THE EFFECTS OF TEMPERATURE AND pH ON THE CONTRACTILE PROPERTIES OF SKINNED MUSCLE FIBRES FROM THE TERRAPIN, PSEUDEMYS SCRIPTA ELEGANS

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SUMMARY

1. Fibre types in the iliofibularis muscle of the freshwater terrapin Pseudemys scripta elegans have been characterized on the basis of their histochemical characteristics, nerve endings and contractile properties. Three types of focally innervated fibres are present, corresponding to the fast glycolytic (Fg), fast oxidative glycolytic (FOG) and slow oxidative (SO) fibre types of other vertebrates.

2. Single fibres or small bundles of fibres representing each histochemical type were identified on the basis of their light scattering properties under dark-field illumination. Fibres were detergent-skinned using Brij 58, and their maximum isometric tensions (P₀) and unloaded contraction velocities (V₀) were determined by the slack test method. At 15°C, fast glycolytic fibres generated maximum isometric tensions of 184 ± 5 kN m⁻² and V₀ values of 5.5 ± 0.3 muscle lengths per second (L₀ s⁻¹). Slow oxidative fibres produced tensions of 70.6 ± 3 kN m⁻² and had V₀ values of 1.3 L₀ s⁻¹. Tensions and V₀ values of fast oxidative glycolytic fibres were between those of Fg and SO fibres.

3. The force-velocity (P–V) characteristics of slow oxidative fibres were studied at 5° and 15°C. Points below 0.6 P₀ on the curves could be fitted by a linear form of Hill's equation. Maximum contraction velocities (Vₘₐₓ) extrapolated from the P–V relationship were 0.62 L₀ s⁻¹ at 5°C and 0.91 L₀ s⁻¹ at 15°C. The curvature of the P–V relationship was relatively independent of temperature over the range 5 to 15°C. Values for Hill's constant a/P₀ were 0.29 and 0.33 at 5°C and 15°C, respectively.

4. The temperature dependence of P₀ and contraction velocity at near zero load (Vᵢ) were studied at constant pH, and under conditions designed to simulate the changes in intracellular pH which occur with temperature in vivo ($ΔpH/ΔT = -0.0186$). Changes in pH in the range 6.6 to 7.8 had no effect on either tension or Vᵢ at temperatures between 0° and 20°C. However, below and above this pH range, both tension and Vᵢ were depressed.

5. It is concluded that pH changes within the normal physiological range (6.7–7.8) have no effect on the temperature dependence of P₀ and Vᵢ.

Key words: Pseudemys scripta elegans skeletal muscle, temperature, pH, muscle fibre types, skinned muscle fibres.
INTRODUCTION

The freshwater terrapin *Pseudemys scripta elegans* remains active between 12° and 40°C, but has a preferred body temperature (PBT) of 30°C (Gatten, 1975). At this temperature, plasma and skeletal muscle pH are about 7.5 and 6.8, respectively (Malan, Wilson & Reeves, 1976). As body temperature rises, there is an increase in respiratory minute volume, oxygen consumption and carbon dioxide excretion, resulting in a decrease in plasma pH, equivalent to about 0.020 pH units/°C (Jackson, 1971). Similar changes occur in other tissues; for example, muscle pH increases from around 6.8 at 35°C to 7.5 at 5°C (Malan *et al.* 1976). It has been suggested that $\Delta$pH/$\Delta$T changes of this magnitude serve to maintain either a constant relative alkalinity ([OH$^-$]/[H$^+$]) or a constant charge state of the histidine groups of proteins (alphastat hypothesis, Reeves, 1972).

