

# Thermal dependence of isotonic contractile properties of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis*

Richard L. Marsh\* and Albert F. Bennett

Department of Developmental and Cell Biology, University of California, Irvine, California 92717, USA

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**Summary.** 1. Contractile properties of the fast-twitch glycolytic (FG) portion of the iliofibularis muscle and sprint running performance were studied at approximately 5 °C intervals from 15–44 °C in the lizard *Dipsosaurus dorsalis*.

2. Maximal running velocity ( $V_R$ ) and stride frequency ( $f$ ) were both greatest when body temperature ( $T_b$ ) was 40 °C, the field-active  $T_b$  in *Dipsosaurus*. At 40 °C  $V_R$  was  $4.3 \pm 0.2$  m/s and  $f$  was  $13.5 \pm 0.5$  s<sup>-1</sup>.

3. Between 25 and 40 °C, the thermal dependencies of  $V_R$  and  $f$  were approximately constant ( $Q_{10}$ 's of 1.31 and 1.36 for  $V_R$  and  $f$ , respectively). Below 25 °C performance declined more markedly with decreasing temperature. At 20 °C strides were qualitatively normal, but  $V_R$  was only half of the value at 25 °C. At 15 °C the lizards were substantially incapacitated, and  $V_R$  was 10% of the value at 20 °C. Stride length was approximately 0.33 m and changed very little with  $T_b$  from 20–44 °C.

4. The time dependent contractile properties of FG muscle were affected more by temperature than was sprint performance. The maximal velocity of shortening at zero load ( $V_0$ ) was 18.7  $l_0/s$  at 40 °C and had a  $Q_{10}$  of 1.7 from 25–40 °C. Maximal power output ( $\dot{W}_{max}$ ) determined from the force-velocity curve was 464 W/kg at 40 °C. Below 40 °C  $\dot{W}_{max}$  varied with temperature with a  $Q_{10}$  of 2–3. The shape of the force-velocity curve changed little with temperature ( $\dot{W}_{max}/P_0 V_0 = 0.11$ ).

5. Between 25 and 40 °C a relatively temperature-independent process must modulate the

effects of temperature on the contractile properties of the muscles that supply the power for burst locomotion. Storage and recovery of elastic energy appears to be a likely candidate for such a process.

6. Below 25 °C, however, the contraction time is prolonged to such an extent that the  $f$  attainable is limited by the minimum time taken to contract and relax the muscles.

## Introduction

Temperature is one of the most important extrinsic factors that influence the locomotory performance of ectothermic animals (Bennett 1980a). Presumably one significant way temperature could affect locomotion is by influencing the speed of contraction of the locomotory muscles. However, relatively few studies have examined the effects of temperature on muscle performance over a range of temperatures relevant to the thermal biology of the animals concerned (Licht 1964; Licht et al. 1969; Bennett 1984; Putnam and Bennett 1982).

Recently the thermal dependence of burst running has been examined in several species of lizards (Bennett 1980b; Christian and Tracy 1981; Huey 1983). These authors have noted that running velocity during burst activity may have a rather low temperature dependence ( $Q_{10} < 1.3$ ) over a broad range of body temperatures. The selective value of this low thermal dependence is clear for ectothermic animals, which often rely on quick bursts of speed to capture prey or avoid capture by predators (Howland 1974; Huey 1983). However, the mechanistic basis for the low  $Q_{10}$  is unknown.

*Abbreviation:* FG fast-twitch, glycolytic (muscle); IF iliofibularis muscle

\* Present address: Department of Biology, Northeastern University, 360 Huntington Ave., Boston, Massachusetts 02115, USA

Information regarding the influence of temperature on the time-related properties of a variety of vertebrate striated muscles indicates that  $Q_{10}$ 's are approximately 2.0 or greater (e.g., Hill 1938; Close 1965; Lännergren 1978; Petrofsky and Lind 1981; Lännergren et al. 1982; Bennett 1984). Recent data on isometric contractile properties of muscles from several species of lizards (Putnam and Bennett 1982) also demonstrated a similar thermal dependence. Nevertheless, the *in vitro* isometric properties may not indicate accurately the function of the muscles *in vivo*. Small lizards may have locomotory muscles that undergo temperature dependent changes in their force-velocity curves allowing a lower temperature dependence of muscles undergoing active shortening, e.g., the curves might become flatter at low temperatures allowing greater power output at intermediate levels of force. Alternatively, the thermal dependence of burst locomotion may be modified by components of the locomotory system other than the contractile elements of the muscles, such as elastic elements in the muscles and tendons, and/or components of the nervous system.

In the present study we have investigated the apparent conflict between data on the thermal dependencies of locomotion on the one hand and of the muscles used in locomotion on the other. Our experimental animal, *Dipsosaurus dorsalis*, is a diurnally active animal that depends on quick bursts of activity to escape predators (Norris 1953; Belkin 1961). Complementary data for this species are available on the thermal ecology (Norris 1953; DeWitt 1967), locomotory performance (Belkin 1961; Bennett 1980b; John-Alder and Bennett 1981), isometric contractile properties of isolated skeletal muscles (Licht 1964; Putnam and Bennett 1982), and the fiber types in the hind-limb muscles (Gleeson et al. 1980; Putnam et al. 1980). The purpose of the present study was to determine the extent to which the thermal dependence of burst locomotion in *Dipsosaurus* is determined directly by the contractile properties of the skeletal muscles that power locomotion.

## Materials and methods

**Animals.** *Dipsosaurus dorsalis* were collected in the vicinity of Palm Springs, Riverside County, California under a scientific collecting permit issued to AFB. Animals used for measurements of running performance were captured in late May and tested within 7 days. These animals were adults with a mean body mass of  $60.9 \pm 2.9$  (SE) g and a snout-vent length of  $119.9 \pm 1.36$  mm ( $n=10$ ). Lizards from which muscles were removed for studies of contractile properties were collected in May through July and kept in captivity for up to two months

before sacrifice. These animals had an average body mass of  $47.0 \pm 3.0$  g and snout-vent length of  $112.7 \pm 2.2$  mm ( $n=25$ ). Lizards were maintained in the laboratory on a diet of lettuce, mealworms, and butterweed (*Senecio sp.*) blossoms.

