MUSCLE ENERGETICS AND THE FENN EFFECT

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ABSTRACT This study suggests and statistically tests a consistent analytical method for isolating the critical variables governing the Fenn effect in frog sartorius muscle. It demonstrates that when the Hill factor ($P_0 - P$) is used to normalize the heat of shortening, or the work, or their sum, the enthalpy of shortening, these reduced energy terms are highly linear functions of the time of shortening. Linear correlation of a given form of reduced energy for a given muscle, against the time of shortening yields correlation coefficients of 0.998 or greater. From the regression equations, equations similar in form to the 1938 Hill force-velocity, and 1964 Hill heat of shortening equations, are deduced. The analysis suggests that the efficiency with which the extra energy of shortening is converted into work is nearly constant over most of the range of fractional loads, and it confirms Fenn's observation that the total extra energy of shortening is about 1.3 times the work. Thus it is also consistent with the results of most of the biochemical studies which correlate the extra breakdown of high energy phosphates entirely with the work done, and no component exclusively with the distance shortened. While the analysis successfully identifies the two factors regulating the release of the energy of shortening, time and the fractional load, there was insufficient data in the literature to develop the form of the relation between the size and the geometry of the muscle and the rate of energy release. Therefore until more data are available it seems unjustified to assume that either b/l_0 and a/P_0 (or the equivalent linear regression constants) are independent of these factors.

INTRODUCTION

In 1923 Fenn (1923, 1924) demonstrated that a fully active muscle, when allowed to shorten, released energy in excess of that released by an active muscle held isometric. The total extra energy released was closely similar for complete cycles (shortening and lengthening) and for the shortening half of the cycle. Thus it is apparent that both the heat and the work of shortening are irreversible expenditures of energy by a contracting muscle.

A. V. Hill (1938) also measured the work energy and the extra heat energy released by muscles allowed to shorten. In his experiments he allowed the muscles to shorten after they had developed full tetanic isometric tension and he caused them to redevelop full isometric tension at a stop applied after a given interval of shortening under a constant load. He measured the total heat associated with shortening, the work between these two isometric positions, and carried out his calculations in such a way that the energy transferred into and out of the series elastic elements was accounted for. The equation $(P + a)v = b\ (P_0 - P)$ published in 1938 summarized his results. The extra energy released from the chemical stores of the muscle during shortening was manifested in two forms, the work done (W) and the heat of shortening (Q_s). The latter appeared to be independent of the load and was a linear function of the distance shortened (S). The operational definition of Q_s has been given by Hill (1964 *a*, p. 307). His description of the determination of Q_s is concise: "... employ a quick release from previously isometric contraction, the muscles being released ... when their tension has been fully developed. The continuation of that isometric contraction, obtained by another stimulus, provided the 'baseline' for the contraction with shortening." Note that no assumptions regarding the relation of Q_s to the isometric, or maintenance, heat is either made or needed. The heat of shortening is defined therefore as extra heat beyond that measured during an isometric contraction. The sum of these two components of energy, expressed in the equation as functions of velocity or as energy rates (Pv and av), were shown to be proportional to a factor $(P_0 - P)$, appropriately called the "Hill factor." This factor can also be written as the product of the isometric force P_0, and the complement of the fractional load $(P_0 - P) = P_0\,(1 - P/P_0)$.

The 1938 Hill equation not only summarized the thermodynamic data but also fit the force-velocity data. The force-velocity data, or the work part of the energetic data, can be used to find the constants a and b independent of the measurements of the heat of shortening.

Carlson et al. (1963) directly measured the extra energy liberated from the chemical stores of an active muscle by analysis of the phosphocreatine (PC) lost from the iodoacetate-poisoned muscles allowed to shorten over that lost from similar muscles held isometric. From the 1938 Hill equation they anticipated finding some PC split which varied with the work done, and a component of PC split which varied with the distance shortened. It appeared that there was only one variable component of PC split and this was proportional to the work done. No statistical case could be made for the existence of a separate variance in the PC split which could be identified with the heat of shortening according to Hill's relation $Q_s = aS$. We will show presently that this result is expected from experiments where muscles are allowed to shorten and evolve both work and heat associated with shortening, and that their evidence should not be construed as support for the hypothesis that the heat of shortening is reversible, i.e., disappears in a complete cycle of shortening and lengthening (Davies et al., 1967). Hill returned to the problem of the heat of shortening in 1964. With more accurate measurements due to improved technical factors (and probably also due to larger muscles) the extra heat liberated by a shortening muscle appeared to increase with the load as well as the distance shortened. Hill could not simply fit the new data for the heat of shortening into the 1938 equation, and summarized it in the relation alpha/P_0 = 0.16 (±0.015) + 0.18 (±0.027) P/P_0, where the numbers in the parentheses are the standard errors and alpha is the heat per centimeter of shortening. Paraphrased, this states that alpha normalized against the isometric force is equal to a constant plus a factor proportional to the fractional load. The new values for alpha and a are not the same, although they are in the same range when the constant a is fitted to the force-velocity data in the 1938 Hill equation (Hill, 1964 *a*).

