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NOT JUST CATALYSTS—MOLECULAR MACHINES IN BIOENERGETICS

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1. INTRODUCTION

In the preceding chapters of this volume, we have viewed the protein-dynamic basis of enzyme action from a number of different perspectives. One common concern is the intimate relationship between the protein and its environment in the achievement of the proper configuration for binding/catalysis processes. The physical complexity of the problem is evident from the diversity of approaches to which these systems are amenable. We are accustomed to thinking of enzyme molecules as free-energy linkage devices, operating at thermodynamic equilibrium with a bulk phase (reservoir, heat bath). Many of the foregoing chapters treat this *modus operandi*. Yet there are indications that some types of enzymes *in organized states* can transduce energy from nonequilibrium external states in the performance of (electro)chemical work (see chapters by Careri and Gratton, Fröhlich, Volkenstein, and Blumenfeld et al.). The relevance of the latter systems is heightened as evidence accumulates regarding the locational specificity of enzyme function in vivo.

Here we discuss some fundamental aspects of enzyme dynamics in relation to the execution of reaction–diffusion processes within structured cellular microenvironments. Enzyme action therein may have rather bizarre qualities compared to the familiar nature manifest by enzymes isolated in bulk solution. Some such enzymes in the "living state" may operate more in the fashion of "molecular machines," driven in a cyclical manner by an external flow of energy. Study of these systems leads us to alter our conceptualization of the very thermodynamic–kinetic basis of many kinds of material transformations as they occur in biological systems. A proper characterization may require analogical thinking, involving concepts from such disciplines as solid-state physics and quantum-statistical mechanics, as well as biochemistry.

2. MOLECULAR ENERGY MACHINES

During the early 1970s the late Dr. Colin W. F. McClare drew attention to the fact that far-from-equilibrium bioenergetic systems, owing to their molecular size, pose unique thermodynamic problems[1–5] (see also reference 6). McClare[1] focused on the conceptual problem in bioenergetics, that "energy appears to be

transferred between single molecules and yet to add up to produce a macroscopic effect." Or, as phrased by Blumenfeld,[7] "The physical peculiarity of living systems consists of the fact that these systems contain devices of molecular dimensions capable of performing mechanical work." Pursuing this issue, McClare coined the term molecular energy machine, to describe a single enzyme molecule that, in a cyclical fashion, acts to couple the energy released by one form of reaction (e.g., the chemical energy of ATP hydrolysis) to an otherwise unfavorable reaction (e.g., "active" ion transport, muscular contraction) without, during the lifetime of its cycle, being exposed to the macroscopic (and thermalizing) environment. In two recent monographs[7,8] Blumenfeld has advanced this theme further, particularly in relation to the properties of protein molecules.

This (microscopic) mechanistic viewpoint is quite essential to an understanding of the nature and function of a variety of bioenergetic transduction processes involving cellular multienzyme systems. As indicated by Blumenfeld,[7] the "first main problem of bioenergetics" is the mechanism of energy coupling of chemical reactions. Continuing, he noted that

> In all cases energy transduction (i.e., the energy coupling of chemical reactions) is actualized with the help of specific macromolecular constructions, by machines of molecular dimensions. The second main problem of bioenergetics is, therefore, the development of a theory explaining the functioning of molecular machines.

Now it must be admitted at the outset that the physical meaning of "energy" (chemical or otherwise) is extremely elusive at the molecular level. Nevertheless, because we shall actually be dealing with (and attempting to make a clear distinction between) what amount to "free" and "unfree" energies (concepts that have some degree of meaning and familiarity at the macroscopic level), we shall retain somewhat loosely the term energy in the following discussion. It will become apparent, however, that at the molecular level, "free" energy is, in fact, far from free.

3. INADMISSIBILITY OF USING THERMAL FLUCTUATIONS FOR DOING USEFUL MOLECULAR WORK

As is well known, there is a heterogeneous molecular distribution of thermal energies in an isolated thermodynamic phase at any constant temperature above absolute zero.[9,10] Of course, this distribution is described by Maxwell–Boltzmann statistics, whereby the average thermal (kinetic) energy of the molecules per degree of freedom is just $\frac{1}{2}k_BT$ (where k_B is the Boltzmann constant).

Early in the development of classical thermodynamics, a paradox became

apparent to James Clerk Maxwell: this energy (velocity) distribution might, in principle, be exploited by a microscopic intelligence, *Maxwell's demon*, which could operate a trapdoor between two isothermal gaseous phases in such a way that spontaneous heat separation might occur and useful external work be done.[11–13] This would, of course, violate the second law of thermodynamics in its usual form, and it became necessary to exorcise Maxwell's demon in a way that would explain exactly why it could not, in fact, operate as proposed by Maxwell. This was duly executed, for instance, by Brillouin,[13–16] who showed that a continuing flow of information, with an energy (negentropy) content greater than that generated by the demon's activities, would be required for its operation. (This is consistent with the view that entropy measures the residual ignorance we have about the microstate of a system.[17])

Nevertheless, as Sir Karl Popper[18] noted, common statements of the second law are highly unsatisfactory (for 13 such statements see reference 19); for example, they do not give a proper explanation for the phenomenon of Brownian motion. As Popper[18] writes,

> Following Einstein's first papers on the Brownian movement [see references 20 and 21] ... we are forced to say that in the Brownian movement, observable heavy particles are sometimes lifted up against the gravitational field of the earth, at the expense of a (slight) cooling down of the liquid. Of course, just as many particles are sinking, thus restoring the lost heat. But this does not matter: the letter of Planck's law is undoubtedly violated, as Einstein in fact stated.

This statement is especially true if we define work simply as energy. And, until we can properly express what "work," "free energy," and "unfree energy" actually mean at the level of the individual macromolecule, we cannot say exactly what it is that the second law does in fact forbid in molecular bioenergetics. It was this most unsatisfactory situation that such workers as McClare[1] and Blumenfeld[7,8] set out to remedy.

4. STORED ENERGY, USEFUL WORK, AND CYCLE TIME

McClare[1] explicitly defined three new terms: stored energy, useful work, and cycle time. Thus the energies in a system at temperature T_1 may be divided into stored and thermal forms. The latter are defined as those energies that exchange with each other and reach equilibrium in a time less than τ, such that they obey a Maxwell–Boltzmann distribution characterized by T_1. Stored energies are those that remain in a different distribution for a time longer than τ, either in a Maxwell–Boltzmann distribution characterized by a higher temperature T_2 (which case, as we shall see, is inappropriate for our purposes), *or* in a different one

such that higher energy states are more populated than states of lower energy. The importance of this point has been stressed (for the case of photosynthetic systems) by Hill and Rich.[22–24] "Stored" energy is taken to mean "any form of energy which, in the interval τ, does not exchange with the translational, rotational, and vibrational energies which normally constitute heat"[1]; stored energy is thus defined relative to a time τ. (Freed[25] has discussed the importance of considering the time scale in radiationless processes exhibiting irreversibility.) Furthermore, *useful molecular work* is done by a system only when one form of stored energy is converted into another. We begin to see what it is that the second law forbids: weights lifted by Brownian motion have not had useful work done on them, *because the same process that lifted them will, as quickly* (and this is the crucial point), *knock them down again.*

Now it may appear that the foregoing discussion contains nothing that is not well established. It is, for example, well known that in order to give a physical meaning to, say, the pH inside a 1-μm diameter spherical bacterial cell (which at pH 8.0 has less than one free hydronium ion per cell), we can introduce time and define

$$\mathrm{pH} = -\frac{1}{\delta t}\int_{t}^{t+\delta t} \log_{10} a_{\mathrm{H}^+}\, dt \qquad (1)$$

This is not, however, a thermodynamic statement of the second law, nor is it McClare's point, for this equation describes only the rapid exchange of protons with those buffering groups with which they *are* in thermal and thermodynamic equilibrium, as in the Brownian motion above. Note, too, that in Eq. (1) δt remains indefinite; and that in any event, owing to the use of the concept of a single ion activity coefficient, pH constitutes an extrathermodynamic parameter.

Now the bioenergetic processes in which our interest lies are, from a macroscopic viewpoint and for all practical purposes, carried out isothermally (because they take place in a relatively large heat bath). Thus the stored energies cannot obey a Maxwell–Boltzmann distribution characterized by a temperature T_2 significantly greater than ambient. Therefore, the stored energies must be arranged in a manner such that states of higher energy are more populated than states of lower energy. One might cite the well-known population inversion that occurs during the pumping of lasers as an example of this type of distribution of energy states.[1,7,26,27] *Our problem, then, is to describe quantally the meaning and nature of this distribution of states in bioenergetic transduction.*

Summarizing thus far, it is recognized[1–8] that the molecular nature of bioenergetic systems raises important conceptual difficulties, owing to the fact that they are continually bombarded with solvent molecules possessed of mean kinetic energy $k_B T$. Yet the second law tells us that the trapping of such kinetic

(thermal) energies for the performance of useful molecular work under isothermal conditions is disallowed. (For an apparent opposite view, see reference 28.) Accordingly, McClare[3,5] proposed that the transfer of "stored" energies in coupled processes (e.g., muscle contraction, active transport) occurs by some type of "resonance process"; there have been developed[3,5–7] certain possible mechanisms for this energy transfer. For our present purposes, it is sufficient to note that *this "resonance" should be interpreted as the intra- and intermolecular resonant exchange of quanta whose energy is greatly different from those to be found in a Maxwell–Boltzmann distribution of molecular energies at the ambient temperature.*

5. WHICH ENZYMATIC SYSTEMS ARE MOLECULAR ENERGY MACHINES?

Blumenfeld[8] posed the following consideration:

> If, to describe a cell, we should have to choose between two extreme models—a clock mechanism and a homogeneous chemical reaction in the gas phase—the choice would be obvious; a cell is much closer to a mechanical device than to a pure statistical system."

The requirement that "usable" energy remain stored away from equilibrium implies that the McClare–Blumenfeld molecular machines cannot use thermal energies *as intermediate forms* of stored energy, for a molecular energy at equilibrium with the surroundings cannot be stored. Thus there is a clear distinction here from conventional *chemical machines*, which involve thermal processes. An example of the latter is a combustion system. It is characterized by such macroscopic parameters as temperature and pressure, involving an enormously large number of molecular (or atomic) degrees of freedom and requiring an averaging over an extensive phase space of particle positions and momenta. By contrast, the working parts of a mechanical system (e.g., a piston hooked up to a combustion chamber) entail relatively few degrees of freedom (i.e., only a small region in the phase space of possible microstates is allowed). In other words, certain regions of the complete phase space "are kinetically unattainable because high potential barriers separate them from the regions determined by the pattern of rigid bonds within the system, i.e., by the system construction."[8]

Of course, one can extend this mechanistic "construction"[8] view of the cell down to the level of the individual macromolecules, namely, proteins. The preceding chapters in this volume do picture the enzyme molecule as a highly specific machine, capable of directing chemical transformations in the microscopic domain of the active center. Yet there is no reason to suppose that the

soluble enzymes of metabolism are molecular energy machines in the sense used by McClare. Even those such as kinases, which use ATP energy to phosphorylate substrates, can act stochastically only as *catalysts*; they cannot trap "energy" and drive chemical reactions in a direction in which they would not otherwise go spontaneously.

