

# Estimation of Theoretical Calorific Relationships as a Teaching Technique. A Review.

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## Abstract

A series of theoretical estimates of the efficiencies of metabolic and physiological processes which can be related to nutritional energetic measurements is presented. The examples and format were developed for, and have proven useful as, teaching aids in undergraduate and graduate courses in bioenergetics and are presented in the hope that others also might find them useful.

A number of the energy transformations and expenditures associated with food utilization for maintenance, growth, and lactation can be explained in physiological and biochemical terms. In undergraduate and graduate courses in Nutrition, we have found that it is helpful to students being introduced to bioenergetics to present examples of metabolic and physiological processes which can be identified with nutritional energetic terms, such as heat increment, net energy, and maintenance. Toward the end of presenting a coherent series of examples of metabolic and physiological processes which can be related to nutritional energetic measurements, the format and examples presented in this paper were developed. This format has been a useful teaching tool in both graduate and undergraduate courses and has been well received by the students. In the hope that others also might find the format useful in attempting to present physiological explanations of classical energetic terms, it is presented in this paper.

A number of reviews relating energy transformations and energy expenditures associated with biological processes are available. Two of these contributed significantly to the development of the philosophy presented in this paper (4, 9). Definitions for the nutritional energetic terms employed were those described in *Energy Metabolism* (2). The primary sources of information on metabolic pathways and biochemical energy relationships were West and Todd (13), Long (11), and Krebs and Kornberg (9).

## Discussion

Release of energy from feedstuffs occurs during digestion, absorption, storage, mobilization, the synthesis of the pyrophosphate bond(s) of

ATP at the expense of the free energy released upon oxidation of foodstuffs and, finally, during hydrolysis of the pyrophosphate bonds of ATP in connection with the performance of physiological work functions. It would be convenient, if it were possible, to discuss examples of each of these physiological processes separately, in order, and relate the examples to specific nutritional energetic terms. Unfortunately, this is not entirely possible because the processes cannot always be separated, are mutually dependent, or are related to different energetic terms. An attempt has been made to consider energy expenditures in the order in which they might be expected to occur for a given feedstuff (i.e., in the order listed above), and to obtain maximum separation of processes that relate to different energetic measurements.

*Digestion.* Two examples of energetic transformations associated with digestion were selected. One of these, energy release due to bond breakage, was selected simply to emphasize the relative insignificance of this type of energy release. The other, estimation of the energy loss associated with the synthesis of pancreatic protein, was selected as a general index of the costs associated with the synthesis of digestive secretions.

The free energy of hydrolysis of peptide bonds is approximately 3 kcal/mole and the average molecular weight of the amino acids within most proteins can be assumed to be approximately 100 g/mole. According to these assumptions, the energy release per 100 g of protein or per mole of peptide bond hydrolyzed during digestion would be 3 kcal. The heat of combustion of 100 g of protein is about 570 kcal and, hence,  $(3/570)$  0.6% of the energy content of proteins is released due to breakage of the peptide bond during digestion. Similarly, the free energy of hydrolysis of glucosidic bonds is approximately 4.3 kcal/mole and the heat of combustion of 100 g of starch is 420 kcal/mole. Starch hydrolysis during digestion releases 2.5 kcal of heat per 100 g starch hydrolyzed or  $(2.5/420)$  0.6% of the heat of combustion of starch.

The conversion of triglycerides to glycerol and fatty acids releases about 0.1% of the energy content of the triglyceride. Losses due to

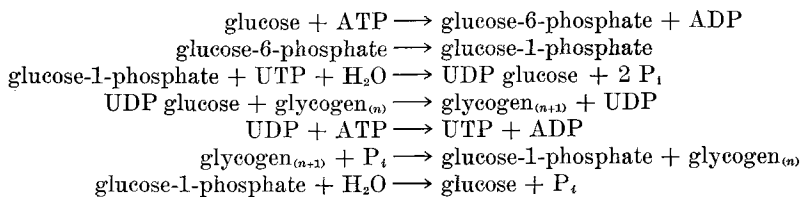
bond breakage occur during digestion and during the mobilization of glycogen, protein, and fat from body tissues during fasting. Since bond breakage occurs during both digestion and mobilization, energy losses due to bond breakage during digestion would not be considered components of heat increment estimates, unless the animal received a high starch diet and during the fasting state was using or mobilizing fat, to provide for its energy requirements. In this case, the difference in heat loss between starch hydrolysis and triglyceride hydrolysis would be a component of the heat increment.