There is a large literature on the effects of temperature on the contractile properties of muscle in lower vertebrates (Hill, 1938; Renaud & Stevens, 1984; Johnston & Brill, 1984; Johnston & Gleeson, 1984). There is evidence that different species and classes have become specialized to match contractile function with preferred body temperature (Putnam & Bennett, 1982; Marsh & Bennett, 1985; Johnston & Altringham, 1985). However, very few of these studies have considered the interaction of pH and temperature. In experiments on whole muscles, intracellular pH will depend upon the rate of temperature change and the pH of the external solution. In experiments specifically designed to investigate the effects of pH on the contractile properties of muscle, the method of changing the external pH also has a bearing on the results obtained (Hill, 1956; Caldwell, 1954; Izutsu, 1972). This can lead to apparently conflicting results; for example, acidosis has variously been reported to increase (Waller & Sowton, 1896; Pannier, Weyne & Leusen, 1970) or decrease (Creese, 1949, 1953; Hill, 1956; Renaud & Stevens, 1984) both twitch and tetanic tension of amphibian muscles.

The effects of temperature and pH on force production and contraction velocity can be studied independently by using demembranated fibre preparations (Schadler, 1967; Fabiato & Fabiato, 1978). The aim of the present study was to compare the temperature dependence of contractile properties of single fibres isolated from the iliofibularis muscle at constant pH, and under conditions where pH was allowed to vary with temperature (as occurs *in vivo*). Since the magnitude of the changes in intracellular pH vary somewhat between tissues (Heisler, Weitz & Weitz, 1976), it was thought of interest to compare results from the different muscle fibre types. Although this species has frequently been used in experiments on acid–base regulation and anoxic tolerance, there have been no previous study on the fibre type composition of its locomotory muscles.

MATERIALS AND METHODS

*Animals*

Terrapins, *Pseudemys scripta elegans* (Cagle, 1946), were obtained from local suppliers and maintained in the laboratory in glass aquaria containing fresh water at
Terrapin muscle

25°C. They were provided with a photothermal gradient on a photoperiodic regime of 12 h light: 12 h dark. A dry concrete block placed 30.5 cm below a 100-W bulb provided an area for basking on. Animals were fed daily on a diet of fresh raw beef or liver. Both sexes were used in the study, with no noticeable differences in the results obtained. The terrapins used for histochemical studies had a body mass of 10.0 ± 0.2 g (N = 12), while those used for mechanical experiments weighed 305 ± 44 g (N = 20) (mean ± s.e.). No change in fibre type composition and distribution was noticed with size and age.

Histochemical methods

Animals were stunned with a sharp blow to the head, decapitated and pithed, and the iliofibularis muscle isolated. Small blocks of whole iliofibularis were mounted perpendicularly in embedding medium (Cryo M-Bed, Bright Ltd, Cambridge) on cryostat chucks and rapidly frozen in isopentane (2-methylbutane) cooled with liquid nitrogen to near its freezing point of −160°C. 10-μm serial sections were cut at −20°C, air-dried at room temperature for at least 1 h, and stained for the activities of succinic dehydrogenase (SDH) (Nachlas et al. 1957), α-glycerophosphate dehydrogenase (α-GPDH) (Wattenberg & Leong, 1960) and myosin ATPase (mATPase), after acid (acid mATPase) or alkaline (alkaline mATPase) pre-incubation (Guth & Samaha, 1969, 1970). Fibres of the same type were traced on quality cartridge paper, using the drawing arm of a microscope (Labophoto, Nikon, Japan) and their relative proportions and cross-sectional areas determined by digital planimetry.

Nerve endings

Fibre types in the iliofibularis were shown to be localized in three discrete regions. Small fibre bundles (10–30 fibres) of each type were isolated under dark-field illumination, pinned to cork strips and stained for regions of high acetylcholinesterase activity (Naik, 1963).

