J. exp. Biol. 122, 223–235 (1986)
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AEROBIC AND ANAEROBIC CORRELATES OF MECHANICAL WORK BY GASTROCNEMIUS MUSCLES OF THE AQUATIC AMPHIBIAN *XENOPUS LAEVIS*

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Accepted 14 January 1986

SUMMARY

1. Isolated, saline-perfused gastrocnemius muscles of *Xenopus laevis* were used to assess the relationships between aerobic and anaerobic metabolism during conditions of rest, isotonic contraction and recovery.

2. The major part (85%) of the energy used during 25 min of isotonic contractions in the saline-perfused muscles was from anaerobic rather than aerobic sources. However, the small contribution made by oxidative metabolism during activity can be attributed, in part at least, to limitations imposed by the rate of perfusion and the low O_2 capacity of the perfusate.

3. The respiratory exchange ratio ($R = \dot{V}_{CO_2}/\dot{V}_{O_2}$) of saline-perfused gastrocnemius muscles of *Xenopus* was 0·82 at rest, increasing to values well above 1·0 during activity. The elevated R value is consistent with liberation of CO_2 by metabolic acid titration of the bicarbonate buffer system of the saline perfusate. Recovery from exercise was characterized as a period of net CO_2 retention (R values of 0·4) presumably reflecting a replenishment of depleted CO_2 stores.

4. Depending on the acid–base status of the venous outflow from the isotonically contracting muscles, hydrogen ions were found to be released at either a greater rate (alkalosis) or slower rate (acidosis) than that of lactate.

INTRODUCTION

It has been suggested (Bennett & Licht, 1973) that amphibians such as grass frogs, engaging in rapid bursts of activity, have high rates of lactic acid production

Key words: amphibian muscle, mechanical work, muscle energetics.

(anaerobic scope), low rates of extra oxygen consumption above resting levels (aerobic scope) and exhaust rapidly. Slow-moving forms such as the bufonid toad, on the other hand, have high aerobic and low anaerobic scopes and do not easily fatigue. *Xenopus* is capable of very rapid movement and can be active in a fairly continuous fashion for 10–20 min, by which time both blood and muscle lactate levels are as high, or even higher (Putnam, 1979; Hutchison & Miller, 1979; Boutilier, Emilio & Shelton, 1986) than those previously recorded in *Rana* (Hutchison & Turney, 1975), *Ambystoma* (Hutchison, Turney & Gratz, 1977) and *Amphiuma* (Preslar & Hutchison, 1978).

The oxygen consumption, the rate of accumulation of lactic acid, and the final concentrations of lactate reached in the muscles and blood of intact animals obviously depend on the power output of the muscles and the exchange of materials between cells, blood and environment. There is no obvious reason why a high power output could not be accompanied by high aerobic as well as high anaerobic scope, but the relationships are very difficult to study in intact animals because levels of activity are difficult to control and measure. In a previous paper (Boutilier *et al.* 1986) we described a newly developed preparation of the gastrocnemius muscle from *Xenopus* in which a system of perfusion greatly enhanced the capacity to contract over a period of time as well as increasing the work output before fatigue. We were able to show that ion and water movements resulting from the quantified contractions were similar to those seen in the intact animal. This preparation seemed to offer the possibility of assessing the relationships between aerobic metabolism, anaerobic metabolism and work under controlled conditions in a way that might be applicable to a wider survey of amphibian species. We therefore undertook the following preliminary experiments to measure changes in oxygen consumption, carbon dioxide production, lactate ions and metabolic protons that occurred during rest, work and recovery.

METHODS

Male and female specimens of *Xenopus laevis* (100–120 g) were obtained from commercial suppliers and held in the laboratory at 20 °C. The gastrocnemius muscle (mean weight, 3·133 g), attached to the knee joint, was prepared as described in detail by Boutilier *et al.* (1986). Each muscle was perfused *via* PP60 cannulae inserted into the femoral artery and vein, all branches of these vessels except for the ones serving the gastrocnemius being tied off. The saline perfusate had the following composition (in mmol l^{-1}): NaCl, 95·0; KCl, 2·4; $CaCl_2$, 2·0; $MgCl_2$, 1·0; $NaHCO_3$, 20·0; glucose, 5·0. It also contained 40 units ml^{-1} carbonic anhydrase (Sigma). The saline was equilibrated with a 1·33 % CO_2–98·67 % O_2 gas mixture and the flow rate through the preparation was held constant at 0·3 ml min^{-1} throughout all stages of the experiment. The knee joint was fixed to a Perspex mount on which the muscle came into contact with stimulating electrodes. The Achilles tendon was attached to a calibrated length transducer (Washington Type T2) whose output was recorded on a Washington Type MD2C pen oscillograph. The muscle and its mount were fixed in the barrel of a 20 ml glass syringe filled with saline. All

experiments were carried out at an air-conditioned room temperature of 22–26°C, which fluctuated not more than ±1°C during a single experiment.

