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A new outlook on the energetics of muscle contraction

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Abstract

Analysis of experimental data on two muscles demonstrates that, in contracting striated muscle, the total rate of ATP splitting, ν_t (number of ATP molecules split per active myosin head per second), comprises of three *separate* components: ν_p, which is required for the generation of the contractile force P which is equal to the external load; ν_v, which is devoted to the development of the velocity of shortening V; and ν_w, which is responsible for the production of the mechanical power (PV). ν_p is proportional to P and ν_v to V, which means that the sliding distance is independent of P. The mechanical power was found to be equal to the free energy change associated with the hydrolysis of ν_w, which means that the thermodynamic efficiency of the power-producing component is practically 100%. It is concluded that ATP hydrolysis is actually three different reactions. The analysis leads to Hill's force–velocity relationship. Its empirical constants a and b are expressed by thermodynamic and molecular parameters. The constant a was found to be inversely proportional to the sliding distance. The same considerations and conclusions should apply also to other muscles and to the movement under load of microtubules interacting with, e.g. kinesin.

Keywords: Hill's equation; Microtubules; Muscle energetics; Muscle mechanochemistry; Sliding distance

1. Introduction

In the following, the various rates will represent the number of ATP molecules hydrolyzed per active myosin head per second, and the muscle considered will be 1 cm long, with a cross-sectional area of 1 cm^2.

The expenditure of ATP during muscle contraction is believed to serve *two* purposes: to develop tension (in an isometric contraction) and to produce work when a loaded muscle shortens. However, muscle spends ATP *also* when it is unloaded, when no tension is generated and no work is done and the velocity of shortening assumes its maximal value, V_m. It, therefore, seems

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that ATP hydrolysis must occur also for the development of velocity. Thus, ATP hydrolysis is required for the performance of *three,* rather than two tasks.

When the muscle contracts under a load P ($O < P < P_o$ where P_o is the isometric force) the velocity V is constant during most of the time, which means, according to Newton's first law, that the *net* force acting on the muscle is zero. This leads to the conclusion that the muscle had developed a contractile force which exactly balances P. However, for approximately a century, it has been taken for granted that the contractile force is *always* P_o, irrespective of the value of P. Just as in the case of an isometric contraction, when each of the active myosin heads hydrolyses ν_o ATP molecules per second, a muscle must spend ATP for the generation of the contractile force also when $O < P < P_o$. It makes sense to believe that the expenditure ν_p will be smaller as P becomes smaller and it should become zero when $P = O$. Thus, contrary to the common belief, the 'payment' for the contractile force $O < P < P_o$ will *not* be ν_o but less.

Since muscle spends ATP also when $P = O$, presumably for the development of the maximal velocity of shortening, there is no reason why it should not split ATP also for developing a *smaller* velocity, i.e. when a load $O < P < P_o$ is attached. The maximal velocity of shortening of different muscles was found to be proportional to the ATPase activity of the actomyosin extracted from the muscle [1]. It is, therefore, quite possible that, in addition to the force-creating process (expenditure ν_p), there exists a *second* process devoted to the development of velocity. It is reasonable to assume that the rate of this process, ν_v, is proportional to V.

It makes sense to believe that now that both the contractile force P and the velocity V have developed and have been paid for, the value of the mechanical power PV, which is the product of P and V, will be both determined and paid for. However, analysis of data published on the rabbit psoas and the frog sartorius muscles, based on the assumption that ν_p and ν_v are proportional, respectively, to P and V, revealed that the sum of ν_p and ν_v was not equal to the total rate, ν_t, for $O < P < P_o$. One could have argued that our assumptions were simply wrong. Surprisingly enough, it was noticed that upon plotting the difference $\nu_w = \nu_t - (\nu_p + \nu_v)$ against V, the shape of the curve was most reminiscent of the PV vs. V plot [2]. The value of ν_w, just like PV, increased with V, from zero at $V = O$ up to a maximum at which $\nu_w \approx 40\%\ \nu_t$ and then decreased down to zero at $V = V_m$. Even more striking was the observation that the values of ν_w as a function of V were quite close to those of $PV/-\Delta G_1$ (at the same V), where ΔG_1 is the total free energy change when each of the myosin heads splits one ATP molecule. The value of the ratio $PV/-\Delta G_1$ ν_w, which represents the thermodynamic *efficiency* η of this *third* process, appeared to be independent of P and its average was 111–115%, which suggests that: (1) payment for P and for V is not sufficient and the muscle must pay, *separately* and *additionally,* for the mechanical power PV; (2) the efficiency of the work-producing process associated with ν_w is most probably 100% for *all* values of $O < P < P_o$ which means that, thermodynamically, it is a *reversible* process; and (3) our assumptions about the proportionality of ν_p and P and of ν_v and V are probably valid.

