Simulation of energy metabolism in the simple-stomached animal

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1. A computer programme is described which simulates energy metabolism in the whole animal. Simulation was based on representation of the animal as a quasi-steady-state system.

2. Input for the programme consisted of the chemical composition of the diet and an estimate of either the maintenance energy requirement or an estimate of energy retention.

3. Simulation was performed by estimating the yield of adenosine triphosphate in the major metabolic pathways operative in simple-stomached animals, and on the utilization of adenosine triphosphate in major anabolic processes.

4. Results obtained from simulation were in close agreement with experimental observations reported by McCracken (1975).

The complex nature of the many reactions involved in energy metabolism in the intact animal has led biological scientists to mathematical modelling to augment their insight of this biological process. Voit (1901) and Rubner (1902) were among the first to propose mathematical expressions to describe heat exchange in the animal. The investigations of Brody & Proctor (1932) and of Kleiber (1932) resulted in refinements of the mathematical model for the basal metabolism of mammals, and the same model can probably be applied to all homoeotherms (Kleiber, 1947). As a result of the efforts of the latter investigators, metabolic body size has been expressed as body-weight $^{0.75}$ (Kleiber, 1965), and many biological measurements and indices have been found to be proportional to metabolic body size (Brody, 1945). Numerous investigators have contributed refinements and extensions to this model of nutritional and metabolic processes in the animal (Miller & Payne, 1963; Hartsook & Hershberger, 1971; Milligan, 1971).

The foregoing mathematical models of energy transformation in the whole animal are of a phenomenological nature. The modelling was based on the measurement of factors which are reflexions of many reactions, and these factors do not necessarily provide information about any specific reaction. An alternate method of mathematical modelling is to develop a model consisting of the differential equations which describe the rates at which nutrients and intermediate metabolites are metabolized (Garfinkel & Heinmets, 1969; Srinivasan, Kadish & Srihar, 1970; Baldwin & Smith, 1971; Mazanov & Nolan, 1976). This approach is based on the concept of the dynamic state of the living organism. Potentially, it is a very useful form of mathematical modelling, but it suffers from a number of practical limitations. A complete and precise model of the dynamic state of energy transformation in an animal requires reliable estimates of the rate constants and reactant concentrations for each differential equation. The term rate constant must be interpreted with caution because it reflects the concentration of an active enzyme, which is itself not a time-invariant in the case of many reactions (Atkinson, 1966; Holzer & Duntze, 1971; Schimke, 1973). Even if estimates were available for all of the rate constants and concentrations, computer hardware which is adequate to provide for the simultaneous numerical solution of the many differential equations needed to define a single mammalian cell completely is not available (Garfinkel & Heinmets, 1969). This type of mathematical modelling of the intact animal has been accomplished by restricting the analysis to the tissues and organs most directly involved in the

process being studied, by representation of several reactions by a single approximate equation, and by supplementing known rate constants and concentrations with reasonable approximation. The judicious use of such approximate equations may result in valid conclusions, but the accuracy and uniqueness of the solutions obtained are always subject to doubt.

The approach to modelling of the energy balance in the whole animal described in this communication consists of computer-based calculation of the amount of ATP formed (mol) during oxidative metabolism, and of calculation of the utilization of ATP for maintenance and for biosynthesis. The calculations were based upon the representation of the animal as a quasi-steady-state system rather than a dynamic system, and on the assumption that the metabolic fate of the dietary components and metabolic intermediates could be represented by a finite number of stoicheiometric expressions. Energy balance was simulated for a diet on the basis of the metabolic stoicheiometries included in the computer programme. The carefully conducted experiments of McCracken (1975) provided a means of comparing well-organized experimental results with the results of computer-based simulation. The results obtained from simulation were in close quantitative agreement with the results and conclusions of McCracken (1975).

METHODS

Description of the computer programme

The computer programme developed to simulate energy balance in the animal was written in FORTRAN IV for use with a Digital Equipment Corporation PDP-10 computer in time-sharing mode. Execution of the programme required approximately 7K core space. A number of assumptions were inherent to this programme. Some of these assumptions are discussed in later sections of the programme description but the most basic assumptions were as follows.

(1) It was assumed that urea is the only nitrogenous excretory product of protein metabolism, and no factor was included in the calculations explicitly for the energy cost of excretion of urea by the kidney.

(2) Ammonia formed during the course of metabolism, with the exception of that formed specifically during urea synthesis, was assumed to be converted to the amide-nitrogen of glutamine with the utilization of 1 mol ATP/mol glutamine formed.

(3) Glutamate dehydrogenase was assumed to play a 'pivotal' role in ammonia formation from amino acids and in glutamate formation.

(4) All nucleotide triphosphates were assumed to be interconvertible, and are expressed as ATP.

(5) It was assumed that 1 mol ATP is required to transfer 1 mol NADH, or the reducing equivalents therefrom, from the cytosol into a mitochondrion, but that the reverse transfer does not require energy (Meijer & Van Dam, 1974).

(6) It was assumed that the transfer of electrons from NADH to NADP to form NADPH requires 1 mol ATP/mol NADPH formed.

(7) The transfer of 1 mol acetyl-coenzyme A (acetyl-CoA) from a mitochondrion to the cytosol was assumed to require 1 mol ATP.

(8) The stoicheiometric expressions itemized in this communication were included in the programme, and they were assumed to summarize the major metabolic pathways with reference to energy balance.

(9) In order to express ATP in units which allow easy comparison with traditional food energy values, it is assumed that 77·3 kJ available energy is required to synthesize 1 mol ATP regardless of the nutrient oxidized to provide the available energy (Schulz, 1975*a*, *b*).

The implication of some of the foregoing stipulations should be clarified. The absorption

and transport of nutrients into the blood stream against a concentration gradient doubtless requires energy. In the case of long-chain fatty acids, transport from the intestinal mucosa to the liver is via the lymphatic system, but energy is required for synthesis of triacylglycerols from the free fatty acids and for formation of the protein coat of the chylomicra (Pike & Brown, 1975). If metabolites are transported from the liver to other tissues, once again, the passage of the metabolite through the cell membrane and into the blood stream no doubt requires energy. The absorption and membrane transport of nutrients required for maintenance appears as one component of the maintenance requirement, but the energy cost of these processes for nutrients in excess of maintenance must be accounted for in an explicit manner. The assumption inherent in the algorithm described here is that each mole of metabolite involved in the biosynthesis of body substance is transported across biomembranes with the expenditure of energy in the form of ATP. The biosynthesis of body substance involves the expenditure of energy for processes other than transport, e.g. the synthesis of nucleic acids, but the energy cost of such ancillary processes is probably relatively small. The programme described here allows the investigator to stipulate the amount (mol) of ATP required for absorption and membrane transport of each mole of nutrient in excess of those required for maintenance. If the investigator does not choose to specify the energy cost of membrane transport, a cost of 2 mol ATP/mol metabolite in excess of maintenance is assumed.

The excretion of urea by the kidneys represents an energy expenditure (Borsook & Winegarden, 1931; Martin & Blaxter, 1965), but it is difficult at the present time to estimate the magnitude of this energy cost. A major portion of the work of the kidneys is a component in the maintenance requirement.

Although glutamate dehydrogenase (*EC* 1.4.1.2) appears to function reversibly in ammonia formation and in glutamate formation (Braunstein, 1957), the purine nucleotide cycle may be more important than glutamate dehydrogenase in ammonia formation from amino acids (Lowenstein, 1972; McGiven & Chappell, 1975). More conclusive evidence is needed before the purine nucleotide cycle can be accepted as the major source of ammonia from amino acids in the intact animal. If the latter were shown to be correct, the yield of ATP from the oxidative metabolism of many amino acids would be slightly lower than estimated by this programme. Appropriate modifications could be inserted in the programme easily.

Conversion of available energy from mol ATP to kcal or kJ was performed because food energy is expressed traditionally in these units. Available energy as ATP was defined as the amount (mol) of ATP formed upon complete oxidation of carbohydrates and fats to carbon dioxide and water and of proteins to urea, CO_2, H_2O and SO_4^{2-}. The enthalpies of combustion of glucose, glycogen and trioleoylglycerol are 15·5, 17·6 and 39·8 kJ/g respectively. The yields of ATP obtained on complete biological oxidation of the foregoing nutrients are 0·200, 0·228 and 0·511 mol ATP/g respectively from which the values of 77·4, 76·9 and 77·7 kJ/mol ATP formed respectively can be calculated. The average value is 77·3 kJ/mol ATP, and this was taken as the kJ equivalent of each mole of ATP derived from any nutrient. Expression of food energy in terms of mol ATP available to the animal on complete biological oxidation of the food was more appropriate than expressing energy values in terms of enthalpy of combustion. This concept appears to have been anticipated by Rubner (1902) who stated: 'The energetic concept of the processes of nutrition is not based upon the fact that someone started to express nutritional materials in calories, but upon the knowledge and proof that the animal organism depends upon the energy values of the nutritional materials and body substances. In spite of the varying chemistry of catabolism which apparently occurs during changes in the form of nourishment, the energy metabolism is the determining factor and focal point around which everything else revolves.'

Data input

The computer programme was dimensioned to allow the analysis of one to ten dietary components. The investigator was given the opportunity to specify the digestibility for each dietary component, or if a digestibility was not specified, a value of 0·95 digestibility was assumed. The input required the amount of each dietary component (g), and for each dietary component the amounts (g) of protein, cellulose, glucose, carbohydrate other than glucose, and fat. The investigator was given the choice between accepting an average amino acid composition for each dietary component or of including the amino acid composition. In the case of fats, any one of three options may have been chosen for each dietary component. The first option was based on the assumption that the composition of the fat in the dietary component can be represented by trioleoylglycerol. The second option provided the investigator the opportunity to specify the amount (g) of polyunsaturated fatty acids. The polyunsaturated fatty acid was assumed to be linoleic acid, and it was assumed to exist as the triacylglycerol. The remainder of the fat in that dietary component was assumed to be represented by dioleoyl-monopalmitoylglycerol. The third option provided the opportunity to specify the fatty acid composition of each dietary component explicitly.

