AUTONOMIC ENERGY CONVERSION

II. An Approach to the Energetics of Muscular Contraction

S. R. CAPLAN

From the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT All discussions of muscle energetics concern themselves with the Hill force-velocity relation, which is also the general output relation of a class of self-regulated energy converters and as such contains only a single adjustable parameter —the degree of coupling. It is therefore important to see whether *in principle* muscle can be included in this class. One requirement is that the muscle should possess a working element characterized by a dissipation function of two terms: mechanical output and chemical input. This has been established by considering the initial steady phase of isotonic and isometric tetanic contraction to represent a stationary state of the fibrils (a considerable body of evidence supports this). Further requirements, which can be justified for the working element, are linearity and incomplete coupling. Thus the chemical input of the muscle may be expected to follow the inverse Hill equation (see Part I). The relatively large changes in activities of reactants which the equation demands could only be controlled by *local* operation of the regulator, and a scheme is outlined to show how such control may be achieved. Objections to this view recently raised by Wilkie and Woledge rest on at least two important assumptions, the validity of which is questioned: (*a*) that heat production by processes other than the immediate driving reaction is negligible, which disregards the regulatory mechanism (possibly this involves the calcium pump), and (*b*) that the affinity of the immediate driving reaction is determined by over-all concentrations. The division of heat production into "shortening heat" and "maintenance heat" or "activation heat" is found to be arbitrary.

INTRODUCTION

The characteristic force-velocity relation for muscle, discovered experimentally by A. V. Hill (1, 2), has a rather striking significance in the nonequilibrium thermodynamic treatment of linear energy converters (3). It seems important to examine the relevance of this finding to muscle energetics, particularly in the light of Pringle's observation that it is "worth enquiring if any conceptual model does really attach to the equation" (4). The Hill equation can be derived directly from the appropriate phenomenological equations without reference to the nature of the mechanism involved, and as such describes the behavior of autonomic linear energy converters

of the simplest canonical class. In this class the information incorporated or "programmed" into the regulator is minimal, comprising only the operational limits of the converter and, implicitly, its degree of coupling. It is therefore necessary to see whether *in principle* muscle can be assumed to belong to a class of energy converters of this kind, and if so what consequences can be deduced.

Recently, in a criticism of this treatment, Wilkie and Woledge discussed the above problem in considerable detail (5). They found that not only is the correct force-velocity relation obtained, but the experimental relation between maximum efficiency and maximum normalized power output is apparently similar to that predicted. On the other hand they concluded that the relation between the rate of the driving reaction and the load is not similar to the predicted one. Furthermore they concluded that the predicted variations in free energy of the driving reaction are so large that they could not actually occur. These criticisms, however, involve some questionable assumptions and require careful reconsideration.

The Hill equation is generally written

$$(P + a)(V + b) = (P_o + a)b = (V_m + b)a \tag{1}$$

where P is the tension and V the initial constant velocity in tetanic shortening; P_o is the "isometric tension" or tension developed when the muscle is prevented from shortening, and V_m is the velocity of contraction when the load is zero. The quantities a and b are purely mechanical constants (2). It follows that the ratios a/P_o and b/V_m are equal; we shall denote them by θ. The most remarkable property of this relation, at first glance, is its symmetry. In this respect it contrasts sharply with an earlier relation proposed by Fenn and Marsh (6)

$$P = P_o\exp(-aV) - kV \tag{2}$$

where a and k are constants, and with the somewhat similar exponential expressions proposed by Polissar (7) and Aubert (8). The symmetry of equation 1 emerges clearly if we normalize it by introducing the variables $\xi_1 = P/P_o$ and $\gamma_1 = V/V_m$ (see Part I, equation 29). It is seen that the functional form of $P(V)$ is identical to that of $V(P)$, a situation reminiscent of the phenomenological relations of non-equilibrium thermodynamics. The normalized equation represents a family of hyperbolae cutting both axes at unity. The curvature of any given hyperbola depends on the quantity θ, which was singled out by Hill (1) as an index of performance for the muscle. Although equation 1 is set aside from the other relations by virtue of both its symmetry and its algebraic simplicity, all were put forward on a largely empirical basis. The choice between them has been described succinctly by Hill (9): "The real question as between the four equations is, which is the most useful? Useful for what? Helping to make a complicated mixture of observed facts more digestible, pointing out where to look for new ones and providing a "take-off" for the next jump into the unknown."

It is argued here that equation 1 fulfils the above criterion of usefulness, and that its probable significance in the description of muscle behavior has been overlooked. The concept of self-regulation in muscle is by no means new—it was known in principle to Heidenhain as early as 1864 (reference 9, p. 9), and was originally proposed explicitly by Fenn (10). Essentially the same idea has been used by Pringle in postulating a property of the contractile component called "activation", which is increased by tension and which controls the velocity of shortening at a given tension (4). The idea of positive feedback was invoked in Hill's study of the effect of tension in prolonging the active state in a twitch (11). Pringle has reviewed the commonly held notions of the control sequence between nerve and muscle (4). In the present treatment we are concerned with a regulator which responds uniquely to the *load*, and the input to the working element of the muscle which interests us is not the series of nerve impulses which maintains tetanus, but rather the input of *chemical energy* controlled by the regulator.

THE MUSCLE AS AN ENERGY CONVERTER

We shall restrict our considerations solely to the *steady-state phase of tetanic contraction*, i.e. the initial phase of both constant velocity and constant tension (including zero velocity, or isometric contraction, as one limiting case, and zero tension, or unloaded contraction, as the other). The thermodynamic analysis can then be applied intuitively, by analogy with equations 1 and 7 of Part I, writing immediately

$$\Phi = V(-P) + vA, \tag{3}$$

where Hill's notation has been used for the output.[1] This dissipation function for muscle will be derived in the next section. It is convenient to consider $-P$ as the equivalent of the output force X_1, taking the direction of contraction as positive, since the spontaneous effect of P is to *stretch* the muscle. Then P_o is directly analogous to the emf $\mathcal{E}$ of an electrochemical cell discussed in Part I. If V is expressed in cm/sec, P must be expressed in dynes. The resistive load, or mechanical resistance $R_L = P/V$, may vary from zero to infinity. The phenomenological relations corresponding to equation 3 contain vectorial cross-coefficients, consequently it is implied that coupling takes place in an anisotropic medium. In this respect the treatment is no different from that of Kedem for active transport (12), indeed muscular contraction may be considered a special case of this, since according to the sliding filament hypothesis metabolic energy is expended to transport actin filaments relative to myosin filaments against mechanical tension. We shall assume that the system can be described, to a good approximation, by linear phenomenological relations with constant coefficients. The assumption of linearity requires justification and will be discussed later. If the affinity of the chemical re-

[1] A list of symbols appears at the end of this paper. (The symbols h and v are used by Wilkie and Woledge (5) to denote chemical reaction rate and velocity of shortening, respectively.)

action, A, remained constant the assumption would be less serious (the enzymatic fuel cell referred to in Part I shows a predictably linear relationship between flows under such conditions), but the affinity is expected to vary. Equations 12 of Part I now take the following form, where for the sake of generality Onsager symmetry has not been introduced:

$$(A_o/P_o) = (\partial A/\partial P)_V = (1/q_{12}^2)(V_m/v_m)$$
$$= (1/q_{12}^2)(\partial V/\partial v)_P = (q_{21}/q_{12})(\partial V/\partial v)_A . \quad (4)$$

The symbols A_o and v_m represent the affinity during an isometric contraction, and the reaction velocity during an unloaded contraction, respectively.

It is possible to use the present approach within the framework of the conventional treatment of heat production. However, in so doing the conventional assumptions must be made. These are (*a*) the affinity is constant, (*b*) the whole muscle is a closed system, and (*c*) the heat production by processes other than the actual contractile process is insignificant.

Heat Production at Constant Affinity

If the affinity remains constant, equation 4 gives

$$A = (1/q_{12}^2)(V_m/v_m)P_o = (q_{21}/q_{12})(\partial V/\partial v)_A P_o . \quad (5)$$

Unless the system is completely coupled, there will be a finite reaction velocity under isometric tension which may be denoted v_o. (In frog sartorius, for example, Infante, Klaupiks, and Davies (13) have demonstrated a steady breakdown of ATP during isometric contractions at 0°C.) On the basis of linear phenomenological relations

$$(\partial V/\partial v)_A = V/(v - v_o). \quad (6)$$

The velocity of reaction is related to the heat of reaction (per mole) h at the given temperature, pressure, and concentrations (14) by

$$-hv = \dot{H} \quad (7)$$

where $\dot{H}$ is the rate of *decrease* of enthalpy, and it is assumed that no other processes contribute to the enthalpy change (for convenience we shall use the notation $\dot{N} \equiv -dN/dt$). Since we are here regarding A as constant, h would not be expected to vary. Combining equations 5, 6, and 7, we obtain

$$A = (q_{21}/q_{12})\{V/(\dot{H} - \dot{H}_o)\}(-h)P_o . \quad (8)$$

As long as v is positive, $-h$ and $\dot{H}$ must have the same sign, so that only their absolute values need concern us.

