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THE EXTENT OF SHORT-TERM AND LONG-TERM COMPENSATION TO TEMPERATURE SHOWN BY FROG AND TOAD SARTORIUS MUSCLE

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SUMMARY

The effect of pH at different temperatures was studied on the force-velocity characteristics of the sartorius muscle to estimate short-term compensation to temperature. The effect of thermal acclimation was also studied in order to estimate long-term compensation. A decrease in either pH or temperature reduced both tetanic tension and shortening velocity. There was a significant pH-temperature interaction for tetanic tension and for maximum mechanical power, but not for V_{max}. It is shown that this pH-temperature interaction was large enough to provide a mechanism for short-term compensation only for the maximum strength (tetanic tension) in both species. Shortening velocities at small loads of the sartorius muscle of frogs and toads acclimated to 25 C were faster than those acclimated to 5 C. The difference between the two acclimation groups increased with test temperature and was almost 1 muscle length per second (m.l. s^{-1}) at 25 C for both species. Acclimation temperature had no significant effect on tetanic tension or on maximum mechanical power at any of the test temperatures. It is proposed that the small capacity for long-term compensation in frog and toad sartorius muscles is related to the strategy employed during winter: frogs and toads hibernate.

INTRODUCTION

Studies of amphibians have shown that there are large variations in their body temperature. During the active season, the body temperature of 112 frogs, *Rana pipiens*, ranged from 18 to 35 C, while that of 84 toads, *Bufo americanus*, ranged from about 11 to 32 C (Brattstrom, 1963). During the inactive season, hibernating leopard frogs are exposed to environmental temperatures of 0–4 C, but are still capable of movements (Emery, Berst & Kodeira, 1972). Amphibians are poikilothermic, so their ability to move must change with temperature because it alters both tetanic tension and shortening velocity (Hill, 1951; Edman, 1979). That is, the contractile activity will be influenced by environmental temperature unless there are some adaptive processes in muscle to compensate for both the daily and seasonal temperature changes.

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The adaptive processes necessary to compensate for daily temperature changes during the active season must be immediate. We define this type of compensation as acute or short-term. One mechanism for short-term compensation is the interaction between pH and temperature. Reeves (1972) showed how the change in blood and tissue pH with body temperature is necessary to maintain the conformation and function of proteins through the constancy of charge state at different temperatures. Reeve's hypothesis was later supported by the observations that the pH optima of many enzymatic reactions change with temperature in the same direction as blood and tissue pH (Hazel, Garlick & Sellner, 1978; Somero, 1981). We reported earlier that the interactive effect of pH and temperature on tetanic tension of toad sartorius muscle in combination with the concomitant change in blood pH that occurs with a change in body temperature is sufficiently large that tetanic tension decreases very little from 25 to 5 °C (Renaud & Stevens, 1981*a*). This is evidence for short-term compensation for tetanic tension.

The type of adaptive processes that are necessary when environmental temperature decreases during winter can be immediate, but seasonal changes in temperature are slow enough to permit permanent adaptive changes in many physiological and biochemical aspects to compensate for the anticipated average temperature change. We define this type of compensation as long-term compensation. It occurs after a period of acclimation to a relatively long, persistent thermal stress. However, in earlier studies on tetanic tension, we found no evidence for long-term compensation in frog and toad sartorius muscle following a period of acclimation to 5 or 25 °C (Renaud & Stevens, 1981*a*,*b*).

Evidence for short- and long-term compensation for shortening velocity is suggested by studies on the activity of myosin ATPase. In short-term compensation, changes in pH optimum with temperature have been reported in the activity of calcium-activated actomyosin ATPase of frog skeletal muscle (Kim, Hwang, Park & Kang, 1977). Evidence for acclimation has been reported for the activity of actomyosin ATPase in goldfish muscles, but not in trout muscles (Penney & Goldspink, 1981). These results suggest that there may be some temperature compensation for the shortening velocity because there is a relationship between the actin-activated myosin ATPase activity and maximum shortening velocity, V_{max} (Barany, 1967).

The main purpose of this study was to estimate the *in vitro* effects of pH and acclimation temperature on the force-velocity characteristics of intact sartorius muscle of *R. pipiens* and *B. americanus*. These two species were chosen because they have different muscle characteristics that correlate well with their different habits, and different jumping and burrowing abilities (Renaud & Stevens, 1983). Our *in vitro* results suggest that there is little *in vivo* capacity to compensate for the effects of temperature on shortening velocity (and mechanical power) of frog and toad sartorius muscle, either in the short term (acute temperature effects) or in the long term (acclimation effects).

MATERIALS AND METHODS

Animals

Frogs, *Rana pipiens*, and toads, *Bufo americanus*, were kept in tanks made wood covered with resins. Each tank was divided in half giving final dimensions of

50 cm × 60 cm and 50 cm deep. Groups of nine animals or less were kept in each division. Aerated water (20 cm deep) was continuously running, cooled or heated. Platforms were also added out of the water. First, each division contained two platforms (each 20 × 25 cm) which were made of transparent Plexiglass. Another platform (20 × 20 cm) made of black Plexiglass was put 5 cm above each transparent platform. Underneath each black platform, separations were added to give three alleys (9 × 20 cm each), so animals could choose to remain either in the dark or under the light. Photoperiod was 12:12 (L:D) and was controlled with a dimmer switch that provided a half-hour transition to approximately natural conditions.

Muscle chamber and physiological saline solution

The experimental arrangement is shown in Fig. 1. Lightweight gold chains were attached to each end of the right sartorius muscle. One gold chain was then tied to a fixed bar, while the other was tied to the torque arm of a muscle ergometer. The muscle was continuously bathed in a physiological saline solution (in mM; 115 NaCl; 3·2 KCl; 1·26 $CaCl_2$; 1·0 $MgSO_4$; 2·0 Na_2HPO_4; 20·0 $NaHCO_3$). The pH of the physiological saline solution (pHe) was changed by modifying the O_2: CO_2 ratio in the gas phase. Plexiglass covers were put on top of the muscle chamber to prevent exchange of CO_2 between the physiological solution and the atmosphere. Recirculation of the physiological saline solution and the temperature control is as described by Renaud & Stevens (1981*b*).

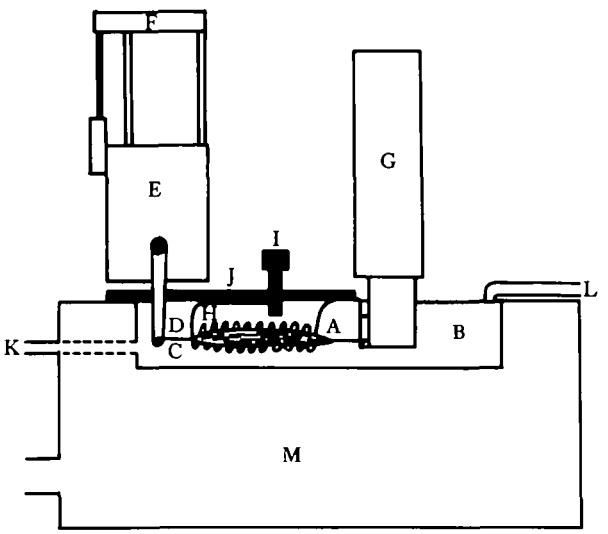

Fig. 1. Schematic illustration of experimental arrangement. A, sartorius muscle; B, muscle chamber; C, gold chains; D, torque arm; E, ergometer; F, control circuitry of the ergometer; G, fixed bar held by a Vernier used to stretch the muscle; H, platinum electrode that has a waveform (only one shown); I, jack to receive electrical wire from stimulator; J, Plexiglass cover placed on top of the physiological saline solution to hold electrodes; K, inlet for bath solution which comes from a stock solution situated above muscle chamber; L, suction drain (solution goes to a controlled water bath before returning to the stock solution); M, jacket for circulation for thermostatically controlled water-glycerol mixture.

