Pure Appl. Chem., Vol. 81, No. 10, pp. 1843–1855, 2009.
doi:10.1351/PAC-CON-08-09-09

Biological calorimetry and the thermodynamics of the origination and evolution of life*

Lee D. Hansen[1,‡], Richard S. Criddle[1], and Edwin H. Battley[2]

[1]Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA; [2]Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA

Abstract: Calorimetric measurements on biological systems from small molecules to whole organisms lead to a new conception of the nature of live matter that has profound consequences for our understanding of biology. The data show that the differences in Gibbs energy (ΔG) and enthalpy (ΔH) are near zero or negative and the difference in entropy (ΔS) is near zero between a random mixture of molecules and live matter of the same composition. A constant input of energy is required to maintain ion gradients, ATP production, and the other functions of living matter, but because cells are organized in a spontaneous process, no energy input is required to maintain the structure or organization of cells. Thus, the origin of life and evolution of complex life forms occurs by thermodynamically spontaneous processes, carbon-based life should be common throughout the universe, and because there is no energy cost, evolution can occur relatively rapidly.

Keywords: calorimetry; enthalpy; entropy; evolution; Gibbs energy; origin of life; thermodynamics; theory.

INTRODUCTION

The energy costs of life formation have long presented questions of great general interest, but these costs have not previously been thoroughly analyzed. Calorimetry has now provided the means for a quantitative analysis. The concept of live matter as a high-energy, low-entropy, metastable state of matter gained general acceptance following publication of the influential book, *What is Life?* by Erwin Schrödinger [1]. (Here, we use the term "live matter" to define matter with the structure of a living organism, but in a state of suspended animation. The term "living matter" is used to define the matter in an organism which is performing the functions of life, i.e., growth, reproduction, movement, etc.) Schrödinger's model for the properties of live matter leads to four, not immediately apparent, conclusions: First, because energy and entropy costs for formation of live matter are assumed to be so energetically unfavorable and therefore so improbable, this model leads to a conclusion that life originated only once on earth and perhaps only once in the entire universe. Second, growth accumulates energy from catabolism into new cells and tissue. Third, all live matter requires a continuous influx of energy to maintain the structure in a metastable state. Fourth, ascending the tree of life achieves an ever-lower-entropy state and higher-energy state and thus imposes a thermodynamic hierarchy on biological classification of species.

---

*Paper based on a presentation at the 20th International Conference on Chemical Thermodynamics (ICCT 20), 3–8 August 2008, Warsaw, Poland. Other presentations are published in this issue, pp. 1719–1959.
‡Corresponding author

However, observations on biological systems challenge Schrödinger's model. Despite the assumed formidable energy and entropy costs, life did originate at least once, and possibly more than once [2]. Second, energy from catabolism is not transferred into new tissue during growth; catabolic reactions are used in driving (i.e., increasing the rates of) anabolic reactions but do not contribute to an overall higher-energy state in the anabolic products [2,3]. Third, live matter exists in a state of suspended animation or in a state with an extremely low rate of respiration for extended lengths of time without energy input (e.g., see [4–7]), for example, microorganisms, resurrection plants, seeds, spores, estivating animals, bdelloids, and inhabitants of desert ephemeral pools. Fourth, experimental data show that life does not always feed on negentropy [8]. And, evolutionary changes occur with frequencies that are difficult to envision if change to more complex life forms requires formation of ever-lower-entropy, higher-energy, metastable states.

Explication of the reaction defining the thermodynamic system in which live matter is formed from nonliving matter resolves the apparent inconsistencies between Schrödinger's model for live matter and these observations. Schrödinger [1] did not explicitly define the reaction used to conclude that the entropy of live matter is lower than that of the "foodstuffs" from which it was formed. The product is clearly living cells, tissues, or an organism, but the state of the live matter and what is meant by "foodstuffs", i.e., the reactants, are not clear. Is the live matter an animal in motion or is it an embryo in a seed in a state of suspended animation? In autotrophs, "foodstuffs" could mean CO_2, H_2O, and N_2, or it could mean the substrates for respiration such as carbohydrates and amino acids. Whether living tissues are in a high- or a low-energy state is relative to the reactants and thus depends on the system considered.

Comparison of a random mixture of molecules in aqueous solution and live matter of the same composition is the appropriate system for resolving the problems with Schrödinger's model, which was derived by assuming the organization of live matter contributes significantly to a low entropy. This assumption is now a widely held concept fundamental to much current thought in biology. However, this concept is incompatible with experimental data on the energy (ΔG), enthalpy (ΔH), and entropy (ΔS) differences between a random arrangement of molecules in aqueous solution and live matter in a state of suspended animation. Results from combustion calorimetry show ΔH is near zero. Results from calorespirometry on living tissue also show ΔH is zero and that catabolism does not increase the overall energy content of anabolic products. Results from titration calorimetry show that ΔG and ΔH are both negative for formation of the noncovalent bonds that structure cells. Results from temperature scanning calorimetry also show these noncovalent bonds are formed exothermically. Results from low-temperature, heat capacity calorimetry show ΔS is near zero. In combination, these results show ΔG is zero or negative for formation of live matter from an aqueous solution of randomly arranged molecules.

These results show that Schrödinger's model conflates the energy required for the functions of life with the energy required for the existence of live matter. A constant input of energy is required to maintain ion gradients, ATP production, and the other functions of living matter, but because cells are organized in a spontaneous process, no energy input is required to maintain the structure or organization of cells. Organisms are open systems with a boundary defined by the physical boundary of the organism, and such a system definition is an appropriate one for analyzing the energy costs for movement, growth, reproduction, and other life functions, but is an inappropriate and misleading choice for comparing the energy and entropy states of live and non-live matter.

