Energy production of rat soleus muscle

C. L. GIBBS AND W. R. GIBSON
Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

Gibbs, C. L., and W. R. Gibson. Energy production of rat soleus muscle. Am. J. Physiol. 223(4): 864-871. 1972.—A thermopile was used to record the heat production of rat soleus muscles, weighing between 16 and 96 mg, at 27 C. The muscles were subjected to isotonic and isometric tetani, 2 sec in duration, and the stimulus frequency was 30 Hz. As well as recording initial energy production, recovery heat evolution was measured and was usually complete 2 min after a 2 sec tetanus. The initial isometric heat rate was 5.1 mcals/g muscle per second; this heat rate varied with muscle length falling when either shortening or stretching of the muscles took place. Most soleus muscles could be stretched so that their tension development fell below 0.1 P0 and the tension-independent initial heat rate at these lengths was 2.3 mcals/g muscle per second. The activation or tension-independent heat (initial and recovery) in response to a single stimulus was estimated to have a mean value of 0.28 mcals/g (recovery heat included), between ¼ and ⅓ of the value found in frog sartorius. In isotonic experiments, minimum energy expenditure (heat + work) occurred with loads equal to 0.4 P0 and maximum expenditure with loads between 0.4 and 0.9 P0. The mechanical efficiency of these muscles (over the complete cycle of contraction, relaxation, and recovery) approached values obtained in amphibian twitch muscles. It was concluded that this mammalian slow muscle has evolved to maintain tension at a low cost and yet to work at a high mechanical efficiency.

Method

Rats were killed either by decapitation or by a blow on the head. A hindlimb was amputated and the soleus muscle was dissected out, while the limb was covered by aerated Krebs-Henseleit solution, containing insulin (0.01 U/ml). The dissected muscles were suspended vertically in a chamber which contained 60 ml of solution. The mechanical arrangement is shown in Fig. 1. The proximal tendon of the soleus muscle was rigidly clamped at the bottom end of the pile, whereas the distal tendon was connected via a light stainless steel tube to the transducers. The muscles were left for 1 hr at 27 C; a Haake NBS thermostat was used to circulate water to the main water bath in which the muscle chamber was suspended.

The resting tension on each muscle was increased until the muscle just developed maximum twitch tension when stimulated. The care was taken not to stretch beyond this region as the plateau of the tetanic tension-length curve ends about this length. Experiments by Close (3) and by Rack and Westbury (25) suggest that the actual sarcomere length at this point will be between 2.6 and 3.0 µ. This length, arbitrarily called l0, which is somewhat longer than the in situ body length, was chosen deliberately because it was found, as reported by Rack and Westbury, that stretch decreases the stimulus frequency at which complete tetanus occurs and thus decreases the stimulus heat in a tetanus. The tension per cross-sectional area in tetanized muscles varied between 2 and 3 kg/cm2, but this value is inflated because the calculation is made on the basis that the individual muscle fibers run the entire length of the muscle, whereas their average length is about 71% l0 (3).

In isotonic experiments muscle displacement was detected using a Brush Instruments Metripak transducer.

THE MYOTHERMIC TECHNIQUE has predominantly been used to investigate the energetics of amphibian fast muscles operating at temperatures close to 0 C. It has been known, however, since the oxygen-consumption studies of Fischer (8) that the isotonic energy output of these muscles differs considerably at higher temperatures. In studies with mammalian cardiac muscle (11, 12) it has been shown that for light-to-medium loads the isotonic energy output, although still interpretable within the classical framework provided by Hill and his colleagues (16-20), is less than that seen in an isometric contraction. There has, however, been no study published on the energetics of mammalian skeletal muscle, except for a recent study by Spande and Schottelius (28) of the effects of muscle fatigue on mouse soleus heat production.

In the present investigation the myothermic technique was adapted for use with rat soleus muscle. The experiments reported in this paper provide evidence which supports the first suggestion and which renders the second unlikely.
most of the experiments a conventional lever system with a 20:1 lever ratio was used, but some of the later experiments were made with a system in which the load was electrically simulated by passing a constant current of the desired magnitude through the coils of a Brush Instruments Mark 200, 80-mm pen motor. Tension was measured using two Ether 350 P type, strain gauges and a bridge circuit. The total system compliance (transducer, stainless steel tube and tie) was 6.5 × 10⁻⁴ cm/g wt. A few quick-release experiments were done using the technique of Wilkie (33) in order to have an estimate of the internal work. In five muscles it averaged .39 kcal/g in an isometric tetanus.

