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Muscle fiber types in tetrapods. A comparative histochemical and morphometric study

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Summary. A comparative histochemical and morphometric study in two groups of homologous muscles from different tetrapods (rat, pigeon, lizard and frog) was performed. On the basis of their fiber diameters and oxidative enzyme activities, an initial correlation between fiber types of all animals is observed, although in the lizard and frog muscles, another fiber type does exists that could not be demonstrated in higher vertebrates. When more than one histochemical techniques are used for the identification of each tetrapod fiber types, the lack of correlation between them becomes obvious. Thus, different animals groups, each showing a characteristic muscle metabolic pattern, could be distinguished.

Key words: Muscle - Tetrapods - Histochemistry - Morphometry

Introduction

Histochemical and physiological studies have demonstrated that the vertebrate skeletal muscles consist of an heterogeneous population of fibers (Dubowitz and Pearse, 1960; Ogata and Mori, 1964; Engel and Irwin, 1967; Edstrom and Kugelberg, 1968; Smith and Ovalle, 1973; Talesara and Mala, 1978). Also, it is generally assumed that in mammals and birds the histochemical techniques for oxidative, glycolytic and ATPasic enzymatic activities show the existence of three main muscle fiber types (Stein and Padykula, 1962; Padykula and Gauthier, 1967; Edgerton and Simpsom, 1969; Brooke and Kaiser, 1970; Barnard et al., 1971). On the other hand, there is not a similar agreement about the number of fiber types in lower vertebrate muscles. Thus, in amphibians Ogata (1958) described three different kinds of muscle fibers, whereas Engel and Irwin (1967) and Smith and Ovalle (1973) identified five fiber types. In reptiles, Talesara and Mala (1978) reported three major fiber types with several subtypes.

Comparative analyses between the muscle fiber types of lower and higher vertebrates are scanty, and the hitherto reported results show important differences between them. While Ogata (1958) and Ogata and Mori (1964) reported, on the basis of the SDH activity, three common histochemical fiber types in fishes, frogs, birds and mammals, Engel and Irwin (1967) pointed out that there are not any mammalian fibers known to have the same histochemical profiles as either the twitch or tonic fibers of the frog. In this sense, Dubowitz and Pearse (1960) demonstrated that the inverse relationship between the respective oxidative and glycolytic activities observed in rat and pigeon muscle fibers does not exist in frogs and fishes.

The purpose of this study is to provide comparative data on the histochemical and morphometric characteristics of muscle fibers between amphibians, reptiles, birds and mammals.

Materials and methods

Five adult animals of both sexes belonging to each of the tetrapod classes were used: Mammals (Wistar rats), Birds *(Columba livia),* Reptiles *(Lacerta lepida)* and Amphibians *(Rana temporaria).* All the animals were fed "ad libitum", and kept in spacious cages. The animals were sacrified by decapitation after ether anesthesia. Two homologous muscle groups were excised from the posterior limb (Table 1).

Muscle specimens were frozen in liquid-nitrogen-cooled isopentane. Serial 10 μm thick cryotome transverse sections were processed for histochemical localization of the following enzyme activities: NADH-tetrazolium reductase

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(NADH-TR) (Scarpelli et al., 1958); menadione-linked-glycerophosphate dehydrogenase (M-GPDH) (Wattenberg and Leong, 1960); phosphorylase (Takeuchi and Kuriaki, 1955; Eranko and Palkama, 1961); alkaline myosin adenosine triphosphatase (pH 9.4) after fixation in buffered formaldehyde (pH 7.4) (Guth and Samaha, 1970; Davies and Gunn, 1972) and acid myosin adenosine triphosphatase (pH 4.6 and 4.2) (Brooke and Kaiser, 1969).

The muscle fiber types of the different animals were identified according to the histoenzymatic oxidative activity (NADH-TR) and the distribution of reaction product (Novikoff et al., 1961; Engel and Irwin, 1967; Barnad et al., 1971). The terminology of Padykula and Gauthier (1967), Guth and Samaha (1969) and Edgerton and Simpson (1969) was used for rat and pigeon fiber types and that of Smith and Ovalle (1973) for lizard and frog.

The cross sectional diameter of each fiber type was measured on NADH-TR stained sections using a granulometric analyzer TGZ-3 (Zeiss) according to Dubowitz and Brooke (1973).