The input information also consisted of the body-weight of the animal and the period (d) on the experiment. Either of two options may have been chosen with respect to the procedure for calculation of energy balance. If estimates were available of the amount of protein, carbohydrate and fat synthesized, the programme would calculate the maintenance energy (ME) requirement. The second option allows the investigator to specify ME requirement in terms of a coefficient and an exponent of body-weight. When the latter option was chosen, the input could include an estimate of the amount of protein synthesized. If such an estimate were not available, the programme would calculate the amount of protein synthesis allowable on the basis of the expression:

$$\text{g protein synthesis} = \frac{\text{mol limiting amino acid in diet}}{\text{mol limiting amino acid/g body protein}}.$$

The investigator was provided the opportunity to specify that the amino acid composition of the synthesized protein was the same as that of average mammalian muscle protein (Block & Weiss, 1956). No net carbohydrate synthesis was simulated unless the investigator specified an amount of lactose or glycogen synthesis. When ME was specified, the amount of fat synthesis simulated was determined by the energy available for fat synthesis. The synthesized fat was assumed to be trioleoylglycerol.

Dietary carbohydrate metabolism

The amount (mol) of glucose derived from carbohydrates other than glucose was calculated by subtracting the amount (g) of cellulose from the total amount (g) of non-glucose carbohydrate and dividing the difference by 162. The amount (mol) of glucose from dietary glucose was calculated directly from the amount (g) of dietary glucose. The available energy in terms of ATP which could be formed from carbohydrate was calculated according to stoicheiometry equation (1) regardless of whether or not a portion of the carbon skeleton of the digested carbohydrate is utilized in biosynthetic reactions:

$$\left.\begin{array}{l} \text{glucose} \longrightarrow 2 \text{ pyruvate} + 2 \text{ NADH}_{\text{cyt}} + 2 \text{ ATP} \\ 2 \text{ NADH}_{\text{cyt}} + 2 \text{ ATP} \longrightarrow 2 \text{ NADH}_{\text{mito}} \\ 2 \text{ NADH}_{\text{mito}} + \text{O}_2 \longrightarrow 6 \text{ ATP} \\ 2 \text{ pyruvate} + 5 \text{ O}_2 \longrightarrow 6 \text{ CO}_2 + 30 \text{ APT} \\ \hline \text{glucose} + 6 \text{ O}_2 \longrightarrow 6 \text{ CO}_2 + 36 \text{ ATP} + 6 \text{ H}_2\text{O} \end{array}\right\} \quad (1)$$

where $NADH_{cyt}$ and $NADH_{mito}$ are cytoplasmic and mitochondrial NADH respectively. The programme simulated the oxidation of glucose which is not utilized for biosynthesis according to stoicheiometry equation (1). The biosynthetic stoicheiometries for glucose are presented in the sections concerned with protein, carbohydrate and fat synthesis (see pp. 244–9).

Dietary amino acid metabolism

Stoicheiometry equation (2) outlines the reactions involved in the formation of urea from glutamate and CO_2. In the remaining amino acid stoicheiometries, this sequence will be presented only as the summation indicated in equation (2):

$$\left.\begin{array}{l} 0{\cdot}5 \text{ glutamate} + 0{\cdot}5 \text{ oxalacetate (OAA)} \longrightarrow 0{\cdot}5 \text{ aspartate} \\ \qquad\qquad + 0{\cdot}5 \ \alpha\text{-ketoglutarate } (\alpha KG) \\ 0{\cdot}5 \text{ glutamate} + 0{\cdot}5 \ NAD_{mito} \longrightarrow 0{\cdot}5 \ \alpha KG + 0{\cdot}5 \ NH_3 + 0{\cdot}5 \ NADH_{mito} \\ 0{\cdot}5 \text{ aspartate} + 0{\cdot}5 \ CO_2 + 0{\cdot}5 \ NH_3 + 2 \text{ ATP} \longrightarrow 0{\cdot}5 \text{ urea} + 0{\cdot}5 \ \text{fumarate}_{cyt} \\ 0{\cdot}5 \ \text{fumarate}_{cyt} \longrightarrow 0{\cdot}5 \text{ OAA} + 0{\cdot}5 \ NADH_{cyt} \\ 0{\cdot}5 \ NADH_{cyt} + 0{\cdot}5 \text{ ATP} \longrightarrow 0{\cdot}5 \ NADH_{mito} \\ NADH_{mito} + 0{\cdot}5 \ O_2 \longrightarrow 3 \text{ ATP} \\ \hline \text{glutamate} + 0{\cdot}5 \ CO_2 + 0{\cdot}5 \ O_2 \longrightarrow 0{\cdot}5 \text{ urea} + \alpha KG + 0{\cdot}5 \text{ ATP} + 0{\cdot}5 \ H_2O \end{array}\right\} \quad (2)$$

A number of amino acids give rise to methyl groups, and their simulated oxidation proceeds as summarized in stoicheiometry equation (3).

$$\left.\begin{array}{l} R\text{-}CH_3 + 0{\cdot}5 \ O_2 \longrightarrow \text{formaldehyde} + R\text{-}H + 2 \text{ ATP} \\ \text{formaldehyde} + \text{tetrahydrofolate } (FH_4) \longrightarrow 5,10\text{-methylene}FH_4 \\ 0{\cdot}5 \ (5,10\text{-methylene}FH_4) + 0{\cdot}5 \ CO_2 + 0{\cdot}5 \ NH_3 + 0{\cdot}5 \ NADH_{mito} \longrightarrow \\ \qquad\qquad 0{\cdot}5 \text{ glycine} + 5 \ FH_4 \\ 0{\cdot}5 \ (5,10\text{-methylene}FH_4) + 0{\cdot}5 \text{ glycine} \longrightarrow 0{\cdot}5 \text{ serine} + 0{\cdot}5 \ FH_4 \\ 0{\cdot}5 \text{ serine} \longrightarrow 0{\cdot}5 \text{ pyruvate} + 0{\cdot}5 \ NH_3 \\ 0{\cdot}5 \text{ pyruvate} + O_2 \longrightarrow 1{\cdot}5 \ CO_2 + 0{\cdot}5 \ NADH_{mito} + 6 \text{ ATP} \\ \hline R\text{-}CH_3 + 1{\cdot}5 \ O_2 \longrightarrow CO_2 + R\text{-}H + 8 \text{ ATP} + H_2O \end{array}\right\} \quad (3)$$

The reactions involved in the simulated oxidation of amino acids to urea, CO_2 and H_2O are summarized in stoicheiometry equations (4) to (24):

$$\text{alanine} + 3 \ O_2 \longrightarrow 2{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 15{\cdot}5 \text{ ATP} + 2{\cdot}5 \ H_2O \quad (4)$$

$$\text{arginine} + 5{\cdot}5 \ O_2 \longrightarrow 4 \ CO_2 + 2 \text{ urea} + 28 \text{ ATP} + 3 \ H_2O \quad (5)$$

$$\text{aspartate} + 3 \ O_2 \longrightarrow 3{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 15{\cdot}5 \text{ ATP} + 2{\cdot}5 \ H_2O \quad (6)$$

$$\text{asparagine} + 3 \ O_2 \longrightarrow 3 \ CO_2 + \text{urea} + 13 \text{ ATP} + 2 \ H_2O \quad (7)$$

$$\text{cysteine} + 4 \ O_2 \longrightarrow 2{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 12{\cdot}5 \text{ ATP} + SO_2^{2-} \quad (8)$$

$$\text{glutamate} + 4{\cdot}5 \ O_2 \longrightarrow 4{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 24{\cdot}5 \text{ ATP} + 3{\cdot}5 \ H_2O \quad (9)$$

$$\text{glutamine} + 4{\cdot}5 \ O_2 \longrightarrow 4 \ CO_2 + \text{urea} + 22 \text{ ATP} + 3 \ H_2O \quad (10)$$

$$\text{glycine} + 1{\cdot}5 \ O_2 \longrightarrow 1{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 6{\cdot}5 \text{ ATP} + 1{\cdot}5 \ H_2O \quad (11)$$

$$\text{histidine} + 5 \ O_2 \longrightarrow 4{\cdot}5 \ CO_2 + 1{\cdot}5 \text{ urea} + 22{\cdot}5 \text{ ATP} + 1{\cdot}5 \ H_2O \quad (12)$$

$$\text{isoleucine} + 7{\cdot}5 \ O_2 \longrightarrow 5{\cdot}5 \ CO_2 + 0{\cdot}5 \text{ urea} + 40{\cdot}5 \text{ ATP} + 5{\cdot}5 \ H_2O \quad (13)$$

leucine + 7·5 O_2 ⟶ 5·5 CO_2 + 0·5 urea + 39·5 ATP + 5·5 H_2O (14)

lysine + 7 O_2 ⟶ 5 CO_2 + 0·5 urea + 36 ATP + 5 H_2O (15)

methionine + 7 O_2 ⟶ 4·5 CO_2 + 0·5 urea + 21·5 ATP + SO_4^{2-} (16)

phenylalanine + 10 O_2 ⟶ 8·5 CO_2 + 0·5 urea + 37·5 ATP + 4·5 H_2O (17)

proline + 5·5 O_2 ⟶ 4·5 CO_2 + 0·5 urea + 29·5 ATP + 3·5 H_2O (18)

hydroxyproline + 5 O_2 ⟶ 4·5 CO_2 + 0·5 urea + 26·5 ATP + 3·5 H_2O (19)

serine + 2·5 O_2 ⟶ 2·5 CO_2 + 0·5 urea + 12·5 ATP + 2·5 H_2O (20)

threonine + 4 O_2 ⟶ 3·5 CO_2 + 0·5 urea + 20·5 ATP + 3·5 H_2O (21)

tryptophan + 11·5 O_2 ⟶ 10 CO_2 + urea + 42 ATP + 4 H_2O (22)

tyrosine + 9·5 O_2 ⟶ 8·5 CO_2 + 0·5 urea + 41·5 ATP + 4·5 H_2O (23)

valine + 6 O_2 ⟶ 4·5 CO_2 + 0·5 urea + 31·5 ATP + 4·5 H_2O. (24)

