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Histochemical Determination of the Fiber Composition of Locomotory Muscles in a Lizard, *Dipsosaurus dorsalis*

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ABSTRACT A histochemical survey was done on the fiber composition of 12 different locomotory muscles in the lizard *Dipsosaurus dorsalis*. Three types of fibers were found in all muscles: (1) fast-twitch-glycolytic (FG); (2) fast-twitch-oxidative-glycolytic (FOG); and (3) tonic fibers. Virtually all locomotory muscles contain some tonic fibers. Most muscles have bulk white regions (containing mostly FG fibers) and distinct red, oxidative regions (with FOG and tonic fibers). These red regions are predominantly located around the joints in the hind limb muscles, and probably serve a postural and joint-stabilizing function. The predominance of FG fibers in the bulk white regions is well-correlated with the rapid, anaerobically supported predator escape behavior of *D. dorsalis*.

In a previous study (Gleeson et al., '80), histochemical techniques were developed for the iliofibularis (IF) muscle of a lizard, *Dipsosaurus dorsalis*. In the present study, we undertook a survey of the fiber composition of 12 locomotory muscles of this lizard. Our purposes in undertaking this study were (1) to determine if our histochemical techniques could be applied to other locomotory muscles; (2) to determine if fiber types not contained within the IF muscle existed in this lizard; (3) to determine if correlations exist between muscle function and fiber compositions; and (4) to assess the relationship between fiber composition of the locomotory muscles and the pattern of locomotion used by this lizard. We also compare our results on the fiber composition in lizard muscles to the results of a growing number of histochemical studies on the fiber types in other groups of vertebrates. A preliminary report of this work has already been made (Putnam et al., '80).

MATERIAL AND METHODS

Adult *Dipsosaurus dorsalis* (mean weight 36.4 gm, range 18.9 to 58.0 gm) of mixed sexes were maintained in the laboratory as previously described (Gleeson et al., '80). Lizards were used within 4 months of capture. All histochemical staining was performed from October through January.

Lizards were sacrificed by decapitation and the skin was removed from one hindlimb. The following muscles were dissected free from the thigh: iliotibialis (IT), femorotibialis (FT), puboischiotibialis (PIT), and iliofibularis (IF). From the shank, the following muscles were removed: extensor digitorum longus (EDL), gastrocnemius (G), peroneus (P), and pronator profundus (PP). A piece of longitudinal tail muscle (T) and the caudofemoralis (CF) were removed from the tail, and a piece of the rectus abdominis (RA) was taken from the abdomen. In addition, the flexor palmaris superficialis (FPS) was removed from the forearm. The penis retractor (PR) was removed from one male lizard. A dissection guide for the location of the hindlimb muscles is shown in Figure 1 (see also Snyder, '54; Romer, '70; Oldham and Smith, '75). The functions of these muscles and homologous muscles in amphibians and mammals are listed in Table 1.

All muscles were trimmed into roughly cylindrical pieces of about 5 mm in diameter and 10 mm high. These muscle pieces were frozen onto cryostat chucks using Tissue Tek II O.C.T. Compound in isopentane cooled in liquid nitrogen to around −160°C. The chucks and frozen muscles were stored for a maximum of 3 days at −20°C. Cross sections of 14 μm thickness were cut on an American Optical Cryo-Cut and the sections were mounted on glass coverslips.

Serial sections were stained for myosin ATPase (mATPase), succinic dehydrogenase (SDH), and α-glycerophosphate dehydrogenase

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TABLE 1. The muscles of the lizard D. dorsalis, *and their functions (included for reference are the amphibian and mammalian homologues of the lizard muscles)*

