ENERGETICS OF ISOMETRIC AND ISOTONIC TWITCHES IN TOAD SARTORIUS

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ABSTRACT Contractile energetics have been studied in twitches of toad sartorius muscle at 6–7°C. Isometric and isotonic energy production has been measured and plotted against a wide range of developed tensions and tension-time integrals. These parameters were varied by altering the isotonic load or by changing the preset isometric length. The isometric tension-independent heat was 1.12 ±0.18 (SD) mcal/g. The isometric heat coefficient Pl_0/H was 12.0 ±1.4 in muscles having twitch to tetanus ratios ranging from 0.4 to 0.6. Isometric enthalpy increased monotonically with tension or tension-time integral but the correlation between isometric heat and these parameters was poor. Isotonic enthalpy consumption was always higher than isometric enthalpy for any given tension or tension-time integral; however, isotonic heat production was consistently less than isometric heat production. The isotonic heat for the highest load (3 g) was not significantly different from the isometric tension-independent heat. Thus isotonic heat production first decreased and then increased with increasing tension or tension-time integral. In the discussion it is shown that the results conflict with all current interpretations of muscle energetics.

INTRODUCTION

Since the classic experiments of Fenn (1923, 1924) and Hill (1938, 1949) there has been an increasing effort on the part of muscle physiologists and biochemists to account for the energy utilization of muscular contraction in terms of more or less independent energy compartments. The historical basis for this drive is understandable: the viscoelastic theory of muscle contraction (Gasser and Hill, 1924) predicted that the total energy of a muscle twitch was fixed and that this energy could be used to perform work or would be "wasted" as heat. Under these conditions the active heat production of isotonic contractions would be expected to be less than that for isometric contractions where no external work was done. Although the work of Fenn (1923, 1924) showed clearly that such was not the case in isolated frog muscles at 0°C, the viscoelastic theory was not completely rejected until the classical paper of Hill (1938) appeared expressing mathematical relationships between force and velocity, heat and shortening in such a way as to indicate that the

dynamic constants of Hill's "characteristic equation" (Hill, 1938) could be derived either from myothermic or mechanical measurements with excellent agreement.

Although this agreement is now known to be experimentally approximate rather than mathematically exact (Hill, 1964 *a*), the model of muscle as a two-component system comprising a contractile element governed by the characteristic equation in series with an undamped elastic element (Hill, 1938) has remained widely accepted from 1938 to the present day. The partition of muscle energy utilization between the various processes such as activation, shortening, the development of tension, or the performance of work remains a subject of some controversy, however (see Discussion).

In the long history of research into muscle energetics, the only paper in which both isometric and isotonic energy production are plotted over the full range of loads appeared in 1967 and described results obtained from cardiac muscle (Gibbs et al., 1967). There still exists no similar published work for skeletal muscle. Nevertheless, the logic of this type of experiment is obvious as discussed by Mommaerts (1969), and it is this approach which has been used in the present work to reinvestigate the contractile energetics of the skeletal muscle twitch. A brief report of part of this work has been communicated (Chapman, 1969).

In this paper the energetics of skeletal muscle twitches are reinvestigated using the sartorius muscle of *Bufo marinus*. It will be shown that the results obtained cannot be interpreted within the framework of any of the existing energetic balance sheets without giving rise to further and more serious problems. The subsequent paper (Chapman and Gibbs, 1972) outlines a more general energetic approach based on the results reported in this paper.

METHODS

Isolated paired sartorii from the toad *Bufo marinus* were used at 6–7°C. The physiological saline solution used in all the experiments contained (in millimoles per liter): NaCl, 111.1; KCl, 2.5; $CaCl_2$, 1.08; NaH_2PO_4, 0.5; Na_2HPO_4, 2.5; and glucose, 10.0. The solution was bubbled with oxygen.

The muscles used had an average mass of 399 mg, ranging from 224 to 543 mg. The resting length under a force of 1 g wt, defined herein as l_0, ranged between 3.2 and 4.1 cm with an average of 3.8 cm.

