MUSCLE FIBER TYPES IN A MIGRATORY

AND A NON-MIGRATORY AVIAN SPECIES

A Thesis

Submitted to

the Division of Biological Sciences

Emporia State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Mark E. La Barge

August, 1990

AN ABSTRACT OF THE THESIS OF

___Mark E. La Barge___ for the ___Master of Science Degree___

in _____Biology_____ presented on _____August 1990_____

Title: ___Muscle Fiber Types in a Migratory and a Non-_____
___migratory Avian Species_____

Abstract approved: _____*Edward C. Rowe*_____

Most vertebrate skeletal muscles are composed of mixed populations of fibers that differ in their biochemical and contractile properties. Three fiber types can be distinguished by standard histochemical staining techniques: β-red fibers, which are rich in mitochondrial enzymes and have an acid-stable myosin ATPase; α-white fibers, which are poor in mitochondrial enzymes and have acid labile myosin ATPase; and α-red fibers, which are "intermediate" between the other two types.

In this study of bird muscle, one running/walking muscle (tibialis anterior) and one flight muscle (pectoralis) were studied in the migratory mourning dove and the non-migratory northern bobwhite. The muscles were fiber-typed and the mean size of each fiber type (in μm^2) and the percentage of muscle occupied by each fiber type determined. Superficial (outermost 3 mm) and deep samples were taken from the pectoralis. The tibialis anterior was sampled through its entire cross section.

β-red fibers were not found in the pectoralis of either species. Deep dove pectoralis is predominantly α-red

(68.3%). Superficial dove pectoralis is predominantly α-white (72.5%). The bobwhite pectoralis is predominantly α-white with a uniform scattering of α-red fibers and no statistical difference between superficial and deep regions. The bobwhite tibialis anterior has all three fiber types, including highly oxidative β-red fibers. The dove tibialis anterior is predominantly α-white.

The fiber composition of the pectoralis muscles of these two species reflects the pattern of usage of the muscles. The non-migratory bobwhite, which uses its pectoralis muscle only for short bursts of flight, contains mostly low-endurance α-white fibers. The pectoralis muscle of the dove, which is utilized during moderately long seasonal migrations, contains mostly higher endurance α-red fibers.

The fiber composition of the tibialis anterior muscle also reflects the pattern of usage in these two species. The tibialis anterior of the bobwhite (which would "rather run than fly") contains high endurance α-red and β-red fibers in addition to α-white fibers. The tibialis anterior of the dove, which contributes little to the seasonal migration of this species, contains predominantly α-white fibers.

R. J. Sokwala
Approved for Major Division

James L. Wolfe
Approved for Graduate Council

ACKNOWLEDGEMENT

I am very grateful to Dr. Robert Klemm and Dr. Georgeanne Whipple (Kansas State University) for demonstrating the techniques used in this study. Their expert advice is greatly appreciated. I am also indebted to Dr. Dwight Moore for advice on the statistical analysis and for help with other aspects of this study too numerous to mention. My thanks go out to Dr. Gaylen Neufeld for stepping in during the middle of this study and providing constructive comments on the manuscript.

I am especially grateful to my major advisor, Dr. Edward Rowe for his encouragement throughout the course of my graduate work. His willingness to locate and order the required chemicals, glassware, and other supplies made this research more troublefree than it would have been without his help.

I am very thankful to my parents, brothers, sister, and my son Brandon. Their love, support, encouragement, and understanding greatly contributed to my success at ESU.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In his review of muscle physiology, Hoyle (1983) complained that a whole generation of muscle physiologists, impressed by the breakthroughs in biochemistry and electron microscopy of muscle, had come to think of all muscles as being alike. However, it is now well understood that there are biochemical and physiological differences between the muscles of different animals, between muscles in the same animal, and between cells within muscles (Barnard et al., 1971; Close, 1972).

Histochemical methods known as fiber-typing provide the most practical way of demonstrating biochemical differences between muscle cells. Recently-developed fiber-typing techniques differentiate muscle into three categories. The β-red fiber type is small in diameter, contracts slowly, is fatigue-resistant but relatively weak, and is associated with muscles that exhibit a high level of endurance. The α-white fiber type has the largest diameter, has the fastest contractile rates, is rapidly fatigable, and is associated with muscles that produce great forces for short periods. The α-red fiber type is intermediate in size and contractile rates, is slowly fatigable, and is associated with muscles that produce moderate amounts of force for short periods. (Burke et al. 1973).

Many avian myology studies have described the proportions of each fiber type for many species, but very few have considered the functional relationship between

fiber types in a muscle and the muscle's role in locomotion.
(Turner and Butler, 1988).

In this study, one flight and one running/walking
muscle were studied in one migratory and one non-migratory
avian species. The muscles chosen were the pectoralis and
the tibialis anterior. The species chosen were the
migratory mourning dove (<u>Zenaida</u> <u>macroura</u>) and the non-
migratory northern bobwhite (<u>Colinus</u> <u>virginianus</u>). Based on
work with other bird species, I hypothesize that the dove
pectoralis is composed of a high percentage of α-red fibers
and the bobwhite pectoralis is composed of a high percentage
of α-white fibers. Furthermore, I believe that dove
tibialis anterior muscle is mainly α-red while bobwhite
tibialis anterior is mainly β-red.

<u>History of Fiber Typing</u>

The following is a brief historical account of
skeletal muscle fiber-typing. Dubowitz and Brooke (1973)
have compiled a detailed history of this topic.

As early as the 1600's, some muscle was catagorized
according to its color. However, researchers in that time
period lacked analytical techniques for detailed
descriptions and progress was slow to develop. In the
1930's, newly-developed stains for enzymes such as
phosphorylases, oxidoreductases, and hydrolases provided a
more reliable way to demonstrate differences in muscle fiber
types. These "modern" histochemical techniques also allowed

the correlation of enzyme content with contractile properties of individual fibers (Dubowitz and Brooke, 1973).

Ogata (1958) stained for the mitochondrial enzyme succinic dehydrogenase (SDH) in the muscles of various animals and described the following three fiber types: a small diameter fiber with strong SDH activity, a large diameter fiber with weak SDH activity, and a fiber with intermediate diameter and SDH activity.

Guth and Samaha (1969) demonstrated that myosin adenosine triphosphatase (ATPase) isozymes in rat, rabbit, and cat muscle could be differentiated with incubation solutions of a specific pH. Preincubation of tissue at pH 9.4 ("routine" method) differentiated "slow" fibers, which stained lightly, from "fast" fibers, which stained darkly. Lowering the pH to 4.35 resulted in a "reversal" of the staining intensities, i.e. the "slow" fibers stained darkly and the "fast" fibers stained lightly (Fig. 1). Brooke and Kaiser (1970) have reported similar results with their "reversed" myosin ATPase method in human muscle.

Recent Techniques

In early muscle-typing studies one section was stained for SDH, an adjacent section was stained for myosin ATPase, and the results for each fiber laboriously combined. In a search for a more convenient technique, Horak (1983) combined two previously standard but separate procedures. The "reversed" ATPase method developed by Guth and Samaha

4

Fig. 1. Histochemical Reactions in Human Muscle (modified from Dubowitz & Brooke, 1973). Density of dots indicate stain intensity.

Treatment	β-Red	α-Red	α-White
Routine myosin ATPase (pH 9.4)	⊙ **********	⬤ **********	⬤ **********
Reversed myosin ATPase (pH 4.3)	⬤ +	○ +	○ +
SDH	⬤	◐	◯
Solomon & Dunn's (1988) combined technique	= ⬤	= ◐	= ◯

(1969) was combined with a method for demonstrating SDH (Lojda, 1965). This combined technique allowed the demonstration of an oxidative enzyme (SDH) and myosin ATPase within a single section.

This technique was later modified by Solomon and Dunn (1988), using Humason's (1979) method for the demonstration of SDH activity and Troyer's (1980) advice for intensifying the stain for SDH by adding phenazine methosulfate. Solomon and Dunn's (1988) procedure utilizes the activity levels of two enzymes to differentiate three fiber types ("combined technique" in Fig. 1) and was the technique used in this study.

The system of nomenclature used in this report (β-red, α-red, α-white) was developed by Ashmore and Doerr (1971) and is the system preferred by Solomon and Dunn (1988). Fiber classifications used by other researchers cited in the discussion section of this report have been translated to their equivalents in this system (Table 1).

