SOME CONSEQUENCES OF THE APPLICATION OF THE SECOND LAW OF THERMODYNAMICS TO CELLULAR SYSTEMS

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SUMMARY

Cellular division is examined in terms of the limitations imposed by the second law of thermodynamics. Entropy changes accompanying cell division are discussed and expressions for these are formulated. The entropy changes are summed and the limitation of the second law ($\Delta S > 0$) is imposed. From the resulting inequality the minimum amount of waste material accompanying cellular synthesis is computed. In addition certain conclusions are drawn as to the maximum rate of cell division.

INTRODUCTION

While the second law of thermodynamics as originally stated in terms of the impossibility of building certain kinds of machines has seemed very remote from biology, nevertheless, this law places very definite restrictions on the operation of living systems. The chief reason for the close relationship between the second law and living systems is the fact that when a cell ingests material from the environment and proceeds to grow into two cells there is a local decrease in entropy[1]. If no violation of the second law is going to occur, we must be able to make some statements restricting the process of cell growth. Recent attempts to apply information theory to living systems[2-4] as well as the intimate relation between information and entropy[5] suggest that the question of the relation between cell growth and the second law of thermodynamics be examined in some detail.

In order to study the problem, we will deal with the following idealized, but experimentally approachable situation. Consider a flask of volume V containing a dilute solution of small nutrient molecules. The flask is in thermal contact within an infinite isothermal reservoir at temperature T. The flask contains a single living cell of volume v. The process we wish to focus attention on is the growth and division of the cell into two similar cells. For convenience assume the further idealization that the cells are spherical during most of their life cycle. The process may be formally represented as

$$1 \text{ cell} + \text{nutrients} \longrightarrow 2 \text{ cells} + \text{waste products}$$

Since the initial cell acts as a catalyst for the overall process, and is effective unchanged at the end of the process, we may simplify the formalism and represent it as

$$\text{nutrients} \longrightarrow \text{cell} + \text{waste products}$$

For cells which grow in simple defined media the above formalism can be stated with some precision. For example for *Escherichia coli* grown in *C* minimal medium supplemented with glucose[6], we may write

$$\begin{aligned}&(3.22\cdot 10^9)\,C_6H_{12}O_6 + (1.94\cdot 10^9)\,NH_3 + (1.69\cdot 10^8)\,H_3PO_4 + (4.8\cdot 10^7)\,H_2SO_4 + \qquad (1)\\ &(9.40\cdot 10^9)\,O_2 \longrightarrow (1)\ \textit{E. coli} + (9.55\cdot 10^9)\,CO_2 + (1.48\cdot 10^9)\,CH_3COOH +\\ &(2.11\cdot 10^9 \text{ End Products} + (13.63\cdot 10^9)\,H_2O\end{aligned}$$

The requirement that we wish to apply to the aforementioned process is that the second law of thermodynamics holds, that is for the entire system including the reservoir the entropy change is greater than or equal to zero for the synthesis of a living cell. Stating the requirement formally, we may write

$$\Delta S \geqslant 0 \qquad (2)$$

To get a more detailed idea of the entropy change, consider that in the process outlined the following occurs: (a) Nutrient is ingested by the cell. (b) This nutrient is either incorporated into the cell or reacted and given off as waste products. (c) Heat is given off by the reactions and is transferred to the isothermal reservoir.

To calculate the entropy changes we will consider cell production to consist of two linked processes.

Process I. Nutrient is concentrated in the cell and converted to cell material.

Process II. Nutrient is converted to waste material. Since nutrient and waste material both end up dissolved in the medium surrounding the cell we need not calculate the entropy change in the concentration of this part of the nutrient and subsequent dilution of the waste material.