Employing the conventional assumption that the whole muscle is essentially a closed system, we may write from the first law

$$\dot{H} = \dot{Q} + PV \quad (PV \text{ in Hill's notation}) \tag{9}$$

since we are dealing with virtually constant volume (15) as well as constant pressure; consequently, if coupling is incomplete,

$$\dot{H} - \dot{H}_o = \dot{Q} + PV - \dot{Q}_o \tag{10}$$

where $\dot{Q}$ is the observed rate of heat production of the muscle. The constant quantity $\dot{Q}_o$ evidently corresponds to the steady-state rate of "maintenance heat" production (1, 2) in an isometric contraction, while the "shortening heat" per unit length of contraction α (1, 2) is given by

$$\alpha = (\dot{Q} - \dot{Q}_o)/V. \tag{11}$$

In general α would be expected to depend on P (or V). Making the further conventional assumption that heat production by metabolic processes which are not *directly* involved in the contractile process is negligible or constant, we may insert equations 10 and 11 into 8 to find

$$A = (q_{21}/q_{12})(\alpha + P)^{-1}(-h)P_o, \tag{12}$$

and, if the system is symmetrical ($q_{12} = q_{21}$),

$$\alpha = (-h)(P_o/A) - P. \tag{13}$$

Thus on the basis of constant affinity of reaction, assuming that α relates only to the mechanochemical process, we would expect $(\alpha + P)$ to remain constant.

Now the constant a of equation 1 was originally identified with α. If indeed α were constant, then obviously $(\alpha + P)$ could not be constant. Recently Hill (2) has shown experimentally that α depends on P; for frog sartorius at 0°C he obtained the average linear relationship

$$\alpha = 0.16P_o + 0.18P. \tag{14}$$

This again rules out the possibility that $(\alpha + P)$ may be constant. It is therefore concluded that the conventional assumptions listed above, taken together with the present phenomenological description, are incompatible with experimental observation.

Heat Production and Work

It is interesting to investigate the thermodynamic basis for another well-known

equation due to Hill. From the definition of α, equation 11, we have

$$\dot{Q} = \dot{Q}_o + \alpha V \tag{15}$$

and hence

$$\dot{H} = \dot{Q}_o + PV + \alpha V. \tag{16}$$

Equation 15 obviously does not represent a fundamental subdivision of the heat production—there are no thermodynamic grounds for supposing that during contraction $\dot{Q}_o$ is associated with one process and αV with another. However, this entirely arbitrary division of the heat production into maintenance heat (a kind of reference heat) and shortening heat permits changes in heat production under different conditions to be characterized by the coefficient α. By considering an unloaded contraction it can be seen, on the basis of coupling, that a zero value for α is an unlikely possibility.

Integrating equation 16 over a short isotonic contraction of length x (in the steady state) gives the result

$$(-\Delta H) = (-Q_o) + W + \alpha x \tag{17}$$

where W is the mechanical work done and $(-Q_o)$ is the maintenance heat which would appear in the same time during a steady isometric contraction. A similar equation was originally written to describe work and heat production in a twitch (16):

$$\mathbf{E} = \mathbf{A} + W + ax \tag{18}$$

where **E** represented the total energy liberated, recently specified as the value obtained "at any moment during shortening" (reference 9, p. 67), **A** represented the "activation heat," and the shortening heat constant was identified with the quantity a of equation 1. (Wilkie (17) has raised the question as to whether a should be replaced by α.) The activation heat was supposed to be that which would be observed on stimulation if shortening of the contractile component of the muscle could be obviated (a nonoperational definition liable to lead to inconsistencies), while maintenance heat in tetanus was concluded to be nothing more than the summated effect of the activation heat produced in response to each of the series of shocks composing the tetanus (18). Although equation 17 strictly refers to steady-state isotonic or isometric tetanic contractions, isotonic twitches show surprisingly steady characteristics over much of their shortening period and records indicate (on the basis of an indirect calculation) an approximately constant rate of production of activation heat (18), so equation 17 might remain a reasonable description for the *steady* interval. On the other hand, if we wish to consider complete contraction-relaxation cycles of isotonic twitches, clearly equation 17 cannot be applicable since $(-Q_o)$ and α are not defined for such cycles. Carlson, Hardy, and Wilkie (19)

did test equation 18 for the full cycle of contraction and relaxation in an isotonic twitch, taking the terms $W + ax$ to refer to the contraction alone. They found that the total energy production E consisted of only two terms, a constant plus a term proportional to, or equal to, the work. No term corresponding to the shortening heat appeared. One view which they considered in interpreting these experiments is that the measured parameters of equation 18 all refer to the contraction phase of the twitch (19); in this view E should be completely determined by the heat evolved in a contraction-relaxation cycle since only the work W which is stored as potential energy during contraction, and known from the shortening record, is degraded to heat during relaxation (18). However, equation 17 would still not apply, since the contraction phase includes both the establishment and the decay of the steady state. A comparable argument based on the continuation of heat production after work has ceased has been made by Hill (20) in an effort to account for the result of Carlson et al. Davies, Kushmerick, and Larson (21) recently concluded that the shortening heat could not be degraded free energy from the splitting of ATP, but attributed it to "reversibly transformed entropy" from some other process. The process postulated was hydrogen-bond formation during coil-helix transitions associated with cyclic movements of the myosin cross-bridges. This suggestion was made to account for the disappearance of the shortening heat in a whole contraction-relaxation cycle. It implies that such cyclic transitions take place reversibly in a thermodynamic sense, which is physically impossible.

THE DISSIPATION FUNCTION OF MUSCLE

It is necessary to examine the grounds for writing equation 3, in order to see whether it represents a complete dissipation function, and whether the appropriate forces and flows have been used. We can arrive at this equation by making use of the approach used for "discontinuous systems" in the study of transport problems by nonequilibrium thermodynamics (22). Fig. 1 shows a diagrammatic representation of a striated muscle in which a single fibril bearing the load appears. Ernst (23) has has discussed in detail the proposition that the myofibril is the smallest functional unit of muscle. Adopting this assumption we shall consider the fibril, or the total assembly of filaments within a fibril, to be the essential working element or "black box" of the system, and for *small* steady tetanic contractions shall suppose it to be in a stationary state (the experimental evidence supporting this will be summarized later). As before, only isometric and isotonic tetani are considered here—not twitches. Attached to the fibril is a reservoir of reagents, compartment I, from which it derives its input. The region denoted as compartment II is the external world, which includes the mass m. Then, for an infinitesimal contraction of the fibril at a temperature T during the stationary state the Gibbs equation for compartment I is

$$dU^{\mathrm{I}} = T\,dS^{\mathrm{I}} - p\,dV^{\mathrm{I}} + \sum_i \mu_i^{\mathrm{I}}\,dn_i^{\mathrm{I}} \tag{19}$$

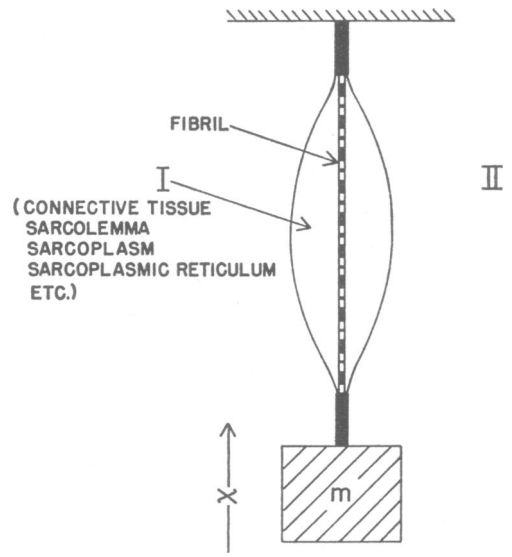


FIGURE 1 The muscle as an energy converter. A single fibril is shown, which represents the "black box" or working element.