Enzymes whose biological habitat is a *bulk solution* appear to be *equilibrium chemodynamical machines*, designed to generate localized free-energy events necessary to drive the bound substrate into the transition state(s).[29,30]. This does not mean that heat is converted into "free" energy, but rather that the transduction of internal energy and heat exchange with the reservoir are part of the same mechanism (i.e., associated with fluctuational interaction of the protein and the solvent). So, the second law is inviolate.[31]

Free-energy linkage in the isolated protein is based ultimately on the equilibrium fluctuational character of the conformational microstates. In order to define "conformational free energy," we see from other chapters in this text (see those by Gavish, Ikegami, and Lumry and Gregory) that a macroscopically observed conformation of a protein corresponds to a *mixture* of a number of instantaneous (microscopic) conformational states in thermal equilibrium.[32,33] Each of these states represents a point (P, R) in the phase space of momenta (P) and generalized coordinates (R) of the protein. The probability of a given state is determined by the Boltzmann factor, $\exp[-E(P, R)/k_BT]$, where $E(P, R)$ is the total (kinetic plus potential) energy of the protein in the state (P, R). One can define the statistical-mechanical *partition function* Z for the protein as

$$Z = (\text{constant}) \int \exp\left(\frac{-F(R)}{k_BT}\right) dR \tag{2}$$

where $F(R)$ is the potential energy.[32] $F(R)$ is a sum of two terms: the intramolecular interaction energy of the macromolecule in the absence of solvent *and* the free energy of solvation. $F(R)$ represents a "potential of mean force" operative on the protein molecule for various configurations of the solvent particles. Then the (Helmholtz) free energy of the protein conformation is defined as $-k_BT \ln Z$.[32,33] If a number of microscopic states contribute to the partition function, then any macroscopic "property" of the system, A (e.g., substrate binding, catalytic constant), is a weighted average over all such states, as follows:

$$\langle A \rangle = \int A \cdot \exp\left(\frac{-F(R)}{k_BT}\right) dR \tag{3}$$

with integration performed over the accessible conformational (coordinate) space.[34]

Lumry and co-workers[35–37] have developed in detail the *enthalpy–entropy compensation* concept as the root of free-energy linkage in enzymatic processes (see first chapter in this volume). In attempting to grasp the thermodynamic basis of enzyme action, as it relates to the macromolecular properties of proteins, let us pursue Lumry's idea. We begin by distinguishing that in a system such as a protein at equilibrium with a bath, Gibbs free energy (G), pressure (P), and temperature (T) must be constant (to a very high order), in contrast to enthalpy (H), entropy (S), and volume (V). It is in fluctuations of the latter quantities that one must seek the dynamic features of enzyme catalysis. In particular, as suggested by Lumry,[36] "There is increasing evidence that enthalpy (H) fluctuations have been exploited by nature in the evolution of many biological functions."

Lumry has elaborated his "motive" view of enzyme dynamics from a *composite* representation of the equation for Gibbs free energy, $G(T)$, due to Max Planck (*Treatise on Thermodynamics*), as presented more recently by Benzinger.[38] At issue is the fundamental point that *G and ΔG* (under isothermal conditions) *can contain no heat terms*. The enthalpy part is decomposed into a sum of two terms: $H(\mathrm{O})$, which is the potential energy and zero-point vibrational energy; and $\langle Q(T)\rangle$, the *mean heat* contained in the system, obtained by integrating the constant-pressure heat capacity, C_p, from 0°K to T. This pure heat part, $\langle Q(T)\rangle$, is called a *compensation enthalpy*, H_{comp}. It describes the fluctuations of internal energy and volume between the system and environment, as can be seen from the relation $C_p(T) = \sigma_H^2/k_B T^2$, with $\sigma_H^2 = \sigma_E^2 + P^2\sigma_V^2 + T\sigma_{EV}$.[36] (The symbol σ^2 denotes the mean-square (variance) value.) In principle (although rarely in reality), any system (e.g., a protein dissolved in solution) in equilibrium with a reservoir could, via a fluctuation, temporarily lose its total heat content (Q). Such a condition implies that, for a chemical change involving the system (e.g., an enzyme-catalyzed reaction), the part ΔH_{comp} has *nothing to do with* the actual mechanism of the chemical reaction; the mechanistic part is specified only by $\Delta H(\mathrm{O})$, the "motive" part.[38] In fact, the second law requires that, in the overall expression of ΔG, ΔH_{comp} be negated (compensated) by a term $T\Delta S_{comp}$ due to a *compensation entropy*. It is found that S_{comp} is just equal to $\langle Q(T)\rangle/T$.[36]

A crucial message from this representation is that the enzyme molecule cannot collimate heat energy (i.e., that from individual molecular, collisional events at the protein–solvent interface) directly to the active site, for the performance of work. If a fluctuation delivers a certain enthalpy to a region of the protein (e.g., the active site), it is "paid for" by an entropy change elsewhere in the macromolecule. *The role of fluctuations is just to set the course of the free-energy transfer in the enzyme.* This condition, of course, fits the role of ΔQ as a bookkeeping device in the first law of thermodynamics.[17]

Thus any functional feature of the enzyme molecule *in bulk solution* is based on the thermal properties of the system at equilibrium with the solvent phase (at temperature T). Because of its size (with much greater density of energy levels), the bulk reservoir controls the situation through its energy-level density (its entropy) and forces upon the system the *canonical distribution* of states (within the internal constraints on the atomic particles in the folded globular protein[7]). Such enzyme systems, whose operation is completely at the whim of a thermalizing heat bath, clearly cannot function as molecular energy machines.

The same considerations hold for (free-energy dissipating) ionophoric membrane channels, both natural ones such as operate in neurotransmission[39] and those formed by polypeptide antibiotics.[40] There is now abundant evidence[41] that stochastic fluctuations are inherent to their activity.

The molecular machines envisioned by McClare require some degree of *structural organization*, with an intrinsic modality for efficient coupling of molecular energy sources with specific molecular work functions. Their microscopic operation is essentially "motive" (in the sense suggested above by Lumry), not involving a heat-exchange step during the intermediary course of the transduction. Membranous systems involved in such processes as ATP synthesis, active ion transport, and, in some respects, muscle contraction, seem to possess *precisely* the attributes described by McClare in his definition of molecular machines. Moreover, there are growing indications that many other enzymatic activities in organized states in vivo may fit this paradigm.

Digressing momentarily, we cannot but construe that a fundamental problem here concerns that of *irreversibility*. It is widely known, and remains a matter of urgent discussion in a variety of apparently disparate disciplines,[42–46] that this problem is intimately bound up with that of the enigma of time's arrow. It constitutes an especially well-drawn, acute, and widely debated problem in terms of the *philosophical status* of descriptions of the degree and nature of determinacy and causality in quantum physical systems,[46–51] and indeed of descriptions of recursive structures generally.[52]

In particular, there is a question as to the validity of the *ergodic hypothesis* for complex molecules (e.g., proteins), as described by Blumenfeld,[7] "when an individual molecule can be regarded as a statistical system with a large number of almost isoenergetic states and a 'memory,' i.e., large enough kinetic barriers separating different regions of the phase space." In statistical mechanics, ergodic theory is devoted to the study of the equivalence of time and ensemble averages for the properties of a system. [As the idea goes, if one waits long enough the path representing the evolution of the system in phase space will pass through (or arbitrarily close to) every point on the energy surface.] There have been problems in attempting to base a description of "equilibrium" on ergodic theory.[53] For one thing, the character of an equilibrium depends on *constraints* and changes when the constraints are changed—indicative of some kind of irreversible "flow." In this regard, Blumenfeld[8] characterizes the mech-

anization of protein function according to "kinetic nonequilibrium states." At least for individual enzymes functioning free in solution the most appropriate designation might be (*quasi-*)*periodic*, whereby a given initial state does recur (although not necessarily with perfect regularity).[53] In such systems the initial state recurs *before* the energy surface is uniformly covered, so there is not an irreversible approach to equilibrium.† We do not pursue these issues further here, save to signify their general relevance to our considerations.

It is noteworthy, that a number of authors[6,7,54,55] have discussed *why irreversibility must be inherent within individual molecular machines* of the type presently under discussion. According to the calculations of Gray and Gonda,[6,54] a molecular energy machine would use up too much energy (information) if it "knew" its position in a free energy-transducing cycle relative to the bulk phase with which it is in geometric contact. Wilkie,[56] in elaborating the state of affairs, presented a view that remains apposite:

> "I would plead for more thought to be given to the problems which arise when the traditionally macroscopic arguments of thermodynamics must be applied to small systems, which, as McClare says, 'use one molecule of ATP at a time.' It is far from clear whether we must modify our basic ideas or not."

6. PROTON-MOTIVATED ATP SYNTHESIS

Consider the oxidation of NADH by molecular oxygen as catalyzed, for instance, by mitochondria. The modified (standard) free-energy change for this reaction is approximately -218 kJ mol^{-1}. The process of ATP hydrolysis (to ADP plus inorganic phosphate) is also exergonic, with a modified (standard) free-energy change of approximately -31 kJ mol^{-1}. The reactions of electron transport and ATP synthesis are catalyzed by *spatially separate* protein complexes embedded (but, to a certain extent, mobile) in the so-called coupling membrane. In some way, free energy released by NADH oxidation may be coupled to the *otherwise endergonic* synthesis of ATP. We may represent this in the form of a space-time diagram, as shown in Figure 1, with the quantum of free energy transferred designated, as has become conventional in this field, by the symbol ~ (squiggle). There exist compounds of diverse chemical structure, termed uncouplers, the addition of which to such a system acts to inhibit

†A system may be both quasi-ergodic and quasi-periodic. As indicated by Berry et al.,[53] "An example of this behavior is provided by a system of two harmonic oscillators with incommensurable frequencies (ratio of frequencies an irrational number). For this case one can establish that the energy surface is uniformly covered by the trajectory of the representative point, yet any initial state recurs with arbitrary precision."

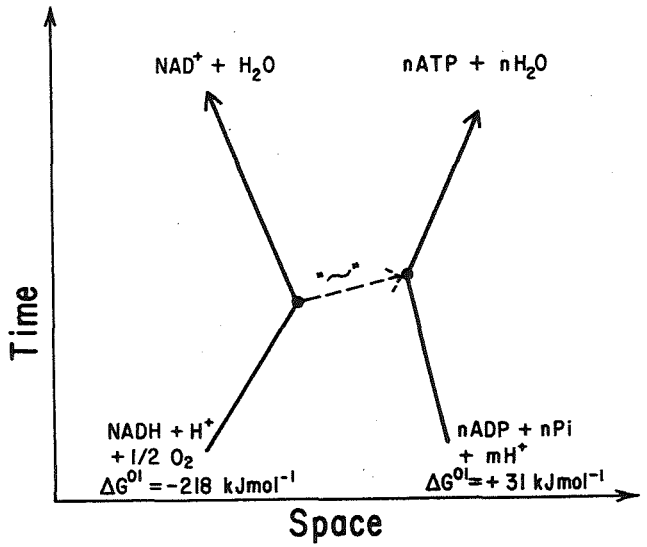


Figure 1. Simplified space-time diagram describing oxidative phosphorylation. Arrows denote (partial) irreversibility. Protons indicated in the ATP synthesis reaction are scalar reactants.

the transfer of free energy between the oxidoreductive and phosphorylative reactions.

