1987

Thermodynamics of embryogenesis

Scott D. Pearson

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THERMODYNAMICS OF EMBRYOGENESIS

Iowa State University

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Thermodynamics of embryogenesis

by

Scott D. Pearson

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Chemical Engineering

Approved:

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In Charge of Major Work

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DEDICATION

This work is dedicated to Kathleen, who has filled my life with joy.
ABSTRACT

Many investigators have attempted to gain insight into the thermodynamics and energetics of growth by correlating various parameters with metabolic activity. Interpretation of these expressions was often difficult. Frequently terms without physical meaning appear. The purpose of this research project is to develop mathematical relationships that adequately describe embryonic energetics, both within the growing organism and in the surrounding microenvironment, using fundamental chemical principles.

In this work two growth models are proposed: one is formulated from equilibrium thermodynamics and kinetics while the other utilizes nonequilibrium thermodynamic principles. The models are based on a small set of basic metabolic reactions that are shown to characterize growing biological systems. The role of the adenosine nucleotides in coordinating and controlling metabolism is included. Theoretical expressions for parameters such as oxygen consumption, heat production, dry tissue growth rate and relative growth efficiency are derived and their utility in assessing metabolic activity is demonstrated.

The interactions between embryonic energetics and the surrounding microenvironment is demonstrated with a specific example. In particular, the heat and mass exchange of eggs incubating in a clutch is considered. Orthogonal collocation on finite elements is used to determine temperature and egg mass profiles in the clutch. The buffering capability of the clutch against thermal oscillations in the surrounding medium is also examined.
I. INTRODUCTION

Recent reviews (Calow 1977, Parry 1983) suggest that the partitioning of material and energy in growing organisms may be represented as in Figure 1. The nutrients supplied from the surroundings are divided between the pathways of energy production and growth. Growth is defined as the net production of new biological molecules by biosynthetic pathways. For complex organisms mass is the parameter most often chosen as a measure of growth. Maintenance is the set of all pathways that describe the nonbiosynthetic energy consuming processes. This includes, for example, the cost of maintaining ionic gradients across membranes. The energy used for growth in an organism is above that needed for maintenance. Inefficiencies of the various pathways results in the production of heat, $\dot{Q}$. All the energy supplied to maintenance reactions is eventually dissipated as heat. In developing the growth model we will only consider organisms whose energy production mechanisms are aerobic. Anaerobic systems may be treated in a similar manner.

A fundamental difficulty associated with modeling a growing biological organism is how to manage the multitude of chemical species and reactions involved. As we shall see considerable simplification is possible because there are only a few pathways through which most metabolites flow. Specific experimental data, on the other hand, for the processes in Figure 1 remain a problem. Currently it is not possible to separate growth and maintenance pathways without disrupting the system.
Figure 1. Energy metabolism for a growing organism
Hence, the partitioning of material and energy is unclear. But certain parameters are readily available: tissue mass, oxygen consumption rate and heat production are examples. Incorporation of such macroscopic observations into a model based on fundamental biochemical principles for a growing system has been elusive.

The approach to the problem of understanding the energetics of growth advocated here is to:

1. identify what factors affect the rates of the processes in Figure 1,
2. discover how the various pathways are integrated,
3. combine the metabolic processes subject to the constraints of the conservation laws of material and energy.

The above steps are used to formulate both a kinetic and a nonequilibrium thermodynamic growth model. As well as allowing evaluation and interpretation of existing data, such a method can suggest additional experiments to further elucidate these processes.

Finally, we consider how the macroscopic effects of a growing organism's energy metabolism influences its microenvironment. In particular, the heat and mass exchange of eggs incubating in a clutch is analyzed and the biological advantage of clutch incubation, as far as environmental regulation, is demonstrated.
II. KINETIC GROWTH MODEL

A. Literature Review

1. Growth models

Walters and Lamprecht (1978) and Grossman and Bohren (1982) have reviewed a wide range of growth models. Three classes are identified: (1) the deterministic, characterized by coupled sets of differential equations that represent the influence of several processes; (2) the stochastic, which are built on probability assumptions; and (3) the parametric, models that essentially introduce parameters without biological meaning in order to fit the data. A few examples will better illustrate the different approaches.

a. Deterministic

The simplest example of a deterministic model often used to describe growth is that of Brody (1945). By assuming a direct proportionality between weight increase and weight, W, at time t

\[
\frac{dW}{dt} = \alpha W(t)
\]

(1)

and then integrating, the so-called logarithmic growth relation is obtained

\[
W(t) = W_0 e^{\alpha t}
\]

(2)
Application of (2) is restricted to certain periods during development. In more complex treatments the growth constant $a$ is a function of time or other parameters. The biochemical basis of this model is difficult to identify.

b. Parametric A parametric model has been proposed by Vleck et al. (1980) for avian embryogenesis. They suggest that the metabolic rate (heat production) can be determined from a balance of the following form:

$$Q = a \cdot GR + b \cdot M + c$$

(3)

where $Q$ is the whole egg heat production, $GR$ is the absolute growth rate based on embryonic wet mass, and $M$ is the wet embryonic mass. The first term on the right in (3) represents the cost of growth and the second the cost of maintenance. The constants $a$, $b$, and $c$ are obtained by multiple regression. The constant $c$ is without physical meaning. The model provides a good fit of the data for several species, however, it overestimates the metabolic rate in embryos less than 2 grams. This can be a significant part of some incubation periods. Justification of (3), like (2), from biochemical principles is unclear.

c. Stochastic The growth equation of Janoschek is an example of a stochastic model (Walters and Lamprecht, 1978). His relation is close to that of target theory where the probability of a nutrient hitting a cell and resulting in growth is calculated. The probability of growth, $P_g$, for $p$ simultaneous reactions with $k$ being the probability of one hit is
If only one of the p reactions suffices to start growth and m targets are considered then

$$P_g = (1 - e^{-kt})^p$$  \hspace{1cm} (4)$$

Now the growth equation of Janoschek, which is underived, is

$$W(t) = W_m(1 - (e^{-kt})^p)$$  \hspace{1cm} (6)$$

where $W_m$ is the maximum weight at the end of development. For most organisms the value of $p$ is greater than 1, while its physical significance is unknown.

There are many other examples of growth equations that are routinely used. They are generally more specific than those mentioned above. It is clear that such approaches are inadequate if the basic relationships that occur in the underlying metabolism are to be understood. The mathematical models proposed in this work are deterministic.

2. Metabolism

In order to identify what factors affect the biological processes of Figure 1 and to understand how they are integrated, it is necessary to review some important features of metabolism. Any number of excellent texts are available for a more thorough treatment (Atkinson, 1977; Krebs and Kornberg 1957).
The set of highly complex and integrated chemical reactions that occur in a biological organism is referred to as metabolism. A major objective of metabolism is to make energy and building blocks available. Many different compounds classified as lipids, proteins and carbohydrates serve as sources. Fortunately, the number of reactions involved and building blocks formed are surprisingly small.

Some of the chemical energy released in catabolic pathways is transferred to a variety of energy carrying molecules. By far the most common is adenosine triphosphate (ATP). The subsequent hydrolysis of ATP is used to drive thermodynamically unfavorable reactions. The critical role of ATP in energy exchanges is a key to elucidating metabolic integration and control. Atkinson (1977) has suggested all the energy carrying molecules can be related to their adenosine nucleotide equivalents, which allows the interactions between catabolic and anabolic reactions to be greatly simplified.

a. Major metabolic pathways and their control
The major pathways for metabolites in biological systems are glycolysis and gluconeogenesis, the citric acid cycle and oxidative phosphorylation, fatty acid synthesis and degradation, and peptide synthesis and degradation. Stryer (1981) and Saier (1987) present excellent discussions of the pathways and their control sites, the important points for our purposes are summarized below and in Table 1.

The conversion of glucose into two molecules of pyruvate is termed glycolysis. The purpose of glycolysis is to provide various building
Table 1. Examples of key metabolic enzymes that are regulated by adenosine nucleotides

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Enzyme</th>
<th>Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis/Gluconeogenesis</td>
<td>Phosphofructokinase</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMP Activates</td>
</tr>
<tr>
<td></td>
<td>Pyruvate Kinase</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td></td>
<td>Pyruvate Carboxylase</td>
<td>ADP Inhibits</td>
</tr>
<tr>
<td>Citric Acid Cycle</td>
<td>Pyruvate Dehydrogenase Complex</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td></td>
<td>Citrate Synthetase</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td></td>
<td>Isocitrate Dehydrogenase</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADP Activates</td>
</tr>
<tr>
<td></td>
<td>α-Ketoglutarate</td>
<td>ATP Inhibits</td>
</tr>
<tr>
<td>Amino Acid Degradation</td>
<td>Glutamate Dehydrogenase</td>
<td>ADP Activates</td>
</tr>
</tbody>
</table>
blocks for biosynthesis and to generate ATP. A reoccurring pattern in metabolism is the regulation of the first irreversible reaction unique to a pathway—the so-called "first committed step". The first committed step in glycolysis is the conversion of fructose 6-phosphate to fructose 1,6-diphosphate by the enzyme phosphofructokinase. A high level of ATP inhibits this step while a high level of AMP activates.

The synthesis of glucose from noncarbohydrate precursors is called gluconeogenesis. Gluconeogenesis is not just a simple reversal of glycolysis; the three irreversible reactions of glycolysis are bypassed. Glycolysis and gluconeogenesis are coordinated so that both pathways are not simultaneously active. Pyruvate carboxylase is inhibited by ADP, while ATP inhibits pyruvate kinase. Consequently, gluconeogenesis is highly active when there are high concentrations of ATP and building blocks.

The citric acid cycle is the process in which acetate (in the form of acetyl-CoA) is oxidized to CO$_2$ and water in mitochondria. The electrons from the acetyl group are initially transferred to either oxidized nicotinamide adenine dinucleotide (NAD$^+$) or flavin adenine dinucleotide (FAD) before they are passed to oxygen through the electron transport chain—i.e., oxidative phosphorylation. The control of the citric acid cycle is determined by the cells need for ATP. The pyruvate dehydrogenase complex, citrate synthetase, isocitrate dehydrogenase, and a-ketoglutarate dehydrogenase are all inhibited by ATP. The dehydrogenase complex is phosphorylated while citrate synthetase experiences an increase in $K_m$ for acetyl-CoA. Isocitrate dehydrogenase
is allosterically stimulated by ADP. The citric acid cycle also provides building blocks for the synthesis of biological molecules.

The electron transport chain mentioned above is tightly coupled to the production of ATP from ADP. In tissue homogenate the concentration of ADP appears to be responsible for regulation. This is termed "respiratory control".

Fatty acid degradation occurs by the removal of two-carbon units in a series of reactions called the β-oxidation pathway. The acetyl-CoA produced either enters the citric acid cycle, if enough oxaloacetate is present, or is diverted to form ketone bodies. The electron acceptors FAD and NAD+ used in β-oxidation are regenerated in oxidative phosphorylation with the concomitant production of ATP. Thus, fatty acid degradation is linked to the need for ATP.

The synthesis of fatty acids takes place by the addition of two-carbon units and is distinct from fatty acid degradation. Elongation is driven by the release of CO2. Acetyl CoA carboxylase is the enzyme for the first committed step. Citrate activates the synthesis of malonyl CoA in eucaryotes. When the levels of ATP and acetyl-CoA are high, fatty acid synthesis increases.

The pathways for synthesis of amino acids and peptides are more diverse than those mentioned above. The nonessential amino acids are regulated by product feedback inhibition. Those amino acids that are not needed for protein synthesis can be converted to glucose or oxidized by the citric acid cycle. Glutamate dehydrogenase, the enzyme that catalyzes the first committed step in amino acid degradation, is
allosterically activated by ADP and GDP. Thus, when the energy content is low catabolism of amino acids increases.

Protein synthesis involves activated amino acid esters which are sequentially joined to the carboxyl end of a growing peptide. The process has three stages: 1) initiation, where the initiator tRNA binds to the mRNA start signal and the association of the ribosomal units; 2) elongation, which is the addition of an amino acid to the chain and a shift to the next codon; and 3) termination, when the stop signal is read and the ribosome releases the peptide. Formation of the aminoacyl-tRNA requires ATP. The initiation complex requires GTP in prokaryotes and GTP and ATP in eucaryotes for formation. Initiation is usually the rate limiting step and is, therefore, a point of control.

The mechanisms for protein degradation are not as clearly understood. Abnormal and short-lived proteins appear to be broken down by an ATP-dependent proteolytic system. The lysosome, a degradative organelle in mammalian cells, is responsible for breakdown of long-lived proteins, membrane proteins, and extracellular proteins (Goldberg, 1983). The regulation of lysosomal degradation is not well defined. In bacterium an insufficiency of ATP for growth causes a rise in ppGpp which dramatically increases proteolysis by an unknown mechanism (Bodley, 1983).

b. Metabolic regularities The production of ATP can be estimated for the complete catabolism of glucose, palmitic acid, and alanine based on the appropriate metabolic pathway being utilized
Table 2. Respiratory data for glucose, palmitic acid, and alanine

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Palmitic Acid</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{(C}_6\text{H}_12\text{O}_6)$</td>
<td>$\text{(CH}_3\text{(CH}<em>2\text{)}</em>{14}\text{COOH})$</td>
<td>$\text{(CH}_3\text{CH(NH}_2\text{)}\text{COOH})$</td>
</tr>
<tr>
<td>$\text{O}<em>2$ consumed, M$</em>{\text{O}_2}$</td>
<td>6</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>(moles/mole substrate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Products</td>
<td>CO$_2$, H$_2$O</td>
<td>CO$_2$, H$_2$O</td>
<td>CO$_2$, H$_2$O, NH$_4^+$</td>
</tr>
<tr>
<td>ATP produced, M$_{\text{ATP}}$</td>
<td>36</td>
<td>130</td>
<td>18</td>
</tr>
<tr>
<td>(moles/mole substrate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{M}_{\text{O}<em>2}/\text{M}</em>{\text{ATP}}$</td>
<td>0.167</td>
<td>0.177</td>
<td>0.167</td>
</tr>
</tbody>
</table>

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The similarity in the ratio of oxygen consumed to ATP produced for the three compounds is not surprising since under aerobic conditions the citric acid cycle and oxidative phosphorylation are used for almost all of the ATP synthesis. We assume that the data for these three chemical species are representative of their respective classes (Erickson and Patel, 1981).

The ATP required to synthesize protein, lipid and carbohydrate may also be estimated. The cost will obviously depend on what building blocks are chosen. For the case where the building blocks are monomeric units of the biomolecules the theoretical ATP cost is (Jobling, 1985); protein - 100 mmole ATP/gm, lipid - 25 mmole ATP/gm, and glycogen - 12 mmole/gm. Thus, for the embryonic chicken whose body composition is roughly 65% protein, 20% lipid and 15% carbohydrate (Romanoff, 1967) the ATP cost of synthesis is about 72 mmole/gm dry tissue.

The purpose of the above review was to illustrate the important role the adenosine nucleotides have in affecting the rates of various pathways and in integrating their actions. In the next section the conservation laws will be formulated, ultimately allowing us to incorporate these features into a growth model.

B. Conservation Laws For Biological Systems

The application of material and energy balances to a growing biological organism is in principle a straightforward procedure. Unfortunately, because of the inherent complexity of biosystems many investigators have either misinterpreted or left important terms out of
the analysis. Here we develop macroscopic forms for the conservation of mass and energy that are applicable to most growing organisms. A more general discussion of conservation laws may be found elsewhere (Bird, Stewart and Lightfoot, 1960).

1. **Mass balance**

Consider a system containing $n$ components which can exchange material with its surroundings thru $m$ ports and in which $r$ chemical reactions are possible. The differential change in mass of component $i$ during the time interval $dt$ is

$$dm_i = \sum_j \delta_j dm_{ij}^F + \sum_k d\xi_{ik}^R$$

where $\delta_j = +1$ for input streams and $-1$ for output streams, $dm_{ij}^F$ is the change in mass of species $i$ due to flow thru port $j$, and $d\xi_{ik}^R$ is the change in $i$ resulting from chemical reaction $k$. This relation expresses the conservation of mass law for a component as the sum of an external part (flow) and an internal part (chemical reaction). A similar splitting will occur when energy conservation is considered.

The molar extent of reaction, $\xi_k$, may be introduced by noting

$$d\xi_{ik}^R = \tilde{M}_i \nu_{ik} d\xi_k$$

with $\tilde{M}_i$ molecular weight of species $i$, $\nu_{ik}$ stoichiometric coefficient of $i$ in reaction $k$ (+ for products and - for reactants), and $d\xi_k = \text{molar extent of the kth reaction}$. The advantage in using the molar extent is
due to the fact it is not tied to any particular component. The mass balance (7) now becomes

$$dm_i = \sum_j^m \delta_{ij} dm_{ij}^F + \sum_k^r \bar{M}_{ik} \nu_{ik} \delta_{ik}$$

(9)

or introducing the time coordinate

$$\frac{dm_i}{dt} = \sum_j^m \delta_{ij} \dot{W}_{ij} + \sum_k^r \bar{M}_{ik} \nu_{ik} \frac{\delta_{ik}}{dt}$$

(10)

where $\dot{W}_{ij}$ is the mass flow rate of $i$ thru port $j$. This is the form of the mass balance that will be applied to growing biosystems. Note that by summing over all $n$ components the total rate of mass exchange is found

$$\frac{dm}{dt} = \sum_i^n \frac{dm_i}{dt} = \sum_i^n \sum_j^m \delta_{ij} \dot{W}_{ij}$$

(11)

which expresses the familiar result that mass is neither created nor destroyed; the mass of a system depends only on exchanges with the surroundings.