where p and V represent pressure and volume, and U and S internal energy and entropy as usual. The corresponding equation for compartment II, representing now the change in total energy of the external world as a consequence of the contraction, is

$$dU^{II} = T\,dS^{II} + p\,dV^{I} + \sum_i \mu_i^{II}\,dn_i^{II} + mg\,dx \tag{20}$$

where x is the distance through which the mass m is raised and g is the acceleration of gravity. The summation term accounts for transfer of matter between compartments II and I, for example oxygen consumption. Since steady contractions only are considered, no inertial terms are involved; the potential energy change brought about in compartment II is included in dU^{II} but that in compartment I is regarded as negligible (i.e. the muscle is regarded as weightless). Summing these two equations and applying the first law, we obtain

$$T\,dS^{total} \equiv T\,d_iS = -mg\,dx - \sum_i \mu_i^{I}\,dn_i^{I} - \sum_i \mu_i^{II}\,dn_i^{II} \tag{21}$$

where d_iS is the "internal entropy production," or total creation of entropy as a consequence of the irreversible processes taking place. By definition the dissipation function is then given by

$$\Phi = T\,d_iS/dt = mg\dot{x} + \sum_j A_j v_j \tag{22}$$

and is positive-definite, although any individual term may be negative ($\dot{x} = -dx/dt$). The summation in equation 22 results from the two summations in equation 21 on introducing affinities and reaction velocities, and refers to a set of independent chemical reactions (diffusional processes which occur within compartment I are readily included in this formalism). We now assume that it is possible to identify among the chemical reactions j a *unique* reaction k which is coupled to the mechanical process of contraction, and that this reaction is not coupled directly to any of the remaining reactions. This is equivalent to separating Φ into two positive-definite parts as follows:

$$\Phi = \Phi_{\text{mech}} + \Phi_{\text{chem}} \tag{23}$$

where

$$\Phi_{\text{mech}} = mg\dot{x} + A_k v_k \tag{24}$$

$$\Phi_{\text{chem}} = \sum_j A_j v_j \qquad (j \neq k). \tag{25}$$

The absence of direct coupling between reaction k and the rest of the reactions j implies that reaction k will not advance if its own affinity is zero and no mechanical force is applied, even though other reactions may have finite affinities and velocities. However, *indirect* effects of some or all of the processes included in Φ_{chem} on the reaction k, and thus on Φ_{mech}, are not excluded. For example, the reactions j may occur in such a way as to maintain A_k at a fixed value. Equations 24 and 3 are obviously identical.

Now it is clear that the fibril cannot be in a truly stationary state while it is shortening. Nevertheless the treatment above will be a good approximation if the fibril is "quasi-stationary," in other words if its state parameters change very slowly in comparison with those of compartment I. There is a considerable body of evidence to support the assumption of quasi-stationarity, indeed this assumption is quite generally made in discussions of tetanic contraction. We shall summarize the evidence briefly under a number of headings.

Mechanical Evidence for Stationarity

In isometric tetanic contractions tension develops to a maximum value, which thereafter remains constant for periods of the order of seconds in the case of frog and toad sartorii at 0°C (24). In isotonic contractions, after an initial short period of adjustment to the load, the velocity of contraction becomes astonishingly constant and may be maintained for 60–70% of the total shortening the muscle undergoes (25). This is all the more remarkable in view of the fact that isometric tension is a function of length, decreasing fairly rapidly at lengths greater or shorter than "rest" length.

Thermal Evidence for Stationarity

Since initial velocity of contraction under isotonic conditions is constant, the well-known fact that shortening heat is directly proportional to distance shortened (1, 26) reflects the constant rate at which heat is produced during shortening. During isometric contractions the heat production also reaches a constant rate (1), the maintenance heat production, which has been associated empirically with the product *ab* (27). Like isometric tension this is a function of length, with a maximum at the rest length (26).

Chemical Evidence for Stationarity

It was mentioned earlier that a linear relationship exists between time and ATP breakdown in isometric contractions of frog sartorius (13). Again there is a length dependence, breakdown being a maximum at rest length. This was established (in the presence of iodoacetate) by measuring the rate of formation of inorganic phosphate, and also by measuring the consumption of ATP directly in sartorii treated with fluorodinitrobenzene to inhibit the creatine phosphoryltransferase. A similar result was obtained for phosphorylcreatine usage in isometric contractions of rectus abdominis (28). Indeed the essential energy-yielding reaction appears to be the splitting of phosphorylcreatine, and the concentration level of ATP remains constant as a consequence of rapid reconstitution (29, 30). Further striking evidence of stationarity is the direct proportionality found between phosphorylcreatine breakdown and mechanical work performed at constant load, using frog rectus abdominis muscles which contracted once or twice to different extents and for different times at 0°C (30).

Structural Evidence for Stationarity

The assumption that the state parameters of a myofibril change relatively slowly during steady contraction does not seem incompatible with what is known of its structure at present. For example, one might expect the configurational entropy of the fibril to undergo a marked change as it shortens. But one of the implications of the sliding filament mechanism is that such changes are minimal. The filaments themselves appear to be rigid (31, 32), and the "entropy of mixing" of actin and myosin when a sarcomere contracts cannot be large. For short contractions, cross-sectional volume elements in the interacting regions of the *A*-band do not have strongly time-dependent characteristics although changes in lattice spacing do occur (31); even the contractile cross-bridges postulated by Davies (33) present a stationary aspect when averaged over small periods of time.

In the above summary of the evidence for stationarity, the first and last items refer to the myofibril. The middle two items, however, refer to the whole muscle.

THE PHENOMENOLOGICAL EQUATIONS FOR MUSCLE: LINEARITY AND INCOMPLETE COUPLING

In the description of muscular contraction considered here it is essential to write linear phenomenological relations with constant coefficients. Since chemical reactions are seldom linear we need to examine the requirements for linearity rather closely. A convenient starting point for this is the enzymatic fuel cell discussed in Part I, which was found experimentally to show linear phenomenological behavior (i.e. the flows were related linearly and the forces were related linearly [34]) despite the absence of linear reaction kinetics. In theory the criterion for linearity of this cell is not that the affinity of the reaction, *as measured in the fuel reservoirs*, should be small in comparison with RT (although this is what one might conclude from equation 21, Part I). It was shown (34) that as long as the *internal* affinity is small, i.e. the reaction in the center compartment is close to equilibrium, linear relations will obtain even if the external affinity is large, providing the system remains linear with respect to *transport* processes. (The latter may be linear over a fairly wide range of forces and flows.) This situation would arise if diffusion through the membranes were rate-limiting.

It has been suggested by Prigogine (35) that when the affinity of a given chemical reaction is large, the reaction may often be split into a certain number of elementary reactions each having a sufficiently small affinity to justify the application of linear phenomenological laws. In the stationary state the velocities of all the elementary reactions become identical, and although the total affinity (the sum of the individual affinities) may be much greater than RT the system remains in the domain of validity of linear relations. If the system is open, but only the initial reactants and final products can be exchanged with the environment, the stationarity condition above includes the influx of reactants and outflux of products; this is in fact the condition of minimum entropy production for a given value of the "external" affinity (reference 35, p. 79). Kinetic schemes for enzymatic reactions invariably consist of a set of consecutive steps. The mechanochemically coupled hydrolysis of ATP in muscle probably involves an elaborate series of intermediate states, commencing with binding of ATP to the H-meromyosin cross-bridges and followed by various interactions and conformational changes before ADP is finally released into the cytoplasm. Such a sequence of reactions has been discussed in detail by Davies (33). The reaction term in Φ_{mech}, equation 24, therefore consists of a series of terms as follows:

$$\Phi_{\text{mech}} = mg\dot{x} + A_\alpha v_\alpha + \sum_{\rho=1}^{r} A_\rho v_\rho + A_\omega v_\omega , \tag{26}$$

where the total affinity, A_k, is given by

$$A_k = A_\alpha + \sum_{\rho=1}^{r} A_\rho + A_\omega . \tag{27}$$

Here the process α may refer to transfer of reactant from cytoplasm or local compartment to the myosin filament, ω may refer to transfer of products from the filament, and a series of r intermediate steps occurs, denoted by ρ. In the stationary state

$$v_\alpha = v_1 = v_2 = \cdots = v_r = v_\omega = v_k \tag{28}$$

and Φ_{mech} collapses to its original form. Our requirement for linearity is realizable if the individual affinities A_ρ are sufficiently small.