To compound the uncertainties surrounding the significance of the measurements of the heat of shortening, Woledge (1968) reported that the extra heat liberated by a tortoise muscle allowed to shorten over that liberated by one kept isometric was simply proportional to the distance shortened, and thus fitted the 1938 results of Hill. Since the tortoise muscles studied by Woledge were similar in weight to the frog muscles studied by Hill in 1938, but have a basic adenosine triphosphatase (ATPase) rate 1/20 of that of frog muscles (Bárány, 1967) it would be more difficult to demonstrate load dependence in 1968 on tortoise than in 1938 on frog. There is no reason to suppose that improved technical factors could allow Woledge more than 10-fold greater sensitivity over the measurements of Hill in 1938 or 1964.

In the next section of this report, Hill's data from 1964 are analyzed in a manner which can be described as a variation of his 1938 equation. We demonstrate that the Hill factor can be used to normalize the heat of shortening (Q_s) or the work (W) or their sum, the enthalpy of shortening (H_s), so that these quantities become obvious linear functions of the time of shortening. Thus the sum of the heat of shortening and the work are still proportional to the Hill factor, but each energy component is also independently proportional to this factor, rather than just the sum as in the 1938 equation. This method of analysis is a quantitative extension of the conclusions of Fenn (1924), since it follows from his recognition that work and heat are continuously generated during contraction, at rates regulated by the load. Following the procedures of Hill, we employ the load rather than the shortening velocity as the independent variable. (A preliminary account of this has been presented by P. W. Brandt, 1970, at the 14th Annual Meeting of the Biophysical Society, Baltimore, Md.)

BASIC ASSUMPTIONS

Our analysis was based on three propositions:

(*a*) Both forms of energy (W and Q_s) released during contraction are predominantly consequences of the chemical reactions that drive muscular contractions throughout the duration of shortening.

(*b*) The rates of these chemical reactions are functions of the fractional load on the muscle. Therefore, at fixed fractional loads the energy release is constant during the time of shortening. This implies that the extra energy release is a linear function of the time of shortening.

(*c*) Both forms of energy release are simple functions of the Hill factor, or the complement of the fractional load, i.e., the energy release is $\propto$ $(P_0 - P)$ or $(1 - P/P_0)$.

Propositions *b* and *c* can be summarized as follows. For the load interval $0 \leq P/P_0 \leq 1.0$, a muscle actively shortening a distance S in a time t will obey the relations:

$$W/(P_0 - P) = b_w t + B_w, \tag{1}$$

$$Q_s/(P_0 - P) = b_q t + B_q. \tag{2}$$

These lines have intercepts on the time axis as defined below.

$$t_w = -B_w/b_w,$$

$$t_q = -B_q/b_q.$$

Since $W/(P_0 - P) = 0$ corresponds to the unloaded muscle, t_w is the time required to traverse S at $v = v_{\max}$, or

$$t_w = S/v_{\max}.$$

The term $Q_s/(P_0 - P)$ should only be zero at $t = 0$, since the extra heat should be liberated for all finite shortening times. For this reason t_q, if significantly different from 0, could be an error term due to frictional effects and heat effects accompanying the start and stop transients.

SOURCE OF DATA

Three sets of complete data were taken from a publication of Hill (1964 *a*). These include the physical dimensions of the muscles: the rest length (l_0) and the wet weight (M); the heat of shortening (Q_s) for four or more different loads (P) over a fixed distance (S), for a time (t); and the isometric forces of the muscles (P_0). These primary data are given in Table I. Since these data were collected on English frog muscles at 0°C, the literature was searched for data on other muscles also treated at 0°C which could be used to confirm at least some of our conclusions. Practically none were found for any temperature, since most authors summarized their data and omitted information such as the weight and the length of the individual muscles, or even the means of these. What could be found, mostly from the publications of Hill, is indicated in the text where appropriate.

