J. exp. Biol. (1979), 82, 35-51
With 10 *figures*
Printed in Great Britain

THE ROLE OF LACTIC ACID ACCUMULATION IN MUSCLE FATIGUE OF TWO SPECIES OF ANURANS, *XENOPUS LAEVIS* AND *RANA PIPIENS*

By ROBERT W. PUTNAM
*Department of Biology, University of California**
Los Angeles, CA. 90024

(*Received* 18 *September* 1978)

SUMMARY

Fatigue produced a marked increase in the lactic acid content of hind-limb muscles, the blood, and the whole animal. After 15 min of rest there was little decline of lactic acid levels but the animals could be stimulated into about 3 min of intense activity. This re-fatigue produced a further increase in lactic acid levels. Gastrocnemius muscles removed from fatigued frogs and stimulated *in vitro* were able to generate initial tensions similar to those in control muscles; total tension was about a third of the control value. *In vitro* stimulation of these muscles from fatigued frogs led to additional accumulation of lactic acid. Fatigue produced little decrease in the glycogen content of muscles in *X. laevis* but a marked decrease in *R. pipiens*. Considerable glycogen stores remained even in the muscles of re-fatigued animals. These data show that accumulation of lactic acid in muscle or blood, depletion of glycogen in muscle, or change in blood pH cannot account for fatigue in these species. Possible other causes of fatigue are discussed.

INTRODUCTION

For short bursts of intense activity, lower vertebrates use primarily anaerobic rather than aerobic metabolism (Bennett, 1978), gaining the advantage that all necessary resources for energy production reside within the muscle and avoiding the time lag involved in the delivery of oxygen (Bennett & Licht, 1972). The use of anaerobic metabolism for longer periods is thought to be limited by the accumulation of end-products, particularly lactic acid (Black *et al.* 1961; Bartholomew, Bennett & Dawson, 1976; Ruben, 1976; Bennett, 1978).

The site of fatigue has variously been claimed to be the central nervous system (Ikai & Steinhaus, 1961), the neuromuscular junction (Landau & Nachsen, 1975) or the muscle itself (Merton, 1954). Since the classic work of Fletcher & Hopkins (1907), lactic acid accumulation has been implicated in muscle fatigue both in intact organisms (Karlsson *et al.* 1975*a*) and isolated muscle preparations (Hill & Kupalov,

* Present address: Department of Developmental and Cell Biology, University of California, Irvine, CA. 92717, USA.

1930; Mainwood & Worsley-Brown, 1975; Fitts & Holloszy, 1976). However, the mechanism by which lactic acid could lead to fatigue is unknown. Many authors (Meyerhof, 1925; Hermansen & Osnes, 1972; Mainwood, Worsley-Brown & Paterson, 1972; Mainwood & Worsley-Brown, 1975) have suggested that the pH changes associated with lactic acid accumulation are responsible for fatigue. Such pH changes can affect Ca^{2+} distribution (Seraydarian, Abbott & Williams, 1961; Eberstein & Sandow, 1963; Nassar-Gentina *et al.* 1978), Ca^{2+} binding to troponin (Fuchs, Reddy & Briggs, 1970), Ca^{2+} activation of myosin-ATPase (Portzehl, Zaoralek & Gaudin, 1969) or glycolytic enzyme activities (Trivedi & Danforth, 1966).

The great discrepancies in the results of different studies may be due to the use of a wide variety of systems, stimulation regimes, experimental organisms and test temperatures. No coordinated study has yet been made pursuing all the various theories of fatigue in both the intact organism and isolated muscle preparations. The present study is intended as the beginning of such an investigation and examines the role of lactic acid accumulation in fatigue of two anuran amphibians: *Xenopus laevis*, an aquatic pipid which tolerates high levels of lactic acid; and *Rana pipiens*, a semiterrestrial ranid, which fatigues rapidly and accumulates high levels of lactic acid. Two approaches have been used: (1) to measure metabolic changes in frogs subjected to fatigue; (2) to assess the performance of muscles removed from fatigued frogs and stimulated directly *in vitro*. A preliminary report of this work has been made (Putnam, 1977).

MATERIAL AND METHODS

X. laevis were collected in southern California, maintained in tanks of water and fed beef spleen periodically. *R. pipiens* were purchased from commercial suppliers and maintained in tanks with water and mealworms available. All animals were held in a room at 16 ± 2 °C with a 12:12 light–dark cycle. They weighed between 30 and 80 g, and only those judged to be in good condition were employed. They were fasted from 3 days to a week before use. The refatiguing experiments were done in the summer, and the isolated muscle experiments were done in the late fall and early winter.

The procedure used to fatigue the frogs has previously been described (Putnam, 1978). Briefly, *X. laevis* were manually induced to swim and to right themselves until they could not do either, when they were said to be fatigued. *R. pipiens* were induced to hop and right, with fatigue defined as the point at which frogs could not return to their original posture after a hop and could not right themselves. Separate groups of frogs were: fatigued (fatigued group); fatigued and allowed to rest for 15 min (recovery group); fatigued, allowed to rest for 15 min and refatigued (refatigued group). In addition, a group of frogs was sacrificed without engaging in any activity (control group). For the resting periods after activity, *X. laevis* were kept in water, while *R. pipiens* were kept in large containers in air. The second activity bout was identical to the first. From each group, some frogs were sacrificed for whole animal lactic acid concentration determination, while tissue samples were obtained from the remaining frogs in the group. For all groups, the time to fatigue was used as an estimate of the amount of activity.

Frogs were sacrificed by double pithing. A ventral incision was made, the ventricle exposed and a mixed venous sample was collected from the ventricle in a 1 ml syringe. A 100 μl sample was added to 200 μl of 0·6 N perchloric acid (PCA), the sample was spun and the supernatant stored at −80 °C for later lactic acid analysis. The remainder of the blood sample was kept on ice in sealed capillary tubes for at most 10 min and then the pH was determined in at least triplicate with a radiometer blood gas analyser. Preliminary experiments indicated that this procedure did not alter the pH of blood. The precision of the pH determination was 0·02 pH units.

After the blood sample was obtained, the skin from one of the hind limbs was cut away and the gastrocnemius, sartorius, gracilis, semimembranosus and triceps femoris muscles were removed and frozen in liquid nitrogen. The order in which the muscles were removed was randomized. These muscles comprised about 75% of the mass of the hind limb. About 5–6 min were required for the complete removal of all these muscles. These muscle samples were stored at −80 °C for at most 1 month before being homogenized.

For the studies of the *in vitro* performance of muscles, the gastrocnemius muscles were removed from frogs which had either not been exercised (control group) or had been fatigued as described above (fatigued group). For a typical experiment, a frog was doubly pithed and the skin removed from the lower portion of the hind limb. The legs were extended with the Achilles tendons touching and the lengths of the gastrocnemius muscles were measured. This length was defined as the resting length. One of the gastrocnemius muscles was removed and quick frozen in liquid nitrogen. The other gastrocnemius muscle was removed with part of the femur and hung in a chamber for stimulation modelled after that of Cerretelli, di Prampero & Ambrosoli, 1972. The femur was held in a bone clamp and the Achilles tendon attached to an inextensible chain hung from a force transducer (Grass FT.03C or Grass FT.10C). The length of the muscle was adjusted to resting length and the chamber closed so that the copper electrodes touched the muscle surface. The electrodes were arranged so that current traversed the muscle diagonally. The force transducer output was displayed on a Grass Model 5 Polygraph.

