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Free energy levels and entropy production in muscle contraction and in related solution systems

(myosin ATPase/actin activation/enzyme kinetics)

TERRELL L. HILL* AND R. M. SIMMONS†

* Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014; and † Department of Physiology, University College London, Gower Street, London WCIE 6 BT, England

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ABSTRACT "Basic" and "gross" free energy levels of a macromolecule such as myosin or Na,K-ATPase, defined in a previous publication, are discussed here for two relatively complicated cases: a six-state kinetic diagram of the sort that could be used to describe the actin activation of myosin-ATPase in solution; and muscle contraction, where a similar kinetic diagram is needed for each value of a positional variable *x*.

This paper is a continuation of a previous publication (1). The first paper contained general considerations and some simple examples. Here we discuss a more complicated illustrative case involving solution kinetics and then turn to the corresponding problem in muscle contraction. The notation is unchanged (1); the reader is assumed to be familiar with the earlier work.

A multi-cycle diagram at steady state

We consider a large ensemble of N independent and equivalent macromolecular systems in solution, each of which has the same kinetic diagram (states, transitions, and rate constants). We let E represent the particular macromolecule of interest. Also, let L = ligand, S = substrate, and P = product. Possible states of a system are then E, ES, EP, LES, etc. Fig. 1 shows the explicit diagram that we consider. In this case there are six states in the diagram; the enzymatic reaction $S \rightarrow P$ provides the thermodynamic drive or chemical force; and the ligand L modifies the enzyme kinetics. For example, E = myosin, S = ATP, P = ADP + P$_i$, L = actin.

The diagram in this case has 14 cycles (Fig. 2). The reaction $S \rightarrow P$ provides the only force ($\mu_S - \mu_P > 0$) but it appears at two places in the diagram. The arrow in cycles *a* through *h* shows both the direction of positive force and the positive direction assigned to the cycle flux. In view, then, of Eqs. 22 and 23a of ref. 1 as applied to each of these cycles, we have $J_a > 0, \ldots, J_h > 0$ at steady state. Cycles *i* through *n* include no force and have zero net steady-state flux. In the actin-myosin (heavy meromyosin or subfragment 1)-ATP system in solution (as well as in muscle), cycle *c* is believed to dominate.

Let us begin by considering the rate of entropy production under transient conditions. This argument can obviously be extended to an arbitrary, more general, diagram. In Eq. 18 of ref. 1, we first separate out the terms in μ_S, μ_P and μ_L:

$$T\frac{d_iS}{dt} = \sum_{ij} J_{ij}\Delta\mu_{ij} + \mu_S(J_{12} + J_{45}) - \mu_P(J_{31} + J_{64}) + \mu_L(J_{14} + J_{25} + J_{36}), \quad [1]$$

where $\Delta\mu_{ij} \equiv \mu_i - \mu_j$. The ij sum is over the nine lines in the diagram. The kinetic equations of the system can be written

$$N\frac{dp_i}{dt} = -\sum_{k_i} J_{ik_i} \quad (i = 1, 2, \cdots, 6) \quad [2]$$

where the sum here is over those (three) states k_i that are connected to state i by lines in the diagram. Using Eq. 2, the ij sum in Eq. 1 becomes

$$\sum_{ij}(J_{ij}\mu_i + J_{ji}\mu_j) = \sum_{i=1}^{6}\mu_i\Big(\sum_{k_i} J_{ik_i}\Big) = -N\sum_i \mu_i\frac{dp_i}{dt}. \quad [3]$$

The second expression results on collecting those terms that belong to each state i. Thus

$$T\frac{d_iS}{dt} = -N\sum_i \mu_i\frac{dp_i}{dt} + \mu_S(J_{12} + J_{45}) - \mu_P(J_{31} + J_{64}) + \mu_L(J_{14} + J_{25} + J_{36}). \quad [4]$$

Eq. 18 of ref. 1 expresses the rate of entropy production as a sum over the transitions of the system while Eq. 4 relates the same quantity to the reaction participants $S, P, L, i = 1, \cdots, 6$ (see, for example, Eq. 7.105 of ref. 2). See also Eq. 31 of ref. 1 (steady state only) for a third mode of expression.

