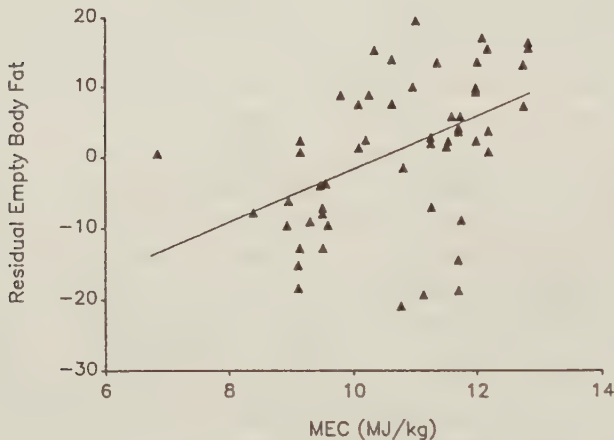


Figure 15.3 Relationship of empty body fat residual (kg) and metabolizable energy concentration (MEC, MJ/kg)

Oltjen *et al.* (1986b) model systematically errs with respect to energy concentration of the diet.

In recent years, attempts have been made to correct for nutrient partitioning and other errors, and integrated models of growth with digestion and/or metabolism have been developed. France *et al.* (1987) proposed a dynamic model of cattle growth based on carbon and nitrogen metabolism. State variables are body protein, lipid, ash, blood acetyl coenzyme A equivalents, blood glucose equivalents and blood amino acids. Inputs of rumen volatile fatty acids are converted stoichiometrically to their equivalent two- or six-carbon metabolites. Synthesis and degradation are represented for each body pool based on animal factors and absorbed nutrient levels. Com-

pared with experimental data, relatively good agreement was observed, although information is limited where both absorbed nutrient profiles and body composition are simultaneously measured. France *et al.* (1987) suggested linking the model with a simple model of rumen digestion (France *et al.*, 1982) to complete the growth prediction system.

In a more complex model, DiMarco *et al.* (1989) extended the growth equations used by Oltjen *et al.* (1986b) to two pools of protein (body and viscera), two associated DNA pools and a body fat pool. Growth is represented as the difference between synthesis and breakdown of each protein and fat pool. Parameters for DNA accretion in each protein pool were estimated using data from a reference steer for which DNA was measured (DiMarco *et al.*, 1987). Protein synthesis and degradation for body and viscera are similarly estimated from the reference steer, but, in addition, synthesis depends on blood amino acid concentration and degradation depends on catabolic hormone level. Lipogenesis is represented by Michaelis-Menten equations, which depend on anabolic hormones, plasma glucose, plasma acetate and fatty acids, and body fat content. Lipolysis is similarly represented and depends on catabolic hormones, plasma fatty acids and body fat content. Empty body weight is the sum of the protein and fat pools plus the water and ash associated with the protein pool (PROT weight/0.243).

Next, DiMarco and Baldwin (1989) integrated their growth model with digestion and metabolism elements. The integrated model represented the digestion of diet nutrients and the products of digestion were input to a metabolism component. Subsequently, key elements of this model were incorporated into the general model of growth and lactation presented and evaluated in Chapters 16 and 17.

Separation of the protein pools by DiMarco *et al.* (1989) account, in part, for variable maintenance requirements demonstrated by Koong *et al.* (1982), wherein relatively smaller viscera was associated with decreased fasting heat production. Also, the metabolism element helped correct errors in prediction of fat gain since the efficiency of each nutrient's use was explicitly represented. Nutrient prioritization as in previous models was not necessary, because equations that represent tissue affinities and the use of metabolites for oxidative and growth functions allow direct competition for their use.

The France *et al.* (1987), DiMarco *et al.* (1989) and similar models can account for interactions among previous plane of nutrition, level of feeding and ration energy concentration. The explicit representation of digestion products suggest that feeds must be represented by their chemical constituents in future systems (Chapter 8). At present, the complexity and lack of identity (additional experimental data are needed to set parameter values with confidence; see Chapters 8, 9, 11, 16 and 17) of these models pose a limit to their practical use (France *et al.*, 1987; DiMarco *et al.*, 1989). However, continuing development of these types of models and computer technol-

ogy and software for their implementation holds great promise for improvements in the effectiveness with which fundamental knowledge of animal function can be applied to improve animal agriculture.

15.2.2 Comparison of models

As was emphasized in Chapter 4, the basic tests that must be applied to models before they are adopted for use in practical animal agriculture are these: does the challenger model yield superior predictions relative to current (defender) models; or, if the systems are approximately equal, does the challenger system offer advantages in terms of (computer) friendliness or application to the advisor/manager that the defender system does not? In the next two sections of this chapter, these issues are developed in specific detail.

Animal responses to different metabolizable energy concentrations in a diet are a way of comparing models (Fig. 15.4). For a 350 kg steer fed 7 kg dry matter, daily gains increased for rations with greater metabolizable energy (ME) concentration in all systems. The TDN system (Winchester and Hedricks, 1953) was least responsive, demonstrating the relative over- and undervaluing of forages and concentrates, respectively. Slopes were nearly parallel for the other systems compared (Lofgren and Garrett, 1968; Fox and Black, 1984; Oltjen *et al.*, 1986b), except for some diminishing increases in rate of gain for the model of Oltjen *et al.* (1986b). Predicted gains for Oltjen *et al.* (1986b) were 0.2–0.4 kg/day greater than those for Lofgreen and Garrett (1968) and Fox and Black (1984), which coincided. This is not unexpected since the Oltjen *et al.* (1986b) system was developed as a dynamic model and, thus, predicts instantaneous gains at a given weight. The previously

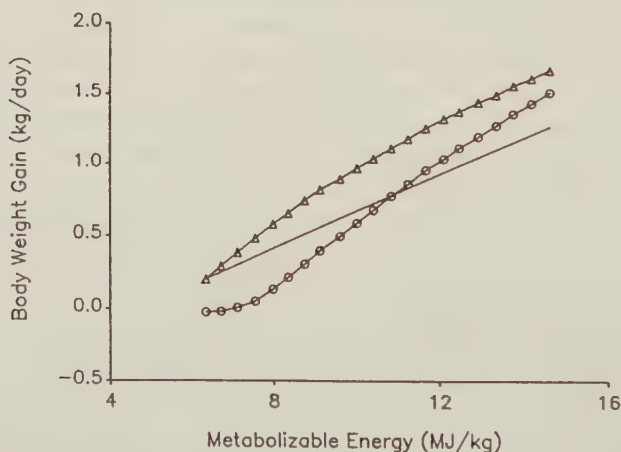


Figure 15.4 The effect of ration metabolizable energy content on daily gain for 350 kg steers fed 7 kg/day for (\) Oltjen *et al.* (1986), (o) Lofgreen and Garrett (1968) and Fox and Black (1984), and (—) Winchester and Hedricks (1953).

developed systems are mostly used by taking the average of initial and final weights for a feeding period. As a result, predicted performance of cattle whose average weights are 350 kg are probably less than their actual gains at 350 kg, a point of nearly maximal growth for medium-frame beef animals. However, if weights less than the average, such as the mean logarithms of initial and final weights or average weights over time, are used for the period, underpredictions of the static models of Lofgreen and Garrett (1968) and Fox and Black (1984) are less.

In another comparison, the models were used to estimate the length of feeding period required for a 193 kg medium-frame weaned steer calf to achieve a given endpoint, either 475 kg or 30% empty body fat (Table 15.1). Two feeding regimes were simulated, a concentrate ration (11.92 MJ ME/kg) fed continuously (C) or a forage-based ration (9.62 MJ ME/kg) fed for 112 days followed by the concentrate ration (FC). Intakes of the rations were those observed by Saubidet and Verde (1976) in an experiment under similar conditions. The differences in predicted days on feed between FC and C for the different models ranged from 38 to 47 days for constant weight, or from 55 to 57 days for constant composition endpoints. The larger differences (55–57 days) between feeding regimens for steers fed to 30% empty body fat illustrates the rather significant effect of ration on body composition at slaughter. This supports the need for accurate models to predict body composition. Although Fox and Black (1984) predict composition, the estimate is a function of body weight alone for steers of similar mature size. Therefore, in their model both steers fed FC for 306 days and those fed C for 249 days weighed 520 kg with equal final body composition. In contrast, in the Oltjen *et al.* (1986b) model, steers fed FC for 242 days weighed 475 kg, while body weights for those fed C for 187 days were 460 kg at the same final body composition.

Table 15.1 Comparison of model estimates of feeding period (days) required for a 193 kg steer calf to achieve a final body weight of 475 kg or 30% empty body fat^a

Model	Days to a common endpoint			
	final weight 475 kg		final composition 30% empty body fat	
	FC ^b	C ^c	FC	C
Winchester and Hedricks (1953)	246	208	—	—
Lofgreen and Garrett (1968)	254	207	—	—
Fox and Black (1984)	254	207	306	249
Oltjen <i>et al.</i> (1986b)	242	200	242	187

^aRations fed and intakes are from Saubidet and Verde (1976).

^bFC is a forage-based ration fed for 112 days followed by a concentrate ration.

^cC is a concentrate ration fed continuously from weaning.

The models were compared for their ability to predict performance of the US Meat Animal Research Center (MARC) Cycle I, II and III steers (Fig. 15.5). These cattle were of diverse breed types, with different mature weights, and fed similar corn-corn silage rations. With the progression of models through time, body-weight estimates were generally less biased and standard errors of prediction (precision) were as good or better than in the previous models (Table 15.2). Also, the correlation between error of final empty weight prediction and mature body weight was less for Fox and Black (1984) and least (i.e. best) for Oltjen (1983). Thus, accounting for frame size in the latter two models improved predictions. These results demonstrate that mechanistic models based on fundamental biological concepts can accommodate greater variance due to frame size observed in production situations and possess greater predictive accuracy and wider application than empirically derived models such as those currently in use.

Table 15.2 Simple correlation coefficients for relationship with residual (predicted–observed) empty body weight^a

Item	Correlation coefficient (r)			
	Oltjen model	Fox and Black model	NRC	ARC
Mature empty body weight	–0.02	–0.08	+0.54	–0.70
Initial FAT ^b	0.0	+0.48	–0.02	–0.59
P ^c	–0.13	–0.32	–0.14	+0.10

^a Adapted from Oltjen (1983), Fox and Black (1987), NRC (1976) and ARC (1980).

^b Empty body fat

^c Proportion of reference energy intake.

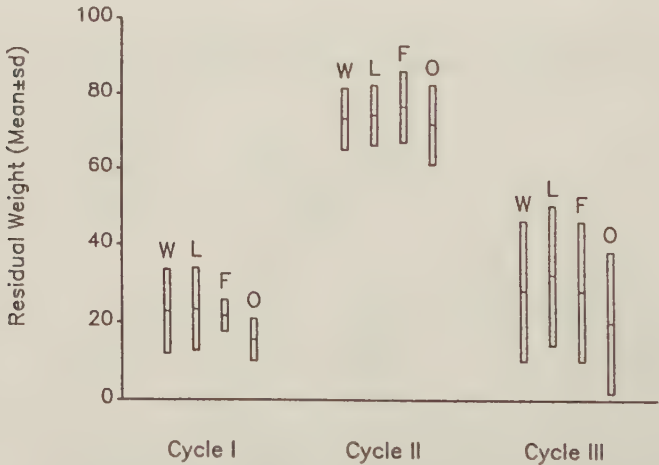


Figure 15.5 Mean residual (predicted–observed) final body weight ($\pm$ SD) of US Meat Animal Research Center steers for W, Winchester and Hedricks (1953); L, Lofgreen and Garrett (1968); F, Fox and Black (1984); and O, Oltjen *et al.* (1986).

15.2.3 Management implications

The models of beef cattle growth discussed in this chapter provide producers with tools that improve cattle management. The inputs needed for and information provided by each system are summarized in Table 15.3. Basically, additional and improved management information comes at the expense of defining more input variables and increasing computation requirements. All of the systems can be used to project performance for a typical feeding period, and the first three systems were developed expressly for that purpose. The Oltjen *et al.* (1986b) and DiMarco *et al.* (1989) dynamic models are especially useful in situations where ongoing collection of feed intake and/or body weight data is routine, such as in a feedlot. The incoming data can be used to recalibrate model predictions using adaptive filtering techniques, which allows increasingly more precise projections of performance as a feeding period progresses (Oltjen and Owens, 1987). This technology is relatively new and its implementation is driven by the economic need for improved performance predictions, such as that required when employing risk management strategies (hedging options). Also, these models are sensitive to conditions that affect composition of gain, thereby providing more robust predictions of gain. This is particularly true over shorter feeding intervals, whereas the preceding systems require longer feeding periods in order for systematic errors to cancel out (Oltjen and Garrett, 1988).

Models of animal performance may be integrated with input costs and output returns for economic evaluations of potential management strategies. Their contributions have been recognized in the cattle feeding industry for many years (Dunbar, 1976; Gill and Burditt, 1989). However, intake predictions for this application remain problematic. Good empirical fits of appropriate data (Plegge *et al.*, 1984; Hicks *et al.*, 1990) are often used, although Hyer *et al.* (1991) have developed a mechanistic model for grazing cattle with and without energy supplements. Nevertheless, these economic models are valuable tools for today's nutritionists and feedlot managers.

Models can be used to evaluate additional management options as sophistication increases, and composition and grades may be predicted. In particular, effects of within and between pen variation (production risk) on profitability have been estimated. Smith *et al.* (1988) simulated normal distributions of initial body weights in uniformly managed pens of 100 feedlot steers with mean initial weights of 318 kg using the model of Oltjen *et al.* (1986b). A typical feedlot diet was fed for 130 days and all steers in a pen were sold the same day on a carcass weight, quality and yield basis using market conditions for 1988 (Fig. 15.6). Results showed, for initial weight variation, that profit is stable up to a standard deviation of about 16 kg or a 5% coefficient of variation (CV), but declines at an increasing rate for pens of cattle with more variation in initial weight. Also, in a separate analysis, initial weight was fixed at 318 kg, but the parameters affecting growth (K2

Table 15.3 Required data and information provided by beef cattle growth prediction systems^a

System information	Required inputs	Outputs	
		biological	management
Winchester and Hedricks (1953)	sex, weight, feed digestibility, intake	weight gain, efficiency	ration energy effects on days to a given weight
Lofgreen and Garrett (1968)	sex, weight, feed energy, intake	weight gain, efficiency	all of the above plus: improved predictions over a range of roughages and concentrate intakes
Fox and Black (1984)	sex, weight, feed energy, intake ^b , frame, previous gain, growth promotant, beef/dairy type	weight gain, efficiency, equivalent weight ^c , growth	all of the above plus: frame size effects on growth, compensatory gain
Oltjen <i>et al.</i> (1986a,b)	sex, weight, feed energy, intake ^b , frame, condition, growth promotant	weight gain, efficiency, composition ^d	all of the above plus: animal and ration effects on composition of growth, days to yield desired fatness
DiMarco <i>et al.</i> (1989)	sex, weight, feed chemical composition, intake, growth promotant	weight gain, efficiency, composition, ^e products of digestion	all of the above plus: effect of individual nutrients on growth and composition, improved compensatory growth characteristics

^aSystems are based on feed energy; other nutrients are not included here.

^bIntake may be calculated or given as input.

^cComposition, quality and yield grade are linear functions of equivalent weight.

^dQuality and yield grade are linear functions of composition.

^eIncludes difference between visceral and non-visceral protein pools.

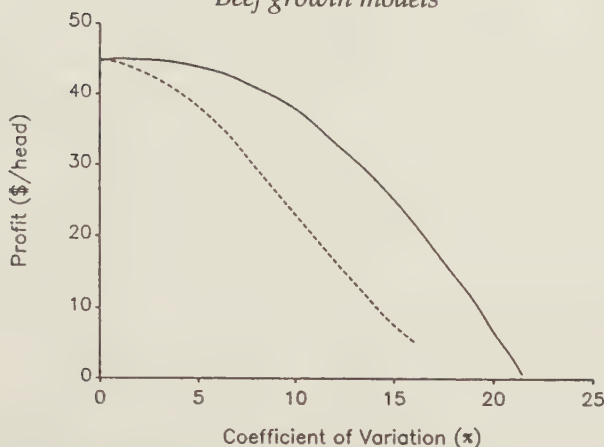


Figure 15.6 The effect of random normal variation of initial body weight (—) or of growth parameters K_2 and a_m (---) on average profit of steers fed in pens of 100, fed and marketed as a group.

and a_m) were generated with independent normal distributions about their means. The growth parameters' deviations resulted in a steadily decreasing profit. For a 10% coefficient of variation of both K_2 and a_m , profit fell \$22 per animal. The first simulation is similar to that of retained ownership of steers of similar genetic makeup, while the second shows the additional cost of mixing increasingly divergent genotypes (although mature weight or frame size was held constant in both analyses). Thus, the cost of animal variation may be directly addressed with the more recent models, which have accurate predictions of appropriate outputs such as composition, quality and yield grades. Further, the economically optimal ration when risk is considered may be different than that formulated for the average animal. That is, rations may need to be formulated so that risk is minimized over an entire group. Hence, rations formulated for the average animal support decreased performance for one-half of the animals. This cost, along with decreased value due to less than optimal body composition, is weighted against cheaper rations (using risk in the formulation may require greater nutrient levels).

Costs of ration variability, another significant source of variation in performance, may also be addressed with these models. The effect of varying ration composition was investigated using the above protocol (Smith *et al.*, 1988). If rations vary from their nominal energy value by 5% (68% of rations within 5% of estimated energy values), the standard deviation of profit per head was \$16 (Fig. 15.7). In other words, about one in six pens of cattle would return at least \$16 per head (about \$1.50/cwt) less than anticipated using the deterministic predictions and 1988 markets.

An area of current feedlot management research, which will become more important if some form of value-based marketing of beef cattle is adopted, concerns grouping cattle according to expected days on feed to a

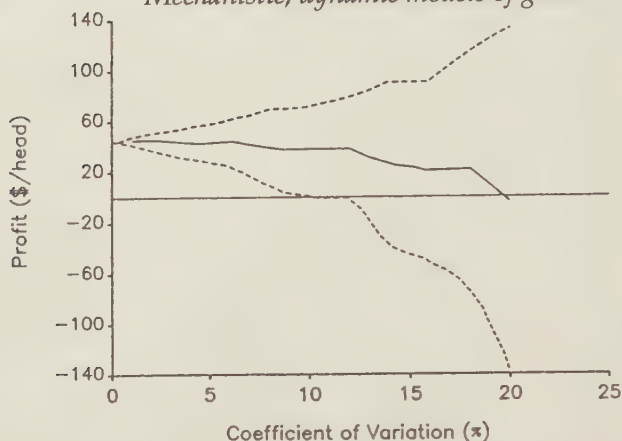


Figure 15.7 The effect of random normal variation of ration energy concentration on mean (—) $\pm$ SD (- -) profit of steers in 100 pens fed and marketed as a group.

common endpoint. Variables with which cattle may be sorted include initial body weight, as suggested above, and fat thickness as determined using ultrasound. Only models that include some measure of composition are useful in exploring possible sorting strategies; however, the current limitation in this research appears to be precise estimation of feed intake for the different groups (Smith, 1990).

The above examples of production risk are independent of price risk. However, stochastic models of market prices are advancing in accuracy in parallel with those for production (J. Trapp, 1990, personal communication). It is anticipated that the two types of models will be linked to achieve an optimal solution between the simultaneous objectives of profit maximization and risk minimization.

Several statements may be made that summarize the progression of models of beef cattle growth. Originally, with each new system, advances in prediction accuracy came about as a result of adding new terms to empirically derived models already accepted and in use. More recently, terms have been added based upon mechanistic concepts (France and Thornley, 1984) and representations of biological functions at lower levels of aggregation. Thus, recent emphasis on the biology of processes involved, and not just another empirical fit or relationship, has extended the accuracy and precision of each new model. This evolutionary process will continue by adapting new research knowledge so that beef cattle growth and performance may be better predicted. The challenge is to deliver these newer systems, which necessarily require many calculations and, hence, computers for implementation. Additional inputs needed for the more complex models may require expert systems to assist the user in gaining additional information available from these systems with ever increasing ranges of applications. The advantage will be their generality and ability to be inte-

grated with other models, thus allowing economic evaluations. This will eventually allow users to automatically search for solutions that approach optimal production strategies.

15.3 SHEEP GROWTH AND METABOLISM MODEL

The general list of modeling objectives in Chapter 3 includes the following:

1. Estimation of parameters which are not directly measurable experimentally;
2. Improved predictions of animal performance;
3. Evaluations of alternative hypotheses for probable adequacies;
4. Extended interpretations of experimental data.

The preceding sections in this chapter illustrate modeling exercises undertaken for improved prediction of animal performance. In that work, incorporation of mechanistic elements into an empirical framework improved predictions of animal performance and also extended the general applicability of the model. Other chapters illustrate estimation of parameters and interpretations of data, as well as design of critical experiments. The modeling exercise described in this section was concerned mainly with evaluation of alternative hypotheses, in this case regarding mechanisms of action of metabolic modifiers.

Metabolic modifiers are biological or synthetic compounds that improve animal performance and/or product composition. For example, commercial applications of sex steroids have been successful for decades. Newer techniques for hormonal manipulation of animal growth include growth hormone and β -adrenergic agonists. A great deal of research has been devoted to elucidating the mechanisms of action of these compounds. The rationale for this work is that if we can understand mechanisms by which a compound improves animal performance and product quality, we will be better able to design novel and socially acceptable methods to achieve the same end. Ricks *et al.* (1984) proposed that the mode of action of clenbuterol (a β -adrenergic agonist) involved stimulation of lipolysis in adipose tissue, which resulted in free fatty acid release and oxidation. This lipid mobilization would therefore supply energy required for increased accretion of lean tissue. In addition, they suggested that decreased muscle protein degradation and/or increased synthesis was responsible for muscle hypertrophy. This catalogue of hypotheses has been neither confirmed nor refuted (Buttery and Dawson, 1987; Kim and Sainz, 1992). In all likelihood, the changes proposed by Ricks *et al.* (1984) occur in response to all types of metabolic modifiers, differing only in relative importance. Experiments using various drugs or hormones, designed to address questions about altered protein or fat turnover, have provided conflicting or inconclusive results. Accordingly, a modeling exercise was undertaken with the following objectives:

1. Integration of existing concepts and data regarding animal growth into a consistent mathematical framework;
2. Evaluation of those same concepts and data for adequacy in describing the growth and body composition of the sheep;
3. Evaluation of alternative hypotheses for probable adequacy in explaining the effects of metabolic modifiers on animal composition.

Due to the greater amount of experimental data available regarding sheep, this species was chosen for the study. As stated previously, the general model of tissue growth developed by Baldwin and Black (1979) confirmed the importance of organ DNA content, DNA unit size and metabolic activity in the control of tissue growth patterns. These concepts were subsequently simplified and applied in models of rat (Oltjen *et al.*, 1985) and steer growth (Oltjen *et al.*, 1986a,b). These models employed very simplistic representations of nutrient entry and utilization and could not be used to satisfy the above listed objectives. Gill *et al.* (1984) developed a model of lamb metabolism to examine effects of varying proportions of nutrients on efficiency of energy utilization. The model was formulated using mechanistic equations representing intermediary metabolism of absorbed nutrients, but empirical relationships were used to define deposition of body protein and fat. This model has been used successfully to examine mechanisms responsible for alterations in energetic efficiency (Black *et al.*, 1987a,b).

The model developed by Gill *et al.* (1984) was considered for this exercise, i.e. evaluate alternative hypotheses regarding mechanisms responsible for effects of growth promotants on lamb growth and body composition. However, our objectives required explicit representations of body fat and protein turnover, as these were the most likely candidates for alteration by anabolic agents. Therefore, we decided to develop a model of lamb metabolism and growth that satisfied the following criteria: simulation of lamb growth from 20 to 40 kg EBW under varying nutritional conditions; representation of fundamental processes associated with growth and metabolism aggregated to the minimal number of necessary pools; and integration of current knowledge of biochemical transactions and metabolic regulation to the whole-animal level.

15.3.1 Model description

(a) General model structure

The structure of the sheep model (BETA) is depicted in Fig. 15.8 and symbols for each variable are given in Table 15.4. The model incorporates less detail than does the lactating cow model presented in Chapter 16. State variables include circulating amino acids (Aa), glucose (Gl), acetate (Ac) and lipids (aggregated as triacylglycerol, Tg). Four protein pools, corresponding to carcass (Pb), viscera (Pv, including all abdominal and thoracic organs),

Table 15.4 Symbols used for quantities in the sheep model designated as BETA in Fig. 15.8

Symbol	Entity
Aa	amino acids
Ac	acetic acid
Ad	ADP
Am	ammonia
At	ATP
Bu	butyric acid
Cd	carbon dioxide
Gl	glucose
Gy	glycerol
La	lactic acid
Ox	oxygen
Pr	propionic acid
Tg	triacylglycerol
Ts	tripalmitin
Tp	triose phosphates
Ur	urea

DNA in:

Db	carcass
Dv	viscera
Dz	other tissues

Protein in (Px):

Pb	carcass
Pv	viscera
Pz	other tissues
Pw	wool

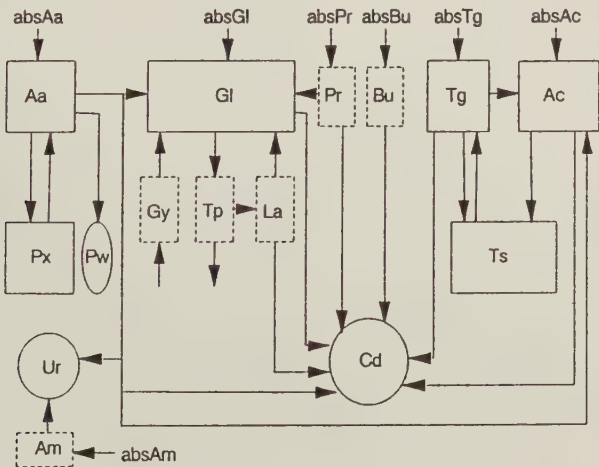


Figure 15.8 Block diagram showing the structure of lamb growth and metabolism model (BETA). Codes are given in Table 15.4.

other tissues (Pz, comprising skin, head and feet) and wool (Pw), are included along with associated DNA pools (Db, Dv and Dz, respectively). Storage fat is represented as tripalmitin (Ts). In addition, several zero pools, in which utilization balances formation at each iteration, are included to maintain stoichiometric balance. These are propionate (Pr), butyrate (Bu), glycerol (Gy), triose phosphates (Tp), lactate (La), ATP (At) and ADP (Ad). These pools do not require differential equations due to their much faster response times relative to other model variables. Changes in the state of the model are calculated as differences between inputs to and outputs from each pool at each iteration.

Equation forms used in BETA to represent rates are either mass action or Michaelis-Menten type, modified to include effects of hormones, metabolic protein mass and multiple substrates or effectors, as in equations 3.20, 3.23, 3.24 and 3.26. These are similar to conventions used in MOLLY (Chapter 16). Several differences between BETA and MOLLY deserve mention. These include the absence in BETA of a rumen and mammary glands, and the growth of wool. In addition, there are differences in equations representing protein synthesis and triacylglycerol turnover, and inclusion of provision for endogenous acetate production. As in MOLLY, maximum velocities for protein synthesis are represented in BETA as a function of tissue DNA contents. Tissue protein rather than empty body weight (raised to an exponent lower than one) is a scalar for V_{AaPx} . This modification was necessary to accommodate the increase in absolute rates as well as the decline in fractional rates of protein synthesis during growth. In MOLLY circulating lipids (Fa) inhibit lipolysis in adipose tissue but have no effect on lipogenesis from acetate; the exact opposite is true in BETA. Another difference between BETA and MOLLY is provision in BETA for entry into the circulation of endogenous acetate derived from incomplete β -oxidation of fatty acids. No endogenous acetate entry was represented in the original model, but this resulted in extremely unstable solutions, particularly at low rates of nutrient entry. Under these conditions, the acetate pool tended toward zero and below. This failure of the model might have been due to the difficulty of solving 'stiff' differential equations, since the acetate pool turns over much more rapidly than, say, the glucose or amino acid pools. This difficulty was overcome by (1) reducing the integration interval to minutes rather than days or (2) inflating the size of the acetate pool by 100-fold (see France and Thornley, 1984). These measures did not stabilize the acetate pool. This was considered to be evidence of the importance of endogenous acetate to homeostatic regulation, justifying inclusion of additional elements. For further details on model construction, parameterization and evaluation the reader is referred to the original publications (Sainz and Wolff, 1990a,b).

(b) Behavior

Patterns of live-weight gain and body composition produced by BETA using

reference inputs showed that the model adequately described normal growth and body composition between 20 and 40 kg empty body weight. This was hardly surprising, since reference parameter values were determined in part from normal growth patterns as described by the Agricultural Research Council (ARC, 1980). Patterns of energy expenditure and deposition of body components, however, were not derived directly from input:output experiments. Instead, they were deduced from estimated nutrient fluxes along with appropriate thermodynamic considerations. Therefore, it was of interest to compare predicted responses to dietary conditions (i.e. maintenance requirements, efficiency of energy utilization for growth, nitrogen balance and body composition) with published estimates under a variety of conditions.

An energy balance experiment was simulated using inputs selected to represent nutrient absorption rates by lambs fed diets varying in forage and concentrate content (Black *et al.*, 1987a,b). Responses to ME intake were linear from one-half to twice maintenance (Sainz and Wolff, 1990a). Predicted efficiencies for growth (k_f) were similar to those of Black *et al.* (1987a,b), but higher than values given by the ARC (1980). Black *et al.* (1987a,b) found that the predicted value of k_f was sensitive to the activity of a postulated ATP-degrading pathway, presumably related to a combination of substrate cycles. This hypothesis has not been rigorously tested *in vivo*. Clearly, uncertainties regarding stoichiometric costs associated with metabolic pathways (e.g. protein degradation) limit the accuracy of quantitative estimates of energy expenditures based on metabolic fluxes.

BETA was also required to accommodate effects of different patterns of volatile fatty acid (VFA) absorption. The data of Orskov *et al.* (1979), from lambs sustained by intraruminal VFA and intragastric casein infusions, overcame uncertainties regarding nutrient entry rates and protein deposition. Those data clearly showed that nitrogen (N) retention decreased in response to increasing acetate:propionate ratio at maintenance and twice maintenance. The response curves produced by BETA were within experimental error of actual estimates at both energy infusion levels (Sainz and Wolff, 1990a). More importantly, BETA was able to accommodate interactions among VFA, glucose and amino acids, and produce an appropriate integrated response (i.e. nitrogen balance). Orskov *et al.* (1979) suggested that propionate spared protein catabolism by acting as a carbohydrate precursor. That hypothesis was supported by the results of this simulation (Sainz and Wolff, 1990a).

In other simulations, BETA exhibited appropriate responses to additional amino acid supply (through infusion or feeding of fish meal). Body protein content increased with level of dietary protein, while the amounts of body fat decreased, in agreement with experimental data (Sainz and Wolff, 1990a). This result was accounted for by the increase in lean tissue deposition, with a concomitant rise in energy expenditure per unit body weight, both of which reduced the amount of energy available for fat deposition.

This conclusion is consistent with the observations of Pullar and Webster (1977), that energy expenditure is a function of lean body mass in Zucker rats, and of Berg and Butterfield (1976), that fat deposition depends on the amount of energy available after supplying the needs for maintenance and lean growth.

BETA was developed in order to evaluate hypotheses about the mode of action of metabolic modifiers. To accomplish this, the model was required to simulate lamb growth from 20 to 40 kg empty body weight under a variety of nutritional conditions; to represent metabolic transformations and their regulation in a simplified but realistic manner; and to accommodate interactions among nutrients, giving reasonable predictions of growth patterns, energy utilization and body composition. BETA performed adequately in these respects and was deemed to be suitable for examination of metabolic changes involved in growth manipulation.

15.3.2 Hypothesis evaluation

In order to evaluate the relative roles of changes in protein or lipid metabolism in growth responses to metabolic modifiers, data sets representing three types of compounds (anabolic steroids, growth hormone (GH) and β -adrenergic agonists) were selected for comparison with BETA predictions. The study by Muir *et al.* (1983) described responses of lambs to diethylstilbestrol (DES, a synthetic estrogen) and to ovine growth hormone (GH). Data from two trials conducted at the Ruakura Animal Research Station in New Zealand using cimaterol were used as representative of responses to β -adrenergic agonists (Sainz and Wolff, 1990b). Data from these studies are summarized in Table 15.5. For each simulation, the model was calibrated so that the reference state corresponded to the growth rate and composition of the experimental controls (Dent and Blackie, 1979). Then, likely impacts upon protein and lipid metabolism were assessed. The available slaughter data provided estimates of protein and fat accretion only. Thus, the data could be used to define parameters of anabolic or catabolic equations but not both. Probable changes were assigned to degradative pathways for simplicity, but synthesis could have been used equally well. Also in the interests of brevity and clarity, the first-order degradation coefficients for carcass protein and fat are referred to in Table 15.6 as K_{PROTEIN} and K_{FAT} , respectively, and are expressed as percentages of reference values.

Once the reference (or control) states were set for each (simulated) experiment, K_{PROTEIN} and K_{FAT} were varied within wide ranges (50–200% of reference) to find the vicinity of the best fit for each of the simulations. Once the region of the minimum was identified, K_{PROTEIN} and K_{FAT} were successively varied by proportional increments of 1.0% within this region. Goodness-of-fit was assessed by calculation of residual sums of squares for carcass protein (R_P) and carcass fat (R_F). Residuals (R) are generally calcu-

Table 15.5 Effects of cimaterol, diethylstilbestrol and ovine growth hormone on growth and carcass protein and fat (kg) in lambs^a

Study	Group	Body weight (kg)	Carcass weight (kg)	Carcass	
				protein (kg)	fat (kg)
Cimaterol ^b (grazing)	initial	24.4	14.4	2.25	3.14
	control	40.3	19.7	3.03	5.42
	treated	42.0	22.4	3.89	4.45
	SE			0.29	0.92
Cimaterol (grain-fed)	initial	28.6	13.0	2.25	2.53
	control	40.2	20.4	2.97	7.00
	treated	41.8	23.1	3.67	6.63
	SE			0.36	1.10
Muir <i>et al.</i> (1983)	initial	27.9	13.1	2.36	2.62
	control	42.9	26.0	4.02	6.49
	DES ^c	45.2	27.7	4.42	6.35
	oGH ^c	43.5	26.4	4.28	5.91

^aAdapted from Sainz and Wolff (1990b).

^bCimaterol dose: 1.8 mg/day.

^cDES, diethylstilbestrol (0.1 mg/day); oGH, ovine growth hormone (7.0 mg/day).

Table 15.6 Effects of cimaterol (Cim), diethylstilbestrol (DES) and ovine growth hormone (oGH) on parameters of protein and fat degradation in the sheep model designated as BETA^a

Simulation/treatment	K _{PROTEIN}		K _{FAT}	
	estimate	SD	estimate	SD
Cim (grazing)	86	2.5	120	5.0
Cim (grain-fed)	87	3.2	111	11.5
DES	94	2.4	133	11.1
oGH	97	2.5	177	13.9

^aTable adapted from Sainz and Wolff (1990b); values expressed as percent of control (reference). See Table 15.5 and text for experimental and simulation details.

lated based on experimental replicates, where P is the response predicted by the model and O_i is the i th observed value:

$$R = \sum (P - O_i)^2 \quad (15.12)$$

When individual observations are not available, a residual may be calculated from group means and variances, as in

$$R = nP^2 + n\bar{O}^2 - 2nP\bar{O} + s^2(n-1) \quad (15.13)$$

where n is the number of observations and $\bar{O}$ and s^2 are the experimental mean and variance. It can be shown that equations 15.12 and 15.13 are equivalent. This allows calculation of the variability of estimates of parameter values

$$V = \mathbf{G}_{ij} R'/(n-p) \quad (15.14)$$

where V is the variance about the estimate, $\mathbf{G}$ is the inverse of the Hessian matrix and p is the number of fitted parameters (France and Thornley, 1984). R_P and R_F were calculated as in (15.13), using group means and variances obtained from the original reports. R_P and R_F were summed to give total residual sums of squares (R_{TOTAL}). Optimal values of K_{PROTEIN} and K_{FAT} were found that produced a minimum value of R_{TOTAL} for each simulation. These estimates were compared with reference values using Student's t -test.

This modeling exercise was designed to evaluate mechanisms that could account for the effects of growth-promoting compounds on carcass composition of lambs. Apparent changes in parameters of equations representing protein and fat turnover were estimated by least-squares optimization. These values, expressed as percentages of the respective reference values, are given in Table 15.6. These analyses do not constitute validation of any given hypothesis, but rather indicate the magnitude of postulated changes that would be adequate to account for the experimental observations. Alternative hypotheses could certainly be formulated, which might upon testing provide better fits than those considered here. In this respect, the solutions presented here are not unique. This study, however, was concerned with testing the hypotheses proposed by Ricks *et al.* (1984), therefore only changes in protein and fat metabolism were examined.

Cimaterol reduced K_{PROTEIN} by 13% in both grazing and concentrate-fed lambs; however, K_{FAT} was significantly increased only in grazing lambs (Table 15.6). DES increased K_{FAT} 33%, while its effect on K_{PROTEIN} was smaller than that of cimaterol. GH treatment produced the greatest change in K_{FAT} (+ 77%), but in contrast to the estrogen and β -agonist, GH did not significantly affect K_{PROTEIN} ($P > 0.05$). These results are consistent with some but not all experimental observations regarding the effects of metabolic modifiers on metabolic fluxes (i.e. protein and lipid turnover). The importance attached to these discrepancies depends upon the relationship between changes in parameter values and predicted metabolic fluxes.

The estimated effects of metabolic modifiers on equation parameters cannot be translated directly into equivalent changes in metabolic fluxes. This is because actual flux rates are determined by substrate supply, physiological/endocrine state and tissue mass, as well as enzymatic capacities and affinities. For example, in simulation 1, cimaterol apparently decreased K_{PROTEIN} (i.e. fractional protein degradation rate), but absolute degradation rate was increased due to greater protein mass (Table 15.7). No effect on protein synthesis was explicitly represented, yet protein synthesis rate was indirectly affected: absolute synthesis rate was 16% higher than reference, while fractional synthesis rate was 9% lower. These results illustrate the difficulty of relating experimental estimates, e.g. of isotope incorporation rates, to metabolic control mechanisms. In addition, isotope incorporation methods are also compromised by uncertainties regarding the appropriate precursor pools (Barnes *et al.*, 1992). These difficulties must be borne in mind when comparing the results of modeling analyses such as this one against experimental observations.

The most cogent statement of hypotheses regarding changes in protein and fat metabolism due to metabolic modifiers was provided by Ricks *et al.* (1984). As mentioned above, many studies have been carried out for the purpose of identifying the mechanisms by which these compounds stimulate deposition of lean tissue. Studies with β adrenergic agonists have shown both increases and decreases in rates of muscle protein synthesis (see Kim and Sainz, 1992). In contrast, effects of β -agonists on lipid metabolism (increased lipolysis and decreased lipogenesis; Dunshea, 1993) are fairly consistent and in accord with the modeling analyses shown here (Table 15.7). Similar lipolytic and antilipogenic effects have been demonstrated for GH (Dunshea, 1993). GH also has consistently been shown to increase rates of protein synthesis with no change in the rate of breakdown of muscle proteins (Boyd *et al.*, 1991). It is worth noting that in the modeling analysis, GH produced an apparent increase in rate of protein synthesis, even though there was no change in the relevant equations. Studies of estrogen treatment and protein metabolism in sheep have failed to demonstrate an increase in synthetic rate (Bohorov *et al.*, 1987), and in fact implicate protein degradation in the anabolic effect. In light of the difficulties described above, the lack of consistent results among experiments is not surprising. Clearly, the multiplicity of interactions among physiological, endocrine and nutritional state that result in a particular flux through a metabolic pathway preclude simple (and simplistic) interpretations of these rates.

