Energetics of Contraction in Phasic and Tonic Skeletal Muscles of the Chicken

JACK A. RALL and B. A. SCHOTTELIUS

From the Department of Physiology and Biophysics, The University of Iowa, Iowa City, Iowa 52242. Dr. Rall's present address is the Department of Physiology, University of California at Los Angeles School of Medicine, Los Angeles, California 90024.

ABSTRACT Comparative energetics of chicken latissimus dorsi muscles, tonic anterior (ALD) and phasic posterior (PLD), were investigated by measuring initial heat production. Heat components were analyzed in terms of the equation:

$$E = A + W + \alpha_F(\bar{\Delta}L) + f(P, t)$$

As the muscles were stretched by increments, heat produced in isometric twitches and tetani decreased in a linear fashion. Two processes are involved: one tension independent, the activation heat, or A; and the other tension dependent, $W_i + \alpha_F(\bar{\Delta}L) + f(P, t)$. In twitches, A, per unit tension, is equivalent in the PLD and ALD. Tension-dependent heat, per unit tension, is greater in the PLD due to W_i; but tension-time-related heat, $f(P, t)$, per unit tension, is similar in both muscles. In tetanic contractions, differences in A and $f(P, t)$, per unit tension, are attributed to the greater $V_{\max}$ in the PLD. The differences in the energetics of isometric contractions in the PLD and ALD, therefore, can be explained by inherent differences in tension development, compliance, and myosin and reticular ATPase activities. Data from isotonic twitches were quantified by means of the equivalent tension technique. Both muscles exhibited an extra heat associated with shortening, $\alpha_F(\bar{\Delta}L)$. In the PLD, the ratio α_F/P_{ot} is greater; it is load independent and ½ the value of a/P_o in both muscles. Enthalpy efficiency, $W_e + W_i/E$, is comparable in both muscles. A Fenn effect is observed only when isotonic energy liberation is compared to a decreasing isometric energy expenditure base line.

INTRODUCTION

Tonic muscles differ in structure and function from phasic muscles. Until recently (Canfield, 1971), there was a paucity of information about the energetic consequences of these differences. Because the anterior latissimus dorsi muscle (ALD) of the chicken is a tonic muscle and the posterior latissimus dorsi muscle (PLD) a phasic muscle (Page, 1969), they offer an opportunity to compare the energetics of nearly homogeneous tonic and phasic muscles from the same animal.

Mommaerts (1969) attempted to reconcile many discrepancies by proposing the following modification of Hill's (1964) energy flux equation:

$$E = A + W + \alpha_F(\bar{\Delta}L) + f(P, t),$$

where E = total initial energy, A = net activation heat, W = work done, $\alpha_F(\bar{\Delta}L)$ = shortening heat redefined with reference to an equivalent force rather than an equivalent length as suggested by Hill (1938), and $f(P, t)$ = heat associated with development and maintenance of tension. The latter term differs from Hill's (1964) feedback heat in that it would be liberated predominantly during the rise of tension and not merely during relaxation.

The above formulation provides a means of comparing energetic data from the ALD and PLD. The net activation heat, A, is probably the enthalpy change of ATP hydrolysis associated with pumping of Ca^{++} back into the sarcoplasmic reticulum (SR) (Homsher et al., 1972; Smith, 1972). Since the ALD fiber has no regularly occurring transverse tubular system and only reduced amounts of SR compared to the PLD (Page, 1969), it is appropriate to ask if these structural differences are reflected in the Ca^{++} pumping mechanism. Such differences might be expressed in the activation heat component of these muscles. Based on the lack of an initial burst of heat in a tetanus, Canfield (1971) has suggested that the activation heat may be absent in the ALD.

Tension-time-related heat, $f(P, t)$, can be evaluated from isometric twitches where the development of tension would predominate or from tetanic contractions where the inclusive effect of development and maintenance of tension would be of major importance. The economy (inverse of the maintenance heat rate) of tension maintenance in the tonic ALD is much greater than in the phasic PLD (Canfield, 1971). This economy could derive from a more efficient conversion of chemical to mechanical energy, as is implied from the data for the slow tortoise rectus femoris muscle (Woledge, 1968). If so, the amount of energy dissipated in tension development in a twitch per unit tension would be less in the tonic ALD. Alternatively, the energy conversion mechanism may function qualitatively the same at the cross bridge level in tonic and phasic muscles. According to Rüegg (1971), V_{max} reflects differences in economy, hence the faster PLD (V_{max} is five times greater than in the ALD) may be less economical because it cycles fivefold more bridges in the same span of time. It would utilize more ATP per unit time in the making and breaking of bridges and the pumping of Ca^{++} back into the SR; and the magnitude of A and $f(P, t)$, per unit tension-time, would be about five times greater in the PLD. Indeed, Goldspink et al. (1970) measured ATP hydrolysis and reported that the ALD was more economical in tension maintenance, but less efficient in performing external work than the PLD. If so, the enthalpy efficiency, $(W_e + W_i)/E$, would be less in the ALD and there would be a greater shortening heat, $\alpha_F(\overline{\Delta}L)$.

We have employed the stretch technique (Homsher et al., 1972; Smith, 1972) to estimate A, and the equivalent tension technique (Gibbs et al., 1967) to determine $\alpha_F(\overline{\Delta}L)$. All the components of the modified equation were quantitated in isometric and isotonic contractions of the PLD and ALD. Subsequently, we compared the energetics of these phasic and tonic muscles.

METHODS

Nerve-Muscle Preparation

Nerve-muscle preparations were obtained from 11–21-day old chicks (Cornish Cross) under Na-pentobarbital anesthesia. The muscles and their innervation are described by Ginsborg (1960 *b*). The ALD was removed, along with its attachment to a small part of the humerus at the peripheral end and to the spinal vertebra at the central end. Mean, blotted muscle weight (35 muscles) was 63.6 ± 2.6 mg (SE of mean); average length was 2.27 ± 0.02 cm. The PLD was isolated also with the spinal vertebra attached; the distal tendon, which inserts into the humerus, formed the other connection. The PLD had a mean weight of 43.9 ± 1.4 mg (22 muscles) and length of 1.82 ± 0.01 cm.

A single nerve-muscle preparation was mounted vertically on the thermopile assembly within the muscle chamber and incubated in avian Ringer for 1 h. The incubating solution contained (millimolar): NaCl, 150; $NaHCO_3$, 20; KCl, 5; $CaCl_2$, 5; $MgCl_2$, 2; and glucose, 11, and was gassed with 95% O_2; 5% CO_2 (Ginsborg, 1960 *a*). During this equilibration period, stimulus parameters were adjusted to supramaximal values through test shocks, and L_o defined as that length where maximum twitch tension was produced. Experiments were performed at room temperature which averaged 20.8° ± 0.1°C.