**Running experiments.** The running track was approximately 5 m long and was fashioned from corrugated cardboard. The running surface was covered with carpet with a very short nap that was marked at 10 cm intervals. The carpeted surface was 20 cm wide and was bordered with 40 cm high walls that prevented the animals from escaping. A darkened box at the end of the track served as a refuge into which the lizards could escape by running the length of the track. For three days prior to the start of the experiments the lizards were run on the track to accustom them to the procedure and eliminate any change in running speed due to familiarity with the track (Bennett 1980b). Two animals that refused to run the length of the track were excluded from the measurements.

During the experimental period the animals were housed in individual boxes with dimensions of  $10 \times 10 \times 30$  cm. Body temperatures from 15 to 44 °C were obtained by placing the boxes in a controlled temperature cabinet. One investigator removed the animals individually from the cabinet, placed them at the end of the track, and induced them to run by pinching the tail. Two trials were conducted in rapid succession, after which the body temperature ( $T_b$ ) was measured cloacally with a Schultheis small animal thermometer. During the runs the animals were filmed from above by another investigator with a super 8 mm movie camera. Framing rates were measured at the beginning and end of each roll of film by filming a stop watch. These rates were 68 frames/s when the lizards  $T_b$  was 25 °C and above, 38 frames/s at a  $T_b$  of 20 °C, and 25 frames/s at a  $T_b$  of 15 °C.

Animals were run in the morning and the afternoon on four successive days. At least 7 h elapsed between the morning and afternoon runs. The  $T_b$ 's in successive runs were approximately 40 °C, 30 °C, 25 °C, 35 °C, 20 °C, 44 °C, and 15 °C.

Frame by frame analysis of the films was performed with a Lafayette motion analyzer. Stride frequency ( $f$ ) was measured by counting frames for at least 4 strides during the fastest portion of the run. Maximum running velocity was determined from frame counts and the 10 cm intervals marked on the track, and is reported as the fastest velocity maintained for at least 50 cm during the runs.

**Muscle contractile properties.** Animals were weighed and then sacrificed by decapitation. The distal tendon of the iliofibularis (IF) muscle was tied with 000 surgical silk and the muscle was removed along with one-half of the pelvic girdle. All muscles except the IF were removed from the dissected pelvis. The IF was pinned at approximately rest length on a wax surface using the surgical silk and the pelvis as attachment points. The muscle was split parallel to the fibers using fine iridectomy scissors under sufficient magnification that the fibers could be seen clearly. Approximately one-half to two-thirds of the muscle was removed and discarded. The portion discarded contained the entire red region and part of the white region (Gleeson et al. 1980). The remaining portion was a bundle of fibers from the white region weighing 20–40 mg and still attached to the pelvis and the distal tendon. A light weight silver chain was tied to the distal tendon using 000 surgical silk. During the dissection procedure the muscles were kept moist at all times with oxygenated Ringer's solution (see below). The IF muscles from both legs were dissected. One of these was used immediately and the other was stored at room temperature in oxygenated Ringer's solution for up to 4 h before use.

The muscle was mounted in a chamber similar to that of Putnam and Bennett (1982) except that the pelvis was tied with 0 surgical silk to a rigid stainless steel rod. The distal end was attached via the silver chain to the transducer of a Cambridge Instruments Model 300 H servo-controlled muscle ergometer. The transducer was mounted on a custom built stand, which allowed the easy adjustment of muscle length with low compliance. Muscle temperature was maintained by submersion in a recirculating flow of oxygenated Ringer's solution from a 1 l reservoir maintained in a water bath. The Ringer's solution was composed of 145 mM NaCl, 4 mM KCl, 20 mM imidazole, 2.5 mM CaCl<sub>2</sub>, and 11 mM glucose. The pH of this solution was adjusted after equilibration at the experimental temperature and saturation with 100% oxygen to values approximating the arterial pH of *Dipsosaurus dorsalis* (Bickler 1981). The pH varied from 7.4 at 44 °C to 7.8 at 15 °C.

Muscles were stimulated via two platinum plate electrodes placed on opposite sides of the muscle. Supramaximal stimuli consisted of 0.2 ms pulses produced by a Grass S-44 stimulator and current amplified with a DC-coupled power amplifier from Southwest Technology Inc. Stimulation frequency during tetanic contractions was sufficient to maintain a smooth tetanus, but low enough to prevent decline in tension during the plateau phase. Frequency varied from 30/s at 15 °C to 500/s at 44 °C. For isometric contractions twitches were recorded within one second before and after a tetanus. Twitches were generally potentiated by the preceding tetanus and the twitch parameters reported are those for potentiated twitches. Isotonic contractions were recorded using tetanic stimulation. A rest period of 8–10 min was interposed between successive tetanic contractions. Force, length, and velocity (the length signal differentiated electronically) were monitored on a Tektronix Model 5111 storage oscilloscope and photographed with Polaroid film.

Muscles were first equilibrated at 40 °C and muscle length and stimulus strength were adjusted to achieve maximal isometric tension in a tetanus ( $P_0$ ). The water bath temperature was then changed to the experimental temperature and the reservoir of Ringer's solution exchanged with one previously equilibrated at the new temperature. A new steady state with regard to temperature was reached within 15 min. Following the temperature change 8 to 10 isotonic contractions were recorded from 0.85–0.01  $P_0$ . Isometric contractions were recorded preceding, in the middle, and following the series of isotonic contractions in order to monitor any decline in  $P_0$ . The force during the isotonic contractions is expressed as a fraction of  $P_0$  assuming that the change in  $P_0$  was linear between successive measurements. Following the measurements the muscle length was measured in place. The muscle was removed from the apparatus, damaged fibers were dissected away, the pelvis and distal tendon were removed, and the remaining fiber bundle was weighed to the nearest 0.1 mg. Cross-sectional area was calculated by dividing the mass of the muscle by the muscle length. The IF is a parallel fibered muscle in which the muscle fibers are at least 95% of the muscle length.