The amount of pancreatic protein secreted by a 200-kg steer was reported to be 86 g/6 hr, or 344 g/day (8). The energy cost associated with the synthesis of this protein can be calculated by assuming that there is 1 mole of amino acid/100 g of pancreatic protein; that the direct cost for protein synthesis is 5 moles of ATP/mole of amino acid incorporated (justified in a later section); and, that the amino acids secreted as pancreatic protein are completely reabsorbed as amino acids and transported back to the pancreas at no net cost. The incorporation of 3.44 moles of amino acid into 344 g pancreatic protein requires the use of  $(5 \times 3.44)$  17.2 moles of ATP. The minimum heat loss associated with the formation and use of 1 mole of ATP is 18 kcal/mole ATP utilized.<sup>1</sup> Hence, the estimated heat loss due to synthesis of pancreatic

protein is  $(17.2 \text{ moles ATP} \times 18 \text{ kcal/mole ATP})$  310 kcal/day in a 200-kg steer. At a daily digestible energy intake of 7,500 kcal/day, the estimated percentage of the steer's daily energy intake lost as heat as a consequence of pancreatic protein secretion is 4.1%. This estimated heat loss would be considered a component of heat increment if pancreatic protein synthesis is assumed to cease during fasting.

*Absorption, storage, and mobilization.* Most physiological texts suggest that the absorption of glucose and amino acids is an active (ATP-requiring) process. If the absorption of 1 mole of glucose from the digestive tract requires the use of 1 mole of ATP, then  $(18 \text{ kcal per mole ATP}/686 \text{ kcal per mole glucose})$  2.6% of the potential energy value of glucose would be expended during absorption.

Animals are periodic eaters and continuous metabolizers. Hence, it is necessary that a considerable portion of the dietary metabolites be stored prior to utilization as sources of energy. Glucose was selected as a representative for calculation of storage costs. Glucose can be stored as either glycogen or fat. Storage as glycogen is most efficient, but the capacity of this system is limited and only about 30% of the dietary carbohydrate may be stored in this form (13). The remainder is either utilized directly or stored as fat. The cost of glycogen storage can be estimated from the following model:



<sup>1</sup> Calculated on the basis of glucose which has a heat of combustion of 686 kcal per mole and yields 38 ATP/mole when totally oxidized according to accepted pathways. When ATP formed from glucose is utilized in processes in which the initial and final states of the system acted on are

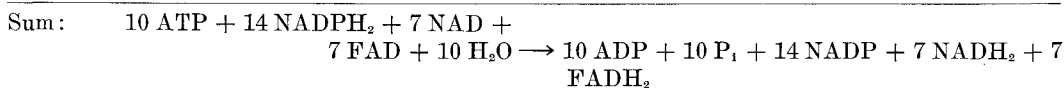
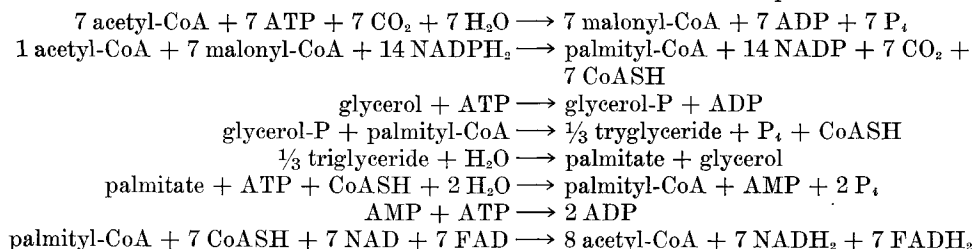
identical (no net work),  $\frac{686 \text{ kcal/mole glucose}}{38 \text{ mole ATP/mole glucose}}$

or 18 kcal/mole ATP are lost as heat. Student confusion about the often-quoted value of 8 kcal/mole ATP, which represents the heat loss upon hydrolysis of the terminal pyrophosphate bond of ATP, must often be dispelled. This value does not include the 10 kcal/mole of ATP lost during the formation of the terminal pyrophosphate bond during glucose oxidation. The sum of the heat losses occurring during the formation (10 kcal/mole) and hydrolysis (8 kcal/mole) of the terminal pyrophosphate bond of ATP is 18 kcal/mole.

