During muscle contraction, the energy from ATP splitting is converted into mechanical work and heat:

$$\text{ATP} \rightarrow \text{ADP} + \text{P}_i + 0.5\text{H}^+ + \text{work} + \text{heat} ,$$ (1)

where P$_i$ is inorganic phosphate. If contraction is to be repeated, as it is during normal muscle function in vivo, the ATP has to be resynthesized. This happens by coupling ATP resynthesis to other processes. In normal muscle, ATP is resynthesized immediately by the reaction catalyzed by the enzyme creatine kinase:

$$\text{H}^+ + \text{PCr} + \text{ADP} \rightarrow \text{Cr} + \text{ATP} ,$$ (2)

where PCr is phosphocreatine and Cr is creatine. The net result of ATP splitting and the creatine kinase reaction is the breakdown of PCr:

$$0.5\text{H}^+ + \text{PCr} \rightarrow \text{Cr} + \text{P}_i + \text{work} + \text{heat} .$$ (3)

Although the concentration of PCr in rested skeletal muscle is a few times greater than that of ATP, there is only enough PCr to fuel a brief burst of severe exercise. Thus, to maintain the ATP supply during sustained exercise and to replenish supplies after the end of exercise, ATP resynthesis is coupled to the oxidation of glycogen and/or fats and/or to the breakdown of glycogen to lactic acid.

Many experiments on various types of muscle have shown that lactic acid is produced during metabolic recovery, even when there is plenty of oxygen available to the muscle (Dawson et al., 1977; Meyer et al., 1991; Phillips et al., 1993; Kemp et al., 1993). However, we recently found in dogfish white muscle what seems to be a simpler pattern of metabolic recovery (Curtin et al., 1997). In these experiments, we used $^{31}$P nuclear magnetic resonance (NMR) to detect the time course of changes in levels of phosphorus-containing metabolites and in pH during metabolic recovery after a brief series of contractions. Approximately half the PCr initially present in the muscle was hydrolyzed during the contractions. The striking feature of the metabolic recovery was its simple time course. The reappearance of PCr mirrored the
Materials and methods

The experiments were performed on bundles of fast, white muscle fibres from the dogfish Scyliorhinus canicula (L.). These fibres are in the main body of the fish and are used during bursts of swimming, rather than for sustained swimming (Bone, 1966; Mos et al., 1990). The bundles were dissected under saline, which contained (in mmol l\(^{-1}\)) NaCl, 292; KCl, 3.2; CaCl\(_2\), 5.0; MgSO\(_4\), 1.0; Na\(_2\)SO\(_4\), 1.6; NaHCO\(_3\), 5.9; urea, 483; and tubocurarine, 1.5 mg l\(^{-1}\). All the experiments were performed at 19°C.

In the preliminary part of each experiment, the strength of the electrical stimulus was adjusted so that all the living fibres were activated. The basis for comparing the performance of bundles containing different numbers of live fibres is explained below; it does not require knowledge of the number of fibres alive and dead. The bundle length was varied to find \(L_0\), the fibre length at which active force was greatest. In the main experiments, bundles were stimulated at \(L_0\) using a constant stimulus pattern. As shown in Fig. 1, the stimulus pattern consisted of 20 isometric twitches at 3 Hz, so the contraction series lasted 6.7 s. This was followed by a recovery period (approximately 2 h) without any stimulation. The stimulus pattern was then repeated, in some cases several times.

Oxygen and heat measurements were made on different muscle fibre bundles in separate experiments. Oxygen measurements were made on 10 fibre bundles from five different fish. In six fibre bundles, oxygen was measured in only one cycle of stimulation and recovery; in the other four bundles, 2–10 cycles of stimulation and recovery were observed. Heat production was measured in experiments on four fibre bundles from four different fish. Measurements were made in 3–6 cycles of stimulation and recovery of each fibre bundle.

The area under the force curves (\(\int P\,dt\)) was measured. At the end of the experiment, the bundle was fixed in ethanol at \(L_0\), and fibre length was measured. The value of \(L_0\)\(P\,dt\) was used as a basis for comparing results from different fibre bundles, which varied in size, number of live fibres and contractile activity.

Oxygen consumption

Oxygen consumption was measured polarographically in a chamber (volume 170 µl) specially designed for small muscle preparations (Elzinga and van der Laarse, 1988). A spinner circulated the saline in the chamber. The response time of the system to a step change in partial pressure of oxygen is approximately 5 s (Elzinga et al., 1984), which includes a circulation time of approximately 1 s. The delay in detecting a change in oxygen consumption by the muscle would include this time and, in addition, some delay due to diffusion of oxygen within the muscle and in any unstirred layer.