We turn now to the special case of a steady state. We examine first the sign of each of the nine J_{ij} (and hence of the $\Delta\mu'_{ij}$) by writing these quantities in terms of $J_a, J_b, \cdots, J_h$, all of which are positive. We can ignore $J_i, \cdots, J_n$ since they are all zero. We find, on inspection of Fig. 2, using simplified notation,

$$\begin{aligned}
J_{12} &= a + d + e + g \\
J_{23} &= a + c + g + h \\
J_{31} &= a + d + f + h \\
J_{45} &= b + c + f + h \\
J_{56} &= b + d + e + f \\
J_{64} &= b + c + e + g \\
J_{14} &= -e + f - g + h \\
J_{25} &= -c + d + e - h \\
J_{36} &= c - d - f + g.
\end{aligned} \quad [5]$$

Obviously, the first six fluxes (and the $\Delta\mu'_{ij}$) are all positive. These are transitions in cycles *a* and *b*, in the direction of the force. But the sign of the three downward fluxes (Fig. 2) is uncertain. Their sum, however, is zero (because the total top and bottom populations in the diagram are constant). Although $\Delta\mu'_{ij}$ is positive for each step in cycles *a* and *b*, this is *not* possible for *all* of the other force-containing cycles (*c* through *h*). This follows from the last three of Eqs. 5. But of

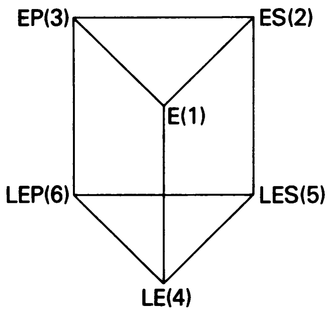

FIG. 1. Kinetic diagram for E = enzyme, S = substrate, P = product. L is a ligand that modifies the enzyme kinetics.

course the *sum* of $\Delta\mu'_{ij}$ around any of the cycles a through h is $\mu_S - \mu_P$. This sum is zero around cycles i through n.

We also note that

$$\begin{aligned} J_{12} + J_{45} \text{ (bind } S) &= J_{23} + J_{56} \ (S \rightarrow P) \\ &= J_{31} + J_{64} \text{ (release } P) \\ &= J_a + J_b + \cdots + J_h \equiv J, \end{aligned} \quad [6]$$

where J is the total flux in *all* cycles. Thus, at steady state, Eq. 4 becomes

$$T\frac{d_iS}{dt} = J(\mu_S - \mu_P), \quad [7]$$

a particularly simple result. This is a special case of Eq. 31, ref. 1.

Numerical Examples Based on Fig. 1. Here we supplement the above discussion with numerical examples that bear some superficial resemblance (but no more than that) to the subfragment 1-F actin–ATP system. Fig. 3a shows the basic free energy levels chosen for the six states. The level $A_4 + \mu_P$ is arbitrarily chosen as zero. The vertical free energy unit used (between pairs of states) is $kT\ln 300 = 5.704\ kT$. The thermodynamic force here, $\mu_S - \mu_P$ (double-headed arrows in Fig. 3), is equal to four of these units, or 22.815 kT (roughly the free energy of ATP hydrolysis at actual concentrations). An extra level has been included at top and bottom of the figure so that all nine transitions in the diagram (Fig. 1) can be indicated by vertical or slanting lines.

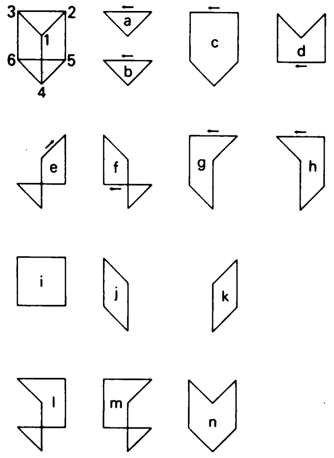

FIG. 2. The 14 cycles that belong to the diagram at upper left. The arrows indicate the direction of positive force and positive flux. The force is zero in the last six cycles and the corresponding steady-state cycle fluxes are also zero.

