

The force exerted by a molecular motor

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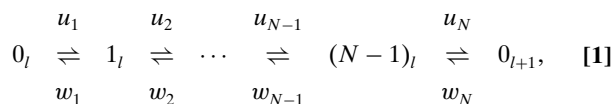
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ABSTRACT The stochastic driving force exerted by a single molecular motor (e.g., a kinesin, or myosin) moving on a periodic molecular track (microtubule, actin filament, etc.) is discussed from a general viewpoint open to experimental test. An elementary “barometric” relation for the driving force is introduced that (i) applies to a range of kinetic and stochastic models, (ii) is consistent with more elaborate expressions entailing explicit representations of externally applied loads, and (iii) sufficiently close to thermal equilibrium, satisfies an Einstein-type relation in terms of the velocity and diffusion coefficient of the (load-free) motor. Even in the simplest two-state models, the velocity-vs.-load plots exhibit a variety of contrasting shapes (including nonmonotonic behavior). Previously suggested bounds on the driving force are shown to be inapplicable in general by analyzing discrete jump models with waiting time distributions.

Molecular motors are protein molecules such as myosin, kinesin, dynein, and RNA polymerase, that move along linear tracks (actin filaments, microtubules, DNA) and perform tasks vital to the life of the organism—muscle contraction, cell division, intracellular transport, and genomic transcription (1–5). Understanding how they operate represents a significant challenge. The hydrolysis of adenosine triphosphate (ATP), with the release of adenosine diphosphate (ADP) and inorganic phosphate (P_i), is known to be the power source for many motor proteins. An activated motor may well be in a dynamical or, better, a stochastic steady state but it *cannot* be in full thermal equilibrium.

Striking *in vitro* experiments observing individual motor proteins moving under controlled external loads (6–11) have stimulated enhanced theoretical work aimed at understanding the mechanisms by which a biological motor functions. From a broad theoretical perspective, a molecular motor is a microscopic object that moves predominantly in one direction along a “polarized” one-dimensional periodic structure, namely, the molecular track (1–11). In recent years, in addition to traditional chemical kinetic descriptions (see, e.g., ref. 12 and references therein) and various more detailed schemes (11, 13, 14), so-called “thermal ratchet” models have been proposed to account for the mechanics: see the review (15).

A common feature of most approaches is that a motor protein molecule is associated with a labeled site l ($= 0, \pm 1, \pm 2, \dots$) on the track and is pictured as being in one of N essentially discrete states j , which may be *free* of (say, $j = 0$) or *bound* to ATP and its various hydrolysis products ($j = 1, 2, \dots, N - 1$). Thus, for a kinesin molecule, K , on a microtubule, M , the ($N = 4$) states identified might be $M \cdot K$, $M \cdot K \cdot ATP$, $M \cdot K \cdot ADP \cdot P_i$, and $M \cdot K \cdot ADP$ (8, 12). Transition rates between these states can be introduced via



where the subscripts indicate that the states j are associated with successive sites, l and $l + 1$, on the track spaced

at distances $\Delta x = x_{l+1} - x_l = d$: this defines the *step size* d . Of course, states $j_l, j_{l+1}, \dots, j_{l+n}$ differ physically only in their spatial displacements $d, 2d, \dots, nd$, along the track. By the same token, the rates u_j and w_j are independent of l (or $x = ld$); however, in the subsequent developments it proves useful to allow for spatially dependent rates $u_j(l)$ and $w_j(l)$.

To properly represent physicochemical reality (that is, microscopic reversibility) *none* of the forward rates, u_j , or backward rates, w_j , may strictly vanish even though some, such as the last reverse rate, w_N , might be extremely small (11, 12). On the other hand, if, as one observes in the presence of free ATP, the motor moves under no external load to the right (increasing x), the transition rates *cannot* (all) satisfy the usual conditions of detailed balance that would characterize thermal equilibrium if Eq. 1 were regarded as a set of chemical reactions (near equilibrium) between effective species j_l (15). [Notice that one may envisage a second-order rate process, e.g., $M \cdot K + ATP \rightleftharpoons M \cdot K \cdot ATP$, to conclude $u_1 = k_1[ATP]$; this can then lead to Michaelis–Menten type rate-vs.-concentration relations (6). However, one might also contemplate a small “spontaneous” or first-order background rate, $u_{1,0} > 0$, that exists even in the absence of ATP.]

Now, within statistical physics, the kinetic scheme in Eq. 1 represents a one-dimensional hopping process of a particle on a periodic but, in general, *asymmetric* lattice. After initial transients, the particle will move (16) with steady (mean) velocity V and diffuse (with respect to the mean position, $\bar{x} = Vt$, at time t) with a diffusion constant D . Complicated, but exact, equations for V and D in terms of u_j and w_j have been obtained for all N (16), as exhibited in the *Appendix*. A dimensionless, overall rate factor that, rather naturally, appears (see Eq. A1), is given by the product

$$\Gamma = \prod_{j=0}^{N-1} \left(\frac{u_j}{w_j} \right) \equiv e^\epsilon. \quad [2]$$

This will play an important role in our discussion. Note, indeed, that viewing Eq. 1 as a standard set of chemical reactions and requiring detailed balance would impose $\Gamma \equiv 1$ (or $\epsilon = 0$), whereas $\Gamma > 1$ (or $\epsilon > 0$) is needed for a positive velocity V . [One might comment, however, (17) that as regards the full chemistry, the complex of motor protein plus track may be regarded simply as catalyzing the hydrolysis of ATP: the reaction rates for this overall process may then be expected to satisfy detailed balance.]