*Statistics and curve fitting.* Parameters are reported as means  $\pm$  SEM. The running performance of the lizards was compared with paired *t*-tests using a STAT-PAC program for the HP-41C calculator. Tetanic tensions of the isolated muscles had skewed distributions and were compared with the non-parametric Mann-Whitney U-test. Force-velocity curves were fit using the nonlinear curvefitting program NLLSQ for the Apple II Plus microcomputer. This program is available from CET Research Group, Ltd., Norman, OK, and is an adaptation of the Levenburg-Marquardt algorithm.

## Results

### *Animal performance*

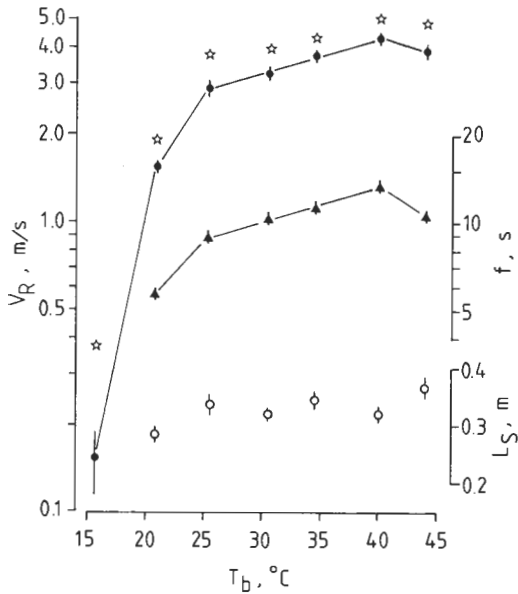
Maximal running velocity ( $V_R$ ) during a four meter burst run and stride frequency ( $f$ ) were both greatest at a body temperature ( $T_b$ ) of 40 °C (Fig. 1). At this temperature, mean  $V_R$  and  $f$  were  $4.28 \pm 0.18$  m s<sup>-1</sup> and  $13.45 \pm 0.49$  s<sup>-1</sup>, respectively ( $n = 10$ ). When  $T_b$  was increased to 44 °C,  $f$  dropped significantly ( $P < 0.001$ , paired *t*-test); whereas, the drop in  $V_R$  was not statistically significant ( $P > 0.05$ ).  $V_R$  and  $f$  are positively correlated with  $T_b$  below 40 °C. Between 25.5 °C and 40 °C the temperature dependence of both parameters was approximately constant with  $Q_{10}$ 's of 1.31 and 1.36 for  $V_R$  and  $f$ , respectively. Below 25.5 °C performance of the animals declined more markedly. Between 20.8 and 25.5 °C the  $Q_{10}$ 's are 3.8 and 2.5 for  $V_R$  and  $f$  respectively; however, the animals at 20.8 °C were still capable of a qualitatively normal stride with only a very slight reduction in stride length ( $L_S$ ) (see below). In contrast, at a mean  $T_b$  of 15.8 °C the lizards were substantially incapacitated. They did not take normal strides at this temperature and thus  $f$  and  $L_S$  are not reported. Limb movements at 15 °C consisted of very small steps, which were only undertaken after considerable prodding.  $V_R$  at 15.8 °C was only 10% of the value at 20.8 °C (Fig. 1).

As can be inferred from the similar temperature dependencies of  $V_R$  and  $f$  over the range of temperatures from 21 to 44 °C,  $L_S$  changed very little with temperature (Fig. 1). Between 25.5 and 40 °C  $L_S$  did not change significantly (paired *t*-test;  $P > 0.05$ ). The mean  $L_S$  for this range of  $T_b$ 's was  $0.328 \pm 0.007$  m. Stride length was significantly higher at 44 °C and significantly lower at 20 °C ( $P < 0.01$ , paired *t*-test), but these changes were small compared to the changes in the time related parameters (Fig. 1).

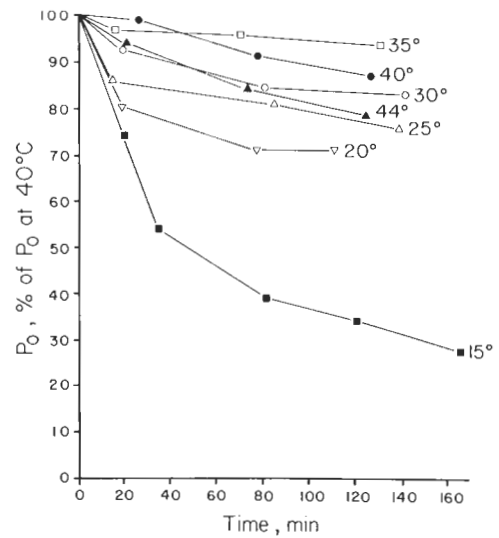
### *In vitro contractile properties*

We found that the twitch force was potentiated by approximately 20% at 40 °C by a preceding tetanus. Additionally, the time to the peak force in the twitch ( $tP_{TW}$ ) was reduced by stimulation immediately preceding the twitch. Because running involves the repetitive activation of muscles and our experiments were designed to compare in vitro and in vivo muscle performance, the values reported for isometric twitches in Table 2 are for contractions potentiated by a previous tetanus.

Because all muscle preparations were first



**Fig. 1.** Relation of body temperature ( $T_b$ ) to maximal running velocity ( $V_R$ , ●), stride frequency ( $f$ , ▲), and stride length ( $L_S$ , ○) in the lizard *Dipsosaurus dorsalis*. Stars indicate the fastest velocity recorded at each temperature. Vertical lines through the means indicate  $\pm 1$  SEM ( $n = 10$ ). Vertical axes for  $V_R$  and  $f$  are logarithmic



**Fig. 2.** Maximum isometric tension ( $P_0$ ) of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis* as a function of time during the course of the contractile measurements. Values are means of 2–4 preparations and are expressed as a percentage of the value recorded at 40 °C (0 time). After the initial equilibration at the experimental temperature, 3–5 isotonic tetani were recorded between each measurement of  $P_0$ .

