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 $^{75}$ (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 reflections 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 stoichiometric expressions. Energy balance was simulated for a diet on the basis of the metabolic stoichiometries 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 stoichiometric 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, 1975a, b).

The implication of some of the foregoing stipulations should be clarified. The absorption
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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, CO₂, H₂O and SO₄²⁻. The enthalpies of combustion of glucose, glycogen and trioleylglycerol are 155, 176 and 398 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:

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

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 stoichiometry equation (1) regardless of whether or not a portion of the carbon skeleton of the digested carbohydrate is utilized in biosynthetic reactions:

\[
\begin{align*}
\text{glucose} & \rightarrow 2 \text{ pyruvate} + 2 \text{ NADH}_{\text{cyt}} + 2 \text{ ATP} \\
2 \text{ NADH}_{\text{cyt}} + 2 \text{ ATP} & \rightarrow 2 \text{ NADH}_{\text{mito}} \\
2 \text{ NADH}_{\text{mito}} + \text{O}_2 & \rightarrow 6 \text{ ATP} \\
2 \text{ pyruvate} + 5 \text{O}_2 & \rightarrow 6 \text{CO}_2 + 30 \text{ ATP} \\
\text{glucose} + 6 \text{O}_2 & \rightarrow 6 \text{CO}_2 + 36 \text{ ATP} + 6 \text{H}_2\text{O}
\end{align*}
\]
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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 stoichiometry equation (1). The biosynthetic stoichiometries for glucose are presented in the sections concerned with protein, carbohydrate and fat synthesis (see pp. 244–9).

Dietary amino acid metabolism

Stoichiometry equation (2) outlines the reactions involved in the formation of urea from glutamate and CO\(_2\). In the remaining amino acid stoichiometries, this sequence will be presented only as the summation indicated in equation (2):

\[
\begin{align*}
0.5 \text{ glutamate} + 0.5 \text{ OAA} & \rightarrow 0.5 \text{ aspartate} + 0.5 \text{ a-KG} + 0.5 \text{ NH}_3 + 0.5 \text{ NADH}_{mito} \\
0.5 \text{ aspartate} + 0.5 \text{ CO}_2 + 0.5 \text{ NH}_3 + 2 \text{ ATP} & \rightarrow 0.5 \text{ urea} + 0.5 \text{ fumarate}_{cyt} \\
0.5 \text{ fumarate}_{cyt} & \rightarrow 0.5 \text{ OAA} + 0.5 \text{ NADH}_{cyt} \\
0.5 \text{ NADH}_{cyt} + 0.5 \text{ ATP} & \rightarrow 0.5 \text{ NADH}_{mito} \\
\text{glutamate} + 0.5 \text{ CO}_2 + 0.5 \text{ O}_2 & \rightarrow 0.5 \text{ urea} + 0.5 \text{ ATP} + 0.5 \text{ H}_2\text{O}
\end{align*}
\]

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

\[
\begin{align*}
\text{R-CH}_3 + 0.5 \text{ O}_2 & \rightarrow \text{formaldehyde} + \text{R-H} + 2 \text{ ATP} \\
\text{formaldehyde} + \text{tetrahydrofolate (FH}_4) & \rightarrow 5,10\text{-methyleneFH}_4 \\
0.5 \text{(5,10-methyleneFH}_4) + 0.5 \text{ CO}_2 + 0.5 \text{ NH}_3 + 0.5 \text{ NADH}_{mito} & \rightarrow 0.5 \text{ glycerol} + 5 \text{ FH}_4 \\
0.5 \text{(5,10-methyleneFH}_4) + 0.5 \text{ glycine} & \rightarrow 0.5 \text{ serine} + 0.5 \text{ FH}_4 \\
0.5 \text{ serine} & \rightarrow 0.5 \text{ pyruvate} + 0.5 \text{ NH}_3 \\
0.5 \text{ pyruvate} + \text{O}_2 & \rightarrow 1.5 \text{ CO}_2 + 0.5 \text{ NADH}_{mito} + 6 \text{ ATP} \\
\text{R-CH}_3 + 1.5 \text{ O}_2 & \rightarrow \text{CO}_2 + \text{R-H} + 8 \text{ ATP} + \text{H}_2\text{O}
\end{align*}
\]

The reactions involved in the simulated oxidation of amino acids to urea, CO\(_2\) and H\(_2\)O are summarized in stoichiometry equations (4) to (24):