Mechanical Arrangements

Isometric Measurements. Muscle pairs were fixed below by screws driven into the acetabula, and the tibial tendons were tied together and attached via a light stainless steel tube to an aluminium isotonic lever. The lever itself was fitted with a pair of Ether 350-ohm, unbound P-type strain gauges for recording tension. The strain gauges formed part of a Wheatstone bridge circuit as described by Jewell et al. (1967). The output of the bridge circuit was amplified with a Brush DC amplifier Model 13-7304-00 (Brush Instruments Div., Clevite Corp., Cleveland, Ohio) and displayed oscillographically using a Grass Model 5D Polygraph (Grass Instrument Co., Quincy, Mass.). The muscles were stimulated directly by shocks of 3 msec duration delivered from the Grass S8 stimulator: the voltage was adjusted

to give a maximal mechanical response (10–15 v). The stimulating cathode was located 25 mm from the pelvic ends of the muscles. Isometric tension was measured at lengths from l_0 down to that for zero developed tension (l_0–1.2 cm). When a muscle was shortened it was always stimulated once at the new length to take up as much slack as possible before a heat measurement was made. It was observed visually that muscles remained at their preshortened lengths probably because of the weak viscous forces existing between the muscle and the thermopile surface.

Isometric force, recorded oscillographically, was normalized to give values of isometric tension or tension-time integral, i.e. $(l_0/m)P$ or $(l_0/m) \int P \cdot dt$ respectively, where l_0 is the muscle resting length in centimeters, m is the muscle mass in grams, P is the isometric force in g wt, and t is time. The tension-time integral was obtained by counting squares enclosed by the isometric myogram on the oscillograph record. This method was more reliable and rapid than the use of a planimeter. Electrical integration of the isometric myogram was not convenient because the potentiometer for balancing the strain gauge Wheatstone bridge circuit was not continuous.

Isotonic Measurements. The pivot of the isotonic lever was connected to a Brush Metripak angular position transducer the output of which was calibrated to measure length changes and was displayed oscillographically. The length signal was differentiated with an RC network of time constant 1 msec to determine the velocity of shortening or lengthening. All the isotonic contractions were afterloaded in random order of magnitude.

Internal Work. 10 muscle pairs were mounted on multielectrode assemblies, stimulated tetanically, and released to various loads to determine the series compliance after the manner of Wilkie (1956). The resulting compliance curves were integrated and normalized to give plots of internal work as a function of tension. Fig. 1 shows the complete set of de-

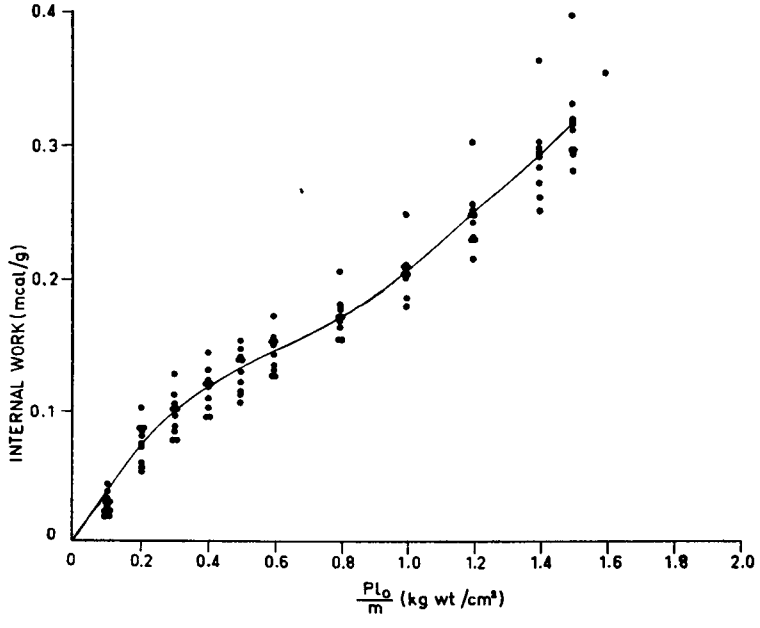

FIGURE 1 Pooled data from 10 tetanized muscles for internal work as a function of tension. Curve drawn by eye.

terminations for the 10 muscles. The average line was drawn by eye and its significance, when extrapolated to twitches of muscles mounted on thermopiles, is that it represents the minimum internal work that would be performed by the muscles as a function of the tension generated.