Stimulations

Muscles were stimulated by passing a current between two electrodes placed on either side of the muscle. The electrodes were made of platinum wire which had a wavelike shape and which were 2·5 cm long. Electrodes were connected to a Grass SIU5 stimulus isolation unit which in turn was connected to a Grass S48 stimulator. Stimulations were rectangular pulses of 3 ms duration with a supramaximal stimulus strength of 10 V. A single pulse (twitch) or a 400-ms train of pulses (tetanus) was given at 100-s intervals. The range of pulse frequencies used to obtain a fused tetanus was 30–40 Hz at 5 °C, 50–60 Hz at 10 °C, 70–80 Hz at 15 °C, 90–100 Hz at 20 °C and 100–120 Hz at 25 °C.

Muscle transducer

An ergometer (Cambridge Technology model 300 H) was used to measure both force and shortening velocities of sartorius muscles. The torque arm of the ergometer was connected to an iron vane which was in the centre of a four-pole magnetic circuit. Increases in the magnetic flux resulted in the application of a torque to the iron vane. The torque arm was also connected to a position detector which was a differential capacitor. Any movement of the torque arm changed the differential current from a 2-MHz 200 VAC signal. In the isometric mode, the force developed by a muscle was measured from the increase of current in the magnetic circuit that was necessary to maintain the position of the torque arm constant. In the isotonic mode, the increase of current in the magnetic circuit was set to a maximum so that the maximum torque applied to the iron vane was less than the maximum isometric force. When the muscle force exceeded the torque on the iron vane, muscle shortening occurred. From external electronics, the force signal output was 1 V/10 g; the position signal output was 1 V mm^{-1}, and the velocity signal output was 0·4 mV mm^{-1} s^{-1}.

Recordings and measurements of response

The force signal was recorded on a Grass polygraph model 79D and a Tektronix oscilloscope type 564B. Length changes of muscles were measured on the oscilloscope from the position signal, while the shortening velocities from the velocity signal output were recorded on the polygraph using a Grass polygraph low-level d.c. preamplifier Model 7P1E.

The force-velocity data were obtained using the after-load and the quick-release techniques described by Jewell & Wilkie (1958). The data were fitted by Hill's (1938) hyperbolic equation:

$$(P + a)\ (V + b) = c,$$

where P is load, V is shortening velocity, a, b and c are constant. The regression analyses used the derivative-free analogue of the Levenberg-Marquardt and Gauss Algorithms for non-linear regression analysis. The maximum shortening velocity, V_{max}, was calculated from the fitted hyperbola by extrapolating to zero load. The ratio P/Po at which maximum mechanical power occurs was calculated from Hill's (1938) equation:

$$P/Po = (a/Po) \times (\sqrt{1 + Po/a} - 1)$$

where P is the load at which maximum mechanical power was observed, Po is the tetanic tension and a is the constant from Hill's hyperbolic equation. From the P/Po ratio and the force-velocity equation, the load (P) and the shortening velocity (V) were obtained. The maximum mechanical power was then calculated as the product P × V.

Protocol

The interactive effects of pHe and test temperature

In the first experiment, the interactive effects of pHe and test temperature were studied on the sartorius muscle to measure its capacity for short-term compensation. Frogs (15–31 g) were obtained from Northwest Laboratory Supply, Guelph, Ontario; their sartorius muscles (42–94 mg) were tested in April 1981. Toads (21–80 g) were caught in April 1980 during their spawning season in Guelph, Ontario; their sartorius muscles (31–117 mg) were tested in January–February 1981. All animals were kept at 25 °C and were fed mealworms every 2 days.

Each muscle was tested at either 5, 15 or 25 °C. Each muscle was first exposed to pHe 9·0 for 30–45 min. The force-velocity curves were then measured at pHe 9·0, 8·0, 7·0 and 6·5 in that order. The partial pressures of carbon dioxide in the gas phase for each pHe level at 5, 15 and 25 °C are given in Table 1. The reversibility of the pHe effects was tested by returning pHe to 8·0 at the end of each experiment. Each pHe change was less than 2 min long. Twitch stimuli were given (1/100 s) during and for 20 min after each pHe change to prevent fatigue. Tetanic stimuli (1/100 s) were then given and the force-velocity curve was measured when the force signal on the oscilloscope screen did not change in shape and magnitude, i.e. tetanic tension had stabilized.

The effect of acclimation temperature

In the second experiment, frogs and toads were acclimated to either 5 or 25 °C in order to estimate the extent of long-term compensation. For this experiment, frogs (6–19 g) and toads (22–45 g) were caught in Guelph, Ontario in September 1980 and April 1981, respectively. Following their capture, frogs and toads were kept at 15–20 °C and were fed mealworms every 2 days prior to the acclimation periods. The

Table 1. *Partial pressure of carbon dioxide in the gas phase required to maintain the pHe constant at different test temperatures*

pHe	Partial pressure of CO_2 (Torr)		
	5 °C	15 °C	25 °C
9·0	0·6	0·6	0·7
8·0	5·6	6·2	7·1
7·0	55·7	62·5	70·8
6·5	176·2	197·5	223·9

The partial pressure was calculated from the Henderson-Hasselbalch equation.
The dissociation constant of carbonic acid and the solubility coefficient in plasma (which has a similar salt composition as the physiological solution) were calculated for each temperature by the method of Reeves (1976).

acclimation periods at 5 or 25 °C were 5 weeks long. The acclimation temperature always refers to the water temperature in the tanks. The difference between the water temperature and air temperature in tanks at 25 °C was less than 1 °C. The temperature of the air in tanks at 5 °C was always 2–4 °C above water temperature. At that latter temperature, most (80 %) frogs remained in water while all toads remained on platforms. These observations were made twice daily (09.00 and 17.00 h). Neither frogs nor toads at 5 °C would feed, but those at 25 °C did.

Frog sartorius muscles (11–48 mg) were tested in March 1981. Five muscles isolated from frogs acclimated to 5 °C and six muscles of frogs acclimated to 25 °C were tested at 15, 20 and 25 °C in that order; two other muscles from frogs acclimated to 25 °C were tested in the reverse direction for the change in test temperature. At all test temperatures, pHe was maintained at 8·0.

Toad sartorius muscles (42–93 mg) were tested in May, June and July, 1981. Sixteen toads were acclimated to each of the two acclimation temperatures of 5 and 25 °C. For each group of 16 toads, the sartorius muscles of eight animals were tested at 5, 10 and 15 °C, while the muscles of the other eight toads were tested at 15, 20 and 25 °C. The reversibility of the test temperature effect was tested as follows: four of the eight muscles tested at 5, 10 and 15 °C were exposed to these test temperatures in that order while the other four muscles were tested in the reverse order. The same protocol was used for the eight muscles tested at 15, 20 and 25 °C. At all test temperatures, pHe was maintained at 8·0.