RESULTS FROM CALORIMETRY

Combustion calorimetry

Recognition that the heat of combustion of organic compounds was approximately constant when expressed per mole of O_2 was a key discovery providing insights that aid understanding of heat rate–growth rate relations. This rule was first discovered for hydrocarbons by Thornton in 1917 and

later found to apply to nearly all organic compounds [9–11]. Thornton's rule is thus a well-established linear correlation between the enthalpy of combustion and the oxidation state of carbon in organic compounds. For the conditions and compounds typically encountered as catabolic substrates in organisms, an average value of –(455 ± 15) kJ mol^{-1} O_2 is applicable, with carbohydrates on the upper end of the range, proteins in the middle, and lipids on the lower end [12]. Thornton's rule is the basis for indirect calorimetry in which the rate of heat production is calculated from the rate of oxygen consumption, a method that is particularly useful for large animals. The relationship may be expressed as

$$R_q = (455 \pm 15)R_{O_2} \tag{1}$$

R_q is the rate of heat production and R_{O_2} is the rate of oxygen consumption. Hess' law can be used to show the relation is also correct for partial oxidation (except when peroxides are formed) even when CO_2 is not a product. In applying this relation to metabolism of a specific organism, heat-producing reactions that do not involve oxygen must be accounted for.

Calorespirometry

The Lavoisiers' study of respiration in a guinea pig [13] showed that respiration was a slow combustion of the guinea pig's food by oxygen, producing CO_2 as a product. The genius in these experiments was in measuring the rate of uptake of a reactant (O_2) and the rate of production of a product (CO_2) to demonstrate that the heat produced was caused by a chemical reaction. Many measurements of metabolic heat rates of organisms have since been made, but the usefulness of these measurements is often limited because no simultaneous measurements of reaction rates were done. The full power of calorimetric measurements of metabolic heat rates can only be realized when the reactions producing the heat are elucidated and their rates measured so that enthalpy and entropy changes can be determined.

Using Thornton's rule and stoichiometric relations for oxidative metabolism, a series of relationships can be developed to describe aerobic respiration in biological systems in a steady state, i.e., no increase, decrease, or change in composition of the biomass is occurring.

$$R_q = (455 \pm 15)(1 - \gamma_s/4)R_{CO_2} \tag{2}$$

$$R_{O2} = (1 - \gamma_s/4)R_{CO_2} \tag{3}$$

γ_s is the oxidation state of carbon in the substrate and R_{CO_2} is the rate of carbon dioxide production. (Note that the chemical scale of oxidation state, not the degree of reduction, is used in the above and following equations.)

If the system is growing or developing, additional terms must be included to account for the production of, or change in the composition of, the biomass [12]. Assuming that substrate is converted only into biomass or CO_2 and that nitrogen is used in metabolism only in a reduced state, the reaction describing such a system is

$$C_{substrate} + \text{compounds of N, P, K, etc.} + xO_2 \rightarrow \varepsilon C_{biomass} + (1 - \varepsilon)CO_2 \tag{4}$$

The variable coefficient is the substrate carbon conversion efficiency. There is an obvious relation between ε and the heat production by reactions producing biomass, allowing measurement of ε by calorimetric methods. When ε is small, more oxidation is required per mole of biomass produced and more heat is evolved. From the stoichiometry, we can write

$$R_A/R_{CO_2} = \varepsilon/(1 - \varepsilon) \tag{5}$$

and

$$R_q = (1 - \gamma_s/4)(455 \pm 15)R_{CO_2} - \Delta H_B R_A \tag{6}$$

R_A is the anabolic rate which can be equated with the rates of growth and development. ΔH_B is the enthalpy change for the reaction

$$C_{substrate} \rightarrow C_{biomass} + yO_2 \quad (7)$$

Rearranging eq. 6 provides an equation for the anabolic rate

$$R_A = [(1 - \gamma_s/4)(455 \pm 15)R_{CO_2} - R_q]/\Delta H_B \quad (8)$$

and combining eqs. 5 and 6 to eliminate R_A provides an equation for the substrate carbon conversion efficiency, ε

$$\varepsilon/(1 - \varepsilon) = [(1 - \gamma_s/4)(455 \pm 15) - R_q/R_{CO_2}]/\Delta H_B \quad (9)$$

Note that y in eq. 7 is positive and ΔH_B is endothermic if the oxidation state of the substrate carbon is more positive (or less negative) than that of the carbon in the biomass, and y is negative and ΔH_B is exothermic if the oxidation state of the substrate carbon is more negative than that of the biomass carbon. If it is assumed that the effects of hydration are negligible, ΔH_B can be determined either from Thornton's rule or by combustion calorimetry, i.e., ΔH_B equals the heat of combustion of substrate per C-mole minus the heat of combustion of biomass per C-mole. If storage materials are excluded, the composition of all living cells is very similar, and therefore live matter has an average oxidation state of carbon of about –0.3. If the substrate is carbohydrate, ΔH_B is thus about 30 kJ $Cmol^{-1}$.

This model has been successfully applied to determine growth rates (R_A) and substrate carbon conversion efficiencies (ε) in many studies of plant tissues [14–18] and in a few studies of insects [19–22]. An example of the data obtained on tomato and cabbage leaf tissue is shown in Fig. 1 [23]. The ability to rapidly determine growth rate and ε as functions of temperature has provided some fundamental insights into the adaptation of plants to temperature [23,24]. In general, R_q increases with temperature up to near the upper temperature limit for growth of the plant. The curve of R_q vs. temperature can sometimes be fit approximately with an Arrhenius function. R_{CO_2} typically goes through a maximum near the midpoint temperature in the temperature range allowing growth. For plants that are well adapted to an environment, the curve of growth rate vs. temperature duplicates the shape of a curve of the reciprocal of temperature frequency (usually plotted as hours at a given temperature) vs. temperature for the environment [24–27]. In climates with a small diurnal variation and in highly unstable climates, the growth rate curve typically has the shape of a skewed parabola with the maximum at the mean kinetic temperature of the environment and crosses zero at the maximum and minimum temperatures of the growth season. In these climates, plants spend roughly half their time growing at temperatures where their growth rate increases with increasing temperature and the other half at temperatures where growth rate declines with increasing temperature. In stable environments with a large diurnal temperature difference such as is found in many semi-desert and desert climates, the growth curve is bimodal with maxima at the kinetic mean temperatures of the day and night [25–27]. Plants in these climates spend almost no time at the mean temperature. The relations between temperature, R_q, R_{CO_2} and growth rate referred to earlier explain the failure of most attempts to find a correlation between growth rate and metabolic rate among different plants. Finding such a correlation depends on the choice of measurement temperature, and can only exist in general if the plants being studied are adapted to temperature patterns similar to the test regimen.