\[
\begin{align*}
\text{alanine} + 3 \text{ O}_2 & \rightarrow 2.5 \text{ CO}_2 + 0.5 \text{ urea} + 15.5 \text{ ATP} + 2.5 \text{ H}_2\text{O} \\
\text{arginine} + 5.5 \text{ O}_2 & \rightarrow 4 \text{ CO}_2 + 2 \text{ urea} + 28 \text{ ATP} + 3 \text{ H}_2\text{O} \\
\text{aspartate} + 3 \text{ O}_2 & \rightarrow 3.5 \text{ CO}_2 + 0.5 \text{ urea} + 15.5 \text{ ATP} + 2.5 \text{ H}_2\text{O} \\
\text{asparagine} + 3 \text{ O}_2 & \rightarrow 3 \text{ CO}_2 + \text{urea} + 13 \text{ ATP} + 2 \text{ H}_2\text{O} \\
\text{cysteine} + 4 \text{ O}_2 & \rightarrow 2.5 \text{ CO}_2 + 0.5 \text{ urea} + 12.5 \text{ ATP} + \text{SO}_4^{2-} \\
\text{glutamate} + 4.5 \text{ O}_2 & \rightarrow 4.5 \text{ CO}_2 + 0.5 \text{ urea} + 24.5 \text{ ATP} + 3.5 \text{ H}_2\text{O} \\
\text{glutamine} + 4.5 \text{ O}_2 & \rightarrow 4 \text{ CO}_2 + \text{urea} + 22 \text{ ATP} + 3 \text{ H}_2\text{O} \\
\text{glycine} + 1.5 \text{ O}_2 & \rightarrow 1.5 \text{ CO}_2 + 0.5 \text{ urea} + 6.5 \text{ ATP} + 1.5 \text{ H}_2\text{O} \\
\text{histidine} + 5 \text{ O}_2 & \rightarrow 4.5 \text{ CO}_2 + 1.5 \text{ urea} + 22.5 \text{ ATP} + 1.5 \text{ H}_2\text{O} \\
\text{isoleucine} + 7.5 \text{ O}_2 & \rightarrow 5.5 \text{ CO}_2 + 0.5 \text{ urea} + 40.5 \text{ ATP} + 5.5 \text{ H}_2\text{O}
\end{align*}
\]
leucine + 7·5 O₂ → 5·5 CO₂ + 0·5 urea + 39·5 ATP + 5·5 H₂O
lysine + 7 O₂ → 5 CO₂ + 0·5 urea + 36 ATP + 5 H₂O
methionine + 7 O₂ → 4·5 CO₂ + 0·5 urea + 21·5 ATP + SO₄²⁻
phenylalanine + 10 O₂ → 8·5 CO₂ + 0·5 urea + 37·5 ATP + 4·5 H₂O
proline + 5·5 O₂ → 4·5 CO₂ + 0·5 urea + 26·5 ATP + 3·5 H₂O
hydroxyproline + 5 O₂ → 3·5 CO₂ + 0·5 urea + 26·5 ATP + 3·5 H₂O
serine + 2·5 O₂ → 2·5 CO₂ + 0·5 urea + 2·5 ATP + 3·5 H₂O
threonine + 4 O₂ → 3·5 CO₂ + 0·5 urea + 20·5 ATP + 3·5 H₂O
tryptophan + 11·5 O₂ → 10 CO₂ + urea + 42 ATP + 4·5 H₂O
tyrosine + 9·5 O₂ → 8·5 CO₂ + 0·5 urea + 41·5 ATP + 4·5 H₂O
valine + 6 O₂ → 4·5 CO₂ + 0·5 urea + 31·5 ATP + 4·5 H₂O.

The available energy in the form of ATP due to dietary protein was calculated from the amino acid composition using the foregoing stoichiometry equations. During the simulated metabolism, those amino acids which are not incorporated into body protein are presumed to be converted to CO₂, urea, glucose and H₂O. The reactions involved in gluconeogenesis are summarized in stoichiometry equations (25) to (43).

Alanine + αKG → glutamate + pyruvate
Glutamate + 0·5 CO₂ + 0·5 O₂ → 0·5 urea + αKG + 0·5 ATP
0·7381 pyruvate + 2·214 ATP + 0·7381 NADH → 0·369 glucose
0·1845 pyruvate + 0·0922 O₂ → 0·5535 CO₂ + 0·7381 NADH + 0·5535 ATP
0·0774 pyruvate + 0·1934 O₂ → 0·2321 CO₂ + 1·160 ATP

Arginine + αKG + 0·5 O₂ → 2 glutamate + urea + 3 ATP
3 glutamate + CO₂ + O₂ → urea + 2 αKG + ATP
αKG + O₂ → CO₂ + OAA + NADH + 6 ATP
OAA + 2 ATP + NADH → CO₂ + 0·5 glucose

Arginine + 2·5 O₂ → CO₂ + 2 urea + 0·5 glucose + 8 ATP

Aspartate + αKG → glutamate + OAA
Glutamate + 0·5 CO₂ + 0·5 O₂ → 0·5 urea + αKG + 0·5 ATP
0·775 OAA + 1·5 ATP + 0·775 NADH → 0·775 CO₂ + 0·3875 glucose
0·1938 OAA + 0·0969 O₂ → 0·775 CO₂ + 0·775 NADH + 0·581 ATP
0·0313 OAA + 0·0782 O₂ → 0·1252 CO₂ + 0·469 ATP

Asparagine → aspartate + NH₃
Aspartate + CO₂ + NH₃ + 4 ATP → urea + fumarate, cyst.
Fumarate, cyst + ATP → OAA + NADH, lipo.
NADH, lipo + 0·5 O₂ → 3 ATP
0·65 OAA + 1·3 ATP + 0·65 NADH → 0·65 CO₂ + 0·325 glucose
0·1625 OAA + 0·0813 O₂ → 0·65 CO₂ + 0·65 NADH + 0·4875 ATP
0·1875 OAA + 0·4657 O₂ → 0·75 CO₂ + 2·813 ATP

Asparagine + 1·05 O₂ → 1·05 CO₂ + urea + 0·325 glucose + 0·05 H₂O.
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cysteine $+$ αKG + NADH$_{mito}$ $+$ 1.5 O$_2$ $\rightarrow$ glutamate $+$ pyruvate $+$ SO$_4^2-$
glutamate $+$ 0.5 CO$_2$ $+$ 0.5 O$_2$ $\rightarrow$ 0.5 urea $+$ αKG $+$ 0.5 ATP
0.5 pyruvate $+$ 0.5 O$_2$ $\rightarrow$ 0.75 CO$_2$ $+$ NADH$_{mito}$ $+$ 0.75 ATP
0.5952 pyruvate $+$ 1.786 ATP $+$ 0.5952 NADH $\rightarrow$ 0.2976 glucose
0.1488 pyruvate $+$ 0.0744 O$_2$ $\rightarrow$ 0.4464 CO$_2$ $+$ 0.5952 NADH $+$ 0.4464 ATP
0.0059 pyruvate $+$ 0.0148 O$_2$ $\rightarrow$ 0.0177 CO$_2$ $+$ 0.0886 ATP

(29)

cysteine $+$ 2.214 O$_2$ $\rightarrow$ 0.7141 CO$_2$ $+$ 0.5 urea $+$ 0.2976 glucose

(30)
glutamate $+$ 0.5 CO$_2$ $+$ 0.5 O$_2$ $\rightarrow$ 0.5 urea $+$ αKG $+$ 0.5 ATP
αKG $+$ O$_2$ $\rightarrow$ CO$_2$ $+$ OAA $+$ NADH $+$ 6 ATP
OAA $+$ 2 ATP $+$ NADH $\rightarrow$ CO$_2$ $+$ 0.5 glucose

