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THE EFFECTS OF MECHANICAL WORK ON ELECTROLYTE AND WATER DISTRIBUTION IN AMPHIBIAN SKELETAL MUSCLE

BY R. G. BOUTILIER

Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1 Canada

M. G. EMILIO

Physiology Laboratory, Department of Cell Biology, Gulbenkian Institute of Science, Oeiras, Portugal

AND G. SHELTON

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England

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SUMMARY

The present experiments were undertaken to confirm whether the increase in haematocrit that consistently accompanies the build-up of lactate in amphibian muscle cells during exercise can be explained in terms of a movement of water from the blood into the active muscles.

Electrically stimulated sartorius and gastrocnemius muscles isolated from *Rana ridibunda* and *Xenopus laevis* had consistently higher total water contents than their paired control muscles. In both instances, it was the intracellular water volume which gave rise to the increase in total muscle water. These results were corroborated *in vivo* by sampling gastrocnemius muscles from exercising and resting *Xenopus laevis*. Analyses of tissue electrolyte levels in the working muscles of each experimental series showed an increase in intracellular [lactate$^-$] and [Na^+]. A corresponding decline in cellular [K^+] occurred in concert with increases in extracellular [K^+].

In saline-perfused gastrocnemii of *Xenopus*, the uptake of vascular water was proportional to the total mechanical work performed. Saline leaving the femoral vein of isotonically contracting gastrocnemius muscles had a greater osmotic pressure than that of the arterial perfusate, whereas arterio-venous osmolality differences of control muscles were negligible. Calculations show that the haemoconcentration during exercise *in vivo* can be attributed at least in part to a net flow of plasma water to osmotically enriched muscle cells.

INTRODUCTION

A significant increase in the haematocrit value of arterial blood of *Xenopus* was seen after 30 min of forced submersion (Emilio & Shelton, 1980). This increase was

Key words: amphibian muscle, mechanical work, water distribution.

associated with a decrease in plasma volume, and it was suggested that water was moving into skeletal muscle tissue where osmotically active metabolic products were accumulating due to the combined effects of hypoxia and activity. Increased haematocrit values related to muscular activity have been observed in other amphibian species (McDonald, Boutilier & Toews, 1980; Boutilier, McDonald & Toews, 1980) as well as in fish where they are also associated, in part at least, with a decline in plasma volume (Black, Manning & Hayashi, 1966; Stevens, 1968; Yamamoto, Itazawa & Kobayashi, 1980). Accumulation of muscle lactate is well documented in exercising fish (reviewed by Jones & Randall, 1978), in amphibians (Bennett & Licht, 1973; Putnam, 1979) and in reptiles (Bennett & Licht, 1972) and there is good evidence that lactate can be formed much more rapidly than it is released to the general circulation. Similar changes in lactate, haematocrit and plasma volume are found in the muscles and blood of exercising mammals (Bergstrom, Guarnieri & Hultman, 1971; Hirche *et al.* 1975; Sahlin, Alvestrand, Brandt & Hultman, 1978) although events during recovery occur more rapidly than they do in lower vertebrates. However, anaerobic metabolism plays a much more important part in vigorous activity in the lower vertebrates with their relatively low levels of aerobic metabolism (Bennett, 1978), so that the effects are likely to be more marked and widespread than they are in birds and mammals.

It is clear, therefore, that during the non-equilibrium states caused by anaerobic activity, movements of water, ions and metabolites can occur between cells, fluid compartments of the body and, in some cases, the external environment (Heisler, 1980; Turner, Wood & Clark, 1983). The relationships are difficult to follow because not only are several, variously related, compartments involved in the exchanges but also activity levels in muscles or groups of muscles are extremely difficult to standardize or quantify in whole animal studies. The present *in vivo* experiments were undertaken to confirm that muscular activity in *Xenopus* caused water and ion movements between blood and muscles as the earlier work would suggest. In addition, *in vitro* experiments were carried out on both perfused and non-perfused muscle preparations to examine these movements in relation to more precisely controlled conditions of activity and of mechanical work output. The perfused muscle preparation was also used to study the metabolite relationships during activity and these results are reported in a subsequent paper (Boutilier, Emilio & Shelton, 1986).

METHODS

Male and female specimens of *Xenopus laevis* (90–120 g) and *Rana ridibunda* (35–55 g) were obtained from commercial suppliers several months before the experiments and were held in tanks at temperatures of 20 and 10 °C respectively. They were fed regularly and were in good condition. The experiments were carried out at an air-conditioned room temperature which ranged from 22 to 26 °C, although during a single experiment the variation was never greater than ± 1 °C.

In vivo *experiments*

Xenopus were anaesthetized in a 0·1 % solution of tricaine methanesulphonate (Sigma) buffered to pH 7 and a femoral artery was cannulated occlusively in each animal, using PP 60 tubing. The animals were allowed at least 24 h to recover from surgery. Two or three hours before the experiments [^{14}C]DMO and [^{60}Co]EDTA were injected into the femoral cannula in order to measure intracellular pH (Waddell & Butler, 1959) and the relative volumes of the tissue water compartments (Ferreira & Swensson, 1979).

