THE JOURNAL OF EXPERIMENTAL ZOOLOGY 214: 293 – 302 (1980)

Histochemical, Enzymatic, and Contractile Properties of Skeletal Muscle Fibers in the Lizard *Dipsosaurus dorsalis*

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ABSTRACT Lizard skeletal muscle fiber types were investigated in the iliofibularis (IF) muscle of the desert iguana *(Dipsosaurus dorsalis)*. Three fiber types were identified based on histochemical staining for myosin ATPase (mATPase), succinic dehydrogenase (SDH), and αglycerophosphate dehydrogenase (αGPDH) activity. The pale region of the IF contains exclusively fast-twitch-glycolytic (FG) fibers, which stain dark for mATPase and αGPDH, light for SDH. The red region of the IF contains fast-twitch-oxidative-glycolytic (FOG) fibers, which stain dark for all three enzymes, and tonic fibers, which stain light for mATPase, dark for SDH, and moderate for αGPDH. Enzymatic activities of myofibrillar ATPase, citrate synthase, and αGPDH confirm these histochemical interpretations. Lizard FG and FOG fibers possess twitch contraction times and resistance to fatigue comparable to analogous fibers in mammals, but are one-half as oxidative and several times as glycolytic as analogous fibers in rats. Lizard tonic fibers demonstrate the acetylcholine sensitivity common to other vertebrate tonic fibers.

Mammalian locomotory muscles are composed of three types of muscle fibers. These fibers can be classified according to their twitch contraction times and their relative activities of oxidative and glycolytic enzymes. By these criteria most mammalian skeletal muscle fibers can be categorized as being fast-twitch-glycolytic (FG), fast-twitch-oxidative-glycolytic (FOG), or a slow-twitch-oxidative (SO) fiber (Peter et al., '72). A fourth type of mammalian skeletal muscle fiber if also recognized. Limited in their distribution, mammalian tonic fibers are found in certain extraocular and middle ear muscles (Hess, '70). Tonic fibers differ from twitch fibers in their morphology and function, most notably in that tonic fibers contract slowly, developing tension in seconds rather than milliseconds, as is typical of twitch fibers. Tonic fibers are not known to exist in mammalian locomotory muscles.

Mammalian twitch fiber types can often be visually discerned. Pale muscles, or regions of muscles, are predominantly FG fibers, while pink or red regions are highly oxidative and possess primarily FOG and SO fibers (Ariano et al., '73; Gonyea and Galvas, '79). Pale and red regions have also been reported in the skeletal muscles of other vertebrates (Fish: Johnston et al., '75; frogs: Ogata and Mori, '64; snakes: Gans et al., '78; birds: Kiessling, '77), although correlations with specific fiber types have not always been made.

The skeletal muscles of iguanid lizards are superficially pale in appearance, but they frequently possess pink or red regions which are medially located or occur near joints. Ultrastructural (Proske and Vaughan, '68; Finol and Ogura, '72) and histochemical studies (Ogata and Mori, '64; John, '66, '70) have shown that lizard muscles possess both twitch and tonic fibers, although their distribution and their similarity to mammalian twitch and tonic fibers are not clear. A detailed review of reptilian muscle ultrastructure and physiology has been compiled by Guthe (in press).

We have characterized the twitch and tonic fibers in the skeletal muscles of the iguanid lizard *Dipsosaurus dorsalis* using standard histochemical, biochemical, and physiological techniques. This comprehensive approach to reptilian fiber typing allows comparison with the classification scheme and characteristics of mammalian muscle fibers, and will provide a framework for investigation of other species of

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0022-104X/80/2143-0293$02.00 © 1980 ALAN R. LISS, INC.

reptiles. In this paper, we report the characteristics of fibers which compose the iliofibularis muscle of the hindlimb. We classify these fibers and compare them to analogous fibers in other vertebrates. In a companion paper (Putnam et al., '80), we histochemically survey 13 locomotory and postural muscles in *Dipsosaurus* and discuss the distribution and probable functions of the different fiber types as they are defined here. A preliminary report of these data has appeared elsewhere (Putnam et al., '80).

MATERIALS AND METHODS

Animals

Desert iguanas (*Dipsosaurus dorsalis*, 19–58 gm) were collected near Palm Springs, California during September 1979 (California Scientific Collecting Permit No. 514). Iguanas held in the laboratory were provided with a photothermal gradient and maintained on a diet of lettuce, dogfood, mealworms, and water. Muscles for comparison were taken from female Wistar rats (Simonsen Labs) of 200–300 gm.

Histochemistry

Muscles were removed from freshly decapitated animals and frozen onto cryostat chucks by plunging them into a 2-methyl-butane-liquid nitrogen bath. Frozen muscles were then stored 1–12 days prior to sectioning. Cut sections (14 μm) were mounted on glass coverslips and air-dried at room temperature (25° C) 0.5–4 hours prior to histochemical treatment.