Theory of the Stains

SDH is an iron-sulfur flavoprotein enzyme that is an integral part of the inner mitochondrial membranes (cristae). This important Krebs cycle enzyme catalyzes the oxidation of succinate to fumarate (Fig. 2). Sodium succinate is provided in the incubation medium. Flavin adenine dinucleotide (FAD) is tightly bound to the SDH molecule. During the oxidation, two electrons (and two

Table 1. Approximately equivalent systems of nomenclature for muscle fiber types.

STUDY	YEAR	SYSTEM OF NOMENCLATURE		
Ogata	1958	Red	Intermediate	White
Dubowitz & Pearse	1960	Type 1	Intermediate	Type 2
Engel	1962	Type 1	-	Type 2
Close	1972	Slow Twitch	Intermediate	Fast Twitch
Dubowitz & Brooke	1973	Type 1	Type 2A	Type 2B
Guth & Samaha	1970	β	$\alpha\beta$	α
*Ashmore & Doerr	1971	β-Red	α-Red	α-White
Solomon & Dunn	1988	β-Red	α-Red	α-White
Peter et al.	1972	Slow Oxidative (SO)	Fast Oxidative Glycolytic (FOG)	Fast Glycolytic (FG)
Burke et al.	1973	Type S	Type FR	Type FF
Suzuki & Tamate	1979	C	A	B
Rosser & George	1986	Slow Tonic (ST)	FOG	FG

* indicates the system used in this report

8

Fig. 2. Succinate Oxidase System and the pathways
         leading to formazan production (modified
         from Dubowitz, 1985). Solid lines
         indicate the normal oxidative pathway;
         dashed lines indicate the pathway when
         visualizing agents are present.

Succinate   Fumarate

SDH-FADH$_2$ ▪ ▪ ▪ ➤ Phenazine Methosulfate

Ubiquinone (CoE Q) ▪ ▪ ➤ Tetranitro Blue Tetrazolium

Cytochrome b   Formazan

Cytochrome c

Cytochrome a

Cytochrome a$_3$

O$_2$

Succinate Fumarate

SDH-FADH$_2$ ▪ ▪ ▪ ▪ ➤ Phenazine Methosulfate

Ubiquinone (CoE Q) ▪ ▪ ➤ Tetranitro Blue Tetrazolium

Cytochrome b   Formazan

Cytochrome c

Cytochrome a

Cytochrome a$_3$

O$_2$
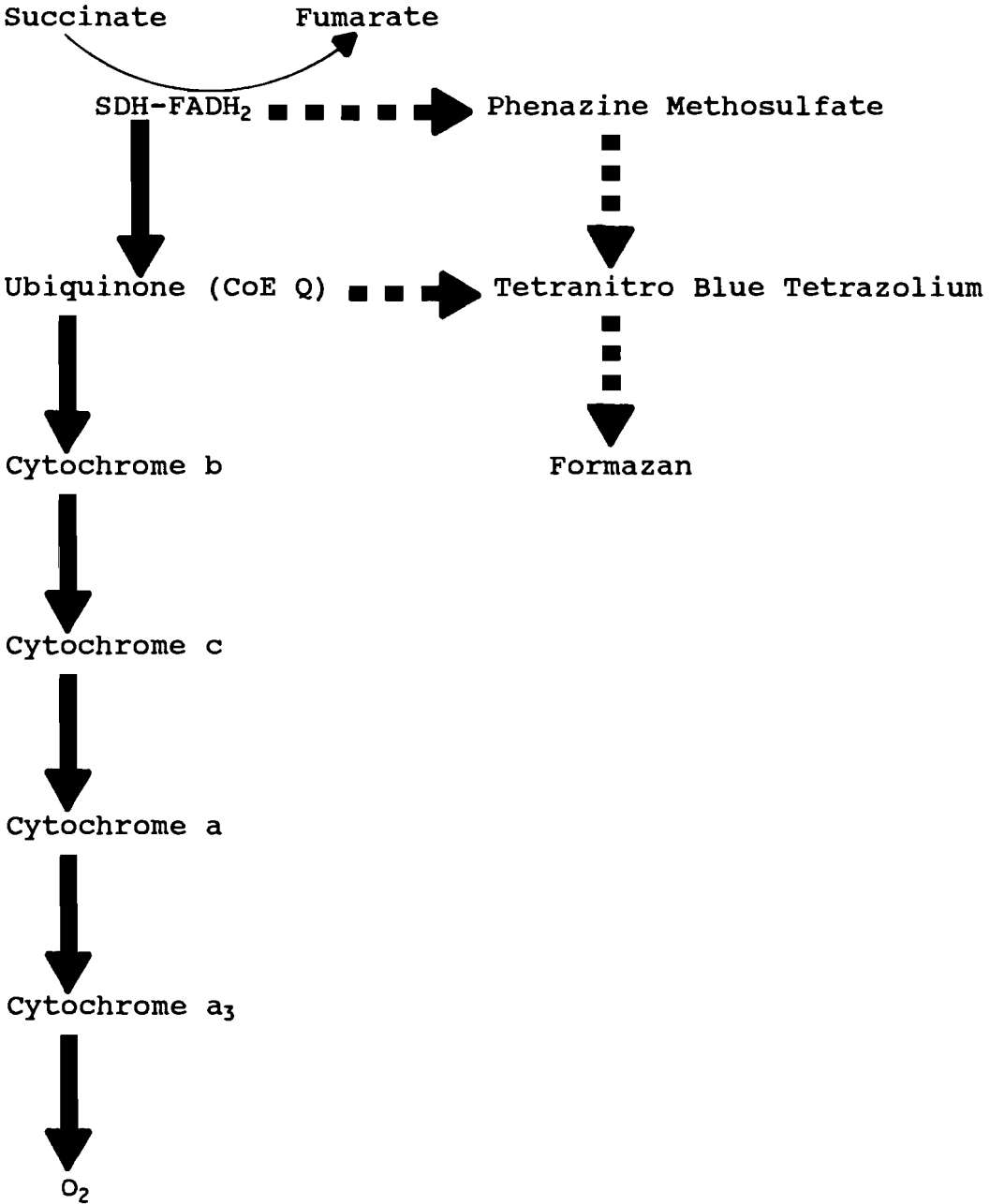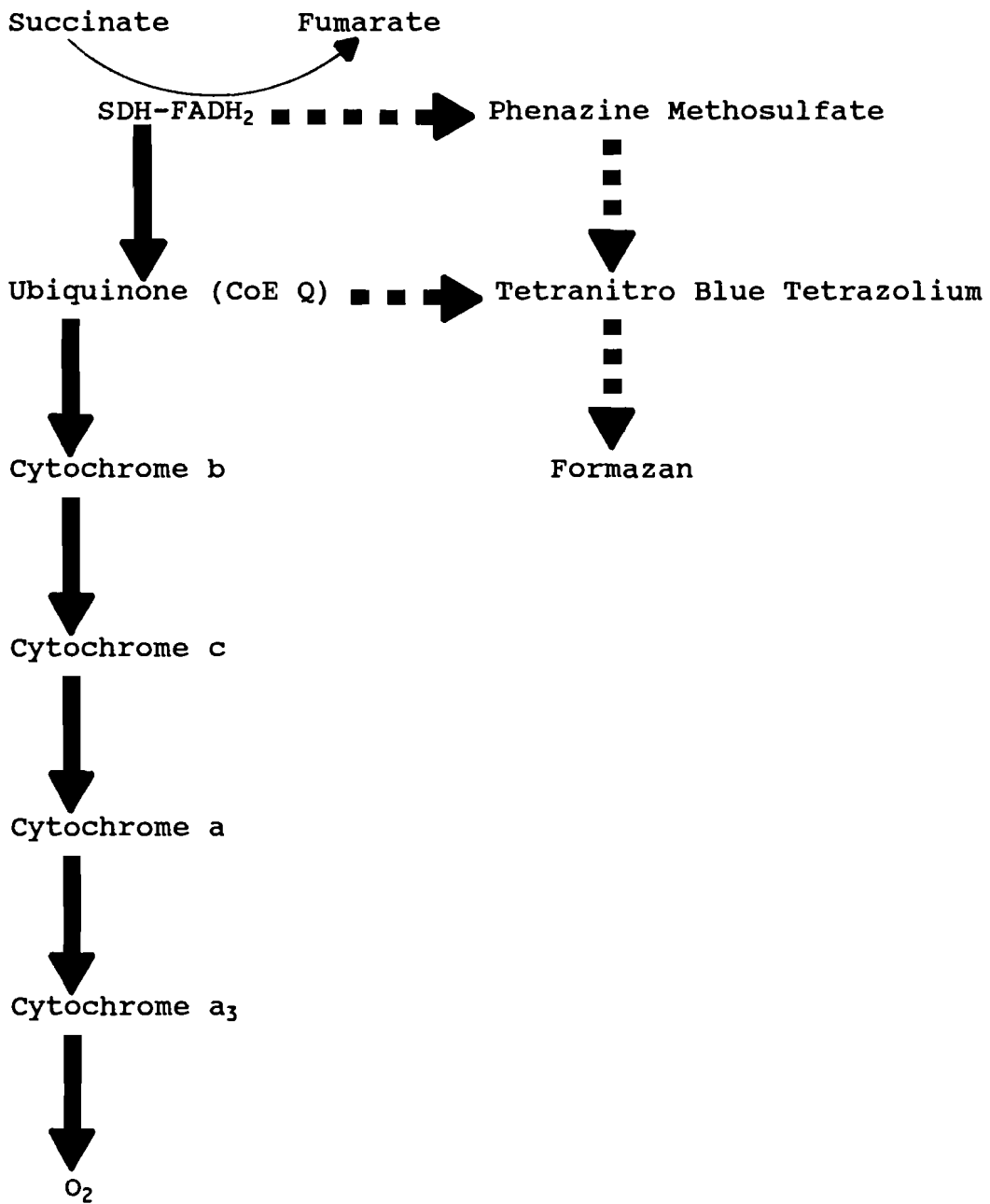
protons) are liberated from the succinate molecule and are temporarily bound to the SDH-FAD complex to produce the reduced form, SDH-FADH$_2$. The electrons are then transferred from the FADH$_2$ part of the complex to the iron-sulfur portion of SDH. These electrons are then passed to an intermediate electron carrier, ubiquinone (coenzyme Q), for transport down the electron transport chain.