The duration and extent of the activity were standardized as far as possible in both the *in vivo* and the *in vitro* studies by using 25- to 30-min exercise periods in all cases, with the levels of activity causing fatigue towards the end of the period. The toads were exercised by giving them mild electric stimuli of 10 ms duration at a frequency of $0{\cdot}6\,s^{-1}$. They responded by making brief swimming movements after each stimulus, moving freely about the tank and occasionally coming to the surface to breathe. At the end of a 30-min period the animals showed signs of fatigue. Immediately following the exercise, a 0·5-ml blood sample was taken from each animal into a gas-tight syringe and the animal was then killed with an anaesthetic overdose. Samples of the gastrocnemius muscles, weighing approximately 0·3 g, were taken as soon as possible with a sharp scalpel. Duplicate tissue samples of both the outer and inner layers of the muscle were immediately frozen in liquid nitrogen for subsequent analysis of lactate concentration. The three or four remaining samples were washed in isotonic sucrose, blotted on filter paper, weighed and dried to a constant weight at 100 °C. They were then placed in scintillation vials containing $0{\cdot}1\,mol\,l^{-1}$ HNO_3 and slowly agitated for 24 h to allow for complete disintegration of the cells. Small quantities were taken for determination of [Na^+], [K^+] and [Cl^-], and the radioactivities of ^{60}Co and ^{14}C in the remaining samples were measured in a gamma counter (Picker-Pace I Gamma System) and scintillation counter (Beckmann LS-1000), respectively. Some 100–150 μl of the blood sample was used to determine pH, P_{CO_2}, and P_{O_2} using Radiometer electrodes and meters, and the remainder of the sample was centrifuged and the plasma frozen. Subsequently, thawed plasma samples were analysed for [Na^+], [K^+], [Cl^-], [lactate$^-$] and osmolality, and the activities of ^{60}Co and ^{14}C were assayed. A second group of animals was subjected to the same procedures but was not exercised so that control values could be determined.

In vitro *experiments*

Xenopus *gastrocnemius muscles*

Toads were anaesthetized as before and the femoral artery and vein in the thigh region of both legs were occlusively cannulated with lengths of PP 60 tubing facing towards the periphery. The blood vessels in both legs were then immediatley washed out with 5 ml of saline containing 50 i.u. heparin ml^{-1}. During the further production of the preparation, the legs were perfused continuously from a constant pressure head of 20–25 cmH_2O, using saline equilibrated with a 1·33 %

CO_2-98·67 % O_2 gas mixture. The saline 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 Roughton-Booth enzyme units of carbonic anhydrase ml^{-1} (Sigma). Branches of the artery and vein not serving the gastrocnemius muscle were isolated and tied off and smaller leaks were stopped by applying tissue adhesive. The knee joint with the cannulated blood vessels and gastrocnemius muscle was then freed from the rest of the leg. The knee was pinned firmly to a length of perspex along which the muscle contacted a row of stimulating electrodes (Fig. 1).

The perspex mount with the preparation was fixed in the barrel of a 20 ml glass syringe filled with saline and the Achilles tendon was connected to a length transducer (Washington Type T2). The muscle was loaded by suspending weights between 30 and 50 g from a long piece of thin elastic attached to the opposite arm of

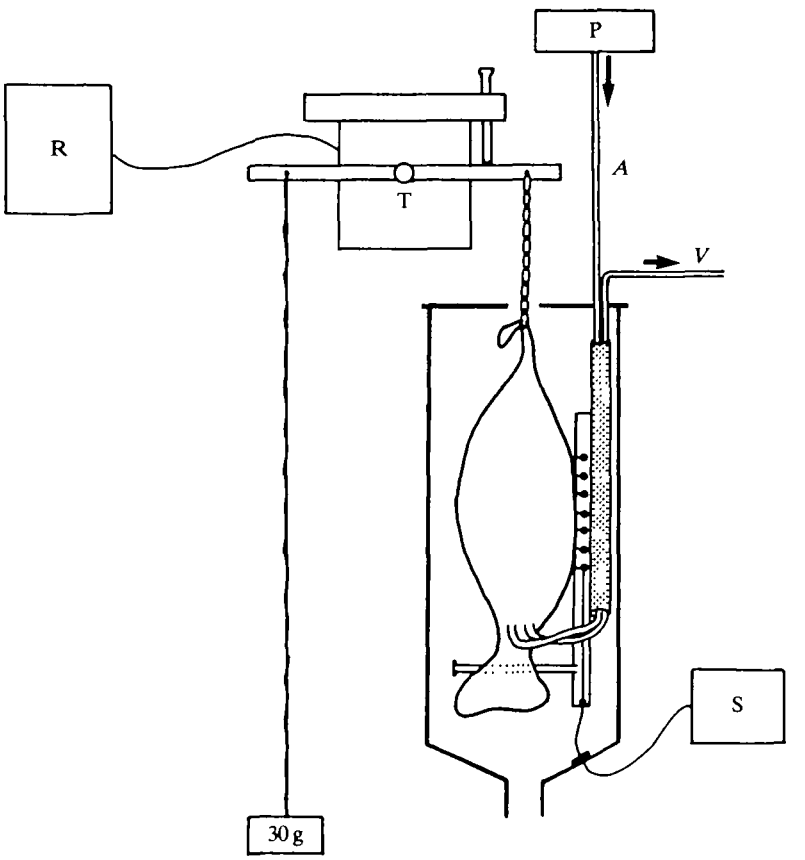

Fig. 1. Diagram of the experimental arrangement for perfused gastrocnemius of *Xenopus*. Isolated muscle fixed to stimulation frame and perfused from pump (P) at constant arterial (*A*) flow of 0·3 ml min^{-1}. Stimulator (S) delivered square-wave impulses causing muscle to contract and shorten against a 30 g load suspended on a long piece of elastic. Length of shortening was measured by an isotonic transducer (T). Control muscles from opposite legs were set up in exactly the same fashion, but without stimulation. R, recorder; *V*, venous flow.

the transducer, equidistant from the pivot (Fig. 1). The elastic avoided inertial complications during the twitch contractions. The afterload stop was adjusted so that the resting muscle was held at its unloaded length. The muscle was stimulated directly at a frequency of $0{\cdot}6\,s^{-1}$, using rectangular pulses 10 ms in duration and of a voltage sufficient to produce maximal twitch contractions. The load and stimulation frequency were chosen so that the muscle would contract for the whole of a 25- to 30-min period, though showing appreciable fatigue at the end of that time. The output of the transducer was calibrated and recorded on a Washington Type MD2C pen oscillograph.