Myosin ATPase activity was assayed in a manner similar to that of Guth and Samaha ('69, '70), but without alkaline or acid preincubation. Unfixed sections were incubated in ATP incubation media (60 mM NaCl, 60 mM Glycine, 24 mM $CaCl_2$, 23 mM NaOH, 3 mM ATP, pH 9.4) at 37° C for 10 minutes in a shaking water bath, then soaked in three 1-minute changes of 1% $CaCl_2$ (25° C), and rinsed in four 30-second changes of pH 9.0 distilled H_2O. Sections were then soaked 3 minutes in 2% $CoCl_2$ (25° C), rinsed as above, and soaked for an additional 3 minutes in 1% $(NH_4)_2S$ (25° C). Stained sections were then rinsed in three 1-minute changes of distilled H_2O and air-dried. Extensive preliminary investigation showed that alkaline and acidic preincubation would not reliably distinguish different fiber types in lizards, as it does in mammals. Alkaline preincubation (pH 10.5, 10 minutes) resulted in a staining pattern identical to that when no preincubation was used. Acid preincubation (pH 4.5, 3 minutes) inactivated some tonic fibers, all FG fibers, and some FOG fibers. Other FOG fibers stained dark. Longer preincubation time or greater acidity inactivated all but the dark FOG fibers.

Succinic dehydrogenase (SDH) was assayed in a manner similar to Nachlas et al. ('57) but with NADH added. Unfixed sections were incubated 2 hours at 37° C in SDH incubation medium which contained succinic acid, 7 mM; NADH, 0.85 mM; nitro blue tetrazolium, 1.2 mM; and Trizma buffer at pH 7.4, 200 mM. Sections were then rinsed 2–5 minutes under running deionized H_2O, dehydrated 5 minutes in 50% acetone, and then air-dried.

Activity of α-glycerophosphate dehydrogenase (αGPDH) was assayed according to the method of Wattenberg and Leong ('60). Sections were incubated 2 hours at 37° C in an incubation medium which contained DL-α-glycerophosphate, 13.9 mM; nitro blue tetrazolium, 1.2 mM; Menadione, 1.4 mM; and Trizma buffer at pH 7.4, 200 mM. Sections were then rinsed and dehydrated as described for SDH assay.

Serial sections stained for all three enzymes were mounted on glass slides with Depex and photographed at 50 × magnification with Kodachrome ASA 64 color slide film.

Our ability to distinguish fast- and slow-twitch fibers with our techniques was confirmed by histochemically staining rat muscles in a manner identical to that used for lizards. Rat soleus, plantaris, and white vastus lateralis muscles were stained for mATPase, SDH, and αGPDH under identical conditions. Our treatment clearly identified the SO, FOG, and FG fibers known to exist in these muscles (Baldwin et al., '72; Ariano et al., '73).

Enzymatic analysis

Muscles were removed from freshly decapitated animals, trimmed of fat and connective tissue, placed in foil envelopes, and frozen between blocks of dry ice. Frozen samples were stored at –20° C until analysis. Both rat and lizard muscles were treated similarly unless otherwise noted.

Citrate synthase and α-glycerophosphate dehydrogenase activities were measured in frozen muscle samples (19–120 mg) which were chilled to –70° C, weighed to the nearest milligram, and transferred to a smooth-surfaced, glass mortar and pestle prechilled to –70° C. Muscles were pulverized to a fine powder and transferred to a 1-ml volume glass-glass tissue homogenizer chilled in ice H_2O. Tissues were

homogenized on ice in 19 × (w/v) 2 mM EDTA in 175 mM KCl, pH 7.4. Homogenates were then slowly frozen to −20° C, thawed three to five times to rupture subcellular compartments, and then centrifuged 3 minutes at 1000 × g (+5° C) to separate connective tissue and other debris. This supernatant was utilized for enzymatic analysis. Preliminary experiments showed that endogenous activity was substantially reduced in 1000 × g supernatants, although enzymatic activity was not reduced relative to crude homogenates. Citrate synthase activity was assayed according to Srere ('69) in a 1-ml volume which contained 0.5 mM oxaloacetate, 0.3 mM acetyl CoA, 0.1 mM 5-5′-dithiobis-(2-nitrobenzoate), 70 mM Tris-HCl buffer, and 5–10 μl of the 1000 × g supernatant.

Cytoplasmic α-glycerophosphate dehydrogenase activity was assayed according to Holloszy and Oscai ('69) in a 1-ml reaction mixture which contained 0.18 mM NADH, 2.9 mM dihydroxyacetone phosphate, 71–75 mM Tris-HCl buffer, and 10–50 μl of the 1000 × g supernatant.