The electrode response was calibrated during each experiment by recording the electrode output in response to saline saturated with three different known concentrations of oxygen, 5%, 10% and air (=20.95%).

The electrode output during repeated cycles of contractions and recovery was divided into individual recordings containing a 300 s baseline period before stimulation, the 6.7 s contraction series, and the 6293 s (approximately 2 h) recovery period. One of these recordings is shown in Fig. 2A. The electrode output falls during the baseline period because oxygen is consumed by the resting muscle fibres and by the electrode itself. The electrode uses oxygen at a rate proportional to the oxygen concentration in the saline.
Each recording (Fig. 2A) was differentiated to give the rate of oxygen consumption (Fig. 2B). An exponential baseline curve of the following form was calculated:

\[ B = I e^{-k(t-150)}, \]

where \( B \) is the rate of oxygen consumption, \( I \) is the value of \( B \) at 150 s, \( t \) is time measured from the start of the recording, and \( k \) is the rate constant for the decay of the baseline rate.

The value of \( I \) was taken to be the mean observed rate during the 300 s before stimulation and centred on 150 s. Similarly, the value of \( F \) was taken to be the mean rate during 300 s of recovery centred on \( t_F \) (see Fig. 2B). The rate constant \( k \) was then calculated from:

\[ k = \log_e \left( \frac{I}{F} \right) / (t_F - 150), \]

where \( t_F \) is the time of \( F \) and was taken to be 3600 s in 19 of 29 recordings. In the other 10 cases, there was a second period of increased (small, but detectable above baseline) oxygen consumption starting before 3600 s. In these cases, an earlier time, before the second period of increased oxygen consumption, was used.

Subtraction of the baseline from the recording of the rate of oxygen consumption gives the rate of consumption by the muscle fibres (Fig. 2C). This rate was integrated to give a recording of the amount of oxygen used by the muscle during recovery (Fig. 2D). An exponential curve was fitted to this recording:

\[ O = A (1 - e^{-T/\tau}), \]

where \( O \) is the amount of oxygen used (nmol), \( A \) is the total amount used in the entire recovery period, \( T \) is the time (s) since the start of recovery, and \( \tau \) (s) is the time constant for recovery oxygen consumption. Table 1 contains a summary of these values.

### Heat production

Heat production was calculated from the temperature change of the fibre bundle measured with a thermopile. The thermopile we used consisted of 48 constantan–chromel thermocouples on a Kapton substrate. It was 12 mm long and contained four thermocouples per millimetre along its length. Recordings were made of the output from a 3 or 4 mm length of thermopile (12 or 16 thermocouples). Each thermocouple produced 34.2 mV per degree K temperature change. To minimize heat

<table>
<thead>
<tr>
<th>Table 1. Results of oxygen consumption measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen used in recovery (nmol)</strong>(^\dagger)</td>
</tr>
<tr>
<td>( \tau ) (s)</td>
</tr>
<tr>
<td>( L_0 )</td>
</tr>
<tr>
<td><strong>Values are means ± S.E.M.</strong></td>
</tr>
<tr>
<td>( \dagger )</td>
</tr>
<tr>
<td>( \dagger )</td>
</tr>
<tr>
<td>( \tau )</td>
</tr>
<tr>
<td>( L_0 )</td>
</tr>
</tbody>
</table>
 artefacts due to condensation, the muscle fibre bundle was covered with a 12μm film of Teflon, as described by Lou et al. (1998).

Force and thermopile output were recorded with fast time resolution (200Hz) during the series of twitches and for a brief time afterwards. Thermopile output was also recorded at a lower time resolution (0.694Hz) for the entire approximately 2h period which included the series of twitches followed by the recovery time without stimulation. Both the fast and slow recordings of thermopile output were converted to heat and corrected for heat loss (Woledge et al., 1985).

We have divided the heat production into the two ‘traditional’ phases: initial heat, which roughly corresponds to the immediate effects of contraction and relaxation, and recovery heat, which is produced after contractile activity is over and corresponds to the time when metabolic processes are resynthesizing the ATP used in contraction. We have chosen to define the recovery period as starting at 4s after the end of stimulation. For a few seconds after the end of stimulation, the initial metabolic activity continues, but at a low rate (see Results). Thus, the exact time chosen for the start of recovery does not have much effect on the results.