The experiments were carried out on identically mounted pairs of muscles from a single animal, one muscle being stimulated and the other remaining inactive. Tissue water compartments were measured by adding the extracellular marker [^{60}Co]EDTA to the saline. During the experimental period the paired muscles were perfused from two 100 ml syringes at a constant rate of $0{\cdot}3\,ml\,min^{-1}$, both syringes being filled with CO_2-O_2 equilibrated saline containing the radioactive marker. After perfusion for a preliminary period of 1·5 to 2 h to ensure an even distribution of the marker, successive 5-min collections of the venous outflow were made for a period of 40–60 min. The samples were collected in weighed scintillation vials which, after reweighing, were stored at −20°C until further analyses of [Na^+], [K^+], [Cl^-], [lactate$^-$] and osmolality could be carried out. The pH of the inflowing and outflowing saline was measured at the end of each 5-min sample using a Radiometer E5021 microelectrode system calibrated with precision buffer solutions. After this sampling period at rest, one of the muscles was then stimulated for 25 min, pH measurements and venous outflow collections continuing as before. Immediately following the exercise period, the experimental and control muscles were dissected from the tendon and knee joint, blotted on filter paper and weighed. As in the *in vivo* experiments, duplicate tissue samples from the muscles were frozen in liquid nitrogen for subsequent lactate analysis. Other replicate samples were weighed, dried to constant weight, and agitated in scintillation vials containing $0{\cdot}1\,mol\,l^{-1}$ HNO_3 for 24 h to allow extraction of the cell contents. The radioactivities of these tissue samples, and of similarly treated samples of the saline perfusates, were measured in the gamma counter. The [Na^+] and [K^+] of the tissue sample extracts were also determined.

Xenopus *and* Rana *sartorius muscle*

Sartorius muscles were separated from both hind limbs of pithed animals and were removed together with a small piece of the pelvic girdle by which each was secured to the perspex mount described above. In the experiments on *Xenopus* sartorius, the muscles were connected to length transducers and bathed in the CO_2-O_2 equilibrated saline. They were weighed accurately before and after activity in order to determine the relationship between weight change and mechanical work. The *Rana* sartorius muscles were immersed in saline containing [^{60}Co]EDTA marker and their tendons fixed in position at the muscle resting length so that any contractions were isometric. Following 2–3 h in the saline to distribute the marker evenly, one muscle

of the pair was stimulated for 30 min. In both types of experiment, single shocks were given to produce maximal twitches at a frequency of $1{\cdot}5\ s^{-1}$, and the muscles became completely fatigued towards the end of the period of stimulation. After activity, duplicate tissue and saline samples were taken in the *Rana* sartorius experiments and processed as before for $[K^+]$, $[Na^+]$ and measurement of water compartments.

Analytical methods

Measurements of $[Na^+]$ and $[K^+]$ were determined on thawed plasma, perfusates and tissue sample extracts by means of an Eppendorf flame photometer. The $[Cl^-]$ was measured by Buchler-Cotlove titration and osmolality was determined in a Knauer osmometer. The concentrations of L-lactate were measured enzymatically, using Boehringer Mannheim test kits and an Eppendorf spectrophotometer to measure the NADH generated, as lactate was oxidized to pyruvate. Before the lactate concentration was determined, the frozen tissue samples were finely ground in a cooled mortar and pestle, weighed and homogenized in known amounts of cooled $HClO_4$ (8% w/v). After centrifuging in the cold for 20 min the supernatant was carefully decanted for analysis. All measurements were made in duplicate within a week following an experiment.

Calculations

The ratio of the extracellular fluid volume of muscle (V_{em}) to the total muscle water (V_m), obtained from the concentrations of $[^{60}Co]$EDTA in muscle water and saline or plasma respectively, was taken to represent fractional extracellular space (Q_{em}). Intracellular fluid volumes were taken as $V_i = V_m - V_{em}$. Ion concentrations in the intracellular fluid were estimated from the amounts measured in the tissue extracts, correcting for the fractional water content of the muscles and Q_{em}, assuming that the concentrations in the intramuscular extracellular space were continuous with those of the saline or plasma.

Intracellular pH (pH_i) of muscle was determined by the transmembrane distribution of $[^{14}C]$DMO according to the equation:

$$pH_i = pK_{DMO} + \log \frac{[DMO]_i}{[DMO]_e} (10^{pH_e - pK_{DMO}} + 1) - 1,$$

where $pK_{DMO} = 6{\cdot}464 - 0{\cdot}00874t$ (Albers, Usinger & Spaich, 1971). Intracellular DMO concentrations ($[DMO]_i$) were calculated according to the relationship: $[DMO]_i = ([DMO]_m - [DMO]_e.Q_{em})/(1 - Q_{em})$ where $[DMO]_m$ and $[DMO]_e$ are the concentrations in the total muscle water and saline or plasma respectively. These relationships were similarly applied to other intracellular ion concentration estimates.

RESULTS

The effects of exercise on blood and muscle constituents in intact Xenopus

The *in vivo* experiments confirmed that exhausting exercise in *Xenopus* caused a blood acidosis which, as expected, was mostly metabolic in origin since it was

accompanied by a substantial fall in plasma [HCO_3^-] (Table 1). Since the toads had access to air, and exercising animals ventilated their lungs frequently, there was only a slight increase in Pa_{CO_2} and the respiratory component of the acidosis was small. Carbon dioxide losses through the skin might also be expected to rise because of the slightly increased gradient, disturbance of the boundary layers of water during activity, and greater blood flow to the skin. The metabolic nature of the acidosis was consistent with the eight-fold increase in blood lactate, six-fold increase in muscle lactate, and the development of a substantial muscle to blood gradient (Table 1).