Stimulation was with pulses 5–20 V in amplitude and 10 ms in duration, delivered at a frequency of 1/s with a Grass SD5 Stimulator. A series of test stimuli were delivered to determine the voltage which would give maximum isometric twitch tension from the muscle. The muscles were stimulated until tension fell to 10% of the initial, maximum twitch tension (P_{max}). These muscles were then removed from the chamber, the femur and the Achilles tendon were cut away, and the muscles were quick frozen in liquid nitrogen. Throughout the experiment the muscles were kept moist using an amphibian Ringers solution (Dunn & Arditi, 1968). About 2–3 min were required to remove the first gastrocnemius muscle and 15 min were required for preparation and stimulation of the other gastrocnemius muscle. Preliminary experiments showed that lactic acid concentrations were not significantly changed in the muscles during the removal time or as a result of the test stimuli. After each muscle was stimulated, the transducer was calibrated by hanging a series of weights from it and this calibration was used to calculate muscle tension generation. The frozen muscles were weighed to the nearest 0·1 mg and cross-sectional area calculated by dividing the mass by the length. All muscle tension values are reported

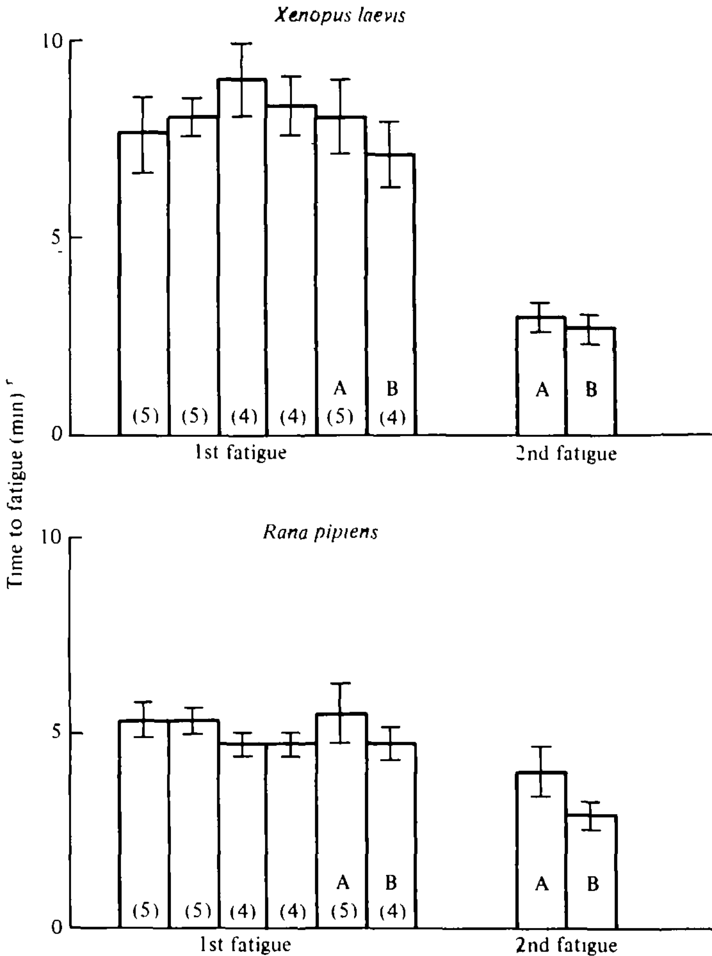

Fig. 1. The time to fatigue in different exercise groups in two species of anuran amphibians. The height of a column is the mean time to fatigue with vertical lines representing ± 1 S.E. The number of animals in each group is given in parentheses. From left to right the groups are: fatigued, muscles removed; recovery, muscles removed; fatigued, whole animal; recovery, whole animal; A, refatigued, muscles removed; B, refatigued, whole animal.

relative to cross-sectional area in units of Newtons per square meter (N/m^2). The frozen muscles were stored in a freezer at −80 °C for at most 1 month before homogenization.

The sample preparation and analyses have previously been described (Putnam, 1978). Briefly, muscles were homogenized in 43 × their weight of cold 0·6 N PCA, and whole animals in 10 × their weight. The supernatants from these homogenates were enzymically assayed for lactic acid (Putnam, 1978). A small (100 mg) sample of frozen muscle was placed in 3 ml of 30% KOH and analyzed for glycogen using the phenol-sulphuric acid technique of Montgomery (1957). The precision of the lactic acid assay was 2% and of the glycogen assay was 5%.

Comparisons of the two means were done using *t* tests (Dixon & Massey, 1969). All comparisons of three or more means were done with a one-way analysis of

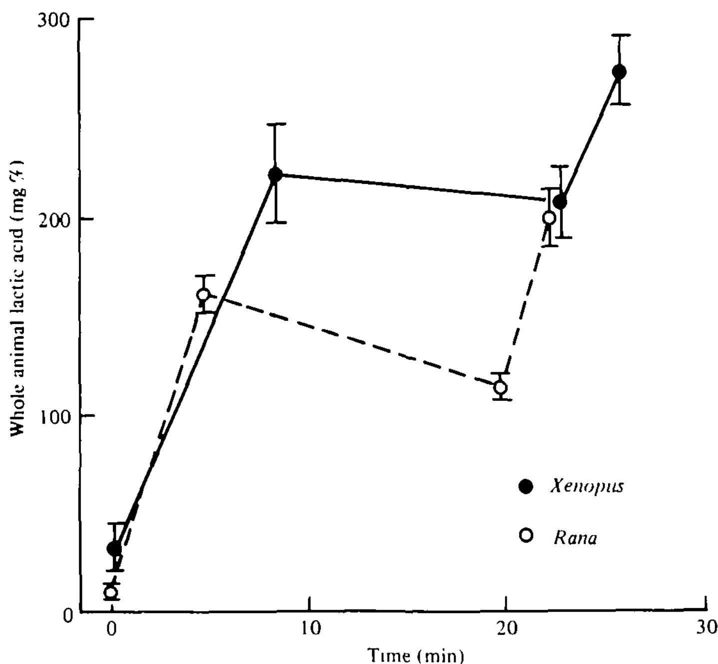

Fig. 2. Whole animal lactic acid content in different groups of exercised frogs. The solid line and filled circles represent values for *X. laevis*, the dotted line and empty circles represent values for *R. pipiens*. The vertical lines are ± S.E. The groups from left to right are: control; fatigued; recovery; refatigued.

variance (ANOVA) with paired comparisons done using Scheffé's procedure (Scheffé, 1953). All *t* tests were two-tailed with a level of significance of 0·05. All values are reported as the mean ± 1 standard error of the mean (S.E.).

RESULTS

Activity. Both *X. laevis* and *R. pipiens* could be readily fatigued. Activity consisted of an initial period of from 1–3 min of intense effort followed by a period of reduced effort leading to complete exhaustion. Activity lasted for 7–8 min in *X. laevis* and for 4–5 min in *R. pipiens*. During the rest period, *X. laevis* usually righted themselves within a minute and remained on the bottom of the container or suspended at the surface. *R. pipiens* resumed an alert posture within 1–2 min but remained in place. Spontaneous movements were rare during rest and never exceeded 2 or 3. During refatigue, both species exhibited intense escape movements for 1–2 min and fatigued within 3 min for *X. laevis* and 4 min for *R. pipiens*. Since time to fatigue was used to assess effort, and different groups of frogs were used for each exercise regime, the times to fatigue in different groups were compared (Fig. 1). An ANOVA revealed no significant differences in the time to initial fatigue among the groups for the two species. The refatigue time was about 40% of the initial fatigue time in *X. laevis* and about 66% in *R. pipiens*.