An unexpected result of this analysis was the great difference in the sensitivity of body composition to parameters associated with protein and fat metabolism. This difference is illustrated by the original cimaterol data, in which the reduction of carcass fat gain observed in grazing lambs was abolished in concentrate-fed lambs. This interaction could not be explained

Table 15.7 Effects of hypothetical changes in protein and fat degradation constants on rates of carcass protein and fat turnover^a

Simulation/group	Protein				Lipolysis	Lipogenesis
	degradation		synthesis			
	day ⁻¹	mole/day	day ⁻¹	mole/day		
1. Control	0.0400	1.005	0.0436	1.097	86.4	101.3
Cim ^b	0.0344	1.102	0.0396	1.269	123.0	86.3
2. Control	0.0400	1.013	0.0457	1.157	75.2	194.0
Cim ^b	0.0348	1.063	0.0428	1.306	92.8	206.5
3. Control	0.0400	1.044	0.0452	1.180	80.4	185.0
DES ^b	0.0376	1.081	0.0441	1.268	108.8	190.3
4. Control	0.0400	1.044	0.0452	1.180	80.4	185.0
oGH ^b	0.0388	1.083	0.0450	1.257	134.4	182.2

^aTable adapted from Sainz and Wolff (1990b).
^bCIM, cimaterol; DES, diethylstilbestrol; oGH, ovine growth hormone.

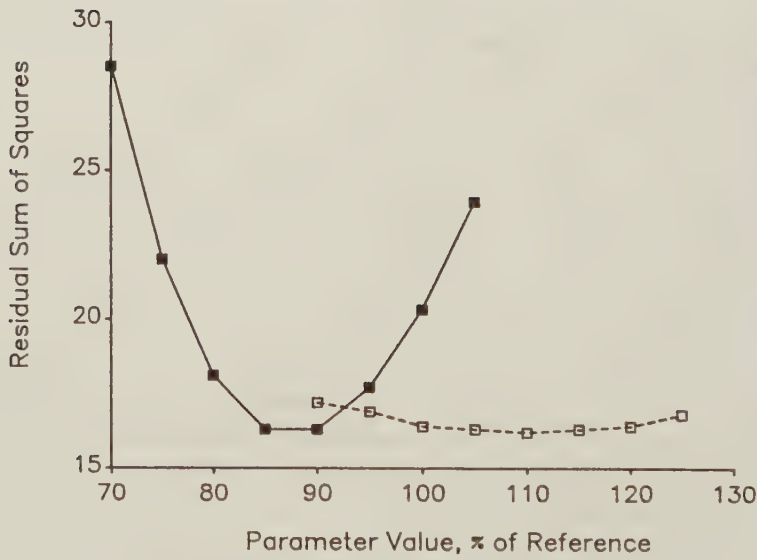


Figure 15.9 Effect of proportional changes in K_{PROTEIN} and K_{FAT} on residual error of model predictions; K_{PROTEIN} (■) and K_{FAT} (□) were varied individually while holding the other parameter at its optimal value.

entirely by differences in diet composition, but was clearly related to energy intake (data not shown). Composition of gain was far less sensitive to parameters of fat metabolism than to those of protein turnover. Indeed, standard errors of estimates of K_{FAT} were two to five times greater than those of K_{PROTEIN} . The lower sensitivity of body composition to changes in fat metabolism is illustrated by Fig. 15.9, which depicts the residual error in simulation 2 versus K_{PROTEIN} and K_{FAT} . These plots show that residual error has a more clearly defined minimum with respect to K_{PROTEIN} than for K_{FAT} . The causes of these differences can be discerned in Table 15.7; note that lipolysis was increased in all simulations. In simulation 1, lipogenesis was decreased (due to low glucose and high fatty acid concentrations) thus markedly reducing fat deposition. In simulations 2 and 3, glucose (and anabolic hormone) concentrations remained high, thus attenuating the increase in lipolysis, lowering fatty acid levels and maintaining lipogenic rates. As a consequence, the response of fat deposition was diminished. Finally, in simulation 4 lipolysis (and fatty acid levels) were increased to such a degree as to prevent the increase in lipogenesis even in the presence of high glucose concentrations. Clearly, interactions among nutrients play a significant role in determining patterns of protein and fat accretion.

The results presented here support the concept that adipose tissue is primarily a storage depot for excess energy. As summarized by Berg and Butterfield (1976), energy surplus to the requirements for maintenance and growth of bone and muscle is deposited as fat. Although this concept is widely accepted, its qualitative nature precludes estimation, for example, of the increase in protein deposition that one might expect from a given amount of energy-yielding substrates released from adipose tissue. The modeling exercise presented differs fundamentally from the simple and empirical 'hydraulic' model of Berg and Butterfield (1976) by incorporating a wide range of biochemical concepts and data. It is therefore encouraging that this metabolically based model yields conclusions similar to those of earlier workers based on extensive empirical observations.

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Lactation

The flow of information downward from whole animal models to tissue and cellular models in the form of constraints and regulation, and the upward flow of information and insight gained in tissue and cellular models to whole animal models serves to facilitate advancement of knowledge and understanding of complex animal systems and identification of areas requiring experimental analysis.

Smith (1970)

16.1 INTRODUCTION

It was noted earlier (Chapters 3 and 4) that Smith (1970) formulated a dynamic model of the metabolism of a lactating cow using the KINSYM modeling language. The model was based solely on mass action equations, was very large and unwieldy, and was unstable, thus it required excessive solution times. Nevertheless, the model was based on a comprehensive analysis of data available at that time (summarized in Chapter 3) and incorporated many relevant concepts regarding ruminant metabolism. Deficiencies in our knowledge of ruminant adipose tissue, mammary gland and liver metabolism were identified as a result of Smith's analysis. Experimentation inspired by this modeling study and the resultant development of detailed models of metabolism in these three tissues were discussed in Chapters 12–14. These models remain imperfect but are providing bases for the identification, design, and interpretation of critical experiments and formulation of less detailed representations of lactating cow metabolism. In this, we were implementing the hierarchical concept of model development, which holds that detailed models of function at one level of organization (e.g. tissue) should provide the bases for construction of models of higher level (e.g. organism) functions. Improvement of the lactating cow model has occurred in parallel with emphases on reducing complexity and computing requirements, improving stability and enhancing the conceptual bases underlying the model.

The basic strategy utilized by Smith (1970) in representing the metabolism of a nutrient in a given tissue is depicted in Fig. 16.1. In the (KINSYM) modeling language, the metabolism of each nutrient utilized by a tissue

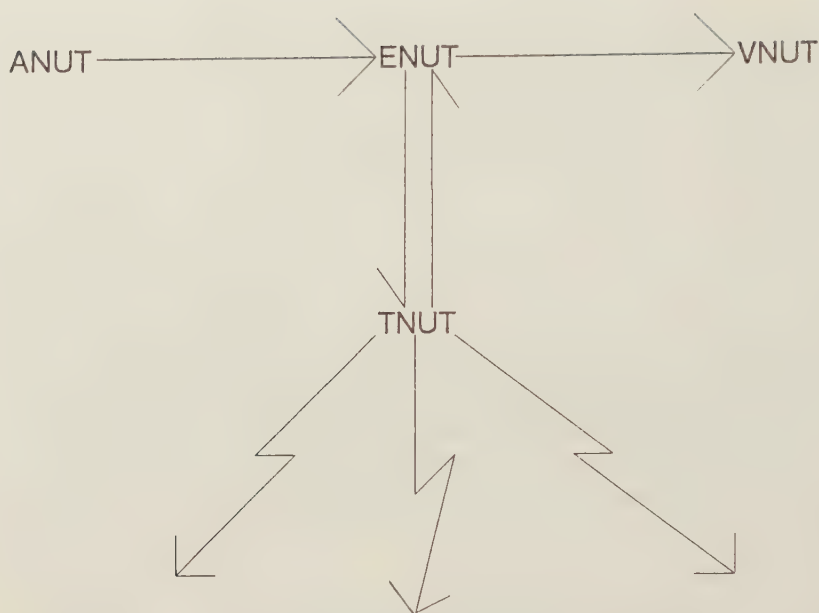


Figure 16.1 Smith model. Codes for abbreviations are arterial substrate concentration (ANUT), extracellular substrate concentration (ENUT), venous substrate concentration (VNUT) and tissue substrate concentration (TNUT).

required estimates of the arterial and venous, extracellular and intracellular pool sizes of each nutrient, blood flow rate to each tissue, and energy expenditure in each tissue. Several problems should be evident:

1. Blood flow estimates vary depending upon tissue energy requirements and this was not accommodated.
2. Tissue and extracellular concentrations of nutrients are variable and, largely, unknown.
3. Basic data available from arteriovenous (A-V) difference and radio-isotope tracer experiments can uniquely define only two or three rather than the required five or six rate constants per nutrient.
4. Provisions for changes over time in rate constants specified for tissues could not be defined and, thus, were not included. Chronic effects of hormones, growth, environment, etc. upon tissue metabolic rates and patterns could not be simulated as would be appropriate throughout a lactation cycle.
5. The minimum number of differential equations required for each tissue were four times the number of nutrients used by that tissue (usually 3–4) plus equations associated with intermediary metabolism summing to a minimum of 15 differential equations per tissue. In addition to the problems with parameterization noted above, large numbers of equations led

to high computation costs, even on today's computers. Turnover rates of intracellular metabolites are very high; up to 50–100 000 per day. In order to avoid unstable solutions, very short integration intervals were required (5×10^{-5} /day), with a corresponding increase in computation requirements.

This was clearly an overparameterized model constructed by experimental scientists with limited modeling experience. As a result of this experience, we identified research needs and gained essential insight into the modeling process. In essence this was a learning rather than a research experience, even though we intended the latter.

Several dynamic, mechanistic models of digestion and metabolism in lactating cows have been reported subsequently. These include the studies of Koong and Lucas (1973), Baldwin *et al.* (1987a–c) and Danfaer (1990). Of these, the model of Koong *et al.* (1982) incorporates the greatest number of empirical elements and the least number of representations of specific biochemical and physiological mechanisms. The model of Danfaer (1990) incorporates the greatest number of mechanistic equations and metabolic and physiological detail. All of these authors clearly stated that their models were research models, imperfect with regard to rigorous predictions of input:output relationships. Also, a great deal of additional modeling and experimental research would be required to adequately simulate the metabolism of lactating cows. On the other hand, evaluations of the several models have shown that dynamic, mechanistic models have several properties that improve their utility relative to static, factorial, empirical models. Most importantly, these models enhance our ability to anticipate effects of previous and current planes of nutrition upon current and future performance and have explanatory power that has helped advance our understanding of ruminant digestion and metabolism. We are not going to discuss the models of Koong *et al.* (1982) and Danfaer (1990) or other models in this chapter. Rather we will discuss a current version of a lactating cow model that evolved from the Smith (1970) model, through the Baldwin *et al.* (1987a–c) model and to the present. Our purposes are to illustrate how research models evolve as a combined experimental and modeling research program progresses. This could be considered a penultimate chapter in the sense that the model presented is the result of modeling and experimental contributions of the many workers and studies summarized in previous chapters of the book. On the other hand, penultimate is much too strong a word as this model does not represent a final integration of current knowledge of ruminant digestion and metabolism. It can and has been used to evaluate hypotheses and predict probable outcomes of possible interventions – genetic, pharmaceutical, nutritional. These evaluations certainly surpass in value the qualitative, intuitive evaluations of 'experts' available at the onset of model development and those available now, even though the

knowledge base available to 'experts' has advanced considerably. As stated by Forrester (1971),

The objective of modeling is to combine the power of the human mind with the power of computers. Only the human mind can formulate a structure or a concept to which data can be fitted. However, the human mind is limited in its ability to analyze or anticipate the quantitative and dynamic behavior of the system described. The computer is ideal for the latter function. ...the computer can trace the behavior of the system through time in a rigorous fashion and thus provide correct – unbiased – implications of the assumptions, concepts, etc. that comprise the model.

In this context, the current model is certainly not penultimate. It represents a flawed attempt to fully utilize the combined powers of the human mind and computers in advancing our understanding of ruminants. It remains a research model in evolution and a transient stage in our continuing efforts to advance our understanding of ruminant digestion and metabolism and fully utilize this knowledge in the resolution of problems facing animal agriculture.

16.2 BACKGROUND ON MOLLY.CSL

Due to early constraints imposed by programming languages, e.g. the maximum of six-letter words in Fortran, most modelers employ acronyms or arbitrary names for entities within models. This habit is not restricted to computer specialists, however. Biochemists, physiologists, nutritionists, medical practitioners etc., also communicate in one or another form of mnemonics, shorthand or slang; perhaps this is a human trait that should not be blamed on programming languages. In models discussed in earlier chapters, many conventional abbreviations and mnemonics were used to identify state variables and metabolic transactions. In contrast, we have been fairly arbitrary, or perhaps just obscure, in naming our models. Well, not completely arbitrary – our model for the growth of male mice was named MICKEY; and our steer growth model was named SAM after a seemingly asexual character in a science fiction novel popular at that time. The first version of the current model of a lactating cow was named COW1 (Baldwin *et al.*, 1987a); how dull. When the digestion (Baldwin *et al.*, 1987c) and metabolism models were joined we named the result MYRTLE. My father once named a heifer calf Myrtle to honor a neighbor woman, but she was offended and my father had to change the calf's name. This woman did not like children and did not give out candy on Halloween, so one might say my name selection for the model was a bit of revenge. When a version of the model with inflated pool sizes (see below) was written to simulate full lactations, the model was named DAISY to indicate a 1-day integration inter-

val. When diskettes with coding for MYRTLE and DAISY were transported home from the UK after my sabbatical, the MYRTLE diskette was scrambled. So as not to tempt fate further, DAISY became the parent version and remained so until early 1992. Over the 6 year period during which DAISY reigned, many piecemeal changes were introduced and the flow of biological logic became disjointed, e.g. DAISY got old; after all, most cows are culled before they complete six lactation cycles. Therefore, the program was reorganized, corrected and reformatted to form MOLLY, named for the very docile, patient cow to whom my father assigned the task of teaching me to milk by hand when I was 8 or 9 years old. Perhaps MOLLY will provide me and associates with a continuing opportunity to learn.

In contrast with the more or less mathematical format used to present models and equations in previous chapters, MOLLY will be presented in ACSL format in this chapter. Statements enclosed by quotes are comments ignored by ACSL, which we include during model development to record our reasoning, calculations, sources, etc. The comments in actual program MOLLY have been edited somewhat to delete slang, identify origins of numbers and add clarity. In ACSL format, each line of comment in a program must start and end with quote marks. When a comment is several lines long, the quote marks on each line are annoying. Therefore, we have eliminated the quote marks within comment blocks leaving only the initial (") and final (") quotes to identify comments. Commentary that was added to extend comments in the program *per se* is presented in italics.

16.2.1 Explanation of coding and identification of state variables

Most entities within the model are identified by a two-letter code, for example, Aa codes for amino acids. A notable exception is that the major body components are identified by single letters – B identifies body or carcass; V identifies viscera and its subcomponents including liver, udder, gastrointestinal tract, kidneys and heart; and F identifies adipose tissue. Transactions are identified as substrate code, product code and body site in which the reaction occurs, e.g. gluconeogenesis from amino acids in viscera (liver) is coded as AaGIV. Kinetic parameters for transactions are identified by prefixes and the transaction code, for example, KAaGIV identifies the affinity constant or K_s for amino acids and VAaGIV the metabolic capacity or $V_{\max}$ for gluconeogenesis from amino acids. Anabolic (AHOR) and catabolic (CHOR) hormones can act as modifiers of either $V_{\max}$ or K_s .

A number of additional prefixes and suffixes are used to identify terms associated with each state variable. These are listed as associated terms in the list of state variables below and include (using Aa as an example):

Aacor	correction factor used to expand pool size of Aa such that integration interval can be increased, for example, to 0.5 day for full lactation simulations (unitless; see Chapter 4)
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CAa	concentration of Aa in plasma (mole/l)
DAa	differential equation for Aa (mole/day)
HCAa	heat of combustion of Aa (Mcal/mole) – when the factor F1 is set to 4.184 (default value in model) values are in MJ/mole. Sometimes HXx is used instead of HCXx
IAa	initial pool size of Aa (mole)
IAaF	factor used to compute IAa based upon body weight (mole/kg)
ICAa	initial concentration of Aa
IVAa	initial volume of distribution of Aa (l)
IVAaF	factor used to compute IVAa from body weight (l/kg)
VAA	volume of distribution of Aa (l)

STATE VARIABLES

Aa	plasma amino acids (mole); associated terms: Aacor, CAa, DAa, HCAa, ICAa, IAaF, IVAa, IVAaF, MWAA, VAA
abEav	absorbed energy average – a rolling average which influences activity of the $\text{Na}^+ \text{K}^+ \text{-ATPase}$ (Mcal, MJ); associated terms: IabEav, DabEav
Ac	plasma acetate (mole); associated terms: Accor, CAC, DAC, HCAC, IAC, IACF, ICAC, IVAC, IVACF, MWAC, VAC
Am	rumen ammonia (mole); associated terms: Amcor, CAM, IAm, IAmF, ICAM, MWAM
As	soluble ash (kg); associated terms: Ascor, CAs, DAs, IAs, IAsF, MWAs, FDAs
Ce	cellulose in small particle pool in rumen; associated terms: Cedor, DCE, HCCe, ICE, ICEF, MWCE
Cs	soluble carbohydrate pool in rumen (mole); associated terms: Cscor, CCs, DCs, ICs, ICsF, MWCS, FDCs (FDOa, FDPe, FDCs)
Fa	plasma lipids, includes non-esterified fatty acids (NEFA) plus triacylglycerol (mole); associated terms: Facor, CFa, DFa, HCFa, IFa, IFaF, IVFA, IVFAF, MWFA, VFA
Fl	long-chain fatty acids in digestive tract (mole); associated terms: Flcor, CFL, DFL, HCFL, IFL, IFIF, MWFL
Gl	plasma glucose (mole); associated terms: Glcor, CGI, DGI, HCGI, IGI, IGIF, IVGI, IVGIF, MWGI, VGI
Ha	α -linked hexose polymers (starch) in rumen small particle pool (kg); associated terms: Hacor, DHa, HCST, IHa, IHaF, MWST
HaMi	microbes associated with starch in small particle pool (kg); associated terms: DHaMi, IHaMi, IHaMiF, HaMicr
Hb	β -linked hexose polymers (holocellulose) in rumen (kg); associated terms: Hbcor, DHb, IHb, IHbF

Hc	hemicellulose in rumen small particle pool; associated terms: Hccor, DHc, HCHc, IHc, IHcF, MWCe
HbMi	microbes associated with Hb (kg); associated terms: DHbMi, IHbMi, IHbMiF, HbMicr
LHOR	lactation hormones which enhance synthesis of udder enzymes (kg); associated terms: DLHOR, ILHOR, BST
Lp	large particles in rumen (kg); associated terms: Lpcor, DLp, ILp, ILpF
Mi	microbes in rumen (kg); associated terms: Micor, DMi, IMi, IMiF, HCMi, LpMi, SpMi, WAMi
MLKave	milk production rolling average over past two weeks (kg/day); associated terms: DMLKav, IMLKav
Ot	'other', insoluble ash plus lignin (kg); associated terms: Otcor, DOT, IOT, IOTF
Pb	protein in body (mole); associated terms: DPb, CPb, HCPx, MWPx, IPb, IPbF
Pi	insoluble protein in rumen (kg); associated terms: Picor, DPI, IPi, IPiF, HCPx, MWPx
PUN	plasma urea (mole); associated terms: PUNcor, DPUN, CPUN, IPUN, IPUNF, IVPUN, IVPUNF, PUNVOL, MWUR, HUR
Pv	protein in viscera (mole); associated terms: DPv, CPv, HCPx, MWPx, IPv, IPvF
RAa	rumen amino acids (mole); associated terms: RAacor, DRAa, IRAa, IRAaF, MWRAa
RAC	rumen acetate (mole); associated terms: RAccor, DRAC, IRAC, IRACF, MWAc, HAc
RBu	rumen butyrate (mole); associated terms: RBucor, DRBu, IRBu, IRBuF, MWBu, HBU
RLa	rumen lactate (mole); associated terms: ULacor, DRLa, IRLa, IRLaF, MWLa, HLa
RLv	rumen liquid volume (l); associated terms: RLvcor, DRLv, IRLv, IRLvF
RPr	rumen propionate (mole); associated terms: RPracor, DRPr, IRPr, IRPrF, MWPr, HPr
TDMIN	total dry matter intake (kg); associated terms: DDMIN
TMLKLm	total lactose production (kg); associated terms: DMLKLm
TMLKPm	total milk protein production (kg); associated terms: DMLKPm
TMLKTm	total milk fat production (kg); associated terms: DMLKTm
TsF	triacylglycerol in adipose tissue (mole); associated terms: DTsF, CTs, ITsF, ITsFF, MWTsF, HCTg

UENZ	udder enzymes (arbitrary units); associated terms: DUENZ, IUENZ
ULm	udder lactose (kg); associated terms: DULm
UMave	milk in udder – rolling average (kg); associated terms: DUMave, IUMave
UPm	udder milk protein (kg); associated terms: DUPm
UTm	udder milk fat (kg); associated terms: DUTm
VAcTsF	$V_{\max}$ for fatty acid synthesis from acetate in adipose tissue (mole/day); associated terms: DVAcTsF, IVAcTs

16.3 THE PROGRAM MOLLY.CSL

'This is an aggregated version of the 550 kg cow described by Smith (1970) and Baldwin and Smith (1971a,b). This version was developed to simulate and analyze overall energy transactions, within day patterns of nutrient use, longer term day-to-day patterns of nutrient use throughout a lactation or growth cycle and as an aid in the design of energy balance experiments.'

'The body weight of the reference cow used to parameterize the model was 550 kg. Empty body weight (EBW) of the initial reference cow was 500 kg; lean body mass (wtB) was 350 kg and includes skin, brain, kidney, muscle, skeleton and minor tissues; adipose tissue (wtF) was 75 kg and comprised of triacylglyceride (Ts = 60 kg) and cytoplasmic elements (wtcytF = 15 kg). Visceral weight (wtV) was 75 kg and included blood, gut, liver, heart and udder. Note that wtB and wtF would be expected to decrease in early lactation, while wtV would increase. The default initial empty body weight (IEBW) has now been changed to 650 kg with corresponding (linear) changes in weights of B, V and F.'

'Nutrient inputs were calculated for a 50:50 forage/concentrate ration and are continuous in the reference (default) state. Milk production was set at 30 kg/day and energy balance to zero. Milk was 3.5, 4.8 and 3.3%, respectively, of fat (expressed as tripalmitin and equivalent to 3.7% of true milk fat), lactose and protein. Input:output comments prior to each subsection of the model refer to this specific feeding/production condition.'

'This version has provisions that accommodate:

1. Different feeding frequencies, e.g. one or more meals per day or continuous feeding;
2. A wide range of diets specified as input by users;
3. Alternative feeding strategies, including specification of amounts of feed offered and conditional changes in rations offered;
4. Provisions for abomasal infusion of casein and BST and T3.'

'The default for this version is set for mid-lactation within day simulations. For long-term simulations, run overlays to increase pool sizes and reset mammary parameters are required.'

16.3.1 Initial section

INITIAL \$ 'section of model'

'BASIC UNITS

TIME IN DAYS

POOL SIZES IN KG OR MOLES

CONCENTRATIONS IN MOLES/KG or LITER

MOLECULAR WEIGHTS IN KG/MOLE

FLUXES IN KG OR MOLES PER DAY'

16.3.2 Physical constants

'MOLECULAR WEIGHTS(MW)in kg/mole

F1 = mixed long-chain fatty acids inplant lipids, Am = ammonia, Wa = water, Ch = choline'

CONSTANT

MWSc = 0.171,MWOa = 0.134,MWPe = 0.187,MWSt = 0.162,MWFDLi = 0.619,...

MWFl = 0.284,MWgy = 0.092,MWHc = 0.132,MWCe = 0.162,MWPs = 0.115,...

MWpi = 0.115,MWNn = 0.322,MWAc = 0.060,MWPr = 0.074,MWbu = 0.088,...

MWva = 0.102,MWch = 0.121,MWCs = 0.180,MWRAa = 0.133,MWUr = 0.060,...

MWAm = 0.017,MWLa = 0.090,MWAs = 0.084,MWfa = 0.256,MWFDFA = 0.885,...

MWCH4 = 0.016,MWAm = 0.014

'HEATS OF COMBUSTION (H_c)

When F1 is set at 1.0, energy values are in Mcal/mole; when set at 4.184, values are in MJ/mole. The HC for palmitate is 2.38. Values for HCMi and HCLg are in Mcal/kg.'

CONSTANT F1 = 1.0

\$ 'Default condition is Mcal/mole.'

HCH4 = 0.211 * F1

\$ HCAc = 0.209 * F1 \$ HCPPr = 0.367 * F1 \$ HCBu = 0.524 * F1

HCGl = 0.673 * F1

\$ HCGy = 0.397 * F1 \$ HCFl = 2.657 * F1

'HCFl is value for stearate'

\$ HCFa = 2.712 * F1

HCCs = 0.673 * F1

\$ HCHc = 0.562 * F1 \$ HCPs = 0.656 * F1

HCNn = 1.2 * F1

\$ HCCh = 0.4 * F1 \$ HCUr = 0.152 * F1

HCOa = 0.3365 * F1

\$ HFDLi = 5.23 * F1 \$ HCLa = 0.336 * F1

HCTg = 7.57 * F1

\$ HCLm = 1.346 * F1 \$ HCAa = 0.627 * F1

HCTp = 0.3365 * F1

\$ HCMi = 5.35 * F1 \$ HCLg = 8.3 * F1

16.3.3 Initial conditions

The initial conditions specified for the model were derived almost exclusively from the data summaries of Smith (1970) which were presented in Chapter 3 as an illustration of the third step in the modeling process – collection of numerical data required to parameterize the mathematical statements.'

'In this context, initial conditions include not only initial pool sizes, body weight, body component weights and metabolite distribution volumes, but also initial fluxes. In the first version of the current model, initial conditions were presented as constants, e.g. iGP = 4.5E – 2. This restricted application of the model to a specific empty body weight (500 kg). Since many of the initial conditions specified are a linear function of body weight over a reasonable range and others vary as a function of body weight raised to, approximately, the 0.75 power (MBW), definitions of initial

conditions were later scaled to body weight or MBW to allow simulations of animals varying in initial body weight. The scaling factors should not be considered to be generally applicable, however. At a body weight of 550 kg, a lactating cow could clearly be thinner or fatter than the average depicted for the reference cow. Growing heifers might simply be growing, be growing and pregnant, or be growing, pregnant and lactating. In all cases, they differ significantly in body composition from the mature, non-pregnant, lactating reference cow. These differences could be quite critical in short-term simulations, in which case the modeler must develop an 'overlay' that corrects the initial conditions to reflect reality. Examples of some appropriate overlays are presented below as comments in the model. In long-term simulations, the model is a bit more robust in the sense that the model can be self-correcting. In other words, after several weeks of simulation, pool sizes adjust. However, body condition (composition) adjustments either take longer or do not occur during a given simulation. When the modeler has data adequate to specify initial conditions more accurately than the default, this should be done.'

'Initial conditions were set to allow for changes in initial empty body weight. This was done by dividing original pool sizes by 500 kg (the initial empty body weight of the reference cow). Thus, the IGI pool that was $4.5E - 2$ became $(4.5E - 2/500.0)$; an initial glucose factor (IGIF) of $9.0E - 5$. The initial nutrient factors can then be multiplied by initial body weight to estimate initial pool size for each nutrient. Initial volumes for blood metabolites were similarly defined and become dynamic variables dependent on EBW changes during solution.'

'FOR THE MID-LACTATION REFERENCE (DEFAULT) STATE'

'Note that the default has been changed from 550 kg to 650 kg BW in this version.'

CONSTANT IEBW = 600.0, MatBW = 650.0, IBW = 650.0, abE = 0.435

BWF = IEBW

labEav = abE * (BWF ** 0.75) * f1

'The term abEav is used in the dynamic section to accommodate effects of energy intake on energy expenditures due to specific functions such as ion transport. The coefficient 0.435 represents absorbed energy at mid-lactation feed intakes and should be adjusted for lower rates of milk production and non-lactating animals.'

CONSTANT IGIf = $9.0E - 5$, IFaf = $5.0E - 4$, IAcf = $1.18E - 3$, IAaf = $7.5E - 5$

CONSTANT IVGIIf = $3.0E - 2$, IVFaf = 1.0, IVAcf = 0.654, IVAaf = $3.0E - 2$

CONSTANT IPbf = 1.18, IPvf = 0.252, ITsf = 0.158

CONSTANT IHaf = $1.7E - 3$, IHbf = $3.28E - 3$, IHcf = $1.5E - 3$, ICef = $1.5E - 3$

CONSTANT ILpf = $6.78E - 3$, IPif = $0.852E - 3$, IOtf = $1.98E - 3$, ICsf = $0.50E - 3$

CONSTANT IAsf = $1.52E - 3$, IAmf = $1.6E - 3$, IMIf = $2.77E - 3$, IFIf = $0.98E - 3$

CONSTANT IRAcf = $8.0E - 3$, IRPrf = $3.5E - 3$, IRBuf = $1.2E - 3$, IRAaf = $0.18E - 3$

CONSTANT IMiHaf = 0.364E - 3, IMiHbf = 0.524E - 3, IPUNf = $7.0E - 3$, IVPUNf = 1.0

CONSTANT IRLaf = $2.0E - 9$, IRLvf = 0.1465, IBDNAf = $1.5E - 4$, IVDNAf = $1.5E - 4$

'IBDNAf and IVDNAf were set at $1.5E - 4$ kg based upon rough estimates of maxima for lactating cows as no direct measurements were available. When data become available these initial values should be corrected. At

these values, the maximum EBW that the reference cow could achieve is 750 kg, but this is not likely under normal feeding conditions.'

'For initiation of full lactation simulations, initial rumen pools must be adjusted to lower intakes characteristic of late pregnancy. Example values for an intake of 7.0 kg * day⁻¹ follow:

CONSTANT IHaF = 0.724E - 3, ILpF = 2.77E - 3, IHbF = 1.48E - 3, IPiF = 0.484E - 3
 CONSTANT IOtF = 0.8E - 3, ICsF = 0.11E - 3, IAsF = 1.0E - 3, IAmF = 1.08E - 3
 CONSTANT IMiF = 1.04E - 3, IFiF = 0.38E - 3, IRaF = 3.89E - 3, IRPrF = 1.66E - 3
 CONSTANT IRBuF = 0.58E - 3, IRAaF = 0.6E - 3, IMiHaF = 0.12E - 3, IMiHb = 0.19E - 3
 CONSTANT IRLaF = 1.0E - 8, IRLvF = 1.4E - 4'

'CORRECTION FACTORS'

'As was discussed in Chapter 4, provisions for inflating pool sizes were introduced into the model to increase the maximum integration interval (MAXT) allowed during solution and, thus, increase solution speeds. For (default) within-day simulation control statements incorporate modest correction factors for the pool sizes of Cs, RAa, Am, Aa and Gl. These are coded *_cor*, as in *Glc*. They are modest in the sense that a MAXT of 0.002 days is used and the pools continue to turn over every 8–10 min and approach a new steady state within 30–40 min. Since experimental measurements at intervals of less than one hour are unusual, these correction factors do not pose a problem in comparing simulated and experimental results, even when the perturbation of the system simulated is fairly dramatic.'

'When the overlay for long-term simulations is used to enable an integration interval of 0.5–1.0 day, some corrections are quite large, and up to two or three simulated weeks can be required to achieve a new steady state if large changes in feed intake or diet are simulated. When a large perturbation of the system is to be simulated, it is suggested that the short-term MAXT and correction factors be used to quickly approach the new steady state, and then the long-term MAXT and correction factors can be invoked to speed the solution. In order to allow this type of switch during a run, new correction factors must be specified during the run to override those defined in this initial section.'

CONSTANT Cscor = 10.0, RAacor = 10.0, Amcor = 10.0, Hacor = 1.0, RLvcor = 1.0
 CONSTANT Hbcor = 1.0, RAacor = 1.0, RPracor = 1.0, RBucor = 1.0, RLacor = 1.0
 CONSTANT Micor = 1.0, Picor = 1.0, Lpcor = 1.0, Otcor = 1.0, Ascort = 1.0
 CONSTANT Fcor = 1.0, MiHacor = 1.0, MiHbcor = 1.0
 CONSTANT Facor = 1.0, Accor = 1.0, Aacor = 10.0, Glcor = 10.0, PUNcor = 1.0
 CONSTANT Hccor = 1.0, Ccor = 1.0

'Computation of initial conditions'

IGl = IGlf * BWF * Glcor	\$ IVGl = IVGlf * BWF * Glcor
IFa = IFaf * BWF * Facor	\$ IVFa = IVFaf * BWF * Facor
IAc = IAcf * BWF * Accor	\$ IVAc = IVAcf * BWF * Accor
IAa = IAaf * BWF * Aacor	\$ IVAa = IVAaf * BWF * Aacor
IPUN = IPUNf * BWF * PUNcor	\$ IVPUN = IVPUNf * BWF * PUNcor
IHa = IHaf * BWF * Hacor	\$ ILp = ILpf * BWF * Lpcor
IHc = IHcf * BWF * Hccor	\$ ICe = ICef * BWF * Ccor
IHb = IHbf * BWF * Hbcor	\$ IPi = IPif * BWF * Picor
IOt = IOtf * BWF * Otcor	\$ IRLa = IRLaf * BWF * RLacor

ICs = ICsf * BWF * Cscor	\$ IAs = IAsf * BWF * Ascor
IAm = IAmf * BWF * Amcor	\$ IMi = IMif * BWF * Micor
IFl = IFlf * BWF * Flcor	\$ IRLv = IRLvf * BWF * RLvcor
IRAc = IRAcf * BWF * RAccor	\$ IRPr = IRPrf * BWF * RPracor
IRBu = IRBuf * BWF * RPracor	\$ IRAa = IRAaf * BWF * RAacor
IMiHa = IMiHaf * BWF * MiHaCR	\$ IMiHb = IMiHbf * BWF * MiHbCR
IBDNA = IBDNAf * BWF	\$ IVDNA = IVDNAf * BWF
IPb = IPbf * BWF	\$ IPv = IPvf * BWF
ITsF = ITsFf * BWF	

'The equations and parameter values for computing mammary metabolic capacity were adopted from Neal and Thornley (1983). Definition of terms:

LHOR = lactation hormone complex in kg
 KLhor = degradation rate constant for Lhor
 VUsyn = enzyme synthetic capacity per cell
 Ucells = (arbitrary) number of secretory cells defines genetic potential of udder to produce milk
 KUsyn = M-M type constant for hormone response
 KUdeg = degradation rate constant for Uenz
 KUdegM = degradation rate constant defining effect of udder milk (UMilk) on Uenz
 Mave = average milk in gland over last 21 days (TaveM = 1/21)
 KMdeg = half response point for degradation due to udder milk
 THETA5 = defines slope of response
 KMinh = factor defining inhibition of milk synthesis by milk
 Mlkmax = maximum mammary capacity for milk'

CONSTANT KLhor = 0.0102, VUsyn = 1.0, KUsyn = 0.2
 CONSTANT KUdeg = 0.1, KUdegM = 0.2, KMdeg = 27.0
 CONSTANT THETA5 = 10.0, TaveM = 0.048, UMLKcr = 1.0

'Default udder parameters for mid-lactation reference state'

CONSTANT ILHOR = 0.395, IUENZ = 6860.0, IUMavf = 10.3, IUTmf = 0.36
 CONSTANT IULmf = 0.494, IUPmf = 0.34, TavMLK = 0.143, Ucells = 1000.

'Parameters for initiation of lactation'

'CONSTANT ILHOR = 1.0, IUENZ = 520.0, IUMavf = 1.0, IUTmf = 0.01
 CONSTANT IULmf = 0.1, IUPmf = 0.01, IMLKav = 10.0, TavMLK = 0.143'

'Administration of exogenous hormones expressed in multiples of endogenous concentrations'

CONSTANT BST = 1.0, T3 = 1.0

'UDDER PARAMETERS'

MLKmax = 30.0 * UMLKcr \$ KMLKI = 3.0 * UMLKcr \$ IUMave = iUMavf * UMLKcr
 IUTm = IUTmf * UMLKcr \$ IUPm = IUPmf * UMLKcr \$ IULm = iULmf * UMLKcr
 END \$ 'OF INITIAL SECTION'

16.3.4 Dynamic, derivative section: digestive elements

DYNAMIC

DERIVATIVE

ALGORITHM IALG = 5 \$'Fourth Order Runge Kutta; see Chapter 4 for alternatives.'

NSTEPS NSTP = 1

CONSTANT CINT = 7.0 \$'Communication interval' MAXTERVAL MAXT = 0.002
\$'Maximum integration interval'

CONSTANT TSTP = 13.99 \$'Time to stop simulation'

TERMT ((T.GE.TSTP).OR.(STFLAG.EQ.1.0))

TIME = T

'The following section is a PROCEDURAL that initiates values for non-state variables and transfers new values that are calculated algebraically from one integration step to the next.'

CONSTANT IOxup = 180.4, IFCM4Z = 44.08, IME = 2.7, IDMILK = 20.0

CONSTANT IpPm = 0.033, IpTm = 0.033, IMLKav = 28.0, IAtAdh = 0.0195

CONSTANT IcFa = 0.5E - 3, IcAc = 1.8E - 3, IcGl = 3.0E - 3, ITCH4 = 1.0E - 9

CONSTANT IDEI = 70.0, IMEI = 60.0

PROCEDURAL (FCM4Z, Oxup1, UMave, rtPOx, VGl, VAc, VFA, VAa, PUNvol, ME, ...

pTm, MLKave, AtAdht, EBW = T, IEBW)

IF (T.LE.1.0E - 6) GO TO P

rtPOx = rtPO \$ pTm = pTm1 \$ FCM4Z = FCM4Z1

AtAdht = atadh1 \$ ME = ME1 \$ Oxup1 = DOx

UMave = UMave1 \$ EBW = EBW1 \$ BW = BW1

VGl = IVGl * EBW * Glcor \$ VFA = IVFAf * EBW * Facor

VAc = IVAcf * EBW * Accor \$ VAa = IVAaf * EBW * Aacor

PUNvol = IVPUNf * EBW

go to Q

P.. rtPOx = 5.3627

Oxup1 = IOxup \$ UMave = IUMave \$ VGl = IVGl

VAc = IVAc \$ VFA = IVFA \$ VAa = IVAa

PUNvol = IVPUN \$ FCM4Z = IFCM4Z \$ ME = IME

RLv = IRLv \$ EBW = IEBW \$ BW = IBW

DMILK = IDMILK \$ MLKave = IMLKav \$ pPm = IpPm

pTm = IpTm \$ AtAdht = IAtAdh

Q.. continue

END \$ 'OF PROCEDURAL'

'A block diagram of the ruminant digestive element of the model with mnemonics and reference fluxes for each transaction is presented in Fig. 16.2. Figure 16.3 represents metabolic elements of the animal portion of the model. These are outputs from simulation runs that are generated by an output subroutine of the model program, which is available upon request. We use these outputs primarily as a diagnostic aid.'