2. **Energy balance**

For the open system described above the differential form of the energy balance can be written (Modell and Reid, 1983)

$$dU = dQ + \sum_i^n \sum_j^m h_{ij} dm_{ij}^F \delta_{ij}$$

(12)

where $dU$ = the change in total internal energy of the system, $dQ$ = heat added to the system from the surroundings ($d$ signifies a path dependent
quantity), and \( h_{ij} \) = specific enthalpy of component \( i \) in stream \( j \). For biological organisms the effects of kinetic and potential energy changes and the volumetric, electrostatic, and electromagnetic forces exerted on the surroundings are usually small so these have been omitted from (12). From the definition of the total enthalpy of the system,

\[
H = U + PV
\]

with \( P \) pressure and \( V \) total volume, it is easily seen that the approximation

\[
dU = dH
\]

results from the assumptions made above. Since biological systems often exhibit ideal solution behavior, the total differential of the enthalpy is

\[
dH = m \left( \frac{\partial H}{\partial T} \right)_m m_1 \, dT + \sum_{i=1}^{n} \left( \frac{\partial H_i}{\partial m_i} \right)_{T,m_j \neq i} m_i \, dm_i
\]

The first term on the right is a result of the thermal effect of temperature changes and the second arises from variations in composition. Equation (14) can be rewritten by recognizing the partial derivatives as the specific heat capacity, \( C_p \), and the specific enthalpy of species \( i \) evaluated at the conditions of the system, \( H_i \),

\[
dH = m C_p \, dT + \sum_{i=1}^{n} H_i \, dm_i
\]

Combining (10), (12), (14) and (16), and noting the molar heat of the \( k \)th reaction is \( \Delta H_k = \sum_{i=1}^{n} H_{i} M_{ik} \), yields
\[
\frac{dT}{dt} = \sum_{i} \Delta H_k \frac{d\xi_k}{dt} + \dot{Q} + \sum_{j} \sum_{i} (h_{ij} - H_i) \frac{dm_i}{dt} \delta_j
\] (17)

Introducing the time coordinate gives the desired form of the overall energy balance

\[
\frac{dT}{dt} = \sum_{i} \Delta H_k \frac{d\xi_k}{dt} + \dot{Q} + \sum_{j} \sum_{i} (h_{ij} - H_i) \frac{w_{ij}}{C_p} \delta_j
\] (18)

Notice that the accumulation of thermal energy in the system is due to both internal and external contributions. The internal part (reaction term) accounts for the stoichiometric energy changes that occur—the metabolism of the organism. This includes, for example, all the catabolic and anabolic pathways as well as membrane transport and muscle contraction processes. The external part of the energy balance has two terms; one arising from thermal conduction the other from the convection of matter. Evaporation of water from an organism is a good example of the latter.

The material and energy balances (10) and (18) yield a set of n+1 equations in n+1 dependent variables: the individual species masses m and the system temperature T. The nr stoichiometric coefficients are specified by the r independent chemical reactions. The equations of state for the system allow expression of the specific enthalpies. Of the remaining n(n+r)+1 independent unknowns the conductive heat transfer and mass exchanges with the surroundings are experimentally known, while the rates of the chemical reactions are often unavailable. Postulating a complete set of reaction rate expressions will close the material and
energy balances. Then combining the above conservation laws with our notions regarding the important role of adenosine nucleotides in metabolic control will provide the framework to examine embryonic energetics.

C. Enzyme Activity

Obtaining reaction rates for the various metabolic pathways is perhaps the most difficult part of the modeling process. For a typical nonbiological system we might expect the rate to be governed by mass action. But an essential feature of biological systems is that the rates of metabolic pathways are mainly determined by the activities of key enzymes (Stryer, 1981). Enzyme activities can be described within the framework of enzyme kinetics.

Allosteric enzymes (characterized by indirect interactions between distinct binding sites) are primarily responsible for the integration of catabolism and anabolism in an organism. The most frequently applied model used to describe allosteric regulation is that of Monod et al. (1965). Here we take advantage of our notion regarding adenosine nucleotide regulation to modify Monod's model to consider the case where substrate concentration remains relatively constant but activator concentration varies.

In the absence of substrate the relationship between an enzyme that has two allowed substrate binding sites and one allowed modulator site is

\[ R_o = T_o + A \]  

(19)
where A is the activator. Assuming the substrate binds primarily with the R form, the equilibrium reactions are

\[ R_o + S = R_1 \]  \hspace{1cm} (20)
\[ R_1 + S = R_2 \]  \hspace{1cm} (21)

and defining the equilibrium constants as

\[ K_R = \frac{2[R_o][S]}{[R_1]} = \frac{[R_1][S]}{2[R_2]} \]  \hspace{1cm} (23)

where the equilibrium constant is assumed to be the same for the first and second substrate.

Now the fraction of sites occupied by the substrate, Y, is just

\[ Y = \frac{[R_1] + 2[R_2]}{2([T_o] + [R_o] + [R_1] + [R_2])} \]  \hspace{1cm} (24)

Substituting in the equilibrium relations (22), (23) and recognizing that in most enzymes \([T_o] >> [R_o]\) (Monod et al., 1965) and also that enzymes are rarely saturated so \([S]/K_R \sim 0.01-1.00\), allows the saturation function to be simplified to

\[ Y = \frac{1 + [S]/K_R}{K_R L} [S][A] \]  \hspace{1cm} (25)

For the case where the substrate concentration is relatively constant the saturation function is proportional to the activator concentration, i.e.,

\[ Y \propto K[A] \]  \hspace{1cm} (26)
This latter assumption follows from the observation that the accumulation of metabolic intermediates is normally negligible.

From (26) the reaction rate per unit volume, \( r \), is obtained by realizing

\[ r = Y r_{\text{max}} \]  \hspace{1cm} (27)

and substituting for the saturation function

\[ r = K'[A] \]  \hspace{1cm} (28)

with \( K' = K r_{\text{max}} \). This is the form we are seeking to describe enzyme activity. Equation (28) suggests that in some cases the rates of metabolic pathways can be modeled with a knowledge of the activator concentration. For a general enzyme with \( n \) substrate sites a derivation similar to the above will yield an analogous expression.

At the present time it is impossible to incorporate into a growth model all the possible metabolic reactions that occur. In order to solve the desired conservation laws a generalized reaction scheme is required. This is the subject to be considered next.

D. Proposed Growth Model

The partitioning of material and energy in a growing organism can be represented by the metabolic network shown in Figure 2. The substrate (nutrient), \( S \), is first activated before it enters intermediary metabolism. The activated building blocks are then used for the production of energy-carrying molecules (ATP) in respiration or for the synthesis of biological macromolecules. In this network we have assumed
Figure 2. Model metabolic network for the partitioning of material and energy in growing biosystems
that the organism is primarily aerobic; oxidative phosphorylation is the mechanism for the synthesis of ATP. Turnover (or utilization in times of need) of the biomolecules results in the production of substrate which can be used to resynthesize or is catabolized. The maintenance pathway is represented as a cycling between two forms of the biosystem: 0 is the state the organism wishes to retain, O is the undesirable state. The coupling between energy producing and energy consuming reactions is shown as a cycling of ATP and ADP.

The major classes of biomolecules (lipids, proteins, and carbohydrates) could be treated separately using three of the above network. Rarely is there sufficient data on tissue composition during growth to be this rigorous so we will assume the relative fractions of lipid, protein, and carbohydrate to remain constant.

1. Rate expressions

Now we consider each of the various reaction pathways in Figure 2 individually as the specific rate equations are developed.

a. Biosynthesis

The biosynthetic reaction is

\[ \ldots + v_{BB}^{BS} BB^* + v_{ATP}^{S} ATP \rightarrow v_{B}^{S} B + v_{ADP}^{S} ADP + v_{Pi}^{S} Pi + \ldots \] (29)

where BB* is an activated building block, B is a biological macromolecule, and \( v_i^S \) are the stoichiometric coefficients. Based on the previous discussion of the role of ATP as an activator in synthesis
reactions, and the allosteric enzyme model (28), the postulated form for the rate equation is

\[ r_s = K_s [ATP] \quad (30) \]

b. **Turnover/utilization** The turnover of biological molecules is a continual process within cells. The reasons for turnover are not well understood. Utilization, on the other hand, is the breaking down of biomolecules in times of poor nutrient supply. The enzymes of the degradative pathways are modulated by ADP levels to a large extent. As a first approximation, we will assume turnover and utilization are controlled by the same signal. Then from Figure 2, the pertinent reaction is

\[ \ldots + v_t^B \rightarrow v_t^S + \ldots \quad (31) \]

with a rate expression of

\[ r_t = K_t [ATP] \quad (32) \]

c. **Maintenance** The maintenance equation can be written as

\[ \ldots + v_m^O + v_m^{ATP} \rightarrow v_m^\bullet + v_m^{ADP} + v_m^{Pi} + \ldots \quad (33) \]

The assumption that underlies the proposed rate expression is that the level of ADP in the system represents the maintenance requirement.
d. Respiration and activation

The remaining two pathways of the metabolic network, respiration and activation, are not independent once the pseudo-steady state hypothesis is made for ATP and building blocks. This assumption follows from the large turnover times observed for these species (Atkinson, 1977). The reaction stoichiometry for respiration is

\[ \ldots + \nu_{BB^*} BB^* + \nu_{O_2} O_2 + \nu_{ADP} ADP + \nu_{Pi} Pi \rightarrow \]

\[ \nu_{ATP} ATP + \nu_{CO_2} CO_2 + \ldots \]  \hspace{1cm} (35)

and for activation

\[ \ldots + \nu_{S} S + \nu_{ATP} ATP \rightarrow \nu_{BB^*} BB^* + \nu_{ADP} ADP + \nu_{Pi} Pi + \ldots \]  \hspace{1cm} (36)

Now applying the material balance (10) to building blocks and neglecting their accumulation

\[ \frac{dBB^*}{dt} = \frac{\nu_{BB^*} d\xi_a}{dt} + \frac{\nu_{BB^*} d\xi_r}{dt} + \frac{\nu_{BB^*} d\xi_s}{dt} = 0 \] \hspace{1cm} (37)

\[ \frac{dATP}{dt} = \frac{\nu_{ATP} d\xi_a}{dt} + \frac{\nu_{ATP} d\xi_r}{dt} + \frac{\nu_{ATP} d\xi_s}{dt} + \frac{\nu_{m} d\xi_m}{dt} = 0 \] \hspace{1cm} (38)

Solving simultaneously and assuming one ATP has been used in activation yields

\[ r_r = -\left(\frac{s}{\nu_{ATP}} + \frac{s}{\nu_{r}}\right)K_m [ATP] - \left(\frac{m}{\nu_{ATP}}\right)K_m [ADP] \] \hspace{1cm} (39)
where (30) and (34) have been substituted for the rates of synthesis and maintenance, and also the definition \( r_k = (1/V)(dS_k/dt) \).

2. Metabolic relationships

With the above rate expressions and the material balances we now formulate relationships for parameters of growing organisms that are readily measurable.

**a. Growth** Consider first a balance on the biological molecules. From (10), (30) and (32)

\[
\frac{dB}{dt} = M_B \nu_B K_s (ATP) + M_B \nu_B K_t (ADP)
\]

or rearranging

\[
\frac{dB}{dt} = M_B \nu_B K_s (1 + \frac{\nu_B K_t}{\nu_B K_s} F)(ATP)
\]

where the phosphorylation ratio \( F \) is

\[
F = \frac{[ADP]}{[ATP]}
\]
and is a measure of the relative energy charge of the system. This ratio has been postulated to be of prime importance in cell metabolism (James, 1971). The phosphorylation ratio appears as a natural consequence of the postulated network. The significance of the phosphorylation ratio in regulating biomolecule accumulation is evident by noting $\frac{v^B}{v^B} < 0$, so the magnitude of $F$ determines whether biomass increases, decreases or remains unchanged. With experimental data on growth rate and nucleotide levels the relative rate of turnover to synthesis can be estimated from (42).

b. Oxygen consumption

The rate of oxygen consumption follows immediately from (39)

$$-rac{dO_2}{dt} = M_0 \left( \frac{v_r}{V_{ATP}} \right) v_{ATP} K_m (1 + \left( 1 + \frac{v_{BB}}{v_{ATP}} \right) \frac{v_{ATP}}{v_{ATP}} s F^{-1}) (ADP) \tag{44}$$

Thus, oxygen consumption is related to the ADP level and the phosphorylation ratio. Evidence from enzymatic studies has also indicated these two parameters are important in controlling respiratory intensity (Jacobus et al., 1982; Kunz et al., 1981). With the phosphorylation ratio constant we would experimentally observe respiratory control in the organism.

The physical significance of terms in (44) can be elucidated:

$$- \frac{O_2}{M_0 (\frac{v_r}{V_{ATP}})} = \text{ratio of the mass of oxygen consumed to the moles of ATP produced in aerobic energy production.}$$

For lipids, carbohydrates and proteins this is about 5.3 mg $O_2$/mmole ATP.
stoichiometric ratio of activated building blocks to ATP in the synthesis reaction. The upper limit on this term would be the case where synthesis is merely the polymerization of monomeric units. For an average tissue composition this value is about 0.25 mmole BB\textsuperscript{*}/mmole ATP (Calow, 1977).

\[
\frac{v_{BB}^*}{v_{ATP}^s} = \frac{v_{ATP}^s}{v_m} K_m^{-1}
\]

ratio of the rate of ATP consumed in biosynthetic reactions to the rate of ATP consumed in maintenance pathways. The ATP consuming rates depend on the particular developmental stage of the organism.

Using experimental data for oxygen consumption rates and nucleotide levels we can then estimate the relative rates of maintenance and biosynthesis.

c. Dependence of oxygen consumption on growth The relationship between oxygen consumption and the accumulation of biomolecules can be found by combining (42) and (44)

\[
-\frac{dO_2}{dt} = M_2 \left( \frac{v_o}{v_{ATP}} \right) \left( \frac{v_{BB}^*}{v_{ATP}^s} \right) \left( 1 + \frac{v_{ATP}^s}{v_m} K_m^{-1} \right) \left( 1 + \frac{v_{BB}^* t}{v_s} \right)
\]
Examination of (45) again shows the importance of the phosphorylation ratio in metabolism. For a constant ratio the growth rate of a biosystem is directly proportional to the rate of oxygen consumption. Experimental data that is not well described by a constant growth coefficient is an indication that substantial changes are occurring in the underlying metabolism and may be accounted for by examining nucleotide levels.

In the last section the metabolic model has been used to obtain theoretical relationships between certain macroscopic parameters of a growing biosystem in order to describe the material transformations. We now turn to the energy equation to formulate expressions for such quantities as heat production and growth efficiency.

3. **Energetic relationships**

Various attempts have been made to describe the heat production of a growing organism from a knowledge of macroscopic parameters. The metabolic relationships developed above can be used to formulate a theoretical expression for heat production.

a. **Heat production** The rate of heat production for a growing biosystem is simply the reaction term (internal contribution) in the energy balance equation (18)

\[
\sum_k \frac{\Delta H}{k} \frac{d\xi_k}{dt} = \sum_i m_i \frac{dT}{dt} + \sum_{i,j} (H_{ij} - \tilde{H}_i) w_{ij} \delta_j
\]

(46)

The thermal, conductive and convective terms on the right-hand side of the (46) are routinely measured in calorimetric experiments. The left-
hand side can be written in terms of the reaction rates for the metabolic network. By comparing the predicted and measured heat production rates the consistency of the model can be confirmed.

Five reaction pathways in Figure 2 contribute to heat production - synthesis, turnover/utilization, maintenance, respiration and activation. Recall that the rates of respiration and activation can be expressed in terms of the synthesis and maintenance rates. Combining these with the appropriate rate equations (30), (32), (34) and the growth relationship (42), and substituting into the reaction term yields after considerable rearrangement

\[ - \frac{1}{\sum_k \Delta H_k} \frac{d \xi_k}{dt} = - (\Delta H_t^R - \frac{1}{B}) \left( \frac{v_B}{\nu \theta} \frac{v_B}{v \theta} F \right) + \Delta H_r^R \left( -\frac{1}{\nu \theta} \right) \]

\[ + \frac{v_B}{\nu \theta} + \frac{v_m}{\nu \theta} F \] + \Delta H_s^R \left( -\frac{1}{\nu \theta} \right) + \Delta H_m^R \left( \frac{v_m}{\nu \theta} \right) \]

\[ \Delta H_s^R \left( \frac{v_m}{\nu \theta} \right) / \left( 1 + \frac{v_B}{\nu \theta} F \right) \]

where the heat of activation has been stoichiometrically included in the heat of synthesis (\(\Delta H_s^R\)) and respiration (\(\Delta H_r^R\)) to simplify the result. The heat production is directly proportional to the rate of dry tissue growth. The coefficient in (47) may be constant or can vary during maturation depending on the phosphorylation ratio F. The enthalpies of reaction and stoichiometric ratios can be estimated based on our knowledge of the particular pathways. Equation (47) can thus be used to
describe the heat production for a developing biosystem while offering insight into the metabolic transformations.

b. **Calorific ratio** Another quantity of energetic interest is the ratio of heat production to oxygen consumption - the calorific ratio. Different theoretical values for the calorific ratio are possible depending on what type of substrate is being catabolized. Departures above the theoretical value arise because heat is generated by anaerobic pathways while deviations below indicate some of the energy is retained in chemical bonds and is not dissipated. It is frequently assumed that the calorific ratio remains constant during growth. A survey of the literature, however, suggests distinct changes do occur during development (Brettel et al., 1980; Romijn and Lokhorst, 1960; Zotin and Lamprecht, 1982). The metabolic model formulated above can account for such variations in the calorific ratio.