Muscle	Function	Amphibian homologue	Mammalian homologue
Caudofemoralis (CF)	retracts femur	caudalipuboischiotibialis	crurococcygeus
Extensor digitorum longus (EDL)	extends phlanges	tibialis anticus longus	ext. digitorum longus ext. hallicus longus peroneus tertius
Flexor palmaris superficialis (FPS)	flexes manus	palmaris longus	flexor digit. sublimis palmaris longus
Femorotibialis (FT)	extends shank	—	vasti
Gastrocnemius (G)	flexes pes	gastrocnemius	gastrocnemius
Iliofibularis (IF)	flexes shank	iliofibularis	gluteus maximus
Iliotibialis (IT)	extends shank	crureus glutaeus	rectus femoris
Peroneus (P)	flexes pes	peroneus	peroneus
Puboischiotibialis (PIT)	flexes knee	sartorius semitendinosus	gracilis
Pronator profundus (PP)	pronates pes	pronator profundus	tibialis posterior
Rectus abdominis (RA)	retracts ribs compresses abdomen	rectus abdominis	rectus abdominis
Longitudinal tail (T)	retracts tail	—	—

(αGPDH) as previously described (Gleeson et al., '80). The mATPase stain was modified from Guth and Samaha ('70) and entailed incubation of sections at 37°C, pH 9.4 for 10 minutes (no preincubation) followed by $CoCl_2$-$(NH_4)_2SO_4$ staining. The SDH stain was modified from Nachlas et al. ('57) and the αGPDH stain was modified from Wattenberg and Leong ('60). Both stains require that sections be incubated for 2 hours at 37°C. After drying, the coverslips with stained muscle sections were mounted with Depex on glass slides, photographed, and scored for fiber type composition. Fibers were divided into three classes based on their staining characteristics—fast-twitch-glycolytic (FG): mATPase dark, SDH light, αGPDH dark; fast-twitch-oxidative-glycolytic (FOG): mATPase, SDH and αGPDH dark; and tonic: mATPase light, SDH dark, and αGPDH moderate. In most muscles, red pockets were visible. Therefore, the fiber composition of a representative region of the bulk of the muscle and from another region representing the red pocket was determined.

In a separate group of lizards, individual muscles were dissected and weighed. The red regions were carefully dissected from the white regions and both regions were weighed. From this, a quantitative estimate was obtained of the proportion of each muscle which the red region comprised.

RESULTS

The intensity and pattern of histochemical staining were similar in all muscles examined and corresponded to those established previously for the IF muscle (Gleeson et al., '80). No fibers which were not clearly assignable to either of the FG, FOG, or tonic categories were found in any muscle. Distinct red and white regions were observed in all muscles except the longitudinal tail muscles. The position and general size of the red regions in various hindlimb muscles are shown in Figure 1. These red regions fall into two broad categories. In some muscles, the red regions consist of a discrete band running the length of the muscle, as in the IF and EDL muscles. In the FT, IT, PIT, G, P,

and CF muscles, the red regions are localized pockets. The red regions of all muscles are medial with respect to the bone. In the hindlimb these regions are localized around the hip, knee and ankle joints. Table 2 shows the relative weights of the red and white regions in these muscles. Distinct red regions could not be dissected from the RA, T, or FPS muscles. In the case of the FPS, the entire muscle appeared red, but on staining a cross section, nearly 40% of the area had a predominance of FG fibers (defined as bulk for consistency with the other muscles). The size of the red region relative to the muscle mass was low (around 15%), did not differ significantly among hindlimb muscles, and was not correlated with gross muscle function in any obvious way. The only exceptions are the PP muscle—an ankle stabilizing muscle (Snyder, '54)—and the FPS. In both these muscles, the red region comprises 55–60% of the muscle mass.

The fiber-type compositions of the white and red regions of the various muscles are shown in Figures 2 and 3. The muscles are categorized by function and location in the body. White regions in all muscles are predominantly FG fibers, containing at most 30% FOG fibers. The red pockets are oxidative, with a roughly equal number of FOG fibers and highly oxidative tonic fibers. No general correlation between fiber composition and muscle location or function was apparent. The penis retractor muscle was composed of 75% FOG and 25% tonic fibers.

DISCUSSION

The three major findings of this study are the presence of tonic muscle fibers in all locomotory muscles examined, the compartmentalization of oxidative regions in most muscles, and the association between tonic and oxidative muscle fibers.