Exercise also caused major changes in the osmotic and ionic equilibria between intracellular and plasma compartments. The intracellular water volume increased significantly and the osmolality of the blood plasma went up (Table 1). There was an

Table 1. *Determinations of ion concentrations in plasma and muscle and of water distribution in muscle made on* in vivo *samples taken from resting and exercising* Xenopus

Arterial plasma Ion concentrations and acid base data		Control ($N = 20$)	30 min exercise ($N = 14$)
pH		7·770 ± 0·036	7·390 ± 0·040*
P_{CO_2} (Torr)		13·8 ± 1·2	15·1 ± 0·6
[HCO_3^-] (mmol l^{-1})		25·3 ± 1·3	11·1 ± 1·2*
[Lactate$^-$] (mmol l^{-1})		1·8 ± 0·1	14·1 ± 1·5*
[Na^+] (mmol l^{-1})		122·0 ± 1·2	128·0 ± 1·4*
[K^+] (mmol l^{-1})		2·48 ± 0·06	3·16 ± 0·02*
[Cl^-] (mol l^{-1})		80·8 ± 0·4	82·9 ± 0·9
Osmolality (mosmol l^{-1})		237·0 ± 1·0	258·0 ± 2·0*
Haematocrit (%)		26·1 ± 1·1	35·9 ± 1·4*
Gastrocnemius muscle **Intracellular ion concentrations**		**Control** **($N = 10$)**	**30 min exercise** **($N = 10$)**
pH		6·860 ± 0·085	6·520 ± 0·076†
[Lactate$^-$] (mmol kg^{-1} cell water)		9·1 ± 1·0	54·6 ± 6·8*
[Na^+] (mmol kg^{-1} cell water)		8·5 ± 1·0	15·3 ± 2·0*
[K^+] (mmol kg^{-1} cell water)		118·2 ± 3·0	105·4 ± 6·9*
[Cl^-] (mmol kg^{-1} cell water)		20·8 ± 2·5	21·9 ± 1·1
Muscle water compartments **(mean muscle weight: wet, 3·230 g; dry, 0·764 g)**		**Control** **($N = 10$)**	**30 min exercise** **($N = 10$)**
Total water (ml H_2O g^{-1} dry wt)	(V_m)	3·23 ± 0·12	3·62 ± 0·13*
Extracellular water (ml H_2O g^{-1} dry wt)	(V_{em})	0·60 ± 0·03	0·63 ± 0·03
Intracellular water (ml H_2O g^{-1} dry wt)	(V_i)	2·62 ± 0·20	2·98 ± 0·22*
Change in intracellular water with exercise (ml H_2O g^{-1} dry wt)	(ΔV_i)	0·36 ± 0·15	

* Significantly different from control at $P < 0·05$: Student's t-test.

† This value must be treated with caution because the distribution of DMO according to pH is time-dependent and it is likely that intracellular pH changes throughout the exercise periods.

increase in plasma $[K^+]$ and a fall in intracellular $[K^+]$, whereas $[Na^+]$ increased in both compartments. The evidence that the haematocrit increase after exercise was due to water movement from plasma to cells is therefore quite good. If it is assumed that the total red cell volume is not changed by exercise, the plasma water loss (ΔH_2O) can be calculated as:

$$\Delta H_2O = PV_{control} - \left(RCV_{control} \cdot \frac{1 - Hct_{ex}}{Hct_{ex}}\right),$$

where $Pv_{control}$ is the resting plasma volume ($1 - Hct_{control} \cdot$ blood volume), $RCV_{control}$ is the resting cell volume ($Hct_{control} \cdot$ blood volume), and Hct_{ex} is the haematocrit after exercise. The mean blood volume of resting, catheterized *Xenopus* at 25 °C is 13·4 ml blood $100\,g^{-1}$ body weight (Emilio & Shelton, 1980). The haematocrit data of Table 1 give a ΔH_2O of 3·66 ml $100\,g^{-1}$ after exercise, which represents a 27 % and 37 % decrease in whole blood and in plasma volumes respectively. The uptake of water by gastrocnemius muscle in an exercised toad caused an overall volume increase of 0·39 ml in a muscle of wet weight 4·23 g. The hind limb musculature of *Xenopus*, which is the major component of the musculature involved in swimming, was weighed after removal from seven anaesthetized and weighed toads that were subsequently killed. It constituted 28 ± 1 % of the body weight. In a 100-g animal there would be a calculated influx of 2·58 ml water into the 28 g of active leg muscle. This is reasonably close to the 3·66 ml calculated from the haematocrit change but suggests that other active muscle groups, or other regions of water loss such as the kidneys, may be involved in the haematocrit increase. There may be recruitment of additional red cells and some cell swelling during activity, though Emilio & Shelton (1980) found no evidence for the former and little for the latter in the haematocrit increase induced by diving.

The effects of contraction on ion distribution in isolated gastrocnemius and sartorius muscles

Preliminary experiments showed that perfusion with saline had a considerable influence on the ability of the gastrocnemius to perform work, greatly extending the time over which muscle twitches could be maintained and increasing the amount of work done (Fig. 2). Non-perfused muscles that were contracting isotonically were found to fatigue very rapidly (Fig. 2A) even when they were in saline equilibrated with 99 % oxygen. Stopping and restarting the perfusion had immediate effects on the onset and elimination of fatigue (Fig. 2B).