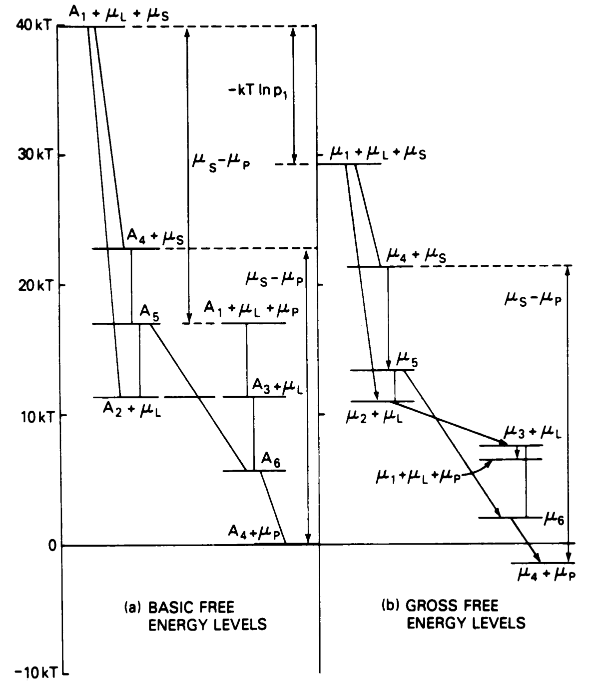

FIG. 3. (a) Basic and (b) gross free energy levels for the numerical example in Fig. 4.

The rate constants are shown in Fig. 4. This is a quite arbitrary set except, of course, inverse rate constants for each line in the diagram must be consistent with the basic free energy level difference already assigned in Fig. 3a (Eqs. 6 and 9 of ref. 1). For example,

$$\frac{\alpha_{56}}{\alpha_{65}} = \frac{\alpha}{\alpha/(9 \times 10^4)} = \exp\left(\frac{A_5 - A_6}{kT}\right) = (300)^2. \quad [8]$$

Also, the rate constants have been selected to make cycle c the dominant cycle (as in the myosin case).

With the rate constants available from Fig. 4, the steady-state probabilities of the six states may be calculated by computer from Eq. 12 of ref. 1 and Eq. 2 (together with $\Sigma_i p_i = 1$). These probabilities are given in Table 1. The transition fluxes $J_{ij}/N\alpha$ then follow from Eq. 12 of ref. 1 and the cycle fluxes $J_n/N\alpha$ from Eqs. 5. These fluxes are also included in

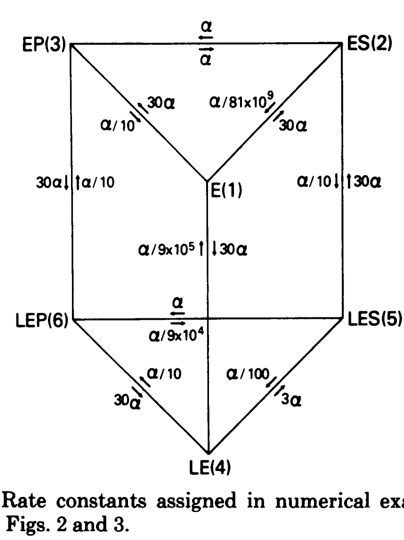

FIG. 4. Rate constants assigned in numerical example corresponding to Figs. 2 and 3.

Table 1. Example in Figs. 3 and 4

i	p_i	ij	$J_{ij}/N\alpha$	$J_{ij}\Delta\mu'_{ij}/N\alpha kT$	n	$J_n/N\alpha$
1	0.000025	12	0.00075	0.01	a	0.00075
2	0.6961	23	0.6736	2.31	b	0.0248
3	0.0225	31	0.0015	0.00	c	0.6721
4	0.2326	45	0.6976	5.54	h	0.00075
5	0.0248	56	0.0248	0.28	sum	0.6984
6	0.0240	64	0.6969	2.39		
		14	0.00075	0.01		
		52	0.6729	1.59		
		36	0.6721	3.79		

Table 1. The important transition fluxes are those belonging to cycle c, which is seen to have the largest cycle flux by far. Cycles d, e, f, and g have negligible fluxes and hence are omitted from the table. The total flux J (Eq. 6) is 0.6984 $N\alpha$ and hence the total rate of entropy production (Eq. 7) is $J(\mu_S - \mu_P) = 15.93\ N\alpha kT$.

Since the five large transition fluxes (those in cycle c) are all about equal, the separate contributions $J_{ij}\Delta\mu'_{ij}$ of these transitions to the total rate of entropy production (Table 1) are approximately proportional to the $\Delta\mu'_{ij}$. The latter quantities can be seen in Fig. 3b.