In these experiments, the test temperature was changed in about 5–10 min. Twitch stimuli were given (1/100 s) during and for 10 min after each change. The tetanic stimuli (1/100 s) were given and the force-velocity curve was measured when the tetanic tension had stabilized.

Statistical analysis

An analysis of variance (ANOVA) was first used to test the significance of acclimation temperature, test temperature, pHe and their interactions. For most experiments, a split-plot design ANOVA was used because muscles were always tested to all levels of one of the treatments (either pHe or test temperature). Moreover, conservative degrees of freedom were used in the split-plots because the order of change of pHe or test temperature was not random. When a main effect or an interaction was significant, multiple comparison tests using the *t*-test according to Steel & Torrie (1980) were done to locate the significant differences. When necessary, the data were transformed to obtain normality and homoscedasticity (most were log transformations). For all statistical analyses, the level of probability for significant change was 1 %.

RESULTS

The interactive effects of pHe and test temperature

A decrease in pHe from 9·0 to 8·0 had a very small effect on the force-velocity curve of toad sartorius muscles at either 5, 15 or 25 °C (Fig. 2). When pHe was further decreased to 7·0 and 6·5, the force-velocity curve was then significantly shifted to th

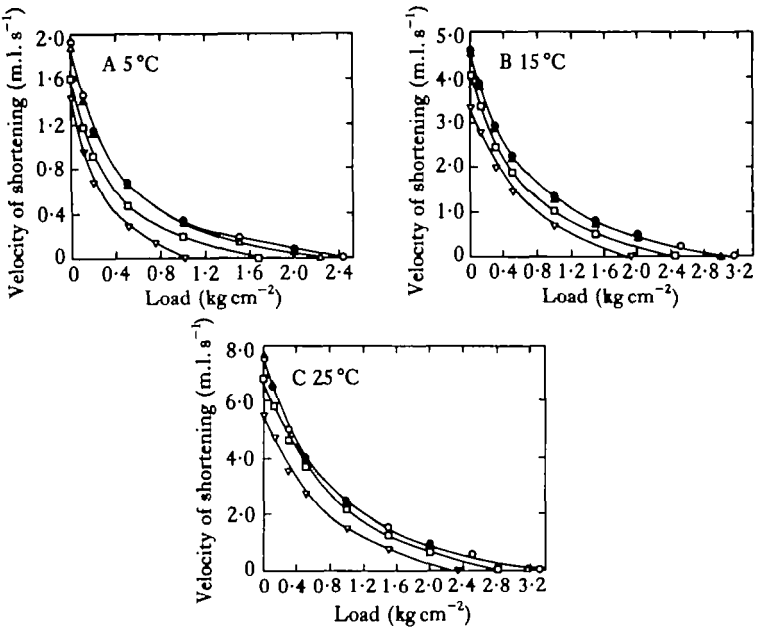

Fig. 2. The interactive effects of pHe and test temperatures on the force-velocity curve of toad (*Bufo americanus*) sartorius muscles. For the force-velocity curves, the shortening velocities (in muscle length per second, m.l. s^{-1}) at different loads were calculated from the fitted hyperbolic equation of each muscle, so the force-velocity curves represent the average of the calculated velocities and not the data *per se*. Test temperatures: (A) 5 °C, (B) 15 °C and (C) 25 °C. The pHe levels are: ○ 9·0, △ 8·0, □ 7·0, ▽ 6·5.

left; i.e. tetanic tension and shortening velocities decreased. The results were the same for frog sartorius muscles (results not shown). The effects of pHe on the force-velocity curve of both frog and toad sartorius muscles had two other characteristics. First, the pHe effects were not uniform along the force-velocity curve: the effects were greater on tetanic tension than on V_{max}, except at 25 °C (Fig. 2). Second, the magnitude of the pHe effects on the force-velocity curves were influenced by test temperatures: the effects appeared relatively greater at low than at high temperatures, especially at high loads. These pHe effects and their interaction with test temperature are studied in greater details with the following three variables: tetanic tension, V_{max} and maximum mechanical power (Table 2).

Tetanic tension

. At pHe 9·0, frog sartorius muscles developed less tetanic tension than did toad sartorius muscles (Table 3). When pHe was decreased from 9·0 to 8·0, tetanic tension of both frog and toad sartorius muscles decreased by less than 10 % (Fig. 3). When pHe was further decreased to 7·0, then tetanic tension of frog sartorius muscles decreased by about 19, 16 and 9 % at test temperatures of 5, 15 and 25 °C, respectively (Fig. 3A). For toad sartorius muscles, the values were about 24, 17 and 11 % (Fig. 3B). Finally at pHe 6·5 the tetanic tension of frog sartorius muscles represented about 60, 66 and 68 % of the tension observed at pHe 9·0 for test temperatures of 5, 15 and 25 °C; while for toad sartorius muscles the values were about 44, 61 and 71 %. Thus, the pHe effects on tetanic tension appeared to be greater at low than at high temperatures, especially

Table 2. *Split-plot ANOVA table of the interactive effect of pHe and test-temperature on the variables measured from the force-velocity curve of frog and toad sartorius muscles*

Treatments	df	Tetanic tension	V_{max}	Maximum mechanical power
A) *Rana pipiens*				
Test temperature	2,18	26·9*	506·7*	452·2*
pHe	1,18	592·4*	81·5*	189·3*
pHe-Test temperature	2,18	9·5*	4·5	3·5
B) *Bufo americanus*				
Test temperature	2,18	34·5*	145·9*	498·8*
pHe	1,18	771·8*	85·6*	293·8*
pHe-Test temperature	2,18	49·6*	0·6	8·8*

Numbers are F values. Conservative degrees of freedom were used in the split-plot (all pHe effects) because the order of pHe changes were not random.
*Significant at 1% level.

Table 3. *The tetanic tension and V_{max} of frog and toad sartorius muscles at pHe 9·0*

Variables	Test temperature (°C)	*Rana pipiens*	*Bufo americanus*
Tetanic tension ($kg\,cm^{-2}$)	5	2·05 ± 0·05	2·44 ± 0·15*
	15	2·70 ± 0·12	3·15 ± 0·13*
	25	2·75 ± 0·10	3·30 ± 0·12*
V_{max} ($m.l.\,s^{-1}$)	5	2·13 ± 0·12	1·93 ± 0·11
	15	5·20 ± 0·11	4·61 ± 0·13*
	25	9·68 ± 0·26	7·46 ± 0·37*

These results are the first values obtained at the beginning of each experiment. Results are given as the mean ± S.E.M. of seven muscles.
*Significantly different from the value for the frog sartorius muscle; *t*-test, $P < 0·01$.

for toad sartorius muscles. In fact, the interaction between pHe and test temperature was significant for both species (Table 2).

An important aspect of this pHe-test temperature interaction was the influence of pHe on the effects of test temperature on tetanic tension. In general, muscles that were exposed at 15 °C developed more tetanic tension than those exposed at 5 °C, while most differences in tetanic tension between 15 and 25 °C were not significant (Fig. 4). However, the Q_{10} values (5–15 °C) for the tetanic tension of frog sartorius muscles increased slightly from pHe 8·0 to 6·5, while for toad sartorius muscles they constantly increased from pHe 9·0 to 6·5 (Table 4). The Q_{10} values between 15 and 25 °C were close to unity, except at pHe 7·0 for frog sartorius muscles and pHe 7·0 and 6·5 for toad sartorius muscles. At these pHe levels, the test temperature effects were at least two-times greater than the effects observed at pHe 8·0 and 9·0 (Fig. 4). In other words, the effects of test temperature on tension appeared to be smaller at high pHe than at low pHe.