An intermediate electron carrier (such as naturally-occurring ubiquinone) is necessary but not always present in sufficient quantities in the section. Therefore, the artificial electron carrier phenazine methosulfate was added to accept the electrons from the SDH molecule and transfer them to a visualizing agent, in this case tetranitroblue tetrazolium (TNBT). If sufficient coenzyme Q is present, the electrons may be shuttled by it to the visualizing agent (Barka and Anderson, 1965, Troyer, 1980, Dubowitz, 1985).

TNBT is a colorless, water-soluble heterocyclic ring compound. When reduced, TNBT forms a pigmented, water-insoluble formazan compound at the enzymic sites (Barka and Anderson, 1965 and Troyer, 1980). The mitochondria stained by this technique are usually positioned adjacent to the A band on either side of the Z line in the intermyofibrillar space (Dubowitz and Brooke, 1973).

Staining intensity depends on mitochondrial SDH concentration and β-red fibers stain the darkest. α-red fibers have moderate amounts of SDH and stain an

intermediate intensity. α-white fibers have the least SDH and stain very little or not at all (Solomon and Dunn, 1988).

The second enzyme detected by the combined technique is myosin ATPase, one of several animal ATPases which differ in intracellular localization, in activators and inhibitors, and in optimal pH. For example, cell membrane ATPase is activated by Na^+ and K^+ ions, requires Mg^{2+}, is inhibited by Ca^{2+} and ouabain, and has an optimal pH of 7.5. In contrast, myosin ATPase is activated by Ca^{2+} and has an optimal pH of 9 (Dubowitz, 1985). Myosin ATPase, like other non-mitochondrial ATPases, catalyzes the hydolysis of ATP to adenosine diphosphate (ADP), hydrogen ion, and inorganic orthophosphate. The energy liberated by this reaction drives muscle contraction.

Myosin ATPase is located in the A band regions of the myofibrils, where it can readily be seen in longitudinal sections (Dubowitz and Brooke, 1973).

Myosin ATPase is detected in the section by the stepwise addition of solutions containing ATP as substrate, then calcium, cobalt, and sulfide. ATP is hydrolyzed by ATPase to produce inorganic phosphate ions, which combine with calcium ion to produce a colorless calcium phosphate precipitate at the sites of enzyme activity. Calcium cations are exchanged with cobalt cations, producing cobalt phosphate. Visualization is achieved by the exchange of

phosphate anions with sulfide. The resulting cobalt sulfide is black and clearly visible (Barka and Anderson, 1965).

The pH at which the myosin ATPase reaction takes place is critical. Human muscle has at least two myosin ATPase isozymes which differ in their pH optima (Fig. 3). Acid or alkaline preincubation solutions at a particular pH can be used to select which of the isozymes is active. Preincubation at pH 4.3 activates the myosin ATPase isozyme of the β-red fibers (so they stain darkly) but fails to activate the ATPase found in α fibers (so they do not stain at all). When this "two fiber" method for myosin is preceded by the SDH method in the combined technique (Fig. 1), the myosin ATPase stain is superimposed on the existing mitochondrial SDH stain. From the two stains three fiber types can be clearly demonstrated (Dubowitz and Brooke, 1973). Horak (1983) has found that the pH which differentiates myosin ATPases may vary between species but remains constant within a species.

Fig. 3. pH sensitivity of human myosin ATPase
         isozymes (modified from Dubowitz and
         Brooke, 1973)

Muscle
Fiber
Type

β-Red

α-Red

α-White

4.0   4.5   5.0   9.5   10.0   10.5   11.0
                      pH of Preincubation Solution

████   indicates a well-defined stain

▒▒▒▒   indicates a progressive loss of stain

MATERIALS AND METHODS

Collection of Animals

The two species examined in this study were the migratory mourning dove and the non-migratory northern bobwhite. All birds were legally collected in Kansas by shooting. Three doves were taken from 1 September 1989 to 10 September 1989. Three quail were taken from 11 November 1989 to 20 December 1989. The muscles examined from both species were the pectoralis and the tibialis anterior.

All of the birds were either dissected immediately after death or were stored at 4° C and dissected within five days post-mortem. Eriksson et al. (1980) showed that human muscle fiber-typing is reliable in specimens stored at 4° C for as long as 10 days post-mortem.

Tissue Preparation

Mid-belly pieces were removed from the tibialis anterior muscle. Superficial (less than 3 mm) and deep samples were taken from the pectoralis, from the proximal 1/2 of the muscle belly approximately 1/2 the distance from the cranial to caudal end of the keel of the sternum (Rosser and George, 1986). All samples were mounted onto circles of sheet cork (1.6 mm thick x 2.5 cm diameter) using Miles O.C.T. embedding medium and/or a 10% solution of gum tragacanth. The tragacanth (specified by Dubowitz and Brooke, 1973) generally provided better support, especially for the smaller samples.

The cork and the sample were quick-frozen by immersion

for approximately 15 seconds into a beaker of isopentane
that had been precooled with liquid nitrogen until it became
gel-like. Freezing for too long can cause large cracks in
the sample and for too short a time can result in cell
destruction by ice crystal formation (Dubowitz, 1985).
After each sample was frozen, it was immediately placed into
a precooled cryostat (-20° C) to prevent thawing. The
isopentane remaining on the sample quickly evaporated at the
higher cryostat temperature and evaporation was judged
complete when the sample lost its shiny appearance. Samples
not to be sectioned that day were placed in pre-cooled
plastic bags and stored at -70° C. Long term storage of the
tissue at this temperature does not lead to enzyme
degradation (Dubowitz, 1985).

Sectioning

Tissues were sectioned in cross-section with an IEC
Damon Microtome-Cryostat. The cork with embedded sample was
mounted on the microtome chuck with a small amount of O.C.T.
To prevent thawing, the tissue was left in the cryostat and
handled only with cold forceps. Sections were cut 12-μm
thick at -20° C. Room temperature cover slips were brought
into close proximity to "attract" the cold sections, which
were then allowed to air dry at room temperatures from 30 to
180 min. (Guth and Samaha, 1970).

Staining

The sections were subjected to the SDH "reversed" myosin-ATPase stain procedure in cover slip jars. All sections were preincubated in an acid solution for 10 min.