We illustrate the foregoing points by indicating certain classical, macroscopically observable properties of oxidative phosphorylation in mitochondria (Fig. 2). The key features of this diagram are that (1) respiration is accelerated in the presence of ADP until such time as the ADP is phosphorylated to the maximum extent possible (a condition termed "static head" in the formalism of nonequilibrium thermodynamics); (2) the transition between the so-called state 3 and state 4 respiration rates (see Fig. 2) is very sharp; and (3) there is a nonvanish-

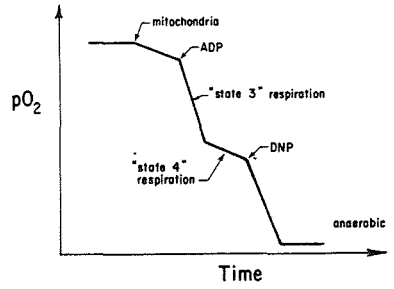
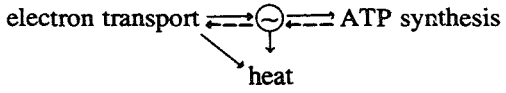


Figure 2. Oxidative phosphorylation by mitochondria. The figure diagrams a typical oxygen-electrode trace of the respiratory activity of well-coupled mitochondria, in the absence or presence of appropriate concentrations of ADP or the uncoupler dinitrophenol (DNP).

ing rate of respiration in the absence of ADP. These features, which, together with the foregoing discussion in this section, are axiomatic and may be gleaned from any textbook of bioenergetics (e.g., reference 57), lead to a descriptive scheme for this process of the following form:



Scheme 1

In conceptual terms, we should like to know more about this ~. In principle, thermodynamics can tell us one thing: Given the reaction stoichiometry, the free energy stored in ~ must be of greater magnitude than that stored in the reaction catalyzed by the ATP synthase and given by the so-called "phosphorylation potential," $\Delta G_p = \Delta G^{\circ\prime} + RT \ln \{[ATP]/([ADP][P_i])\}$. Our special problem, in considering models such as that in Scheme 1, arises in particular from the following experimentally derived observations[58–67]: The free-energy transfer between the reactions of electron transport and ATP synthesis seems to occur at the level of the *individual* protein complexes, and thus the ~ is not adequately described by a macroscopic free-energy term. More explicitly, let us consider reactions of the form

$$\text{ETC} + \text{NADH} + \tfrac{1}{2}\,\text{O}_2 + \text{H}^+ \longrightarrow \text{ETC}^* + \text{NAD}^+ + \text{H}_2\text{O}$$

$$\text{ETC}^* + \text{ATPase complex} \longrightarrow \text{ETC} + \text{ATPase complex}^*$$

$$\text{ATPase complex}^* + \text{ADP} + \text{P}_i + n\text{H}^+ \longrightarrow \text{ATPase complex} + \text{ATP} + \text{H}_2\text{O}$$

where starred (*) terms indicate, for want of a better word, some type of out-of-equilibrium energized state of the protein complex. If the free-energy transfer occurs at the level of the *individual* molecule, we cannot sensibly treat intermediates such as "electron transport complex*" (ETC*) as possessing an "ensemble" free-energy content. (We do not here consider the detailed chemistry of the ATP synthetic reaction, nor the fact that the free-energy transferring step probably acts to drive pre-formed ATP from the enzyme molecule.)

The critical point is that, owing to the inherent "energy leaks" in this type of nonequilibrium system, the traditional method of statistical physics when applied to "small" ergodic systems,[68] of taking an average over (an arbitrary) time of the energy in a single molecule and equating it with the ensemble average, fails.[7] *If you wait too long, the excess free energy in a particular electron transport complex has leaked away to heat and cannot be used to make ATP.* This fact becomes especially clear if a trace of uncoupler is present, because the macroscopically observable rates of ATP synthesis are decreased more efficiently by uncouplers when either the input flux (rate of electron transport) *or the output flux* (number of active ATP synthase molecules) is decreased by appropriate treatments.[61–67]

Thus, exactly as pointed out by McClare and Blumenfeld and occasionally discussed by others in relation to the problem of electron-transport phosphorylation,[69,70] we can only average the energetic properties of the "high-energy" intermediates under consideration over a time smaller than that of the individual turnover times of the ATP synthase molecules under consideration. In other words, these "small" systems are not ergodic.

Such reasoning raises many questions. Although it is easy to ascribe a *macroscopic* thermodynamic value to, say, the free energy stored as ATP (because all the ATP molecules are in the same macroscopic thermodynamic phase and the notion of concentration as being the number of molecules per unit volume retains validity), how do we describe the excess free energy in a *single* protein molecule ("intermediate") that can transfer free energy to a single ATP synthase but that does not apparently "talk to" any of the other ATP synthase molecules? If such an electron transport protein complex increases its internal energy as a result of a favorable thermal fluctuation, is it allowed to use this to make ATP when it would otherwise have insufficient energy for this purpose? If so, it must avoid making ATP on another occasion. How might it obtain knowledge of this fact? (Experimentally, it does not behave thus.) What is the distribution of *usable* (transferable) free energies in an ensemble of *noninteracting* "energized" protein complexes, which may nevertheless constitute a macroscopically (e.g., spectroscopically) observable "intermediate"? Does the inherent irreversibility in such processes arise because *not one individual* ATP-synthesizing enzyme cycle is allowed to pass through a state of lesser energy than that equivalent to a single quantum of ATP at the prevailing phosphorylation potential? Immediately following the addition of ADP to a suspension of respiring mitochondria (see Fig. 2), the work that must be done in making one ATP molecule (per pair of electrons moving down the respiratory chain) is very much less than that which must be done just prior to the state 3–state 4 transition, owing to the different ATP/(ADP + P_i) ratios prevailing. How does the molecular machine know how to divert an *exactly* correct proportion of the free energy available toward the performance of useful work (in making ATP), and how *exactly* does it succeed either in "dumping" the rest as heat or in retaining it within the system for other purposes or later turnovers?

It is widely recognized that the operation of certain electron transport complexes is more or less tightly coupled to the transfer of electrical charge (pro-

tons) across the plane of the coupling membrane in which the complexes are embedded. Purportedly without loss of generality,[71] one can draw up a scheme for a mitochondrial redox H^+ pump that can bind and release H^+ in a vectorial fashion (Fig. 3), although we find it difficult to see how such a model might conform to the so-called "loop" concept.[70,72] As nicely illustrated by the use of Hill diagrams (see Fig. 3), we may distinguish one coupled and two uncoupled cycles of such a redox-linked H^+ pump.[71,73,74] (For a "localized" version of Fig. 3, see reference 67.) The nonproductive cycles of such an energy converter are referred to as molecular slip.[71,75–77] One may readily draw a comparable (simplified) Hill diagram for a proton-motive ATP synthase. (Note that we do not consider the degree of concertedness of the multiple H^+-binding reactions that are required to account for the observable stoichiometries.)

The molecular nature of slip processes is, mechanistically, relatively easy to comprehend in the case of a redox-linked H^+ pump; slip occurs either when a

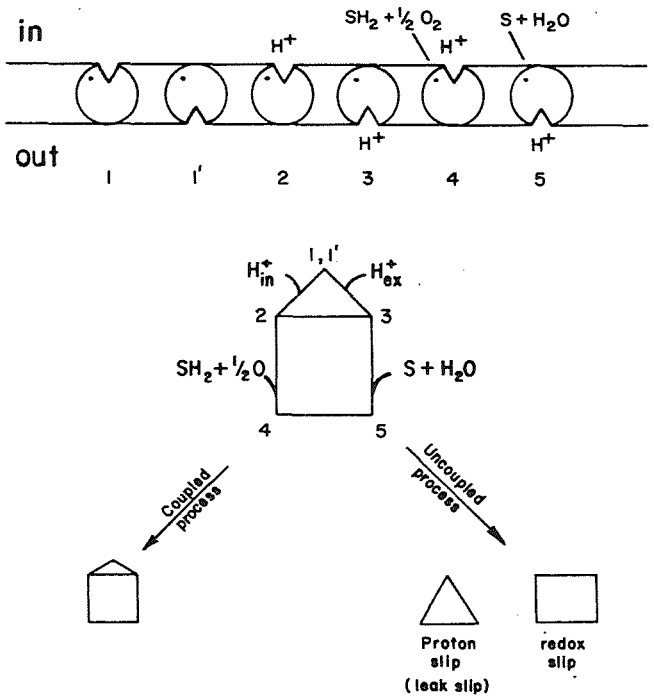


Figure 3. Hill diagram illustrating the coupled and uncoupled (slip) cycles of a redox-linked proton pump. Top, conformational substates of membrane-localized proton pump. Bottom, cycle diagram indicating pathways of protein–ligand (substrate) dynamics. (Modified after Hill[68] and Stucki[71]).

proton is not pumped during a redox cycle *or* when it returns across the coupling membrane without a concomitant reversal of the electron transport reactions. On consideration of the overall process of oxidative phosphorylation, it is evident that neither slip cycle could lead to the synthesis of ATP if ATP synthesis is normally coupled to the net passage of protons back through the ATP synthase energy converter. Now it is usual,[77] following the chemiosmotic coupling concept,[78] to treat, say, a mitochondrial preparation in a typical experimental setup as being equivalent to two homogeneous thermodynamic phases separated by a macroscopic (but inert) coupling membrane, so that all the protons pumped by a particular redox-linked H^+ pump (primary H^+ pump) may be used, stochastically, by *any* ATP synthase (secondary H^+ pump). In such a case, the proton electrochemical potential, which would be equivalent to the ~ of Scheme 1, is indeed possessed of a delocalized, ensemble character and appropriately described using conventional nonequilibrium thermodynamics. Although we would aver that the concept of slip is crucial to the understanding of such molecular energy machines (they alone, in contrast to pure catalysts, possess it, where it is manifest both as irreversibility and as "failures" in free-energy transfer), nevertheless, the macroscopic treatments of these energy converters available to date would seem to be inapplicable to situations in which the free-energy transfer is *not* an ensemble, delocalized process.

Let us perform a thought experiment concerning electron transport phosphorylation in bacterial chromatophores, presented previously.[69,70] A suspension of bacterial chromatophores may be energized by a flash of light lasting approximately 10 μs. The suspension is immersed in an aqueous phase that contains ADP and inorganic phosphate (and a negligible amount of ATP). The cyclic proton-motive redox reactions set up some kind of energized state ("primary macroerg"[7]), often described as a membrane potential ($\Delta\psi$). Macroscopic thermodynamics tells us that we cannot make any ATP unless the product $z \cdot \Delta\psi$ is greater than the phosphorylation potential ΔG_p, where z is the number of protons that pass through the ATP synthase during the coupled synthesis of one molecule of ATP. Thus as we decrease the number of active electron transport chains we require more and more flashes to generate a $\Delta\psi$ sufficient to synthesize *any* net ATP, if the free energy (membrane potential) is delocalized over the whole chromatophore. In contrast, if the free energy generated by electron transport is quantized (localized), the ability to generate any ATP is relatively independent of the number of active-electron transport chains. (Note that we are considering conditions in which the phosphorylation reaction has an insignificant effect upon the prevailing ΔG_p.) Although the foregoing experiment has not yet been performed exactly, the available evidence does, notably, indicate (as discussed elsewhere in this context) that both the $P_i|2e^-$ ratio in a number of systems[64,67,79] and the rate of photosynthetic carbon dioxide fixation in plant chloroplasts[24] are independent, over a wide range, of the rate of electron trans-

port and thus that such systems should indeed be deemed molecular energy machines.