Heat Measurements

Two thermopiles of the type designed by Ricchiuti were used (Ricchiuti and Mommaerts, 1965). Their respective characteristics were: length of active region, 17.5 mm each; length of protective region, 6.5 and 6.0 mm; number of active junctions, 140 and 135; sensitivity, 4.95 and 4.50 mv/°C. The thermoelectric output of the piles was amplified with a chopper-stabilized Astrodata Nanovolt Amplifier, Model 120 (Astrodata, Inc., Anaheim, Calif.). The amplifier output was filtered such that upper 3 db point occurred at 25 Hz and the 6 db point at 56 Hz. The output was fed into a Tektronix 502A oscilloscope (Tektronix, Inc., Beaverton, Ore.) via a device for electronic heat loss correction. The Y-plates of the oscilloscope were connected to the inputs of a Grass Polygraph or a Brush Mark 280 pen recorder. The entire recording system was calibrated to within 1% accuracy.

Heat Loss Correction

The heat loss of the muscle-thermopile system was practically exponential and was corrected for by an integrating circuit with adjustable time constant. The appropriate time constant was determined by examining the time course of decay of the thermoelectric signal recorded from the muscle when heated with a radio frequency current. Heat losses ranged from 1.2 to 4.0%/sec.

Stimulus Artifact

The Nanovolt amplifier was usually momentarily blocked by electrical interference from the stimulus and it was necessary to determine the earliest moment after the stimulus artifact at which the oscillograph heat record was completely reliable. This was ascertained by connecting the output of the stimulator to the input of the Astrodata amplifier and applying a 3 msec pulse across the input. The magnitude of the pulse was adjusted to block the amplifier in a similar fashion to the worst block produced by the stimulus artifact in any of our physiological experiments. This stimulus together with a known AC signal was fed into the Astrodata amplifier and it was found that in this "worst case" situation it took 250 msec before the AC signal was recorded faithfully on the oscillograph. The 250 msec period of unreliability was caused by several factors. The maximum saturation time of the Astrodata Amplifier even with this severe overload is about 40 msec, but this effect is magnified by the filter networks used in conjunction with the Astrodata and the polygraph amplifiers and by the transient saturation of the polygraph amplifier by the artifact. In actual practice if the stimulus artifact is kept small, by having well insulated thermopiles, then the period of unreliability with our equipment can be reduced to about 30 msec (see Fig. 3). Since all our records were read 5 sec after the stimulus was applied to a muscle there is no possibility of signal error produced by the stimulus.

There remains the possibility that the stimulus artifact, on being integrated by the heat loss corrector, would have produced an error in the final value of the initial heat deflection which was read seconds after the stimulus. This possibility was ruled out by the following procedure. With the integrator set to correct for a loss of 4%/sec a simulated artifact somewhat larger than the worst encountered experimentally was applied directly to the Nanovolt amplifier in the absence of any other signal. The deviation from base line produced by integrating this

large simulated artifact was less than 2% of the average myothermic signals. The stimulus energy was generally about 5% of the isometric heat at l_0 and was subtracted before making the illustrations. The stimulus energy in each experiment was subtracted from the total energy values to determine all the means and standard deviations quoted in the text. The magnitude of the stimulus energy was determined by measuring the current and voltage in the stimulating circuit during the 3 msec pulse.