In terms of the previous notation the calorific ratio is the following

\[
\text{calorific ratio} = \frac{- \sum_{k} \Delta H_k^R \frac{d \xi_k}{dt}}{- \frac{dO_2}{dt}}
\]

(48)

Combining (45), (47) and (48) gives the desired expression

\[
\frac{- \sum_{k} \Delta H_k^R \frac{d \xi_k}{dt}}{- \frac{dO_2}{dt}} = \Delta H_{E}^R \frac{-1}{O_2} + \Delta H_{S}^R \frac{-1}{\frac{v_{ATP}}{v_s}} + \Delta H_{T}^R \frac{1}{v_t}
\]

\[
= \Delta H_{E}^R \frac{1}{v_{O_2}} + \Delta H_{S}^R \frac{1}{v_{ATP}} + \Delta H_{T}^R \frac{1}{v_t}
\]
The first term on the right represents the energy liberated during the synthesis of ATP and the second term accounts for the heat released as ATP is utilized. Note that the calorific ratio is a function of the phosphorylation ratio; as F increases the heat produced per gram of oxygen consumed also increases while the converse is also true. The calorific relationship in (49) provides a theoretical basis to analyze the experimental data of a growing organisms metabolism without resorting to empirical parameters.

4. Efficiency

There are currently many different notions regarding the efficiency of a biological process. Unfortunately, some are unique to the particular system or depend on the reference state chosen. From a thermodynamic viewpoint the gross efficiency of a chemically reacting process can be defined as the ratio between the rates of free energy input and output for the system. If the system is a growing biological organism then we can identify the input free energy as that resulting from respiratory pathways and the output free energy as that associated with the synthesis reactions. In this case the growth efficiency is
where the free energy changes for the reactions \( \Delta G^R \) are determined as if they are not coupled to the synthesis or hydrolysis of ATP (indicated by an \(^*\)). The free energy change in biological systems can be approximated by the enthalpy difference for the reaction (Erickson and Patel, 1981). Then using the rate expressions for synthesis (30) and respiration (39), the efficiency of growth is

\[
\eta_{\text{growth}} = \frac{-\Delta G^R_s}{-\Delta G^R_r} \frac{d\xi_s}{dt} / \frac{d\xi_r}{dt}
\]  

(50)

The growth efficiency is inversely related to a linear function of the phosphorylation ratio. As \( F \) increases the efficiency decreases while a decreasing \( F \) implies an increasing efficiency. The maximum efficiency follows directly from (51)

\[
\eta_{\text{growth}}^{\text{max}} = \frac{-\Delta H^R_s}{-\Delta H^R_r} \frac{1}{v_{S_{\text{ATP}}}} \cdot \frac{1}{1 + \frac{v_{BB^*}}{v_{S_{\text{ATP}}}} + \frac{v_{m_{\text{ATP}}}}{v_{S_{\text{ATP}} K_{s}}} K_{s}}
\]  

(52)

and corresponds to the situation where maintenance requirements are negligible. Dividing (51) by (52) yields an expression for the relative growth efficiency.
5. Metabolic applications

a. Brain and liver (chicken) The dry tissue growth rate for the liver and brain of the chicken (Romanoff, 1967) during days 9-21 are plotted in Figure 3 versus the ATP content. Both sets of data are well described by the growth equation developed earlier (42)

\[
\frac{dB}{dt} = 176.80(\text{ATP}) \quad r^2 = 0.987 \quad (54)
\]

\[
\frac{dB}{dt} = 588.56(\text{ATP}) \quad r^2 = 0.965 \quad (55)
\]
Figure 3. Linear relationship between dry tissue growth rate and ATP content for the brain and liver of the developing chick embryo.
where the units on the coefficients are mg tissue/mg ATP. The
phosphorylation ratio appears to be constant during the growth of these
organs. Whether the difference in slopes is a result of a higher rate of
liver tissue synthesis or a relatively lower turnover cannot be
determined since ADP data are unavailable.

b. **Escherichia coli** The nonlinear dependence of growth rate on
ATP concentration for *E. coli* in a chemostat is also described by (42).
Swedes et al. (1975) have measured the generation time and adenosine
nucleotide level of an adenosine autotroph. For this system (42)
becomes:

\[
\frac{dB}{dt} = (1 - 3.72F)^{0.23}(ATP)
\]

with a \(r^2=0.996\). The experimental data and (56) are plotted in Figure 4.
The predicted turnover rate is very high, averaging about 50% of
synthesis. Substantial shifts in the balance of metabolism are reflected
in the changing phosphorylation ratio. Direct comparison between the *E.
coli* data and chicken data are not possible because chemostat biomass
concentrations were not reported.

c. **Oryzias latipes** The relationship between oxygen consumption
and the adenosine nucleotides as expressed in (44) can be applied to the
developing egg of the teleost *Oryzias latipes*. Ishida (1951) has
measured oxygen consumption while Taguchi (1962) has determined adenosine
nucleotide levels during the growth of *Oryzias latipes*. The
Figure 4. Nonlinear relationship between *E. coli* growth rate and ATP content resulting from changing phosphorylation ratios.
Figure 5. Oxygen consumption of Oryzias latipes during incubation. Predicted oxygen consumption due to maintenance is also shown.
incubation time for *Oryzias* eggs is 11 days. Equation (44) in this case is

\[
- \frac{dO_2}{dt} = (648.1 + 3672.1F^{-1})(ADP)
\]

and \( r^2 = 0.993 \). The experimental data are shown in Figure 5 along with the above equation. The predicted oxygen consumption due to maintenance reactions is also plotted.

From the figure it is clear that as hatching approaches the maintenance costs become a larger fraction of the total, i.e., synthesis of biomolecules is reduced. The total oxygen consumption determined from (57) overestimates that measured by Ishida during the first few days. This could be an indication that a significant amount of ATP is produced anaerobically.

d. *Xenopus laevis* Another example of a changing phosphorylation ratio during growth can be found by examining the development of *Xenopus laevis*. The incubation period for this toad is typically 40 hrs. Equation (45) gives the predicted relationship between oxygen consumption and dry tissue growth rate as a function of phosphorylation ratio. Rearranging (45) and assuming a relatively small turnover rate allows the phosphorylation ratio to be estimated from oxygen consumption and growth data

\[
F = \left\{ \left[ - \frac{dO_2}{dt} / (dB/dt) \right] - \frac{O_2}{v_{ATP}} \frac{v_{ATP}}{v_{BB^*}} \frac{\nu_s}{\nu_r} \right\} - M_2 \left( \frac{\nu_r}{\nu_{ATP}} \right) (1 + \frac{\nu_s}{\nu_{ATP}})
\]
Figure 6. Predicted phosphorylation ratio during growth of *Xenopus* L. Experimental data for *Bufo* a. are shown for comparison.
Dontosva and Grudnitskii (1977) have measured the respiration and growth of Xenopus as well as tissue composition during development. The average body composition is 30% lipid, 30% protein and 40% carbohydrate which means the ATP cost of synthesis is about 43.6 mmole ATP/gm tissue.

Figure 6 shows the estimated phosphorylation ratio for Xenopus using (58) and assuming $v_{m}^{ATP}/v_{m}^{K_{m}} = 17$. Unfortunately, no nucleotide ratio data has been measured for Xenopus during growth. Moreno et al. (1976), however, have determined adenine nucleotide contents during the stages of gastrula and neurula for Bufo arenarum. The experimental phosphorylation ratio for Bufo is also shown in Figure 6 for comparison with the predicted Xenopus ratio. The trend calculated from (58) for Xenopus is very similar to that observed in Bufo. The increasing phosphorylation ratio implies a substantial increase in relative maintenance costs occurs throughout development.

e. Spinal cord (chicken) There is some indication in the literature that oxygen concentration affects growth (Arshavsky, 1983; Bauman, 1984; Visschedijk, 1980; Kutchia and Steen, 1971). Here we derive a relationship between oxygen concentration and growth based on the metabolic model and apply it to the spinal cord of the developing chick embryo.

Recall from (39) that the interrelationships between respiration and
the other pathways were determined. If the rate of respiration is limited by the availability of oxygen within the organism, then a rate expression of the following form is suggested

$$r_{\text{res}} = K_{\text{res}}[O_2]$$

Combining this with (35) allows the concentration to be related to measurable parameters.

$$[O_2] = \frac{-\frac{dO_2}{dt}}{K_{\text{res}}}$$

(60)

Stokes (1982) has measured oxygen tension in the spinal cord of the avian embryo over the last six days of incubation. The measurements were made with a small recessed-tip oxygen microelectrode. To apply (60) we need oxygen consumption rates and system volumes. If, however, the phosphorylation ratio is approximately constant, as was shown for the whole brain (54), then from (45) the tissue growth rate is related to $O_2$ consumption by a constant. So the growth data of Romanoff (1967) for the spinal cord can be used to estimate respiration. Also, the total wet mass of the cord is a measure of the system volume since the fraction of water remains relatively constant (Romanoff, 1967). For the chicken spinal cord, (60) is

$$P_{O_2} = 80.0 \frac{(\frac{dM}{dt})}{M}$$

(61)

and $M$ is the wet mass in mg. The units on the coefficient in (61) are torr.day. The correlation coefficient $r^2$=0.973. Oxygen tensions from
Figure 7. Partial pressure of oxygen in the spinal cord of the chick embryo during the last quarter of incubation.
Stokes and (61) are shown in Figure 7. The good agreement confirms the hypothesis regarding oxygen limitation during embryogenesis, at least for the spinal cord of the chicken. This behavior of the tissue pO$_2$ was not anticipated using standard blood gas analysis (Tazawa, 1980).

f. Whole egg (chicken)  Respiratory measurements of the whole avian egg have often been attributed to the embryo alone. This is a result of in vitro experiments on membrane oxygen consumption which indicated the contribution of these tissues was small (Romanoff, 1967). Furthermore, the data suggested a peculiar relationship existed between the growth of extraembryonic tissues and their oxygen consumption which could not be explained.

Returning to (45) we see that the respiration of the whole egg can be expressed as a function of the dry tissue growth rates of embryo and membranes (here the consumption by the yolk, etc., has been neglected), i.e., since

$$\begin{align*}
- \frac{dO_2}{dt}_{\text{whole}} &= (- \frac{dO_2}{dt}_{\text{embryonic}}) + (- \frac{dO_2}{dt}_{\text{extra- embryonic tissue}}) \\
\text{and using (45),}
- \frac{dO_2}{dt}_{\text{whole}} &= (x \frac{dB}{dt}_{\text{embryonic tissue}}) + (x \frac{dB}{dt}_{\text{extra- embryonic tissue}})
\end{align*}$$

(62)
Previously we saw that the phosphorylation ratio was relatively constant in the chicken brain and liver. As a first approximation assume this also holds for all the living tissue ($\chi$ constant).

Using the oxygen consumption data of Romijn and Lockhorst (1960) and the dry tissue data from Romanoff, (63) takes the form for the chicken egg

\[
\frac{dO_2}{dt}\bigg|_{\text{whole}} = 0.93 \frac{dB}{dt}\bigg|_{\text{embryonic tissue}} + 1.41 \frac{dB}{dt}\bigg|_{\text{extra-embryonic tissue}}
\]

and the units for the coefficients are mg $O_2$/mg tissue. The correlation coefficient $r^2=0.995$. Equation (64) is plotted in Figure 8 along with the data from Romijn and Lockhorst. By including the respiration of extraembryonic membranes and using dry tissue growth rates, an accurate description is apparent even in early embryogenesis. This is in contrast to the model of Vleck et al. (1980).

It is interesting that the coefficient for the membranes is 1.5 times that of the embryo. The reason for this is seen if $\chi$ is examined,

\[
\chi = \frac{v_{O_2}}{v_{ATP}} \frac{v_{ATP}}{v_{BB*}} \left( 1 + \frac{v_{m \text{ ATP}}}{v_{s \text{ ATP}}} \frac{v_{m \text{ F}}}{v_{s \text{ F}}} \right)
\]

We can estimate $\chi$ for the embryo. Because of the yolk and albumen that supply protein, lipids, and carbohydrates to the living tissues, the
Figure 8. Oxygen consumption by the whole chicken egg during incubation.
synthesis reactions are largely polymerizations. Earlier the cost of ATP required to synthesize a gram of embryonic tissue was estimated to be 72 mmoles, and the ratio of building blocks to ATP about 0.25. From Table 2 we observed that the oxygen to ATP in respiration is about 1/6. And finally assuming the turnover rate is 10% of synthesis while ATP use for maintenance is equal gives the estimated coefficient for the embryonic tissues from (63) to be

\[
\chi = \left(32 \frac{mg \ O_2}{mmole \ O_2}\right) \left(-\frac{1 \ mmole \ O_2}{6 \ mmole \ ATP}\right) \frac{-72 \ mmole \ ATP(1 + 0.25 + 1)}{gm \ tissue} \frac{(1 - 0.10)}{X = 0.96 \ gm \ O_2/gm \ tissue}
\]

This is in excellent agreement with the value of 0.93 gm O_2/gm tissue obtained in (64). Now making the same assumptions as above for the extraembryonic membranes, we immediately see that the fraction of ATP needed for maintenance must be larger (by a factor of 2) or the relative turnover is greater. A possible explanation for an increased maintenance requirement cost may be due to the role of the membranes in transport processes, or because their increased surface to volume ratio necessitates more energy to regulate intracellular pH and ionic composition. In any case, the metabolic model allows us to interpret differences in oxygen requirements for various growing tissues.

The fraction of oxygen consumed by the embryo (from (64)) as a function of incubation time is plotted in Figure 9. The experimental data of Romanoff (based on membrane respiration) and Kucera et al. (1984) are also shown. The low fraction of consumption predicted for the
Figure 9. Fraction of whole chicken egg oxygen consumption due to the embryo during incubation
embryo in early incubation is in good agreement with that measured by Kucera. Apparently the values obtained from in vitro membranes are not applicable in vivo. The figure also shows that by the 15th day the respiration of the whole egg is primarily due to the embryo.

An average oxygen concentration in the tissues of the developing chick can now be estimated using the embryonic oxygen consumption determined above and (60). The rate constant is approximated by recognizing the tissue $pO_2$ should be about 10-20 torr based on the limitations of mitochondrial function and from circulatory considerations. In Figure 10 the result is plotted over days 10 to 18. The embryonic tissue concentration appears relatively stable with a slight maximum around day 15. This corresponds with a maximum specific growth rate of the embryo.

The oxygen conductance between the embryonic blood and tissues can also be estimated. The oxygen flow rate to the tissues can be expressed as the product of a concentration difference and a conductance (Weibel, 1984):

$$\frac{dO_2}{dt} = G(p^B_{O_2} - p^T_{O_2})$$

where the conductance $G$ is determined by the geometry of transport and the diffusivity of oxygen. The partial pressure of oxygen in the capillary bed, $p^B_{O_2}$, in this case is the average of chorioallantoic vein and artery pressures. This value is plotted in Figure 10. The resulting conductance from (67) is also shown. The oxygen conductance is seen to
Figure 10. Oxygen partial pressure in the capillary bed and embryonic tissue during chick incubation. Predicted oxygen conductance is also shown.
increase rapidly through day 15 before leveling off. A physical explanation may be that of an increasing vascularization of embryonic tissues. The additional surface area results in a larger conductance. In the days just prior to hatching, however, the tissues may mature to the point where significant changes in vascularization do not occur. In any case, the five-fold change in oxygen conductance may explain how the chick meets its increasing demand for oxygen.

6. Energetic applications

The heat production of incubating avian eggs can be described with the energetic equation (47) developed earlier. In this case the heat production is represented by the sum of two contributions: one due to the embryo and the other resulting from the extraembryonic tissues

\[
\frac{\Delta H_k}{k} \frac{d\xi_k}{dt} = (\frac{dB}{dt})_{\text{embryonic tissue}} + (\frac{dB}{dt})_{\text{extra-embryonic tissue}}
\]  

(68)

Again we assume the phosphorylation ratio is relatively constant. Romijn and Lokhorst (1960) have measured the heat production of a developing chick egg over the whole period of incubation using a compensation calorimeter. Using the growth data from Romanoff (1967) and the calorific data of Romijn and Lokhorst, (68) takes the form

\[
\frac{\Delta H_k}{k} \frac{d\xi_k}{dt} = 3.4 \frac{dB}{dt}_{\text{embryonic tissue}} + 4.5 \frac{dB}{dt}_{\text{extra-embryonic tissue}}
\]  

(69)
Figure 11. Heat production by the whole chicken egg during incubation
with the coefficients in kcal/gm tissue. The correlation coefficient 
\[ r^2 = 0.994. \]
The regression is shown in Figure 11 along with the data from 
Romijn and Lokhorst. Accounting for the heat production of the 
extraembryonic tissues allows an accurate description throughout 
incubation. The difference in the values for the embryonic and 
extraembryonic tissues can be explained by examining the calorific 
coefficient in more detail.