7. LONG-RANGE ENERGY CONTINUA IN INTERMEDIARY METABOLISM

Today, cell biology presents us with a rather simple, biphasic view of cellular infrastructure: *a solid phase*, encompassing extensive membranous reticulation as well as a hyaloplasmic lacework of cytoskeletal elements and an interlocking microtrabecular lattice; and *a soluble, aqueous phase* (albeit containing a considerable amount of "structured" water). Accumulating empirical and theoretical considerations indicate that the majority (if not all) of the enzymes of intermediary metabolism operate in vivo in association with particulate structures,[80–83] with the so-called soluble phase relegated to such subservient roles as thermal buffering and distribution of common substrates, regulatory substances, and salt ions.

As prophesied so accurately some 50 years ago by the late R. A. Peters,[84] biochemistry is now at a point in its development where we have reached the limit for the application of ordinary statistical, mass-action relationships. Accordingly, we must substitute (in Peters' words) a more anatomical view, based on control by surfaces. Within the confines of localized microenvironments, engendered by the various organizational modes in vivo, traditional "macroscopic" descriptions of metabolic processes break down. A reason for the "theoretical flimsiness" of modern biochemistry, as suggested by Blumenfeld,[7] is that it "uses for theoretical description of biochemical processes nineteenth century physical chemistry, i.e., the concepts developed to describe the behavior of low-molecular compounds in gaseous phase and dilute solutions."

For example, the usual approach is inadequate for depiction of the *vectorialized material flow* ("metabolic channeling") in reaction–diffusion systems exhibiting the kind of molecular inhomogeneity and anisotropy that many (perhaps all) of those in vivo do. Moreover, the idea of a "bulk concentration" will not apply in these cellular microenvironments. Here, concentrations of enzymes and their respective substrates are, in many cases, of the same order of magnitude. It is probable that there are "molecular channels," which may be structural or functional in both space and time, in the organized multienzyme systems of the cell, wherein each individual enzyme is subject to a *local, "quantized" substrate concentration*.[80] This situation is analogous to the problem of electron transport phosphorylation discussed above, where the "molecular (proton) channels" have been termed "protoneural networks."[67,70,72,85]

More explicitly, let us consider a multienzymatic metabolic process such as glycolysis. A flux-generating step catalyzed by enzyme E_1 turns substrate S_0 into S_1, the substrate for the next reaction, and so on. If a structured compartment contains an ensemble of substrate molecules, each of which may be metabolized by *any* of the enzymes of a given type within this compartment at random (in other words, the diffusion of substrate within the compartment is much faster than the catalytic turnover of the enzyme in question), it seems legitimate to refer to the "concentration" of the substrate as being defined by the number of molecules in the compartment divided by the volume of the compartment. However, if a given substrate produced by a *particular* enzyme is metabolized solely by a *particular* enzyme molecule catalyzing the next reaction, then the concept of "concentration" as usually construed does not hold. Although this problem has been recognized, and attempts have been made to effect rectification[67,82,85,86] (see review in reference 80), an adequate resolution is not yet at hand.

Analogously, in terms of the electron transport phosphorylation problem discussed in Section 6, we may draw a scheme such as that in Figure 4. This diagram indicates that there may be a directed "channeling" of free energy between *individual* electron transport and ATP synthase proton pumps. Accordingly, the idea has been developed[67,70,72,91,100] that specialized proteinaceous devices, distinct from the primary and secondary proton pumps, serve to channel the "energized" protons between their membrane-localized sources and sinks. This picture is illustrated crudely in Figure 5.

There are compelling kinetic *and* thermodynamic reasons for abandoning the traditional "macroscopic" view of intermediary metabolism, in favor of the kind of "molecular machine" picture suggested by McClare and Blumenfeld.[87] This distinction is heightened further when we ponder the likelihood of *long-range energy continua* permeating structured enzyme systems in vivo. An appreciation of this possibility has been gained from a growing realization of the "reactivity" of the protein matrix surrounding the enzyme active site (see references 29, 30, 88 and references cited therein). A number of workers[35,73,80,89–91] have drawn attention to the potential for transduction of chemical free energy in the function of multienzyme complexes. By virtue of the

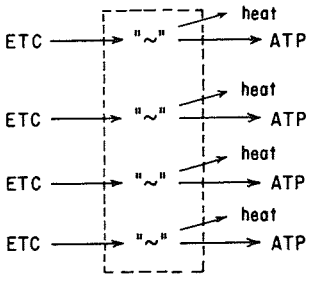


Figure 4. "Microcompartmentation." The figure illustrates the distinction between models in which *all* electron transport complexes (ETC) in a given closed-membrane vesicle can transfer free energy (~) to *all* ATP synthases (ATP), and those in which they cannot. The dotted line indicates ensemble ("pool") behavior of the "energized state."

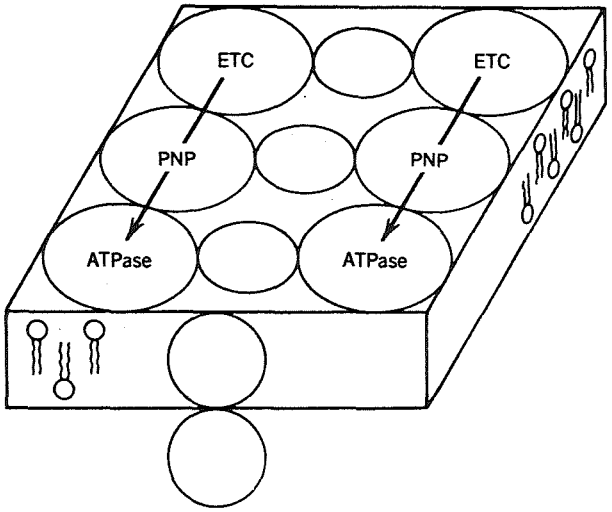


Figure 5. Diagram of free-energy transfer (as "energized" protons) between individual electron transport (ETC) and ATP synthase (ATPase) complexes, utilizing molecular channels contained in associated "protoneural" proteins (PNP). It must be stressed that the complexes are rather mobile in the coupling membrane, and that the purpose of this diagram is only to emphasize the general role of the protoneural proteins as channels for proton flow. (Modified from Kell and Morris[70]).

existence of a transition-state barrier, essentially every type of elementary chemical process yields product molecules *initially* with a nonequilibrium energy distribution, that is, excited vibrational and/or electronic states. For homogeneous systems in bulk solution, the fate of such excited states is rapid relaxation ("thermalization") via collisional deactivation. With intermediate-substrate species confined ("channeled") in a multienzyme aggregate, some of this energy may be retained in the protein superstructure and used in subsequent catalytic and/or substrate-translocation steps.[80,82] Lumry and Biltonen[35] have argued that the protein matrix in these organized enzyme systems serves as a free-energy "buffer" in coordinating the chemical flow in metabolic sequences. (A possible role of this kind of organization as a *thermal* buffer in thermophilic organisms is discussed by Kell and Westerhoff.[67]) Such organized metabolic designs clearly cannot be described as conventional chemical machines.

Organization of enzyme sequences at cytosol-particulate interfaces juxtaposes them to potential *external* energy devices (e.g., strong electric fields, mobile protonic states). As seen in the chapter by Prof. Fröhlich (see also references 92 and 93), the activities of localized enzymes may couple to electric fields (and other energy sources) via excitation of metastable states in the protein. The modality involves longitudinal electric dipolar oscillations of *hydrogen bond units*, which are modeled as active *phonons* (i.e., quasi-particle lattice vibrations). Such systems can store external energy in specific modes (and subsequently do work with it), via a phonon-condensation phenomenon, if the energy is "pumped" into them above a critical level (rate). Coherent hydrogen bond phonons (e.g., in α-helical regions), arising from external "pumping," might propagate under some conditions in the form of dispersionless wave packets (*solitons*). These soliton modes have been proposed as transduction devices in the operation of proton-motivated molecular engines and ATP-linked muscle contraction, among others.[91,94–100] We might note that McClare[2] was one of the earliest workers to describe a possible role of phonon modes in ATP-associated molecular engines.

Berry[101] has offered a novel *electrochemical interpretation of cell metabolism*, which advances the idea that proton current ("proticity"), generated by local redox (or photoredox) processes and ATP-cleavage reactions, flows through "intracellular circuits" in structured matrices, specific for various metabolic functions in the cell. Structural proteins in membranes (e.g., "protoneural" proteins—see Section 6) and microtrabecular lattices, as well as multienzyme systems adsorbed thereto, might sustain this proton transmission. Recently, Welch and Berry[88] extended this view, drawing further attention to a possible physiological connection between the central bioenergetic concept of "proticity" *and* the universal role(s) of "mobile protons" in enzyme structure, function, and evolution. It was suggested that organized proton flow in *localized* enzyme aggregates can coordinate protochemical, as well as conformational-dynamic, aspects of enzyme catalysis—in the manner of a molecular energy machine.

8. PROCESS ATOMISM IN THE FUNCTION OF MOLECULAR ENERGY MACHINES

As discussed in preceding sections, the unique feature of the McClare–Blumenfeld molecular engines is the capability of storing free energy (over some relevant time period) for the performance of useful molecular work. The critical point is that the transduction process be insulated from the "thermalizing" bulk phase, *or* in some manner sustained in a nonequilibrium state. The physical designs treated in Sections 6 and 7 can fit this characteristic. Localized proton flow, as per the "protoneural" view of electron transport phosphorylation, as well as the Berry notion of proton-motivated metabolic processes, would require "wires" (though this is an inappropriate term, because it implies a purely passive role). Hydrogen bond chains, extending through (and between) conjoined proteins (possibly involving structured water), have been proposed to serve such a role.[81,97,98,101–104] Such chains could conduct "active quanta" rapidly and

with essentially *no loss in energy* (within nonpolar cores of globular proteins and through hydrophobic protein–protein interfaces).

The phonon modality in the Fröhlich-type scheme is maintained in a nonequilibrium energy distribution by the external "pump," in conjunction with energy exchange along the oscillating units (hydrogen bonds). Energy may be stored transiently (say, over the period of an event in the catalytic cycle), by coupling the electrical polarization process to elastic deformation in the system (e.g., conformational transition in the protein—see chapters by Careri and Gratton, Gavish, Volkenstein, and Blumenfeld et al.). However, it should be stressed that such systems do not, of themselves, require long-term *spatial* structure.