These considerations do not imply, however, that $-\dot{x}$ bears a fixed relation to v_k, i.e. that the ratio $-\dot{x}/v_k$ is constant under all conditions. If it were, the muscle would be fully coupled ($q = 1$) (36); in this case (and *only* in this case) the reaction would be brought to a halt in an isometric contraction, and reversed in all stretches during tetanus. On the other hand, if the muscle is incompletely coupled the reaction could in principle still be brought to a halt and ultimately reversed—but only by a tension greater than isometric. (The smaller the value of q, the larger the increase in tension above isometric needed for this purpose.) Tensions insufficiently greater than isometric would only slow down the reaction. It was shown by Abbott, Aubert, and Hill (37) that all of the work done on a muscle, when it is stretched while being stimulated, may be absorbed. Infante, Klaupiks, and Davies (38) showed that stretching an activated fluorodinitrobenzene-poisoned frog sartorius caused a reduction in the breakdown of ATP by as much as 90% "even though" the tension developed was about 70% greater than the isometric tension at rest length (no net resynthesis was observed). The breakdown of ATP during isometric contractions has been commented on earlier. On the basis of these observations it is concluded that muscle is an incompletely coupled energy converter. This is a further essential requirement if it is to be regarded as an autonomic energy converter of the type considered here (3). (It is tempting to suppose that in the "catch" muscle of molluscs q is very nearly unity.)

THE MUSCLE AS AN AUTONOMIC ENERGY CONVERTER

According to the view taken in this paper, the force-velocity relation (equation 1) contains only a single adjustable parameter when normalized—the degree of coupling of the working element of the muscle.[2] The tighter the coupling, the greater the curvature of the Hill hyperbolae; this is readily seen on a normalized plot (3). A characteristic of the Hill equation is that at *high* degrees of coupling the power output remains essentially constant over a considerable range of load resistance (3). This is roughly the case in muscle, as was pointed out by Fenn and Marsh (6). In frog sartorius θ has a mean value of about 0.25 (1), which corresponds to a q

[2] It has been shown that in frog sartorius the Hill equation applies to contractions initially below rest length providing the isometric tension appropriate to a given initial length is used; *a and b are constant for all lengths* (*39*, *40*).

of 0.89 and an η_{max} of 38% if Onsager symmetry is assumed. On the basis of work and heat measurements using a Levin-Wyman ergometer, which maintains constant shortening speed, Hill (27) found the maximum "mechanical efficiency" of frog sartorius to be 39.4%. More recently he obtained a maximum value of about 45% for maintained isotonic contractions (41). This corresponds to a q of 0.92 and a θ of 0.17; usually in these sartorii the range of θ extends from 0.2 to 0.3.

Caution must be exercised, however, in interpreting the measurements quoted above, since they refer to the ratio $PV/(\dot{Q} + PV)$ where the quantities are those appearing in equation 9. On the other hand, the efficiency is, in general terms, the ratio $-J_1X_1/(\Phi_{mech} - J_1X_1)$. As Φ_{mech} and $\dot{Q}$ are not identical, the two ratios do not coincide. The former *may* be a reasonable approximation to the latter, providing (*a*) the heat production associated with Φ_{chem} is negligible or can be corrected for, (*b*) the muscle is virtually a closed system during the experiment, and (*c*) the absolute value of dS^I/dt is negligibly small in comparison with that of dS^{II}/dt, since d_iS/dt is given by the sum of these quantities (see equations 19–22). Proviso *c* is least likely to hold, and the assumption that it does so is weakest in the region of maximum efficiency. For example, in the hypothetical limit of a completely-coupled muscle operating reversibly under a tension only infinitesimally greater than or less than isometric, the efficiency is unity, $\Phi_{mech} = 0$, and $\dot{Q} = T\,dS^{II}/dt = -T\,dS^I/dt$. A detailed discussion of the problem of measuring efficiency has been given by Wilkie (42), who suggested that the ratio (work)/(heat + work) could be corrected by multiplying the denominator by a factor Y, defined in our terms as $Y = A/(-h)$ (see equation 7). Unfortunately, although measurements of work and heat cannot be translated into efficiency without assuming a value for Y, it is not a characteristic constant of the driving reaction if A is not constant. The three conventional assumptions mentioned earlier must be invoked.

The inverse Hill equation, relating the affinity and velocity of the driving reaction, can be written as follows (cf. equation 32, Part I):

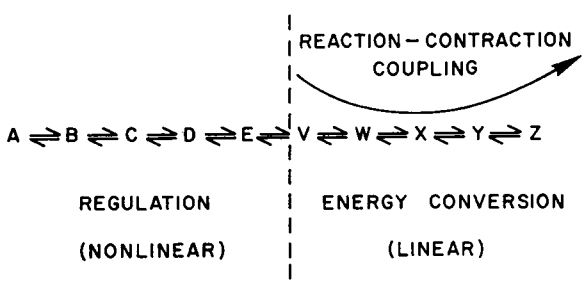
$$\{(A/A_m) - (v - v_o)/v_m\}\{(v/v_o) - (A - A_m)/A_o\} = 1. \qquad (29)$$

As before, the subscripts o and m refer to isometric and unloaded tetanic contractions, respectively. Equation 29 may demand rather large variations in the affinity, especially if the degree of coupling is high. (This is most easily seen in Figs. 3 and 4, Part I.) The implication is that the regulator brings about large changes in the activities of the reactants or products (or both) of the driving reaction. Clearly this could only take place *locally*. The possibility that substantial changes occur in the local concentrations or activities of adenine nucleotides, i.e. that they participate in muscular contraction in compartmentalized form, has been raised by several workers (43–45). Yagi and Mase (45) showed that the Michaelis constant for ATP in the coupled reaction of myosin-ATPase and creatine kinase was one hundredth to one fiftieth of that of myosin-ATPase alone. They suggested therefore that ATP

may be localized around a composite active site (or "compartment") composed of the two enzymes, giving rise to a higher effective concentration for the reaction. The formation of a myosin-phosphate complex as a "key" reaction has been demonstrated by Tonomura and Kanazawa (46). Local variations in pH may also occur.

It is illuminating to consider the hydroelectric system described in Part I as an analog, however distant. The reservoir shown in the figure might, for example, correspond to the creatine phosphate-ATP (equilibrium) pool, in which case the regulator would correspond to the local compartments. The valve behavior may be attributed to regulation by calcium, which in this interpretation controls the rate of a process "in series" with the mechanochemically coupled set of reactions. The constant-velocity pump is less readily identified, although Dydyńska and Wilkie have claimed that fluorodinitrobenzene-poisoned muscles do not merely draw on their store of ATP to meet their energetic requirements, but continue to support complicated chemical changes, including some which can regenerate ATP (47). A more satisfactory analog is obtained by dispensing with the constant-velocity pump and introducing the additional high-level "primary" reservoir. The latter now corresponds to the main creatine phosphate-ATP pool, while the secondary and tertiary reservoirs represent local compartments or intermediate stages in the hydrolysis. The electroosmosis cell obviously corresponds to the stages directly involved in reaction-contraction coupling.

In chemical terms, the type of self-regulatory system under consideration is most clearly illustrated by a schematic sequence of consecutive reactions as follows:



(A more general reaction chain of this kind is discussed by Essig and Caplan[3] in relation to active transport.) The sequence of reactions directly involved in contraction, corresponding to the reaction terms in equation 26, need not necessarily appear at the end of the chain. However, the scheme shown emphasizes the relationship of the "regulator" to the "converter": the former is essentially in series with the latter and controls the input derived from an energy source (cf. reference 3, Fig. 3). Under stationary conditions, stationary-state coupling as described by equation 28 applies to the whole chain of reactions, i.e. both to regulator and to converter. The

[3] Essig, A., and S. R. Caplan. 1968. *Biophys. J.* In press.

total affinity for the change $A \rightarrow Z$ ($A_{tot} = \mu_A - \mu_Z$) is regarded as constant (fixed by the creatine phosphate-ATP pool in muscle), but that portion of the affinity responsible for the change $V \rightarrow Z$ ($A_{conv} = \mu_V - \mu_Z$) is determined by the regulator according to the mechanical load. When the load is changed, a corresponding change occurs in some or all of the chemical potentials in the series μ_B to μ_Y. (The regulator might also act as a "buffer" for the converter against minor variations in A_{tot}.) In this interpretation the nonlinearity of the over-all system resides entirely in the regulator, i.e. in the reaction sequence $A \rightarrow V$ (driven by the affinity $A_{reg} = \mu_A - \mu_V$). In glycerin-extracted fiber models this sequence may have, to a large extent, been removed; such models apparently do not follow the Hill equation (48). It will be seen that

$$A_{tot} = A_{reg} + A_{conv} \tag{30}$$

and likewise for the heats of reaction

$$h_{tot} = h_{reg} + h_{conv}. \tag{31}$$

If no other processes occur we would write

$$\Phi_{chem} = vA_{reg} \tag{32}$$

$$\Phi_{mech} = V(-P) + vA_{conv}. \tag{33}$$

The corresponding phenomenological relations are

$$A_{reg} = \mathbf{f}(v) \quad \text{(a non-linear function)} \tag{34}$$

and

$$-P = R_{11}V + R_{12}v \tag{35}$$

$$A_{conv} = R_{21}V + R_{22}v. \tag{36}$$

Equation 34, the equation of the regulator, may be identified with equation 29 by using equation 30 to relate A_{reg} to A_{conv}. The degree of coupling of the converter, assuming Onsager reciprocity, is (36, 49)

$$q_{conv} = -R_{12}/\sqrt{R_{11}R_{22}} \quad \text{(a positive quantity)}. \tag{37}$$