(31)
glutamate $+$ 1.5 O$_2$ $\rightarrow$ 1.5 CO$_2$ $+$ 0.5 urea $+$ 0.5 glucose $+$ 4.5 ATP $+$ 5 H$_2$O

(32)
0.5 glycine $\rightarrow$ 0.5 CO$_2$ $+$ 0.5 NH$_3$ $+$ 0.5 NADH$_{mito}$ $+$ 0.5 (5,10-methyleneFH$_4$)
0.5 glycine $+$ 0.5 (5,10-methyleneFH$_4$) $\rightarrow$ 0.5 serine
0.5 αKG $+$ 0.5 NH$_3$ $+$ 0.5 NADH$_{mito}$ $\rightarrow$ 0.5 glutamate
0.5 glutamate $+$ 0.5 OAA $\rightarrow$ 0.5 aspartate $+$ 0.5 αKG
0.5 aspartate $+$ 0.5 CO$_2$ $+$ 0.5 NH$_3$ $+$ 2 ATP $\rightarrow$ 0.5 urea $+$ 0.5 fumarate$_{cyt}$
0.5 fumarate$_{cyt}$ $+$ 0.5 ATP $\rightarrow$ 0.5 OAA $+$ 0.5 NADH$_{mito}$
0.5 NADH$_{mito}$ $+$ 0.25 O$_2$ $\rightarrow$ 1.5 ATP
0.3095 pyruvate $+$ 0.0286 ATP $+$ 0.3095 NADH $\rightarrow$ 0.1548 glucose
0.0774 pyruvate $+$ 0.0387 O$_2$ $\rightarrow$ 0.2322 CO$_2$ $+$ 0.3095 NADH $+$ 0.2322 ATP
0.1131 pyruvate $+$ 0.2828 O$_2$ $\rightarrow$ 0.3393 CO$_2$ $+$ 1.697 ATP

glycine $+$ 0.5715 O$_2$ $\rightarrow$ 0.5715 CO$_2$ $+$ 0.5 urea $+$ 0.51548 glucose $+$ 0.571 H$_2$O

(33)
histidine $\rightarrow$ glutamate $+$ 2 NH$_3$ $+$ 5,10-methyleneFH$_4$
5,10-methyleneFH$_4$ $+$ NADPH $\rightarrow$ 5,10-methyleneFH$_4$
0.5 (5,10-methyleneFH$_4$) $+$ 0.5 CO$_2$ $+$ 0.5 NH$_3$ $+$ 0.5 NADH$_{mito}$ $\rightarrow$ 5 glycine
0.5 (5,10-methyleneFH$_4$) $+$ 0.5 glycine $\rightarrow$ 0.5 serine
0.5 αKG $+$ 0.5 NH$_3$ $+$ 0.5 NADH$_{mito}$ $\rightarrow$ 0.5 glutamate
1.5 glutamate $+$ 1.5 OAA $\rightarrow$ 1.5 aspartate $+$ 1.5 αKG
1.5 aspartate $+$ 1.5 CO$_2$ $+$ 1.5 NH$_3$ $+$ 6 ATP $\rightarrow$ 1.5 urea $+$ 1.5 fumarate$_{cyt}$
1.5 fumarate $\rightarrow$ 1.5 OAA $+$ 1.5 NADH$_{cyt}$
NADH $+$ ATP $\rightarrow$ NADPH
0.5 NADH$_{cyt}$ $+$ 0.5 ATP $\rightarrow$ 0.5 NADH$_{mito}$
αKG $+$ O$_2$ $\rightarrow$ CO$_2$ $+$ OAA $+$ NADH $+$ 6 ATP
OAA $+$ 2 ATP $+$ NADH $\rightarrow$ CO$_2$ $+$ 0.5 glucose

histidine $+$ 2 O$_2$ $+$ 1.4 H$_2$O $\rightarrow$ 1.5 CO$_2$ $+$ 1.5 urea $+$ 0.5 glucose $+$ 2.5 ATP
### Metabolic Pathways

#### (34) Isoleucine Metabolism

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>isoleucine + αKG + 3·5 O$_2$</td>
<td>2 CO$_2$ + succinate + glutamate + 20 ATP</td>
</tr>
<tr>
<td>glutamate + 0·5 CO$_2$ + 0·5 O$_2$</td>
<td>0·5 urea + αKG + 0·5 ATP</td>
</tr>
<tr>
<td>succinate + 0·5 O$_2$</td>
<td>OAA + NADH + 2 ATP</td>
</tr>
<tr>
<td>OAA + 2 ATP + NADH</td>
<td>CO$_2$ + 0·5 glucose</td>
</tr>
<tr>
<td>isoleucine + 4·5 O$_2$</td>
<td>2·5 CO$_2$ + 0·5 urea + 0·5 glucose + 20·5 ATP + 2·5 H$_2$O</td>
</tr>
</tbody>
</table>

#### (35) Methionine and Homocysteine Metabolism

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>methionine + 1·5 O$_2$</td>
<td>CO$_2$ + homocysteine + 5 ATP</td>
</tr>
<tr>
<td>homocysteine + serine</td>
<td>cysteine + α-ketobutyrate + NH$_3$</td>
</tr>
<tr>
<td>cysteine + αKG + NADH$_{slo}$ + 1·5 O$_2$</td>
<td>glutamate + pyruvate + SO$_4^{2-}$</td>
</tr>
<tr>
<td>glutamate + PEP</td>
<td>serine + αKG</td>
</tr>
<tr>
<td>0·5 αKG + 0·5 NH$<em>3$ + 0·5 NADH$</em>{slo}$</td>
<td>0·5 glutamate</td>
</tr>
<tr>
<td>0·5 glutamate + 0·5 OAA</td>
<td>0·5 aspartate + 0·5 αKG</td>
</tr>
<tr>
<td>0·5 aspartate + 0·5 CO$_2$ + 0·5 NH$_3$ + 2 ATP</td>
<td>0·5 urea + 0·5 fumarate$_{est}$</td>
</tr>
<tr>
<td>0·5 fumarate$_{est}$ + 0·5 ATP</td>
<td>0·5 OAA + 0·5 NADH$_{slo}$</td>
</tr>
<tr>
<td>α-ketobutyrate</td>
<td>succinate + NADH$_{slo}$</td>
</tr>
<tr>
<td>succinate + 0·5 O$_2$</td>
<td>OAA + NADH + 2 ATP</td>
</tr>
<tr>
<td>OAA + 2 ATP + NADH</td>
<td>CO$_2$ + 0·5 glucose</td>
</tr>
</tbody>
</table>