**Table 1.** Isometric contractile properties of the fast-twitch glycolytic region of the iliofibularis muscle from the lizard *Dipsosaurus dorsalis*

$T_M$ (°C)	$P_0$ (kN/m <sup>2</sup> )	$P_{TW}/P_0$	$tP_{TW}$ (ms)	$t90\%P_0$ (ms)	$dP/dt$ (% $P_0$ /ms)	$t50\%R$ (ms)	$n$
44	205	$0.35 \pm 0.04$	$9.0 \pm 1.4$	$12.6 \pm 0.5$	$12.38 \pm 0.49$	$8.3 \pm 0.85$	4
40	218	$0.34 \pm 0.02$	$11.2 \pm 0.3$	$15.8 \pm 0.5$	$10.36 \pm 0.39$	$10.3 \pm 0.39$	17
35	208	$0.26 \pm 0.02$	$14.3 \pm 0.7$	$23.1 \pm 0.9$	$7.39 \pm 0.21$	$12.4 \pm 1.10$	4
30	199	$0.37 \pm 0.03$	$22.7 \pm 0.3$	$31.9 \pm 1.7$	$5.13 \pm 0.39$	$17.6 \pm 0.89$	4
25	188	0.58	30.8	39.5	4.30	29.0	2
20	165	$0.72 \pm 0.04$	$53.5 \pm 2.7$	$55.3 \pm 1.4$	$2.79 \pm 0.12$	$49.7 \pm 4.4$	3
15	157	$0.67 \pm 0.05$	$85.5 \pm 2.8$	$106.0 \pm 5.9$	$1.50 \pm 0.14$	$112.0 \pm 6.5$	4

Values expressed  $\pm 1$  SEM

$T_M$  = muscle temperature

$P_0$  = maximum tetanic tension; based on the value for 40 °C and percentage drop in tension upon transfer for 20 min to the temperature indicated (see text for explanation)

$P_{TW}/P_0$  = ratio of twitch to tetanic tension

$tP_{TW}$  = time from onset of force to peak tension in a twitch

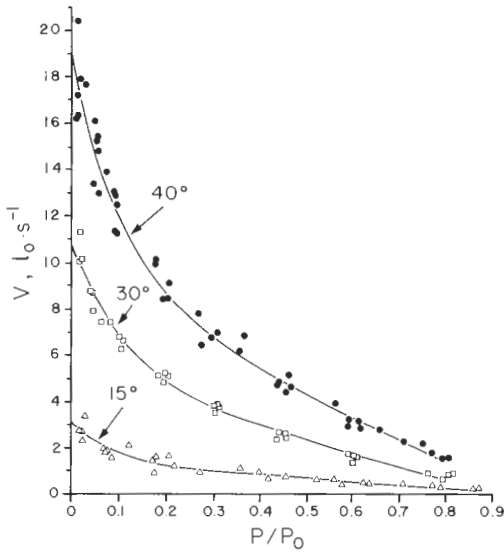
$t90\%P_0$  = time from onset of force to 90% of maximum tetanic tension

$dP/dt$  = maximum rate of rise in tetanic tension

$t50\%R$  = time from peak twitch tension to 50% relaxation

equilibrated at 40 °C, the most accurate estimates of isometric properties were obtained at this temperature. The overall mean maximum isometric tension ( $P_0$ ) for 25 preparations at 40 °C was  $202 \pm 9.2$  kN/m<sup>2</sup> (range 118 kN/m<sup>2</sup> to 293 kN/m<sup>2</sup>). The mean  $P_0$  for preparations used immediately after removal from the animal ( $218 \pm 10.3$  kN/m<sup>2</sup>;  $n = 15$ ) was significantly greater than the  $P_0$  for preparations that were stored for several hours at

room temperature ( $177 \pm 14.2$  kN/m<sup>2</sup>;  $n = 10$ ) ( $P < 0.05$ ; Mann-Whitney U-test). The estimate of  $P_0$  at all other temperatures (Table 1) was based on the mean  $P_0$  at 40 °C in the fresh preparations and the relative decrement in tension after 15–20 min at the experimental temperature (Fig. 2). The proportional decrement at various temperatures was relatively constant compared to the absolute level of tension between various preparations. Thus, this



**Fig. 3.** Force-velocity relations of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis* recorded at 3 different muscle temperatures. Curves were fit by a nonlinear method (see Table 2 for the constants)

estimate of  $P_0$  factors out variations in the starting level of tension at 40 °C. Above 30 °C  $P_0$  was almost constant and it decreased regularly as  $T_b$  was lowered below 30 °C (Table 1, Fig. 2). The stability of the preparations, as judged by the decline in tension after the initial equilibration period, was approximately the same at all temperatures except 15 °C (Fig. 2). At this temperature tension continued to fall rapidly during the course of the measurements and reached values of only 30% of the tension at 40 °C. The ratio of twitch tension to tetanic tension ( $P_{TW}/P_0$ ) increased at temperatures lower than 35 °C due to the fall in  $P_0$  combined with an increase in  $P_{TW}$  (Table 1).

All of the time-dependent isometric properties measured were affected by temperature throughout the range of temperatures studied (Table 1). The maximum rate of rise in tension ( $dP/dt$ ) and the inverse of the time to 90% of tetanic tension ( $1/t_{90\%P_0}$ ) showed very similar thermal dependencies. The  $Q_{10}$  of these parameters was greater than 3 below 20 °C and 1.9 over the range of temperatures from 20 to 40 °C. The thermal dependence of the time course of relaxation, as indicated by the  $1/2$  relaxation time during an isometric twitch ( $t_{50\%R}$ ), declined with increasing temperature between 15 °C and 40 °C.