The data in Table I were collected by Hill from experiments described as after-loaded isotonic contractions. In this method pairs of frog sartorius muscles are mounted on a

TABLE I

DATA FROM HILL (1964 *a*)

Muscle	Time	Load	Work	Heat	Total energy
1	0.10	24.0	12.0	10.2	22.2
	0.16	42.0	21.0	13.3	34.3
	0.24	60.0	30.0	15.9	45.9
	0.34	74.0	37.0	18.3	55.3
	0.54	96.0	48.0	20.2	68.2
	0.78	111.0	55.5	21.7	77.2
2	0.15	2	1.4	7.6	9
	0.288	18.1	12.67	12.9	25.57
	0.415	35.8	25.06	16.7	41.76
	0.637	53.4	37.38	19.3	56.68
	1.05	71.3	49.91	23.8	73.71
3	0.16	6.5	4.55	18.4	22.95
	0.257	33.6	23.52	21	44.52
	0.36	53.6	37.52	24.2	61.72
	0.502	73.6	51.52	26.4	77.92

	Muscle		
	1	2	3
P_0, *g*	155	110	180
S, cm	0.5	0.7	0.7
M, g	0.232	0.199	0.250
l_0, *cm*	3.2	3.1	3.45

thermopile, connected to an isotonic lever or an ergometer lever at one end, and fixed at the other end.

The muscle and the thermopile are immersed in an ice bath (0°C) and the muscle drained by lowering the bath level shortly before the experimental run is made. The muscle is then tetanically stimulated while held isometric at slightly above rest length, until P_0 is attained and the heat rate is relatively constant. Then it is released to shorten a fixed distance, under a given load, or if it is attached to an ergometer lever instead of a isotonic lever it is released at a given velocity. After it has shortened the predetermined distance, the stimulus is continued until the muscle reattains full isometric force. The shortening time is recorded, the difference in the heat evolved above the projected isometric heat is calculated, and the work is calculated or read off of the ergometer record. A critical factor is the calculation of the extra heat to be associated with the shortening, and the reader is referred to Hill (1964 *a*) for details on this. We used the results of Hill's calculations, and did not alter them in any way, but we have used his values for the heat of shortening directly rather than his alpha values.

Our basic method of analysis consisted of dividing each energy quantity, W or Q_s or the sum of the two, by the Hill factor and correlating the result with the shortening time. The pairs of data, in centimeters and seconds, were correlated with a standard linear regression program in a Wang 370-2 calculator (Wang Laboratories, Inc., Tewksbury, Mass.), and the statistical information used to calculate correlation coefficients (r), slopes (b), intercepts (B), and confidence intervals.

RESULTS

A plot of the processed data for muscle 1 is presented in Fig. 1 and the statistical information from the linear regression program is listed in Table II. The ordinate (in centimeters) of Fig. 1 is the energy, either the heat, the work, or their sum, divided by the Hill factor and the abscissa is the time (in seconds) taken by the muscle to shorten the fixed distance. The heat of shortening is the solid circles, the work is the open circles, and the sum of the two, the enthalpy, is the open triangles. A high degree of linearity is evident in the plot. The correlation coefficients, slopes, and intercepts of all the data from the three muscles, along with the 95% confidence intervals, are listed in Table II.

Because the data are so linear when treated in the above manner we used the energy data to find P_0 by programming the calculator to develop the P_0 which gave the highest degree of correlation. The P_0's found for muscles 2 and 3 were not significantly different from those measured by Hill, while that found for muscle 1 was 105% of that measured by Hill. This illustrates the sensitivity of the analysis to the Hill factor.

A rate of enthalpy release during shortening $\mathring{H}_s = b_h P_0 (1 - P/P_0)$ can be derived from equations 1 and 2, where $b_h = b_q + b_w$. If P_0 is proportional to the cross-

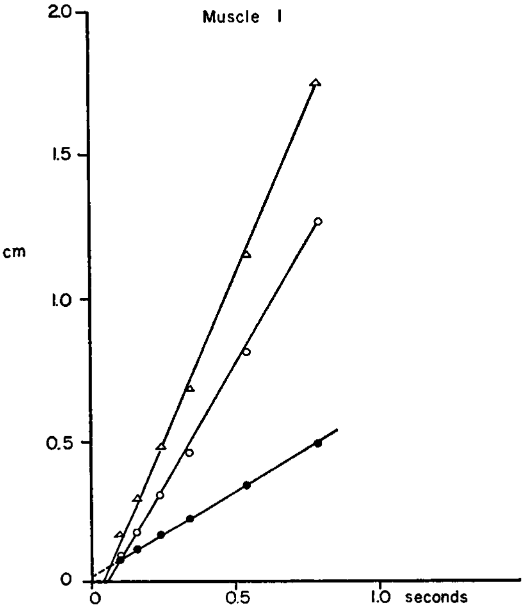

FIGURE 1 Energy data for muscle 1 (Table I) reduced according to equations 1 and 2. △, enthalpy of shortening; ○, work; ●, heat of shortening.