The available energy in the form of ATP due to dietary protein was calculated from the amino acid composition using the foregoing stoicheiometry equations. During the simulated metabolism, those amino acids which are not incorporated into body protein are presumed to be converted to CO_2, urea, glucose and H_2O. The reactions involved in gluconeogenesis are summarized in stoicheiometry equations (25) to (43).

alanine + αKG ⟶ glutamate + pyruvate
glutamate + 0·5 CO_2 + 0·5 O_2 ⟶ 0·5 urea + αKG + 0·5 ATP
0·7381 pyruvate + 2·214 ATP + 0·7381 NADH ⟶ 0·369 glucose
0·1845 pyruvate + 0·0922 O_2 ⟶ 0·5535 CO_2 + 0·7381 NADH + 0·5535 ATP
0·0774 pyruvate + 0·1934 O_2 ⟶ 0·2321 CO_2 + 1·160 ATP
alanine + 0·7856 O_2 ⟶ 0·2855 CO_2 + 0·5 urea + 0·369 glucose + 0·286 H_2O (25)

arginine + αKG + 0·5 O_2 ⟶ 2 glutamate + urea + 3 ATP
3 glutamate + CO_2 + O_2 ⟶ urea + 2 αKG + ATP
αKG + O_2 ⟶ CO_2 + OAA + NADH + 6 ATP
OAA + 2 ATP + NADH ⟶ CO_2 + 0·5 glucose
arginine + 2·5 O_2 ⟶ CO_2 + 2 urea + 0·5 glucose + 8 ATP (26)

aspartate + αKG ⟶ glutamate + OAA
glutamate + 0·5 CO_2 + 0·5 O_2 ⟶ 0·5 urea + αKG + 0·5 ATP
0·775 OAA + 1·55 ATP + 0·775 NADH ⟶ 0·775 CO_2 + 0·3875 glucose
0·1938 OAA + 0·0969 O_2 ⟶ 0·775 CO_2 + 0·775 NADH + 0·581 ATP
0·0313 OAA + 0·0782 O_2 ⟶ 0·1252 CO_2 + 0·469 ATP
aspartate + 0·6851 O_2 ⟶ 1·175 CO_2 + 0·5 urea + 0·3875 glucose + 0·175 H_2O (27)

asparagine ⟶ aspartate + NH_3
aspartate + CO_2 + NH_3 + 4 ATP ⟶ urea + $fumarate_{cyt}$
$fumarate_{cyt}$ + ATP ⟶ OAA + $NADH_{mito}$
$NADH_{mito}$ + 0·5 O_2 ⟶ 3 ATP
0·65 OAA + 1·3 ATP + 0·65 NADH ⟶ 0·65 CO_2 + 0·325 glucose
0·1625 OAA + 0·0813 O_2 ⟶ 0·65 CO_2 + 0·65 NADH + 0·4875 ATP
0·1875 OAA + 0·4657 O_2 ⟶ 0·75 CO_2 + 2·813 ATP
asparagine + 1·05 O_2 ⟶ 1·05 CO_2 + urea + 0·325 glucose + 0·05 H_2O (28)

$$\text{cysteine} + \alpha\text{KG} + \text{NADH}_{\text{mito}} + 1{\cdot}5\ \text{O}_2 \longrightarrow \text{glutamate} + \text{pyruvate} + \text{SO}_4^{2-}$$
$$\text{glutamate} + 0{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{O}_2 \longrightarrow 0{\cdot}5\ \text{urea} + \alpha\text{KG} + 0{\cdot}5\ \text{ATP}$$
$$0{\cdot}25\ \text{pyruvate} + 0{\cdot}125\ \text{O}_2 \longrightarrow 0{\cdot}75\ \text{CO}_2 + \text{NADH}_{\text{mito}} + 0{\cdot}75\ \text{ATP}$$
$$0{\cdot}5952\ \text{pyruvate} + 1{\cdot}786\ \text{ATP} + 0{\cdot}5952\ \text{NADH} \longrightarrow 0{\cdot}2976\ \text{glucose}$$
$$0{\cdot}1488\ \text{pyruvate} + 0{\cdot}0744\ \text{O}_2 \longrightarrow 0{\cdot}4464\ \text{CO}_2 + 0{\cdot}5952\ \text{NADH} + 0{\cdot}4464\ \text{ATP}$$
$$0{\cdot}0059\ \text{pyruvate} + 0{\cdot}0148\ \text{O}_2 \longrightarrow 0{\cdot}0177\ \text{CO}_2 + 0{\cdot}0886\ \text{ATP}$$
$$\text{cysteine} + 2{\cdot}214\ \text{O}_2 \longrightarrow 0{\cdot}7141\ \text{CO}_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}2976\ \text{glucose} \quad (29)$$

$$\text{glutamate} + 0{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{O}_2 \longrightarrow 0{\cdot}5\ \text{urea} + \alpha\text{KG} + 0{\cdot}5\ \text{ATP}$$
$$\alpha\text{KG} + \text{O}_2 \longrightarrow \text{CO}_2 + \text{OAA} + \text{NADH} + 6\ \text{ATP}$$
$$\text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow \text{CO}_2 + 0{\cdot}5\ \text{glucose}$$
$$\text{glutamate} + 1{\cdot}5\ \text{O}_2 \longrightarrow 1{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 4{\cdot}5\ \text{ATP} + 5\ \text{H}_2\text{O} \quad (30)$$

$$\text{glutamine} \longrightarrow \text{glutamate} + \text{NH}_3$$
$$\text{glutamate} + \text{OAA} \longrightarrow \text{aspartate} + \alpha\text{KG}$$
$$\text{aspartate} + \text{CO}_2 + \text{NH}_3 + 4\ \text{ATP} \longrightarrow \text{urea} + \text{fumarate}_{\text{cyt}}$$
$$\text{fumarate}_{\text{cyt}} + \text{ATP} \longrightarrow \text{OAA} + \text{NADH}_{\text{mito}}$$
$$\text{NADH}_{\text{mito}} + 0{\cdot}5\ \text{O}_2 \longrightarrow 3\ \text{ATP}$$
$$\alpha\text{KG} + \text{O}_2 \longrightarrow \text{CO}_2 + \text{OAA} + \text{NADH} + 6\ \text{ATP}$$
$$\text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow \text{CO}_2 + 0{\cdot}5\ \text{glucose}$$
$$\text{glutamine} + 1{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \text{urea} + 0{\cdot}5\ \text{glucose} + 2\ \text{ATP} \quad (31)$$

$$0{\cdot}5\ \text{glycine} \longrightarrow 0{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{NH}_3 + 0{\cdot}5\ \text{NADH}_{\text{mito}} + 0{\cdot}5\ (5,10\text{-methyleneFH}_4)$$
$$0{\cdot}5\ \text{glycine} + 0{\cdot}5\ (5,10\text{-methyleneFH}_4) \longrightarrow 0{\cdot}5\ \text{serine}$$
$$0{\cdot}5\ \text{serine} \longrightarrow 0{\cdot}5\ \text{pyruvate} + 0{\cdot}5\ \text{NH}_3$$
$$0{\cdot}5\ \alpha\text{KG} + 0{\cdot}5\ \text{NH}_3 + 0{\cdot}5\ \text{NADH}_{\text{mito}} \longrightarrow 0{\cdot}5\ \text{glutamate}$$
$$0{\cdot}5\ \text{glutamate} + 0{\cdot}5\ \text{OAA} \longrightarrow 0{\cdot}5\ \text{aspartate} + 0{\cdot}5\ \alpha\text{KG}$$
$$0{\cdot}5\ \text{aspartate} + 0{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{NH}_3 + 2\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{fumarate}_{\text{cyt}}$$
$$0{\cdot}5\ \text{fumarate}_{\text{cyt}} + 0{\cdot}5\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{OAA} + 0{\cdot}5\ \text{NADH}_{\text{mito}}$$
$$0{\cdot}5\ \text{NADH}_{\text{mito}} + 0{\cdot}25\ \text{O}_2 \longrightarrow 1{\cdot}5\ \text{ATP}$$
$$0{\cdot}3095\ \text{pyruvate} + 0{\cdot}9286\ \text{ATP} + 0{\cdot}3095\ \text{NADH} \longrightarrow 0{\cdot}1548\ \text{glucose}$$
$$0{\cdot}0774\ \text{pyruvate} + 0{\cdot}0387\ \text{O}_2 \longrightarrow 0{\cdot}2322\ \text{CO}_2 + 0{\cdot}3095\ \text{NADH} + 0{\cdot}2322\ \text{ATP}$$
$$0{\cdot}1131\ \text{pyruvate} + 0{\cdot}2828\ \text{O}_2 \longrightarrow 0{\cdot}3393\ \text{CO}_2 + 1{\cdot}697\ \text{ATP}$$
$$\text{glycine} + 0{\cdot}5715\ \text{O}_2 \longrightarrow 0{\cdot}5715\ \text{CO}_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}1548\ \text{glucose} + 0{\cdot}571\ \text{H}_2\text{O} \quad (32)$$