Stimulation

When recording heat production, the muscles were stimulated through their nerves, since the correction for warming of the muscle by the stimulus can be intolerably large with direct stimulation (Canfield, 1971). Nerve stimulation produced about 10% less tension; but, as verified in curarized nerve-muscle preparations, produced no heating artifacts. Muscles were stimulated directly in quick-release experiments. Nerve stimulation was via silver wire electrodes of 25 μm diameter; direct stimuli were applied through three platinum electrodes, 55 μm thick, arranged in the plane of the thermopile as described by Hill (1965). Capacitor discharges were employed (0.5 μF for direct and 0.05 μF for nerve stimulation).

Mechanical Measurements

The spinal attachment of the ALD or PLD was mounted in a Lucite clamp affixed to the bottom of the thermopile frame at an angle approximating its *in situ* position with the wing extended. Connection of the ALD to the transducers was via fine stainless-steel tubing and a stainless-steel hook inserted through the core of the humerus. The PLD tendon was tied directly to a hook with a small piece of silk thread; the hook, in turn, was attached to the stainless-steel tubing leading to the transducers.

Isometric tension was detected by an 8 ounce Statham strain gauge (Statham Instruments, Inc., Oxnard, Calif.). Length changes were measured by a Sanborn (Sanborn Co., Waltham, Mass.) linear variable differential transformer (LVDT 7DC-DT-050) whose core was attached to a light magnesium lever. Movement of the lever displaced the core within the LVDT generating a voltage output proportional to muscle length. The lever ratio was 18:1, and the equivalent mass of the lever and connectors was 740 mg for the PLD and 940 mg for the ALD. Isometric and isotonic system compliances were 1.3 and 2.5 μm/g weight, respectively. Length or tension changes were monitored on an Offner (Offner Electronics Inc., Schiller Park, Ill.) type RS dynograph recorder.

Quick-release experiments were performed as described by Jewell and Wilkie (1958). The muscles were stimulated repetitively; after peak tension was developed, a relay-activated stop was withdrawn and the muscles shortened against a preselected load. Stress-strain curves obtained were graphically integrated to determine internal work. Force-velocity relations were derived from the slow component of the quick-release records. The force-velocity equation was rearranged into the form:

$$\frac{P}{P_o} = \frac{b}{L_o}\left[\frac{L_o(1 - P/P_o)}{V}\right] - \frac{a}{P_o}$$

and data linearized by plotting P/P_o vs. $L_o(1 - P/P_o)/V$. The constants a/P_o and b/L_o were determined from the intercept and slope, respectively, of the resulting line.

Heat Measurements

Heat production was measured with a thermopile constructed by a modification of Hill's technique (1965). 1 mil constantan and chromel wires were flattened to about twice their original diameter, overlapped, and electrowelded to form individual couples. These couples, placed eight to each millimeter, were sandwiched between two pieces of 12.5 μm Kapton insulation (Kapton, provided by the E. I. DuPont de Nemours and Co., Wilmington, Del., has a heat capacity 70% that of mica), and held in place with epoxy. This thermopile had a 16 mm active region bounded by 1 mm protective regions and a physical thickness of approximately 50 μm. The active region consisted of four, 4-mm sections which could be used individually or in series. At 20°C, sensitivity of the whole thermopile was 7.64 mV/°C. Output of the thermopile was amplified by a Dana series 3500 amplifier (Dana Laboratories, Inc., Irvine, Calif.) and displayed on an Offner dynograph recorder along with length or tension changes.

Thermopile output was converted to millicalorie per gram as described by Hill and Woledge (1962). Records were corrected for thermopile heat capacity, some 3–5% of the total heat capacity, and for an exponential heat loss, about 5% of the heat produced in an isometric twitch. Because the overall correction was small, heat loss was normalized to a total muscle-thermopile heat capacity. Expressed in this fashion, the heat loss coefficient (Wilkie, 1968) is 2.89 mcal/deg per s ($n = 14$) for the ALD and 2.54 ($n = 8$) for the PLD. The error introduced by neglecting the variation of heat loss with length, particularly at stretched lengths, in a single twitch is less than 2% of the total initial heat production. In tetanic contractions where heat loss becomes significant, it was determined at each length where heat was measured and was compensated accordingly. Only total heat quantities were important in these experiments; thus no attempt was made to correct the time-course of the heat records for conduction delays.

Since temperature was recorded from only a portion of the muscle length, it is important to show that the part sampled is representative of the whole length. In the ALD, temperature was monitored from 70% of the muscle length during an isometric contraction at L_o and 53% during an isotonic contraction. Temperature elevation recorded with the whole thermopile (16 mm) during an isometric twitch was compared to that measured in shortening muscles when only the lower 12 mm of thermopile was used. The average temperature difference between the two active regions was 1.2 ± 1.7% ($n = 6$). Other combinations of active regions (lower ½ vs. whole, lower ½ vs. lower ¾, upper ¾ vs. whole, and center ½ vs. upper ¾) gave an average difference of 0.5 ± 1.5% ($n = 4$). In the PLD, temperature was recorded from 88% of the muscle length in an isometric contraction at L_o and 66% in an isotonic contraction. The mean recorded temperature difference between the lower 12 mm and the whole thermopile was 2.3 ± 1.1% ($n = 10$). As muscles are stretched, the the muscle length sampled declines, but so does the tension which causes thermal inequalities. At extreme stretch, 50% of the muscle length was monitored from the ALD and 65% from the PLD.

To test for sampling errors due to unequal temperature distribution occurring with relaxation in isotonic twitches, temperature measured by the lower ½ of the thermopile was compared to that of the lower ¾. In an experiment with the ALD, the isometric condition plus four loads ranging from 0.2 to 0.7 P/P_{ot} were examined. The average difference was 1.8 ± 1.3% of the temperature elevation measured from each active part. The same type of experiment with the PLD yielded an average difference of 0.8 ± 2.3% for observations at two different loads. In all isotonic experiments, data from two to three twitches were averaged and no alteration of heat output was ever observed. Thermal inequalities do not appear to have affected the results of this investigation.

Plan of Experiments

Our experiments are divided into three categories: (1) energetics of isometric twitches, (2) energetics of isometric tetanic contractions, and (3) energetics of isotonic twitches.