TABLE II

STATISTICAL RESULTS OF LINEAR REGRESSION TEST OF DATA FROM TABLE I IN $E/(P_0 - P) = bt + B$, WHERE $E = Q_s$ OR W, OR $Q_s + W = H_s$

Data*	Muscle		
	1	2	3
Heat (Q_s)			
r	0.999	0.998	0.999
b_q	0.605 ±0.01	0.605 ±0.04	0.42 ±0.03
B_q	0.02 ±0.005	−0.03 ±0.02	0.04 ±0.01
t_q	−0.03 ±0.01	0.05 ±0.04	−0.01 ±0.003
Work (W)			
r	0.999	0.998	0.9999
b_w	1.72 ±0.08	1.45 ±0.12	1.34 ±0.03
B_w	−0.097 ±0.035	−0.248 ±0.07	−0.186 ±0.01
t_w	0.056 ±0.02	0.171 ±0.05	0.139 ±0.01
v_{max}	8.85 ±3	4.1 ±1	5.05 ±0.3
Enthalpy (H_s)			
r	0.999	0.998	0.9999
b_h	2.32 ±0.08	2.05 ±0.13	1.76 ±0.03
B_h	−0.077 ±0.04	−0.277 ±0.09	−0.148 ±0.01
t_h	0.033 ±0.02	0.138 ±0.05	0.084 ±0.006

* r, correlation coefficient; b, in centimeters per second; v_{max}, maximum velocity in centimeters per second; t, time in seconds; B, in centimeters. Subscripts refer to form of energy, and E is energy in gram-centimeters.

sectional area, b_h should be proportional to the length of the muscle, as was supposed by Hill (1938) since presumably the energy released per unit time is directly proportional to the total mass of active tissue. For the three muscles analyzed here, however, b_h correlated significantly with the mass but not with the length. In an attempt to resolve this the literature was searched for other data where b, the length, and the mass of individual muscles were reported for frog sartorius muscles shortening at 0°C. Data on a total of six "English" frogs and four "American" frogs were correlated separately and together. Both correlation series were significant with mass, but not significant with length. The data on English frogs are given in Table III. From these data it appears that at 0°C b_h (or Hill's b) is related to the mass in grams by a proportionality constant of about 6 cm/g per sec.

The b values computed from Woledge (1968, Table I) were similarly correlated against mass and length, giving correlation coefficients of 0.31 and 0.58 respectively, neither of which is statistically significant at the 90% level. We conclude from these analyses that evidence for constancy of b/l_0 is lacking and that there may be a correlation between b and muscle mass. The source of this latter correlation is unknown, and in the absence of further experimental work speculation would serve no useful purpose.

TABLE III

DATA FOR ENGLISH FROGS

Source	Muscle weight	b*	l_0
	mg		*cm*
Hill, 1964 *a*	232	2.13	3.2
Hill, 1964 *a*	199	2.25	3.1
Hill, 1964 *a*	250	1.82	3.45
Hill, 1938	102	1.12	3.45
Hill, 1938	165	1.03	3.8
Abbott and Wilkie, 1953	97	1.19	3.0

Linear regression results	Correlation coefficient (r)
(a) b is proportional to l_0	$r = -0.48$
(b) b is proportional to mass	$r = +0.77$

* To estimate Hill's b we used the relation $b = b_w/(1 + B'_w)$ for the first three muscles; the remaining b values are given in the references. We note for muscles 1–3 $b_h \simeq b$ ±10%.

The maximum velocity v_{max} predicted by equation 1 is found by setting the load to 0 and assuming that the time axis intercept is that time required to shorten the fixed distance with no load as previously described. The values for the maximum velocity v_{max} are listed in Table II under the division labeled "work data." The ergometer-tested muscle is rather higher and has a larger 95% confidence interval than the other two muscles, which were tested with isotonic lever systems. In any case the maximum velocities calculated from the work data according to equation 1 are within the normal range for frog sartorius muscles shortening at 0°C. For example with Hill's 1938 constants for b = 1 cm/sec, a/P_0 = 0.25, v_{max} is 4 cm/sec.