Initial heat was calculated from the fast recording (200Hz) of thermopile output. The recovery heat produced in the first 170s of recovery was calculated from the fast recording (200Hz) and the rest (up to 61.6 min) from the slow recording (0.694Hz). Total heat is defined as the sum of initial heat and recovery heat.

Simulations of oxygen diffusion and heat flow

Oxygen diffusion in the muscle

We have simulated oxygen diffusion within the muscle only since this is likely to be the slowest step between oxygen use by the muscle and its detection by the oxygen electrode. As described above, the oxygen chamber and the circulation system were designed for rapid and effective mixing of the saline within the chamber.

One-dimensional finite-element analysis of oxygen diffusion within the muscle fibre bundle was carried out using a cylindrically symmetrical model consisting of 20 concentric shells. The boundary conditions were no flow at the inner boundary (the centre of the cylinder) and constant oxygen concentration at the outer boundary (the surface of the ‘muscle’). The time interval used for the simulation was 0.01 s. The radius of the cylinder of ‘muscle’ was taken as 0.319 mm, which gives a cross-sectional area for the cylinder equal to the average cross-sectional area of the muscle preparations used in the experiments reported here. The diffusion coefficient for oxygen in muscle at 20°C was taken as 0.91×10⁻³ mm² s⁻¹ (Hill, 1965). A baseline (pre-contraction) distribution of oxygen within the tissue was calculated by running the simulation with a constant rate of oxygen consumption, representing the resting metabolic rate, for 50 s before the start of recovery. Recovery was then simulated by an increase in the rate of oxygen consumption starting at its maximum rate instantly and declining with a time constant of 844 s (the mean value of the time constants observed for oxygen consumption in the experiments, Table 1).

Heat flow

A one-dimensional analysis of heat flow within the muscle and the thermopile was made using a rectangular coordinate model. The muscle was represented as 10 slices of ‘thickness’ (in the direction of heat flow) 0.05 mm and area 0.64 mm², and the thermopile as 40 slices of the same thickness and of area 0.0024 mm². Boundary conditions were no heat flow at the outer boundary (the surface of the muscle not in contact with the thermopile) and constant temperature at the inner boundary (the junction of the thermopile with its frame). The thermal diffusivity of the muscle was taken as 0.135 mm² s⁻¹ (Hill, 1937) and that of the thermopile as 1.8 mm² s⁻¹. This latter value was chosen to match the time constant for heat loss of the model to that observed in the experiments (8.79±0.28 s, mean ± S.E.M., N=4). The time interval used for the simulation was 0.0003 s. The temperature of each slice was calculated at each time interval, and thermopile output was simulated as the temperature at the boundary of the muscle and the thermopile.

A baseline (pre-contraction) distribution of temperature within the muscle and thermopile was calculated by running the simulation for 50 s with a constant rate of heat production in the muscle. Recovery heat production was then simulated by an abrupt increase in heat rate followed by an exponential decline with a time constant of 844 s. The simulated signal was then corrected for heat loss in the same way as the actual signals obtained in the muscle experiments.

For comparison between the simulations and the actual experimental results, the simulated results were expressed relative to the total amount of oxygen consumption or recovery heat.

Results

Comparison of oxygen and heat recordings

Fig. 2C shows an example of the time course of the rate of oxygen consumption. The broken vertical line corresponds to the start of recovery (4s after the end of stimulation). The rate is high initially and has declined to zero by approximately 2000 s. The mean time constant for oxygen consumption during recovery was 844±47 s (mean ± S.E.M.) (Table 1).

Fig. 3 shows an example of the rate of heat production with the same pattern of stimulation and recovery as was used for the oxygen measurements. During the initial period (contraction + relaxation) the heat rate is very high and off the scale of Fig. 3, which is scaled to show the recovery heat rate during most of the recovery period. As with oxygen consumption, the recovery heat rate declined steadily. In this particular recording, recovery heat production was completed in approximately 2800 s. There was some variation among the fibre bundles, but in all of them the recovery heat production was complete by 3600 s (60 min).
Table 2. Results of heat production measurements