**Heat Measurements**

The thermopile was made using the method developed by Ricchiuti (26), and it possessed a groove that contained the active junctions. It had an output of 4.73 mV/C; its active region was 10 mm long, and there were two protective regions — the top one 8 mm long and the bottom one 1 mm. The output from the thermopile was taken to an Astro data Inc. 120-nV amplifier whose frequency response was reduced by a filter network such that the upper 3-dB point occurred at 25 Hz and the 6-dB point at 56 Hz. The heat loss from the muscle thermopile system was always exponential and averaged 10.1%/sec (range 6.3–15.9). The high average heat loss was caused by the small size of the preparations and the fact that there was a muscle on only one side of the pile.

The groove in the thermopile was essential to keep the muscle in contact with the active junctions, but it did produce certain complications in that it trapped a relatively large volume of fluid when the bath chamber was drained. The difference between the true and drained weights of the muscle averaged close to 20% compared with values of 6–8% reported for frog sartorii. The heat capacity of the thermopile was not negligible and has been shown experimentally to be equivalent to 5.5 mg of artificial muscle. For these reasons a heat calibration was made with each muscle by liberating a condenser discharge of known energy (10.2 mcal) into each preparation. Difficulties with this method have recently been reported by Woledge (36) and by Wilkie (35), although in the past it has been used successfully by Aubert (2) and by Hill and Woledge (21) to determine the sensitivity of their thermopiles. Woledge (36) has suggested that electrode polarization at low voltages may be one reason for low values he has obtained recently. In our hands this technique seems satisfactory, but to obtain consistent results it is essential for the stimulating electrodes to be positioned only over the active region of the thermopile. Our experiments suggest that heating between the stimulating electrodes is far from uniform and if these electrodes are in the usual position, that is, at either end of the pile (i.e., active plus protective regions), then the active region of the pile records from the cooler region of the heated muscle. To test the method, artificial muscles were made using the method described by Aubert (2), and it was found that provided these muscles were made the exact length of the active region of the thermopile, then good results were achieved. This is shown in Fig. 2 where the calibrating condenser has been discharged into artificial muscles of different weights. The results suggest that the thermopile has a heat capacity equivalent to 5.5 mg of artificial muscle. In this series of observations, there is only one large discrepancy with a muscle that weighed 82 mg, and this was probably caused by the artificial muscle not being in contact with all the active region of the thermopile. In experiments with real muscles the "trapped" solution prevents this type of error.

**Stimulus Heat**

Figure 1 shows that quite a range of stimulating arrangements were possible. In practice, the flexible cathodal electrode was always positioned on top of the muscle and situated directly above the top edge of the active region of the pile. The flexible anodal electrode was positioned over the lower edge of the active region of the pile; this electrode was

\[
\text{Q} = \text{heat in muscle} + \text{heat in thermopile} = M_m \cdot C_m \cdot \Delta T + M_p \cdot C_p \cdot \Delta T
\]

where \(M_m\) and \(M_p\) are muscle and pile mass, respectively; \(C_m\) and \(C_p\) are specific heats of muscle and pile, and \(\Delta T\) is measured temperature increment. The intercept of plot, on \(Q/\Delta T\) ordinate, provides an estimate of \(M_p \cdot C_p\).
not used to stimulate the muscle during experiments but was used in the condenser calibration. In the experimental situation the electrode nearest the bottom edge of the pile, and in the same plane as the pile, was used as the anodal electrode so that the muscle was stimulated transversely. This method of stimulation seemed to produce maximum activation at the lowest stimulating voltage. In a tetanus a large proportion of the initial heat recorded is due to the stimulus; a correction was made for this artifact by placing the muscle, at the end of the experiment, into an equimotic solution that had a KCl concentration of 100 mM. This solution rendered the muscle inexcitable and when the muscle was stimulated the stimulus heat could be measured. The stimulus heat averaged 14% of the initial heat at maximum tension or about 30% of the heat seen at zero tension. This inexcitable preparation was later heated using the two surface electrodes with the condenser discharge to provide the calibration for the experiment. The muscle was marked with fine ink lines at the level of each electrode; it was then removed from the pile, stretched to its experimental length on wax, and sectioned where marked. The blotted weights of a) the whole muscle, and b) that portion of the muscle on the active region of the pile were obtained. In attempting to dissect out only the muscle between the two stimulating electrodes, there is the possibility of error; but if this can be assumed to be normally distributed about the true value over all the experiments, then the other errors that might arise are ones which will underestimate the heat liberated in the calibration run, and this will cause the calculated physiological heat values to be too high.