$$\text{histidine} \longrightarrow \text{glutamate} + 2\ \text{NH}_3 + 5,10\text{-methylidyneFH}_4$$
$$5,10\text{-methylidyneFH}_4 + \text{NADPH} \longrightarrow 5,10\text{-methyleneFH}_4$$
$$0{\cdot}5\ (5,10\text{-methyleneFH}_4) + 0{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{NH}_3 + 0{\cdot}5\ \text{NADH}_{\text{mito}} \longrightarrow 5\ \text{glycine}$$
$$0{\cdot}5\ (5,10\text{-methyleneFH}_4) + 0{\cdot}5\ \text{glycine} \longrightarrow 0{\cdot}5\ \text{serine}$$
$$0{\cdot}5\ \text{serine} \longrightarrow 0{\cdot}5\ \text{pyruvate} + 0{\cdot}5\ \text{NH}_3$$
$$0{\cdot}5\ \text{pyruvate} + \text{O}_2 \longrightarrow 1{\cdot}5\ \text{CO}_2 + 0{\cdot}5\ \text{NADH}_{\text{mito}} + 6\ \text{ATP}$$
$$0{\cdot}5\ \alpha\text{KG} + 0{\cdot}5\ \text{NH}_3 + 0{\cdot}5\ \text{NADH}_{\text{mito}} \longrightarrow 0{\cdot}5\ \text{glutamate}$$
$$1{\cdot}5\ \text{glutamate} + 1{\cdot}5\ \text{OAA} \longrightarrow 1{\cdot}5\ \text{aspartate} + 1{\cdot}5\ \alpha\text{KG}$$
$$1{\cdot}5\ \text{aspartate} + 1{\cdot}5\ \text{CO}_2 + 1{\cdot}5\ \text{NH}_3 + 6\ \text{ATP} \longrightarrow 1{\cdot}5\ \text{urea} + 1{\cdot}5\ \text{fumarate}_{\text{cyt}}$$
$$1{\cdot}5\ \text{fumarate} \longrightarrow 1{\cdot}5\ \text{OAA} + 1{\cdot}5\ \text{NADH}_{\text{cyt}}$$
$$\text{NADH} + \text{ATP} \longrightarrow \text{NADPH}$$
$$0{\cdot}5\ \text{NADH}_{\text{cyt}} + 0{\cdot}5\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{NADH}_{\text{mito}}$$
$$\alpha\text{KG} + \text{O}_2 \longrightarrow \text{CO}_2 + \text{OAA} + \text{NADH} + 6\ \text{ATP}$$
$$\text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow \text{CO}_2 + 0{\cdot}5\ \text{glucose}$$
$$\text{histidine} + 2\ \text{O}_2 + 1{\cdot}4\ \text{H}_2\text{O} \longrightarrow 1{\cdot}5\ \text{CO}_2 + 1{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 2{\cdot}5\ \text{ATP} \quad (33)$$

$$\left.\begin{array}{l}\text{isoleucine} + \alpha\text{KG} + 3{\cdot}5\ O_2 \longrightarrow 2\ CO_2 + \text{succinate} + \text{glutamate} + 20\ \text{ATP} \\ \text{glutamate} + 0{\cdot}5\ CO_2 + 0{\cdot}5\ O_2 \longrightarrow 0{\cdot}5\ \text{urea} + \alpha\text{KG} + 0{\cdot}5\ \text{ATP} \\ \text{succinate} + 0{\cdot}5\ O_2 \longrightarrow \text{OAA} + \text{NADH} + 2\ \text{ATP} \\ \text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow CO_2 + 0{\cdot}5\ \text{glucose} \\ \hline \text{isoleucine} + 4{\cdot}5\ O_2 \longrightarrow 2{\cdot}5\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 20{\cdot}5\ \text{ATP} + 2{\cdot}5\ H_2O\end{array}\right\} \quad (34)$$

$$\left.\begin{array}{l}\text{methionine} + 1{\cdot}5\ O_2 \longrightarrow CO_2 + \text{homocysteine} + 5\ \text{ATP} \\ \text{homocysteine} + \text{serine} \longrightarrow \text{cysteine} + \alpha\text{-ketobutyrate} + NH_3 \\ \text{cysteine} + \alpha\text{KG} + \text{NADH}_{\text{mito}} + 1{\cdot}5\ O_2 \longrightarrow \text{glutamate} + \text{pyruvate} + SO_4^{2-} \\ \text{pyruvate} + 2\ \text{ATP} \longrightarrow \text{PEP} \\ \text{glutamate} + \text{PEP} \longrightarrow \text{serine} + \alpha\text{KG} \\ 0{\cdot}5\ \alpha\text{KG} + 0{\cdot}5\ NH_3 + 0{\cdot}5\ \text{NADH}_{\text{mito}} \longrightarrow 0{\cdot}5\ \text{glutamate} \\ 0{\cdot}5\ \text{glutamate} + 0{\cdot}5\ \text{OAA} \longrightarrow 0{\cdot}5\ \text{aspartate} + 0{\cdot}5\ \alpha\text{KG} \\ 0{\cdot}5\ \text{aspartate} + 0{\cdot}5\ CO_2 + 0{\cdot}5\ NH_3 + 2\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{fumarate}_{\text{cyt}} \\ 0{\cdot}5\ \text{fumarate}_{\text{cyt}} + 0{\cdot}5\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{OAA} + 0{\cdot}5\ \text{NADH}_{\text{mito}} \\ \alpha\text{-ketobutyrate} \longrightarrow \text{succinate} + \text{NADH}_{\text{mito}} \\ \text{succinate} + 0{\cdot}5\ O_2 \longrightarrow \text{OAA} + \text{NADH} + 2\ \text{ATP} \\ \text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow CO_2 + 0{\cdot}5\ \text{glucose} \\ \hline \text{methionine} + 3{\cdot}5\ O_2 \longrightarrow 1{\cdot}5\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 0{\cdot}5\ \text{ATP}\end{array}\right\} \quad (35)$$

$$\left.\begin{array}{l}\text{phenylalanine} + \alpha\text{KG} + \text{NADPH} + O_2 \longrightarrow \text{glutamate} + p\text{-hydroxyphenylpyruvate} \\ p\text{-hydroxyphenylpyruvate} + 2\ O_2 \longrightarrow CO_2 + \text{acetoacetate} + \text{fumarate} \\ \text{glutamate} + 0{\cdot}5\ CO_2 + 2\ \text{ATP} \longrightarrow 0{\cdot}5\ \text{urea} + \alpha\text{KG} + \text{NADH} \\ \text{NADH} + \text{ATP} \longrightarrow \text{NADPH} \\ \text{acetoacetate} + 4\ O_2 \longrightarrow 4\ CO_2 + 23\ \text{ATP} \\ \text{fumarate} \longrightarrow \text{OAA} + \text{NADH} \\ \text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow CO_2 + 0{\cdot}5\ \text{glucose} \\ \hline \text{phenylalanine} + 7\ O_2 \longrightarrow 5{\cdot}5\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 19\ \text{ATP} + 1{\cdot}5\ H_2O\end{array}\right\} \quad (36)$$

$$\left.\begin{array}{l}\text{proline} + O_2 \longrightarrow \text{glutamate} + 5\ \text{ATP} \\ \text{glutamate} + 0{\cdot}5\ CO_2 + 0{\cdot}5\ O_2 \longrightarrow 0{\cdot}5\ \text{urea} + \alpha\text{KG} + 0{\cdot}5\ \text{ATP} \\ \alpha\text{KG} + O_2 \longrightarrow CO_2 + \text{OAA} + \text{NADH} + 6\ \text{ATP} \\ \text{OAA} + 2\ \text{ATP} + \text{NADH} \longrightarrow CO_2 + 0{\cdot}5\ \text{glucose} \\ \hline \text{proline} + 2{\cdot}5\ O_2 \longrightarrow 1{\cdot}5\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5\ \text{glucose} + 9{\cdot}5\ \text{ATP}\end{array}\right\} \quad (37)$$

$$\left.\begin{array}{l}\text{hydroxyproline} + O_2 \longrightarrow \text{alanine} + \text{glyoxylate} + 5\ \text{ATP} \\ \text{glutamate} + \text{glyoxylate} \longrightarrow \text{glycine} + \alpha\text{KG} \\ \text{glycine} + 0{\cdot}5715\ O_2 \longrightarrow 0{\cdot}5715\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}1548\ \text{glucose} \\ \text{alanine} + \alpha\text{KG} \longrightarrow \text{glutamate} + \text{pyruvate} \\ 0{\cdot}8\ \text{pyruvate} + 2{\cdot}4\ \text{ATP} + 0{\cdot}8\ \text{NADH} \longrightarrow 0{\cdot}4\ \text{glucose} \\ 0{\cdot}2\ \text{pyruvate} + 1\ O_2 \longrightarrow 0{\cdot}6\ CO_2 + 0{\cdot}8\ \text{NADH} + 0{\cdot}6\ \text{ATP} \\ \hline \text{hydroxyproline} + 1{\cdot}671\ O_2 \longrightarrow 1{\cdot}171\ CO_2 + 0{\cdot}5\ \text{urea} + 0{\cdot}5548\ \text{glucose} \\ \qquad\qquad + 3{\cdot}2\ \text{ATP} + 0{\cdot}171\ H_2O\end{array}\right\} \quad (38)$$