If we write equation 34 as

$$A_{reg} = R_{reg}v \tag{38}$$

where R_{reg} is implicitly, if not explicitly, a function of v, we may replace equation

36 by

$$A_{tot} = R_{21}V + (R_{22} + R_{reg})v. \tag{39}$$

The degree of coupling of the *over-all system* (regulator plus converter) in some condition of operation is then

$$q_{tot} = -R_{12}/\sqrt{R_{11}(R_{22} + R_{reg})} \tag{40}$$

and clearly $q_{tot} < q_{conv}$. Thus for the over-all system a reduced degree of coupling is the price paid for regulation. One can indeed view the over-all system as an energy converter with a variable degree of coupling, and look upon q_{tot} as the quantity which adjusts itself to the load. This view would not necessarily require q_{conv} to be constant, but its constancy is an essential property of the class of energy converters considered here. It is worth noting that if R_{reg} were constant, so that the over-all system behaved linearly, regulation of a kind would still occur and A_{conv} would increase with the load. However, such a system would be unnecessarily inefficient, since it requires extremely low values of q_{tot} to achieve anything like a uniform output over a wide range of loads.

To present this picture clearly in terms of muscle chemistry is not possible at present, since too little is understood of the system. However, one might consider for purposes of illustration the following hypothetical scheme, which is an adaptation of the molecular mechanism recently proposed by Pringle (50). The successive stages are:

(*a*) Cytoplasmic ATP is bound (or compartmentalized) in the form of Mg ATP at a site on the HMM-actin cross-bridge. This occurs at the end of the "active" or forward stroke, which may involve contraction, rotation, or a combination of the two. Any bound ADP or P_i previously present at the site is displaced, and the bridge detaches from the actin filament (this may be an allosteric effect).

The binding of ATP at an HMM-actin site, and the consequent displacement of ADP and P_i, is *inhibited in the absence of Ca.* The inhibitor is possibly the EGTA-sensitizing factor isolated by Ebashi (51, 52); when Ca binds to this regulatory protein, inhibition ceases.

(*b*) The detached cross-bridge reverts rapidly to a conformation or orientation permitting the formation of a new actin bond (possibly mediated by Ca as suggested by Davies (33)).

(*c*) An alteration occurs in the binding of the ATP, giving rise to an energetically more favorable configuration. This is accompanied by a conformational change in the cross-bridge (the active stroke) and generates tension if binding to actin has occurred. As a consequence the ATP is properly placed with respect to the ATPase site.

(*d*) The ATP is split by the ATPase. This releases the configurational constraint

on the cross-bridge, but initially both ADP and P_i remain bound (or compartmentalized) with the actin bond intact.

(*e*) The tension in the cross-bridge relaxes to a low value for the remainder of the forward stroke (it is assumed that the cross-bridges do not move synchronously). Some release of ADP and P_i, and detachment from actin, may occur. However, for the most part the presence of bound ADP stabilizes the actin bond until binding of ATP (or perhaps phosphorylation of the ADP) takes place once again.

In this scheme steps *a* and *b* correspond to the regulator (which is thus a Ca-sensitive control mechanism), while steps *c*, *d*, and *e* correspond to the energy converter. The converter reaction is essentially

$$(ATP)_{bound} + H_2O \rightleftharpoons (ADP)_{bound} + (P_i)_{bound}$$

and its degree of coupling depends chiefly on the extent to which actin bond formation takes place. The affinity of this reaction is partly determined, in this model, by the local Ca level. The cyclic nature of the process would be reflected in the values of the phenomenological coefficients, but does not appear explicitly in the dissipation function (53).

It was pointed out in Part I that an energy converter of $q = 0.9$ would be well-regulated if, for example, $\xi_2^l = 0.6$, the maximum change in the affinity being about 2-fold. A 10-fold change in the activity ratio for a reaction at 0°C is equivalent to a change of 1.26 kcal/mole in its affinity. Hence, if we allow a 100-fold change in the local activity ratio (say a 100-fold change in P_i activity) we can have a 2-fold change in affinity covering the range 2.5 to 5 kcal/mole. If we allow a 1000-fold change in the local activity ratio (say 100-fold in P_i activity and 10-fold in ATP/ADP ratio), the range which can be covered becomes about 4–8 kcal/mole. This is perfectly consistent with an over-all affinity of approximately 11 kcal/mole, the in vivo value suggested by Wilkie and Woledge (5).

CRITICISMS OF WILKIE AND WOLEDGE

The objections raised by Wilkie and Woledge (5), referred to in the introduction, rest primarily on the following assumptions:(*a*) the velocity of the driving reaction during steady-state shortening can be inferred from the observed rates of heat and work production and the experimental estimates of the enthalpy of creatine phosphate breakdown, and (*b*) the affinity of the driving reaction depends on the concentrations of the reactants and products as determined by chemical analysis of the muscle.

Assumption *a* involves disregarding the processes in Φ_{chem}, among which the operation of the regulator itself is included. Some considerations on the basis of this assumption were discussed earlier, leading to equations 12 and 13. Wilkie and Woledge themselves point out that at present it is virtually impossible to say how great the expenditure of ATP by the calcium pump must be. They comment further

that "... certainly evolutionary pressure would keep it as low as possible. Moreover, there seems no reason at all why the rate of calcium pumping should vary with the load." If the calcium pump is an important component of the regulator, however, there is every reason why its rate should vary with the load, and the consequences of evolutionary pressure are not obvious. Davies and coworkers[4] believe, on the basis of a variety of experimental approaches, that ATP usage by the calcium pump is about 25% of the total rate, and increases in very lightly loaded isotonic contractions.

Assumption *b* overlooks the necessity for the input to the working element to be the output of the regulator, which is in effect a "preconverter". Since gross changes in the sarcoplasmic concentrations of the reactants involved in the driving reaction are improbable, regulation must be achieved on the basis of changes in the *local activities* of these reactants.

An analysis of the quantitative evaluation of this treatment presented by Wilkie and Woledge is given in the Appendix. It is shown that five of the six examples which they chose for examination (taking q to be 0.9 and assigning different representative values to the factor Y) are, on a priori grounds, inconsistent with the observed ability of muscle to regulate its output over a wide range of loads. The sixth is free of this inconsistency, and when interpreted according to the views discussed above it lends no support to their conclusion that muscle is not an energy converter of the type under consideration.

DISCUSSION

It is interesting to compare the predictions of the "thermodynamic" Hill equation with some of the rather scanty data available on reaction kinetics in muscle. What are needed are rate measurements carried out during *stationary* contraction in tetanus under different loads. Cain, Infante, and Davies (30, 54) have studied the usage of high-energy phosphate during isotonic contractions, and it is claimed (54) that their results "show clearly that during single contractions the breakdown is associated with the work done by the muscle and not with the shortening *per se*" (Wilkie (17) has criticized this claim). They found the total phosphorylcreatine breakdown, in frog rectus abdominis muscles at 0°C which did approximately constant work but shortened by widely varying amounts, to be almost independent of the extent of shortening; the breakdown in muscles which did varying amounts of work while shortening by a constant amount was directly proportional to the external work done. These results are identical in our terms, and can be written

$$J_2/J_1 \propto (-X_1) \tag{41}$$

or, more specifically,

$$v/V \propto P. \tag{42}$$

[4] Davies, R. E. Private communication.

It is evident that this proportionality relation cannot hold at the limits; however it may hold approximately at moderate tensions. In an isometric contraction (one limiting case) the left-hand side is infinity, since v is not zero (Davies and coworkers (54, 55) have shown that there is "a continuous to and fro dithering of the myofilaments within the sarcomeres"). On the other hand, in an unloaded contraction (the other limiting case) the left-hand side is not zero, since v is not zero. Indeed Davies (33) has pointed out, on the basis of a molecular theory of muscle contraction, that the ATP breakdown would be expected to take place at a very rapid rate; moreover, according to Wilkie (17) unloaded shortening is accompanied by a large consumption of energy. In Fig. 2 the normalized quantities are used and the ratio γ_2/γ_1 is plotted against ξ_1 for $q = 0.9$; it is seen that when ξ_2^l is in the neighborhood of unity there is rough proportionality in the range $\xi_1 = 0.3$ to 0.6.