The rate of perfusion and the pressure in the arterial cannula were parameters of major importance. At normal arterial pressures (25–35 cmH_2O; Emilio & Shelton, 1972) there was noticeable oedema of the muscle, due probably to the lack of large molecules such as proteins in the perfusate. The perfusion system ultimately used was one in which a constant flow of perfusate was delivered from a motor-driven syringe. Blood flow rates of approximately 0·1 ml min^{-1} to the whole leg were measured in resting, anaesthetized *Xenopus*. A perfusion rate of 0·3 ml min^{-1} was chosen therefore because it probably exceeded the rate for the gastrocnemius muscle

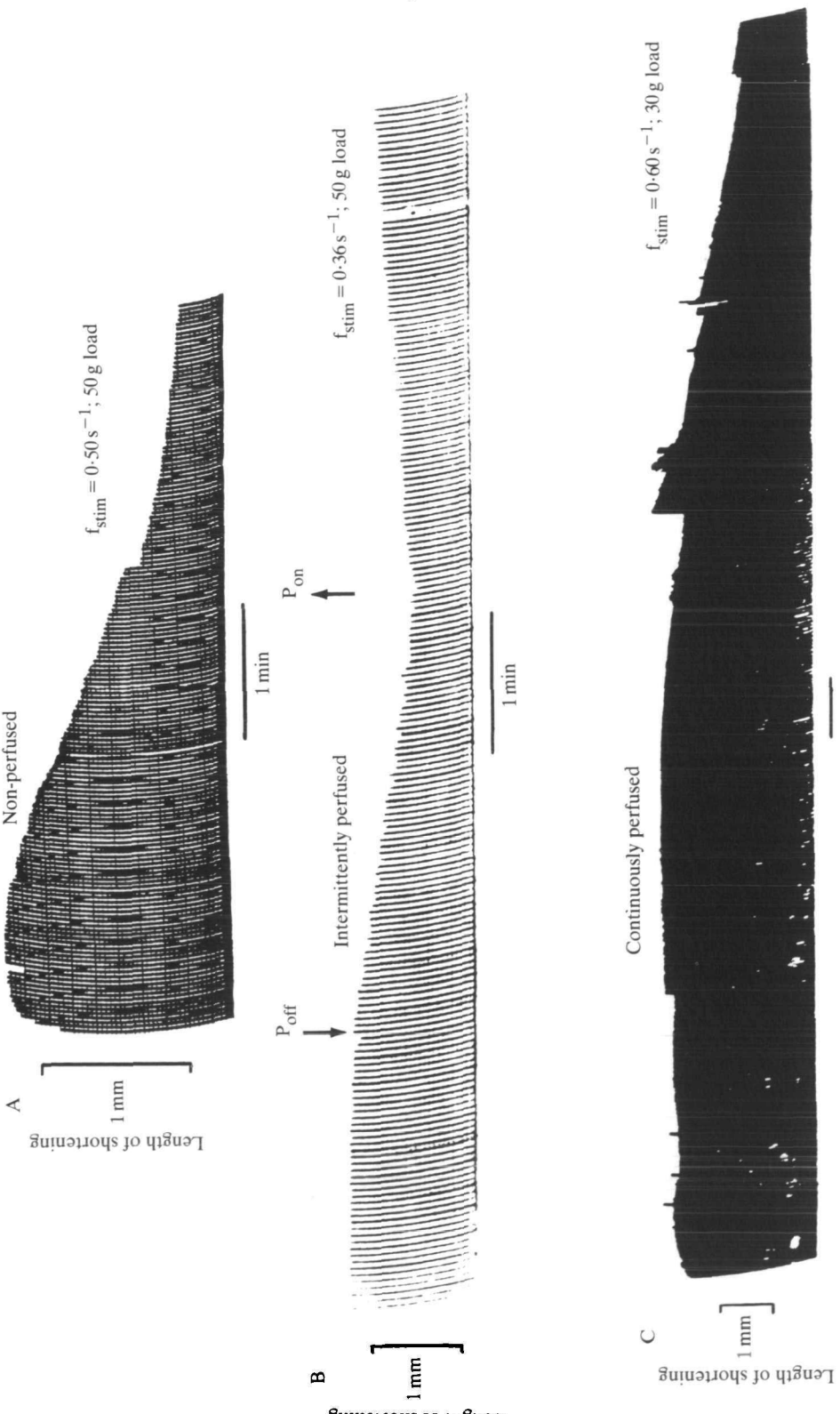

Fig. 2. Experimental recordings of length of muscle shortening in non-perfused (A), intermittently perfused (B) and continuously perfused (C) gastrocnemius muscles of *Xenopus laevis*. Note that length of shortening is perfusion-dependent; panel B shows immediate decline in length measurement when perfusion stopped (P_{off}) and increase when perfusion resumed (P_{on}). Amount of work (force × length) declines when muscles are non-perfused (panel A *versus* C) or intermittently perfused (B). f_{stim}, the frequency of square-wave stimulation; load, weight lifted by muscle under conditions shown in Fig. 1.

in vivo even during activity and, at this rate, resting muscle did not change weight substantially over several hours.

The arterial, venous and control concentrations of the major ions in the perfusate, together with its osmolality, are shown in Fig. 3 for periods before and during 25 min of muscle contraction. Muscle performance during each 5-min period is also shown, expressed as fractions of the total work done in 25 min. In absolute terms the total work varied considerably and unpredictably in different muscles, from 0·1 to 0·4 J g^{-1} muscle. To some extent this was due to varying rates of fatigue but there was also variation between muscles in the extent of shortening during a twitch. In the controls, and in the experimental muscles before stimulation, the ionic composition of the venous perfusate remained essentially the same as that in the arterial cannula. When stimulation began, lactate concentrations in the perfusate increased progressively from 0 to 3·2 mmol l^{-1} and the arterio-venous pH difference went up from 0·18 to 0·80 pH units. These results will be described in more detail in a later paper (Boutilier *et al.* 1986). Stimulation also resulted in a significant increase in $[K^+]$ and in osmolality (Fig. 3). In addition, there was an increase in venous $[Na^+]$ which was not significant at the 0·05 level, and no obvious change occurred in $[Cl^-]$.