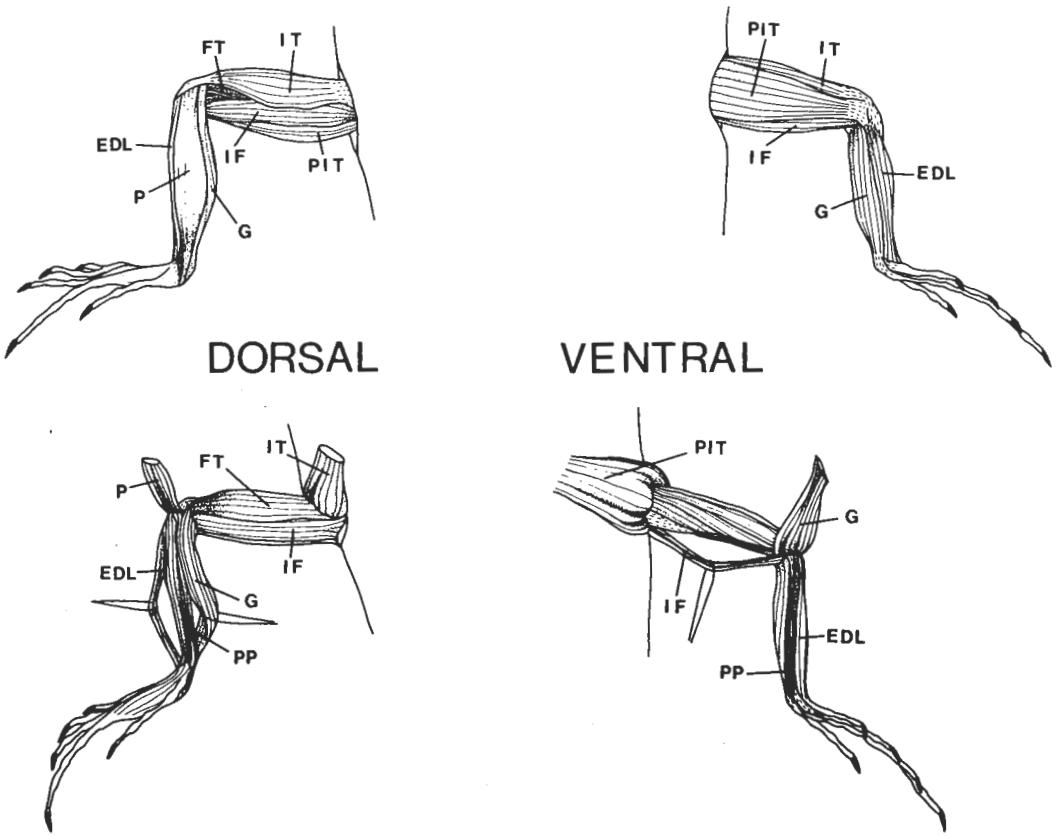

Fig. 1. Dorsal and ventral view of the muscles of the hindlimb of the lizard, *D. dorsalis*. The red regions are indicated with heavy shading. For a key to muscle names see Table 1.

TABLE 2. Muscle mass (as a % of body mass) and the relative size of the red region (as a % of the mass of a given muscle) in various muscles of D. dorsalis *(values reported are the mean (range in parenthesis) for three lizards whose average mass was 45.1 gm (range 34.0 to 55.1 gm))*

	Muscles									
	CF	EDL	FPS	FT	G	IF	IT	P	PIT	PP
Muscle mass (% body mass)	1.21 (1.01–1.35)	0.09 (0.06–0.10)	0.10 (0.07–0.13)	0.29 (0.25–0.35)	0.65 (0.51–0.76)	0.20 (0.14–0.30)	0.29 (0.24–0.33)	0.17 (0.12–0.23)	1.09 (0.95–1.18)	0.07 (0.06–0.07)
Red region mass (% muscle mass)	12.0 (8.7–15.1)	25.7 (11.9–39.5)	— —	23.6 (16.2–31.3)	8.9 (4.4–14.7)	23.7 (19.4–30.8)	16.7 (16.6–17.0)	11.7 (11.4–12.1)	10.8 (3.3–13.4)	54.6 (51.5–57.3)