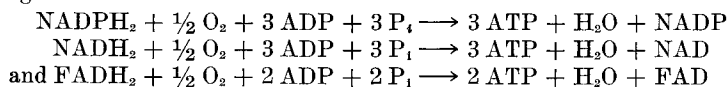
The estimated energy cost associated with the intermediate storage of glucose as glycogen would be  $(2 \times 18 \text{ kcal/mole ATP}) / (686 \text{ kcal/mole glucose})$  approximately 5%. A portion of the heat loss associated with glycogen storage would occur soon after the consumption of food and, hence, would be considered a component of heat increment estimates. The portion of the energy loss associated with the mobilization of glucose from glycogen (4.1 kcal/mole) would occur during fasting and would not be considered a component of heat increment estimates.

The model discussed below for estimation of the energy costs associated with storage of glucose as fat is more complex than the one proposed for storage of glucose as glycogen. The general model employed involves the conversion

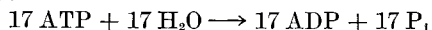
of glucose to acetyl-CoA which is converted to fat and then reformed after fat mobilization. The energy relationships associated with the conversion of glucose to acetyl-CoA were not considered in the calculation of energy losses in the model, because these would occur with or without intermediate storage of glucose as fat. The detailed model is as follows:



Considering:



The sum can be simplified to:



The energy cost associated with storage of glucose as fat can be estimated from the relationship:

$$\begin{aligned}
 \frac{17 \text{ moles of ATP} \times 18 \text{ kcal/mole ATP}}{4 \text{ moles of glucose} \times 686 \text{ kcal/mole glucose}} \\
 \times 100 = 8.3\%
 \end{aligned}$$

Based upon estimates of the oxygen consumption of epididymal fat pads, Ball (1) calculated that the heat loss associated with the storage of glucose as fat was 10.6%.

The estimated costs of absorption of glucose from the intestine (2.5%), plus the costs of pancreatic secretion (4.1%) and storage of glucose as glycogen (5%) and fat (10%), yield estimates of heat increment close to observed heat increments of 11% associated with the feeding of carbohydrate to animals under maintenance conditions. The estimated energy costs of storage appear to be the major component of the heat increment of carbohydrate utilization and their contribution would be expected to vary, dependent upon the amount and form of glucose storage.

The heat increment associated with the utilization of protein and amino acids as sources of energy is much larger than the heat increment associated with carbohydrate utilization (20-40%) and, hence, the energy losses are much more difficult to account for. Krebs (10) has attempted to formulate the assumptions re-

quired for such calculations. In addition to the energy cost for urea synthesis (2 ATP per atom of protein nitrogen excepting arginine), he has considered energy losses due to partial oxidation and excretion (12%) and the extent of coupling of oxidative processes with phosphorylation. His models result in calculated heat increments for protein utilization for en-

ergy of about 20%. Costs of digestion and absorption were not included in the estimates. Grisolia and Kennedy (5) presented several alternate views concerning the heat increment of amino acid utilization.

*Formation of ATP.* Two major pathways of carbohydrate utilization operate in animals. One of these involves glycolysis via the Embden-Meyerhof pathway and terminal oxidation via the tricarboxylic acid pathway, and the other involves a combination of hexose monophosphate cycle and hexose monophosphate glycolysis coupled with terminal oxidation via the tricarboxylic acid pathway. The relative contributions of these alternate pathways vary with species, diets, tissues, and physiological status. Their relative activities can be assessed in intact animals (7) and in tissues (6). The efficiencies of ATP formation via these alternate pathways are approximately equivalent; hence, the intermediate aspects of the two pathways will not be considered. Total oxidation of glucose via Embden-Meyerhof glycolysis and TCA cycle oxidation yields 38 moles of ATP. Current estimates of the energy content of the terminal pyrophosphate bond of ATP indicate an energy content of about 8 kcal per mole (11). This means that during the oxidation of 1 mole of glucose ( $8 \times 38$ ), 244 kcal of energy are effectively trapped in the form of ATP. Since the heat of combustion of glucose is 686

kcal per mole, the efficiency of ATP formation during the oxidation of glucose is (244/686) 44%. Thus, 56% of the potential energy available from glucose is lost as heat during ATP synthesis. Based upon similar reasoning, the heat losses associated with the oxidation of palmitate, propionate, and acetate for the formation of ATP are 57, 61, and 62%, respectively. These energy losses are components of the basal energy expenditure of animals.