Determination of contractile properties

Solutions

Relaxing solution had the following composition (in mmol l⁻¹): imidazole-HCl, 20; creatine phosphate, 10; EGTA, 10; KCl, 90; MgCl₂, 6.8; ATP, 6; and 20 units ml⁻¹ of creatine phosphokinase. Activating solutions were made by the addition of CaCl₂ to relaxing solution. Skinning solution contained 1% polyoxyethylene-20-cetyl ether (Brij 58) in relaxing solution. One set of solutions had its final pH set to 7.2 at 20°C and pH was allowed to vary with temperature according to the pH/temperature relationship of imidazole of −0.018 pH units/°C. This is similar to the ΔpH/ΔT value of −0.0186 pH units/°C obtained for Pseudemys scripta elegans skeletal muscle in vivo (Malan et al. 1976). The other set of solutions had its pH adjusted at each temperature (electrode calibrated with Radiometer phosphate standard at each temperature). Free ion concentrations of the solutions were calculated from apparent dissociation constants using an iterative computer program incorporating corrections for pH and temperature (Nicol, 1985). The solution
composition was adjusted to give a pCa of 4.56, pMg of 3.00, pMgATP of 2.27 and an ionic strength of 180 mmol\textsuperscript{-1}.

**Isolation of fibres**

Small bundles of 20–50 fibres were dissected from the intermediate and inner regions of the iliofibularis muscle in ice-cold terrapin Ringer which had the following composition (in mmol\textsuperscript{-1}): NaCl, 75; NaHCO\textsubscript{3}, 40; KCl, 4.1; CaCl\textsubscript{2}, 2.8; MgCl\textsubscript{2}, 2.2; Na\textsubscript{2}HPO\textsubscript{4}, 1.1; pH 7.4 at 10°C. Single or small bundles of FOG and SO fibres were dissected on a cooled stage in a drop of relaxing solution under silicone oil (BDH MS 550). Individual fibre types were identified by their light-scattering under dark-field illumination.

Fibre segments, 2000–2400 \textmu m in length, were transferred to the apparatus using fine jewellers' forceps. Their ends were wrapped around two stainless steel hooks and secured using Plexiglas acetone glue (Altringham  &  Johnston, 1982).

**Measurement of contractile properties**

**Experimental protocol**

Fibres were skinned for 15 min. Sarcomere length was measured by laser diffraction and set to 2.3 \textmu m. Length and diameter were measured in situ using a high-powered microscope. Fibres were transferred to relaxing solution for 5 min prior to being maximally activated at each pH and temperature. Force was measured using a strain gauge (AE801, AME Horton, Norway), average sensitivity 0.38 V mN\textsuperscript{-1} and a compliance of <4 \mu m mN\textsuperscript{-1}. Experiments were carried out at 0–20°C and pH 6.0–8.0. Each fibre acted as its own control and was sequentially activated at pH 7.2, the test pH, and pH 7.2 again (Fig. 1). In some cases maximum isometric tension decreased with successive activations and only those fibres showing less than 5% decline in force between the first and last activations were used. An interpolation technique was used to correct for the decline in tension. Fibres were only activated at one temperature. Attempts to activate them at a second temperature and temperatures above 20°C were not successful due to fibre breakage. Since fast glycolytic fibres could not be activated more than once without a >5% decline in tension, they were not used in pH studies.

![Fig. 1. An isometric force record from a fast oxidative glycolytic fibre (0°C) illustrating the experimental protocol used in the pH studies.](image-url)
**Slack test determination of unloaded contraction velocity**

Unloaded contraction velocity ($V_0$) was determined by the slack test method using a servo-controlled length transducer (Johnston & Sidell, 1984). Fibres were maximally activated, and on attainment of steady tension a series of releases (<2·0 ms) was given so as to abolish tension, and the time to take up slack was measured. Following each release the fibre was re-extended to its original length. Records were analysed by drawing a line parallel to and above the zero force line by an amount equivalent to the noise level of the transducer. The time to take up slack ($\Delta t$) was measured from the point at which the force record crossed this line. At this point, force was rapidly increasing, allowing a more accurate measurement of $\Delta t$. A typical record illustrating the experimental protocol is shown in Fig. 2. Unloaded contraction velocity was determined from the slope of a plot of the applied length change ($\Delta L$) versus $\Delta t$.