Fig. 3b shows the gross free energy levels for this model, calculated from the basic levels (Fig. 3a), the state probabilities in Table 1, and Eq. 3 of ref. 1. That is, the i-th basic level is lowered by an amount $-kT\ln p_i$ to obtain the i-th gross level. The single-arrowed lines in Fig. 3b belong to those six transitions (in cycles a and b) for which $\Delta\mu'_{ij}$ is necessarily positive. Note that although $\Delta A'_{31}$ is negative, the steady-state probabilities p_1 and p_3 turn this around and make $\Delta\mu'_{31}$ positive (as it must be).

As a second example, suppose the concentration of substrate c_S is reduced by a factor of 300, with no other change in the system. This is a step nearer to equilibrium, for if c_S is reduced by a factor of $(300)^4$, then $\mu_S - \mu_P = 0$ and the steady-state kinetic system will be operating at equilibrium. In the $c_S/300$ case, all rate constants in Fig. 4 remain unchanged except α_{12} and α_{45}, which are both reduced by a factor of 300. In Fig. 3a, the basic free energy levels are the same except that the top two levels (states 1 and 4, because of the μ_S term) are both lowered by one unit, $kT\ln 300$. The left-hand side of Table 2 shows the new steady-state probabilities and gives the only three significant cycle fluxes. Most systems are now in state 4 because of the small rate constant α_{45}. The transition fluxes (omitted from the table) follow from Eqs. 5. The total flux J is reduced here to 0.00986 $N\alpha$ and the total rate of entropy production is lowered to 17.111 $kTJ = 0.169\ N\alpha kT$. These much smaller values reflect the fact that this steady state is closer to equilibrium than in the previous case (Figs. 3 and 4).

Table 2. Effect of reduction of c_S

$c_S \times (1/300)$				$c_S \times (1/300)^4$	
i	p_i	n	$J_n/N\alpha$	i	p_i^e
1	5.65×10^{-7}	b	0.00035	1	3.69×10^{-8}
2	0.00983	c	0.00949	2	1.11×10^{-5}
3	0.000328	h	0.00002	3	1.11×10^{-5}
4	0.9859	sum	0.00986	4	0.9967
5	0.000350			5	3.69×10^{-8}
6	0.00361			6	0.00332

The gross free energy levels have the same general appearance as in Fig. 3b, but are somewhat compressed, with the top level ($\mu_1 + \mu_L + \mu_S$) at 19.84 kT and the bottom level ($\mu_4 + \mu_P$) at -0.01 kT.

In the equilibrium case referred to above (a third example), again only α_{12} and α_{45} are changed: both are reduced from the values in Fig. 4 by a factor of $(300)^4$. In this case, the top two levels in Fig. 3a are lowered by four units, $kT\ln(300)^4$, so that they now coincide with the levels $A_1 + \mu_P + \mu_L$ and $A_4 + \mu_P$ (since $\mu_S = \mu_P$). Thus there is only a single set of six basic free energy levels, namely, the bottom six levels in Fig. 3a, and there is a Boltzmann probability distribution among these levels, as given on the right-hand side of Table 2. State 4 is practically the only state occupied. The flux J and rate of entropy production are both zero. The compression of the gross free energy levels (mentioned above) is now complete, because all states are at the same gross level, $-kT\ln p_4 = -0.0033\ kT$ (because we have taken $A_4 + \mu_P \equiv 0$).

If, instead of reducing the value of c_S as in the two preceding examples, we had changed the value of c_L or c_P, then the basic levels in Fig. 3a that include μ_L or μ_P, respectively, would have been altered, with corresponding changes in the α_{ij} (for transitions 14, 52, 36 or for 31, 64, respectively).

Extension to muscle contraction: Single actin site models

In this section we extend the previous discussion to the problem of muscle contraction. The situation is somewhat more complicated here because the biochemical kinetics depend on a structural variable x. Furthermore, this same variable brings external mechanical work into the analysis. Incidentally, as applied to muscle, "steady-state" as used in previous sections refers here to a steady isometric contraction (and any value of x). A steady isotonic contraction at a finite velocity v requires separate analysis (see below).

To avoid undue notational complexity, we consider only single actin site models (3, 4): at any instant a given myosin cross-bridge has accessible to it (we assume) only one actin site. Multi-site models do not differ in any fundamental way (4); multiple sites simply lead to a proliferation of states in the diagram and possibly to a change in the repeat distance (below).

The longitudinal location of the actin site relative to the cross-bridge is specified by a variable x. The actin site repeat distance is d; the origin of the x-axis is chosen for convenience so that an integral from $-d/2$ to $+d/2$ may be used in taking averages over x.