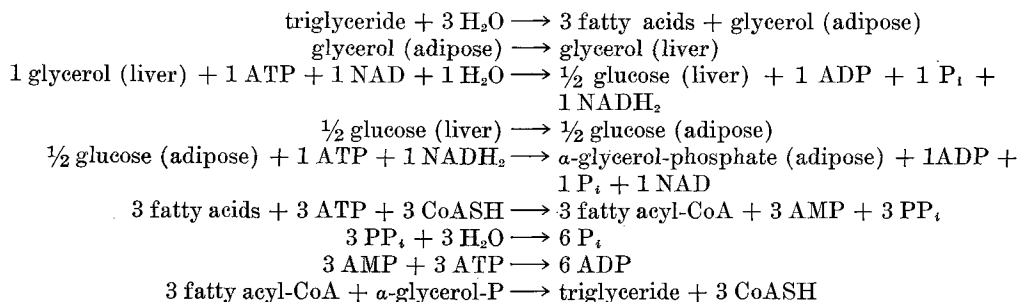
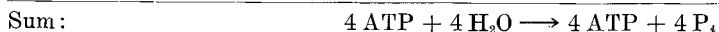
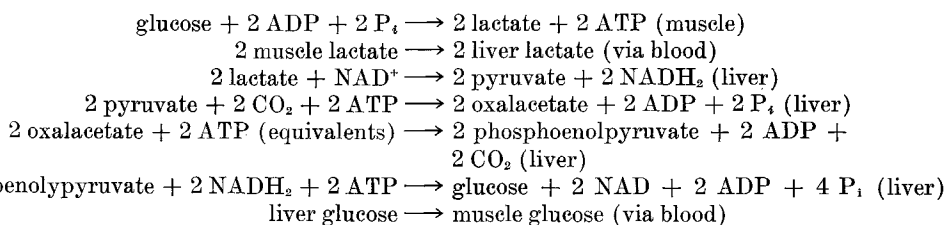
*Utilization of ATP for maintenance processes.* Many of the work functions associated with the maintenance of living systems have not been described in sufficient detail such that the energy (ATP) costs associated with them can be estimated. The number of ATP's utilized in connection with maintenance of muscle tone, heart action, kidney reabsorption, transmission of nerve impulses, protein resynthesis, and ion transport across membranes cannot be estimated accurately. Three examples which represent only a small portion of the total energy costs associated with maintenance have been developed. No attempt at accounting for the total energy expenditures of maintenance was made. One of the examples was the operation of the Cori cycle, another was turnover of triglyceride in the adipose tissue, and the third was the replacement of intestinal epithelium.

The model employed for estimation of the energy cost associated with turnover of the Cori cycle follows:

According to the model, the cost per turn of the Cori cycle would be (18 kcal/mole ATP  $\times$  4 mole ATP) 72 kcal. Reichard (12) reported that in normal, human resting subjects, 25 mg of glucose were recycled via the Cori cycle per hr per kg body weight, or that 42 g of glucose would be recycled each day in a 70-kg resting human. This represents about 0.25 mole of glucose and an energy expenditure of (72 kcal/mole glucose cycled  $\times$  0.25 mole glucose) 20 kcal per day. If the maintenance requirement of resting, fasting human subjects is estimated to be 2,000 kcal per day, then about 1% of the energy expenditure would be due to Cori cycle activity. Similar extrapolations of results obtained with subjects with cancer indicate that approximately 5% of the energy expended by these subjects was due to Cori cycle activity. In working, normal human subjects the energy expenditure associated with the Cori cycle would be expected to be considerably greater than that observed in resting subjects.

The following model was utilized to estimate the energy costs associated with the turnover of triglycerides in adipose tissue:

The turnover of 1 mole of triglyceride (at 18 kcal/mole of ATP) results in an energy loss of 144 kcal. The energy expenditure in adipose tissue alone is 126 kcal/mole of triglyceride turned over. Ball (1) estimated the rate of



turnover of triglyceride in adipose tissue and found that this cost accounted for the total oxygen consumption of adipose tissue *in vitro*. On the basis of measurements of the mass of adipose tissue in rats and estimates of the triglyceride turnover rate, Ball estimated that the energy expenditure associated with turnover of triglycerides amounted to approximately 15% of the digestible energy intake at maintenance.