(a) Feed composition and physical properties

'Essential inputs to the digestive element of the model are listed throughout the model (see Chapter 6 also). Obviously, the requirement that a diet be described in this detail imposes a severe limitation upon practical application of the model, since detailed data on diet composition are very few. Basic reasons for requiring such

DAISYP

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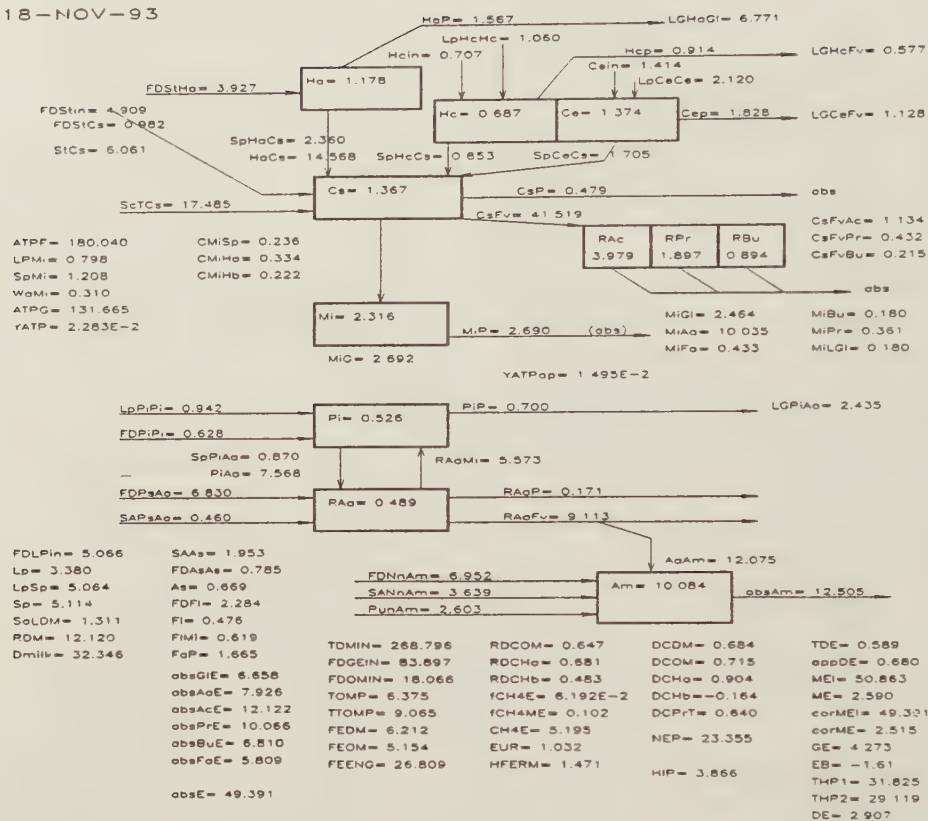


Figure 16.2 Diagrammatic representation of digestive element of the lactating cow model. Entities enclosed by boxes represent state variables and arrows indicate fluxes. Codes for abbreviations are defined in text and Baldwin *et al.* (1987b).

detail were 2-fold. The first was due to the fact that a primary reason why products of fermentation vary among diets is that the several components of the feed – soluble carbohydrates, starch, hemicellulose, cellulose, etc. – are utilized by different microbes whose fermentation products inherently differ. This concept has been widely accepted for a number of years and was amply demonstrated in the discussion of the Murphy *et al.* (1982a) model discussed in Chapter 8. The results of Murphy *et al.* (1982a) were used to define the stoichiometric coefficients used in this model.'

'The second reason underlying the detailed description of diet required as input to the digestive element is the requirement that input/output relationships in the model obey the first law of thermodynamics. Otherwise, the model could clearly not be used to investigate energetic relationships. This is easily achieved in the empirical, static, factorial models discussed in previous chapters because measurements underlying the systems are calculated by difference, as in the following.'

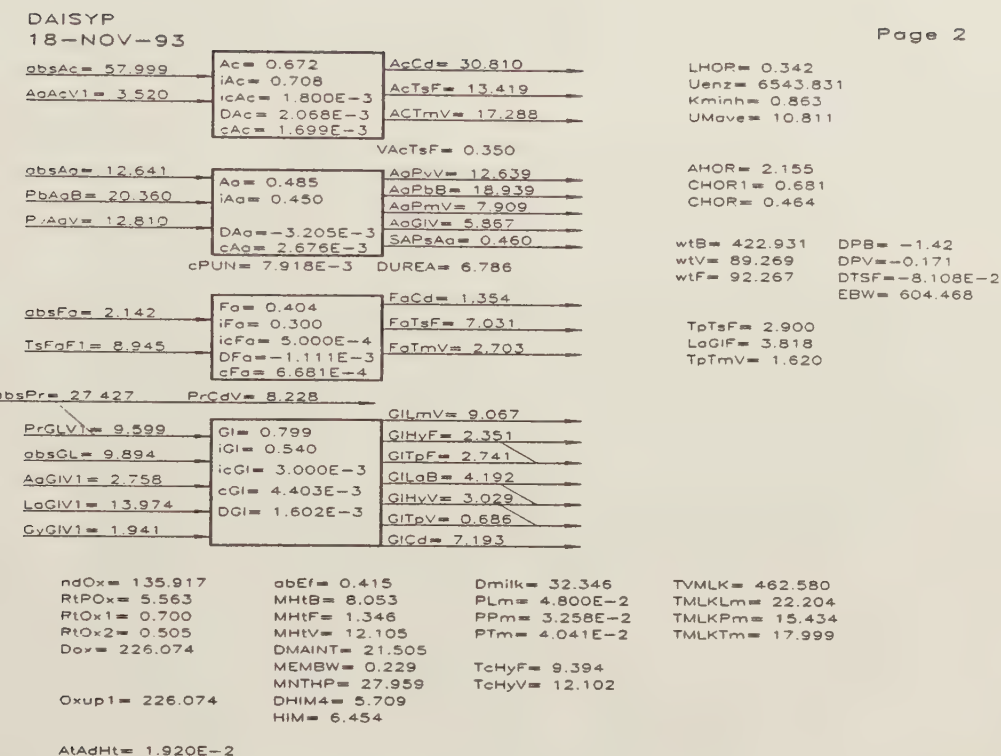


Figure 16.3 Diagrammatic representation of the metabolism model. Boxes indicate state variables. Arrows indicate fluxes.

'In comparative slaughter experiments such as,

$$HP = IE - FE - GE - UE - RE$$

and in indirect calorimetric experiments such as,

$$RE = IE - FE - GE - UE - HP.'$$

'Mechanistic models, by definition, incorporate specific precursor:product relationships and, as a result, energy contents of specific precursors and specific products must be explicitly represented. Murphy et al. (1982a) determined that the proportions of products of fermentation from soluble carbohydrates, organic acids and pectins did not differ. However, the heats of combustion of these feed components differ significantly. Thus, they clearly cannot be treated in aggregate across feed-stuffs. Similar considerations apply to plant lipids (Li) as opposed to yellow grease

(Fat), to hemicellulose (Hc), to cellulose (Ce), and to organic acids (Ac, Bu, La) in silage.'

'Definition of physical characteristics of feed components, including estimates of starch solubility or availability to microbes and the particle size factor, are essential to accommodation of physical and temporal availabilities of nutrients to the rumen microbes.'

'The reference diet is 50% concentrate:50% chopped alfalfa hay fed at 15 kg/day on a continuous basis (0.625 kg/h). This is equivalent to an intake of 3% of BW (calculated at a EBW of 500 kg). This intake is almost adequate to keep the reference cow in energy balance.'

'The prefix fD indicates the fraction in feed of each nutrient in kg/kg dry matter. Codes are soluble carbohydrate (Sc), organic acids (Oa), pectins (Pe), lactate (La), lipid (Li), starch (St), hemicellulose (Hc), cellulose (Ce), soluble protein (Ps), insoluble protein (Pi), non-protein nitrogen (Nn), lignin (Lg), soluble ash (As), insoluble ash (Ai), volatile fatty acids as in silage (Ac,Bu) and organic matter (OM). Stsol is the fraction of starch that is soluble and must be input, since it is a physical characteristic of feed. For steam-rolled and ground cereal grains, this seems to be an adequate index of temporal availability of starch. For whole grains, milo and corn in particular, this is not adequate and rumen digestion coefficients are too high. PSF is the proportion of feed that enters the small particle pool directly and is a property of feeds and processing procedures.'

CONSTANT fDSc = 0.06, fDOa = 0.05, fDPe = 0.06, fDLa = 0.0, fDLi = 0.04, ...
 fDSt = 0.25, fDHc = 0.09, fDCe = 0.18, fDPS = 0.04, fDPI = 0.08, ...
 fDNn = 0.03, fDLg = 0.04, fDAs = 0.04, fDAi = 0.04, fDAc = 0.0, ...
 fDBu = 0.0, fDUr = 0.0, Stsol = 0.2, PSF = 0.4
 fDOM = 1.0 - fDAi - fDAs

CONSTANT OBSME = 2.52, OBSDE = 3.00, OBSCH4 = 0.287

'In evaluations of the model, it is useful to input observed values, when available, so that these can be graphed versus model-predicted values in outputs from simulation runs. There are a number of observed values that can be defined as inputs to the model if desired. In this case, observed ME, DE and CH₄ values can be input. Model estimates of DE should always be below observed values, because microbial nucleic acids are all excreted in feces while, in reality, a significant portion of these are excreted in urine. Thus, simulated FE values are too high and UE values too low.'

'This procedural allows fat to be added from control language without recalculating proportions of other feed components. Thus, fDFAT is specified by setting FATADD to the proportion in the diet.'

CONSTANT FATADD = 0.0

PROCEDURAL (fDFAT = FATADD)

fDFAT = 0.0

IF (FATADD.GT.0.0) GO TO 2

GO TO 3

2.. factor = (1 - FATADD)

fDFAT = FATADD

fDPe = factor * fDPe

\$ fDSc = factor * fDSc

\$ fDLa = factor * fDLa

\$ fDOa = factor * fDOa

\$ fDLi = factor * fDLi

fDSt = factor * fDSt	\$ fDHc = factor * fDHc	\$ fDCe = factor * fDCe
fDPs = factor * fDPs	\$ fDPi = factor * fDPi	\$ fDNn = factor * fDNn
fDLg = factor * fDLg	\$ fDAs = factor * fDAs	\$ fDAi = factor * fDAi
fDac = factor * fDac	\$ fDBu = factor * fDBu	\$ fDOM = 1.0 - fDAi - fDAs

GO TO 3

3.. CONTINUE

END

\$ 'OF PROCEDURAL'

CWC = fDHc + fDCe + fDLg

FDGE1 = fDLi * 8.45 + fDSt * 4.154 + fDHc * 4.26 + fDCe * 4.154 + fDNn * 5.7

FDGE2 = (fDPs + fDPi) * 5.7 + fDLg * 8.3 + fDac * 3.48 + fDBu * 5.95

FDGE3 = FDSC * 3.94 + FDFAT * 9.63

FDGE = (fDOa * 2.51 + fDPe * 3.6 + fDLa * 3.73 + FDGE1 + FDGE2 + FDGE3) * F1

'LARGE PARTICLE FRACTION OF FEED AND COMPOSITION'

'For simplicity, the decision was made to represent the large particle fraction as an aggregate state variable comprised of Ce, Hc, Lg, etc. rather than consider large particle pools of each component as a separate state variable. Implicit assumptions are that large particles cannot pass from the rumen, that no hydrolysis and fermentation of large particle components can occur, and that the conversion of large particles to small particles be totally dependent upon rumination. For subsequent equations it is essential that the composition of large particles be calculated.'

fLp = (fDCe + fDHc + fDLg + fDAi + fDPi) * (1.0 - PSF) \$ 'Proportion of Lp'

fLpHc = fDHc * (1.0 - PSF)/fLp \$ 'proportion of hemicellulose'

fLpCe = fDCe * (1.0 - PSF)/fLp \$ 'proportion of cellulose'

fLpPi = fDPi * (1.0 - PSF)/fLp \$ 'proportion of insoluble protein'

fOt = fDLg + fDAi \$ 'combines lignin and silicates as Ot'

fLpOt = fOt * (1.0 - PSF)/fLp \$ 'proportion of Ot'

fLgOt = fDLg/fOt

'TOTAL SOLUBLE SUGAR EQUIVALENT IN FEED

Combines Oa,Pe, lipid glycerol and Sc as one fraction'

'As noted above and in Chapter 8, Murphy et al. (1982a) found that fermentation patterns of Oa, Pe, etc., were very similar. They must be specified separately as inputs to the model for energetic reasons, but for purposes of simulating their fermentation can be combined into a common (Sc) pool.'

*'The algebra presented below converts kilograms of nutrients metabolized via a common pool to a common molecular weight basis. They were calculated as follows: (171 g Sc/mole Sc) * (0.5 mole Sc/mole Oa) * (134 g Oa/mole Oa)⁻¹ = (85.5 g Sc/mole Oa) * (134 g Oa/mole Oa)⁻¹ = 0.638 g Sc/g Oa.'*

CONSTANT OaScSC = 0.638, PeScSC = 0.914, LiScSC = 0.138,

CONSTANT LaScSC = 0.95, HcCeCE = 1.02, FdFaSC = 0.097

OaSc = fDOa * OaScSC

PeSc = fDPe * PeScSC

LiSc = fDLi * LiScSC

FATSC = FdFAT * FdFaSC

fDScT = fDSc + OaSc + PeSc + LiSc + FATSC

'fDtot is a check to assure that DM/kg equals 1.0'

fDtot = fdsc + fdoa + fdpe + fdla + fdli + fdfat + fdst + fdhc + fdce + fdlg + fdps...

+ fdpi + fdnn + fdur + fdas + fdai + fdac + fdbu

'This provision was added to enable simulation of experiments where protein was infused per abomasum.'

CONSTANT INFPRT = 0.0 \$ 'INFPRT = ABOMASAL INFUSION OF CASEIN'
'EXPRESSED IN KG/DAY'

(b) Feeding management and strategies

'Alternatives available for specifying feed inputs are:

RFEED1 – Multiple meals per day at intakes specified by user; during continuous feeding RFEED1 = 0.0.

RFEED2 – A specified feeding rate; can input total intake as observed in an experiment or an estimate of feed intake required for maintenance to which increments for lactation, growth and/or pregnancy can be added. Specified as default model input at 9.0 kg/day.

RFEED3 – An allowance for feed dry matter intake per unit of milk. Default for model is FDMMLK = 0.33.

RFEED4 – Equation developed based upon voluntary feed intakes of lactating cows in southeastern US (Brown *et al.*, 1977).

RFEED5 – Equation derived from NRC (1989) equations for ME requirements of lactating cows (RFD5.A) with restrictions on ME intake due to NDF content of the ration offered (RFD5.B) according to Mertens (1985). RFD5.B is invoked when it is less than RFD5.A.

RFEED6 – Feeds the animal as a function of metabolic body weight. Utility of this provision is limited to simulation experiments in which this feeding strategy was used.

RFEED7 – Maintenance requirements of pregnant heifers and cows expressed as a function of days pregnant.

RFEED8 – Requirements of pregnant cattle for fetal growth as a function of days pregnant.

RFEED9 – Maintenance requirement of growing heifers.

RFED10 – ME requirement for weight gain in heifers.'

'Computation of RFEED1'

'For multiple feedings per day you must specify FDTM,FDINT and FDRAT (default values are 0.0). Feeding time (FDTM) is the time spent eating in each feeding interval (FDINT). FDRAT is kg dry matter consumed in each FDINT. Hourly feeding yields a FDINT of (1/24)0.04167. Summary equations are not always valid when using this mode.'

CONSTANT FDTM = 0.0,FDINT = 0.0,FDRAT = 0.0


```
PROCEDURAL (RFEED1 = T,FDTM,FDINT,FDRAT)
  RFEED1 = 0.0
  IF(FDRAT.EQ.0.0) GO TO 9
  IF(AMOD(TIME,FDINT).LE.FDTM) GO TO 8
  GO TO 9
```

```
8.. RFEED1 = FDRAT/FDTM
  GO TO 9
```

```
9.. CONTINUE
  END                                $ 'OF PROCEDURAL'
```

'Computation of RFEED2 AND RFEED3'

Basal feeding rate – RFEED2 – and kilogram feed allowed per kilogram milk – FDMLK – must be specified. This alternative (RFEED2 + RFEED3) is the default for full lactation studies.'

'The provision (Tavmlk, MLKave) in the section below was added originally to smooth feeding allowances for milk production over a period of time as opposed to feeding based upon milk production on a given day; the former approach is normally used during experiments. It turns out that this provision inadvertently has the property of lagging increases in feed intake in early lactation while milk production is increasing rapidly. Also, the delayed feed intake response which occurs several weeks after initiation of BST treatment is accounted for by this provision. Since models of feed intake that accommodate mechanistic explanations (and we know of none) for lags in feed intake responses to rapid changes in milk production, this provision has some useful, though fortuitous, properties.'

```
CONSTANT TavMLK = 0.072,RFEED2 = 9.0,FDMLK = 0.33
  RFEED3 = MLKave * FDMLK
  DMLKav = TavMLK * (DMILK - MLKave)
  MLKave = INTEG(DMLKav,iMLKav)
  'Computation of RFEED 4 and 5'
  RFEED4 USES THE BROWN ET AL. (1977) EQUATION W/O THE SEASON EFFECT
  RFEED5 USES THE MERTENS (1985) EQUATIONS
  THESE ARE CONTROLLED BY SETTING RFD4F OR RFD5F = 0.0(OFF)
  OR = 1.0(ON).
  CONSTANT RFD4F = 0.0,RFD5F = 0.0,SEAF = 0.61,LAMMAX = 0.4,KLAM = 85.0
  CN = 20.854 - 27.076 * ((FCM4Z/2.204)/(MatBW ** 0.75)) $'LOWER LIMIT' 'FOR FIBER'
  CFF = CN * 0.018001 - CN ** 2 * 0.000557 $'CRUDE FIBER FACTOR' DMIN = 2.7182 *
  * (SEAF - 0.000827 * T + 0.148073 * ALOG(T + 0.00001) + ... 0.33922 * ALOG(DMILK) +
  0.09926 * DMILK * PTM + 0.0675 * MatBW/(100.0 + CFF)) RFEED4 = DMIN
  RFEED5 = AMIN1(RFD5A,RFD5B)
  RFD5A = LAMDA * MatBW/NDF
  LAMDA = (1.0 + LAMMAX/((1.0 + KLAM/T + 1.0E - 4)))/100.0
  NDF = fDHc + fDCe + fDLg
  'CODING OF NRC (1989) DAIRY CATTLE EQUATION'
  NELREQ = NELMNT + NELLAC + NELD + NELP
  NELMNT = 0.08 * BW ** 0.75
  NELLAC = NELLKG * DMILK
  NELLKG = 0.3512 + 0.0962 * PTM * 100.0
  NELD = 5.12 * DBW
  PROCEDURAL (DBW = DWTB,DWTV,DWTTsF)
```

DBW = DWTB + DWTV + DWTTsF

IF(DBW.LT.0.0) DBW = 0.0

IF(DBW.GT.0.5) DBW = 0.5

END \$ 'OF PROCEDURAL'

CONSTANT PDAYS = 305

'The default specification of PDAYS at 305 means that the reference cow is not pregnant during default simulations of a lactation. If otherwise must specify.'

PROCEDURAL (NELP = T,BW,PDAYS)

IF(T.LT.PDAYS) NELP = 0.0

IF(T.GE.PDAYS) NELP = 0.024 * BW ** 0.75

END \$ 'OF PROCEDURAL'

MEREQ = NELREQ/0.645

RFD5B = MEREQ/obsME

'Computation of RFEED6 which feeds at 2.0% of body weight'

CONSTANT FEEDBW = 0.02,RFD6F = 0.0

'Computation of RFEED 7 and 8.'

ENERGY REQUIREMENTS FOR PREGNANCY (FETUS & GRAVID UTERUS)

according to Moe and Tyrell (1972) and Ferrell *et al.* (1976).'

CONSTANT RFD7F = 0.0,RFD8F = 0.0

CONSTANT dconc = 90 \$ 'day (post partum) of conception'

dpreg = t - dconc \$ 'days pregnant'

PROCEDURAL (RFEED7,RFEED8 = T,DPREG,obsME,BW,NELMNT)

MTME = NELMNT/0.645

RFEED7 = MTME/obsME

RFEED8 = 1.0E - 8 \$ 'sets FMEREQ prior to conception to 1.0E - 8'

IF (T.LE.DCONC) GO TO 71

'MOE and TYRRELL equation (1972)'

MTME = (BW ** 0.75) * (0.567 * exp(0.0174 * dpreg))/1000

RFEED7 = MTME/obsME

'FERRELL - gross energy in gravid uterus at efficiency of 0.14 gives ME requirement in Kcal/day - (0.14/1000 = 0.14E - 3) converts to Mcal/day'

FMEREQ = (69.73 * exp((0.0323 - 2.75E - 5 * dpreg) * dpreg)) * ... (0.0323 - 2 * 2.75E - 5 * dpreg)/0.14/1000.0

RFEED8 = FMEREQ/obsME

71..CONTINUE

END \$ 'OF PROCEDURAL'

'Computation of RFEED9 and RFED10'

Feeding strategy for heifers from NRC (1989) up to breeding age.'

CONSTANT MNT = 0.077,LWG = 0.27,RFD9F = 0.0,RFD10F = 0.0

PROCEDURAL (RFEED9,RFED10 = RFD9F,rfed10f,MNT,...

LWG,obsME,ME,pTm,MLKave,BW)

RFEED9 = 1.0E - 8 \$ RFED10 = 1.0E - 8

IF ((RFD9F.eq.0.0).AND.(RFED10F.eq.0.0)) GO TO 73

RFEED9 = ((MNT * (BW ** 0.75)) + (MLKave * (10 * pTm + 0.35)))/...

((1.37 * ME) - (0.138 * (ME ** 2)) + (0.0105 * (ME ** 3)) - 1.12)

RFED10 = (0.0686 * (BW ** 0.75) * (LWG ** 1.119))/...

((1.42 * obsME) - (0.174 * (obsME ** 2)) + (0.0122 * (obsME ** 3)) - 1.65)

GO TO 73

73.. CONTINUE

END \$ 'OF PROCEDURAL'

```

RFEED = RFEED1 + RFEED2 + RFEED3 + RFEED4 * RFD4F + ...
RFEED5 * RFD5F + RFEED6 * RFD6F + RFEED7 * RFD7F + ...
RFEED8 * RFD8F + RFEED9 * RFD9F + RFEED10 * RFD10F
FDDMIN = RFEED $ DMIN = FDDMIN
FDDOMIN = FDDMIN * fDOM
TDMIN = INTEG(DDMIN,1.0E - 8)
'FEEDING STRATEGIES'
LOGICAL FLAGGG

```

'This section provides for conditional changes in diet or other inputs based upon preset criteria and current status of the system. This is achieved by setting STFLAG to 1.0, which stops the simulation. Then the user can change the diet or other inputs and then continue the simulation with the CONTIN command.

STRAT1	provides for diet changes after 98 and 210 days of lactation.
STRAT2	provides for a diet change after 140 days of lactation.
STRAT3	provides for a diet change when milk production drops below 29.5 kg/day.
STRAT4	provides for a diet change when milk production drops below 29.5 and EBW is greater than 560 kg.
STRAT5	provides for changes when milk production drops below 20.0 kg/day.'

```

PROCEDURAL (STFLAG = T,DMILK,EBW,FLAGGG)
CONSTANT STRAT1 = 0.0,STRAT2 = 0.0,STRAT3 = 0.0,STRAT4 = 0.0,...
STRAT5 = 0.0,STRAT6 = 0.0,STRAT7 = 0.0
CONSTANT FLAGGG = .FALSE.

```

'If flag is set, skip testing for this iteration, just reset to zero.'

```

IF (STFLAG.EQ.1.0) GOTO 41
IF(STRAT1.EQ.1.0 .AND. (T.EQ.98.0 .OR. T.EQ.210.0))STFLAG = 1.0
IF(STRAT2.EQ.1.0 .AND.(T.EQ.140.0)) STFLAG = 1.0
IF(STRAT3.EQ.1.0 .AND.(DMILK.LT.29.5) .AND. (T.GT.50.0) .and. .not. FLAGGG)
GOTO 47
IF(STRAT4.EQ.1.0 .AND. (DMILK.LT.29.5).AND.(EBW.GT.560.0) .AND. (T.GT.50.0) .and.
.not. FLAGGG) GOTO 47
IF(STRAT5.EQ.1.0 .AND.(DMILK.LT.20.0) .AND. (T.GT.50.0) and not FLAGGG) GOTO
47 GOTO 45
41..STFLAG = 0.0
GOTO 45
47.. STFLAG = 1.0
FLAGGG = .TRUE.
GOTO 45
45..CONTINUE
END

```

\$'OF PROCEDURAL '

(c) Stoichiometric coefficients for fermentation

'For fermentation, three sets of coefficients are included. One is for, largely, forage diets (FORSET). The second is for 50:50 forage:concentrate diets

(MIXSET). The third set is for, largely, concentrate diets (CONSET). FORSET and CONSET are from Murphy *et al.* (1982a). MIXSET is a composite of the other two, since these apply to less than 50% and more than 50%, respectfully. Valerate was separated to 1/2Bu + 1Pr to maintain carbon and hydrogen balance. Values are in moles of VFA produced per mole of hexose fermented. One mole of glucose gives 2 moles of Ac or Pr but only one mole of butyrate. Amino acid fermentation stoichiometry is also from Murphy *et al.* (1982a). Even though the solution was non-unique (Chapter 8), it maintains C, H and O balance. The high proportion of propionate formed reflects Pr + 1/2 Bu from BCFA. CH₄ is very highly dependent upon the proportion of BCFA produced. Stoichiometric coefficients for protein are expressed as moles VFA produced per mole AA fermented with an average 5.08 carbons per amino acid. Stoichiometries:'

PROCEDURAL (ScAcAc, ScPrPr, ScBuBu = FORSET, MIXSET, CONSET)

CONSTANT FORSET = 0.0,MIXSET = 1.0,CONSET = 0.0

IF(FORSET.EQ.1.0) GO TO 10

IF(MIXSET.EQ.1.0) GO TO 11

IF(CONSET.EQ.1.0) GO TO 12

'FORSET'

10..ScAcAc = 1.38 \$ ScPrP = 0.40 \$ ScBuB = 0.11 \$ ScLaL = 1.0E - 8

StAcAc = 1.20 \$ StPrPr = 0.34 \$ StBuBu = 0.23 \$ StLaLa = 1.0E - 8

HcAcAc = 1.14 \$ HcPrPr = 0.40 \$ HcBuBu = 0.23

CeAcAc = 1.32 \$ CePrPr = 0.20 \$ CeBuBu = 0.24

GO TO 13

'MIXSET'

11..ScAcAc = 1.14 \$ ScPrPr = 0.43 \$ ScBuBu = 0.215 \$ ScLaLa = 1.0E - 8

StAcAc = 1.00 \$ StPrPr = 0.52 \$ StBuBu = 0.24 \$ StLaLa = 1.0E - 8

HcAcAc = 1.13 \$ HcPrPr = 0.49 \$ HcBuBu = 0.19

CeAcAc = 1.45 \$ CePrPr = 0.20 \$ CeBuBu = 0.17

GO TO 13

'CONSET'

12..ScAcAc = 0.90 \$ ScPrPr = 0.46 \$ ScBuBu = 0.32 \$ ScLaLa = 1.0E - 8

StAcAc = 0.80 \$ StPrPr = 0.70 \$ StBuBu = 0.25 \$ StLaLa = 1.0E - 8

HcAcAc = 1.12 \$ HcPrPr = 0.58 \$ HcBuBu = 0.15

CeAcAc = 1.58 \$ CePrPr = 0.20 \$ CeBuBu = 0.11

GO TO 13

13.. AaFvAc = 0.60,AaFvPr = 0.60,AaFvBu = 0.25,LaAcAc = 0.88,LaPrPr = 0.12

CONTINUE

END

\$ 'OF PROCEDURAL'

'ADJUSTMENT OF STOICHIOMETRIC COEFFICIENTS FOR RPH'

'Stoichiometric coefficients are affected by pH. This is probably the reason Murphy *et al.* (1982b) found a relationship between amount of concentration in a ration and the stoichiometric coefficients. Therefore, provisions were added by Argyle and Baldwin (1988) for the pH to affect these coefficients. Data to do this were barely adequate. For this reason, the equations that should probably be sigmoidal are linear. Similarly the switch to a 'pure' lactate fermentation (below) at pH 5.4 may not be correct, since it is prob-

ably a logarithmic increase starting at 5.5. The changes in stoichiometric coefficients produced by the pH corrections parallel those suggested by the equation to CONSET as proportions of concentrate in the ration increased as formulated by Murphy *et al.* (1982b).'

```
PROCEDURAL (ScAc,ScPr,ScBu,ScLa,StAc,StPr,StBu,StLa = RpH,ScAcAc,...
  ScPrPr,ScBuBu,ScLaLa,StAcAc,StPrPr,StBuBu,StLaLa)
  ScAc  = ScAcAc $ ScPr  = ScPrPr $ ScBu  = ScBuBu $ ScLa=ScLaLa
  StAc  = StAcAc $ StPr  = StPrPr $ StBu  = StBuBu $ StLa=StLaLa
  IF(RpH.GE.6.2) GO TO 24
  IF(RpH.LE.5.4) GO TO 23
  ScAc  = 0.70 + ((RpH - 5.4)/0.8) * (ScAcAc - 0.70)
  ScPr  = 0.50 + ((RpH - 5.4)/0.8) * (ScPrPr - 0.50)
  ScBu  = 0.40 + ((RpH - 5.4)/0.8) * (ScBuBu - 0.40)
  StAc  = 0.66 + ((RpH - 5.4)/0.8) * (StAcAc - 0.66)
  StPr  = 0.82 + ((RpH - 5.4)/0.8) * (StPrPr - 0.82)
  StBu  = 0.26 + ((RpH - 5.4)/0.8) * (StBuBu - 0.26)
GO TO 24
23..  ScAc = 0.0 $ ScPr = 0.0 $ ScBu=0.0
      StAc = 0.0 $ StPr = 0.0 $ StBu=0.0
      ScLa = 2.0 $ StLa = 2.0
24.. CONTINUE
      END                                $ 'OF PROCEDURAL'
```

(d) *Rumination, salivation and water dynamics.*

'RUM is the proportion of time spent ruminating per unit time. There are separate equations for twice-daily feeding and continuous or multiple feedings. Rumination is shut off during feeding when the animal is fed twice daily. In continuous feeding, the animal ruminates during feeding, because rumination is needed for large particles to be broken down into small particles. Since the default is continuous feeding, RUMEQ is set to zero and ruminating rate (RUMF = RUM) to 0.33. When discrete feeding periods are used (FDINT does not equal FDTM), one should implement the rumination equation of Murphy *et al.* (1982b) by setting RUMF = 0.0 and RUMEQ = 1.0. This also enables resting salivation and drinking for the water balance equations below.'

```
DAY = 1.0
CWCf = 1.0 * (0.174 + 0.5085 * CWC)
CONSTANT RUMEQ = 0.0, RUMF = 0.333
CONSTANT MEAN1 = 0.0, MEAN2 = 0.054, AMP1FT = 0.1251, AMP2FT = 0.190
PROCEDURAL (RUM,RUMcor,RESTSA,RESTWA = RUMEQ,CWCf,DAY,...
  TIME, AMP2FT,RUMF,FDINT,FDDMIN)
  RUM = RUMF
  REST = 1.0 - RUMF          $ RUMcor = 1.0
  RSTcor = 1.0              $ NOFEED = 1.0
  RESTSA = 0.85 * (EBW ** 0.75) * REST
  RESTWA = 1.4 * FDDMIN * 0.75
  IF(RUMEQ.EQ.0.0) GO TO 84
  IF(FDINT.EQ.0.5) GO TO 83
```

```

AMPONE = AMP1FT * cos(6.283 * AMOD(TIME, DAY) + 1.9109)
RUM = (CWCf + MEAN1) - AMPONE
REST = (1.0 - (CWCf + MEAN1)) + AMPONE
RESTSA = 6.0 * (RFEED/FDINT) * REST * RSTcor
GO TO 84
83.. IF(FDDMIN.GT.0.0) NOFEED = 0.0
AMPTWO = AMP2FT * cos(6.283 * (2 * AMOD(TIME, FDINT)) + 5.1)
RUM = ((CWCf + MEAN2) - AMPTWO) * NOFEED
REST = ((1.0 - (CWCf + MEAN2)) + AMPTWO) * NOFEED
RSTcor = FDINT/(FDINT - RUMF/2)
RUMcor = FDINT/(FDINT - FDTM)
RESTWA = 0.0
RESTSA = 0.0
GO TO 84
84.. CONTINUE
END                                $ 'OF PROCEDURAL'

```

'Salivation, drinking, water flow through the rumen wall; rumen soluble, particulate and total rumen dry matter (RDM) are all variables that influence rumen volume. Rumen volume can be calculated based upon RDM/0.11, which is the default, or based upon water dynamics and osmolality when the rumen liquid volume equation (RLVEQ) is set to 1.0. The empirical equation for OSWA is not generally applicable and should not be used for continuous feeding and unusual diets, e.g. high salt, NaHCO_3 , and, thus should be closely monitored when RLVEQ is set to 1.0.

'SALIVATION'

At RLVEQ = 0.0, the equation $\text{RESTSA} = 6.0 * (\text{RFEED}/\text{FDINT}) * \text{REST} * \text{RSTcor}$ from above leads to the secretion of 60 l/day. When the animal is not eating, RFEED/FDINT is zero and RESTSA = 0.0. While the animal is ruminating, saliva flow is 85 l/day when RUM = 0.33 and EBW = 500kg.'

```

RUMSA = 2.41 * (EBW ** 0.75) * RUM
EATSA = 3.2 * FDDMIN                                $ 'l/kg FDDMIN'
SAin = EATSA + RESTSA + RUMSA                        $ 'Total saliva flow'

```

'DRINKING'

'Drinking functions are based on 4.7 l/kg DM, with 70% being consumed during eating and only 75% of water consumed enters the rumen.'

```

EATWA = 3.30 * FDDMIN * 0.75
RESTWA = 1.40 * (RFEED/FDINT) * 0.75 * RUMcor * NOFEED
DRNKWA = EATWA + RESTWA

```

'Rumen volume based upon rumen dry matter CRDM'

```

SOLDM = Cs * MWCs/Cscor + Am * MWAm/Amcor + RPr * MWPr/RPRcor + RBu *
MWBu...

```

```

/RBucor + RAa * MWRAa/RAacor + As/Ascor + Fl * MWFl/Flcor + ...

```

```

RAc * MWAc/RAacor + RLa * MWLa/RLacor

```

```

RDM = Lp/Lpcor + Sp + SOLDM + Mi/Micor RUMVOL = RDM/0.11

```

'WATER DYNAMICS'

```

CONSTANT RLVEQ = 0.0

```

```

CONSTANT OsMolF = 1.70 $ 'OSMOLALITY FACTOR'

```

PROCEDURAL (RLv,PRDM = RDM,RLvEQ,Sain,DRNKWA)

IF (RLVEQ.EQ.1.0) GO TO 28

RLv = RDM/(0.11 - RDM)

WAin = Sain + DRNKWA

WAout = RLv * KWAP/RLvcor

Go to 30

28.. WAin = Sain + DRNKWA

WAout = RLv * KWAP/RLvcor

'Rumen Fluid Osmolality. As (soluble ash) multiplied by a factor to give moles of ions. MWAs = 0.084 was picked from NaHCO_3 '

ROsMol = (Cs/Cscor + Fl/Flcor + Am/Amcor + RAc/RAccor + ...

RPr/RPcor + RBu/RBucor + RLa/RLacor + RAa/RAacor * OsMolF...

+ (As/MWAs)/AScor * OsMolF)/RLv

OsWA = 0.74 * ((ROsMol - 0.280) * 1000) - 41.0

\$ 'From Dobson *et al.* (1970)'

DRLv = WAin - WAout + OsWA

RLv = INTEG(DRLv,IRLv)

\$ 'Rumen liquid volume'

PrDM = RDM/(RDM + RLv)

\$ 'percent rumen dry matter'

GO TO 30

30.. CONTINUE

END

\$ 'OF PROCEDURAL'

DLRATE = WAout/RLv

'RUMEN pH'

'Rumen pH influences stoichiometry of fermentation (above) and hydrolytic rate constants for cellulose and hemicellulose. This is most relevant when meal rather than continuous feeding is implemented. However, in the default it is left on (RpHCON = 1.0, FIXDpH = 0.0). When RpHCON = 0.0, fixed pH (FIXDpH = 6.8) must be specified.'

CONSTANT VFAeff = 0.015, RpHCON = 1.0, FIXDpH = 0.0

RpH = (7.20 - (VFAeff * cVFA + 0.0015 * (cRLa * 1000))) * RpHCON + FIXDpH

TVFA = RAc/RAccor + RPr/RPcor + RBu/RPcor

cVFA = TVFA/(RLv/RLvcor) * 1000 \$ 'cVFA in mMoles/liter'

(e) Passage rate constants

CONSTANT KSPP = 1.33, KWAP = 3.5

KSPP = 2.67 * (FDDMIN/(EBW ** 0.75)) + 1.00

KWAP = 1/((0.028 * (EBW ** 0.75)/FDDMIN) + 0.16)

'KSPP and KWAP are fractional turnover rates/day. Equations derived by regression equations'

(f) Large and small particle pools (Lp and Sp in kg)

CONSTANT KLpSp = 4.50 \$ 'Questions such as Should KLpSp be a function of the physical properties of feeds; should KLpSp be a function of fermentation rate; and, should entry be lagged for hydration are unresolved at present.'

DLp = FDLpin - LpSp

FDLpin = fLp * FDDMIN \$ 'LARGE PARTICLES'

$$LpSp = KLpSp * Lp/Lpcor * RUM$$

$$Lp = INTEG(DLp, ILp)$$

'SMALL PARTICLE POOL SIZE (in kg)'

$$Sp = Ha/Hacor + Hb/Hbcor + Pi/Picor + Ot/Otcor \$ 'SMALL PARTICLES'$$

$$SpPer = Sp/(Sp + Lp)$$

'Provisions for association of microbes with small particle Ha(MiHa) and Hb(MiHb) were added to prevent increases in microbes from Ha fermentation from increasing the digestion of Hb (due to more microbes) and vice versa, i.e. to give specificity to microbes associated with small particles based on the substrate upon which they grew.'