In the first group of experiments, tension-independent and tension-dependent heat were estimated; and force-velocity and stress-strain characteristics were evaluated. Heat production in single isometric twitches at lengths greater than L_o was measured and plotted against developed tension. During a measuring period, or series, the muscle was stimulated at 60–90-s intervals. Generally, a series started at L_o, went to longer lengths, and then returned to L_o; the order did not make a difference. Two to three twitches were examined at each length. Subsequently, the incubating medium was reintroduced around the muscle for 20–30 min. Quick-release experiments were performed on the same muscle to determine internal work and the constants of the force-velocity relation. In these, a 5 min rest interval followed each tetanic contraction.

In the second group of experiments, tension-dependent and tension-independent heat production in isometric tetanic contractions was analyzed. Heat was measured at various stretched lengths and plotted against the time-integral of developed tension. Both muscles were stimulated at a rate that produced maximum fused tension: the ALD at 40 Hz and the PLD at 90 Hz. During a series, two contractions were elicited at a selected muscle length and the results averaged. Then the muscle was rested for 10–15 min in the incubation solution.

The third group of experiments employed the equivalent tension technique (Gibbs et al., 1967) to estimate the thermal effect of shortening and work, and to determine enthalpy efficiency. Heat was recorded and plotted against tension developed in isometric twitches at stretched lengths, and against the load lifted by the muscle in isotonic twitches. During an isotonic measurement series, muscles were stimulated every 60–90 s, starting and ending with an isometric twitch. In between, loads were applied randomly to the muscle. The results from two to three contractions at each load were averaged. Usually, a 5 mm protective region was employed for isotonic heat measurements.

RESULTS

1. *Energetics of Isometric Twitches*

GENERAL MECHANICAL AND THERMAL CHARACTERISTICS In a typical experiment (Fig. 1), the PLD developed more tension and produced more heat, as shown by a 3 mdeg temperature elevation compared to 0.7 mdeg in

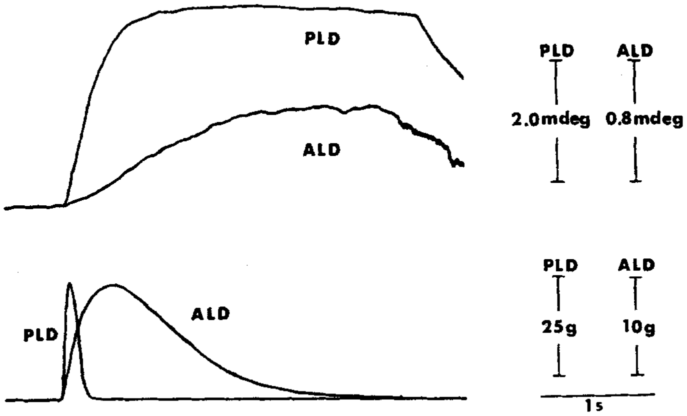

FIGURE 1. Heat and tension records for isometric twitches of the PLD and ALD. The PLD developed 2.5 times more tension (lower traces) and produced 4.5 times more heat (upper traces). Time-to-peak twitch tension was four times less in the PLD.

the ALD. Table I displays average values for mechanical and thermal characteristics of the PLD ($n = 10$) and ALD ($n = 20$). Time-to-peak tension and half-relaxation times were four- to sixfold less in the PLD. Mean twitch tension per cross-sectional area, $P_{ot}L_o/M$, in the PLD was 2.4 times greater than in the ALD, whereas twitch-to-tetanus ratios were comparable. Heat per unit mass (Q_o) produced by the PLD was 3.8 times greater; the resulting isometric heat coefficient, $P_{ot}L_o/Q_o$, was about 0.7 that in the ALD.

TENSION-INDEPENDENT AND TENSION-DEPENDENT HEAT PRODUCTION As muscles were stretched incrementally, heat produced in an isometric twitch decreased in a linear fashion. In a typical experiment for an ALD (Fig. 2), both heat and tension decreased as the muscle was stretched from L_o to 1.4 L_o. In analyzing these results (Fig. 3), heat is plotted against tension, P_s, developed at stretched lengths as a fraction of the tension, P_{ot}, at L_o. Although heat output decreased with stretch, the data indicates, after least-squares regression analysis, that appreciable heat would be present at zero tension. In both muscles, the analysis suggests that there are two components of heat production: one tension independent, A, and the other tension dependent, K. In all experiments, the ALD muscles could be stretched reversibly, and on the average, 90% of the tension was eliminated. The mean correlation coefficient was 0.984. In the PLD muscles, which are more resistant to stretch, only 55% of the tension was eliminated, but a linear relaxation existed as shown by an average correlation coefficient of 0.991.

TABLE I

SUMMARY OF MECHANICAL AND THERMAL PROPERTIES FOR TWITCHES OF PLD AND ALD MUSCLES

	PLD ($n = 10$)	ALD ($n = 20$)
Time to peak tension (ms)	79±6*	319±12
Time for half relaxation (ms)	82±6	508±17
a/P_o, tetanus	0.27±0.01	0.11±0.002
V_{max}, tetanus (L_o/s)	9.8±0.4	1.9±0.1
$P_{ot}L_o/M$ (kg/cm^2)	1.12±0.04	0.46±0.02
Twitch/tetanus ratio	0.61±0.02	0.53±0.01
Q_o (mcal/g)	2.80±0.12	0.74±0.03
$P_{ot}L_o/Q_o$	9.40±0.30	14.50±0.30
A (mcal/g)	0.49±0.03	0.19±0.01
K, or $W_i + \alpha_F(\bar{\Delta}L)_i + f(P, t)$ (mcal/g)	2.32±0.10	0.55±0.03
W_i (mcal/g)	0.94±0.06	0.11±0.01
Maximum W_e/E	0.44±0.03	0.55±0.04
α_F/P_{ot}	0.14±0.02	0.07±0.02

* Mean ± SE of mean.

Tension-independent heat was 18% of that produced in an isometric twitch at L_o in the PLD and 26% in the ALD (Table I). On an absolute basis, these values are markedly different, but so was the tension developed. If A values are expressed relative to maximum twitch tension per cross-sectional area, $P_{ot}L_o/M$, as suggested by Homsher et al. (1972), they are the same within 7%. At L_o, the tension-dependent heat, K, is 4.2 times greater in the PLD (Table I), but expressed relative to $P_{ot}L_o/M$, it is only 1.8 times larger. Components of the tension-dependent heat, W_i, $\alpha_F(\overline{\Delta L})_i$, and $f(P, t)$, can be separated partially by determining W_i due to stretching of the compliant elements in series with the contractile element (Table I). The series compliance in an isometric twitch averaged 10% of L_o for the PLD and 3.4% of L_o for the ALD.