Myofibrillar ATPase activity was measured using a technique modified from that of Baldwin et al. ('77b). Muscle samples (12–80 mg) were weighed and pulverized as described above. Muscles were homogenized in a solution of 250 mM sucrose, 100 mM KCl, and 5 mM EDTA adjusted to pH 6.5 at +5° C (10 ml/gm muscle). The homogenate was centrifuged at 1100 × g for 10 minutes (5° C) and the supernatant was discarded. The pellet was resuspended and washed (10 ml/gm) twice in 0.5% Triton-X solution (175 mM KCl, 2 mM EDTA, 0.5% Triton-X 100, pH 6.8 at 5° C) followed by two washings (10 ml/g) in 150 mM KCl (pH 7.0, 5° C). Each wash was followed by centrifugation of 1100 × g for 10 minutes at 5° C. Following the final wash, the myofibrillar pellet was resuspended in 15 × (w/v) 150 mM KCl in 30 mM Tris (pH 7.4, 5° C). Protein concentration of this suspension was then determined by the Biuret technique and the final protein concentration adjusted to 5 mg/ml with KCl-Tris.

Ca^{++}-activated myofibrillar ATPase activity was assayed in a 2-ml reaction volume which contained 100 μl myofibrillar protein solution, 200 μl of $MgSO_4$-$CaCl_2$ solution (10 mM $MgSO_4$, 0.1 mM $CaCl_2$, 20 mM Na^+ azide in 30 mM Tris, pH 6.9, 40° C), 200 μl ATP solution (50 mM in 30 mM Tris, pH 7.0, 25° C), and 1.5 ml 30 mM Tris-HCl buffer. The ATPase reaction was stopped after 2 minutes with the addition of 1.0 ml 10% TCA. A 2-ml sample of this mixture was then analyzed for phosphate according to Fiske and Subbarow ('25).

Acidity and temperature of the three enzymatic reaction mixtures were adjusted to approximate the intracellular conditions of both the rat and *Dipsosaurus* muscle. Muscle pH was assumed to be 0.6 pH units below resting blood pH based on muscle-blood pH differences reported by Reeves ('77). Rat enzymes were therefore assayed at pH 6.9 and 37° C while *Dipsosaurus* enzymes were assayed at pH 6.9 and 40° C. The preferred body temperature of this ectotherm is 40° C (DeWitt, '67). Assays were performed in a thermostated recording Beckman Model 25 spectrophotometer. Enzyme activities are expressed as U/gm protein (U = μmole product formed/minute).

Contractile properties

Isometric twitch and tetanic properties were measured in iliofibulari muscles removed from decapitated animals. The iliofibularis muscle of each limb was exposed, the distal tendon tied with surgical silk (2–0), and the tendon cut. The muscle was freed from the animal along with the attached ilium of the pelvic girdle and placed in Ringer's solution (155 mM NaCl, 4mM KCl, 2 mM $CaCl_2$, 2 mM phosphate buffer, pH = 7.2, 25° C).

The iliofibularis (IF) is a cylindrically shaped muscle composed of parallel fibers running its entire length. It possesses a discrete red region which is medially parallel to the femur. To measure the contractile properties of the red and white regions of the IF, the red region fibers of one muscle were dissected free, leaving an intact white region for study. The white region of the contralateral IF was similarly removed and the remaining red region used for contractile studies. The order in which the red and white IF were studied was randomized.

The iliofibularis (white or red) was attached to a Grass FTO3C force transducer with an inextensible chain tied to the distal tendon. The ilium was tied to a large glass rod and the muscle then lowered into a 300 ml thermostated (40 ± 1° C) bath of aerated Ringer's solution. Force transducer output was displayed on a Textronix Model RM 564 dual-beam storage oscilloscope and recorded on a Grass Model 79D polygraph.

Contractile properties were measured after 10 minutes of thermal equilibration. The responses to single stimuli (40–60 volts, 1 msec duration) were recorded. The muscle was stimulated through two platinum-wire surface

electrodes using a Grass SD9 stimulator. Contraction time (CT) was measured from the initiation of a mechanical response to the peak of the twitch on the oscilloscope. Half-relaxation time (1/2 RT) was the time from peak mechanical response to the point during recovery when tension had fallen to one-half maximum twitch tension. Ten twitches or less were sufficient to obtain these data. Maximum tetanic tension and fusion frequency were then obtained by stimulating the muscle at high frequencies (40–60 Hz) for short duration (1–3 seconds). The lowest frequency at which the tension curve appeared smooth was defined as the fusion frequency; the tension generated at this frequency was defined as the maximum tetanic tension. After a brief rest, muscles were then twitched at a frequency of 1 pulse/second (1 msec pulse duration) for 5 minutes. The fatigue index of the muscle was defined as the ratio of the final to initial twitch tension generated during this 5-minute stimulus regime.