The simplest or “minimal” physical models have $N = 2$, and one can then calculate analytically not only the steady state behavior but also the full transient responses, specifically, the probabilities, $P_j(l; t)$, of being in state j_l (“at” site l) at time t having started, say, at site $l = 0$ in state $j = 0$ at time $t = 0$ (see ref. 17). In ref. 17 only the special (limiting) cases with $w_N \equiv w_2 = 0$ were treated; but as seen below, this limit can be misleading, and so for completeness (and for possible comparisons with experiment and simulation), we present the general $N = 2$ results (see *Appendix*). In particular, the

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velocity and diffusion constant for $N = 2$ are

$$V = \frac{(u_1 u_2 - w_1 w_2) d}{u_1 + u_2 + w_1 + w_2} \equiv (\Gamma - 1) \omega d, \quad [3]$$

$$D = \frac{1}{2} [\Gamma + 1 - 2(\Gamma - 1)^2 \omega / \sigma] \omega d^2, \quad [4]$$

where $\Gamma = u_1 u_2 / w_1 w_2$ (as in Eq. 2), and for convenience, we have introduced the associated overall rates

$$\sigma = u_1 + u_2 + w_1 + w_2, \quad \omega = w_1 w_2 / \sigma. \quad [5]$$

One can, of course, envisage more complicated schemes than Eq. 1, with various internal loops, parallel pathways, etc.‡ In all cases, however, there will be a well defined (zero-load) steady-state velocity V and a diffusion constant D (independent of the particular states, j) which are susceptible to estimation by simulation even though their explicit mathematical expressions may be intractable. Furthermore, in real systems both V , as often demonstrated (6, 8–11), and D (7) are susceptible to experimental measurement.

Now there arises an obvious but crucial question, namely: “What (mean) driving force, f , will such a general motor protein model exert as it moves along the track?” That is the issue we address here from a theoretical standpoint.

Analysis

Maximum Driving Force. The hydrolysis of one ATP molecule releases a free energy ΔG_0 of about 0.50×10^{-19} J [corresponding to 7.3 Kcal/M or $12 k_B T$ at typical *in vitro* temperatures, T (3)]. If all of this free energy could be converted into mechanical energy and move the motor protein through a distance $\Delta x = d$, the step size (going from state 0_l to 0_{l+1} : see Eq. 1), the force exerted would be

$$f_{\max} = \Delta G_0 / d. \quad [6]$$

Accepting that one molecule of ATP is sufficient to translocate the motor protein by one step (7), this expression clearly represents the maximal driving force that can be exerted. For a kinesin moving on a microtubule (6–12) with $d \approx 8.2$ nm (9) it yields $f_{\max} \approx 6.2$ pN. If f is the driving force actually realized, the efficiency of a motor protein may sensibly be defined by $\mathcal{E} = f / f_{\max}$.

Einstein Force Scale. Consider a small (“mesoscopic”) particle with “instantaneous” position $x(t)$ and velocity $v(t)$ that undergoes one-dimensional Brownian motion in a fixed, slowly varying external potential, $\Phi(x)$. Under a constant external force, $F = -(d\Phi/dx)$, the particle diffuses with a diffusion constant which, for long times, t , satisfies

$$D \approx [\langle x^2(t) \rangle - \langle x(t) \rangle^2] / 2t, \quad [7]$$

where $\langle \cdot \rangle$ denotes a statistical average (18–21). In addition, the particle experiences an (effective) frictional force, $f_E = \zeta v(t)$, where ζ is a friction coefficient determined by the environment (18–21). In a steady state, the friction balances the external force, F , leading to a drift motion, $\langle x(t) \rangle \approx Vt$, with mean velocity $V = F / \zeta = f_E / \zeta$. Now, by definition, Brownian motion takes place within *full thermal equilibrium*: that fact dictates (18–21) the Einstein relation $\zeta = k_B T / D$, which, in turn, implies the result

$$f_E = k_B T V / D. \quad [8]$$

‡Thus a backwards reaction directly from, say, state j_l^+ to 0_l could account for “futile” ATP hydrolysis, i.e., without forward motion of the motor (13); but note that within $N = 2$ models (which enforce $j^+ = 1$) the phenomenon may be described simply by including the futile-hydrolysis parallel reaction rate in the backward rate w_1 .

In the present context this is an appealing formula in that it sets a *force scale* in terms only of the velocity, V , and the diffusion constant, D , predicted by a motor-protein model (or observed in an experiment or simulation); one might call it the “Einstein scale.” However, because an activated molecular motor is *not* a Brownian particle and *cannot* be described by thermal equilibrium, there are no grounds for expecting f_E to be related to the proper driving force, f . Nevertheless, we will show that in a certain limit such a Brownian motion “mimic” of an activated motor protein does provide an appropriate prediction for f . Indeed, ref. 17 accepted the identification $f = f_E$ without discussion and used Eq. 8 to estimate driving forces for restricted ($w_2 = 0$) $N = 2$ models: the values of f so obtained were not unreasonable in comparison with experiments (17).