It was decided arbitrarily to multiply all the records of skeletal muscle heat production by 1.05 to allow for the heat capacity of the thermopile. This procedure is comparable with that used by Hill (1938) and Aubert (1956), but is likely to be a too generous allowance for experiments in which relatively bulky muscles (approximate mass 0.5 g) were used. In these cases the multiplication factor could have led to an overestimation of heat production by as much as 2%. This means that the uncertainty in heat measurements due to errors in determining the sensitivity of the thermopile, calibration errors in the amplifying and recording network, and inefficiency of the thermopile could have been as great as 4% with a bias towards overestimation.

Organ Bath

Temperature stability was achieved by using a large capacity water bath, the temperature of which was controlled with a Haake model NBS Ultrathermostat coupled to a Haake model K11 refrigeration unit. Experimental temperatures in the range 6–7°C were used because the slower time course of the mechanical events at these temperatures allows more accurate measurements of tension-time integrals. The muscles would not survive below 5°C, and at temperatures above 10°C the stimulus had to be raised to an extent that gave rise to objectionable stimulus heat artifacts.

Periods of heat measurements were restricted to 20 min, after which the physiological saline solution was returned to the muscle for at least 15 min before draining to make another set of heat measurements. The muscles were stimulated once every 90 sec between observation periods and the heats of single twitches were recorded at least 90 sec apart. In the isometric experiments the muscle was first stimulated at l_0 and then the muscle was shortened (the first two steps were usually 2 mm and subsequent shortenings were 1 mm). In the isotonic experiments at l_0 the different loads were applied in a random sequence.

RESULTS

Energetics of Isometric Twitches

The relationships between heat and tension, heat and tension-time integral, were determined in 22 muscles and typical results from two muscles (upper and lower) are illustrated in Fig. 2. These curves were obtained by recording the heat production and tension of single twitches at different muscle lengths (see Methods). The solid circles represent total energy (including internal work) and the open circles represent total energy minus internal work extrapolated from the curve of Fig. 1. It was generally found that subtraction of internal work destroyed any significant correlation between heat and either tension or tension-time integral for values of tension less than about 50% of the value at l_0. The curves of heat vs. tension were always more curvilinear than those for heat vs. tension-time integral.