Since the two processes are linked, the linkage can be expressed by a parameter f, where f represents the number of grams of waste product produced per gram of cell produced. If the cell mass is then m, fm grams of material will be given off in the synthesis of a single cell. The entropy changes are as follows:

1. The entropy decrease involved in condensing the nutrient molecules in the cell. This may be approximated by $\Delta S_c = -mR/M \log Cc/Cm$, where M is the average molecular weight of the nutrients, Cc is the concentration of material in the cell (g/l) and Cm is the concentration of nutrient in the medium. This expression is derived by assuming that the entropy can be approximated by the entropy of an ideal gas, that $V \gg v$, the volume of the container is much larger than the volume of the cell, and that $Cc \gg Cm$, the material is much more concentrated in the cell than in the nutrient solution.

2. The entropy decrease involved in ordering the nutrient molecules into a cellular structure. At the moment we may represent this as ΔS_0. This is a quantity related to calculated values of the information content of a cell. We may use the relationship

$$\Delta S_0 = kI \ln 2 \qquad (3)$$

where k is Boltzmanns constant and I is the information increase of the cell over the unordered component clusters of atoms. For convenience in later expression we shall define a normalized information density as $i = I/mN$ where N is Avagadro's number. The entropy change is then

$$\Delta S_0 = -kmNi \ln 2 \qquad (4)$$

3. The entropy change (probably increase) in converting nutrients to waste

products. If this is ΔSw entropy units per gram then the total change in the growth of a cell will be $fm\Delta Sw$.

4. The entropy change $\Delta Q/T$ involved in the transfer of heat to or from the reservoir. This change is given by

$$\frac{\Delta Q}{T} = -\frac{(m\Delta Hc + fm\Delta Hw)}{T}$$

where ΔHc is the enthalpy per gram for cell synthesis and ΔHw is the enthalpy per gram of waste produced. Thus, summing all the entropy changes, we get

$$\Delta S = -\frac{mR}{M}\log\frac{Cc}{Cm} - mRi\ln 2 + fm\Delta Sw - \frac{(m\Delta Hc + fm\Delta Hw)}{T} \geqslant 0 \qquad (5)$$

Rearranging terms we get

$$-\frac{RT}{M}\log\frac{Cc}{Cm} - RTi\ln 2 - f\Delta Fw - \Delta Hc \geqslant 0 \qquad (6)$$

where ΔFw is the free energy change per gram of waste produced. Since the first two terms are negative and ΔHc may be in principle either positive or negative (we will later attempt an approximate calculation of this quantity) but is probably small, the second law demands that ΔFw is negative and that f has a certain minimum value given by

$$f \geqslant -\frac{1}{\Delta Fw}\left[\frac{RT}{M}\log\frac{Cc}{Cm} + RTi\ln 2 + \Delta Hc\right] \qquad (7)$$

The quantity f measures the minimum price that must be payed in converting unorganized medium into a highly organized cell. Before attempting to compute minimum values of f we must have some idea of the value of ΔHc, the enthalpy of formation of a cell. We reconsider the chemical equation (1) which we have written for the formation of an *E. coli*. We then must split this into two equations, one describing the cellular synthesis and the other describing the metabolic formation of waste products.

The equation describing cellular synthesis is

$$\begin{aligned}&(0.97\cdot 10^9)\,C_6H_{12}O_6 + (1.73\cdot 10^9)\,NH_3 + (0.196\cdot 10^9)\,H_3PO_4 + \\ &(0.048\cdot 10^9)\,H_2SO_4 \longrightarrow 1\ \textit{E. coli} + 4.03\cdot 10^9\,H_2O\end{aligned} \qquad (8)$$

Including $4.03\cdot 10^9$ water molecules along with the synthesis of the cell is somewhat arbitrary; it is done to balance the chemical equations. (We shall later correct the enthalpy for this factor.) The result of this is to make the estimate of ΔHc too high. The atomic composition of the *E. coli* cell is $5.83\cdot 10^9$ carbon, $9.38\cdot 10^9$ hydrogen, $2.69\cdot 10^9$ oxygen, $1.73\cdot 10^9$ nitrogen, $0.169\cdot 10^9$ phosphorus and $0.048\cdot 10^9$ sulfur. If we know the types and distribution of bonds on both sides of the equation we can use the available data on heat of formation of bonds[7,8] to calculate the ΔHc of the process. From the macromolecular composition of the *E. coli* cell, its bond distribution has been computed[9,10]. The data are presented in Table I. The value of ΔHc as computed from these data is — 682 cal/g. If we consider the formation of water as a linked reaction and use an approximate method to evaluate the ΔH of this reaction, the ΔHc is reduced to a value of — 124 cal/g.