It should, perhaps, be mentioned in passing that ref. 22 (see also ref. 13) invokes an Einstein relation in an analysis of “protein friction.” However, this is a rather different context in which many “blocked” motor proteins are attached to a substrate and a rigid microtubule diffuses, apparently freely, close by in the medium above. Quantitative arguments (13, 22) explain the large frictional slowdown observed (relative to an Einstein-relation estimate) as due to weak protein binding and unbinding.

Barometric Formulation. Although the identification of the motor driving force f with f_E is unjustified, it would be desirable to have a soundly based, general expression for f that, like f_E , does not entail any intrinsic modifications or extensions of the motor model or of the associated physicochemical picture. To that end, consider the placement of an “impassable block” or *barrier* on the molecular track, say, between sites L and $L + 1$ ($\gg 1$) or at distance $x = D = l_0 d$ from the origin $x = 0$ (fixed, as we suppose, by where the motor starts). Such a barrier may be realized theoretically by decreeing that all states j_l for $l \geq L + 1$ are inaccessible; this may be achieved simply by setting one of the local forward rate constants, say, $u_{j+1}(l = L)$, equal to zero. No other rate constants need be modified; but if, perhaps to take cognizance of some aspects of a realistic barrier, further nearby rate constants are changed, it will have no consequences for the main conclusions.

It is intuitively clear that running a molecular motor up to such a barrier will lead [provided it does not detach from the track or “freeze” irreversibly, as might happen in practice (6, 10)] to a stationary probability distribution, $P_j(l, t \rightarrow \infty) = P_j^\infty(L - l)$, in which $z = (L - l)d = D - x$ measures the distance back from the barrier. On very general theoretical grounds one should expect this distribution to decay exponentially with increasing z (except for possible deviations close to the barrier) so that, explicitly, one has

$$P_j^\infty(z/d) \approx A_j e^{-\kappa z}. \quad [9]$$

The (positive) decay constant κ should, in principle, be experimentally measurable (although this may be difficult if κd is large). The amplitude ratios A_j / A_0 must depend on the various rate ratios, u_i / w_i , while A_0 is set simply by normalization.

To justify this surmise for the kinetic scheme in Eq. 1 (although it is of general validity), note that the mean flow between adjacent states 0_l and $(N - 1)_{l-1}$ and between j_l and $(j - 1)_l$ [for $j = 1, 2, \dots, (N - 1)$] must vanish for a stationary distribution. Balancing local forward and backward rates thus yields

$$\begin{aligned} u_N(l - 1) P_{N-1}^\infty(L - l + 1) &= w_N(l - 1) P_0^\infty(L - l), \\ u_j(l) P_{j-1}^\infty(L - l) &= w_j(l) P_j^\infty(L - l), \end{aligned} \quad [10]$$

for $j = 1, 2, \dots, (N - 1)$. Starting from an initial nonzero value $P_j^\infty(0)$, one can then recursively determine $P_{j-1}^\infty(0)$, $P_{j-2}^\infty(0), \dots, P_0^\infty(0)$, $P_{N-1}^\infty(1)$, $P_{N-2}^\infty(1), \dots$. By induction, this leads directly to Eq. 9 [since the $u_j(l)$, and $w_j(l)$ become independent of l for, say, $l < L - l_0$ where l_0 is a fixed integer].

Most crucially one finds (with the notation of Eq. 2) that the decay constant is simply

$$\kappa = (\ln \Gamma)/d = \epsilon/d. \quad [11]$$

Now, to interpret these results, consider a dilute gas of molecules of mass m moving in a gravitational field that acts “downwards” along the z axis. Each molecule then has a weight $f_G = mg$; in addition, the equilibrium density distribution is given by (23)

$$\rho(z) = \rho(0)e^{-mgz/k_B T}, \quad [12]$$

where $\rho(0)$ is the density at the level $z = 0$. [Any deviations arising close to the “lower” wall (at $z \approx 0$) due to molecular size, structure, etc., have been neglected.] Comparing this well known barometric formula with the distribution Eq. 9 leads us to identify the driving force f of the molecular motor with

$$f_B = k_B T (\ln \Gamma)/d = k_B T \epsilon/d. \quad [13]$$

The subscript B here serves merely to indicate the barometric analogy underlying our identification. By comparison with Eq. 6 for f_{\max} , we may expect $\epsilon \lesssim \Delta G_0/k_B T$ for a real molecular motor.

Before studying this result in relation to extensions of Eq. 1 needed to describe a motor functioning under external loads, let us compare f_B with f_E .

Barometric vs. Einstein Scale. Suppose the molecular motor operates close to equilibrium in the sense that $\epsilon = \ln \Gamma$ is small. Then, on expanding in ϵ at fixed ω/σ , Eqs. 2–5 and 13 yield

$$f_B/f_E = 1 + \left[\frac{1}{12} - (\omega/\sigma)\right]\epsilon^2 - \frac{1}{2}(\omega/\sigma)\epsilon^3 + \dots, \quad [14]$$

for $N = 2$. Evidently, the coefficient of ϵ vanishes identically! Furthermore, one finds $0 < \omega/\sigma \leq \frac{1}{16}$ so that the coefficient of ϵ^2 is small, lying between $\frac{1}{48}$ and $\frac{1}{12}$. Consequently, and as anticipated, the Einstein scale approximates the barometric result very well when the motor operates sufficiently close to equilibrium. Indeed, for $\Gamma < 10$, calculations show that f_B can exceed f_E by no more than 44%. Furthermore, the series truncated at $O(\epsilon^2)$ in Eq. 14 is reasonably accurate up to $\epsilon \approx 5$ ($\Gamma \approx 150$) where one has $1.473 < f_B/f_E < 2.535$; beyond that, the bounds $\frac{1}{4}\epsilon < f_B/f_E \lesssim \frac{1}{2}\epsilon$ are effective.