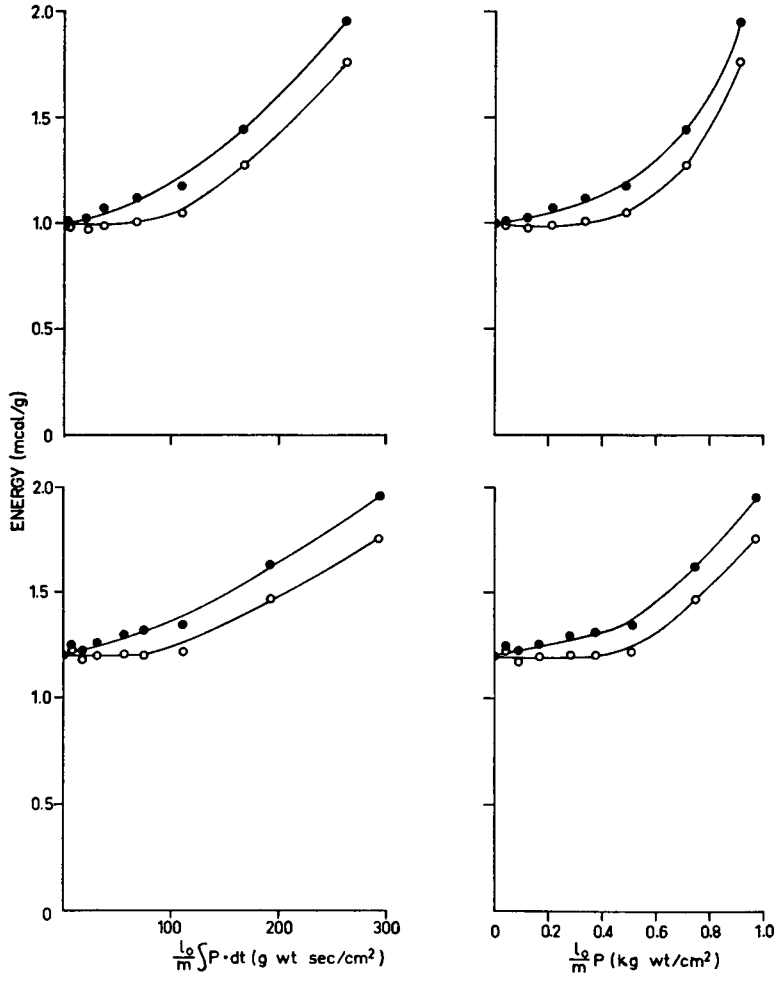

FIGURE 2 Isometric energy curves for two muscles (upper and lower) plotted against tension-time integral (left) and tension (right). Filled circles, total enthalpy; open circles, total enthalpy minus contractile element work. Upper curves: mass, 420 mg; l_0, 3.35 cm. Lower curves: mass, 499 mg; l_0, 3.75 cm. These curves have been obtained by shortening the muscles using the procedure described in the text. Each point represents one twitch at a particular muscle length, the point on the far right of each plot being the response obtained at l_0.

The intercept of the isometric energy curves with the ordinate will be referred to as the tension-independent heat at this stage: in the 22 muscles the intercept had a mean value of 1.12 $\pm$0.18 (SD) mcal/g. This value compares with similar values of tension-independent heat production for frog sartorius reported by Hill (1949) and Gibbs et al. (1966). The isometric heat coefficient Pl_0/H, where H is expressed in g wt cm, had a mean value of 12.0 $\pm$1.4 (SD) comparable with the values found for frog muscle using instruments with minimum compliance (Hill, 1965). The

difference between the isometric heat at l_0 and the tension-independent heat was of the order of 1 mcal/g, compared with differences of the order of 2 mcal/g for frog muscle at 0°C (e.g., see Bendall, 1969). The larger difference in frog muscle is probably associated with the large twitch to tetanus ratio of frog sartorius at 0°C. The toad sartorius at 7°C had a twitch to tetanus ratio ranging from 0.4 to 0.6. The maximal tetanic tensions were in the range 2–3 kg wt/cm².

Energetics of Isotonic Twitches

Fig. 3 shows original oscillograph records obtained during an isotonic contraction of a muscle against an afterload of 25 g: the preload was 1.5 g. The upper record (*P*) is of contractile force which rose isometrically to a plateau during which the muscle shortened and relaxed against the load as indicated by the records of length (*L*) and velocity (*V*). In these records shortening and rate of shortening appeared as downward deflections. The heat record (*H*) was the thermoelectric output of the thermopile in microvolts; the stimulus artifact marked the moment of stimulation and the heat record was unreliable for 30–250 msec (see Methods). The final mechanical event was isometric relaxation. The isotonic plateau in the force record contains a slight "ringing" due to absence of damping, and a slight curvature due to a small decrease in mechanical advantage as the muscle shortened and thereby changed the orientation of the angular position transducer. When the muscle relaxed against the load, the external work performed by the muscle in lifting the load was partly returned to the muscle as heat and was partly dissipated as degraded kinetic energy