Results

All examined muscles showed a heterogeneus population of fibers that could be identified by their enzyme activities and diameters. In lizard and frog fibers the position of the nuclei was also considered. The results obtained for each one of the species are shown in tables 2 to 5.

Rat: Three main fiber types could be identified: 1) Small red fibers, with a high NADH-TR activity and a dispersed reaction product showing a tendency to form subsarcolemic aggregates. 2) Large white fibers, showing low NADH-TR activity and a mainly subsarcolemmal-distributed reaction product. 3) Intermediate fibers, exhibiting an enzyme activity and diameter between those of the red and white fibers, but lacking of subsarcolemmal agregates of diformazan (Fig. 1).

On serial sections red fibers showed moderate M-GPDH and phosphorylase activities, high alkaline (pH 9.4) and low acid (pH 4.2) ATPase activities. However, white fibers exhibited high M-GPDH and phosphorylase activities and moderate alkaline and low acid ATPase activities. Intermediate fibers generally showed low M-GPDH, phosphorylase and alkaline ATPase activities and very intense acid ATPase staining (Fig. 2).

Pigeon: The histochemical profiles of the pigeon muscles were essentially similar to those of the rat (Fig. 3), although some minor differences are evident. Thus, the three pigeon fiber types were more homogeneously NADH-TR satained than those of the rat muscles (Fig. 4). Also, the diameter differences between each one of the three pigeon fiber types are less wide than in rat, since the intermediate pigeon fibers are similar or slightly smaller than the red fibers (Table 3).

Moreover, while the rat white fibers are formaldehyde-sensitive with moderate alkaline ATPase activity, the pigeon white fibers are formaldehyde-resistent and show high alkaline ATPase activity (Fig. 5). Also, acid ATPase activities (pH 4.6) of both red and white rat and pigeon fibers were different. Since rat red and white fibers exhibit respectively a low and moderate activity, in the pigeon we found an inverse pattern (Fig. 6).

Lizard: Four fiber types were identified: Large fibers (Type 1), showing inner nuclei and low NADH-TR activity with subsarcolemmal diformazan aggregates. Small fibers (Type 4), showing subsarcolemmal nuclei and low NADH-TR activity without aggregates of diformazan. Types 2 and 3 showed diameters ranging between those of the fiber types 1 and 4. Their nuclei were deeply seated in the sarcoplasm and also under the cell membrane. Both fiber types showed high NADH-TR activity with subsarcolemmal aggregates of diformazan. The enzyme activity is most intense in smallest fibers (Type 3) (Fig. 7).

In serial sections, type 1 fibers show low M-GPDH activity, high phosphorylase activity, moderate alkaline (pH 9.4) and very low acid (pH 4.6 and 4.2) ATPase staining. Type 4 fibers exhibited a low enzyme activity with all techniques used. Both fiber types 2 and 3 showed high M-GPDH activity, moderate phosphorylase activity and high alkaline and acid (pH 4.6) ATPase staining, with the last decreasing slightly at pH 4.2 (Fig. 8).

Frog: In the frog muscles four fiber types could also be identified. Their histochemical profiles were similar to that of the lizard (Figs. 9 and 10), but some differences in the diameter and ATPase activities can be pointed out. Thus, each frog fiber type is thicker than the respective lizard one; the lizard type 4 fiber is the smallest one (Table 4), but in the frog this type shows a diameter greater than that of types 2 and 3 (Table 5).

ATPase activities of the frog fibers are more sensitive to low pH than the lizard ones. Thus, all frog fiber types show very low ATPase staining at pH 4.2 and at PH 4.6 is comparatively lower than that found in lizards (Fig. 11).