**Relaxation Heat**

During isotonic relaxation, the energy which appears as mechanical work is converted to heat (the relaxation heat) when the muscle lowers the load. The heat record is therefore really a total enthalpy record, but there are two factors that can produce errors. First, at 27 C the load hits the afterload stop with a high velocity so that for certain loads a considerable proportion (up to 30%) of the energy of the load is dissipated as heat in the afterload stop. Second, the relaxation heat may not always be distributed evenly along the muscle (20), and depending upon the area of the muscle being sampled by the thermopile, it would be possible to see too little or too much heat. With the smaller muscles, 40 mg or less, any such error is probably minimal because the active region of the pile is about the length of the individual muscle fibers. The magnitude of the relaxation heat in these smaller muscles was about the correct size, but the early appearance of recovery heat made really accurate measurements difficult. With the larger muscles there was evidence in three preparations that the relaxation heat was too small, and the isotonic results from these muscles were neglected.

**Recovery Heat**

Another reason for using a mammalian muscle and operating at higher temperatures is that it enables recovery metabolism to be studied more rapidly than at 0 C. It was found that at 27 C the recovery heat appeared so rapidly that it was difficult to clearly separate the initial and recovery heats; after a 2-sec tetanus, the recovery heat phase was essentially complete within 1–2 min. The experimental records were electrically corrected for heat loss, and there were two factors that could produce errors. First, there could have been a change in the resting heat rate or the bath-reference temperature could have altered. Occasionally, a muscle did seem to change its resting energy production, but such a change was immediately obvious as the corrected heat record never reached a plateau; it either continued to rise or to fall. Tests showed that the bath temperature stability was more than adequate for periods in excess of 10 min. The second factor that could have caused an error in the detected heat production was an incorrectly set heat loss correction or a nonexponential heat loss. The exponential nature of the heat loss is easily demonstrated by briefly heating the muscle with high-frequency current (100 kHz) and plotting the decline in muscle temperature with time. In order to ensure that the heat loss was set correctly and that temperature stability was satisfactory, the muscle was heated as described above for 100–300 msec, and in such a situation the heat record reached a plateau (within a few seconds). This plateau level had to be maintained for times in excess of 2 min.

**Experimental Protocol**

In the isometric experiments there were two aims: 1) to examine the relationship between the rate of heat production (meal/g muscle per second) and tension development (kg/cm²) at different muscle lengths, and 2) to measure the rate of heat production when little or no external tension was being developed. In order to make this measurement, the preparations were stretched to lengths where tension development was less than 0.1 P₀. These experiments were always performed last as there is the possibility of muscle damage at these extended lengths. If on return to its normal length a muscle failed to eventually develop at least 90% of its prestretch tension, then the heat records were rejected. By stimulating the relaxed muscle at different frequencies, it was possible to obtain an estimate of the activation heat (see RESULTS and DISCUSSION).

**Isotonic Experiments**

In the isotonic experiments in order to minimize the effects of fatigue (5–15% depending upon the preparation), during any given recording period the experiments were run in a mirror-image fashion and the results were averaged. The contractions were afterloaded 2-set tetani, and the loads were chosen to be close to 0.1, 0.2, 0.4, 0.6, and 0.8 P₀. The energy output of each muscle was expressed in milli-calories per gram of muscle. The mechanical efficiency of each muscle was determined by dividing the external work by the total enthalpy (work + initial and recovery heats) and expressing the result as a percentage.

**Curve Fitting**

Previous work (36) has shown that mechanical efficiency can be related to certain parameters of the Hill (16) force-velocity equation: \(P + a)v = b(P₀ - P)\) in which \(P\) = load, \(v\) = speed of shortening, \(P₀\) = maximum tetanic tension, and \(a\) and \(b\) are constants. This equation was fitted to
ENERGY PRODUCTION

FIG. 3. Heat and mechanical records in a 2-sec tetanus at 27°C. Top trace monitors stimulus; second trace records heat (corrected electrically for heat loss); third trace records muscle tension; fourth trace shows muscle shortening under a 15-g load. Time calibration is given between traces 2 and 3. Initially marks are 1 set apart, but in latter part of record time base is changed and horizontal bar represents 1 min. Muscle weighed 17.4 mg and its length was 16.8 mm.