<table>
<thead>
<tr>
<th></th>
<th>Number of observations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial heat (mJ)</td>
<td>0.658±0.043</td>
</tr>
<tr>
<td>Recovery heat (mJ)†</td>
<td>1.124±0.129</td>
</tr>
<tr>
<td>Total heat (mJ)</td>
<td>1.781±0.163</td>
</tr>
<tr>
<td>$L_0\int Pdt$ (mm N s)</td>
<td>0.872±0.073</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.
*Four different muscle fibre bundles were used. Of the total variance, 40% was within groups of observations made with the same bundle.
†During 61.6 min of recovery.
Total heat = initial heat + recovery heat.
$L_0$ is the length at which active force was maximum; $\int Pdt$ is the integral of force and time.

achieved by expressing the oxygen consumption (or heat production) as a fraction of the total oxygen consumed (or heat produced) by that muscle fibre bundle. The recordings show that oxygen use gradually increases in rate for 50–100 s before starting the slow decline that continues for the rest of the recovery period. The heat recordings show more complex behaviour. The heat recording reaches a peak at the end of stimulation. This is followed by a few seconds of heat absorption, after which the heat production starts and continues for the rest of recovery. Similar observations of a period of heat absorption at the end of a contraction, referred to as ‘negative delayed heat’, have been made on frog muscle (Hartree, 1932; Hill, 1961, 1965). We have chosen to define the start of the recovery period at 4 s after the end of stimulation, which approximately coincides with the end of the period of heat absorption. The amount of heat absorbed is 2.0±0.6% (mean ± S.E.M., N=4) of the recovery heat and 3.3±1.4% (N=4) of the initial heat. This could be due to the occurrence of a net heat-absorbing metabolic process during this time. Since the quantity is sufficiently small that it would not affect our conclusions, we have not at this stage undertaken further investigation of this feature of the recordings.

Oxygen consumption (Fig. 4A) starts at a low rate that continues to increase for over 50 s, whereas the rate of heat production (Fig. 4B) reaches its maximum value much sooner, within approximately 10 s after recovery starts. Fig. 5 compares the rate of oxygen consumption and the rate of heat production during the whole period of recovery. In this figure, time is replaced on the abscissa by the progress of recovery, expressed as the fraction of recovery completed. The results show a reasonably good match between the time course of the rates of oxygen consumption and of recovery heat production, except at the start of recovery. The largest discrepancy occurs early in recovery when the rate of heat production is greater than the rate of oxygen consumption. Two-way analysis of variance (ANOVA) was used to test the significance of the difference between oxygen and heat rates during the first 0.30 fraction of recovery (the first six points from each series in Fig. 5). The rate of heat production was significantly higher than the rate of oxygen consumption ($P=0.034$, $F=4.56$). It will be shown in the Discussion that this difference is likely to be due to the rate of diffusion of oxygen being less than that of heat, rather than to a difference in the time at which oxygen is used and heat is produced by the muscle.

Total quantities

Fig. 6 shows that oxygen consumption and heats (total and recovery) were correlated with contractile activity, measured as $L_0\int Pdt$. We used the observed slope, heat/($L_0\int Pdt$), from the heat experiments and the $L_0\int Pdt$ produced in each recording of oxygen consumption to predict the amounts of heat that would be produced in each of the oxygen experiments. Each heat value was plotted against the corresponding oxygen consumption, and the best-fitting scaling factor (kJ heat mol⁻¹ oxygen used) was found by linear regression.

---

Recovery oxygen and heat 1205

Fig. 3. An example of the rate of heat production (digitised record). The broken vertical line indicates the start of recovery (at 4 s after the end of stimulation). The vertical scale was chosen to show the rate of recovery heat during most of the recovery period (compare with the rate of oxygen consumption in Fig. 2C). The heat rate during the first 60 s of recovery is very much higher and is not shown.

Table 2 summarizes the mean values of heat produced during the series of twitches (initial heat), recovery heat and total (initial + recovery) heat. The table also includes the mean value of $L_0\int Pdt$ for the series of twitches, which is a measure of fibre bundle size and contractile activity.