serine ⟶ pyruvate + NH_3
0·5 αKG + 0·5 NH_3 + 0·5 $NADH_{mito}$ ⟶ 0·5 glutamate
0·5 glutamate + 0·5 OAA ⟶ 0·5 aspartate + 0·5 αKG
0·5 aspartate + 0·5 CO_2 + 0·5 NH_3 + 2 ATP ⟶ 0·5 urea + 0·5 $fumarate_{cyt}$
0·5 $fumarate_{cyt}$ + 0·5 ATP ⟶ 0·5 OAA + 0·5 $NADH_{mito}$
0·5952 pyruvate + 1·786 ATP + 0·5952 NADH ⟶ 0·2076 glucose
0·1488 pyruvate + 0·0744 O_2 ⟶ 0·4464 CO_2 + 0·5952 NADH + 0·4464 ATP
0·2560 pyruvate + 0·6399 O_2 ⟶ 0·768 CO_2 + 3·838 ATP
serine + 0·7144 O_2 ⟶ 0·7144 CO_2 + 0·5 urea + 0·2976 glucose + 0·7144 H_2O (39)

threonine ⟶ α-ketobutyrate + NH_3
αKG + NH_3 + $NADH_{mito}$ ⟶ glutamate
glutamate + 0·5 CO_2 + 0·5 O_2 ⟶ 0·5 urea + αKG + 0·5 ATP
α-ketobutyrate ⟶ succinate + $NADH_{mito}$
succinate + 0·5 O_2 ⟶ OAA + NADH + 2 ATP
OAA + 2 ATP + NADH ⟶ CO_2 + 0·5 glucose
threonine + O_2 ⟶ 0·5 CO_2 + 0·5 urea + 0·5 glucose + 0·5 ATP + 0·5 H_2O (40)

tryptophan + NADPH + 3·5 O_2 ⟶ 2 CO_2 + alanine + NH_3 + α-ketoadipate
α-ketoadipate + O_2 ⟶ 2 CO_2 + acetoacetyl-CoA + NADH + 5 ATP
NADH + ATP ⟶ NADPH
acetoacetyl-CoA + 4 O_2 ⟶ 4 CO_2 + 24 ATP
alanine + αKG ⟶ glutamate + pyruvate
glutamate + OAA ⟶ aspartate + αKG
aspartate + CO_2 + NH_3 + 4 ATP ⟶ urea + $fumarate_{cyt}$
$fumarate_{cyt}$ ⟶ OAA + NADH
pyruvate + 3 ATP + NADH ⟶ 0·5 glucose
tryptophan + 8·5 O_2 ⟶ 7 CO_2 + urea + 0·5 glucose + 21 ATP + H_2O (41)

tyrosine + αKG + 2 O_2 ⟶ CO_2 + glutamate + acetoacetate + fumarate
glutamate + 0·5 CO_2 + 0·5 O_2 ⟶ 0·5 urea + αKG + 0·5 ATP
acetoacetate + 4 O_2 ⟶ 4 CO_2 + 23 ATP
fumarate ⟶ OAA + NADH
OAA + 2 ATP + NADH ⟶ CO_2 + 0·5 glucose
tyrosine + 6·5 O_2 ⟶ 5·5 CO_2 + 0·5 urea + 0·5 glucose + 21·5 ATP + 1·5 H_2O. (42)

valine + αKG ⟶ glutamate + α-ketoisovalerate
glutamate + 0·5 CO_2 + 0·5 O_2 ⟶ 0·5 urea + αKG + 0·5 ATP
α-ketoisovalerate + 2 O_2 ⟶ 2 CO_2 + propionyl-CoA + 11 ATP
propionyl-CoA + CO_2 ⟶ succinate
succinate + 0·5 O_2 ⟶ OAA + NADH + 2 ATP
OAA + 2 ATP + NADH ⟶ CO_2 + 0·5 glucose
valine + 3 O_2 ⟶ 1·5 CO_2 + 0·5 urea + 0·5 glucose + 11·5 ATP + 1·5 H_2O. (43)

Dietary fat metabolism

Simulation of dietary fat metabolism was facilitated by the assumption that fat could be represented entirely as triacylglycerol. With this assumption, the only further information which was necessary was the number of C atoms and the number of unsaturated bonds in each fatty acid. The stoicheiometric expressions for energy yield, respiratory exchange, and

intermediary metabolism required calculation of the amount (mol) of triacylglycerol, and the molecular weight was calculated using equation (44). (In equations (44) to (53), N is the number of C atoms/fatty acid, and U is the number of double bonds in the fatty acid.)

$$\text{Molecular weight} = 42N + 134 - 6U. \quad (44)$$

The amount (mol) of O_2 consumed and the amount (mol) of CO_2 produced for the complete oxidation of fats were calculated using equations (45) and (46).

$$O_2 \text{ consumed} = 4{\cdot}5N + 0{\cdot}5 - 1{\cdot}5U, \quad (45)$$

$$CO_2 \text{ produced} = 3N + 3. \quad (46)$$

The yield of ATP/mol triacylglycerol was slightly different for a triacylglycerol which contained a fatty acid with an even number of C atoms than for the triacylglycerol which contained a fatty acid with an uneven number of C atoms. If the fatty acid contained an even number of C atoms, the yield of ATP (mol) was calculated according to the following equation.

$$\text{ATP yield} = 25{\cdot}5N - 1 - 6U. \quad (47)$$

Equation (48) provided for calculation of the energy yield/mol triacylglycerol consisting of a fatty acid with an uneven number of C atoms:

$$\text{ATP yield} = 25{\cdot}5N - 2{\cdot}5 - 6U. \quad (48)$$

Equations (44) to (48) provided the stoicheiometries for the complete oxidation of fat, and the available energy due to dietary fat was calculated on this basis. The algorithm followed in the simulated metabolism of fat calculated the stoicheiometry for conversion of triacylglycerol to mitochondrial acetyl-CoA and glucose. O_2 consumption (mol) for this process was calculated with the following equation:

$$O_2 \text{ consumed} = 1{\cdot}5N - 2{\cdot}5 - 1{\cdot}5U. \quad (49)$$

The yields in mitochondrial acetyl-CoA (mol) and ATP (mol/mol triacylglycerol) with a fatty acid with an even number of C atoms are given in equations (50) and (51).

$$\text{mitochondrial acetyl-CoA} = 1{\cdot}5N, \quad (50)$$

$$\text{ATP} = 7{\cdot}5N - 19 - 6U. \quad (51)$$

The corresponding stoicheiometries for triacylglycerol containing a fatty acid with an uneven number of C atoms are:

$$\text{mitochondrial acetyl-CoA} = 1{\cdot}5N - 4{\cdot}5, \quad (52)$$

$$\text{ATP} = 7{\cdot}5N - 26{\cdot}5 - 6U. \quad (53)$$

The stoicheiometry for glucose synthesis from triacylglycerol containing even number C atom fatty acids was 0·5 mol glucose formed/mol triacylglycerol, and for uneven numbered C acids 2 mol glucose formed/mol triacylglycerol.

Biosynthesis of protein

The energy cost of protein synthesis in terms of ATP was assumed to be 2 mol ATP/mol amino acid residue for formation of aminoacyl-tRNA, 1 mol ATP for elongation of the peptide chain by one amino acid residue, and 1 mol ATP for translocation of the peptidyl chain from the A-site to the P-site of the 70S ribosome at the time at which each amino acid residue is incorporated into the protein (Haselkorn & Rothman-Denes, 1973). Thus, 4 mol ATP (actually, 2 mol ATP and 2 mol GTP) are required for the formation of each peptide

bond. The cost of membrane transport of amino acids was estimated by multiplying each amino acid incorporated into protein by the transport factor for amino acids.

The programme included a check to prevent protein synthesis in excess of the limiting essential amino acid. If the diet did not contain an adequate supply of non-essential amino acids to provide for protein synthesis, the synthesis of needed non-essential amino acids was simulated according to stoicheiometry equations (54) to (64). According to these stoicheiometries, the ammonia for amino acid synthesis was transported to the site of amino acid synthesis as glutamine, and the glutamine utilized was regenerated within the stoicheiometric equation:

$$\left.\begin{array}{l} 0{\cdot}5\ \text{glucose} \longrightarrow \text{pyruvate} + \text{NADH}_{\text{cyt}} + \text{ATP} \\ \text{NADH}_{\text{cyt}} + \text{ATP} \longrightarrow \text{NADH}_{\text{mito}} \\ \text{glutamine} \longrightarrow \text{glutamate} + \text{NH}_3 \\ \alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} \longrightarrow \text{glutamate} \\ \text{pyruvate} + \text{glutamate} \longrightarrow \text{alanine} + \alpha\text{KG} \\ \text{glutamate} + \text{NH}_3 + \text{ATP} \longrightarrow \text{glutamine} \\ \hline 0{\cdot}5\ \text{glucose} + \text{NH}_3 + \text{ATP} \longrightarrow \text{alanine} + \text{H}_2\text{O} \end{array}\right\} \quad (54)$$

$$\left.\begin{array}{l} 0{\cdot}5\ \text{glucose} \longrightarrow \text{pyruvate} + \text{NADH}_{\text{mito}} \\ \text{pyruvate} + \text{CO}_2 + \text{ATP} \longrightarrow \text{OAA} \\ \text{glutamine} \longrightarrow \text{glutamate} + \text{NH}_3 \\ \text{glutamate} + \text{OAA} \longrightarrow \text{aspartate} + \alpha\text{KG} \\ \alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} \longrightarrow \text{glutamate} \\ \text{glutamate} + \text{NH}_3 + \text{ATP} \longrightarrow \text{glutamine} \\ \hline 0{\cdot}5\ \text{glucose} + \text{CO}_2 + \text{NH}_3 + 2\ \text{ATP} \longrightarrow \text{aspartate} + \text{H}_2\text{O} \end{array}\right\} \quad (55)$$