'In the first version of the model, cellulose (Ce) and hemicellulose (Hc) were dealt with in aggregate as holocellulose (Hb). Later, it was recognized that this simplification led to some significant errors due to the fact that fermentation products from these two entities differ significantly, as can their relative rates of hydrolysis when a wide range of feedstuffs were considered. Therefore, the decision was made to treat Ce and Hc as separate state variables. In some sections of the model, including the following depiction of the association and release of microbes from small particles of fiber, this separation could have added considerable complexity. Therefore, the simple, original representation and consideration of Hb as a state variable dependent upon Ce and Hc hydrolysis was retained (see section on fiber digestion). Some subtle errors arise from the facts that the Ruminococci hydrolyze Ce and Hc and ferment the products of hydrolysis, while Fibrobacter succinogens hydrolyzes both but only ferments the products of cellulose hydrolysis. In the absence of detailed models of the rumen ecology we must accept these subtle errors, which may well be small at this level of aggregation.'

$$CONSTANT KMiHa = 1.56, KMiHb = 0.41, VMiHa = 0.85$$

$$CONSTANT VMiHb = 0.85, KMiHaF = 0.095, KMiHbF = 0.041$$

$$Csin = ScTCs + StCs + HaCs + HcCs + CeCs$$

\$ 'Fractions of Cs entry'

$$fCsHa = HaCs/Csin$$

\$ 'attributed to Ha and Hb'

$$fCsHb = (HcCs + CeCs)/Csin$$

\$ 'hydrolysis.'

$$HaMiP = (HaMi/MiHaCr) * KSPP$$

$$HbMiP = (HbMi/MiHbCr) * KSPP$$

\$ 'Passage of microbes in'

$$OtMiP = OtP * cMiSp$$

\$ 'association with Sp'

$$PiMiP = PiP * cMiSp$$

$$SpMiPi = OtMiP + PiMiP + HaMiP + HbMiP$$

$$CsMiG = MiG * (CsFv * CsFvAT/ATPF)$$

\$ 'Proportions of microbial'

$$HaMiG = CsMiG * fCsHa$$

\$ 'growth attributable to'

$$HbMiG = CsMiG * fCsHb$$

\$ 'Cs formed from Ha and Hb' 'hydrolysis.'

$$cMiHa = (HaMi/MiHaCr)/(Ha/Hacor)$$

\$ 'Concentration (kg/kg) of'

$$cMiHb = (HbMi/MiHbCr)/(Hb/Hbcor)$$

\$ 'microbes associated with' 'Ha and Hb.'

$$SpMiHa = KMiHa * Ha/Hacor * cMiSp$$

\$ 'Microbes attached'

$$SpMiHb = KMiHb * Hb/Hbcor * cMiSp$$

$$HaMiR = cMiHa * SpHaCs$$

\$ 'Microbes already associated with Sp potentially released due to hydrolysis of particulate substrates.'

$$HbMiR = cMiHb * SpHcCs + cMiHb * SpCeCs$$

$$\text{HaMiF} = \text{VMiHa}/(1.0 + \text{KMiHaF}/((\text{Ha}/\text{Hacor})/\text{Sp}))$$

\$ 'Fraction of potentially released microbes actually released.'

$$\text{HbMiF} = \text{VMiHb}/(1.0 + \text{KMiHbF}/((\text{Hb}/\text{Hbcor})/\text{Sp}))$$

\$ 'Dependent on fractions of Ha and Hb in Sp where maximum retention on Sp is 0.85 of those potentially released.'

$$\text{MiHaMi} = \text{HaMiF} * (\text{HaMiG} + \text{HaMiR})$$

\$ 'Microbes on particles' 'and'

$$\text{MiHbMi} = \text{HbMiF} * (\text{HbMiG} + \text{HbMiR})$$

\$ 'those grown from Ha at Hb hydrolysis and fermentation which remain in association with SP.'

$$\text{DHaMi} = \text{SpMiHa} + \text{MiHaMi} - \text{HaMiP} - \text{HaMiR}$$

$$\text{DHbMi} = \text{SpMiHb} + \text{MiHbMi} - \text{HbMiP} - \text{HbMiR}$$

$$\text{HaMi} = \text{INTEG}(\text{DHaMi}, \text{iMiHa})$$

$$\text{HbMi} = \text{INTEG}(\text{DHbMi}, \text{iMiHb})$$

'Starch (St in kg) or α -hexose (Ha in kg) hydrolysis and passage'

$$\text{CONSTANT KHaCs} = 6.0$$

$$\text{DHa} = \text{FDStHa} - \text{HaP} - \text{SpHaCs}$$

$$\text{FDStin} = \text{fDSt} * \text{FDDMIN}$$

$$\text{FDStCs} = \text{FDStin} * \text{Stsol}$$

$$\text{FDStHa} = \text{FDStin} - \text{FDStCs}$$

$$\text{HaP} = \text{Ha}/\text{Hacor} * \text{KSPP}$$

$$\text{SpHaCs} = \text{KHaCs} * \text{Ha}/\text{Hacor} * \text{cMiHa}$$

'Note that all equations for the hydrolysis of components of small particles are mass action in nature. Units in these equations are KHaCs in day⁻¹ times Ha in kilogram times cMiHa in kg/kg = kg/day. The premise is that once a microbe is associated with a particle, substrate concentration is saturating and, thus, the rate of hydrolysis is a function of hydrolytic capacity (kHaCs) per unit of microbes in association with the substrate. Also, the equation above for the association and release (HaMiR and HaMiF) from particulate substrates sets a maximum for associated microbes (MiHaMi) dependent upon the amount of substrate available. Thus, the equations are mass action in form but are highly constrained such that implicit maximal rates of hydrolysis are specified.'

$$\text{RDCHa} = 1.0 - \text{HaP}/\text{FDStin}$$

$$\text{Ha} = \text{INTEG}(\text{DHa}, \text{iHa})$$

'Holocellulose (Hb = Hc + Ce; Hc + Ce in kg) hydrolysis and passage'

Rates of hemicellulose and cellulose hydrolysis vary with type of forage. The following procedural allows for adjustment of KHcCs1 and KCeCs1 to grasses, legumes and corn silages. The default setting is for legumes.'

$$\text{CONSTANT Grass} = 0.0, \text{Legume} = 1.0, \text{CornSi} = 0.0, \text{GKCeCs} = 9.0$$

$$\text{CONSTANT GKHCs} = 7.0, \text{LKCeCs} = 6.0, \text{LKHcCs} = 6.0, \text{CKCeCs} = 9.0$$

$$\text{CONSTANT CKHCs} = 9.0$$

$$\text{PROCEDURAL} (\text{KCeCs1}, \text{KHcCs1} = \text{Grass}, \text{Legume}, \text{CornSi}, \text{GKCeCs}, \dots$$

$$\text{GKHCs}, \text{LKCeCs}, \text{LKHcCs}, \text{CKCeCs}, \text{CKHCs})$$

$$\text{IF}(\text{Grass.EQ.1.0}) \text{GO TO 14}$$

```

IF(Legume.EQ.1.0)
GO TO 15 IF(CornSi.EQ.1.0) GO TO 16
'Grass'
14.. KCeCs1 = GKCeCs $ KHcCs1 = GKHCs
GO TO 17
'Legume'
15.. KCeCs1 = LKCeCs $ KHcCs1 = LKHcCs
GO TO 17
'CornSi'
16.. KCeCs1 = CKCeCs $ KHcCs1 = CKHCs
GO TO 17
17.. Continue
END

```

\$ 'OF PROCEDURAL'

'The following equations were devised to accommodate RpH effects on fiber digestion. The equation should probably be sigmoidal from pH 7.0 to pH 5.5, with the steepest decrease between 6.2 and 5.5; but, there are not enough data to fit that form.'

```

PROCEDURAL (KHcCs, KCeCs = RPH, KHcCs1, KCeCs1)
KHcCs = KHcCs1 $ KCeCs = KCeCs1
IF(RpH.GE.6.2) GO TO 22
KHcCs = KHcCs - (KHcCs * 1.875 * (6.2 - RpH))
KHcCs = AMAX1(KHcCs,0.0)
KCeCs = KCeCs - (KCeCs * 1.875 * (6.2 - RpH))
KCeCs = AMAX1(KCeCs,0.0)
GO TO 22

```

22.. CONTINUE

END \$ 'OF PROCEDURAL'

'The effects of added dietary fat on organic matter digestibility depicted below were added 12/90 but are very tentative, as the linear slope was derived from $\pm$ fat data.'

CONSTANT KfatHb = 0.06

```

DHc = Hcin + LpHcHc - SpHcCs - HcP
RHcin = fDHc * FDDMIN
Hcin = RHcin * PSF
LpHcHc = LpSp * fLpHc
SpHcCs = KHcCs * (1 - (FDFAT/fDLi * KFATHb)) * Hc/Hccor * cMiHb
HcP = KSPP * Hc/Hccor
RDCHc = 1.0 - HcP/RHcin
Hc = INTEG(DHc,IHc)
DCe = Cein + LpCeCe - SpCeCs - CeP
RCein = fDCe * FDDMIN
Cein = RCein * PSF
LpCeCe = LpSp * fLpCe
SpCeCs = KCeCs * (1 - (FDFAT/fDLi * KFATHb)) * Ce/Cecor * cMiHb
CeP = KSPP * Ce/Cecor
RDCCe = 1.0 - CeP/RCein
Ce = INTEG(DCe,ICe)
DHb = Hbin + LpHbHb - SpHbCs - HbP
Hbin = Cein + Hcin
LpHbHb = LpCeCe + LpHcHc
SpHbCs = SpCeCs + SpHcCs

```

$$\text{RDCHb} = 1.0 - \text{HbP}/(\text{FDDMIN} * (\text{fDHc} + \text{fDCe}))$$

$$\text{Hb} = \text{INTEG}(\text{DHB}, \text{IHb})$$

Insoluble protein (Pi in kg) hydrolysis and passage

$$\text{CONSTANT KPiAa} = 7.0, \text{KFATPi} = 0.03$$

'KPiAa is probably a variable across feedstuffs and would be a variable if there were data to formulate an appropriate equation. Tentative effects of added dietary fat on protein degradability were added in 12/90. This effect is poorly supported.'

$$\text{DPi} = \text{FDPiPi} + \text{LpPiPi} - \text{SpPiAa} - \text{PiP}$$

$$\text{FDPiPi} = \text{fDPi} * \text{FDDMIN} * \text{PSF}$$

$$\text{LpPiPi} = \text{LpSp} * \text{fLpPi}$$

$$\text{SpPiAa} = \text{KPiAa} * (1 - (\text{FDFAT}/\text{fDLi} * \text{KFATPi})) * \text{Pi}/\text{Picor} * \text{cMiSp}$$

$$\text{PiP} = \text{KSPP} * \text{Pi}/\text{Picor}$$

$$\text{TPRTin} = (\text{fDPs} + \text{fDPi} + \text{fDNn}) * \text{FDDMIN}$$

$$\text{RDCPRT} = (\text{TPRTin} - \text{PiP} - (\text{RAaP} * \text{MWAA}))/\text{TPRTin}$$

$$\text{Pi} = \text{INTEG}(\text{DPi}, \text{IPi})$$

Lignin and insoluble ash (Ot in kg) passage

$$\text{DOt} = \text{LpOt} + \text{fDOt} - \text{OtP}$$

$$\text{LpOt} = \text{LpSp} * \text{fLpOt}$$

$$\text{FDOt} = \text{FDDMIN} * \text{fOt} * \text{PSF}$$

$$\text{OtP} = \text{KSPP} * \text{Ot}/\text{OTcor}$$

$$\text{Ot} = \text{INTEG}(\text{DOt}, \text{IOt})$$

(g) Soluble pools

Soluble carbohydrates (Sc in kg; Cs in Moles)

$$\text{CONSTANT VCsFv} = 1000, \text{KCsFv} = 0.009$$

$$\text{DCs} = \text{ScTCs} + \text{StCs} + \text{HaCs} + \text{HcCs} + \text{CeCs} - \text{CsFv} - \text{CsMi} - \text{CsP}$$

$$\text{cCs} = (\text{Cs}/\text{Cscor})/(\text{RLv}/\text{RLvcor})$$

'These equations convert kg of carbohydrates to moles of hexose equivalents.'

$$\text{ScTCs} = \text{FDSCT} * \text{FDDMIN}/\text{MWSc}$$

$$\text{StCs} = \text{FDStCs}/\text{MWSt}$$

$$\text{HaCs} = \text{SpHaCs}/\text{MWSt}$$

$$\text{HcCs} = \text{SpHcCs}/\text{MWHc} * 0.833$$

$$\text{CeCs} = \text{SpCeCs}/\text{MWCe}$$

$$\text{CsFv} = \text{VCsFv} * \text{WaMi}/(1.0 + \text{KCsFv}/\text{cCs})$$

'Note that all equations associated with the fermentation and utilization of soluble nutrients are of the Michaelis-Menten type in form. In a number of cases, including soluble carbohydrates (Cs), amino acids and peptides (Aa), concentrations of the nutrients in rumen fluids are far below the apparent affinities of microbes for the nutrient and, thus, mass action equations would serve equally well under most conditions. Two observations led to adoption of the equation form used: there are and have been experiments run using diets and conditions where concentrations of soluble nutrients have been high enough to approach saturation (see Argyle and

Baldwin, 1989, for examples). Some of these have been adequate to parameterize non-linear equations. In general, equations depicting saturation kinetics are more stable and help to avoid aberrant solutions that are sometimes associated with the use of mass action kinetics.'

$$\text{CsMi} = \text{MiG} * (\text{CsMiG1} * \text{G1} + \text{CsMiG2} * \text{G2})$$

$$\text{CsP} = \text{KWAP} * \text{Cs/Cscor Cs} = \text{INTEG}(\text{DCs,ICs})$$

Amino acid (RAa in moles) metabolism

$$\text{CONSTANT VRAaFv} = 407.0, \text{cSaPs} = 0.002, \text{KRAaFv} = 0.0064$$

$$\text{DRAa} = \text{FDPsAa} + \text{PiAa} + \text{SaPsAa} - \text{RAaFv} - \text{RAaMi} - \text{RAaP}$$

$$\text{cRAa} = (\text{RAa/RAacor})/(\text{RLv/RLvcor})$$

$$\text{FDPsAa} = \text{fDPS} * \text{FDDMIN/MWPs}$$

$$\text{PiAa} = \text{SpPiAa/MWPs}$$

$$\text{RAaP} = \text{KWAP} * \text{RAa/RAacor}$$

$$\text{SaPsAa} = \text{cSaPs} * \text{SAin}$$

$$\text{RAaFv} = \text{VRAaFv} * \text{WaMi}/(1.0 + \text{KRAaFv/RAa})$$

$$\text{RAaMi} = \text{MiG} * \text{AaMiG2} * \text{G2}$$

$$\text{RAa} = \text{INTEG}(\text{DRAa,IRAa})$$

Ammonia (Am in moles) metabolism

$$\text{CONSTANT NnAmAM} = 3.8, \text{AaFvAM} = 1.325, \text{KAmabs} = 12.4, \text{UrAmAM} = 2.0$$

$$\text{CONSTANT VPUNAm} = 5.67\text{E} - 2, \text{KPUNAm} = 0.007, \text{KIAm} = 0.003$$

$$\text{DAm} = \text{FDNnAm} + \text{AaAm} + \text{SaNnAm} + \text{PUNAm} - \text{absRAm} - \text{AmMi} + \text{FdUrAm}$$

$$\text{FDUrAm} = \text{fDUR} * \text{FDDMIN} * \text{UrAmAM/MWUr}$$

$$\text{cAm} = (\text{Am/Amcor})/(\text{RLv/RLvcor})$$

$$\text{cPUN} = (\text{PUN/PUNcor})/\text{PUNvol}$$

$$\text{FDNnAm} = \text{fDNn} * \text{FDDMIN} * \text{NnAmAM/WNn}$$

$$\text{AaAm} = \text{RAaFv} * \text{AaFvAm}$$

$$\text{SaNnAm} = \text{cPUN} * \text{SAin} * \text{UrAmAM}$$

$$\text{PUNAm} = (\text{VPUNAm} * (\text{EBW} * * 0.75)/(1.0 + \text{KPUNAm/cPUN} + \text{cAm/KiAm})) * \text{UrAmAM}$$

'Pun transport across rumen wall inhibited by Am.'

$$\text{absRAm} = \text{KAmabs} * \text{Am/Amcor}$$

$$\text{AmMi} = \text{MiG} * (\text{AmMiG1} * \text{G1} + \text{AmMiG2} * \text{G2}) \text{ Am} = \text{INTEG}(\text{DAm,iAm})$$

Soluble ash (As in kg)

$$\text{CONSTANT fSaAs} = 0.0085, \text{KAsabs} = 58.0$$

$$\text{DAs} = \text{FDAsAs} + \text{SaAs} - \text{AsP} - \text{absRAs}$$

$$\text{FDAsAs} = \text{fDAs} * \text{FDDMIN}$$

$$\text{SaAs} = \text{fSaAs} * \text{SAin}$$

$$\text{AsP} = \text{As/Ascor} * \text{KWAP}$$

$$\text{absRAs} = \text{KAsabs} * \text{cAs}$$

$$\text{cAs} = (\text{As/Ascor})/(\text{RLv/RLvcor})$$

$$\text{As} = \text{INTEG}(\text{DAs,IAAs})$$

Lipids (Fl,Fa in moles)

$$\text{CONSTANT FDLiFl} = 1.8, \text{FDLiCh} = 0.133, \text{FDFaFl} = 3.0$$

$$\text{DFI} = \text{FDFI} + \text{FDFI1} - \text{FIMi} - \text{FaP}$$

$$\text{FDFI} = \text{fDLi} * \text{FDDMIN/MWFILi} * \text{FDLiFl}$$

$$\text{FDFI1} = \text{FDFAT} * \text{FDDMIN} * \text{FDFaFl/MWFIa}$$

$$\text{FIMi} = \text{MiG} * \text{FIMiG}$$

FaP = KWAP * FI/Flcor
 FI = INTEG(DFI,IFI)

Volatile fatty acids and lactate (RAc,RPr,RBu,RLa in moles)

'Rate constants for absorbtion of all VFA may not be equal as assumed here.
 KabsLa is set low to allow maximal La fermentation and create acidosis if
 desired, but the rate constants must be considered to be ill-defined.'

CONSTANT KabsAc = 10.5,KabsPr = 10.5,KabsBu = 10.5,KabsLa = 0.1

'ACETATE'

DRAc = FDFvAc + CsAc + RAaAc + RLAc - absRAc - RAcP

FDFvAc = fDAc * FDDMIN/MWAc

CsAc = CsFv * CsFvAc

RLAc = RLafv * LaAcAc

CsFvAc = ScAc * fScCs + StAc * fStCs + HcAcAc * fHcCs + CeAcAc * fCeCs

fScCs = ScTCs/Csin

fStCs = (StCs + HaCs)/Csin

fHcCs = HcCs/Csin

fCeCs = CeCs/Csin

RAaAc = AaFvAc * (RAaFv + (0.76 * FDNnAm))

absRAc = KabsAc * RAc/RAccor

cRAc = (RAc/RAccor)/(RLv/RLvcor)

RAcp = (RAc/RAccor) * KWAP

RAc = INTEG(DRAc,IRAc)

MPAc = (RAc/RAccor)/TVFA

'PROPIONATE'

DRPr = CsPr + RAaPr + RLAPr - absRPr - RPrP CsPr = CsFv * CsFvPr

RLAPr = RLafv * LaPrPr

CsFvPr = ScPr * fScCs + StPr * fStCs + HcPrPr * fHcCs + CePrPr * fCeCs

RAaPr = AaFvPr * (RAaFv + (0.76 * FDNnAm))

RPrp = (RPr/RPracor) * KWAP

cRPr = (Rpr/RPracor)/(RLv/RLvcor)

absRPr = KabsPr * RPr/RPracor

RPr = INTEG(DRPr,IRPr)

MPPr = (RPr/RPracor)/TVFA

'BUTYRATE'

DRBu = CsBu + RAaBu + FDFvBu - absRBu - RBuP

CsBu = CsFv * CsFvBU

CsFvBU = ScBu * fScCs + StBu * fStCs + HcBuBu * fHcCs + ...

CeBuBu * fCeCs

RAaBu = AaFvBu * (RAaFv + (0.76 * FDNnAm))

FDFvBu = fDBu * FDDMIN/MWBu

absRBu = KabsBu * RBu/RBucor

RBuP = (RBu/RBucor) * KWAP

cRBu = (RBu/RBucor)/(RLv/RLvcor)

RBu = INTEG(DRBu,IRBu)

MPBu = (RBu/RBucor)/TVFA

'LACTATE'

CONSTANT KLaFv = 0.5

DRLa = CsLa + FDFvLa - RLAP - absRLa - RLafv

CsLa = CsFv * CsFvLa

CsFvLa = ScLa * fScCs + StLa * fStCs \$'assumes no Hc,Ce,Aa, go to La'

FDFvLa = FDDMIN * fDLA/MWL

RLAP = (RLa/RLacor) * KWAP

$$\begin{aligned} \text{cRLa} &= (\text{RLa}/\text{RLacor})/(\text{RLv}/\text{RLvcor}) \\ \text{RLaFv} &= \text{kLaFv} * \text{CMiWA} * \text{RLa}/\text{RLacor} \\ \text{absRLa} &= \text{RLa} * \text{KabsLa}/\text{RLacor} \\ \text{RLa} &= \text{INTEG}(\text{DRLa}, \text{IRLa}) \end{aligned}$$

(h) Microbial growth and passage

'Early dynamic models of rumen digestion such as those of Waldo et al. (1972) and Nolan (1975) were restrictive, in that they considered either carbohydrate fermentation and passage alone or nitrogen metabolism alone. As a result, when applying the models of carbohydrate fermentation and passage, an implicit assumption was that nitrogen availability was adequate; when models of nitrogen metabolism were used, the implicit assumption was that energy (from carbohydrate) was not limiting or limiting to a constant extent. Yet these models were very useful in describing and comparing individual data sets, e.g. do these diets differ because fermentation rates differ or because passage rates differ, etc. When interactions occur across diets or between energy and nitrogen availabilities, microbes must be considered explicitly in the model, because effects of nitrogen availability upon carbohydrate fermentation are mediated, in large part, via effects upon microbial growth and vice versa.'

'There are about 20 prominent microbial species in the rumen. Interactions among these are very complex and have not been modeled, probably because of an untested presumption that current concepts and data are not adequate to support such a modeling analysis. Lacking a detailed modeling analysis of the rumen ecosystem to guide the formulation of simplified representations of microbial functions in models of the ruminant digestive process, a number of alternative approaches to depict microbial growth and function have been proposed. The simplest approach was to consider the microbes in aggregate as one multifunctional group (Beever et al., 1981; France et al., 1982). The assumption that underlies this approach is that the primary cause of variation in the microbial population is diet composition. Further, this approach implies that the primary or most important result of changes in the microbial population at this level of aggregation is the effect upon products of fermentation. The analyses of Murphy et al. (1982a) were undertaken to support implementation of this approach. A problem associated with this approach has been that when a highly available carbohydrate source such as starch was provided in the diet, the increase in microbial mass results in unacceptably high rates of hemicellulose and cellulose digestion (Baldwin et al., 1987b). This led to development of the equations presented above to largely restrict the capacity for hydrolysis of starch, hemicellulose and cellulose to microbes that grew in association with each respective substrate. This approach also produces a lag (while associated microbes proliferate) in hydrolysis of insoluble substrates similar to that which occurs in reality.

This current representation is simpler than the conceptually similar approach utilized by Baldwin et al. (1977) and Murphy et al. (1986), where four differential equations for each insoluble substrate were required. With this number of equations, parameter values could not be defended on the basis of available data. A third approach, which was utilized by Baldwin et al. (1970) and more recently by Fox et al.

(1988) and Beever et al. (1981), is to explicitly subdivide the microbial population into groups such as those that ferment soluble carbohydrates, starch and holocellulose. When associated and free populations of these groups are represented, rigorous parameterization, again, becomes a problem. An associated problem discussed by Baldwin et al. (1970) was deciding what proportion of hydrolytic products formed from particulate substrates are fermented by the amylolytic and cellulolytic populations and what proportion by the soluble carbohydrate fermenters. Also, specification of a specific set of stoichiometric coefficients for each of the three sub-groups is not adequate, because the mix of microbes in each group vary with diet. The analyses of Murphy et al. (1982a) and other data discussed in Chapter 8 clearly indicate that this is true. In the face of these uncertainties and complexities, we believe that the modified single population represented herein is in keeping with the degree of aggregation required by our objectives and our ability to parameterize the equations based upon available data. Changes or additions will be required when data and modeling analyses indicate these are needed. This necessity has not yet been encountered.'

'Estimates of microbial growth require, at a minimum, specification of microbial composition (a known variable considered as a constant herein), precursors of each component, ATP yields from fermentation, microbial maintenance requirements and factors that influence the net efficiency of microbial growth.'

'MICROBIAL COMPOSITION (kg/kg)'

'NOTE: Organic Matter only. These values are from Reichl and Baldwin (1975).'

CONSTANT MiPiPi = 0.572, MiNnNn = 0.095, MiHaHa = 0.212, MiLiLi = 0.121

'Lipid composition in mole/mole'

CONSTANT MiLiFa = 1.2, MiLiBu = 0.5, MiLiPr = 1.0, MiLiCh = 0.8, MiLiG1 = 0.5

MWMiLi = 0.632 \$ HMiLi = 4.53 * F1

'Two alternative sets of stoichiometric factors for growth are considered: G1 in which amino acids and peptides are not used; and, G2 in which up to one-half of microbial protein can be derived from amino acids and peptides dependent on their availability.'

CONSTANT CsMiG1 = 6.133, AmMiG1 = 7.33, HyMiG1 = 2.71, CdMiG1 = 0.518

CONSTANT FIMiG = 0.23

CONSTANT CsMiG2 = 2.133, AmMiG2 = 1.12, KYATAa = 0.0001, AaMiG2 = 4.97, ...

HyMiG2 = -0.42, CdMiG2 = -0.05

G1 = 1.0 - G2

G2 = 0.5/(1.0 + KYATAa/cRAa)

PROCEDURAL (MiMaAd = RPH) \$ 'Effect of pH on the microbial maintenance requirement.'

MiMaAd = 20 \$ 'moles/kg/day'

IF(RPH.GE.6.2) GO TO 26

IF(RPH.LE.5.4) GO TO 25

MiMaAd = MiMaAd + (MiMaAd * ((0.8 - (RPH - 5.4))/(0.8))

GO TO 26

25.. MiMaAd = 40

26.. CONTINUE

END

\$ 'OF PROCEDURAL'

CONSTANT RYATP = 0.013, KFgAm = 0.0012, LaFvAT = 1.0

CONSTANT KFATFg = 0.03

'Microbial growth (MiG) is a function of ATP derived from fermentation (ATPF), ATP used in maintenance functions (ATPM) and modifiers of the efficiency of use of ATP available for growth including YATP, which is a function of amino acid availability, ammonia concentration and dietary fat.'

$MiG = MiG - MiP$

$MiG = ATPG * YATP * FgAm * FgFa$

$ATPG = ATPF - ATPM$

$ATPF = CsFv * CsFvAT + RAaFv * AaFvAT + 0.76 * FDNnAm * AaFvAT + RLaFv * LaFvAT$

$ATPM = Mi/Micor * MiMaAD$

$FgAm = 1.0 / (1.0 + KFgAm/cAm)$

$FgFa = 1 + (FDFAT/fDLi * KFATFg)$

$YATP = 0.012 + RYATP / (1.0 + KYATAa/cRAa)$

$YATPAP = MiG/ATPF$

$MiP = SpMiP + WAMiP$

$SpMiP = KSPP * SpMi$

$WAMiP = KWAP * WAMi$

'Distribution of microbes among water, Sp and Lp is dependent upon fractions of total dry matter in each pool.'

$LpMi = ((Lp/Lpcor)/(RDM - Mi)) * Mi/Micor$

$SpMi = (Sp/(RDM - Mi)) * Mi/Micor$

$WAMi = (Mi * SOLDM/(RDM - Mi))/Micor$

$cMiSp = SpMi/Sp$

$cMiWa = WAMi/SOLDM$

$Mi = INTEG(DMi, iMi)$

(i) Methane production

CONSTANT AaFvHY = 1.14

$DTCH4 = DDCH4$

$DCsFvH = CsFvAc * 2.0 - CsFvPr * 1.0 + CsFvBu * 2.0$

$DCsHy = CsFv * DCsFvH$

$DRAaHy = RAaFv * AaFvHy$

$DHyMi = HyMiG1 * G1 + HyMiG2 * G2$

$DHyFIF = FDFI * 2.0$

$DTHy = DCsHy + DRAaHy - DHyMi - DHyFIF + 2.0 * RLaAc - RLaPr$

$DDCH4 = DTHy/4.0$

(j) Lower gut digestion (LG) equations are basically defined by digestion and fermentation coefficients input by user

CONSTANT LGDCHa = 0.7, DCMiPi = 0.75, DCMiLi = 0.7, LGDCHb = 0.1

CONSTANT LGDCPi = 0.4, LGDCAs = 0.85, LGDcAi = 0.10, LGDCFa = 0.9

$LGHaGl = HaP * LGDCHa/MWSt$

$MiGl = MiP * MiHaHa * LGDCHa/MWSt$

$MiAa = MiP * MiPiPi * DCMiPi/MWPi$

$MiLiDg = MiP * MiLiLi * DCMiLi/MWMLi$

$MiFa = MiLiDg * MiLiFa$

$LGFaDg = LGDCFa * FaP$
 $MiBu = MiLiDg * MiLiBu$
 $MiPr = MiLiDg * MiLiPr$
 $MiLGl = MiLiDg * MiLiGl$
 $MiCh = (MiP * MiLiLi/MWMLi) * MiLiCh$
 $LGHcFv = HcP * LGDChb/MWHc * 0.833$
 $LGHcAc = LGHcFv * HcAcAc$
 $LGHcPr = LGHcFv * HcPrPr$
 $LGHcBu = LGHcFv * HcBuBu$
 $LGCEfV = CeP * LGDChb/MWCE$
 $LGCEAc = LGCEfV * CeAcAc$
 $LGCEPr = LGCEfV * CePrPr$
 $LGCEBu = LGCEfV * CeBuBu$
 $LGPIAa = PiP * LGDCPi/MWPI$
 $LgAs = LgDcAs * AsP$
 $LgAi = LgDcAi * OtP * fDAi/fOt$

(k) Computation estimates of rumen and overall digestion coefficients and energetic relationships

'FECES'

$FEHa = HaP * (1.0 - LGDCHa)$
 $FEHb = HbP * (1.0 - LGDCHb)$
 $FEHc = HcP * (1.0 - LGDCHb)$
 $FECE = CeP * (1.0 - LGDCHb)$
 $FMiPi = MiP * MiPiPi * (1.0 - DCMiPi) \$KG.$
 $FMinN = MiP * MinNn$
 $FMiLi = MiP * MiLiLi * (1.0 - DCMiLi)$
 $FEFa = FaP * (1 - LGDCFa) * MWFI$
 $FEPI = PiP * (1.0 - LGDCPi)$
 $FEASH = AsP * (1.0 - LGDCAs) + OtP * fDAi/fOt * (1.0 - LgDcAi)$
 $FEMiHa = MiP * MiHaHa * (1.0 - LGDCHa)$
 $FELg = OtP * fDLg/fOt$
 $FEPT = FMiPi + FMinN + FEPI$
 $FECh = ((fDLi * FDDMIN/MWFDLi) * FDLiCh) * MWCh + MiCh * DCMiLi * MWCh$
 $FEOM = FEHa + FEHb + FEPT + FMiLi + FELg + FECh + FEMiHa + FEFa$
 $FEENG = (FEHa * 4.154 + FEHb * 4.154 + (FMiPi + FEPI) * 5.7 + FMiLi * 7.2 + ...$
 $FELg * 8.3 + FECh * 3.31 + FMinN * 5.7 + FEMiHa * 4.154 + FEFa * 9.53) * F1$
 $FEDM = FEOM + FEASH$
 $SOLDMP = SOLOMP + AsP$
 $TOMP = SOLOMP + HaP + HbP + PiP + OtP * fLgOt$
 $TTOMP = TOMP + MiP \$ 'RUMEN DIGESTION COEFFICIENTS'$
 $RDCOM = 1.0 - TOMP/FDOMIN \$ 'FOR TRUE ORGANIC MATTER'$
 $RDCOMa = 1.0 - TTOMP/FDOMIN \$ 'FOR APPARENT ORGANIC' MATTER$
 $TStin = FDDMIN * FdSt$
 $DCHa = (TStin - FEHa)/TStin$
 $DCHb = (Hbin - FEHb)/Hbin$
 $DCLg = ((fDLg * FDDMIN) - FELg)/(fDLg * FDDMIN)$
 $DCPRT = (TPRTin - FEPT)/TPRTin$
 $DCOM = (FDOMIN - FEOM)/FDOMIN \$ 'DIGESTION COEFFICIENTS'$
 $DCDM = 1.0 - FEDM/FDDMIN \$ 'FOR: ORGANIC AND DRY MATTER'$
 $'Computation of digestion coefficients for energy and energy terms.'$
 $FDGEin = FDGE * FDDMIN + INFPRT * 5.7$

```

AccGEI = INTEG(FDGEin,1.0E - 8)
TDE = absE/FDGEin
appDE = (FDGEin - FEENG)/FDGEin $ 'APPARENT DIGESTIBLE ENERGY'
DEI = FDGEin - FEENG $ 'DIGESTIBLE ENERGY INTAKE'
DE = DEI/FDDMin $ 'DIGESTIBLE ENERGY'
AccDEI = INTEG(DEI,1.0E - 8)
CH4E = DTCH4 * HCH4 $ 'APPARENT AND CORRECTED'
EUR = DUREA * HcUR $ 'METABOLIZABLE ENERGY'
MEI = (FDGEin - CH4E - EUR - FEENG)
AccMEI = INTEG(MEI,1.0E - 8)
ME1 = MEI/FDDMIN
GE = FDGEin/FDDMIN
HFERM = FDGEin - absE - FEENG - CH4E - EUR
corMEI = MEI - HFERM
corME = corMEI/FDDMIN

```

16.3.5 Interface of digestion and animal elements

'This section computes the absorbtion of nutrients from gut in moles. This is the input to the animal model.'

```

absGl = LGHaGl + CsP + MiGl + MiLGl
absAa = MiAa + LGPiAa + RAaP + INFPRT/0.110
absAc = absRAc + LGHcAc + LgCeAc + RAcP
absPr = absRPr + MiPr + LGHcPr + LgCePr + RPrP
absBu = absRBU + LGHcBu + LgCeBu + MiBu + RBuP
absAm = absRAM
absFa = (MiFa + LGFaDg) * MWFI/MWFa

```

'The ratio MWFI/MWFa converts stearate (FI) from gut to palmitate in animal.'

```

absAs = absRAs + LgAs + LgAi
absLa = absRLa + RLaP
absAcE = absAc * HCAc
absPrE = absPr * HCP
absBuE = absBu * HCBu
absFaE = absFa * HCFa
absAaE = absAa * HCAa
absGLE = absGl * HCGl
absLaE = absLa * HCLa
AbsE = absAcE + absPrE + absBuE + absFaE + absAaE + absGLE + absLaE

```

16.3.6 Dynamic elements of the animal submodel

'A basic premise underlying formulation of the animal element of the lactating cow model was that efficiencies of utilization of the specific absorbed nutrients inherently differ as discussed in Chapters 5 and 6. Thus, in keeping with our modeling objective, it was essential the animal submodel consider specific chemical entities and their respective rates of utilization for alternative processes explicitly (Baldwin et al., 1987a).'

'A number of simplifying assumptions were made to keep the model simple and achieve reasonable solution speeds with then current computers. All absorbed

butyrate was assumed to be oxidized. Ketone bodies are not represented and, thus, the model cow cannot become ketotic (we have a version in which ketosis can occur). Minerals and micronutrients are not represented, and thus their availability is assumed to be adequate. When this is not so, the model will fail to simulate experimental data. Similarly, amino acids are considered in aggregate, with the result that responses to experimental manipulations of the balance of amino acids supplied, e.g. administration of rumen-protected lysine and methionine, cannot be simulated (again, there is a current version with this capacity). State variables, transactions and reference fluxes are presented in Fig. 16.2. Bases for initial calculations presented as comments in the model refer to the initial model version for the reference cow (Chapter 13). Some drift from these conditions has occurred over the years but, in general, original and current values are quite close. As above, reasons for significant changes from the original and added comments are presented in quotes.'

'A significant change from the original was changing many $V_{\max}$ specifications from constants to variables ($V_{\max}F * EBW^{0.75}$) so as to allow for a range of initial empty body weights.'

(a) Mammary gland elements

'Several inputs to this section were presented and discussed as initial conditions in the initial section.'

```

CONSTANT MLKINT = 1.0, MLTM = 1.0 $ 'Milking'
PROCEDURAL (KMILK = MLKINT, MLTm, TIME)
KMILK = 0.0
IF (AMOD (TIME, MLKINT).LE. MLTm) GO TO 50
GO TO 51
50.. KMILK = 2.91/UMLKcr
GO TO 51 51.. CONTINUE
END                                     $ 'OF PROCEDURAL'
DLHOR = - KLHOR * LHOR $ 'LACTATION HORMONE'
LHOR = INTEG (DLHOR, ILHOR)
DUENZ = Usyn - Udeg $ 'UDDER ENZYMES'
Usyn = VUsyn * Ucells * LHOR * BST / (KUsyn + LHOR * BST)
Udeg = UENZ * (KUdeg + KUdegM * ((UMave/KMdeg) ** THETA5 / (1.0 + ...
UMave/KMdeg) ** THETA5))
DUMave = TaveM * (UMILK - UMave) $ 'RETAINED MILK EFFECTS'
UMave = INTEG (DUMave, IUMave)
KMinh = (MLKmax - UMILK) / (MLKmax - UMILK + KMLKI)
UENZ = INTEG (DUENZ, IUENZ)

```

(b) Anabolic and catabolic hormones

```

AHOR = (cGI/IcGI) ** theta2
AHOR1 = (cGI/IcGI) ** theta4
CHOR = (IcGI/cGI) ** theta3
CHOR1 = (IcGI/cGI) ** theta4

```

'Metabolic hormones such as insulin, glucocorticoids, glucagon, catecholamines, etc., clearly fulfil a central role in the coordination of animal metabolism in rumi-

nants as well as non-ruminant species. Specific roles for many of the metabolic hormones have been well studied in other species, particularly rodents, but not ruminants. Studies with ruminants have been adequate with some notable exceptions, to assure that mechanisms of action of the metabolic hormones are the same as those observed in rats, but also have shown that the magnitude of responses in ruminants are very low relative to rodents. For example, respective maximum responses of ruminant adipocytes to insulin, glucagon, and catecholamines are 1.3-, 2.0- and 2.0-fold as compared to 4.0-, 8.0- and 6.0-fold in rodents (Yang and Baldwin, 1973a,b; Vernon, 1980). Acute effects of glucagon upon rates of gluconeogenesis in ruminant hepatocytes were marginal (Looney et al., 1987) and chronic effects of adrenalectomy on rates of gluconeogenesis in sheep liver were absent (Ely and Baldwin, 1976), while gluconeogenic responses in rats to glucocorticoids and gluconeogenesis are very prominent. Since rates of gluconeogenesis are maximal in fed ruminants and in fasted rats, one might expect species differences. Effects of glucocorticoid insufficiency on rat mammary glands are highly significant (Louis and Baldwin, 1975) and absent in sheep mammary glands (Ely and Baldwin, 1976).'