The shapes of the curves of growth rate vs. temperature, together with eq. 5 which expresses growth rate as a product of a rate and an efficiency, lead to a postulate that total growth of adapted plants over time and temperature of the growth season is proportional to the ratio $T_{mean}/(T_{max} - T_{min})$ of the environment [24]. These relationships are capable of quantitatively explaining the global distribution of plant species [24], and with sufficient data on the respiratory properties of plants could be used to predict the effects of climate change on plant distributions.

Some other advances in the plant sciences that have been achieved through application of the model embodied in eqs. 8 and 9 are the following. Application of eqs. 8 and 9 to rapidly determine

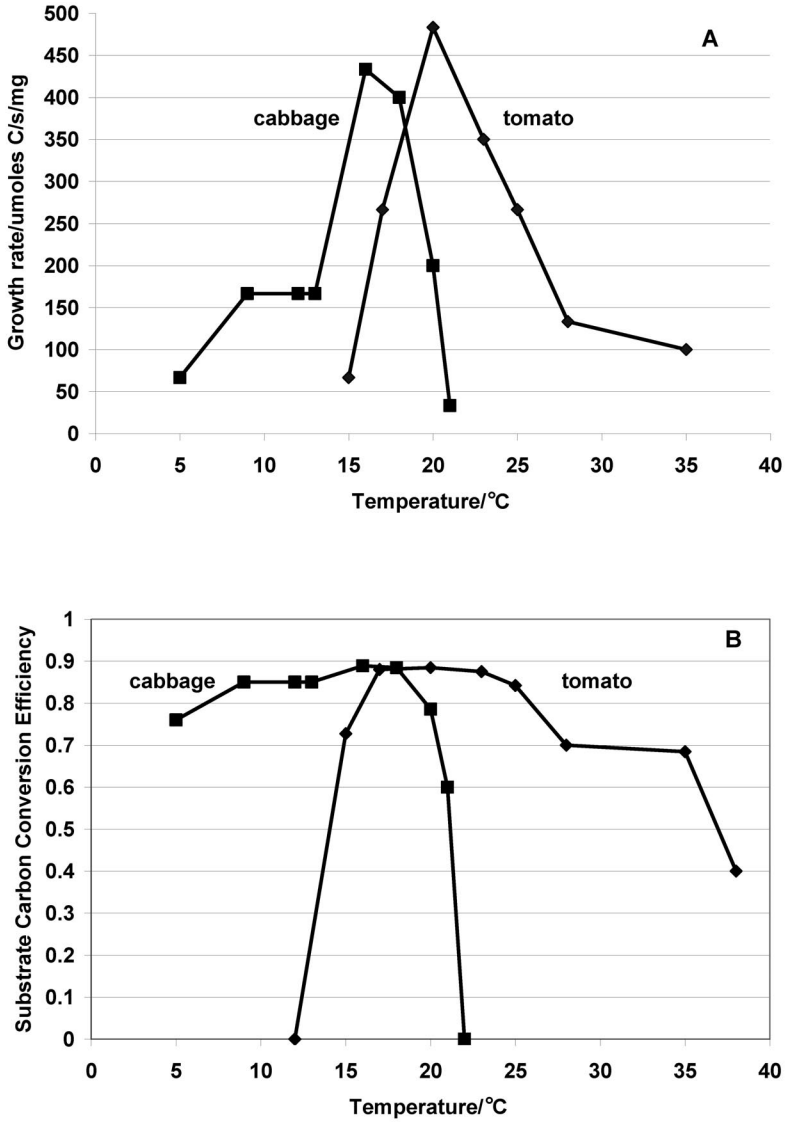

Fig. 1 Growth rates (A) and substrate carbon conversion efficiencies (B) of tomato and cabbage leaf tissue calculated from measurements of R_q and R_{CO_2}. Recalculated and plotted from data in [23].

growth rates and metabolic efficiencies has shown that interpretation of the ubiquitous linear relation between mass specific R_{CO_2} and growth rates of leaf tissue of different ages from the same plant [28,29] in terms of growth and maintenance coefficients is incorrect [30]. "Maintenance energy" is an appealing mental concept, but entirely lacks physical reality. The decrease in both heat rates and growth rates with age is caused by dilution of the tissue with inactive components, not by an actual decrease in either rate for the total tissue. Because growth of young leaf tissue of herbaceous perennials is under strong selection by environmental temperature, the temperature dependence of the metabolic heat rate in new tissue is negatively correlated with increasing altitude and latitude (as proxies for environmental temperature) of the native range of the plant [31]. Because this selection pressure diminishes as the growth season progresses, this correlation is not found in older leaf tissue [32,33]. Because ε was very difficult to determine by the methods available prior to development of the calorimetric method and be-

cause of confusion caused by the incorrect interpretation of the growth coefficient from the slope of the correlation between R_{CO_2} and R_G as an efficiency, variability in ε both with species and with conditions has largely been ignored in considering the effects of conditions on the growth of plants. For cabbage, Fig. 1 shows that ε increases slowly with increasing temperature in the lower part of the growth temperature range and then drops abruptly above 20 °C. For tomato, ε decreases slowly with increasing temperature in the upper part of the growth temperature range and decreases rapidly at temperatures below 15 °C [23]. Because growth rate is proportional to $\varepsilon/(1 - \varepsilon)$, see eq. 5, small changes in ε cause large changes in total growth over time. The sharp decreases in ε at high temperature in cabbage and at low temperature in tomato signal severe temperature stress at and beyond these temperatures.