The standard free enthalpies of reaction for the synthesis, 
turnover, maintenance and respiration pathways are -6.8 kcal/mole, -4 
kcal/mole, -7.3 kcal/mole and -10.7 kcal/mole, respectively (Lehninger, 
1982). Lipid is assumed to be the substrate catabolized in respiration. 
Using the same values determined in the growth coefficient calculation 
for turnover ratio, etc., gives the estimated calorific coefficient for 
embryonic tissues to be

\[ \zeta = \frac{4(-8.68)(-0.1) + 10.7(1 + 0.25) + 6.8 + (10.7 + 7.3)(1)}{(0.9)(-0.072)^{-1}} \]

\[ \zeta = -3.1 \text{ kcal/gm} \] (70)

This agrees well with the value obtained by regression in (69). If the 
same calculation is made for the extraembryonic tissues the relative 
amount of ATP used in maintenance must be about twice as large. This 
result is consistent with what we determined by examining the whole egg 
 oxygen consumption.

The ratio between heat production and oxygen consumption in chick 
 eggs does not appear to remain constant during incubation (Romijn and
Lokhorst, 1960). The changes in the calorific quotient have often been attributed to embryonic metabolism alone. What has not been recognized by previous investigators is the contribution of the extraembryonic tissues to the observed calorific ratio. Such a contribution can be examined using the metabolic model.

From (49) the observed whole egg calorific quotient is written as the sum of an embryonic and extraembryonic term

\[
\left(\frac{1}{k} \sum_{\xi} \frac{\Delta H}{k} \frac{d\xi}{dt}\right) \left(-\frac{dO_2}{dt}\right) = \beta[\psi]_{\text{embryonic}} + (1 - \beta)[\psi]_{\text{extra-embryonic}}
\]

where \(\beta\) is the fraction of oxygen consumed by the embryo and \(\psi\) is the calorific quotient for the particular tissue. The \(\psi\)'s in (71) are constants since \(F\) was assumed constant during chick development. The calorific quotients can be calculated from the parameters used in the heat production and oxygen consumption sections. Equation (71) is then

\[
\left(\frac{1}{k} \sum_{\xi} \frac{\Delta H}{k} \frac{d\xi}{dt}\right) \left(-\frac{dO_2}{dt}\right) = 3.6\beta + 3.2(1 - \beta)
\]

and the coefficients are in kcal/gm oxygen. Figure 12 shows the experimental data of Romijn and Lokhorst along with the theoretical calorific quotient from (72). Calculating a ratio from experimental data significantly increases the uncertainty in the result, thus, the error
Figure 12. Calorific coefficient for the whole chicken egg during incubation.
bars in Figure 12 may easily be ± 50%. Even taking this into account, the correspondence between theory and experiment is not that good. However, with the exception of days 8 thru 10 the upward trends are similar. While no firm conclusions can be drawn for the incubating chick egg, we can infer that the metabolism of the extraembryonic tissues plays a significant role in determining the observed whole egg calorific quotient.

7. Incubation length effects

The influence of a prolonged incubation time on avian metabolism can be evaluated by comparing the growth of wedge-tailed shearwater (Puffinus pacificus chlororhynchus) and chicken embryos. The wedgetailed shearwater has an incubation period more than twice as long as the chicken, 52 days versus 21 days, and consumes 45% more oxygen (Ackerman et al., 1980). In spite of this the hatchling mass is similar in both species. In an integral sense the cost of producing a gram of wedge-tailed shearwater tissue appears larger. This is consistent with the notion that maintenance requirements scale with the length of incubation (Whittow, 1980; Vleck et al., 1980; Ackerman et al., 1980; Parry, 1983; Bucher and Bartholomew, 1984; Pettit et al., 1984; Jobling, 1985). Further insight, however, can be gained by applying the metabolic growth model to incubating wedge-tailed shearwater eggs.

Proceeding as we did for the whole chicken egg, the respiration of the shearwater egg is expressed as a function of embryonic and extraembryonic dry tissue growth rates
The phosphorylation ratio is again assumed constant. The two growth coefficients in (73) are determined by regressing the oxygen consumption and embryonic growth data of Ackerman et al., along with an estimate of the extraembryonic growth rate. The membrane estimates are obtained by scaling the growth rate of chick extraembryonic tissues to the shearwater incubation period. This is a reasonable approach because the chick and shearwater egg sizes are similar. The water content of the tissues is assumed comparable to values reported for other species (Romanoff, 1967). Then for the shearwater egg over days 13 thru 41 (prior to the first indication of pipping) the oxygen consumption relationship is

$$\frac{dO_2}{dt|_{\text{whole egg}}} = (x \frac{dB}{dt|_{\text{embryonic tissue}}} + (x \frac{dB}{dt|_{\text{extra-embryonic tissue}}}$$ (73)$$

with the coefficients expressed in mg oxygen/mg dry tissue. The correlation coefficient $r^2 = 0.991$. The metabolic model provides a good description of the shearwater embryonic data.

The wedge-tailed shearwater growth coefficients are roughly the same as those determined for the chicken (64). This is illustrated in Figure 13 where the shearwater whole egg oxygen consumption is predicted from chicken growth coefficients. The agreement with the experimental data is quite good. The physical significance of this is that the relative maintenance costs are identical for the two species up to pipping. This
Figure 13. Oxygen consumption of the whole shearwater egg during incubation.
is in contrast to what was expected based on the net oxygen consumption of the hatchling.

The additional oxygen consumption of the shearwater can be attributed to the relatively long period of little or no growth between pipping and hatching compared to the chick. The biological reason for this delay is unclear. We can conclude, however, that two distinct metabolic periods are evident in wedge-tailed shearwater and chick embryos: a metabolically comparable prepipping stage and a dissimilar postpipping stage. This example does illustrate the misleading conclusions regarding maintenance costs that can be made if only integral data are examined.

8. Effects of incubation at altitude

The incubation of embryos under conditions of chronic hypoxia has been studied by several researchers. However, their experiments were primarily directed at observing alterations in development or viability; energetic effects that may accompany growth at high altitude remain unclear. Quantitative energetic investigations can provide information regarding fundamental metabolic changes or can indicate embryonic compensation. Here we apply the metabolic growth model to the incubation of chick eggs at altitude and compare the results to sea level incubation.

Smith et al. (1969) has measured first generation embryonic growth at an elevation of 3100 m (524 mm Hg). The hatchling mass is about 2/3 of a sea level hatchling and the incubation period is prolonged by 15%.
Figure 14. Heat production of the whole chicken egg at high altitude
Lokhorst and Romijn (1965) report heat production data for the chick at a simulated altitude of 507 mm Hg with the use of a low pressure calorimeter. The calorific coefficients can be determined with the heat production relationship derived above (assuming $F$ is constant)

$$\sum_{k} \Delta H_{k} \frac{d \xi_{k}}{dt} = 3.3 \frac{dB}{dt}|_{\text{embryonic}} + 4.4 \frac{dB}{dt}|_{\text{extra-embryonic tissue}}$$

with a correlation coefficient $r^2=0.991$. From (75) we see that the metabolic model provides a good description of the heat production. The calorific coefficients in (75) are essentially the same as for the case of incubation at sea level (69), Figure 14. Thus we can conclude that the relative metabolism for chick embryos is largely unaffected by the hypoxic conditions of incubation at high altitude. A reduction in oxygen partial pressure in the environment slows the entire metabolic network without preference to a particular pathway. The tissue oxygen concentration can be calculated for incubation at altitude in exactly the same manner described for sea level incubation. It turns out that the embryonic tissue concentrations are within 5% for the two cases. This suggests that the high altitude embryo has compensated for the reduced oxygen driving force, probably by increasing vascularization of the tissues or increasing cardiac output.

9. **Relative growth efficiency**

The relative growth efficiency is a useful measure of the degree to which metabolism is dedicated to synthesis processes. Here we consider
the relative efficiencies for the teleost *Oryzias* and a chick during development.

The relative growth efficiency for *Oryzias* can be calculated from the theoretical expression (53) and the data of Taguchi (1962) and Ishida (1951). From (57) the stoichiometric rate constant ratio of maintenance to synthesis is 0.22 during incubation. The relative efficiency of growth for *Oryzias* is then

\[ \eta_{rel} = \frac{1.25}{1.25 + 0.22F} \] (76)

and is plotted in Figure 15. The relative efficiency increases slightly before rapidly decreasing as hatching approaches. Through the first 9 days of incubation the relative efficiency never falls below 50%.

For the whole chicken egg the relative growth efficiency is due to both embryonic and extraembryonic contributions. Returning to the definition of efficiency (50), the whole egg efficiency is

\[ \eta_{\text{whole egg}} = \frac{(-\Delta G)^{\text{embryonic}} S}{(-\Delta G)^{\text{embryonic}} S + (-\Delta G)^{\text{extraembryonic}} S} \] (77)

Assuming the stoichiometry is the same in the embryo and membranes, and using the respiration equation (39), the relative growth efficiency for the whole chicken egg is

\[ \eta_{rel} = \frac{\frac{BB^*}{v_{s_{S}}} + \frac{BB^*}{v_{s_{ATP}}}}{1 + \frac{1}{v_{s_{S}}} + \frac{1}{v_{s_{ATP}}}} \]
Figure 15. Predicted relative growth efficiency for *Oryzias* l. and the whole chicken egg throughout incubation.
and $\beta$ is the fraction of oxygen consumed by the embryo. Notice that the phosphorylation ratio is taken to be a constant based on our previous discussions, so substituting in for the ratios in (78) yields the desired expression for the whole chicken egg.

\[
\eta_{rel} = \frac{1.25/(1.25 + \frac{\beta}{2.25} + \frac{1 - \beta}{1.62})}{\frac{\beta}{2.25} + \frac{1 - \beta}{3.25}}
\]  

Equation (79) is also plotted in Figure 14. The relative growth efficiency for the chicken egg increases steadily throughout incubation even though the relative efficiency of the embryo is unchanged.

The most obvious difference between the relative growth efficiencies of *Oryzias* and the chick lies in the higher average level maintained by the teleost. The metabolism of *Oryzias* is directed more toward biomolecule synthesis than it is in the chick. Further experimental data are needed to determine whether these efficiencies are characteristic of their respective species.
In the previous sections we have formulated a kinetic growth model and developed various theoretical expressions for parameters of material or energetic interest. We have shown the model to be consistent with a wide range of available growth data. In addition, the quantities that need to be measured in order to be able to compare growth have been identified. It seems clear that the metabolic model provides a good foundation for analysis and comparison of growth in aerobic biological organisms.
III. NONEQUILIBRIUM THERMODYNAMIC GROWTH MODEL

The growth model that we have considered thus far is based on classical equilibrium thermodynamics and an appropriate kinetic description (since the concepts of time and rate of processes are absent from classical thermodynamics). An alternative development can be shown within the framework of nonequilibrium thermodynamics. The advantage of the latter approach is that it does not depend on detailed kinetic knowledge. Additionally, the extensive coupling which exists between the various irreversible processes occurring in a growing biological system is, in principle, taken into account.

In this section nonequilibrium thermodynamics will be used to formulate metabolic relationships for oxygen consumption and dry tissue growth rate. We will find these expressions to be similar to those obtained from the previous kinetic analysis. Phenomenological coefficients will also be calculated for a specific organism.

Following an introduction to nonequilibrium thermodynamics and a brief review of prior growth models we will consider how to improve the metabolic description of developing organisms. A more thorough discussion of nonequilibrium thermodynamics may be found elsewhere (Prigogine, 1967; DeGroot and Mazur, 1962; Katchalsky and Curran, 1965).

A. Introduction to Linear Nonequilibrium Thermodynamics

Central to the framework of nonequilibrium thermodynamics is the development of an equation relating the rate of change of entropy in a system to the irreversible processes, or fluxes, which occur. From
equilibrium thermodynamics the entropy change for an isolated system during a spontaneous process is constrained to be nonnegative

\[ dS \geq 0 \]  

(80)

In the more general case the entropy change can be split into two terms (similar to the material and energy balances): one due to external interactions with the environment and the other resulting from internal contributions

\[ dS = dS_\text{in} + dS_\text{e} \]  

(81)

Comparing (80) and (81) it is clear that the entropy production of a system can never be negative

\[ dS_\text{in} \geq 0 \]  

(82)

Notice that no thermodynamic restrictions can be applied to the external interactions.

The form of (81) suggests an entropy balance can be applied to an arbitrary system volume element. Without going into the mathematical formalisms this turns out to be the case. In order to be able to use the resulting entropy expression for calculations we must assume that even though the system is nonequilibrium as a whole, we can define entropy in any arbitrary subsystem. Then by using the Gibb's equation entropy can be defined with the same independent variables as in the equilibrium case. The local equilibrium assumption is reasonable provided the deviations from equilibrium are not too large.
The explicit form of the entropy equation is obtained by inserting the conservation relationships for mass, energy and individual chemical species. Two contributions to the entropy change in a system are apparent: entropy flow and entropy production. The entropy flow consists of a term associated with heat flow and one with material diffusion across the system boundaries. The entropy production contains terms arising from heat conduction, diffusion of material, momentum transfer, and chemical reaction. Structurally the entropy production expression is simply a sum of products of forces and fluxes

\[ \frac{dS}{dt} = \frac{1}{T} \sum_k J_k X_k \]  

(83)

where \( J_k \) are the flows (heat, chemical reactions, etc.) and \( X_k \) are the corresponding forces (temperature gradient, chemical potential gradient, etc.). The choice of force-flux products are rather arbitrary so long as algebraic equivalence is preserved. By examining the dissipation function, (83), the important flows and forces for a given application can be identified.

From (82) and (83) we see that there is a thermodynamic constraint on the relationship between the \( X_k \) 's and \( J_k \) 's. Since we have no indication that a unique relationship exists, a linear set of phenomenological equations relating forces and flows is postulated. This assumption is not very restrictive since experimental evidence suggests for many processes linear phenomenological laws (e.g., Fourier's, Fick's and Ohm's laws) are adequate. For other processes (chemical reactions,
etc.) large overall deviations from linearity may be observed. Prigogine (1962) points out that linear phenomenological relations between chemical reaction rates and affinities are still valid for successive reactions even if the net reaction appears nonlinear so long as the elementary steps are sufficiently reversible. The fact that biological systems are often characterized by sequential reactions close to equilibrium indicates linear nonequilibrium thermodynamic methods may be appropriate.

The general linear relationship between the forces and flows is the following:

\[ J_k = \sum_i L_{ik} X_k \]  \hspace{1cm} (84)

with \( L_{ik} \) termed phenomenological coefficients. There are a few constraints that are placed on (84) and the \( L_{ik} \)'s. In an isotropic medium only driving forces of the same or two orders different can influence the flux considered (Curie principle). Also, so as not to violate thermodynamics (82), the coefficients must satisfy the following inequalities:

\[ L_{ii} > 0 \] \hspace{1cm} (85)

\[ L_{ii} L_{kk} \geq \frac{1}{4} (L_{ik} + L_{k1})^2 \] \hspace{1cm} (86)

And finally, Onsager showed that the cross coefficients are symmetric with respect to force-flow interactions:

\[ L_{ik} = L_{ki} \] \hspace{1cm} (87)
These are referred to as Onsager's reciprocity relations and are strictly valid only in the linear phenomenological regime.

With the above background we can now consider previous nonequilibrium thermodynamic models of growing organisms.

B. Review of Nonequilibrium Thermodynamic Growth Models

Perhaps the simplest nonequilibrium thermodynamic model has been proposed by Zotina and Zotin (1967):

\[ J_g = L_g (T_m - t) \]  \hspace{1cm} (88)

Where \( J_g \) is the specific growth rate of the organism and \( T_m \) is the time when growth ceases. Their model assumes growth is determined by a single force

\[ X_g = T_m - t \]  \hspace{1cm} (89)

From an inspection of the dissipation function it is difficult to justify using a time difference as a thermodynamic driving force. Additionally, how the metabolic factors are coupled to determine growth is not apparent in this model. While (89) does adequately describe the growth of fish and humans, it is really more of an empirical observation.

An alternative model was developed by Keller (1984) for biological systems that grow by isothermal mass exchange with the surroundings. For simplicity the system is taken to be a single component. The proposed growth relation is the phenomenological expression

\[ J_g = L_g \left( \frac{\mu_g^S - \mu_g^1}{T} \right) \]  \hspace{1cm} (90)
with $\mu^S$ and $\mu_1$ the chemical potentials of species 1 in the surroundings and system respectively. Keller assumes the phenomenological coefficient is related to the surface area of the organism

$$L_g = L^* g M^{2/3}$$

(91)

where $M$ is the mass. In general, linear phenomenological coefficients may depend on parameters which define the state of the system but not the flows or forces of interest. Equation (90) is, therefore, a nonlinear nonequilibrium thermodynamic model and the linear formalisms no longer apply. The final assumption made is that the driving force is constant, making (90)

$$J = L^* g M^{2/3}$$

(92)

Unfortunately, Keller provides no examples of organisms whose growth can be described by (92). Such a model may work when growth is simply an accumulation of water but it lacks a regulatory mechanism to limit $J$ at long times. This model also obscures the effects of the underlying metabolic processes on the observed parameters of growing biological systems.