2. Evaluation of ν_t, ν_p,ν_v, ν_w and the efficiency η from the rate of ATP hydrolysis by the rabbit psoas muscle

Recently, He et al. [2] measured ν_t as a function of V for permeabilized rabbit psoas muscle fibers. Contractions were elicited by the photolytic release of ATP from caged ATP. Since usually the generation of the contractile force P (to counter-balance the load attached) is the primary event, ν_t has been replotted against P/P_o (employing Hill's empirical force–velocity equation in its reversed form, i.e. P vs. V and the values of its constants as given by He et al. [2]). As can be seen from Fig. 1, ν_t decreases linearly with P/P_o), i.e.

$$\nu_t = \nu_m - k(P/P_o) = \nu_m - (\nu_m - \nu_o)(P/P_o)$$

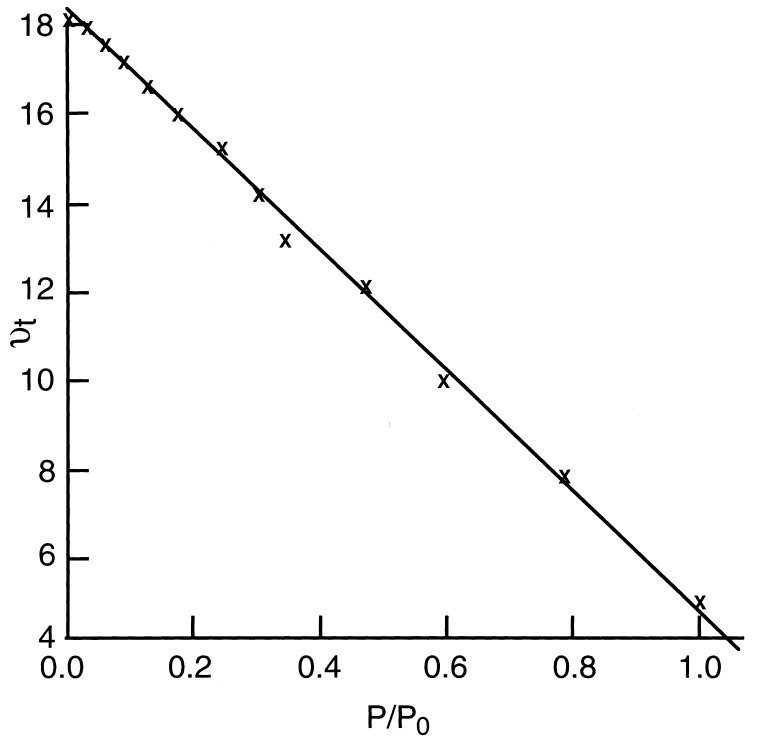

Fig. 1. ν_t vs. P/P_o.

$$= \nu_o(P/P_o) + \nu_m(1 - P/P_o) \quad (1)$$

ν_m and ν_o are the values of ν_t at $P = 0$ and $P = P_o$, respectively. The first term is proportional to P, which is in line with the assumption made above. Hence,

$$\nu_p = \nu_o(P/P_o) \quad \text{or} \quad P = (\nu_p/\nu_o)P_o \quad (2)$$

If, indeed, ν_v and V are proportional to each other then

$$\nu_v = \nu_m(V/V_m) \quad \text{or} \quad V = (\nu_v/\nu_m)V_m \quad \text{and}$$
$$\Delta l \equiv V/\nu_v/\nu_m \quad (3)$$