#### (36) Phenylalanine Metabolism

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenylalanine + αKG + NADPH + O$_2$</td>
<td>glutamate + p-hydroxyphenylpyruvate</td>
</tr>
<tr>
<td>p-hydroxyphenylpyruvate + 2 O$_2$</td>
<td>CO$_2$ + acetoacetate + fumarate</td>
</tr>
<tr>
<td>glutamate + 0·5 CO$_2$ + 2 ATP</td>
<td>0·5 urea + αKG + NADH</td>
</tr>
<tr>
<td>NADH + ATP</td>
<td>NADPH</td>
</tr>
<tr>
<td>acetoacetate + 4 O$_2$</td>
<td>4 CO$_2$ + 23 ATP</td>
</tr>
<tr>
<td>fumarate</td>
<td>OAA + NADH</td>
</tr>
<tr>
<td>OAA + 2 ATP + NADH</td>
<td>CO$_2$ + 0·5 glucose</td>
</tr>
</tbody>
</table>

#### (37) Proline Metabolism

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>proline + O$_2$</td>
<td>glutamate + 5 ATP</td>
</tr>
<tr>
<td>glutamate + 0·5 CO$_2$ + 0·5 O$_2$</td>
<td>0·5 urea + αKG + 0·5 ATP</td>
</tr>
<tr>
<td>αKG + O$_2$</td>
<td>CO$_2$ + OAA + NADH + 6 ATP</td>
</tr>
<tr>
<td>OAA + 2 ATP + NADH</td>
<td>CO$_2$ + 0·5 glucose</td>
</tr>
<tr>
<td>proline + 2·5 O$_2$</td>
<td>1·5 CO$_2$ + 0·5 urea + 0·5 glucose + 9·5 ATP</td>
</tr>
</tbody>
</table>

#### (38) Hydroxyproline Metabolism

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxyproline + O$_2$</td>
<td>alanine + glyoxylate + 5 ATP</td>
</tr>
<tr>
<td>glutamate + glyoxylate</td>
<td>glycine + αKG</td>
</tr>
<tr>
<td>glycine + 0·5715 O$_2$</td>
<td>0·5715 CO$_2$ + 0·5 urea + 0·1548 glucose</td>
</tr>
<tr>
<td>alanine + αKG</td>
<td>glutamate + pyruvate</td>
</tr>
<tr>
<td>0·8 pyruvate + 2·4 ATP + 0·8 NADH</td>
<td>0·4 glucose</td>
</tr>
<tr>
<td>0·2 pyruvate + 1 O$_2$</td>
<td>0·6 CO$_2$ + 0·8 NADH + 0·6 ATP</td>
</tr>
<tr>
<td>hydroxyproline + 1·671 O$_2$</td>
<td>1·71 CO$_2$ + 0·5 urea + 0·5548 glucose + 3·2 ATP + 0·171 H$_2$O</td>
</tr>
</tbody>
</table>
Simulation of energy metabolism

serine $\rightarrow$ pyruvate $+$ NH$_3$

$0.5\;\alpha$KG $+$ $0.5\;\text{NH}_3$ $+$ $0.5\;\text{NADH}_{\text{mito}}$ $\rightarrow$ $0.5\;\text{glutamate}$

$0.5\;\text{glutamate} + 0.5\;\text{OAA} \rightarrow 0.5\;\text{aspartate} + 0.5\;\alpha$KG

$0.5\;\text{aspartate} + 0.5\;\text{CO}_2 + 0.5\;\text{NH}_3 + 2\;\text{ATP} \rightarrow 0.5\;\text{urea} + 0.5\;\text{fumarate}_{\text{cyt}}$

$0.5\;\text{fumarate}_{\text{cyt}} + 0.5\;\text{ATP} \rightarrow 0.5\;\text{OAA} + 0.5\;\text{NADH}_{\text{mito}}$

$0.5952\;\text{pyruvate} + 1.786\;\text{ATP} + 0.5952\;\text{NADH} \rightarrow 0.2076\;\text{glucose}$

$0.1488\;\text{pyruvate} + 0.0744\;\text{O}_2 \rightarrow 0.4464\;\text{CO}_2 + 0.5952\;\text{NADH} + 0.4464\;\text{ATP}$

$0.2560\;\text{pyruvate} + 0.6399\;\text{O}_2 \rightarrow 0.768\;\text{CO}_2 + 3.838\;\text{ATP}$

$0.952\;\text{pyruvate} + 0.7144\;\text{O}_2 \rightarrow 0.7144\;\text{CO}_2 + 0.5\;\text{urea} + 0.7144\;\text{H}_2\text{O}$

$\text{Dietary fat metabolism}$

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 stoichiometric 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.)

\[
\text{Molecular weight} = 42N + 134 - 6U. \tag{44}
\]

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).

\[
\begin{align*}
O_2 \text{ consumed} &= 4.5N + 0.5 - 1.5U, \tag{45} \\
CO_2 \text{ produced} &= 3N + 3. \tag{46}
\end{align*}
\]

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.

\[
\text{ATP yield} = 25.5N - 1 - 6U. \tag{47}
\]

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

\[
\text{ATP yield} = 25.5N - 2.5 - 6U. \tag{48}
\]

Equations (44) to (48) provided the stoichiometries 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 stoichiometry for conversion of triacylglycerol to mitochondrial acetyl-CoA and glucose. $O_2$ consumption (mol) for this process was calculated with the following equation:

\[
O_2 \text{ consumed} = 1.5N - 2.5 - 1.5U. \tag{49}
\]

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).

\[
\begin{align*}
\text{mitochondrial acetyl-CoA} &= 1.5N, \tag{50} \\
\text{ATP} &= 7.5N - 19 - 6U. \tag{51}
\end{align*}
\]