FIG. 4. Total (initial and recovery) energy production (E) and external work (W) are plotted against load where loads are exact fractions of maximum isometric tension $P_0$. A: result for a single muscle which weighed 70.2 mg and was 22.5 mm long. B: mean and SE for 18 muscles are plotted for (E). Standard errors for (W) were too small (< .25 meal/g) to be plotted.

the experimental data from each muscle by the method of least squares. The value of $P$ as the ordinate was plotted against $(P_0 - P)/v$ as the abscissa; this is the straight-line fit of the Hill equation, and the intercept of the line on the ordinate provides a measure of $(a/b)$.

RESULTS

Isotonic Experiments

For every muscle the total enthalpy liberation in after-loaded isotonic contractions was maximal with loads in the 0.4–0.8 $P_0$ range and was minimal with the lightest loads used (0.1 $P_0$). A typical isotonic recording is shown in Fig. 3. The record is not retouched, but the heat due to the stimulus determined at the end of the experiment has been superimposed on the heat trace. Many features of rat soleus heat production resemble those described by Hill (16) for frog sartorius muscle; there is a rapid initial heat phase which accompanies the mechanical response and a pronounced relaxation heat phase that occurs when the muscle lowers the load. This initial heat phase is followed by the much slower phase of recovery heat production (note the change in time scale); it is difficult to determine exactly the transition point between the initial and recovery heat, but the results so far suggest that the recovery energy/initial energy ratio in soleus muscle is approximately one which is in agreement with results from frog sartorius muscle (17). In Fig. 4A the total enthalpy (total heat + work) and the work are plotted against different loads (loads expressed as a fraction of $P_0$) for one muscle, and in Fig. 4B the mean values for 18 muscles have been plotted. The mechanical efficiencies at the different load levels are shown in Table 1. The isotonic efficiencies are as high as those reported for frog sartorius (18), and yet in these experiments the soleus efficiencies are not maximal values. This can be seen from Fig. 3 where in a 2-sec tetanus muscle shortening plateaus long before the tetanus is complete. This means that the work output of the muscle is finished while the heat production continues as long as the muscle is stimulated. A few experiments using shorter duration tetani showed that the maximum efficiency values can easily be increased by 10–20%. The Hill force-velocity relationship (16) was fitted to the isotonic data, and the mean value of $a/P_0$ for the 18 muscles was 0.22.

Isometric Experiments

Effect of length. Muscles were gradually shortened down, and at selected lengths the muscles were tetanized and their heat production and tension development were measured. The usual type of relationship obtained is shown in Fig. 5 (solid circles). The relationship between rate of heat production and developed tension was nearly rectilinear. Although statistically significant curvature was frequently detected when second-degree polynomials were fitted (31), the curvature was neither consistently upward nor downward and was relatively unimportant so that straight lines were considered adequate to represent the dependence of heat production on tension development. In measuring the rate of heat production during the initial energy phase, the average heat rate over the 2 sec of stimulation was measured. As the soleus muscle does not show evidence of a labile heat phase (2), the heat rate is practically constant over this time.

<table>
<thead>
<tr>
<th>Table 1 Mechanical efficiency vs. load</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P/P_0$</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of preparations is 18. These efficiency values include the recovery heat component.
was performed satisfactorily (no irreversible damage), the equation:

\[ Q = 2.307 + 1.219 T \]

where \( Q \) = heat rate, mcal/g muscle per second, and

\[ T = \text{tension per cross-sectional area, kg/cm}^2 \]