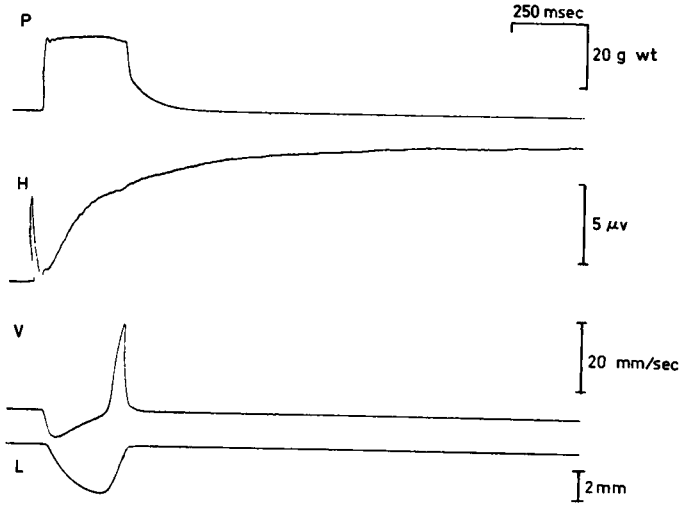

FIGURE 3 Oscillograph record of an afterloaded isotonic contraction. *P*, isometric force; *H*, heat production; *V*, velocity of lengthening (upward deflection); *L*, length of muscle (shortening downward).

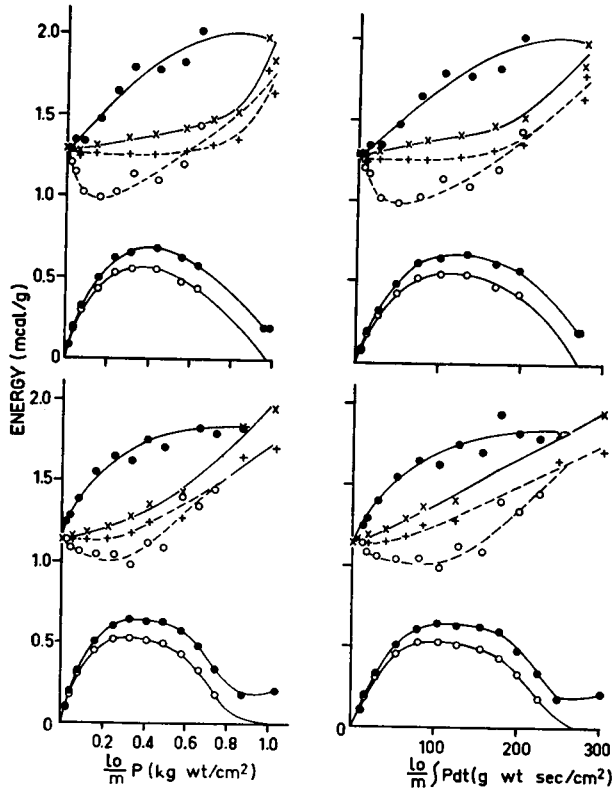

FIGURE 4 Isotonic (circles) and isometric (crosses) energy curves for two muscles (upper and lower) plotted against tension (left) and tension-time integral (right). Further explanation in text. Upper curves: mass, 474 mg; l_0, 3.80 cm. Lower curves: mass, 461 mg; l_0, 3.80 cm.

as the lever hit the afterload stop; this latter part of the dissipated energy does not appear in the total heat recorded by the thermopile and has to be added to it in order to obtain an accurate value for total energy utilization during isotonic contractions. The kinetic energy ranged from negligible values to values as high as 7% of the total energy. The kinetic energy imparted to the load by the muscle during isotonic shortening was always less than 2% of the total energy. This estimate allows for the equivalent mass of the isotonic lever and connecting rods (820 mg). This kinetic energy was, of course, returned to the muscle as heat during isotonic deceleration in the shortening phase.

Isotonic energy curves were determined in 14 muscles and representative results are shown for two muscles (upper and lower) in Fig. 4. Energy has been plotted as a function of tension (left) and tension-time integral (right). In each graph the set of curves with energy intercepts of the order of 1.0–1.5 mcal/g represents total isotonic energy utilization (filled circles), total isotonic energy minus contractile

element work (open circles), total isometric energy utilization (×) and total isometric energy minus internal work (+). The curves emanating from the origin in each graph represent isotonic contractile element work (filled circles) and isotonic external work (open circles). Contractile element work is the sum of internal and external work, the internal work being obtained from Fig. 1. The isometric energy curves were determined by the method described in the preceding section.

(*a*) In the limit of zero contractile element work all four energy curves approached the ordinate with similar values of energy utilization.