$$0{\cdot}5\ \text{glucose} + \text{CO}_2 + 2\ \text{NH}_3 + 3\ \text{ATP} \longrightarrow \text{asparagine} + 2\ \text{H}_2\text{O} \quad (56)$$

$$\left.\begin{array}{l} \text{methionine} + \text{R-H} + 3\ \text{ATP} \longrightarrow \text{R-CH}_3 + \text{homocysteine} \\ \text{R-CH}_3 + 1{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \text{R-H} + 8\ \text{ATP} \\ \text{homocysteine} + \text{serine} \longrightarrow \text{cysteine} + \alpha\text{-ketobutyrate} + \text{NH}_3 \\ \alpha\text{-ketobutyrate} + 0{\cdot}5\ \text{O}_2 \longrightarrow \text{succinate} + 3\ \text{ATP} \\ \text{succinate} + 0{\cdot}5\ \text{O}_2 \longrightarrow \text{OAA} + \text{NADH}_{\text{mito}} + 2\ \text{ATP} \\ \text{OAA} + \text{ATP} \longrightarrow \text{CO}_2 + \text{PEP} \\ \text{glutamate} + \text{PEP} \longrightarrow \text{serine} + \alpha\text{KG} \\ \alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} \longrightarrow \text{glutamate} \\ \hline \text{methionine} + 2{\cdot}5\ \text{O}_2 \longrightarrow 2\ \text{CO}_2 + \text{cysteine} + 9\ \text{ATP} + \text{H}_2\text{O} \end{array}\right\} \quad (57)$$

$$\left.\begin{array}{l} \text{glucose} \longrightarrow 2\ \text{pyruvate} + 2\ \text{NADH}_{\text{mito}} \\ \text{pyruvate} + \text{CO}_2 + \text{ATP} \longrightarrow \text{OAA} \\ \text{pyruvate} + 0{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \text{acetyl-CoA} + 3\ \text{ATP} \\ \text{acetyl-CoA} + \text{OAA} + 0{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \alpha\text{KG} + 3\ \text{ATP} \\ \text{glutamine} \longrightarrow \text{glutamate} + \text{NH}_3 \\ \alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} \longrightarrow \text{glutamate} \\ \text{NADH}_{\text{mito}} + 0{\cdot}5\ \text{O}_2 \longrightarrow 3\ \text{ATP} \\ \text{glutamate} + \text{NH}_3 + \text{ATP} \longrightarrow \text{glutamine} \\ \hline \text{glucose} + \text{NH}_3 + 1{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \text{glutamate} + 7\ \text{ATP} + 3\ \text{H}_2\text{O} \end{array}\right\} \quad (58)$$

$$\text{glucose} + 2\ \text{NH}_3 + 1{\cdot}5\ \text{O}_2 \longrightarrow \text{CO}_2 + \text{glutamine} + 6\ \text{ATP} + 4\ \text{H}_2\text{O} \quad (59)$$

$0{\cdot}5$ glucose $\longrightarrow$ PEP + $NADH_{cyt}$
glutamate + PEP $\longrightarrow$ serine + αKG
serine + R-H $\longrightarrow$ glycine + $R\text{-}CH_3$
$R\text{-}CH_3 + 1{\cdot}5\ O_2 \longrightarrow CO_2 + R\text{-}H + 8$ ATP
$NADH_{cyt}$ + ATP $\longrightarrow$ $NADH_{mito}$
glutamine $\longrightarrow$ glutamate + NH_3
αKG + NH_3 + $NADH_{mito}$ $\longrightarrow$ glutamate
glutamate + NH_3 + ATP $\longrightarrow$ glutamine
$0{\cdot}5$ glucose + $NH_3 + 1{\cdot}5\ O_2 \longrightarrow CO_2$ + glycine + 6 ATP + 2 H_2O (60)

glucose $\longrightarrow$ 2 pyruvate + 2 $NADH_{cyt}$ + 2 ATP
$NADH_{cyt}$ + ATP $\longrightarrow$ $NADH_{mito}$
NADH + ATP $\longrightarrow$ NADPH
pyruvate + CO_2 + ATP $\longrightarrow$ OAA
pyruvate + $0{\cdot}5\ O_2 \longrightarrow CO_2$ + acetyl-CoA + 3 ATP
acetyl-CoA + OAA $\longrightarrow$ CO_2 + αKG + $NADH_{mito}$
glutamine $\longrightarrow$ glutamate + NH_3
αKG + NH_3 + $NADH_{mito}$ $\longrightarrow$ glutamate
glutamate + $NADH_{mito}$ + ATP $\longrightarrow$ glutamic semialdehyde
glutamic semialdehyde + NADPH $\longrightarrow$ proline
glutamate + NH_3 + ATP $\longrightarrow$ glutamine
glucose + $NH_3 + 0{\cdot}5\ O_2 \longrightarrow CO_2$ + proline + ATP + 3 H_2O (61)

glucose + $NH_3 + 0{\cdot}5\ O_2 \longrightarrow CO_2$ + proline + ATP
proline + αKG + $O_2 \longrightarrow CO_2$ + hydroxyproline + succinate + ATP
succinate + $O_2 \longrightarrow$ OAA + 5 ATP
$0{\cdot}5$ glucose $\longrightarrow$ pyruvate + $NADH_{mito}$
$NADH_{mito} + 0{\cdot}5\ O_2 \longrightarrow$ 3 ATP
pyruvate + $0{\cdot}5\ O_2 \longrightarrow CO_2$ + acetyl-CoA + 3 ATP
acetyl-CoA + OAA + $0{\cdot}5\ O_2 \longrightarrow CO_2$ + αKG + 3 ATP
$1{\cdot}5$ glucose + NH_3 + 4 $O_2 \longrightarrow$ 4 CO_2 + hydroxyproline + 16 ATP + 6 H_2O (62)

$0{\cdot}5$ glucose $\longrightarrow$ PEP + $NADH_{cyt}$
$NADH_{cyt}$ + ATP $\longrightarrow$ $NADH_{mito}$
glutamine $\longrightarrow$ glutamate + NH_3
glutamate + PEP $\longrightarrow$ serine + αKG
αKG + NH_3 + $NADH_{mito}$ $\longrightarrow$ glutamate
glutamate + NH_3 + ATP $\longrightarrow$ glutamine
$0{\cdot}5$ glucose + NH_3 + 2 ATP $\longrightarrow$ serine (63)

0.5 glucose $\longrightarrow$ pyruvate + $NADH_{cyt}$ + ATP
NADH + ATP $\longrightarrow$ NADPH
phenylalanine + NADPH + $O_2 \longrightarrow$ tyrosine
pyruvate + $2.5\ O_2 \longrightarrow 3\ CO_2 + 15$ ATP
$0{\cdot}5$ glucose + phenylalanine + $O_2 \longrightarrow 3\ CO_2$ + tyrosine + 15 ATP + 3 H_2O (64)

Biosynthesis of carbohydrate

This programme allowed the investigator to specify an amount of either lactose or glycogen synthesis. The amount (mol) of glucose required for synthesis of lactose was calculated as follows.

$$\text{glucose required} = \text{lactose (g)}/171. \tag{65}$$

The amount (mol) of glucose required for glycogen synthesis was calculated by the following equation:

$$\text{glucose required} = \text{glycogen (g)}/162. \tag{66}$$

Equation (67) summarizes the stoicheiometry for lactose synthesis:

$$\left.\begin{array}{l} \text{glucose} + \text{ATP} \longrightarrow \text{glucose-6-phosphate (G6P)} \\ \text{G6P} \longrightarrow \text{glucose-1-phosphate (G1P)} \\ \text{G1P} + \text{UTP} \longrightarrow \text{uridine diphosphoglucose (UDPG)} \\ \text{UDPG} \longrightarrow \text{UDPGalactose} \\ \text{UDPGalactose} + \text{glucose} \longrightarrow \text{lactose} + \text{UDP} \\ \hline 2\ \text{glucose} + 2\ \text{ATP} \longrightarrow \text{lactose} + \text{H}_2\text{O} \end{array}\right\} \tag{67}$$

The following is the stoicheiometry for glycogen synthesis:

$$\left.\begin{array}{l} \text{glucose} + \text{ATP} \longrightarrow \text{G6P} \\ \text{G6P} \longrightarrow \text{G1P} \\ \text{G1P} + \text{ATP} \longrightarrow \text{UDPG} \\ \text{UDPG} + (\text{glucosyl})_{n-1} \longrightarrow (\text{glucosyl})_n \\ \hline \text{glucose} + (\text{glucosyl})_{n-1} + 2\ \text{ATP} \longrightarrow (\text{glucosyl})_n + \text{H}_2\text{O} \end{array}\right\} \tag{68}$$

Biosynthesis of fat

Before simulation of fat synthesis, a check was made of the energy status of the system in terms of ATP. When a deficit of ATP existed, the programme simulated oxidation of available substrates until the deficit ATP had been replenished. The sequence of oxidation of available substrates was mitochondrial acetyl-CoA, glucose, and body fat respectively. At this point in the simulated metabolism, the amino acids which were not required for protein synthesis had been utilized in gluconeogenesis in accordance with stoicheiometries (25) to (43). Trioleoylglycerol was assumed to be representative of body fat. The oxidation of mitochondrial acetyl-CoA was simulated according to equation (69), glucose was oxidized in accord with stoicheiometry equation (1), and the stoicheiometry for trioleoylglycerol is summarized in equation (70).

$$\text{acetyl-CoA}_{\text{mito}} + 2\ \text{O}_2 \longrightarrow 2\ \text{CO}_2 + \text{CoAH} + 12\ \text{ATP} + \text{H}_2\text{O}, \tag{69}$$

$$\text{trioleoylglycerol} + 80\ \text{O}_2 \longrightarrow 57\ \text{CO}_2 + 452\ \text{ATP} + 52\ \text{H}_2\text{O}. \tag{70}$$

It was assumed that body fat is synthesized from acetyl-CoA or glucose, or both. Maintenance of animals for relatively long periods of time on a diet which contains an unusual fatty acid composition results in deposition of body fat which reflects the fatty acid composition of the diet to some extent, but this is a slow process (Egwim & Kummerow, 1972). The assumption that fatty acid synthesis occurs by de novo synthesis exclusively did not appear to introduce significant error in the calculation of energy balance for the rat as judged from McCracken's results. In other species de novo synthesis might be a less significant process.