Measurements were taken from the resting muscle for a period of at least 30 min, following which the preparation was stimulated for 25 min, the stimuli being single 10-ms pulses at a frequency of $0{\cdot}6\,s^{-1}$ and a voltage sufficient to give maximal twitches. The muscle contracted throughout the period of stimulation but was showing appreciable fatigue at the end of that time. Recovery was followed over varying lengths of time as specified in the text.

In some of the experiments described here (Series 1) the arrangements were identical to those detailed in the previous paper, with output from the venous cannula passing directly to a vial in which successive collections of saline were made over 5-min periods. Small samples taken towards the end of each period were used to determine venous pH by means of a calibrated Radiometer E5021 electrode system. The 5-min collections were stored at −20°C until such time as the lactate concentrations could be measured enzymatically, using Boehringer Mannheim test kits. At the end of the experiment, the muscle was weighed and samples taken and frozen at −20°C, also for subsequent lactate analysis. These experiments ended immediately after the stimulation period and the events during recovery were not examined. Full details of the procedures are given in the preceding paper (Boutilier *et al.* 1986).

In most of the experiments described in this paper (Series 2), the saline outflow from the muscle passed directly *via* the venous cannula into the thermostatted measuring cuvettes of serially arranged Radiometer P_{O_2} and P_{CO_2} electrodes. The continuous output of Radiometer PHM 27 display meters, connected to these electrodes, was permanently recorded by means of a Philips PM 8252 flat bed recorder (Fig. 1). Both electrodes were calibrated before and after the 2–2·5 h experimental period and corrections were applied to the recorded data on the assumption that any drift, which was always small, was linear throughout the intervening time. The time delays for a given bolus of saline to pass through the electrode measuring system were negligible in relation to the overall experimental time periods and the rates of change in venous P_{O_2} and P_{CO_2} (Boutilier, 1981). From the gas electrodes the perfusate entered a weighed volume of fluid in a small vial containing a combined pH electrode (GK 2320c) and the output tip of an automatic NaOH dispenser (Fig. 1). Both were connected to a Radiometer titration assembly (TTT11, PHM 27 meter, and SBR 2C titrigraph) set at a sensitivity that allowed constant end-point titration to the pH of the reference, set at the level of the arterial perfusate. Thus, at constant P_{CO_2} the volume of NaOH titrant added to the vial over a period of time would be equivalent to the H^+ ions resulting from metabolic (i.e. non-carbonic acid) additions to the perfusate from the muscle in that time. Successive 5-min collections of the outflow were made as it left the pH-stat and their volume measured, corrections being made for the volume of titrant added to the initial volume of perfusate. The collections were stored at −20°C until the lactate concentrations could be measured enzymatically as before (Boutilier *et al.* 1986). These experiments were continued after the stimulation period for 40–90 min so that recovery could be assessed.

Calculation of the geometric time delays due to cannula lengths and diameters, chamber volumes and pen writing differentials allowed compensating corrections to be applied to the results. The composite time constants, due to electrode response times and mixing in the analytical chambers of the O_2, CO_2 and pH-stat, were determined experimentally by applying step changes in the relevant parameter to the total system. This was carried out after an experiment by connecting the perfusion syringe and arterial catheter, already filled with a suitably constituted perfusate, to the venous catheter, thus bypassing the muscle. The overall responses to such step changes could be described adequately by single exponentials and appropriate corrections were applied to the results.

Paired gastrocnemius muscles from both legs of an animal were prepared and set up in identical sets of equipment. Only one of the muscles was stimulated to contract; the other was used as a control in assessing the changes caused by exercise.

Mean data are presented as $\bar{x} \pm 1$ standard error of the mean.