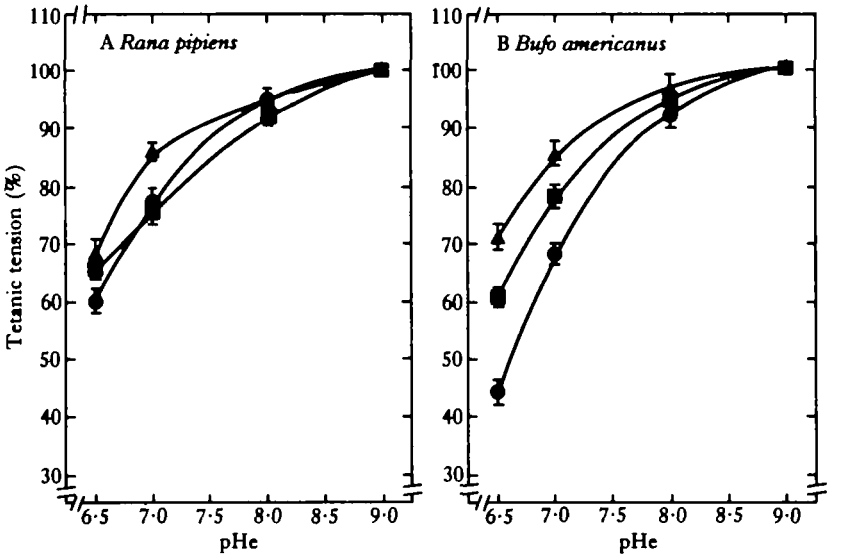

Fig. 3. The interactive effect of pHe and test temperature on the tetanic tension of (A) frog and (B) toad sartorius muscles. For each test temperature the tetanic tension observed at pHe 9·0 was taken as 100 %. Vertical bars represent the S.E.M. of seven muscles. Test temperatures: ● 5 °C, ■ 15 °C, ▲ 25 °C.

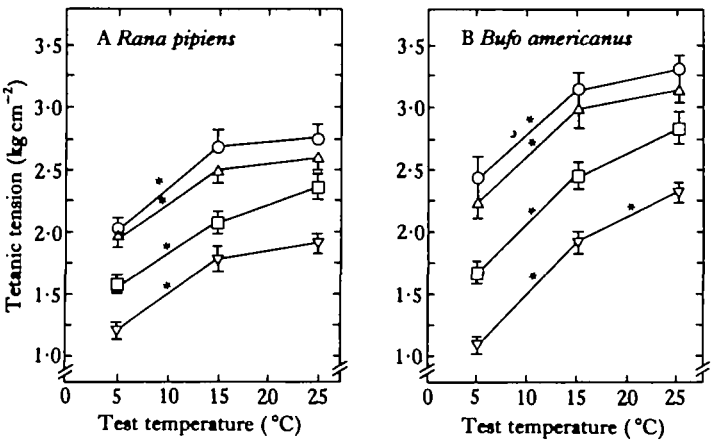

Fig. 4. The effects of test temperature on the tetanic tension of (A) frog and (B) toad sartorius muscles at different pHe. Vertical bars represent the S.E.M. of seven muscles. The pHe values are: ○ 9·0, △ 8·0, □ 7·0, ▽ 6·5. * Significant change; multiple comparison tests, t-test $P < 0{\cdot}01$.

V_{max}

At pHe 9·0, the values of V_{max} for frog sartorius muscles were faster than those for toad sartorius muscles (Table 3). A decrease in pHe from 9·0 to 8·0 at 5 or 15 °C reduced the mean V_{max} of both frog and toad sartorius muscles by less than 5 %, while a further decrease to pHe 7·0 and 6·5 significantly reduced V_{max} (Fig. 5). Also, at these two test temperatures and for both species the pHe effects were smaller on V_{max} than on tetanic tension (compare Figs 3 and 5). When muscles were tested at 25 °C, on the other hand, the mean V_{max} reached a peak at about pHe 8·0. Such a peak was not

Table 4. *The effects of pHe on the Q_{10} values of tetanic tension, V_{max} and maximum mechanical power*

Test temperature (°C)	pHe	Q_{10}		
		Tension	V_{max}	Maximum mechanical power
A *Rana pipiens*				
5–15 °C	9·0	1·32	2·44	3·31
	8·0	1·28	2·46	3·27
	7·0	1·31	2·46	3·61
	6·5	1·46	2·45	3·62
15–25 °C	9·0	1·02	1·86	1·83
	8·0	1·04	2·00	2·10
	7·0	1·15	1·97	2·01
	6·5	1·05	1·72	1·62
B *Bufo americanus*				
5–15 °C	9·0	1·29	2·39	3·77
	8·0	1·33	2·43	3·82
	7·0	1·48	2·50	4·25
	6·5	1·79	2·35	4·81
15–25 °C	9·0	1·05	1·62	1·89
	8·0	1·06	1·66	1·92
	7·0	1·15	1·67	2·15
	6·5	1·21	1·64	1·96

The Q_{10} values were calculated from the means because for each test temperature there was a different group of muscles.

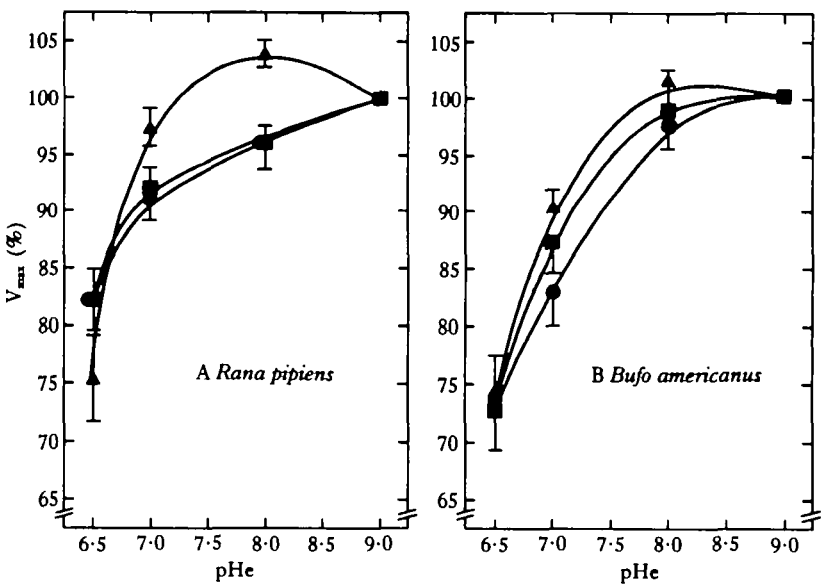

Fig. 5. The interactive effects of pHe and test temperature on the V_{max} of (A) frog and (B) toad sartorius muscles. Test temperatures: ● 5 °C, ■ 15 °C, ▲ 25 °C.

observed for tetanic tension. Another difference between V_{max} and tetanic tension was that the interaction between pHe and test temperature was not significant for V_{max} (Table 2). In other words, the differences in the pHe effects on V_{max} at 5, 15 and 25 °C were not significant.