where Δl is usually defined as the sliding distance associated with the velocity-producing process. Usually Δl is defined as V/ν_t. The value of this Δl decreases continuously upon increasing P from V_m/ν_m at $P = 0$ to zero at $P = P_o$ (as can be seen from Table 1 upon dividing V by its corresponding ν_t value). The continuous decrease of this sort of Δl does not make any sense since it suggests that the value of the step distance (to which Δl is proportional) [3] varies with P. The interaction between a myosin head and actin must, however, be very well-defined topologically and sterically (continuously varying with time) so that the step distance *must* be constant and independent of P (see also the end of the discussion).

However, a simple examination shows that $\nu_v = \nu_m(V/V_m)$ [Eq. (3)] cannot be equal to the second term in Eq. (1) if ν_t was composed of ν_p and ν_v only. Furthermore, inspection of Table 1 reveals that the sum of ν_p and ν_v, i.e. $\nu_o(P/P_o) + \nu_m(V/V_m)$ is smaller than ν_t for $O < P < P_o$ and the difference $\nu_t - \nu_v - \nu_v$ assumes a maximum at the region where the maximal power is observed [2]. The difference vanished at both $P = O$ and $P = P_o$, just like the power. We define the difference as

$$\nu_w = \nu_t - \nu_p - \nu_v \quad \text{or} \quad \nu_t = \nu_p + \nu_v + \nu_w \quad (4)$$

Table 1
Analysis of the data for the rabbit psoas muscle according to He et al. [2]

V	P/P_o	ν_t	ν_p	ν_v	$\nu_t-\nu_p-\nu_v=\nu_w$	$PV/-\Delta G_1$	η
0.0	1.0	5.1	5.1	0.0	0.0	0.0	0.0/0.0
0.1	0.77	7.9	3.9	1.5	2.5	3.36	1.34
0.2	0.59	10.4	3.0	2.9	4.5	5.13	1.14
0.3	0.47	12.1	2.4	4.4	5.3	6.15	1.16
0.4	0.34	13.2	1.7	5.9	5.6	5.90	1.05
0.5	0.30	14.3	1.5	7.4	5.4	6.52	1.20
0.6	0.24	15.2	1.2	8.8	5.2	6.25	1.20
0.7	0.18	16.0	0.9	10.3	4.8	5.45	1.13
0.8	0.13	16.6	0.7	11.8	4.1	4.52	1.10
0.9	0.09	17.2	0.5	13.2	3.5	3.52	1.00
1.0	0.06	17.6	0.3	14.7	2.6	2.61	1.00
1.1	0.03	18.0	0.15	16.2	1.65	1.43	0.87
1.21	0.0	18.1	0.0	18.1	0.0	0.0	0.0/0.0

It was, therefore, tempting to compare ν_w to $PV/-\Delta G_1$ where ΔG_1 is the free energy change occurring when each of the active myosin heads splits one ATP molecule. This is given by $\Delta G_1 = \Delta G_m \cdot c$ where

$$\Delta G_m = \Delta G^o + \mathrm{RT}\ln[\mathrm{ADP}][\mathrm{Pi}]/[\mathrm{ATP}] \quad (5)$$

ΔG_m is the molar free energy change and ΔG^o is the standard molar free energy change of the ATP hydrolysis reaction, and c is the molar concentration of the active myosin heads divided by 10^3 (for a 1-cm^3 muscle). However, in the following ΔG^o will be employed instead of ΔG_m because the concentrations on the r.h.s. of Eq. (5) are unknown. Since $\Delta G^o = -7.21$ kcal mol^{-1} [4] (for conditions approximating those existing in He et al.'s experiments) and $c = 10^{-3} \times 0.145$ mM according to He et al. we get

$$\Delta \mathrm{G}_1 = \Delta \mathrm{G}^{\mathrm{o}} \cdot c = -4.4 \times 10^4 \quad \text{(in c.g.s. units)} \quad (6)$$