'These observations clearly indicate that additional data on the actions of metabolic hormones in ruminants are required, as current data are not adequate to support the development of detailed models of metabolic hormone actions in ruminants and, certainly, require that their actions be represented very simply in ruminant metabolism at the whole-animal level. These observations led to two decisions about how metabolic hormones function in this model. The first was that the metabolic hormones be represented in two aggregate groups – anabolic (AHOR) and catabolic (CHOR). The second was that both homeostatic and homeorhetic processes operate to assure that adequate nutrient supplies be available to tissues for performance of their functions. Glucose is a prominent indicator of such adequacy and thus could be used to drive the relative signals for anabolic and catabolic responses as in the equations below.'

(c) Lipid metabolism (Fa, Ts)

'Inputs to the storage triacylglyceride pool (Ts) are fatty acid esterification ($FaTsF * FaTsTS = 6 * 0.33 = 2.0$) and lipogenesis from acetate ($AcTs * AcTsTS = 16 * 0.042 = 0.667$). Output is lipolysis ($TsFaF = 2.667$). Inputs to the fatty acid plus triacylglyceride pool (Fa) are absorption ($absFa = 1.0$) from gut and lipolysis ($TsFaF * TsFaFA = 2.667 * 3 = 8.0$). Outputs are fatty acid (re)esterification ($FaTsF = 6.0$), incorporation into milk fat ($FaTmV = 1.8$) and oxidation ($FaCd = 1.2$).'

'The current equation for TsFa differs from the original, where energy balance was a major effector of lipolytic rates. The change was forced by the observation that in early lactation, when simulated, cows were in severe negative energy balance; lipolytic rates were too high, which resulted in elevated blood lipids and uptake by the udder. As a result, milk fat percentages were elevated above normal, thus exacerbating the negative energy balance. In the current equation, catabolic hormones and thyroxine can elevate the V_{max} for lipolysis. As in the original equation the high

value of THETA1 (= 5.0) assures that the concentration of triglyceride (cTs) does not become limiting until about 80% of Ts has been mobilized. The added term cFa/k1TsFa represents a negative feedback of fatty acids on lipolysis. Several observations support this effect.'

'cFa is $0.5E - 3$ and cGl is $3.0E - 3$. KFaTsF was set at $1.67E - 4$ to make cFa close to saturating and K1FaTs at $2.0E - 3$ to make the reaction responsive to Gl changes. Aggregation confounds KFaTmV and VFaTmV so these were set to produce $1/2 V_{\max}$ in the reference state.'

CONSTANT TgFaFa = 3.0, AcAcTs = 24.0, VTsFaF = $5.0E - 2$, KTsFaF = 0.2

CONSTANT theta1 = 5.0, MWTs = 0.806

CONSTANT KFaTsF = $5.0E - 4$, K1FaTs = $2.0E - 3$, K1TsFa = $5.0E - 4$

CONSTANT VFaTsF = 0.113, KFaTmV = $5.0E - 4$, VFaTmV = $0.75E - 3$

CONSTANT FaTgTg = 0.333, AcTgTg = 0.041667, K1FaTm = $1.5E - 3$

CONSTANT theta2 = 2.0, theta3 = 2.0, theta4 = 1.0, P1 = 2.0

CONSTANT EXP10 = 2.0

WTCYTF = $15.0/500 * bwf$

'STORAGE TRIACYLGLYCEROL METABOLISM'

DTsF = FaTsF1 + AcTsF1 - TsFaF

TsFaF = VTsFaF * (EBW * 0.75) * CHOR1 * T3/(1.0 + (cFa/K1TsFa) * EXP10...
+ (KTsFaF/cTs) * theta1)

cTs = TsF/WTF

FaTsF1 = FaTsF * FaTgTg

AcTsF1 = AcTsF * AcTgTg

WTF = WTCYTF + MWTs * TsF

DWTTsF = DTsF * MWTs

TsF = INTEG(DTsF, ITsF)

'PLASMA LIPID METABOLISM'

DFa = absFa + TsFaF1 - FaTsF - FaTmV - FaCd

FaTsF = VFaTsF * (EBW * 0.75)/(1.0 + KFaTsF/cFa + K1FaTs/(AHOR * cGl))

cFa = Fa/VFA

TsFaF1 = TsFaF * TgFaFa

FaTmV = (VFaTmV * UENZ * KMinh)/(1.0 + KFaTmV/cFa + K1FaTm/cGl)

Fa = INTEG(DFa, IFa)

(d) Acetate metabolism (Ac)

'Inputs are from absorbtion (absAc = 65.9) and gluconeogenesis (AaGIV * AaAcAc = $4 * 0.6 = 2.4$). Output is oxidation (AcCd = 35.5) and lipogenesis in adipose and mammary (AcTsF = 16.0, AcTmV = 16.8). AaAcAa set at 0.6 to replace 0.27 KB on an equal -P basis. Must correct final output of Cd for excess produced by this compromise. Respective Ks values for Ac and Gl in adipose and mammary are similar at 1.5–2.0 and 0.8–1.0 mM. Thus set at $1.8E - 3$ and $1.0E - 3$ for both tissues. Chronic regulation of adipose lipogenic capacity (VAcTsF) was included to stimulate effects of energy balance throughout lactation.'

CONSTANT K1VAct = 0.06, K2VAct = 0.268, IVAcTs = 0.35

DVAcTs = K1VAct * AHOR1 - K2VAct * VAcTsF

VAcTsF = INTEG(DVAcTs, IVAcTs)

CONSTANT KAcTsF = $1.8E - 3$, VAcTmV = $10.0E - 3$...

$K1AcTs = 1.0E - 3, AaGIaC = 0.6, KAcTmV = 1.8E - 3, K1AcTm = 1.0E - 3$
 $Dac = absAc + AaAcV1 - AcTsF - AcTmV - AcCd$
 $AaAcV1 = AaGIV * AaGIaC$
 $AcTsF = Vmax / (1.0 + KAcTsF/cAc + K1AcTs / (AHOR * cGI))$
 $Vmax = VAcTsF * (EBW ** 0.75)$
 $cAc = Ac/VAc$
 $AcTmV = VAcTmV * UENZ * KMinh / (1.0 + KAcTmV/cAc + K1AcTm/cGI)$
 $Ac = INTEG(DAc, IAc)$
 'MILK FAT SECRETION (UTm, DMLKTm, TMLKTm)'
 $CONSTANT MWTm = 0.806$
 $DUTm = (AcTmV1 + FaTmV1) * MWTm - DMLKTm$
 $DMLKTm = UTm * KMILK$
 $UTm = INTEG(DUTm, IUTm)$
 $TMLKTm = INTEG(DMLKTm, 1.0E - 8)$

(e) DNA accretion, not a variable in original model

'Equations adapted from DiMarco and Baldwin (1989).'

$CONSTANT ExpB2 = 1.19, KDNAb = 7.36E - 4, ExpV2 = 0.96, KDNAv = 1.7E - 4...$
 $BDNAmx = 0.112, VDNAmx = 0.110$
 $DBDNA = (KDNAb * ExpB2) * ((BDNAmx - BDNA) / BDNAmx)$
 $DVDNA = (KDNAv * ExpV2) * ((VDNAmx - VDNA) / VDNAmx)$
 $BDNA = INTEG(DBDNA, IBDNA)$
 $VDNA = INTEG(DVDNA, IVDNA)$

(f) Amino acid and nitrogen metabolism

'Amino acid metabolism (Aa) including protein turnover in Body (Pb) and Viscera (Pv). Inputs to Aa are absAa (12.6), PbAa (10.0) and PvAa (8.2). The interspecific protein degradation at rate of $18 \text{ g/BW}^{0.75}$ from Reeds (1989) indicates a rate of 2.0 kg/day or (/0.110) 18.2 moles/day in the reference animal. Data (Sainz *et al.*, 1986) on lactating rats indicate this estimate may be low. Outputs are AaPbB (10), AaPv (8.2), AaPm (8.6) and gluconeogenesis (AaGIV = 4.0). Biosynthetic reactions set at $1/2 V_{max}$ in reference state. Capacity for AaGIV is very high (five times) relative to flux in the fed (reference) state and was set there. Computation of mass in B and V assumes that protein is 25% dry matter (fractional dry weight = fdwt) and that remaining weight is constant (otwtB, otwtV). KPbAaB and KPvAaV were adjusted 05/01/91 to make equations functions of Pb and Pv rather than cPb and cPv. VAaGIV was also scaled to body size (BWF).'

'Provisions for energy use associated with pregnancy were not included in the original model. When these were added to energy requirements as in RFEED8, a provision for removal of amino acids for protein accretion at the rate of 0.05 of the fetal ME requirement (Aapreg) was added.'

$CONSTANT VAaPbB = 300, VAaPvV = 200, KAaPbB = 2.5E - 3, KAaPvV = 2.5E - 3...$
 $VAaPmV = 2.89E - 3, VAaGIV = 0.228, KAaGIV = 10.0E - 3, KPbAaB = 0.030...$
 $KPvAaV = 0.090, fDWT = 0.25, AaGIUr = 0.62, KAaPmV = 2.1E - 3, MWpB = 0.110, VSIZf$
 $= 80.0$

$$\text{OtWTB} = 103.6/500 * \text{BWF}$$

$$\text{OtWTV} = 22.2/500 * \text{BWF}$$

Amino acid metabolism and protein turnover

$$\text{DAa} = \text{absAa} + \text{PbAaB} + \text{PvAaV} - \text{AaPbB} - \text{AaPvV} - \text{AaPmV} - \text{AaGIV} - \text{SaPsAa} - \text{Aapreg}$$

$$\text{DPb} = \text{AaPbB} - \text{PbAaB}$$

$$\text{DPv} = \text{AaPvV} - \text{PvAaV}$$

$$\text{PbAaB} = \text{KPbAaB} * \text{Pb}$$

$$\text{PvAaV} = \text{KPvAaV} * \text{Pv}$$

$$\text{cPb} = \text{Pb}/\text{WTB}$$

$$\text{cPv} = \text{Pv}/\text{WTV}$$

$$\text{WTB} = \text{Pb} * \text{MWPb}/\text{fDWT} + \text{OtWTB} \text{ DWTB} = \text{DPb} * \text{MWPb}/\text{fDWT}$$

$$\text{WTV} = \text{Pv} * \text{MWPv}/\text{fDWT} + \text{OtWTV}$$

$$\text{DWTV} = \text{DPV} * \text{MWPb}/\text{fDWT}$$

$$\text{AaPbB} = \text{VAaPbB} * \text{BDNA}/(1.0 + \text{KAaPbB}/(\text{AHOR} * \text{cAa}))$$

$$\text{AaPvV} = \text{VAaPvV} * \text{VDNA}/(1.0 + \text{KAaPvV}/(\text{AHOR} * \text{cAa}))$$

$$\text{PbFsR} = \text{AaPbB}/\text{Pb}$$

$$\text{PvFsR} = \text{AaPvV}/\text{Pv}$$

$$\text{PbFdR} = \text{PbAaB}/\text{Pb}$$

$$\text{PvFdR} = \text{PvAaV}/\text{Pv}$$

$$\text{AaPmV} = \text{VAaPmV} * \text{UENZ} * \text{KMinh}/(1.0 + \text{KAaPmV}/\text{cAa})$$

$$\text{AaGIV} = \text{VAaGIV} * (\text{EBW} * * 0.75)/(1.0 + \text{KAaGIV}/(\text{CHOR} * \text{cAa}))$$

$$\text{cAa} = \text{Aa}/\text{VAa}$$

$$\text{Aapreg} = \text{FMEREQ} * 0.05/\text{HcAa}$$

$$\text{Pv} = \text{INTEG}(\text{DPv}, \text{IPv})$$

$$\text{Pb} = \text{INTEG}(\text{DPb}, \text{IPb})$$

$$\text{Aa} = \text{INTEG}(\text{DAa}, \text{IAa})$$

'AMMONIA AND UREA METABOLISM'

$$\text{CONSTANT AmUrUr} = 0.5, \text{KPUNU} = 857.1$$

$$\text{DPUN} = \text{AaUr} + \text{AmUr} - \text{PUNRAm} - \text{SaNRAm} - \text{DUREA}$$

$$\text{AaUr} = \text{AaGIV} * \text{AaGIUr}$$

$$\text{AmUr} = \text{absAm} * \text{AmUrUr}$$

$$\text{PUNRAm} = \text{PUNAm} * \text{AmUrUr}$$

$$\text{SaNRAm} = \text{SaNnAm} * \text{AmUrUr}$$

$$\text{DUREA} = \text{KPUNU} * \text{cPUN}$$

$$\text{PUN} = \text{INTEG}(\text{DPUN}, \text{IPUN})$$

'MILK PROTEIN SECRETION (UPm, DMLKPm, TMLKPm)'

$$\text{CONSTANT MWAA} = 0.133$$

$$\text{DUPm} = \text{AaPmV} * \text{MWAA} - \text{DMLKPm}$$

$$\text{DMLKPm} = \text{UPm} * \text{KMILK}$$

$$\text{UPm} = \text{INTEG}(\text{DUPm}, \text{IUPm})$$

$$\text{TMLKPm} = \text{INTEG}(\text{DMLKPm}, 1.0\text{E} - 8)$$

(g) Glucose metabolism (Gl)

'Entries are from propionate (9.84), lactate (4.05), glycerol (3.59), Aa (1.88) and absorption (4.7). Outputs are to lactose (8.3); the glycerol moiety of triacylglycerol (0.95), pentose cycle (1.47) and TpCd (2.04/2) in V; to Cd (4.3) and lactate (La, 4.9/2) in B, and to pentose cycle (2.8/2), triacylglycerol (2.667/2), La (3.2/2) and TpCd ((1.4 + 0.47)/2) in adipose tissue.'

'Reported (Forsberg *et al.*, 1985) $K_{G,LmV}$ for Gl for lactose synthesis is 8–10 mM so set at $9.0E - 3$. $NADPH_2$ required from the pentose cycle for fatty acid synthesis is calculated in terms of moles Gl (GIHy) used, since reaction rate is computed as acetate used per Ac incorporated. Stoichiometry for pentose cycle used is Gl to $3Cd + 6NADPH_2 + Tp$. Thus, the stoichiometric coefficient (GIGIHy) is calculated as $(1.75 NADPH_2/AcFa)/6NADPH_2$ per glucose used ($= 0.292$). These must be multiplied by the fraction of $NADPH_2$ generated via the pentose cycle specified as input for that tissue (fGIHyF and fGIHyV) and AcTs flux to get actual pentose cycle flux. Related calculations are of ATP equivalents of amount of $NADPH_2$ generated in the tricarboxylic acid cycle by the NADP-linked isocitrate dehydrogenase and of the reduction in oxygen consumption associated with this. These are in the oxidative metabolism section. In adipose tissue, GITpF was set to provide Tp in excess of that required for fatty acid esterification. This accommodates the possibility that Tp generation via GIHy can be less than TpTs, and provides sufficient Tp for oxidation (TpCd) and conversion to lactate (La) as has been observed in this tissue. Lactate from B and F are quantitatively converted to Gl in V. *In accord with concepts presented in Chapter 4, glycerol and lactate are considered to be zero pools in this section.*'

CONSTANT AaGIGl = 0.47, PrGIGl = 0.5, LaGIGl = 0.5, GyGIGl = 0.5...

VGILmV = $3.9E - 3$, KGILmV = $3.0E - 3$, VGITpF = $3.78E - 2$, KGITpF = $3.0E - 3$,...

VGITpV = $9.46E - 3$, KGITpV = $3.0E - 3$, fLaCdF = 0.226, KGILaB = $1.5E - 3$,...

VGILaB = $4.61E - 2$, fLaCdB = 0.246, GLGIHy = 0.292, pGIHyF = 0.6,...

pGIHyV = 0.6, fPrGl = 0.7, TgGyGy = 1.0, GILaLa = 2.0, GIHyTP = 1.0,...

GITpTp = 2.0, TpTpTs = 1.0, GIGyGy = 2.0, KAaLmV = $2.0E - 3$,...

DGI = upGl + AaGIV1 + PrGIV1 + LaGIV1 + GyGIV1 - GILmV - ...

GIHyF - GIHyV - GITpF - GITpV - GILaB - GICd

upGl = $0.10 * absGl$

AaGIV1 = AaGIV * AaGIGl

PrGIV1 = PrGIV * PrGIGl

PrGIV = absPr * fPrGl

LaGIV1 = (LaGIF + LaGIB + RLaGl) * LaGIGl

RLaGl = absRLa + RLaP + GGILa

GGILa = $0.90 * absGl * GILaLa$

GyGIV = (TsFaF + FaTmV1) * TgGyGy

GyGIV1 = GyGIV * GyGIGl

GILmV = VGILmV * UENZ * $KMinh/(1.0 + KGILmV/cGl + KAaLmV/cAa)$

cGl = Gl/VGl

GIHyF = AcTsF * fGIHyF * GIGIHy

GIHyV = AcTmV * fGIHyV * GIGIHy

'pGIHyF and pGIHyVb are not constants and should become dependent variables when appropriate equation forms are deduced using tissue models.'

fGIHyF = pGIHyF

fGIHyV = pGIHyV

GITpF = VGITpF * (EBW * 0.75)/(1.0 + KGITpF/cGl)

GITpV = VGITpV * (EBW * 0.75)/(1.0 + KGITpV/cGl)

GILaB = VGILaB * (EBW * 0.75)/(1.0 + KGILaB/cGl)

TpinF = GIHyF * GIHyTP + GITpF1

GITpF1 = GITpF * GITpTp


```

TpinV = GILHyV * GILHyTp + GITpV1
GITpV1 = GITpV * GITpTp
LainB = GILaB1
GILaB1 = GILaB * GILaLa
TpLaF = TpinF - TpTsF
TpCdV = TpinV - TpTmV
TpTsF = (FaTsF1 + AcTsF1) * TPTpTs
TpTmV = (FaTmV1 + AcTmV1) * TPTpTs
AcTmV1 = AcTmV * AcTgTg
FaTmV1 = FaTmV * FaTgTg
LaCdF = TpLaF * fLaCdF
LaGIF = TpLaF - LaCdF GIGyT = (TpTsF + TpTmV) * GyGIGI
LaCdB = LainB * fLaCdB
LaGIB = LainB - LaCdB
GI = INTEG(DGL,Igi)
'LACTOSE SECRETION (ULm, DMLKLm,TMLKLm) plus TOTAL MILK YIELD'
CONSTANT GILmLm = 0.5,MWLm = 0.342,PCLm = 0.048
DULm = GILmV * GILmLm * MWLm - DMLKLm
DMLKLm = ULm * KMILK
DMILK = DMLKLm/PcLm
ULm = INTEG(DULm,IULm)
TMLKLm = INTEG(DMLKLm,1.0E - 8)
TVMLK = TMLKLm/PcLm
UMILK = ULm/PcLm

```

(h) Oxidative metabolism (Ox,Cd)

'Computations based on energy needs expressed as rate of ADP formation (AtAd_g), oxygen (Ox) uptake calculated from P/O ratio (PO), and Ac and Fa oxidation rates from ratios of Michaelis–Menten type equations assuming V_{max} for oxidation of each are equal. See Chapter 4 for details regarding oxidative metabolism and the treatment of ATP and ADP as zero pools.'

```

CONSTANT GICdAT = 38.0,AcCdAT = 10.0,FaCdAT = 129.0,GyGIAT = 2.0,...
PrCdAT = 18.5,TpCdAT = 20.0,LaCdAT = 18.0,TpLaAT = 2.0,GILaAT = 2.0,...
BuCdAT = 25.0,HyAtAT = 3.0
CONSTANT AaPxAD = 5.0,GILHyAD = 1.0,GITpAD = 2.0,LaGIAD = 3.0, ...
TpTgAD = 9.0,AcFaAD = 2.875,TcHyAD = 5.25,GILmAD = 1.0,PrGIAD = 2.0,...
abGIAD = 1.0,abAaAD = 1.0
CONSTANT OxAcCd = 2.0,OxPrCd = 3.5,OxBuCd = 5.0,OxGICd = 6.0,...
OxLaCd = 3.0,OxTpCd = 3.0,OxFaCd = 23.0,OxPrGI = 1.0
CONSTANT AcCdCD = 2.0,PrCdCD = 3.0,BuCdCD = 4.0,GICdCD = 6.0,...
GILHyCD = 3.0,FaCdCD = 16.0,LaCdCD = 3.0,TpCdCD = 3.0

```

'Computation of effects of feed intake (absorbed energy) on basal energy expenditures. Absorbed energy is averaged (abEave) over 20 days (TavabE = 1/20). Absorbed energy factor (abEf) is expressed in units of metabolic body weight. This implements concepts introduced in Chapter 6 that basal energy expenditures vary with feed intake.'

```

CONSTANT TavabE = 0.05
DabEav = TavabE * (absE - abEave)
abEave = INTEG(DabEav,IabEav)
abEf = abEave/(EBW * 0.75)

```

$AtAd = AtAdB + AtAdF + AtAdV$
 $AdAt = AdAtB + AdAtF + AdAtV$
 $EBW1 = WTB + WTF + WTV$
 $BW1 = EBW1 + RUMvol$

ATP use and heat production in body (B)

'Energy expenditures in reference state expressed as ATP utilization are basal (294) and protein synthesis (50) for a sum of 344. Mandatory ATP generation from glucose because brain and kidney are in this element is $GILaB * GILaAT$ ($4.75 * 2.0 = 9.5$) plus $LaCdB * LaCdAT$ ($4.6 * 18 = 82.8$) for a sum of 92.3. Basal energy expenditures also vary with thyroid status.'

CONSTANT $kbasB = 2.634$, $KNaB = 0.119$, $HYAcFa = 1.75$, $AaGIH = 0.554$

$AtAdB = basalB + AtAdB1$
 $AtAdB1 = AaPbB * AaPxAD$
 $basalB = (kbasB + KNaAtB) * wtB * 0.75$
 $KNaAtB = 0.8 + KNaB * T3 * abEf$
 $AdAtB = AdAtB1 + AdAtB2$
 $AdAtB1 = GILaB * GILaAT$
 $AdAtB2 = LaCdB * LaCdAT$
 'Heat production in body'
 $basHtB = basalB * AtAdHt$
 $AaPbHt = AtAdB1 * AtAdHt$
 $MHtB = basHtB + AaPbHt$

ATP use and heat production in adipose tissue (F)

'Energy expenditures ($AtAdF = 169.2$) are $basalF$ (41.0); $GIHyF$ (2.8); $GItpF$ (4.0); $TpTsF * TpTsAD$ ($2.667 * 9$ (6 ATP to form acyl CoA plus 3 for $NADH_2$ to reduce Tp) = 24); $AcTsF * (tcHyAD * (1.0 - fGIHyF) = 16 * (5.25 * (1 - 0.6) + 2.875) = 16 * (2.1 + 2.875) = 79.6$; $tcHyAD$ is the cost of $NADPH_2$ from TCA cycle expressed in ATP per acetate ($1.75 * 3 = 5.25$); and, ATP cFa is cost in ATP/Ac converted to Fa * ($(8Ac - 8AcCoA (= 16AtAd) + 7AcCoA - 7Mal - CoA * (= 7AtAd)) * 8 = 2.875$). Oxidation of Tp yields 18.66 ATP and 6.4 ATP are generated in $TpLaF$. Reduction in oxygen uptake due to $NADPH_2$ generation in Tc is $HYAcFa * (1.0 - fGIHyF) = 1.75 * 0.4 = 0.7$ in this version.'

CONSTANT $kbasF = 1.12$, $KNaF = 0.107$
 $AtAdF = basalF + AtAdF1 + AtAdF2 + AtAdF3 + AtAdF4$
 $basalF = (kbasF + KNaAtF) * wtF * 0.75$
 $KNaAtF = 0.3 + KNaF * T3 * abEf$
 $AtAdF1 = GIHyF * GIHyAD$
 $AtAdF2 = GItpF * GItpAD$
 $AtAdF3 = TpTsF * TpTgAD$
 $AtAdF4 = AcTsF * (tcHyAD * (1.0 - fGIHyF) + AcFaAD)$
 $AdAtF = AdAtF1 + AdAtF2$
 $AdAtF1 = TpLaF * TpLaAT$
 $AdAtF2 = LaCdF * LaCdAT$
 'HEAT PRODUCTION IN ADIPOSE'
 $basHtF = basalF * AtAdHt$
 $HtF2 = AtAdF2 * AtAdHt$
 $HtF3 = AtAdF3 * AtAdHt - AcTsH4$
 $MHtF = basHtF + HtF2 + HtF3$

ATP use and heat production of viscera (V)

'Energy expenditures are basalV (339), TpTsV (* TpTsAD = 1.3 * 9 = 11.7), AcTmV (16.8 * (see F) = 83.6), AaPrV (41), AaPmV (43), GILmV (16.6), GIHyV (1.47), GITpV (0.4), LaGl (16.2), absGl (3.59) and absAa (12.6).'

'ATP use in fetal growth (AtAd16) set to equal 95% of ME intake for gestation. Five percent was removed as Aa used for protein synthesis above. Other nutrients are considered to be lost from the body in proportion to their availabilities and are accounted as heat losses in this scenario. Because of this, heat production in pregnancy is inflated by about 10% of FMERREQ.'

'ATP formation in viscera is from GyGIV (14.4), TpCdV (40.8), PrCdV (156), BuCd (245).'

CONSTANT KbasV = 3.5, KNaV = 0.20, kidwrk = 0.378, hrtwrk = 0.096, ...

reswrk = 0.3333, ATAmUr = 4.0

AtAdV = basalV + AtAdV1 + AtAdV2 + AtAdV3 + AtAdV4 + AtAdV5 + AtAdV6 + ...

AtAdV7 + AtAdV8 + AtAdV9 + AtAd10 + AtAd11 + AtAd12 + AtAd13 + AtAd14 + ...

AtAd15 + AtAd16

basalV = (kbasV + KNaAtV) * wtV * * 0.75

KNaAtV = 3.2 + KNaV * T3 * fdomin

AtAdV1 = TpTmV * TpTgAD

AtAdV2 = AcTmV * (tcHyAD * (1.0 - fGIHyV) + AcFaAD)

AtAdV3 = AaPvV * AaPxAD

AtAdV4 = AaPmV * AaPxAD

AtAdV5 = GILmV * GILmAD

AtAdV6 = GIHyV * GIHyAD

AtAdV7 = GITpV * GITpAD

AtAdV8 = LaGIV * LaGIAD

LaGIV = LaGIF + LaGIB + RIAGI

AtAdV9 = PrGIV * PrGIAD

AtAd10 = absGl * abGIAD

AtAd11 = absAa * abAaAD

AtAd12 = kidwrk * EBW * * 0.75

AtAd13 = hrtwrk * Oxup1

AtAd14 = reswrk * Oxup1

AtAd15 = (AaUr + AmUr) * ATAmUr

AtAd16 = (1.0 - effprg) * FMERREQ/AtAdHt

AdAtV = AdAtV1 + AdAtV2 + AdAtV3 + AdAtV4 + AdAtV5

AdAtV1 = GyGIV * GyGIAT

AdAtV2 = TpCdV * TpCdAT

AdAtV3 = PrCdV * PrCdAT

AdAtV4 = BuCdV * BuCdAT

PrCdV = absPr * (1.0 - fPrGl)

BuCdV = absBu

AdAtV5 = GILa * GILaAt

'Heat production in viscera'

basHtV = basalV * AtAdHt \$ 'basal'

HtV2 = AtAdV3 * AtAdHt \$ 'Protein TO'

HtV3 = AtAdV7 * AtAdHt \$ 'Gl to Tp'

HtV4 = AtAdV8 * AtAdHt \$ 'La to Gl'

HtV5 = AtAd12 * AtAdHt \$ 'kidney work'

HtV6 = AtAd13 * AtAdHt \$ 'heart work'

HtV7 = AtAd14 * AtAdHt \$ 'respiration'

HIV8 = ATAd15 * AtAdHT \$ 'urea synthesis'

MHTv = basHtV + HtV2 + HtV3 + HtV4 + HtV5 + HtV6 + HtV7 + HIV8

Oxidative metabolism of glucose, acetate and fatty acids. 'These equations implement the zero pool concept for adenine nucleotides discussed in Chapter 4.'

CONSTANT KAcCd = 2.0E - 3, KGICd = 20.0E - 3, KFaCd = 2.21E - 3

ndAt = AtAd - AdAt

ndOx = ndAt/rtPOx

rtOx1 = cGl * (cAc + KAcCd)/(cAc * (cGl + KGICd/AHOR))

rtOx2 = cFa * (cAc + KAcCd)/(cAc * (cFa + KFaCd))

GICd = ((ndOx * rtOx1)/(rtOx1 + rtOx2 + 1.0))/OxGICd

FaCd = ((ndOx * rtOx2)/(rtOx1 + rtOx2 + 1.0))/OxFaCd

AcCd = (ndOx/(rtOx1 + rtOx2 + 1.0))/OxAcCd

rtPO = (AcCd * AcCdAT + FaCd * FaCdAT + GICd * GICdAT)/(AcCd * OxAcCd...
+ FaCd * OxFaCd + GICd * OxGICd)

TcHyF = AcTsF * HyAcFa * (1.0 - fGIHyF) \$ 'correction for NADPH generated'

TcHyV = AcTmV * HyAcFa * (1.0 - fGIHyV) \$ 'by ICD in TCA cycle'

DOx = (LaCdB + LaCdF) * OxLaCd + TpCdV * OxTpCd + PrCdV * OxPrCd + ...

BuCdV * OxBuCd + AcCd * OxAcCd + FaCd * OxFaCd + GICd * OxGICd...

- TcHyF - TcHyV

'Calculation of heat equivalent of ATP. Note that HcLa and HcTp being very close to 1/2 glucose are set exactly to that so energy changes in glycolysis are not represented.'

AtHt1 = (LaCdB + LaCdF) * HcLa

AtHt2 = TpCdV * HcTp

AtHt3 = GyGIV1 * (GIgYGY * HcGy - HcGl)

AtHt4 = PrCdV * HcPr

AtHt5 = BuCdV * HcBu

AtHt6 = GICd * HcGl

AtHt7 = AcCd * HcAc

AtHt8 = FaCd * HcFa

AtHt = AtHt1 + AtHt2 + AtHt3 + AtHt4 + AtHt5 + AtHt6 + AtHt7 + AtHt8

AtAdHt = AtHt/AtAd

16.3.7 Summary equations

'Equations that follow are summary equations for specific evaluation purposes. A number of these have been deleted to constrain the length of an already long chapter. Short notes to the effect that such equations are present in the original program have been substituted.'

(a) Milk energy and composition

ELm = DMLKLm * 2.0 * HcGl/MWLm

EPm = DMLKPm * HcAa/MWAa

ETm = DMLKTm * HcTg/MWTm

REMLK = ELm + EPm + ETm

PLm = DMLKLm/DMILK

PPm = DMLKPm/DMILK

PTm1 = DMLKTm/DMILK

(b) Energy balance and nitrogen retention

'THPI is estimated in the program by summing heat production associated with each transaction. This is a long section and is not presented here. THPI and THP2 constitute a cross-check on equations and should be equal within roundoff error.'

$$DBE = DPb * HcAa$$

$$DFE = DTsF * HcTg$$

$$DVE = DPv * HcAa$$

$$EB = DBE + DFE + DVE$$

$$THP2 = MEI - EB - REMLK$$

$$Nret1 = Nbody + Nmilk$$

$$Nabs = (AbsAa * AaFvAm * MWN) + (AbsAm * MWN) \$ 'AbsAa and AbsAm in moles'$$

$$Nur = Durea * UrAmAm * MWN$$

$$Nret2 = Nabs - Nur$$

$$Nbody = (DAa + DPb + DPv) * AaFvAm * MWN NMILK = DMLKpm * 0.16$$

(c) Methane emissions

'Methane calculated from model values generated above and by empirical equations.'

$$CH4Efd = CH4E/FDDMIN$$

$$fCH4E = CH4E/FDGEin$$

$$fCH4DE = CH4E/DEI$$

$$fCH4ME = CH4E/MEI$$

$$TCH4 = INTEG(DTCH4,ITCH4) \$ 'TCH4 is in moles'$$

$$CH4KGY = TCH4 * 0.016$$

$$CH4MLK = CH4KGY/TVMLK$$

$$TCH4E = TCH4 * HCH4$$

$$netME = AccMEI/TDMIN$$

$$CH4GEI = TCH4E/AccGEI$$

$$CH4DEI = TCH4E/AccDEI$$

'Methane calculated (BCH4) using Blaxter and Clapperton (1965) equation.'

$$fFIM = FDDMIN/((100 * EBW ** 0.75)/ME)$$

$$mult = MEI/(f1 * 0.110 * EBW ** 0.75)$$

$$BCH4 = (1.30 + 0.112 * appDE * 100 + mult * (2.37 - 0.050 * appDE * 100)) * f1$$

$$TBCH4 = INTEG(BCH4,1.0E - 8)$$

'Methane calculated according to the equation of Moe and Tyrrell (1979).'

$$MCH4 = (3.406 + 0.510 * (FDDMIN * (FdSt + FdSc + FdOa + FdPe))...$$

$$+ 1.736 * (FDDMIN * FDHc) + 2.648 * (FDDMIN * FDCe)) * f1/4.184$$

$$TMCH4 = INTEG(MCH4,1.0E - 8)$$

'A procedural that provides for estimates of income over feed costs under alternative milk pricing systems is not presented here.'

'A procedural that calculates energy expenditures associated with each energy transaction in the model and sums these to obtain the estimate of THPI mentioned above was deleted as was a procedural for estimation of RQ.'

END \$ 'OF DERIVATIVE'

END \$ 'OF DYNAMIC'

END \$ 'OF PROGRAM'

Evaluations and uses of the model are presented in Chapter 17.

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Evaluation and use of a growth and lactation model

with Lane Ely and K. C. Donovan

There has been a tendency to apply excessively simple economic and applied mathematics to complicated biological problems and, in the process, lose the richness that's implicit in the biology. And, the potential economic gains that are available if the richness is taken into account are lost too.

J. R. Black (personal communication)

17.1 INTRODUCTION

The objectives of this chapter are to illustrate the behavior of the lactating dairy cattle model described in Chapter 16, present sensitivity analyses of effects of varying some key parameter values in the digestion and animal elements and, finally, illustrate the types of data that a dynamic, mechanistic model can generate in support of risk analyses appropriate to evaluations of alternate feeding management strategies as a component of enterprise management.

17.2 BEHAVIORAL ANALYSES

As discussed in previous chapters, behavioral analyses of models are normally undertaken for two reasons. The first is to determine whether or not a model contains provisions adequate to simulation of variance observed in reality. The second is to determine whether or not a model can accurately simulate reality or, as it stands, be used for predictive purposes. Both positive and negative examples will be considered in this section, which illustrate limitations and strengths of the model. Effects of diet and feeding management strategies will be considered in the final section of the chapter. Sensitivity analyses, similarly, can be used for several purposes. One of these is to determine what data are required to estimate parameters not directly measurable using current methods. A related use of sensitivity

analyses is evaluations of possible effects errors in specifying input parameter values can have upon behavior of not only the specific metabolic equation in which it is used, but also behavior of the total system modeled. This later use of sensitivity analyses will be illustrated in this section.

The digestive element of the Baldwin *et al.* (1987b,c) model was expressly developed to provide best estimates of rates of nutrient availability to the animal element (Baldwin *et al.*, 1987a,c), which was the primary focus of that modeling study. It was clearly stated (Baldwin *et al.*, 1987b) that when experimental data on rumen digestion coefficients, microbial growth yields and digestible and metabolizable energy values of feeds used in a specific study, proportions of volatile fatty acid (VFA) formed, etc., were available, model parameters should be adjusted accordingly to provide more accurate estimates of nutrient availabilities to the simulated animal. Several specific limitations of the digestion element were identified. One was that rate constants for hydrolysis of hemicellulose and cellulose were based on data for dry legumes and may not apply to dried grasses, silages or fresh forages. A second was that rumen digestion coefficients for starch on high concentrate diets were too high. The stoichiometric coefficients for high concentrate diets did not account for systematic errors in predicting molar proportions of acetate and propionate production as percentage of concentrate in the diet increased. This source of systematic error was identified by Murphy *et al.* (1982), as discussed in Chapter 8.

Addition of provisions for monitoring rumen pH in the model and representing effects of pH upon fermentation rates and patterns in the model (Chapter 16; Argyle and Baldwin, 1988; Baldwin and Argyle, 1988) helped correct errors in predicting rumen digestion coefficients at high concentrate intakes. As rumen pH decreased (Fig. 17.1a) from 6.25 to 5.5, the respective rumen digestion coefficients for cellulose, starch and organic matter decreased from 0.52, 0.69 and 0.73 to 0.0, 0.56 and 0.59. As a result of these changes and a direct effect of pH on microbial maintenance requirements, microbial growth (Fig. 17.1b) decreased from 2.55 to 1.43 kg/day. Also, cellulose passage increased from 1.54 to 3.24 kg/day (not shown) and the overall ME value of the feed decreased from 10.9 to 9.4 MJ/kg.

Estimates of digestible and urinary energy output by the model (Chapter 16) contain a systematic error due to the simplified representation that microbial nucleic acids are all output in feces rather than a portion in urine and the remainder in feces. This simplification is canceled out in estimates of ME output by the model. Thus, it is valid to challenge ME but not DE values produced by the model. The result of such a challenge is presented in Fig. 17.2(a). There are a number of possible reasons for differences between observed and predicted values in a comparison of this type, including experimental and animal variance, errors in evaluating data on diet composition in formulating diet inputs to the model and model errors. In view of these potential sources of error, the r^2 value of 0.85 indicates acceptable agreement and the lack of discernible systematic error of prediction is

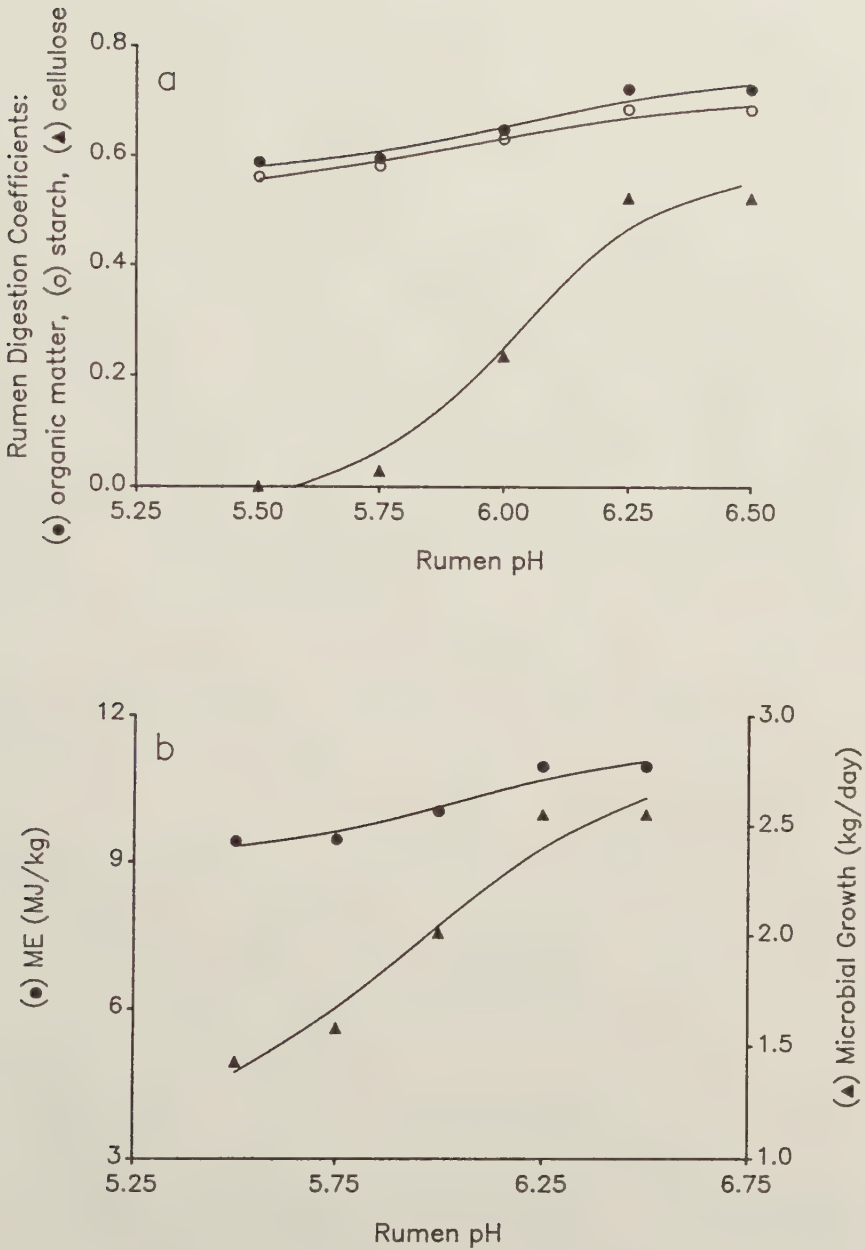


Figure 17.1 Effect of rumen pH on several digestive functions. Effects of pH were simulated by setting FIXPH (Chapter 16) to values specified on the x-axis. The model was for a 600 kg lactating cow fed 18.0 kg of the default diet specified in Chapter 16. Data are for day 14 of simulation.