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

\[
\begin{align*}
\text{mitochondrial acetyl-CoA} &= 1.5N - 4.5, \tag{52} \\
\text{ATP} &= 7.5N - 26.5 - 6U. \tag{53}
\end{align*}
\]

The stoichiometry 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
Simulation of energy metabolism

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 stoichiometry equations (54) to (64). According to these stoichiometries, 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 stoichiometric equation:

\[
\begin{align*}
0.5 \text{glucose} & \rightarrow \text{pyruvate} + \text{NADH}_{\text{mito}} + \text{ATP} \\
\text{NADH}_{\text{mito}} + \text{ATP} & \rightarrow \text{NADH}_{\text{mito}} \\
\text{glutamine} & \rightarrow \text{glutamate} + \text{NH}_3 \\
\alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} & \rightarrow \text{glutamate} \\
\text{pyruvate} + \text{glutamate} & \rightarrow \text{alanine} + \alpha\text{KG} \\
\text{glutamate} + \text{NH}_3 + \text{ATP} & \rightarrow \text{glutamine} \\
0.5 \text{glucose} + \text{NH}_3 + \text{ATP} & \rightarrow \text{alanine} + \text{H}_2\text{O} \\
\end{align*}
\]

(54)

\[
\begin{align*}
0.5 \text{glucose} + \text{pyruvate} + \text{NADH}_{\text{mito}} & \rightarrow \text{OAA} \\
\text{glutamine} & \rightarrow \text{glutamate} + \text{NH}_3 \\
\text{glutamate} + \text{OAA} & \rightarrow \text{aspartate} + \alpha\text{KG} \\
\alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} & \rightarrow \text{glutamate} \\
\text{glutamate} + \text{NH}_3 + \text{ATP} & \rightarrow \text{glutamine} \\
0.5 \text{glucose} + \text{CO}_2 + \text{NH}_3 + 2 \text{ATP} & \rightarrow \text{aspartate} + \text{H}_2\text{O} \\
\end{align*}
\]

(55)

\[
\begin{align*}
\text{methionine} + \text{R-H} + 3 \text{ATP} & \rightarrow \text{R-CH}_3 + \text{homocysteine} \\
\text{R-CH}_3 + 1.5 \text{O}_2 & \rightarrow \text{CO}_2 + \text{R-H} + 8 \text{ATP} \\
\text{homocysteine} + \text{serine} & \rightarrow \text{cysteine} + \alpha\text{-ketobutyrate} + \text{NH}_3 \\
\alpha\text{-ketobutyrate} + 0.5 \text{O}_2 & \rightarrow \text{succinate} + 3 \text{ATP} \\
\text{succinate} + 0.5 \text{O}_2 & \rightarrow \text{OAA} + \text{NADH}_{\text{mito}} + 2 \text{ATP} \\
\text{OAA} + \text{ATP} & \rightarrow \text{CO}_2 + \text{PEP} \\
\text{glutamate} + \text{PEP} & \rightarrow \text{serine} + \alpha\text{KG} \\
\alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} & \rightarrow \text{glutamate} \\
\text{methionine} + 2.5 \text{O}_2 & \rightarrow 2 \text{CO}_2 + \text{cysteine} + 9 \text{ATP} + \text{H}_2\text{O} \\
\end{align*}
\]

(56)

\[
\begin{align*}
\text{glucose} & \rightarrow 2 \text{pyruvate} + 2 \text{NADH}_{\text{mito}} \\
\text{pyruvate} + \text{CO}_2 + \text{ATP} & \rightarrow \text{OAA} \\
\text{pyruvate} + 0.5 \text{O}_2 & \rightarrow \text{CO}_2 + \text{acetyl-CoA} + 3 \text{ATP} \\
\text{acetyl-CoA} + \text{OAA} + 0.5 \text{O}_2 & \rightarrow \text{CO}_2 + \alpha\text{KG} + 3 \text{ATP} \\
\text{glutamine} & \rightarrow \text{glutamate} + \text{NH}_3 \\
\alpha\text{KG} + \text{NH}_3 + \text{NADH}_{\text{mito}} & \rightarrow \text{glutamate} \\
\text{NADH}_{\text{mito}} + 0.5 \text{O}_2 & \rightarrow 3 \text{ATP} \\
\text{glutamate} + \text{NH}_3 + \text{ATP} & \rightarrow \text{glutamine} \\
\text{glucose} + \text{NH}_3 + 1.5 \text{O}_2 & \rightarrow \text{CO}_2 + \text{glutamate} + 7 \text{ATP} + 3 \text{H}_2\text{O} \\
\text{glucose} + 2 \text{NH}_3 + 1.5 \text{O}_2 & \rightarrow \text{CO}_2 + \text{glutamine} + 6 \text{ATP} + 4 \text{H}_2\text{O} \\
\end{align*}
\]

(57)

(58)

(59)
\[
0.5 \text{ glucose} \rightarrow \text{PEP + NADH}_{\text{cvt}} \\
\text{glutamate + PEP} \rightarrow \text{serine + } \alpha \text{KG} \\
\text{serine + R-H} \rightarrow \text{glycine + R-CH}_3 \\
\text{R-CH}_3 + 1.5 \text{ O}_2 \rightarrow \text{CO}_2 + \text{R-H} + 8 \text{ ATP} \\
\text{NADH}_{\text{cvt}} + \text{ATP} \rightarrow \text{NADH}_{\text{nilo}} \\
\text{glutamine} \rightarrow \text{glutamate + NH}_3 \\
\alpha \text{KG + NH}_3 + \text{NADH}_{\text{nilo}} \rightarrow \text{glutamate} \\
\text{glutamate + NH}_3 + \text{ATP} \rightarrow \text{glutamine} \\
\]