These specific results are limited to $N = 2$; but we suspect (and have checked for $N = 3$) that the vanishing of the $O(\epsilon)$ term in Eq. 14 is independent of N . Likewise, we expect f_B always to rise steadily above f_E when ϵ increases. Indeed, on recalling Eq. 2 for Γ , one observes from Eq. 13 that f_B is unbounded above and so, with an injudicious assignment of rate constants, it may even exceed f_{\max} (as given in Eq. 1)! Conversely, one may show from Eqs. 3, 4, and 8 that f_E for $N = 2$ is bounded above by $4k_B T/d$ (17). However, we will demonstrate below that this bound on f_E is rather artificial and does not apply for models that account directly for the discreteness of ATP hydrolysis.

Stalling Force Measured by Spring Compression. In a typical experiment on motor proteins (6–10), optical tweezers are used to carry a silica bead coated with a few molecules of the motor protein up to the molecular track. Then a single motor binds to the track and starts to move, exerting a force against the opposing load, F , as it pulls the bead towards a side of the optical trap. The external force F is a linear function of the displacement from the trap center, and the constant of proportionality can be measured. Thus the trap and bead work like a calibrated spring acting on the molecular motor. To represent such an experiment the load-free scheme embodied in Eq. 1 must, clearly, be extended.

To this end, suppose the motor moves in a slowly varying external potential, $\Phi(x)$, so that in translocating from site l to

$l + 1$, additional mechanical work $\Delta\Phi(x = ld) = \Phi(x + d) - \Phi(x)$ must be done (relative to the load-free situation). Of course, this corresponds to imposition of a local external force, $F(x) = \Delta\Phi(x)/d$, directed negatively. For an (ideal) optical trap of spring constant K we may take

$$\Phi(x) = \frac{1}{2}Kx^2, \quad F(x) = K(x + \frac{1}{2}d). \quad [15]$$

In such a situation the motor should, in effect, compress the spring and, as t increases, attain a stationary distribution, say $P_0^S(l)$, where, for simplicity, we focus only on the (free) states 0_j . This distribution should peak at some l_S , corresponding to a mean (or most probable) compression of the spring by a displacement $x_S = l_S d$. Then the measured “stalling force” would be $f_S = Kx_S$.

Now it is physically clear that under any local load, $F(x)$, the transition rates, $u_j(l)$ and $w_j(l)$, must change. If, as traditional, one views the chemical transitions between successive states, j and $j + 1$, as proceeding in quasiequilibrium over various free energy barriers (13), one expects (in leading approximation) the rates to change exponentially with $F(x)d/k_B T$. But how the exponential loading factors should be distributed among the various reaction processes, $j \rightleftharpoons (j + 1)$, is far from clear: indeed, this distribution is of considerable interest in understanding the motor mechanism at a microscopic level. Without prejudice, therefore, we will explore the *quasiequilibrium hypothesis* that under a local load, F , the local transition rates change in accord with

$$\begin{aligned} u_j &\Rightarrow u_j^{(F)} = u_j^{(0)} e^{-\theta_j^+ Fd/k_B T}, \\ w_j &\Rightarrow w_j^{(F)} = w_j^{(0)} e^{+\theta_j^- Fd/k_B T}. \end{aligned} \quad [16]$$

The distribution factors, θ_j^+ and θ_j^- , need not be of uniform sign; but we certainly expect the overall factor,

$$\theta = \sum_{j=1}^N (\theta_j^+ + \theta_j^-), \quad [17]$$

to be positive, implying an opposition to motion. Indeed, should the motor undergo diffusion in thermal equilibrium when *not* activated by ATP (as suggested parenthetically in the introductory discussion of Eq. 1), detailed balance considerations would dictate $\theta = 1$. As a *supplement* to our quasiequilibrium hypothesis this value of θ is also plausible for an activated motor that operates not too far from equilibrium. Notice that a *negative* θ_j^+ or θ_j^- simply means that the corresponding forward rate, u_j , is *enhanced*, or the reverse rate, w_j , is *diminished* by the internal molecular strain induced in the motor by the load.

Accepting Eq. 16 we can find the stationary distribution $P_0^S(l)$ with the aid of the rate-balance Eq. 10 [replacing $P_j^S(L-l)$ by $P_j^S(l)$ and the rates u_j and w_j in accord with Eq. 16]. The most probable motor location, l_S , follows by equating $P_0^S(l)$ and $P_0^S(l + 1)$, which leads directly to the condition

$$\Gamma^{(F)}(l) \equiv \prod_{j=1}^N [u_j^{(F)}(l)/w_j^{(F)}(l)] = \Gamma^{(0)} e^{-\theta F(x)d/k_B T} = 1. \quad [18]$$

Solving this determines $x_S = l_S d$ and thence yields the measured spring or stalling force

$$f_S = k_B T (\ln \Gamma)/\theta d = k_B T \epsilon/\theta d, \quad [19]$$

where we have, of course, identified the zero-load rate factor, $\Gamma^{(0)}$, with the original rate factor Γ in Eq. 2.