Fig. 1

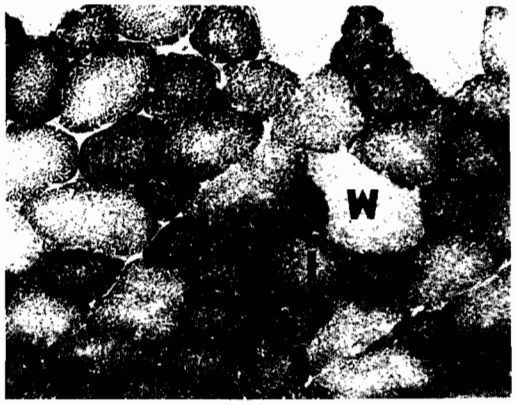


Fig. 2

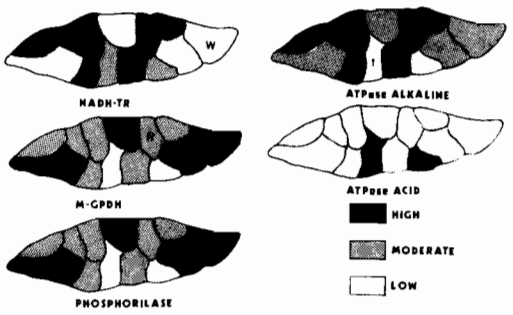


Fig. 3

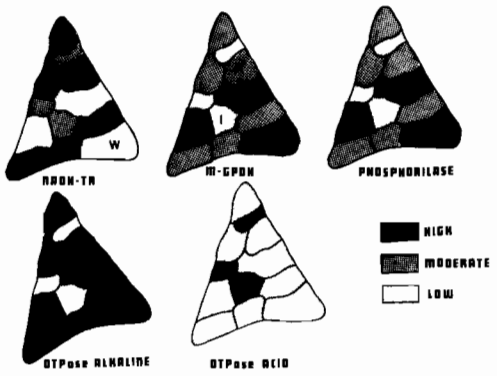
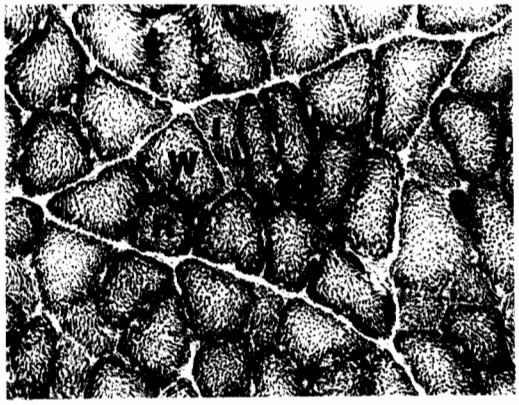


Fig. 4



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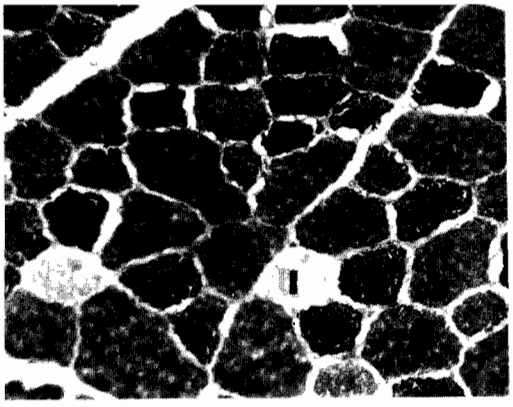


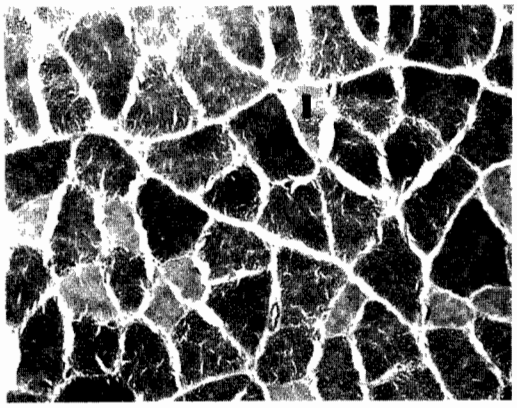
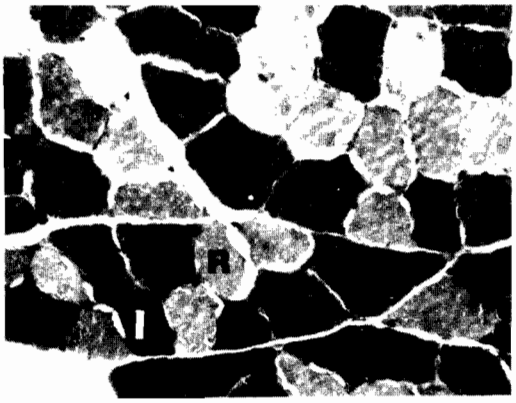