Figure 3 gives representative force-velocity data at 3 temperatures to illustrate the variability in the data and the accuracy of our fitted curves (see below). Relative variability was greatest at 15 °C. The data at all temperatures deviated from the

**Table 2.** Force-velocity relations of the fast-twitch glycolytic region of the iliofibularis muscle of *Dipsosaurus dorsalis*. Constants are from the equation:  $V = ae^{-\beta P/P_0} + \gamma(1 - P/P_0)$  where,  $V$  is shortening velocity in  $l_0/s$ ,  $P$  is force in  $kN/m^2$ , and  $P_0$  is maximum isometric force in  $kN/m^2$ . Also given are the extrapolated velocity at zero force,  $V_0$ , and the power ratio,  $\dot{W}_{max}/V_0P_0$ . The power ratio is an index of the degree of curvature of the force-velocity relation

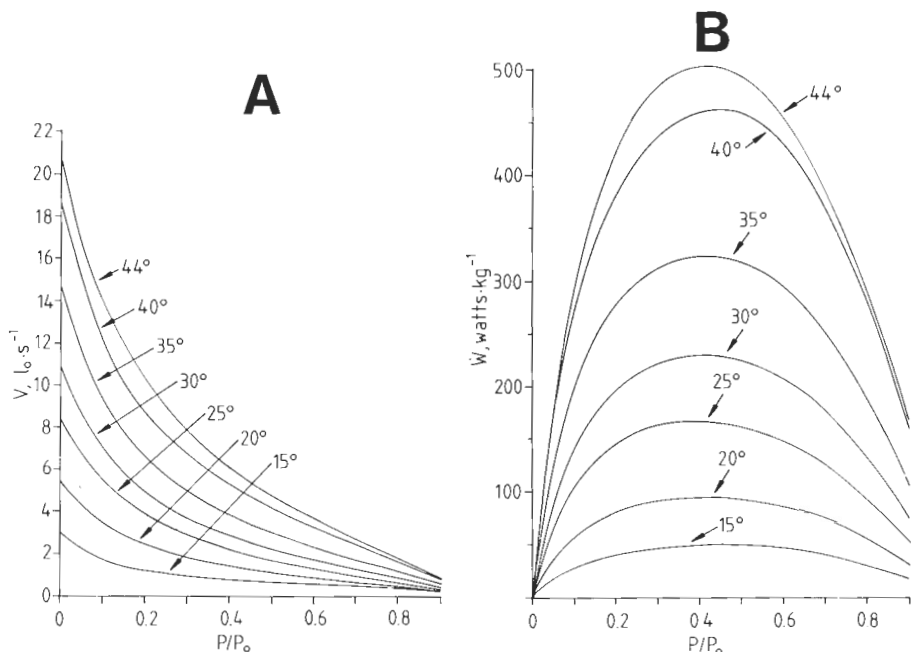
$T_M$ (°C)	$\alpha$ ( $P_0/s$ )	$\beta$	$\gamma$ ( $P_0/s$ )	$V_0$ ( $P_0/s$ )	$\dot{W}_{max}$	$\dot{W}_{max}/V_0P_0$
44	$11.72 \pm 0.39$	$5.81 \pm 0.46$	$8.34 \pm 0.44$	20.1	505	0.13
40	$10.84 \pm 0.49$	$7.51 \pm 0.87$	$7.93 \pm 0.44$	18.7	464	0.11
35	$9.01 \pm 0.71$	$7.01 \pm 1.33$	$5.59 \pm 0.68$	14.6	325	0.11
30	$6.62 \pm 0.27$	$6.86 \pm 0.71$	$4.12 \pm 0.28$	10.7	230	0.11
25	$5.36 \pm 0.41$	$6.39 \pm 1.23$	$3.02 \pm 0.44$	8.38	167	0.11
20	$3.41 \pm 0.16$	$7.29 \pm 0.86$	$2.10 \pm 0.15$	5.50	95	0.10
15	$1.75 \pm 0.21$	$10.08 \pm 2.56$	$1.28 \pm 0.12$	3.03	51	0.11

hyperbolic equation of Hill (1938) particularly at high levels of force. The following equation was fit to the data using a nonlinear curve fitting procedure as outlined above in "Materials and Methods":

$$V = ae^{-\beta P/P_0} + \gamma(1 - P/P_0)$$

Using this equation in the nonlinear regression procedure resulted in a 27% lower residual sums of squares of the regression than using the Hill equation in the same procedure (Marsh and Bennett, unpublished). The fitted constants for our alternative equation are given in Table 2 and the curves shown graphically in Fig. 4A. Figure 4B shows the curves of instantaneous power ( $\dot{W}$  in Watts/kg) calculated from the force-velocity curves.

The shape of the force-velocity curves showed little change from 15 to 40 °C. The similarity in shape is reflected in the constancy of the power ratio ( $\dot{W}_{max}/V_0P_0$ ), which has a mean value of approximately 0.11 in all curves from 15 to 40 °C (Table 2). The power ratio is inversely proportional to the degree of curvature of the force-velocity relation. The force-velocity curve at 44 °C was somewhat flatter (Fig. 4B) and yielded a power ratio of 0.13. Neither extrapolated velocity at zero force ( $V_0$ ) or the maximum power ( $\dot{W}_{max}$ ) change greatly between 40 and 44 °C ( $Q_{10} = 1.2$  for both) (Table 2). Below 40 °C both  $V_0$  and  $\dot{W}_{max}$  decreased regularly, with  $Q_{10}$ 's between 25 and 40 °C of 1.7 and 2.0, respectively. Below 25 °C the  $Q_{10}$ 's for both parameters increased (Table 2). The thermal dependence of power over the interval from 15 to 20 °C is a minimal estimate, because the value for  $P_0$  obtained immediately after equilibration at the experimental temperature was used in the calcula-



**Fig. 4A, B.** Isotonic contractile data of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis*. **A** Force-velocity curves fit by a nonlinear method to data collected at 7 different muscle temperatures (see Table 2 for the constants). **B** Power output derived from the force-velocity curves

tions. At 15 °C  $P_0$  continued to drop during the experiment (Fig. 2).