DISCUSSION

Regulation of Shortening Energy by the Load

The essence of the Fenn effect is that the load imposes some constraint on the rate of energy release by the muscle. How this may fit into a mechanism of muscular shortening in which the thick and thin myofilaments move past one another through some kind of energetic interaction involving cross bridges (A. F. Huxley, 1957) is presently speculation. It is plausible, however, that an immobilized bridge will fail to hydrolyze ATP. Although we have a detailed kinetic analysis in preparation, at this time we restrict ourselves to a quote by Hill on the physical significance of what we call the Hill factor. Hill said in 1938, when the concept of the cross bridges was unknown, "We can imagine that when the force in the muscle is high the affinities of more of these points [Hill meant sites of energy release] are satisfied by the attractions they exert on one another, and that fewer of them are available to take part in chemical transformations.... The rate at which chemical transformations would occur, and therefore at which energy would be liberated, would be directly proportional to the number of exposed affinities or catalytic groups and so would be a linear function of the force exerted by the muscle, increasing as the force diminished." Whatever the molecular mechanism underlying muscular contraction may prove to be, the present correlations demonstrate that the factors which regulate the release of energy as work also regulate the release of energy as the heat of shortening.

When the heat of shortening, the work, or the sum of the two is divided by the Hill factor and this quotient is then correlated by a linear regression program with the time of shortening, a near-perfect correlation is found for data from a group of three muscles studied by Hill in 1964. We propose that this pattern is correct for all frog sartorius muscles and probably describes the majority of striated skeletal muscles. This hypothesis seems worthy of testing because of the unusually high correlation coefficients (0.998+) and the small 95% confidence intervals attached to the slopes of the regression lines.

It was impossible to find sufficient data on individual muscles so that the correlation between our constant b_h and the muscle length or mass could be convincingly tested. Part of our purpose then, in presenting this analysis, is to stimulate those who have more detailed data than has been published to test some of it with the analysis described here.

The physical significance of the parameters in equations 1 and 2 is clarified by writing them in terms of their intercepts on the time axis, t_w and t_q (equations 1 *a* and 2 *a*). As described earlier, t_w is the time for unloaded muscle to traverse S at $v = v_{\max}$.

$$\frac{W}{P_0 - P} = b_w(t - t_w), \tag{1 a}$$

$$\frac{Q_s}{P_0 - P} = b_q(t - t_q). \tag{2 a}$$

The intercept t_q is on the average not significantly different from zero, as anticipated. We are thus led to the simple relationships 1 *b* and 2 *b*:

$$\frac{W}{P_0 - P} = b_w t + B_w' S, \tag{1 b}$$

$$\frac{Q_s}{P_0 - P} = b_q t, \tag{2 b}$$

where

$$B_w' = \frac{B_w}{S} = \frac{-b_w}{v_{\max}}.$$

Force-Velocity Relation

A relation between force and velocity is easily deduced by dividing both sides of equation 1 *b* by *S*. This gives a convenient form for data fitting, a function of P as a linear function of v^{-1}.

$$\frac{P}{P_0 - P} = \frac{b_w}{v} + B'_w . \tag{3}$$

Before applying equation 3 numerically, it is appropriate to put this force-velocity equation into the classic form of Hill's 1938 equation. If both sides of equation 3 are multiplied by $(P_0 - P)\,v$ and terms proportional to v are put on the left-hand side, equation 3 *a* is obtained.

$$[P(1 + B'_w) - P_0 B'_w]v = b_w(P_0 - P). \tag{3 a}$$

If we define

$$-\frac{a}{P_0} = \frac{B'_w}{1 + B'_w} \quad \text{and} \quad \frac{b_w}{1 + B'_w} = b,$$

and substitute them into equation 3 *a*, Hill's equation is obtained.

$$(P + a)v = b(P_0 - P). \tag{4}$$

Hill (1970) has published new force-velocity data in reduced form, i.e., v/l_0 vs. P/P_0. Equation 3 fits these data with a correlation coefficient $r = 0.996$ (see Fig. 2). The constants of the equation are $b_w/l_0 = 0.329$ and $B'_w = -0.184$. These