These extensive results from calorespirometric studies demonstrate the capability of these methods and models to accurately describe the reactions in living organisms. Furthermore, eq. 8 provides a ready means for direct determination of ΔH_B in living tissues from measurements of R_q, R_{CO_2}, and R_A. The slope of a plot of $\Delta H_B R_A$ calculated from measured R_q and R_{CO_2} rates (see eq. 8) vs. independently measured R_A values measured as growth rate on the same tissue at the same temperature is equal to ΔH_B. Measurements on oat shoots shown in Fig. 2 [3] show that ΔH_B thus measured in live tissue gives the same results as measurements on dry tissue made by combustion calorimetry and with values calculated from Thornton's rule, thus validating the assumption that heats of hydration do not make a significant contribution to ΔH_B. ΔH_B values may be used to obtain the enthalpy change for the overall anabolic growth reaction

$$C_{substrate} \rightarrow (1 - y)C_{biomass} + yCO_2 \tag{10}$$

by Hess' law. The results demonstrate the enthalpy change for this reaction is near zero.

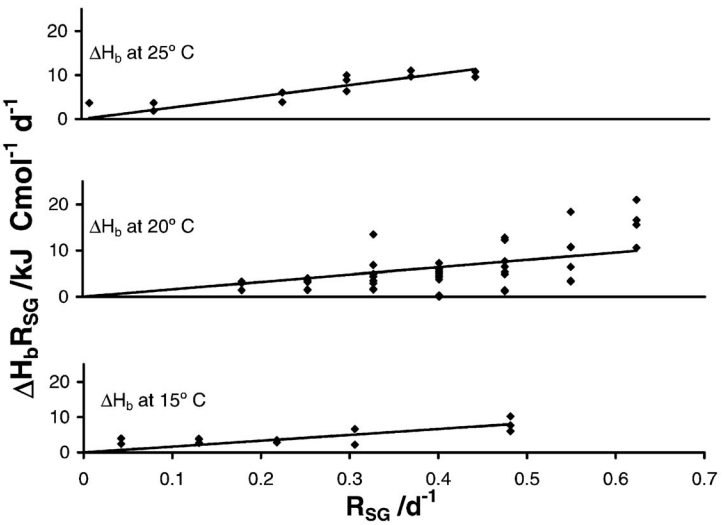

Fig. 2 $\Delta H_B R_G$ values calculated from measurements of R_q and R_{CO_2} plotted vs. directly measured growth rates of oat shoots. ΔH_B values are obtained as the slopes of the fitted lines. Reprinted by permission from ref. [3].

Titration calorimetry

Because titration calorimetry simultaneously measures both ΔG and ΔH for a reaction, it is the method of choice for study of noncovalent interactions between molecules found in living systems, e.g., between proteins and substrates or cofactors. The calorimetric method is also uniquely suited for studies of systems with small equilibrium constants or where other methods (e.g., potentiometry, fluorescence) do not work well because of the high concentrations of reactants required to obtain measurable reac-

tion, or are not applicable because of the physical characteristics of the system (e.g., spectrophotometry in colorless or cloudy solutions). The van't Hoff method for determination of ΔH (i.e., from the slope of a plot of lnK vs. T^{-1}) is particularly inaccurate for the types of reactions for which the calorimetric method is well suited. Details of the method have been described in several books and chapters [34–40], and it is now a widely accepted method. Titration calorimeters with detection limits of a few nanowatts and titrate volumes of 200 µl are now commercially available for studies of noncovalent interactions of cellular components. Bibliographies of recent work have been published [41–44], and review of the results shows that the noncovalent bonds between the components of cells are largely, if not exclusively, formed in spontaneous, exothermic reactions, i.e., ΔG and ΔH are both negative under the conditions in cells and tissues.

Temperature scanning calorimetry

Temperature scans of whole cells of two species of bacteria are shown in Fig. 3 [45]. Thermal disruption of the structures of ribosomes, DNA, and cell wall components at these temperatures only break noncovalent bonds, but account for thermal inactivation of the microorganisms. All of the events in the curves in Fig. 3 and in similar data in references [46–50] are endothermic, again showing the noncovalent bonds that structure cells are formed exothermically. Measurements of the heats of thermal denaturation of biopolymers too numerous to cite here all show that ΔH for thermal disruption of these structures is also endothermic.

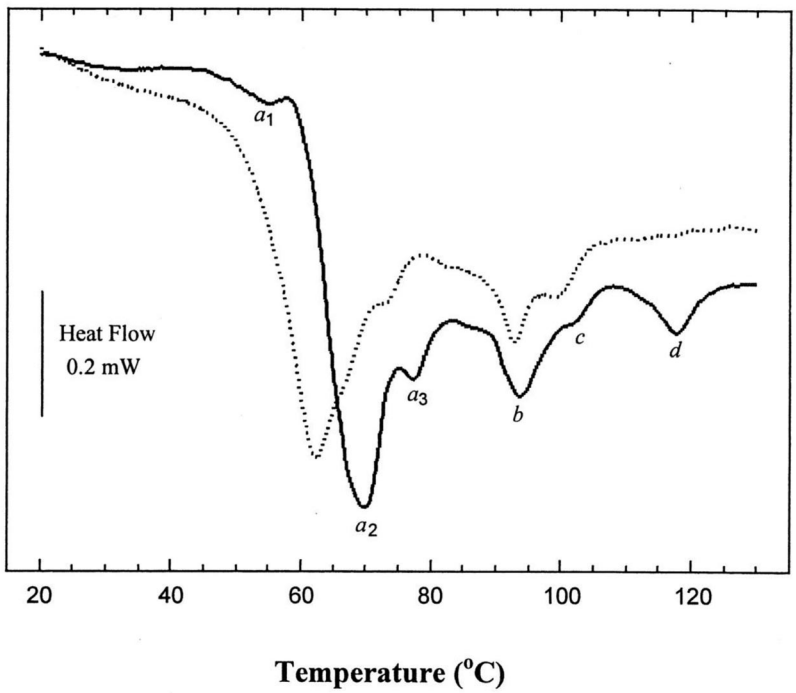

Fig. 3 Scans of bacterial cells by differential scanning calorimetry. The solid line is for *E. coli* and the dotted line is for *Lactobacillus plantarum*. The a, b, c, and d indicate endothermic events where the structures of ribosomes (a), DNA (b) and cell walls (c and d) are thermally disrupted. Reprinted by permission from ref. [45].