Whole-body lactic acid concentrations. As shown in Fig. 2, lactic acid accumulated

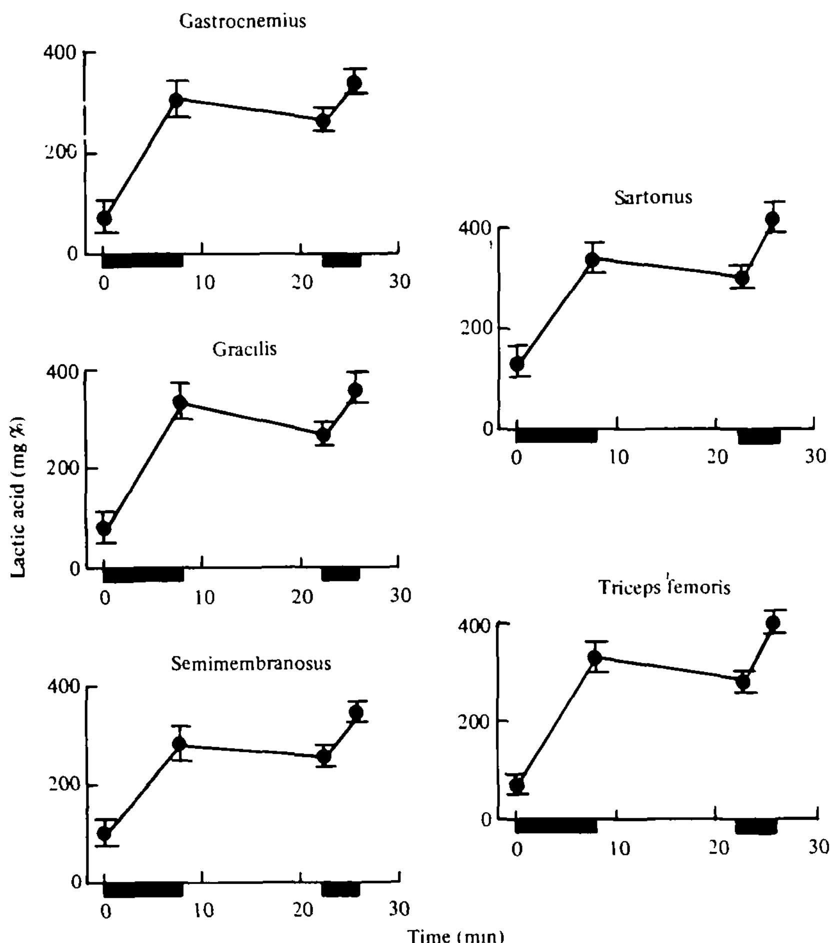

Fig. 3. The lactic acid level in five hindlimb muscles in different groups of exercised *X. laevis*. The black bars on the abscissa represent periods of activity (7·7 min for fatigue and 3·1 min for refatigue). Vertical lines represent ± 1 S.E.

to high levels during the initial fatigue. After 15 min recovery, there was no change in lactic acid content in *X. laevis* but a significant decline in *R. pipiens*. Further accumulation occurred during the second exercise bout.

Muscle lactic acid concentrations. Figs. 3 and 4 show muscle lactic acid contents in the different groups. The general pattern was for a large accumulation of lactic acid during the initial fatigue, some disappearance during recovery, and further accumulation during the second exercise bout to levels in excess of those after initial fatigue. An ANOVA for the five muscles revealed that the lactic acid levels were similar within each group for each species. The results of the statistical analysis across groups for each muscle showed that in *X. laevis* significant recovery of lactic acid levels occurred in the gastrocnemius, gracilis and triceps femoris muscles while in *R. pipiens* significant recovery occurred in the gastrocnemius, sartorius and semimembranosus muscles. However, in all cases muscle lactic acid content was lower after recovery than after fatigue, suggesting that some level of lactic acid remov

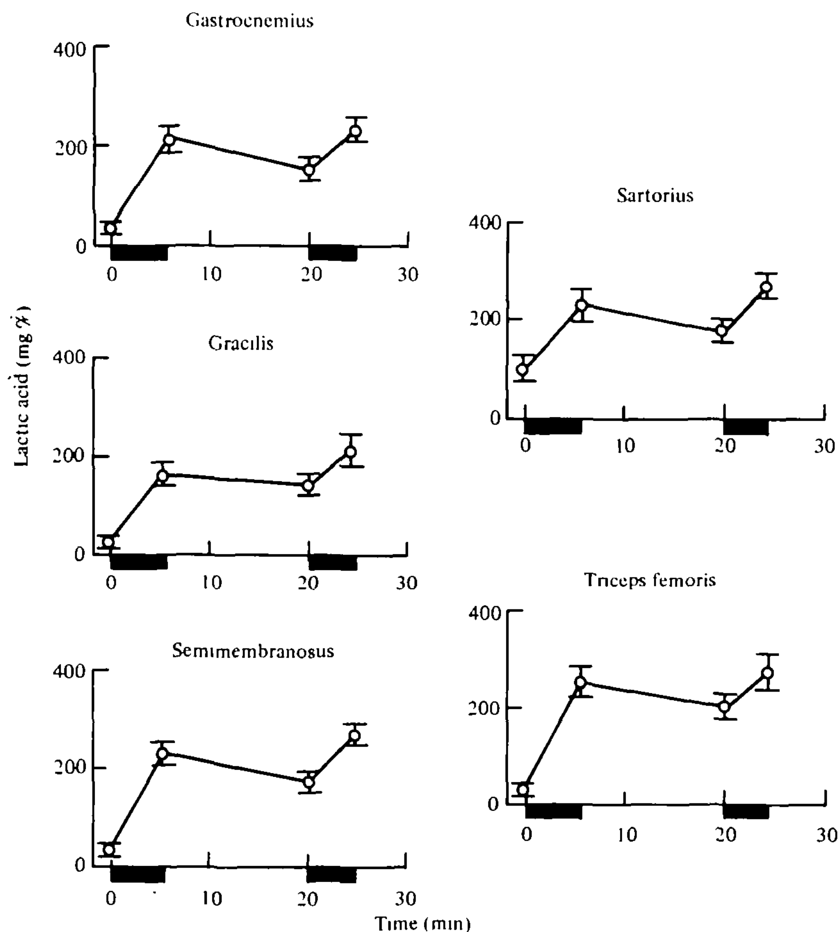

Fig. 4. The lactic acid level in five hindlimb muscles in different groups of exercised *R. pipiens*. Other symbols as in Fig. 3. The time to fatigue was 5·3 min and the refatigue time was 4·2 min.

occurred in all the muscles. Comparing lactic acid contents in specific muscles after the second fatigue with values after initial fatigue, significantly higher levels were found in the sartorius, triceps femoris and semimembranosus muscles of *X. laevis*, and the gracilis and semimembranosus muscles of *R. pipiens*. Again, though, lactic acid content in all the refatigued muscles exceeded that in muscles after initial fatigue, suggesting that higher muscle levels of lactic acid were being attained during refatigue. In all cases, the lactic acid accumulation during refatigue (lactic acid in refatigued group compared to recovery group) was statistically significant.