Calculations

The rate of O_2 consumption by the muscle ($\dot{V}_{O_2}$) was determined as follows:

$$\dot{V}_{O_2} = \dot{V} \times \alpha O_2 \, (Pa_{O_2} - Pv_{O_2}),$$

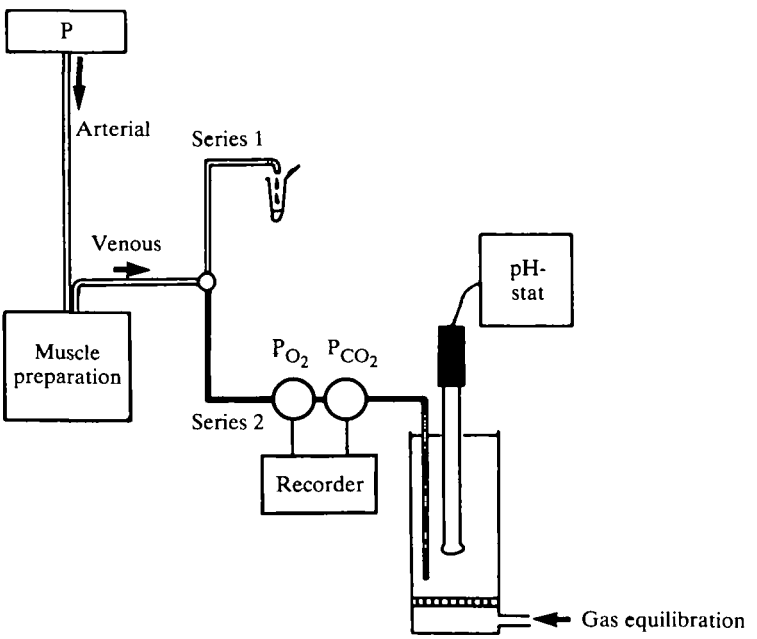

Fig. 1. Diagram of the experimental arrangement for perfused gastrocnemius of *Xenopus*. Muscle preparation for measurement of isotonic work as illustrated in Boutilier, Emilio & Shelton (1986). Muscle perfused from pump (P) at constant arterial flow of 0·3 ml min^{-1}. Venous outflow passes through serially connected P_{O_2} and P_{CO_2} electrodes, then into a titration vial (pH-stat) containing a combined pH electrode and the output tip of an automatic NaOH dispenser. Further details in text.

where $\dot{V}$ is the flow rate of the perfusate and αO_2 is the solubility coefficient for oxygen in a 0·119 mol l^{-1} NaCl solution at 25 °C (0·0274 ml ml^{-1} atm^{-1}; Altman & Dittmer, 1971).

The rate of production of CO_2 by the muscle ($\dot{V}_{CO_2}$) was calculated as the overall amount of CO_2 added to the perfusate (as measured by the rise in P_{CO_2} above arterial value) minus that which could be attributed to the addition of metabolically derived H^+ ions (stoichiometrically equivalent to the fall in bicarbonate concentration and therefore to the amount of titrant added in the pH-stat):

$$\dot{V}_{CO_2} = \{\dot{V} \times \alpha CO_2 \times (Pv_{CO_2} - Pa_{CO_2})\} + ([OH^-] \times \dot{V}),$$

where $([OH^-] \times \dot{V})$ is the concentration of NaOH titrant added times the perfusate flow rate (corrected for the volume of titrant added for the time interval considered), and αCO_2 is the solubility coefficient for carbon dioxide in a 0·119 mol l^{-1} NaCl solution at 25 °C (0·746 ml ml^{-1} atm^{-1}; Altman & Dittmer, 1971).

The total lactate production of the muscle ($lactate^-_{tot}$) was calculated as the sum of lactate transferred to the venous perfusate ($[lactate^-]_v \times V_v$) and the bathing solution volume ($[lactate^-]_b \times V_b$) together with that which was left in the muscle ($[lactate^-]_m \times$ wet $weight_m$) at the end of the control or stimulation period:

$$lactate^-_{tot} = ([lactate^-]_m \times W_m) + \sum_{i=1}^{n}([lactate^-]_v \times V_v) + ([lactate^-]_b \times V_b).$$

RESULTS

Series 1: lactic acid formation during activity

The effect of activity on the pH and lactate concentrations in the perfusate are shown in Fig. 2. The venous pH in the resting state was some 0·18 units lower than that in the arterial cannula. There was an exponential fall in pH during stimulation and the difference increased to 0·80 units towards the end of the period of activity. The venous lactate concentration was never higher than 0·15 mmol l^{-1} in the perfusate from resting muscle and was not significantly different from zero. During activity the concentration rose continuously, reaching a mean value of 3·15 ± 0·63 mmol l^{-1} at the end of stimulation, at which time it was still rising. The final mean concentration of lactate inside the six muscles immediately after exercise was 30·60 ± 3·06 mmol kg^{-1} (5·58 ± 0·91 mmol kg^{-1}, after 30 min of rest in the paired control muscles).