It has been estimated that 500 g of protein must be synthesized per day by an adult ruminant, to support the rapid replacement of the cells of the intestinal epithelium. On the basis of this estimate and the estimate for the ATP requirement for protein synthesis presented in connection with the discussion of the cost of synthesis of pancreatic proteins, it can be estimated that (5 ATP/100 g protein synthesized  $\times$  18 kcal/mole ATP  $\times$  500 g protein) 450 kcal/day must be expended in the replacement of the intestinal epithelium. This replacement would require the expenditure of approximately 6.0% of the daily energy intake of a 200-kg steer.

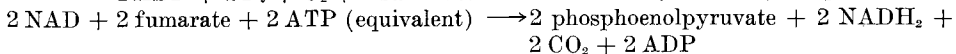
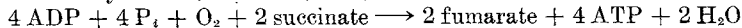
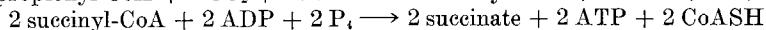
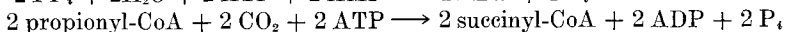
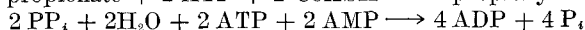
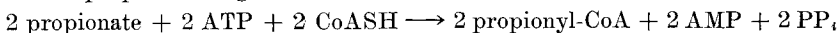
*Energy utilization for productive processes.* Functions associated with productive performance represent another type of work function requiring energy. A somewhat different treatment has been developed for this type of function, in order that the results of calculations based on biochemical pathways can be compared to estimates of the efficiency of utilization of metabolizable energy (ME). In the maintenance condition, it was considered that the efficiency of ME utilization for maintenance relates to the efficiency with which foodstuffs are utilized as substitutes or replacements for body

stores, and that the heat increment reflects energy expenditures associated with the utilization of foodstuffs in excess of those associated with the utilization of body stores, such as glycogen and fat. In the case of production, it was considered that the efficiency of ME utilization reflects the efficiency with which foodstuffs are incorporated into the product, while heat increment estimates reflect the energy expenditures required for the incorporation of foodstuffs into the product. To conform with this latter definition, input (metabolizable energy) : output (energy in product:net energy); relationships have been employed in the construction of models for estimation of the metabolic costs of productive processes.

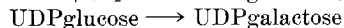
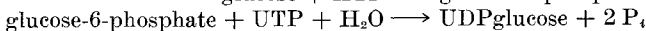
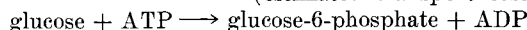
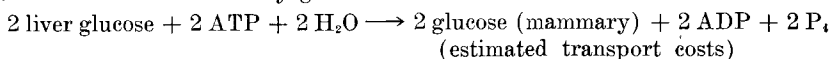
The example of productive function selected for detailed discussion was milk synthesis. To consider this function in terms of intermediary metabolism, a number of simplifying assumptions were necessary. It was assumed that the ratio of acetate to propionate absorbed from the rumen and available for milk synthesis was 2.5 to 1; that the balance of amino acids absorbed was ideal for milk synthesis; and, that only energy costs occurring after absorption need be considered in relating metabolizable energy to net energy. A number of additional assumptions were made in connection with the formulation of portions of the model. These will not be mentioned separately.

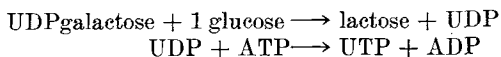
The synthesis of lactose was considered to occur totally at the expense of propionate which, after absorption, was converted to glucose in the liver and then transported to the mammary gland for conversion to lactose, according to the following model for the synthesis of 1 mole of lactose:

1. Conversion of propionate to glucose in liver



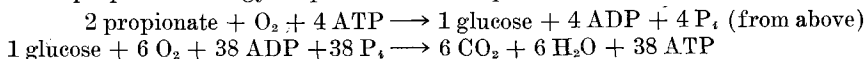
2. Synthesis of lactose in the mammary gland





Sum: 2 liver glucose + 4 ATP  $\longrightarrow$  1 lactose + 4 ADP + 4 P<sub>i</sub>  
 Net ATP requirement for the conversion of 4 propionate to 1 lactose = 12 ATP.