## Discussion

### Sprint performance

Bennett (1980b) found that several species of small lizards, including *Dipsosaurus dorsalis*, had a low thermal dependence of burst running velocity over a rather broad range of body temperatures. We repeated the measurements of running velocity of *Dipsosaurus* during a sprint ( $V_R$ ) to attempt to confirm this observation and to gather new data on stride frequency ( $f$ ) and stride length ( $L_S$ ) during these runs. Using a longer track than in the earlier study and picking the segment of the run at which  $V_R$  was maximal resulted in higher estimates of  $V_R$  at each temperature than the values recorded by Bennett (1980b). However, the similarity in the pattern of thermal dependence between the two studies is striking. Bennett (1980b) found a mean  $Q_{10}$  between 25 and 40 °C of 1.38 compared to 1.3 in the present study. Both studies also indicate that  $V_R$  is much more affected by temperature in the interval between 20 and 25 °C ( $Q_{10}$ 's of 4.2 and 3.8 in the earlier and in the present study, respectively). Changes in  $f$  account for most of the variation in  $V_R$  with temperature; whereas  $L_S$  is approximately constant over most of the range of  $T_b$ 's studied. Only between 20 and 25 °C does a significant change in  $L_S$  parallel the change in  $V_R$ , and even in this case the difference in  $L_S$  accounts for only 20% of the shift in  $V_R$ .

In the present study we were interested in assessing the possible physiological basis for the thermal dependence of maximal running performance. Therefore, we must necessarily evaluate the likelihood that the observed thermal dependence might reflect a behavioral artifact rather than a shift in physiological capacities. We feel that the running velocities attained by *Dipsosaurus* in our study approach the maximum speeds possible in this species. Both the mean velocity (4.2 m/s) and the individual maximal velocity (5.1 m/s) recorded at a body temperature of 40 °C are faster than the velocities recorded in the laboratory for any species of lizard weighing less than 100 g (Bennett 1980b; Huey 1982; Huey and Hertz 1982). The fastest speeds recorded in the field (Belkin 1981) are, however, somewhat higher than our laboratory measurements. On the other hand, what is critical to the interpretation of our data is not that we recorded the absolute top speed of each animal, but that the performances at each temperature are a similar proportion of the top speed. Several lines of evidence support this supposition. First, the behavior of the lizards was qualitatively similar at all temperatures except 15 °C (see Results). Second, the pattern of thermal dependence was very similar to that found by Bennett (1980b) despite the difference in absolute level of performance. Third, the  $Q_{10}$ 's calculated from the fastest animal at each temperature are similar to those based on the mean values (Fig. 1). These animals may be presumed to be demonstrating the best performance possible under the conditions of the experiment. The  $Q_{10}$  of

$V_R$  of these animals is 1.2 in the interval from 25 to 40 °C and 4.1 between 20 and 25 °C. We conclude that the change in  $V_R$  with temperature in our experiments is very likely an expression of changing physiological capacities and not a behavioral artifact of our laboratory measurements.

### *In vitro contractile properties*

We measured the contractile properties of a portion of the iliofibularis muscle. Our preparation consisted of only fast-twitch glycolytic (FG) fibers (Gleeson et al. 1980). The muscles of the hind limb of *Dipsosaurus* are composed predominantly of FG fibers (Putnam et al. 1980), and presumably these fibers are responsible for providing the power to sustain anaerobically fueled bursts of activity in this species (Bennett and Dawson 1972).

The temperature dependencies of the isometric contractile properties of the FG fibers of *Dipsosaurus* (Table 1) are similar to those found for the whole iliofibularis and gastrocnemius muscles by Putnam and Bennett (1982). The absolute values of some of the parameters do differ between the two studies. These differences are likely attributable to the use of a preparation consisting of a single fiber type in the present experiments and possibly to other methodological differences.

The shape of the force-velocity curves that we measured did not change with muscle temperature except at the highest temperature used (Table 2). This result agrees in general with data on amphibian and mammalian muscles (Hill 1928; Close 1965; Cecchi et al. 1978; Lännergren 1978). Shortening velocity and power output of *Dipsosaurus* FG fibers both change with temperature throughout the range of temperatures studied (Fig. 4, Table 2). The  $Q_{10}$  of  $V_o$  is within the range measured for other vertebrate twitch muscles (Hill 1938; Close 1965; Cecchi et al. 1978; Lännergren 1978; Petrofsky and Phillips 1981; Lännergren et al. 1982; Bennett 1984).

Because isometric contractile properties of skeletal muscle are substantially easier to measure than are isotonic properties, it is useful to evaluate which of the time-related isometric properties are the best indicators of the isotonic properties. The average thermal dependence of  $V_o$  over the entire range of temperatures studied does not precisely match that of any of the isometric parameters; the  $Q_{10}$  from 15 to 44 °C is 1.92, 2.07, 2.08, and 2.17 for  $V_o$ ,  $dP/dt$ ,  $1/t90\%P_o$ , and  $1/tP_{TW}$ , respectively. The significance of this comparison is limited by

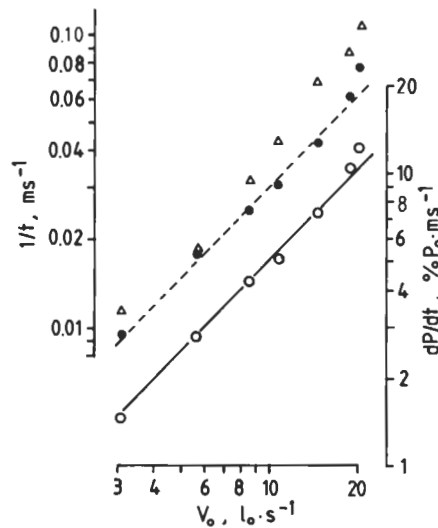
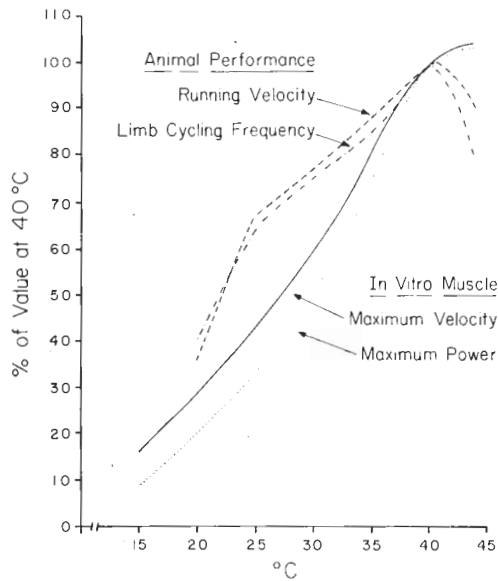