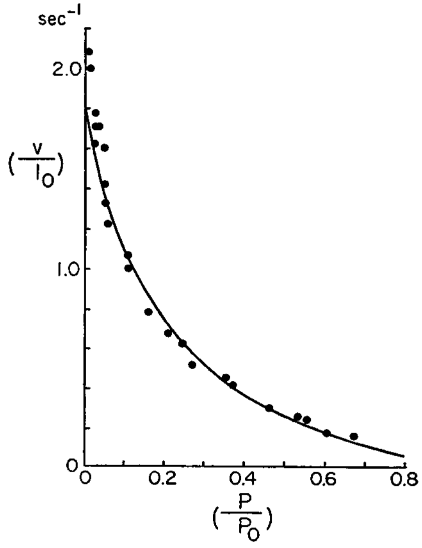

FIGURE 2 Force-velocity relation: reduced data (v/l_0) of Hill (1970). Line is calculated from equation 3, constants given in text.

values give $a/P_0 = 0.225$ and $b/l_0 = 0.40$. Hill (1970) fit his equation to these data with $a/P_0 = 0.26$ and $b/l_0 = 0.42$. It is obvious that the 1938 Hill equation prediction for $v_{\max} = (b/a)P_0$ is the same as our prediction from the time intercept t_w, since $v_{\max} = (b/a)P_0 = -b_w/B'_w$.

Heat Release per Centimeter Shortening α

Hill's equation (Hill, 1964 *a*) relating the heat released per centimeter to fractional load is given as equation 5.

$$\frac{\alpha}{P_0} = 0.16 + 0.18\frac{P}{P_0}. \tag{5}$$

Combination of equations 2 *b* and 3 gives a relation for α similar in form to equation 5. Equation 2 *b* is first multiplied by $(P_0 - P)/S$ to give α.

$$\alpha = b_q\frac{P_0 - P}{v}.$$

The term $(P_0 - P)/v$ is obtained from equation 3.

$$\frac{P_0 - P}{v} = \frac{P(1 + B'_w) - B'_w P_0}{b_w},$$

$$\therefore \frac{\alpha}{P_0} = \frac{b_q}{b_w}\left[\frac{P}{P_0}(1 + B'_w) - B'_w\right]. \tag{6}$$

Average values from the three muscles, $b_q/b_w = 0.36$, $-B'_w = 0.27$, lead to equation 6 *a*, which is of the form of equation 5 but with quite different constants.

$$\frac{\alpha}{P_0} = 0.10 + 0.26\frac{P}{P_0}. \tag{6 a}$$

A portion of the difference between the coefficients of equation 6 *a* and those given by Hill is due to the variability in heat data and the fact that Hill averaged seven muscles to obtain the constants of equation 5. Another portion of the difference is due to the techniques in data reduction. For the three muscles (Table II) the ratios calculated by Hill of the coefficient of P/P_0 to the constant term are 2.4, 3.1, 1.2 (average = 2.2). Our correlation gives for these ratios 4.0, 1.8, 2.9 (average = 2.9). The significance to biochemical studies of the near-proportionality to load of the heat liberation will be discussed shortly.

Enthalpy of Shortening H_s and Shortening Efficiency

The most accessible link between muscle energetics and biochemistry is through the enthalpy of hydrolysis of creatine phosphate (CP) or ATP. The sum of heat and work during a contraction must equal the enthalpy decrease in the contracting muscle. Since the relation between maintenance heat and energy liberation during work is not well understood, we shall discuss only the enthalpy of shortening, i.e., the sum of the shortening heat and the work. This term is calculated from the sum of equations 1 *a* and 2 *a*.

$$\frac{H_s}{P_0 - P} = b_h(t - t_h), \tag{7}$$

where $b_h = b_w + b_q$ and $t_h = -[(B_w + B_q)/(b_w + b_q)]$.

An important concept for muscle energetics is the shortening efficiency, W/H_s, given by equation 8

$$\frac{W}{H_s} = \frac{b_w}{b_h}\left(\frac{t}{t_w} - 1\right) \Big/ \left(\frac{t}{t_w} - \frac{t_h}{t_w}\right). \tag{8}$$

If we define $R = P/(P_0 - P)$ and substitute equation 3 into equation 8, a relation between shortening efficiency and fractional load is obtained.

$$\frac{W}{H_s} = \frac{b_w}{b_h}\left[\frac{R}{R - B'_w\left(1 - \frac{t_h}{t_w}\right)}\right]. \tag{8 a}$$

Average values for these ratios derived from Table II, $t_h/t_w = 0.69$, $b_w/b_h = 0.735$, and $-B'_w = 0.27$, were substituted into equation 8 *a* to give the numerical relationship 8 *b*.

$$\frac{W}{H_s} = \frac{0.735R}{R + 0.084} \tag{8 b}$$

Equation 8·*b* was used to predict the efficiency as a function of the fractional load and a comparison of the prediction with calculated efficiencies from the data in Table I is given in Fig. 3. The agreement is excellent and encourages us to assert that certain generally held concepts of muscular efficiency should be reexamined.