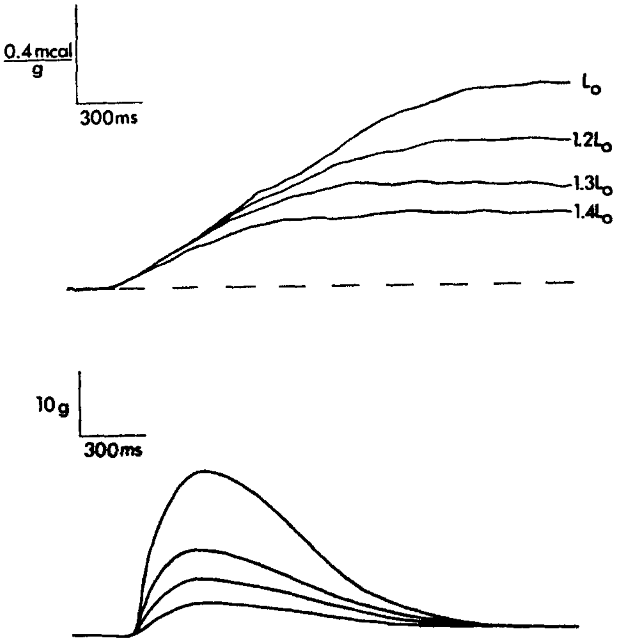

FIGURE 2. Decrease in heat and tension with stretch in the ALD preparation. As this ALD was stretched from L_o to 1.4 L_o, heat, upper traces, decreased by 65% and tension lower records, diminished by 82%.

TENSION-DEPENDENT AND TENSION-INDEPENDENT HEAT VS. MAXIMUM TWITCH TENSION If the slope of the heat vs. tension curve represents tension-dependent heat, K, it should be proportional to maximum twitch tension, $P_{ot}L_o/M$. This heat was so correlated in both the PLD ($r = 0.83$) and ALD ($r = 0.88$). In contrast, A did not show a significant correlation with maximum twitch tension, $r = 0.62$ for the PLD and 0.36 for the ALD. Because A per $P_{ot}L_o/M$ was essentially the same in both muscles, these results have been pooled to increase the tension range available for analysis. Under these circumstances, A was directly proportional to $P_{ot}L_o/M$ ($r = 0.94$). However, the meaning of this dependence should be emphasized. In a given preparation, A should be a function of the amount of Ca^{++} released (Homsher et al., 1972; Smith, 1972); it is assumed to remain unchanged as tension decreases with stretch and is, therefore, tension independent. This assumption constitutes the basis of the stretch technique. But in dual pulse-potentiation experiments (Smith, 1972), for example, A increased in parallel with the tension increase caused by a second shock, i.e. more Ca^{++} was apparently released by a second shock, producing more tension. These results indicate that the amount of Ca^{++} released, of which A is a function, varies but only with maximum twitch tension (Smith, 1972).

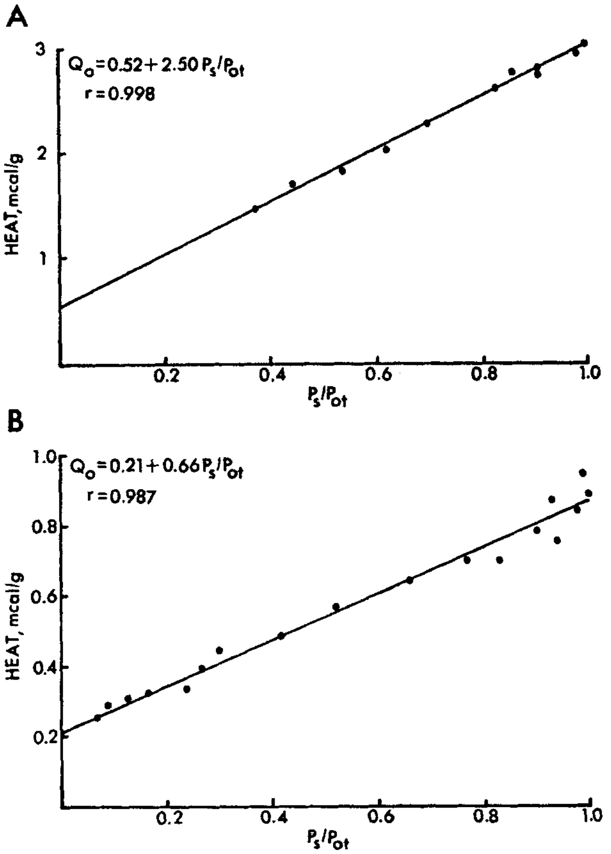

FIGURE 3. Comparison between isometric heat and twitch tension in a PLD (*a*) and an ALD (*b*) stretched beyond L_o. In this PLD, heat decreased in a linear fashion as a function of tension. The equation is the linear regression relation between heat, Q, and developed tension, P_s/P_{ot}, at various muscle lengths; r is the correlation coefficient. According to this interpretation 18% and 25% of the total initial heat at L_o was independent of tension in the PLD and ALD, respectively.

TWITCH-TO-TETANUS RATIO VS. LENGTH Evidence for the assumption that A is length independent is obtained from the variation of the twitch-to-tetanus ratio with muscle length (Smith, 1972). Assuming that the maximum fused tetanus tension represents the fully active state and that activation in the tetanus is length independent, any change in the twitch-to-tetanus ratio would presuppose a change in the degree of activation of the twitch. In the ALD the twitch-to-tetanus ratio decreased progressively, from 0.52 to 0.28, with increasing length beyond L_o. This 46% reduction compares to a 37% decrease for frog semitendinosus muscle (Smith, 1972). The decrease was probably due to a shortening of the ends of the stretched muscle at the expense of the central region (Gordon et al., 1966). This would cause a slower rise in tetanus tension; in fact, time-to-peak tension increased threefold at extreme lengths. After correcting for this effect, as suggested by Gordon et al. (1966), the twitch-to-tetanus ratio was found to be length independent. In identical experiments on the PLD muscles that were of necessity stretched less, the decrease in twitch-to-tetanus ratio was smaller, about 15–20%. This decrease also was accounted for by the change in time-to-peak tension in the tetanus.

Energetics of Isometric Tetanic Contractions

Tension is not maintained by the repetitively stimulated PLD (Canfield, 1971). Consequently, mechanical data were expressed as the time-integral of tension, $\int P(t)dt$. Both the ALD and PLD exhibited a linear decrease in total initial heat as they were stretched and stimulated tetanically beyond L_o. The extrapolated value of A was 42% of the total initial heat in a 2.0 s tetanus in the PLD and 49% in a 8.8 s tetanus in the ALD (Table II).