The total amount of work performed by the different muscles varied over a substantial range due to differences in rates of fatigue and in the extent of shortening during a twitch. No experiments were specifically directed towards varying the work done. However, it was found from these spontaneous fluctuations that those muscles carrying out most work produced the greatest total amount of lactate (Fig. 3A) and also contained the highest concentration at the end of the stimulation period (Fig. 3B).

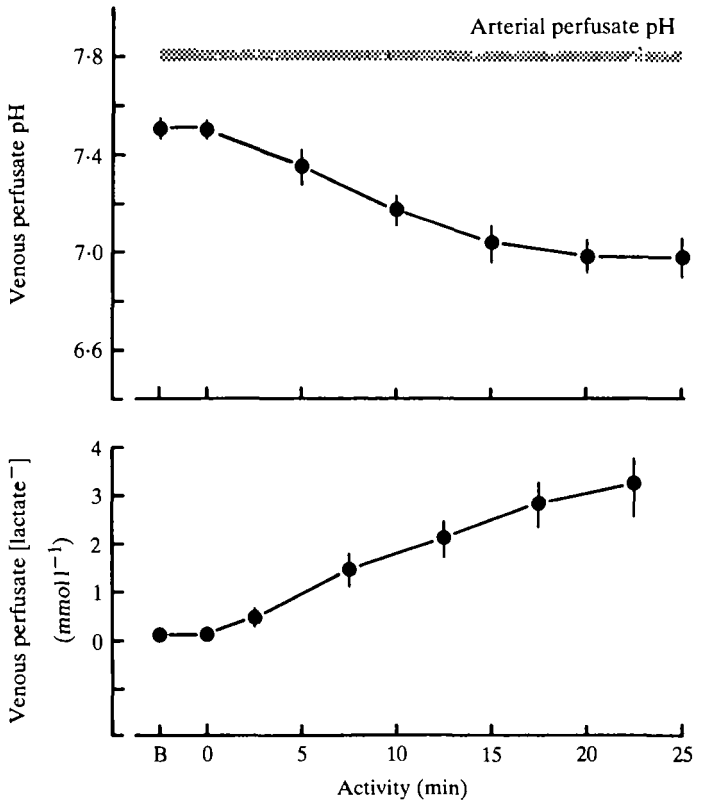

Fig. 2. Mean (±1 S.E.M.) values for pH and lactate concentration in the venous outflow of saline perfused gastrocnemius muscles ($N = 7$) at rest and during 25 min of isotonic contractions. Shaded area in pH plot represents ±1 S.E.M. of the mean arterial perfusate pH. The arterial perfusate was lactate-free. B = mean values for 1-h period of rest prior to activity.

Series 2: aerobic and anaerobic correlates of activity and recovery

Lactate and hydrogen ion release

The rates of H^+ and lactate ion release, before, during and after the period of stimulation are shown in Fig. 4. The accuracy of these estimations is limited by the delay factors and the time constants for change in the pH-stat and collecting system. During the first 10 min of activity, H^+ efflux from the muscle exceeded that of the lactate ion, but as the pH in the extracellular fluid fell (Fig. 2), the reverse relationship was found. During recovery, H^+ emerged from the muscles at a slightly greater rate than the lactate ions.

Oxygen uptake and carbon dioxide production

Outputs from the P_{O_2} and P_{CO_2} electrodes, after corrections for delays and time constants which are both very small, are shown in Fig. 5. Activity in the muscle caused the P_{O_2} to fall to very low levels in the venous perfusate and increased the P_{CO_2} to about 100 Torr. The calculated levels of O_2 consumption ($\dot{V}_{O_2}$), CO_2 production ($\dot{V}_{CO_2}$) and the respiratory exchange ratio ($R = \dot{V}_{CO_2}/\dot{V}_{O_2}$) are shown in Fig. 6 for the

resting, active and recovering muscles. There was considerable variation in the values obtained during activity from the eight muscles used, as shown by the large standard errors. The estimates of $\dot{V}_{CO_2}$ were clearly less straightforward than those of $\dot{V}_{O_2}$ in that the OH^- additions by titration had to be taken into account. Towards the end of the stimulation period, about 75% of the P_{CO_2} increase was due to the addition of metabolically derived protons.