Recently, Westerhoff et al. (1982) proposed a nonequilibrium thermodynamic model for bacterial growth which incorporates the coupling of catabolism and anabolism by the intracellular ATP pool. In their model the forces are the free energies of reaction for generalized catabolic, anabolic and ATP hydrolysis reactions while the flows are the corresponding reaction rates:
\[ J_c = L_c \{(\Delta G_c - \Delta G_c^\#) + v_p^c(\Delta G_p - \Delta G_p^\#)\} \quad (93) \]

\[ J_a = L_a \{(\Delta G_a - \Delta G_a^\#) + v_p^a(\Delta G_p - \Delta G_p^\#)\} \quad (94) \]

\[ J_p = L_p \{(\Delta G_p - \Delta G_p^\#)\} \quad (95) \]

and the parameters \(\Delta G^\#\) depend on the magnitude of the flow. In particular, \(\Delta G^\#\) is defined as follows: when \(\Delta G\) is high (low), i.e., the flow is independent of the force, \(\Delta G^\#\) equals \(\Delta G\) minus a constant, the effect being \(\Delta G - \Delta G^\#\) is constant. When the force and flux are not independent, \(\Delta G^\#\) is taken to be a constant so that \(\Delta G - \Delta G^\#\) is proportional to \(\Delta G\). Such a scheme is an attempt to approximate the response of an enzyme catalyzed reaction to changes in substrate concentration. Rearranging (93) through (95) gives the microbial growth equation

\[ J_a = C_1(-J_c) + C_2 \quad (96) \]

where \(C_1\) is the growth yield and \(C_2\) is the maintenance coefficient. This expression is in agreement with some experimental results for microbial systems.

The main difficulties with (96) are the seemingly arbitrary driving forces that are buried in the coefficients and the lack of metabolic regulation. These issues will be addressed in the next section as we develop a linear nonequilibrium thermodynamic model to describe the growth of biological systems.
C. Linear Nonequilibrium Thermodynamic Growth Model

The first step in applying nonequilibrium thermodynamic principles to growing organisms is to examine the dissipation function (83). The force-flow pairs present in biosystems are numerous: concentration gradients/material diffusion, temperature gradients/heat conduction, pressure gradients/bulk flow, etc. Fortunately, considerable simplification of the entropy production equation is possible. The primary dissipative processes identified in biological systems are chemical reactions (Prigogine, 1962). For example, Briedis and Seagrave (1984) estimated the relative importance of heat conduction in avian eggs and showed it was negligible. In biological systems (83) is approximately

\[ \frac{dS_i}{dt} = \frac{1}{T} \sum_r J_r X_r \]  

(97)

and the summation is over all the chemical reactions. The forces in (97) are chemical affinities. The general metabolic network developed earlier (Figure 2) in the kinetic analysis can be used to reduce the reaction complexity.

Before writing down the phenomenological relations for the force-flux pairs in (97) consider the following simplified description of a biological organism. The growing system is assumed to consist of a metabolically active region where bioreactions occur and a nonactive region (Figure 16). All the biosynthetic, catabolic and maintenance pathways are considered to be located in the active region. The size of
Figure 16. Simplified biological organism for nonequilibrium thermodynamic model
the active region is defined to be proportional to the energy carrier content of the entire system. And finally, separate catabolic and anabolic areas are recognized to exist within the active region. For example, in eucaryotic organisms the mitochondria may be considered the catabolic area.

For this general biosystem an appropriate set of phenomenological expressions is

\[ J_c = L_c A_c + L_{ca} A_a \]  

(98)

\[ J_a = L_{ac} A_c + L_a A_a \]  

(99)

\[ J_t = L_t A_t \]  

(100)

and the units are J (moles Y/gm active region.hr). A few points can be made about (98)–(100). First, since we are applying linear nonequilibrium thermodynamics the phenomenological coefficients are constants. Secondly, only the catabolic and anabolic reactions are coupled; turnover is assumed to be uncoupled to other processes. Lastly, the other flows in the metabolic network (Figure 2) are combinations of the above:

\[ J_m = \left( \frac{v_{m,ATP}}{v_{m}} \right) J_c - \left( \frac{v_{m,ATP}}{v_{m}} \right) J_a \]  

(101)

\[ J_g = \left( \frac{v_{g,ATP}}{v_g} \right) J_a - \left( \frac{v_{g,ATP}}{v_g} \right) J_t \]  

(102)
where $J_m$ is the maintenance flow and $J_g$ is the growth flow (i.e., the net biosynthesis).

The two expressions desired for oxygen consumption and dry tissue growth rate are (98) and (102) respectively. To obtain the working relationships for experimental data the chemical affinities of the reaction pathways must be examined in some detail.

1. Reaction pathways

   a. Anabolic The stoichiometry for the anabolic pathway is the same as the synthesis reaction considered earlier with substrate activation included:

   $$\ldots + v^S_a S + v^ATP_a ATP \rightarrow v^B_a B + v^ADP_a ADP + \ldots$$  \hspace{1cm} (103)

   The affinity of the anabolic reaction is

   $$A_a = -\sum_i v^a_i \mu^a_i$$  \hspace{1cm} (104)

   with $\mu^a_i$ the chemical potential of species $i$ evaluated in the anabolic compartment of the biosystem. Since the chemical potential of any component can be expressed relative to some reference state (Modell and Reid, 1983)

   $$\mu_i = G^i + RT\ln \frac{f_i}{f_i^0}$$  \hspace{1cm} (105)
where \( \mu_i^0 = G_i^0 \) and the fugacity of \( i \) in the standard state is \( f_i^0 \).

Additionally, biological solutions are typically dilute enough to allow the fugacity ratio in (105) to be approximated by the species concentration. Equation (104) then can be written as:

\[
A_a = -\Delta G_a^0 - RT \ln \frac{v^B}{v^S} \cdot \frac{[B]^a}{[S]^a} \cdot \frac{v^{ADP}}{v^{ATP}} \cdot \frac{[ADP]^a}{[ATP]^a} \cdot [\ldots]_a
\]

(106)

with \( \Delta G_a^0 \) the standard free energy of reaction. Assuming the substrate and product concentrations are relatively constant (as we did for the kinetic model), and the reactions are normalized to the participating nucleotides, (106) is

\[
A_a = -\Delta G_a^0 - RT \ln \frac{[ADP]}{[ATP]} = -\Delta G_a^0 - RT \ln (F_a)
\]

(107)

Note that the affinity is related to the phosphorylation ratio in the anabolic area of the organism, \( F_a \).

b. Catabolic

In the catabolic reaction the substrate is oxidized while ATP is aerobically synthesized from ADP

\[
\ldots + v^S_c S + v^O_2_c O_2 + v^{ADP}_c ADP \rightarrow v^{ATP}_c ATP + v^{CO_2}_c CO_2 + \ldots
\]

(108)

The affinity for catabolism is again defined as the stoichiometric sum of the chemical potentials. Following the same method as in the anabolic case.
and the catabolic affinity is influenced by the mitochondrial phosphorylation ratio. Bohnensack et al. (1982) have suggested that ln(F_c) is approximately constant relative to an order of magnitude change in F_a in rat liver mitochondria. If we assume this is generally true, then the catabolic affinity can be considered constant

\[ A_c = -\Delta G_c^\circ + RT \ln (F_c) \]  

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\[ A_c = -\Delta G_c = \text{constant} \]  

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**c. Turnover/utilization**  
Turnover is the breaking down of biomolecules to substrates. The stoichiometry is simply

\[ \ldots + v_t^B + v_t^\text{ADP} \text{ADP} \longrightarrow v_t^S + v_t^\text{ATP} \text{ATP} + \ldots \]  

Hence the turnover affinity, after (105) and (106), is

\[ A_t = -\Delta G_t^\circ + RT \ln (F_a) \]  

and the phosphorylation ratio is evaluated in the anabolic compartment.

Using the above expressions for the chemical affinities the phenomenological equations can be evaluated and explicit relationships for the oxygen consumption and growth rate of developing organisms determined.
2. Metabolic relationships

a. Oxygen consumption  The rate of catabolism in terms of the affinities (107) and (111) is

\[ J_c = L_c (\Delta G_c) + L_{ca} (\Delta G_a^c - RT \ln(F_a)) \]  

(114)

Now the logarithm can be approximated by the series expansion

\[ \ln(x) = 2 \left( \frac{x - 1}{x + 1} \right) + \frac{1}{3} \left( \frac{x - 1}{x + 1} \right)^3 + \ldots \]  

(115)

Since the normal range of \( F_a \) is \( 0.1 \leq F_a \leq 1.0 \), the higher ordered terms are small compared to the first, so

\[ \ln(F_a) = 2 \left( \frac{F_a - 1}{F_a + 1} \right) \]  

(116)

or in total moles of ATP and ADP present in the anabolic area,

\[ \ln(F_a) = 2 \left( \frac{ADP - ATP}{ADP + ATP} \right) \]  

(117)

Substituting this back into (114) gives the catabolic flow

\[ J_c = \left( L_c (\Delta G_c) + L_{ca} (\Delta G_a^c) \right) - 2L_{ca} RT \left( \frac{ADP - ATP}{ADP + ATP} \right) \]  

(118)

Pfaller et al. (1984) measured mitochondrial and cytoplasmic adenosine nucleotide levels in rat renal cells and showed the majority is found in the cytoplasm. Assuming this is true in general allows the nucleotide ratio in (118) to be approximated using total amounts.
The total oxygen consumption for the growing biosystem is obtained by multiplying the catabolic flow by the molecular weight and stoichiometric coefficient of oxygen, and the ratio of the mass of the active region to the moles of nucleotides, \( \omega \) (which is a constant from the definition of the active area), or

\[
- \frac{dO_2}{dt} = (-v_0^0)M_{O_2} \omega J_c
\]  

(119)

Combining (118) and (119), the oxygen consumption is

\[
- \frac{dO_2}{dt} = M_{O_2} (\beta_0 + \beta_1(F^{-1}))(ADP)
\]  

(120)

with the constants defined as

\[
\beta_0 = (-v_0^0)\omega(L_c(-\Delta G_c) + L_{ca}(-\Delta G^*) - 2RT)
\]  

(121)

\[
\beta_1 = \beta_0 + 4L_{ca}(-v_0^0)M_{O_2} \omega RT
\]  

(122)

Equation (120) states that the rate of oxygen consumption is determined by both the amount of ADP in the system and the ADP to ATP ratio. Unfortunately, it is more difficult to attach a physical interpretation to the nonequilibrium thermodynamic constants in (121) and (122) than it was for the kinetic model.

Notice that the oxygen consumption relationship in (120) is of the same form as previously obtained from a kinetic analysis. Comparison of (120) and the corresponding kinetic expression (44) requires the phenomenological coefficients to be
It is clear that the phenomenological coefficients depend on the state of
the system.

As an example, the coefficients in (123) and (124) can be estimated
for the developing egg of the teleost Oryzias latipes. Using the values
from (57)

\[ L_{ca} = 1.4 \times 10^{-7} \text{ moles O}_2 \cdot \text{moles ATP/J \cdot hr \cdot gm \cdot 500 eggs} \]

\[ L_c = 1.5 \times 10^{-7} \text{ moles O}_2 \cdot \text{moles ATP/J \cdot hr \cdot gm \cdot 500 eggs} \]

Here \(1/\omega\) was assumed to be 2x10^-4 moles/gm and the \(\Delta G\)'s were approximated
by the standard enthalpies of reaction.

b. Growth The growth rate relationship is found by substituting
into the growth flow (102) the appropriate expressions for anabolism and
turnover (107) and (113)

\[ J_g = \left( \frac{a}{b} \right) \left( L_{ca} \left( -\Delta G_c \right) + L_a \left( \left( -\Delta G^* \right) - RT \ln \left( F_a \right) \right) \right) \]

(125)
The logarithm of the phosphorylation ratio is again expanded in an infinite series and all but the first term dropped. Multiplying the growth flow by the molecular weight and stoichiometric coefficient of biomolecules, and the mass of active region per mole of nucleotide, \( \omega \), yields the total dry tissue growth rate

\[
\frac{dB}{dt} = \tilde{M}_B \omega (v^B_a(L_{ac}(-\Delta G_c) + L_a(-\Delta G^*_a)) + v^B_{t,t}(-\Delta G^*_t) - 2(v^B_{t,t} - v^B_{a,a})RT + [v^B_a(L_{ac}(-\Delta G_c) + L_a(-\Delta G^*_a)) \\
+ v^B_{t,t}(-\Delta G^*_t) + 2(v^B_{t,t} - v^B_{a,a})RT]F(ATP)
\] (126)

Examining (126) we see the dry tissue growth rate is determined by both the total amount of ATP in the organism and the phosphorylation ratio. The nonequilibrium thermodynamic formulation suggests the same form as that obtained from a kinetic analysis,

\[
\frac{dB}{dt} = (\beta g^0 - \beta g^F)ATP
\] (127)

Thus, (126) also describes the experimental data considered earlier for the growth of chick brain and liver, and \textit{E. coli}.

Expressions for the phenomenological coefficients follow directly from (42) and (126)

\[
L_a = \frac{v^B_{K,t} - v^B_{K,s}}{2v^B_{a,a}} \omega (-\Delta G^*_a) - \frac{v^B_{m}}{v^ATP}K_m - \frac{v^m}{v^ATP}(1 + \frac{v^s}{v^ATP})K_s
\] (128)
Estimates of these coefficients can be made from simultaneous respiratory and growth data.

The objective of the above sections has been to formulate a linear nonequilibrium thermodynamic growth model that adequately reflects the underlying metabolism of biosystems. We found the relationships for oxygen consumption and dry tissue growth rate depended explicitly on the phosphorylation ratio and the ADP or ATP level just as in the kinetic analysis. Values for phenomenological coefficients during *Oryzias latipes* development were also calculated. Thus, we can conclude that the nonequilibrium thermodynamic model is substantiated by a comparison with the previously developed kinetic model and experimental data.
IV. A COMPUTATIONAL ANALYSIS OF HEAT AND WATER TRANSPORT IN EGG CLUTCHES

It has been recognized for some time that the external physical environment in which the eggs of certain species incubate has a profound effect on the material and energy exchanges which occur between the egg and the environment. Numerous experimenters have investigated incubation at different temperatures and hydric conditions, but the analysis has been hampered by the lack of a suitable model that accounts for the interactive exchange of heat and water. Recently, Ackerman et al. (1985b) proposed a model that adequately describes energy and water fluxes for single, partially buried parchment-shelled reptile eggs. With their model they were able to examine the variations in egg temperature and mass for incubation in sand or vermiculite at various water potentials. The model demonstrated the important effect of substrate thermal conductivity on the exchange processes. In practice, however, most large reptiles do not bury single eggs but employ clutches containing anywhere from 10-200 eggs. It is clear that the individual egg model cannot be directly applied to eggs within clutches where packing geometry and inter-egg effects now become important.

The purpose of this paper is to extend the single egg model to cases where the eggs are incubating in a clutch. The clutch models are constructed by applying conservation equations for energy and mass with the appropriate auxiliary conditions. The resulting relationships are numerically solved using orthogonal collocation on finite elements and an implicit multi-step integration scheme to yield temperature, water vapor
pressure and egg mass profiles. Two different clutch types are simulated: in one case the interior spaces are assumed to contain only vapors (air and water), while the second case considers the interstices to be filled with the substrate that surrounds the clutch. The physical parameters of the clutch and substrate can be changed to investigate a wide variety of nest conditions. In particular, we examine the effect of daily temperature oscillations in the surrounding medium on heat and water transport within the clutch. This approach may begin to demonstrate the significance of the clutch in regulating the microenvironment of developing eggs.

A. Techniques and Analysis

The two types of clutch environments, vapor or substrate-filled interstices, are modeled in a similar fashion. Both use generalized balances for energy and mass. Consider first the system where the substrate occupies the spaces between the incubating eggs.

1. External medium interstices

The partial differential equations used to describe the clutch containing substrate are:

\[
\frac{\partial T}{\partial t} = \alpha \nabla^2 T + \frac{1}{\rho C_p} \frac{\partial}{\partial t} R_t
\] (130)

\[
\frac{\partial \rho}{\partial t} = R_m
\] (131)

with auxiliary conditions

1) \( T(r,0) = T_0 \)
ii) \( \rho[r,0] = \rho_0 \)

iii) \( \frac{3T}{\partial r} \bigg|_{r=0} = 0 \) \hspace{1cm} (132)

v) \(-k\frac{3T}{\partial r} \bigg|_{r=R} = h(T[R,t] - T_s)\)

The clutch radius is \( R \), its temperature is \( T \), and the mass density is \( \rho \). The thermal diffusivity is \( \alpha \), with a heat capacity of \( C_p \), rate of thermal energy production per unit volume (i.e., the metabolic heat production of the eggs) of \( R^m_t \), and a rate of transport of water vapor across the egg shell per unit volume of \( R^m_m \). In writing these relations we have ignored convection of energy, evaporative heat transfer, and furthermore assumed that the primary barrier to water exchange is the egg shell. This later assumption is reasonable when the surrounding medium fills the interstices of the clutch since the soil dynamics for biologically realistic conditions are several orders of magnitude greater than the water uptake by an egg. Although there is no significant evaporative heat transfer within the clutch associated with the movement of water from liquid phase to vapor then diffusing across the egg shell and subsequently condensing, there will be a small heat loss to the surroundings from a net water vapor flux into the substrate. The product \( \rho C \) is assumed to be constant. The mass balance relation assumes that mass changes within the clutch result solely from the transfer of water. This is appropriate if we restrict the model to cases where the masses of
O₂ consumed and CO₂ produced are equivalent (R.Q.=0.72) and the properties of the substrate in the clutch are relatively constant. If we wish to consider the more complicated situations Equation (131) and its associated auxiliary conditions need to be modified.