A correction was not employed for the fact that in tetanically stimulated stretched muscle preparations the final tension is characteristic of the ends

TABLE II

ENERGETIC EXPENDITURES IN THE ISOMETRIC TETANUS OF PLD AND ALD MUSCLES

	PLD ($n = 2$)	ALD ($n = 5$)
$\frac{L_o}{M}\int P(t)dt$ (kg-s/cm^2)	0.85±0.03*	8.19±0.48
Q_o (mcal/g)	11.89±0.88	18.33±1.17
A (mcal/g)	5.00±0.53	9.07±0.81
K (mcal/g)	6.89±0.35	9.26±0.67
$W_i + \alpha_F(\tilde{\Delta} L)_i$ (mcal/g)‡	1.19	0.35
r§	0.898±0.017	0.984±0.001

* Mean ± SE of mean.

‡ Estimated values; determined from mean stress-strain relationships and mean shortening heat coefficients derived from other experiments.

§ Correlation coefficient for Q vs. $\int P(t)dt$.

of the muscle, whereas the heat is measured predominantly from the center. It was not apparent how such a correction should be applied to the PLD muscle which does not maintain tension. Because our main interest concerned relative differences between the PLD and ALD, the effect of omitting such a correction, which alters quantities for both muscles in the same direction, should be small. In the ALD, where the correction could be applied, the magnitude of the intercept was 3.5% underestimated and that of the slope 3.5% overestimated. These differences are within measurement error.

Normalization of data per cross-sectional area, P_oL_o/M, is valid if tension is maintained; otherwise, its decrease with time can be taken into account by presenting data as $Q/\frac{L_o}{M}\int P(t)dt$, i.e., heat produced per kilogram of tension developed per cross-sectional area and maintained for a unit time. Differences between results expressed in this manner cannot be due to tension, rate of fatigue, or duration of contraction. Normalized activation heat, $A/\frac{L_o}{M}\int P(t)dt$, was 5.3 times larger; and the normalized tension-time-related heat, $f(P, t)/\frac{L_o}{M}\int P(t)dt$, was 6.2 times greater (Table II) in the PLD than in the ALD.

3. *Energetics of Isotonic Twitches*

GENERAL MECHANICAL AND THERMAL CHARACTERISTICS Heat and shortening records of isotonic and isometric twitches from a PLD and an ALD are compared in Fig. 4. The PLD liberated 2.9 mcal/g of energy isometrically and only 9% more in an isotonic twitch, at 0.35 P/P_{ot}, resulting in maximum external work. The ALD expended 0.8 mcal/g in an isometric twitch, whereas heat produced isotonically, with a load of 0.5 P/P_{ot}, was 20% greater. In the PLD, because of the velocity of relaxation, a sizeable fraction of the energy of the load is dissipated as degraded kinetic energy when the lever hits the afterstop, and is not recorded as heat by the thermopile. All records were corrected for this effect, which can be as high as 16% of the total energy liberated in an isotonic twitch. In the ALD, the energy of the load is quantitatively (within 2%) returned to the muscle as heat.

ENERGY LIBERATION IN ISOTONIC AND ISOMETRIC TWITCHES According to Mommaerts (1969), total initial energy liberated in an isotonic twitch equals:

$$E = [A + \alpha_F(\bar{\Delta}L)_i + W_i + f(P, t)] + \alpha_F(\bar{\Delta}L)_e + W_e,$$

where $[A = \alpha_F(\bar{\Delta}L)_i + W_i + f(P, t)]$, or Q_i, is the energy produced in an isometric twitch or the isometric phase of an isotonic twitch. Each of these components, Q_i, $\alpha_F(\overline{\Delta L})_e$, and W_e, was estimated. Total initial energy, E, liberated by shortening muscles was recorded from 10 PLD and 10 ALD preparations. Moreover, the isometric heat-vs.-tension relationship, Q_i, was determined for each muscle by the stretch technique. Typical results for a PLD and an ALD are shown in Fig. 5 where energy, E, and isotonic heat production, $E - W_e$, have been plotted as a function of tension, P/P_{ot}. In the PLD (Fig. 5 *a*) the maximum isotonic energy expended was 13% greater than the maximum isometric energy, Q_o, at L_o, whereas in the ALD (Fig. 5 *b*) it was 56% greater. Both muscles displayed, at lighter loads, an extra heat production, $(E - W_e) - Q_i$, that corresponded to $\alpha_F(\overline{\Delta L})_e$. In all experiments

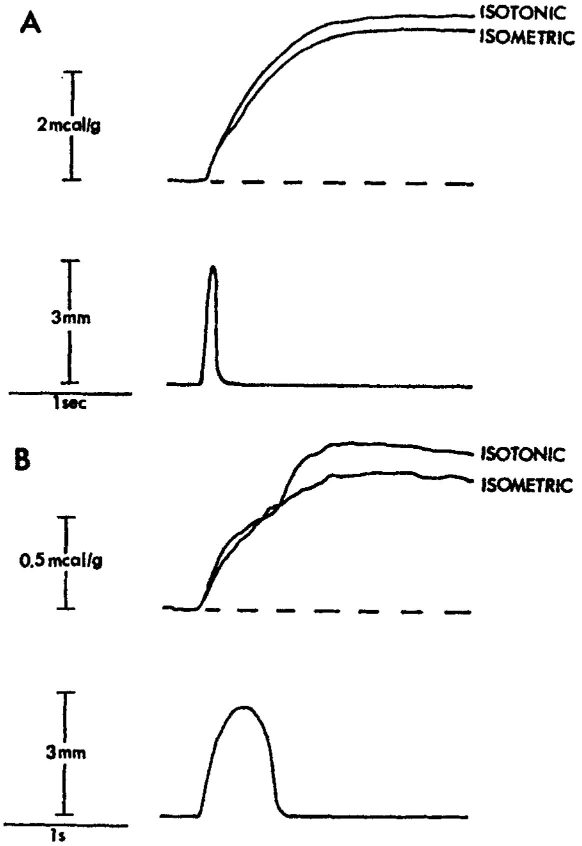

FIGURE 4. Heat and shortening records for isotonic and isometric twitches of the PLD and ALD. (*a*) The PLD liberated 9% more energy in the isotonic twitch at 0.35 P/P_{ot} (upper trace). Time lag in the heat records is apparent on comparison with the shortening (lower trace). (*b*) The ALD produced 20% more energy in the isotonic twitch elicited at 0.5 P/P_{ot}. The hump in the isotonic heat trace is due to energy dissipated when the load is lowered (relaxation heat).