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[*] Bond distributions are calculated from the known information on the composition and the structural formulae of the constituents. Some relevant information is presented in ref. [9,10].

TABLE I

DISTRIBUTION OF COVALENT BONDS IN NUTRIENT MEDIUM AND IN *Escherichia coli*

Nutrients		*Cell and cellwater*	
C—H	$54.2\cdot 10^8$	C—H	$71.03\cdot 10^8$
C—C	$48.5\cdot 10^8$	C—C	$37.94\cdot 10^8$
C—N	0.0	C—N	$28.75\cdot 10^8$
N—H	$51.9\cdot 10^8$	N—H	$17.01\cdot 10^8$
C=O	0.0	C=O	$13.37\cdot 10^8$
C—O	$67.9\ \cdot 10^8$	C—O	$12.25\cdot 10^8$
P—O	$5.07\cdot 10^8$	P—O	$5.14\cdot 10^8$
O—H	$53.5\ \cdot 10^8$	O—H	$5.79\cdot 10^8$
C=C	0.0	C=C	$3.00\cdot 10^8$
C=N	0.0	C=N	$3.00\cdot 10^8$
P=O	$1.69\cdot 10^8$	P=O	$1.71\cdot 10^8$
C—S	0.0	C—S	$0.75\cdot 10^8$
S—H	0.0	S—H	$0.17\cdot 10^8$
S—S	0.0	S—S	$0.04\cdot 10^8$
S=O	$0.96\cdot 10^8$	S=O	0.0
S—O	$0.96\cdot 10^8$	S—O	0.0
		Water	
		O—H	$80.6\cdot 10^8$

We may now attempt to evaluate f minimum, by substituting observed and calculated values for the parameters in equation (7).

(a) Cc is the order of 250 g/l while Cm is the order of 5 g/l. (Cm is under the experimenters control and may be varied over a wide range. We shall later make use of this fact.) M may be taken as the order of 100 and we shall perform the calculation for $T = 300°$. The term $RT/M \log Cc/Cm$ is then 23.4 cal/g. (b) The term $RTi \ln 2$ is 159 cal/g based on the calculated information content of a 10^{-13} g cell as $4\cdot 10^{10}$ bits[4]. (c) ΔHc has been calculated as — 124 cal/g. The quantity is very dependent on the nutrient medium the cells are grown in. In a very enriched medium ΔHc probably approaches zero. (d) The principle linked reaction in the case just discussed is the conversion of glucose to carbon dioxide and water. For a first approximation we may assume that ΔFw is the free energy of glucose oxidation. This free energy is — 691,000 cal/mole or 1860 cal/g.

Substituting in equation (7), we get

$$f \geqslant \frac{[58.4]}{1860} = 0.03 \qquad (9)$$

Note that the quantity in the parenthesis is positive, indicating f is positive, that is that linked reactions must exist. While the calculated f value seems small, it is a minimum. The assumed value of ΔFw is high because the other linked reactions have a lower ΔF than the glucose oxidation. Experimentally for the example chosen f has a value 4.95, that is to say for each gram of cellular material synthesized 4.95 g of waste product (principally CO_2 and water) are given off. Thermodynamically f min is the order of 1/100th this value indicating of course that the process of *coli* duplication in minimal medium produces far more entropy than is required to satisfy the second law. From some published value on the heat given off by dividing bacterial cells[11] we previously calculated[4] that *coli* cells produced about 3.3 times the entropy required by a balance of the second law. Since the thermal measurements were made on cells growing in a complex medium, it is difficult to compare the two figures except to note that the cells seem to be vastly more efficient (as entropy sinks) when growing in a complex medium.