When the shortening and stretch results are compared, it is found that the tension-independent heat rate is higher in the stretch experiments and the slope of heat-versus-tension relationship is lower. The differences are not statistically significant (0.05 < \( P < 0.1 \)), but possible reasons for these tendencies which tend to be the reverse of those seen in frog sartorius muscle at 0 0 C are examined in the DISCUSSION. The present experiments have shown that the rat soleus muscle is a preparation that can be used satisfactorily with a slightly modified thermopile technique. Even with the larger muscles (> 60 mg), the rate of fatigue was not excessive over the course of an experiment and there was generally good recovery upon reequilibration of the preparations after each recording session. Muscles with weights in the 20–60 mg range were most suited to the present thermopile, but larger muscles can be used, although care must be taken in isotonic experiments to check that the relaxation heat is being accurately measured. The use of a groove to maintain good contact between the muscle and the active junctions is satisfactory, but it does produce one problem in that fluid is trapped when the muscle chamber is drained and this slows the measured heat output and also makes it necessary to calibrate each experiment individually. Measurements at 27 C have certain advantages in that mammalian muscle is working closer to its normal temperature by the number of stimuli to give an estimate of the heat per single stimulus. In experiments with frog sartorius muscles using paired stimuli, Gibbs, Mommarts, and Ricchiuti (13) have shown, however, that the value so obtained is dependent upon the stimulus interval. It was therefore decided to stimulate stretched muscles, which were developing less than 0.1 \( P_o \) tension, with a constant number of stimuli (30), but to apply these stimuli at rates ranging from 50 Hz to 2 Hz. Stretched rather than shortened muscles were used because there are less complications in the interpretation of the results. It was then possible to get an estimate of the activation heat per stimulus at the different rates and to plot out activation heat-versus-stimulus interval curves (AHSI curves). In Fig. 6 the mean results obtained with 12 muscles are graphed. Notice that the heat liberated per stimulus was lowest at high rates of stimulation and gradually rose to reach a plateau value when the stimulus rate was decreased. The plateau level provided a reasonably accurate estimate of the single-stimulus activation heat. The value so obtained was 0.28 mcal/g, and it includes the recovery heat; it must therefore be halved to make a valid comparison with values of about 1.0 mcal/g obtained in frog and toad sartorii.

**DISCUSSION**

Now the first term in the above equations represents the tension-independent heat rate, whereas the tension coefficient represents the slope of the tension-dependent heat. When the shortening and stretch results are compared, it is found that the tension-independent heat rate is higher in the stretch experiments and the slope of heat-versus-tension relationship is lower. The differences are not statistically significant (0.05 < \( P < 0.1 \)), but possible reasons for these tendencies which tend to be the reverse of those seen in frog sartorius muscle at 0 0 C are examined in the DISCUSSION. The experimentally determined average initial heat rate was 5.1 mcal/g, muscle per second.

**Activation heat.** The soleus muscle at 27 C has a twitch tetanus ratio of about 0.2, and basically for this reason not much heat is produced in response to a single stimulus. It is possible, however, to get an accurate estimate of the tension-independent or activation heat in this muscle. Theoretically, the total heat produced in a tetanus of a fully stretched muscle could be measured, and this heat could be divided into its heat components. The present experiments have shown that the rat soleus muscle is a preparation that can be used satisfactorily with a slightly modified thermopile technique. Even with the larger muscles (> 60 mg), the rate of fatigue was not excessive over the course of an experiment and there was generally good recovery upon reequilibration of the preparations after each recording session. Muscles with weights in the 20–60 mg range were most suited to the present thermopile, but larger muscles can be used, although care must be taken in isotonic experiments to check that the relaxation heat is being accurately measured. The use of a groove to maintain good contact between the muscle and the active junctions is satisfactory, but it does produce one problem in that fluid is trapped when the muscle chamber is drained and this slows the measured heat output and also makes it necessary to calibrate each experiment individually. Measurements at 27 C have certain advantages in that mammalian muscle is working closer to its normal temperature by the number of stimuli to give an estimate of the heat per single stimulus. In experiments with frog sartorius muscles using paired stimuli, Gibbs, Mommarts, and Ricchiuti (13) have shown, however, that the value so obtained is dependent upon the stimulus interval. It was therefore decided to stimulate stretched muscles, which were developing less than 0.1 \( P_o \) tension, with a constant number of stimuli (30), but to apply these stimuli at rates ranging from 50 Hz to 2 Hz. Stretched rather than shortened muscles were used because there are less complications in the interpretation of the results. It was then possible to get an estimate of the activation heat per stimulus at the different rates and to plot out activation heat-versus-stimulus interval curves (AHSI curves). In Fig. 6 the mean results obtained with 12 muscles are graphed. Notice that the heat liberated per stimulus was lowest at high rates of stimulation and gradually rose to reach a plateau value when the stimulus rate was decreased. The plateau level provided a reasonably accurate estimate of the single-stimulus activation heat. The value so obtained was 0.28 mcal/g, and it includes the recovery heat; it must therefore be halved to make a valid comparison with values of about 1.0 mcal/g obtained in frog and toad sartorii.