In general, the effects of test temperature were much larger on V_{max} than on tetanic tension, while the influence of pHe on the test temperature effects was much smaller (Fig. 6). There was no significant difference in the effects of test temperature on the V_{max} of frog sartorius muscle between pHe 9·0 and 7·0. At pHe 6·5 the absolute increases in V_{max} with test temperature were smaller (Fig. 6A). For toad sartorius muscles, the effects of test temperature on V_{max} appeared to be smaller at pHe 7·0 and 6·5 than at pHe 9·0 and 8·0 (Fig. 6B). For both species however, the Q_{10} values were quite similar at different pHe levels (Table 4).

Maximum mechanical power

The P/Po ratios of the maximum mechanical power were quite constant between muscles tested under similar conditions as shown by the small standard error of the mean in Table 5. These ratios were smaller for toad sartorius muscles than for frog sartorius muscles and they increased as pHe was decreased from 9·0 to 6·5.

Maximum mechanical power, like V_{max}, was greater in frog sartorius than in toad sartorius muscles. The pHe effects on the maximum mechanical power of frog sartorius muscles were similar to those on V_{max}. That is, when pHe was first decreased from 9·0 to 8·0, the maximum mechanical power decreased slightly at 5 and 15 °C, but it increased at 25 °C (Table 5A). When pHe was further decreased to 7·0 and 6·5, the maximum mechanical power decreased significantly at all test temperatures. Finally, the interaction between pHe and test temperature was not significant for the

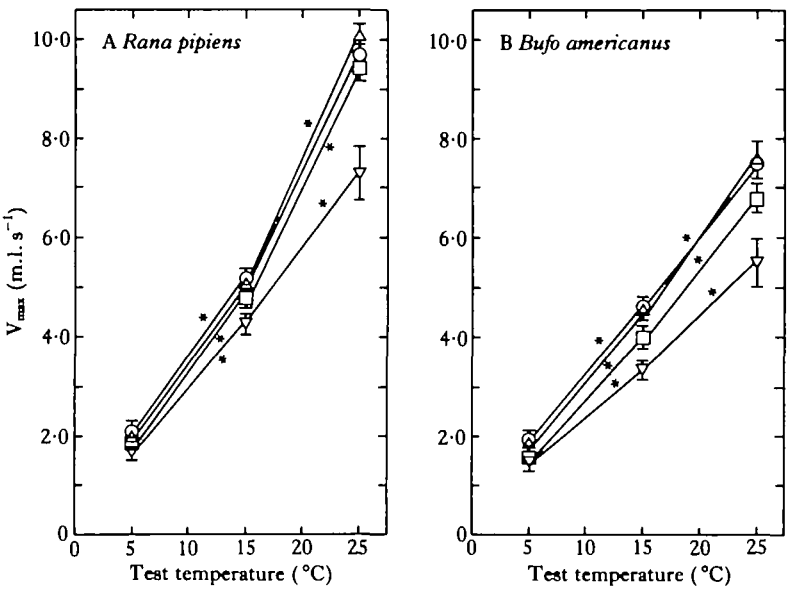

Fig. 6. The effects of test temperature on the V_{max} of (A) frog and (B) toad sartorius muscles at different pHe. pHe values are: ○ 9·0, △ 8·0, □ 7·0, ▽ 6·5.

Table 5. *The interactive effects of pHe and test temperature on the maximum mechanical power of frog and toad sartorius muscles*

Temperature (°C)	pHe	Maximum power ($kg\,cm^{-2} \times m.l.\,s^{-1}$)	P/Po
A *Rana pipiens*			
5	9·0	0·51 ± 0·03	0·336 ± 0·004
	8·0	0·48 ± 0·02	0·344 ± 0·005
	7·0	0·38 ± 0·02*	0·352 ± 0·005
	6·5	0·29 ± 0·01*	0·369 ± 0·006
15	9·0	1·69 ± 0·07	0·343 ± 0·003
	8·0	1·57 ± 0·06	0·350 ± 0·004
	7·0	1·37 ± 0·06	0·371 ± 0·003
	6·5	1·05 ± 0·07*	0·371 ± 0·007
25	9·0	3·09 ± 0·15	0·335 ± 0·001
	8·0	3·30 ± 0·15	0·334 ± 0·004
	7·0	2·76 ± 0·13	0·347 ± 0·004
	6·5	1·70 ± 0·11*	0·351 ± 0·008
B *Bufo americanus*			
5	9·0	0·35 ± 0·02	0·268 ± 0·006
	8·0	0·33 ± 0·02	0·277 ± 0·006
	7·0	0·24 ± 0·01*	0·298 ± 0·006
	6·5	0·16 ± 0·01*	0·317 ± 0·009
15	9·0	1·32 ± 0·05	0·300 ± 0·006
	8·0	1·26 ± 0·03	0·303 ± 0·005
	7·0	1·02 ± 0·03*	0·321 ± 0·007
	6·5	0·77 ± 0·02*	0·344 ± 0·010
25	9·0	2·49 ± 0·14	0·314 ± 0·006
	8·0	2·42 ± 0·13	0·312 ± 0·009
	7·0	2·19 ± 0·13	0·339 ± 0·010
	6·5	1·51 ± 0·13*	0·343 ± 0·011

The P/Po ratios at which the maximum powers were observed are also given. Results are given as the mean ± S.E.M. of seven muscles.
*Significantly different from pHe 9.0; $P < 0·01$.

maximum mechanical power of frog sartorius muscles (Table 2). For toad sartorius muscles, however, the interaction was significant and similar to the interaction observed for tetanic tension. In other words, the pHe effects were relatively greater at 5 than at 25 °C. For example, at pHe 7·0 the maximum mechanical power of toad sartorius muscles was about 69 and 88 % of that at pHe 9·0 at the test temperatures of 5 and 25 °C, respectively.

The maximum mechanical power was the variable that was the most affected by test temperature, i.e. its Q_{10} values were the highest (Table 4). The pHe had its greatest influence on the Q_{10} values of toad sartorius muscles where the Q_{10} values for maximum mechanical power increased as pHe was decreased from 9·0 to 6·5. This pHe effect on the Q_{10} values of the maximum mechanical power of toad sartorius muscles was similar to the effect on tetanic tension.

Reversibility of the pHe effects

After muscles had been tested at pHe 6·5, the pHe was then returned to 8·0 and

Table 6. *Reversibility of the pHe effects*

Variables	Test temperature (°C)	*Rana pipiens* Start	*Rana pipiens* End	*Bufo americanus* Start	*Bufo americanus* End
Tetanic tension ($kg\,cm^{-2}$)	5	1·95 ± 0·05	1·84 ± 0·04*	2·24 ± 0·13	2·18 ± 0·14
	15	2·50 ± 0·10	2·35 ± 0·08*	2·99 ± 0·16	3·13 ± 0·14
	25	2·60 ± 0·13	2·55 ± 0·14	3·17 ± 0·13	3·01 ± 0·11
V_{max} ($m.l.\,s^{-1}$)	5	2·04 ± 0·10	1·93 ± 0·09	1·88 ± 0·10	1·74 ± 0·07
	15	5·01 ± 0·09	4·58 ± 0·12*	4·57 ± 0·20	4·32 ± 0·19
	25	9·79 ± 0·10	9·15 ± 0·38	7·57 ± 0·35	6·60 ± 0·43*
Maximum mechanical power ($kg\,cm^{-2} \times m.l.\,s^{-1}$)	5	0·48 ± 0·02	0·45 ± 0·03	0·33 ± 0·02	0·31 ± 0·02
	15	1·57 ± 0·06	1·44 ± 0·08	1·26 ± 0·03	1·27 ± 0·03
	25	3·03 ± 0·15	2·77 ± 0·18	2·42 ± 0·13	2·22 ± 0·16

All results were obtained at pHe 8·0. Results under 'Start' are the results obtained before muscles were exposed to pHe 6·5 at the start of the experiment. Results under 'End' are those obtained after muscles were exposed to pHe 6·5 at the end of the experiment. Results are given as the mean ± S.E.M. of seven muscles.