**Isotonic lever releases**

The contraction velocity of lightly loaded (<0·02P/$P_0$) fibres was determined using an isotonic lever (Altringham & Johnston, 1982). Initial mean velocity ($V_i$) was calculated by extrapolating the rate of shortening over the first 50 ms (Fig. 3). Contraction velocity decreased with shortening, causing the traces to be curved. The degree of curvature was greater at higher loads and in slow fibres.

**Statistical analysis**

All the results are reported as means ± s.e. The histochemical results were analysed using a basic quantification and statistical computer program obtained

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**Fig. 2.** (A) A typical record illustrating the determination of unloaded contraction velocity by the slack test method. A series of length steps (L) starting at 200 µm and increasing by 50 µm during each step is shown. Each step is of sufficient magnitude to abolish force; P, redevelopment of force. (B) A plot of the applied length step versus the time taken for the beginning of force redevelopment. Unloaded contraction velocity is determined from the slope.
RESULTS

Histochemistry

Macroscopically, the iliofibularis muscle can be differentiated into a pale outer and a red inner region. On staining fibres for acid and alkaline mATPase, SDH and \( \alpha \)-GPDH activities, three distinct fibre types could be differentiated (Fig. 4).

One type stains intensely for alkaline mATPase and lightly for acid mATPase, SDH and \( \alpha \)-GPDH activities (Fig. 4; Table 1). This type corresponds to fast glycolytic (Fg) fibres and has the largest average diameter of 25.7 ± 0.2 \( \mu \)m. Due to their low \( \alpha \)-GPDH activity, we have used the term Fg to differentiate them from FG fibres, which have a high \( \alpha \)-GPDH activity. They constitute the majority of the fibres in the pale outer region (Figs 4, 5). The second type corresponds to fast oxidative glycolytic (FOG) fibres and stains lightly for acid mATPase and intensely

from Hewlett-Packard. A Student's \( t \)-test was used to compare the results at various test pH values with those at pH 7.2.
for alkaline mATPase, SDH and α-GPDH. The fibres are intermediate in both diameter and location, forming the majority of the fibres in the intermediate region of the muscle (Figs 4, 6). The third type, which stains intensely for the activities of acid mATPase and SDH and lightly for alkaline mATPase and α-GPDH activities, is similar to mammalian slow oxidative (SO) fibres (Fig. 5). They have the smallest mean diameter of $14.6 \pm 0.4 \mu m$ and constitute 80% of the fibres in the red region of the muscle (Fig. 6). Their proportions as a percentage of the whole cross-section of

Fig. 4. Serial cross-sections of whole iliofibularis muscle stained for the activity of alkaline myosin ATPase (A) and succinic dehydrogenase (SDH) (B). Fg, fast glycolytic; FOG, fast oxidative glycolytic; SO, slow oxidative fibres. Note the tendency towards regional localization of the different muscle fibre types. (C–E) Teased muscle fibres stained for acetylcholinesterase activity to localize nerve terminals. Note the similarity in endplates for all three fibre types. Scale bars: A,B, 150 μm; C,D, 25 μm; E, 15 μm.
Table 1. Location, histochemical characteristics and contractile properties of the three fibre types in the iliofibularis muscle of the terrapin Pseudemys scripta elegans