The statements for the auxiliary conditions (132) are straightforward: the first two are initial conditions for temperature and mass density, the third results from symmetry considerations, and the last expresses the idea that the heat flux at the surface of the clutch is continuous. The thermal conductivity of the clutch is k, the heat transfer coefficient for transfer from the clutch to the surrounding medium is h, and the temperature of the surrounding is Tₛ. For the case where we wish to simulate the daily fluctuations in substrate temperature, boundary condition (iv) is modified by allowing Tₛ to vary sinusoidally.

To simplify the analysis the egg clutch is represented by three concentric spheres—each a homogeneous mixture of eggs and substrate. This allows the properties of the clutch to vary between elements; i.e., accounting for changes such as egg surface area and substrate content resulting from differences in egg packing density.

Now applying orthogonal collocation on finite elements (130) and (131) are solved for the temperature distribution and water uptake by the clutch. The method of orthogonal collocation is discussed elsewhere (Finlayson, 1980). We begin by evaluating the partial differential equations at the collocation points in each of the three elements. For each element, using Lagrangian cubic polynomials, the energy balance (130) becomes:
where I, J are the local numbering system indices, H is the element width, and \( B_{IJ} \) and \( A_{IJ} \) are numerical approximations to second and first derivatives respectively. The metabolism of the eggs, \( R^e \), is input using an empirical relationship based on the particular species being modeled.

The boundary conditions for (133) are now:

1) \[ \sum_J A_{IJ} T_J = 0 \] (134)

2) \[ -\frac{1}{H} \sum J A_{IJ} T_J = \frac{h}{k_3} (T_{10} - T_g) \] (135)

We also required flux continuity between elements, yielding two more relationships of the following form:

\[ \left( \frac{k}{H} \sum J A_{IJ} T_J \right)_{\text{element } k-1} = \left( \frac{k}{H} \sum J A_{IJ} T_J \right)_{\text{element } k} \] (136)

Notice we have transformed the original linear parabolic partial differential equation for the energy balance into a series of ordinary differential equations with known initial conditions.

To evaluate the water uptake by the eggs during incubation we proceed in a slightly different manner. The appropriate expression for \( R_m \) in (131) was shown by Ackerman et al. (1985b) to be

\[ R_m = k \frac{A}{g} (P - P_e) \] (137)
where $k$ is the mass transfer coefficient for water vapor transport across an egg shell, $A$ is the shell surface area per unit clutch volume, and $P$ and $P_e$ are the water vapor partial pressures in the spaces between eggs and the eggs themselves, respectively. Since we have assumed the vapor phase is in equilibrium with either the substrate or egg fluid we can use the product of a vapor pressure-temperature relation (e.g., the Antoine equation) and an activity coefficient to determine water vapor pressures. Equation (137) is a function of both space and time coordinates because vapor pressure depends on temperature. We can only evaluate temperatures at discrete points within the clutch (i.e., at the collocation points) so we use numerical quadrature to integrate the space dimension for each element. For element $k$, (131) and (137) become:

$$\frac{dM}{dt}_{\text{element } k} = 4\pi k g A_k \sum_j W_j (P_j - P_{e,j}) r_j^2$$

$$M(t=0) = M_0$$

where the $W$'s are the geometrically appropriate weighting factors for the collocation points and depend on the integration limits in a straightforward manner. Thus, (138) is a set of three ordinary differential equations describing the rate of mass change for the three elements. Equations (133)-(136) and (138) are then assembled in a global fashion and solved simultaneously subject to the initial conditions in (132) and (138). Any number of software packages are available to solve the four algebraic and nine ordinary differential equations. Because the equations are moderately stiff we have chosen an implicit method based on Gear's method (see Appendix).
2. Vapor phase interstices

For a clutch in which the interstices are free of substrate and filled with a vapor mixture of air and water, the conservation equations for energy and mass are

\[
\frac{\partial T}{\partial t} = \alpha \nabla^2 T + \frac{1}{\rho c_p} \left( R_t - \Delta H_{\text{vap}} k g (P_e - P) \right) \tag{139}
\]

\[
\frac{\partial \rho_{H_2O}}{\partial t} = \nabla^2 \rho_{H_2O} + k g (P_e - P) \tag{140}
\]

and the auxiliary conditions are

i) \( T[r,0] = T_0 \)

ii) \( \rho_{H_2O}[r,0] = \rho_{H_2O}^0 \)

iii) \( \frac{\partial T}{\partial r} \bigg|_{r=0} = 0 \) \tag{141}

iv) \( \frac{\partial \rho_{H_2O}}{\partial r} \bigg|_{r=0} = 0 \)

v) \( -k \frac{\partial T}{\partial r} \bigg|_{r=R} - \Delta H_{\text{vap}} \frac{\partial \rho_{H_2O}}{\partial r} \bigg|_{r=R} = h(T[R,t] - T_s) \)

vi) \( \rho_{H_2O}[R,t] = \rho_{H_2O}^{\text{sat}}[T] \)

with \( \Delta H_{\text{vap}} \) the specific heat of vaporization for water, \( \rho_{H_2O} \) is the concentration of water, and \( D \) is the effective diffusion coefficient.
for water in the clutch. Again in writing these expressions convective transport has been ignored. An evaporative heat transfer term, however is included in (139) since there is in this case a net evaporation of water within the clutch. For the mass balance we need only consider the vapor phase and can treat the transport into/out of the eggs as a sink/source. The mass change for any region of the clutch is then determined by the difference between the flux out and into the area of interest.

The auxiliary conditions are similar to the previous case: we have initial conditions for temperature and water concentration, symmetry statements for energy and mass flux, a flux continuity at the outer edge of the clutch for heat transfer (where we now allow condensation/evaporation to occur) and finally a Dirichlet-type boundary condition at the outer edge of the clutch for water concentration (the vapor at this point is in equilibrium with the surrounding medium). To examine the effect of temperature oscillations in the surrounding we merely vary $T_s$ sinusoidally in (141).

The egg clutch is represented as before—three homogeneous rings of eggs and vapor phase. The method of orthogonal collocation is applied to (139) and (140) at the interior collocation points in each element, the boundary conditions are transformed and expressions for flux continuity between elements for mass and energy are written. Three additional ordinary differential equations describing the rate of mass change of each element as a flux difference into and out of the element are also constructed. Having done this it turns out that we need to
simultaneously solve a system of seven algebraic equations and fifteen ordinary differential equations that are initial-value problems. The system is in general stiff so we again use Gear's method to obtain temperature and water vapor concentration profiles and mass changes for each of the three elements (see Appendix).

B. Simulation Results And Discussion

Typical results are shown in Figure 17 for incubation of 116 *Chelonia mydas* eggs in an external medium filled clutch. A summary of the parameters used is shown in Table 3. Over the 60 day incubation period for *C. mydas* the computed temperature in the sand clutch increases by 1.25 °C on the average, with the largest increase during the last 20 days of incubation. The profile is relatively flat throughout the clutch; only a 0.5 °C temperature drop from the center to the outer edge is predicted on day 60. Both of these results are due to the high effective thermal conductivity of the sand/egg type clutch.

In contrast, the computed temperature profiles for the vapor phase interstices type clutch shown in Figure 18 are quite different. In this case the maximum temperature rise is over 4 °C at the center of the clutch with a 2.5 °C drop to the edge. Since the thermal conductivity of air is an order of magnitude less than the thermal conductivity of an egg, the bulk of the energy transport occurs through the eggs and results in a relatively slower dissipation rate compared to the sand filled clutch.
Table 3. *Chelonia mydas* clutch physical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>k</strong>, Thermal Conductivity</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>0.00460 J/cm sec °K</td>
</tr>
<tr>
<td>Sand</td>
<td>0.01250 J/cm sec °K</td>
</tr>
<tr>
<td>Air</td>
<td>0.00026 J/cm sec °K</td>
</tr>
<tr>
<td><strong>ΔH_vap</strong>, Heat of Vaporization</td>
<td>2435.6 J/gm</td>
</tr>
<tr>
<td><strong>h</strong>, Heat Transfer Coefficient</td>
<td>~ 0.00090 J/cm^2 sec °K</td>
</tr>
<tr>
<td><strong>ρ</strong>, Density</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>1.20 gm/cm^3</td>
</tr>
<tr>
<td>Sand</td>
<td>1.60 gm/cm^3</td>
</tr>
<tr>
<td>Air</td>
<td>0.0013 gm/cm^3</td>
</tr>
<tr>
<td><strong>C_p</strong>, Heat Capacity</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>~ 4.18 J/gm °K</td>
</tr>
<tr>
<td>Sand</td>
<td>~ 2.09 J/gm °K</td>
</tr>
<tr>
<td>Air</td>
<td>1.00 J/gm °K</td>
</tr>
<tr>
<td><strong>ε</strong>, Egg Volume Fraction</td>
<td></td>
</tr>
<tr>
<td>Center element (4 eggs)</td>
<td>0.2670</td>
</tr>
<tr>
<td>Middle element (20 eggs)</td>
<td>0.4052</td>
</tr>
<tr>
<td>Outer element (32 eggs)</td>
<td>0.4525</td>
</tr>
<tr>
<td><strong>Y</strong>, Water Potential</td>
<td>- 802.0 kPa</td>
</tr>
<tr>
<td><strong>k_g</strong>, Egg Shell Mass Transfer Coefficient</td>
<td>~ 3.836 x 10^-7 gm/cm^2 sec torr</td>
</tr>
<tr>
<td>Sand interstices</td>
<td>1.918 x 10^-7 gm/cm^2 sec torr</td>
</tr>
<tr>
<td>Air interstices</td>
<td></td>
</tr>
<tr>
<td><strong>D</strong>, Diffusion Coefficient (H_2O)</td>
<td>0.2975 cm^2/sec</td>
</tr>
<tr>
<td><strong>T_s</strong>, Temperature of Surroundings</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>28.0 °C</td>
</tr>
<tr>
<td>Daily oscillation</td>
<td>28 - 3cos(2πt) °C</td>
</tr>
<tr>
<td><strong>Q</strong>, Metabolism</td>
<td>178/(1+exp(-0.1(t-52.1))) J/egg cm^3 day</td>
</tr>
</tbody>
</table>

---

Figure 17. Predicted temperature profile in a sand-type clutch of 116 C. mydas eggs on days 20, 40, and 60 of incubation (Ts = 28 °C)
Figure 18. Predicted temperature profile in an air-type clutch of 116 C. mydas eggs on days 20, 40, and 60 of incubation (Ts = 28 C)
The computed water vapor profiles for the air/egg clutch are shown in Figure 19. The increase in water vapor pressures within the clutch parallels the predicted temperature rise. The relative humidity, however, decreases as one moves from the edge to the center of the nest at any given time. Additionally, the relative humidity decreases at any position in the clutch as incubation proceeds. This latter effect is most extreme in the center of the nest where the relative humidity falls from 99.4% on day 20 to 98.6% on day 60. The edge of the nest does not experience as great a decrease in relative humidity because of its proximity to the surrounding medium.

Thus, from the predicted water vapor profiles for the air/egg nest we expect both a net water flux into the external environment over the course of incubation and the mass loss per egg to be greatest at the center (since the driving force for water exchange across the egg shell is largest in the clutch center where the relative humidity is lowest). The computed mass change for an egg located in each of the three clutch elements is shown in Figure 20. An egg located in the center of the nest is predicted to lose more than 3.0 gm while an egg at the edge actually increases mass slightly through day 40 before returning to nearly its initial mass. The mass changes, however, are relatively small—on the order of a few percent for a majority of the eggs.

For the sand type clutch the computed mass increase for an egg located in each of the three elements is shown in Figure 23. When sand occupies the interstices the eggs are predicted to gain mass at a nearly constant rate, reaching an average increase of 21 gms, irrespective of
Figure 19. Predicted water vapor partial pressure in the interstices of an air-type clutch on days 20, 40, and 60 of incubation (Ts = 28 C)
Figure 20. Predicted mass change for a C. mydas egg incubating in one of three locations in an air-type clutch.
Figure 21. Predicted mass change for a *C. mydas* egg incubating in one of three locations in a sand-type clutch.
location in the clutch. In this case there is no position dependence for egg mass change because it was assumed at the outset that soil dynamics were not rate limiting (the relative humidity in a sand egg clutch is 100% everywhere).

We now can compare the predictions of our sand and air-type clutch models to the single egg model of Ackerman et al. for fully buried and exposed reptile eggs. Table 4 lists the single egg results using the physical parameters in Table 3. The computed temperature rise for single eggs is not as large as for the clutch simulations. This is not unexpected since the higher surface-to-volume ratio of a single egg affords better heat transfer characteristics. The mass change for a fully buried single egg is predicted to be 13.2 gm, which is only 63% of the value calculated for a sand clutch. This difference results from the way the driving force for water vapor transport across the shell is handled in the models. For the single egg model the water vapor pressure of the surroundings is fixed for the entire calculation; as the temperature rises the driving force across the shell decreases. In the clutch model the water vapor pressure of the sand within the clutch varies according to its temperature, so the driving force will increase as clutch temperature increases. Which method is most appropriate is unclear. On the other hand, the predicted mass change for a fully exposed single egg (at 99.4% relative humidity) is in good agreement with an egg incubating in the center of an air-type clutch. In summary, it seems clear that the thermal behavior of a singly-buried egg is significantly different than a clutch egg, but the egg mass changes are comparable.
Table 4. Single egg model results (C. mydas)

<table>
<thead>
<tr>
<th>Day</th>
<th>Mass (gm)</th>
<th>Temperature (°C)</th>
<th>Mass (gm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>50.0</td>
<td>28.000</td>
<td>50.0</td>
<td>28.00</td>
</tr>
<tr>
<td>20.0</td>
<td>49.8</td>
<td>28.006</td>
<td>55.1</td>
<td>28.024</td>
</tr>
<tr>
<td>40.0</td>
<td>49.2</td>
<td>28.035</td>
<td>59.9</td>
<td>28.030</td>
</tr>
<tr>
<td>60.0</td>
<td>46.7</td>
<td>28.117</td>
<td>63.2</td>
<td>28.068</td>
</tr>
</tbody>
</table>
The model makes one other interesting prediction for incubation in an air clutch, namely, the mass transfer coefficient for water exchange across the egg shell is not as important in determining mass changes as the water diffusivity of the nest (diffusion is rate limiting). In fact, if the coefficient is decreased by an order of magnitude the predicted egg mass changes are quite similar to those in Figure 22. This suggests that mass changes for eggs in an air-type nest are highly dependent on the particular packing density rather than the egg shell conductance.

The previous clutch calculations were obtained using a constant surroundings temperature. To examine the extent that incubation of eggs in a clutch acts as a buffer against variations in environmental conditions experienced by the surroundings we simulated temperature oscillations in the substrate (sinusoidal amplitude of 3°C and a period of 1 day). The boundary conditions affected are (132-iv) and (141-v) for the sand and air clutch, respectively.

In both clutch types the predicted average temperature profiles within the nest for fluctuating surroundings temperature did not differ from the constant temperature cases shown in Figures 17 and 18. The egg mass changes yielded a similar result. Thus the average behavior is unchanged. If a particular day is examined, however, the buffering role of the clutch is more apparent. Figure 22 illustrates the temperature variations predicted for an egg incubating in either the center, middle or outer element of the sand-type clutch on day 45. The input surroundings temperature and the predictions from the single egg model
Figure 22. Predicted egg temperature on day 45 for three different locations in a sand-type C. mydas clutch. The surrounding substrate is oscillated around 28 °C with a period of one day and an amplitude of 3 °C. Computed results for a single egg are shown for comparison.
Figure 23. Predicted egg temperature on day 45 for three different locations in an air-type C. mydas clutch. The surrounding substrate is oscillated around 28 C with a period of one day and an amplitude of 3 C. Computed results for a single egg are shown for comparison.
are also shown. An egg in the center of the clutch experienced a
temperature variation of only 2.5°C while the surroundings varied by 6°C;
an amplitude ratio of 0.42. The other two elements in the sand clutch
are also moderately damped. Note that the clutch response lags behind
the surroundings by roughly 6 hours. In contrast, the single egg
especially tracks with the oscillations in the surrounding medium
(amplitude ratio = 1.0, phase = 0.0). Figure 23 shows the corresponding
calculations on day 45 for an air clutch and a fully exposed single egg.
The temperature amplitude of an egg located in the clutch center is
damped (amplitude ratio = 0.60), but not as effectively as in the sand
clutch. The outer element of the air clutch is undamped. The phase
shifts are slightly larger for each element. Again the single egg is
relatively undamped compared to the surroundings and oscillates in phase.

The different responses for the sand and air-type clutches are
easily understood when the larger thermal mass and higher thermal
conductivity of the sand clutch is considered. The most significant
observation is undoubtedly the buffering of egg temperatures in a clutch
compared to single eggs. This is attributable to the better heat
transfer characteristics of a single egg resulting from its higher
surface-to-volume ratio. The biological advantage of clutch incubation
as far as temperature regulation is concerned, seems clear—clutch
incubation moderates the microenvironment of the eggs.

C. Simulation Conclusions

Models have been developed that describe the simultaneous heat and
mass transfer of eggs in air and external media-filled clutches. In both
cases conservation relationships for energy and mass were solved numerically using orthogonal collocation on finite elements and an implicit integration scheme to yield temperature, water vapor pressure and egg mass profiles throughout the nest. The computational method is flexible enough to handle a wide range of physical properties for the clutch or external environment.

Several conclusions are significant from the computational results for incubation of _C. mydas_ eggs:

(1) The temperature at the center of the nest rose 1.25°C and 4.00°C for the sand and air-type clutch, respectively. Egg mass changes for the air clutch were relatively small while for the sand clutch a 21.0 gm increase is predicted.