It is striking that this expression for the stalling force (which depends on the quasiequilibrium hypothesis, Eq. 16, that is needed to extend the original kinetic model) agrees

precisely with the barometric expression Eq. 13 for f_B , provided one accepts the natural, near-equilibrium evaluation $\theta = 1$. We regard this overall consistency as strengthening both approaches.

Velocity vs. Load. The extended rate constants $u_j^{(F)}$ and $w_j^{(F)}$ given in Eq. 16 also serve to provide a relation for the motor velocity, $V(F)$, as a function of a steady load force, F [and, equally, for the load-dependent diffusion constant, $D(F)$]. For arbitrary N one may appeal to Eq. A1, which shows, as expected, that the stalling load, F_S , which brings $V(F)$ to zero, agrees with Eq. 19, i.e., $F_S = f_S$. To write an explicit result for $N = 2$ in an illuminating form, we introduce the reduced force and modified load factors

$$\eta = F/F_S \quad \text{and} \quad \Delta_j^\pm = \frac{1}{2} - (\theta_j^\pm/\theta). \quad [20]$$

Then from Eqs. 3, 16, and 19 we obtain

$$\frac{V(F)}{V(0)} = \frac{\sigma \sinh[\frac{1}{2}\epsilon(1-\eta)]/\sinh(\frac{1}{2}\epsilon)}{u_1 e^{-\Delta_1^+ \epsilon \eta} + u_2 e^{-\Delta_2^+ \epsilon \eta} + w_1 e^{\Delta_1^- \epsilon \eta} + w_2 e^{\Delta_2^- \epsilon \eta}}, \quad [21]$$

where, naturally, $V(0)$ is simply the no-load result of Eq. 3; thus the right hand side reduces to unity when $\eta = 0$ (and vanishes as $\eta \rightarrow 1$).

Now for ϵ small (say, $\lesssim 2$), so that the motor is operating not too far from equilibrium, one has $V(F) \approx V(0)(1-\eta)/(1+c\epsilon\eta)$. This represents a *hyperbolic* force law which will be *concave* or *convex* depending on the sign, + or -, of c : see the illustrative examples in Fig. 1. For small c the law is close to *linear* and, in fact, c vanishes whenever $u_1\Delta_2^+ + u_2\Delta_1^+ = w_1\Delta_2^- + w_2\Delta_1^-$. This condition has many solutions; for example, if the backward rates are small, so that $\delta = (w_1 + w_2)/(u_1 + u_2) \lesssim 0.1$, say, the loading scheme $\theta_1^+ = \theta_2^+ \approx \frac{1}{2}\theta/(1+\delta)$ yields a near-vanishing c .

On the other hand, if u_1 greatly exceeds u_2 , w_1 , and w_2 , the reduced (V, F) plots become insensitive to u_1 . Then if, as mentioned (6), one has $u_1 \approx k_1[\text{ATP}]$, the plots will become independent of the ATP concentration (6). Furthermore, if ϵ is large but $(\theta_2^+/\theta)\epsilon = 1$, the (V, F) plots will be close to linear.

Although straight, convex, and concave velocity-load plots are readily generated, other reasonable values of the six parameters: ϵ , w_1/u_1 , w_2/u_1 , and θ_2^+/θ , θ_1^-/θ and θ_2^-/θ , yield plots exhibiting points of inflection of either sense, as shown in Fig. 1. However, plots with *negative* inflection points, such

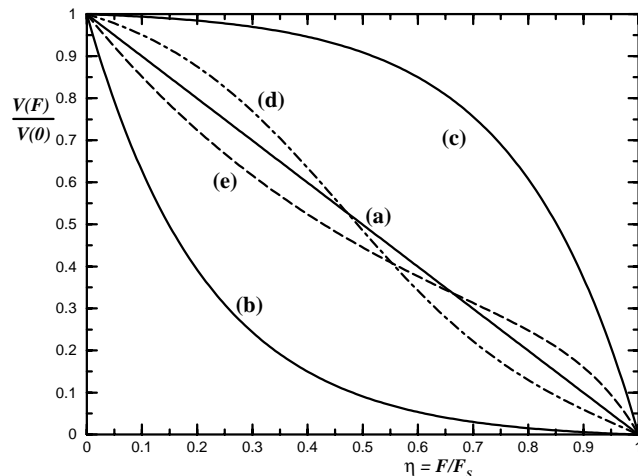


FIG. 1. Examples of velocity-load plots for $N = 2$ models with various parameter sets $\{\epsilon; (w_1, w_2)/u_1; (\theta_2^+, \theta_1^- = \theta_2^-)/\theta\}$: (a) $\{10^{-2}; 0.99, 0.99; \frac{1}{2}, 0\}$, (b) $\{9.2; 10^{-2}, 10^{-2}; \frac{1}{2}, 0\}$, (c) $\{10^{-2}; 10^{-2}, 10^{-2}; 0, \frac{1}{2}\}$, (d) $\{11.1; 10^{-4}, 0.15; 0, \frac{1}{2}\}$, (e) $\{23.0; 10^{-5}, 10^{-5}; 0.07, 0.43\}$. Note that $V(0)$ is the velocity at zero load (Eq. 3) while F_S denotes the stalling load.

as (e), are realized in relatively small regions of the parameter space. If negative θ_2^+ or θ_1^+ are admitted (see after Eq. 17) the velocity may initially *rise* when a load is imposed! Plots with two inflection points are then also allowed. Thus if one could determine plausible values for the no-load transition-rate ratios, experimental (V, F) plots might, at least for an $N = 2$ model, throw some light on the load distribution parameters, θ_j^\pm .