Efficiency is usually defined as work divided by total energy output, including the maintenance heat or isometric heat (Hill, 1964 *b*). We choose to focus attention on the energy fluxes associated with shortening, and therefore our values are not immediately comparable with published efficiencies. We have done this on the assumption that the enthalpy of shortening is more related to the molecular events underlying shortening, i.e. energy transductions whereby chemical energy is converted into external work, than is the maintenance heat. Equation 8 *a* implies that efficiency of shortening is monotonically increasing with the fractional load up to 1 and it indicates that the upper limit is b_w/b_h, which for the muscles in Table I ranges from 0.69 to 0.76.

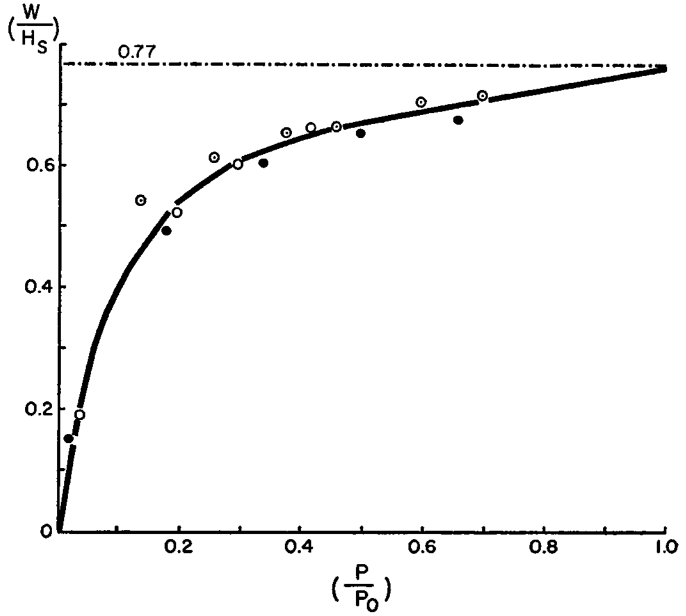

FIGURE 3 Efficiency of shortening as a function of fractional load for the three muscles of Table I. The line is calculated from equation 8 *b*, and the assumption that the limiting efficiency is 0.77. ⊙, muscle 1; ●, muscle 2; ○, muscle 3.

In accord with the original observations of Fenn (1923) the extra energy of shortening is nearly proportional to the work over a range of fractional loads from approximately 0.2–1. His conclusions that the total "extra energy" of shortening is about 1.3 times the work implies a shortening efficiency of 0.77 or almost exactly the value predicted from our analysis of Hill's data.

The efficiency data on the tortoise muscle (Woledge, 1968) which includes the maintenance heat nearly fits our description of the efficiency of shortening because the maintenance heat rate for tortoise is 50 times smaller than that of the frog, while the rate of liberation of shortening energy is about 10 times less. Thus the enthalpy used in the calculation of the efficiency of tortoise includes a relatively smaller maintenance heat and the over-all efficiency is relatively larger. This suggests to us that the efficiencies of the frog and the tortoise shortening mechanisms are about the same.

If the correlation described here is generally applicable it will be virtually impossible for a biochemist to define a component of high energy phosphate split, which can be exclusively correlated with the heat of shortening. Since the efficiency falls only to ½ its maximum value at $P/P_0 = 0.05$, $Q_s \propto W$ with an error less than 33% over most of the range of experimental loads employed. Therefore the extra energy of shortening and the hydrolysis of high energy phosphate must be nearly proportional to the work at any finite load. This is in fact the result of all the biochemical studies to date (Mommaerts et al., 1962; Carlson et al., 1963; Davies et al., 1967).

Further insight into the regulation of energy release by the Hill factor is gained by plotting the energy rates as functions of the fractional load. If we multiply equation 3 by $v/v_{max}(1 - P/P_0)$ we obtain

$$\mathring{W}/(P_0 v_{max}) = -B'_w(1 - v/v_{max})(1 - P/P_0), \qquad (9)$$

which can be evaluated by substituting $v = b_w/(R - B'_w)$. The heat rate $\mathring{Q}_s$ can be found by differentiating equation 2 which gives

$$\mathring{Q}_s/P_0 v_{max} = (b_q/v_{max})(1 - P/P_0), \qquad (10)$$

if we set B_q equal to zero.