The sensitivities of the white and red regions of the iliofibularis to acetylcholine were measured in muscles which were treated as above but were not used to determine fatigue index. One ml of an acetylcholine solution (3 mg/ml) was added to the 300-ml bath in the region of the muscle and the contractile response recorded.

Tensions are reported as gm/cm^2 cross-sectional area. Cross-sectional area was calculated by dividing muscle weight by its length. All data are reported as mean ± SEM.

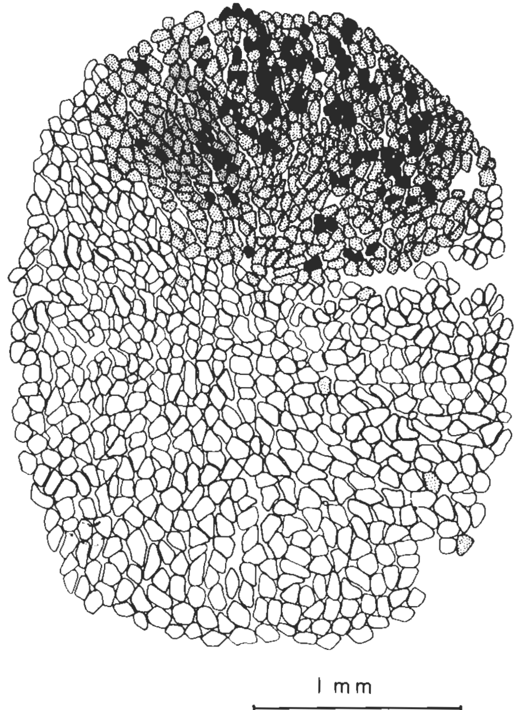

Fig. 1. Cross section of an entire iliofibularis muscle of *Dipsosaurus* illustrating the distribution of fiber types. Clear fibers demonstrate high mATPase and αGPDH activities, low SDH. These fibers are termed FG fibers and comprise the white iliofibularis. Stippled fibers (FOG) have high activities for all three enzymes. Dark fibers (tonic) have low mATPase, high SDH, and αGPDH activities. FOG and tonic fibers compose the red iliofibularis.

RESULTS

Histochemical characteristics

Histochemical staining of the *Dipsosaurus* iliofibularis for myosin ATPase (mATPase) activity demonstrates two classes of muscle fibers. The majority of fibers stain darkly for mATPase, indicating high enzyme activity. A small percentage of fibers stain very lightly under the same conditions. The mATPase-light fibers are small in diameter and occur medially in the iliofibularis near the femur.

Succinic dehydrogenase (SDH) activity is used as an index of the oxidative capacity of muscle fibers. Histochemical staining for SDH activity reveals a distinct region of high SDH activity (Fig. 1). This oxidative pocket lies nearest to the femur and corresponds to the visibly red region observed during dissection. Fibers which possess high SDH activity include not only those with low mATPase activity, but also a subset of high mATPase fibers.

All fibers within the iliofibularis demonstrate substantial α-glycerophosphate dehydrogenase (αGPDH) activity when stained for this enzyme. Fibers within the oxidative zone described by SDH staining appear slightly less dark when stained for αGPDH than fibers in the white region, but the difference is not great.

The staining pattern in the muscle demonstrates three fiber types in *Dipsosaurus* skeletal muscle (Fig. 1) — two fiber classes which are fast-twitch in their contractile properties and one fiber type which our contractile data (presented below) indicate is a tonic fiber. The bulk of the iliofibularis, white or pale in appearance, is composed of fibers which stain darkly for mATPase and αGPDH, lightly for SDH. We have labeled these fibers fast-glycolytic (FG) fibers, adopting the classification system for mammalian fibers proposed by Peter et al. ('72). The majority of fibers within

the red portion of the iliofibularis histochemically stain darkly for all three enzymes. We classify these fibers as fast-twitch-oxidative-glycolytic (FOG) fibers. The third fiber type present in *Dipsosaurus* iliofibilaris muscles is a tonic fiber, histochemically characterized as having low mATPase activity, moderate-to-high SDH and αGPDH activities, and restricted to the medially located red region of the muscle.

Fiber type abundance and distribution

The iliofibularis of *Dipsosaurus* is a cylindrical muscle composed of 900–1,000 fibers. The muscle illustrated in Figure 1 contains 979 fibers of which 493 are in the white region, 486 in the red region. The white region comprises approximately 70% of the cross-sectional area and mass of the iliofibularis, and is composed largely of FG fibers. The white region of muscles from seven lizards possessed 96–100% FG fibers, the remainder being FOG fibers. The FG fibers of the white iliofibularis are large, with diameters between 111 and 143 μm. The red region is a mixed compartment, with FOG fibers representing 59–77% of the total red region fiber population. Tonic fibers represented 21–33% and FG fibers represented between 0 and 10% of all red zone fibers (Fig. 2). Fiber diameters of FOG and tonic fibers within the red region are similar, ranging between 54 and 81 μm.