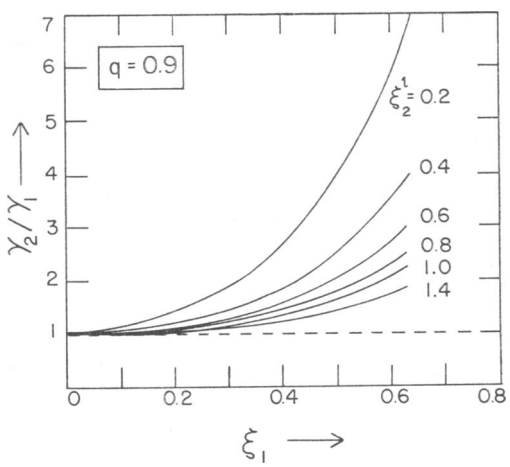


FIGURE 2 Normalized plot of the ratio of reaction velocity to contraction velocity (γ_2/γ_1) vs. tension (ξ_1) for a series of values of the normalized reaction affinity in an unloaded contraction (ξ_2^l). The degree of coupling is 0.9.

An unexpected linear relation (which may be derived from equations 29 and 47, Part I) is that

$$(\gamma_2 - \gamma_2^s)/\gamma_1 = 1 - (1 - q^2 + q^2\xi_1)/\xi_2^l. \tag{43}$$

This relation is plotted in Fig. 3 for $q = 0.9$, and indicates that the ratio of the increase in reaction velocity in an isotonic (as compared with a steady isometric) contraction, to the contraction velocity, *decreases* linearly with the tension. Now if the fibrils behaved as thermodynamically ideal systems, the heat of reaction would be independent of changes in the concentrations, and hence the affinity. Then, using the notation of equations 7 and 8, we could write

$$(v - v_o)/V = (\dot{H} - \dot{H}_o)/(-h)V \tag{44}$$

and introducing equations 10 and 11

$$(v - v_o)/V = (\alpha + P)/(-h), \tag{45}$$

i.e.

$$\alpha = (-h)(v - v_o)/V - P \tag{46}$$

which is equivalent to equation 13 if A is constant. Equation 45 is readily transformed into

$$(\gamma_2 - \gamma_2^s)/\gamma_1 = V_m(\alpha + P_o\xi_1)/(-h)v_m . \tag{47}$$

Equation 47 can only be consistent with equation 43 if α *decreases* linearly with P, a condition which also followed on the basis of constant affinity, equation 13. As was pointed out, this does not correspond to reality. The assumptions of constant heat of reaction, and of a heat production reflecting only the mechanochemical process, can hardly be expected to hold for an open, nonideal system with a regulator controlling the affinity.

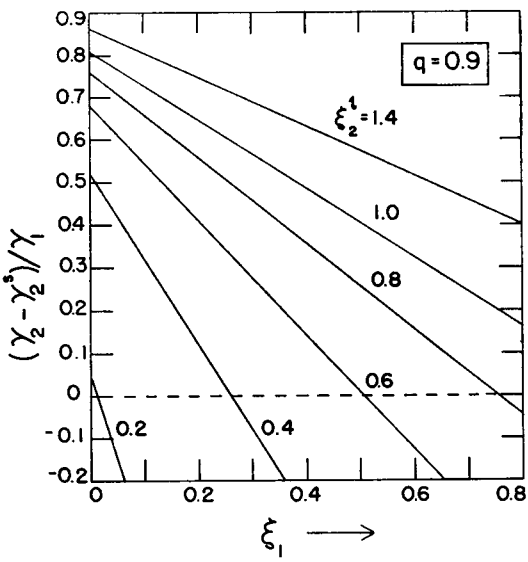


FIGURE 3 Normalized plot of the ratio of the difference between reaction velocities in isotonic and isometric contractions to the contraction velocity ($(\gamma_2 - \gamma_2^s)/\gamma_1$) vs. tension ($\xi_1$) for a series of values of the normalized reaction affinity in an unloaded contraction (ξ_2^1). The degree of coupling is 0.9.

The regulator which we suppose to be present in muscle corresponds exactly to the idea Fenn had in mind when he wrote, in 1923, ". . . there is another regulatory mechanism within each individual fibre which, within certain limits, is able to adapt the energy output to the work done. . . . The other mechanism is independent of the nervous system and works merely by virtue of the fundamental nature of the muscle machine, whatever that may be . . . The energy liberated . . . can be modified by the nature of the load which the muscle discovers it must lift . . ." (10). The processes involved in regulation are accounted for in Φ_{chem}, equation 25. Small changes in affinity correspond to quite large changes in concentration or activity, and these can only be envisaged in terms of *local* concentrations or ac-

tivities at the reaction sites. In view of the important role played by calcium in excitation, it is conceivable that the local affinity could be regulated in some way by the calcium level. This view is not inconsistent with the theory of Pringle (50). (It has recently been suggested by Cohen and Longley (56) that reversible binding of bivalent cations by tropomyosin may have a regulatory function in muscle contraction.) A detailed discussion of feedback regulation at the molecular level, and the interaction between metabolite-modulated enzymes, has been given by Atkinson (57).

If the above considerations have a bearing on muscle, it must behave as a regulated linear energy converter with constant coefficients. Alternatively, one may consider muscle to be an energy converter with variable coefficients. Quite possibly the difference between these two descriptions would be found to lie only in the choice of the "black box" or working element; it would certainly be surprising if a system conforming to the second description, and characterized by the Hill equation, could not be reinterpreted in terms of the first. The idea of self-adaptive regulation requires the velocity of shortening to depend on the tension and nothing else, an assumption which is well established by mechanical observations, as for example in the elegant method described by Macpherson (58) for determining the force-velocity relation from the initial stages of two isometric contractions. Another method for determining the force-velocity relation, which has not been described, is to allow the muscle to contract against a series of purely dissipative mechanical resistances, i.e. dashpots. The regulator would then be called upon to respond directly to the load $R_L = P/V$, instead of to P alone as in an isotonic contraction or to V alone as in a Levin-Wyman ergometer. Among the relations which describe the *chemical* behavior of the system, the linear dependence of tension on both velocity of contraction and velocity of reaction (equation 35) should be readily testable. If linearity can be established, it is possible in principle to determine all the parameters in equations 35 to 40, as q_{conv} is known from the force-velocity curve.

Since the theoretical problem of autonomic energy conversion hinges on the operational limits, it is in effect a "boundary-value" problem, and no conclusion can be drawn as to the behavior, even under stationary conditions, when the limits are exceeded. The hydroelectric model described in Part I, however, would function as a constant driving force machine for currents greater than short-circuit current, and as a constant driving flow machine if operated against voltages greater than open-circuit voltage. This behavior has no generality, although on general grounds discontinuities would be expected at the limits. Katz (59) demonstrated a strong mechanical discontinuity at P_o in frog sartorius under conditions of slow, uniform lengthening with tensions in excess of P_o. The velocity of extension increased linearly with P, but extraordinarily slowly, as if the regulator, by suitably increasing the affinity, were attempting to prevent the very occurrence of stretching. It would be interesting to know whether a parallel phenomenon exists at the other limit. This would require some means of "pushing" the muscle during contraction.

CONCLUSIONS

The muscle fibril is shown to be a "working element" characterized by a dissipation function of two terms: mechanical power output and chemical power input. There is a considerable body of evidence that during at least the initial phase of isometric or isotonic tetanic contraction the fibril attains an essentially stationary state. (Such stationarity probably occurs frequently in vivo, as in Wilkie's demonstration of the Hill force-velocity relation in human muscle [60].)

Since the mechanochemically coupled hydrolysis of ATP in muscle probably involves a series of intermediate steps, each of low affinity, the application of linear phenomenological relations to the initial phase of tetanic contraction may not be unjustified. If so, the fibril may be treated as a linear energy converter. Its degree of coupling (36) must be less than unity, as shown, for example, by the continued breakdown of ATP during isometric tetanic contraction.

The muscle may in principle belong to the class of simple autonomic (i.e. self-regulated) energy converters (3), since it apparently satisfies the requirements of stationarity, linearity, and incomplete coupling. If so the Hill force-velocity relation contains only a *single* adjustable parameter—the degree of coupling.