To establish the optimum acid preincubation pH, a preliminary study was conducted for each species. The sections were exposed to several pH values to find the pH that gave maximum differentiation. The optimum pH was found to be between 4.05 and 4.10 in both species. After completion of the acid preincubation, all sections were subjected to the procedure given below. The composition of each solution is shown in the Appendix.

SDH - ATPase COMBINED STAIN PROCEDURE

1. Acid Preincubation Solution: 10 min. incubation time at room temperature; pH 4.05 to 4.10.

2. Rinse Solution: Two 1 min. rinses at room temperature; pH 7.8.

3. SDH Solution: 45 min. at 37° C.

4. Rinse in distilled water for 30 sec.

5. Incubation (ATP) Medium: 30 min at 37° C; pH 9.4.

6. 1% $CaCl_2$: 3 changes of 30 sec. each.

7. 2% $CoCl_2$: 3 min.

8. Distilled water: 4 changes for 30 sec. each.

9. 2% $(NH_4)S_2$: 3 min.

10. Rinse in running tap water for 3 min.

11. Rinse in distilled water for 3 min.

12. Rinse in 50% EtOH for 2 min.

13. Drain, dry, and mount with glycerol gel.

Analysis of Slides

Three randomly chosen microscope fields for each muscle site in each bird were photographed to produce 5x7 in. photomicrographs. These were analyzed to determine the mean fiber area (in μm^2) for each fiber type and the percentage of the total muscle cross-section occupied by each fiber type.

A polar planimeter was used to measure the cross-sectional area of 30 fibers (10 from each photomicrograph) for each fiber type, in each muscle, bird, and species. All planimetry measurements were made in triplicate to obtain a mean value (in arbitrary planimeter units), which was later converted to μm^2.

To determine the percentage of each muscle's cross-section occupied by each fiber type, a transparency with 300 points arranged in a 17 cm X 21 cm grid was randomly placed over each micrograph. The points that fell onto each fiber type were tallied and a percentage of total area calculated.

Statistical Analysis

A two-way analysis of variance was used to test for difference in mean cross-sectional area and percentage of each fiber type between the muscles and between the species. Significant interaction between muscle and species were also determined. Statistical analyses were performed using

Biostat I statistical package (Pimentel and Smith, 1986).

RESULTS

Number of Fiber Types In Each Muscle

The combined SDH and myosin-ATPase staining technique
provided clear differentiation of the α-white, α-red, and β-
red fiber types. The lightly stained α-white and the
intermediately stained α-red fibers were found in the
pectoralis and the tibialis anterior muscles of both species
(Fig. 4-8). The darkly stained β-red fiber was found only
in the bobwhite tibialis anterior muscle (Fig. 8).

Pattern of Distribution

In dove pectoralis the larger-diameter α-white fibers
tended to border each fascicle and the smaller-diameter α-
red fibers tended to be clustered toward the center of each
fascicle (Fig. 4 and 5). The α-white and the α-red fibers
within the bobwhite pectoralis (Fig. 6) seemed to be
randomly scattered when compared to the same fibers within
the dove pectoralis (Fig. 4 and 5). The β-red fibers within
the bobwhite tibialis anterior (Fig. 8) are often surrounded
by α-red fibers and have few, if any contacts with α-white
fibers. The α-red fibers of the dove tibialis anterior
(Fig. 7) are often clumped together in groups of closely
packed fibers.

Percentage Composition of Muscle Cross-section

By using the previously described "point" method, the
percentage of the cross section occupied by each fiber type
was calculated for the specified location for each muscle