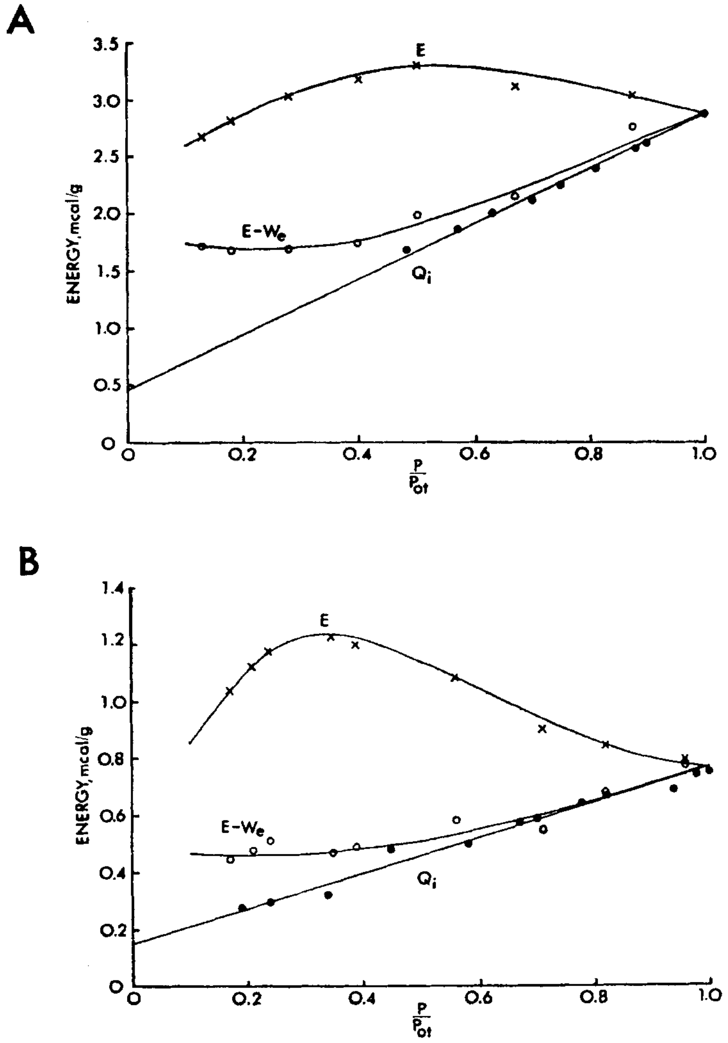

Figure 5. Relationships among isotonic energy, E; isotonic heat, $E - W_e$; isometric heat, Q_i; and load, P/P_{ot}, for a PLD and an ALD preparation. (a) In this PLD, there is an extra heat associated with shortening, $(E - W_e) - Q_i$, and a slight increase in the maximum isotonic energy above the isometric heat production at L_o. (b) The maximum isotonic energy, E, is much greater than the isometric heat at L_o in this ALD preparation, and there is extra heat associated with shortening in the lighter load range.

(Table III), maximum isotonic energy was 1.14 times greater than Q_o in the PLD and 1.44 times greater in the ALD. Extra heat was associated with shortening in both muscles. For the PLD, $E - W_e$ was greater ($P < 0.05$) than Q_i at fractional loads up to, and including, 0.6 P/P_{ot}. This was true in the ALD up to, and including, 0.5 P/P_{ot}. Maximum mechanical efficiency, W_e/E, was greater ($P < 0.05$) in the ALD (Table I). It occurred at 0.3

TABLE III

MEAN VALUES AND STANDARD ERRORS OF TOTAL INITIAL ENERGY (E), EXTERNAL WORK (W_e), ISOMETRIC HEAT (Q_i) AT VARIOUS FRACTIONAL LOADS (P/P_{ot}) FOR THE PLD AND ALD MUSCLES

	Fractional load (P/P_{ot})									
Energy (mcal/g)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
ALD										
(n = 10) E	0.81	0.93	1.02	1.04	1.00	0.95	0.88	0.83	0.77	0.72
	±0.03	±0.03	±0.04	±0.03	±0.03	±0.03	±0.03	±0.02	±0.03	±0.02
W_e	0.30	0.48	0.56	0.55	0.49	0.40	0.30	0.19	0.09	0
	±0.02	±0.02	±0.03	±0.03	±0.02	±0.02	±0.02	±0.02	±0.01	
Q_i	0.24	0.29	0.35	0.40	0.46	0.51	0.56	0.62	0.67	0.72*
	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.02	±0.02	±0.02	±0.02
PLD										
(n = 10) E	2.80	3.09	3.27	3.34	3.36	3.30	3.18	3.11	3.03	2.96
	±0.15	±0.17	±0.17	±0.17	±0.16	±0.16	±0.15	±0.15	±0.14	±0.13
W_e	0.78	1.25	1.43	1.45	1.31	1.08	0.81	0.52	0.24	0
	±0.04	±0.07	±0.08	±0.09	±0.09	±0.08	±0.07	±0.05	±0.03	
Q_i	0.74	0.99	1.24	1.38	1.72	1.98	2.22	2.46	2.71	2.96*
	±0.04	±0.04	±0.05	±0.13	±0.07	±0.08	±0.10	±0.11	±0.12	±0.13

* $Q_i = Q_o$ at P/P_{ot} = 1.0.

P/P_{ot} in the ALD and between 0.3 and 0.4 P/P_{ot} in the PLD (Table III). To examine the dependence of α_F on load, α_F/P_{ot} was plotted against P/P_{ot} and found to be load independent. Also, α_F/P_{ot} was significantly less ($P < 0.05$) in the ALD (Table I).

DISCUSSION

1. *Mechanical Properties of PLD and ALD Muscles*

The results from our mechanical studies are in broad qualitative agreement with those of Canfield (1971), but there are distinct quantitative differences. In general, both studies suggest that the tonic ALD is best suited for prolonged tension maintenance, which is consistent with its probable postural role (Cambier, 1969). The phasic PLD seems designed to maximize power output. Quantitatively, we found the maximum tetanus tension, P_oL_o/M, to be twofold greater in the PLD than the ALD. Canfield reported that P_oL_o/M was the same for both muscles. Results for the ALD are similar in both studies. The difference in tension-generating capacity of the PLD may be related to the fact that the total creatine content, often considered an estimate of a muscle's ability to generate tension, is threefold greater in the PLD (Malvey et al., 1971). Canfield has observed, and we have verified, that isometric tetanic tension increases with increasing frequency of stimulation above fusion in both muscles. This observation might be explained by recruitment of more fibers with increasing rate of stimulation. Hence, it could be asked whether these muscles are fully activated with a single shock. We found twitch tensions that were two to five times greater in the ALD and 6–12 times greater in the PLD than those in Canfield's study. This data would suggest that, though possibly not completely activated, the muscles we examined were activated more fully than those employed by Canfield. Since our corrected twitch-to-tetanus ratio is length independent, the degree of activation is independent of length. Furthermore, our data have been expressed per unit tension for comparative purposes (Tables I and II), and the conclusions presented here should not be affected by the possibility of incomplete activation.