Equation (7) suggests an experimental check under certain extreme conditions. We may rewrite (7) as an equality:

$$f = \frac{-1}{\Delta Fw}\left[\frac{RT}{M}\log\frac{Cc}{Cm} + RTi\ln 2 + \Delta Hc\right] + f' \tag{10}$$

where f' is a measure of the extra entropy produced or the inefficiency of the cell in ordering its constituents with the least possible change in the surroundings.

Now it seems clear that f' will not depend strongly on Cm, the concentration of nutrients, because any effects due to medium concentration should be covered in the term $RT/M \log Cc/Cm$. We have already pointed out that Cm is under the control of the experimenter. We may then measure f as a function of Cm and plotting f *versus* $\log Cc/Cm$ the slope will be $RT/M\Delta Fw$ which gives an opportunity to measure the effective value of ΔFw. In cases where f' is the dominating term it will be difficult to determine the effect due to concentration but for cases of very enriched medium where f seems to be closer to f min the effect should be observable.

The necessary production of heat involved in the entropy balance imposes further restrictions on cell duplication. Specifically, since exothermic processes are a necessary part of cell duplication, the rate of duplication must be slow enough to dissipate the heat or else thermal inactivation of cell components will result. This may be seen by rewriting equation (5) as

$$\Delta Q \geqslant \frac{RT}{M}\log\frac{Cc}{Cm} + RTi\ln 2 - fT\Delta Sw \tag{11}$$

where ΔQ equals $(\Delta Hc + f\Delta Hw)$. Now $m\Delta Q$ is the amount of heat produced in the dividing cell system in a single division time, I. If the average volume of the system during the division process is $3/2\ v$, the average rate of heat production per unit volume in the cell system, designated q, is $2/3\ m\Delta Q/v\tau$. If the average radius of the cell during the process is a, then assuming that the temperature steady state is reached rapidly in comparison to the change in size, we can use the theory of thermal conductivity to compute the temperature at the center of the sphere, Ti,

$$Ti = T + \frac{qa^2}{K} \tag{12}$$

where K is the thermal conductivity and T is the temperature of the environment. Substituting for q we get

$$\Delta T = Ti - T = \frac{-2\,ma^2}{3\tau vK}(\Delta Hc + f\Delta Hw) \tag{13}$$

In general if the cell is going to avoid disruptive effects due to heat $\Delta T < \beta$ where β is some temperature rise which empirically interferes with cell replication. Applying this to equation (13) and rearranging terms

$$\frac{\tau}{a^2} > -\frac{2\rho}{3\beta K}(\Delta Hc + f\Delta Hw) \tag{14}$$

where ϱ is the density of the cell m/v. Substituting reasonable values of the parameters[*], we get

$$\tau > (12{,}400 + 181{,}200\,f)a^2$$

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[*] $K = 0.0014$ cal deg/cm sec; $\varrho = 1.05$ g/cm^3; $\beta = 5°$; $\Delta Hc = -124$ cal/g; $\Delta Hw = -1812$ cal/g.

Table II presents τ as a function of a for some extreme values of f. In principle Table II should hold for a tissue mass as well as a cell, provided that conduction is the only method available for getting rid of heat. It is then clear that as a cell or tissue gets larger than a millimeter or so in size it must develop methods of getting rid of heat or it must divide at a rather slow rate. This limitation stems only from the second law of thermodynamics and the auxiliary fact that living systems are subject to thermal inactivation.

TABLE II

MINIMUM DIVISION TIME OF CELLS AS A FUNCTION OF SIZE AND METABOLIC EFFICIENCY

a	For $f = 0.1$	For $f = 10$
1 μ	0.0003 sec	0.02 sec
10 μ	0.03 sec	2 sec
100 μ	3 sec	200 sec
1 mm	5 min	300 min
1 cm	5000 min	500 h
10 cm	833 h	2000 days

The main conclusion to be drawn at this point is that thermodynamic analysis can be useful in a detailed analysis on some living processes. At present experimental data is not oriented toward this type of treatment. The analysis itself is of course capable of much greater sophistication. Open fields for such investigation are irreversible thermodynamics[12] and the application of statistical mechanics to biological system[10].

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