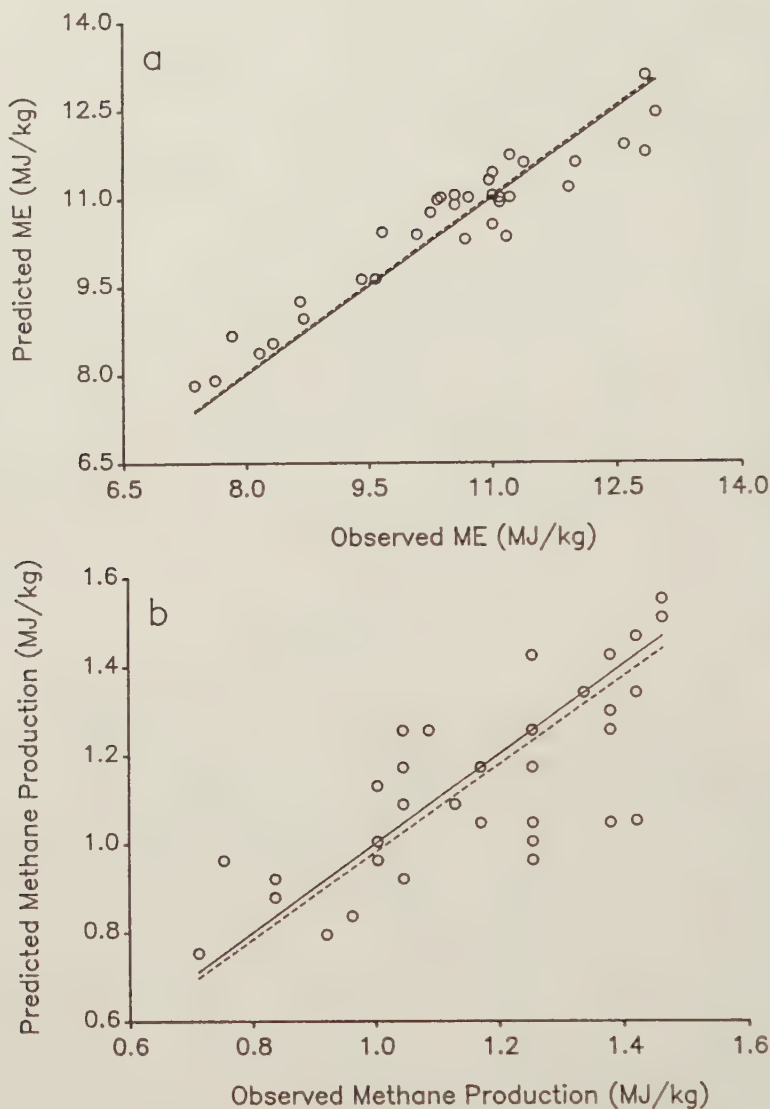


Figure 17.2 Comparisons of predicted versus observed estimates of (a) metabolizable energy and (b) methane production. Regression equations for best fit lines (—) were $y = a + bx$ with r^2 values for ME and CH_4 of 0.85 and 0.60, respectively. Regression equations for lines of equality (---) were $y = bx$. The 34 experimental estimates were reported by Armsby and Fries (1915), Armstrong (1964), Armstrong *et al.* (1958, 1964), Blaxter (1967), Blaxter and Clapperton (1965), Colovos *et al.* (1970, 1979), Coppock *et al.* (1964), Flatt *et al.* (1967), Graham, *et al.* (1958), Moe *et al.* (1972), Moe and Tyrrell (1976, 1979a,b), Moe *et al.* (1973a,b), Tyrrell and Moe (1972) and Tyrrell *et al.* (1976) for diets including high and low quality legume, corn silage, corn meal, soybean meal, and high and low quality grass hays.

gratifying. The addition of provisions for the use of different hydrolytic rate constants for cell-wall constituents of legumes, grasses and silages, for pH effects, and for the separate consideration of hemicellulose and cellulose clearly contributed to an improvement in model behavior, since r^2 values over the range of diets depicted in Fig 17.2(a) in the absence of these changes were in the 0.4–0.5 range.

Estimates of methane emissions across feeds are influenced by a number of factors, including diet composition, digestion in the rumen, patterns of VFA formed and microbial growth yields. Because of the complex interplay of factors that influence methane emissions, a comparison of observed and predicted estimates for the diets used to formulate Fig. 17.2(a) was undertaken. The results are presented in Fig. 17.2(b). Causes of differences among observed and predicted values are the same as those noted for ME estimates above, although it should be noted that animal variance expressed as coefficients of variation are significantly greater for methane as compared to ME estimates. Percentages of digestible energy lost as methane range from 6 to 13% and are calculated in the model (Chapter 16) based upon hydrogen left over after VFA patterns and yields and microbial growth are computed. As a result, small errors in computing rumen digestion coefficients and fermentation products other than methane can have major (percentage) effects upon estimates of methane loss. In view of these considerations, the r^2 value of 0.60 with only a small bias from the line of equality (Fig. 17.2b) is very encouraging. The semi-empirical equations (e.g. the equations contain mechanistic elements but were fit statistically) of Blaxter and Clapperton (1965) and Moe and Tyrrell (1979b) are commonly utilized to estimate methane emissions by ruminant livestock. When these equations are applied to predict methane emissions for the same data set used in Fig. 17.2(b), r^2 values of prediction were significantly below 0.6. Exact r^2 values are not presented because direct comparisons are inappropriate. The Blaxter and Clapperton (1965) equation was fit to forage diets and the Moe and Tyrrell (1979b) equation was fit to mixed, (largely) dairy cattle diets. Thus, these should not be challenged using rations beyond those within range with the data sets used to fit the equations.

Diets containing whole and cracked corn were excluded from the comparisons presented in Fig. 17.2(a and b). The model presented in Chapter 16 incorporates the concept that starch solubility influences rate of starch hydrolysis. This was implemented by specifying as an input the fraction of dietary starch that is water soluble (StSol), different rate constants for hydrolysis of soluble and insoluble starch, and differing rate constants for passage of soluble nutrients, including starch with water (StSol * k_{WAP}) and small particle (Sp) material (SpSt * k_{SP}). The rate terms k_{WAP} and k_{SP} were constants in the original model. Modifications in water dynamics (Argyle and Baldwin, 1988) and addition of dry matter intake as an effector of k_{SP} (Chapter 16; Knapp, unpublished) enabled adequate simulations of RDChA estimates obtained at moderate intakes of corn, barley and wheat

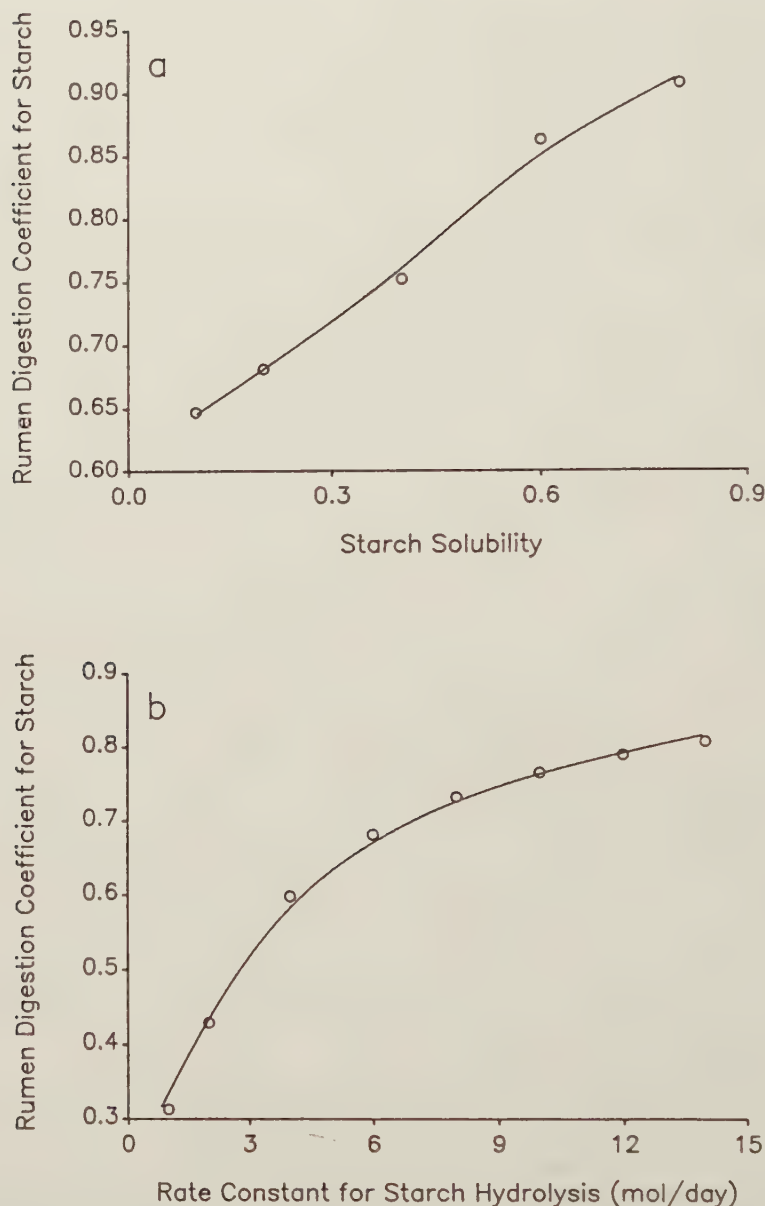


Figure 17.3 Effects of (a) starch solubility (StSol) and (b) changes in the rate constant for particulate starch hydrolysis (k_{HaCs}) on the rumen digestion coefficient for starch (RDCHa). Estimates of starch solubility were obtained using the default value of 6.0 for k_{HaCs} . Effects of varying k_{HaCs} were estimated using a StSol value of 0.2 for the default diet (Chapter 16). Other simulation inputs were as described for Fig. 17.1.

over the range from 0.65 to 0.9 (Fig. 17.3a). However, at the high feed intakes of high producing dairy cows fed milo and cracked corn-based rations (Herrera-Saldana *et al.*, 1990; Klusmeyer and Clark, 1991), RDCHa estimates were in the range of 0.45 and could not be simulated. These observations can be reproduced if, in addition to specifying starch solubility as a determinant of RDCHa, different values for the hydrolytic rate constants for starch (k_{HAcS}) are input for each diet (Fig. 17.3b). This is not at all satisfying from the mechanistic point of view. This is acceptable in applied models and was the approach adopted in formulating the Cornell Net Carbohydrate and Protein System (Search:Agriculture, 1990) wherein values for RDCHa are specified as input and differ dependent upon intake and the grains and processing procedures used. Ewing and Johnson (1987) developed a dynamic simulation model of corn starch digestion. In this, particulate starch digestion, passage and size reduction rates were described for five particle sizes. When *in vitro* and *in situ* (nylon bag) data were used to parameterize the model, rumen and total tract starch digestibilities were significantly underestimated, which indicated that *in vivo* estimates are essential to the development of improved representations of the digestion of not only corn but all cereal starches. These observations clearly emphasize the need to identify and quantitatively evaluate the chemical and physical properties of starch that influence degradation in the rumen and incorporate these into the model.

The use of high (added) fat diets in the dairy cattle industry is relatively recent. Initial simulations of effects of high fat intakes were compatible with early experimental data in the sense that milk yields and partial efficiencies of milk production were increased, while milk proteins yields were decreased slightly. However, as experimental data accumulated, it became apparent that the model was producing correct answers for the wrong reasons. Rumen digestion coefficients for fiber components were reduced in simulations due to decreases in amounts of microbes in the rumen, but the decreases were, generally, not as great nor variable as those observed experimentally. Further, the decreases in milk protein yields simulated were due to decreases in amounts of fermentable substrates available in the rumen and concomitant decreases in microbial growth yields, while accumulating experimental data indicated that microbial growth yields were not depressed by high fat diets. This led to modifications in the model (Chapter 16), which included addition of feed fat (FdFat) as an inhibitor of cellulose and hemicellulose (Hc, Cs) hydrolysis as in equation 17.1 and as a positive effector of microbial growth (equations 17.2 and 17.3).

$$U_{\text{Hc,Cs}} = k_{\text{Hc,Cs}} * (1.0 - (\text{FdFat} * \text{FdLi}^{-1} * k_{\text{FaHb,Cs}})) * \text{Hc} * \text{cMiHb} \quad (17.1)$$

$$\text{MiG} = (\text{ATP}_F - \text{ATP}_M) * Y_{\text{ATP}} * \text{FAM}_G * \text{FFa}_G \quad (17.2)$$

$$\text{FFa}_G = 1.0 + \text{FdFat}/\text{FdLi} * k_{\text{Fa,ATg}} \quad (17.3)$$

From a mechanistic point of view, these equations must be considered

arbitrary since they are not based upon direct experimentation. Direct observations are not available that FdFat effects the rate constant for hemicellulose ($k_{\text{Hc,Cs}}$) as well as cellulose hydrolysis, rather than reduces the concentration of microbes associated with cell walls (cMiHb). Similarly, well substantiated data indicate that availability of ammonia (FAM_G) is an effector of microbial growth yields, while direct FdFat effects on the efficiency of microbial growth are virtually nonexistent. One can postulate that high fat diets decrease protozoa and protozoal predation on bacteria with a result of increased net yields, however, direct measurements are not available. In view of these uncertainties, parameter values used to implement the FdFat effects were set in a very conservative fashion.

Behavior of the model with these equations incorporated is illustrated in Fig. 17.4(a and b). When the values of $k_{\text{FaHb,Cs}}$ and $k_{\text{Fa,ATg}}$ in the model were set to the default value of 0.03, fat effects were minimal, because reductions in rate constants for cellulose and hemicellulose hydrolysis were balanced or offset by the increase in microbial growth (results not presented). Results for the $k_{\text{FaHb,Cs}}$ values of 0.06 and 0.09 are presented in Fig. 17.4(a and b) to illustrate the simulated responses to feed fat. When values for $k_{\text{FaHb,Cs}}$ were set at 0.06 and 0.09, respectively, decreases in the rumen digestion coefficients for cellulose (RDCCe) were 0, 8 and 18% at the highest (8%) rate of fat addition (Fig. 17.4a). Over the same range of diets, respective reductions in concentration of blood lipids (cFA) and overall digestion of organic matter were 0, 8 and 11% and 0, 1.5 and 3.4% (Fig. 17.4a). Further, the responses were not linear with regard to dietary fat content. As expected, diet ME values, microbial growth and the transfer of blood lipids increased as dietary fat increased (Fig. 17.4b).

The characteristic depression in percentage protein in milk associated with fat feeding was not observed in the simulation analyses. Cant *et al.* (1993a,b) presented data suggesting that the depression in milk protein yields on high fat diets may be attributable, at least in part, to reduced rates of blood flow to the mammary glands. The current model is based on the concept that metabolic activities of organs and/or products of metabolism are primary determinants of local rates of blood flow (Chapter 6). The results of Cant *et al.* (1993a,b), might be compatible with this concept if one argues that the efficiency of milk fat synthesis is increased in fat-fed cows to the extent that venous bicarbonate produced per liter of milk synthesized is reduced. This causes, in turn, a decrease in blood flow per liter of milk and a decrease in amounts of amino acids available per liter of milk produced. The model, as currently configured, cannot respond to a subtle change in signal such as this. Clearly, further experimental and modeling research are essential to resolution of these issues.

The limitations in the starch and feed fat elements of the model were presented early in this chapter to emphasize that the development of research models is an evolutionary process and behavioral analyses are an important tool for use in identifying priorities for further research.

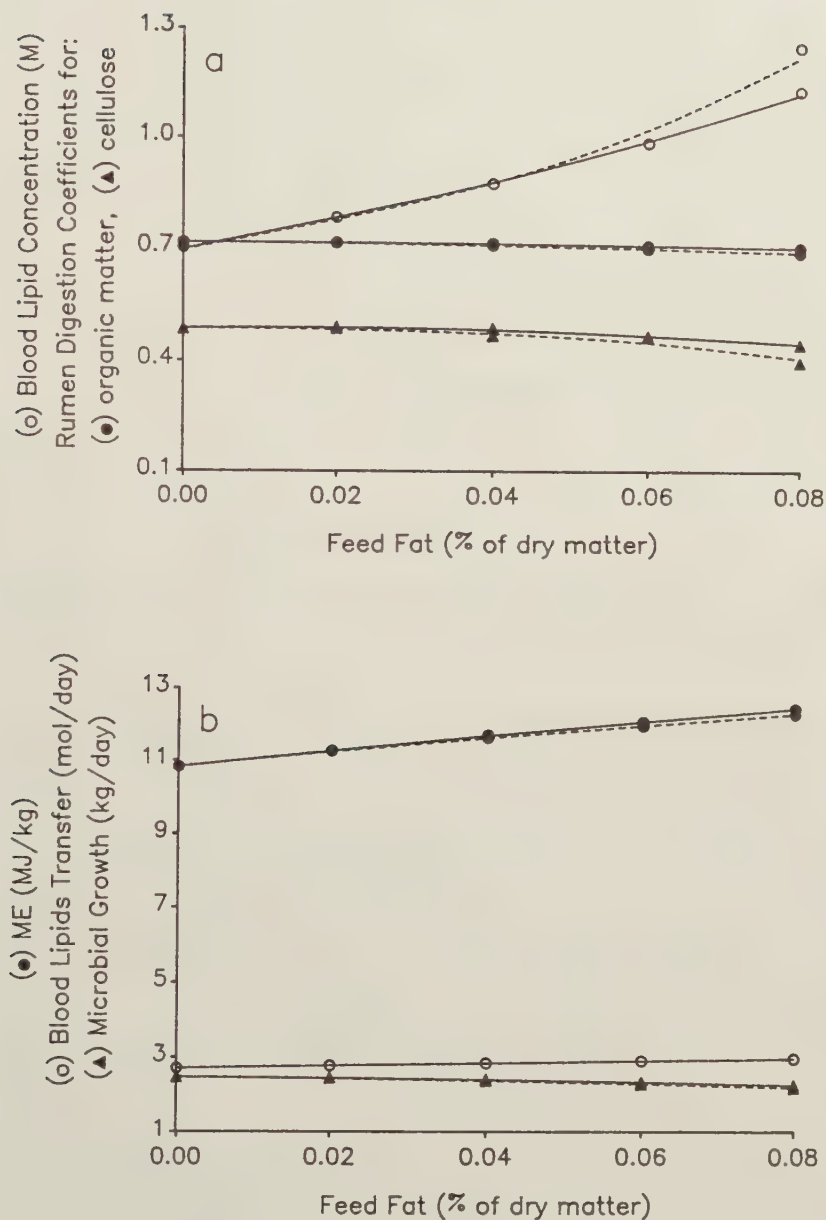


Figure 17.4 Behavior effects of dietary fat when $K_{FaHb,Cs}$ is set to 0.06 (—) and 0.09 (---) upon (a) rumen digestion coefficients for organic matter and cellulose and blood lipid concentration; and (b) metabolizable energy, blood lipid transfer and microbial growth. Feed fat (FdFat) inputs were calculated as substitutions of fat for starch in the default diet in Chapter 16. Other simulation conditions are as described for Fig. 17.1.

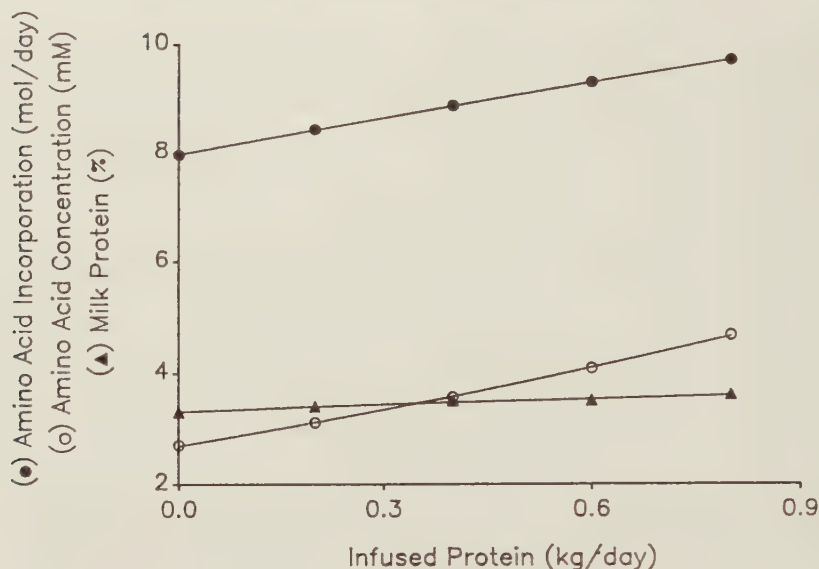
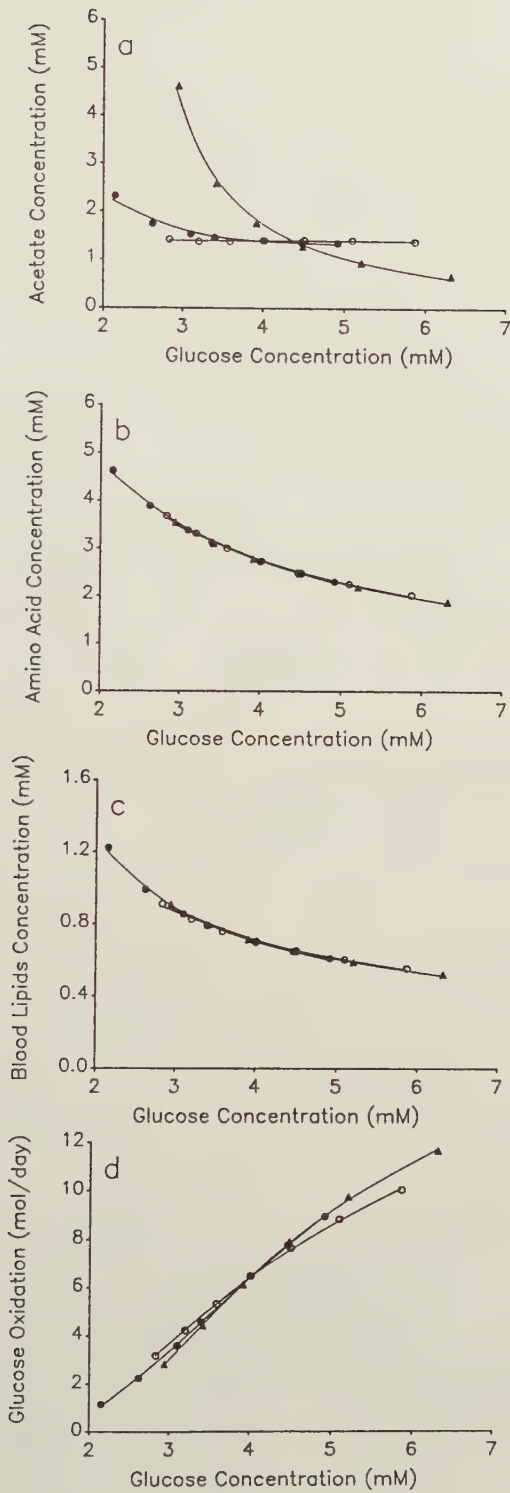
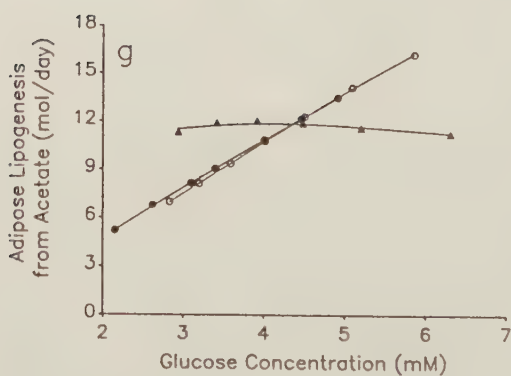
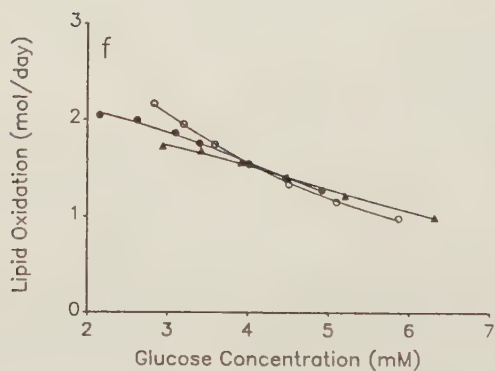
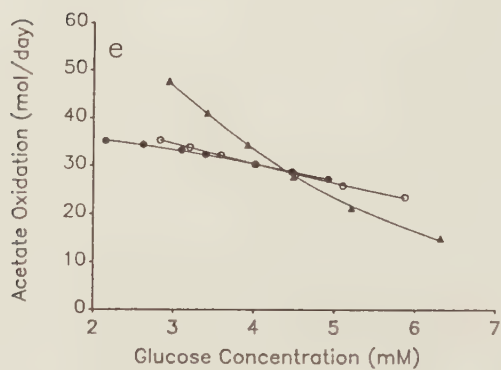


Figure 17.5 Effects of protein infusions per abomasum on milk protein synthesis. Infusions were in addition to 18 kg/day of default diet fed to a 600 kg lactating dairy cow as described in legend to Fig. 17.1.

Whitelaw *et al.* (1986) among others reported that supplementation of lactating cows with formaldehyde-treated casein or casein per abomasum increased milk production 10–20%. Simulation results indicated increases in circulating amino acid concentrations and amino acid incorporation into milk protein (Fig. 17.5). The latter observation along with the indication that milk protein percentage did not change significantly indicates a milk yield increase of about 20%. In contrast, Cant *et al.* (1993a) observed a small increase in milk protein percentage when casein was infused and a lesser increase in milk production. All studies indicate an increase in milk protein yields when casein is infused. Some indicate that this is due to increases in milk yields and others that this is due to increases in milk protein percentage (Cant *et al.*, 1993a). Thus, this simulation result and the variable experimental results require further examination.

The several panels in Fig. 17.6 are different from but somewhat analogous to those presented by Baldwin *et al.* (1987a) to illustrate behavior of the original model. The analyses were repeated using the current version because of the many changes incorporated as the model evolved. Effects of three manipulations of the model were undertaken. In the first case, the differential equation for glucose was set to zero ($DGI = 0.0$) and glucose concentration (cGI) was specified as input. Availabilities of other nutrients were those normally produced during the digestion of 18 kg/day of the default ration (Chapter 16) fed to a 650 kg cow in mid-lactation. In the second test case, the equation for propionate absorption was set to values





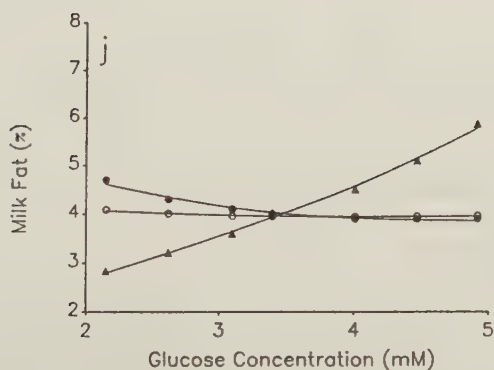
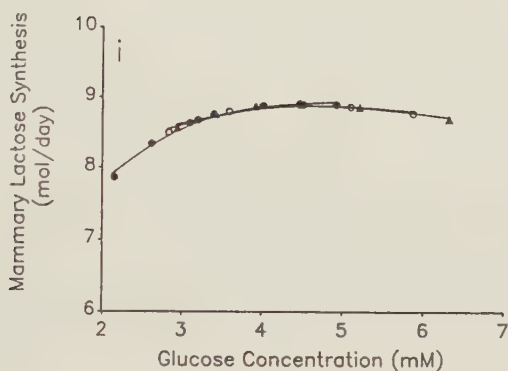
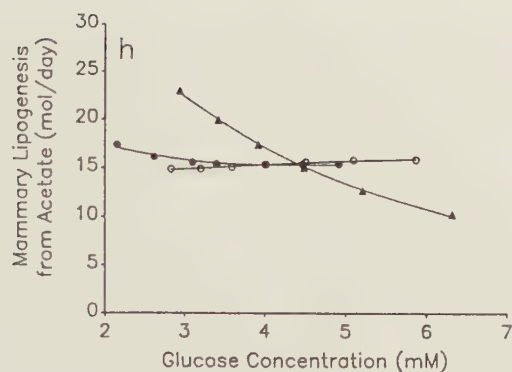


Figure 17.6 Effects of glucose concentrations (cGl) in blood produced by specifying cGl as input (case 1, ●), infusing propionate (case 2, ○) and isoenergetic substitution of propionate for acetate (case 2, ▲). Other inputs were as described in text and legends to Fig. 17.1.

specified as input. This manipulation altered both propionate absorption rates and energy availability. The third test case involved specifying both acetate and propionate absorption to produce isoenergetic changes in the availabilities of these two nutrients.

Baldwin *et al.* (1980) noted that, although data compiled from radiotracer experiments conducted both *in vivo* and *in vitro* and some, but certainly not all, arteriovenous difference studies of tissue nutrient uptakes (see Chapter 13) clearly indicate that Michaelis–Menten saturation-type kinetic equations best fit most data sets, considerable variance is not accounted for when such equations are applied to data compiled from experiments with different diets, animals and conditions. By way of supporting the concept that dynamic, mechanistic simulation models can be utilized as an effective tool in research, Baldwin *et al.* (1980) suggested that interactions among nutrients contribute to the unexplained variance and that such models should/must contain provisions adequate to explain this variance. In accord with this view, Baldwin *et al.* (1987a) undertook the behavioral analyses. Similar analyses are presented in Fig. 17.6. It is impossible to generate the large number of experimental data essential to quantitative assessments of model behavior depicted in the figure. The questions addressed are:

1. Does the model contain provisions (note that most equations are based on lower level data and concepts, e.g. mechanistic) adequate to accommodate observed variance in the intact system?
3. Are the simulated interactions among nutrients consistent with both informed conceptual analyses and the sparse data sets currently available?

A redeeming virtue of the model predictions presented is that most can be challenged using existing experimental technologies and such challenges are totally appropriate. Unfortunately, the required experimental challenges are quite laborious and expensive.

The response profiles depicted in Fig. 17.6 indicate that the model contains provisions capable of accommodating a range of interactions among metabolites, hormones and energy status and can explain variance (scatter in experimental data), which was the challenge posed above. The response profiles will only be discussed in a general sense here, since detailed analyses would be redundant with concepts, interactions and responses discussed in previous chapters.

Effects of varying glucose concentrations (cGl) on concentrations of acetate (cAc), amino acids (cAa) and blood lipids (cFA) are presented in Fig. 17.6(a–c). These effects are important because concentration changes influence the rates of many reactions in the model. In the first test case, cAc decreased as glucose increased, because lipogenesis from acetate in adipose tissue (AcTsF) increased (Fig. 17.6g). This increase more than offset decreases in acetate oxidation (AcCd; Fig. 17.6e) and mammary lipogenesis from acetate (AcTm; Fig. 17.6h). The latter decrease caused a small depres-

sion of milk fat percentage (PTm) (Fig. 17.6j). In the second case, changes in AcCd, adipose (AcTsF) and mammary gland (AcTm) lipogenesis balanced out such that cAc was not altered. The isoenergetic substitution of propionate for acetate (case 3) produced, as might be expected, the greatest changes in cAc, AcCd, AcTm and PTm (Fig. 17.6a, e, h and j) and had only a minor effect on AcTsF (Fig. 17.6g). All increases in cGl caused amino acid concentrations (cAa) to decrease (Fig. 17.6b). Milk yields and milk protein content were not changed significantly by the simulated treatments (not shown). Thus, the major cause of the decrease in cAa was increased rates of synthesis of visceral and body proteins. Concentrations of blood lipids (cFA) also decreased as glucose increased in all three cases (Fig. 17.6c). Basic causes were decreased lipolysis and increased fatty acid re-esterification in adipose tissue at higher glucose concentrations. As circulating glucose concentrations increased glucose oxidation (GlCd) increased (Fig. 17.6d), while AcCd and lipid oxidation (FaCd) decreased (Fig. 17.6d, e and f). These changes were driven primarily by relative changes in concentrations, but as emphasized above, these changes clearly illustrate that the model has the capacity to explain the scatter of data observed when results of multiple experiments are compiled to evaluate effects of nutrient concentrations in blood upon rates of nutrient oxidation. Rates of lactose synthesis in mammary tissue were not very sensitive to changes in cGl (Fig. 17.6i). This lack of sensitivity reflects the fact that amino acids are required for the synthesis of α -lactalbumin, which is required for lactose synthesis (Chapter 16). As a result, the increases in glucose concentration are counterbalanced by the associated decreases in cAa.

Simulated effects of acetate concentration upon metabolism are presented in Fig. 17.7. These results were produced by setting the differential equation for acetate to zero and specifying the acetate pool size as input. Availabilities of other nutrients were simulated for a 650 kg lactating cow fed 18 kg/day of the default diet (Chapter 16). As increases in the acetate pool size were specified, energy availability to the animal increased. Thus, the responses depicted reflect both changes in acetate concentration and energy balance. As acetate concentration was increased, acetate oxidation (AcCd) and both adipose (AcTsF) and mammary gland (AcTm) lipogenic rates increased. Glucose oxidation decreased as would be expected (not shown). Rates of blood lipid incorporation into milk fat (FaTmV) were relatively unaffected, therefore the increased percentage fat in milk (PTm) is totally attributable to the increase in mammary gland lipogenesis.

Several effects of varying feed intake are presented in Fig. 17.8. Rates of body energy loss (–EB) and fatty acid oxidation (FaCd) decreased as feed intake increased. The mobilization of body energy was not adequate to sustain milk production (milk energy; NEp). The concentration of acetate in blood (cAc) increased as rates of absorption of energy (absE) and acetate (absAc) increased. These responses are consistent with what a worker in the

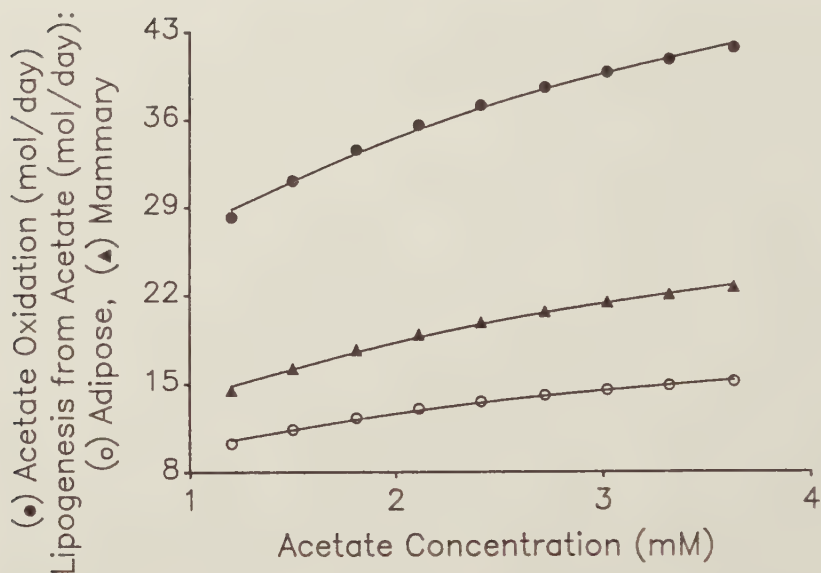


Figure 17.7 Effects of acetate concentration (cAc) in blood specified as input. Other inputs as described for Fig. 17.1.

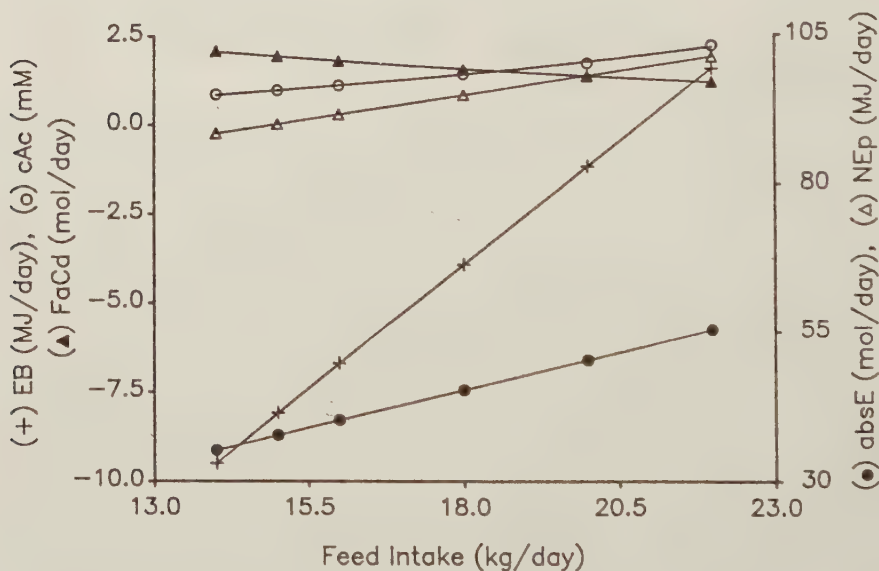


Figure 17.8 Effects of varying feed intakes of default diet. Other simulation conditions as described for Fig. 17.1. Codes are defined as body energy loss (EB), fatty acid oxidation (FaCd), energy absorption rate (absE), blood acetate concentration (cAc) and milk energy (NEp)

area would expect and agree, in general magnitude, with experimental data. However expected these results may be, it is essential that such simulations be included in behavioral analyses to assure that overall quantitative relationships are appropriate.