Muscle glycogen content. The changes in the glycogen content in the muscles as a result of activity are shown in Fig. 5. In *X. laevis*, there was no significant change. In *R. pipiens* initial fatigue produced a decline in glycogen content to around 30% of control values and there was further decline during refatigue. Only in the gracilis muscle was there significant recovery during the rest period. The lowest mean glycogen content for any muscle was 250 mg%.

Blood lactic acid and pH. Fig. 6 presents the results of blood lactic acid and pH

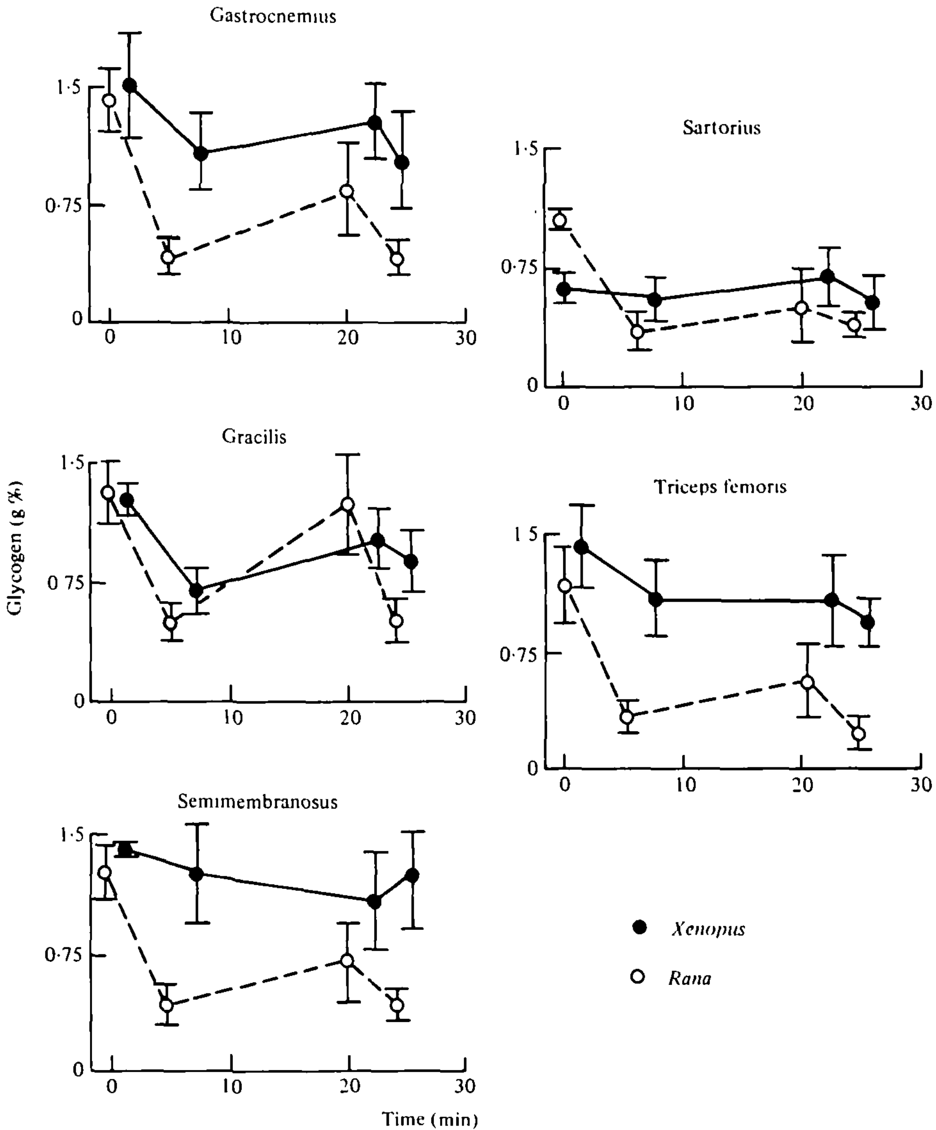

Fig. 5. Glycogen content in five hindlimb muscles from various groups of exercised *X. laevis* and *R. pipiens*. Symbols as in Fig. 2.

measurements in the different groups of frogs. In *X. laevis*, blood lactic acid increased during the initial fatigue, was not modified during recovery, and increased further during refatigue. These increases in blood lactic acid were paralleled by decreases in blood pH. In *R. pipiens*, a similar pattern was observed except that blood lactic acid increased significantly during the recovery period while blood pH remained unchanged.

In vitro *muscle performance*. Tracings of tension generated by stimulated gastrocnemius muscles from control and fatigued frogs are shown in Figs. 7 and 8. These tracings are chosen to represent best the average length of the stimulation period, average P_{max}, and average total tension generated by a particular group. For ea

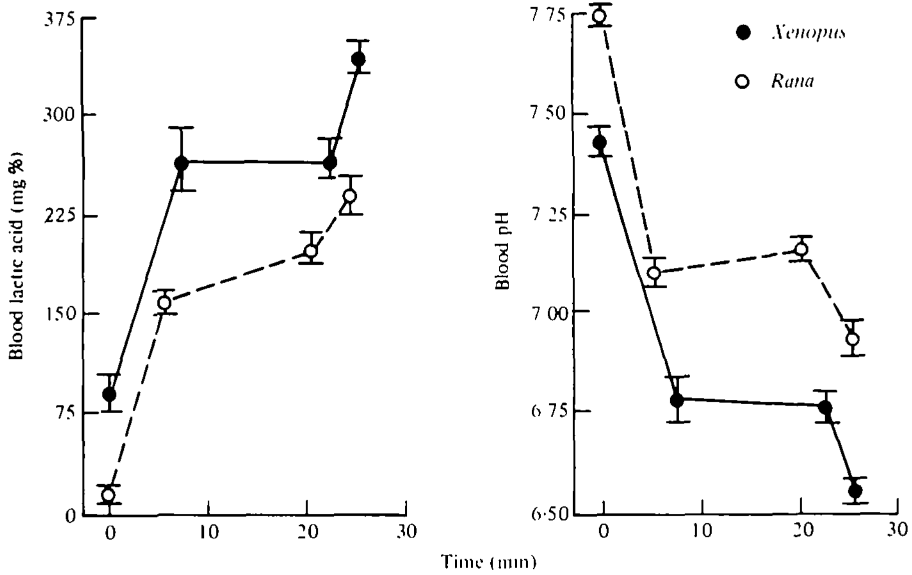

Fig. 6. Blood lactic acid content and blood pH in various groups of exercised *X. laevis* and *R. pipiens*. Symbols as in Fig. 2.