Tonic muscle fibers have been reported in locomotory muscles of lampreys (Meyer, '79), elasmobranchs (Bone, '66), fish (Hidaka and Toida, '69; Hess, '70), amphibians (Lännergren and Smith, '66; Engel and Irwin, '67; Smith and Ovalle, '73), reptiles (Hess, '63; Proske and Vaughan, '68; John, '70; Ridge, '71; Finol and Ogura, '77), and birds (Hess, '61; Kiessling, '77; Simpson, '79), but not in mammals (Ariano et al., '73; Burke, '78). However, tonic fibers have been found in the extraocular and ear muscles of a variety of mammals (for review see Hess, '70). The significance of the apparent evolutionary loss of tonic fibers from mammalian locomotory muscles is unknown but the similarities between amphibian tonic fibers and mammalian intrafusal fibers have been discussed (Kuffler and Vaughan Williams, '53).

Tonic fibers are not believed to be directly involved in locomotion (Hess, '70; Goldspink, '77), so their widespread presence in the locomotory muscles of *D. dorsalis* is notable. In birds, flight muscle is composed solely of twitch fibers, although other wing muscles associated with posture and leg muscles contain tonic fibers (Hess, '70; Simpson, '79). It may be that flight requires such rapid and powerful contractions that the major flight muscles must contain only fast-twitch fibers. Anuran amphibians appear to be more similar to *D. dorsalis*, containing some tonic fibers in virtually all of their locomotory muscles (Smith and Ovalle, '73; Putnam, unpublished data). The widespread presence of tonic fibers in the locomotory muscles of lower vertebrate tetrapods may indicate a locomotory role for these fibers. Tonic fibers in the red muscle of fishes have been implicated in slow-speed locomotion (Bone, '66; Hidaka and Toida, '69). Alternatively, tonic fibers in terrestrial vertebrates would function as limb deccelerators by being stretched during activation by antagonistic muscles, or they could be used for postural support (Kuffler and Vaughan Williams, '53). Simpson ('79) attributes a joint-stabilizing function to tonic fibers in the postural muscles of bird wings. The prevalence of tonic fibers around the joints of the hindlimbs suggests a similar function in reptiles (see below and Fig. 1). The prolonged contraction and oxidative nature of tonic fibers suit them for either slow locomotion or prolonged functioning without fatigue.

The presence of distinct oxidative regions in locomotory muscles has been reported for fish (Boddecke et al., '59), amphibians (Lännergren and Smith, '66), and mammals (Gonyea and

Galvas, '79). Two types of red regions are seen in lizard hindlimb muscles. In the IF and the EDL muscles, the red zones run the length of the muscle on the medial surface, parallel with the white regions. In the G, P, IT, PIT, and FT muscles, the oxidative pockets are found in only a portion of the muscle and are localized near the joints (Fig. 1). The concentration of these localized red regions across joints suggests that maintained contraction of these fibers may serve to strengthen or stabilize the joints. The oxidative nature of the fibers and the presence of tonic fibers would allow for prolonged activity of the fibers in these pockets. A postural role for oxidative regions is indicated by the predominantly FOG and tonic fiber composition of the FPS muscle. When standing, *D. dorsalis* normally holds the anterior portion of its body elevated off the ground (Norris, '53). Continuous activity would be required by the FPS during this period, and the large number of tonic fibers in this muscle would be an adaptation for this prolonged activity. The long, red zones (in IF and EDL) may serve as limb stabilizers or be directly involved during locomotion. In particular, the red region of the IF could be used during low-speed thigh retraction or as a deccelerator of the limb in the recovery phase of the step cycle during high-speed locomotion. Alternatively, this red region may be continuously contracted during locomotion and thus function as an elastic tendon running from the thigh to the knee.