The work rates and the heat rates, and their sum, the rate of enthalpy liberation, are plotted as functions of the fractional load in Fig. 4. The heat rate increases linearly with the decrease in load, while the work rate reaches a maximum at 0.35 P_0 and is zero at the two limits. This work rate determines the nonlinear component of the enthalpy rate curve. Fig. 8 of Woledge (1968) presents an experimentally derived highly nonlinear heat rate vs. load curve. Whether this is due to the difficulty of heat measurements on tortoise or represents a real difference between tortoise and frog is uncertain, as we have discussed in the Introduction. Our enthalpy rate curve has a maximum at $P/P_0 \simeq 0.28$; by contrast, the enthalpy rate curve is linear in the Hill equation of 1938, because the calculated falloff in work rate with load is compensated by a calculated increase in heat rate (Woledge, 1968).

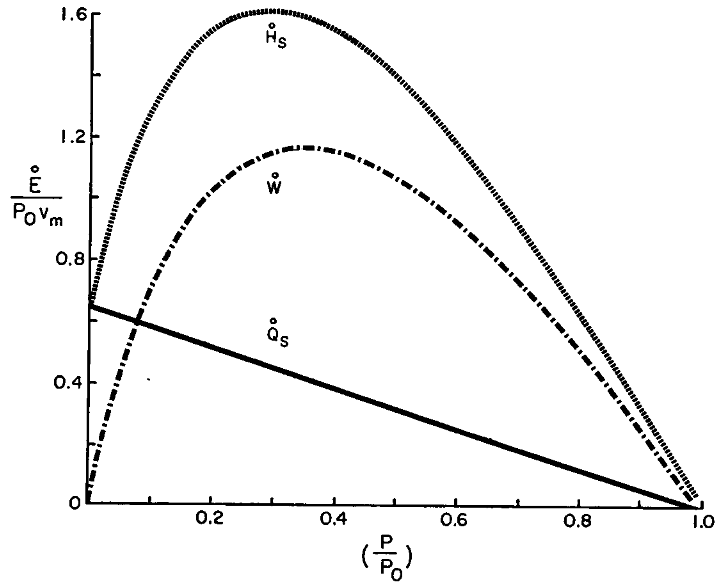

FIGURE 4 Calculated energy of shortening rates normalized with $P_0 v_{max}$ and according to equations 9 and 10. $\mathring{H}_s$ is enthalpy of shortening rate, $\mathring{W}$ is mechanical power, and $\mathring{Q}_s$ is heat of shortening rate. The rates are plotted as functions of the fractional load.

When the Hill equation for heat of shortening (1964 *a*) is combined with the 1938 Hill equation treated as a work rate equation, the resulting calculated enthalpy rate is also nonlinear (Woledge, 1968).

In our treatment the enthalpy rate decreases approximately in parallel to the capacity of the muscle to perform work. The thermodynamic data place a restriction on models of muscle contraction: the predicted enthalpy rate must have a maximum near $P/P_0 = 0.30$. The falloff in enthalpy liberation rate at small fractional loads (high velocities) suggests to us that some kinetic process in the energetic cycle is limiting at low fractional loads. How this observation is predicted by various models of muscular contraction is the subject of another report (Orentlicher and Brandt, manuscript in preparation).

Our analysis agrees with Hill's (1964 *a*) that the heat of shortening is not the same as the constant a multiplied by the distance shortened. Our equations yield no formal fixed relation between the quantities of energy predicted by equations 1 and 2. There exists an empirical relation that b_q is about 0.36 of b_w and B_q is probably zero. It is thus possible to estimate from equation 2 the heat of shortening if b_w is estimated from the force-velocity data and relation.

We conclude that the data support these two propositions: (*a*) that all forms of shortening energy are liberated at a constant rate for a fixed load, and (*b*) all shortening energy rates vary with load in proportion to the Hill factor $(P_0 - P)$. These propositions are expressly stated in equations 1 and 2. Equation 1 also leads to a force-velocity relation, equation 3, which is suitable for linear regression analysis of data, and is shown to be algebraically equivalent to the Hill equation of 1938. Because many forms of data can be correlated with these equations they should be useful to students of muscle biophysics.

No correlation was found between the constant b_h (or Hill's constant b) and muscle length; rather a stronger statistical case could be made for a correlation between b and the muscle mass. Until more data are available it seems unjustified to assume that either b/l_0 or a/P_0 is a universal constant, or to ascribe molecular significance to these parameters.

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