Comparison of the time courses of oxygen consumption and recovery heat production

Fig. 4 shows the time course of oxygen consumption and of heat production during the first 170 s of recovery. The recordings were normalized to take account of differences due to variations in the sizes of the muscle fibre bundles. This was done by expressing the oxygen consumption (or heat production) as a fraction of the total oxygen consumed (or heat produced) by that muscle fibre bundle. The recordings show that oxygen use gradually increases in rate for 50–100 s before starting the slow decline that continues for the rest of the recovery period. The heat recordings show more complex behaviour. The heat recording reaches a peak at the end of stimulation. This is followed by a few seconds of heat absorption, after which the heat production starts and continues for the rest of recovery. Similar observations of a period of heat absorption at the end of a contraction, referred to as ‘negative delayed heat’, have been made on frog muscle (Hartree, 1932; Hill, 1961, 1965). We have chosen to define the start of the recovery period at 4 s after the end of stimulation, which approximately coincides with the end of the period of heat absorption. The amount of heat absorbed is 2.0±0.6% (mean ± S.E.M., N=4) of the recovery heat and 3.3±1.4% (N=4) of the initial heat. This could be due to the occurrence of a net heat-absorbing metabolic process during this time. Since the quantity is sufficiently small that it would not affect our conclusions, we have not at this stage undertaken further investigation of this feature of the recordings.

Oxygen consumption (Fig. 4A) starts at a low rate that continues to increase for over 50 s, whereas the rate of heat production (Fig. 4B) reaches its maximum value much sooner, within approximately 10 s after recovery starts. Fig. 5 compares the rate of oxygen consumption and the rate of heat production during the whole period of recovery. In this figure, time is replaced on the abscissa by the progress of recovery, expressed as the fraction of recovery completed. The results show a reasonably good match between the time course of the rates of oxygen consumption and of recovery heat production, except at the start of recovery. The largest discrepancy occurs early in recovery when the rate of heat production is greater than the rate of oxygen consumption. Two-way analysis of variance (ANOVA) was used to test the significance of the difference between oxygen and heat rates during the first 0.30 fraction of recovery (the first six points from each series in Fig. 5). The rate of heat production was significantly higher than the rate of oxygen consumption ($P=0.034$, $F=4.56$). It will be shown in the Discussion that this difference is likely to be due to the rate of diffusion of oxygen being less than that of heat, rather than to a difference in the time at which oxygen is used and heat is produced by the muscle.

Total quantities

Fig. 6 shows that oxygen consumption and heats (total and recovery) were correlated with contractile activity, measured as $L_0\int Pdt$. We used the observed slope, heat/($L_0\int Pdt$), from the heat experiments and the $L_0\int Pdt$ produced in each recording of oxygen consumption to predict the amounts of heat that would be produced in each of the oxygen experiments. Each heat value was plotted against the corresponding oxygen consumption, and the best-fitting scaling factor (kJ heat mol⁻¹ oxygen used) was found by linear regression.
Fig. 7A shows the result for recovery heat versus oxygen consumption; the best-fitting slope is 287 kJ mol$^{-1}$. Fig. 7B shows the result for total heat (initial + recovery) versus oxygen consumption; the best-fitting slope is 451 kJ mol$^{-1}$.

**Discussion**

The white fibres of dogfish have a low mitochondrial content, only 1% of the fibre volume is mitochondria (Bone et al., 1986). This is also a characteristic of fast, white fibres from a wide range of animal species. Recovery of fast fibres from a number of species has been investigated using $^{31}$P-NMR (frog, Dawson et al., 1977; cat, Meyer et al., 1991; rat, Phillips et al., 1993; humans, Kemp et al., 1993). These studies showed that, in these muscles, the intracellular pH becomes more acid than the resting, pre-contraction pH because of the lactic acid that is formed by glycolysis. These results suggested that the metabolic profile of these muscles is low oxidative activity and relatively high glycolytic activity, and that glycolysis contributes to the resynthesis of PCr during metabolic recovery in these muscles, even when there is an abundant supply of oxygen.

However, recovery of white fibres of dogfish does not follow this pattern. We found no evidence of lactic acid accumulation in white muscle fibres from dogfish in a recent investigation of recovery metabolism using $^{31}$P-NMR (Curtin et al., 1997). Resynthesis of PCr was very slow (half-times in the range 24–52 min), which is consistent with the low mitochondrial content and the absence of glycolysis as an additional recovery process synthesizing PCr.

A simple hypothesis that can explain the observations...
Recovery oxygen and heat

regarding recovery of white fibres from dogfish is that the PCr broken down during contraction is resynthesized by oxidative phosphorylation, without glycolytic production of lactic acid. The experiments reported here were performed to test this hypothesis directly by measuring oxygen consumption and comparing it with the recovery heat production. Are the new results compatible with this simple hypothesis? We will consider this from three points of view. (i) Is oxygen used? (ii) Is the 'stoichiometric' relationship between total oxygen use and heat production consistent with this hypothesis? (iii) Does the time course of recovery heat match that of the oxygen consumption with appropriate 'stoichiometry'?  