Low-temperature, heat-capacity calorimetry

Low-temperature, heat-capacity calorimetry has been widely applied to determine entropies of many substances of importance to biology [51]. The relation between heat capacity (C_p) and entropy (S) is

$$S_T - S_0 = \int_0^T (C_P/T)\mathrm{d}T \quad (11)$$

T is absolute temperature, C_P is the heat capacity at constant pressure and S_0 is the entropy at absolute zero temperature. Of particular interest in this paper are data on whole, dry cells of *E. coli*, *S. cerevisiae*, rice shoots, and oat shoots [52,53] which show ($S_T - S_0$) values of these to be essentially the same as a weighted sum of the entropies of the components.

THERMODYNAMICS OF THE ORIGINATION AND EVOLUTION OF LIFE

Do energy and entropy differ between an unorganized mixture of molecules and live matter?

The reaction

mixture of complex molecules in aqueous solution → live matter of the same composition (12)

which compares a random mixture of peptides, lipids, carbohydrates, etc. to organized live matter in a state of suspended animation is the relevant reaction for considering whether live matter is in a high-energy, low-entropy, metastable state or in an equilibrium state. As will be shown, ΔG, ΔH, and ΔS for this reaction are all near zero. References [3,51] provide the most direct evidence for this conclusion, but many other data support such a conclusion. To reiterate, we use the term "live matter" to distinguish the state of suspended animation from "living matter" which is not in a state of suspended animation and is performing the functions of life, i.e., growth, reproduction, movement, etc. Equation 12 includes all of the mass of reactants and products and focuses only on the question; do the energy and entropy differ between an unorganized mixture of molecules and live matter? Equation 12 is further defined as a closed system, only heat energy crosses the system boundary, not mass. Catabolism is not included in the system defined by eq. 12. Catabolic reactions in vivo act in controlling the rates of anabolic reactions, mostly by phosphorylation of reactants, but the phosphate groups are largely removed during formation of the living materials and thus do not contribute to the energy state of the products. This was recognized long ago by Blum [2] when he wrote "In an overall sense the ATP-ADP system may be regarded as behaving as a catalyst which lowers the free energy of activation by acting in a cyclical manner". Catalysts do not introduce energy into reaction products. Energy from catabolism is used for movement, transport of materials, etc., but only the formation of live matter from preformed molecules of the same composition, not these other energy-requiring life functions are included in eq. 12. Because Schrödinger included catabolism in his system, a continuous energy input for these functions was required, but this continuous input of energy is used to do thermodynamic work on or to increase the entropy of the surroundings, not to increase the energy content or decrease the entropy of the live matter.

ΔG is the appropriate measure of the energy difference between the live matter and reactants in reaction 12, however, ΔG for reaction 12 cannot be measured directly, and therefore must be calculated from eq. 13 and measured values of ΔH and ΔS.

$$\Delta G = \Delta H - T\Delta S \quad (13)$$

ΔG, ΔH, and ΔS are state functions, with values dependent only on the difference between the starting and ending states, not on the process used to get from reactants to products. Reviews of literature relevant to the thermodynamics of reaction 12 [8,54] suggest that, under some conditions, ΔG, ΔH, and ΔS are all near zero for this process. The most direct evidence for this comes from two studies that

show that ΔH is indeed zero for the anabolic reaction in living plant tissues (see Fig. 2 and eq. 10) [3] and that the entropy of organic matter depends only on the composition [51] and thus that $\Delta S \approx 0$ for reaction 12.

ΔH for reaction 12 can be determined directly by calorimetry (i.e., by combustion calorimetry of dried material and by metabolic calorimetry of living tissues and organisms as explained above) or from elemental analysis of tissues and organisms and application of Thornton's rule. ΔH can also be determined for the reverse of reaction 12 by calorimetric methods, i.e., by temperature scanning (see Fig. 3) or some other change in the surroundings that causes dissociation of the live matter into separate molecules by disruption of noncovalent bonds. The results from heats of combustion and Thornton's rule show that ΔH for reaction 12 is near zero in dried, but viable cells as well as in dried, dead tissue. Arguments that ΔH for reaction 12 is endothermic because the heat of combustion of biomass per C-mole is greater than the heat of combustion of substrate per C-mole are incorrect because they neglect the stoichiometry of the combustion reaction. The difference in the heats of combustion gives ΔH_B for reaction 7. The actual anabolic reaction in most aerobic systems is reaction 10 for which ΔH equals zero. The results from DSC experiments on organelles and whole cells (see Fig. 3) indicate that the reverse of reaction 12 is endothermic, and thus that reaction 12 is exothermic. The conclusion that the enthalpy change for reaction 12 is near zero or exothermic is thus based on a large body of data collected with several methods. The conclusion is also readily generalized to all plants, animals, and microorganisms.