*Significant difference between the values at the end of the experiment; paired *t*-test; $P < 0·01$.

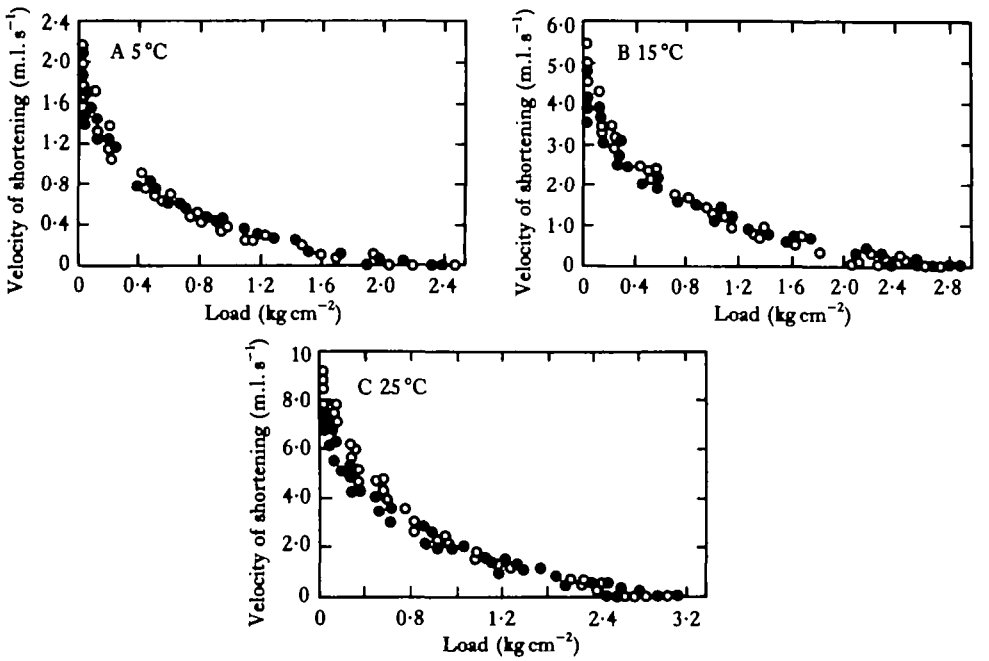

Fig. 7. The effects of acclimation temperature on the force-velocity curve of toad (*Bufo americanus*) sartorius muscles. All data are obtained at pHe 8·0. Each point represents an individual result: Test temperatures: (A) 5 °C; (B) 15 °C; (C) 25 °C. Acclimation temperature: ● 5 °C; ○ 25 °C.

variables were measured again. In Table 6 we compare the results at pHe 8·0 before and after muscles were exposed to pHe 6·5. In most cases the differences were less than 10 % and there were few significant differences. A difference of 13 % was observed with the V_{max} of toad sartorius muscle tested at 25 °C.

The effect of acclimation temperature

There was little or no clear separation of the shortening velocities at different loads between the two acclimation groups for both frog and toad sartorius muscles. An

Table 7. *The effects of acclimation temperature on tetanic tension, V_{max} and maximum mechanical power of frog and toad sartorius muscles at pHe 8·0*

Test temperature (°C)	Acclimation temperature (°C)	*N*	Tetanic tension ($kg\,cm^{-2}$)	V_{max} ($m.l.\,s^{-1}$)	Maximum mechanical power ($kg\,cm^{-2} \times m.l.\,s^{-1}$)	Maximum mechanical power P/Po
A *Rana pipiens*						
15	5	5	2·59 ± 0·18	5·43 ± 0·28	2·00 ± 0·16	0·375 ± 0·009
	25	8	2·31 ± 0·06	5·37 ± 0·13	1·71 ± 0·09	0·368 ± 0·006
20	5	5	2·64 ± 0·18	7·30 ± 0·28	2·79 ± 0·19	0·373 ± 0·006
	25	8	2·43 ± 0·07	7·35 ± 0·24	2·50 ± 0·11	0·370 ± 0·007
25	5	5	2·62 ± 0·20	9·17 ± 0·13	3·31 ± 0·19	0·366 ± 0·008
	25	8	2·44 ± 0·09	10·11 ± 0·35	3·29 ± 0·19	0·359 ± 0·007
B *Bufo americanus*						
5	5	8	2·18 ± 0·05	1·86 ± 0·08	0·39 ± 0·01	0·305 ± 0·005
	25	8	1·96 ± 0·15	1·99 ± 0·06	0·34 ± 0·03	0·289 ± 0·007
10	5	8	2·50 ± 0·04	2·90 ± 0·14	0·73 ± 0·03	0·313 ± 0·006
	25	8	2·31 ± 0·15	3·23 ± 0·10	0·69 ± 0·05	0·299 ± 0·004
15	5	16	2·70 ± 0·05	4·31 ± 0·09	1·21 ± 0·03	0·317 ± 0·003
	25	16	2·54 ± 0·09	4·84 ± 0·11*	1·23 ± 0·04	0·312 ± 0·003
20	5	8	2·88 ± 0·07	5·85 ± 0·26	1·69 ± 0·07	0·310 ± 0·007
	25	8	2·71 ± 0·10	6·35 ± 0·20*	1·77 ± 0·05	0·315 ± 0·004
25	5	8	2·84 ± 0·08	7·55 ± 0·17	2·36 ± 0·08	0·324 ± 0·006
	25	8	2·69 ± 0·10	8·51 ± 0·24*	2·46 ± 0·09	0·322 ± 0·006

The P/Po ratios at which the maximum mechanical powers were observed are also given. Results are given as the mean ± S.E.M. (*N*, number of muscles).
*Significant difference between the two acclimation temperatures; $P < 0·01$.

Table 8. *Split-plot ANOVA table of the interactive effects of acclimation temperature (Acc. Temp.) and test temperatures (Test Temp.) on the variables measured from the force-velocity curve of frog and toad sartorius muscles*

Treatments	df	Tetanic tension	V_{max}	Maximum mechanical power
A *Rana pipiens*				
Acc. Temp.	1,12	2·7	0·0	2·4
Test Temp.	1,12	2·2	266·3*	206·3*
Acc.-Test Temp.	1,12	1·0	0·3	0·4
B *Bufo americanus*				
Acc. Temp.	1,30	1·2	15·2*	0·3
Test Temp.	4,56	38·8*	863·9*	632·1*
Acc.-Test Temp.	4,56	0·3	0·3	2·2

Numbers are F values.
*Significant at 1 % level.

example is shown from the results obtained with toad sartorius muscles at the test temperatures of 5, 15 and 25 °C in Fig. 7.