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fast glycolytic</th>
<th>Fast oxidative glycolytic</th>
<th>Slow oxidative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>peripheral</td>
<td>intermediate</td>
<td>central</td>
</tr>
<tr>
<td>Macroscopic appearance</td>
<td>pale</td>
<td>intermediate</td>
<td>red</td>
</tr>
<tr>
<td>Dark-field illumination</td>
<td>clear</td>
<td>white</td>
<td>clear</td>
</tr>
<tr>
<td>Fibre diameter ($\mu$m$^2$)</td>
<td>25.7 ± 0.2</td>
<td>19.0 ± 0.2</td>
<td>14.6 ± 0.4</td>
</tr>
<tr>
<td>Staining characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline myosin ATPase</td>
<td>+++</td>
<td>+++</td>
<td>(+)</td>
</tr>
<tr>
<td>Acid myosin ATPase</td>
<td>(+)</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>+</td>
<td>++</td>
<td>+ +</td>
</tr>
<tr>
<td>$\alpha$-Glycerophosphate</td>
<td>+</td>
<td>+++</td>
<td>+ +</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Contractile properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum isometric tension (kN m$^{-2}$)</td>
<td>183.0 ± 5</td>
<td>120.4 ± 3</td>
<td>70.6 ± 3</td>
</tr>
<tr>
<td>Maximum contraction velocity (L$_0$ s$^{-1}$)</td>
<td>5.5 ± 0.3 (17)</td>
<td>3.0 ± 0.1 (16)</td>
<td>1.3 ± 0.1 (19)</td>
</tr>
</tbody>
</table>

Contractile properties were studied at 15°C. All values represent mean ± S.E. Mean number of fibres (N) used in mechanical studies is given in brackets.

+++= intense, ++= moderate, + = light, (+) = no staining to light staining. $L_0$, muscle length.

the iliofibularis were 54% for Fg fibres, 31% for FOG fibres and 15% for SO fibres (Fig. 7).

Under dark-field illumination, the majority of the Fg and SO fibres scatter little or no light, making them appear transparent. In contrast, most FOG fibres scatter significant amounts of the incident light, giving them a milky appearance. The difference in their appearance under dark-field illumination, together with their location in the muscle, was used to identify individual fibre types for mechanical studies.

*Nerve endings*

All three fibre types in the iliofibularis muscle have one or two endplates which consist of discrete, elongated finger-like processes (Fig. 4). In Fg fibres they occurred at any point along the fibre length and most of the fibres had two endplates. However, in FOG and SO fibres endplates were located close to the fibre tendons and were usually single.

*Contractile properties*

The contractile properties of the three fibre types are summarized in Table 1. At 15°C, the maximum isometric force generated by fast glycolytic fibres was
183 ± 5 kN m"2. This is 2-6 times higher than that generated by the slow oxidative fibres. They also contract four times faster with an unloaded contraction velocity (V₀) of 5·5 ± 0·3 muscle lengths s⁻¹ (L₀ s⁻¹). Fast oxidative glycolytic fibres have isometric tensions and unloaded contraction velocities intermediate between those of Fg and SO fibres (Table 1).

**Fig. 5.** Serial sections of fibres from the outer pale region of the iliofibularis muscle stained for the activities of myosin ATPase at pH 9-4 (A), SDHase (B), myosin ATPase at pH 4-8 (C); and the intermediate region stained for the activities of myosin ATPase at pH 4-8 (D), SDHase (E) and α-GPDH (F). SO, slow oxidative fibres; FOG, fast oxidative glycolytic fibres; Fg, fast glycolytic fibres. Scale bar, 50 μm.
The force-velocity (P–V) relationship of slow oxidative and fast oxidative glycolytic fibres

The force–velocity characteristics of slow fibres were determined at 5°C and 15°C, while those for fast oxidative glycolytic fibres were studied at 5°C. Points below 0·6 P₀ on the P–V curve could be fitted to a linear form of Hill’s (1938) equation:

\[(P + a)V = b(P₀ - P),\]

where P is load, P₀ is maximum isometric tension, V is velocity, and a and b are constants. However, points above 0·6 P₀ deviated in a characteristic manner; at low temperatures they deviated upwards and at higher temperatures downwards. In SO fibres, a/P₀ values of 0·29 and 0·33 were obtained at 5°C and 15°C, respectively, suggesting that the curvature of the P–V relationship is relatively independent of temperature over the range 5°C to 15°C (Fig. 8). In FOG fibres an a/P₀ value of 0·41 was obtained at 5°C (Fig. 9). Vₘₐₓ for SO fibres was 0·62 L₀ s⁻¹ at 5°C and 0·91 L₀ s⁻¹ at 15°C, compared with 0·77 L₀ s⁻¹ for FOG fibres at 5°C (Figs 8 and 9, respectively).
Effects of temperature and pH on tension generation and contraction velocity in fast oxidative glycolytic and slow oxidative fibres

Experiments were conducted at constant pH, and under conditions mimicking the known change in pH with temperature for terrapin skeletal muscles in vivo. Tension
and $V_1$ were independent of pH in the range 6.6 to 7.8 at all temperatures. However, above and below this range, both parameters are depressed. This decline in tension and $V_1$ is not uniform in the two fibre types (Figs 10, 11).