(*b*) The curve of isotonic energy minus contractile element work dipped significantly below its isometric counterpart at light to moderate loads before reapproaching the latter at heavy loads.

(*c*) The dipping of the isotonic curve referred to in point *b* resulted in some of the values of isotonic energy minus contractile element work being appreciably lower than the tension-independent heat determined by the isometric method.

This behavior can be quantitated for the 14 muscles in the following way:

(*a'*) The mean difference between the total isotonic energy utilization for the lightest load (3 g) and the tension-independent heat production, expressed as a percentage increase of the tension-independent heat, was 10.2 ±8.2% (SD).

(*b'*) The mean difference between the isotonic energy minus contractile element work for the lightest load and the tension-independent heat production expressed as for *a'* was −0.4 ±−6.6%.

c' The minimum value of the isotonic energy minus contractile element work curve was 11.4 ±5.0% lower than the tension-independent heat production.

DISCUSSION

The current problems and controversies of contractile energetics have been discussed most recently in two general muscle reviews (Peachey, 1968; Bendall, 1969) and in an exhaustive specialized review by Mommaerts (1969). The main controversy has centered around the existence of the "shortening heat," classically described by A. V. Hill (1938, 1949, 1964 *a*) but questioned by Carlson et al. (1963) and McCarter and Ramsey (1968), while the existence of the activation heat (Hill, 1949; Gibbs et al., 1966; Homsher and Ricchiuti, 1969) has also been questioned by Jöbsis and Duffield (1967). From these various differences of opinion have emerged three main equations purporting to account for the initial enthalpy of a muscle twitch:

$$E = A + W \text{ (Carlson et al., 1963)}, \tag{1}$$

$$E = A + W + a \cdot x + h \text{ (Hill, 1964 } b\text{)}, \tag{2}$$

$$E = k_1 W + k_2 \int P \cdot \mathrm{d}t + k_2 \int S \cdot \mathrm{d}t \text{ (after Jöbsis and Duffield, 1967)} \tag{3}$$

where A = activation heat, W = contractile element work, a = shortening heat

constant, x = distance shortened, h = heat associated with the persistence of tension, $\int P \cdot dt$ = force-time integral, $\int S \cdot dt$ = shortening-time integral, and k_1 k_2, and k_3 are constants.

Mommaerts (1969) has shown that the difference between equations 1 and 2 is more apparent than real because A as defined by Carlson et al. (1963) is not the true activation heat, but contains in addition heats due to shortening and the presence of tension which happen to sum to a roughly constant term in frog sartorius at 0°C. Therefore Mommaerts has suggested a more general formulation of equation 1:

$$E = A + W + a \cdot x + f(P, t) \text{ (Mommaerts, 1969).} \qquad (1\,a)$$

A comparison of equations 1, 2, and 3 shows that although the compartmentalization of energy flux is a controversial subject, all three mutually exclusive interpretations are agreed in treating work (W) as an essential but mathematically independent component of the energy flux. It is the purpose of the remaining discussion in this and the following paper to show that this treatment is not essential, and may be wrong.

Now it is explicit in equation 1 and at least implicit in equations 2 and 3 that when extra energy (adenosine triphosphate, ATP) is "mobilized" for the performance of work, the chemomechanical transduction occurs such that all the "work" enthalpy in fact appears as real work. It is odd that only last year was the singularity of this strange relationship pointed out for the first time (Mommaerts, 1969). As it stands it means either that the enthalpy and free energy of ATP hydrolysis in vivo are equal and the chemomechanical transduction process is 100% thermodynamically efficient, or that the free energy of ATP hydrolysis in vivo is greater than the corresponding enthalpy and the thermodynamic efficiency of transduction is such as to produce a coincidental equality between enthalpy and real work. It could be argued that the $a \cdot x$, h, and $k_2 \int P \cdot dt$, $k_3 \int S \cdot dt$ terms of equations 2 and 3 respectively allow for thermodynamic inefficiency of transduction, but, if this is so, it is misleading to make W mathematically (and therefore, by implication, energetically) independent of these terms.