The function of the FOG fibers in the oxidative pockets is unclear. Gonyea and Galvas ('79) observed oxidative pockets, containing twitch

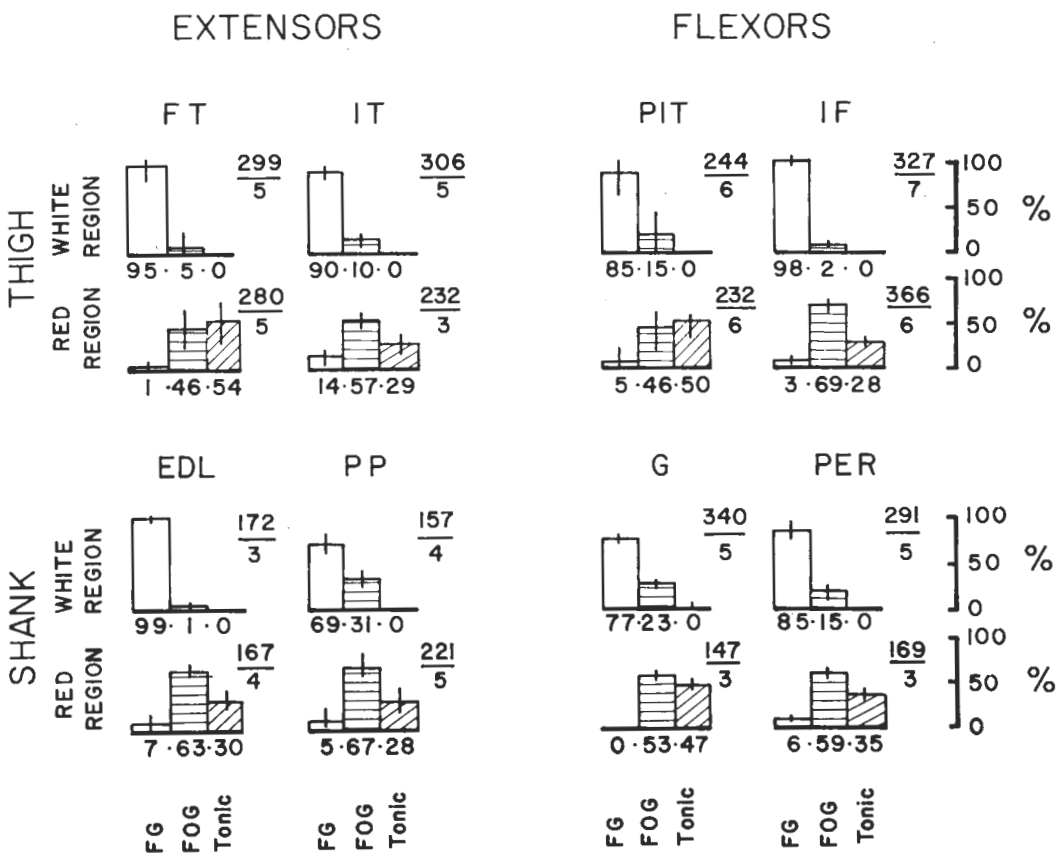

Fig. 2. The fiber composition of the red and white regions of the muscles in the hindlimb of *D. dorsalis*. For each region, from left to right, the histograms indicate the mean percentage fiber composition of FG, FOG, and tonic fibers, respectively. The vertical line within each histogram indicates the range of percentage composition for that fiber. The numbers below each histogram are the numerical value of the mean percentage composition for that fiber. The numbers to the side are the mean number of fibers counted per section over the sample size (number of animals) for each muscle.

fibers, in the muscles of various mammals. They found separate innervation bundles to oxidative and nonoxidative regions and differences in the spindle content of the different regions and suggested that oxidative regions are preferentially used for controlled, precise movement. The FOG fibers in the red pockets of lizards are fast-twitch fibers (Gleeson et al., '80) and therefore are probably involved in locomotion. *D. dorsalis* can maintain slow rates of walking for prolonged periods (John–Alder and Bennett, unpublished data) and may use oxidative fibers during such locomotion. Thus, we envision a prolonged postural and stabilizing function for the fibers in the red pockets, with the FOG fibers possibly active during low-speed locomotion and some tonic fibers serving a passive role during locomotion.