\[
0.5 \text{ glucose} + \text{NH}_3 + 1.5 \text{ O}_2 \rightarrow \text{CO}_2 + \text{glycine} + 6 \text{ ATP} + 2 \text{ H}_2\text{O} \\
\text{glucose} \rightarrow 2 \text{ pyruvate} + 2 \text{ NADH}_{\text{cvt}} + 2 \text{ ATP} \\
\text{NADH}_{\text{cvt}} + \text{ATP} \rightarrow \text{NADH}_{\text{nilo}} \\
\text{NADH + ATP} \rightarrow \text{NADPH} \\
\text{pyruvate + CO}_2 + \text{ATP} \rightarrow \text{OAA} \\
\text{pyruvate + 0.5 O}_2 \rightarrow \text{CO}_2 + \text{acetyl-CoA + 3 ATP} \\
\text{acetyl-CoA + OAA} \rightarrow \text{CO}_2 + \alpha \text{KG + NADH}_{\text{nilo}} \\
\text{glutamine} \rightarrow \text{glutamate + NH}_3 \\
\alpha \text{KG + NH}_3 + \text{NADH}_{\text{nilo}} \rightarrow \text{glutamate} \\
\text{glutamate + NADH}_{\text{nilo}} + \text{ATP} \rightarrow \text{glutamic semialdehyde} \\
\text{glutamic semialdehyde + NADPH} \rightarrow \text{proline} \\
\text{glutamate + NH}_3 + \text{ATP} \rightarrow \text{glutamine} \\
\]

\[
\text{glucose + NH}_3 + 0.5 \text{ O}_2 \rightarrow \text{CO}_2 + \text{proline} + \text{ATP} + 3 \text{ H}_2\text{O} \\
\text{glucose + NH}_3 + 0.5 \text{ O}_2 \rightarrow \text{CO}_2 + \text{proline} + \text{ATP} \\
\text{proline + } \alpha \text{KG + O}_2 \rightarrow \text{CO}_2 + \text{hydroxyproline + succinate + ATP} \\
\text{succinate + O}_2 \rightarrow \text{OAA + 5 ATP} \\
0.5 \text{ glucose} \rightarrow \text{pyruvate + NADH}_{\text{nilo}} \\
\text{NADH}_{\text{nilo}} + 0.5 \text{ O}_2 \rightarrow 3 \text{ ATP} \\
\text{pyruvate + 0.5 O}_2 \rightarrow \text{CO}_2 + \text{acetyl-CoA + 3 ATP} \\
\text{acetyl-CoA + OAA + 0.5 O}_2 \rightarrow \text{CO}_2 + \alpha \text{KG + 3 ATP} \\
1.5 \text{ glucose + NH}_3 + 4 \text{ O}_2 \rightarrow 4 \text{ CO}_2 + \text{hydroxyproline + 16 ATP + 6 H}_2\text{O} \\
0.5 \text{ glucose} \rightarrow \text{PEP + NADH}_{\text{cvt}} \\
\text{NADH}_{\text{cvt}} + \text{ATP} \rightarrow \text{NADH}_{\text{nilo}} \\
\text{glutamine} \rightarrow \text{glutamate + NH}_3 \\
\text{glutamate + PEP} \rightarrow \text{serine + } \alpha \text{KG} \\
\alpha \text{KG + NH}_3 + \text{NADH}_{\text{nilo}} \rightarrow \text{glutamate} \\
\text{glutamate + NH}_3 + \text{ATP} \rightarrow \text{glutamine} \\
0.5 \text{ glucose + NH}_3 + 2 \text{ ATP} \rightarrow \text{serine} \\
\]

\[
0.5 \text{ glucose} \rightarrow \text{pyruvate + NADH}_{\text{cvt}} + \text{ATP} \\
\text{NADH + ATP} \rightarrow \text{NADPH} \\
\text{phenylalanine + NADPH + O}_2 \rightarrow \text{tyrosine} \\
\text{pyruvate + 2.5 O}_2 \rightarrow 3 \text{ CO}_2 + 15 \text{ ATP} \\
0.5 \text{ glucose + phenylalanine + O}_2 \rightarrow 3 \text{ CO}_2 + \text{tyrosine + 15 ATP + 3 H}_2\text{O} \\
\]
Simulation of energy metabolism

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.

\[ \text{glucose required} = \frac{\text{lactose (g)}}{171}. \]  

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

\[ \text{glucose required} = \frac{\text{glycogen (g)}}{162}. \]

Equation (67) summarizes the stoichiometry for lactose synthesis:

\[
\begin{align*}
\text{glucose} + \text{ATP} & \rightarrow \text{glucose-6-phosphate (G6P)} \\
\text{G6P} & \rightarrow \text{glucose-1-phosphate (G1P)} \\
\text{G1P} + \text{UTP} & \rightarrow \text{uridine diphosphoglucone (UDPG)} \\
\text{UDPG} & \rightarrow \text{UDPGalactose} \\
\text{UDPGalactose} + \text{glucose} & \rightarrow \text{lactose} + \text{UDP}
\end{align*}
\]

The following is the stoichiometry for glycogen synthesis:

\[
\begin{align*}
\text{glucose} + \text{ATP} & \rightarrow \text{G6P} \\
\text{G6P} & \rightarrow \text{G1P} \\
\text{G1P} + \text{ATP} & \rightarrow \text{UDPG} \\
\text{UDPG} + (\text{glucosyl})_{n-1} & \rightarrow (\text{glucosyl})_n \\
\text{glucose} + (\text{glucosyl})_{n-1} + 2 \text{ ATP} & \rightarrow (\text{glucosyl})_n + \text{H}_2\text{O}
\end{align*}
\]

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 stoichiometries (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 stoichiometry equation (1), and the stoichiometry for trioleoylglycerol is summarized in equation (70).

\[
\begin{align*}
\text{acetyl-CoA}_{\text{mito}} + 2 \text{ O}_2 & \rightarrow 2 \text{ CO}_2 + \text{COAH} + 12 \text{ ATP} + \text{H}_2\text{O}, \\
\text{trioleoylglycerol} + 80 \text{ O}_2 & \rightarrow 57 \text{ CO}_2 + 452 \text{ ATP} + 52 \text{ H}_2\text{O}.
\end{align*}
\]

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 stoichiometries presented in equation (71), and finally exclusively from glucose according to the stoichiometries summarized in equation (72).