Theoretically speaking, the hydrogen bond scheme in protein α-helices seems ideally designed to function as a guide for soliton wave packets, whereby chemical-energy release, electric pulses, and so on can be transformed into localized excitation energy that is actively transported through the system. A hallmark of the soliton modality is the coherent way in which it can transduce energy *rapidly and without dispersion or dissipation.* It does appear more than happenstance that the energy level required to excite this modality in α-helices is just in the range of that released by ATP hydrolysis.[95–97]

Emerging from the conceptualizations in Sections 6 and 7 is the basic notion that molecular machines can operate effectively in cellular bioenergetic processes *only if* the energy is handled in discrete parcels (over the cycle period of the engines). Regarding the protein-dynamic basis of these machines, Blumenfeld[7] stated aptly,

> In the case of complicated macromolecular constructions, a situation may occur when a transition between two states differing from one another by their geometry and by their scheme of secondary bonds requires a strictly definite sequence of many small changes, every one of which can be described as a quantum jump.

This notion applies to processes that generate *and* utilize cellular energy. As we have seen from the foregoing, these "parcels" may consist of *material particles* (e.g., "active" protons) or *quantum-statistical quasi-particles* (e.g., phonons, solitons). Embracing photoredox bioenergetic phenomena as well, we could include *excitons* also in the latter group. These quasi-particles are likely to be involved in the migration (channeling) of trapped electromagnetic radiation in the light-harvesting complexes of phototrophic organisms.[96]

In 1972 two groups independently coined the term conformon (*conform-* from "conformation" and *-on* signifying a particle) to denote localized conformational deformations in biological macromolecules. Volkenstein (in this volume; see also reference 105) specified the conformon in relation to local electronic/protonic interactions between the enzyme active site and the protein matrix during specific catalytic events. Local electronic/protonic displacements generate

conformational changes in the protein. Accordingly, "the change can be treated as the excitation of long-wave phonons, and the system electron [or proton] plus local conformational deformation of the macromolecule behaves similarly to a polaron." The Volkenstein conformons are relatively short-range, and their energy dissipates rapidly. Green and Ji,[106] on the other hand, defined the conformon as "the free energy associated with a localized conformational strain in biological macromolecules," and said, "The conformon is to protein systems under physiological conditions what the phonon is to inorganic crystal lattice structures." Of primary concern to these authors[90,91,100,106] was the conceptualization of *mobile free-energy packets* (*quanta*), associated with protein deformation, which effect generally the free-energy exchange between *coupled* biological reactions. More recently, Ji[100] extended the definition of the conformon to specify "genetically determined local conformational strains of biological macromolecules, each endowed with specific biological functions including ligand binding, catalysis, free energy storage and transfer." As discussed elsewhere,[29,100] the conformon notion may be part and parcel of several current models of energy transduction in enzymes. However, its relationship to such specific modalities as phonons, solitons, polarons, and so forth remains to be elucidated.

9. THE PARTICULATE NATURE OF ATP FREE-ENERGY CURRENCY

We must also include the diffusible, stable *chemical particles* that "carry" free energy in cell metabolism—the primary example being ATP.[107] The *immediate* sources of cellular energy are of course (photo)redox processes (and, secondarily, ATP hydrolysis). The aforementioned modalities, involving "active" protons and quasi-particles, represent forms of immediate coupling between source and sink. However, many cellular processes (particularly in larger cells, with spatial compartmentation of specific functions) require *indirect coupling*. As phrased by Reich and Sel'kov,[108] compounds such as ATP serve as "intermediate forms of energy so that rigid direct coupling can be avoided, and the use of energy becomes versatile both in time and in purpose." As is well known, ATP functions as a *dual* energy currency. By virtue of the phosphate group-transfer potential, ATP is a *chemical energy* currency in reactions such as the following:

$$\mathrm{ATP} + \mathrm{X} \rightleftharpoons \mathrm{ADP} + \mathrm{X\text{-}P} + n\mathrm{H}^+$$

This design indicates clearly how the energy-producing (photo)redox processes (via electron transport phosphorylation) can couple to endergonic chemical reactions of cellular metabolism. The high phosphate-transfer potential of ATP

is used, directly or indirectly, to "activate" the reactants in those reactions. And, there is the role of ATP as an *ion-motive currency*. One finds this reaction functioning in proton injection and cotransport (or counterexchange) of metabolites, among other processes. Importantly, these two currencies are to some extent interconvertible, via the proton-motive H^+–ATPase.

From the considerations in preceding sections, we might specify a third role of ATP, as a *mechanical energy currency*. This relates to its potentiality for exciting the quasi-particle (e.g., phonon, soliton) modes in proteins. Here, ATP hydrolysis generates an impulsive force in "wave guides," such as α-helices. The "parcel" of vibrational energy so provided was termed the ATP excitation by McClare.[2] The protons released upon ATP hydrolysis may play the key role in generating these quasi-particle "excitations."[98,103] Such a quasi-particle may be akin formally to the ATP *proton–soliton* proposed by Scott.[104]

In our view, therefore, ATP is regarded simply as a stable transducer of three possible *bioenergetic parcels:* "active" phosphates, "active" protons, and macromolecular "excitations." Although it is a *diffusible* energy carrier, the local processes that *make and use* ATP befit the molecular machine model.

We seem here to be faced with an apparent paradox. On the one hand, we have argued that the free-energy transfer in processes such as electron transport phosphorylation (see Section 6) is a quantized, nonthermal (and nonstochastic) process; yet on the other hand, we admit to the existence of a *macroscopically observable* irreversibility (incomplete degree of coupling, or slip) in such processes, which may be varied by changes in such classically stochastic (macroscopic) parameters as temperature or pH. During the time corresponding to an average turnover of a coupled H^+–ATP synthase enzyme (approximately 1–10 ms), the enzyme explores a multitude of conformational substates, not all of which lead to a successful free-energy transfer. To such an extent, there is a stochastic element in electron transport phosphorylation (see chapter by Careri and Gratton). Yet this does *not* mean that the process of free-energy *transfer* is stochastic in nature; it is only the unsuccessful free-energy transferring events that exhibit this property, and such events lead merely to thermalization of the input force (see Section 11).

Now there exist in nature some processes (e.g., the growth of certain fermentative bacteria) whose formal catabolic reaction stoichiometry must be construed as being coupled to the synthesis of less than 1 mol of ATP.[109] In such cases (and probably for many other systems), a number of quanta must evidently be stored in some out-of-equilibrium state *before* free-energy transfer to an ATP synthase is possible. Yet here too the free-energy transfer step *itself* is quantized. Such examples show that the idea of *the one* biological quantum (e.g., ATP at the prevailing phosphorylation potential) is probably not helpful in general, however useful it may be in discussions of isolated, simplified systems in vitro.

10. THE QUANTUM-MECHANICAL ESSENCE OF BIOENERGETIC PROCESSES

Through the molecular machine motif, we gain a deeper understanding of the microscopic nature of bioenergetic phenomena. Underlying the energy continua of the living cell must be discrete "particles." We find a situation rather analogous to that in modern physics, whence we learn that *every elementary particle* (including massless "particles" such as the photon) is the quantum of action in a specific field. Relativistic quantum field theory has led to the dissolution of the matter–energy (and particle–field) duality in physics. As stated by Pagels,[110] "There isn't anything to material reality except the transformation and organization of field quanta—that is all there is." At the level of elementary particles, all forces and all interactions are mediated by the exchange of quanta. (For example, the electromagnetic field that holds electrons and protons together in atoms is actually mediated by the constant exchange of *photons*.) With this primacy of process at the microscopic realm, we are reminded that "space-time and matter are semimacroscopic statistical constructs akin to temperature and entropy."[111]

By reducing bioenergetics to its particulate form, we recognize clearly that *biological machines are quantum mechanical.*[2] The dynamic character of the enzyme macromolecule provides the "medium" for the quantal flow of energy. The list of particles (and quasi-particles) suggested in preceding sections is not meant to be exhaustive. As more information is acquired about the molecular aspects of bioenergetic transductions, the character (and number) of such particles will require refinement. And it should be emphasized that direct experimental evidence for these transduction modalities (particularly those discussed in Section 7) is likely to be difficult to obtain using the more conventional techniques. Transposing an enzyme molecule, from its "cytosociological habitat"[82] in vivo to a bulk solution in vitro, would tend to mask any quantum-mechanical function in the guise of "classical" (Maxwell-Boltzmann) statistical behavior. Although we have gained some clues from enzymes isolated in vitro, as to their roles in molecular machines, the holistic modalities must be studied with cytological substructures (compare Section 7).[64,88,92,99,112]

11. SOME THERMODYNAMIC–KINETIC ASPECTS OF MOLECULAR MACHINES

Quantification of the *flow* in molecular energy machines cannot be pursued meaningfully until we know the exact manner by which the system (together with the external pump) competes against the thermal environment. To illustrate something of the nature of this problem, let us discuss the conformon model

treated by Volkenstein in this volume. We consider a protein I which serves as an intermediary carrier of electrons (or protons). It could be a component of an electron transport (or proton-pump) chain, *or* an organized enzyme system driven by a flow, say, of "active" protons. We have the flow process

$$D_\infty \overset{W_2(x)}{\rightarrow} I \overset{W_1(x)}{\rightarrow} A_\infty$$

where D_∞ and A_∞ are the constant source and sink, respectively, of electrons (or protons). The rate constants $W_1(x)$ and $W_2(x)$ are explicit functions of the conformational coordinate x of the protein I (see Fig. 6). Following Volkenstein (this volume), we write the following kinetic equations:

$$\frac{\partial P_1(x,t)}{\partial t} = \frac{\partial}{\partial x}\left[D_1(x)\left(\frac{\partial P_1(x,t)}{\partial x} + \frac{1}{k_B T} P_1(x,t)\frac{\partial U_1(x)}{\partial x}\right)\right] - W_1(x)\,P_1(x,t) + W_2(x)\,P_2(x,t)$$

$$\frac{\partial P_2(x,t)}{\partial t} = \frac{\partial}{\partial x}\left[D_2(x)\left(\frac{\partial P_2(x,t)}{\partial x} + \frac{1}{k_B T} P_2(x,t)\frac{\partial U_2(x)}{\partial x}\right)\right] + W_1(x)\,P_1(x,t) - W_2(x)\,P_2(x,t) \quad (4)$$

where $P_1(x, t)$, $U_1(x)$, and $D_1(x)$ are the probability distribution function, conformational potential energy, and conformational "diffusion" coefficient, respectively, for the protein I in the reduced (or protonated) state; and the same with $P_2(x, t)$, $U_2(x)$, and $D_2(x)$ for the oxidized (or deprotonated) state. The reader will recognize that Eqs. (4) represent a Fokker-Planck equation supplemented with external chemical-flow terms. This type of molecular energy machine contains a *stochastic degree of freedom*, owing to the strong effect of the thermal fluctuations of the medium on the conformational movements within the protein. The movement of an electron (or proton) from point x_2 to x_1 (see Fig. 6) can be regarded as the movement of a quasi-particle—*the conformon* (see the chapter by Volkenstein in this volume). The system of Eqs. (4) is actually *the equation of motion of this quasi-particle*.