Fig. 5. Time-related isometric contractile properties (O =  $dP/dt$ ,  $\Delta$  =  $1/tP_{TW}$ ,  $\bullet$  =  $1/t90\%P_o$ ) of the fast-twitch glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis* plotted as a function of the  $V_o$  measured at different temperatures. Lines drawn have a slope of 1.0 on the log-log coordinates and indicate equal thermal dependence of the X- and Y-variables

changes with temperature in the  $Q_{10}$ 's of all variables (calculated from data in Tables 1 and 2). More accurate direct comparisons of the thermal dependence could involve calculation of the  $Q_{10}$ 's over smaller intervals (e.g., see Putnam and Bennett 1982). We have found, however, that the overall correlations are easier to visualize by plotting the log of each variable versus log  $V_o$  (Fig. 5). If temperature affects a variable to the same degree as it affects  $V_o$ , the slope of this log-log plot will be 1.0. Figure 5 indicates that thermal effects on the isometric properties indicating the rate of rise of tension in a tetanus ( $dP/dt$  and  $1/t90\%P_o$ ) are similar to the effect on  $V_o$  between 15 and 40 °C. In contrast, over most of the range of temperatures studied, the time course of the twitch is more influenced by temperature than is  $V_o$ , and the resulting slope (Fig. 5) is greater than 1.0. The equality of the thermal effects on  $V_o$  and the rate of rise of tension in the tetanus does not hold above 40 °C. The continued change in  $dP/dt$  despite little change in  $V_o$  may indicate a change in muscle stiffness (Jewell and Wilkie 1958).

### *Relation of animal performance to muscle performance*

We will restrict our discussion of the relation between muscle performance and animal perfor-



**Fig. 6.** Summary of the effects of temperature on running performance and on the isotonic contractile properties of fast-twitch glycolytic skeletal muscle in the lizard *Dipsosaurus dorsalis*

mance to temperatures of 20°C and above. At this temperature and above the running behavior is qualitatively similar. The incapacitation seen at 15°C may be related to the drastically reduced ability of the muscles to produce force (Fig. 2), but, in view of the very limited movements possible at this temperature, neural factors may also be involved.

The thermal dependence of performance during a burst run by *Dipsosaurus* does not parallel the thermal dependence of the time-related properties of the muscles that power locomotion. The magnitude of the difference between the actual performance of the animals and the performance predicted from the isotonic contractile properties is indicated in Fig. 6. At 25°C  $V_R$  is 1.5 and 1.9 times the values that would be predicted from  $V_o$  and  $\dot{W}_{max}$ , respectively.

The lower thermal dependence of  $V_R$  and  $f$  than of power production by the muscles might be predictable if total power input during rapid locomotion was not directly proportional to velocity. In this case a large decrease in the power input from the muscles would lead to a smaller change in  $V_R$ . No data are presently available on the mechanics and energetics of sprint locomotion in lizards. However, Heglund et al. (1982a) have shown that the mass-specific total power input during running in a variety of quadrupedal and bipedal mammals and birds is relatively independent of body size and mode of locomotion. Their results (their Eq. 1) indicate that *Dipsosaurus* sprinting at 25°C with a

velocity of 2.88 m/s would require 60% of the total power required to sustain 4.25 m/s at 40°C. This predicted power requirement at 25°C is almost double the value expected given the thermal dependence of power production by the isolated muscles during isotonic contractions.

Clearly, if our measures of running performance reflect physiological capacity, as we have argued above, then some other aspect of the locomotory system must modulate the effect of temperature on the skeletal muscles. Two possible mechanisms may be suggested to explain the low thermal dependence of burst running in lizards: 1) recruitment of more muscle fibers at low temperatures and 2) involvement of elastic storage in powering sprint running. Any mechanism put forward must involve the peripheral systems supplying power input. The neuronal circuits that determine the oscillatory activation of the skeletal muscles obviously cannot drive the system at a frequency higher than that determined by the ability to supply power during each stride (Fedak et al. 1982; Heglund et al. 1982b).

The first possible mechanism to explain the low  $Q_{10}$  of  $V_R$  is increased fiber recruitment at low temperatures. According to this hypothesis, lizards running at 40°C would have to have a considerable reserve of inactive motor units that would be recruited during running at low body temperatures. This mechanism is necessary to explain the low thermal dependence of  $V_R$  if one assumes that the active shortening of the muscles provides the major source of power to sustain locomotion. Given this assumption and the measured force-velocity properties, we have calculated that to achieve a  $Q_{10}$  of 1.3 between 25 and 40°C a lizard must activate approximately 70% more cross-sectional area at 25°C than it does at 40°C. (This proportional increase in recruitment at 25°C would provide a maximum power output of 60% of the value at 40°C and would yield 70% of the value at 40°C for power calculated as the integral under the force-velocity curve). Increased fiber recruitment at low temperatures has been hypothesized to play a role in the temperature independence of some locomotory parameters during slow sustained walking in lizards (Rome 1982) and slow sustained swimming in fish (Loughna et al. 1983). However, during a sprint run it is difficult to explain why a lizard at 40°C would fail to activate a substantial proportion of its muscle fibers when by activating them it could attain even higher velocities. This is especially paradoxical as *Dipsosaurus* are rarely active above ground with body temperatures as low as 25°C (Norris 1953). The possible advantage of



having a substantial power reserve for use on the occasions when it must be active at low temperatures would likely be counterbalanced by the disadvantage of carrying substantially more weight than would otherwise be required when running with body temperature near the preferred level.

A possible test of the hypothesis of differential fiber recruitment would be an examination of glycogen depletion after burst running at different temperatures to determine if some motor units are not activated at high  $T_b$ 's. Preliminary data collected by T.T. Gleeson (personal communication) indicate, to the contrary, that glycogen depletion following a burst run at 40 °C occurs throughout the population of FG fibers. Such data are not definitive, however, because motor units might be activated alternately resulting in glycogen depletion in all units even though only a fraction of the units are active at any one time.

