Energy production of rat extensor digitorum longus muscle

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The myothermic technique was employed to investigate the energy production of the fast-twitch extensor digitorum longus (EDL) muscle of the rat. Both isotonic and isometric tetani were studied. The total energy liberated during an isotonic tetanus was found to be maximal with loads approximately 0.6 P0 (where P0 is the peak isometric tension) and was always minimal for the lightest loads. The mechanical efficiency of these muscles appeared to be quite low (maximally 7.3–14.2% with the stimulus parameters employed). In isometric experiments, the maintenance heat rate varied with muscle length. At l, length, the heat rate was 32.6 mcal/g muscle per second, and it fell with tension when the muscles were either stretched or shortened. In stretched muscles the tension-independent heat rate was 11.3 mcal/g muscle per second and the activation heat, in response to a single stimulus, was estimated to be 0.57 mcal/g muscle over both the initial and recovery phases. These values are considerably higher than comparable values reported for the slow-twitch soleus muscle, and it is suggested that the greater energy expenditure of the fast-twitch EDL muscle reflects higher myosin ATPase activity and a more extensive and active sarcoplasmic reticulum. It is concluded that the fast-twitch EDL muscle sacrifices energetic economy in return for speed of response.

Mammalian skeletal muscles may be classified as being either of the fast- or slow-twitch type. These two kinds of muscles are distinguished on the basis of differences in the time relations of their isometric responses and, consequently, the frequency of stimulation necessary to provoke a tetanus. This difference between the two muscle types has prompted many varied investigations into their properties. Included among these are histological and histochemical studies as well as extensive investigations of the dynamic properties of these muscles and the relations between neural influences and muscle type. These aspects of mammalian fast- and slow-twitch muscles have recently been reviewed by Close (11).

Functionally, slow muscles are generally concerned with sustained postural contractions, whereas fast muscles are involved in rapid phasic movements. On the basis of their biochemical studies, Goldspink, Larson, and Davies (17, 18) suggested that slow muscles have evolved to maintain isometric tension at a low-energy cost, and the results of a recent investigation into the energy production of rat soleus muscle (15) certainly support this view. Fast muscles could, however, be expected to be more concerned with rapidity of response rather than energetic economy. In the present experiments the myothermic technique has been employed to investigate the energy expenditure of mammalian fast twitch muscles during tetanic contractions. The extensor digitorum longus (EDL) muscle from the hindlimb of the rat was the preparation used, since this muscle appears to be quite representative of mammalian fast-twitch muscles (10, 22).

A preliminary report of some of this work has been published elsewhere (33).

METHODS

Both male and female rats between the ages of 27 and 40 days were used. Animals of this age were chosen, since all the limb muscles in the rat are uniformly slow at birth; whereas in the adult they are differentiated into fast- and slow-twitch muscles. The differentiation between the two types is essentially complete by 4 weeks after birth (10), and the EDL muscles from rats of this age are still small enough to be maintained in good condition in the isolated state (12, 24). The muscles used had an average weight of 39.4 mg within a range from 27.5 to 55.4 mg and varied in length from 17.0 to 22.5 mm with an average length of 19.7 mm. The average tetanic tension developed by these muscles was 39.0 ± 12.1 g SD.

Rats were killed by a blow to the head and a hindlimb was amputated. The EDL muscle was dissected out while the limb was immersed in Krebs-Henseleit solution that was continuously bubbled with 95% O2-5% CO2. The solution also contained insulin (0.01 U/ml). Following dissection the muscle was arranged on the thermopile and suspended vertically in a chamber containing 60 ml of the above solution. A control vessel (Ilaake model NBS thermostat) was used to circulate water to an experimental water bath in which the muscle chamber was suspended. All experiments were conducted at 27 C. Once the muscle was set up in the chamber, a 45-min equilibration period was allowed before experimentation started.

The proximal tendon of the muscle was firmly clamped at the bottom end of the thermopile, whereas the distal tendon was attached above via a light stainless steel tube to the transducers. In the isotonic experiments length changes were detected using a Brush Metripak transducer. The
loads against which the muscles were required to contract were electrically simulated by passing the appropriate current through the coils of a Brush Mark 200 pen motor, thus providing a source of constant torque at the fulcrum of the lever. Tension was measured using two Ether 350 P type strain gauges and a bridge circuit (25). The total compliance of the transducer, stainless steel tube, and silk tie was $6.5 \times 10^{-4}$ cm/g-wt. All recordings were made with a Brush Mark 260 pen recorder.