The results for tetanic tension, V_{max} and maximum mechanical power are given in Table 7 and the results of the ANOVA on these variables are given in Table 8. Acclimation temperature had no significant effect on tetanic tension and maximum mechanical power of both frog and toad sartorius muscles. The V_{max} of sartorius muscles of toads acclimated to 25 °C was always faster than of those acclimated to 5 °C. The difference between the two acclimation groups increased with test temperature and was almost 1 m.l. s^{-1} at 25 °C (Table 7B). When tested at 25 °C, the V_{max} of sartorius muscles of frogs acclimated to 25 °C was also 1 m.l. s^{-1} faster than those acclimated to 5 °C, but the difference was not significant (Table 7A).

DISCUSSION

Muscle characteristics and species activities

Under each experimental condition used in this study, frog sartorius muscles developed less tetanic tension, but shortened faster and developed greater maximal mechanical power than toad sartorius muscles. In an earlier study, we concluded that these differences in contractile characteristics of the sartorius muscle between these two species correlated well with their habits, jumping ability, burrowing activities and endurance in the field (Renaud & Stevens, 1983). *Rana pipiens* is a species which relies on its jumping ability to escape predators, and faster shortening velocities and greater maximum mechanical power are two important muscle characteristics for maintaining a great jumping ability. *Bufo americanus*, on the other hand, does not rely on its jumping ability to escape predators, and it seems that the species has sacrificed rapidity for strength, and high power output at heavy loads for burrowing activity and greater endurance to muscle fatigue.

Force production and speed of contraction are largely influenced by temperature, suggesting that the environmental temperature has a strong effect on the activities of the two species in the field. The purpose of this study was to estimate the extent of compensation to temperature in sartorius muscle. We will first discuss the short-term compensatory capacity for daily temperature changes in sartorius muscles and then discuss long-term compensation as a result of a period of acclimation. Emphasis will be given to the muscle characteristics that are important for each of the two species as described above. We show that the pH-temperature interaction is important for short-term compensation only for tetanic tension, while the effects of acclimation temperature are too small to be considered biologically important for any thermal compensation.

The pH-temperature interaction as a mechanism for short-term compensation

The effect of temperature on muscular contraction is often studied in conditions in which the pH of the physiological solution bathing the muscle is maintained at 7·0–7·1 (see for example, Edman, 1979; Bressler, 1981). However, for many amphibian species, blood pH is higher than 7·0 and blood and tissue pH increase as temperature decreases (Reeves, 1969, 1972). Moreover, there is an interaction between the pH

effects and test temperature effects and we showed that in some cases this interaction was such that pHe influenced the temperature effects (Table 4). It is therefore important to consider both the pH change and the interaction when estimating change in muscular contractility with temperature.

To illustrate this, we first use the tetanic tension of toad sartorius muscle. If we calculate the effect of temperature at constant pHe 7·0 (as in previous studies) then between 25 and 15 °C tetanic tension decreases by 13 %, and between 25 and 5 °C it decreases by 41 %. At constant pHe 8·0, a pH closer to blood pH, the percentages are 7 and 29 % (Fig. 4B). To calculate the changes that might occur *in vivo*, we must first fit the data to a binomial equation (by regression analysis) and then extrapolate tension to the blood pH that is normal for each temperature (taken from Reeves, 1969). The effect of temperature on tetanic tension *in vivo* is now expected to be as follows: from 25 to 15 °C and a concomitant change in blood pH from 7·6 to 7·85, tetanic tension decreases from about 3·07 to 2·94 kg cm^{-2} (−4·2 %) and from 25 to 5 °C (pH 7·6 to 8·1) it drops from 3·07 to 2·31 kg cm^{-2} (−25 %); the Q_{10} values are 1·27 from 5 to 15 °C and 1·04 from 15 to 25 °C. If the same calculations are made for frog sartorius muscles, the corresponding Q_{10} values *in vivo* are 1·22 and 1·05. Most of these Q_{10} values are lower than those calculated when pH is constant (Table 4).

Thus, the change in blood pH with body temperature in combination with the pH-temperature interaction effectively reduces the magnitude of the temperature effect on the tetanic tension of frog and toad sartorius muscles providing a mechanism for short-term compensation. This short-term compensation for tetanic tension is of considerable importance for toads which depend on their strength to maintain their digging ability maximum at different environmental temperatures. High mechanical power at loads greater than the P/Po ratio of the maximum mechanical power is also important for toads (Renaud & Stevens, 1983). Although we showed results only for maximum mechanical power, from the force-velocity curves in Fig. 2 we can calculate that from 25 to 15 °C at constant pHe 7·0, the mechanical power at 1·5, 2·0 and 2·5 kg cm^{-2} drops by about 60, 64 and 98 %, respectively. If blood pH is considered the percentages are 49, 54 and 64 %, i.e. the pH-temperature interaction also provides short-term compensation for the mechanical power at heavy loads in toad sartorius muscle.

Fast shortening velocities and large mechanical power at small loads are two important muscle characteristics during a jump (Calow & Alexander, 1973; Renaud & Stevens, 1983). There was evidence that the pH optimum for both V_{max} and maximum mechanical power of frog sartorius muscles is lower at 25 than at 5 and 15 °C (Fig. 5, Table 5). This is in agreement with the study of Kim *et al.* (1977) who reported that the pH optimum for the activity of calcium-activated actomyosin ATPase of frog skeletal muscle decreases as temperature increases. However, it is evident from Fig. 6 and Table 5 that the pH effects on V_{max} and maximum mechanical power between 9·0 and 7·0 are too small compared to the temperature effects to suggest any short-term compensation for shortening velocities (and mechanical power) at small loads as described for tetanic tension. This means that the speed of both frogs and toads in the field during the active season varies tremendously since their body temperatures vary between 11 and 35 °C (Brattstrom 1963).

The acclimation temperature as a mechanism for long-term compensation

The lack of acclimation temperature effect on tetanic tension is in agreement with earlier reports that acclimation temperature has no major effect on the isometric myogram of both frog and toad sartorius muscles (Renaud & Stevens, 1981*a*,*b*). We suggested from studies on the ATPase activity of myosin that there might be some effects of acclimation temperature on shortening velocities (see Introduction).

Inverse compensation was observed for V_{max} in this study: at 25 °C the V_{max} of sartorius muscles of both frogs and toads acclimated to 25 °C was about 1 m.l. s^{-1} faster than of those acclimated to 5 °C (Table 5). However, V_{max} is an intrinsic property of a muscle that has little biological importance because *in vivo* a muscle is shortening against a load. To be of any biological importance the difference in V_{max} must also be observed at slower shortening velocities. It is obvious from Fig. 7 and Table 7 that the effects of acclimation temperature occur only at high velocities. Rome (1983) also reported a lack of acclimation temperature effect on the force-velocity curve of frog sartorius muscles at pH 7·1.

The lack of acclimation effect on the sartorius muscle cannot be attributed to the absence of thermal acclimation in amphibians. Even though the magnitude of acclimation effects in amphibians are generally smaller than in fish, complete, partial or inverse compensation has been reported for metabolic rate and for critical maximum temperature of whole animals and tissues (including muscles) in many anuran species (Reick *et al.* 1960; Brattstrom & Lawrence, 1962; Bishop & Gordon, 1967; Dunlap, 1971; Harri, 1973; Hutchison & Maness, 1979).