Enzymatic analysis

The enzymatic activities of citrate synthase, α-glycerophosphate dehydrogenase, and myofibrillar ATPase in the red and white portions of the lizard iliofibularis are compared in Figure 3. The red iliofibularis of *Dipsosaurus* has approximately seven times the citrate synthase activity relative to the white iliofibularis (207 ± 18 versus 30 ± 3 U/gm protein), reflecting the greater oxidative capacity in the red region. In contrast, the white IF possesses approximately five times the α-glycerophosphate dehydrogenase activity relative to the red IF (530 ± 33 versus 98 ± 11 U/gm protein). The enzyme α-glycerophosphate dehydrogenase is used as an index of glycolytic capacity. The myofibrillar ATPase activity of the white region (190 ± 15 U/gm protein) is approximately twice that of the red IF (98 ± 9 U/gm protein). The differences between the red and white iliofibularis for all three enzymes are highly significant ($P < 0.0001$, *t*-tests).

The activities of these enzymes in *Dipsosaurus* iliofibularis are compared to those of rat soleus, plantaris, and white vastus lateralis muscles in Figure 3. In rat, the soleus muscle is 80–90% SO fibers, the plantaris 55% FOG fibers and 40% FG fibers, and the white vastus 95–100% FG fibers (Baldwin et al., '72; Ariano et al., '73). The white iliofibularis exhibits five to eight times the αGPDH activity of rat glycolytic fibers (FG + FOG). Citrate synthase activity in the lizard red IF is roughly half that found in the rat soleus, which is predominately oxidative. Myofibrillar ATPase activity in the lizard iliofibularis muscle approximates that found in the slow-twitch fibers of the rat soleus muscle.

Physiological properties

Twitch and tetanic properties. The contractile properties of the red and white regions of *Dipsosaurus* iliofibularis muscles were measured in muscles from lizards of both sexes with a mean body weight of 34 ± 2 gm. Muscles ranged from 1.4 to 2.7 cm in length with mean cross-sectional areas of the red and white regions of 0.013 ± 0.002 cm^2 and 0.027 ± 0.003 cm^2, respectively. Surgical separation of white from red regions prior to measuring contractile tension damages fibers on the periphery of both fiber bundles; thus, active cross-sectional areas are somewhat less and tensions per cross-sectional area somewhat greater than those actually reported here.

The contractile properties of the red and white iliofibularis are summarized in Table 1. Both twitch and tetanic tensions generated in the red IF are less than that in the white region, although the red IF has a twofold greater tetanic-twitch ratio than the white IF. At 40° C, both regions generate peak twitch tension in less than 27 msec; the red region, however, takes 50% longer to reach one-half relaxation than the white region.

The muscle fibers of the red region show substantial fatigue resistance relative to fibers in the white region. After a standardized 5-minute stimulation regime, fibers in the red region still generated approximately 80% of their initial peak tension. In contrast, white region fibers fatigued readily and after 5 minutes of stimulation generated only 30% of their initial tension.

ACh sensitivity. The red and white regions of four iliofibulari muscles were tested for acetylcholine (ACh) sensitivity to detect the functional presence of tonic fibers. The white region, shown histochemically to contain 98–100% FG fibers, showed no contractile response

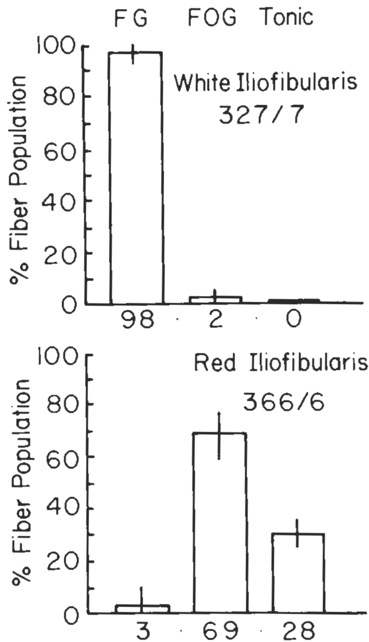

Fig. 2. Distribution of fiber types in *Dipsosaurus* red and white iliofibularis. Numbers along horizontal axis represent percentages of the total population within each region composed of each fiber type. Ratios denote mean number of fibers counted per animal/sample size.

to repeated acetylcholine applications. Application of acetycholine to the bath containing the red iliofibularis resulted in a contractile response in each of four muscles. In three muscles, repeated ACh application caused a slow contracture which generated tensions of 29–130% of the twitch tension stimulated electrically just prior to ACh application. In contrast to the milliseconds required by the muscle to twitch and relax, ACh contraction at 40° C required 12–15 seconds to generate peak tension, and another 20–35 seconds to reach one-half relaxation. Acetylcholine sensitivity was also demonstrated in a forearm muscle of *Dipsosaurus*, the flexor palmaris superficialis (FPS). Histochemical evaluation of the red regions of both the FPS and iliofibularis demonstrate an abundance of FOG fibers in addition to tonic fibers (Fig. 1; Putnam et al., '80).