**DISCUSSION**

Now the first term in the above equations represents the tension-independent heat rate, whereas the tension coefficient represents the slope of the tension-dependent heat. When the shortening and stretch results are compared, it is found that the tension-independent heat rate is higher in the stretch experiments and the slope of heat-versus-tension relationship is lower. The differences are not statistically significant (0.05 < \( P < 0.1 \)), but possible reasons for these tendencies which tend to be the reverse of those seen in frog sartorius muscle at 0 0 C are examined in the DISCUSSION. The experimentally determined average initial heat rate was 5.1 mcal/g, muscle per second.

**Activation heat.** The soleus muscle at 27 C has a twitch tetanus ratio of about 0.2, and basically for this reason not much heat is produced in response to a single stimulus. It is possible, however, to get an accurate estimate of the tension-independent or activation heat in this muscle. Theoretically, the total heat produced in a tetanus of a fully stretched muscle could be measured, and this heat could be divided into its heat components.
and recovery heat is liberated so rapidly that alterations in base-line resting heat production are not a problem. At 0°C, where amphibian recovery heat takes 15-30 min to be liberated, changes in base-line add greatly to the uncertainty of the measurement.

Isotonic Experiments

There are several interesting points in regard to the isotonic energy production of the soleus muscle. In particular, the total-energy-versus-load curve has an overall shape that is located between that found in frog and toad sartorii at 0°C and that found in cardiac muscle at 20-30°C (11). The overall shape of the curve resembles that found by Fischer (8) in oxygen-consumption studies on frog sartorius at 12°C. Similarly shaped oxygen consumption-versus-load curves have recently been obtained for mammalian skeletal muscles (29, 30). It can be seen from Fig. 4 that for light loads the isotonic energy production does not exceed the isometric heat production at the standard length. There is, however, a distinct Fenn effect for most afterloads, and if the “equivalent” isometric heat level for each load is taken as the base line (24), then there is evidence of additional energy liberation for all loads lifted.

Another interesting point, found in these experiments, is the comparatively high efficiency values. There is little point in getting involved in the current arguments over the definition of efficiency (34, 37), since in these experiments we only wish to make a comparison with frog sartorius results. However, it is generally agreed that provided oxidation of glycogen is fuelling the primary chemical reactions, then it is reasonable to employ work/total enthalpy liberation as a definition of mechanical efficiency over the complete cycle of contraction, relaxation, and recovery. It is worthwhile to emphasize here, however, that even if there were any calibration errors and we believe that the evidence is good that these were small, then the true efficiency values must be higher than those reported here. That is to say that any calibration error of the type that has been found by some workers (35, 36) must lead, as Hill and Woolf (21) have pointed out, to an overestimation of heat production which would reduce the calculated efficiency value. Analysis of the force-velocity data from 18 muscles has revealed a mean value of 0.22 for the a/Po ratio. This suggests that the force-velocity relationship will be more curved than it is in frog sartorius muscle, and this curvature can be expected to correlate with increased mechanical efficiency (36). In his very extensive studies on rat soleus muscles, Close (3, 4) has also reported lower a/Po ratios for soleus muscles (means ranging between 0.17 and 0.25).

From the shape of the energy-versus-load curves, it can be seen that for light-to-moderate loads there is less energy being liberated than in the frog sartorius preparation at 0°C (6). This comparative energy decrease presumably takes place because one or more of the classically identified heat components has lower values than for sartorius muscle. Although this point has not been investigated in these experiments, it would seem that the shortening heat component is reduced. As in cardiac muscle (11), the isotonic heat rate rarely exceeds the isometric heat rate recorded at the standard length, whereas this is a regular occurrence with frog and toad sartorii. We are not saying that the reduced shortening heat component is the only cause of the high efficiencies. Mechanical efficiency, for example, would be raised if the work output could be maintained with a comparatively lower maintenance heat rate, but, if this was the sole reason for the efficiency increase, then the overall configuration of the energy-versus-load plot would not be expected to vary.