The thermal dependence of tension and velocity can be expressed by the terms $R_{10}$ and $Q_{10}$, respectively (Bennett, 1984), and are obtained from the formulae:

$$R_{10} \text{ or } Q_{10} = (R_2/R_1)^{10/(T_2-T_1)} ,$$

where $R_1$ and $R_2$ are either velocities ($Q_{10}$) or tensions ($R_{10}$) at temperatures $T_1$ and $T_2$, respectively, $T_2$ being greater than $T_1$. The thermal dependence of both parameters in the two fibre types decreased as the preferred body temperature (25–30°C) was approached. For example, in SO fibres the $R_{10}(0–10°C)$ and $Q_{10}(0–10°C)$ ranged between 1.9 and 2.0, decreasing to 1.2–1.3 between 10° and 20°C.

**DISCUSSION**

**Fibre types**

Reptilian limb muscles are composed mainly of fast twitch fibres (Guthe, 1981). These can be further subdivided, on the basis of their staining characteristics for aerobic and glycolytic enzymes, into fast oxidative glycolytic (FOG) and fast glycolytic (FG/Fg) fibre types. In the iliofibularis muscle of both terrapin (Fig. 5) and the water monitor lizard (Gleeson, 1983), FOG fibres stain much more intense
Fig. 10. Effect of temperature and pH on maximum isometric tension of fast oxidative glycolytic (A) and slow oxidative (B) fibres. Values represent mean ± S.E.M. (6—10 fibres). * Significantly different from pH 7.2 at P < 0.05 level.

Fig. 11. Effects of temperature and pH on contraction velocity at zero load (V₀) of fast oxidative glycolytic (A) and slow oxidative (B) fibres. Values represent mean ± S.E.M. (6—10 fibres). * Significantly different from pH 7.2 at P < 0.05 level.
for marker enzymes of glycogenolysis than the more numerous Fg fibres. This contrasts with the situation in most mammalian muscles in which low-oxidative fast fibres stain intensely for glycolytic enzymes (Peter et al. 1972). Spurway (1980) has suggested that the terms FG and Fg be used to distinguish fast fibres with, respectively, high and moderate/low glycolytic capacities. An alternative source of energy for Fg fibres is phosphocreatine hydrolysis which is capable of providing very high levels of ATP turnover but only for short periods (Hochachka, 1985). Reptiles recruit their Fg fibres to run at high speed (Marum & Armstrong, 1978), achieving amongst the highest anaerobic power outputs of any vertebrates (Gleeson, Mitchell & Bennett, 1980a). Significantly, maximum speed declines over a few seconds, which is consistent with the time course for depletion of phosphocreatine stores and the fatigue of Fg motor units (John-Alder & Bennett, 1981). In addition, various slow fibres have been identified in reptilian muscles on the basis of innervation pattern (Hess, 1970), histochemical myosin ATPase staining activity (Gleeson, Nicol & Johnston, 1984) and contraction speed (Morgan & Proske, 1984). These fibres can be divided into those that are focally or multiply innervated. All the slow fibres in the iliofibularis muscle of *Pseudemys scripta* are focally innervated (Fig. 4).