17.3 SENSITIVITY ANALYSES

As emphasized in Chapter 4, sensitivity analyses are undertaken for a number of reasons. An important reason is to determine whether or not and how sensitive solutions of a model are to key parameter values. This issue will be addressed in this section. A lack of (expected) sensitivity would be held suspect as would excessive sensitivity. Either of these results normally leads to re-examination of the model. Neither of these results is infrequent. They are usually unexpected and, thus, are important in model evaluations. An intermediate result is more acceptable and reassuring but, of course, does not in and of itself constitute proof that a given equation and associated parameter values adequately capture the degree of reality required by a given modeling objective. In this sense, 'undesired' results in sensitivity analyses help identify defects in concepts and data incorporated into a model and, as a result, are useful. 'Desired' results are nice but not overly informative, except for the very important fact that the accuracy with which a parameter value must be defined assures acceptable simulation outputs. 'Undesired' results of sensitivity analyses were encountered and addressed during development of the model presented in Chapter 16. Therefore, emphasis in this section will be upon the accuracy with which parameter values in the model must be known.

17.3.1 Digestion elements

The unique ability of ruminants, conferred by virtue of microbial digestion in the forestomachs, to convert plant fiber to human edible foods is dependent upon two rate constants in the model; the respective rate constants for cellulose (kCeCs) and hemicellulose (kHcCs) hydrolysis by microbes associated with particulate forage cell walls. Effects of varying kCeCs upon rumen digestion coefficients (RDC_{__}) for cellulose (RDC_{Ce}), hemicellulose (RDC_{Hc}) and starch (RDC_{Ha}) are depicted in Fig 17.9. The increase in RDC_{Ce} was, of course, expected. The lesser increase in RDC_{Hc} reflects the fact that cellulolytic microbes growing in association with small particles hydrolyze both cellulose and hemicellulose. The even more modest increase in RDC_{Ha} reflects the growth of microbes other than the cellulolytics on hydrolytic products of hemicellulose and cellulose hydrolysis. The response profiles are not linear because the positive feedback produced as a result of increased fiber digestion results in increased amounts of microbes closely associated with the cell wall fraction. These, in turn, increase rates of cellu-

lose and hemicellulose digestion and microbial growth up to a maximum determined by the rates of entry of cell wall constituents to the small particle pool, rates of hydrolysis and rates of passage (Chapter 16). Given that a positive feedback exists, one might expect that the rate constants for cellulose and hemicellulose hydrolysis would have to be specified with a high degree of accuracy for each feedstuff. This would create great difficulty in developing a generally applicable model, since a great number of data not currently available would have to be collected. In fact, collection of the large data set implied by acceptance of this notion would be economically unfeasible. The values specified for k_{CeCs} in the current model are 6.0 for leguminous and 9.0 for grass forages. These two values were used to develop the regression relationships between observed and predicted ME estimates presented in Fig. 17.2(a). The high r^2 associated with that regression relationship indicates that errors associated with failures to specify exactly correct values for k_{CeCs} and k_{HcCs} probably make only a minor contribution to variance not explained by the model. Similarly, the sensitivity analyses presented indicate that reasonable but not excessive accuracy is required in specification of these two parameter values. Otherwise, a much more complex set of equations, input parameter values and descriptions of interactions among individual microbes and individual fiber types would be required.

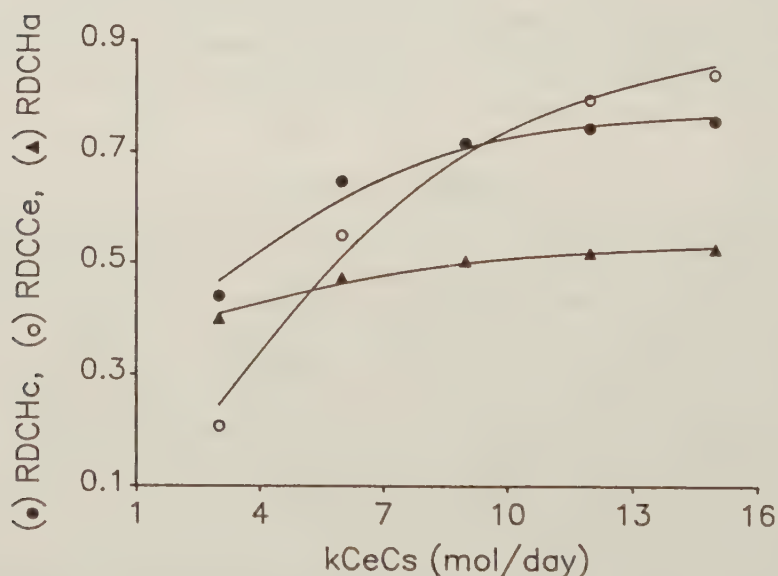


Figure 17.9 Analyses of effects of varying the hydrolytic rate constant (k_{CeCs}) on rumen digestion coefficients (RDC) for cellulose (Ce), hemicellulose (Hc) and starch (Ha). Diet and other conditions as described for Fig. 17.1.

Rates of nutrient and microbial passage from the rumen have been the subject of considerable experimental and modeling research and controversy over the years (Matis, 1972; Van Soest, 1982; Baldwin and Bywater, 1984; Chapter 11). Effects of quite a number of factors including plant species, processing, rates of particle size reduction due to rumination and hydrolysis of cell wall components, and particle density have been studied. Several issues relevant to this complexity of factors were discussed above in connection with Fig. 17.3. As a result, there is a strong motivation towards development of relatively complex equation forms to simulate passage and we have yielded to this temptation a number of times. However, none of the equation forms we devised performed better than the simple mass action equation in the current model, where passage is a function of a rate constant for small-particle passage (k_{spp}) and the mass of small particles in the rumen. There is an optional equation for scaling k_{spp} to relative feed intake in the model (Chapter 16). This can be implemented when intakes are low, e.g. during the dry period or when the model is used to simulate growth. Effects of varying k_{spp} upon several digestive processes are shown in Fig. 17.10. The range of fractional rate constants tested was 0.8 to 2.0 day^{-1} . The model default value is 1.33 day^{-1} or 5.5%/h. Solutions are clearly sensitive to changing k_{spp} over the 2.5-fold range tested. As the passage-rate constant increased, starch (HaP) and cellulose (CeP) passage increased 2.7-fold. Microbial growth (MiG), the rumen digestion coefficient for cellulose (RDCCe), absorption of acetate (absAc) and adipose tissue lipogenesis (AcTsF) decreased 30, 70, 41 and 42%, respectively. On the other hand, the model is not overly sensitive to k_{spp} , because the simulated ME value only decreased 17% and milk production (not shown) was not depressed significantly (Fig. 17.10). Based upon the sensitivity analyses presented for k_{CeCs} in Fig. 17.9 and effects of k_{spp} in Fig. 17.10, it should be clear that when the model produces errant estimates of ME or rumen and overall digestion coefficients for fiber components, particularly for some specific forage or feedstuff, adjustments easily can be made in the model to accommodate some unusual property of the feed. However, the complexity of the interactions illustrated in Fig. 17.3 with regard to feed fat effects indicates that caution should be used in adjusting even this, highly aggregated, model.

Diet crude protein is input to the model (Chapter 16) as three fractions, i.e. non-protein nitrogen, soluble true protein and insoluble true protein. The most important rate constant defining nitrogen metabolism in the rumen is the rate constant for insoluble protein hydrolysis ($k_{Pi,Aa}$), because it influences amounts of dietary protein that pass intact from the rumen and amounts of amino acids and peptides available in the rumen (RAa) which, in turn, enhance microbial growth yields (MiG). Effects of varying $k_{Pi,Aa}$ from 3.0 to 14.0 day^{-1} are presented in Fig. 17.11. As expected, when $k_{Pi,Aa}$ is increased RAa, MiG and incorporation of amino acids and peptides into microbes (RAaMi) increase, and diet protein passage PiP decreases. The net effect of increased MiG and decreased PiP was a 10% decrease in amino

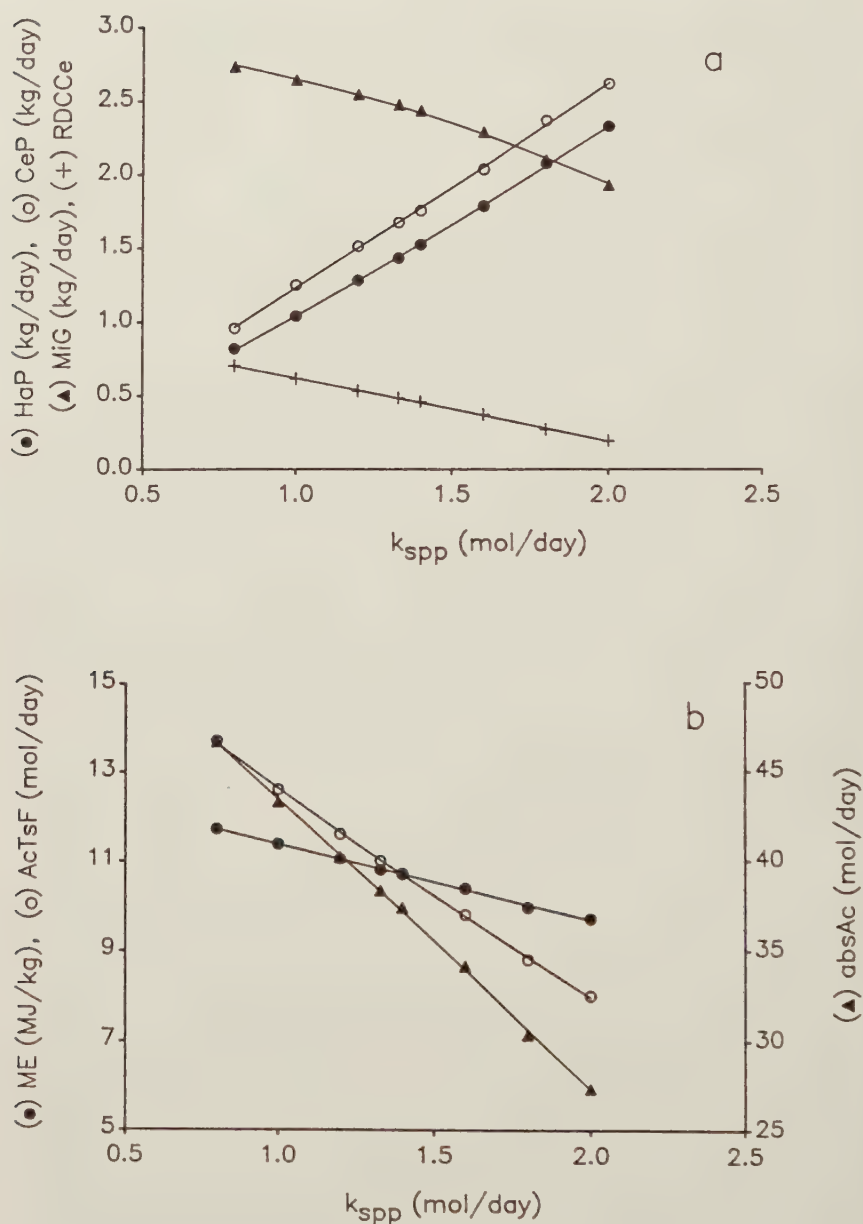


Figure 17.10 Effects of rate constant for small particle passage (k_{spp}) upon (a) starch (HAP) and cellulose (CeP) passage, microbial growth (MiG), rumen digestion coefficient for cellulose (RDCCe) and (b) ME absorption of acetate (absAc) and adipose tissue lipogenesis (AcTsF). Simulation conditions other than k_{spp} specified as input were as described for Fig. 17.1.

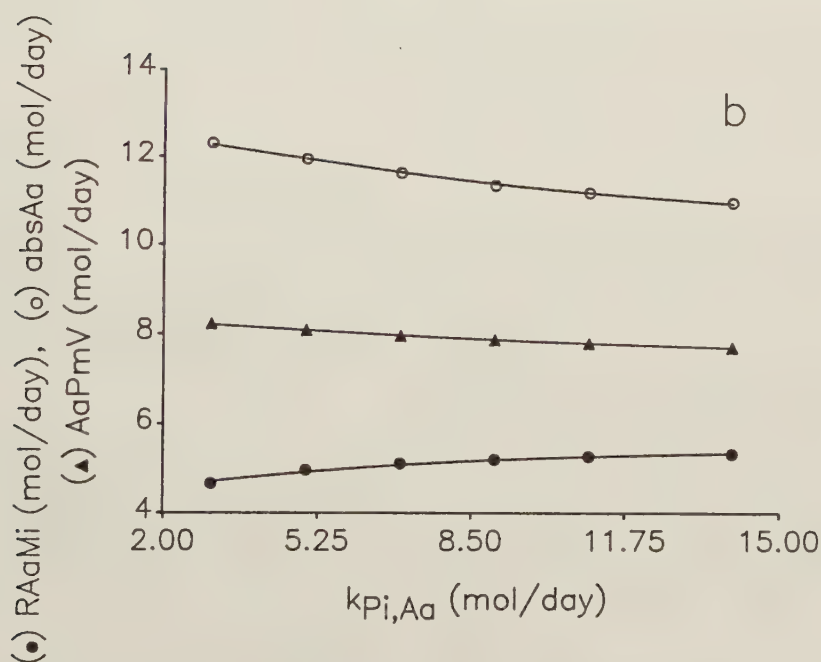
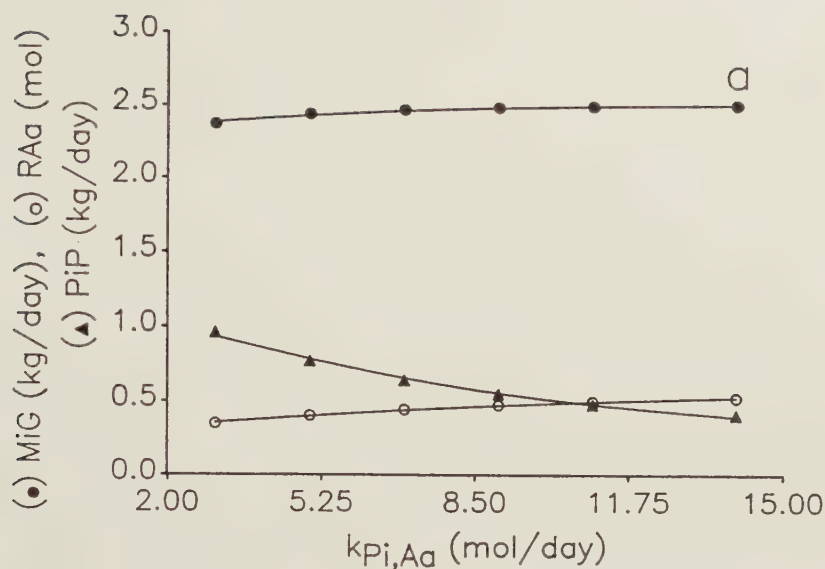


Figure 17.11 Effects of rate constant for insoluble protein hydrolysis ($k_{Pi,Aa}$) on (a) amino acids and peptides available in the rumen (RAa), microbial growth (MiG) and dietary protein passage (PiP), and (b) incorporation of amino acids and peptides into microbes (RAaMi), milk protein synthesis (AaPmV), and amino acid absorption (absAa). Excepting $k_{Pi,Aa}$ values specified as input, simulation conditions as described for Fig. 17.1.

acid absorption (absAa) by the animal and a 6% decrease in milk protein synthesis (AaPmV; Fig. 17.11b). The decrease in AaPmV was reflected in smaller decreases in milk yield and percentage of protein in milk (results not shown). This analysis indicates a relatively modest level of sensitivity that is well within range of the accuracy with which rates of hydrolysis of insoluble protein can be measured in feedstuffs.

Russell *et al.* (1992) emphasized the importance of variation in microbial maintenance requirements (MiMAd) as a determinant of microbial growth yields (MiG) and the availability of amino acids (absAa) to the host animal. Effects of varying MiMAd in the lactating cow model are depicted in Fig. 17.12. The range of values assigned MiMAd in this analysis essentially covers the range of values observed in individual microbes under various conditions, including low pH values. Increases in MiMAd over the range tested decreased ME, MiG, absAa, RDCHa and RDCHb by 6, 40, 25, 15 and 38%, respectively. The decreases in microbial growth and holocellulose and starch digestion would have been greater if rumen concentrations of amino acids and peptides (RAa) had not increased and caused a concomitant increase in the efficiency of microbial growth. The results of this sensitivity analysis emphasize the importance of MiMAd as a determinant of rumen digestion and, further, that a high priority should be assigned to research directed at augmentating the current dearth of data available on rumen microbial maintenance requirements.

In the discussion above of analyses of sensitivity of model solutions to $k_{Pi,Aa}$ and MiMAd, effects of amino acids and peptides upon microbial growth rates and yields were considered central to the responses noted. The major parameter that influences responses to amino acids and peptides is the affinity constant KYAtAa. Effects of varying KYAtAa are presented in Fig. 17.13. As this affinity constant increased, mass of microbes in the rumen (Mi) decreased 20% and microbial growth (MiG) decreased (18%). The overall effect of the changes upon the rumen digestion coefficient for organic matter (RDCOM) was modest.

The simulation results presented in Figs 17.11–17.13 illustrate behavior of the microbial component of the digestive element. The results are in general agreement with experimental data, both qualitatively and quantitatively. However, relevant experimental data are very sparse, which emphasizes an urgent need for further experimental and modeling analyses of this critical portion of the ruminant digestive elements.

17.3.2 Animal elements

Mammary metabolic capacity (UENZ) in the lactating cow model is the primary determinant of milk production (Chapters 13 and 16). Therefore, an analysis of effects of UENZ upon simulated patterns of nutrient use was undertaken. In other simulations, UENZ was allowed to decrease continuously throughout the 2 week simulation periods because it is a state variable

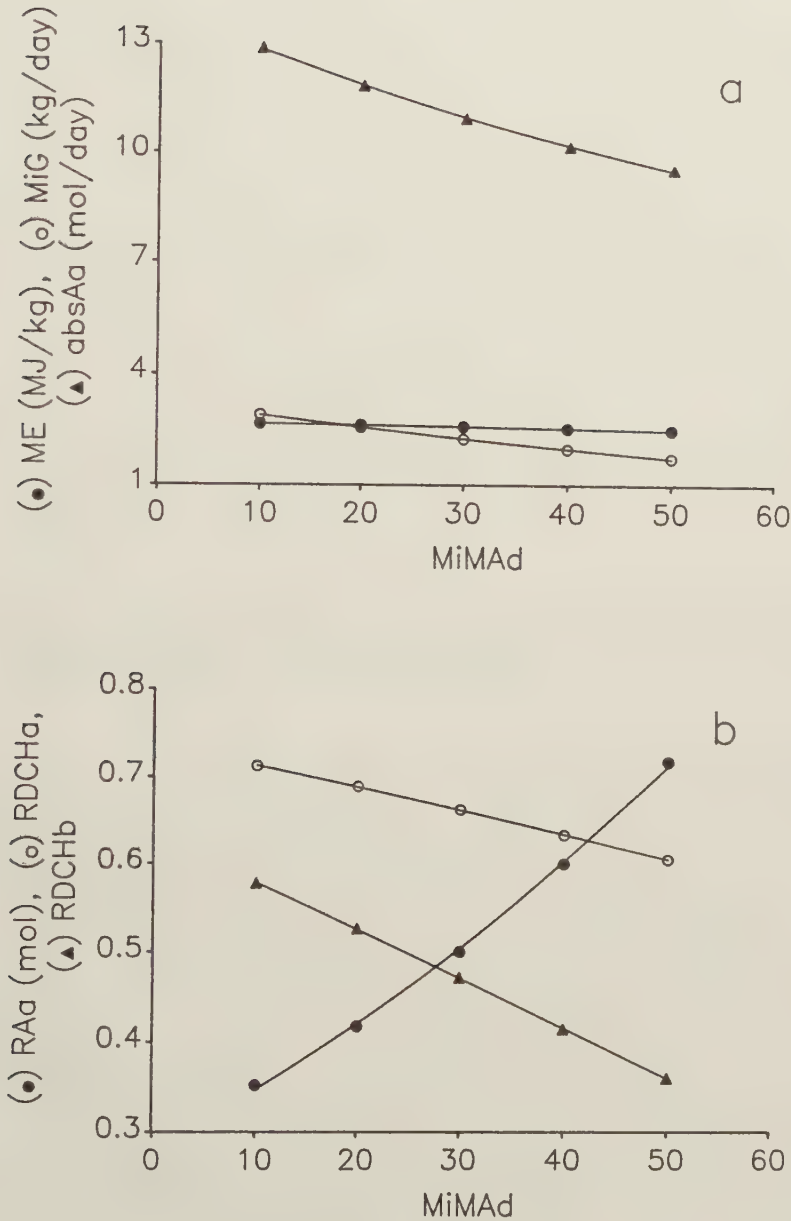


Figure 17.12 Effects of rumen microbial maintenance requirements (MiMAd) on (a) metabolizable energy (ME), microbial growth yields (MiG) and amino acid availability (absAa), and (b) amino acids and peptides available in the rumen (RAa) and rumen digestion coefficients (RDC) for starch (H_a) and holocellulose (H_b). Simulation conditions as described for Fig. 17.1.

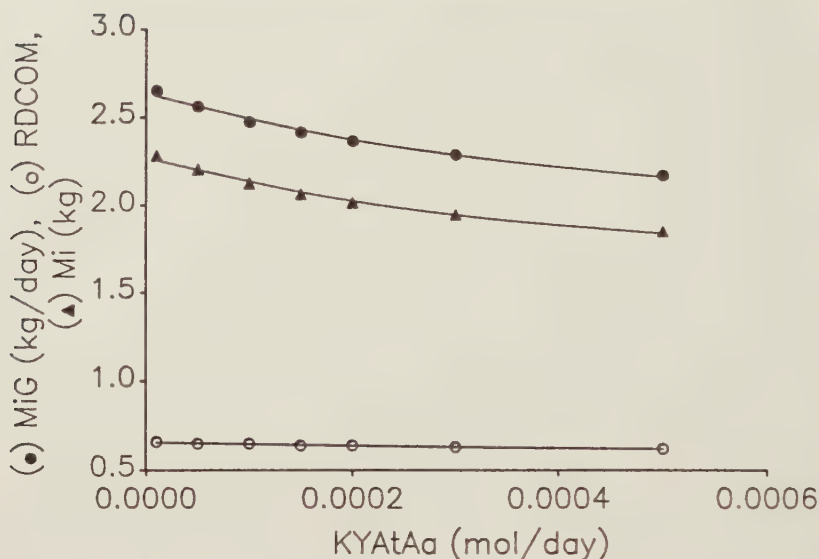


Figure 17.13 Responses to varying the affinity constant from amino acid effects (KYAtAa) upon microbial growth (MiG), microbial mass (Mi) and the rumen digestion coefficient for organic matter (RDCOM). Simulation conditions as described for Fig. 17.1.

defined by the equations of Neal and Thornley (1983), which were adopted during development of the current model. For purposes of the simulations of UENZ effects, DUENZ was set to zero such that initial values of UENZ specified as input were held constant throughout each simulation. Also, in order to simplify interpretations of the simulation results, feed intake was held constant at 18 kg/day. This means that animals were overfed at low values of UENZ and underfed when milk production (DMILK) was high. Results of the simulations are presented in Fig. 17.14. As UENZ increased from 2000 to 14 000 (7-fold), DMILK increased 4.4-fold, a curvilinear response. At the highest rate of milk production – 50.9 kg/day – the simulated animal's energy balance was –79.1 MJ/day. Clearly, DMILK would have decreased rapidly in prolonged simulations because of depletion of body energy reserves. Over the range of UENZ values tested, rate of acetate oxidation (AcCd) remained relatively constant, while the decrease in rate of lipogenesis in the mammary glands (AcTm) was essentially counterbalanced by the decrease in rates of fatty acid synthesis (AcTs) in mammary adipose tissue (Fig. 17.14a). The decrease in glucose availability for oxidation as an energy source (not shown) attributable to the increase in milk production was balanced by an increase in fatty acid oxidation (FaCd) and a decrease in rate of fatty acid re-esterification (FaTs) in adipose tissue (Fig. 17.14b).

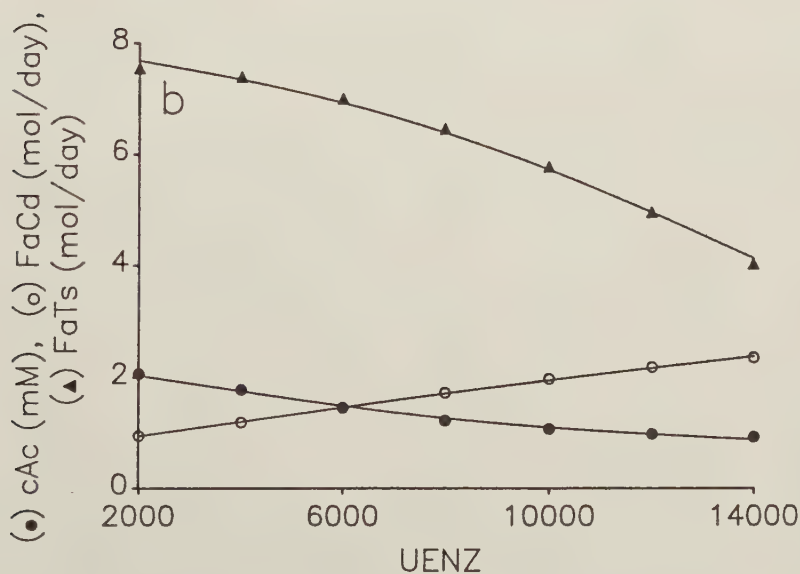
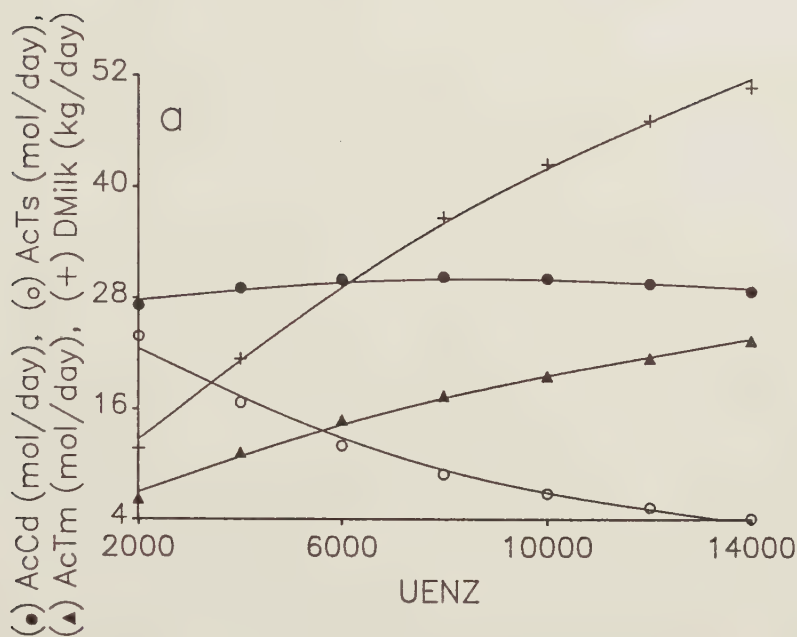


Figure 17.14 Effects of mammary gland metabolic capacity (UENZ) upon (a) acetate oxidation (AcCd), rate of mammary lipogenesis (AcTm) adipose lipogenesis (AcTs) and daily milk production (DMILK), and (b) blood acetate concentration (cAc), fatty acid oxidation (FaCd) and adipose fatty acid re-esterification (FaTs). Simulation conditions as described for Fig. 17.1.

A stated objective in formulation of the animal element of the cow model was 'to evaluate the use of *in vitro* data to parameterize mechanistic models' of metabolism. As discussed in previous chapters, it was already clear that $V_{\max}$ values determined in *in vitro* studies are usually significantly lower than those observed *in vivo*. Thus, the primary focus of this objective was on apparent affinity (k_s) values estimated *in vitro*. In a highly interactive model such as MOLLY, which was constructed for the explicit purpose of helping to explain the complex interactions among tissues that determine overall input:output relationships characteristic of real animals, relative affinities and metabolic capacities of tissues competing for common nutrients are central to model behavior and acceptability. Relative metabolic capacities of tissues can be scaled on the bases of independent data as discussed in Chapters 9, 10, 12, 13, 14 and 15. Relative affinities for nutrients are expensive, difficult and are often impossible to define *in vivo* because of the limited ranges of concentrations of nutrients that can be achieved under physiologically meaningful and acceptable conditions. A greater range of conditions can be created in studies of individual tissues *in vitro*. Thus, evaluations of effects of k_s values assigned to tissues on overall model behavior became an important focus of sensitivity analyses.

Effects of varying the apparent affinities of mammary ($k_{Ac,Tm}$) and adipose ($k_{Ac,Ts}$) tissue independently of one another are presented in Fig. 17.15. In the simulations presented both $k_{Ac,Tm}$ and $k_{Ac,Ts}$ were varied from 1.0 to 3.0 mM. The default value in both cases is 1.8 mM. Both sets of changes produced changes in concentrations of acetate (cAc); therefore, cAc was used as the independent variable in the plots presented. The response patterns depicted are essentially what one would expect. As the relative affinities of adipose and mammary tissues for acetate shifted to favor one tissue over the other, relative rates of lipogenesis and milk fat percentages (PTm) shifted accordingly. However, over the range of potential error in specifying the respective k_s values (1.8 ± 0.8 mM), effects on rates of lipogenesis in the two tissues were quite modest. Effects on acetate oxidation, glucose concentrations and milk production were all less than 5% (results not shown). Thus, model behavior is quite robust regarding these two affinity values, which supports the view that values derived from *in vitro* experiments are adequate for use in the model.

Effects of varying the affinity ($k_{Gl,Lm}$) of the lactose synthetase complex from 1.0 to 6.0 mM upon model behavior are presented in Fig. 17.16. As was noted in Chapter 13, results of arteriovenous studies indicate that glucose uptake by mammary tissue is relatively insensitive to the concentration of glucose in blood plasma. This was interpreted as indicating that glucose uptake is determined by factors other than glucose concentration, such as acetate availability from blood. Perhaps, the 6-fold range of $k_{Gl,Lm}$ tested was too large. At the extreme $k_{Gl,Lm}$ values of 1.0 and 6.0 mM, percentages of protein (PPm) and fat (PTm) in milk were too low and high, respectively (Fig. 17.16a). Comparing the full range of $k_{Gl,Lm}$ values, at higher values

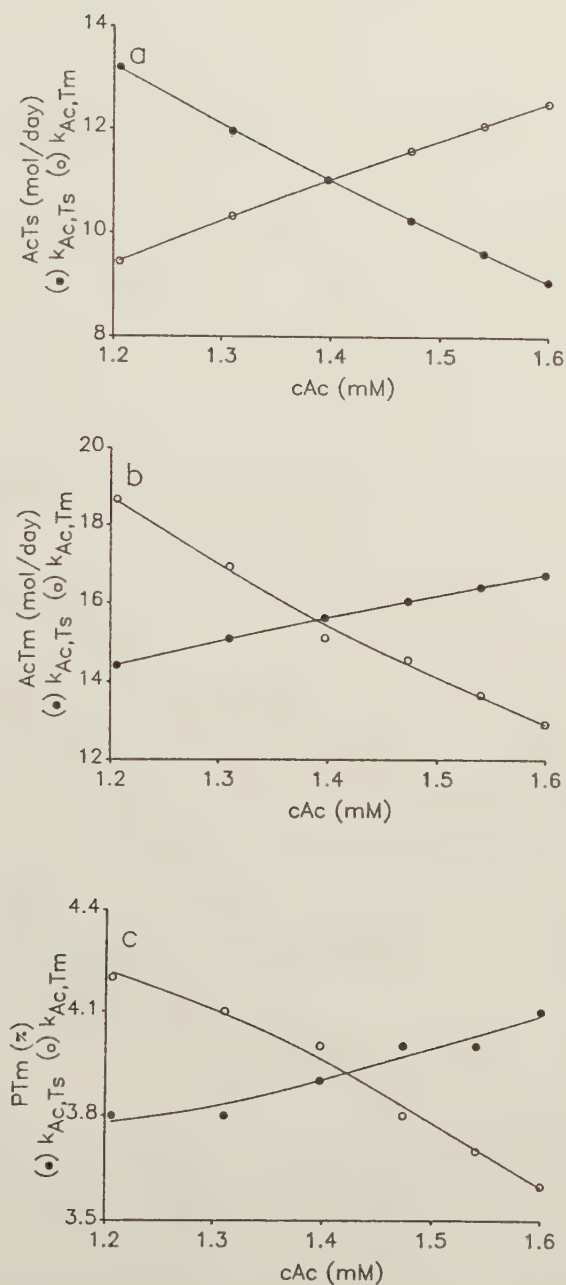


Figure 17.15 Effects of varying relative affinities of adipose ($k_{Ac,Ts}$) and mammary ($k_{Ac,Tm}$) tissues for acetate concentration (cAc) upon simulated animal performance relationships for (a) adipose tissue (AcTs), (b) lipogenesis in mammary (AcTm) and (c) milk fat percentage (PTm). Simulation conditions as described for Fig. 17.1.

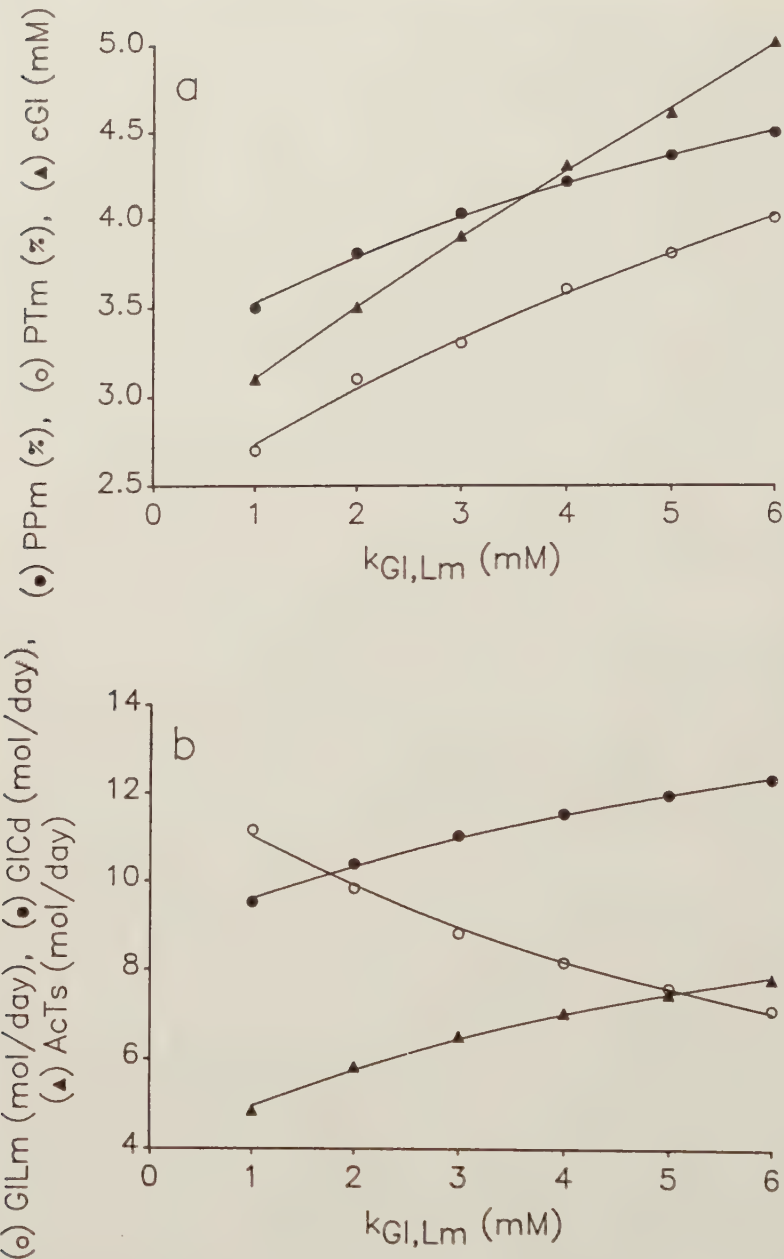


Figure 17.16 Simulated effects of affinity of the lactose synthetase complex for glucose ($k_{GI,Lm}$) upon (a) milk protein (PPm), milk fat percentages (PTm) and glucose concentration (cGI), and (b) lactose synthesis (GLm), glucose oxidation (GLCd) and lipogenesis from acetate in adipose tissue (AcTs). Simulation conditions as described for Fig. 17.1.

lactose synthesis (GILm) and milk production (not shown) increased 36%, glucose oxidation (GICd) increased 37% and lipogenesis from acetate in adipose tissue (AcTs) decreased 22.7%, indicating significant sensitivity to k_{GLm} not only at the mammary gland but also at the systemic level (Fig. 17.16b). This result is in apparent conflict with the lack of a glucose concentration effect upon mammary gland arteriovenous data (Miller *et al.*, 1991). However, the range of glucose concentrations observed in that study was basically 2.5 and 4.0 mM. Effects of specifying values for k_{GLm} between 2.0 and 4.0 mM were relatively modest, which suggests that if the current value of 3.0 mM is correct $\pm 30\%$, there is no conflict between the sensitivity analysis and the arteriovenous difference data. The implied requirement that k_{GLm} be specified with this accuracy is clearly worthy of additional experimental and modeling analyses.

Results of the final sensitivity analysis to be presented are in Fig. 17.17. In this analysis, the affinity of the mammary gland for amino acids ($k_{\text{Aa,Pm}}$) was varied. This constant is critical to model behavior, since it has significant effects upon milk protein synthesis and percentage ($U_{\text{Aa,Pm}}$, PPM), body protein synthesis ($U_{\text{Aa,Pb}}$), milk production (DMILK) and gluconeogenesis from amino acids ($U_{\text{Aa,Gi}}$). Most of the responses are essentially linear. As discussed in Chapters 10, 13 and 16, both total amino acid availabilities and availabilities of specific (limiting) amino acids to the udder can increase PPM, milk production or both. A single, highly aggregated affinity constant cannot provide for simulations of these alternatives. This is why emphasis in our recent experimental research and in revision of the mammary gland (Chapter 13) and lactating cow (Chapter 16) models has been placed upon the formulation of equations that enable improved simulations of amino acid metabolism in mammary tissue. Thus, the sensitivity analyses presented in Fig. 17.17 are presented as an example of the use of models and sensitivity analyses to identify weaknesses in a model and our current data and, thus, help set research priorities.