The mean $\dot{V}_{O_2}$ of resting muscle was $3{\cdot}03\times10^{-2}$ μmol g^{-1} wet wt min^{-1} at 25°C, falling within the range reported for resting sartorius muscle equilibrated in saline ($1{\cdot}36\times10^{-2}$–$4{\cdot}95\times10^{-2}$ μmol g^{-1} min^{-1} at 20–25°C; Hill, 1965; Mahler, 1978). The corresponding $\dot{V}_{CO_2}$ value was $2{\cdot}45\times10^{-2}$ μmol g^{-1} min^{-1}, resulting in a respiratory exchange ratio (R) of $0{\cdot}82\pm0{\cdot}04$ for muscles at rest (Fig. 6). Within the first 10 min of activity, $\dot{V}_{CO_2}$ began to exceed $\dot{V}_{O_2}$, and the R value was greater than unity. This continued to be true throughout the period of stimulation, although there was marked variability between muscles. During recovery, $\dot{V}_{CO_2}$ fell quite quickly to resting levels, whereas $\dot{V}_{O_2}$ remained above the pre-exercise values for periods in excess of the 40–90 min adopted in the protocol of this group of experiments. As a result, the R values fell to levels somewhat lower than those found in resting muscles.

Muscle energetics during activity

The total mechanical work, measured as force times distance of shortening, was similar in both series of experiments ($0{\cdot}211\pm0{\cdot}051$ J g^{-1} wet wt, series 1; $0{\cdot}235\pm0{\cdot}047$ J g^{-1}, series 2), and so we assume the partitioning of energy from aerobic and anaerobic sources to have been similar as well. With these provisions, the

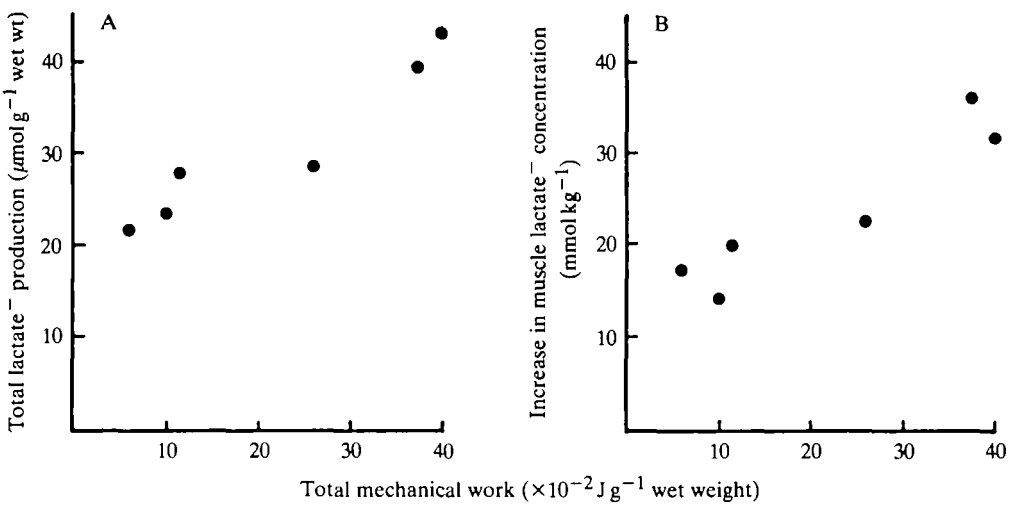

Fig. 3. Relationship between the total mechanical work performed per gram wet weight of muscle and (A) the total lactate production, (B) the increase in muscle lactate concentration. These experiments ended immediately after the period of mechanical work. Each data point represents one paired experiment where the production or concentration changes of resting muscles have been subtracted from those of the stimulated muscles.

energetic equivalents of the lactate produced in series 1 experiments (Fig. 3A) and oxygen consumed in series 2 experiments (Fig. 6) have been used to estimate the efficiency of the contracting muscles and the relative importance of aerobic and anaerobic sources of energy (Table 1). Efficiencies, calculated as the total work output divided by the sum of the energetic equivalents of the O_2 consumed and lactate produced, were about 5–8%. In the muscle preparations, perfused as described, it is estimated that approximately 15% of the energy input during activity comes from metabolism involving the extra O_2 consumed, the rest coming from processes leading to the formation of lactate.