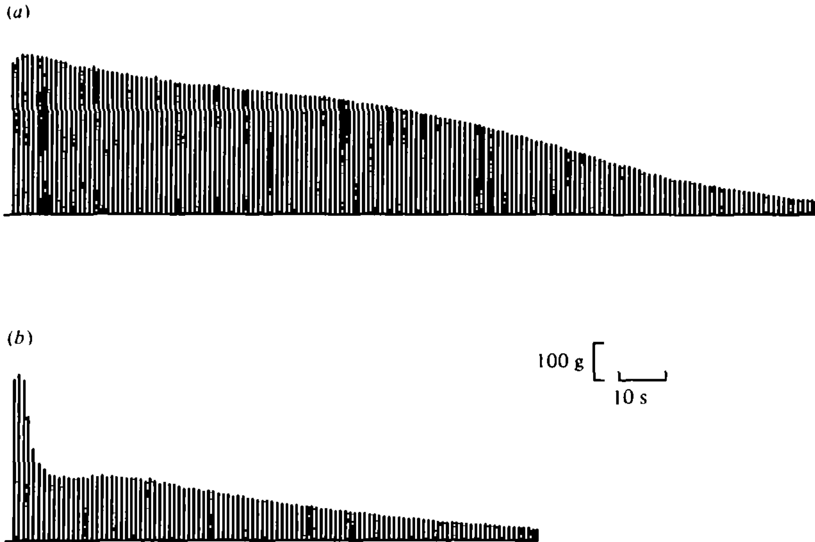

Fig. 7. Tension tracings of gastrocnemius muscles from *X. laevis* stimulated *in vitro*. (*a*) Muscle from a control frog. (*b*) Muscle from a fatigued frog.

muscle, the stimulation period was divided into ten periods of equal duration and the tension at the end of each period was averaged across all the muscles in a group. The average time of stimulation for muscles from fatigued animals was expressed as a percentage of the average stimulation time for muscles of control animals from the same species. The results of this analysis of tension *v.* % time to fatigue are shown in Figs. 9 and 10. The initial tension values in these plots are the tensions generated [illegible]the first twitch of the muscles. Values of P_{max} were calculated from the maximum

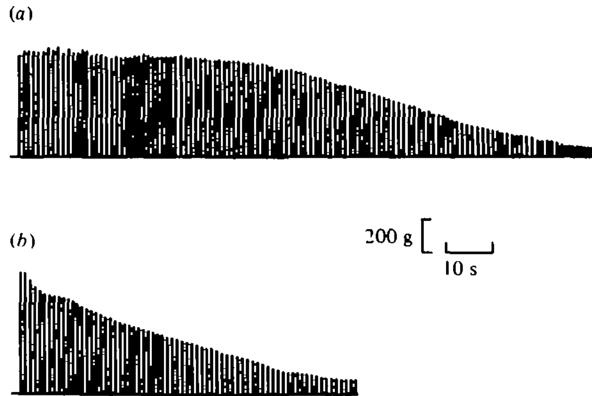

Fig. 8. Tension tracings of gastrocnemius muscles from *R. pipiens* stimulated *in vitro*. (*a*) Muscle from a control frog. (*b*) Muscle from a fatigued frog.

Table 1. *Performance parameters of excised gastrocnemius muscles from* X. laevis *and* R. pipiens

(Control muscles were from unexercised frogs and fatigue muscles were from fatigued frogs. See text for explanation of the various parameters. The values are the mean, ± 1 S.E. and the number of determinations in parentheses. An * indicates that the fatigue and control values were different at the 0·05 level of significance.)

	X. laevis		*R. pipiens*	
	Control	Fatigue	Control	Fatigue
P_{max} (N/m²) × 10⁻²	990	860	2190	1860
	±70 (7)	±50 (10)	± 60 (7)	±130 (11)
Total tension (N/m²) × 10⁻⁴	920	288*	1806	706*
	±98 (7)	±42 (6)	±133 (7)	±120 (7)
Number of twitches	168	109*	135	83*
	± 7 (7)	± 8 (6)	± 13 (7)	± 6 (7)

twitch height in a muscle recording and total tension was calculated by summing all the tensions for an entire stimulation period. Table 1 reports the values for P_{max}, total tension, and number of twitches for muscles from the various groups. Gastrocnemius muscles from fatigued *X. laevis* generated about the same initial tension as those from rested frogs, but generated tension rapidly fell to about 50% of initial values and then decayed more slowly (Fig. 9, Table 1). The gastrocnemius muscle from the fatigued *X. laevis* continued to twitch for nearly 2 min and generated about a third of the total tension of muscles from rested frogs. By comparison, the gastrocnemius muscles from fatigued *R. pipiens* also generated initial tensions similar to muscles from control frogs, but tension declined more slowly. The rate of decline of tension in muscles from fatigued animals was similar to the rate of decline in muscles from control animals, with the only difference being the presence of an initial plateau in control muscles (Fig. 10). The muscles from fatigued *R. pipiens* were able to generate tension for about 1½ min and generated about 40% of the total tension of control muscles. Table 2 shows the corresponding lactic acid contents of the muscles from the various groups. In stimulated muscles from fatigued frogs,

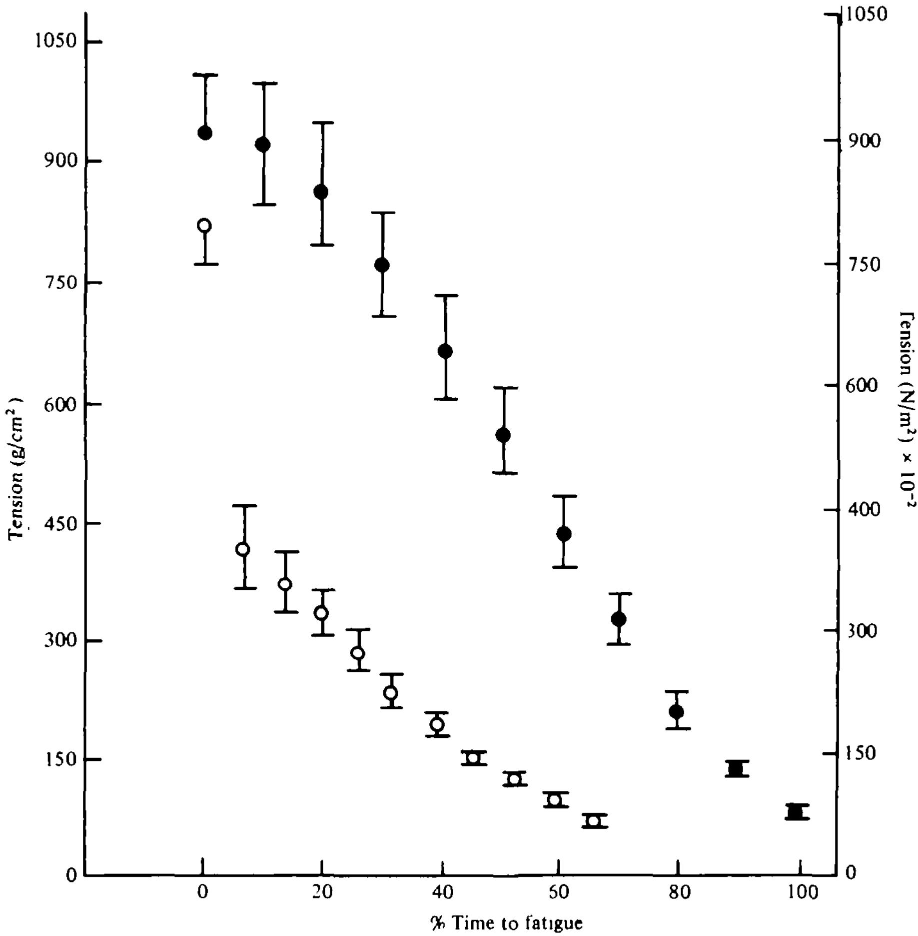

Fig. 9. Tension generated by excised gastrocnemius muscles from *X. laevis* versus time. ●, Muscles taken from unexercised control frogs; ○, muscles taken from fatigued frogs. Vertical lines represent ± 1 S.E. See text for explanation of percentage time to fatigue.