It was striking to see (Table 1) how close the values of ν_w and $PV/-\Delta G_1$ were (P_o was taken as 190 kNm^{-2} from He et al's work). It was, therefore, not surprising to observe that the value of the efficiency η, defined as

$$\eta = PV/-\Delta G_1 \nu_w \quad (7)$$

was (Table 1) close to one (average value 1.11) and practically *independent* of P/P_o, unlike the efficiency $PV/-\Delta H$ (where $-\Delta H$ is the *total* change in enthalpy) which is usually evaluated, that assumes a *maximum.*

Since η cannot be larger than 1.00, the real value of ΔG must be larger than ΔG_1. It is, therefore, naturally tempting to think of the possibility that η is actually 100%, which would mean that the work-producing process associated with ν_w is a *reversible* process. In the following I shall assume that, indeed, $\eta = 100\%$ and, therefore, ΔG_1 will have to be multiplied by 1.11, i.e.

$$\Delta G \equiv 1.11\Delta G_1 = -1.11 \times 4.4 \times 10^4 = -4.9 \times 10^4 \quad \text{and then} \quad (8)$$

$$\eta \equiv PV/-\Delta G \cdot \nu_w = 1 = 100\% \quad (9)$$

In Fig. 2 both $PV/-\Delta G$ and ν_w were plotted against V. Apparently, a single curve should fit the calculated points of both, in line with Eq. (9).

Actually, if we take η to be equal to 100% then the total molar free energy change ΔG_m [Eq. (5)] will be given by $1.11 \times (-7.21) = -8.0$ kcal mol^{-1}, i.e. RTln[ADP][P$_i$]/[ATP] (in the same equation) will be equal to $-(8.0 - 7.21) = -0.8$ kcal mol^{-1}. In other words: our analysis might enable us to evaluate this parameter in vivo for different muscles under different conditions.

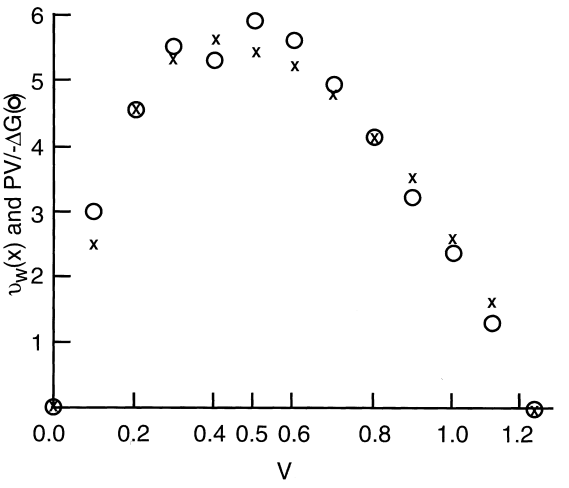

Fig. 2. $v_w(x)$ and $PV/-\Delta G$ vs. V.

3. Evaluation of the various ν values and of η from heat and work measurements on the frog sartorius muscle

The kinetics of ATP hydrolysis in contracting muscle has been followed for many years by measuring the rate of total enthalpy change, ΔH, i.e.

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

where Q is the rate of heat evolution. According to Hill [5] Q is the sum of two terms:

$$Q = Q_o + \alpha V \tag{11}$$

where Q_o is the maintenance heat, i.e. the rate of isometric heat production and αV is the heat of shortening. This division is arbitrary and has historical roots: it was quite surprising and unexpected at the time to observe that a shortening muscle produced more heat than an isometrically contracting one. The proportionality factor α was originally thought to be constant but [5] was found later on to be given by

$$\alpha = 0.16P_o + 0.18P \tag{12}$$

for the frog sartorius muscle. Hence,

$$-\Delta H = Q_o + (0.16P_o + 0.18P)V + PV$$
$$= Q_o + (0.16\ P_o + 1.18P)\mathrm{V} \tag{13}$$