The change in venous $[K^+]$ was produced by a significant rise in K^+ efflux from 5·6 mmol g^{-1} muscle min^{-1} to 40·0 mmol g^{-1} muscle min^{-1} over the first 5 min of stimulation, the efflux remaining at approximately this level until the end of stimulation. An empirical relationship between changes in the venous $[K^+]$ and $[H^+]$ has been reported in exercising mammalian muscles (Tibes *et al.* 1974; Steinhagen *et al.* 1976; Hirche, Schumacker & Hagemann, 1980) and this was also found to be the case with the frog gastrocnemius, though the scatter in the results was more pronounced (Fig. 4). At the end of the period of stimulation the intracellular $[K^+]$ was significantly reduced (Table 2), as it was in the *in vivo* experiments (Table 1). Intracellular $[Na^+]$ increased but to a much smaller extent than $[K^+]$ fell. Because of the low intracellular $[Na^+]$, contamination from the extracellular space makes accurate measurements difficult by the techniques employed.

Similar reductions in intracellular $[K^+]$ were found in the unperfused sartorius muscles of *R. ridibunda* after stimulation for a period of 25 min and there was a slight rise in $[Na^+]$ (Table 2). The electrolyte movements seem to be similar in *Rana* and *Xenopus*, though flux rates may be different *in vivo* and in perfused and unperfused *in vitro* preparations.

The effects of contraction on muscle water compartments in isolated gastrocnemius and sartorius muscles

The results of the $[^{60}Co]$EDTA determinations on perfused gastrocnemius and unperfused sartorius muscles are given in Table 2. The volumes of muscle water compartments are all standardized to one gram dry weight of muscle since this has the advantage of using a 'fixed' reference. The amount of water in both intra- and extramuscular compartments was found to be significantly higher in the *in vitro* (Table 2) than in the *in vivo* (Table 1) experiments, in spite of the precautions to

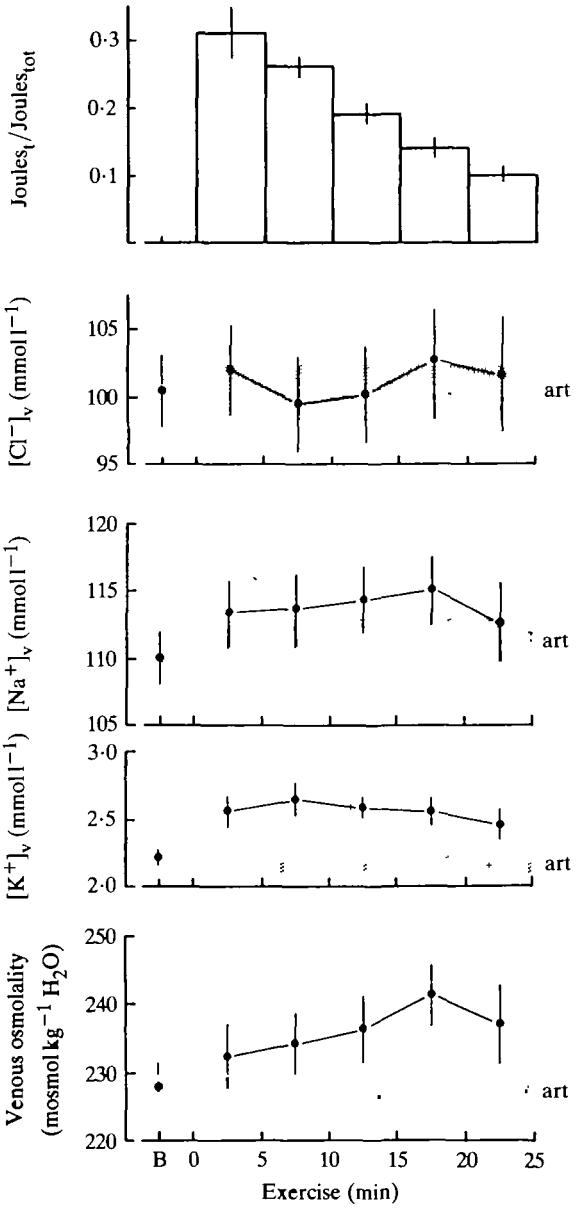

Fig. 3. Top panel: muscle performance normalized as the ratio of the mechanical work done in a 5-min block of time (Joules$_t$) over the total work performed in 25 min (Joules$_{tot}$) (means ± 1 S.E.M.). Lower panels: mean arterio-venous differences in $[Na^+]$, $[K^+]$, $[Cl^-]$ and osmolality ($N = 7$ perfused gastrocnemius muscle pairs from *Xenopus*) over a 25-min period (0–25 on time scale) of isotonic work (as in Fig. 1). Shaded areas = ± 1 S.E.M. of the mean arterial (art) perfusate concentrations. Venous (v) concentrations shown for 5-min collection intervals (mean ± 1 S.E.M.) during 0–25 min exercise period. Mean values before exercise (B) are for inactive control muscle measurements made over 25-min period when paired muscle was being stimulated.

prevent obvious signs of tissue oedema. Moreover, the perfused gastrocnemius clearly contained more water than the unperfused sartorius. The effects of perfusion pressures and of the low colloid osmotic pressure of the perfusate must be of major importance in establishing new ionic and osmotic equilibria, even in the resting muscles. The use of pairs of control and stimulated muscles is therefore essential in these experiments. Similar differences in water compartments, though smaller in extent, were observed in tissue samples from rat diaphragm in saline and *in vivo* (Heisler & Piiper, 1972). Swelling of the extracellular compartment may also account for the large differences between the *in vivo* and *in vitro* concentrations of intracellular $[Na^+]$ and $[K^+]$ found in *Xenopus* gastrocnemius (Tables 1, 2). Such estimates are bound to be influenced by contamination from the extracellular space, which in the isolated muscles will increase the likelihood of estimating higher $[Na]_i$ and lower $[K]_i$ than those seen *in vivo*.