When an excess of ATP existed, synthesis of body fat (trioleoylglycerol) was simulated

first using mitochondrial acetyl-CoA and minimal glucose according to the stoicheiometries presented in equation (71), and finally exclusively from glucose according to the stoicheiometries summarized in equation (72).

$$\left.\begin{array}{l} 24\ \text{acetyl-CoA}_{\text{mito}} + 24\ \text{ATP} \longrightarrow 24\ \text{aceytl-CoA}_{\text{cyt}} \\ 24\ \text{acetyl-CoA}_{\text{cyt}} + 42\ \text{NADPH} + 21\ \text{ATP} \longrightarrow 3\ \text{palmitate} \\ 3\ \text{palmitate} + 6\ \text{ATP} \longrightarrow 3\ \text{palmityl-CoA} \\ 3\ \text{palmityl-CoA} + 3\ \text{acetyl-CoA}_{\text{mito}} + 6\ \text{NADPH} \longrightarrow 3\ \text{stearyl-CoA} \\ 3\ \text{stearyl-CoA} + 3\ \text{NADPH} + 3\text{O}_2 \longrightarrow 3\ \text{oleoyl-CoA} \\ 0{\cdot}5\ \text{glucose} + \text{NADH} + \text{ATP} \longrightarrow \alpha\text{-glycerol-PO}_4 \\ 3\ \text{oleoyl-CoA} + \alpha\text{-glycerol-PO}_4 \longrightarrow \text{trioleoylglycerol} \\ 17{\cdot}333\ \text{acetyl-CoA}_{\text{mito}} + 8.667\ \text{O}_2 \longrightarrow 34{\cdot}667\ \text{CO}_2 + 34.667\ \text{ATP} + 52\ \text{NADH} \\ 51\ \text{NADH} + 51\ \text{ATP} \longrightarrow 51\ \text{NADPH} \\ \hline 44{\cdot}333\ \text{acetyl-CoA}_{\text{mito}} + 0.5\ \text{glucose} + 11.667\ \text{O}_2 + (68{\cdot}333 + Z)\ \text{ATP} \\ \quad \longrightarrow 34{\cdot}667\ \text{CO}_2 + \text{trioleoylglycerol} + 44{\cdot}333\ \text{CoASH} \end{array}\right\} \quad (71)$$

where $Z = 44{\cdot}333 \times$ transport factor for fat/9

$$\left.\begin{array}{l} 14\ \text{glucose} \longrightarrow \alpha\text{-glycerol-PO}_4 + 27\ \text{pyruvate} + 26\ \text{NADH} + 26\ \text{ATP} \\ 25\ \text{pyruvate} \longrightarrow 25\ \text{CO}_2 + 25\ \text{acetyl-CoA}_{\text{mito}} + 25\ \text{NADH} \\ 51\ \text{NADH} + 51\ \text{ATP} \longrightarrow 51\ \text{NADPH} \\ 2\ \text{pyruvate} + \text{O}_2 \longrightarrow 2\ \text{CO}_2 + 2\ \text{acetyl-CoA}_{\text{mito}} + 6\ \text{ATP} \\ 24\ \text{acetyl-CoA}_{\text{mito}} + 24\ \text{ATP} \longrightarrow 24\ \text{acetyl-CoA}_{\text{cyt}} \\ 24\ \text{acetyl-CoA}_{\text{cyt}} + 42\ \text{NADPH} + 21\ \text{ATP} \longrightarrow 3\ \text{palmitate} \\ 3\ \text{palmitate} + 6\ \text{ATP} \longrightarrow 3\ \text{palmityl-CoA} \\ 3\ \text{palmityl-CoA} + 3\ \text{acetyl-CoA}_{\text{mito}} + 6\ \text{NADPH} \longrightarrow 3\ \text{stearyl-CoA} \\ 3\ \text{stearyl-CoA} + 3\ \text{NADPH} + 3\ \text{O}_2 \longrightarrow 3\ \text{oleoyl-CoA} \\ 3\ \text{oleoyl-CoA} + \alpha\text{-glycerol-PO}_4 \longrightarrow \text{trioleoylglycerol} \\ \hline 14\ \text{glucose} + 4\ \text{O}_2 + (70 + zz)\ \text{ATP} \longrightarrow 27\ \text{CO}_2 + \text{trioleoylglycerol} \end{array}\right\} \quad (72)$$

where $zz = 14 \times$ transport factor for glucose.

When neither a deficit nor a surplus of ATP existed, fat synthesis was simulated according to the stoicheiometry summarized in equation (73) until the available mitochondrial acetyl-CoA was expended or until the stipulated amount of fat had been synthesized, and according to the stoicheiometry in equation (74) until all glucose was expended or until the designated amount of fat had been synthesized.

$$\left.\begin{array}{l} 44{\cdot}333\ \text{acetyl-CoA}_{\text{mito}} + 0{\cdot}5\ \text{glucose} + 11{\cdot}667\ \text{O}_2 + (68{\cdot}333 + Z)\ \text{ATP} \\ \qquad \longrightarrow 34{\cdot}667\ \text{CO}_2 + \text{trioleoylglycerol} \\ (68{\cdot}333 + Z)/12\ \text{acetyl-CoA}_{\text{mito}} + (68{\cdot}333 + Z)/6\ \text{O}_2 \longrightarrow \\ \qquad (68{\cdot}333 + Z)/6\ \text{CO}_2 + (68{\cdot}333 + Z)\ \text{ATP} \\ \hline (44{\cdot}333 + (68{\cdot}333 + Z)/12)\ \text{acetyl-CoA}_{\text{mito}} + 0{\cdot}5\ \text{glucose} + \\ \quad (11{\cdot}667 + (68{\cdot}333 + Z)/6)\ \text{O}_2 \longrightarrow (34{\cdot}667 + (68{\cdot}333 + Z)/6)\ \text{CO}_2 \\ \qquad + \text{trioleoylglycerol} \end{array}\right\} \quad (73)$$

where Z is as defined in equation (71).

$$\left.\begin{array}{l} 14\ \text{glucose} + 4\ \text{O}_2 + (70 + zz)\ \text{ATP} \longrightarrow 27\ \text{CO}_2 + \text{trioleoylglycerol} \\ (70 + zz)/36\ \text{glucose} + (70 + zz)/6\ \text{O}_2 \longrightarrow (70 + zz)/6\ \text{CO}_2 + (70 + zz)\ \text{ATP} \\ \hline (14 + (70 + \text{zz})/36)\ \text{glucose} + (4 + (70 + zz)/6)\ \text{O}_2 \longrightarrow \\ \qquad (27 + (70 + zz)/6)\ \text{CO}_2 + \text{trioleoylglycerol} \end{array}\right\} \quad (74)$$

where zz is as defined in equation (72).

Stoicheiometry equations (71) and (74) provided for the energy cost of fat synthesis, but there were additional energy-requiring processes associated with fat synthesis. The NADPH for fat synthesis was presumed to arise from reactions catalysed by the malic enzyme or isocitrate dehydrogenase, or both. McGilvery has presented a similar stoicheiometry in which NADPH was presumed to arise from the glucose-6-phosphate dehydrogenase reaction (McGilvery, 1970). The two procedures have negligible effect on the energetics. Goodridge measured the activities of these enzymes in different physiological states and concluded that glucose-6-phosphate dehydrogenase was the least important of the three in lipogenesis (Goodridge, 1968).

Calculation of energy balance

If estimates of the amount of protein and fat synthesis were available, the ME requirement was equal to the energy remaining after the estimated body synthesis had been simulated, and the metabolism of all remaining substrates had been simulated in accord with the stoicheiometries presented in the previous discussion. An estimate of protein synthesis could be made from measurement of N balance, or in the absence of an estimate of protein synthesis, the programme would simulate protein synthesis equal to that allowed by the limiting essential amino acid. If an estimate of fat synthesis was not available, an estimate of the ME requirement must be provided. The prescribed ME was subtracted before simulation of fat synthesis, and the amount of fat synthesis was determined by the substrates and energy available. The remaining parameters of energy balance were calculated using equations (75) to (78).

$$\text{Heat production} = (\text{dietary available energy} + \text{available energy in body substance oxidized}) - (\text{available energy in body substance synthesized}); \quad (75)$$

$$\text{Heat increment} = \text{heat production} - \text{ME}; \quad (76)$$

$$\text{Partial efficiency of maintenance} = (\text{maintenance energy}) \div (\text{heat production} - \text{cost of synthesis of body substance}); \quad (77)$$

$$\text{Partial efficiency of retention} = (\text{available energy of body substance synthesized}) \div (\text{available energy of body substance synthesized} + \text{cost of synthesis of body substance}). \quad (78)$$

DISCUSSION

The most appropriate criterion for evaluation of any procedure which would simulate animal energy metabolism is comparison of results obtained by simulation with the observations reported in carefully-conducted animal experiments. McCracken (1975) has reported the results of a thorough study of the effect of eating pattern on the energy metabolism of young rats. The results of that study provided an opportunity to compare computer simulation with experimental results. Exp. 2 in McCracken's (1975) study was a 3×3 factorial experiment which consisted of diets containing three levels of protein at three levels of energy intake. The proportions of available energy present as protein were 0·047 (groups 1, 4 and 7), 0·093 (groups 2, 5 and 8), and 0·18 (groups 3, 6 and 9). The body-weights were estimated from the heat production and heat production/d per kg taken from Table 8 and the increases in body protein and fat were taken from Table 7 of McCracken (1975).