ΔS for a reaction under isothermal conditions can be estimated by determining C_P of both reactants and products as functions of temperature and applying the relation in eq. 11. Equation 14

$$\Delta S = \sum(S_T - S_0)_{\text{products}} - \sum(S_T - S_0)_{\text{reactants}} \tag{14}$$

is then used to calculate the entropy change. Because S_0 cannot be measured and the third law only applies to crystalline substances, it must be assumed the S_0 terms cancel. Application of the heat capacity method for determination of the entropy of dried yeast cells, *E. coli* cells, oat shoots, and rice shoots demonstrates that the value of $S_T - S_0$ does not differ significantly from a weighted sum of the values for proteins, starch, cellulose, and other nonliving biomaterials. This result is not surprising because the heat capacity arises primarily from vibrational energy of C–H, C–C, C–O, C–N, and N–H covalent bonds and noncovalent bonds such as hydrogen bonds and salt bridges which are essentially the same and present in the same quantity in the reactants and products of reaction 12. Thus, we conclude that ΔS for reaction 12 is approximately zero.

Measurements of C_p over the temperature range from near 0 K to room temperature must be performed on dried material, and therefore it might be argued that the entropy of hydration may make a negative contribution to S_T [55]. However, the enthalpy of hydration of dried tissues has been determined to be near zero [56], and, as shown above, ΔH_B is the same in dry and living tissue, both of which argue, because of the enthalpy-entropy compensation that commonly occurs in hydration of biomolecules [57], that the entropy of hydration is also near zero. Thus, in that portion of the entropy arising from the heat capacity term, the C_p and enthalpy of hydration values indicate no significant entropy difference between living and nonliving materials of the same proximate composition. This leaves only differences in S_0, the zero point entropy, as a possible source for the postulated low entropy in living organisms relative to the entropy in nonliving biomaterials.

Morowitz [58], Volkenstein [59], Riedl [60], and others have proposed that the information content of cells contributes to a low entropy through what has been termed "informational entropy". If "informational entropy" can be calculated with the Boltzmann equation

$$S = R\ ln\ w \tag{15}$$

where R is the gas constant and w is the probability of a given state within an ensemble of states, and is thus equivalent to thermodynamic entropy, it must appear in S_0. While it is true that the information content of cells is based on ordered macromolecular sequences that enable them to maintain and pass

on genetic information, it does not follow that information content and thermodynamic entropy are equivalent and that the two can be summed [61,62]. That information content is not equivalent to thermodynamic entropy also follows from a lack of common units for the two. The thermodynamic or Boltzmann entropy is a mathematical function describing the microscopic motions of and energy distribution among atoms and molecules. Informational or Shannon entropy does not have the physical reality characteristic of material bodies. Only when multiplied by the temperature for which the Boltzmann entropy has been calculated, does the product become a physical quantity. Information content or Shannon entropy is represented by a similar mathematical function, but there is no way of converting it to a physical quantity.

A "gedanken" experiment can be used to show that equating information content to a thermodynamic entropy leads to an incorrect result. Consider two identical pieces of copper foil containing 1 mol of Cu atoms. Cu is chosen because it has a sharp melting point and has been used to inscribe written messages, thus making this gedanken experiment actually doable if someone wishes to test our conclusions. On one foil we shall write the genetic code for an *E. coli* cell using the usual A, T, G, C designations for the DNA bases. On the other foil, we shall write the same number of characters, but in a random arrangement. Then we shall measure the melting points of the foils. The melting point of pure Cu is 1356 K, the $\Delta H_{\text{melting}}$ is 13 kJ mol^{-1}, and the $\Delta S_{\text{melting}}$ is 4.58 J mol^{-1} K^{-1} [63]. Since the melting point is equal to the ratio $\Delta H_{\text{melting}}/\Delta S_{\text{melting}}$ (see eq. 13 with $\Delta G = 0$) and the entropies and enthalpies of the liquids from the two foils must be the same, assuming $\Delta H_{\text{melting}}$ is not affected by information, the foil with the genetic information on it must melt at a lower temperature if "information content" lowers the entropy of the foil. Assuming an *E. coli* cell requires 4×10^{10} bits of information [58], the first Cu foil would melt at 848 K, i.e., $\Delta S_{\text{melting}}$ is increased by $Rln2$ [64]. If the genetic code and random sequence were written on 1 g of Cu instead of 1 mol, the Shannon entropy would remain the same, but because the mass of Cu is reduced, the melting point would be 35 K. This is an absurd result, disproving the assumption that informational entropy is equivalent to thermodynamic entropy. It is as though a 5 Gb hard drive would melt if it was fully loaded with an information-laden sequence of binary digits. A similar gedanken experiment can be done with DNA by comparing the ΔS for hydrolysis of DNA from an organism and DNA with the same GC content, but with any random sequence of base pairs. Hydrolysis to the nucleotides destroys the information, so the "information theory" would predict hydrolysis of organismal DNA would be easier, i.e., ΔS and ΔG would be more negative, than for hydrolysis of random DNA. Again, an incorrect conclusion since it is well known that large DNAs with the same GC content have essentially the same melting point and enthalpy of melting whether from bacteria, humans, or chemical synthesis. The argument that a structure with a "highly ordered sequence" possesses lower entropy than an analogous compound with a random distribution of the constituents is a misinterpretation of the statement commonly made in thermodynamics that a highly ordered structure has lower entropy than a less-ordered structure. This statement applies to comparisons of crystal structures and of crystals with their melts, but not to a comparison of random and organismal DNA since both kinds of DNA have the same "crystal structure". Entropy thus depends on the structure, not on the sequence of the DNA. Information does not translate into thermodynamic entropy because information is given by the sequence of symbols, bits, or bases; thermodynamic entropy depends only on the number of physical entities and how energy is distributed among them, not on their sequence.

Since both ΔH and ΔS for reaction 12 are approximately zero, eq. 13 shows that ΔG must also be approximately zero, or, for a condition in which no products are present or are removed continuously, ΔS must be positive and ΔG must be a large negative value. Combined evidence from several sources thus indicates that the entropy and energy of living cells, tissues, and organisms does not differ significantly from a random assemblage of the molecules from which they are made. The consequences to understanding the origin and evolution of living cells, tissues, and organisms are profound.