Fig. 6



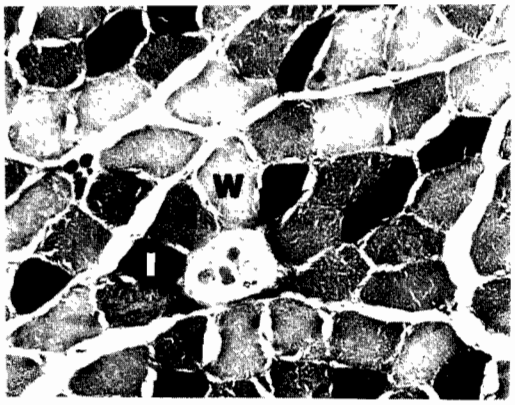


Fig. 7

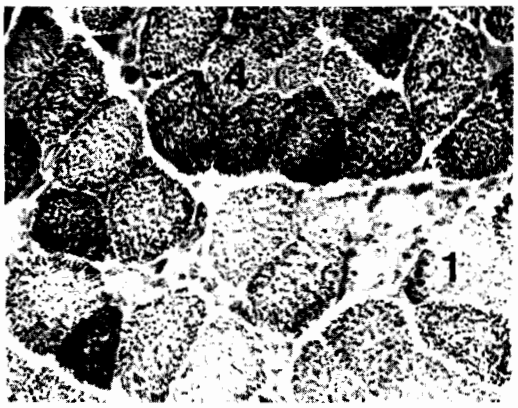


Fig. 8

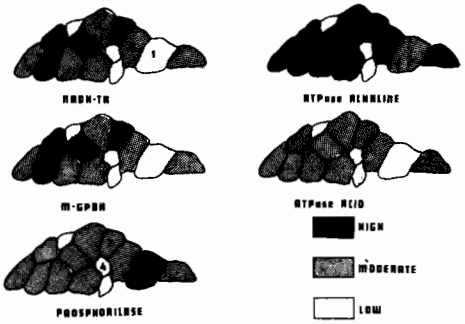


Fig. 9

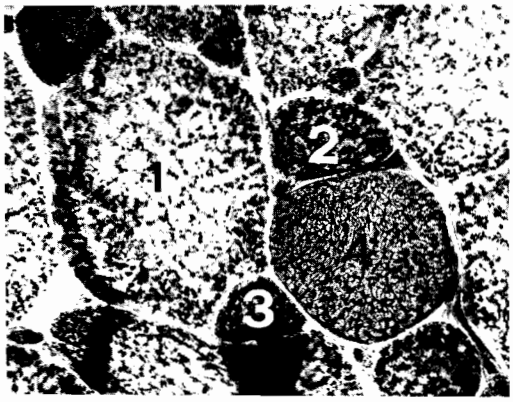


Fig. 10

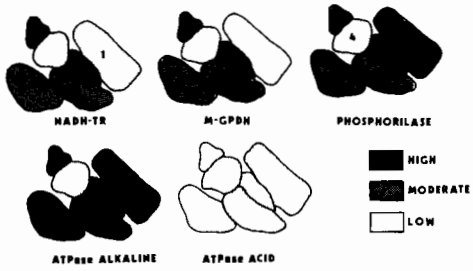
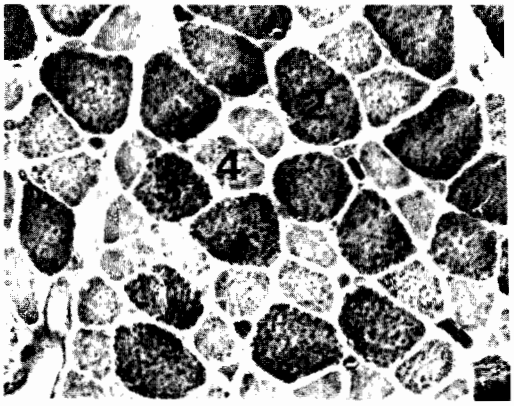