The former hypothesis assumes that all of the power to sustain  $V_R$  is produced by active shortening of the contractile elements in the muscles. If a portion of the total power were derived from a temperature-independent process, however, the low thermal dependence of  $V_R$  might be explained even if fiber recruitment were constant. Elastic storage and recovery of energy during each stride may provide such a temperature-independent process. The storage of energy in elastic elements occurs during the initial contact phase of the stride when muscles are activated while lengthening (Goslow et al. 1981). Subsequently, the body is accelerated upward and forward partly due to the release of energy from elastic structures in the tendons and the muscles. On the basis of present data it is difficult to determine the likelihood that elastic elements play a role in damping the effects of temperature on muscle function in lizards. Several studies have indicated that elastic storage and recovery of energy play an important role in rapid running in large mammals (Cavagna et al. 1971; Asmussen and Bonde-Petersen 1974a, b; Alexander and Vernon 1975; Cavagna and Kaneko 1977), but the quantitative contribution of these processes to the total power input in small animals has been questioned (Biewener et al. 1981).

The thermal dependence of the elastic properties of muscles and tendons have not been extensively investigated, but at least some of these properties are relatively insensitive to temperature (Rigby et al. 1958; Jewell and Wilkie 1958). Under certain in vitro conditions, prestretching a muscle can result in a rather low  $Q_{10}$  (< 1.4) of total energy release (Cavagna et al. 1981). Unfortunately, in man, where the contribution of elastic storage of

energy to the power input during rapid running is well characterized, no studies exist of velocity during sprinting as a function of muscle temperature. Pedalling velocity and power output in bicycle ergometry, which involves no storage and recovery of elastic energy (Asmussen and Bonde-Petersen 1974b), show thermal dependencies similar to that expected from isolated muscles (Bergh and Ekblom 1979). Clearly, the role of elastic storage during a burst run by *Dipsosaurus* cannot be fully evaluated on the basis of available data. Equally clearly, however, this mechanism does have the potential to reduce the thermal dependence of locomotory performance. It would be particularly interesting to know if ectothermic vertebrates, such as lizards, have any morphological or kinematic specializations that increase the storage of elastic energy.

#### *Limitation of locomotory performance at low temperatures*

If one accepts as a working hypothesis that the reacceleration of the body in each stride (and thus ultimately the stride length and stride period) is determined partly by thermally independent parameters, e.g., inertial and elastic properties, and partly by the thermally dependent contractile velocity of the muscles ( $Q_{10}$  values of 1.7 to 2.0), then the intermediate value of 1.3 for the  $Q_{10}$  of  $V_R$  is reasonable. Why then does the effect of temperature increase dramatically below 25 °C? The increase in  $Q_{10}$  over this range of temperatures may be related to a limit imposed by the duration of the state of activation of the contractile apparatus. Following even a single stimulus (twitch contraction) the contractile machinery is fully active for a period of time considerably longer than the stimulus (Hill 1970). How might the kinetics of a twitch relate to in vivo function? First, the time to peak tension in an isometric twitch should not be taken to indicate the time required to develop tension in vivo. If the muscle is stretched following a single stimulus, tension rises more quickly and to higher levels than during an isometric twitch (Hill 1970). On the other hand, the time from the stimulus until the muscle can be lengthened again without redeveloping tension, i.e., the duration of the state of activation, will limit how soon the return stroke of the limb can occur without interference between antagonistic muscles. Preliminary data on the FG fibers of *Dipsosaurus* (R.L. Marsh, unpublished) and data on frog muscle (Jewell and Wilkie 1960) suggest that the time from onset of force to 50% relaxation in an isometric twitch ( $t_c$ ) approximates

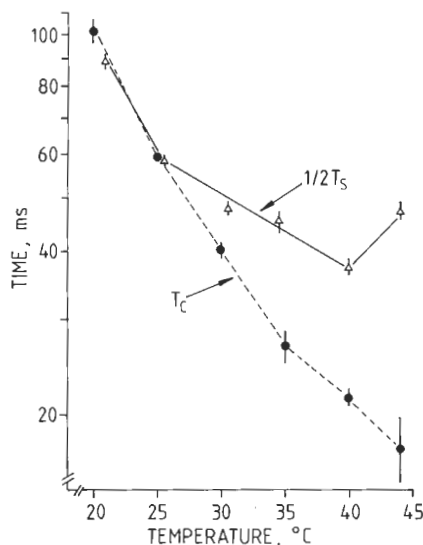


Fig. 7. Effects of temperature on the contraction time of fast-twitch glycolytic skeletal muscle ( $t_c$  = time from the onset of force to 50% relaxation in an isometric twitch) and on the time required for the power stroke of the hind-limb ( $1/2 t_s$ ) in the lizard *Dipsosaurus dorsalis*

the duration of the state of activation. This suggestion is supported by our isotonic data, which indicate that the time from the last stimulus until the muscle can be lengthened by a force equal to 10%  $P_0$  is approximately equal to  $t_c$ . In sprint running our films indicate that the propulsive and return strokes each occupy approximately 50% of the stride period ( $t_s$ ). Thus the muscles must be capable of contracting and relaxing in  $1/2 t_s$ . In Fig. 7  $t_c$  and  $1/2 t_s$  are plotted as a function of temperature. At high temperatures  $1/2 t_s$  is considerably longer than  $t_c$ , suggesting that the muscle could be activated by short tetani without limiting  $t_s$ . However, at low temperatures the higher thermal dependence of  $t_c$  compared to that for  $t_s$  causes the times to approach one another. The curves intersect at a temperature near 25°C, and below this intersection the thermal dependence of  $t_s$  increases greatly, paralleling that for  $t_c$ . We believe these data support the hypothesis that  $t_c$  may limit  $t_s$  at low temperatures and thus be responsible for the greater effect of temperature on  $V_R$  below 25°C.

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