\[
\begin{align*}
24 \text{acetyl-CoA}_{\text{mito}} + 24 \text{ATP} &\rightarrow 24 \text{acetyl-CoA}_{\text{cyt}} \\
24 \text{acetyl-CoA}_{\text{cyt}} + 42 \text{NADPH} + 21 \text{ATP} &\rightarrow 3 \text{palmitate} \\
3 \text{palmitate} + 6 \text{ATP} &\rightarrow 3 \text{palmitoyl-CoA} \\
3 \text{palmitoyl-CoA} + 3 \text{acetyl-CoA}_{\text{mito}} + 6 \text{NADPH} &\rightarrow 3 \text{stearyl-CoA} \\
3 \text{stearyl-CoA} + 3 \text{NADPH} + 3 \text{O}_2 &\rightarrow 3 \text{oleoyl-CoA} \\
0.5 \text{glucose} + \text{NADH} + \text{ATP} &\rightarrow \alpha\text{-glycerol-PO}_4 \\
3 \text{oleoyl-CoA} + \alpha\text{-glycerol-PO}_4 &\rightarrow \text{trioleoylglycerol} \\
17.333 \text{acetyl-CoA}_{\text{mito}} + 8.667 \text{O}_2 &\rightarrow 34.667 \text{CO}_2 + 34.667 \text{ATP} + 52 \text{NADH} \\
51 \text{NADH} + 51 \text{ATP} &\rightarrow 51 \text{NADPH} \\
44.333 \text{acetyl-CoA}_{\text{mito}} + 0.5 \text{glucose} + 11.667 \text{O}_2 + (68.333 + Z) \text{ATP} &\rightarrow 34.667 \text{CO}_2 + \text{trioleoylglycerol} + 44.333 \text{CoASH}
\end{align*}
\]

where \(Z = 44.333 \times \text{transport factor for fat/9}\)

\[
\begin{align*}
14 \text{glucose} &\rightarrow \alpha\text{-glycerol-PO}_4 + 27 \text{pyruvate} + 26 \text{NADH} + 26 \text{ATP} \\
25 \text{pyruvate} &\rightarrow 25 \text{CO}_2 + 25 \text{acetyl-CoA}_{\text{mito}} + 25 \text{NADH} \\
51 \text{NADH} + 51 \text{ATP} &\rightarrow 51 \text{NADPH} \\
2 \text{pyruvate} + \text{O}_2 &\rightarrow 2 \text{CO}_2 + 2 \text{acetyl-CoA}_{\text{mito}} + 6 \text{ATP} \\
24 \text{acetyl-CoA}_{\text{mito}} + 24 \text{ATP} &\rightarrow 24 \text{acetyl-CoA}_{\text{cyt}} \\
24 \text{acetyl-CoA}_{\text{cyt}} + 42 \text{NADPH} + 21 \text{ATP} &\rightarrow 3 \text{palmitate} \\
3 \text{palmitate} + 6 \text{ATP} &\rightarrow 3 \text{palmitoyl-CoA} \\
3 \text{palmitoyl-CoA} + 3 \text{acetyl-CoA}_{\text{mito}} + 6 \text{NADPH} &\rightarrow 3 \text{stearyl-CoA} \\
3 \text{stearyl-CoA} + 3 \text{NADPH} + 3 \text{O}_2 &\rightarrow 3 \text{oleoyl-CoA} \\
3 \text{oleoyl-CoA} + \alpha\text{-glycerol-PO}_4 &\rightarrow \text{trioleoylglycerol} \\
14 \text{glucose} + 4 \text{O}_2 + (70 + zz) \text{ATP} &\rightarrow 27 \text{CO}_2 + \text{trioleoylglycerol} \\
\end{align*}
\]

where \(zz = 14 \times \text{transport factor for glucose}\).

When neither a deficit nor a surplus of ATP existed, fat synthesis was simulated according to the stoichiometry 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 stoichiometry in equation (74) until all glucose was expended or until the designated amount of fat had been synthesized.

\[
\begin{align*}
44.333 \text{acetyl-CoA}_{\text{mito}} + 0.5 \text{glucose} + 11.667 \text{O}_2 + (68.333 + Z) \text{ATP} &\rightarrow 34.667 \text{CO}_2 + \text{trioleoylglycerol} \\
(68.333 + Z)/12 \text{acetyl-CoA}_{\text{mito}} + (68.333 + Z)/6 \text{O}_2 &\rightarrow (68.333 + Z)/6 \text{CO}_2 + (68.333 + Z) \text{ATP} \\
(44.333 + (68.333 + Z)/12) \text{acetyl-CoA}_{\text{mito}} + 0.5 \text{glucose} + \\
(11.667 + (68.333 + Z)/6) \text{O}_2 &\rightarrow (34.667 + (68.333 + Z)/6) \text{CO}_2 + \text{trioleoylglycerol}
\end{align*}
\]

where \(Z\) as defined in equation (71).

\[
\begin{align*}
14 \text{glucose} + 4 \text{O}_2 + (70 + zz) \text{ATP} &\rightarrow 27 \text{CO}_2 + \text{trioleoylglycerol} \\
(70 + zz)/36 \text{glucose} + (70 + zz)/6 \text{O}_2 &\rightarrow (70 + zz)/6 \text{CO}_2 + (70 + zz) \text{ATP} \\
(14 + (70 + zz)/36) \text{glucose} + (4 + (70 + zz)/6) \text{O}_2 &\rightarrow (27 + (70 + zz)/6) \text{CO}_2 + \text{trioleoylglycerol}
\end{align*}
\]

where \(zz\) as defined in equation (72).
Stoichiometry 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 stoichiometry 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 stoichiometries 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).