Fig. 4.   Cross-section of superficial dove
          pectoralis. The stippled arrow points to
          an α-red fiber and the open arrow points
          to an α-white fiber (calibration bar =
          100μm).
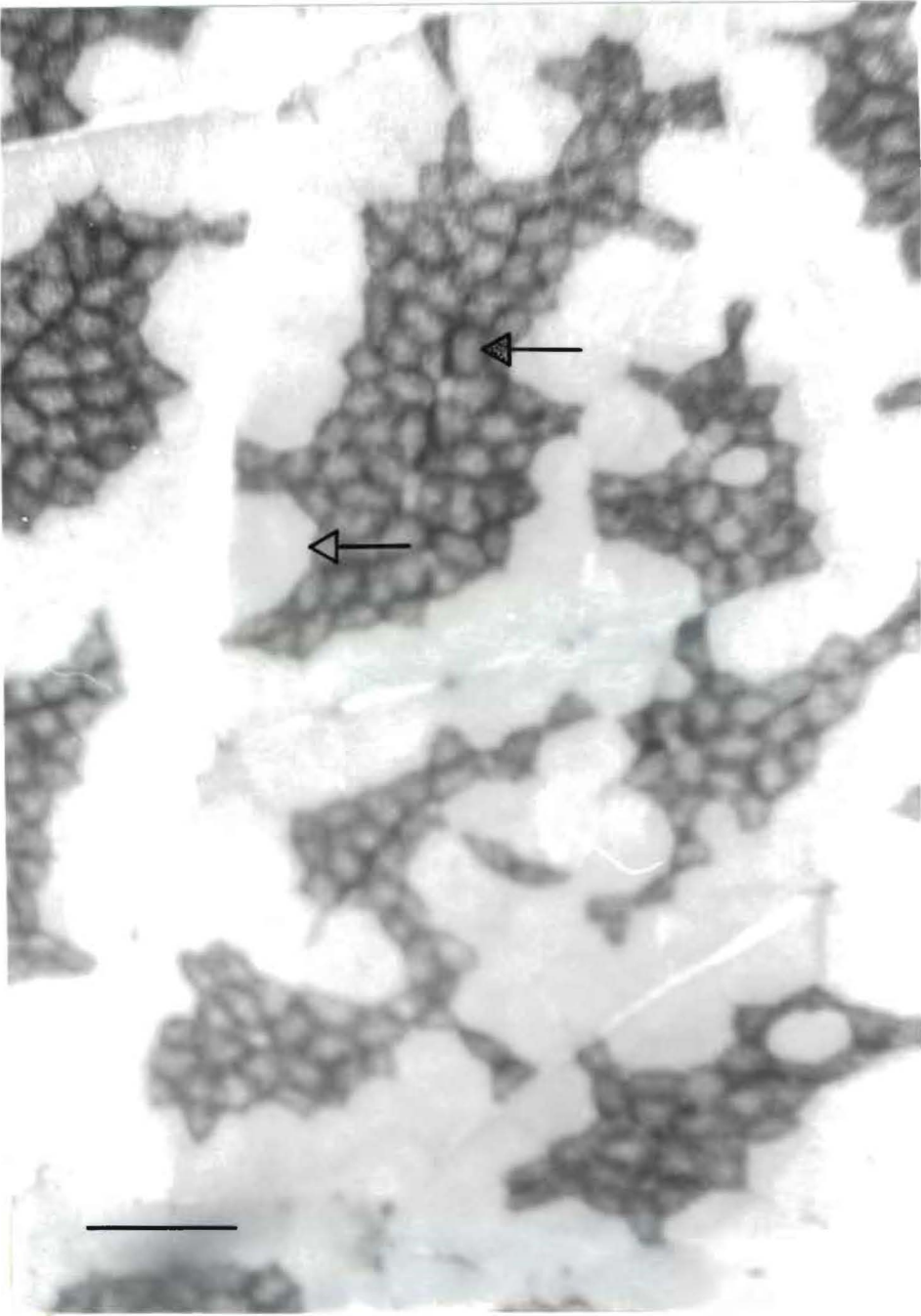
Fig. 5. Cross-section of deep dove pectoralis.
The stippled arrow points to an α-red
fiber and the open arrow points to an α-
white fiber (calibration bar = 100μm).
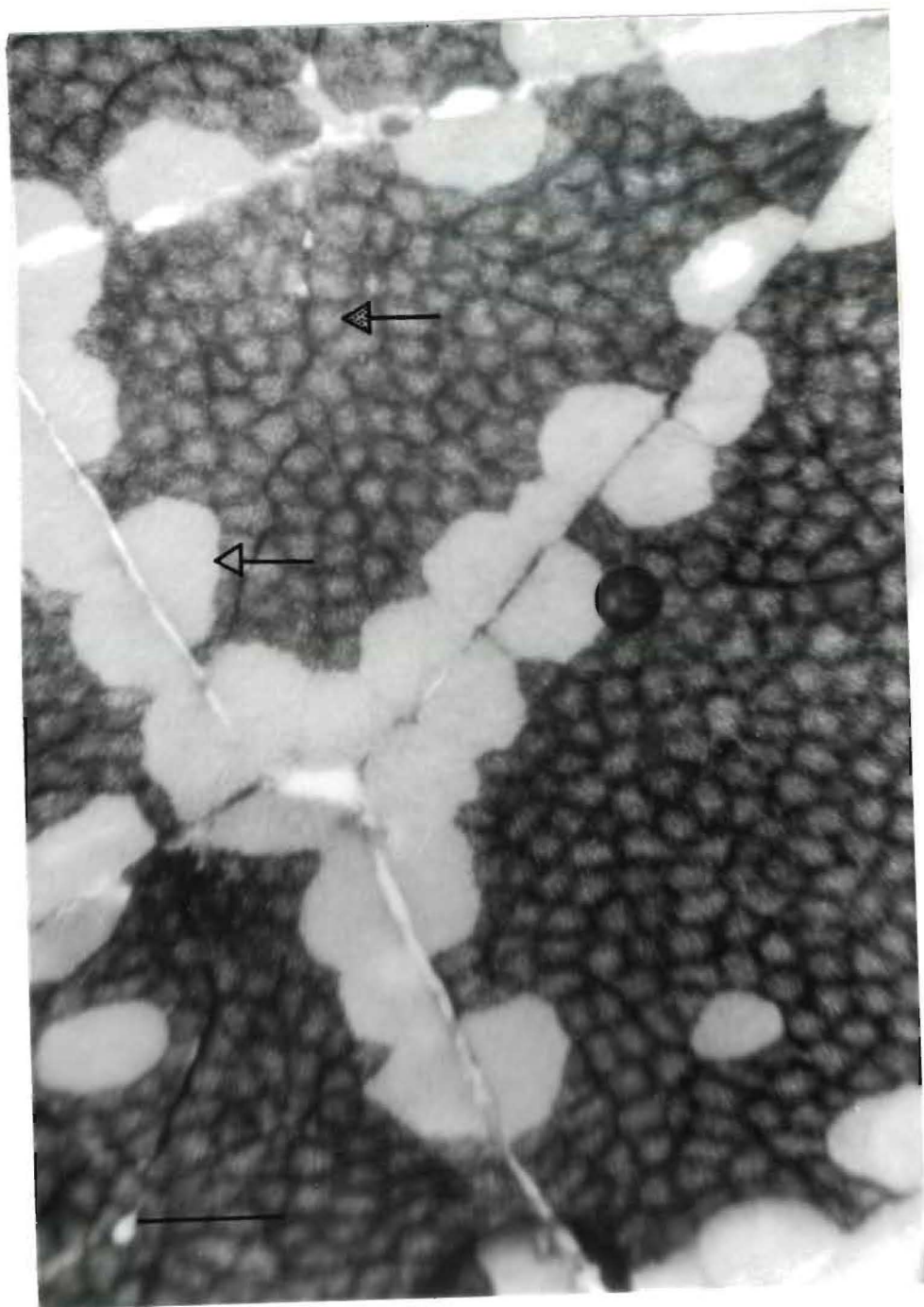
Fig. 6. Cross-section of deep bobwhite pectoralis.
         The stippled arrow points to an α-red
         fiber and the open arrow points to an α-
         white fiber (calibration bar = 100μm).
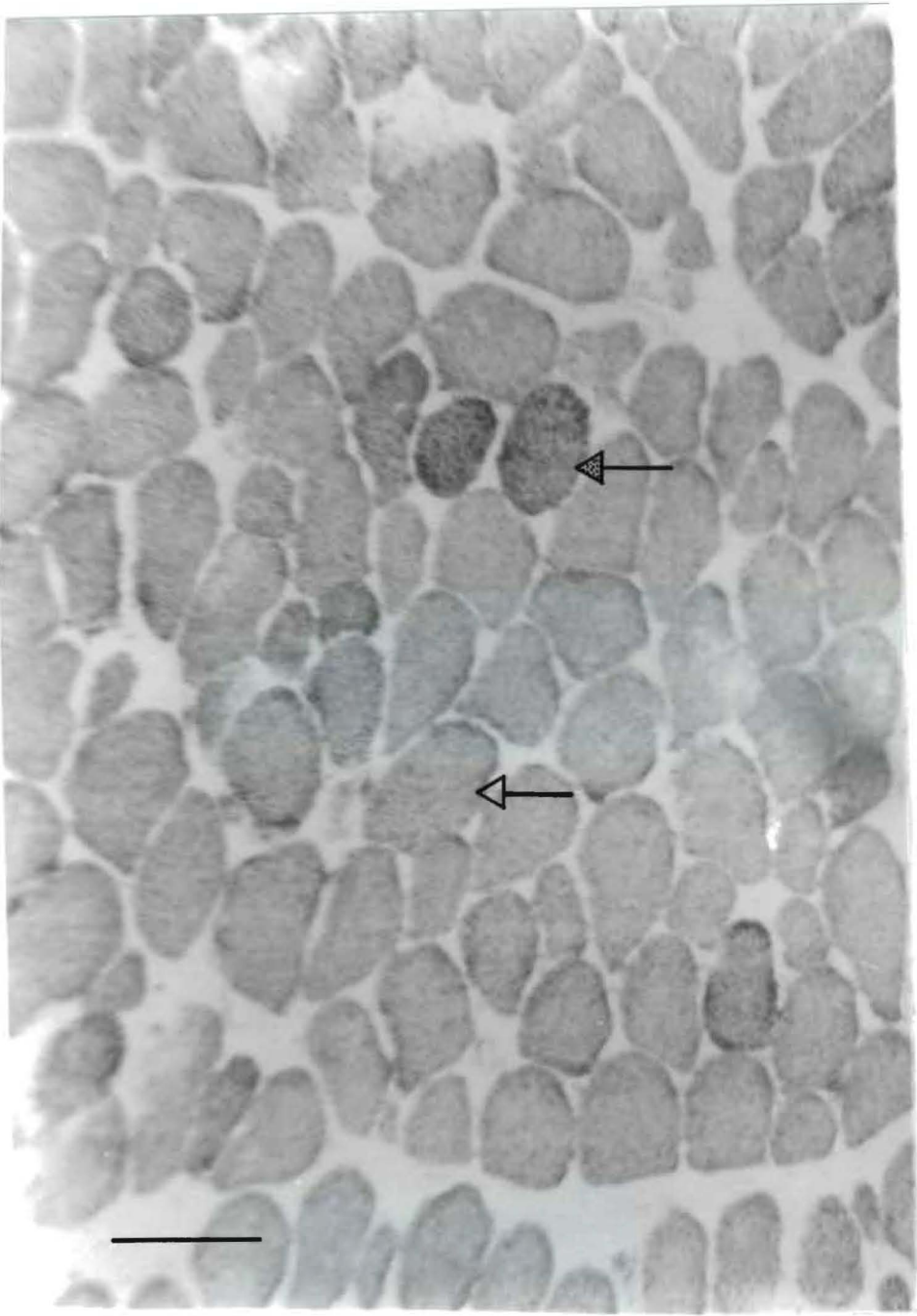
Fig. 7.   Cross-section of dove tibialis anterior.
          The stippled arrow points to an α-red
          fiber and the open arrow points to an α-
          white fiber (calibration bar = 100μm).
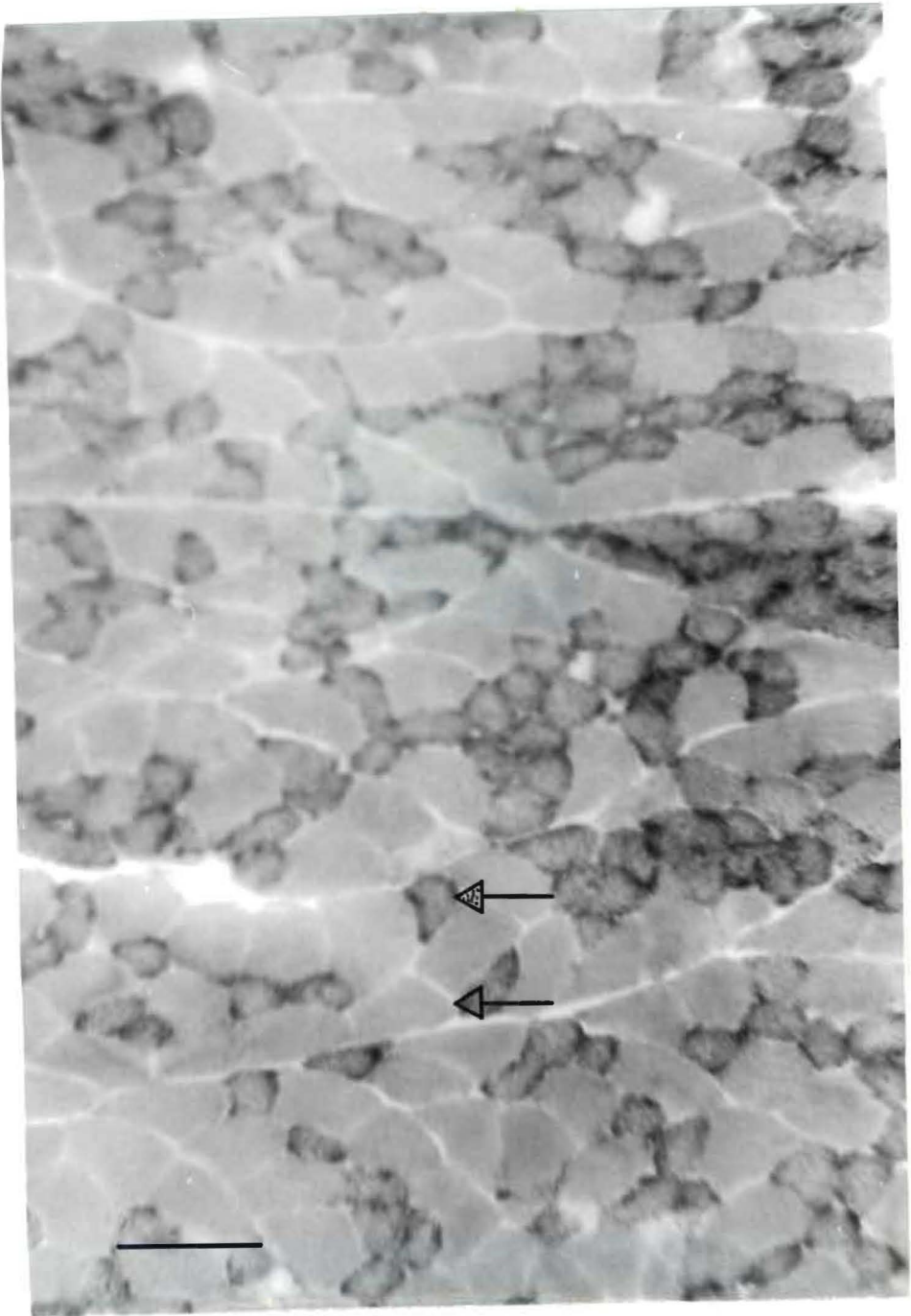
Fig. 8. Cross-section of bobwhite tibialis
         anterior. The closed arrow points to a β-
         red fiber, the stippled arrow points to an
         α-red fiber, and the open arrow points to
         an α-white fiber (calibration bar =
         $100\mu m$).
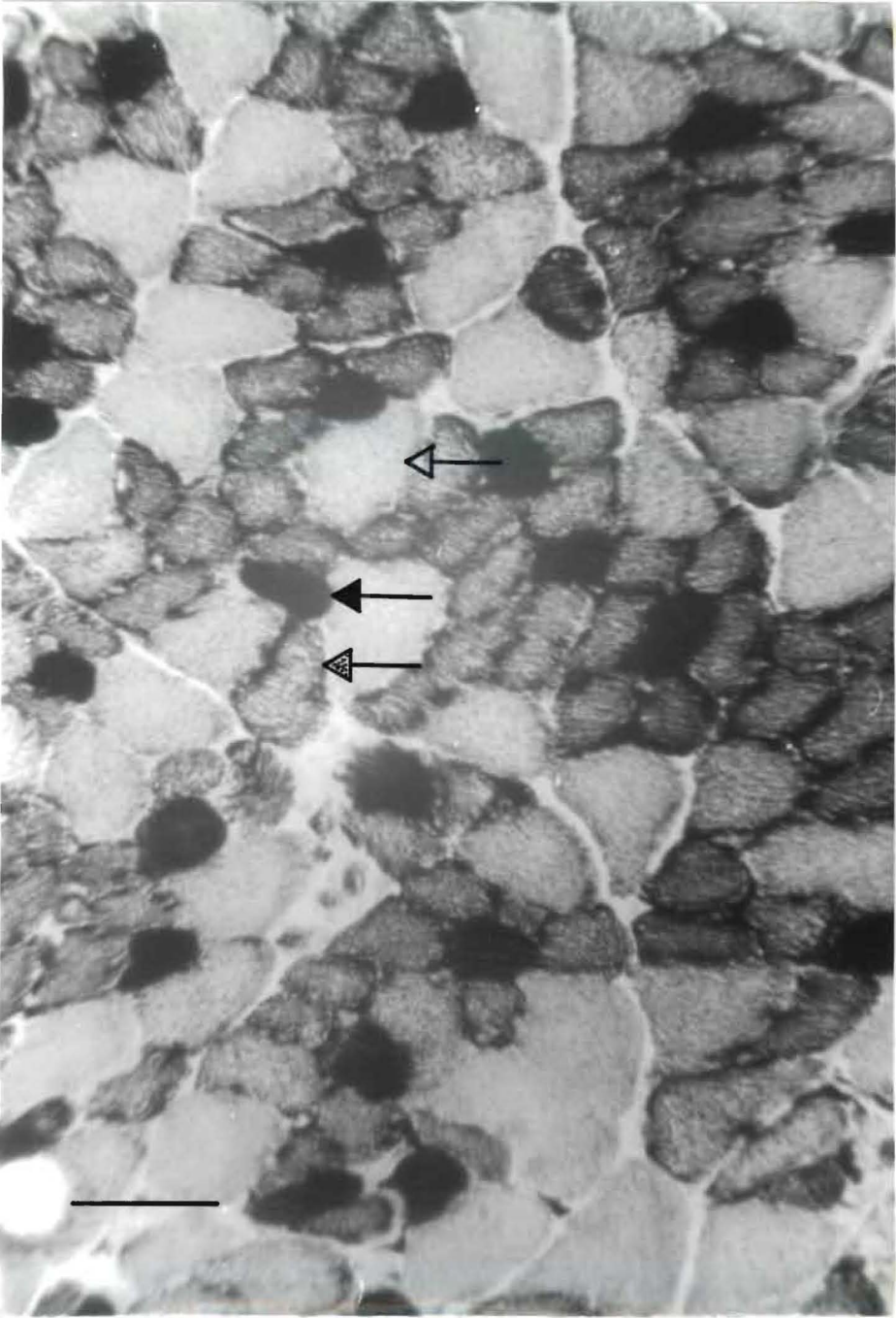
Table 2. Dove and bobwhite pectoralis. Mean fiber size and percentage of sample cross-section occupied by each of the fiber types (means ± S.D.).