DISCUSSION

The major part of the energy used during the period of activity in the experiments described here is derived from anaerobic rather than aerobic sources (Table 1). In terms of the concepts proposed for intact animals by Bennett & Licht (1973) and Bennett (1978), the isolated and perfused gastrocnemius muscle of *Xenopus* seems to have a high anaerobic capacity and probably a high anaerobic and low aerobic scope. However, it seems likely that the small contribution to the energy input made by oxidative metabolism during activity, as shown by the relatively modest (2·5-fold)

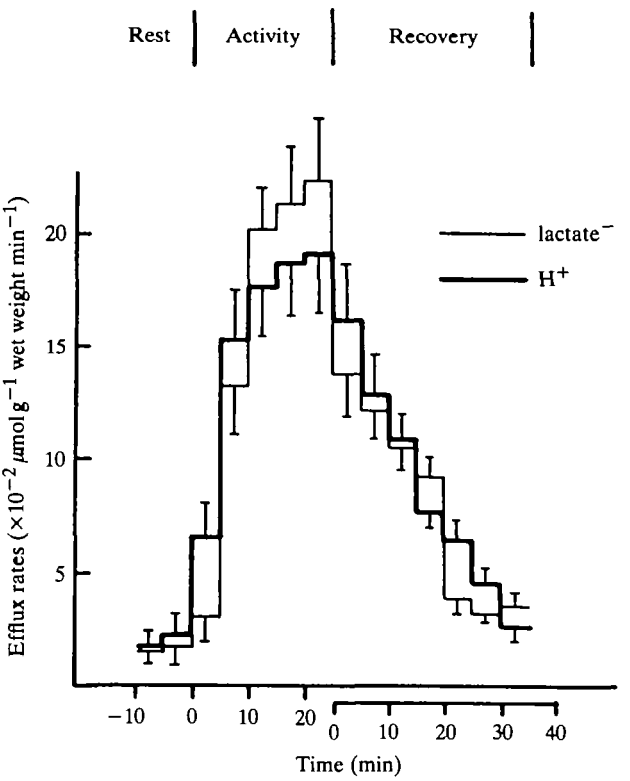

Fig. 4. Mean (±1 S.E.M.) efflux rates of hydrogen and lactate ions for periods before, during and after 25 min of isotonic work by gastrocnemius muscle pairs ($N = 8$).

Table 1. *Total mechanical work and energetic equivalents of oxygen consumed and lactate produced during 25 min of isotonic contractions (0·6 s^{-1}) of* Xenopus *gastrocnemius muscles perfused with saline*

Gastrocnemius muscle wet weight (g)*	3·133 ± 0·149
Total mechanical work (J g^{-1})*	0·224 ± 0·051
Energetic equivalent of lactate produced (J g^{-1})†	2·79 ± 0·24
Energetic equivalent of oxygen consumed (J g^{-1})‡	0·41 ± 0·24
% Efficiency of muscle	7·1 ± 0·84

* Mean of series 1 and series 2 experiments.
† Series 1 experiments (six muscle pairs).
‡ Series 2 experiments (eight muscle pairs).
Values are means ± 1 S.E.M.

increase in O_2 consumption, could be attributed to the limitations imposed by the rate of perfusion and the low O_2 capacity of the perfusate. The extraction of O_2 from the perfusate was virtually complete in all the experiments (e.g. Fig. 5) and it is clear that perfusion with a higher capacity medium, such as blood itself, would be necessary if *in vivo* conditions were to be reproduced. Even so, the aerobic contribution to the total energetic cost of activity in the isolated muscles here (approx. 15 %, Table 1) is virtually identical to that found for intact *Xenopus* when exercised to exhaustion at comparable temperatures (approx. 16 %, Hutchison & Miller, 1979).