As is well known, the ADP formed by the hydrolysis of ATP is rephosphorylated by phospho-creatine (PC_r). Hence, the rate of PC_r splitting is equal to the total rate of ATP hydrolysis, $\nu_{t.}$ Assuming that ΔH is due to PC_r splitting only

$$-\Delta H = -\Delta H_m \cdot c \cdot \nu_t \tag{14}$$

where ΔH_m is the molar enthalpy change associated with PC_r splitting and ν_t is the total rate of this process we obtain

$$\nu_t = [\mathrm{Q_o} + (0.16P_o + 1.18P)V]/\Delta H_m \cdot c \tag{15}$$

Hence at $P = P_o$

$$\nu_o = Q_o / -\Delta H_m \cdot c \tag{16}$$

whereas at $P = 0$

$$\nu_m = \nu_o + 0.16\ P_o V_m / -\Delta H_m \cdot c \tag{17}$$

V depends on P according to Hill's empirical equation [5]

$$V = b[P_o - P]/[P + a]$$
$$= b[1 - P/P_o]/[P/P_o + a/P_o] \tag{18}$$

Where b and a are empirical constants so that the values of ν_t for different values of P can be derived employing Eqs. (15) and (18).

The numerical values of the constants used in the following for the frog sartorius of *Rana pipiens* at 0°C are [5–7]: $P_o \cong 200$ kN m^{-2}; $V_m = 2.24$; $a/P_o = 0.25$; $b = (a/P_o)\ \ V_m = 0.56$; $\Delta H_o = P_o V_m/16$.

The 'in vivo' value of ΔH_m was taken to be 11 kcal mol^{-1} [5] and the value of $c = 0.236 \times 10^{-3}$ mM was based on He et al.'s [2] value of 0.145 mM for the concentration of active myosin heads in the rabbit psoas muscle, taking into consideration the fact that the sarcomere length in their experiments was 2.7 μm while in the work on the frog sartorius [6,7] the muscle was at rest length, i.e. the sarcomeres length was 2.2 μm. Different

Table 2
Analysis of the data for the frog sartorius muscle (see text)

P/P_o	V	ν_t	ν_p	ν_v	ν_w	$PV/-\Delta G_t$	η
0.0	2.24	9.48	0.00	9.48	0.00	0.00	0.00/0.00
0.1	1.43	10.00	0.26	6.07	3.67	4.14	1.17
0.2	0.99	9.80	0.52	4.21	5.07	4.57	0.94
0.3	0.71	9.32	0.78	3.21	5.33	6.16	1.20
0.4	0.52	8.60	1.04	2.18	5.38	5.95	1.15
0.5	0.37	7.78	1.30	1.58	4.90	5.40	1.15
0.6	0.26	6.85	1.56	1.11	4.18	4.55	1.14
0.7	0.18	5.82	1.82	0.75	3.25	3.57	1.15
0.8	0.11	4.80	2.08	0.45	2.27	2.46	1.13
0.9	0.05	3.70	2.34	0.21	1.15	1.26	1.15
1.0	0.00	2.60	2.60	0.00	0.00	0.00	0.00/0.00

sarcomere lengths are associated with different degrees of overlap of the actin and the myosin filaments and, therefore, with different concentrations of active myosin heads.

In Table 2 the values of V and of ν_t are given for various P/P_o values. Using Eqs. (2) and (3), the corresponding values of ν_p and ν_v were derived. In the next column $\nu_w = \nu_t - \nu_p - \nu_v$ is presented. As can be seen, the value of ν_w as a function of P assumes a maximum at $P/P_o = 0.4$, i.e. *exactly* where (from ATP hydrolysis measurements) it assumed a maximum for the rabbit psoas muscle (Table 1).

In the next column the values of $PV/-\Delta G_1$ are given with $\Delta G_1 = -10.74 \times 10^4$. This value was obtained as follows: the PC_r splitting reaction $PC_r \Leftrightarrow Cr + Pi$ is the sum of the ATP hydrolysis reaction $ATP \Leftrightarrow ADP + Pi$ and the creatine phosphokinase reaction ($PC_r + ADP \Leftrightarrow Cr + ATP$). Hence, its standard molar free energy change for PC_r splitting is the sum of those of these two reactions: -7.21 [4] and -2.51 [5], respectively, i.e. -9.72 kcal mol^{-1}.