Species	Fiber Type	Mean Fiber Size: Cross-sectional Area (μm^2)	Percentage of Cross-section of muscle
Dove (superficial)	α-Red	516.4 ± 109.5	27.1 ± 9.0
	α-White	4444.4 ± 672.9	72.5 ± 8.9
(deep)	α-Red	516.4 ± 78.2	68.3 ± 11.7
	α-White	4397.4 ± 751.1	31.2 ± 11.5
Bobwhite (superficial)	α-Red	2300.4 ± 226.0	8.5 ± 3.2
	α-White	4804.4 ± 422.5	82.1 ± 3.1
(deep)	α-Red	2269.2 ± 219.0	7.9 ± 2.4
	α-White	4835.7 ± 344.3	82.2 ± 2.0

Table 3. Dove and bobwhite tibialis anterior. Mean fiber
size and percentage of sample cross-section occupied by
each of the fiber types (means ± S.D.).

Species	Fiber Type	Mean Fiber Size: Cross-sectional Area (μm^2)	Percentage of Cross-section of muscle
Dove	α-Red	719.9 ± 140.8	31.1 ± 5.2
	α-White	1643.2 ± 203.4	68.9 ± 5.1
Bobwhite	β-Red	1658.8 ± 266.0	6.3 ± 3.3
	α-Red	1846.6 ± 250.4	47.8 ± 5.9
	α-White	4444.4 ± 547.7	42.0 ± 5.5

(Tables 2 and 3). For some muscles the sum of the component fiber types do not add up to 100%. This is due to the presence of vascular, nerve, and connective tissues between the muscle fibers.