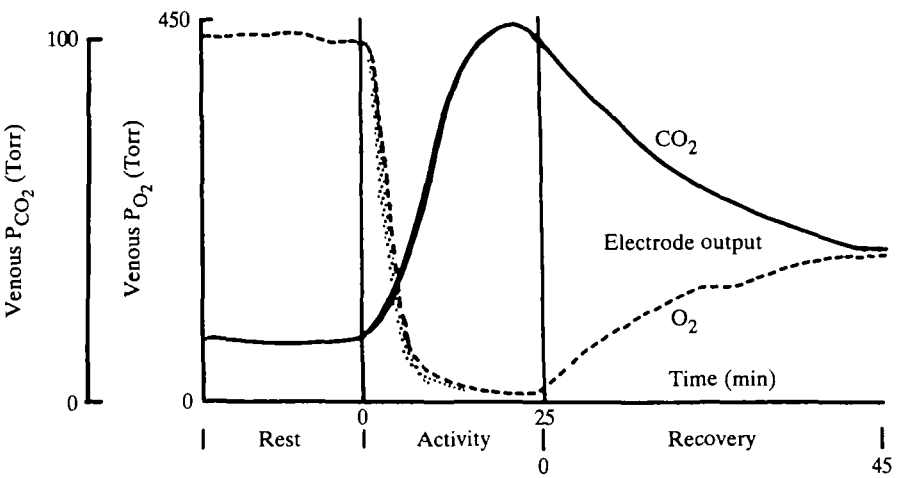

Fig. 5. P_{O_2} and P_{CO_2} electrode outputs of a single experiment on perfused gastrocnemius muscle at rest, during 25 min of isotonic contractions and 45 min recovery. Electrodes were measuring gas tensions in continuous venous outflow as in Fig. 1. Arterial perfusate had a P_{O_2} of 615 Torr and a P_{CO_2} of 12·5 Torr. Computer analysis corrected for any time delays in the electrode measuring system in relation to the time periods and frequencies at which venous P_{O_2} and P_{CO_2} levels were changing (i.e. correction for electrode response delays). At periods of most rapid change (i.e. activity), the thickness of the P_{CO_2} line and the shaded area about the P_{O_2} line encompass the levels of error involved.

The elevation of the respiratory exchange ratio (R) to values above 1 during exercise (Fig. 6) was consistent with the production of CO_2 by metabolic acid titration of bicarbonate in the saline. The recovery phase was characterized by net CO_2 retention and decline in R values as bicarbonate stores were replenished. This period of bicarbonate recovery would tend to lessen any further intracellular pH changes and function also in restoring muscle performance (Jones, Sutton, Taylor & Toews, 1977).

High levels of anaerobic energy production in isolated gastrocnemius muscles can result in substantial accumulations of lactate ions (30–40 mmol kg^{-1}) in the fatiguing

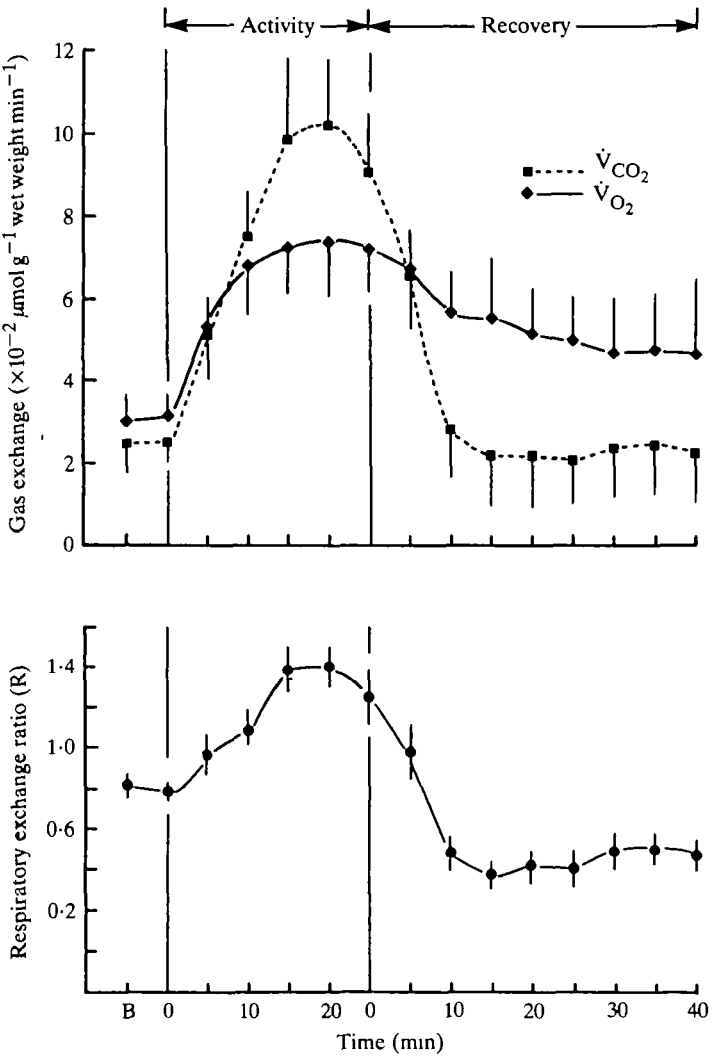

Fig. 6. Mean (±1 S.E.M., $N = 8$) changes in the oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$) and respiratory exchange ratio, R ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) for periods before, during and after 25 min of isotonic work by isolated gastrocnemius muscles of *Xenopus*. B = mean value for 1-h period of rest prior to activity.