We assume that the reader of this section is familiar in a general way with the necessary theoretical formalism (3) for models of this type. Most details of the formalism will not be repeated here.

In effect, we have a different ensemble of cross-bridges, all of equal size, for each equal interval dx between $x = -d/2$ and $x = +d/2$. We shall use Fig. 1 ($E = M$ = myosin cross-bridge, $S = T$ = ATP, $P = D$ = ADP + P$_i$, $L = A$ = actin site) as a prototypal biochemical diagram; cycle c in Fig. 2 presumably dominates in this diagram (5).

The free energies A_i of the unattached states (i = 1, 2, 3) are constants while the A_i of attached states (i = 4, 5, 6) are functions of x. "Attached" refers here to the AM link. All rate constants α_{ij} are in general functions of x except those between pairs of unattached states. For given t, $p_i(t,x)$ is the fraction of cross-bridges at x in state i. We have a set of basic and a set of gross free energy levels of the sort shown in Fig. 3, at each x, where the chemical potentials μ_i are defined as before (Eq. 3 of ref. 1). However, for both the basic and gross free energy levels, μ_L is here omitted from Fig. 3 because $L = A =$ actin site is now part of the permanent myofilament structure and is not a ligand in solution with a concentration. The role of actin is in this respect analogous to that of a solid adsorbent with sites for the binding of an adsorbate. The thermodynamic force $\mu_T - \mu_D$ in Fig. 3 is the same for all x.

Eq. 18 of ref. 1 and the following paragraph are relevant here: $J_{ij}(t,x)\Delta\mu'_{ij}(t,x) \geq 0$ for all transitions at any t and x. But definite directional statements about J_{ij} and $\Delta\mu'_{ij}$ separately are more difficult (as we have already seen, even without x dependence).

In isometric contractions, an ensemble at any x has no interaction with the other x-ensembles. Therefore, the prior conclusions for single-cycle models and multi-cycle models apply here at each x without change. No external work is involved. Under nonisometric conditions, however, the different x-ensembles are not independent of each other and external work must be taken into account.

The interdependence of x-ensembles is implicit in the appropriate kinetic equations. These are (3)

$$\left(\frac{\partial p_i}{\partial t}\right)_x - v(t)\left(\frac{\partial p_i}{\partial x}\right)_t = -\sum_{k_i} J_{ik_i}(t,x)\,(i = 1, 2, \cdots, 6) \quad [9]$$

where $v(t)$ is the velocity of contraction and, for convenience, from this point on we omit the factor N in Eq. 12 of ref. 1 and Eq. 2 so that J_{ik_i} is a mean flux *per cross-bridge* at x. In a steady isotonic contraction with v = constant, we have

$$\left(\frac{\partial p_i}{\partial t}\right)_x = 0 = v\,\frac{\partial p_i}{\partial x} - \sum_{k_i} J_{ik_i}(x)\,(i = 1, 2, \cdots, 6). \quad [10]$$

This equation illustrates the interaction between ensembles at different x values: Eq. 10 may be regarded as the steady-state kinetic equation for the components of a chemically reacting system with hypothetical *one-way* (x decreasing; $v > 0$), one-dimensional diffusion (diffusion coefficient = v) subject to periodic boundary conditions (what "goes out" at $x = -d/2$ "comes in" at $x = +d/2$). The "diffusion" mixes the x-ensembles when $v > 0$, the more so the larger the value of v.

We see from Eq. 10 that, in a steady isotonic contraction, the total flux into each state i at x, owing to transitions, is *not* equal to zero; but it *is* equal to zero if we average over x:

$$0 = -\sum_{k_i} \overline{J}_{ik_i} \qquad (i = 1, 2, \cdots, 6) \quad [11]$$

where the term in v drops out because of the periodic boundary conditions. We have used, in Eqs. 11, the definition

$$\overline{J}_{ij}(t) \equiv \frac{1}{d}\int_{-d/2}^{+d/2} J_{ij}(t,x)dx. \quad [12]$$

It is easy to show from the set of Eqs. 11 that

$$\overline{J}_{12} + \overline{J}_{45} = \overline{J}_{23} + \overline{J}_{56} = \overline{J}_{31} + \overline{J}_{64}, \quad [13]$$

just as in Eq. 6. But here x averaging is required and we cannot make use of the diagram method (because $v \neq 0$).