According to Eq. (6), $\Delta G_1 = \Delta G^{o} \cdot c = -9.72 \times 4.2 \times 10^{10} \times 0.236 \times 10^{-3} \times 10^{-3} = -9.65 \times 10^4$ (in c.g.s. units). As with the rabbit psoas muscle, the values of ν_w and the corresponding $PV/-\Delta G_1$ are close. It is, therefore, not surprising that the thermodynamic efficiency η [Eq. (7)] is close to 1, independently of P (Table 2). Since the value of η cannot be larger than 1.00, the (constant) thermodynamic efficiency of the frog sartorius muscle is, as in the case of the rabbit psoas muscle, possibly 100%. This would then mean that the actual value of ΔG is larger than that employed by approximately 15% (as we saw in the case of the rabbit psoas muscle). The same applies to ΔG_m which should be $= -1.15 \times 9.65 = -11.1$ kcal mol^{-1}.

Hence, the heat and work measurements have led to exactly the same conclusions as the chemical ones which, were carried out on a different muscle. In both cases: (1) the ATP splitting reaction appears to be composed of three separate processes; (2) the mechanical power-producing process is reversible; and (3) the sliding distance Δl is constant, independent of P.

The equality of $PV/-\Delta G$ and ν_w [from Eq. (9) with $\eta = 1.00$] means that the assumption that ν_t is proportional to $-\Delta H$ [Eq. (14)] was justified, i.e. the heat and work production are fully paid for by the splitting of PC_r and no additional chemical reactions are involved. In other words: there is no 'unexplained heat' (or missing heat). Apparently, possible changes in the conformations of other protein species such as titin and actin do not contribute in total to the heat. It also justifies the use of the controversial [5] value of -11 kcal mol^{-1} for ΔH_m.

It is clear that both ν_p and ν_v are dissipated as heat. Does ν_w also contribute to the heat production? We have seen above that the value of ΔG_m for the PC_r splitting reaction is probably -11.1 kcal mol^{-1}, which is practically equal to -11.0 kcal mol^{-1} for ΔH_m. Hence, from Gibbs equation, $T\Delta S_m = \Delta H_m - \Delta G_m = 0$. Thus, the power-

producing reaction does not involve any net change in entropy. The same should hold also for ν_p and ν_v. Since $-\Delta H_m \cdot c \cdot \nu_w = -\Delta G_m \cdot c \cdot \nu_w = PV$ [Eq. (9)] and $-\Delta H_m \cdot c \cdot \nu_w = Q_w + PV$ [Eq. (10)] (where Q_w is the heat evolved by the hydrolysis of ν_w) we get $Q_w = O$. The power-producing reaction is thus not accompanied by any heat change.

In conclusion, the enthalpy changes associated with the splitting of ν_p and ν_v are fully converted into heat whereas that of ν_w is fully used as work. Thus [see Eqs. (2) and (3)]

$$\begin{aligned} Q &= Q_p + Q_v = -\Delta H_m \cdot c[\nu_o(P/P_o) + V/\Delta l] \\ &= -\Delta H_m \cdot c[\nu_o(P/P_o) + \nu_w(V/V_m)] \\ &= Q_o(P/P_o) + Q_m(V/V_m) \end{aligned} \quad (19)$$

where Q_m is the rate of heat production at $P = O$.

In summary, from the study of ATP splitting and heat changes as functions of P, which have been performed on two different skeletal muscles, the total rate of ATP splitting (ν_t) appears to be composed of three components: ν_p designed for the generation of tension; ν_v used for developing the velocity; and ν_w which *reversibly* produces work at a thermodynamic efficiency of 100%. The fact that the work-producing process is reversible is most unexpected and exciting but at this stage still a mystery, possibly hiding a fundamental principle, which characterizes all biological energy converters.