The time-course of the mechanical response in single isometric twitches (Table I) indicated that the PLD was four to six times faster than the ALD. By comparison, Canfield (1971) found an eightfold difference. In agreement with Canfield, V_{max} at 20°C was five times greater in the PLD than in the ALD, but the ALD and PLD were three times faster in our studies (Table I). The value of a/P_o from the force-velocity relation was smaller in the ALD than the PLD (Table I). This result is contrary to that reported by Canfield who found no difference, but qualitatively similar to the results of Goldspink et al. (1970). Since the chickens in the two studies were the same age and techniques employed were similar, we can only suggest that there may be specific differences between the strains of animals utilized.

Energetics of Isometric Contractions in PLD and ALD Muscles

Normalized to an equivalent tension, net activation heat was the same in the PLD (0.44) and ALD (0.41 mcal/g per kg per cm^2). This relationship of A to $P_{ot}L_o/M$ can be extended to other muscles. Values from frog semitendinosus muscle of 0.46 and 0.57 mcal/g per kg per cm^2 were reported by Homsher et al. (1972) and Smith (1972), respectively. Such results indicate that a muscle developing a twitch tension of 1 kg/cm^2 expends about 0.5 mcal/g of energy in the resequestration of Ca^{++}, according to the interpretation of Homsher et al. (1972) and Smith (1972). Thus, the energy requirement for Ca^{++} pumping, per unit tension, in an isometric twitch of the ALD and PLD is similar and is comparable to that observed in frog muscle despite the fact that the SR is less developed in the ALD than in the PLD or frog muscle. Moreover, a positive correlation appears to exist between the speed of relaxation, maximum tension generating capacity, and the morphological features of the Ca^{++} releasing and accumulating apparatus in the ALD and PLD.

Our observations appear to contradict the suggestion, based on the initial burst of heat in a tetanic contraction, that there may be no "activation heat" in the ALD (Canfield, 1971). He observed, as we did, that this fast phase was small or absent. Such an initial burst of heat probably corresponds in part to the fast, reversible phase of the activation heat (Homsher et al., 1972). It is apparently a step in the excitation-contraction coupling process, perhaps the heat of neutralization of H$^+$ released by troponin on binding with Ca^{++} (Homsher et al., 1972 and Woledge, 1971), and would be less pronounced in any muscle activated less abruptly and generating less tension than phasic muscles of the frog. These are characteristics of the ALD. Indeed, the initial burst of heat in a tetanus may not be an infallible measure of net activation heat.

Tension-dependent heat, K, in an isometric twitch probably represents energy liberation accompanying the interaction of actin and myosin at the cross bridge level (Homsher et al., 1972; Smith, 1972). The isometric heat coefficient, $P_{ot}L_o/Q_p$, for the PLD is less than that for the ALD because of its larger tension-dependent heat per unit tension (Table I). This implies that the ALD consumes less energy than the PLD in generating the same tension. However, these differences in tension-dependent heat can be accounted for entirely by differences in internal work, W_i. Subtracting W_i plus the internal shortening heat from the total initial heat and re-expressing the isometric heat coefficient as $P_{ot}L_o/[A + f(P, t)]$, yields values of 17.8 for the ALD and 17.4 for the PLD. Both estimates are greater than the maximum of 13 predicted for frog sartorius muscle (Hill, 1965), but less than a value of 21 suggested for toad sartorius muscle (Hill and Howarth, 1959). An isometric heat coefficient of 20 can be calculated from the data of Homsher et al. (1972) for frog semitendinosus muscle (*Rana pipiens*), whereas an estimate of 11 is obtained from Smith's (1972) data on frog semitendinosus muscle (*Rana temporaria*). Since A per unit tension is similar in these semitendinosus muscles, the twofold variation in $P_{ot}L_o/[A + f(P, t)]$ must be mainly due to a difference in $f(P, t)$. This suggests that for a given isometric twitch tension the *Rana temporaria* semitendinosus muscle hydrolyzes more ATP at the cross bridge level for every mole of ATP used in pumping Ca^{++} back in the SR. However, in the PLD and ALD the ratio of ATP consumed at the cross bridges to that dissipated in Ca^{++} pumping apparently is the same. Thus, the ALD is no more economical than the PLD in generating the same isometric twitch tension (under comparable compliance conditions) despite developing that tension four to six times more slowly.

The stoichiometry between A and $f(P, t)$ appears to change in tetanic contractions of the ALD and PLD. Here, A/Q_o was larger and $f(P, t)/Q_o$ less than predicted from the twitch data. The augmented contribution of the tension-independent heat could be due to energy dissipation by a membrane Na^+ pump. This suggestion is rendered plausible by the observation that in 3-wk old chickens the ALD and PLD fiber diameters are less than 20 μm (Ginsborg, 1960 *b*). Thus, in the chicken muscles we investigated, there probably was at least three- to fourfold more membrane surface area, and presumably more Na^+ pumping sites dissipating energy, per unit muscle volume than observed in a typical frog sartorius muscle (assuming fiber diameters of 60–70 μm).

Normalized activation heat, $A/\frac{L_o}{M}\int P(t)dt$, and tension-time-related heat, $f(P, t)/\frac{L_o}{M}\int P(t)dt$, are five to six times larger in the PLD than ALD. Since myosin ATPase activity is reflected in V_{max} (Bárány, 1967) and V_{max} is approximately five times greater in the PLD (Table I), the PLD apparently cycles cross bridges five times faster and thus requires five times as much Ca^{++} to be resequestered in unit time. Consequently, in a tetanus it should liberate, as observed, five times more activation heat and tension-time-related heat in a given time. Differences in the energetics of isometric contractions in the phasic PLD and tonic ALD, therefore, can be attributed to inherent variations in tension development, compliance, and myosin and reticular ATPase activities.

3. *Energetics of Isotonic Contractions in PLD and ALD Muscles*

Goldspink et al. (1970) have suggested that muscles which maintain tension economically are inherently inefficient in the performance of external work. Specifically, they found that the ALD was relatively inefficient in performing external work, $W_e/E = 0.21$. However, as remarked by Canfield (1971), inspection of their data reveals that W_e probably was not studied at the optimum fractional load, 0.3 P/P_{ot}, but nearer to 0.7 P/P_{ot}. At this fractional load, our results indicate an efficiency of about 0.30. We find that after accounting for W_i, the maximum enthalpy efficiency, $(W_e + W_i)/E$, is comparable in the ALD (0.57) and the PLD (0.51). Thus, economy of tension maintenance is unrelated to maximum efficiency in the ALD and PLD.