Integration of Eqs. (4), with the assumption that the reaction rate depends rather specifically on the macromolecular conformation (namely, W_1 and W_2 are delta functions—see Fig. 6), yields for the rate ($\overline{W}$) of the steady-state process the following form[113]:

$$\overline{W}^{-1} = \tau_1(x_2) + \tau_2(x_1) + [W_1 \kappa P_1^{(0)}(x_1)]^{-1} + [W_2 \kappa P_2^{(0)}(x_2)]^{-1} \quad (5)$$

where $\tau_1(x_2)$ and $\tau_2(x_1)$ are the mean times of "diffusion" from x_1 to x_2 and

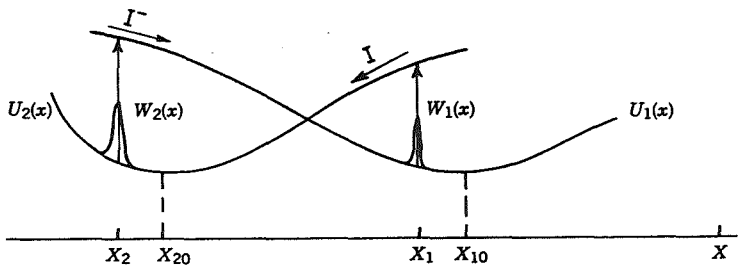


Figure 6. A molecular machine model for electron-/proton-conformational interaction involving "conformon" diffusion. Group I obtains an electron (or proton) from group D_∞ in a conformation characterized by coordinate x_2. A change in charge state of group I leads to a change in the conformational potential and to a transition to the potential surface $U_1(x)$. Part of the energy of reduction (or protonation) of I is stored in the form of conformational stress. This is followed by conformational relaxation of group I^- over the potential surface $U_1(x)$, which has the nature of limited diffusion. When group I^- reaches the point x_1, electron (proton) transfer to group A_∞ takes place, and the system returns to the potential surface $U_2(x)$. Part of the reaction energy can also be stored in the form of conformational stress of the uncharged group I. Conformational relaxation subsequently occurs over the potential surface $U_2(x)$, and so on. (Adapted from Shaitan and Rubin[113]; see also reference 115.)

back, over the potential surfaces $U_1(x)$ and $U_2(x)$, respectively. These two parameters are given by

$$\tau_1(x_2) = \int_{x_1}^{x_2} \frac{dx}{D_1(x)\,P_1^{(0)}(x)} \quad (6)$$

and

$$\tau_2(x_1) = \int_{x_1}^{x_2} \frac{dx}{D_2(x)\,P_2^{(0)}(x)}$$

with $P_1^{(0)}$ and $P_2^{(0)}$ *equilibrium* distribution functions defined as

$$P_{1,2}^{(0)}(x) = P_{1,2}^{(0)}(0) \cdot \exp\left(-\frac{U_{1,2}(x) - U_{1,2}(0)}{k_B T}\right) \quad (7)$$

The remaining two terms in Eq. (5) characterize the rates of the (electro-proto-) chemical transformations (with κ a constant).

Equation (5) shows the competition between the external energy pump and the random (thermal) walk of the conformational "diffusion." (Similar considerations may apply to the thermally activated proton-pump model of Nagle and Morowitz.[102,103,115]) If such a stochastic feature is integral to our molecular energy machines, then the following conditions should obtain: (1) the macro-

molecule must be able to store in particular degrees of conformational freedom (i.e., prevent thermalization) of a certain amount of the reduction/oxidation (or protonation/deprotonation) energy over the time periods $\tau_1(x_2)$ and $\tau_2(x_1)$[7,8]; and (2) there must be rather precise energetic/conformational coupling, or gating, between D_∞ and I and between I and A_∞.[70] The appearance of slip in the operation of proton pumps may be a reflection of some imprecision in the matching (i.e., the delta-function form of W_1 and W_2 assumed above is too restrictive for the "real" system). Moreover, variability of the coupling process may have regulatory implications (see Sections 6 and 9).

Alternatively, some types of molecular energy machines in organized states in vivo may be entirely *deterministic*, in the sense that the course of the microconformational changes is governed by specific coupling (namely, "chemical fixation" to) the external pump.[113] The importance of this case is heightened by consideration of the high microviscosity potentially extant in vivo. If the mechanics were purely stochastic, some of the relevant protein-dynamic modes might be overdamped in viscous microenvironments.[30]

Consideration of reaction/diffusion processes ultimately leads one to focus on the very physicochemical nature of such concepts as *rate* and *driving force*. The operation of molecular machines of the kind suggested in Sections 6 and 7 requires some rethinking of the phenomenological flow-force relations for chemical processes in vivo. Here we wish to point out some of the problems.

Customarily, the laws of chemical kinetics tell us that the rate (velocity) of a reaction in bulk solution is given as the product of the concentrations and a unitary rate constant. Of course, this product is just the probability of *finding* a reactant molecule (or an encounter pair, in the case of a bimolecular reaction) in solution, multiplied by the probability that such a molecule (or encounter pair) actually *reacts*. We usually think of the term "concentration" very simplistically, as a scalar quantity reflecting the random statistical distribution of solutes in the bulk phase. (For example, one might calculate the probability of finding an enzyme–substrate complex in solution, according to the Poisson distribution.[114]) As indicated in Section 7, the concept of "concentration" in the usual macroscopic sense breaks down for organized enzyme systems. Notionally, we must define "concentration" according to the physical *context* in which a given reaction occurs.[80,81,86]

The chemical rate constant, say, for the enzymatic reaction ES → E + P, is specified in the general form

$$k = \nu W(h^{\ddagger}, T) \tag{8}$$

where ν is a frequency term and W is the probability of finding a molecule(s) with the critical energy (enthalpy, $h^{\ddagger}$) required for the reaction. The most familiar depiction of Eq. (8) is the empirical Arrhenius form

$$k = A \exp\left(\frac{-E_a}{k_B T}\right) \tag{9}$$

where E_a is an "activation energy" (enthalpy) and A is the frequency factor. (The exact nature of A depends on the molecularity of the reaction, as well as the kind of reaction-rate theory one adopts.) A grasp of Eq.(8) is vital to a comprehension of the workings of the molecular machines. It represents the probability that a reactant molecule(s) has the proper *energetic configuration* for the reaction process (expressed as the fraction of existing reactant molecules), multiplied by the rate at which such an "activated" molecule proceeds across the reaction boundary. One recognizes immediately that the exponential term in Eq. (9) reflects a *Maxwell–Boltzmann distribution* for the energy state of reactant species. This statistical distribution pertains to the so-called "classical limit," applicable at high temperature *or* at low density (i.e., where the number of potentially available molecular-quantum states is much greater than the number of particles in the system). This condition applies to the vast majority of physicochemical systems studied under laboratory situations, including isolated enzymatic processes in vitro. Considering their inherent restriction on molecular degrees of freedom, though, it is apparent that *molecular machines cannot be described by Maxwell–Boltzmann statistics.*

By virtue of the quantal nature of the McClare–Blumenfeld "machines," one must use some more exact formalism than that of Eq. (9). In quantum physics, all particles (and quasi-particles) are categorized as *either* "fermions" *or* "bosons," depending on whether the particles fit Fermi-Dirac *or* Bose-Einstein statistics, respectively. (Both go over to Maxwell–Boltzmann in the classical limit.) For example, electrons and protons are fermions, whereas photons and phonons are bosons. The quantum-statistical counterpart of the equilibrium distribution function in Eq. (9) is

$$\bar{n}_i = \frac{\lambda e^{-\epsilon_i/k_B T}}{1 \pm \lambda e^{-\epsilon_i/k_B T}} \tag{10}$$

where $\bar{n}_i$ is the average number of particles in the ith quantum state, ϵ_i is the energy of the ith state, and λ is an absolute activity, given as

$$\lambda = e^{\mu/k_B T} \tag{11}$$

with μ the chemical potential. With (+) we have Fermi–Dirac and with (−) Bose–Einstein statistics. (Electronic states in metals and electromagnetic radiation are two well-known phenomena that are described by quantum statistics.)

Certainly, the various "bio-quanta" discussed in preceding sections are, in principle, amenable to quantum-statistical description. However, one cannot

proceed by simple substitution of Eq. (10) into Eq. (8). For the distribution in Eq. (10)—just as that in the Maxwell–Boltzmann form used in Eq. (9)—represents an *equilibrium situation.* In our molecular machines, the particles are not allowed to sample an equilibrium distribution of energy states, because of non-equilibrium constraints imposed by the organizational mode of the system and/or by external energy sources. (Recall that it is this *status rerum* that potentiates the utilization of stored energies in the performance of useful work.) Thus with a molecular machine plugged into an external energy source (pump), one might obtain W of Eq. (8) (i.e., the fraction of reactant species possessing some critical energy) by solving (for $\bar{n}_i$) a *stationary-state equation* containing the sources and sinks for the relevant quanta. Means for approaching this problem (e.g., for phonon modalities) are treated in the chapters by Careri and Gratton and Fröhlich in this volume. Similar calculations might be possible for other "bioquanta," such as "active" protons,[115] insofar as they are energetically separable from protein modes.

We might approach the frequency term ν in Eq. (8) in a like manner. Although the active-site configuration dictates the requisite energetic character (E_a) of an enzyme–substrate transition state (i.e., W in Eq. (8)), it is *the protein matrix* (in dynamic interaction with the surrounding medium) that determines the rate at which that state is reached. As indicated in Section 5, the frequency factor, for an enzyme protein at thermal equilibrium with its surroundings (in bulk solution), depends on transient *thermal* excitation of the relevant quanta.[29,30,88] When the enzyme is organized as part of a molecular machine the conformational-dynamic participation of the protein may be *driven* by a cyclical (external) flow of quanta.[7,8,26,88,92,93,95,97,98,102,103]

The idea of "rate" aside, we must also consider the thermodynamic *driving force* on chemical processes, apropos of molecular machines. The latter concept is embodied in the *chemical affinity*, $\mathcal{A}$. For a reaction such as S → P, the affinity is given as

$$\mathcal{A} = -\left(\frac{\partial G}{\partial \xi}\right)_{T,P} = \mu_s - \mu_p \tag{12}$$

where the partial derivative (at constant temperature and pressure) is the free-energy (G) change per extent of reaction (ξ)—what is usually called ΔG. The μ's are the respective chemical potentials of S and P. With the usual assumptions of ideality, unit concentration (activity) for the standard state, and so on, Eq. (12) takes the familiar (molar) form

$$\left(\frac{\partial G}{\partial \xi}\right)_{T,P} = \left(\frac{\partial G}{\partial \xi}\right)^{\circ}_{T,P} + RT \ln \frac{[P]}{[S]} \tag{13}$$

where the superscript (°) denotes the *standard* free-energy change. Equation (12) is the more rigorous expression and the more suited for analysis of processes in vivo.

As is well known in physical chemistry,[116,117] the chemical potential, μ_i, of a molecular species i is composed of two terms, one dependent upon, and one independent of, its concentration. The concentration-dependent part (which does not relate to the chemical nature of the molecule) is obtained from the configurational entropy of the system, which is defined according to the number of microscopic states compatible with the macroscopic configuration (e.g., positional degeneracy). For molecules dissolved in aqueous solution, this part is approximated crudely as $RT \ln C_i$, where C_i is the molar concentration of i. *For bound ligand*, this contribution is usually calculated statistically according to the number of possible ways of distributing (randomly) a given amount of ligand over a number of binding sites.[117] Molecular machines, with their channeling characteristic (see Sections 6 and 7), manifest a much reduced configurational entropy, as a result of the aforementioned notion of, for want of a better term, localized concentration. This feature obviously leads to a higher *effective* chemical potential for reaction processes in organized states.