The chemical input of the muscle may be expected to follow the inverse Hill equation (see Part I). This demands a considerable variation in the affinity of the reaction if the degree of coupling is high. The corresponding activity changes could only be brought about *locally* by the regulator. It is possible to devise a scheme showing that this is feasible, for example an adaptation of the molecular mechanism recently proposed by Pringle (50). The nonequilibrium thermodynamic parameters of the system are all experimentally accessible.

The objections to this view recently raised by Wilkie and Woledge (5) rest on at least two questionable assumptions. One of these neglects heat production by all processes other than the immediate driving reaction. (This disregards the operation of the regulator itself, which possibly includes the calcium pump.) The other assumption considers the affinity of the immediate driving reaction to depend on *over-all* concentrations of reactants and products, which are unlikely to be subject to large variations. However, there is evidence that substantial changes may occur in local activities.

The division of heat production into "shortening heat" and either "maintenance heat" or "activation heat" is entirely arbitrary. The conventional assumptions made in considering heat production (affinity of reaction constant, muscle a closed system, heat production by processes other than the actual contractile process negligible), when taken together with the phenomenological description, are incompatible with experimental observation. However, if the system is assumed closed the following result may be obtained for a short isotonic contraction of length x in the steady state:

$$(-\Delta H) = (-Q_o) + W + \alpha x.$$

H represents enthalpy, W the mechanical work done, $(-Q_o)$ the heat which would appear in the same time during a steady isometric contraction, and α the shortening heat per unit length of contraction (at a given tension). This is similar to the equation given by Hill to describe work and heat production in a twitch (16).

APPENDIX

The thermodynamic treatment used by Wilkie and Woledge rests not only on the two assumptions discussed in the above consideration of their criticisms, but also on two further assumptions: (*c*) the muscle can be regarded as a closed system, and (*d*) the enthalpy change for the contraction-coupled ATP splitting in vivo is -11 kcal/mole, a constant value. Assumption *c* is implicit, evidently based on the supposition that absorption of O_2 and loss of CO_2 by the muscle may be neglected during the stationary phase of contraction. Assumption *d* is based on a measured value of -11 kcal/mole for creatine phosphate splitting, and refers to the *over-all* reaction. However, a heat of reaction independent of possible changes in the activities of reactants and products would be expected only in ideal solutions.

Wilkie and Woledge have attempted to calculate both the velocity of reaction and the affinity as functions of tension for a muscle of $q = 0.9$. In so doing they realized that "predictions from Caplan's theory" describing *input* quantities involve a second adjustable parameter, which we have characterized as $\xi_2{}^l$. On the basis of their assumptions they were able, with considerable ingenuity, to introduce the factor Y for isometric or unloaded contractions instead. The curves they present, which are thus predictions from their own modification of the theory, are computed for six cases, the following three values being assigned to either Y_o or Y_m: 0.5, 1.0, 2.0. It is obviously important to examine the relationship between Y_o or Y_m and $\xi_2{}^l$.

From equation 25, Part I, the following two relations can be derived:

$$P_oV_m/A_ov_o = \xi_2{}^l q^2/(1 - q^2) \tag{A1}$$

$$P_oV_m/A_mv_m = q^2/\xi_2{}^l. \tag{A2}$$

Wilkie and Woledge quote experimental evidence for frog muscle showing that

$$P_oV_m \approx 16\dot{Q}_o \approx 4.12\dot{Q}_m\,. \tag{A3}$$

They also introduce relations which depend explicitly on their assumptions *a* and *c* (corresponding to the conventional assumptions *c* and *b* mentioned in the earlier treatment of heat production above), and which may be written as follows:

$$\dot{Q}_o/A_ov_o = \dot{H}_o/A_ov_o = -h_o/A_o = 1/Y_o \tag{A4}$$

$$\dot{Q}_m/A_mv_m = \dot{H}_m/A_mv_m = -h_m/A_m = 1/Y_m\,. \tag{A5}$$

Combining equations A1, A3, and A4 we obtain

$$\xi_2{}^l q^2/(1 - q^2) = 16/Y_o\,, \tag{A6}$$

while equations A2, A3, and A5 give

$$q^2/\xi_2{}^l = 4.12/Y_m\,. \tag{A7}$$

If $q = 0.9$, equations A6 and A7 lead to the conclusion that

$$\xi_2{}^l \approx 4/Y_o \approx Y_m/5. \tag{A8}$$

The values of $\xi_2{}^l$ which correspond to the six choices for Y made by Wilkie and Woledge are tabulated below:

Y	0.5	1.0	2.0
$\xi_2{}^l (Y = Y_o)$	8	4	2
$\xi_2{}^l (Y = Y_m)$	0.1	0.2	0.4

It is seen that the isometric choices all correspond to $\xi_2{}^l > 1$, while two of the unloaded choices correspond to $\xi_2{}^l \ll 1$. The only choice which is consistent with the criterion for optimal regulation discussed in Part I (high output power over a wide range of load resistance, at moderate to good efficiency) is $Y_m = 2.0$, corresponding to $\xi_2{}^l = 0.4$ (the maximum variation in reaction velocity would be about 2-fold). This is also the only choice which even approximately fits both the isometric and the unloaded limiting values of the reaction velocity as calculated from measurements of heat and work production ($\xi_2{}^l = 0.6$ fits somewhat better). However, the shape of the "experimental" curve for reaction velocity vs. tension is very different from that of the "theoretical" curve, which is not surprising in view of the assumptions underlying the computation. The choice of $Y_m = 2.0$, or $\xi_2{}^l = 0.4$, is found by Wilkie and Woledge to represent a variation in affinity ranging from about 20 to 55 kcal/mole (which would require changing the activity ratio by a factor of about 10^{30} at 0°C). This range of affinities is dictated primarily by their assumption *d*, i.e. Y_m is taken to represent a ratio of quantities characterizing the *over-all* process rather than the converter process alone. A tenth of the above values would represent a more reasonable affinity range.

SYMBOLS

A Affinity of a chemical reaction, defined by equation 9, Part I.
a Mechanical constant of muscle, according to equation 1.
b Mechanical constant of muscle, according to equation 1.
E Potential difference between the terminals of an electrochemical cell.
$\mathcal{E}$ Electromotive force of an electrochemical cell, defined by equation 13, Part I.
$\mathfrak{F}$ Faraday constant.
G Gibbs free energy.
g Acceleration of gravity.
H Enthalpy.
h Heat of reaction.
$\mathbf{h}$ Programming function for regulator.
I Electric current.
J Generalized flow ($J^l_{1,2} = (J_{1,2})_{X_1=0}$; $J^s_2 = (J_2)_{J_1=0}$).
k Subscript denoting mechanochemically linked reaction, according to equation 24.
L Phenomenological conductance coefficient; subscript denoting load.
l Superscript denoting level flow.
m Subscript denoting unloaded contraction; mass.
n_i Number of moles of ith kind.
o Subscript denoting isometric contraction.

P Tension ($P_o = (P)_{V=0}$).
p Pressure.
Q Heat ($-Q_o = (-Q)_{V=0}$, the maintenance heat).
q Degree of coupling, defined by equations 2 and 3, Part I.
R Phenomenological resistance coefficient.
R_L Load resistance ($R_L = -X_1/J_1$).
R Gas constant.
S Entropy.
s Superscript denoting static head.
T Temperature.
t Time.
U Internal energy.
V Velocity of shortening ($V_m = (V)_{P=0}$).
V Volume.
v Velocity of reaction, defined by equation 11, Part I.
X Generalized force ($X^s_{1,2} = (X_{1,2})_{J_1=0}$; $X_2{}^l = (X_2)_{X_1=0}$).
x Distance contracted by muscle.
Y Wilkie's factor ($Y = -A/h$).
α Shortening heat, defined by equation 11.
γ Normalized generalized flow ($\gamma_1 = J_1/J_1{}^l = V/V_m$; $\gamma_2 = J_2/J_2{}^l = v/v_m$, $\gamma_2{}^s = J^s_2/J_2{}^l = v_0/v_m$).
Δ Difference, in particular according to the definitions 31 and 38, Part I.
η Efficiency ($\eta = -J_1X_1/J_2X_2$).
θ Index of performance ($\theta = a/P_o = b/V_m$).
μ_i Chemical potential of ith species.
ν_i Stoichiometric coefficient of ith species.
ξ Normalized generalized force ($\xi_1 = X_1/X^s_1 = P/P_o$; $\xi_2 = X_2/X^s_2 = A/A_o$, $\xi_2{}^l = X_2{}^l/X^s_2 = A_m/A_o$).
Φ Dissipation function.
$\cdot$ $\dot{N} \equiv -dN/dt$.
1, 2 Subscripts denoting output and input processes, respectively.