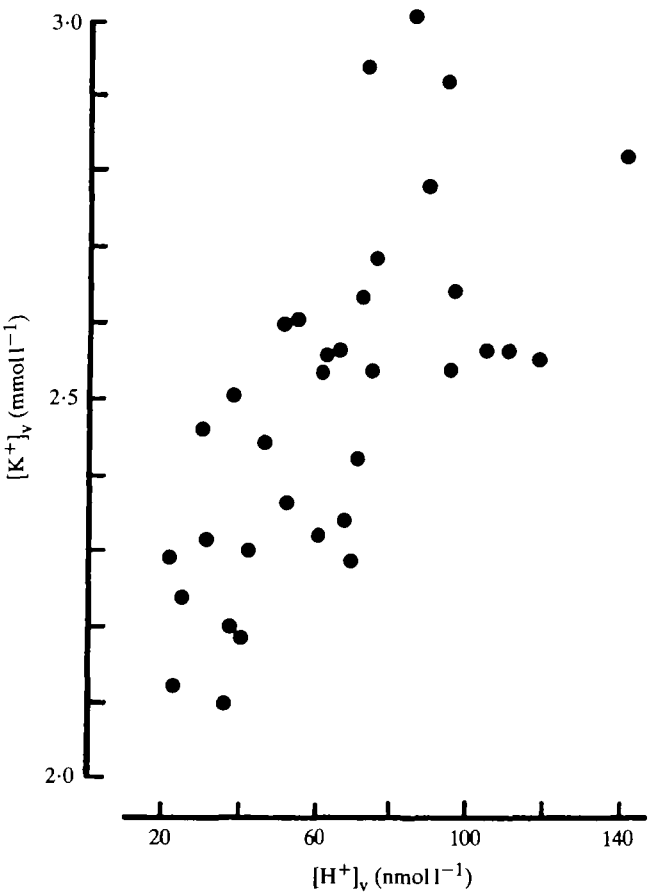

Fig. 4. Relationship between venous concentrations of potassium ions ($[K^+]_v$) and venous hydrogen ion activity (i.e. venous pH) in isotonically working gastrocnemius of *Xenopus*. Each point represents measurements of venous perfusate collected over a 4- or 5-min period ($N = 7$ muscles).

Table 2. *Changes in muscle water compartments and intracellular ion concentrations in control and stimulated pairs of* Xenopus *gastrocnemius and* Rana *sartorius muscles* in vitro

		Xenopus laevis – perfused gastrocnemius muscle ($N = 7$) (mean muscle weight: wet, 3·133 g; dry, 0·545 g)		*Rana ridibunda* – unperfused sartorius muscle ($N = 6$) (mean muscle weight: wet, 0·713 g; dry, 0·150 g)	
		Control	Stimulated	Control	Stimulated
Muscle water compartments					
Total water (ml $H_2O\,g^{-1}$ dry wt)	(V_m)	4·49 ± 0·27	4·91 ± 0·12*	3·82 ± 0·08	4·15 ± 0·03*
Extracellular water (ml $H_2O\,g^{-1}$ dry wt)	(V_{em})	1·26 ± 0·16	1·27 ± 0·08	0·88 ± 0·08	0·75 ± 0·04*
Intracellular water (ml $H_2O\,g^{-1}$ dry wt)	(V_i)	3·23 ± 0·22	3·65 ± 0·20*	2·94 ± 0·03	3·40 ± 0·05*
Change in intracellular water with activity (ml $H_2O\,g^{-1}$ dry wt)	(ΔV_i)	0·42 ± 0·16		0·46 ± 0·14	
Intracellular ion concentrations					
$[Na^+]$ (mmol kg^{-1} cell water)		20·3 ± 1·8	29·5 ± 5·4*	20·4 ± 0·8	22·0 ± 0·8**
$[K^+]$ (mmol kg^{-1} cell water)		103·2 ± 6·7	76·4 ± 2·9*	148·2 ± 2·0	126·6 ± 1·8*

* Significantly different from control at $P < 0·05$: Student's t-test.
** Significantly different from control at $P < 0·10$: Student's t-test.

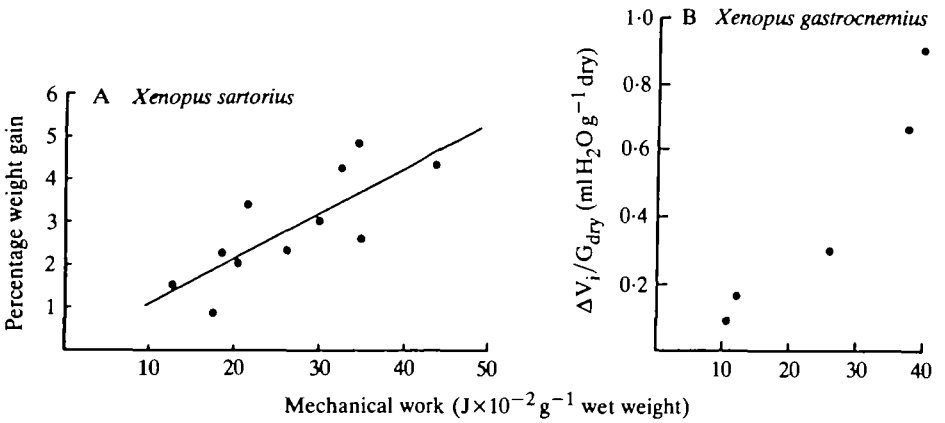

Fig. 5. Relationship between the total mechanical work performed (in Joules × 10^{-2} g^{-1} wet weight) and; (A) the percentage water uptake of saline-equilibrated sartorius muscles of *Xenopus* and (B) the water volume change in the intracellular compartment (ΔV_i) of *Xenopus* gastrocnemius. Each point represents one experiment where changes of the control muscle have been subtracted from those of the stimulated muscle.

The stimulated muscles always had water contents that were higher than control values, due to significant increases of some 13–16% in the intracellular compartments (Table 2). These percentage changes were similar to those seen in the intracellular compartments of gastrocnemius muscles *in vivo* (Table 1). In perfused gastrocnemius, the extracellular water volume did not change, whereas it fell slightly in the unperfused sartorius muscles (Table 2).