We tested the possibility that FOG fibers rather than our presumed tonic fibers were sensitive to ACh by testing the ACh sensitivity of the peroneus muscle. The peroneus is a lower hindlimb muscle whose white region contains FG and FOG fibers, but no tonic fibers (Putnam et al., in press). The twitch and tetanic contractile properties of the peroneus are summarized in Table 1. In four experiments, the FG and FOG fibers of the peroneus showed no sensitivity to acetylcholine application.

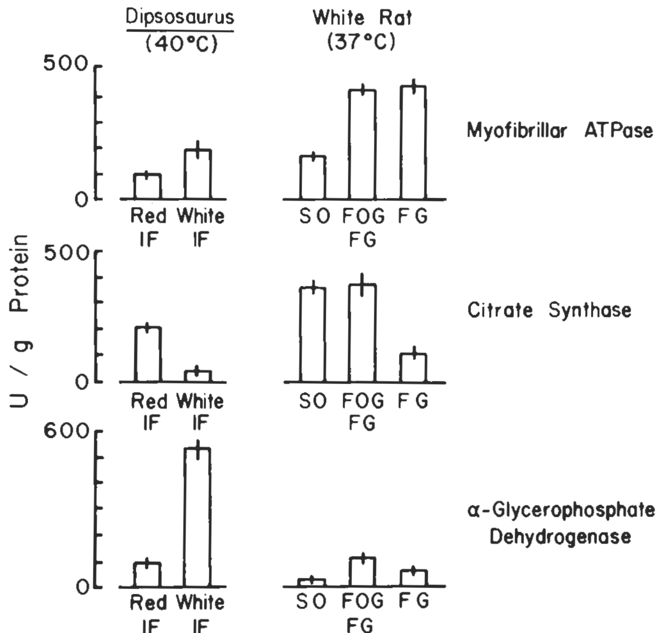

Fig. 3. Myofibrillar ATPase, citrate synthase, and α-glycerophosphate dehydrogenase activities in muscles of *Dipsosaurus* and rat. Red and white iliofibularis as defined in Figure 1. Rat fibers are derived from the following hindlimb muscles: SO, soleus; FOG and FG mix, plantaris; FG, white vastus lateralis. Temperatures denote enzyme reaction and body temperatures.

DISCUSSION

Fiber types in Dipsosaurus

Histochemical analysis of *Dipsosaurus* iliofibularis muscle for myosin ATPase, SDH, and αGPDH has allowed us to characterize three distinct fiber types according to their contractile speeds (Bárány, '67) and by their oxidative and glycolytic capacities. Enzymatic and contractile data support our histochemical interpretation.

Fast-glycolytic (FG) fibers of the white iliofibularis are large muscle fibers which stain darkly for mATPase and αGPDH. The enzymatic and contractile profile of this fiber type is based on the properties of the white IF, which is nearly 100% FG fibers (Fig. 2).

Fast-twitch-glycoltyic fibers possess five times the glycolytic activity and twice the mATPase activity as fibers making up the red IF, indicating that these fibers are adapted to high rates of energy utilization. This view is supported by the high rate of tension generation and rapid fatigue of these fibers (Table 1). The low oxidative activity of FG fibers suggest that a large portion of the energy they utilize during vigorous contraction is generated via anaerobic glycolysis. This fiber type represents the majority of muscle fibers in *Dipsosaurus* (Putnam et al., '80), and the enzymatic profile of this fiber closely reflects the large anaerobic capacity that this animal utilizes during vigorous muscular activity (Bennett and Dawson, '72).

Fast-twitch-oxidative-glycolytic (FOG) fibers are smaller in diameter than FG fibers and have high histochemical activities of all three enzymes. SDH and αGPDH staining intensities are quite dark, so one may infer that FOG fibers are metabolically the most active of all three fiber types. Enzymatic analysis of lizard FOG fibers was limited by our inability to locate a pure FOG region in any of 13 *Dipsosaurus* skeletal muscles (Putnam et al., '80). The biochemistry of the FOG fiber is reflected in the enzymatic activity of the 3:1 mix of FOG and tonic fibers in the red iliofibularis. The similar intense staining of FOG and tonic fibers for SDH in the red IF, coupled with the high enzymatic citrate synthase activity in that region of the muscle (Fig. 3), suggest that lizard FOG and tonic fibers are several times more oxidative than lizard FG fibers.