Let us turn now to the rate of entropy production *per cross-bridge*, averaged over x, and under arbitrary transient conditions:

$$T\frac{d_iS}{dt} = \frac{1}{d}\int_{-d/2}^{+d/2} \sum_{ij} J_{ij}(t,x)\Delta\mu'_{ij}(t,x)dx \geq 0, \quad [14]$$

where the sum is over the nine lines in the diagram (Fig. 1). We can separate out the terms in μ_T and μ_D (as in Eq. 1), use Eqs. 3 and 9, and find

$$T\frac{d_iS}{dt} = -\frac{1}{d}\int_{-d/2}^{+d/2} \sum_i \mu_i \left[\frac{\partial p_i}{\partial t} - v(t)\,\frac{\partial p_i}{\partial x}\right]dx + \mu_T(\overline{J}_{12} + \overline{J}_{45}) - \mu_D(\overline{J}_{31} + \overline{J}_{64}). \quad [15]$$

To examine the terms in $v(t)$, we replace μ_i by $A_i + kT\ln p_i$. For unattached states (i = 1,2,3), A_i = constant. Then both integrals (A_i, $\ln p_i$) are zero (periodic boundary conditions, integration by parts). For attached states (i = 4,5,6), the $\ln p_i$ integral is also zero but the A_i integral (integration by parts) introduces the force $\bar{F}_i$, since $F_i(x) = \partial A_i(x)/\partial x$. Thus

$$T\frac{d_iS}{dt} = -\frac{1}{d}\int_{-d/2}^{+d/2} \sum_i \mu_i(t,x)\,\frac{\partial p_i}{\partial t}\,dx - \overline{F}(t)v(t) + \mu_T[\overline{J}_{12}(t) + \overline{J}_{45}(t)] - \mu_D[\overline{J}_{31}(t) + \overline{J}_{64}(t)] \geq 0, \quad [16]$$

where $\bar{F} = \bar{F}_4 + \bar{F}_5 + \bar{F}_6$. The "reaction participant" terms (i = 1, • • •, 6; T,D) are essentially as in Eq. 4, but a new feature here is the appearance of the rate of performance of external work, $\bar{F}v$. The work term obviously originates, mathematically, from the x-ensemble "mixing" term, $-v\partial p_i/\partial x$, in Eq. 9. The interpretation of Eq. 16: not all of the macroscopic free energy drop in the over-all reaction system (terms in μ_i, μ_T, μ_D; see Eq. 7.105 of ref. 2) is wasted or dissipated (Td_iS/dt); some of it is converted into external work ($\bar{F}v$). Or to turn this statement around: the macroscopic free energy drop of all participants arises not only from dissipative transitions but also from external work done by the system.

In a steady isotonic contraction, Eq. 16 simplifies to

$$T\frac{d_iS}{dt} = \overline{J}(\mu_T - \mu_D) - \overline{F}v \geq 0, \quad [17]$$

where $\bar{J}$ (ATP flux) is defined as either of the expressions in brackets in Eq. 16 (see Eq. 13). This same relation (Eq. 17) is introduced in a very different way on pp. 291 and 336 of ref. 3.

Under *steady* conditions, it is easy to see that we also have

$$T\frac{d_iS}{dt} = \frac{1}{d}\int_{-d/2}^{+d/2} \sum_{ij} J_{ij}\Delta A'_{ij}dx = \overline{J}(\mu_T - \mu_D) - \overline{F}v \geq 0 \quad [18]$$

because all $\ln p_i$ terms in Eq. 14 make no contribution to the final result. Thus, so to speak, the dissipation owing to all transitions ij, averaged over x, can be monitored on either the gross free energy levels (Eq. 14) or on the basic free energy levels (if v = constant). The latter point of view is used, in effect, on pp. 328–329 of ref. 3. The contribution of the transition ij (averaged over x) to the entropy production is clearly

$$\frac{1}{d}\int_{-d/2}^{+d/2} J_{ij}\Delta\mu'_{ij}dx = \frac{1}{d}\int_{-d/2}^{+d/2} J_{ij}\Delta A'_{ij}dx \geq 0. \qquad [19]$$

But this ij contribution cannot be further separated into a work and an ATP flux term (as in Eq. **18**) because work is associated with states, not transitions.

Finally, let us consider what can be said about the sign of the nine $\bar{J}_{ij}$ and $\overline{\Delta\mu'}_{ij}$ in Fig. 1 in a steady isotonic contraction, where, as in Eq. **12**, we define

$$\overline{\Delta\mu'}_{ij} \equiv \frac{1}{d}\int_{-d/2}^{+d/2} \Delta\mu'_{ij}dx. \qquad [20]$$

We limit the discussion to systems for which $\bar{F} \geq 0$ and $v \geq 0$ (other cases are possible).