Originally, Hill (1938) compared the time-course of isotonic energy liberation to an isometric energy expenditure base line at the same muscle length and ascribed the difference to shortening heat. In this frame of reference, neither the PLD nor ALD exhibits (Fig. 6), in a twitch, a net shortening heat,

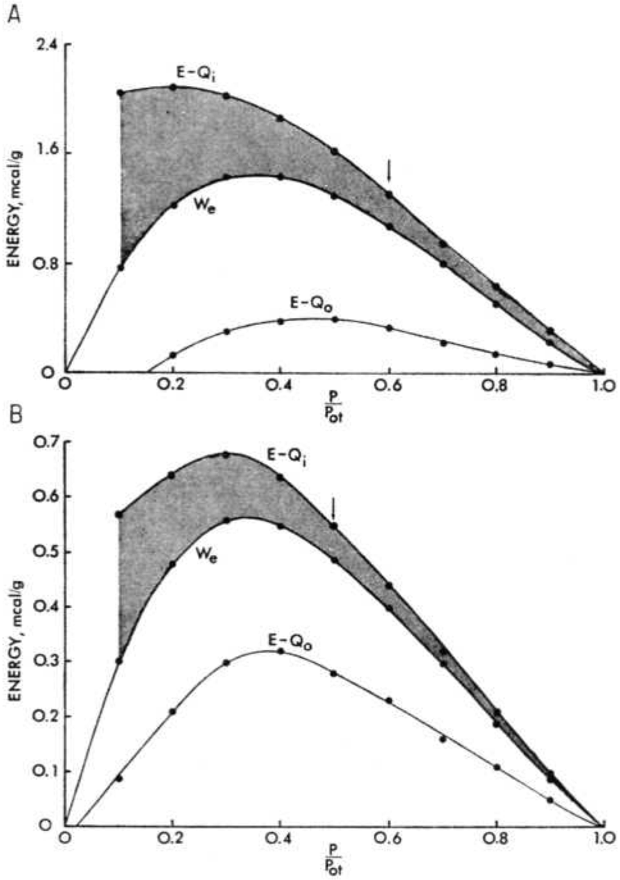

FIGURE 6. The Fenn effect: a comparison among mean values of external work, W_e; energy above isometric heat produced at L_o, $E - Q_o$; energy above isometric heat produced in stretched muscle preparations, $E - Q_i$; and load for the PLD and ALD. (*a*) When W_e is compared to $E - Q_o$ in the PLD, the enthalpy efficiency during external work is greater than unity. The Fenn effect is only observed when W_e is compared to $E - Q_i$. Shortening heat is represented by the shaded area. The arrow signifies that $E - Q_i$ is significantly greater than W_e at this and all lighter loads. (*b*) The same general conclusions as in (*a*) apply to the ALD.

or displays a Fenn effect. (As it has become known, the Fenn effect implies that external work, W_e, equals the extra energy liberated above that produced at L_o, i.e., $E - Q_o$.) Thus, the shortening heat either (*a*) does not exist in these chicken muscles (external work is performed at greater than unity enthalpy efficiency), (*b*) is reversed during relaxation, (*c*) is masked by the presence of a feedback heat (Hill, 1964), or (*d*) is inadequately described for the twitch by the Hill definition. The first possibility seems unlikely on theoretical grounds (Mommaerts, 1969), whereas the second was untestable in the PLD, due to the slow system response, and not observed in the ALD (note in Fig. 4 that the difference between the early isotonic and isometric heat rates was often discernible). However, a net shortening heat does persist over the whole contraction-relaxation cycle in frog muscle stimulated tetanically (Aubert and Lebacq, 1971). Though not examined in our muscle studies, feedback heat is absent in frog semitendinosus muscle which does not exhibit a net shortening heat in a twitch (Homsher et al., 1972; Homsher, personal communication). The last alternative may be the most appropriate.

Gibbs et al. (1967) suggested a redefinition of the shortening heat from the point of view of an equivalent mechanical output, i.e., an equivalent tension or force base line. (To distinguish this quantity from $\alpha(\overline{\Delta}L)$ as defined by Hill, we have used the notation $\alpha_F(\overline{\Delta}L)$ [*F* for force] throughout.) Consideration of the shortening heat from this frame of reference is compelling for the following reasons: (*a*) Aubert (1956) has shown, and the results of this investigation imply, that tension and not length is the main determinant of energy liberation in a contracting muscle. (*b*) It eliminates the discrepancy that arises when comparing isotonic twitches and tetani in frog muscles. Over the whole contraction-relaxation cycle of a tetanic contraction, a net shortening heat is evolved (Aubert and Lebacq, 1971); yet none is observed over the same cycle in a twitch (Carlson et al., 1963). Mommaerts (1969) has argued and Homsher, Mommaerts, and Ricchiuti (personal communication) have verified that the shortening heat is independent of base line chosen (tension or length) in a tetanic contraction, whereas in a twitch it is discernible only with an equivalent tension base line. (*c*) It alleviates the uneasy conclusion that external work is performed at 100% or greater enthalpy efficiency throughout the total load range (Mommaerts, 1969) and may explain the large variability in the isotonic energy versus load relations summarized by Woledge (1971, his Fig. 9).

Our equivalent tension base line analysis suggests that a net shortening heat is produced over the whole contraction-relaxation cycle in both the phasic PLD and tonic ALD (Fig. 6), and thus that this heat may be metabolically derived (Aubert and Lebacq, 1971). Also, it leads to a redefinition of the Fenn effect (Mommaerts, 1970) and, consequently, unmasks the Fenn effect in the ALD and PLD (Fig. 6).

The lesser value of a/P_o (Table I) for the ALD dictates that the curvature of the force-velocity relation must be greater than that for the PLD. Thus, at any fractional load, P/P_o, the fractional velocity, V/V_{max}, is less in the ALD. There may be a relationship between this "slowness" and the dissipation of energy as shortening heat (Woledge, 1968). This view is consistent with the values of α_F/P_{ot} and a/P_o for the PLD and ALD. This slowness is different than the slowness attributed to myosin ATPase activity, since ATPase activity differences are accounted for in the normalization procedure.

It is a pleasure to thank Dr. E. Homsher for stimulating discussions and critically reading the manuscript.
This investigation was supported by Public Health Service Research Grant NS 08550 from the National Institute for Neurological Diseases and Stroke.

Received for publication 19 July 1972.

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