**Muscle length.** The initial length of each muscle was set to be optimal for twitch tension development, and this was then regarded as the $l_o$ length. Bahler, Fales, and Zierler (3) found that for rat gracilis anticus muscle at 17.5°C the length for a maximal tetanus was somewhat less than the length for a maximal twitch. However, Close and Iloh (12) report that the optimal length is the same for twitch and tetanic contractions of rat EDL muscles at 20°C, and this also appeared to be the case in the present experiments.

**Stimulation.** The muscles were stimulated transversely between two platinum electrodes. This method of stimulation appeared to produce maximum activation of the muscles. Other electrode arrangements required higher stimulating voltages to produce the same mechanical response. During the experiments the muscles were subjected to 1-sec tetani at a stimulus frequency of 60 Hz. Direct stimulation at this frequency produced fully fused tetani.

**Heat measurements.** The thermopile used in these experiments has been described in detail by Gibbs and Gibson (15). It had an output of 4.73 mv/°C and was used in conjunction with an Astrodota 120-nv amplifier, the frequency response of which was reduced to 20 Hz by a filter network on its output. Heat loss from the muscle thermopile system was always exponential. It ranged from 9.7 to 12.8 sec$^{-1}$ and was corrected for electrically.

The thermopile possessed a groove that contained the active junctions. This groove ensured that the muscle kept in good contact with the active junctions, but it did result in the complication of trapping some solution when the muscle chamber was drained prior to making heat measurements. The amount of solution trapped varied from preparation to preparation but generally represented between 20 and 30% of the blotted weight of the muscle. This, coupled with the fact that the thermopile itself had an appreciable heat capacity (equivalent to 5.5 mg of muscle), made it necessary to perform an independent heat calibration on each preparation. The calibration was achieved by liberating a condenser discharge of known energy into the muscle, which had previously been rendered inexcitable by electrocution or soaking in 100 mM KCl. During the experiments the muscles were subjected to isotonic tetani at a frequency of 80 Hz. Direct stimulation at this frequency produced fully fused tetani.

**Isotonic experiments.** The isotonic contractions were afterloaded 1-sec tetani, the muscle lifting a variety of loads. In each case these loads were chosen to be near 0.1, 0.2, 0.4, 0.6, and 0.8 P, where $P$ is the peak isometric tension developed by the muscle. For each contraction the total heat production of the muscle over the initial and recovery phases was measured. The load and the shortening were also recorded, and consequently the external work done by the muscle could be determined. When the muscle lowers the load upon relaxation, the energy that appeared as mechanical work is returned to the muscle as heat (the relaxation heat), and the heat record is therefore a total enthalpy record (i.e., work + total heat). One problem that does arise, however, is that upon relaxation the lever hits the afterload stop at a relatively high velocity so that for some loads a considerable proportion (up to 50%) of the energy of the load is dissipated as heat in the afterload stop. The mechanical efficiency of the muscle was determined by dividing the external work by the total enthalpy and expressing the results as a percentage.

**Isometric experiments.** In the isometric experiments the relationship between the rate of heat production and the tension developed was investigated. The isometric tension developed by a muscle is dependent on the length of the muscle and two approaches were taken. Tension was altered either by shortening the muscle down from its $l_o$ length or by stretching the muscle beyond $l_o$. In each case the rate of initial heat production was measured for different levels of tension development.

A particular aspect of these experiments was to measure the rate of heat production when there was little or no external tension developed. In order to make this measurement, muscles were stretched to lengths where tension development was less than 0.1 P. Unfortunately, there is the possibility of muscle damage at these extended lengths, and if damage was evident (as indicated by poor performance of the muscle on return to $l_o$), the heat records were rejected. By stimulating these stretched muscles at varying frequencies, an estimate of the activation heat was obtained. The basis of this method, which was used by Gibbs and Gibson (15) on soleus muscles, is considered in results.
RESULTS

Isotonic experiments. In Fig. 1 the total energy liberated and the external work done in isotonic contractions are plotted against different loads. The loads have been expressed as fractions of $P_0$ and the results are the means from 17 muscle preparations. It was a consistent observation in each case that maximum energy production occurred with loads approximately 0.6 $P_0$. Minimum energy expenditure, on the other hand, always occurred with the lightest loads used. The work output follows the characteristic bell shape, being zero when there is no load and when there is no shortening (i.e., at $P_0$).