Discrete Jump Models. As mentioned above, the Einstein force scale obtained from the kinetic scheme, Eq. 1, is subject to a fairly stringent bound. Analyzing the expression $f_E/k_B T = V/D$ (see Eq. 8) for the case $N = 2$ (using Eqs. 3–6) we can prove that for all rates $u_j, w_j > 0$, the Einstein scale satisfies $f_E/k_B T \leq 2N/d$; the maximum for $N = 2$ is realized for uniform rates $u_j = u_0 \gg w_j = w_0$ (all j), and we believe the same condition yields the bound as stated generally for all N . (The uniformity condition can be understood heuristically since in such a case there are no distinguishing rate-limiting steps in the cycle. The $N = 2$ model studied in ref. 17 also respects the *lower* bound $f_E/k_B T > 2/d$; but this is attributable to the special limiting situation, $w_2 = 0$, studied there which cannot be literally true.)

Our purpose here is to demonstrate that these bounds on f_E are related to the continuous-time picture of the rate process embodied in the kinetic master equations based on Eq. 1; in essence, these force a minimum value of the diffusion constant D . To see this most directly, consider an $(N = 1)$ -state model with master equation

$$\begin{aligned} \frac{\partial P_0}{\partial t}(l, t) = & uP_0(l-1, t) + wP_0(l+1, t) \\ & - (u+w)P_0(l, t), \end{aligned} \quad [22]$$

where we have put $u_1 = u \geq w_1 = w > 0$. Then one finds

$$V = (u-w)d, \quad D = \frac{1}{2}(u+w)d^2, \quad [23]$$

(see, e.g., ref. 16). Note the lower limit $D > \frac{1}{2}ud^2$, which is approached when $w/u \rightarrow 0$. This leads directly to the bound $f_E/k_B T < 2/d$.

By contrast, consider a *discrete* event sequence in which a forward or backward jump is attempted at (mean) time intervals $\Delta t = \tau$ (triggered, one might picture for a molecular motor, by the arrival of individual ATP molecules). If $\check{P}_0(l; n)$ is the probability that the (motor) particle is at site l after n jump attempts, one has (19, 20, 24)

$$\begin{aligned} \check{P}_0(l; n+1) = & p_+ \check{P}_0(l-1; n) + p_0 \check{P}_0(l; n) \\ & + p_- \check{P}_0(l+1; n), \end{aligned} \quad [24]$$

where p_+ and p_- are the probabilities of completing a positive or negative step while $p_0 = 1 - p_+ - p_-$ is the probability of remaining at the same site. If one sets $p_+ = u\tau$ and $p_- = w\tau$, and identifies the time as $t \approx n\tau$, this discrete master equation reduces to the continuous form, Eq. 22, in the limit $\tau \rightarrow 0$ (24).

Now the mean displacement $\langle x \rangle_n$ after $n = 1$ attempts is clearly $(p_+ - p_-)d$ so that the mean velocity is

$$V = (p_+ - p_-)d/\tau = (u-w)d. \quad [25]$$

Note that the identifications appropriate to the continuous limit yield agreement with Eq. 23. To compute D we may use Eq. 7 with only a *short* time interval, specifically $t = \tau$, since, by assumption, successive jump attempts are uncorrelated. Thus, from $\langle x^2 \rangle_1 = (p_+ + p_-)d^2$ we obtain

$$\begin{aligned} D = & \frac{1}{2}(d^2/\tau)[p_+ + p_- - (p_+ - p_-)^2] \\ = & \frac{1}{2}[u + w - (u-w)^2\tau]d^2. \end{aligned} \quad [26]$$

To see that D now has no positive lower bound, we may specialize to the case $p_0 = 0$ or consider the limit $p_- (= w\tau) \ll p_+$: then one finds $D \propto (d^2/\tau)p_+(1-p_-)$ which becomes indefinitely small when p_+ approaches unity. Hence no upper bound on f_E exists in such a discrete jump model. Indeed, it is intuitively clear that in the limit $p_+ = 1$ (so that $p_0 = p_- = 0$) the particle moves essentially ballistically at speed d/τ with no dispersion.

Notice also that the barometric formulation can be applied directly to the jump model by using Eq. 24. It leads precisely to the previous form, Eq. 13, but with $\Gamma = p_+/p_-$; however, this agrees exactly with the continuous-time ($N = 1$) result $\Gamma = u/w$ when, as above, one puts $p_+ = u\tau$ and $p_- = w\tau$. In addition, the ratio $R(\epsilon) = f_B/f_E$ obeys Eq. 14 but with, in leading order, (ω/σ) replaced by $\frac{1}{2}p_- = (1-p_0)/(2(\Gamma+1))$. For $\epsilon > 2$ one has $\frac{1}{2}p_- < 0.06$ and $R(\epsilon)$ varies much as discussed above for the continuous case.