17.4 BIOECONOMIC ANALYSES

The lactating cow model presented in Chapter 16 has been subjected to fairly extensive testing. Some results were discussed in earlier parts of this and previous chapters. Even though it was developed primarily for research purposes, the lactating cow model has a number of desirable qualities. Responses to changes in diet and intake in terms of estimates of ME and balances among products of digestion are appropriate, as are responses in blood nutrient concentrations to dietary and other perturbations and effects of these upon patterns of nutrient utilization. Nitrogen and energy balance relationships compare favorably with experimental data for a range of diets. Most importantly, the model has the capacity to simulate effects of

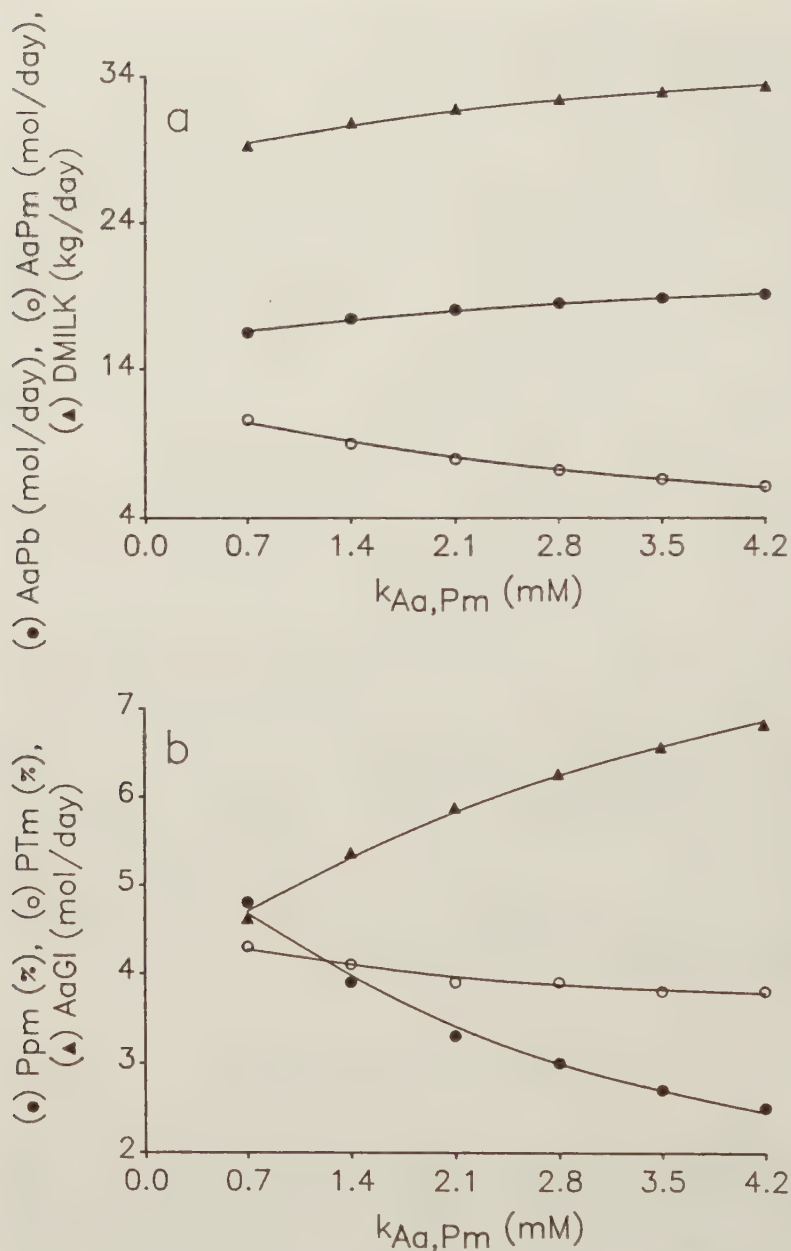


Figure 17.17 Effect of varying the aggregate mammary gland affinity constant for amino acid use for protein synthesis ($k_{Aa,Pm}$) upon (a) body protein synthesis (AaPb), protein synthesis (AaPm) and milk production (DMILK), and (b) milk protein percentage (PPm), milk fat percentage (PTm) and gluconeogenesis from amino acids (AaGl). Simulation conditions as described for Fig. 17.1.

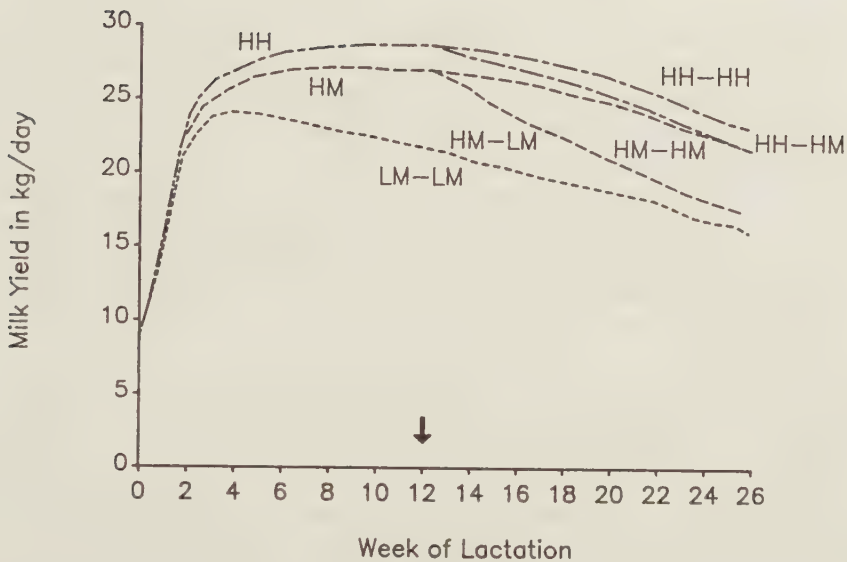


Figure 17.18 Effect of different feeding strategies upon lactational performance. L_ indicates a feeding rate of 5 kg per day plus 1 kg feed per 3 kg milk averaged over the previous 3 weeks. H_ indicates a feeding rate of 8 kg feed per day plus 1 kg feed per 3 kg average daily milk yield. _M indicates the standard forage:concentrate (50:50) ration at 15% crude protein. _H indicates standard ration was adjusted to 18% crude protein with fishmeal. Changeovers of diet and feeding strategy occurred at week 12 of lactation.

previous and current nutritional management strategies upon subsequent performance in dairy cattle. This is illustrated in Fig. 17.18.

The present lactating cow model has a number of limitations and a number of strengths, which might identify it as a prototype. It produces reasonably accurate simulations of effects of current feeding strategies upon subsequent performance, an attribute not shared with static models be they either highly empirical or highly mechanistic. Therefore, it was chosen for the bioeconomic evaluations presented in this section to illustrate applications of dynamic models in optimization of management strategies.

As an example of uses and benefits that can be gained from use of a dynamic dairy cow model, two areas will be examined. The first will be an evaluation of dry matter intake (DMI) equations. The second area will address an evaluation of feeding management strategies utilizing 100-cow herd simulations.

17.4.1 Evaluation of dry matter intake equation

A number of equations have been developed over the years to predict what a cow should eat. The NRC (1987) publication, *Predicting Feed Intake of Food*

Producing Animals, contains summaries of data for predicting dry matter intake, and factors that influence dry matter intake including milk production, body weight, environment, hormones, diet composition and physical activity. Key factors that limit dry matter intake in dairy cattle are physical fill and energy density of the feed (ME/kg). Conrad *et al.* (1964) and Conrad (1966) demonstrated that dry matter intake increased as dry matter digestibility increased until energy demands were met. Thereafter, dry matter intake remains constant or decreases as excess energy is available in relation to animal requirements. Conrad (1971) developed the following equation to describe dry matter intake:

$$\text{DMI} = 5.4W * (500 * F)^{-1} \quad (17.4)$$

where DMI is dry matter intake (kg/day), W is body weight (kg) and F is the portion of undigestible energy in the dry matter.

Several additional equations have been developed over the years (Table 17.1). The equations use various factors to determine the DMI that should be provided to support a level of milk production. The equations are static for the situation described at discrete levels of production. For evaluation, three of the equations were compared using the dynamic cow model over a full lactation.

The first of the intake equations was based upon NRC (1978) where allocations were made for maintenance and milk production. The equation used was as follows:

$$\text{DMI} = \text{FD}_{\text{maint}} + \text{FD}_{\text{milk}} \quad (17.5)$$

$$\text{FD}_{\text{maint}} = 8 \text{ kg} \quad (17.6)$$

$$\text{FD}_{\text{milk}} = 0.33 \text{ Milk} \quad (17.7)$$

where DMI is dry matter intake (kg), FD_{maint} is DMI for maintenance (kg), FD_{milk} is DMI for milk production and Milk is daily milk production (kg).

The second equation was based upon the concept that physical fill or capacity sets an upper or maximum limit on intake. This was linked to the fiber components of the diet, especially the indigestible fiber fraction. Mertens and Ely (1979, 1982) developed a model of fiber digestion and passage from the digestive tract. Disappearance from the digestive tract was influenced by rate of digestion, the portion of indigestible and potentially digestible fiber, physical size of particles in the diet, and intake. The model indicates that rate of passage and the indigestible fiber fraction have more influence than does rate of digestion on maximum intake. Following this analysis, Mertens (1985) developed equations based on NDF (neutral detergent fiber) in feeds to predict maximum intake. The following equation was used in the evaluations:

$$\text{NDF} = (1 - A) * \text{CNDF} + A * \text{FNDF} \quad (17.8)$$

where NDF is NDF content of the total diet, A is the forage fraction of the

Table 17.1 Comparisons of intake predictions (%body weight/day) for 600 kg dairy cows with zero body weight changes

4 % fat corrected milk (kg)	Equation reference number							
	a	b	c	d	e	f	g	h
10	1.87	2.2	2.53	2.12	—	2.50	2.52	2.1
15	1.94	2.4	2.69	2.42	2.25	2.82	2.79	2.5
20	2.08	2.7	2.87	2.72	2.55	3.09	3.07	2.9
25	2.27	3.0	3.03	3.02	2.84	3.32	3.34	3.2
30	2.52	3.2	3.20	3.32	3.13	3.50	3.62	3.5
35	2.82	3.4	3.37	3.56	3.43	3.64	3.89	3.8
40	3.14	3.6	3.53	—	3.72	3.74	4.17	4.1
45	3.48	3.8	3.70	—	3.94	3.79	4.44	4.4

^aBrown *et al.* (1977).

^bNational Research Council (1987).

^cAgricultural Research Council (1980).

^dMertens (1985) grass (65% NDF).

^eMertens (1985) legume (50% NDF)

^fMcCullough (1981).

^gConrad (1971).

^hNational Research Council (1989).

diet, CNDF is the NDF content of the concentrate and FNDF is the NDF content of the forage. Intake capacity (C) was assumed to be 1.1% of body weight per day of NDF. DMI is calculated as the minimum of either the fill capacity or the energy requirement of the animal based upon the energy content of the ration. (Equations implementing these concepts are identified as the MERTEN'S feeding strategy in Chapter 16).

Additional equations have been developed that utilize several factors such as body weight, milk production and nutrient density or content as variables. Brown *et al.* (1977) developed a prediction equation for DMI (kg/day) based upon live weight (W), milk fat in kg/day (FAT), milk yield in kg/day (DMILK), days in milk (DIM) and crude fiber content (CF):

$$\begin{aligned} \ln \text{DMI} = & 0.519 - (0.000827 \text{ DIM}) + (0.148 \text{ LN DIM}) + \\ & (0.33922 * \text{LN Milk}) + (0.099266 \text{ FAT}) + (0.000675 \text{ W}) \\ & + (0.018001 \text{ CF}) - (0.000557 \text{ CF}^2) \end{aligned} \quad (17.9)$$

The coefficients in the equations were based upon 4839 observations of 28-day intakes in university feeding trials. Field observations demonstrated that this equation predicted lower dry matter intakes than observed for high producing cows. Subsequently, Smith *et al.* (1989) adopted the equations to reflect high milk production and effects of season. Using a sine function for season, the transition from season to season (SEAF) was as follows:

$$\begin{aligned} \text{DMI} = & 2.7182 * (\text{SEAF} - 0.000827 * \text{DIM} + 0.14807 * \text{LN}(\text{DIM}) \\ & + 0.33922 * \text{LN Milk} + 0.0009926 * \text{Milk} * \text{FAT} \\ & + 0.0675 * \text{BW} + \text{CFF}) \end{aligned} \quad (17.10)$$

$$\text{SEAF} = -(0.24 * \sin 0.0166 (\text{DTJ}) + 1.01) + 0.586 \quad (17.11)$$

and

$$\text{CFF} = (\text{CN} (0.018001 - \text{CN}^2) * 0.00057) \quad (17.12)$$

where DTJ is Julian date, Fat became percent Fat; BW is body weight (kg/100), CFF is a crude fiber factor and CN is the lower limit for crude fiber. This equation is identified as the ELY equation in Chapter 16, because he programmed it for the model, and as the DART equation in this chapter. DART simply identifies the consortium of scientists who contributed to development of the equation. In the comparisons presented below, the season factor was set at a constant that would equal a March 15 feeding date.

The dairy cow model was run with the NRC, MERTEN'S and DART intake equations with four different diets (Table 17.2). The diets varied in concentrate to forage ratios to produce differences in energy density. Diet 1 was essentially a 60:40 concentrate:forage diet with a crude protein content of 15%. Diet 2 was the same as diet 1 but with an increased protein content (18%) achieved by adding fishmeal to increase bypass protein. The

Table 17.2 Diet composition (%) used in cow lactation model simulations

Item	Diet			
	1	2	3	4
Concentrate:forage	60:40	60:40	30:70	0:100
Crude protein	15	18	15	15
Soluble protein	4	2	4	4
Insoluble protein	8	13	8	8
NPN	3	3	3	3
Soluble carbohydrate	6	5	6	6
Organic acids	5	4	5	5
Pectin	6	5	6	3
Lipid	4	4	4	4
Hemicellulose	9	9	12	15
Cellulose	18	18	25	32
Lignin	4	4	4	7
Soluble ash	4	4	4	4
Insoluble ash	4	4	4	4
Starch	25	25	15	5
Soluble starch	2	2	2	2
Small particles	4	4	4	2
\$/kg	0.123	0.138	0.110	0.091

forage content of diet 3 was 70% and diet 4 was 100% forage. Protein contents of diets 3 and 4 were 15%. With increasing forage content, hemicellulose, cellulose and lignin increased and starch decreased in the diets. Costs of the ration (\$/kg) were calculated for a corn silage and alfalfa hay forage base.

Runs were made for average and high potential rates of production. The high production cow had a 50% greater capability for milk production than did the average cow. Each cow was simulated with each diet and intake equation for a 305 day lactation.

Because the equations for feed intake per day were devised as static equations and the evaluations were dynamic, in initial simulations body weight in the equations acted as an accelerator either upward or downward. As the simulated cow got larger she could eat more and therefore continue to increase her intake until she became overly fat. In contrast, as the simulated cow lost body weight on poor rations, she could eat less which, in turn, accelerated weight losses until simulated death occurred due to exponential overflows in the computer. To prevent this, reference body weight in the equations was set to 650 kg. This was weight at calving for the mature model cow. This was the reference for computation of maintenance requirements such that simulated body weight at a given time did not act as an accelerator either upward or downward. For the NDF equation to function properly, a maximum daily body weight gain was specified.

For the simulations, maximum daily body weight gain was set at 0.5 kg/day. This prevented the NDF equation from spiralling upward to produce a huge cow. To soften the effect of daily milk production changes upon intake, daily milk production was computed as a running 3 day average.

Diet 4 would not support full lactations for the high cow with any of the intake equations.

Total milk production and dry matter intakes for the full simulated lactations are in Table 17.3. On all diets and for all of the intake equations, the high cow produced more milk than did the average cow. The high cow had a greater dry matter intake than the average cow for each of the intake equations, but the NDF and DART equations had greater dry matter intakes for the average cow than the NRC equation had for the high cow. The NRC equation had the lowest milk productions and dry matter intakes for all combinations of diets and cows. The NDF and DART equations were similar, with the NDF equation yielding greater intakes and milk yields with some diets and DART for others. The results were confounded with milk production potential, such that no generalizations with regard to use of the two equations were evident.

Table 17.3 Milk production and dry matter intake over a full lactation for a high producing and an average cow with different intake equations

Intake equation	Cow	Diet			
		1	2	3	4
Milk production (kg)					
NRC ^a	AVG	6206	7106	5693	4899
NDF ^b	AVG	6788	7530	6376	4786
DART ^c	AVG	6887	7675	6353	5479
NRC	HIGH	8125	9430	7415	
NDF	HIGH	9453	10361	7877	
DART	HIGH	9106	10167	8381	
Dry matter intake (kg)					
NRC	AVG	4774	5064	4610	4359
NDF	AVG	6788	5507	5499	4547
DART	AVG	5567	5751	5387	5062
NRC	HIGH	5388	5806	5162	
NDF	HIGH	6667	6693	5785	
DART	HIGH	6349	6540	6121	

^aNational Research Council (1989).

^bMertens (1985).

^cMcCullough (1981)

Final body weights for each simulated cow on the diets are presented in Table 17.4. With a starting weight of 650 kg and a gain of 50 kg for pregnancy, the final weights should be 700 kg. The NRC equation with the

Table 17.4 Final body weights (kg) after a full lactation^a

Intake	Cow	Diet			
		1	2	3	4
NRC	AVG	703	727	617	472
NDF	AVG	778	773	744	603
DART	AVG	806	815	714	569
NRC	HIGH	660	698	562	
NDF	HIGH	808	798	678	
DART	HIGH	780	789	683	

^aSee footnote to Table 17.3.

average cow and diet 1 yielded estimates very close to that desired, with a slight increase in diet 2. On diets 3 and 4 with the average cow and on diets 1 and 3 with the high cow, the NRC equation yielded low body weights. The NDF equation produced high body weights, except for diet 4 with the average cow and diet 3 with the high cow. The DART equation produced body weights above 700 kg in all cases, except for diet 4 with the average cow and diet 3 with the high cow. Diets 3 and 4 demonstrate the effects of lower nutrient densities in rations and the need for the cow to utilize excessive body tissue for milk production.

Traditionally, diets are evaluated economically by calculating income over feed cost (IOFC). Income is milk income. In this example, milk was priced at \$0.26/kg of 3.5% fat milk with a fat differential of \$0.0024/0.1% change of fat percent. Feed prices used for each diet are in Table 17.2. IOFC are reported in Table 17.5.

A cost for body weight change was also calculated. This was used to evaluate the use of feed for body condition gain that would be used in the next lactation. Body weight cost was calculated based upon the assumption that 1 kg body weight is equal to 8 kg milk (NRC, 1989). This value needs to be evaluated to accommodate the very large weight gains on some of the diets. Profit was calculated as milk income minus feed cost minus body weight cost. In this analysis, cows gaining weight would have a lower profit than those losing weight. Please note that for simplicity, each diet was fed for the full lactation. If the model had been interfaced with an economic optimization routine, one or more ration changes during the lactation cycle would have been indicated. Calculated profits for the lactations are reported in Table 17.5. IOFC and profit generally reflect the milk production data, with the highest profit being associated with the highest production. Diet 2 produced the highest IOFC and profit.

If diet 1 is considered to be a standard or reference production level, differences from diet 1 can be used to evaluate the effect of adding protein (diet 2) or increasing forage and decreasing energy (diet 3 and diet 4). Val-

Table 17.5 Income over feed costs (IOFC) and profit for full lactations^a

Intake	Cow	Diet			
		1	2	3	4
IOFC (\$)					
NRC	AVG	983	1098	939	856
NDF	AVG	1112	1191	1089	829
DART	AVG	1133	1222	1085	987
NRC	HIGH	1411	1609	1329	
NDF	HIGH	1710	1819	1431	
DART	HIGH	1631	1775	1547	
Profit (\$)					
NRC	AVG	1234	1367	1109	965
NDF	AVG	1381	1456	1333	1008
DART	AVG	1410	1507	1334	1156
NRC	HIGH	1644	1883	1495	
NDF	HIGH	1989	2099	1649	
DART	HIGH	1901	2053	1799	

^aSee footnote to Table 17.3.**Table 17.6** Differences from diet 1 for milk production, feed, feed cost, intake over feed costs and profit for the average cow^a

<i>Intake</i>	<i>Diet</i>		
	2	3	4
Milk (kg)			
NRC	900	-513	-1307
NDF	742	-912	-2002
DART	788	-534	-1408
Feed (kg)			
NRC	290	-164	-415
NDF	100	92	-860
DART	184	-184	-505
Feed costs (\$)			
NRC	111	-80	-190
NDF	95	-60	-251
DART	109	-92	-224
IOFC (\$)			
NRC	114	-44	-127
NDF	80	-22	-282
DART	89	-48	-146
Profits (\$)			
NRC	33	-125	-269
NDF	75	-48	-373
DART	86	-76	-254

^aSee footnote to Table 17.3.

ues for the average cow are in Table 17.6. Increasing protein (diet 2) resulted in more milk, more feed eaten, increased feed cost, increased IOFC and increased profit. Increasing forage content with decreasing energy (diet 3 and 4) resulted in less milk, less feed, lowered feed costs, lower IOFC and lower profits. Even though money is saved in feed costs with these changes, returns are actually lower with less milk and body weight. Values for the high production cow are in Table 17.7 and follow the same trend as the average cow but with differences of greater magnitude. This emphasizes the fact that production level affects the results of management decisions.

Table 17.7 Differences from diet 1 for milk production, feed, feed cost, income over feed costs and profit for high production cow^a

Intake	Diet	
	2	3
	Milk (kg)	
NRC	1305	-710
NDF	908	-1576
DART	1061	-725
	Feed (kg)	
NRC	418	-226
NDF	26	-882
DART	191	-227
	Feed costs (\$)	
NRC	138	-94
NDF	103	-184
DART	121	-107
	IOFC (\$)	
NRC	198	-81
NDF	109	-279
DART	143	-83
	Profits (\$)	
NRC	239	-148
NDF	110	-339
DART	151	-102

^aSee footnote to Table 17.3.

To further evaluate the equations, daily milk production and daily DMI were graphed for each of the diets and equations. Milk production for the average cow on diet 1 (Fig. 17.19), dry matter intake for the average cow on diet 1 (Fig. 17.20), milk production for the high cow on diet 1 (Fig. 17.21) and dry matter intake for the high cow on diet 1 (Fig. 17.22) show the lactation curves produced. The relative rankings for the intake equations are the same as for the full lactation data. With the high fiber diets (diet 3 and diet 4), the NDF equation differs from the NRC and DART equations. Feed intake does not peak sharply and flatten, as shown for the high cow on diet

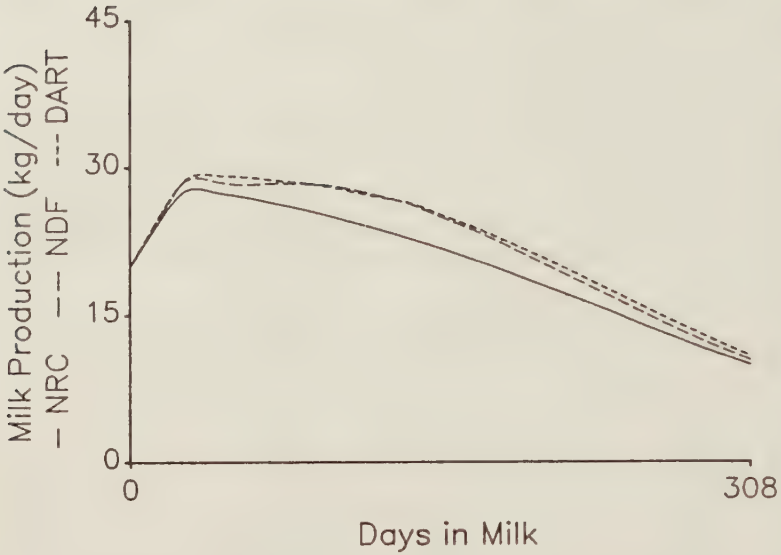


Figure 17.19 Milk production for the average cow on diet 1 (60:40 concentrate:forage ratio, 15% protein).

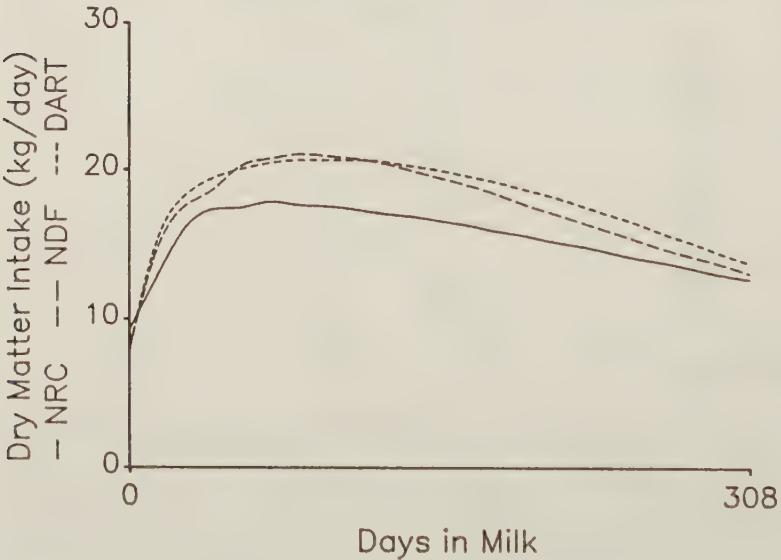


Figure 17.20 Dry matter intake of average cow on diet 1 (60:40 concentrate:forage ratio, 15% protein).

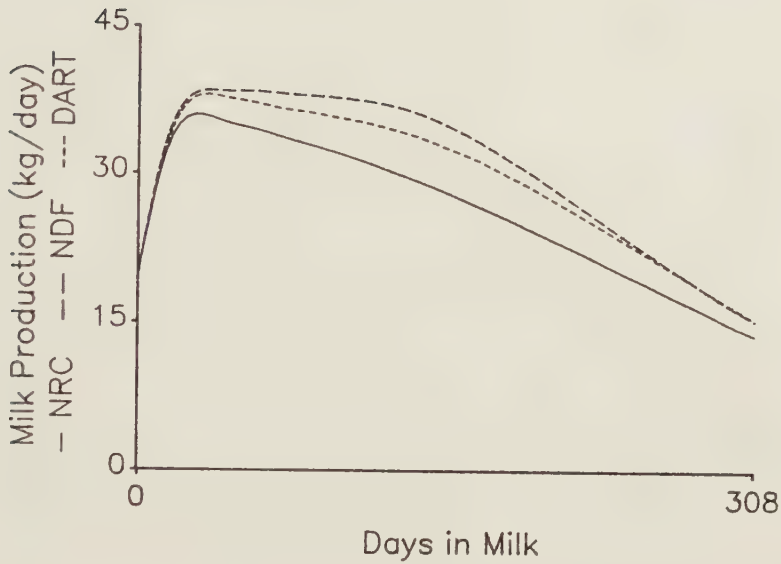


Figure 17.21 Milk production of high cow on diet 1 (60:40 concentrate:forage ratio, 15% protein).

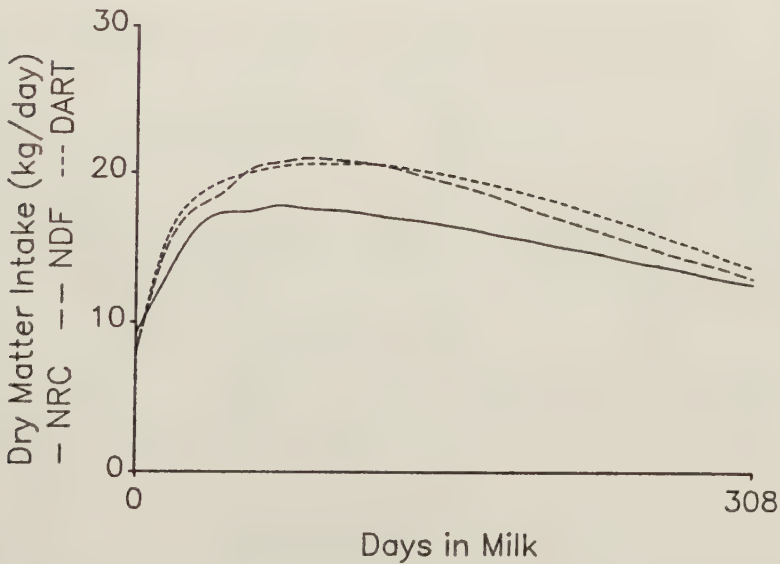


Figure 17.22 Dry matter intake of high cow on diet 1 (60:40 concentrate:forage ratio, 15% protein).

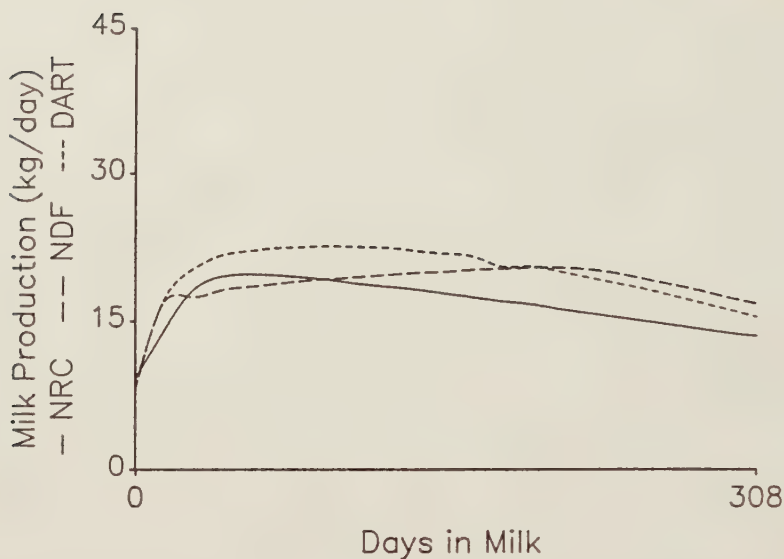


Figure 17.23 Dry matter intake of high cow on diet 3 (30:70 concentrate:forage ratio, 15% protein).

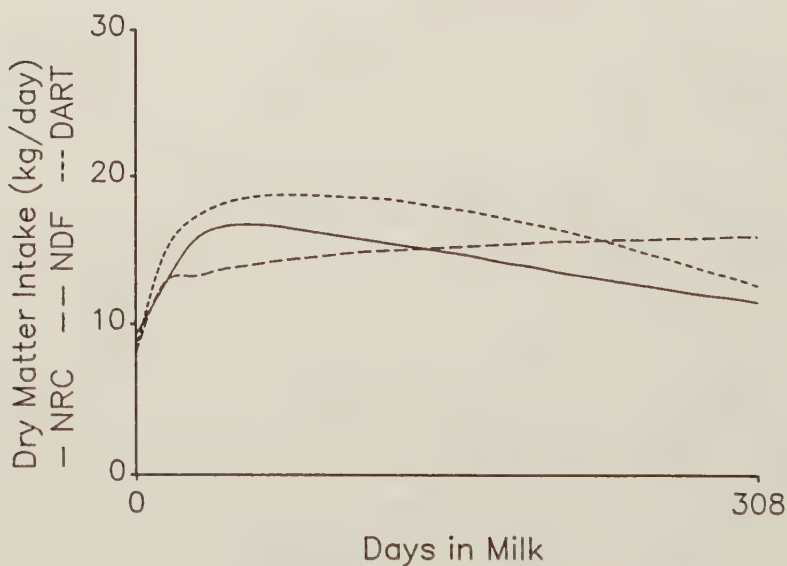


Figure 17.24 Dry matter intake of average cow on diet 4 (100 forage, 15% protein).

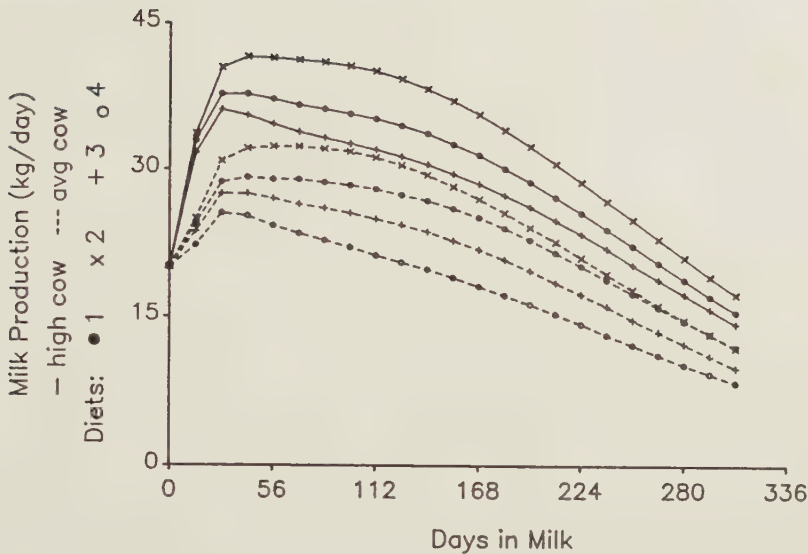


Figure 17.25 Milk production response for the DART intake equation for average cow on diets 1, 2, 3 and 4; and for high cow on diets 1, 2 and 3.

3 (Fig. 17.23) and the average cow on diet 4 (Fig. 17.24). Additional data must be generated with high fiber diets at high levels of milk production to further evaluate the NDF and DART equations and the simulated milk production responses presented in these figures.

Figure 17.25 has the milk production responses for the DART equation on all diets for both cows. The rank of the curves is the same as for lactation yields with the highest being the high cow on diet 2 followed by high cow diet 1, high cow diet 3, average cow diet 2, average cow diet 1, average cow diet 3 and average cow diet 4. The high cow's lactation curve peaks higher and decreases more sharply and faster than does the average cow as compared with lactation curve analyses of DHIA data. Comparing the three equations, the NRC equation underestimates intake while the DART and NDF equations are very similar, with the major difference occurring on high fiber diets. The DART equation may be underestimating the rumen capacity limit of these diets in early lactation.

17.4.2 Evaluation of feeding strategies

One management question that is of concern to the dairy industry is when is the best time to change rations. Using diets 1, 2 and 3, various strategies were evaluated. For these simulations, individual lactation curves for 100-cow herds were generated. The milk production potential for the herd was the same as the high cow (relative udder metabolic capacity expressed as

Table 17.8 Evaluation of alternative herd feeding strategies^a

<i>Strategy</i>	<i>Diet</i>	<i>Milk (kg)</i>	<i>FCM (lb)</i>	<i>DMI (kg)</i>	<i>EBW (kg)</i>	<i>Profit (\$)</i>
0	1	9044 ^b	19648 ^a	6295 ^c	690 ^{bc}	1888 ^c
0	2	1027 ^g	20007 ^a	6619 ^e	701 ^f	2179 ⁱ
0	3	8324 ^e	18155 ^c	6069 ^d	585 ^g	1708 ^h
3	1→3 T = 140	9021 ^b	19593 ^a	6292 ^c	690 ^b	1884 ^c
6	1→3 T = 98	8502 ^d	18776 ^b	6123 ^d	598 ^d	1766 ^g
11	1→3 M = 20	9019 ^b	19587 ^a	6286 ^c	690 ^{bc}	1883 ^c
12	1→3 M = 29.5	9015 ^b	19580 ^a	6285 ^c	689 ^b	1882 ^c
7	2→1 T = 98	9899 ^{ac}	19761 ^a	6447 ^a	694 ^c	2061 ^e
2	2→1 T = 140	9830 ^a	19636 ^a	6381 ^b	691 ^b	2047 ^{ab}
8	2→3 T = 98	9397 ^f	18943 ^b	6298 ^c	605 ^e	1940 ^f
1	2→1→3 T = 98 T = 210	9829 ^a	19654 ^a	6455 ^a	653 ^a	2022 ^a
4	2→1 M = 29.5	10079 ^c	19709 ^a	6483 ^a	690 ^b	2035 ^{ad}
10	2→1 M = 20	10071 ^c	19693 ^a	6475 ^a	690 ^{bc}	2034 ^{ae}
9	2→1→3 M = 29.5 M = 20	10095 ^c	19747 ^a	6483 ^a	689 ^b	2031 ^{ae}
Pooled standard error		63	155	21	1	14

^aStrategies indicated by 0 involved feeding the simulated herd of 100 cows diets 1, 2 or 3 for the whole lactation. Strategy 1 involved feeding diet 2 for 98 days, diet 1 from 98 to 210 days of lactation and diet 3 from 210 days to the end of lactation. Strategies 2 and 3, respectively, involved starting lactation with diet 2 or 1 and switching to diet 1 or 3 for the remainder of the lactation. Strategy 4 simulated feeding individual cows diet 2 until their milk production dropped below 29.5 kg/day. Strategies 6, 7 and 8, respectively, involved changing from diet 1 to diet 3, diet 2 to diet 1 or diet 2 to diet 3 at day 98 of lactation. Strategy 9 involved changing individual cows from diet 2 to diet 1 when milk production dropped below 29.5 kg/day. Respective changes for strategies 10 and 11 were to change from diets 2 to 1 and 1 to 3 when milk production dropped below 20 kg/day. Strategy 12 indicated a shift from diet 1 to 3 when milk production dropped below 29.5 kg/day.

Values with differing superscripts within a column were significantly different from one another according to the Student–Newman–Kuhl test ($P < 0.9/05$). Strategy 1 was arbitrarily assigned the superscript 'a' for purposes of a common reference point.

Ucells [Chapter 16] was 1500). Individual cow lactation potentials were randomly generated using a range of Ucells of 1500 ± 200 . The resulting herd of 100 cows had an average of 1500 Ucells $\pm$ a standard deviation of 156. Everything else in the model was the same as in previous runs for individual cows. For this evaluation, only the DART intake equation was used.

Strategy 0 was feeding single diets for the whole lactation. Milk production (in kg), total volume of milk corrected to 4.0% fat (FCM in pounds), dry matter intake (DMI in kg), empty body weight (EBW in kg) and profit (in dollars) averages for the simulated 100-cow herd (Table 17.8) were slightly lower than those for the single-cow estimates presented above.

Alternative strategies evaluated were based upon diet switches at different days in milk or rates of milk production (see Table 17.8 footnote). Our purpose in formulating Table 17.8 was to illustrate the utility of using a dynamic, mechanistic model to evaluate the probable impact of alternative feeding management strategies upon profitability in herds with a known milk production potential and genetic variance. Interested readers can compare among strategies they prefer for herds with a production potential of about 9000 kg/lactation. However, we would emphasize that with herds with genetic potentials above or below this reference, rankings will differ. Therefore, our discussion of the results will be limited and should not be considered generally applicable. Two trends are evident in the simulation outputs. Starting lactations with diet 2 (higher protein strategies 7, 2, 8, 1, 4, 10, 9) instead of diet 1 (strategies 6, 3, 11, 12) resulted in higher averages for milk production, dry matter intake, profit and fat-corrected milk (Table 17.8). The increased protein input during early lactation to cows of above average genetic potential paid dividends. The second trend that was evident was that the longer a more nutrient dense diet was fed (or the diet switch was made later in lactation), increased performance and profit resulted.

This limited evaluation demonstrates the value of the use of dynamic models to evaluate alternative feeding strategies and enable researchers or managers to test these in the real world. As there are an infinite number of feeding strategies, this approach could be used to identify those that are most critical. In order to improve the strategies, inclusion of body condition scores based upon body composition could be built into the decision switches illustrated in this example.

Dynamic, mechanistic lactating dairy cow models allow the generation of data for evaluations of animal performance and managerial decisions. A large number of variables can be examined to give direction to decision making for researchers and managers. As teachers of animal science and lactation courses, we wish to note that the performance evaluations presented in Tables 17.1–17.7 extend significantly beyond those one can compile from available literature to illustrate the complex matrix of decisions a dairy manager or nutritionist must address. Current practitioners may address these issues intuitively or through the use of static equation systems,

but both approaches are, at most, semiquantitative when used in evaluations of lactation and economic performance. In our view, dynamic models are absolutely essential to economic evaluations of risks associated with current decisions because these clearly influence subsequent performance. When a proven, dynamic, mechanistic model becomes available to students and practitioners to generate data such as those presented in Table 17.8 and, further, to enable cause-and-effect analyses of underlying reasons for the observed responses, we will have created a superior instrument for both the teaching and application of our science. This is the challenge we pose.

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Modeling Ruminant Digestion and Metabolism

R.L. Baldwin

This important book is the most comprehensive and authoritative work on the evolution and application of models for use in support of nutritional and animal science research. The book commences with a discussion of modeling philosophy, continuing with the evolution of models in energy and nitrogen metabolism.

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