Table 2. *Lactic acid content (mg %) of gastrocnemius muscles from* X. laevis *and* R. pipiens

(Control muscles were from unexercised frogs while fatigue muscles were from fatigued frogs. Unstimulated muscles were immediately removed and quick-frozen while the stimulated muscles were removed and stimulated *in vitro* to fatigue before being quick-frozen. The values are the mean, ± 1 S.E. and the number of determinations in parentheses. For each group, stimulated values are significantly different from unstimulated values at the 0·05 level.)

	Control		Fatigue	
	Unstimulated	Stimulated	Unstimulated	Stimulated
X. laevis	135 ± 15 (7)	407 ± 15 (7)	319 ± 24 (6)	413 ± 16 (6)
R. pipiens	25 ± 12 (6)	407 ± 16 (6)	264 ± 15 (7)	433 ± 4 (7)

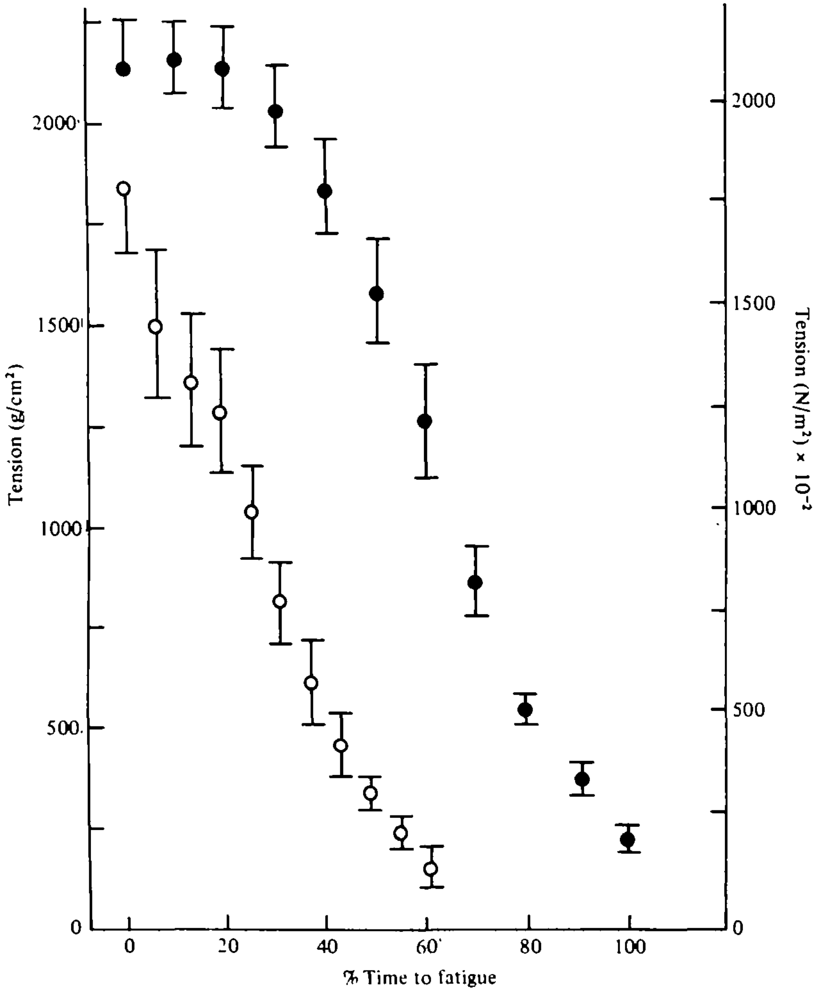

Fig. 10. Tension generated by exercised gastrocnemius muscles from *R. pipiens* versus time. Symbols as in Fig. 9.

there was a marked additional accumulation of lactic acid, and the stimulated muscles from both species accumulated similar levels of lactic acid, whether or not the frog had been initially active.

DISCUSSION

Behavioural and metabolic recovery after fatigue. The lactic acid concentrations that were observed after fatigue are similar to other reported values for these species (Bennett & Licht, 1974; Hutchison & Turney, 1975; Hillman, 1977; Putnam, 1978). The rate of removal of lactic acid from the body during recovery in anuran amphibians is a slow process requiring several hours (Bennett & Licht, 1973; Hutchison & Turney, 1975). In this study, after 15 min of rest, lactic acid levels showed no decline in *X. laevis* and decreased about 25% in *R. pipiens*. The difference may be due to the observed differences in behaviour during recovery. While *X. laevis* most frequently remained submerged during recovery and were not ventilating the

lungs, *R. pipiens* recovered in air and gular pumping was both strong and frequent. It is apparent from Fig. 1 that both species show a significant recovery of the ability to be active. The results contrast with those obtained in similar studies with salamanders: Martin Feder (personal communication) found significant behavioural recovery in *Batrachoseps attenuatus* after 30 min of recovery, but measured no change in lactic acid content of the body; and Bennett & Licht (1973) restimulated this species 135 min after an initial fatigue and found body lactic acid levels similar to values after the initial fatigue. In the two anurans studied here, however, lactic acid concentrations associated with initial fatigue are not the maximum values which can be tolerated and behavioural recovery can occur without lactic acid disappearance.

The role of muscle lactic acid in organismal fatigue. Changes in the lactic acid content of individual muscles (Figs. 3, 4) were measured to determine if fatigue is due to lactic acid accumulation to a certain critical value beyond which the muscles cannot function. The lactic acid accumulation in the muscles during the initial fatigue in the two species parallels the change in whole body lactic acid contents. A modest decrease in lactic acid concentration during recovery (of about 40–50 mg %) is evident in all the muscles from both species. Calculations using lactic acid efflux values from sartorius muscles (Mainwood *et al.* 1972; Mainwood & Worsley-Brown, 1975) show that most of this reduction (about 35 mg %) should occur by diffusion. The results of whole animal lactic acid analysis showed that net disappearance of lactic acid (presumably by oxidation and/or gluconeogenesis) occurred only in *R. pipiens*. The fate of lactic acid accumulated during activity is a problem which warrants further study.

The pattern of higher lactic acid concentrations in the muscles of refatigued frogs when compared to muscles from fatigued frogs is different from the pattern found in studies using muscle biopsy techniques on active humans. In intermittent fatigue studies (Karlsson *et al.* 1975*a*) and in studies with pre-exercise of certain muscle groups (Karlsson *et al.* 1975*b*) the lactic acid levels in the muscles attained similar levels with repeated fatigue. These studies suggest there is a critical lactic acid value in human muscles which is associated with fatigue and that either lactic acid *per se* or some correlated factor such as pH change is responsible for fatigue.

It is clear that the lactic acid contents of the muscles associated with the initial fatigue in the frogs are not maximum values beyond which further lactic acid accumulation or activity is impossible. The conclusion that lactic acid accumulation *per se* does not limit muscle performance is further supported by the results of the *in vitro* muscle preparations. These muscles from fatigued frogs could generate maximum tensions which were similar to control muscles, total tensions which were 30–40% of total tensions from control muscles, and could generate tension for 60–65% of the time of control muscles (Table 1). While it is not known what tension output the muscles must have in order for the frog to be active, it is clear that these muscles from fatigued frogs have contractile systems which are still capable of generating a great deal of tension. It is also clear that the presence of 'fatiguing' levels of lactic acid in these muscles does not prevent the glycolytic enzymes from producing energy anaerobically with further lactic acid accumulation.