Fig. 11



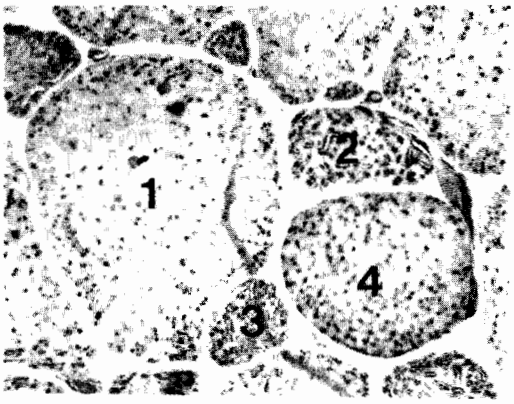


Fig. 1. Fiber types of rat muscle. NADH-TR. X 200
Fig. 2. Histoenzymatic profile of rat muscle fibers.
Fig. 3. Histoenzymatic profile of pigeon muscle fibers.
Fig. 4. Fiber types of pigeon muscle. NADH-TR. X 200
Fig. 5. Note formaldehyd-sensitivity differences between rat and pigeon white fibers. ATPase alkaline (pH 9.4). X 200
Fig. 6. Note activity differences between red and white rat fibers and those of pigeon muscle. ATPase acid (pH 4.6). X 200
Fig. 7. Fiber types of lizard muscle. NADH-TR. X 200
Fig. 8. Histoenzymatic profile of lizard muscle fibers.
Fig. 9. Fiber types of frog muscle. NADH-TR. X 200
Fig. 10. Histoenzymatic profile of frog muscle fibers.
Fig. 11. pH-sensitivity differences between lizard and frog muscle fibers can be noticed. ATPase acid (pH 4.6). X 200

Table 1. Groups of homologous muscles selected for this study.
(Romer and Parsons, 1977)

	ANIMAL	MUSCLE
GROUP 1	RAT	Tibialis anterior (TA)
	PIGEON	Tibialis anterior (TA)
	LIZARD	Tibialis anterior (TA)
	FROG	Extensor cruris brevis (ECB)
GROUP 2	LIZARD	Extensor digitorum comunis (EDC)
	FROG	Tibialis anticus longus (TAL)

Table 2. Enzyme activities and diameters of the rat muscle fiber types.

	RED	INTERMEDIATE	WHITE
NADH-TR	High	Moderate	Low
M-GPDH	Moderate	Low	High
Phosphorylase	Moderate	Low	High
ATPase alkalina (9.4)	High	Low	Moderate
APTase acid (4.6)	Low	High	Moderate
ATPase acid (4.2)	Low	High	Low
Diameters (μm)	31.87 $\pm$ 2.95	34.56 $\pm$ 3.42	49.03 $\pm$ 5.18

Table 3. Enzyme activities and diameters of the pigeon muscle fiber types.

	RED	INTERMEDIATE	WHITE
NADH-TR	High	Moderate	Low
M-GPDH	Moderate	Low	High
Phosphorylase	Moderate	Low	High
ATPase alkaline (9.4)	High	Low	High
APTase acid (4.6)	Moderate	High	Low
ATPase acid (4.2)	Low	High	Low
Diameters (μm)	33.75 $\pm$ 1.75	31.97 $\pm$ 0.29	43.77 $\pm$ 1.85

Table 4. Enzyme activities, diameters and location of the nuclei of the lizard muscle fiber types.

	TYPE 1	TYPE 2	TYPE 3	TYPE 4
NADH-TR	Low	Moderate	High	Low
M-GPDH	Low	Moderate	High	Low
Phosphorylase	High	Moderate	Moderate	Low
ATPase alkaline (9.4)	Moderate	Hig	High	Low
APTase acid (4.6)	Very Low	High	High	Low
ATPase acid (4.2)	Very Low	Moderate	Moderate	Low
Diameters (μm)	111.5 $\pm$ 18.06	80.1 $\pm$ 11.84	71.1 $\pm$ 6.23	65.55 $\pm$ 11.57
Location of the nuclei	Inner	Inner or Subsarcolemmal	Iner or Subsarcolemmal	Subsarcolemmal

Table 5. Enzyme activities, diameters and location of the nuclei of the frog muscle fiber types.

	TYPE 1	TYPE 2	TYPE 3	TYPE 4
NADH-TR	Low	Moderate	High	Low
M-GPDH	Low	Moderate	High	Low
Phosphorylase	High	Moderate	Moderate	Low
ATPase alkaline (9.4)	High	High	High	Low
ATPase acid (4.6)	Very Low	Moderate	Moderate	Low
ATPase acid (4.2)	Low	Low	Low	Low
Diameters (μm)	134.77 ± 8.92	97.98 ± 0.25	54.54 ± 4.56	99.3 ± 1.18
Location of the nuclei	Inner	Inner or Subsarcolemmal	Inner or Subsarcolemmal	Subsarcolemmal

Table 6. Fiber mean diameters in the animals studied.