A positive interaction exists between the species and muscle depth within the pectoralis for each species. The pectoralis of both species will therefore be discussed separately. The superficial regions of dove pectoralis are composed of a significantly higher percentage (F = 26.92; d.f. = 1,24; P = 0.001) of α-white fibers than the deep portions. The deep regions of the dove pectoralis contain a significantly (F = 26.92; d.f. = 1,24; P = 0.001) higher percentage of α-red fibers than the superficial regions. This difference in the percent composition between superficial and deep parts of the muscle is obvious and can be seen in Fig. 4 and 5.

No significant difference (F = 26.92; d.f. = 1,24; P = 0.001) in percent composition was detected for α-white and α-red fibers within the deep and superficial regions of the bobwhite pectoralis (Table 2 and Fig. 6).

For the tibialis anterior, the percent of α-white fibers differ between dove and bobwhite (Table 3 and Fig. 7 and 8). A small percentage of the darkly-staining β-red fibers (6.3 %) are found in bobwhite but not in dove (Table 3 and Fig. 7 and 8).

The mean fiber size (cross-sectional area in μm^2) for each fiber type (obtained with the planimeter) is listed in Tables 2 and 3. The α-white fibers of the dove and bobwhite pectoralis do not differ significantly (F = 0.14; d.f. = 1,348; P = 0.714) in size between the superficial and deep regions of the muscle. Similarly, the α-red fibers of dove and bobwhite pectoralis do not differ significantly (F = 0.112; d.f. = 1,348; P = 0.744) in size between superficial and deep regions of the muscle.

This uniformity within each species can be seen in Figures 4-6. However, when the fibers in the dove are compared to the analogous fibers in the bobwhite, mean fiber size obviously differs. For example, dove α-red fibers are significantly smaller (F = 11581.89; d.f. = 1,348; P = 0.00) than bobwhite α-red fibers and dove α-white fibers are significantly smaller (F = 312.33; d.f. = 1,348; P = 0.00) than bobwhite α-white fibers. No species X muscle depth interaction exists with regards to α-white (P = 0.22) and α-red (P = 0.08) fiber size. This indicates that size differences between species is uniform across muscle depth.

The β-red fibers from bobwhite tibialis anterior (Table 3) are significantly smaller than the α-red fibers, and the α-red fibers are significantly smaller than the α-white fibers.

DISCUSSION

Adaptations for Flight

Marsh (1984) has shown that the avian pectoralis provides power for the downstroke of the wing during flight. For most species, the pectoralis consists entirely of α-white fibers (George and Berger, 1966). However, the pectoralis in some species has both α-white and the α-red fiber types or, in a very few species all three types, including the β-red (Lundgren and Kiessling, 1988).

Rosser and George (1986) found that the pectoralis of the red-tailed hawk, the double-crested cormorant, and the domestic chicken all have three fiber types, including the highly oxidative β-red fiber. These last are usually found deep within the pectoralis in what has been called the "deep red strip". The function of the β-red fibers in the pectoralis of these species may be stabilization of the shoulder joint during soaring (in the hawk), in diving (in the cormorant), and in postural maintenance (in the chicken). The lack of β-red fibers in both dove and bobwhite pectoralis probably reflects the fact that these birds must actively flap their wings to produce enough force in order to stay airborne.

Talesara and Goldspink (1978) have proposed that the power requirements for flight are so great that most bird species cannot afford the added weight of "slow" fibers that do not contract fast enough to contribute to the generation of force during flight.

The α-white fibers in the superficial dove pectoralis comprise 72.5% of the total cross-sectional area, with the remainder consisting of α-red fibers. George and Berger (1966) found similar proportions in the superficial pectoralis of the domestic pigeon. Rosser and George (1986) believe that this thin layer of "fast" fibers may provide an advantage during take-off when maximum power is needed. Deeper α-red fibers are most likely active during both moderate and intense flight and the superficial α-whites only during intense flight. Henneman (1974) found a similar pattern of use in slow versus fast fibers in cat medial gastrocnemius and soleus muscles.

"Superficial" in this study refers to only the outermost 3 mm of tissue. Therefore, the α-red component of the dove pectoralis (comprising 68.3% in the deep regions) represents the majority of fibers in the whole muscle. The α-red fibers in the deep areas of bird pectoralis are thought to be the most important fiber type for sustained flight (Rosser and George, 1986).

The mourning dove migrates within the confines of the North American continent and can therefore be considered a short distance migrator. The doves in this study were most likely hatched in North Dakota and collected while wintering in Kansas, or were hatched in Kansas and had not yet departed for the southern tier of states (Finck, E.J. pers. comm.). The aerobic requirement for migration is

substantial (Lundgren and Kiessling, 1988) and is fulfilled
by the presence of many α-red fibers that are very oxidative
but still possess "fast twitch" characteristics necessary
for flight.

The mean cross-sectional fiber area in avian pectoralis
fibers is related to their metabolic properties. Lundgren
and Kiessling (1988) measured the area of pectoralis fibers
in long-distance, short distance, and partial/non-migratory
species. They found that as the migration distance
increased, the mean fiber size of α-red fibers decreased.
This inverse relationship occurred in all four short-
distance migrators that were studied and included the
blackbird (<u>Turdus</u> <u>merula</u>) and the goldcrest (<u>Regulus</u>
<u>regulus</u>). A mean α-red fiber area of 981 μm^2 was calculated
for all members of this group. Dove pectoralis α-red fibers
of this study have a mean cross-sectional area of 516 μm^2.
Shorter diffusive distances from the capillaries are a
direct result of this smaller fiber size and lead to
increased aerobic metabolism (Henneman, 1974).

Northern bobwhite are ground-dwelling, non-migratory,
galliformes that can make rapid escape flights at speeds
that may exceed 64 km/h for short distances (Bent, 1963).
The bobwhite pectoralis has a larger percentage of α-white
fibers than does the mourning dove (Fig. 5 vs. Fig. 6).
With both depths combined, only 8.2% of the bobwhite
pectoralis is occupied by α-red fibers. The rarity of the

α-red and the high percentage (82%) of the α-white fiber is comparable to the percentages found by Talesara and Goldspink (1978) in the non-migrating ring-necked pheasant (89% α-white and 11% α-red by number). The high percentage of α-white fibers in bobwhite and pheasant pectoralis represents an adaptation for maximum but short-lived bursts of power. This burst of power can occur because the myosin ATPase within the α-white fibers has the ability to split ATP rapidly. The ground dwelling habit of this bird demands the capability of taking flight quickly to avoid ground predators. However, this rapid burst of power is short in duration because the α-white fibers have few mitochondria to replenish the ATP stored in the muscle.

Muscle fibers involved in short duration contraction with high power production tend to be larger fibers. The α-red fibers in the pectoralis of the partial/non-migratory yellowhammer (Emberiza citrinella) are considered large for passerines (mean = 1411 μm^2) and are not well adapted for the sustained repetitive contractions that are characteristic of migratory flight (Lundgren and Kiessling, 1988). The non-migratory bobwhite of this study has pectoralis α-red fibers that are even larger (2284.8 μm^2, deep and superficial combined) and these are presumably even less adapted for migration. The bobwhite pectoralis α-white fibers are still larger (4820 μm^2, deep and superficial combined), and provide short duration bursts of power.

Hennemen (1974) proposed that large muscle fibers in in general, are activated only when larger increments of tension are needed.

Adaptations for Ground Locomotion

The tibialis anterior in carinate birds (including dove and bobwhite) is responsible for flexion of the tarsometatarsus or foot (Getty, 1975). Any species-specific differences in ground locomotion could therefore be expected to influence the percentages of fiber types in their tibialis anterior muscle.

Turner and Butler (1988) studied leg muscles in the tufted duck (Aythya fuligula), which are adapted for sustained underwater swimming. The lateral gastrocnemius muscle was determined to be 15% β-red fibers and 85% α-red. They concluded that the presence of the β-red fiber and the high percentage of α-red fibers provides an aerobic advantage to this duck when swimming and diving. The leg muscles of cursorial birds could be expected to have the same need for an increased oxidative capacity.