The author is indebted to Professor D. R. Wilkie and to Dr. R. C. Woledge for allowing him to see their manuscript prior to publication, and for valuable discussions. Thanks are also due to Drs. F. Rothstein and A. Essig, for giving their time and energy unstintingly in order to help the author over a formidable communication barrier.
This investigation was supported in part by Atomic Energy Commission Contract No. AT (30-1)-2453, and by Public Health Service Research Career Development Award No. 1 K03 GM35292-01, 02 from the National Institute of General Medical Sciences.

Received for publication 16 November 1967 and in revised form 13 June 1968.

REFERENCES

1. HILL, A. V. 1938. *Proc. Roy. Soc. Ser. B.* **126**:136.
2. HILL, A. V. 1964. *Proc. Roy. Soc. Ser. B.* **159**:297.
3. CAPLAN, S. R. 1966. *J. Theoret. Biol.* **11**:63.
4. PRINGLE, J. W. S. 1961. *Symp. Soc. Exptl. Biol.* **14**:41.
5. WILKIE, D. R., and R. C. WOLEDGE. 1967. *Proc. Roy. Soc. Ser. B.* **169**:17.
6. FENN, W. O., and B. S. MARSH. 1935. *J. Physiol. (London)* **85**:277.
7. POLISSAR, M. J. 1952. *Am. J. Physiol.* **168**:766.

8. AUBERT, X. 1956. Le Couplage Énergétique de la Contraction Musculaire. Éditions Arscia, Brussels, Belgium. 233.
9. HILL, A. V. 1965. Trails and Trials in Physiology. Edward Arnold Ltd., London, England. 137.
10. FENN, W. O. 1923. *J. Physiol. (London)* **58**:175.
11. HILL, A. V. 1964. *Proc. Roy. Soc. Ser. B.* **159**:589.
12. KEDEM, O. 1961. *In* Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Academic Press, Inc., New York. 87.
13. INFANTE, A. A., D. KLAUPIKS, and R. E. DAVIES. 1964. *Biochim. Biophys. Acta.* **88**:215; see also Maréchal, G. 1964. Le Métabolisme de la Phosphorylcréatine et de L' Adénosine Triphosphate durant la Contraction Musculaire. Éditions Arscia, Brussels, Belgium.
14. PRIGOGINE, I., and R. DEFAY. 1954. Chemical Thermodynamics. English edition, translated by D. H. Everett. Longmans, Green and Co., Ltd., London, England.
15. BASKIN, R. J., and P. J. PAOLINI. 1966. *J. Gen. Physiol.* **49**:387.
16. HILL, A. V. 1949. *Proc. Roy. Soc. Ser. B.* **136**:220.
17. WILKIE, D. R. 1966. *Ann. Rev. Physiol.* **28**:17.
18. HILL, A. V. 1949. *Proc. Roy. Soc. Ser. B.* **136**:195.
19. CARLSON, F. D., D. J. HARDY, and D. R. WILKIE. 1963. *J. Gen. Physiol.*, **46**:851.
20. HILL, A. V. 1963. *J. Physiol. (London)* **170**:18 P; 1964. *Proc. Roy. Soc. Ser. B.* **159**:596.
21. DAVIES, R. E., M. J. KUSHMERICK, and R. E. LARSON. 1967. *Nature.* **214**:148.
22. DE GROOT, S. R., and P. MAZUR. 1962. Non-Equilibrium Thermodynamics. North-Holland Publishing Co., Amsterdam, The Netherlands. Chap. 15.
23. ERNST, E. 1963. Biophysics of the Striated Muscle. Akadémiai Kiadó, Budapest, Hungary. 51.
24. GASSER, H. S., and A. V. HILL. 1924. *Proc. Roy. Soc. Ser. B.* **96**:398; HILL, A. V. 1949. *Proc. Roy. Soc. Ser. B.* **136**:399.
25. SANDOW, A. 1961. *In* Biophysics of Physiological and Pharmacological Actions. A. M. Shanes, editor. American Assoc. for the Advancement of Science, Washington, D. C. 413.
26. ABBOTT, B. C. 1951. *J. Physiol. (London)* **112**:438.
27. HILL, A. V. 1939. *Proc. Roy. Soc. Ser. B.* **127**:434; HILL, A. V., and R. C. WOLEDGE. 1962. *J. Physiol. (London)* **162**:311.
28. INFANTE, A. A., D. KLAUPIKS, and R. E. DAVIES. 1964. *Nature.* **201**:620.
29. CARLSON, F. D., and A. SIGER. 1960. *J. Gen. Physiol.* **44**:33.
30. CAIN, D. F., A. A. INFANTE, and R. E. DAVIES. 1962. *Nature.* **196**:214.
31. ELLIOTT, G. F., J. LOWY, and B. M. MILLMAN. 1965. *Nature.* **206**:1357.
32. HUXLEY, H. E., W. BROWN, and K. C. HOLMES. 1965. *Nature.* **206**:1358.
33. DAVIES, R. E. 1963. *Nature.* **199**:1068.
34. BLUMENTHAL, R., S. R. CAPLAN, and O. KEDEM. 1967. *Biophys. J.* **7**:735.
35. PRIGOGINE, I. 1961. Thermodynamics of Irreversible Processes. John Wiley and Sons, Inc., New York. 59.
36. KEDEM, O., and S. R. CAPLAN. 1965. *Trans. Faraday Soc.* **61**:1897.
37. ABBOTT, B. C., X. M. AUBERT, and A. V. HILL. 1950. *J. Physiol. (London)* **111**:41P.
38. INFANTE, A. A., D. KLAUPIKS, and R. E. DAVIES. 1964. *Science.* **144**:1577; DAVIES, R. E. 1964. *Proc. Roy. Soc. Ser. B.* **160**:480.
39. ABBOTT, B. C., and D. R. WILKIE. 1953. *J. Physiol. (London)* **120**:214.
40. MATSUMOTO, Y. 1967. *J. Gen. Physiol.* **50**:1125.
41. HILL, A. V. 1964. *Proc. Roy. Soc. Ser. B.* **159**:319.
42. WILKIE, D. R. 1960. *Progr. Biophys. Biophys. Chem.* **10**:259; 1954. *Progr. Biophys. Biophys. Chem.* **4**:288.
43. MOMMAERTS, W. F. H. M., A. J. BRADY, and B. C. ABBOTT. 1961. *Ann. Rev. Physiol.* **23**:529.
44. SERAYDARIAN, K., W. F. H. M. MOMMAERTS, and A. WALLNER. 1962. *Biochim. Biophys. Acta.* **65**: 443.
45. YAGI, K., and R. MASE. 1962. *J. Biol. Chem.* **237**:397; 1965. *In* Molecular Biology of Muscular Contraction. S. Ebashi et al., editors. American Elsevier Publishing Co., New York. 109.
46. TONOMURA, Y., and T. KANAZAWA. 1965. *J. Biol. Chem.* **240**:PC 4110.
47. DYDYŃSKA, M., and D. R. WILKIE. 1966. *J. Physiol. (London)* **184**:751.

48. ULBRECHT, G., and M. ULBRECHT. 1953. *Biochim. Biophys. Acta.* **11**:138.
49. CAPLAN, S. R. 1966. *J. Theoret. Biol.* **10**:209.
50. PRINGLE, J. W. S. 1967. *Progr. Biophys. Mol. Biol.* **17**:1.
51. EBASHI, S. 1963. *Nature.* **200**:1010; see also Ebashi, S. 1965. *In* Molecular Biology of Muscular Contraction. S. Ebashi et al., editors. American Elsevier Publishing Co., New York. 197.
52. PERRY, S. V. 1967. *Progr. Biophys. Mol. Biol.* **17**:325.
53. KATCHALSKY, A., and R. SPANGLER. 1968. *Quarterly Rev. Biophys.* **1**:127.
54. DAVIES, R. E. 1965. *In* Muscle. W. M. Paul et al., editors. Pergamon Press, Oxford, England. 49.
55. LARSON, R. E., M. J. KUSHMERICK, D. H. HAYNES, and R. E. DAVIES. 1968. *Biophys. J. Soc. Abstr.* **8**:A-8.
56. COHEN, C., and W. LONGLEY. 1966. *Science.* **152**:794.
57. ATKINSON, D. E. 1965. *Science.* **150**:851.
58. MACPHERSON, L. 1953. *J. Physiol. (London)* **122**:172.
59. KATZ, B. 1939. *J. Physiol. (London)* **96**:45.
60. WILKIE, D. R. 1950. *J. Physiol. (London)* **110**:1 249.