4. Derivation of Hill's equation and the physical meaning of its constants

For the rabbit psoas muscle, combining Eqs. (1)–(3) and (9) we get $\nu_v + \nu_w = \nu_m \cdot (V/V_m) + PV/-\Delta G = \nu_m\,(1 - P/P_o)$. Hence,

$$V = [-\Delta G \cdot \nu_m/P_o][P_o - P]/[+(-\Delta G/\Delta l] \quad (20)$$

which is equivalent to Hill's force–velocity equation [Eq. (18)]

with $b = (-\Delta G/P_o) \cdot \nu_m$ and

$$a = -\Delta G/\Delta l \text{ or}$$

$$a/P_o = -\Delta G/(P_o\ \Delta l) \quad (21)$$

Both $-\Delta G$ and P_o are proportional to the concentration of the active myosin heads, which should diminish when the degree of overlap of the actin and myosin filaments is reduced by stretching the muscle. Thus, $-\Delta G/P_o$ must be constant and, therefore, b is proportional to ν_m (which is characteristic of the muscle), and the constant a/P_o is proportional to $1/\Delta l$, which is a molecular parameter. Since, for the rabbit psoas muscle, $-\Delta G = 4.9 \times 10^4$ [Eq. (8)], $\Delta l = V_m/\nu_m = 1.21/18.1 = 0.067$ (see Table 1) and $P_o = 190$ k Nm^{-2} we obtain [from Eq. (21)] $a/P_o = 0.39$, which compares nicely with He et al.'s value (obtained by fitting Hill's equation) of 0.42. For Hill's constant $b = -\Delta G \cdot \nu_m/P_o$ we get 0.47, compared to He et al.'s 0.51.

We can write the expression for a/P_o somewhat differently: $a/P_o = -\Delta G/(P_o \cdot \Delta l) = (-\Delta G/P_o) \times (V_m/\nu_m)^{-1}$ [see Eq. (3)] or: $a/P_o = (P_o V_m/-\Delta G \nu_m)^{-1}$, which is the ratio of the imaginary maximal power which could be obtained if both P and V attained their maximal value at the same time, divided by the maximal change in free energy, which really occurs at $P = 0$ when ν_t is maximal (Fig. 1). The parameter a/P_o can thus be considered as (efficiency)$^{-1}$.

5. Discussion

The hydrolysis of ATP by active muscle is composed of *three different* processes: (a) the generation of contractile force which is equal to the external load P, i.e. the contractile force is equal to P_o *only* in isometric contractions; (b) the development of velocity, not only the velocity of shortening of unloaded muscle; and (c) the production of mechanical power when $O < P < P_o$. The last process appears to be thermodynamically *reversible*, i.e. its efficiency is 100% for all values of $O < P < P_o$. In living muscle, the enthalpy change associated with processes (a) and (b) is fully converted into heat. This is not a 'waste'

because: (1) the generation of P_o and V_m is sometimes necessary even though no work is performed; and (2) when $O < P < P_o$ the work-producing reaction (associated with ν_w) cannot occur if it is not preceded by the 'creation' of both P and V.

The three processes follow each other in the sequence $\nu_p \rightarrow \nu_v \rightarrow \nu_w$. The value of the imposed load determines the values of both ν_p and V (through the load-velocity equation). The value of ν_v is linked to V. Once the muscle 'knows' what the values of P and V are, it proceeds to the splitting of ν_w for the production of the mechanical output PV.

The three processes differ not only kinetically (i.e. the three ν values differ numerically except when $P = O$ and $P = P_o$) but also thermodynamically (i.e. in the case of ν_p and ν_v, the enthalpy change is fully converted into heat whereas in the case of ν_w it is fully transformed into mechanical work. We may, therefore, speculate that the three processes, which appear to be three different *chemical reactions,* possess three different *molecular mechanisms.* As in solution $P = O$, only ν_v exists. Hence, the numerous works which have been carried out on the kinetics (and equilibria) of ATP hydrolysis in solution [usually by F-actin and heavy meromyosin (HMM) or its subfragment-1] relate only to ν_v and, therefore, have nothing to do with the tension developing reaction ν_p and with the work producing reaction ν_w in active muscle. Since ν_v is associated with the generation of velocity, the velocity of the actin filaments must be accelerated by their interaction with HMM also in *solution*, in the presence of MgATP, as has indeed, been demonstrated [8].