The question arises of which of tension and tension-time integral is the better parameter against which to plot isometric energy utilization. No clear answer is apparent because, when internal work is taken into account, much of the correlation between heat production and either tension or tension-time integral is lost.

The high value of the isometric heat ratio Pl_0/H in muscles with a twitch to tetanus ratio of 0.4–0.6 is perhaps indicative of a greater economy of energy utilization in toad sartorius muscle compared with that of the frog at 0°C where the isometric heat coefficient tends to be slightly lower with a much greater twitch to tetanus ratio.

The present results also show clearly that the total initial enthalpy of isotonic

twitches was always greater than that of isometric twitches developing either the same tension or the same tension-time integral. One might be tempted to call this a demonstration of the Fenn effect were it not for the fact that the extra energy mobilized for isotonic work in these experiments was not even sufficient to account for the work alone, whereas the Fenn effect is usually taken to imply the mobilization of extra energy in excess of the work, the excess being generally known as the shortening heat (Hill, 1938, 1949 *a*; Mommaerts, 1969). In the present experiments the total enthalpy minus work for isotonic contractions was always considerably less than that for isometric contraction. Although this is the first time such an effect has been described it should be noted that the form of the isotonic total enthalpy curves is similar to that obtained by Jöbsis and Duffield (1967) for toad sartorius using a biochemical technique, and Fischer (1931) and McCarter and Ramsey (1968) for frog sartorius using oxygen consumption methods. The only other instance of an enthalpy minus work curve which dips below both its extreme values is that of Hill (1964 *c*) where heat is plotted as a function of shortening velocity for contractions at constant shortening velocity using an ergometer; however, in those experiments Hill obtained values of total enthalpy at light loads comparable with the enthalpy of isometric twitches at l_0, as did Carlson et al. (1963). This was taken to mean that a large shortening heat component was present in the lightly loaded isotonic contractions of the frog sartorius at 0°C (Mommaerts, 1969).

In the present experiments there is no room to accommodate a shortening heat component in the isotonic contractions because the isotonic heat curves always fell below the isometric ones and the enthalpy of lightly loaded isotonic contractions was comparable with the isometric tension-independent heat production, rather than with the isometric heat at l_0. Clearly, isometric contractile element shortening could never be greater than isotonic contractile element shortening at the same tension.

The only other possible interpretation already in the literature is that of Jöbsis and Duffield (1967), who account for the enthalpy of twitches by the relation given in equation 3. The constants were evaluated for four of the muscles by fitting the total energy, tension, and shortening data empirically to three simultaneous equations and solving for k_1, k_2, and k_3 after the manner of Jöbsis and Duffield (1967). These workers obtained a value of about 80 for the ratio of the energy cost of 1 cm-sec of shortening-time integral to the cost of 1 g wt-sec of force-time integral (F. F. Jöbsis, personal communication) whereas the four muscles analyzed in the present work gave ratios of 68, 70, 62, and 175 respectively for the same quantities. The muscle weights were 499, 474, 398, and 224 mg, respectively, the first three corresponding to the range used by Jöbsis (personal communication). It is difficult to compare these ratios as the quantities P, S, and W were not normalized for the analysis. Two further difficulties arise from this type of analysis. First, the constant k_1 had values of 1.2, 1.7, 1.5, and 1.4 respectively for the four muscles analyzed in this way. This suggests that there is a "work heat" component of the heat production

in addition to the heat associated with shortening and force. Secondly, this analysis does not allow for a heat of activation in the classical sense (Hill, 1949) whereas the existence of a heat component independent of myofilament activity is indicated from energetic considerations (see following paper).

As they stand, the present results only add to the confusion, without resolving the controversies already mentioned. Rather than invoking some special mechanisms to interpret the behavior of toad sartorius at 6–7°C it seems more profitable to develop a general approach to muscle energetics that has a wider applicability to all types of muscles. This is attempted in the following paper (Chapman and Gibbs, 1972).

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