The association between the FOG and tonic fibers is striking. Kuffler and Vaughan Williams ('53) demonstrated that tonic fibers in frog IF muscles maintained a higher contraction tension if the twitch fibers in the IF were briefly tetanized first than if only the tonic fibers were activated. Such a synergism may be functioning between the FOG and tonic fibers in the red regions of lizard hindlimb muscles. Alternatively, the benefit of the segregation of oxidative elements may be in the fact that a virtually pure FG region remains. Since FG fibers represent the bulk of lizard hindlimb muscles and are used predominantly during activity, the white regions may function optimally if only FG fibers are present.

The fiber composition of locomotory muscles is well-correlated with the activity pattern of an organism. In fish, the extent of lateral red muscle correlates with the extent of activity, so that active swimming fish like salmon have a great deal of red muscle, whereas fish which only "sprint," such as perch or bullhead, have only white myotomal muscle (Boddecke et al., '59). In mammals, muscles which must function for prolonged periods are enriched in oxidative fibers. For instance, the ankle extensor muscles of skunks, which wander great distances during nocturnal foraging, contain only FOG and SO fibers, and completely lack FG fibers (Frederick and Goslow, '76). In addition, the content of slow fibers is high in the muscles of slow-moving mammals like the sloth (Goffart, '71), the slow loris (Ariano et al., '73), and the potto (Marechal et al., '76). The lizard *Dipsosaurus dorsalis* is an herbivore. It is highly territorial and forages slowly in a confined area. It can sustain only low-speed locomotion (around 1 km/hour) (John–Alder and Bennett, unpublished data). It can also undertake impressive bursts of activity, accelerating to 8 km/hour in less than 1 second (Bennett, '80) and attaining

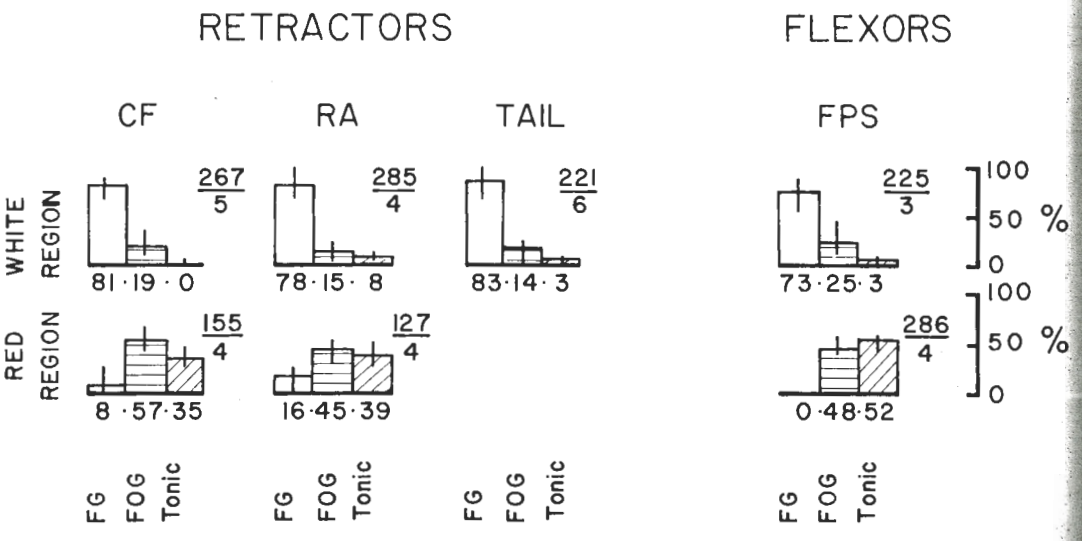

Fig. 3. The fiber composition of the red and white regions of a variety of other body muscles of *D. dorsalis*. All notations as in Figure 2.

speeds averaging 26 km/hour (Belkin, '61). During activity, the lizard relies heavily on anaerobic metabolism (Bennett and Dawson, '72). The predominance of FG fibers in the hind limbs of *D. dorsalis* is entirely consistent with such a rapid, anaerobically supported pattern of locomotion. The locomotory muscles of the lizard *D. dorsalis* may thus be a "two gear" system. The red regions are well adapted for postural support and perhaps low-speed locomotion over sustained periods. The bulk white regions are best suited to high-speed burst activity which is supported by glycolytic metabolism.

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