3. Conversion of propionate energy to provide ATP requirements



Sum: 2 propionate + 7 O<sub>2</sub> + 34 ADP + 34 P<sub>i</sub>  $\longrightarrow$  6 CO<sub>2</sub> + 6 H<sub>2</sub>O + 34 ATP  
 Net ATP yield per mole of propionate = 17.

At 17 moles ATP/mole propionate oxidized (12/17) 0.70 mole of propionate must be oxidized to provide the ATP required for the synthesis of lactose.

Input: 4.70 moles of propionate @ 367 kcal per mole = 1,730 kcal.

Output: 1 mole of lactose @ 1,350 kcal per mole = 1,350 kcal.

$$\text{Efficiency} = \frac{\text{output}}{\text{input}} \times 100 = 78\%$$

The estimated metabolic efficiency of lactose synthesis from propionate is 78%.

The synthesis of milk protein was assumed to occur from an almost perfect balance of amino acids, with propionate serving as a source of energy, or ATP. The costs associated with uptake, transport, and rearrangement of amino acids were assumed to be nil. Based on present concepts of protein synthesis it can be estimated that 2 ATP are required for the activation of 1 mole of amino acid to form acyl-s-RNA; that 1 ATP equivalent (GTP) is expended during peptide bond formation; and, that about 2 ATP equivalent are required for regeneration of transfer and other RNA after the synthesis of 100 g of protein. Hence, the estimated ATP requirement for the synthesis of 100 g of milk protein is 5.0 moles of ATP. From 3 above, it can be estimated that 0.295 mole of propionate

must be oxidized to satisfy this ATP requirement. Hence, the input-output relationship is as follows:

Input: 567 kcal/mole of average amino acid + 108 kcal/0.295 mole of propionate = 675.

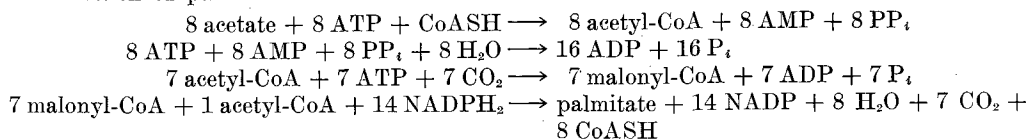
Output: 570 kcal/mole of protein = 570.

Efficiency = 82%.

The estimated metabolic efficiency for milk protein synthesis from amino acids and propionate was 82%.

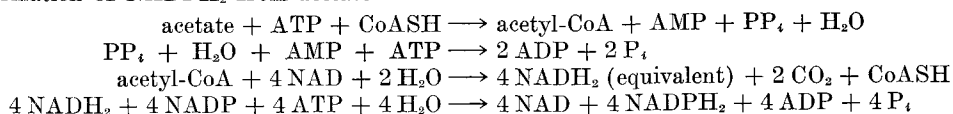
To simplify estimation of the metabolic efficiency of fat synthesis, it was assumed that the efficiency of palmitate incorporation into milk fat is representative of over-all efficiency and that acetate is the sole precursor of palmitate, according to the following model:

1. Formation of palmitate



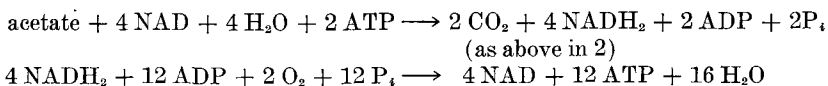
Sum: 8 acetate + 23 ATP + 14 NADPH<sub>2</sub>  $\longrightarrow$  palmitate + 14 NADP + 23 ADP + 23 P<sub>i</sub>  
 23 ATP + 14 NADH<sub>2</sub> must be obtained from acetate, to provide for the synthesis of palmitate.

2. Formation of NADPH<sub>2</sub> from acetate



Sum:  
 acetate + 6 ATP + 6 H<sub>2</sub>O + 4 NADP  $\longrightarrow$  2 CO<sub>2</sub> + 6 ADP + 6 P<sub>i</sub> + 4 NADPH<sub>2</sub>  
 @ 4 NADPH<sub>2</sub> per acetate need (14/4) 3.5 moles of acetate plus (3.5 × 6) 21.0 additional moles ATP to provide 14 NADPH<sub>2</sub> for palmitate synthesis.