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

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

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

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

**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 $3 \times 3$ 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.
Table 1. *Energy balance values obtained by simulation using results from Expt 2 of McCracken (1975)*

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Proportion of available energy</th>
<th>Partial efficiency</th>
<th>Simulated value (kJ/d per W${^{0.75}}$)</th>
<th>Experimental value†</th>
<th>Proportion of available energy</th>
<th>Proportion of available energy</th>
<th>Partial efficiency</th>
<th>Proportion of available energy</th>
<th>Proportion of available energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.204</td>
<td>0.799</td>
<td>0.965</td>
<td>433</td>
<td>4.542</td>
<td>0.859</td>
<td>0.7474</td>
<td>0.141</td>
<td>0.800</td>
</tr>
<tr>
<td>2</td>
<td>4.601</td>
<td>0.877</td>
<td>0.987</td>
<td>455</td>
<td>4.715</td>
<td>0.910</td>
<td>0.4715</td>
<td>0.090</td>
<td>0.801</td>
</tr>
<tr>
<td>3</td>
<td>4.802</td>
<td>0.934</td>
<td>0.996</td>
<td>458</td>
<td>4.974</td>
<td>0.968</td>
<td>0.6312</td>
<td>0.123</td>
<td>0.802</td>
</tr>
<tr>
<td>4</td>
<td>4.737</td>
<td>0.522</td>
<td>0.911</td>
<td>460</td>
<td>5.930</td>
<td>0.654</td>
<td>3.139</td>
<td>0.346</td>
<td>0.811</td>
</tr>
<tr>
<td>5</td>
<td>4.895</td>
<td>0.544</td>
<td>0.918</td>
<td>452</td>
<td>6.027</td>
<td>0.660</td>
<td>2.971</td>
<td>0.330</td>
<td>0.809</td>
</tr>
<tr>
<td>6</td>
<td>4.703</td>
<td>0.534</td>
<td>0.920</td>
<td>420</td>
<td>5.832</td>
<td>0.662</td>
<td>2.979</td>
<td>0.338</td>
<td>0.806</td>
</tr>
<tr>
<td>7</td>
<td>4.444</td>
<td>0.424</td>
<td>0.896</td>
<td>436</td>
<td>6.362</td>
<td>0.581</td>
<td>4.598</td>
<td>0.419</td>
<td>0.813</td>
</tr>
<tr>
<td>8</td>
<td>4.122</td>
<td>0.379</td>
<td>0.862</td>
<td>365</td>
<td>5.927</td>
<td>0.545</td>
<td>4.944</td>
<td>0.455</td>
<td>0.812</td>
</tr>
<tr>
<td>9</td>
<td>4.273</td>
<td>0.401</td>
<td>0.869</td>
<td>368</td>
<td>6.006</td>
<td>0.564</td>
<td>4.639</td>
<td>0.436</td>
<td>0.810</td>
</tr>
</tbody>
</table>

* 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 $436 \pm 25 \text{ kJ/d per kg}^{0.75}$, while the average value from simulation was $427 \pm 37 \text{ kJ/d per kg}^{0.75}$. A number of features of the results presented in Table I 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).

\[
\text{'Partial efficiency of retention'} = \frac{\text{available energy retained}}{\text{heat production} + \text{available energy retained} - \text{ME}} = \frac{\text{available energy retained}}{\text{available energy retained} + \text{total heat increment}}. \tag{79}
\]

Values from Table I 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 0.730 $\pm$ 0.009 was calculated which differs from the average partial efficiency of retention of 0.807 $\pm$ 0.005 presented in Table I. 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)

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Maintenance (mol ATP)</th>
<th>Retention (mol ATP)</th>
<th>Total (mol ATP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1507</td>
<td>0.1874</td>
<td>0.3381</td>
</tr>
<tr>
<td>2</td>
<td>0.0587</td>
<td>0.1172</td>
<td>0.1759</td>
</tr>
<tr>
<td>3</td>
<td>0.0165</td>
<td>0.1556</td>
<td>0.1721</td>
</tr>
<tr>
<td>4</td>
<td>0.4610</td>
<td>0.7320</td>
<td>1.193</td>
</tr>
<tr>
<td>5</td>
<td>0.4293</td>
<td>0.7027</td>
<td>1.132</td>
</tr>
<tr>
<td>6</td>
<td>0.4110</td>
<td>0.7180</td>
<td>1.129</td>
</tr>
<tr>
<td>7</td>
<td>0.6600</td>
<td>1.058</td>
<td>1.718</td>
</tr>
<tr>
<td>8</td>
<td>0.6610</td>
<td>1.144</td>
<td>1.805</td>
</tr>
<tr>
<td>9</td>
<td>0.6430</td>
<td>1.090</td>
<td>1.733</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Oxygen consumed (mol)</th>
<th>Carbon dioxide produced (mol)</th>
<th>Respiratory quotient</th>
<th>Urea-N excreted (g)*</th>
<th>Proportion of N excreted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7911</td>
<td>0.7353</td>
<td>0.952</td>
<td>0.0822</td>
<td>0.490</td>
</tr>
<tr>
<td>2</td>
<td>0.8188</td>
<td>0.7566</td>
<td>0.924</td>
<td>0.1118</td>
<td>0.344</td>
</tr>
<tr>
<td>3</td>
<td>0.8639</td>
<td>0.7639</td>
<td>0.884</td>
<td>0.1483</td>
<td>0.237</td>
</tr>
<tr>
<td>4</td>
<td>0.9928</td>
<td>1.023</td>
<td>1.03</td>
<td>0.0673</td>
<td>0.234</td>
</tr>
<tr>
<td>5</td>
<td>1.020</td>
<td>1.023</td>
<td>1.00</td>
<td>0.1590</td>
<td>0.286</td>
</tr>
<tr>
<td>6</td>
<td>1.006</td>
<td>0.9736</td>
<td>0.968</td>
<td>0.3432</td>
<td>0.320</td>
</tr>
<tr>
<td>7</td>
<td>1.058</td>
<td>1.136</td>
<td>1.07</td>
<td>0.1069</td>
<td>0.308</td>
</tr>
<tr>
<td>8</td>
<td>0.9921</td>
<td>1.064</td>
<td>1.07</td>
<td>0.1591</td>
<td>0.237</td>
</tr>
<tr>
<td>9</td>
<td>1.033</td>
<td>1.048</td>
<td>1.01</td>
<td>0.4794</td>
<td>0.370</td>
</tr>
</tbody>
</table>

* 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.

REFERENCES