(i) Oxygen use

The results show that oxygen was used during the recovery period after every contraction series (see Table I for mean values).

(ii) Comparison of heat production with oxygen use

If all the PCr split during contraction was resynthesized during recovery by oxidative phosphorylation, then the only net change during a cycle of contraction and recovery would
be the use of oxygen and conversion of substrate into CO₂ and water. If the substrate were carbohydrate and it was completely oxidized, the total energy output during contraction + recovery would be 473 kJ mol⁻¹ of oxygen used (Carpenter, 1939). If the substrate were fat, the energy produced would be 439 kJ mol⁻¹ of oxygen (Carpenter, 1939). In fact, we found that the amount of energy produced per mole of oxygen used was 451±34 kJ mol⁻¹. This is within the range of the expected values for the hypothesis that metabolic recovery is wholly oxidative. In other words, the amount of heat we observe matches well with that expected from the amount of oxygen used. However, the precision of our determination is not sufficient to distinguish between the possible substrates, glycogen and fat.

Our results are consistent with the hypothesis of recovery metabolism being completely oxidative. However, if we consider the upper 95 % confidence limit of the heat production, there is more heat than the amount expected from oxidation. How much glycolysis would be required to produce this excess heat? Table 3 sets out the calculation of the maximum amount of glycolysis that would be consistent with our results. For this calculation, we will assume that fat, rather than carbohydrate, is the substrate for oxidation, since fat produces less heat and would thus leave more to be accounted for by glycolysis. In Table 3, the top line gives our observed upper limit of heat production, 521 kJ mol⁻¹ O₂, and the maximum energy that can be produced by the oxidation of fat, the molar enthalpy change (ΔH₀), which is 439 kJ mol⁻¹ O₂. The difference, 82 kJ mol⁻¹ O₂, is the ‘excess’ heat that hypothetically could have been produced by glycolysis in our experiment. The second line converts these heats to fractions of the total observed heat. The third line shows the amount of PCr that could be synthesized by oxidation and by glycolysis per unit of total observed heat. The PCr from oxidation is found by dividing the heat from oxidation by the ΔH₀ for formation of PCr by oxidation of fat, 74.4 kJ mol⁻¹ PCr. Similarly the PCr from glycolysis is found by dividing the ‘excess’ heat by the ΔH₀ for formation of PCr by glycolysis, 63.3 kJ mol⁻¹ PCr. The total PCr is the sum of that from oxidation and that from glycolysis. In the bottom line, the amounts of PCr from the different sources are shown as fractions of the total PCr synthesized. The calculation shows that glycolysis could produce at most 17.9 % of the total PCr during recovery.

(iii) Time course of recovery

If the simple hypothesis is correct, then the time course of oxygen use and of recovery heat production should match. However, if another reaction such as glycolysis were occurring during part of the recovery period, it would produce or absorb heat in addition to that from oxidation, and the summed heat would diverge from that expected for oxidation alone. As shown in Fig. 5, the rates of oxygen consumption and heat production match during most of the recovery period. However, there is mismatch in the early part of recovery. Heat production reaches its maximum rate much earlier than does the oxygen consumption. This discrepancy in rates is seen at the beginning of recovery during the first 100 s, as the first 5–20 % of recovery metabolism occurs. Is this evidence of an extra heat-producing reaction in addition to oxidation early in recovery?

Oxygen and heat must move between the sites of metabolism inside the muscle fibres and the oxygen electrode and thermopile, respectively, for the changes to be recorded. As explained in the Materials and methods section, the slowest steps are likely to be those depending on diffusion of oxygen and heat within the muscle. Therefore, we have explored the possibility that the lag between the detection of oxygen consumption and the detection of heat production in the early part of recovery is due to the slower diffusion of oxygen than of heat. In other words, oxygen is used within the muscle fibres and heat is produced at the same time, but we only detect the oxygen use when oxygen has left the solution around the muscle by diffusing into the muscle (which is slow) to replace its diffusion into the muscle.