The mechanical efficiency of the isotonic contractions at different load levels is shown in Table 1, and it is emphasized that these are the efficiencies over the entire cycle of contraction, relaxation, and recovery. These efficiency values are quite low when compared to those reported for other muscles (e.g., 15, 20). However the values obtained in the present experiments are not maximal, since in a 1-sec tetanus the work output of these EDL muscles is essentially complete early in the tetanus, whereas heat production will continue as long as the muscle is stimulated. The maximum efficiency attained in the 1-sec tetani ranged between 7.3 and 14.2% with a mean of 9.5%, and this maximum always occurred with a load near 0.4 $P_0$. In some experiments tetani of shorter duration (0.5 sec) were employed, and this generally increased the maximum efficiency values by between 60 and 70%. Figure 2 shows the effect of shorter tetani on the efficiency of a muscle contracting isotonically.

Isometric experiments. The initial heat rate was measured during isometric tetani at various muscle lengths (and hence levels of tension development). Figure 3 shows the typical relationship obtained between initial heat rate and tension development. The upper curve, fitted to the filled circles, was obtained by shortening the muscle down from its $l_n$ length, whereas the lower curve (open circles) was obtained from the same muscle stretched beyond $l_n$. Due to the risk of damaging the muscle by stretching it, the experiments in which the muscles were foreshortened were always performed first, followed by the stretching. Consequently, it is possible that the lower displacement of the stretch curve in Fig. 3 is at least partially due to muscle fatigue. However, it has previously been reported that the maintenance heat rate in an isometric contraction generally extrapolates to a higher tension independent value.
when the muscle is shortened than when it is stretched (1, 2, 13, 27). This is understandable, since, even in a muscle shortened down to a length at which it can no longer develop active tension, there is still the strong possibility that some bonds will form between the contractile proteins giving rise to the production of heat associated with the actomyosin-ATPase reaction. In a stretched muscle, on the other hand, there should be no overlap of thick and thin filaments, and the tension-independent heat would not be contaminated by any actomyosin-linked reaction.

In 22 muscles studied the relationship between heat rate and tension development was adequately fitted by a straight line, the tension-independent heat rate generally representing about 40% of the peak maintenance heat rate obtained at 

The mean value of the peak isometric heat rate for the 22 muscles was 32.6 kcal/g muscle per second and the tension-independent value, obtained from stretched muscles only, was 11.3 kcal/g muscle per second.

If the heat produced in a tetanus is divided by the number of stimuli in the tetanus, a value for the heat per single stimulus is obtained. In a fully stretched muscle, developing no active tension, this value should represent the heat associated with the activation of the muscle. It is not, however, the activation heat that would be measured if a single stimulus was given to the muscle, since, as Gibbs, Ricchiuti, and Mommaerts (16) have shown, the value obtained decreases as the stimulus interval is decreased. This means that in a tetanus the apparent activation heat per stimulus will be considerably smaller than in the single stimulus situation. Unfortunately, in these muscles the magnitude of the activation heat in response to a single stimulus is not great enough to permit accurate measurement. Therefore, to obtain a better estimate of the activation heat, a constant number of stimuli was given to stretched muscles at varying frequencies as in a previous paper (15). Forty stimuli were applied to to the stretched muscles at frequencies ranging from 80 to 2.5 Hz, and the total heat liberated was measured. After correction for stimulus heat artifact and any heat associated with active tension development, in cases where this could not be completely eliminated, the heat per stimulus was determined. Plotting the heat per stimulus against the stimulus interval gave the curve shown in Fig. 4, which represents the mean results obtained from 13 muscles. The heat liberated per stimulus is lowest at high frequencies of stimulation and rises to a plateau as the stimulus frequency is decreased. This plateau value of the heat may be taken as a reasonable estimate of the single-stimulus activation heat. For the EDL muscles studied, this value was 0.57 kcal/g muscle, and it must be kept in mind that this includes the associated recovery heat.