muscles after 25 min activity (Fig. 3B). These levels are not appreciably different from those found in gastrocnemius muscles after intact animals have been exercised to exhaustion (Putnam, 1979; Boutilier *et al.* 1986). There are difficulties in ascribing the development of fatigue simply to the accumulation of lactate, since recovery of muscle performance can occur with little change in lactate levels (Putnam, 1979). However, the stoichiometrically produced H^+ ions cause a fall in intramuscular pH (Boutilier *et al.* 1986), and the effect that this has on Ca^{2+} distribution is thought to be important in fatigue development (Mainwood, Worsley-Brown & Paterson, 1972; Mainwood & Lucier, 1972; Nassar-Gentina, Passonneau, Vergara & Rapoport, 1978).

Lactate production during activity eventually leads to large concentration differences between muscle cells and blood (Putnam, 1979; Boutilier *et al.* 1986) or muscle and perfusate, indicating that there are probably rate-limiting steps involved in removal of the ion across the cell membrane and/or the interstitial space. There is some uncertainty as to the relative rates of efflux of the lactate and the stoichiometrically generated H^+ ions. Benadé & Heisler (1978) found that the efflux rates for H^+ exceeded those for lactate by 14 times in rat diaphragm and 50 times in frog sartorius muscle, after the muscles were transferred to saline following fatigue-induced stimulation in a gaseous environment. In other experiments on the active gastrocnemius of dog, H^+ ions were released at the same rate as lactate$^-$ if the blood perfusate was alkalotic or at a lower rate if the blood was acidotic (Hirche *et al.* 1975). Similar findings have also been reported (Mainwood & Worsley-Brown, 1975) for frog sartorius muscle preparations that were continuously superfused with saline. In general, our experiments support these latter results, the relative efflux of H^+ exceeding lactate$^-$ when pH_v was high with the reverse occurring as the pH_v decreased during exercise (Fig. 4). Caution is needed in these interpretations because the relationships are complex (cf. Holeton & Heisler, 1983). The absence of protein buffers in the saline perfusate leads to much greater pH_v changes than occur *in vivo* (Boutilier *et al.* 1986) and so to smaller gradients and probably to restricted H^+ release. The saline-perfused preparation may also favour lactate ion washout, as compared to *in vivo* conditions, since the arterial perfusate was lactate-free, thus increasing the gradient from the cells. However, low external pH may also decrease the rate of lactate ion release (Mainwood & Worsley-Brown, 1975). Changes in water distribution between intra- and extracellular compartments during muscle contractions (Boutilier *et al.* 1986) may also be important in determining the relative efflux rates of H^+ and lactate ions, as might differences in the rates of muscle perfusion. It is clear that more detailed studies are needed to resolve the differences that exist in results obtained under a variety of experimental conditions.

The estimates of efficiency in the conversion of chemical into mechanical energy are very low in the gastrocnemius preparation as compared with those for the frog sartorius muscle using measurements of heat production (Hill, 1939, 1964) or the hydrolysis of ATP (Kushmerick & Davis, 1969). The experiments described here were obviously capable of determining only that energy involved in shortening and some energy wastage must have occurred before shortening began and after it ended.

More importantly, it seems very likely that, at loads which were far below the maximum isometric tension, the velocities of contraction would be well above those required for greatest efficiency. Further experiment, with muscles shortening against different loads, would be necessary to confirm this suggestion.

The perfused gastrocnemius preparation clearly enables detailed studies to be made of the exchanges between muscle cells and blood system when the muscle is stimulated to levels of activity which can be experimentally controlled. The use of a simple saline as the perfusion medium limits the pressures and rates of perfusion (Boutilier *et al.* 1986) and, because of its low capacities for the respiratory gases, also restricts gas exchange, acid–base equilibria and aerobic metabolism. These problems are resolvable by perfusing the system with blood and the further use of this amphibian preparation in modelling and investigating *in vivo* processes will almost certainly require a development of this type.

We wish to thank Ms Wivi Svensson for expert technical assistance. Financial support from the Commonwealth Scholarship Commission and the Gulbenkian Institute are gratefully acknowledged.

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