The contractile properties of FOG fibers are represented by the twitch characteristics of the red iliofibularis (Table 1). Tonic fibers do not respond mechanically to the brief (1 msec) electrical stimulus (Luff and Proske, '79) of the type used in this study; thus, the twitch characteristics of the red IF are due to FOG activity alone. The key contractile properties of the FOG fiber are its rapid twitch-contraction-time (CT) and its high fatigue resistance. The CT of FOG fibers is fast, supporting their high histochemical mATPase activity. When repeatedly stimulated to twitch, FOG fibers show nearly three times the resistance to fatigue relative to FG fibers. FOG fatigue resistance is reflective of its higher oxidative capacity.

The tonic fibers of Dipsosaurus stain lightly for mATPase, dark for SDH, and moderate for

TABLE 1. Contractile properties of two hindlimb muscles in Dipsosaurus[1]

Contractile characteristic	Illiofibularis Red region	Illiofibularis White region	Peroneus White region
Twitch CT (msec)	25 ± 1 (9)	21 ± 1[2] (9)	23 ± 1 (4)
Twitch 1/2 RT (msec)	28 ± 3 (9)	18 ± 1[3] (9)	18 ± 3 (4)
Tetanic tension (gm/cm²) (P_0)	523 ± 85 (9)	756 ± 81[2] (9)	1,533 ± 188 (4)
Fusion freq (Hz)	55 ± 1 (9)	58 ± 2[2] (9)	56 ± 1 (4)
Twitch tension (gm/cm²) (P_t)	136 ± 31 (9)	409 ± 49[3] (9)	705 ± 85 (4)
P_0/P_t	4.4 ± 0.5 (9)	1.9 ± 0.1[3] (9)	2.20 ± 0.21 (4)
Fatigue index	0.78 ± 0.07 (5)	0.29 ± 0.07[3] (5)	—
Acetylcholine sensitivity	+ (4)	— (4)	— (4)

[1]40° C, x ± SEM (sample size).
[2]White region different than red region: $P < 0.05$.
[3]White region different than red region: $P < 0.005$.

αGPDH. This histochemical pattern is similar to that of mammalian slow-twitch-oxidative fibers (Barnard et al., '71; Peter et al., '72). Our interpretation of this fiber type as tonic is based on its contractile properties. Slow and prolonged contraction in response to acetylcholine application is a characteristic attributed to amphibian and mammalian tonic fibers (Kuffler and Vaughan-Williams, '53; Hess and Pilar, '63; Lännergren and Smith, '66; Engel and Irwin, '67; Lehmann and Schmidt, '79). The red region of the iliofibularis is acetylcholine-sensitive, as are other muscles which contain fibers with the above histochemical characteristics. Regions of muscles which do not contain this type of fiber, on the other hand, do not respond to ACh. The tetanic-twitch tension ratio of the red IF also suggests the presence of tonic fibers. Lizard tonic fibers have been shown to respond only to electrical stimulation of 10 Hz or greater (Proske and Vaughan, '68); thus, tonic fibers may be recruited during tetanic tension determination but not during twitch tension determination. This would result in high tetanic-twitch tension ratios (P_0/P_t) for muscles containing tonic fibers. We believe the high P_0/P_t ratio of the red iliofibularis relative to the white region indicates the presence of tonic fibers in the red IF. A similar distribution of tonic fibers is found in the iliofibularis of amphibians (Kuffler and Vaughan-Williams, '53; Lännergren and Smith, '66; Smith and Ovale, '73; Luff and Proske, '79).

Enzymatic analysis of tonic fibers was restricted by their low density distribution. The data reported in Figure 3 can only be interpreted as suggesting that lizard tonic fibers possess myofibrillar ATPase and αGPDH activities substantially lower than those found in FG and FOG fibers.

Close examination of the staining intensities revealed that approximately 30–50% of the fibers which we consider as tonic stained slightly more intensely for mATPase than the other tonic fibers. These fibers may represent a mammalianlike SO group, although the difference in staining intensity was very subtle and not always present. Another possibility is that these fibers represent a second type of tonic fiber, similar to the multiply innervated tonic fiber identified ultrastructurally by Finol and Ogura ('72, '77) and physiologically by Proske and Vaughan ('68). We made no attempt to characterize these fibers biochemically or functionally. Single motor-unit analysis, which has been used successfully in characterizing different mammalian fibers (Close, '67; Burke et al., '71; Frederick et al., '78) might further differentiate this low mATPase fiber type. We have been intentionally conservative in our classification of lizard fiber types, and do not feel that a qualitative technique such as histochemistry should be used alone to distinguish between very similar fiber types.