**DISCUSSION**

This discussion will be mainly concerned with a direct comparison between the results obtained in these experiments and those reported by Gibbs and Gibson (15) for rat soleus muscle at the same temperature; the soleus being typical of the slow-twitch type mammalian skeletal muscle, whereas the EDL is a characteristic fast-twitch muscle.

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The overall shape of the total energy versus load curve for EDL, shown in Fig. 1, is very similar to that obtained for soleus. However, the magnitude of the total energy liberated is considerably greater in the case of EDL. From Fig. 1 it can be seen that the total energy curve lies between 39 and 57 kcal/g muscle. The corresponding limits for soleus muscle are 15 and 22 kcal/g muscle (see Fig. 4 of ref. 15). The soleus results arose from isotonic tetani 2 sec in duration and at a stimulus frequency of 30 Hz compared with 1-sec tetani and 80 Hz for the EDL results. In both cases the frequency of stimulation was that needed to pro-

**TABLE 2. Comparative data from fast-twitch and slow-twitch muscles**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Fast twitch, kcal/g muscle</th>
<th>Slow twitch, kcal/g muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Stimulus frequency, Hz</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>Tetanus duration, sec</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Isometric heat rate at 10, kcal/g muscle per second</td>
<td>32.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Tension independent heat rate, kcal/g muscle per second</td>
<td>11.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Estimated activation heat, kcal/g muscle</td>
<td>0.57</td>
<td>0.28</td>
</tr>
<tr>
<td>Maximum mechanical efficiency, %</td>
<td>9.5</td>
<td>10.5</td>
</tr>
</tbody>
</table>

* The efficiency values are those obtained with the stimulus conditions shown. For both muscles this value could be increased by using shorter duration tetani. † Data for the soleus muscle are from Gibbs and Gibson (15).

**TABLE 3. Total energy versus load**

<table>
<thead>
<tr>
<th>Load, P/P₀</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDL</td>
<td>39.0</td>
<td>44.3</td>
<td>53.1</td>
<td>57.1</td>
<td>55.5</td>
<td>51.2</td>
</tr>
<tr>
<td></td>
<td>±2.1</td>
<td>±2.2</td>
<td>±2.6</td>
<td>±2.8</td>
<td>±2.7</td>
<td>±2.9</td>
</tr>
<tr>
<td>Soleus*</td>
<td>15.1</td>
<td>17.7</td>
<td>20.7</td>
<td>21.2</td>
<td>19.6</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>±0.8</td>
<td>±1.0</td>
<td>±1.3</td>
<td>±1.4</td>
<td>±1.2</td>
<td>±1.9</td>
</tr>
</tbody>
</table>

Values are in kcal/g muscle and represent the means ± SE. * Data for soleus muscle are taken from Gibbs and Gibson (15). Stimulus rates and durations are as in Table 2.
duce a fused tetanus. A considerable discrepancy between the total isotonic energy production of the two muscles is evident. Examination of the external work versus load curves, however, reveals that the work output of EDL is only slightly greater than that of soleus.

The above differences in isotonic energy production of the two muscles lead to a consideration of their efficiencies. Although the current definition of muscle efficiency is far from satisfactory (34, 37), in making comparisons with other results it seems acceptable to use work/total enthalpy as a definition of mechanical efficiency over the whole cycle of contraction, relaxation, and recovery. Using this the 1-sec tetani provided a maximum efficiency of only 9.5%, but for reasons stated earlier this could be markedly increased by shortening the duration of the tetani, and it appears that the maximum mechanical efficiency of these EDL muscles could be expected to be around 16%. Gibbs and Gibson (15) reported a mean value of 18.5% for the maximum efficiency of soleus muscles performing 2-sec isotonic tetani. They also found that shorter tetani produced somewhat higher values, and from their results the maximum mechanical efficiency of soleus muscle would appear to be about 23%, indicating that the slow-twitch soleus muscle is more efficient in performing external work than the fast-twitch EDL muscle.