Origin and evolution of life

Assuming live matter is in a high-energy, low-entropy state and equating information and entropy make it difficult to define a mechanism by which the complex structures and arrangements of molecules in cells could transpire. But spontaneous generation of self-replicating molecules from substrate building blocks in the same energy and entropy state requires only suitable reaction conditions and time. The first "live matter" need be only a molecule with the ability to self-replicate, probably with clay or quartz or some other optically active mineral as a catalyst, and no special conditions of energy gradients or high temperature are necessary. There is a high probability that such conditions and systems existed on the early earth [65]. Once such a system exists, natural selection can begin to operate to select the fittest molecules, i.e., molecules with the highest reproductive rate. This "first" system would no longer exist on earth because it would have been rapidly replaced with live matter that was more complex and more fit, i.e., with more efficient reproductive ability. A near-zero difference in energy and entropy between substrates and some form of self-replicating matter thus changes generation of living matter from a highly improbable event to an event that, given time, is inevitable (i.e., what is thermodynamically possible is also inevitable). A near-zero difference in energy and entropy between live and non-live matter thus eliminates one of the more vexing problems in understanding the origin of life. Thus, it is also highly probable that carbon-based life will develop on any planet having the right conditions, i.e., the presence of liquid water [66] to support the reactions we call life.

Not equating information content with thermodynamic entropy and accepting a near-zero energy difference between non-live and live matter also removes any entropy and energy constraints to evolutionary changes. Evolutionary changes are continuous and too numerous to be consistent with increasing energy cost with increasing complexity of the organism. The absence of energy and entropy costs is consistent with spontaneous exploration of the fitness landscape, allows for stasis, and facilitates rapid filling of empty niches following extinction events. Mutational events, genetic recombination, and environmental change result in perturbations which, without a requirement to overcome large energy costs during the transition, can rapidly relax to an altered state. The path of evolution on other planets as well as on earth is constrained by extant conditions. As is true on earth for different environments, conditions on other planets may only allow microorganisms or organisms that can live in niches sheltered from conditions that would destroy their component molecules or prevent them from functioning.

REFERENCES

1. E. Schrödinger. *What is Life?*, Cambridge University Press, Cambridge (1967).
2. H. F. Blum. *Time's Arrow and Evolution*, 2nd ed., pp. 110, 173, 202, Harper Torchbook, New York (1962).
3. D. Ellingson, A. Olson, S. Matheson, R. S. Criddle, B. N. Smith, L. D. Hansen. *Thermochim. Acta* **400**, 79 (2003).
4. C. P. Hayden. "Suspended animation: A brine shrimp essay", in *Remaking Life & Death*, S. Franklin, M. Lock (Eds.), pp. 193–225, James Currey Ltd., Oxford (2003) and refs. therein.
5. F. A. Hoekstra, E. A. Golovina, J. Buitink. *Trends Plant Sci.* **6**, 431 (2001).
6. C. Walters, L. M. Hill, L. J. Wheeler. *Integr. Comp. Biol.* **45**, 751 (2005).
7. D. Bartels. *Integr. Comp. Biol.* **45**, 696 (2005).
8. U. von Stockar, J. S. Liu. *Biochim. Biophys. Acta* **1412**, 191 (1999).
9. W. M. Thornton. *Philos. Mag*. **33**, 196 (1917).
10. S. A. Patel, L. E. Erickson. *Biotechnol. Bioeng*. **23**, 2051 (1981).
11. J. L. Cordier, B. M. Butsch, B. Birou, U. von Stockar. *Appl. Microbiol. Biotechnol*. **25**, 305 (1987).
12. L. D. Hansen, C. Macfarlane, N. McKinnon, B. N. Smith, R. S. Criddle. *Thermochim. Acta* **422**, 55 (2004).