Isometric Heat Production

It has been a consistent finding in myothermic investigations of frog and toad sartorii that the initial heat rate in isometric contractions depends upon muscle length and/or tension development (1, 2, 7). The myothermic results have been given impressive biochemical support by the work of Sandberg and Carlson (27). All these studies have shown that the maintenance heat rate in an isometric contraction extrapolates to a higher tension-independent value when the muscle is shortened than when it is stretched. The most probable reason for this result is that in a stretched muscle there should be little or no contamination of the tension-independent heat by heat from the actomyosin ATPase reaction. In shortened muscles there is, however, a strong possibility that there will be some bonds formed between the contractile proteins, and hence some internal shortening and work can take place with the production of heat.

The question therefore arises as to why the usual result has not been obtained in the present experiments; indeed, often the stretched muscles produced the highest tension-independent heat rate. The answer probably can be found in recent experiments upon frog muscles at 20°C (5, 32). These experiments seem to show that the activation of a muscle, particularly at higher temperatures, is length dependent. It may be that in frog muscle the decline in activation as the muscle is shortened is overshadowed by the remaining myofibrillar ATPase, whereas in mammalian muscle the decline in activation is so strong as to further depress the myofibrillar ATPase.

Following the work of Aubert (2) with frog sartorius, it has been customary to divide maintenance heat into a labile and a stable fraction. In this investigation there has not been any attempt to follow the maintenance heat rate over long periods of time, but our experimental records show little evidence of the pronounced step in heat rate at the start of a tetanus, a feature which is quite pronounced in the heat records of Hill (16) and Aubert (2). In our records heat production during an isometric tetanus rises practically linearly after a brief delay, presumably caused by the solution trapped in the groove with the muscle. The absence of a labile component is another factor which would make for greater muscle efficiency.

Activation Heat

As mentioned in RESULTS, it is possible to obtain an estimate of the activation heat per stimulus and to show that the magnitude of this heat is dependent upon the interval between stimuli (Fig. 6). Now the resultant AHSI curve is very similar to those obtained by Gibbs, Ricchiuti, and Mommaerts (13) for frog sartorii. In our original paper using the two-stimulus method (13), a second heat step
seen at longer stimulus intervals was tentatively identified as being due to the additional mechanical response caused by the second stimulus. For our earlier explanation to be valid, the second heat step should disappear when the mechanical response is prevented. As can be seen from Fig. 6, in a fully stretched muscle where there is no myosin-actin overlap, the second heat step does indeed disappear.

There is, however, a large difference in the magnitude of the activation heats of frog sartorius and rat soleus muscle, and this difference is, of course, reflected in the maintenance heat rates of the two muscles. In skeletal muscle, Gibbs et al. (13) reported that the activation heat was about 1.2 mcal/g muscle, and more recent experiments upon stretched frog semitendinosus muscles (22) provide a value close to 1.0 mcal/g. Now these results do not include a recovery heat component, whereas the present soleus muscle experiments do include one. The initial activation heat of rat soleus muscle is therefore about 0.14 mcal/g muscle, a factor 5-10 times smaller than for frog sartorius. Following Hill (19) we can regard the tetanic maintenance heat, or more correctly, the tension-independent maintenance heat, as being the summed activation heat in response to the individual stimuli. Now in the present investigation the initial heat rate during a tetanus is about 5 mcal/g muscle per second, whereas frog sartorii have a heat rate in the range 50-70 mcal/g muscle per second (assuming a Q10 of 3.0 and using results from Aubert (2)). Even if one suspects that the soleus initial heat rate should be compared with the stable maintenance heat rate of the frog, this would only halve the above rate and leave us with an energy expenditure still 7 times greater than the rat.

In conclusion, the results would seem to show that mammalian slow muscle has evolved to maintain tension at a low cost. The suggestion has been made recently that slow muscles have evolved for maintaining isometric tension efficiently, whereas fast muscles have evolved for efficiency in doing external work (14, 15). These authors also suggested on the basis of their biochemical investigation that mammalian slow-twitch muscles are a compromise between true slow muscles, which have a slow rate of shortening and a low isometric efficiency and fast muscles, which have a high cost of tension maintenance and a high isometric efficiency. It appears, however, that one of the successful adaptations of mammals has been the evolution of fast muscles that can maintain tension at a reasonable cost and can perform work at high efficiency.

We thank Mr. H. Vogelsanger for technical help and Miss D. Harrison and Miss S. Woolley for preparing the figures. This work was supported by Grant 69/4676 from the National Health and Medical Research Council of Australia.

Received for publication 24 January 1972.

REFERENCES