Comparisons to other vertebrate fiber types

Examination of *Dipsosaurus* iliofibularis muscle identifies two types of fast-twitch fibers and one tonic fiber type. A similar fiber type composition is found in other muscles of *Dipsosaurus* (Putnam et al., '80) and in other vertebrates. Two twitch and one tonic fiber types have been reported in anuran amphibians (Lännergren and Smith, '66; Engel and Irwin, '67; Luff and Proske, '79) and in snakes (Talesara, '73; Talesara and Mala, '76). Two twitch and two tonic fiber types were identified in the lizards *Tiliqua, Cnemidophorus,* and *Iguana,* based on ultrastructural or electrophysiological characteristics (Proske and Vaughan, '68; Finol and Ogura, '72, '77). There is abundant additional evidence for the presence of both twitch and tonic fibers in lizards (reviewed by Hess, '70; Guthe, in press), although most other studies were not designed to discriminate differences within these two classes of fibers. Two twitch and one tonic fiber types also compose the muscles of several bird species (Kiessling, '77; Suzuki and Tamate, '79).

Fast-twitch fibers common to both lizards and mammals (FG, FOG) appear functionally similar. Twitch contraction times and fatigue resistances of lizard FG and FOG fibers are very similar to their mammalian analogs (Table 2). Reptilian fast-twitch fibers differ from analogous fibers in mammals primarily in their metabolic capacities. Citrate synthase and αGPDH activities indicate that *Dipsosaurus* twitch fibers are, in general, roughly one-half as oxidative and several times more glycolytic than the analogous fibers found in rats (Fig. 3). The oxidative and glycolytic capacities of these fibers faithfully reflect the aerobic scopes and anaerobic capacities of the animals to which they belong. Maximal rates of mass-specific oxygen consumption in adult *Dipsosaurus* and rats differ by a factor of three (*Dipsosaurus:* 2.2 ml $O_2 \cdot g^{-1} \cdot hour^{-1}$, Bennett and Dawson, '72; Rat: 6 ml $O_2 \cdot g^{-1} \cdot hour^{-1}$, Shepherd and Gollnick, '76). Although maximum rates of anaerobic metabolism have not been directly compared in the two species, 2 minutes of vigorous activity in *Dipsosaurus* re-

TABLE 2. Twitch contraction time and fatigue resistance[1] in analogous muscle fibers from different vertebrates

	FOG[2]		FG[3]		SO[4]		
	CT[5] (msec)	Fatigue resistance	CT (msec)	Fatigue resistance	CT (msec)	Fatigue resistance	Reference
Cat (36° C)[6]	40	> 0.75	34	< 0.25	73	> 0.75	Burke et al. ('71)
Guinea pig (37° C)[6]	19	—	21	—	82	—	Barnard et al. ('71)
Rat (35–38° C)[6]	19	0.50	11	0.10	35	1.00	Close ('67) Edström and Kugelberg ('68)
Skunk (36° C)[6]	36	High	—	—	50–64	High	Frederick et al. ('78)
Xenopus (21–25° C)[6]	37–54	0.55–0.93	29	0.03–0.10	—	—	Lännergren and Smith ('79)
Dipsosaurus (40° C)[6]	25	0.78	21	0.29	—	—	This study

[1]Fatigue resistance defined as fraction of initial twitch standardized stimulus regime for set period: Period = 30 seconds, *Xenopus;* 5 minutes, *Dipsosaurus;* 2 minutes, cat; 10 minutes, rat.
[2]Fast-twitch-oxidative-glycolytic fiber.
[3]Fast-twitch-glycolytic fiber.
[4]Slow-twitch-oxidative fiber.
[5]Contraction time.
[6]Muscle temperature.

sults in blood lactic acid concentrations nearly twice that found in rats after 5 minutes (Bennett and Dawson, '72; Baldwin et al., '77a).

There are no fibers in mammalian locomotory muscles that are analogous to the tonic fibers in *Dipsosaurus.* Lizard tonic fibers are similar to amphibian tonic fibers in their distribution within the iliofibularis, their response to high-frequency electrical stimulation, and their acetylcholine sensitivity (Kuffler and Vaughan-Williams, '53; Lännergren and Smith, '66; Engel and Irwin, '67; Lehmann and Schmidt, '79). They differ from amphibian tonics in that they histochemically exhibit higher oxidative and glycolytic enzyme activities than are typical for amphibian tonic fibers (Lännergren and Smith, '66; Engel and Irwin, '67). In this regard, they more closely reflect avian tonic fibers (Kiessling, '77; Suzuki and Tamate, '79). Overall, reptilian tonic fibers have characteristics of both the contractile properties of amphibian tonics and a metabolic profile similar to tonic fibers in birds.

ACKNOWLEDGMENTS

We thank Dr. Ken Baldwin for biochemical assistance and Dr. Bob Josephson for the generous loan of equipment. This work was supported by the following grants: NSF PCM 77-24208 and NIH K04 AM00351 to A.F.B.; NIH AM06350-01 to T.T.G.

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