13. A. Lavoisier, P. LaPlace. *Mémoire sur la Chaleur*, Mémoires de l'Academie des Sciences, Paris (1780); [English translation in: M. L. Gabriel, S. Fogel. *Great Experiments in Biology*, pp. 85–93, Prentice Hall, Englewood Cliffs, NJ (1955)].
14. L. D. Hansen, M. S. Hopkin, D. R. Rank, T. S. Anekonda, R. W. Breidenbach, R. S. Criddle. *Planta* **194**, 77 (1994).
15. L. D. Hansen, M. S. Hopkin, D. K. Taylor, T. S. Anekonda, D. R. Rank, R. W. Breidenbach, R. S. Criddle. *Thermochim. Acta* **250**, 215 (1995).
16. R. S. Criddle, L. D. Hansen. In *Handbook of Thermal Analysis and Calorimetry*, *Vol. 4: From Macromolecules to Man*, R. B. Kemp (Ed.), pp. 711–763, Elsevier, Amsterdam (1999).
17. L. D. Hansen, R. S. Criddle, B. N. Smith. In *Plant Respiration: From Cell to Ecosystem*, H. Lambers, M. Ribas-Carbo (Eds.), pp. 17–30, Springer, Dordrecht (2005).
18. C. Macfarlane, M. A. Adams, L. D. Hansen. *Proc. R. Soc. London* **269**, 1499 (2002).
19. E. B. Acar, B. N. Smith, L. D. Hansen, G. M. Booth. *Environ. Entomol.* **30**, 811 (2001).
20. E. B. Acar, D. D. Mill, B. N. Smith, L. D. Hansen, G. M. Booth. *Environ. Entomol.* **33**, 832 (2004).
21. E. B. Acar, D. D. Mill, B. N. Smith, L. D. Hansen, G. M. Booth. *Environ. Entomol.* **34**, 241 (2005).
22. J. J. Joyal, L. D. Hansen, D. R. Coons, G. M. Booth, B. N. Smith, D. D. Mill. *J. Therm. Anal. Calorim.* **82**, 703 (2005).
23. R. S. Criddle, B. N. Smith, L. D. Hansen. *Planta* **201**, 441 (1997).
24. R. S. Criddle, L. D. Hansen, B. N. Smith, C. Macfarlane, J. N. Church, T. Thygerson, T. Jovanovic, T. Booth. *Pure Appl. Chem.* **77**, 1425 (2005).
25. V. W. McCarlie, L. D. Hansen, B. N. Smith, S. B. Monsen, D. J. Ellingson. *Russ. J. Plant Phys.* **50**, 205 (2003).
26. S. Matheson. *Metabolic Adaptation of Oats to Climatic Temperature*, Brigham Young University Honors Thesis (2000).
27. T. A. Ward. *Guiding Restoration Efforts Through Metabolic Properties of Native Grass Populations and Reptile Abundance and Species Richness*, Brigham Young University M. S. Thesis (2007).
28. J. H. M. Thornley. *Nature* **227**, 304 (1970).
29. J. S. Amthor. *Respiration and Crop Productivity*, Springer Verlag, New York (1989).
30. S. Matheson, D. J. Ellingson, V. W. McCarlie, B. N. Smith, R. S. Criddle, L. Rodier, L. D. Hansen. *Funct. Plant Biol.* **31**, 929 (2004).
31. R. S. Criddle, M. S. Hopkin, E. D. McArthur, L. D. Hansen. *Plant, Cell Environ.* **17**, 233 (1994).
32. M. G. Tjoelker, J. Oleksyn, P. B. Reich. *Global Change Biol*. **7**, 223 (2001).
33. O. K. Atkin, M. G. Tjoelker. *Trends Plant Sci*. **8**, 343 (2003).
34. L. D. Hansen, R. M. Izatt, J. J. Christensen. In *New Developments in Titrimetry*, J. Jordan (Ed.), pp. 48–62, Marcel Dekker, New York (1974).
35. J. Barthel. *Thermometric Titrations*, pp. 47–49, 138–149, John Wiley, New York (1975).
36. H. J. V. Tyrrell, A. E. Beezer. *Thermometric Titrimetry*, pp. 77–78, Chapman and Hall, London (1968).
37. D. J. Eatough, E. A. Lewis, L. D. Hansen. In *Analytical Solution Calorimetry*, K. Grime (Ed.), pp. 137–161, John Wiley, New York (1985).
38. J. L. Oscarson, R. M. Izatt, J. O. Hill, P. R. Brown. In *Solution Calorimetry*, K. N. Marsh, P. A. G. O'Hare (Eds.), pp. 222–227, Blackwell, Oxford (1994).
39. J. E. Ladbury, B. Z. Chowdhry. *Biocalorimetry*, John Wiley, Chichester (1998).
40. J. E. Ladbury, M. L. Doyle. *Biocalorimetry 2*, John Wiley, Chichester (2004).
41. A. Ababou, J. E. Ladbury. *J. Mol. Recog*. **20**, 4 (2007).
42. A. Ababou, J. E. Ladbury. *J. Mol. Recog*. **19**, 79 (2006).
43. M. J. Cliff, A. Gutierrez, J. E. Ladbury. *J. Mol. Recog*. **17**, 513 (2004).

44. M. J. Cliff, J. E. Ladbury. *J. Mol. Recog*. **16**, 383 (2003).
45. J. Lee, G. Kaletunç. *Appl. Environ. Microbiol*. **68**, 5379 (2002).
46. J. Lee, G. Kaletunç. *J. Appl. Microbiol*. **93**,178 (2002).
47. G. Kaletunç, J. Lee, H. Alpas, F. Bozoglu. *Appl. Environ. Microbiol*. **70**, 1116 (2004).
48. J. R. Lepock. *Methods* **35**, 117 (2005).
49. H. T. T. Nguyen, J. E. L. Corry, C. A. Miles. *Appl. Environ. Microbiol*. **72**, 908 (2006).
50. I. Milek, M. Črnigoj, N. P. Ulrih, G. Kaletunç. *Can. J. Microbiol*. **53**, 1038 (2007).
51. E. H. Battley, J. R. Stone. *Thermochim. Acta* **349**, 153 (2000).
52. E. H. Battley, R. L. Putnam, J. Boerio-Goates. *Thermochim. Acta* **298**, 37 (1997).
53. J. Boerio-Goates. Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA (personal communication).
54. E. H. Battley. In *Handbook of Thermal Analysis and Calorimetry, Vol. 4, From Macromolecules to Man*, R. B. Kemp (Ed.), pp. 219–266, Elsevier, Amsterdam (1999).
55. T. Sousa, R. Mota, T. Domingos, S. A. L. M. Kooijman. *Phys. Rev. E* **74**, 051901 (2006).
56. P. Duboc, I. Marison, U. von Stockar. In *Handbook of Thermal Analysis and Calorimetry, Vol. 4, From Macromolecules to Man*, R. B. Kemp (Ed.), p. 281, Elsevier, Amsterdam (1999).
57. R. Lumry, S. Rajender. *Biopolymers* **9**, 1125 (1970).
58. H. J. Morowitz. *Biochim. Biophys. Acta* **40**, 340 (1960).
59. M. V. Volkenstein. *Physical Approaches to Biological Evolution*, pp. 253–255, Springer-Verlag, Berlin (1994).
60. R. Riedl. *Order in Living Organisms*, pp. 17–26, John Wiley, New York (1978).
61. E. D. Schneider, D. Sagan. *Into the Cool: Energy Flow, Thermodynamics and Life*, pp. 19–24, University of Chicago Press, Chicago (2005).
62. E. H. Battley. *Biotechnol. Bioeng*. **41**, 422 (1993).
63. R. C. Weast (Ed.). *Handbook of Chemistry and Physics*, 55th ed., p. D-56, CRC Press, Boca Raton (1974).
64. P. Nelson. *Biological Physics: Energy, Information, Life*, updated 1st ed., W. H. Freeman, New York (2008).
65. S. D. Senanayake, H. Idriss. *Proc. Natl. Acad. Sci. USA* **103**, 1194 (2006).
66. T. D. Brock, G. K. Darland. *Science* **169**, 1316 (1970).