(2) The difference in temperature rise calculated for the two clutch types is due to the thermal conductivity of the material filling the clutch interstices. Sand has an order of magnitude greater thermal conductivity and is therefore able to dissipate heat to the surroundings at a higher rate. The egg mass changes for the nests demonstrates the effect of two extremes in water vapor transport: for the air clutch the rate limiting step is diffusion within the nest while for the sand clutch the transport across the egg shell is limiting.

(3) In comparing the single egg model with the clutch model the calculated egg mass changes are comparable but the temperature profiles are quite different because of the better heat transfer characteristics of the single egg.

(4) It seems clear that the clutch plays a significant role in regulating the microenvironment of incubating eggs. This is particularly
evident in the air clutch which buffers the egg mass for a wide variety of egg shell mass transfer coefficients. We have also seen that the clutch will moderate the thermal oscillations that might occur in the surroundings.
V. CONCLUSIONS

The theoretical approach presented in this paper has been aimed at elucidating embryonic energetics. We have developed two growth models which are based on fundamental biochemical principles and also examined energetic effects during clutch incubation. In the models, the complex metabolism of growing biological systems is adequately described by considering generalized pathways for processes of synthesis, turnover/utilization, respiration, activation, and maintenance. Macroscopic parameters such as growth rate, oxygen consumption, heat production, and growth efficiency are shown to depend on the adenosine nucleotide levels in the organism. Both models are substantiated by experimental data. These theoretical expressions allow us to examine and interpret the growth of different organisms, or growth in various environments.

From the kinetic model several specific conclusions are apparent: 1) the tissue growth rates of brain and liver in the embryonic chicken and also the growth of *E. coli* are well described by ATP levels and the phosphorylation ratio; 2) the oxygen consumption rate of *Oryzias latipes* is similarly related to the amount of ADP and the phosphorylation ratio during incubation; 3) the oxygen tension in the spinal cord of the chick embryo can be estimated and agrees with the experimentally determined trend during the last several days of incubation; 4) The oxygen consumption and heat production of the extraembryonic tissues is shown to be significant in early development and appears to have higher
maintenance costs than embryonic tissues; 5) there was shown to be no effect on the underlying metabolism for a longer incubation period (chick vs. shearwater) or for incubation at high altitude (sea level chick vs. 3100m chick); 6) the growth efficiency depends on the phosphorylation ratio and is significantly higher in *Oryzias* than the chick.

The nonequilibrium thermodynamic growth model also yielded relationships for oxygen consumption and growth rate that depended on the phosphorylation ratio and the ATP or ADP level. In addition, phenomenological coefficients were calculated for *Oryzias* during growth. While not offering as much metabolic insight as the kinetic model, the nonequilibrium thermodynamic model is a significant improvement over attempts of previous investigators.

And finally, the relationship between embryonic energetics and the surrounding microenvironment has been examined for the particular case of eggs incubating in a clutch. The temperature behavior of eggs in a clutch was predicted to be markedly different than that of a single egg. The medium that filled the clutch interstices (sand or vapor) had a profound effect on the heat and mass transfer characteristics of the nest. A biological advantage of clutch incubation was shown to be the ability to buffer the microenvironment.

Obviously, the simplified models that we have developed here will not always apply. It is hoped, however, that this approach will serve as a starting point for future modeling attempts that incorporate more complex networks.
VI. RECOMMENDATIONS

The objective of this research project was to develop a framework in which embryonic energetics could be evaluated and interpreted. While the simple relationships derived here have allowed us to examine a wide range of existing data, they also suggest areas where continued work would be useful.

1) Experimental studies need to be undertaken that emphasize the measurement of parameters used in the kinetic model (primarily dry tissue growth rates and adenosine nucleotide levels) during the course of normal or abnormal growth. Microbial organisms would seem to be a particularly good system to concentrate on for this analysis. As well as supplying specific information on the underlying metabolic processes, this will test the model limitations.

2) The metabolic model was developed to describe the energetics associated with the formation of tissue—but it could equally describe the production of any other biological product. It would be interesting to apply the kinetic relationships to microbial systems in order to understand how macroscopic parameters can be manipulated to optimize the desired product.

3) The kinetic model can be expanded by allowing other types of energy metabolism. For example, the incorporation of anaerobic or photosynthetic pathways would greatly increase the utility of the model.
4) Second law considerations for growing bioorganisms have been of interest for some time. The nonequilibrium thermodynamic model can be used to approach questions such as the time course of entropy production rates and stationary states.

5) The clutch simulation models could be improved by adding a more accurate description of soil dynamics under various water contents. In addition, the polar asymmetry of a clutch in the field could be taken care of by adding another space coordinate, although at this stage the effort might be better directed at experimental measurements of egg clutches.


VIII. ACKNOWLEDGEMENTS

I would like to thank Dr. Richard Seagrave for his continuous support and guidance throughout all phases of this project. I also wish to express my appreciation to Dr. Ralph Ackerman whose enthusiastic work in this area was in large part responsible for both my initial interest and many of the ideas which were subsequently developed.
IX. APPENDIX: EGG CLUTCH SIMULATION PROGRAMS

The computer programs listed below solve the coupled energy and mass equations for incubation in an external medium or an air-type clutch.

The method of OCFE is discussed in Finlayson (1980), and the nomenclature used in the programs is as similar to this as possible.

The sand clutch program is:

1. THIS PROGRAM USES OCFE METHOD AND AN IMPLICIT INTEGRATION PACKAGE TO SOLVE FOR TEMPERATURE AND MASS PROFILES IN A SAND CLUTCH. THE ENERGY AND MASS EQUATIONS ARE:

   \[
   \frac{dT}{dt} = a(\Delta) T + RE \\
   \frac{dM}{dt} = KG*\alpha*(PC-PE)
   \]

   WITH AUXILIARY CONDITIONS:

   \[ @ r=0, \frac{dT}{dt} & \frac{dM}{dt} = 0 \]
   \[ @ r=13.9cm -k(\frac{dT}{dr}) = h(T - TS) \]
   \[ @ t=0, T = To & M = Mo \]

   WHERE

   \[ RE = \text{METABOLIC HEAT PRODUCTION, J/ EGG CM3 DAY} \]
   \[ a = \text{THERMAL DIFFUSIVITY, CM2/DAY} \]
   \[ KG = \text{EGG SHELL MASS TRANSFER COEFFICIENT, GM/CM2 DAY TORR} \]
   \[ \alpha = \text{EGG SURFACE AREA / CLUTCH VOLUME, CM2/CM3} \]
   \[ M = \text{MASS, GM} \]
   \[ k = \text{THERMAL CONDUCTIVITY, J/CM DAY C} \]
   \[ PC = \text{PARTIAL PRESSURE OF H2O IN INTERSTICES, TORR} \]
   \[ PE = \text{PARTIAL PRESSURE OF H2O IN EGG, TORR} \]
   \[ h = \text{HEAT TRANSFER COEFFICIENT, J/CM2 DAY C} \]
   \[ T = \text{TEMPERATURE, C} \]

   THREE ELEMENTS OF WIDTH 5.3, 4.3, AND 4.3 CM ARE USED AND CUBIC LAGRANGIAN BASIS FUNCTIONS HAVE BEEN CHOSEN FOR EACH ELEMENT. THE TEMPERATURE VARIABLES ARE:

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>RADIAL POSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>0.0</td>
</tr>
<tr>
<td>Y(1)</td>
<td>(@ ORTH. ROOT)</td>
</tr>
<tr>
<td>Y(2)</td>
<td></td>
</tr>
<tr>
<td>Y2</td>
<td>5.3</td>
</tr>
</tbody>
</table>
THE MASS VARIABLES Y(7), Y(8), AND Y(9) CORRESPOND TO THE MASSES OF THE THREE ELEMENTS FROM CENTER TO OUTER. PARAMETERS CAN BE CHANGED IN THE DATA STATEMENT IN SUBROUTINE FCN.

INTEGER N,METH,MITER,INDEX,IWK(9),IER,K
COMMON Y1,Y2,Y3,Y4,K3,BI,P1,P2,P3,P4,P5,P6,P7,P8,P9,P10
REAL Y(9),WK(180),X,TOL,XEND,H,K3,Pl,P2,P3,P4,P5,P6,P7,P8
EXTERNAL FCNJ
N = 9
SET INITIAL TIME AND INITIAL CONDITIONS
X = 0.0
Y(1) = 28.0
Y(2) = 28.0
Y(3) = 28.0
Y(4) = 28.0
Y(5) = 28.0
Y(6) = 28.0
Y(7) = 623.615
Y(8) = 3082.36
Y(9) = 7534.52
SET INTEGRATION PARAMETERS FOR DGEAR (IMSL)
TOL = 0.00001
H = 0.00000001
METH = 2
MITER = 2
INDEX = 1
START INTEGRATION LOOP - SPECIFYING THE TIMES AT WHICH OUTPUTS ARE DESIRED WITH XEND
DO 10 K = 1,3
  XEND = FLOAT(K)*20.0
  CALL DGEAR (N,FCN,FCNJ,X,H,Y,XEND,TOL,METH,MITER,INDEX,IER)
  IF(IER.GT.128)THEN
    PRINT DGEAR ERROR MESSAGE AND CHECK WHERE YOU ARE IF FAILED
    PRINT,'IER GT 128' ,Y,XEND,H,X
    STOP
  ELSE
    SATISFY AUXILLIARY CONDITIONS FOR LATEST SOLUTION
    CALL FCN(N,X,Y,YPRIME)
    PRINT OUTPUT WHEN SUCCESSFUL THEN CONTINUE
    PRINT,'PROFILE AT TIME' ,XEND,Y1,Y(1),Y(2),Y2,Y(3),Y(4),
    # Y3,Y(5),Y(6),Y4
    PRINT,'MASS',Y(7),Y(8),Y(9)
THIS SUBROUTINE EVALUATES THE ODE'S FOR USE IN DGEAR
THE VARIABLES USED IN THE DATA STATEMENTS ARE THE FOLLOWING:

- R1, R2, ... = POSITION OF COLLOCATION POINTS NORMALIZED TO CLUTCH RADIUS
- H1, H3, H3 = ELEMENT WIDTH (CENTER TO OUTER) NORMALIZED TO CLUTCH RADIUS
- ALPHA01, ... = THERMAL DIFFUSIVITY OF ELEMENT 1 (CENTER), CM2/DAY
- K10, ... = THERMAL CONDUCTIVITY OF ELEMENT 1 (CENTER), J/CM DAY C
- TS = TEMPERATURE OF SURROUNDINGS, C
- S1, S2, S3 = EGG SURFACE AREA/CLUTCH VOLUME, CM2/CM3
- BI = BIOT NUMBER
- A11, A12, ... = OC APPROXIMATIONS TO FIRST DERIVATIVE
- B21, B22, ... = OC APPROXIMATIONS TO SECOND DERIVATIVE
- AW = WATER ACTIVITY COEFFICIENT IN EGG
- C1, C2, C3 = ANTOINE VAPOR PRESSURE CONSTANTS FOR WATER
- KG = EGG SHELL MASS TRANSFER COEFFICIENT, GM/CM2 DAY TORR
- A1, A2, A3 = EGG AREA/CLUTCH VOLUME, CM2/CM3
- W1, W2, ... = WEIGHTING FACTORS FOR NUMERICAL INTEGRATION

THE DATA IN THE DATA STATEMENTS ARE FOR THE INCUBATION OF 116 C. MYDAS EGGS.
# 2.43577,1.883683,0.1497/

C SPECIFY THE HEAT PRODUCTION/UNIT AREA*EGG

Q = 43.4062/(1+EXP(-0.1*(X-52.1)))

C MAKE CONDUCTIVITIES FUNCTIONS OF METABOLISM (IF DESIRED)

K1 = K10
K2 = K20
K3 = K30
ALPHA1 = ALPH01
ALPHA2 = ALPH02
ALPHA3 = ALPH03

C SET THE MATRIX FOR THE BOUNDARY AND FLUX CONTINUITY REQUIRED

B(1) = (-1.0)*(A12*T(1)+A13*T(2))
B(2) = (K2/H2)*(A12*T(3)+A13*T(4))-(K1/H1)*(A42*T(1)+A43*T(2))
B(3) = (K3/H3)*(A12*T(5)+A13*T(6))-(K2/H2)*(A42*T(3)+A43*T(4))
B(4) = A42*T(5)+A43*T(6)-H3*BI*TS

A(1,1) = 11
A(1,2) = A14
A(1,3) = 0.0
A(1,4) = 0.0
A(2,1) = K1*A41/H1
A(2,2) = (K1/H1)*A44-(K2/H2)*A11
A(2,3) = -K2*A14/H2
A(2,4) = 0.0
A(3,1) = 0.0
A(3,2) = K2*A41/H2
A(3,3) = (K2/H2)*A44-(K3/H3)*A11
A(3,4) = -K3*A14/H3
A(4,1) = 0.0
A(4,2) = 0.0
A(4,3) = -A41
A(4,4) = -A44-H3*BI

C SET PARAMETERS FOR EQUATION SOLVER

MM = 1
NN = 4
IA = 4
IDGT = 0

C SOLVE FOR ELEMENT END TEMPS USING ALGEBRAIC SOLVER LEQT1F

CALL LEQT1F (A,MM,NN,IA,B,IDGT,WKAREA,IER)

C RETRIEVE SOLUTION

T1 = B(1)
T2 = B(2)
T3 = B(3)
T4 = B(4)

C SPECIFY THE ODE'S TO BE SOLVED

YPRIME(1) = (ALPHA1/H1**2)*((B21*T1+B22*T(1)+B23*T(2)+B24*T2)
# +(2*ALPHA1/(H1*RI1))*(A21*T1+A22*T(1)+A23*T(2)+A24*T2)+S1*Q

YPRIME(2) = (ALPHA1/H1**2)*((B31*T1+B32*T(1)+B33*T(2)+B34*T2)
# +(2*ALPHA1/(H1*RI2))*(A31*T1+A32*T(1)+A33*T(2)+A34*T2)+S1*Q
The air clutch program is:

1 C THIS PROGRAM USES OCFE METHOD AND AN IMPLICIT INTEGRATION PACKAGE
2 C TO SOLVE FOR TEMPERATURE AND MASS DISTRIBUTIONS IN AN AIR CLUTCH.
3 C THE ENERGY AND MASS EQUATIONS ARE:
4 C
\[ \frac{dT}{dt} = a(\Delta l)^2 T + \left( 1/RHO*Cp \right) (RE - HVAP*KG*A*(PE - PC)) \]
\[ \frac{dCH2O}{dt} = D(\Delta l)^2 CH2O + KG*A*(PE - PC) \]

WITH AUXILIARY CONDITIONS:

\[ @ r=0, \frac{dT}{dt} & \frac{dCH2O}{dt} = 0 \]
\[ @ r=13.9cm, -k(\frac{dT}{dr}) = h(T - TS) \]
\[ @ t=0, T = T_0 & CH2O = CH2O_0 \]
\[ @ r=13.9cm, CH2O = CH2O(SAT'D AT T(r=13.9)) \]

WHERE

\[ RE = METABOLIC HEAT PRODUCTION, J/ EGG CM3 DAY \]
\[ a = THERMAL DIFFUSIVITY, CM2/DAY \]
\[ KG = EGG SHELL MASS TRANSFER COEFFICIENT, GM/CM2 DAY TORR \]
\[ A = EGG SURFACE AREA / CLUTCH VOLUME, CM2/CM3 \]
\[ k = THERMAL CONDUCTIVITY, J/CM DAY C \]
\[ PC = PARTIAL PRESSURE OF H2O IN INTERSTICES, TORR \]
\[ PE = PARTIAL PRESSURE OF H2O IN EGG, TORR \]
\[ h = HEAT TRANSFER COEFFICIENT, J/CM2 DAY C \]
\[ T = TEMPERATURE, C \]
\[ HVAP = SPECIFIC HEAT OF VAPORIZATION FOR WATER, J/GM \]
\[ RHO = DENSITY, GM/CM3 \]
\[ Cp = HEAT CAPACITY, J/GM C \]
\[ CH2O = WATER VAPOR CONCENTRATION, GM/CM3 \]

THREE ELEMENTS OF WIDTH 5.3, 4.3, AND 4.3 CM ARE USED AND CUBIC LAGRANGIAN BASIS FUNCTIONS HAVE BEEN CHOSEN FOR EACH ELEMENT. THE TEMPERATURE AND CONCENTRATION VARIABLES ARE:

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>RADIAL POSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>CH2O</td>
</tr>
<tr>
<td>Y1, Y5</td>
<td>0.0</td>
</tr>
<tr>
<td>Y(1), Y(7)</td>
<td>(@ ORTH. ROOT)</td>
</tr>
<tr>
<td>Y(2), Y(8)</td>
<td>.</td>
</tr>
<tr>
<td>Y2, Y6</td>
<td>5.3</td>
</tr>
<tr>
<td>Y(3), Y(9)</td>
<td>.</td>
</tr>
<tr>
<td>Y(4), Y(10)</td>
<td>.</td>
</tr>
<tr>
<td>Y3, Y7</td>
<td>9.6</td>
</tr>
<tr>
<td>Y(5), Y(11)</td>
<td>.</td>
</tr>
<tr>
<td>Y(6), Y(12)</td>
<td>.</td>
</tr>
<tr>
<td>Y4, Y8</td>
<td>13.9</td>
</tr>
</tbody>
</table>