It might be objected that our arguments have more or less assumed that the jump attempts occur regularly at times $n\tau$ whereas, more realistically, there should be some distribution, say $\psi(t)$, of waiting times between one event and the next. Then τ would be the mean time between attempts, defined by

$$\tau = \bar{t} \quad \text{with} \quad \bar{t}^n = \int_0^\infty t^n \psi(t) dt. \quad [27]$$

Such a model may be studied along the lines of Montroll and Scher (24). Provided $\psi(t)$ decreases sufficiently fast when $t \rightarrow \infty$ that the second moment \bar{t}^2 is finite, the analysis for V and D can be carried through: it shows again that D is unbounded below while $f_E \propto V/D$ is unbounded above. Indeed, Eq. 25 for V remains valid while Eq. 26 for D gains a factor $(1-\Theta)$ before each squared term, $(p_+ - p_-)^2$ and $(u-w)^2$. The parameter $\Theta = (\bar{t}^2 - \bar{t}^2)/\bar{t}^2 \geq 0$ measures the relative width or “spread” of the waiting time distribution $\psi(t)$: e.g., for $\psi(t) \propto t^{\nu-1}e^{-\gamma t}$ with $\nu, \gamma > 0$, one has $\Theta = \nu/\gamma$ and $\Theta = 1/\nu$. The sharp distribution originally envisaged corresponds to the limit $\nu \rightarrow \infty$.

Finally, note that we can also analyze precisely multistate versions of these discrete jump models with waiting times.

Discussion and Summary

To understand the driving force, f , exerted by a molecular motor that takes steps of size d on a molecular track, we have analyzed a broad class of stochastic models: in particular, Eqs. 1 and 2 embody a general, “linear” reaction sequence. In the presence of a constant free energy source, the motor will achieve a steady velocity $V (> 0)$ but with fluctuations about the mean position, $\langle x(t) \rangle = Vt$, described by a diffusion constant, D , that may be measured by observing the variance (see Eq. 7). Table 1 lists various force scales that then arise and their relation to f .

By way of a concrete numerical illustration, consider a kinesin molecule moving on a microtubule (2–4, 6–10, 12) for which $d \approx 8.2$ nm (9). Svoboda, Mitra, and Block (6) observed $V \approx 670$ nm/s when $[\text{ATP}] = 2$ mM and measured the variance from which we obtain $D \approx 1395$ nm²/s. At $T = 300$ K these data yield $f_E \approx 2.0$ pN. On the other hand, the observed stalling force was $f_S \approx 5-6$ pN (6, 7), significantly larger than f_E , as we have argued it should be. Note also, comparing with the maximal force, $f_{\text{max}} \approx 6.2$ pN, that the observed efficiency \mathcal{E} , is in the range 80–95%. [This observational estimate does not allow for the possible “wastage” of ATP by futile hydrolysis (13) without translocation of the motor: recall the remark in the footnote to the sentence following Eq. 5, above.]

Table 1. Forces related to a Molecular Motor

Force	Notation and Relations	Eq.
Maximum driving force	$f_{\text{max}} = \Delta G_0/d > f$	6
Einstein Scale	$f_E = k_B T V/D < f$	8
Gravitational force	$f_G = mg$	12
Barometric force	$f_B = k_B T \kappa \cong f$	12, 13
Stalling force	$f_S = k_B T \epsilon / \theta d \stackrel{?}{=} f$	19
Load and stalling load	$F, F_S, \eta = F/F_S$	20

The “barometric” force scale, f_B , arises by considering an obstacle that blocks the motor’s motion on the track: the resulting statistically stationary distribution decays with the distance z from the obstacle as $e^{-\kappa z}$ (see Eq. 9). It would be interesting (although difficult) to measure κ and to compare f_B , so derived, with the observed stalling force f_S .

For the general ($N = 2$)-state model (see Eqs. 1–5) with transition rates u_1, u_2, w_1 , and w_2 one has $f_B = (k_B T/d) \times \ln(u_1 u_2 / w_1 w_2)$. For kinesin (from *Drosophila*) Gilbert and Johnson (12) studied the kinetics by using chemical-quench flow methods. Assuming $[\text{ATP}] = 2$ mM their data show that $u_1 = 3800$ s⁻¹, $u_2 = 15$ s⁻¹, and $w_1 = 200$ s⁻¹ represent a sensible map on to an $N = 2$ model; however, w_2 proved unobservably small. Merely for illustration, therefore, suppose $w_2 = u_2/100 = 0.15$ s⁻¹. This gives $V \approx 116$ nm/s and $D \approx 474$ nm²/s, which yield $f_E \approx 1.0$ pN (at $T = 300$ K), while the rates give $f_B \approx 3.8$ pN. The agreement with the results of Svoboda *et al.* is not impressive: nevertheless, the orders of magnitude, the inequality $f_B > f_E$, and the rough equality $f_B \approx f_S$ are in full accord with our analysis.