Only analysis of active muscles led to the *conclusion* that the total ATP used during muscle contraction is made of three separate fractions. The analysis also provided us with the rates of each of these reactions as functions of P. The solution studies cannot in principle be sufficient for the evaluation and characterization of the kinetics and energetics of muscle *fiber* contraction. If we want to elucidate the processes underlying muscular contraction there is no other way but to analyze the kinetics of contraction of muscle itself in terms of the rate of ATP splitting as a function of the load and that is exactly what we have done. For this purpose some assumptions had to be made and I believe that these were eventually justified.

Since ν_p is employed just for the development of force and, as ν_w is not expected to cause any increase in either P or V (which had already been 'paid' for by ν_p and ν_v, respectively), it may be concluded that when ν_p and ν_w are utilized the myosin heads interact with actin without any relative movement taking place. On the other hand, when ν_v is split, the interaction of the myosin heads with actin causes the relative movement of the two filaments. The distance covered must be related to Δl. Thus, when $0 < P < P_o$, the splitting of ATP may occur simultaneously in *two* different pathways, not just one as is generally taken for granted. Only one involves movement whereas the other does not. They have *different kinetics* and the rates of both depend (*differently*) on P. Only one exists at each end of the P region (at $P = 0$ and at $P = P_o$).

The division of the total ATP into fractions may be reminiscent of Hill's division of the heat production into maintenance heat and heat of shortening (Q_o and αV, respectively, [Eq. (11)]). However, Hill's distinction between the two heats has no physical significance and, as stated above, αV is just the difference between the total heat Q and the heat evolved under isometric conditions. Many attempts have been made to reveal what lies behind the heat of shortening but these led nowhere and just created confusion [5]. Had α been constant then one could say that the heat of shortening αV is the equivalent of $\nu_v = (1/\Delta l)V$ [Eq. (3)], but according to Eq. (12), for frog sartorius, α depends on P, moreover, the heat of shortening is not conspicuous in several muscle types [9].

ΔH_o and the corresponding ν_o [Eq. (16)] have been considered to be part and parcel of Q not only under isometric conditions but also for all other values of P (including $P = O$) as if the 'payment' for force production is always the same and equal to that of the isometrically contracting muscle. Thus, He et al. [2] state that 'in this calculation (that of the maximal efficiency), the energy input *includes* the ATP hydrolyzed to

maintain the isometric state' (even though at that point $P/P_o = 0.51$). Our analysis suggests that the expenditure for the generation of this force is not v_o but rather less [Eq. (2)]: $v_p = (P/P_o) \cdot v_o = 0.51$ v_o, i.e. only half of v_o.

The analysis performed above, and the conclusions derived from it, should apply to *all* striated muscles, and logically to smooth muscles as well.

As we have seen [Eq. (21)], $a/P_o = (\Delta G/P_o)/\Delta l$. The ratio $(-\Delta G/P_o)$ may change upon changing the temperature T if ΔG and P_o depend differently on T. It is, therefore, not surprising that the value of a/P_o changes with temperature [5].

It has been reported [10] that the value of Δl changes upon varying the sarcomere length (s.l.) at which shortening starts, which suggests the possibility that Δl is a function of the s.l. Inspection of Tables 1 and 2 reveals a difference between the values of Δl for the two muscles investigated: while it is equal to 0.067 for the rabbit psoas it assumes the value of 0.236 for the frog sartorius. Since the s.l. was 2.7 μm for the first and 2.2 μm for the second, it appears that Δl might decrease upon increasing the s.l. Thus, from Eq. (21) (i.e. a/P_o is inversely proportional to Δl) we may anticipate that, for the same muscle under the same environmental conditions, the value of a/P_o should increase upon increasing the s.l. This was, indeed, reported by Edman [11].

In all probability, our analysis should apply also to microtubular engines, which are so similar to the actomyosin systems. Particularly in view of the recently published report that the value of the step distance for kinesin stepping along microtubules is constant over a wide range of loads [12]. The step distance is 8 nm, which is the length of the tubulin dimer, whereas the value for muscle is 2.75 nm which is half the diameter of the actin monomer [3,13].

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