### Table 3. How much phosphocreatine could be produced by glycolysis during recovery?

<table>
<thead>
<tr>
<th>Total observed</th>
<th>From oxidation of fat</th>
<th>From glycolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat (kJ k⁻¹ total observed heat)</td>
<td>521 a</td>
<td>439 b</td>
</tr>
<tr>
<td>Heat (kJ mol⁻¹ total observed heat)</td>
<td>1.00 (=521/521)</td>
<td>0.843 (=439/521)</td>
</tr>
<tr>
<td>PCr formed (mol kJ⁻¹ total observed heat)</td>
<td>0.01133 (=0.01133+0.00248)</td>
<td>0.01133 (=0.843/74.4 c )</td>
</tr>
<tr>
<td>PCr formed (as fraction of total)</td>
<td>0.01381 (=0.01381/0.01381)</td>
<td>0.01133/0.01381</td>
</tr>
</tbody>
</table>

aThe total observed heat per mole O₂, 521 kJ mol⁻¹, represents the maximum value and is the mean + 95 % confidence limit (see Fig. 7B).
b439 kJ mol⁻¹ O₂ used is the molar enthalpy value for oxidation of fat (Carpenter, 1939).
c74.4 kJ mol⁻¹ PCr formed is the molar enthalpy value for oxidation of fat expressed per mole of phosphocreatine (PCr), see b and assuming PCr/O₂ =5.9.
d63.3 kJ mol⁻¹ PCr formed is the molar enthalpy value for glycolysis expressed per mole of PCr (Meyerhof, 1930; Curtin and Woledge, 1978, p. 725). Assume PCr/lactic acid=1.5.
that used. Similarly, heat produced by oxidation (or any other process) is only detected when it has diffused from the muscle into the thermopile (but this is relatively fast) and produced a temperature change there. Is the diffusion of oxygen within the muscle sufficiently slow to account for the shape of the oxygen consumption recording shown in Fig. 4A?

Fig. 8 shows simulations (see Materials and methods) of the time course of oxygen consumption and heat production for a muscle preparation having dimensions equal to the average values for the muscle preparations used in the experiments. The simulations are based on the assumptions that recovery metabolism starts at its maximum rate at the onset of recovery and that its rate then declines exponentially. There is a clear delay between the increase in the rate of oxygen consumption by the muscle at the start of recovery and the time when the oxygen electrode would detect it. In contrast, the simulation shows that recovery heat production would be detected almost immediately by the thermopile.

Fig. 9 shows the results of the simulations expressed as rates and plotted against the amount of recovery that has occurred. These plots can be compared with the experimental results in Fig. 5, and seem very similar. Both in the experimental recordings and in the simulation, the differences in rates are at the beginning of recovery where the detected rate of heat production is higher than the detected rate of oxygen consumption. We conclude that the difference we observe between the oxygen and heat recordings can be explained by the slower diffusion of oxygen than heat. Taking this into account, our observations are consistent with the hypothesis that recovery metabolism is wholly oxidative.

Efficiency of recovery

Our results can be used to estimate the efficiency of the oxidative phosphorylation occurring during the recovery process. When the substrate is carbohydrate, a maximum of 6.5 phosphorylations can occur per O$_2$ used (Kushmerick, 1977). However, it could be that fewer phosphorylations occur per O$_2$ used during actual recovery.

If the P:O$_2$ ratio is at its maximum value of 6.5, the total quantity of energy produced by oxidation is 473 kJ mol$^{-1}$ O$_2$. Of this total energy, 221 kJ is used to synthesize PCr, and the rest (252 kJ) is released as heat. This situation corresponds to 100% efficiency, defining efficiency as the proportion of the energy potentially available to synthesize PCr that is actually used in that process (221/221). Another way of expressing this
is that the P:O₂ ratio is 100 % of its theoretical maximum value (6.5/6.5).

If the P:O₂ ratio during recovery were less than 6.5, then more heat would be produced per mole of O₂ used, since less energy would be taken up by PCr synthesis. In the extreme, when no PCr synthesis occurs, the efficiency of recovery would be 0 % and all the 473 kJ of energy per mole of O₂ would be released as heat.

In Fig. 10, the thin lines show the relationship between oxygen use and heat production that would exist for different values of the efficiency of recovery as defined above. The observations reported in this study are shown in red. Although the individual values are quite widely distributed, a mean value of the efficiency can be determined by regression analysis, and this gives a value of 84.0±20.1 % (±95 % confidence limits). Overall, the efficiency for oxidative recovery in these muscle fibres seems to be quite high. We are aware of no other measurement of this quantity in intact muscle fibres with which this result can be compared.

We thank the Biotechnology and Biological Sciences Research Council (UK) for financial support.

References