THE MASS VARIABLES Y(13), Y(14), AND Y(15) CORRESPOND TO THE THREE ELEMENTS FROM CENTER TO OUTER AND ARE CALCULATED AS A FLUX DIFFERENCE INTO/OUT-OF THE ELEMENT. PARAMETERS CAN BE CHANGED IN THE DATA STATEMENT IN SUBROUTINE FCN.
56 C
57 C
58 INTEGER N,METH,MITER,INDEX,IWK(15),IER,K
59 COMMON Y1,Y2,Y3,Y4,K3,BI,P1,P2,P3,P4,P5,P6,P7,P8,P9,P10,Y5
60 # ,Y6,Y7,Y8,CREF
61 REAL Y(15),WK(390),X,TOL,XEND,H,K3,P1,P2,P3,P4,P5,P6,P7,P8
62 # ,P9,P10,YPRIME(15)
63 EXTERNAL FCN,FCNJ
64 N = 15
65 C SET INITIAL TIME AND INITIAL CONDITIONS
66 X = 0.0
67 Y(1) = 28.0
68 Y(2) = 28.0
69 Y(3) = 28.0
70 Y(4) = 28.0
71 Y(5) = 28.0
72 Y(6) = 28.0
73 Y(7) = 1.0
74 Y(8) = 1.0
75 Y(9) = 1.0
76 Y(10) = 1.0
77 Y(11) = 1.0
78 Y(12) = 1.0
79 Y(13) = 200.0
80 Y(14) = 1500.0
81 Y(15) = 4100.0
82 Y8 = 1.
83 CREF = 2.7371E-5
84 C SET INTEGRATION PARAMETERS FOR DGEAR (IMSL)
85 TOL = 0.001
86 H = 0.0000000001
87 METH = 2
88 MITER = 2
89 INDEX = 1
90 C START INTEGRATION LOOP - SPECIFYING THE TIMES AT WHICH OUTPUTS ARE
91 C DESIRED WITH XEND
92 DO 10 K = 1,3
93 XEND = FLOAT(K)*20.0
94 CALL DGEAR (N,FCN,FCNJ,X,H,Y,XEND,TOL,METH,MITER,INDEX,
95 # IWK,WK,IER)
96 IF(IER.GT.128)THEN
97 C PRINT DGEAR ERROR MESSAGE AND CHECK WHERE YOU ARE IF FAILED
98 PRINT,'IER GT 128',Y,XEND,H,X
99 STOP
100 ELSE
101 C SATISFY AUXILIARY CONDITIONS FOR LATEST SOLUTION
102 CALL FCN(N,X,Y,YPRIME)
103 C PRINT OUTPUT WHEN SUCCESSFUL THEN CONTINUE
104 PRINT,'PROFILE AT TIME',XEND,Y1,Y(1),Y2,Y(2),Y3,Y(3),Y4,
105 # Y3,Y(5),Y(6),Y4
106 PRINT,'CONCENTRATION',Y5,Y(7),Y(8),Y6,Y(9),Y(10),Y7
124

107     #  ,Y(11),Y(12),Y8
108     PRINT,'MASS',Y(13),Y(14),Y(15)
109     ENDIF
110     10 CONTINUE
111     STOP
112     END
113     C
114     C
115     C
116     C THIS SUBROUTINE EVALUATES THE ODE'S FOR USE IN DGEAR
117     C
118     C THE VARIABLES USED IN THE DATA STATEMENTS ARE THE FOLLOWING:
119     C
120     C     R1, R2, ... = POSITION OF COLLOCATION POINTS NORMALIZED
121     C     TO CLUTCH RADIUS
122     C     H1, H3, H3 = ELEMENT WIDTH (CENTER TO OUTER) NORMALIZED
123     C     TO CLUTCH RADIUS
124     C     ALPHAO1, ... = THERMAL DIFFUSIVITY OF ELEMENT 1 (CENTER),
125     C     CM2/DAY
126     C     K10, ... = THERMAL CONDUCTIVITY OF ELEMENT 1 (CENTER),
127     C     J/CM DAY C
128     C     TS = TEMPERATURE OF SURROUNDINGS, C
129     C     S1, S2, S3 = EGG SURFACE AREA/CLUTCH VOLUME, CM2/CM3
130     C     BI = BIOT NUMBER
131     C     A1, A12, ... = OC APPROXIMATIONS TO FIRST DERIVATIVE
132     C     B21, B22, ... = OC APPROXIMATIONS TO SECOND DERIVATIVE
133     C     AW = WATER ACTIVITY COEFFICIENT IN EGG
134     C     C1, C2, C3 = ANTOINE VAPOR PRESSURE CONSTANTS FOR WATER
135     C     KG = EGG SHELL MASS TRANSFER COEFFICIENT,
136     C     GM/CM2 DAY TORR
137     C     A1, A2, A3 = EGG AREA/CLUTCH VOLUME, CM2/CM3
138     C     W1, W2, ... = WEIGHTING FACTORS FOR NUMERICAL INTEGRATION
139     C     DIFIO, ... = DIFFUSIVITY OF WATER VAPOR IN CENTER ELEMENT,
140     C     CM2/DAY
141     C     HVAP = HEAT OF VAPORIZATION FOR WATER, J/GM
142     C     R = GAS CONSTANT,
143     C     RHCP1, ... = PRODUCT OF DENSITY AND HEAT CAPACITY OF
144     C     CENTER ELEMENT, J/CM3 C
145     C
146     C THE DATA IN THE DATA STATEMENTS ARE FOR THE INCUBATION OF 116
147     C. MYDAS EGGS.
148     C
149     C
150     SUBROUTINE FCN(N,X,T,YPRIME)
151     INTEGER N
152     COMMON T1,T2,T3,T4,K3,NI,P1,P2,P3,P4,P5,P6,P7,P8,P9,P10
153     # ,T5,T6,T7,T8,CREF
154     REAL T(N),YPRIME(N),X,K10,K20,K30,A(7,7),B(7),WKAREA(49)
155     # ,K1,K2,K3,P1,P2,P3,P4,P5,P6,P7,P8,P9,P10,KG,HVAP
156     DATA R1,R2,R3,R4,R5,R6/0.0806,.3813,.3093,.3094/,
157     # 0.934/,.3093,.3094/,
158     # .7525,.3093,.3094/,
159     # .7525,.3093,.3094/,
DATA SI,S2,33/0.6280,0.5080,0.4758/,BI/5.621/,All,A12,A13,A14,
// A21,A22,A23,A24,A31,A32,A33,A34,A41,A42,A43,A44/-7.0,8.196,# -2.196,1.0,-2.732,1.732,1.732,-0.732,0.732,-1.732,-1.732,
# 2.732,1.0,2.196,-8.196,7.0/,B21,B22,B23,B24,B31,B32,B33,
# B34/16.39,-24.0,12.0,-4.392,-4.392,12.0,-24.0,16.39/
DATA AW/0.994/,CI,C2,C3/8.10765,1750.286,235.0/,KG/0.01657/,Al,A2,A3/0.3726,0.5654,0.6314 /,DIF10,DIF20,DIF30/12560.70,10192.5,9381.96/,HVAP/2430.58/,R/62360./,RHCPl,RHCP2,RHCP3/
# 0.593,1.113,1.327/

SPECIFY THE HEAT PRODUCTION/UNIT AREA*EGG
Q = 89.1757*(1.5/2.15)**2/(1+EXP(-0.1*(X-52.1)))

MAKE CONDUCTIVITIES FUNCTIONS OF METABOLISM (IF DESIRED)

K1 = K10
K2 = K20
K3 = K30
ALPHA1 = ALPHOl
ALPHA2 = ALPH02
ALPHA3 = ALPH03
DIF1 = DIF10
DIF2 = DIF20
DIF3 = DIF30

SET THE MATRIX FOR THE BOUNDARY AND FLUX CONTINUITY REQUIRED

B(1) = (-1.0)*(A12*T(1)+A13*T(2))
B(2) = (K2/H2)*(A12*T(3)+A13*T(4))-(K1/H1)*(A42*T(1)+A43*T(2))
B(3) = (K3/H3)*(A12*T(5)+A13*T(6))-(K2/H2)*(A42*T(3)+A43*T(4))
B(4) = (A42*T(5)+A43*T(6)-H3*BI*TS+(DIF3*HVAP*CREF/K3)*
# (A42*T(11)+A43*T(12)+A44*T8)
B(5) = (-1.0)*(A12*T(7)+A13*T(8))
B(6) = (DIF2/H2)*(A12*T(9)+A13*T(10))-(DIF1/H1)*(A42*T(7)+A43*T(8))
# A43*T(10))

A(1,1) = A11
A(1,2) = A14
A(1,3) = 0.0
A(1,4) = 0.0
A(1,5) = 0.0
A(1,6) = 0.0
A(1,7) = 0.0
A(2,1) = K1*A41/H1
A(2,2) = (K1/H1)*A44-(K2/H2)*A11
A(2,3) = -K2*A14/H2
A(2,4) = 0.0
A(2,5) = 0.0
A(2,6) = 0.0
A(2,7) = 0.0
A(3,1) = 0.0
A(3,2) = K2*A41/H2
A(3,3) = (K2/H2)*A44-(K3/H3)*A11
A(3,4) = -K3*A14/H3
A(3,5) = 0.0
\[ A(3,6) = 0.0 \]
\[ A(3,7) = 0.0 \]
\[ A(4,1) = 0.0 \]
\[ A(4,2) = 0.0 \]
\[ A(4,3) = -A41 \]
\[ A(4,4) = -A44-H3*BI \]
\[ A(4,5) = 0.0 \]
\[ A(4,6) = 0.0 \]
\[ A(4,7) = -DIF3*HVAP*CREF*A41/K3 \]
\[ A(5,1) = 0.0 \]
\[ A(5,2) = 0.0 \]
\[ A(5,3) = 0.0 \]
\[ A(5,4) = 0.0 \]
\[ A(5,5) = A11 \]
\[ A(5,6) = A14 \]
\[ A(5,7) = 0.0 \]
\[ A(6,5) = DIF1*A41/H1 \]
\[ A(6,6) = (DIF1/H1)*A44-(DIF2/H2)*A11 \]
\[ A(6,7) = -DIF2*A14/H2 \]
\[ A(6,4) = 0.0 \]
\[ A(6,3) = 0.0 \]
\[ A(6,2) = 0.0 \]
\[ A(6,1) = 0.0 \]
\[ A(7,5) = 0.0 \]
\[ A(7,6) = DIF2*A41/H2 \]
\[ A(7,7) = (DIF2/H2)*A44-(DIF3/H3)*A11 \]
\[ A(7,4) = 0.0 \]
\[ A(7,3) = 0.0 \]
\[ A(7,2) = 0.0 \]
\[ A(7,1) = 0.0 \]

C SET PARAMETERS FOR EQUATION SOLVER

MM = 1
NN = 7
IA = 7
IDGT = 0

C SOLVE FOR ELEMENT ENDS USING ALGEBRAIC SOLVER LEQT1F

CALL LEQT1F (A,MM,NN,IA,B,IDGT,WKAREA,IER)

C RETRIEVE SOLUTION

T1 = B(1)
T2 = B(2)
T3 = B(3)
T4 = B(4)
T5 = B(5)
T6 = B(6)
T7 = B(7)

C USE ANTOINE EQUATION FOR WATER VAPOR PRESSURE

P1 = AW*(10.0**((C1-C2)/(T1+C3)))
P2 = AW*(10.0**((C1-C2)/(T(1)+C3)))
P3 = AW*(10.0**((C1-C2)/(T(2)+C3)))
P4 = AW*(10.0**((C1-C2)/(T2+C3)))
P5 = AW*(10.0**(C1-C2/(T3+C3)))
P6 = AW*(10.0**(C1-C2/(T4+C3)))
P7 = AW*(10.0**(C1-C2/(T3+C3)))
P8 = AW*(10.0**(C1-C2/(T5+C3)))
P9 = AW*(10.0**(C1-C2/(T6+C3)))
P10 = AW*(10.0**(C1-C2/(T4+C3)))

T8 = P10*18./(AW*R*(T4+273.15)*CREF)

YPRIME(l) = (ALPHA1/H1**2)*(B21*T1+B22*T(1)+B23*T(2)+B24*T2)
# + (2*ALPHA1/(H1*R1))*(A21*T1+A22*T(1)+A23*T(2)+A24*T2)+S1*T

# -(HVAP*KG*A1/RHC1)*(P2-R*(T(1)+273.15)) = T(7)*CREF/18.)

YPRIME(2) = (ALPHA2/H2**2)*(B31*T2+B32*T(2)+B33*T(3)+B34*T3)
# + (2*ALPHA2/(H2*R2))*(A31*T2+A32*T(2)+A33*T(3)+A34*T3)+S2*T

# -(HVAP*KG*A2/RHC2)*(P3-R*(T(2)+273.15)) = T(8)*CREF/18.)

YPRIME(3) = (ALPHA3/H3**2)*(B21*T3+B22*T(3)+B23*T(4)+B24*T4)
# + (2*ALPHA3/(H3*R3))*(A21*T3+A22*T(3)+A23*T(4)+A24*T4)+S3*T

# -(HVAP*KG*A3/RHC3)*(P4-R*(T(3)+273.15)) = T(9)*CREF/18.)

YPRIME(4) = (ALPHA2/H2**2)*(B31*T3+B32*T(3)+B33*T(4)+B34*T4)
# + (2*ALPHA2/(H2*R4))*(A31*T3+A32*T(3)+A33*T(4)+A34*T4)+S2*T

# -(HVAP*KG*A2/RHC4)*(P5-R*(T(4)+273.15)) = T(10)*CREF/18.)

YPRIME(5) = (ALPHA3/H3**2)*(B21*T4+B22*T(4)+B23*T(5)+B24*T5)
# + (2*ALPHA3/(H3*R5))*(A21*T4+A22*T(4)+A23*T(5)+A24*T5)+S3*T

# -(HVAP*KG*A3/RHC5)*(P6-R*(T(5)+273.15)) = T(11)*CREF/18.)

YPRIME(6) = (ALPHA3/H3**2)*(B31*T4+B32*T(4)+B33*T(5)+B34*T5)
# + (2*ALPHA3/(H3*R6))*(A31*T4+A32*T(4)+A33*T(5)+A34*T5)+S3*T

# -(HVAP*KG*A3/RHC6)*(P7-R*(T(6)+273.15)) = T(12)*CREF/18.)

YPRIME(7) = (DIF1/(13.9*H1)**2)*(B21*T5+B22*T(5)+B23*T(6)+B24*T7)
# +B24*T6+(2*DIF1/(H1*R1*(13.9)**2))*(A21*T5+A22*T(5)+A23*T(6)+A24*T7)

# -(HVAP*KG*A1/RHC1)*(P7-R*(T(5)+273.15)) = T(7)*CREF/18.)

YPRIME(8) = (DIF1/(13.9*H1)**2)*(B31*T6+B32*T(6)+B33*T(7)+B34*T8)
# +B34*T7+(2*DIF1/(H1*R2*(13.9)**2))*(A31*T6+A32*T(6)+A33*T(7)+A34*T8)

# -(HVAP*KG*A2/RHC2)*(P8-R*(T(7)+273.15)) = T(8)*CREF/18.)

YPRIME(9) = (DIF2/(13.9*H2)**2)*(B21*T6+B22*T(7)+B23*T(8)+B24*T9)

# -(HVAP*KG*A1/RHC3)*(P9-R*(T(8)+273.15)) = T(9)*CREF/18.)

YPRIME(10) = (DIF2/(13.9*H2)**2)*(B31*T7+B32*T(8)+B33*T(9)+B34*T10)

# -(HVAP*KG*A2/RHC4)*(P10-R*(T(9)+273.15)) = T(10)*CREF/18.)

YPRIME(11) = (DIF3/(13.9*H3)**2)*(B21*T7+B22*T(9)+B23*T(10)+B24*T11)
# +B24*T10+(2*DIF3/(H3*R5*(13.9)**2))*(A21*T7+A22*T(9)+A23*T(10)+A24*T11)

# -(HVAP*KG*A3/RHC5)*(P11-R*(T(10)+273.15)) = T(11)*CREF/18.)
YPRIME(12) = \frac{DIF3}{(13.9 \cdot H3)^2} \cdot (B31 \cdot T7 + B32 \cdot T(11) + B33 \cdot T(12)) + \frac{2 \cdot DIF3}{H3 \cdot R6 \cdot (13.9)^2} \cdot (A31 \cdot T7 + A32 \cdot T(11) + A33 \cdot T(12)) + T(12) + A34 \cdot T8) + KG \cdot A3 \cdot \left(\frac{P9}{CREF} - R \cdot (T(6) + 273.15) \cdot T(12) / 18\right)

YPRIME(13) = 25.395 \cdot DIF1 \cdot (A41 \cdot T5 + A42 \cdot T(7) + A43 \cdot T(8) + A44 \cdot T6)

YPRIME(14) = 83.3180 \cdot DIF2 \cdot (A41 \cdot T6 + A42 \cdot T(9) + A43 \cdot T(10) + A44 \cdot T7)

YPRIME(15) = 174.673 \cdot DIF3 \cdot (A41 \cdot T7 + A42 \cdot T(11) + A43 \cdot T(12) + A44 \cdot T8)

RETURN
END

DUMMY SUBROUTINE FOR DGEAR

SUBROUTINE FCNJ(N,X,Y,PD)
INTEGER N
REAL Y(N),PD(N,N),X
RETURN
END

ENTRY