The concentration-independent part, designated μ_i°, is a function of the actual molecular species i under consideration, *including the free energy contributed by interaction with its environment.* In the function of molecular machines, with their intrinsic molecular channels, intermediate compounds may not equilibrate with the bulk solution. For compartmentalized substrates (products), the "environment" is a microcavity whose properties differ significantly from the bulk phase. Hence the applicable form of μ_i° for Eq. (12) is $\mu_{i,c}^\circ$, which we call the inherent free energy of the channeled substrate (product), "solvated" by the organized enzyme system. Moreover, it is $\mu_{i,c}^\circ$ that relates directly to the free-energy transduction modality. *The value of $\mu_{i,c}^\circ$ changes while the molecule is associated with the structure*, during the transduction process (whereas the concentration-dependent term in μ_i remains the same). One should note that it is not possible to define explicitly the chemical potential of a protein-bound ligand *during the course of transduction.*[118,119]

Following such approaches as by Hill,[73] Lumry,[36] and Tanford,[117] we might model the transduction process in an elementary way. We suppose that the enzyme complex (as part of an organized molecular machine) "binds," at two distinct sites, a substrate molecule (S) and a source (Z) (e.g., ATP, proton-injector) of transducing "particles" (quanta). Free energy is transferred from Z to S through the medium of the protein aggregate. We might formalize this process in the form ZES ⇌ (Z ~ ζ ~ S) ⇌ ZEP, where the intermediate state (Z ~ ζ ~ S) represents the "energized" modality.[91,100] Then we approximate the standard free-energy change as

$$\left(\frac{\partial G}{\partial \xi}\right)^{\circ}_{T,P} = (\mu^{\circ}_{P,c} - \mu^{\circ}_{S,c}) + (\mu^{\circ}_{\zeta} - \mu^{\circ}_{Z}) \qquad (14)$$

where the second term on the right-hand side is the free-energy change (per catalytic cycle) generated by the flow of quanta (ζ) from the "external source" (Z).

The quantity $(\partial G/\partial \xi)^{\circ}_{T,P}$ (or $\Delta\mu^{\circ}$) is probably a more useful *thermodynamic* parameter for the analysis of free-energy transduction in molecular machines. Yet it is a very elusive quantity from the experimental viewpoint and difficult to define except at the beginning and end of transduction. Conventional studies of isolated enzyme reactions in vitro can give only an *overall* ΔG value, which yields no information on the free-energy transduction phenomenon.[36,117,119]

In the case of molecular machines plugged into external energy sources (pumps) in vivo, the appropriate chemical potentials (namely, μ°) must be obtained more exactly from the kind of nonequilibrium quantum-statistical method indicated above. From the form of $\bar{n}_i$ (found by solving a flow equation containing the sources and sinks for the relevant quanta—see above), one can extract the chemical potential [compare Eqs. (10) and (11)] as a function of the pump rate, internal quantal-exchange rate, number of particles, and so on.[26] Furthermore, the involvement of protons (or other charges) in the given reaction process implies that the second term on the right-hand side of Eq. (14) will contain an electro(proto)chemical contribution (see chapters by Careri and Gratton and Blumenfeld et al.). This contribution would not be realized from the conventional way in which we study isolated enzymatic processes in vitro (i.e., "unplugged" from their natural energy source[88,101]).

Evidently many present-day conclusions regarding such matters as the rate, nonequilibrium character, and efficiency of metabolic processes are potentially in error.[101] Part of the problem arises from the lack of appreciation, until recent years, of the nature of enzymes as "chemodynamical machines"[35] and part comes from the customary manner by which Eq. (13) is (mis)used: (1) in attempting to establish bulk solution mass-action ratios (while neglecting participation of "local" metabolites); and (2) in the estimation of *standard* free-energy changes under highly artificial solution conditions in vitro.

12. THE SECOND LAW OF THERMODYNAMICS FOR MOLECULAR MACHINES

McClare[1] suggested that an improper view of classical thermodynamics (namely, a "macroscopic" interpretation of the second law) has led us to look at bioenergetic phenomena in the wrong way. Introducing the notions of temporality (τ), stored energy, and useful work, he showed that biological systems are composed of molecular machines that require *quantum explanations*. Macroscopic phenomena manifested by the living cell represent simply *the summed effects of individual molecular machines*. Apropos of these machines, McClare[1] phrased the second law as follows:

> It is impossible to devise an engine, of any size whatever, which, acting in a cycle which takes a time τ, shall produce no effect other than the extraction of energies, which have equilibrated with each other in a time less than τ, from a reservior at one temperature and the conversion of these energies into a form in which they would remain stored for longer than τ, either at a higher temperature, or in a population inversion.

Stemming from our "bio-quantum-mechanical" discourse in the foregoing, we can give a "quantal" corollary to McClare's statement. To develop this, we ask the reader, once again, to digress with us into the realm of the parental science of quantum physics. Consider the physics of the *vacuum state*. Despite the rigor of the first law of thermodynamics (conservation of energy), the Heisenberg uncertainty relations indicate that energy is "uncertain" on short scales of time and distance. Quantum field theory has shown that quanta (e.g., particle–antiparticle pairs, photons) can go in and then out of reality transiently in empty space. They have a "virtual existence" (the time period being determined by their energy, according to the uncertainty relations), but in the end they must cancel out. These virtual quanta become *real* (i.e., generate macroscopic effects) only if sufficient *external energy* is applied to the vacuum. (There is even physical argument[120] that the universe in toto is one gigantic fluctuation of the vacuum.)

We find an analogous situation for enzymes that function as energy-transducing molecular machines. Let us imagine the enzyme protein "unplugged" from its energy source and placed in (and at thermal equilibrium with) a bulk solution. Now the protein matrix of this kind of enzyme has the *inherent capability* of sustaining the relevant quantal modes. On occasion, various quanta (e.g., mobile protons, phonon vibrations) will be found in the protein molecule. However, their existence is fleeting, ephemeral. The creation/destruction of these "virtual quanta" is dictated purely by the equilibrium-fluctuational interaction of the protein with the medium (solvent). The fact that such enzymes, when isolated in bulk solution in vitro, catalyze reactions at high rates could be construed as evidence in itself for the existence of the quantal "wave guides." Some of the single-quantum events (e.g., proton hopping[102,103]) in proteins can be excited readily by thermal energy (i.e., $k_B T$). With regard to charged protein molecules, for example, it was indicated some 30 years ago[121] that the fluctuational behavior of a protein with no net dipole moment would still result in its possession of a net *mean square* dipole moment. Obviously, such fluctuational

motions of charged groups on a protein, in equilibrium with its surroundings, are not associated with the emission of electromagnetic radiation that might be used for the performance of useful work. Any such excitations should be classed as virtual quanta.

We now write a simple corollary to McClare's statement of the second law, as follows:

> *It is impossible for a molecular energy machine to perform useful work with virtual quanta.*

The "capacity to perform useful work" is enshrined in the Gibbs free-energy function,† which can be expressed as

$$G = \sum_i n_i \mu_i \tag{15}$$

with the chemical potential in the form

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_j(i \neq j)} \tag{16}$$

If a given particle (quantum) species i is to contribute to some work process (e.g., alteration of the mass-action ratio of a chemical reaction) executed by the system, its chemical potential (μ_i) must be *maintained* at a requisite value over the course (τ) of the reaction cycle. In systems at equilibrium with the surroundings, the chemical potential of any *nonconserved* particle (e.g., virtual quantum) is identically zero.[116] Therefore, the transduction term ($\mu_\zeta^0 - \mu_z^0$) in Eq. (14) is nonvanishing *only if* the virtual quanta are reified by the presence of an external energy source.

13. MOLECULAR ENERGY MACHINES AND BEYOND

Thermodynamics presents us with two kinds of "order principle," which govern the organization and dynamic behavior of matter.[122] In the equilibrium regime, we find the so-called *Boltzmann order principle*. In this realm, the notion of "decrease in free energy" has stood to characterize much of the general behavior of systems such as those of biological interest. To see the significance of this Boltzmann principle, consider the Helmholtz free-energy function,

$$F = E - TS \tag{17}$$

†It was suggested by McClare[1] that molecular energy machines should be *enthalpy driven* (i.e., governed by the ΔH part of ΔG), because the actual process of energy conversion here would seem to be *faster than heat flow*–which is specified in the $T\Delta S$ part of ΔG.

where E is the internal energy and S the entropy of the system. Equation (17) reflects a competition between energy and entropy. At lower temperature the TS term becomes negligible, and the minimum value of F imposes structures corresponding to minimum energy and low entropy. Contrarily, at higher temperature the energy becomes more uniformly distributed over all states, corresponding to high entropy. One calculates the probability (P_i) of occupation of states by the (Maxwell-)Boltzmann distribution,

$$P_i = \exp\left(\frac{-E_i}{k_B T}\right) \tag{18}$$

(or, under more restrictive conditions, by the quantum-statistical analogue—see Section 11). Accordingly, the *Boltzmann order principle* tells us that, in equilibrium regimes, *the only way to achieve organization* (i.e., preferred energy states) *is to lower the temperature*. In biology this principle is evident, for example, in the formation of stable macromolecular structures. However, it cannot explain the intricate *dynamic* organization of matter under the conditions in which living systems exist. McClare,[2] in echoing a point made long ago by Schrödinger (*What Is Life?*, Cambridge University Press, 1944), noted a puzzling situation: although living systems are nonequilibrium in nature, their global organizational features do, indeed, resemble low-temperature states (see also reference 17).

Advances in the *far-from-equilibrium* branch have demonstrated the existence of another kind of order principle, the *dissipative structure* concept, developed largely by the Brussels school.[46,122] Here, one sees how global spatiotemporal organization can evolve from chaos, under certain nonlinear conditions for the reaction–diffusion processes composing the system. This dissipative modality no doubt is intimately associated with the very evolution *and* maintenance of the "living state."[27,46,122–125] These "dissipative structures" arise from symmetry-breaking instabilities. Ordering of the supramolecular assemblages in our molecular energy machines (while requiring dissipation of free energy for the biosynthesis) is not dictated by such instability conditions. This organization is usually maintained by *kinetic constraints*, that is, by the high potential barriers engendered by bonding patterns.[8]

How does the idea of molecular energy machines relate to these order principles? To answer this question, we must explore more deeply the uniqueness and teleonomic creativity of the living state as a distinct state of matter. It is the *creation and utilization of requisite (electro)chemical potentials* of mass components that actually sets apart matter in the living state from that in inanimate forms. This sort of view connotes a *conservative* character for the energetic machinery of cell metabolism. In our "machines," we see the evolutionary design of organized supramolecular regimes, which preclude the randomizing influence of Eq. (18) on intermediary metabolism and which main-

tain efficient channeling of cellular energy for useful work.[126] Yet superimposed on this design is the trend of *increasing intensity of free-energy dissipation*, which accompanies evolutionary increase in complexity.[80,122,127] (In cosmology, such paradoxes are sometimes resolved by postulating an inflationary universe.[128] Similar reasoning has been applied to the evolution of the biosphere.[129])

A way to remove this dilemma is found in an argument by Wittenberger.[130] He recognized, first, that an increasing capacity for energy production (or dissipation), measured intensively, is the *main trend* of evolution. However, this is seen most often at the level of complex, integrative functions. In large measure, this "dissipation" can be attributed to the energy expenditures required for building up and maintaining internal order and structural complexity, an increase in which features is associated with evolutionary progress. On the contrary, the tendency toward greater economization of energy appears most frequently *at the level of elementary, component processes*. Accordingly, the structuralization of cellular metabolism, while entailing high rates of "dissipation" (per unit mass), might be viewed from the level of the individual reactions as a trend of making metabolism more *energetically profitable*.[80,126,127]

Thus we discern two "thermodynamic branches" relevant to the evolution of cellular infrastructure. There is a "dissipative branch," which potentiates the appearance of global organization in the system, and there is a "conservative branch," which governs the operation of energetic processes *within the microscopic confines of preexisting organized states*. The free-energy transduction in these states can be quite efficient. That is, the left-hand side of Eq. (14) may be close to zero, though the term $(\mu^{\circ}_{P,c} - \mu^{\circ}_{S,c})$ is large and positive and the term $(\mu^{\circ}_{i} - \mu^{\circ}_{z})$ large and negative. (Of course, there will be dissipation at some point in the operation of the external energy pump. The key point is that the *intermediary* steps of the "coupling" process itself do not go through a heat stage.)