Table 1 summarizes energy balance as determined by simulation. The maintenance requirement, heat production, energy retention and heat increment as their equivalent in mol ATP and as the proportion of dietary available energy are tabulated for each group in McCracken's (1975) Expt. 2. The ME (/d per kg $^{0.75}$) as estimated from McCracken's (1975) results for each group is included in Table 1 for comparison with the simulation data. The

Table 1. *Energy balance values obtained by simulation using results from Expt 2 of McCracken* (1975)

	Maintenance energy					Heat production		Retention			Heat increment	
Group no.	mol ATP*	Proportion of available energy	Partial efficiency	Simulated value (kJ/d per $W^{0.75}$)	Experimental value†	mol ATP	Proportion of available energy	mol ATP	Proportion of available energy	Partial efficiency	mol ATP	Proportion of available energy
1	4·204	0·799	0·965	433	426	4·542	0·859	0·7474	0·141	0·800	0·3381	0·064
2	4·601	0·877	0·987	455	437	4·715	0·910	0·4715	0·090	0·801	0·1759	0·034
3	4·802	0·934	0·996	458	465	4·974	0·968	0·6312	0·123	0·802	0·1721	0·034
4	4·737	0·522	0·911	460	462	5·930	0·654	3·139	0·346	0·811	1·193	0·132
5	4·895	0·544	0·918	452	445	6·027	0·670	2·971	0·330	0·809	1·132	0·125
6	4·703	0·534	0·920	420	436	5·832	0·662	2·979	0·338	0·806	1·129	0·128
7	4·644	0·424	0·896	436	453	6·362	0·581	4·598	0·419	0·813	1·718	0·157
8	4·122	0·379	0·862	365	388	5·927	0·545	4·944	0·455	0·812	1·805	0·166
9	4·273	0·401	0·869	368	413	6·006	0·564	4·639	0·436	0·810	1·733	0·163

* Mol ATP can be converted to kJ by multiplication by 77·3.
† Calculated from data of McCracken assuming partial efficiency of retention of 0·7.

estimates of ME from McCracken's (1975) results were calculated assuming a constant partial efficiency of retention of 0·7. The average value for ME calculated from McCracken's (1975) results was 436 ± 25 kJ/d per $kg^{0.75}$, while the average value from simulation was 427 ± 37 kJ/d per $kg^{0.75}$. A number of features of the results presented in Table 1 are noteworthy in addition to the close agreement between the results obtained in experiments and those obtained by simulation. The definition of partial efficiencies presented in equations (77) and (78) differs from the definition given these partial efficiencies in experiments involving whole animals (Kleiber, 1961). Equations (77) and (78) define the partial efficiency of maintenance and the partial efficiency of retention properly, but these definitions require information which is not discernible in experiments with whole animals. In contrast to equation (78), the partial efficiency of retention in experiments with whole animals is calculated according to equation (79).

$$\text{'Partial efficiency of retention'} = (\text{available energy retained}) \div (\text{heat production} + \text{available energy retained} - \text{ME}) = (\text{available energy retained}) \div (\text{available energy retained} + \text{total heat increment}). \quad (79)$$

Values from Table 1 can be used to calculate 'partial efficiency of retention' according to equation (79). When this was done for each of the nine groups, a value of $0{\cdot}730 \pm 0{\cdot}009$ was calculated which differs from the average partial efficiency of retention of $0{\cdot}807 \pm 0{\cdot}005$ presented in Table 1. The value of 0·73 is in close agreement with the values of 0·70 and 0·72 calculated by McCracken (1975), and with the values quoted by other investigators for simple-stomached animals (Verstegen, Close, Start & Mount, 1973; Kleiber, 1961). The difference between the two estimates of partial efficiency of retention resides in the fact that heat increment consists of at least two components. One component is the heat increment associated with energy retention. This component consists of (1) the sum of ATP required for membrane transport of the metabolites involved in the net synthesis of body substance, (2) the ATP required for the synthesis of nucleic acids to direct protein synthesis, (3) the ATP required of the synthesis of covalent bonds, and (4) the ATP required for all other reactions associated with the synthesis of body substance. Another component of the total heat increment is that associated with metabolic processes, such as gluconeogenesis, which result in lowering the yield of ATP during metabolism, but processes which are not related uniquely to the net synthesis of body substance. The latter component of heat increment is here designated the heat increment of maintenance. Only the heat increment of retention is considered properly in calculating the partial efficiency of retention, and only the heat increment of maintenance is considered properly in calculating the partial efficiency of maintenance. In experiments with whole animals, it is generally not possible to estimate the magnitude of the individual components of heat increment, for heat increment is estimated as the difference between heat production and ME. Thus, the 'partial efficiency of retention' in those experiments is defined by equation (79) rather than by the correct definition given by equation no. (78).

Simulation provides a means of separating total heat increment into its components. Table 2 presents the distribution of the total heat increment into its components for each of the nine groups of McCracken (1975). At the present time it is not possible to check the accuracy of the distribution of heat increment, but the accuracy of the total heat increment is indicated by the close agreement between the 'partial efficiency of retention' estimated by simulation using equation (79) and the estimates of partial efficiency of retention calculated from values obtained in whole animal experiments.

The procedure now employed in the programme will tend toward higher values for the heat increment of retention because amino acids which were not utilized in protein synthesis were assumed to give rise to maximal gluconeogenesis. This is in accord with the effect of

Table 2. *Distribution of heat increment from simulation values obtained using results from McCracken* (1975)

Group no.	Heat increment: Maintenance (mol ATP)	Heat increment: Retention (mol ATP)	Heat increment: Total (mol ATP)
1	0·1507	0·1874	0·3381
2	0·0587	0·1172	0·1759
3	0·0165	0·1556	0·1721
4	0·4610	0·7320	1·193
5	0·4293	0·7027	1·132
6	0·4110	0·7180	1·129
7	0·6600	1·058	1·718
8	0·6610	1·144	1·805
9	0·6430	1·090	1·733

Table 3. *Respiratory exchange and nitrogen excretion values obtained by simulation using results from Expt 2 of McCracken* (1975)

Group no.	Oxygen consumed (mol)	Carbon dioxide produced (mol)	Respiratory quotient	Urea-N excreted (g)*	Proportion of N excreted
1	0·7711	0·7353	0·952	0·0822	0·490
2	0·8188	0·7566	0·924	0·1118	0·344
3	0·8639	0·7639	0·884	0·1483	0·237
4	0·9928	1·023	1·03	0·0673	0·234
5	1·020	1·023	1·00	0·1590	0·286
6	1·006	0·9736	0·968	0·3432	0·320
7	1·058	1·136	1·07	0·1069	0·308
8	0·9921	1·064	1·07	0·1591	0·237
9	1·033	1·048	1·01	0·4794	0·370

* Simulated N excretion values did not include endogenous N excretion.

proteins and amino acids on specific dynamic action (Brody, 1945). The programme could be modified readily to provide the investigator the option of stipulating the extent of gluconeogenesis.

The ME requirement for groups 8 and 9 in Table 1 was lower than for other groups when ME was calculated from McCracken's (1975) results and also when estimates were made from simulation values. On the basis of limited observations, it is not possible to determine if the decrease is real, and if so, what the explanation of the lowered energy requirement might be. In the simulation algorithm, it was assumed that triacylglycerols are synthesized from acetyl-CoA and glucose rather than by direct incorporation of fatty acids into triglycerides. Synthesis from acetyl-CoA results in higher heat increment of retention with the result that the estimate of ME from simulation will be lower than if synthesis of triacylglycerols occurred from incorporation of existing free fatty acids. The close agreement between estimates from experiments and those from simulation suggests that the assumption included in the simulation algorithm does not introduce a significant error. The effect of lowering the heat increment of retention can be investigated in the simulation procedure. When this was done with groups 8 and 9 of McCracken (1975), the estimates of ME increased to 392 and 393 kJ/d per kg $^{0.75}$, while the partial efficiency increased to 0·85.

Simulation provides data on respiratory exchange and N balance. The respiratory exchange and N balance results from simulation of the nine groups are presented in Table 3. Values necessary for calculation of N balance are usually collected in metabolic studies of this nature, but respiratory values are not often collected in this type of study. Respiratory values would provide an additional basis for checking the validity of the results from simulation.

Estimates made on the basis of simulation were here compared with results from experiments in which protein and fat synthesis were measured. If an estimate of net N retention and ME were available, simulation would provide a means of estimating the retention of energy as body fat for a given diet. The utility of the computer programme described here is enhanced by the ease with which it can be applied to metabolic studies in which the precision of estimates of diet composition may vary considerably. It can be applied to diets in which the chemical composition of one or all of the dietary components is known only in terms of crude protein, crude fat and crude carbohydrate. Where the precise chemical composition is known for one or all of the dietary components, the additional information is utilized in the simulation.

A listing of the computer programme here described together with input instructions may be obtained from the author. The author wishes to express his appreciation to Dr E. J. Davis for many helpful discussions and suggestions during the preparation of this manuscript. This study was supported in part by the Grace M. Showalter Residuary Trust.

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