Bobwhite are very good at ground locomotion and would, as the hunters say, "rather run than fly". Whole coveys often run or walk considerable distances while searching for food (Bent, 1963). Some cursorial species have maximal oxygen consumptions that are very near to those found in flying birds (Butler, 1982). If this is true in the bobwhite, the presence of the highly oxidative β-red fiber

(6.3%) and oxidative α-red fiber (47.8%) would most likely
create a greater oxygen demand, but provide a sustainable
energy source and enhance the ability of this species to
fill the ground-dwelling niche.

The dove tibialis anterior has a composition of 30% α-
red and 70% α-white fibers, approximately the reverse of the
deep dove pectoralis. The high percentage of endurance α-
red fibers in dove flight muscle makes functional sense in
light of the importance of the flight muscle to migration.
The lower percentage of α-red fibers in the tibialis
anterior relates to the fact that this species makes little
sustained use of its leg muscles.

LITERATURE CITED

LITERATURE CITED

Ashmore, C.R. and L. Doerr. 1971. Comparative aspects of
      muscle fiber types in different species. Exp. Neurol.
      31, 408-418.

Barka, T. and P.J. Anderson. 1965. Histochemistry: Theory,
      Practice, and Bibliography. Harper & Row, New York. 660
      pp.

Barnard, R.J., V.R. Edgerton, T. Furukawa, and J.B. Peter.
      1971. Histochemical, biochemical and contractile
      properties of red, white and intermediate fibers. Am.
      J. Physiol. 220, 410-414.

Bent, A.C. 1963. Life Histories Of North American
      Gallinaceous Birds. Dover Pub., New York. 490 pp.

Brooke, M.H. and K.K. Kaiser. 1970. Three "myosin adenosine
      triphosphatase" systems: the nature of their pH
      lability and sulfhydryl dependence. J. Histochem.
      Cytochem. 18, 670-672.

Burke, R.E., D.N. Levine, P. Tsairis, and F.E. Zajac. 1973.
      Physiological types and histochemical profiles in motor
      units of the cat gastrocnemius. J. Physiol. 234, 723-
      745.

Butler, P.J. 1982. Respiration during flight and diving in
      birds. In Exogenous and Endogenous Influences on
      Metabolic and Neural Control (ed. A.D.F. Addink and N.
      Spronk), pp. 103-114. Oxford: Pergamon Press.

Close, R.I. 1972. Dynamic properties of mammalian skeletal
      muscles. Physiol. Rev. 52, 129-197.

Dubowitz, V. 1985. Muscle Biopsy: A Practical Approach.
Bailliere Tindall, Philadelphia. 720 pp.

_____ and M.H. Brooke. 1973. Muscle Biopsy: A modern
approach. W.B. Saunders Company, Philadelphia.
475 pp.

_____ and A.G.E. Pearse. 1960. A comparative
histochemical study of oxidative enzyme and
phosphorylase activity in skeletal muscle. Histo. 2,
105.

Engel, W.K. 1962. The essentiality of histo- and
cytochemical studies of skeletal muscle in the
investigation of neuromuscular disease. Neuro. 12, 778-
794.

Eriksson, P., A. Eriksson, M. Ringquist, and L. Thornell.
1980. The reliability of histochemical fibre typing of
human necropsy muscles. Histo. 65, 193-205.

George, J.C. and A.J. Berger. 1966. Avian Myology.
Acedemic Press, New York. 500 pp.

Getty, R. 1975. Sisson and Grossman's The Anatomy of the
Domestic Animals. W. B. Saunders, Philadelphia. 2095
pp.

Guth, L. and F.J. Samaha. 1969. Qualitative differences
between actomyosin ATPase of slow and fast mammalian
muscle. Exp. Neurol. 25, 138-152.

_____ 1970. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. 28, 365-367.

Henneman, E. 1974. Peripheral mechanisms involved in the control of muscle. In Mountcastle, V. B. (Editor). Medical Physiology; Volume One. C. V. Mosby Company. 617-635.

Horak, V. 1983. A successive histochemical staining for succinate dehydrogenase and "reversed"-ATPase in a single section for the skeletal muscle fibre typing. 78, 545-553.

Hoyle, G. 1983. Muscles and Their Neural Control. John Wiley & Sons, New York. 689 pp.

Humason, G.L. 1979. Animal Tissue Techniques; 4th ed. San Francisco. W. H. Freeman. ?? pp.

Lojda, Z. 1965. Remarks on histochemical demonstration of dehydrogenases. II. Intracellular localization. Folia Morphol. 13, 84-96.

Lundgren, B.O. and K.H. Kiessling. 1988. Comparative aspects of fibre types, area, and capillary supply in the pectoralis muscle of some passerine birds with differing migratory behaviour. J. Comp. Physiol. 158, 165-173.

Marsh, R.L. 1984. Adaptations of the gray catbird _Dumetella carolinensis_ to long-distance migration: flight muscle hypertrophy associated with elevated body mass.

Physiol. Zool. 57, 105-117.

Ogata, T. 1958. A histochemical study of the red and white muscle fibres. I. Activity of the succinoxidase system in muscle fibres. Acta Medica Okayama 12, 216.

Peter, J.B., R.J. Barnard, V.R. Edgerton, C.A. Gillespie, and K.E. Stempel. 1972. Metabolic profiles of three fiber types of skeletal muscle in Guinea pigs and rabbits. Biochem. 11, 2627-2633.

Pimentel, R.A. and J.D. Smith. 1986. BIOSTAT I. Sigma Soft, Placentia, CA.

Rosser, B.W.C. and J.C. George. 1986. The avian pectoralis: histochemical characterization and distribution of muscle fiber types. Can. J. Zool. 64, 1174-1185.

Solomon, M.B. and M.C. Dunn. 1988. Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine and porcine skeletal muscle. J. Anim. Sci. 66, 255-264.

Suzuki, A. and H. Tamate. 1979. Histochemical properties and fiber type composition of the pectoral and thigh muscles of the Japanese quail. Acta Histochem. Cytochem. 12, 69-74.

Talesara, G.L. and G. Goldspink. 1978. A combined histochemical and biochemical study of myofibrillar ATPase in pectoral, leg and cardiac muscle of several species of bird. Histochem. Journ. 10, 695-709.

Troyer, H. 1980. Principles and Techniques of
    Histochemistry. Little, Brown & Company, Boston. 431
    pp.

Turner, D. L. and P. J. Butler. 1988. The aerobic capacity
    of locomotory muscles in the tufted duck, _Aythya
    fuligula_. J. Exp. Biol. 135, 445-460.

APPENDIX

COMPOSITION OF SOLUTIONS

Acid Preincubation Solution:

    0.18 M $CaCl_2$ 100.0 ml

    glacial acetic acid   3.0 ml

    distilled water   900.0 ml

    add above together and adjust to appropriate pH

Rinse Solution:

    tris-(hydroxymethyl)-aminomethane   12.1 g

    0.18 M $CaCl_2$ 100.0 ml

    distilled water   900.0 ml

    add above together and adjust to pH 7.8

*SDH solution:

    a) dissolve 25 mg tetranitroblue tetrazolium
       (TNBT) in 1.25 ml N,N-dimethylformamide

    b) dissolve 2.5 g sodium succinate in 50.0 ml
       distilled water

    c) add above solutions together and then pour
       in 100.0 ml 0.2 M Tris buffer (pH 7.4)

*ATP incubation medium:

    1.5 M Sigma 221 Buffer   10.0 ml

    0.18 M $CaCl_2$ 15.0 ml

    KCL   0.555 g

ATP   0.228 g

distilled water   120.0 ml

Add the above together, adjust pH to 9.4, bring

final volume to 150 ml and filter before use.

* indicates those solutions that need to be made fresh

Mark E La Barge
Signature of Graduate Student

Edward C Rowe
Signature of Major Adviser

I, _____ Mark Edward La Barge _____ , hereby submit this thesis to Emporia State University as partial fulfillment of the requirements for an advanced degree. I agree that the Library of the University may make it available for use in accordance with its regulations governing materials of this type. I further agree that quoting, photocopying, or other reproduction of this document is allowed for private study, scholarship (including teaching) and research purposes of a nonprofit nature. No copying which involves potential financial gain will be allowed without written permission of the author.

Mark E La Barge
Signature of Author

10 July 1990
Date

Muscle Fiber Types in a Migratory and a Non-migratory Avian Species
Title of Thesis

Jackie Tollert
Signature of Graduate Office Staff Member

July 10, 1990
Date Received