By introducing *time* into the performance of useful work by molecular energy machines (see Section 3), McClare showed how efficiency and large velocity are compatible with thermodynamic considerations.[4] This is an important point, for evolutionary biology recognizes that a "morphofunctional unit" has dimensional character in both space *and* time. Although increase in spatial complexity represents the morphological aspect of evolution, increase in velocity of component processes constitutes the *physiological aspect*.[131] These "machines" are reminiscent of the old vitalistic notion of *entelechy*, introduced by Driesch.[132] He suggested that "entelechy acts by affecting the detailed *timing* of microphysical processes, by 'suspending' them and releasing them from suspension whenever required for its purposes."[133] As applicable to our molecular energy machines, we might employ the contemporary hypothesis that "entelechy orders physicochemical systems by influencing physically indeterminate events

within the statistical limits set by energetic causation. *To do so, it must itself be patterned spatiotemporally*" [italics ours].[133] Hence it seems justified to resurrect the concept of "entelechy" in the context of our present knowledge of cell metabolism, by replacing the idea of "hidden variables" (which shadowed the concept in its original vitalistic interpretation)† with the now well-founded supposition of a functional organization of enzymatic processes extant in vivo (see Section 4). This picture is consonant with the "hierarchical control" theory of Pattee,[134] according to which "hierarchical constraints classify degrees of freedom to achieve selective behavior." McClare[4] dubbed this state as a *new level of organization in biology*: "a tuned resonance between energy levels in different molecules that enables bioenergetic machines to operate rapidly and yet efficiently." A similar concept is implicit in the writings of Szent-Györgyi.[135,136]

The concept of molecular energy machines, as well as the exciting advances in the "dissipative structure" model, lead us to a new understanding of "energy" and "energetic transformations" in living systems. Naively, one thinks of matter as the substance of things, and *energy as the moving principle—a cause of change*. Yet physics teaches us that there is really no distinction between matter and energy; they are intertwined as one entity, whose motion (change) is dictated, or ordered, by *local fields*. The dissipative structure idea[122] has demonstrated that matter in far-from-equilibrium regimes may exist in unique spatiotemporal states, a condition suggestive of a *field structure*. This situation holds as well for the microscopic realm of molecular energy machines. For example, the energy of ATP hydrolysis cannot be thought of as "driving" a work function (e.g., muscle contraction), unless there is an organized framework (e.g., myosin-ATPase) to provide for the energy transduction. Likewise, the flow of electrons from NADH to oxygen cannot "drive" ATP synthesis,

†In the present century, the inclusion of "hidden variables" has been regarded by most scientists as the bane of a physical theory. Perhaps a reason for this situation lies in our apprehension at giving up the reductionistic idea of the world as a superposition of (apparently) autonomous individual processes, in favor of an intrinsic "connectedness." In contemporary science, "hidden variables" are discussed primarily in the realm of quantum physics. Despite the spectre of a death knell cast by recent experimental results,[144] there remains a conviction among many physicists as to the role of an "implicate order" in the physical world.[48,50] For a biologist, it might be amusing to note that the currently popular *gauge field theories* in physics make use of such notions as "hidden gauge invariance" and "hidden symmetry" as intrinsic to the structure of the physical world. These theories, which have been developed for all the basic forces (with the exception of gravitation), are founded on certain mathematical symmetry relations. In order to construct a tractable and consistent model of particle interactions, it has proved necessary to assume that the vacuum state of nature is itself asymmetric (quantum-mechanically speaking). This assumption entails the postulate that in the physical vacuum there are actually present extra, "background" fields—the so-called *Higgs fields*—which break ("hide") the symmetry of a gauge field, for example, by allowing certain gauge bosons to acquire mass while keeping others massless.[145]

unless there is a structured (namely, lipoprotein) matrix to allow for energetic coupling. For "energy," of itself, cannot "select" a course of action. The *ordering* of change depends on the existence of a spatial substratum—*a field*.

We would maintain that the organizational design of molecular machines in vivo defines a *bio-ergonic field*†. The field imposes restrictions on the energetic course of microphysical processes therein. This feature qualifies it according to the usual definition of a "field" in classical physics. Furthermore, following the discourse in preceding sections, our field has a "quantum-mechanical" character that qualifies it by analogy with quantum physics. Moreover, the bio-ergonic field has a *relativistic* nature as well.[82] Yet, as described by Prigogine,[46] "biological space" is atypical of the Euclidean geometrical form used in classical physics. In living systems we have an organized space "in which every event proceeds at a moment and in a region that make it possible for the process to be coordinated as a whole. This space is functional, not geometrical ... In this space the events are processes localized in space and time and not merely trajectories."[46]Although the bio-ergonic field does indeed have such a functional form, we would contend that it is also characterized by definite trajectories. A distinguishing feature of our field lies in the existence of an underlying *material substratum,*[84] which directs the spatiotemporal course (trajectory) of molecular events therein.[87] Not only are the enzymes contained in these organized systems simply the catalysts of specific chemical transformations, but also they (together with conjoined cytomatrix proteins and/or lipoprotein arrays) form the very substratum that is the "scene of the action."[83] Clearly, a thorough knowledge of the dynamic properties of proteins is vital to our understanding of the quantum-mechanical basis of the molecular machines that effect the flow of energy in these local fields.

The development of a true molecular theory of the living state can be fostered by the use of analogical thinking and common motifs from the parental sciences of physics and chemistry. Conventional biochemistry, despite its great wealth of accumulated knowledge, is of itself inadequate for the task at hand. As stated by Thom[137] in this regard,

> [The] whole geometrical and spatial aspect of biochemical reactions eludes the power of biochemical explanation; usually the realization of an enzymatic reaction *in vitro* is considered a great success if it has so far been thought to be specific to living matter, when it should rather be deplored because, when all is said and done, an animal will never be a test tube.

It is hoped that the emergence of the concept of molecular energy machines will contribute to the progressive unification of biology and physics. According

†From the Greek *ergon*, work. This is a "microphysical field," not to be confused with the so-called "morphogenetic fields" which have been implicated in the generation of macroscopic structure and form in developing biological systems.[132]

to the present situation in physics, all of the four basic forces (fields) of nature are characterized by the exchange of specific boson particles.[138] Analogical reasoning would suggest the identification of appropriate bosons in the proteinaceous molecular energy machines (see the detailed discussion by Ji[100]). As is obvious from a number of chapters in this volume, *hydrogen bond units* may hold the answer because of their central role in protein structure *and* dynamics (see also references 88, 139).

We admit that the dialectical course discussed herein adds a seemingly bewildering degree of microscopic complexity to the sciences of biochemistry and cell biology. There is a similar state of affairs in elementary particle physics, as we come to realize that the subatomic world is an extremely intricate, replete "particle jungle." As phrased succinctly by Prigogine,[46] "Belief in the 'simplicity' of the microscopic level now belongs to the past." So appears to be the case in molecular biology. Thinking analogically, we would agree with Thom,[137] in that

> "Biology may perhaps [have labored] under the same delusion as physics: the belief that the interaction of a small number of elementary particles embraces and explains all macroscopic phenomena, when, in fact, the finer the investigation is, the more complicated the events are, leading eventually to a new world to be explained in which one cannot discern among the enormous set of new phenomena the relevant factors for macroscopic ordering."

The *topological problem,*[137] entailed in the passage from the "local" level to the "global" one, is far from resolution at present.

We would like to close with a speculation (again based on analogical thinking with physics) as to the relationship between enzymes and the aqueous medium, and the evolution of enzymes therefrom. Modern science has replaced the Aristotelian adage, "Nature abhors a vacuum," with the notion that "the vacuum is all of physics."[110] Indeed, the vacuum contains (in a "virtual" sense; see Section 7) all known quantum particles (as well as those yet to be discovered). Moreover many theoretical and experimental physicists today are spending their time studying "nothing at all"—the vacuum state. But as noted by Pagels,[110] "that nothingness contains all of being." In a sense the vacuum represents the *ground state* of material being, out of which elementary particles appear as "excitations."[50] There is an analogous situation in the living state. One might state, as a dictum, that "the nature of living systems abhors molecular chaos." The more we pry into the microscopic workings of the "living state," the more we realize that the chaos of the bulk (aqueous) solution phase is antipathic to the coherence and order of life. Molecular energy machines represent the antithesis of that chaos. Yet they are "chemodynamical engines"[35] which *evolved out of that chaos*. Biological evolution is an anagenetic pro-

cess,[140] which implies that it builds on (and adds to) existing form and "remembers" the lessons learned at each stage in the evolutionary course.

Water manifests (in a "virtual" sense) some of the same *quanta* (e.g., mobile protons, collective motions[141]) involved in the operation of molecular energy machines. It has been suggested[35,88,142] that the enzyme molecule is a "micro-colloid" engineered in evolution, from the very primordial condition, to capitalize on such energy "modalities" of the aqueous state. (Is it mere happenstance, for example, that the H–O–H bending mode of water has the same frequency as that of an α-helix soliton in proteins[104]?!) From the chapters of Blumenfeld, Fröhlich, and Volkenstein in this volume, we realize that a role of the protein matrix is to provide a stable "ground-state structure" from which excited modes can be applied to specific chemical processes in a selective and controlled fashion. Water may be regarded as the "mater and matrix" of all of life,[143] just as the vacuum is the "mater and matrix" of all of physical being. In fact, *water is the very "ground state" of life*. As emphasized by Szent-Györgyi,[143] the redox state of water provides the ultimate "gauge" for bioenergetic transformations. Just as physicists are learning more of the microscopic nature of inanimate material processes by turning attention to the vacuum state, so may biophysicists gain more understanding of the nature of biomolecular processes by turning attention to the properties of water (see Chapters by Lumry and Gregory and Gavish.)

By comparing the features of biological molecular energy machines and the characteristics of the surrounding "molecular chaos," as well as by perceiving the mode of interaction between these machines and the ambient condition, we may discover a kind of interrelationship (or perhaps complementarity) that leads to a deeper appreciation of the essence of the living state—as a triumph of Apollon over Dionysos.

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