ANIMALS	DIAMETERS (μm)
RAT	40.99 ± 3.25
PIGEON	38.65 ± 1.45
LIZARD	87.55 ± 11.70
FROG	100.88 ± 10.79

ABBREVIATIONS

I:	Intermediate fiber type
NADH-TR:	NADH-tetrazolium reductase
R:	Red fiber type
W:	White fiber type
1:	Type 1 fiber
2:	Type 2 fiber
3:	Type 3 fiber
4:	Type 4 fiber

Discussion

Histochemical profile of the fibers: From a comparative point of view we have found in muscles of all species studied several fiber types that can be identified on the basis of the different histoenzymatic techniques employed.

A possible correlation between the fiber diameters and oxidative activities can be pointed out. Thus, generally large fibers show low NADH-TR activity; small fibers exhibit high NADH-TR activity and fibers whose diameters are between those of the two first show intermediate NADH-TR activity. This results agree in some way with those reported by Ogata (1958) and Ogata and Mori (1964) on the SDH activity in fishes, frogs, birds and mammals. In all the studied species, these authors reported the presence of three main fiber types that they classified as white, red an intermediate. However, in the frog and lizard muscles, a fourth fiber type not included in Ogata's classification can be identified. It shows low oxidative enzyme activity but its diameter is generally smaller than that of the highly oxidative fibers. This fourth fiber type, not present in the rat and pigeon, has been also reported in the frog as "tonic fibers" by Engel and Irwin (1967) and as "small pale fibers" or "type 4 fibers" by Smith and Ovalle (1973).

When more than one histochemical tecnique are used for the identification of each fiber type the lack of correlation between the fiber types in the tetrapod classes becomes obvious. As is shown in tables 2 to 4, two main groups of animals may be considered: the first one comprising the rat and pigeon and the second one including the lizard and frog. Thus, those rat and pigeon fibers showing moderate oxidative activity, exhibit low M-GPDH, phosphorylase and alkaline ATPase activities and may correspond to the intermediate

fibers of Edgerton and Simpson (1979). In contrast, the same fiber type of lizard and frog shows moderate M-GPDH and phosphorylase activities and high ATPase staining and may correspond to the type 2 fibers of Smith and Ovalle (1973). Moreover, in the lizard and frog muscles, another fiber type (Type 4) not present in the rat and pigeon muscles, exhibiting low oxidative, phosphorylase, M-GPDH and alkaline ATPase activities can be found. This lack of correlation between muscle fiber types of fishes, amphibians, birds and mammals was reported by Dubowitz and Pearse (1960) on the basis of oxidative and phosphorylase activities. Likewise, on several muscles of the frog posterior limb, Engel and Irwin (1967) pointed out this fact based upon histophysiological studies.

On the other hand, the lizard and frog fibers of types 2 and 3 show both stable alkaline and acid ATPase activities. This fact has been noticed by Talesara and Mala (1978) in a comparative study between frog, lizard and turtle fibers. Usually, in birds and mammals each one of this two ATPase isoenzymes appear located in different fiber types, excepting the ocular muscles, as was reported by Yellin (1969).

Muscle fiber diameter: When the mean diameters of rat, pigeon, lizard and frog muscle fibers are compared (Table 6), the highest values correspond to those of the frog, while the smallest appear in rat and pigeon muscles. We did not found any previous data comparing the diameter ranges of fibers belonging to homologous muscles of different tetrapod classes. Within the same species, several authors demonstrated that this character depends not only upon genetic influences but on other factors as well. Thus, Goldspink (1964, 1965) found that physical training and nutrition may change muscle fiber mean diameter. When only one class of tetrapods is considered, body size constitutes another factor. So Hill (1956) postulated that fiber diameter would change as the square root of the linear size of the body do. Later on, this hypothesis has been supported by other reports on birds and mammals muscles (George and Naik, 1959; Gauthier and Padykula, 1966; Stickland, 1977). However, other authors (Davies and Gunn, 1972; Gunn, 1978) did not find such a relationship. Our results clearly show that the muscle fibers of cold-blood tetrapods are much larger than those of warm-blood ones.

Previous and also our own data suggest essential differences between lizard and frog muscle metabolic activities and that of the rat and pigeon; therefore it is not possible to generalize histophysiological consequences to both animal groups.

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