## 3. Formation of ATP from acetate



Sum: acetate + 2 O<sub>2</sub> + 10 ADP + 10 P<sub>i</sub> → 10 ATP + 2 CO<sub>2</sub> + 12 H<sub>2</sub>O

8 moles of acetate were utilized directly in the synthesis of palmitate, at 10 ATP/mole acetate, 2.3 moles of acetate must be oxidized to provide the ATP required for palmitate synthesis, 3.5 moles of acetate must be utilized to provide the TPNH required for palmitate synthesis, and 2.1 moles of acetate must be oxidized to provide ATP for NADPH<sub>2</sub> synthesis. The input-output relationship is as follows:

Input: 15.9 moles of acetate @ 209 kcal/mole = 3,320 kcal.

Output: 1 mole of palmitate @ 2,398 kcal/mole = 2,398 kcal.

Efficiency = 72%.

The estimated metabolic efficiency of milk fat synthesis is 72%.

The over-all metabolic efficiency of synthesis of 100 g of milk is shown in Table 1. The net input was 95.2 kcal and the output was 72.8 kcal, with an over-all estimated efficiency of milk synthesis of 76%. This estimate appears to compare favorably with observed efficiencies of utilization of metabolizable energy for milk synthesis of 70% (2). In the formulation, it is evident the estimates of metabolic efficiency developed were maximal; since only direct metabolic costs associated with the synthesis of the major components of milk were considered. Energy costs associated with the secretion of minor milk components and maintenance of the mammary glands were not considered. The energy expenditures associated with maintenance of the gland might be relatively large, since recent estimates of the rate of turnover of cytoplasmic protein in the mammary glands of rats indicated that up to 3% of the cytoplasmic protein turned over per hour (3). This high rate of turnover may not apply to cows; however, if it does the efficiency estimate above would be reduced several per cent. In view of an estimated efficiency of milk synthesis of 76%, as compared to real efficiency estimates as high as 70%, it seems reasonable to suggest that milk secretion can occur at close to maximal theoretical efficiency and with a minimal heat increment.

The theoretical efficiency estimates obtained for protein and fat synthesis in the above formulation for milk synthesis can also be employed to estimate theoretical efficiencies for growth and fattening. The theoretical efficiency of growth in rapidly growing lambs is estimated in Table 2 to be 78%, which compares favorably with estimates of efficiency of utilization of ME supplied above maintenance for growth in young animals of greater than 70% (2). An alternate means of expressing the comparison of observed and theoretical efficiencies can be devised, based upon the fact that when productive performance is considered the difference between input above maintenance expressed as metabolizable energy (ME) and output expressed as energy in product or net energy (NE) represents the heat increment (HI = ME - NE). According to this relationship the estimated, theoretical heat increment of growth is (100 - 78) 22% and the observed heat increment is (100 - 70) 30%, indicating that almost (22/30) 75% of the energy costs (heat loss) associated with growth can be accounted for as biochemical costs. This means of expression emphasizes the functional nature of heat increment and helps dispel notions that the term heat increment implies waste.

The examples of various types of metabolic

TABLE 1  
Estimation of efficiency of synthesis of  
100 g of milk

Milk component	%	Output (kcal)	Efficiency	Input
Protein	3.3	19.0	0.84	22.6
Fat	3.7	34.6	0.72	48.0
Lactose	4.8	19.2	0.78	24.6
Net		72.8	0.76	95.2

TABLE 2  
Estimation of efficiency of growth in young lambs

Composition of gain	Gain	Efficiency	Estimated ME required above maintenance
(g)	(kcal)		
Protein	926	0.84	6,280
Fat	414	0.72	5,400
Total	1,340	0.78	11,680

energy expenditures associated with the performance of life functions presented in this paper have proven useful in attempts to acquaint students with the physiological significance of nutritional estimates of caloric efficiencies. Metabolic models are presented which account for major portions of the energy transformations associated with the heat increment of feeding carbohydrate to animals under maintenance conditions and the estimated efficiencies of utilization of ME for milk synthesis and growth. Only a minor portion of the energy transformations related to maintenance was accounted for. In this latter case, a great deal of additional physiological and biochemical data must be accumulated before meaningful models can be formulated. It should be clear that the format presented herein was constructed to serve as a teaching tool, that the simplifying assumptions made in its formulation were often naïve and sometimes invalid, and that because of this they should not be interpreted as representing the final explanations of the physiological significances of the classical nutritional terms to which they are compared.

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