The lack of a strong correlation between the level of lactic acid and loss of performance in the animal or in the muscles does not imply that lactic acid accumulation

has no effect on frogs. Clearly, the performance of the whole animal and of isolated muscles are both decreased by fatiguing exercise. Lactic acid accumulation might be the cause of the initial fatigue and during recovery the muscles might undergo some form of compensation which allows further lactic acid accumulation. Alternatively, fatigue may be due to some factor other than lactic acid content, and this factor may undergo recovery with a more rapid time course than lactic acid removal. From the present data it cannot be determined which of the possible alternatives is responsible for fatigue, but the observation that the performance of both the intact frog and isolated muscle preparations are impaired is suggestive of a peripheral origin of fatigue.

Fatigue in isolated muscles. Gastrocnemius muscles from both species, when stimulated directly to fatigue, accumulated lactic acid to a concentration of around 415 mg %, whether the muscle was taken from a fatigued frog (thus having elevated lactic acid levels) or not. This level cannot be accounted for by glycogen depletion since preliminary experiments showed that at least 200 mg % of glycogen remained in these muscles (unpublished data). These data suggest that there is an upper critical level of lactic acid in the gastrocnemius muscles of these two species which is associated with fatigue. Caution must be exercised, however, in interpreting the above data as proof that lactic acid accumulation *per se* leads to fatigue. Fitts & Holloszy (1976) showed a strong correlation between lactic acid level and decline of tension in stimulated frog sartorius muscles, but these two parameters were poorly correlated during recovery. Their results show that fatigue is not a simple function of lactic acid concentration and that muscle performance is probably affected by some secondary effect of the accumulation of lactic acid. Much of the data from the literature on fatigue in isolated frog muscle preparations points to the involvement of decreased pH and its effect on Ca^{2+} distribution (see Eberstein & Sandow, 1963; Nassar-Gentina *et al.* 1978; Mainwood & Lucier, 1972; Mainwood *et al.* 1972). The data on stimulated muscles in the present study are not inconsistent with this theory. It is doubtful that this theory can explain fatigue in the intact organism, however, since the isolated muscles are able to withstand greater lactic acid accumulation (presumably resulting in larger changes in pH) than are seen in the muscles of fatigued frogs. That the cause of fatigue may differ in intact organisms and isolated muscles is an interesting possibility which warrants further consideration.

Glycogen utilization. Another possible explanation for fatigue in exercising frogs is depletion of metabolic substrates, particularly the phosphagens and glycogen. While the phosphagens (ATP and creatine phosphate) were not measured in this study, many studies with isolated muscle preparations stimulated to fatigue have shown that ATP concentrations are maintained while creatine phosphate concentrations fall more rapidly than the decline in tension (Cerretelli *et al.* 1972; Mainwood *et al.* 1972; Nassar-Gentina *et al.* 1976; Fitts & Holloszy, 1976). However, the concentrations of these phosphagens may change differently in response to activity of the organisms than in isolated muscle preparations and thus this possible cause of fatigue cannot be discounted. The results of the present study show that glycogen depletion cannot account for fatigue during short bursts of intense activity. In *X. laevis* there was no significant decline in muscle glycogen content with fatigue,

and although there was a marked decrease in muscle glycogen for *R. pipiens*, the lowest values measured were 250 mg %. This amount of glycogen could still lead to the production of 500 mg % of lactic acid. In addition, initial glycogen contents exceed 1 g % in all muscles. In *R. pipiens* the glycogen decrease is not accompanied by a comparable increase in lactic acid, so that an increase in metabolic intermediates (such as glucose-6-phosphate), which could still serve as a source of energy for muscle contraction, is likely in these muscles. Further study has also shown the presence of substantial glycogen reserves in the muscles of fatigued frogs (Putnam, 1978). In contrast, similar experiments with fish show virtual depletion of muscle glycogen upon fatigue (Black, Robertson & Parker, 1960; Black *et al.* 1961; Pritchard, Hunter & Lasker, 1971).

Blood lactic acid and pH. Bartholomew *et al.* (1976), in a study of freely active Galapagos marine iguanas (*Amblyrhynchus cristatus*), found that blood lactic acid or blood pH may lead to fatigue, possibly by an effect on the nervous system. In the present study, for both *X. laevis* and *R. pipiens*, levels of blood lactic acid and pH associated with initial fatigue do not prevent further activity after 15 min of rest, which demonstrates that in anurans there is not a critical blood lactic acid or pH value that is associated with fatigue. Similar results have been reported for blood pH of intermittently exercised humans (Hermansen & Osnes, 1972). Exercise and the resultant metabolic acidosis will affect other blood parameters, such as P_{CO_2} and bicarbonate levels, and these parameters may correlate better with performance capacities of the organism. In *R. pipiens* during recovery, the observation that blood lactic acid and pH both increase suggests that the frogs excrete excess CO_2 across the lungs and/or skin, which would lead to lower blood bicarbonate levels. Assessing the possible involvement of changes in blood parameters with fatigue must await detailed studies of blood physiology during activity in anurans.

Ecological consequences of lactic acid accumulation. Reduced performance capability due to lactic acid accumulation would be of ecological significance if the animal was less able to escape predators during the recovery period. In various lower vertebrates the loss of activity capabilities has been reported to be prolonged (Bennett & Licht, 1973; Bartholomew *et al.* 1976). The two species of anurans used in this study, then, appear to be unusual among the lower vertebrates in that they could re-engage in intense activity after only 15 min of rest. It is unlikely that these anurans are as active in their natural environment as during the fatigue period in this study. However, even with the large lactic acid accumulation observed in laboratory activity, these frogs would still be able to engage in considerable activity within a short time. Therefore, the ecological consequences of lactic acid accumulation must be minor.

To summarize, these data indicate that in two species of anuran amphibians, behavioural recovery can occur without lactic acid disappearance. Fatigue does not appear to be due to lactic acid accumulation *per se*, glycogen depletion, or blood lactic acid or pH changes. Fatigue may be due to central nervous system inhibition of activity (perhaps to maintain a certain degree of behavioural capacity in reserve), some metabolic change in the muscle which recovers more quickly than lactic acid disappearance, or an electrical change at the neuromuscular junction or the muscle fibre membrane. In isolated gastrocnemius preparations there appears to be an upper

critical maximum value for lactic acid accumulation which may lead to fatigue by decreased pH effects on Ca^{2+} distributions.

I wish to thank Dr M. S. Gordon for giving me the opportunity to do this study, for logistical support, for advice and for critically reading this manuscript. I want to thank Drs G. A. Bartholomew, R. Edgerton and M. Letinsky for generously loaning me equipment. For advice and for critically reading the manuscript I thank Drs A. F. Bennett, A. J. Brady and E. Homsher. A special debt is gratefully acknowledged to Drs Stanley Hillman, Robert Freel, Phillip Withers and Christopher Loretz for help in all aspects of this work and friendship. This work is part of a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at U.C.L.A., and was funded by a University of California Patent Fund Grant and a Chancellor's Intern Fellowship.