The most interesting aspect of the isometric results is the high maintenance heat rate of EDL as compared to soleus muscles. The peak isometric heat rate of EDL was 32.6 meal/g muscle per second, and this is a factor of some sixfold greater than the 5.1 meal/g muscle per second reported for soleus muscle (15). Again it is stressed that these results were obtained at the same temperature. A similar discrepancy exists between the tension-independent heat rates of the two muscles. In comparing these values it must be kept in mind that the EDL results came from muscles tetanized with a frequency of 80 Hz, whereas the soleus muscles were stimulated with a frequency of 30 Hz. Nevertheless, these different stimulus rates alone cannot begin to account for the discrepancy in heat rates.

The heat rates have not been normalized in terms of tension per cross-sectional area because of the difficulty in determining the actual fiber length of these muscles. Both soleus and EDL are fusiform in shape due to the staggered attachment of the fibers to long tendons, and as Hill (22) has pointed out, this makes it extremely hard to see where the fibers end. Close (10) gives the fiber length as about 61% of the muscle length for EDL and 70% for soleus. The average tension per cross-sectional area for the EDL muscles in this investigation was 2.90 kg/cm² (determined on the basis of total muscle length, not fiber length), and the corresponding value, obtained on the same basis by Gibbs and Gibson (15), for the soleus muscles was 2.44 kg/cm². If these are corrected for fiber length using Close's estimates (10) and the heat rate values are divided by the estimated tension per cross-sectional area, the heat rates become 17.9 meal/g muscle per kg per cm² per second for EDL and 3.0 meal/g muscle per kg per cm² per second for soleus. This represents only a slight (6.6%) narrowing of the observed differences between the two muscles.

The activation heat was estimated to have a magnitude of 0.57 mcal/g muscle, and again this is considerably higher than the corresponding value of 0.28 mcal/g muscle for soleus muscle. A higher activation heat component must necessarily result in a higher tension-independent heat rate, and this is consistent with what was observed. Both these values for the activation heat contain the initial and the associated recovery heat. Although no attempt was made to determine accurately the magnitude of the recovery heat, it did appear from the experimental records that the ratio of recovery heat/initial heat was about 1. This would be in good agreement with the results of Hill (21) and would mean that the initial activation heat in rat EDL muscles is about 0.29 meal/g muscle. The recent studies of Smith (28) and Homsher, Mommaerts, Ricchiuti, and Wallner (23) have shown that activation heat can be measured successfully in stretched muscles and that it represents about 25-30% of the heat produced at t₀. They agree on a value of around 1.0 meal/g muscle for the initial activation heat in frog and toad muscle.

In seeking to understand the greater heat production of the fast-twitch EDL muscle as compared to the slow-twitch soleus, the mechanically linked heat, which presumably represents actomyosin activity, will be considered first and separately from the heat of activation, which is independent of actomyosin activity.

The rate at which the actin-activated ATPase activity of myosin with the speed of contraction (4, 6). This correlation was further substantiated by comparative studies of the enzymic properties of developing mammalian skeletal muscles (7, 31) and similar investigations into the effects of nerve cross union (3, 8). All these results pointed to a direct relationship between the intrinsic speed of contraction and myosin ATPase activity. In accordance with this, the myosin ATPase activity of rat EDL muscles is considerably higher than that of soleus muscles (5). If ATP is the prime energy source in muscle contraction, then greater myosin ATPase activity, and hence greater breakdown of ATP, must represent a greater energy expenditure during contraction. The high heat rate of EDL as compared to soleus muscle can therefore be considered to relate, at least in part, to their comparative myosin ATPase activities.

Activation heat is now generally accepted as the thermal accompaniment of the movement of calcium out from and back into the sarcoplasmic reticulum (9, 23, 27, 28), the reaccumulation of calcium involving an ATP-dependent transport system (19, 32). There are some interesting differences between the sarcoplasmic reticulum of fast and slow muscles. Fast muscle appears to contain at least twice as much sarcoplasmic reticulum as slow muscle (14, 26), and kinetic studies on fragmented sarcoplasmic reticulum in vitro have shown the maximum rate of uptake of calcium by fast muscle fragments to be approximately 4 to 11 times greater than the rate for slow muscle fragments (14, 29, 30, 38). If the accumulation of calcium by the sarcoplasmic reticulum involves the hydrolysis of ATP (19, 37), then the greater activity as well as the augmented sarcoplasmic
Not only does it expend a great deal more energy than the slow-twitch soleus muscle in developing and maintaining tension, but it also sacrifices isotonic efficiency.

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