The morphology of the nerve endings is similar to that found in type 1–4 fibres of *Xenopus* (Lannergren & Smith, 1966), tortoise extrafusal fibres (Crowe & Ragab, 1970), and costocutaneous muscle of the garter snake (Ridge, 1971). In contrast, only multiply innervated fibres are found in the iliofibularis muscle of the desert iguana, *Dipsosaurus dorsalis* (Gleeson & Johnston, 1986). Nothing is known about the electrophysiological properties of these fibres, although they do respond to acetylcholine with a prolonged contracture (Gleeson, Putnam & Bennett, 1980b). In the scalenus muscle of the blue tongue lizard, there appear to be two classes of multiply innervated slow fibres, one which does not conduct action potentials and another rarer type which is capable of both graded potentials and twitch contractions (Proske & Vaughan, 1968). In spite of these differences in nerve endings and electrophysiological properties, all slow fibres in reptiles stain moderately/intensely for aerobic enzymes (Gleeson et al. 1984). There is little direct information about how fibre recruitment patterns vary with particular types of locomotory behaviour in any reptile (Marum & Armstrong, 1978). It has been suggested that multiply innervated slow fibres in squamate lizards may have a postural function in stabilizing joints (Hess, 1970) or may provide the power for slow movements associated with display behaviour and walking at low speeds (Morgan & Proske, 1984; Johnston, 1985). Cole (1955) reported the presence of both focally and multiply innervated fibres in an unnamed terrapin muscle referred to as the paravertebrals in Guthe's (1981) review of reptilian muscle. This suggests that the presence of different types of slow fibres may be related to function rather than phylogeny. Terrapins spend most of their time in water, and when on land often bask resting on their plastron (Cagle, 1946). Thus the limb muscles have little need of fibres specialized for postural support and contain only twitch fibres, whereas the paravertebrals which function 'tonically' to hold the head upright during swimming contain multiply innervated slow fibres.
Effects of temperature and pH on contractile properties

In a study of the iliofibularis muscle of the desert iguana, *Dipsosaurus dorsalis*, Johnston & Gleeson (1986) found that unloaded contraction velocities at 25°C were $1.3-1.9 L_0 s^{-1}$ for multiply innervated slow fibres, $2.0-7.4 L_0 s^{-1}$ for FOG fibres and $5.8-8.7 L_0 s^{-1}$ for Fg fibres. While there was an overlap in the contraction speeds of fast fibres with different metabolic characteristics, slow fibres were found to constitute a distinct population. Once a correction is made for the different temperatures of study, a similar spectrum of contraction speeds is apparent for the various fibre types in the iliofibularis muscle (Table 1). This is surprising in view of the differences in slow fibre characteristics and locomotory behaviour of the two species.

There is no simple relationship between the thermal dependence of locomotion and that of contractile properties of isolated skeletal muscles (Johnston & Gleeson, 1984; Marsh & Bennett, 1985). In lizards, at temperatures above 15°C, maximum running speed and stride frequency are less temperature-dependent than either the maximum shortening velocity or power output of the iliofibularis muscle (Marsh & Bennett, 1985). It has been postulated that other relatively temperature-independent factors, such as the storage and recovery of elastic energy by muscles and tendons, serve to modulate the thermally dependent properties of muscle fibres (Bennett, 1985; Marsh & Bennett, 1985). Nevertheless, it is clear that the thermal dependence of many properties of isolated muscles, including maximum tension generation (Putnam & Bennett, 1982; Johnston & Altringham, 1985), twitch contraction times, and relaxation rates (Putman & Bennett, 1982), reflect the normal or preferred body temperatures (PBT) of the species. For example, at low temperatures skinned fibres from cold-tolerant ectotherms generate much higher tensions than homologous fibres from reptiles and mammals (Stephenson & Williams, 1984; Johnston & Altringham, 1985; Johnston & Gleeson, 1986). At temperatures approaching the PBT, maximum tetanic tension (live fibres) and maximum Ca$^{2+}$-activated force (skinned fibres) generally become relatively temperature-independent (Marsh & Bennett, 1985; Stephenson & Williams, 1984). For example, values of $Q_{10}(10-20°C)$ for $V_i$ and $P_0$, in chemically skinned muscle fibres, are significantly lower in *Pseudemys* which has a PBT of 30°C than for comparable fibre types in the iliofibularis muscle of the desert iguana (Johnston & Gleeson, 1984) which has a PBT of 40°C (Marsh & Bennett, 1985).