When $v = 0$, the diagram method can be applied *at each* x, as in Eqs. **5** and **6**. Thus the first six $J_{ij}(x)$ in Eq. **5** [and the corresponding $\Delta\mu'_{ij}(x)$] are positive at every x. On integrating over x, these six $\bar{J}_{ij}$ (and $\overline{\Delta\mu'}_{ij}$) are necessarily positive. The other three $\bar{J}_{ij}$ and $\overline{\Delta\mu'}_{ij}$ are uncertain in sign; it is even possible that one or more corresponding pairs would not agree in sign. But the sum of the three $\bar{J}_{ij}$ must be zero (as it is at every x). Just as in the previous section, $\overline{\Delta\mu'}_{ij}$ is positive for all the steps in cycles a and b but the same cannot be said for *all* of cycles c through h.

If $v > 0$, we have Eqs. **11** and **13**, on integrating over x. Each pair ($= \bar{J}$) in Eq. **13** is surely positive, as a consequence of Eq. **17**. We also have

$$\bar{J}_{14} + \bar{J}_{25} + \bar{J}_{36} = 0. \qquad [21]$$

But the sign of the nine individual $\bar{J}_{ij}$'s is not obvious. The same is true of the nine $\overline{\Delta\mu'}_{ij}$'s. Also, $\bar{J}_{ij}$ and $\overline{\Delta\mu'}_{ij}$ need not always agree in sign. It is easy to see that the *sum* of the $\overline{\Delta\mu'}_{ij}$ around any of the cycles a through h is $\mu_T - \mu_D$. This sum is zero around the other cycles (i through n).

If $v > 0$ and only a single cycle is important, say cycle c (Fig. 2), 236452, then one can go further (see Eq. **11**):

$$\bar{J} = \bar{J}_{23} = \bar{J}_{36} = \bar{J}_{64} = \bar{J}_{45} = \bar{J}_{52} > 0. \qquad [22]$$

That is, *after* x-averaging, each step has equal and positive flux. At individual values of x, these $J_{ij}(x)$ are not only not equal (Eq. **10**), but negative values are possible. For example, consider $J_{52}(x)$ near $x = +d/2$ when $v > 0$. Because $p_2 \neq 0$ and $p_3 \neq 0$ while $p_4 = p_5 = p_6 = 0$ (attached states) at $x = +d/2$, the rate of the transition $5 \rightarrow 2$ is zero. Therefore, both $J_{52}(x)$ and $\Delta\mu'_{52}(x)$ must be negative near $x = +d/2$. The larger v, the greater the x interval over which these negative values will persist before becoming positive. These comments obviously resemble those made in ref. 1 concerning a transient with t near $t = 0$.

In the single-cycle, $v > 0$ case, the sum of the $\overline{\Delta\mu'}_{ij}$ around the cycle is $\mu_T - \mu_D$. Despite Eq. **22**, it is not certain that all of the individual $\overline{\Delta\mu'}_{ij}$'s are positive. For example, it seems possible that $\overline{\Delta\mu'}_{52}$ might be negative when v is large. Of course all of the $\overline{\Delta\mu'}_{ij}$ must be positive if $v = 0$.

As can be seen from the above discussion, x-averaging of J_{ij} and $\Delta\mu'_{ij}$ (at arbitrary v) does not lead to any particularly simple or fundamental thermodynamic relations involving $\bar{J}_{ij}$ and $\overline{\Delta\mu'}_{ij}$. For example, the ij entropy production in Eq. **19**, $\overline{J_{ij}\Delta\mu'_{ij}}$, is not equal to $\bar{J}_{ij}\overline{\Delta\mu'}_{ij}$. Although $\bar{J}_{ij}$ is an operational quantity, $\overline{\Delta\mu'}_{ij}$ does not seem to have any fundamental significance. Correspondingly, there does not appear to be any correct way of giving an x-averaged kinetic account of this system (i.e., using x-averaged rate constants, state probabilities, etc.). Many of the fundamental parameters of a model are functions of x, and this level of detail cannot be escaped in a rigorous treatment.

Numerical illustration of this section, using an explicit model of muscle contraction, will be reserved for a later publication.

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