Although Lagerspetz (1977) showed that compensatory temperature acclimation effects on the metabolic rate and nervous system are observed mainly with winter frogs and not with summer frogs, thermal acclimation has been reported for the excitability of the sartorius muscle of 'spring' frogs and for the tetanic tension of the sartorius muscle of 'summer' frogs (Hajdu, 1951; Ushakov & Zander, 1961). In our laboratory, acclimation experiments were made during winter, spring or summer. We did not observe any large effects of acclimation for isometric and isotonic contraction of muscles (Renaud & Stevens, 1981*a*,*b* and this study).

Therefore, this study provides further evidence for our suggestion (Renaud & Stevens, 1981*b*) that the lack of acclimation temperature effect is due to strategies employed by *R. pipiens* and *B. americanus*: they hibernate. During hibernation the need for rapid movement is almost nil, and so is the need for compensation.

In conclusion, the results obtained in this study from frog and toad sartorius muscle provide evidence that the pH-temperature interaction was large enough to provide short-term compensation for maximum force. This is an important compensation for toads which often bury themselves in the ground. The rapidity of movements, on the other hand, appears to be affected by changes in environmental temperature because neither the pH-temperature interaction nor thermal acclimation are strong enough to counteract the effects of temperature on shortening velocities. Frogs must then keep themselves warm behaviourally to maintain a great jumping ability to escape predators. Brattstrom (1979) had suggested that many frog species are in fact fairly active thermoregulators, interplaying between water economy and thermoregulation

to allow them to be more active. This would be less important for toads because their defences against predators are static (Dickerson, 1906).

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REFERENCES

BARANY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. In New York Heart Association, *The Contractile Process*, pp. 197–218. Boston: Little, Brown and Company.

BISHOP, L. G. & GORDON, M. S. (1967). Thermal adaptation of metabolism in Anuran amphibian. In *Molecular Mechanisms of Temperature Adaptations*, (ed. C. L. Prosser). Washington, D.C.: *Am. Assocn Adv. Sci.* **84**, 263–280.

BRATTSTROM, B. H. (1963). Preliminary review of the thermal requirements of amphibians. *Ecology* **44**, 238–255.

BRATTSTROM, B. H. (1979). Amphibian temperature regulation studies in the field laboratory. *Am. Zool.* **19**, 345–356.

BRATTSTROM, B. H. & LAWRENCE, P. (1962). The rate of thermal acclimation in anuran amphibians. *Physiol. Zool.* **35**, 148–156.

BRESSLER, B. H. (1981). Isometric contractile properties and instantaneous stiffness of amphibian skeletal muscle in the temperature range from 0 to 20 °C. *Can. J. Physiol. Pharmac.* **59**, 548–554.

CALOW, L. J. & ALEXANDER, R. MCN. (1973). A mechanical analysis of a hind leg of a frog (*Rana temporaria*). *J. Zool., Lond.* **171**, 243–321.

DICKERSON, M. C. (1906). *The Frog Book*. New York: Dover Publications Inc.

DUNLAP, D. G. (1971). Acutely measured metabolic rate-temperature curves in the cricket frog, *Acris crepitans*. *Comp. Biochem. Physiol.* **38**A, 1–16.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and the relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol., Lond.* **291**, 143–159.

EMERY, A. R., BERAT, A. H. & KODEIRA, K. (1972). Under ice observations of wintering sites of leopard frogs. *Copeia* **1972**, 123–126.

HAJDU, S. (1951). Observations on the temperature dependence of the tension developed by the frog muscle. *Archs int. Physiol.* **59**, 58–61.

HARRI, M. N. E. (1973). The rate of metabolic temperature acclimation in the frog, *Rana temporaria*. *Physiol. Zool.* **46**, 148–156.

HAZEL, J. R., GARLICK, W. S. & SELLNER, P. A. (1978). The effects of assay temperature upon the pH optima of enzymes from poikilotherms: a test of the imidazole alphastat hypothesis. *J. comp. Physiol.* **123**, 97–104.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B.* **126**, 136–195.

HILL, A. V. (1951). The influence of temperature on the tension developed in an isometric twitch. *Proc. R. Soc. B.* **138**, 349–354.

HUTCHISON, V. H. & MANESS, J. D. (1979). The role of behavior in temperature acclimation and tolerance in ectotherms. *Am. Zool.* **19**, 367–384.

JEWELL, B. R. & WILKIE, D. R. (1958). An analysis of the mechanical components in frog's striated muscle. *J. Physiol., Lond.* **143**, 515–540.

KIM, H. J., HWANG, A. R., PARK, Y. S. & KANG, D. H. (1977). pH-temperature dependence of the Ca^{++}-ATPase activity in actomyosin systems of rabbit and frog skeletal muscle. *Korean J. Physiol.* **11**, 59–65.

LAGERSPETZ, K. Y. H. (1977). Interactions of season and temperature acclimation in the control of metabolism in *Amphibia*. *J. ther. Biol.* **2**, 223–231.

PENNEY, P. K. & GOLDSPINK, G. (1981). Short temperature acclimation in myofibrillar ATPase of a stenotherm *Salmo gairdneri* Richardson and a eurytherm *Carassius auratus*. *J. Fish Biol.* **18**, 715–721.

REEVES, R. B. (1969). Role of body temperature in determining the acid-base status in vertebrates. *Fedn Proc. Fedn Am. Socs exp. Biol.* **28**, 1204–1208.

REEVES, R. B. (1972). An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. *Respir. Physiol.* **14**, 219–236.

REEVES, R. B. (1976). Temperature-induced changes in blood acid-base status: pH and P_{CO_2} in a binary buffer. *J. appl. Physiol.* **40**, 752–761.

RENAUD, J. M. & STEVENS, E. D. (1981*a*). The interactive effects of temperature and pH on the isometric contraction of the toad sartorius muscle. *J. comp. Physiol.* **145**, 67–71.

RENAUD, J. M. & STEVENS, E. D. (1981*b*). Effect of acclimation temperature and pH on contraction of frog sartorius muscle. *Am. J. Physiol.* **240**, R301–R309.

RENAUD, J. M. & STEVENS, E. D. (1983). A comparison between the contractile performance of frog and toad sartorius muscle. *J. comp. Physiol.* **151**, 127–131.

RIECK, A. F., BELLI, J. A. & BLASKOVICS, M. E. (1960). Oxygen consumption of whole animal and tissues in temperature acclimated amphibians. *Soc. exp. Biol. Med.* **103**, 436–439.

ROME, L. C. (1983). The effects of long term exposure to different temperatures on the mechanical performance of frog muscles. *Physiol. Zool.* **56**, 33–44.

SOMERO, G. N. (1981). pH-temperature interaction on proteins: principles of optimal pH and buffer system design. *Mar. biol. Lett.* **2**, 163–178.

STEEL, R. G. D. & TORRIE, J. H. (1980). *Principles and Procedures of Statistics. A Biometrical Approach.* Montreal: McGraw-Hill Book Company.

USHAKOV, B. P. & ZANDER, N. V. (1961). Adaptations of muscle fibers of lake frogs in warm habitat to the temperature factor. *Biofizika* (English Transl.: Biophysics New York) **6**(3), 52–56.