The amount of water taken up by the muscle during activity varied over a substantial range and could be correlated in a general way with the total amount of mechanical work performed. In the unperfused sartorius muscles of *Xenopus*, total wet weight increased, relative to paired control muscles, as a linear function of mechanical work carried out in the 30-min stimulation period (Fig. 5A). The increase in intracellular water content of perfused gastrocnemius muscles also seems to be related to the amount of work performed (Fig. 5B), though there are too few points on the graph to allow a reliable line to be fitted to the data.

DISCUSSION

In *Xenopus*, exhausting exercise causes changes in lactate concentration, both in the arterial plasma and in the active muscles, that are among the largest reported for amphibians. The blood values are roughly equivalent to those found after activity in *Amphiuma* (Preslar & Hutchison, 1978), *Ambystoma* (Hutchison, Turney & Gratz, 1977), and *Rana* (Hutchison & Turney, 1975) and are much higher than in *Bufo* (McDonald *et al.* 1980) and *Cryptobranchus* (Boutilier *et al.* 1980). The muscle lactate concentrations after activity are twice as large as those found in *Ambystoma* (Hutchison *et al.* 1977) and *Rana* (Hutchison & Turney, 1975) and a little higher than those seen in *Amphiuma* (Preslar & Hutchison, 1978). They are also somewhat higher than those previously reported for *Xenopus* by Putnam (1979), though in all

these experiments the methods of inducing activity, the levels of activity and the time taken for the animals to become fatigued, are extremely variable and illustrate the intrinsic difficulties of whole animal studies. However, there can be no doubt that *Xenopus* is a very active amphibian, capable of extensive anaerobiosis during activity. The bearing that these results have on the suggestion of an inverse relationship between anaerobic and aerobic scopes in amphibians (Bennett, 1978; Bennett & Licht, 1973) will be discussed in a subsequent paper (Boutilier *et al.* 1986).

The increase in haematocrit that consistently accompanies the build-up of lactate in the muscle cells during these whole animal studies can be explained in terms of a movement of water from the blood into the active muscles. The experimental results from intact *Xenopus* show that the increase in muscle water during activity is sufficient to account for a large part, if not all, of the haematocrit change. Muscle swelling is known to be associated with activity in mammalian skeletal muscle (Fenn, 1936; Sréter, 1963) and is also thought to be caused by the intracellular build-up of the osmotically active lactate ion (Bergstrom *et al.* 1971; Sahlin *et al.* 1978). However, needle biopsies taken from the muscles of humans during exhausting exercise showed that both intra- and extracellular compartments increased in volume (Bergstrom *et al.* 1971; Sahlin *et al.* 1978), whereas in the present studies no changes were seen in the volume of the extracellular compartment in *Xenopus* gastrocnemius and in *Rana* sartorius the extracellular volume actually went down (Table 2).

This view of water movement occurring from blood to muscle cells, due to the high accumulation of lactate ions in the latter, is further substantiated by the experiments on isolated muscle preparations. Notwithstanding the fact that the unstimulated controls of perfused gastrocnemius and unperfused sartorius muscles contained, respectively, 39% and 18% more water per unit dry weight than the gastrocnemius *in vivo*, there was a strikingly similar shift of water following fatigue induced by stimulation to that seen in the *in vivo* experiments (Tables 1, 2). Moreover, there is an indication that the amount of water taken up is proportional to the amount of work done (Fig. 5) and probably to the quantity of lactate produced. The isolated muscles, therefore, behave in the same way as do those in the intact animal and there is no evidence that control systems, other than those at the cellular level, are involved in this water movement.

However, the mechanisms underlying the uptake of water by exercising muscle are still not fully understood. Plasma osmolality also increases after exercise but only by 9% as compared to the 38% change in haematocrit (Table 1). There are clearly electrolyte adjustments between muscles and blood, and probably regulatory changes in other parts of the body, such as the kidney, as well. There is a net Na^+ influx into active muscle cells as shown by the increase in intracellular $[Na^+]$, both *in vivo* (Table 1) and *in vitro* (Table 2), a change also found in mammals (Bergstrom *et al.* 1971; Sahlin *et al.* 1978; Rooth, 1966; Gelli, Bergstrom, Hultman & Thalme, 1969). The decrease in intracellular $[K^+]$ and the corresponding rise in venous $[K^+]$ during the intra- and extracellular acidoses associated with exercise (Tables 1, 2; Fig. 3) is again consistent with other observations on amphibian and mammalian muscles (Fenn & Cobb, 1935; Fenn, 1936; Brown & Goott, 1963; Mainwood &

Lucier, 1972; Tibes *et al.* 1974; Hazeyama & Sparks, 1979; Hirche *et al.* 1980). The correlation between venous $[K^+]$ and $[H^+]$ during exercise in this (Fig. 4) and other studies (Tibes *et al.* 1974; Adler & Fraley, 1977; Hirche *et al.* 1980) suggests that the Na^+/K^+ pump of the muscle cell membrane may be inhibited by the rise in hydrogen ion activity, although depletion of energy-rich phosphates (Steinhagen *et al.* 1976) or increases in passive fluxes cannot be ruled out at this stage. A major unknown in all these interpretations is the composition of the interstitial fluid and its resistance to the transfer of ions between cells and plasma or perfusate. In the dog gastrocnemius, for example, K^+ concentrations and H^+ activities between interstitial space and plasma were found to vary in a complicated and transient fashion during exercise (Steinhagen *et al.* 1976; Hirche *et al.* 1980).

The isolated and perfused muscle preparation seems to behave in substantially the same way as muscles *in vivo* and so to have considerable potential for the further study of water and ion exchange during exercise. The rate and amount of work can be accurately quantified and the composition of the perfusate can be controlled. Simple saline perfusates give rise to problems, causing tissue oedema and restricting the range of perfusion pressures and flow rates that can be used. These and other problems will be discussed in a later paper (Boutilier *et al.* 1986).

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