More recently, Higuchi *et al.* (9) obtained data (for bovine brain kinesin) leading us to $u_1 \approx 1400$ s⁻¹ and $u_2 \approx 45$ s⁻¹, in only rough agreement with the values derived from ref. 12. The *ad hoc* assumption $w_1/u_1 \approx w_2/u_2 \approx 1/100$ yields $V \approx 354$ nm/s and $D \approx 1370$ nm²/s, closer to observations (6). Likewise, $f_E \approx 1.1$ pN and $f_B \approx 4.7$ pN now accord better with the direct experiments (although these values do depend logarithmically on w_1 and w_2). While the general theoretical picture is supported, further experiments on standardized kinesin samples would clearly be valuable and could provide more stringent tests.

To discuss the velocity $V(F)$ of a motor under a load F , the transition rates must be modified: an appropriate *quasiequilibrium* hypothesis is embodied in Eqs. 16 and 17 where the load-distribution factors, θ_j^\pm , recognize that the various transitions in a motor protein probably accept quite different fractions of the total stress. Indeed, some forward rates might even be *accelerated*, which could provide a mechanism to conserve, e.g., ATP under “no-load” conditions. It is natural to take the overall load-distribution factor θ (Eq. 17) as unity which leads to the equality of f_S and f_B (see Eq. 19 *et seq*). However, $\theta = 1$ can be doubted for real motors and might well be tested by experiment or simulation.

Even for a two-state model, the expression for $V(F)$ is quite complex (see Eq. 21). As seen in Fig. 1, the six independent parameters permit velocity-load plots of varied shapes (including nonmonotonic forms not shown). Certain types, such as (e) characterize small regions of the parameter space, but, in general, the variation of V with F may reveal comparatively little about the motor mechanism or parameter values.

Negative, i.e., assisting loads ($F < 0$) are predicted to speed up the motor and this has been observed (10). Conversely, under super-stalling loads ($F > F_S$), *backwards velocities* are predicted; single reverse steps of kinesin have then been seen (10), but no steady reverse velocities have been reported. This probably reflects very small terminal reverse rates, w_N (12). Indeed, these transitions presumably describe second (or higher) order chemical reactions controlled by the low concentrations of hydrolysis products. The frequently ob-

[§]The specific results quoted in ref. 24, equations 75 for $\nu = \frac{1}{2}$ and $\nu = 2$, are in error (and the factor 4 in equation 76 should read 2). Dr. Harvey Scher has kindly acknowledged the need for these corrections.

served process of detachment from the track (6, 10) should also be included in a fuller account.

The adequacy of the stochastic models encompassed by Eq. 1 is challenged by lower bounds on the diffusion constant, D , which yield upper bounds on f_E . For kinesin at $T = 300$ K this bound is 2.03 pN for any ($N = 2$)-state model. The data of Svoboda *et al.* (6, 7) essentially meet this, but were the bound violated, one might conclude that an $N \geq 3$ kinetic model was needed. However, models in which the transitions are described by *discrete jumps* occurring after certain waiting times are *not* susceptible to these constraints. Such models might well prove more realistic, although at present the simpler kinetic representations seem adequate. Nevertheless, it should be noted that the main principles we have enunciated are not restricted to the $N = 2$ sequential kinetic models specifically analyzed. Consequently, the observation of significant violations would indicate serious deficiencies in the general understanding of motor mechanisms.

Appendix

For a one-dimensional hopping model with N states and arbitrary transition rates u_j and w_j , as introduced in Eq. 1, Derrida (16) obtained the exact steady-state behavior. For the drift velocity he found

$$V = \frac{d}{\sum_{j=1}^N r_j} \left(1 - \prod_{j=0}^{N-1} \frac{w_j}{u_j} \right), \quad [\text{A1}]$$

where d is the spatial period (or step-size) while

$$r_j = \frac{1}{u_j} \left(1 + \sum_{k=1}^{N-1} \prod_{i=1}^k \frac{w_{j+i-1}}{u_{j+i}} \right). \quad [\text{A2}]$$

The expression for the diffusion constant (16) is similar but more complex and less illuminating.

For $N = 2$, solutions can be obtained for all times following the procedure outlined in ref. 17. Thus, the probability that the particle is at site l in state j_l ($j = 0, 1$) after a time t having started at the origin, $l = 0$, is

$$P_j(l, t) = \int_{-\pi}^{\pi} \frac{dq}{2\pi} e^{-iq(l+j/2)} \left[\Xi_+(q) e^{\lambda_+(q)t} - \Xi_-(q) e^{\lambda_-(q)t} \right] \quad [\text{A3}]$$

with rate parameters $\lambda_+(q)$ and $\lambda_-(q)$ given by

$$\lambda_{\pm}(q) = \frac{1}{2} \left[-\sigma \pm \sqrt{\sigma^2 + 4u_1u_2(e^{2iq} - 1) + 4w_1w_2(e^{-2iq} - 1)} \right], \quad [\text{A4}]$$

while σ is in Eq. 5 and the coefficient functions are

$$\Xi_{\pm}(q) = \frac{\lambda_{\pm}(q) + u_1 + w_2}{\lambda_{\pm}(q) - \lambda_{\mp}(q)} \left(1 + \frac{\lambda_{\pm}(q) + u_1 + w_2}{u_2 e^{iq} + w_1 e^{-iq}} \right). \quad [\text{A5}]$$

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