Temperature-dependent changes in the force—velocity curve offer another possibility for adjusting muscle characteristics to a particular thermal regime. For example, a reduction in the curvature of the P—V relationship would result in an increase in velocity and hence power output at low loads. A measure of the curvature of the P—V relationship is given by Hill’s constant $a/P_0$ (which is inversely related to the degree of curvature). The $a/P_0$ values observed for terrapin fast and slow fibres at 5°C and 15°C lie within the range reported for similar fibres from other vertebrates. For example, 0.2 and 0.4 for rat slow and fast muscles, respectively, at 30°C (Ranatunga, 1982) and 0.35 and 0.26 for types 1 and 3 fibres, respectively, from
Xenopus at 10°C (Lännergren, Lindblom & Johansson, 1982). The effects of temperature on the P–V relationship vary between slow and fast muscles (Ranatunga, 1982) and between species. In teleost skinned fibres $a/P_0$ values decrease with increasing temperature (Johnston & Altringham, 1985), whereas in whole rat muscles the opposite effect occurs (Ranatunga, 1982). In contrast, for intact fibres in Dipsosaurus (Marsh & Bennett, 1985) and Xenopus (Lännergren, 1978), and skinned fibres in Pseudemys (Fig. 8), the shape of the P–V curve is similar over a wide range of temperatures. Therefore no clear picture of the significance of these results emerges.

Muscle pH in resting Pseudemys at the PBT is approximately 6.8, rising to 7.3 at 10°C, which corresponds to the minimum temperature for active locomotion (Cagle, 1946). $P_0$ and $V_i/V_{\text{max}}$ for both SO and FOG fibres are relatively independent of pH change over this range. These observations are comparable to those reported for demembranated fibres from frog muscles (Schadler, 1967; Kentish & Nayler, 1977; Fabiato & Fabiato, 1978). The stability of these parameters over a wide pH range in ectotherms may be important as muscle pH varies significantly with body temperature (Malan et al. 1976; Heisler et al. 1976). Outside this pH range both tension and contraction velocity in the two fibre types are depressed (Figs 10, 11). It is known that the basic pattern of acid–base regulation can be modified by other factors such as exercise (Gatten, 1975) and diving (Penney, 1974). Pseudemys scripta elegans is remarkably tolerant of anoxia (Clark & Miller, 1973). It can survive a 24-h forced dive in deoxygenated water at 22°C (Penney, 1974) and up to 2 weeks submerged in oxygenated water at 16–18°C (Robin, Vester, Murdaugh & Millen, 1964). Under these conditions, energy supply is provided by anaerobic glycolysis resulting in a lactate acidosis (Penney, 1974). For example, at 22°C a 24-h submergence results in an increase in plasma lactate concentration from 1 to 37 mmol l$^{-1}$ (Penney, 1974) and 15 h in 100% nitrogen reduces the pH of cardiac muscle to 6.6 (Clark & Miller, 1973). The contractile performance of demembranated fibres in this species appears to be unaffected by the levels of acidosis likely to be encountered during prolonged dives.

Our results have implications for experiments designed to investigate the thermal dependence of contractile properties using skinned muscle fibres. It is clear that at saturating Ca$^{2+}$ concentrations the effects of temperature on $P_0$ and $V_i/V_{\text{max}}$ are similar under conditions of constant pH and under conditions where pH is allowed to vary with temperature within the physiological range. However, this is not likely to be the case at sub-saturating Ca$^{2+}$ concentrations, because an increase in temperature or a decrease in pH are both known to shift the pCa–force relationship to higher Ca$^{2+}$ concentrations (Fabiato & Fabiato, 1978; Godt & Lindley, 1982).

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