REFERENCES

BARTHOLOMEW, G. A., BENNETT, A. F. & DAWSON, W. (1976). Swimming, diving and lactate production of the marine iguana, *Amblyrhynchus cristatus*. *Copeia*, pp. 709–720.

BENNETT, A. F. (1978). Activity metabolism of the lower vertebrates. *Ann. Rev. Physiol.* **40**, 447–469.

BENNETT, A. F. & LICHT, P. (1972). Anaerobic metabolism during activity in lizards. *J. comp. Physiol.* **81**, 277–288.

BENNETT, A. F. & LICHT, P. (1973). Relative contributions of anaerobic and aerobic energy production during activity in amphibia. *J. comp. Physiol.* **87**, 351–360.

BENNETT, A. F. & LICHT, P. (1974). Anaerobic metabolism during activity in amphibians. *Comp. Biochem. Physiol.* **48** A, 319–327.

BLACK, E. C., ROBERTSON, A. C., HANSLIP, A. R. & CHIU, WING-GAY (1960). Alterations in glycogen, glucose and lactate in rainbow and Kamloops trout, *Salmo gairdneri*, following muscular activity. *J. Fish. Res. Bd Can.* **17**, 487–500.

BLACK, E. C., ROBERTSON, A. C. & PARKER, R. R. (1961). Some aspects of carbohydrate metabolism in fish. In *Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals* (ed. A. W. Martin), pp. 89–124. Seattle: University of Washington Press.

CERRETELLI, P., DIPRAMPERO, P. E. & AMBROSOLI, G. (1972). High energy phosphate resynthesis from anaerobic glycolysis in frog gastrocnemius muscle. *Am. J. Physiol.* **222**, 1021–1026.

DIXON, W. J. & MASSEY, F. J., JR. (1969). *Introduction to Statistical Analysis*, 3rd ed. San Francisco: McGraw-Hill.

DUNN, A. & ARDITI, J. (1968). *Experimental Physiology*. San Francisco: Holt, Rinehart and Winston.

EBERSTEIN, A. & SANDOW, A. (1963). Fatigue mechanisms in muscle fibres. In *The Effect of Use and Disuse on Neuromuscular Function* (ed. E. Gutmann and P. Hnick), pp. 515–534. New York: Elsevier Publishing Co.

FITTS, R. & HOLLOSZY, J. (1976). Lactate and contractile force in frog muscle during development of fatigue and recovery. *Am. J. Physiol.* **231**, 430–433.

FLETCHER, W. M. & HOPKINS, F. G. (1907). Lactic acid in amphibian muscle. *J. Physiol., Lond.* **35**, 247–303.

FUCHS, F., REDDY, V. & BRIGGS, F. N. (1970). The interaction of cations with the calcium binding site of troponin. *Biochim. biophys. Acta* **221**, 407–409.

HERMANSEN, L. & OSNES, J.-B. (1972). Blood and muscle pH after maximal exercise in man. *J. appl. Physiol.* **32**, 304–308.

HILL, A. V. & KUPALOV, P. (1930). Anaerobic and aerobic activity in isolated muscle. *Proc. R. Soc. Lond.* B **105**, 313–322.

HILLMAN, S. S. (1977). Some aspects of the physiology of desiccation and physiological correlates of differential desiccation tolerance in anuran amphibians. Ph.D. thesis, University of California, Los Angeles.

HUTCHISON, V. H. & TURNEY, L. D. (1975). Glucose and lactate concentrations during activity in the leopard frog, *Rana pipiens*. *J. comp. Physiol.* **99**, 287–295.

IKAI, M. & STEINHAUS, W. (1961). Some factors modifying the expression of human strength. *J. appl. Physiol.* **16**, 157–163.

KARLSSON, J., FUNDERBURK, C. F., ESSEN, B. & LIND, A. (1975*a*). Constituents of human muscle in isometric fatigue. *J. appl. Physiol.* **38**, 208–211.

KARLSSON, J., BONDE-PETERSEN, F., HENRIKSSON, J. & KNUTTGEN, H. G. (1975*b*). Effects of previous exercise with arms or legs on metabolism and performance in exhaustive exercise. *J. appl. Physiol.* **38**, 763–767.

LANDAU, E. M. & NACHSEN, D. A. (1975). The interaction of pH and divalent cations at the neuromuscular junction. *J. Physiol., Lond.* **251**, 775–790.

MAINWOOD, G. W. & LUCIER, G. E. (1972). Fatigue and recovery in isolated frog sartorius muscles: the effects of bicarbonate concentration and associated potassium loss. *Can. J. Physiol. Pharmacol.* **50**, 132–142.

MAINWOOD, G. W., WORSLEY-BROWN, P. & PATERSON, R. (1972). The metabolic changes in frog sartorius muscles during recovery from fatigue at different external bicarbonate concentrations. *Can. J. Physiol. Pharmacol.* **50**, 143–155.

MAINWOOD, G. W. & WORSLEY-BROWN, P. (1975). The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J. Physiol., Lond.* **250**, 1–22.

MERTON, P. A. (1954). Voluntary strength and fatigue. *J. Physiol., Lond.* **123**, 553–564.

MEYERHOF, O. (1925). *Chemical Dynamics of Life Phenomena*. Philadelphia: Lippincott.

MONTGOMERY, R. (1957). Determination of glycogen. *Archs Biochem. Biophys.* **67**, 378–386.

NASSAR-GENTINA, V., PASSONNEAU, J., VERGARA, J. & RAPOPORT, S. (1976). Relation of fatigue state in frog single muscle fibers to concentrations of glycogen, phosphocreatine (Pcr) and ATP. *Biophys. J.* **16**, 206a.

NASSAR-GENTINA, V., PASSONNEAU, J., VERGARA, J. & RAPOPORT, S. (1978). Metabolic correlates of fatigue and recovery from fatigue in single frog muscle fibers. *J. gen. Physiol.* **72**, 593–606.

PORTZEHL, H., ZAORALEK, P. & GAUDIN, J. (1969). The activation by Ca^{2+} of the ATPase of extracted muscle fibrils with variation of ionic strength, pH and concentration of MgATP. *Biochim. biophys. Acta* **189**, 440–448.

PRITCHARD, A., HUNTER, J. & LASKER, R. (1971). The relation between exercise and biochemical changes in red and white muscle and liver in the jack mackerel, *Trachurus symmetricus*. *Fishery Bull.* **69**, 379–386.

PUTNAM, R. W. (1977). Relationship between fatigue and changes of lactate and glycogen in muscles of anuran amphibians. *Am. Zool.* **17**, 893.

PUTNAM, R. W. (1978). The role of lactic acid in activity metabolism and fatigue of anuran amphibians. Ph.D. thesis, University of California, Los Angeles.

RUBEN, J. A. (1976). Aerobic and anaerobic metabolism during activity in snakes. *J. comp. Physiol.* **109**, 147–157.

SCHEFFÉ, N. A. (1953). A method for judging all contrasts in the analysis of variance. *Biometrika* **40**, 87–104.

SERAYDARIAN, M. W., ABBOTT, B. C. & WILLIAMS, E. (1961). Studies on the frog's sartorius at different stages of activity. *Biochim. Biophys. Acta* **46**, 355–363.

TRIVEDI, B. & DANFORTH, W. (1966). Effect of pH on the kinetics of frog muscle phosphofructokinase. *J. biol. Chem.* **241**, 4110–4111.